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An undescribed karyotype for Thaptomys (2*n*=50) and the mechanism of differentiation from *Thaptomys nigrita* (2*n*=52) evidenced by FISH and Ag-NORs

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Abstract - *Thaptomys nigrita* is a monotypic species with 2*n*=52 from Akodontini tribe. The karyotype is composed by 25 pairs of autosome being 24 acrocentric decreasing in size and a small metacentric pair. X and Y are respectively a medium size acrocentric and a small submetacentric.
In this paper we report for the first time a karyotype with 2*n*=50 for an undescribed species of genus *Thaptomys*. This new karyotype is composed by 24 pairs of acrocentric autosomes decreasing in size; X and Y chromosomes are respectively a large acrocentric and a small submetacentric; Heterochromatic blocks are observed in the pericentromeric regions of all autosomes and X chromosome, whereas the long arm of the Y is entirely heterochromatic. Multiples Ag-NORs are located at the telomeric regions of the long arm of the autosomes, and a single chromosome pair (24) presents Ag-NORs in both telomeric regions, which is similar to the pattern observed in the metacentric autosome pair 25 of *Thaptomys nigrita* with 2n=52. It can be suggested that this pair 24 has undergone a pericentric inversion and originated the acrocentric pair in *Thaptomys* sp. with 2n=50. G-banding pattern and interstitial telomeric signal (ITS) by FISH suggest that the karyotype differentiation between the karyomorphs with 2*n*=52 in *Thaptomys nigrita* and 2*n*=50 of *Thaptomys* sp. was due to a tandem fusion involving respectively pairs 2 and 24 from the former resulting in pair 2 of the latter. We propose that this new karyotype with 2*n*=50 belongs to a new and cryptic species for the genus *Thaptomys*, since these two entities seem to be morphologically indistinguishable and the geographic localization plus the chromosome rearrangements can represent a reproductive barrier between these two forms.

Key words: Rodentia; *Thaptomys;* karyotype; cryptic species; tandem fusion.

INTRODUCTION

Of the approximately 4.600 species of living mammals (WILSON and REEDER 1993), about 1.115 are known from the Neotropics (PATTERSON 1994) and approximately 80% are endemic to that region. Unknown species have been recognized by careful and extensive fieldwork in previously poorly sampled areas and new species are being discovered at an impressive rate (PATTERSON 1994).

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Fig. 1 – Geographical distribution of *Thaptomys nigrita* (A-O) and *Thaptomys* sp. (✦) in southeastern Brazil. (A) Sapiranga, RS- 29° 38'S, 51° 00'W; (B) Torres, RS- 29° 20'S, 49° 43'W (Castro, 1989); (C) Itapetininga, SP- 23° 35'S, 48° 03'W (Souza, 1981); (D) Iguape, SP- 24° 42'S, 47° 33'W (Souza, 1981; Fagundes, 1993); (E) Salesópolis, SP- 23° 31'S, 45°` 50'W (Geise, 1995); (F) São Bernardo do Campo, SP- 23° 41'S, 46° 33'W (Present paper); (G) Biritiba – Mirim, SP- 23° 34'S, 46° 02'W (Present paper); (H) Parati, RJ- 23° 10'S, 44° 47'W; (I) Itatiaia, RJ- 22° 27'S, 42° 58'W; (J) Teresópolis, RJ- 22° 27'S, 42° 58'W; (K) Sumidouro, RJ- 22° 12'S, 42° 43'W; (L) Nova Friburgo,RJ- 22° 19'S, 42° 20'W; (M) Castelo, ES- 20° 30'S, 41° 04'W; (N) Cachoeiro do Itapemirim, ES- 20° 31'S, 40° 59'W (Geise, 1995); (O) Santa Teresa, ES- 19° 56'S, 40° 36'W (Paresque, 2001); ✦ Una, BA- 15° 17'S, 39° 04'W (Present paper).

South American sigmodontine rodents have fascinated and challenged researchers due to the diversity of forms and the difficulty in assessing relationships. Their taxonomy is uncertain at all levels, from species boundaries to tribal grouping (SMITH and PATTON 1999).

