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Cytogenetics and systematic approach on a new *Oryzomys* species, of the *nitidus* group (Sigmodontinae, Rodentia) from Northeastern Brazil

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Abstract — The diploid number $2n$=76 was found in five specimens (four males and one female) of a new species of *Oryzomys* belonging to the *nitidus* group (Sigmodontinae, Rodentia) from Pacoti, state of Ceará, Northeastern Brazil. Cytogenetic data included CBG and GTG-banding, Ag-NOR staining as well as fluorescence *in situ* hybridization (FISH) with $(T_2AG_3)_n$ as probe, which revealed exclusive signals in all telomeres of all chromosomes. The karyotype comprises two large pairs (1 and 3) and four small metacentric pairs (34 to 37); 31 acrocentric pairs from large to small (pairs 2, and pairs 4 to 33). The X chromosome is a large subtelocentric and the Y is a medium size chromosome with two different morphologies: acrocentric and submetacentric. Pairs 1 and 3 are the result of Robertsonian rearrangements when compared with the $2n$=80 karyotypes observed in *Oryzomys russatus, O. nitidus* and *O. emmonsae*. Interstitial telomeric sites (ITS) after FISH were not detected in those specific chromosomes.

Key words: FISH, *Oryzomys*, Robertsonian rearrangements, Rodentia.

INTRODUCTION

Oryzomys is one of the most important and diverse genus in tropical forest communities of South America. It belongs to the subfamily Sigmodontinae, and includes about 40 species (MUSSER and CARLETON 1993; WEKSLER 1996; MUSSER *et al.* 1998; PERCEQUILLO 1998). This genus presents the widest geographic distribution within sigmodontine rodents, occurring from southeastern United States to northern Argentina. As a consequence of its diversity and extensive geographic distribution, the genus was never entirely reviewed until the middle of 90's, when some efforts to revise the group finally emerged (WEKSLER 1996; MUSSER *et al.* 1998; PERCEQUILLO 1998).

Cytogenetic information has been a powerful tool in the last decades in sigmodontine species recognition and definition (GARDNER and PATTON 1976; SILVA and YONENAGA-YASSUDA 1997, 1998a,b; CHRISTOFF *et al.* 2000; BONVICINO *et al.* 1998), including *Oryzomys*. Most of *Oryzomys* species have diploid numbers that vary from $2n$ = 34 to $2n$ = 80 (PEREZ-ZAPATA and AGUILERA 1996; BONVICINO *et al.* 1998; see also MUSSER *et al.* 1998, table 13, p. 80-81). As an example of the diagnostic value of karyotypical data is the discrimination, along with morphological and molecular evidences, of eastern and western Amazonian samples of *Oryzomys megacephalus* in two distinct taxa: *O. megacephalus* and *O. perenensis* (PATTON *et al.* 2000).

In this paper we present the cytogenetic data of a new species of *Oryzomys* (PERCEQUILLO and WEKSLER in prep.) from northeastern Brazil, including conventional staining, CBG, GTG-banding and Ag-NOR, as well as FISH data, and offer some comments on its affinities among *nitidus* group.

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MATERIALS AND METHODS

Material

Seven specimens of *Oryzomys* sp. nov. were collected from Pacoti (04°13'26"S, 38°55'19"W), Serra de Baturité, Ceará state, northeastern Brazil. The animals were trapped between 5th and 18th of October, 1998 and voucher specimens were deposited at the Museu de Zoologia, Universidade de São Paulo (MZUSP). Cytogenetical data were obtained from five specimens.

The region of Serra de Baturité, a highland plateau reaching about 1000 meters of altitude surrounded by lowland plains, shows a mesic environment, an ombrophilic open forest with humid fitoclimate (BRASIL 1973), located in the Caatinga domain. The Caatinga is a semi-arid environment that extends for 800.000 square kilometers in northeastern Brazil and presents a xeromorphic vegetation, characterized by small and deciduous trees, and various species of cacti. These forests that occurs at Caatinga are always associated with highlands and hills and are maintained by orographic precipitation, locally called "brejos". The flora and fauna of these forests exhibit Amazon and Atlantic elements (SNETHLAGE 1922), as a result of the Pleistocene connection between the both forests (VIVO 1997). This is in contrast with the mammalian Caatinga fauna, which is a faunal subset of Brazilian Cerrado (MARES *et al.* 1985).