Akodontini is the second tribe of Sigmodontinae in species richness, and according to REIG (1986) includes genera *Akodon, Microxus, Bolomys, Oxymycterus, Lenoxus, Blarinomys, Podoxymys, Juscelinomys, Chelemys* and *Notiomys*. MUSSER and CARLETON (1993) added to Akodontini the genera *Thalpomys, Chroeomys* and *Geoxus*. HERSHKOVITZ (1998) based on morphological data recognized the subgenus *Thaptomys* formally included in *Akodon* as a full genus (type species *Thaptomys nigrita*). SMITH and PATTON (1999) based on cytochrome *b* sequencing provided the phylogenetic support for the generic status of *Thaptomys*, and recognized the genera *Akodon, Thaptomys, Bolomys, Oxymycterus, Lenoxus, Blarinomys, Brucepattersonius, Podoxymys, Juscelinomys, Thalpomys, Scapteromys, Kunsia* and *Bibimys* as belonging to Akodontini.

Thaptomys is a monotypic genus from southeastern Brazil found in the states of Rio Grande do Sul, Paraná, São Paulo, Rio de Janeiro and Espírito Santo (YONENAGA 1972; SOUZA 1981; CASTRO 1989; GEISE 1995; FAGUNDES 1993; PARESQUE 2001) (Fig. 1).

Cytogenetic analyses have been supplied important data concerning chromosome organization, function, duplication, variation and evolution in rodents. Additionally, several chromosomal characters are diagnostic at species level for many rodent groups. In association to

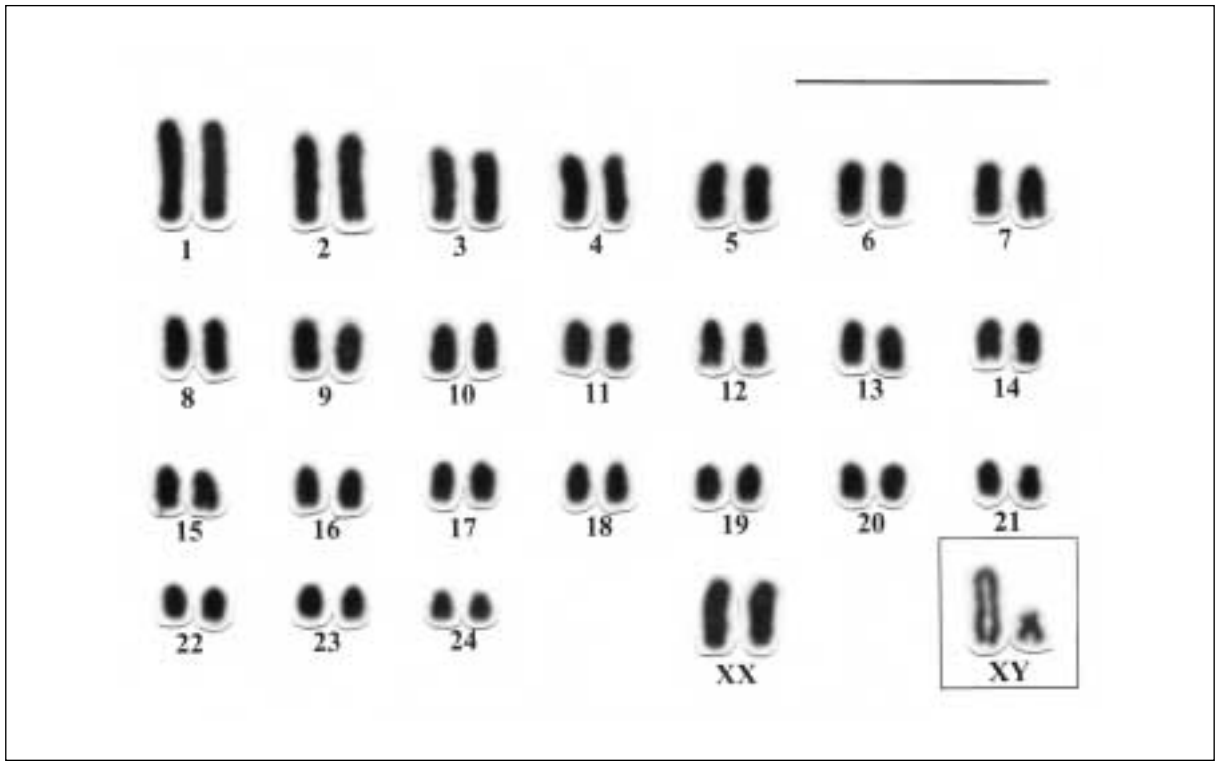

Fig. 2 – Karyotype conventionally stained of a male of *Thaptomys* sp., 2*n*=50. In set, sex chromosomes of a male. Bar 10μm.

morphological and molecular studies, chromosomes give support to elucidate mechanisms of species differentiation, contributing to phylogenetic studies in Neotropical rodents.

In this paper we describe a new karyotype for *Thaptomys* collected in Una, state of Bahia, Brazil, that is morphologically indistinguishable from *Thaptomys nigrita* with 2*n*=52. We present conventional and Ag-NOR staining, CBG and GTG-banding, and we compare GTG-pattern and FISH data of 2*n*=50 and 2*n*=52 karyotypes of *Thaptomys*.

MATERIAL AND METHODS

Four males and one female of *Thaptomys* sp. from Una, state of Bahia, Brazil (Fig.1), were cytogenetically analysed. All of them previously identified as *Thaptomys nigrita*.

Metaphases were obtained from bone marrow and fibroblasts derived from ear biopsies, which were cultured in Dulbecco's modified Eagle's medium supplemented with 20% fetal bovine serum, according to conventional procedures. Cells were spread onto clean slides, air dried, and stored at –20°C until use. GTG, CBG-banding and Ag-NOR staining were based on routine cytogenetic procedure.

For *in situ* hybridization, DAKO Telomere PNA FISH Kit/Cy3 (code No. K 5326) was used following the recommended protocol. During the pretreatment, slides were immersed in TBS for about 2 minutes, and then in 3.7% formaldehyde in TBS for exactly 2 minutes; the slides were washed again in TBS and were immersed in a pre-treatment solution for 10 minutes following by another TBS washing and then in cold ethanol series (70%, 85% and 96%). For denaturation and hybridization, 10 μL of Telomere PNA Probe/Cy3 were added to the slides and covered with 18x18 mm coverslip. The slides were placed in a pre-heated incubator adjusted to 80°C and after that, in the dark at room temperature for 30 minutes. In order to remove the coverslips, the slides were immersed in a rinse solution and them washed in a wash solution pre-heated at 65°C, and once more immersed in the same cold ethanol series as previously. Onto each slide it was applied 2x10μL of mounting solution (Vectashield antifade supplemented with 0.1 μg/mL DAPI). FISH images were observed using a Zeiss Axiophot fluorescence microscope with RBG filter sets. Images were saved one by one in the computer, and analysed after overlapping both chromosome and signal images.