Chromosomal preparations and banding patterns

Chromosomal preparations were obtained from four males and one female during the fieldwork from bone marrow and spleen after an *in vivo* colchicine treatment. Fibroblast cultures from ear biopsy of two out of that four males and the female were estab-

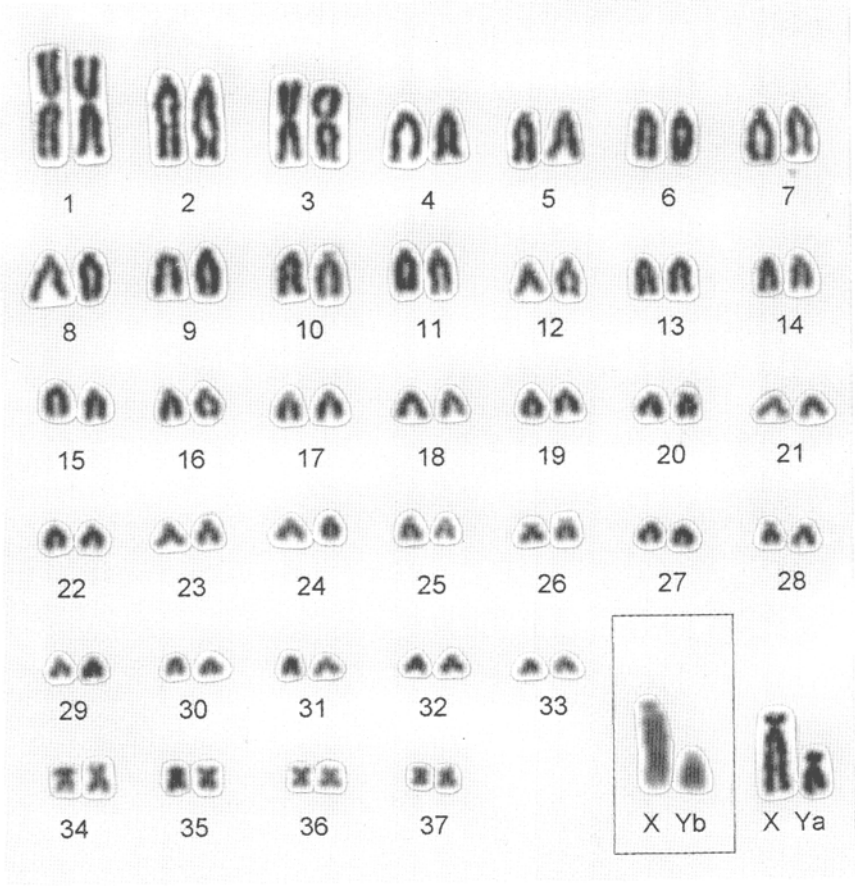

Fig. 1 — Karyotype conventionally stained of *Oryzomys* sp. nov. with $2n=76$; Ya is a submetacentric chromosome. In the rectangle, sex chromosomes from other specimen: Yb is acrocentric.