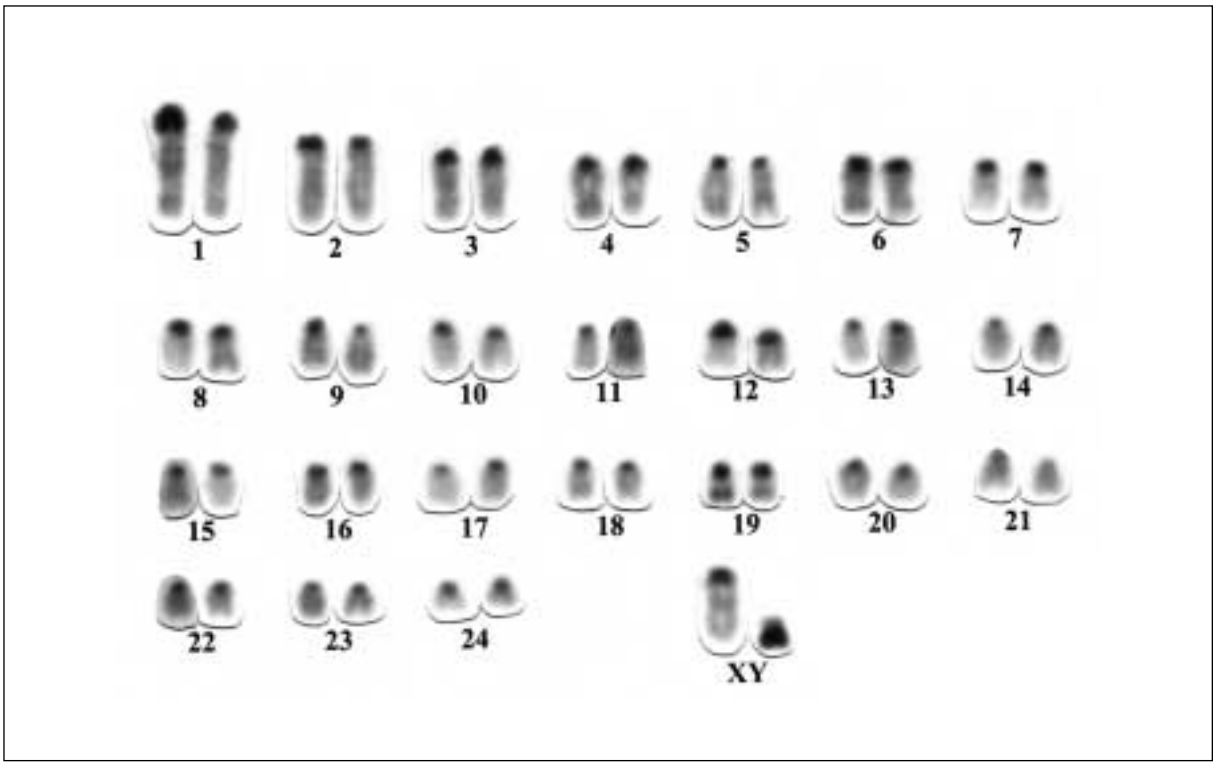

Fig. 3 – C-banded metaphases of a male of *Thaptomys* sp. Pair 1 heteromorphic for the size of C-bands.

RESULTS

The new karyotype presents 2*n*=50, with 24 autosome acrocentric pairs decreasing gradually in size; the Y chromosome is a small submetacentric and the X is a large acrocentric, indistinguishable from other autosomes in conventional staining (Fig. 2).

C-banded metaphases revealed the pericentromeric regions of all chromosomes and the long arm of Y chromosome strongly stained (Fig. 3). Pairs 1 and 9 were found heteromorphics in size for constitutive heterochromatin (Fig. 4).

G-banding patterns allowed to recognize all the homologous and to establish the X chromosome as a large acrocentric due to the two interstitial stained bands in the long arm (which are typical in X chromosomes of mammals) (Fig. 5).

Multiples Ag-NORs ranging from 2 to 12, located at the telomeric regions of the long arm of autosomes were observed. One small acrocentric pair presented signals at both telomeres (Fig. 6).

After fluorescence *in situ* hybridization with telomeric probe (TTAGGG)n, signals were observed in all telomeres (Fig. 7A). One interstitial telomeric signal (ITS) was observed proximally in the long arm of chromosome 2 of *Thaptomys* sp. (TSP 2) (Fig. 7A and 7B).

Both karyotypes, 2*n*=50 of *Thaptomys* sp. and 2*n*=52 of *Thaptomys nigrita,* are composed of 24 pairs of acrocentric autosomes decreasing in size. *Thaptomys nigrita* (2*n*=52) however pre-

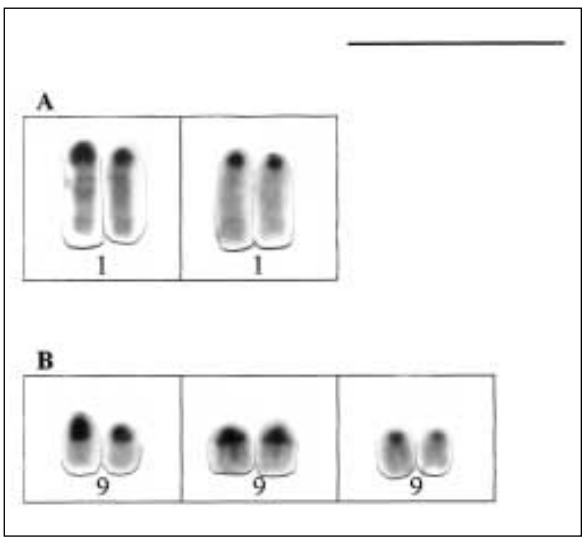

Fig. 4 – Heteromorphic C-banded chromosome pairs of *Thaptomys* sp.: (A) Pair 1; (B) Pair 9. Bar 10μm.