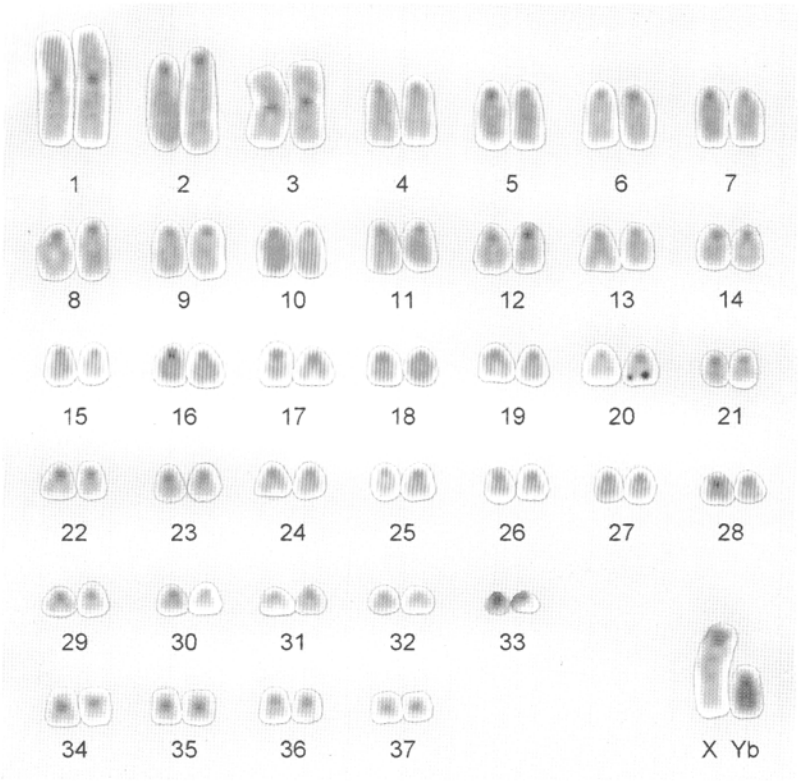

Fig. 2 — CBG banding pattern of *Oryzomys* sp. nov.

lished in laboratory using Dulbecco's modified Eagle medium (DMEM) supplemented with 20% fetal bovine serum. Analysis were carried out after Giemsa, CBG, GTG and Ag-NOR staining, according to routine cytogenetical techniques (SUMNER 1972; SEABRIGHT 1971; HOWELL and BLACK 1980).

Fluorescence in situ hybridization (FISH)

For localization of telomeric sequences, the digoxigenin-labeled (TTAGGG)n deoxynucleotide was used as a probe following the Oncor's protocol (catalog number P5097-DG.5). Hybridization signals were detected by incubation with fluorescein isothiocyanate (FITC)-labeled anti-digoxigenin and the slides were counterstained with propidium iodide in fluorescence antifade solution. Chromosome signals were visualized using a Zeiss Axiophot microscope equipped with a FITC filter and photographed using Ektachrome 400 (Kodak) color slide film.

RESULTS

The diploid number $2n$=76 and fundamental number (FN) = 86 were found. The autosomal complement in the karyotype comprises two large pairs (1 and 3) and four small metacentric pairs (34 to 37), and 31 acrocentric pairs (2 and 4 to 33) which decrease gradually in size. The X was a large subtelocentric and the Y was a medium-size chromosome with two different morphologies: submetacentric (Ya) and acrocentric (Yb). The X and Ya were perfectly distinctive from the autosomes (Fig. 1).

CBG-banding revealed small heterochromatic bands in the pericentromeric regions of all autosomal pairs. The short arm of X and the Y were entirely heterochromatic (Fig. 2).

Analysis of 21 metaphases after Ag-NOR staining revealed intra and interindividual variability with NORs located predominantly on the end of the short arm of small acrocentrics, ranging from 4 to 9 with mean = 6.3 and mode = 7 (Fig 3).

In the Figure 4 we present two GTG-banded karyotypes: Figure 4a shows the GTG-banded chromosomes of the new species with $2n$=76; in the Figure 4b is presented the banding pattern of *O. russatus* ($2n$=80) from São Paulo state (Brazil). Comparison of GTG-banded chromosomes between these two karyotypes revealed the following specific homoelogies, besides all the rest of the complement: concerning the chromosome 1 of $2n$=76, 1q and 1p are equivalent respectively to chromosome 2 and 7 from $2n$=80 karyotype; and regarding to chromosome 3 of $2n$=76, 3q and 3p are probably equivalent to chromosome 12 and 14 from $2n$=80, respectively.