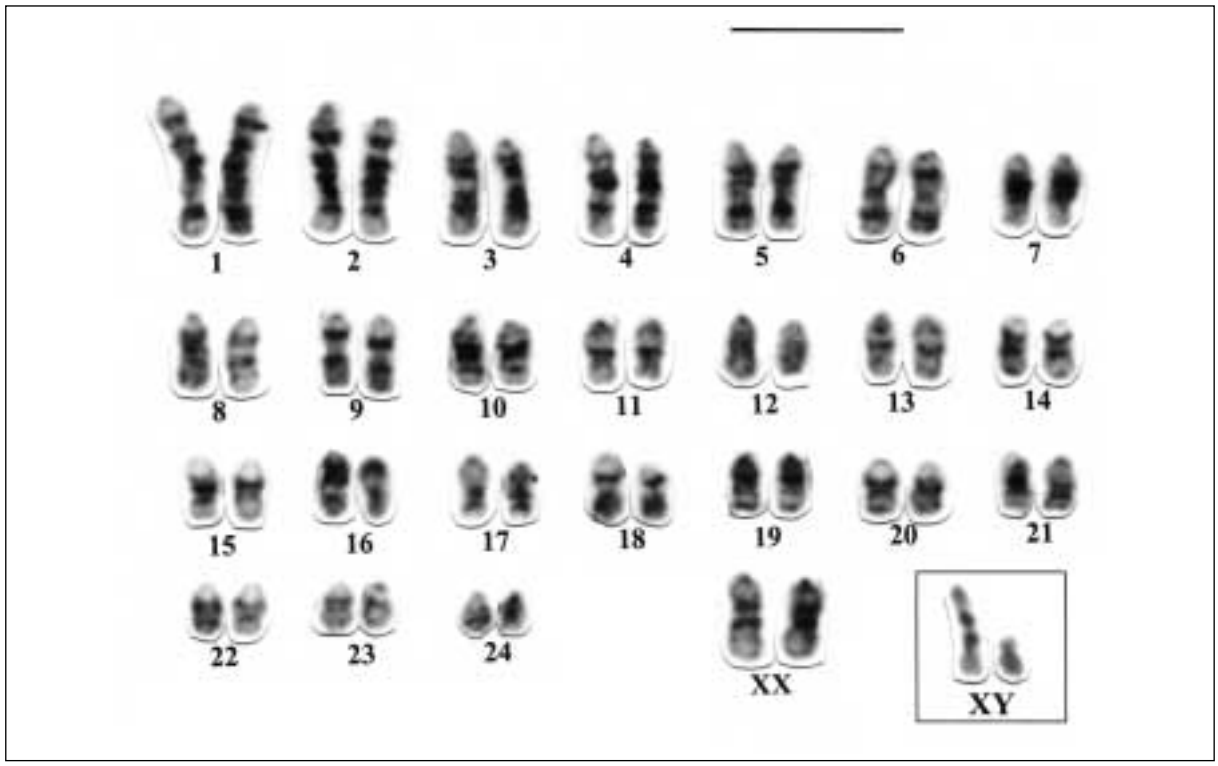

Fig. 5 – GTG-banded karyotype of a female of *Thaptomys* sp (2*n*=50), from Una, Bahia State, Brazil. In set, sex chromosomes of a male. Bar 10μm.

sents a small metacentric pair which is absent in *Thaptomys* sp. with 2*n*=50 (Fig. 8).

C-banding pattern is similar in both karyomorphs. The polymorphism found in pair 1 of *Thaptomys* sp. (2*n*=50) has never been described for *Thaptomys nigrita*. On the other hand, the polymorphism related to size of the pericentromeric heterochromatic block for pair 9 had been observed for *Thaptomys nigrita* (FAGUNDES 1993).

Ag-NORs are located in the same telomeric region of the long arm of autosomes in

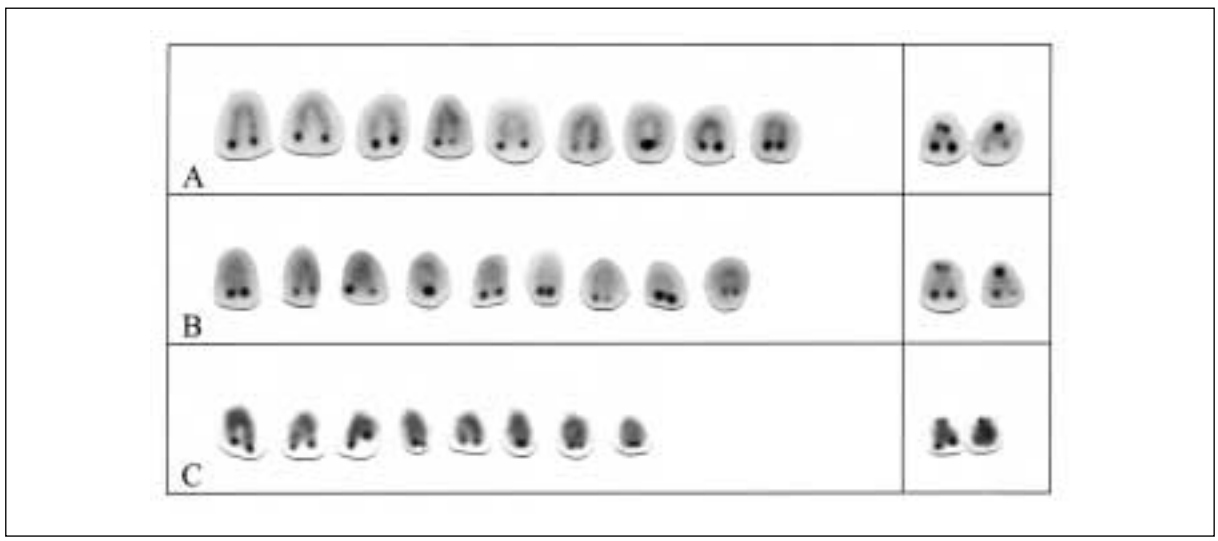

Fig. 6 – Multiple Ag-NORs at the telomeric regions of the long arms in acrocentric autosomes of *Thaptomys* sp. (2*n*=50) in three metaphases. Inset, the acrocentric pair with Ag-NORs in both telomeric regions.