Hybridization signals after FISH were observed exclusively in the telomeres of all chromosomes of $2n$=76 (Fig. 5a) and it was also observed in all chromosomes of the $2n$=80 karyotype from *O. nitidus* (Fig. 5b) trapped in Cláudia, Mato Grosso state (Brazil).

DISCUSSION

In recent and independent taxonomic revisions of genus *Oryzomys*, WEKSLER (1996) and PERCEQUILLO (1998), recognized a new species from Serra de Ibiapaba, Ceará, northeastern Brazil. Based on morphological and morphometrical characters and comparisons with *O. nitidus*, *O. macconnelli*, *O. lamia*, *O. legatus* and *O.russatus*, they found that the Ibiapaba sample was distinct from these taxa. Morphological comparisons of the samples from Serra de Ibiapaba and Serra de Baturité evidenced that these specific samples from both localities belong to the same species. The diploid number $2n$=76 and FN= 86, herein presented, is another evidence for the discrimination of this geographic sample as a distinct species.

The five species mentioned above, plus the sample from Ibiapaba, are grouped by WEKSLER (1996) in the *nitidus* group. MUSSER *et al.* (1998) in a revision of almost the same taxa defined a *nitidus* group very similar in

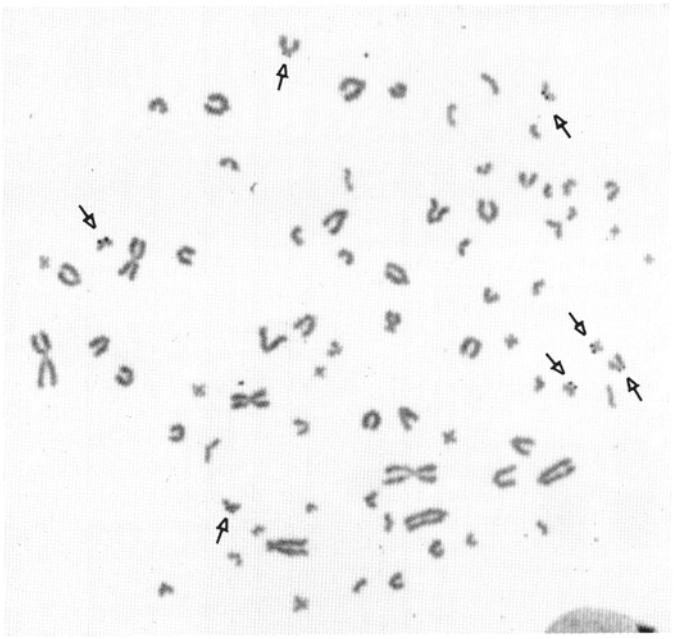

Fig. 3 — NORs-bearing chromosomes of *Oryzomys* sp. nov. Total of 7 Ag-NORs (arrows).