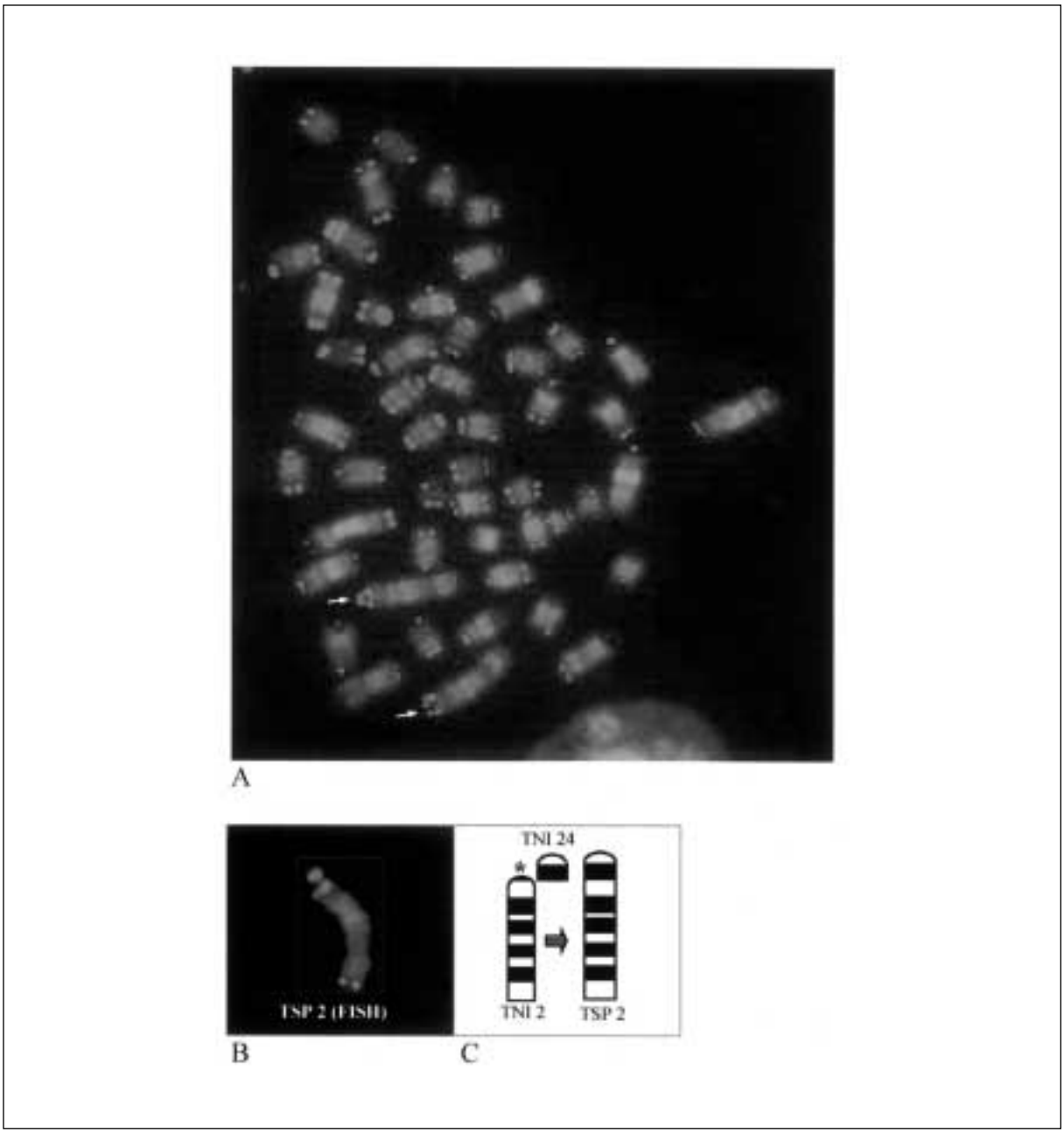

Fig. 7 – (A) Metaphase of *Thaptomys* sp. after fluorescence *in situ* (FISH) of telomeric probes: arrows indicate 2 (TSP2) with interstitial telomeric signal (ITS) proximally in the long arm; (B) Detail of chromosome 2 of TSP2 evidenced hybridization showing the (ITS); (C) An idiogram of tandem fusion rearrangement involving chromosomes 2 and 24 of *T. nigrita* (TNI 2+TNI24) resulting in chromosome 2 of *T.* sp. (TSP2) *asterisk = centromere.

both karyomorphs. A single autosome pair of each karyotype presented Ag-NORs in both telomeres: in *Thaptomys nigrita* this pair (25) is the metacentric (SOUZA 1981; CASTRO 1989; FAGUNDES 1993) and it is a small acrocentric in *Thaptomys* sp. (pair 24). Another difference is the presence of Ag-NOR just in the short arm of the acrocentric pair 24 of *Thaptomys nigrita*. None chromosome exhibited this sort of signal in the short arm of *Thaptomys* sp.

Pairs 1 and pairs 3 to 7 matched in both karyotypes after GTG banding. The difference between these two karyotypes (2*n*=50 and 2*n*=52) was detected in both size and number of GTG-bands of the autosome pair 2: pair 2 of *Thaptomys* sp. with 2*n*=50 is larger in size and exhibited five interstitial dark bands

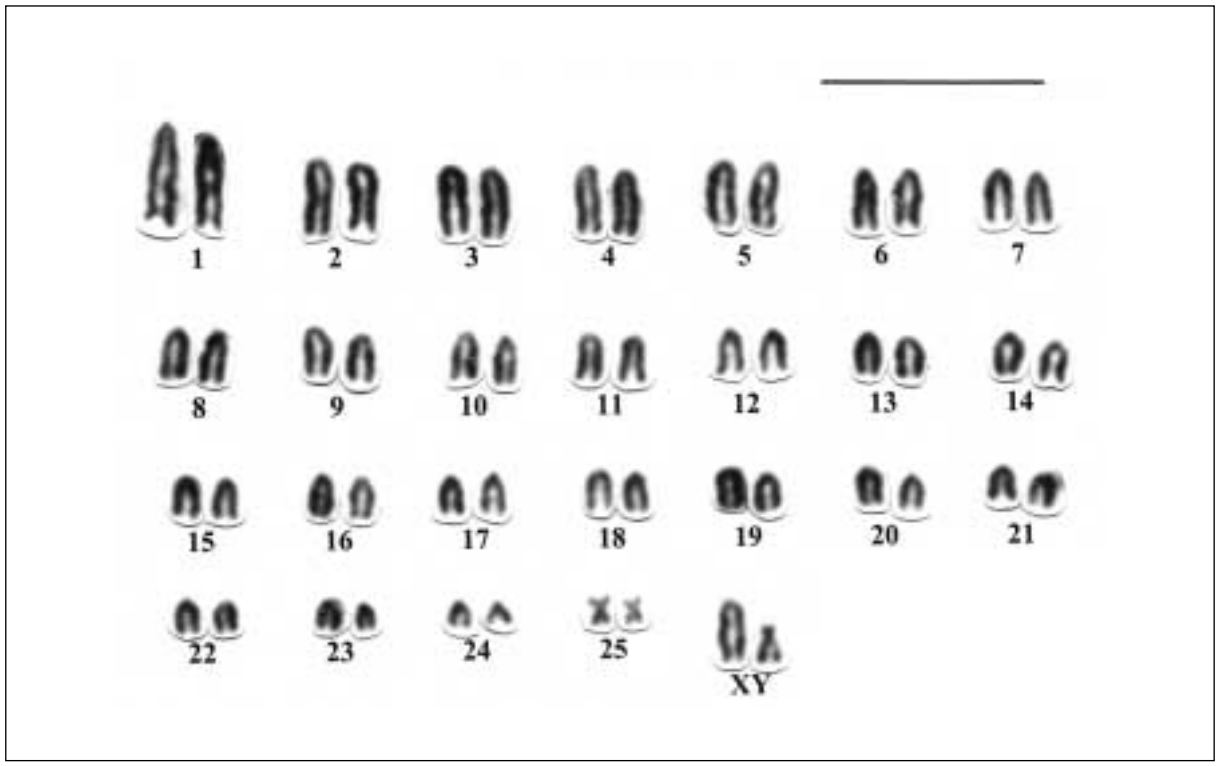

Fig. 8 – Karyotype of a male of *Thaptomys nigrita*, 2*n*=52 after conventional staining. Bar 10μm.

whereas pair 2 of *Thaptomys nigrita* (2*n*=52) is smaller and shows four interstitial positive G-bands besides a centromeric band.