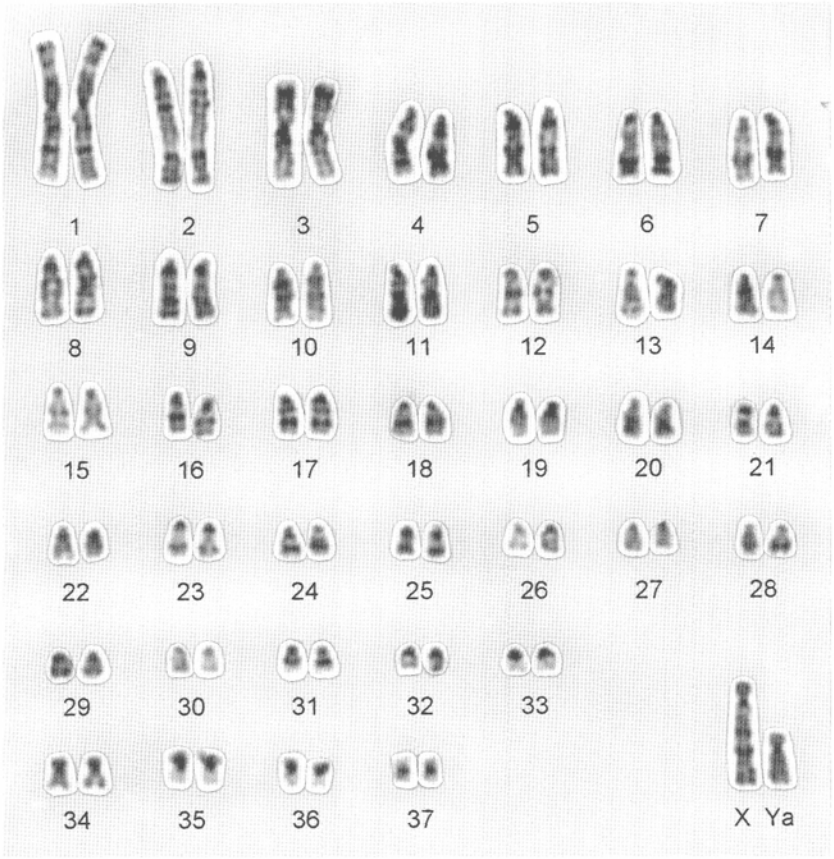

Fig. 4a — GTG banding patterns of *Oryzomys* sp. nov. with $2n$=76 from Ceará state.

composition as that one described by WEKSLER (1996) which includes *O. russatus, O. macconnelli, O. nitidus* and a new species, *O. emmonsae*. They synonymized *O. lamia, O. legatus, O. intermedius, O. kelloggi, O. physodes, O. coronatus* and *O. moojeni* under the name *O. russatus*. However, the synapomorphic character furnished by WEKSLER (1996) for the group, the parafosset on second upper molar teeth, is highly polymorphic in almost all species of the *nitidus* group and highly homoplastic occurring in members of *megacephalus* groups. MUSSER *et al.* (1998) did not furnish any diagnostic characters to *nitidus* group.

In a cladistic analysis of 22 species of *Oryzomys*, PERCEQUILLO (1998) found only one sinapomorphy for the *nitidus* group, long and dense ungeal tuft in feet toes, which is highly homoplastic. However, this author also provided some characteristics for the group, such as presence of squamosoalisfenoid groove and sphenofrontal foramen (pattern 1; Voss 1988), simple palate, with simple posterolateral pits, incisive foramina occupying from 58% to 66% of diastema, and sphenopalatine vacuities absent or only small vacuities around presfenoid-basisphenoid suture (PERCEQUILLO in prep.). As this new species presents these characters, we include it in the *nitidus* group.

Oryzomys russatus, O. nitidus, O. emmonsae and O. macconnelli are the species from which the diploid number are known in the *nitidus* group. The karyotype of *O. russatus* from São Paulo (Brazil) has $2n$=80 and FN=86 (SILVA 1994, described as *O. nitidus*) and the same karyotype is observed in *O. nitidus* from Peru

(GARDNER and PATTON 1976) and *O. emmonsae* from Pará state, Brazil (MUSSER *et al.* 1998). Although *O. lamia* has been considered as synonym of *O. russatus* by MUSSER *et al.* (1998), the karyotype presented $2n$=58 and FN=82 (BONVICINO *et al.* 1998) and probably represents a different species from *O. russatus*. *Oryzomys macconnelli* with $2n$=64, FN=64, was found in Peru (GARDNER and PATTON, 1976) and in Mato Grosso state, Brazil (SILVA *et al.* 2000); $2n$=64, FN=70 was observed in samples from Amazonas state, Brazil (MUSSER *et al.* 1998), and $2n$=76, FN=85 from Venezuela (MUSSER *et al.* 1998).

The $2n$=76 karyotype of the new species presented here is quite similar to that one with $2n$=80 observed in *O. russatus*, *O. nitidus* and *O. emmonsae*, except due to pairs 1 and 3 which are involved in fusion/fission mechanisms when compared with $2n$=80 karyotype. G-banding pattern comparison between the karyotypes of *Oryzomys* sp. nov. and *O. russatus* showed that the long and short arms of chromosome 1 from $2n$=76 are homoeologous, respectively, to chromosome 2 and chromosome 7 of the $2n$=80 karyotype; and long and short arms of chromosome 3 of $2n$=76 are probably equivalent, respectively, to chromosome 12 and 14 of $2n$=80 karyotype. And, although we observed that these two metacentric pairs are involved in fusion/fission rearrangements, no interstitial telomeric sites (ITS) were detected.

The *O. macconnelli* karyotype with $2n$=76 from Venezuela (MUSSER *et al.* 1998) is different from $2n$=76 of *Oryzomys* sp. nov. because

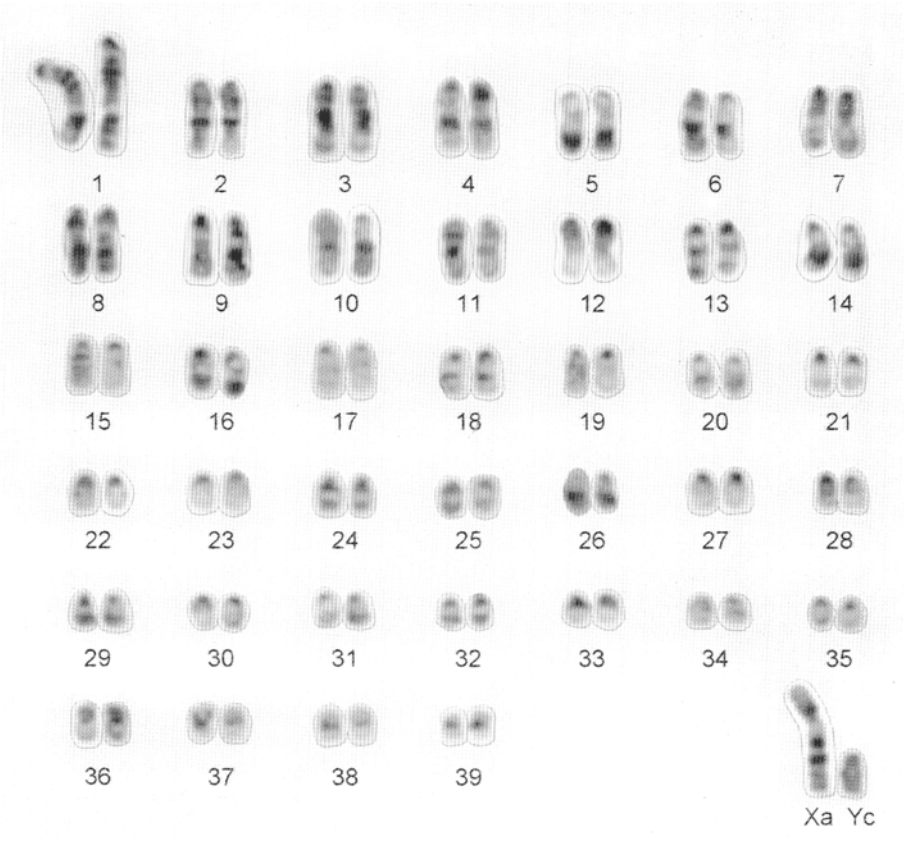

Fig. 4b — *Oryzomys russatus* from São Paulo state, with $2n$=80 and sex chromosomes XaYc according to SILVA (1994).