DISCUSSION

Thaptomys was firstly characterized as a monotypic genus from southeastern Brazil found in the states of Rio Grande do Sul, Paraná, São Paulo, Rio de Janeiro and Espírito Santo (YONENAGA 1972; SOUZA 1981; CASTRO 1989; GEISE 1995; FAGUNDES 1993; PARESQUE 2001). Our specimens, extending the distribution of the genus farther north to the state of Bahia (Fig. 1), although morphologically similar to *T. nigrita* have a distinct karyotype that is fully a diagnostic. This new evidence and the allopatric nature of their karyotypes is highly suggestive that these specimens are members of an undescribed cryptic new species of *Thaptomys.*

The species *Akodon cursor* (2*n*=14) and *Akodon montensis* (2*n*=24, 25) occur in Brazil and present karyotypes as diagnostic feature, since these two entities are cryptic species and therefore indistinguishable based on morphological data. These species, sympatric at some regions in the state of São Paulo, Brazil, hybridized along a hybrid zone. This textbook example of cryptic species, exemplify the importance of chromosome analysis as a first step to refine systematic studies and revealing unsuspected diversity (YONENAGA 1975; YONENAGA-YASSUDA 1979; SBALQUEIRO and NASCIMENTO 1996; CHRISTOFF 1997; FAGUNDES *et al.* 1997; FAGUNDES *et al.* 1998).

Chromosomal analyses of the specimens of *Thaptomys* from Una, showed 2*n*=50 and according to preliminary analyses these animals are morphologically indistinguishable from *Thaptomys nigrita* with 2*n*=52 (Christoff, pers. comm.). The karyotype therefore represents a diagnosable feature to separate these two other entities of Akodontini tribe.

We detected the absence of a small metacentric pair in the karyotype with 2*n*=50 from *Thaptomys* sp. after conventional staining. Therefore a chromosome rearrangement must be involved in the chromosomal morphologic differentiation of this small metacentric pair.

Presence of Ag-NORs in both arms at the telomeric region of one small autosomic pair of

Thaptomys sp. (2*n*=50) and in the autosomic metacentric (pair 25) of *Thaptomys nigrita* led us to suggest that these pairs are homoeologous and that a pericentric inversion should be the chromosomal rearrangement responsible by the differences between these two pairs (acrocentric in 2*n*=50 and metacentric in 2*n*=52). In both *Akodon* and *Bolomys* genus, sister groups of *Thaptomys*, a characteristic small metacentric pair is found.

The same G-band pattern was observed for pairs 1 and pairs 3 to 7 between those two karyomorphs. The difference in size between pairs 1 and 2 in both karyotypes can be the result of a tandem fusion rearrangement involving chromosome 2, that has lost its centromeric region, and chromosome 24 of *Thaptomys nigrita* (TNI 2 + TNI 24), which has originated chromosome 2 of *Thaptomys* sp. (TSP 2) with 2*n*=50. The existence of an extra interstitial G-band in TSP 2, when compared with TNI 2, could be related to the tandem fusion mechanism of TNI 2 + TNI 24 (Fig 7C).

G-banding pattern of the pairs involved in the rearrangement in addition to the interstitial telomeric signal (ITS) observed in TSP 2 plus the absence of a small acrocentric pair in *Thaptomys* sp. (which is $AgNO_3$ positive just in the short telomeric arm) support the hypothesis of tandem fusion rearrangement involving pairs 2 and 24 of *T. nigrita*, resulting in pair 2 of *Thaptomys* sp.

Although we are in favor of the tandem fusion hypothesis for the origin of chromosome 2 and the lower diploid number of the new species herein presented, we cannot eliminate the reverse possibility of a fission mechanism. Only an explicit phylogenetic framework can solve this problem.

YONENAGA-YASSUDA *et al.* (1988) recognized two tandem fusions as the main mechanism involved in the differentiation of two karyotypes (2*n*=52 and 2*n*=56) of *Nectomys*. In this case, G-bands showed that each single tandem fusion occurred with loss of centromeric region of one acrocentric. These two distinct karyotypes have been considered as belonging to two different species (BONVICINO *et. al.* 1996; SILVA and YONENAGA-YASSUDA 1998). Similarly as observed in *Nectomys,* the chromosomal rearrangements found involving *T. nigrita* and *Thaptomys* sp. karyotypes could represent two different species.

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