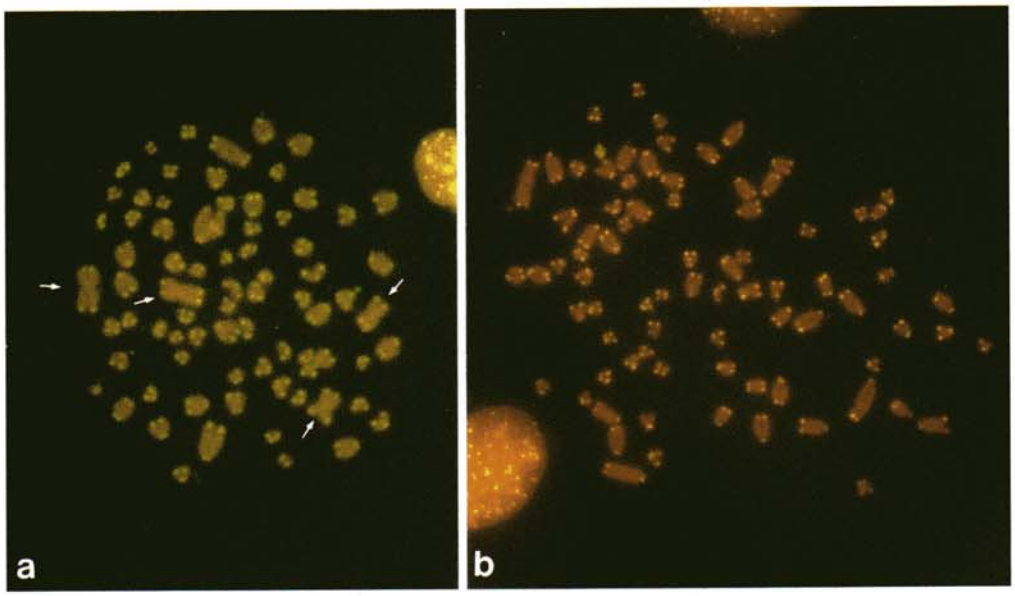

Fig. 5 — FISH with telomeric (TTAGGG)n probes: telomeric signals are evident at all chromosome telomeres. (a) *Oryzomys* sp. nov. with $2n$=76 from Pacoti, Ceará state; large metacentric pairs 1 and 3 (arrows), which are involved in fusion/fission rearrangements, do not show interstitial telomeric sites (ITS). (b) *O. nitidus* with $2n$=80 from Mato Grosso state.

in the former there are no large metacentric chromosomes (there is just a heteromorphic medium pair) and there are five metacentric small pairs while in the latter karyotype, two large metacentrics were observed as well as four small metacentric pairs. Sex chromosomes were also distinctive between both karyotypes.

In spite of being a small sample, we recognized two different Y chromosome morphologies in *Oryzomys* sp. nov., reinforcing the polymorphism of sex chromosomes detected in other Oryzomyini genera (SILVA and YONENAGA-YASSUDA 1998b).

In fact, our data reinforce the importance of karyotyping such a huge group as *Oryzomys*, in order to identify and recognize species, since they share morphological similarities, differing among each other by few anatomical characters, some of then polymorphic, and by morphometrical characters (WEKSLER 1996; MUSSER *et al.* 1998; PERCEQUILLO 1998). The description of this species is being prepared by PERCEQUILLO and WEKSLER, as well the morphological comparisons among members of *nitidus* group.

WEKSLER (1996) and PERCEQUILLO (1998) provided cladograms for species of *Oryzomys* which included the new species here discussed. In both cladograms, the new species appeared as sister group to *O. lamia*, a central Brazilian species. When this information is contrasted against biogeographical hypothesis concerning the evolution of the Caatinga mammal fauna, it seems to us that this case would represent speciation within a previously widely distributed open vegetation Cerrado-Caatinga mammal fauna. However, the karyotypic data reveals that the affinity of *Oryzomys* sp. nov. may be to either one of its rainforest neighbor species, namely *O. russatus* (Atlantic Forest) and *O. emmonsae* and *O. nitidus* (Amazonian rainforest).

On the other hand, both WEKSLER (1996) and PERCEQUILLO (1998) do not found any synapomorphy for the clade *O. lamia* + *Oryzomys* sp. nov. Both trees showed the sister group relationship above as a result of consensus analysis and no character supported the clade. It is important to notice that these hypothesis of phylogenetic relationships were based on a limited number of species of *Oryzomys* and probably will change with the inclusion of additional taxa. Moreover, the inclusion of cytogenetic and molecular data in future analysis will certainly enhance our comprehension of relationships within *Oryzomys*.

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