



Thaptomys Thomas 1915 (Rodentia, Sigmodontinae, Akodontini) with karyotypes $2n = 50$, $FN = 48$, and $2n = 52$, $FN = 52$: Two monophyletic lineages recovered by molecular phylogeny

Karen Ventura¹, Maria José de Jesus Silva² and Yatiyo Yonenaga-Yassuda¹

¹*Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo, São Paulo, SP, Brazil.*

²*Laboratório de Ecologia e Evolução, Instituto Butantan, São Paulo, SP, Brazil.*

Abstract

A novel karyotype with $2n = 50$, $FN = 48$, was described for specimens of *Thaptomys* collected at Una, State of Bahia, Brazil, which are morphologically indistinguishable from *Thaptomys nigrita*, $2n = 52$, $FN = 52$, found in other localities. It was hence proposed that the $2n = 50$ karyotype could belong to a distinct species, cryptic of *Thaptomys nigrita*, once chromosomal rearrangements observed, along with the geographic distance, might represent a reproductive barrier between both forms. Phylogenetic analyses using maximum parsimony and maximum likelihood based on partial cytochrome *b* sequences with 1077 bp were performed, attempting to establish the relationships among the individuals with distinct karyotypes along the geographic distribution of the genus; the sample comprised 18 karyotyped specimens of *Thaptomys*, encompassing 15 haplotypes, from eight different localities of the Atlantic Rainforest. The intra-generic relationships corroborated the distinct diploid numbers, once both phylogenetic reconstructions recovered two monophyletic lineages, a northeastern clade grouping the $2n = 50$ and a southeastern clade with three subclades, grouping the $2n = 52$ karyotype. The sequence divergence observed between their individuals ranged from 1.9% to 3.5%.

Key words: Atlantic Rainforest, cytochrome *b*, endemism, molecular phylogeny, *Thaptomys*.

Received: April 27, 2009; Accepted: November 24, 2009.

Although many of the recently proposed redefinitions of generic groupings within the rodent subfamily Sigmodontinae were based on phylogenetic analyses using molecular markers (Smith and Patton, 1991, 1993, 1999, 2007; D'Elía 2003; D'Elía *et al.*, 2003), much of the debate regarding its taxonomy and systematics has been historically focused on less inclusive taxonomic categories, based on morphological characters (Reig, 1987). This situation is especially true regarding the establishment of some taxa which are morphologically similar to *Akodon* (*e.g.*, *Deltamys*, *Hypsimys*, *Microxus*, *Thaptomys* and *Thalpomys*).

The genus *Thaptomys* Thomas, 1915 was described to allocate *Hesperomys subterraneus* Hensel, 1873 (*e.g.*, *Mus nigrita* Lichtenstein, 1829). Massoia (1963) clarified the identity of *H. subterraneus*, *Thaptomys* type-species, as a synonym of the previously named *M. nigrita*, reporting a larger area of occurrence for the species. This taxon has been usually referred to as a subgenus of *Akodon* (Ellerman, 1941; Cabrera, 1961) or even as a full synonym of the

nominate subgenus (Reig, 1987). In taxonomic reviews, Hershkovitz (1990, 1998) has reasserted the singular morphological traits of *Thaptomys* and assigned the generic status in accordance to Thomas (1915).

Molecular systematic studies carried out by Smith and Patton (1999), using cytochrome *b* sequences, provided evidence from a phylogenetic perspective for the genus *Thaptomys* and led to the conception that the tribe Akodontini is formed by *Akodon* (including *Deltamys*, *Microxus* and *Hypsimys*), *Thaptomys*, *Necromys* (referred to as *Bolomys*), *Oxymycterus*, *Lenoxus*, *Blarinomys*, *Brucepattersonius*, *Podoxymys*, *Juscelinomys*, *Thalpomys*, *Scapteromys*, *Kunsia* and *Bibimys*. So far, *Thaptomys* is a monotypic genus (Musser and Carleton, 2005), containing the species *T. nigrita*, distributed along the Brazilian east coast, eastern Paraguay and northeastern Argentina. Karyotyped samples with diploid number ($2n$) = 52, fundamental number of autosomal arms (FN) = 52, are recorded for different localities of the Brazilian Atlantic Rainforest in the States of Rio Grande do Sul, Paraná, São Paulo, Rio de Janeiro and Espírito Santo (reviewed in Ventura *et al.*, 2004).

Ventura *et al.* (2004) described a new karyotype with $2n = 50$, $FN = 48$, for specimens of *Thaptomys* sp. (TSP)

collected at Una, State of Bahia, Brazil, which are morphologically indistinguishable from *T. nigrita* (TNI) with $2n = 52$, found in other Brazilian localities. G-banding patterns and interstitial telomeric signals (ITS) detected by fluorescent *in situ* hybridization (FISH) suggested that the differentiation of the diploid number between both karyotypes is due to a tandem rearrangement involving pairs TNI 2, TNI 24, and pair TSP 2. It has been hence proposed that this new karyotype with $2n = 50$ could belong to a distinct species, cryptic of *Thaptomys nigrita*. The chromosomal rearrangements could promote errors in meiotic drive and, along with the geographic distance, might represent reproductive barriers between both forms.

Molecular phylogenetic analyses using maximum parsimony and maximum likelihood, based on partial cytochrome *b* sequences of 18 karyotyped *Thaptomys* specimens with karyotypes $2n = 52$ and $2n = 50$ from eight different localities of the Atlantic Rainforest (Table 1 and Figure 1), were performed, attempting to establish the relationships among the individuals along the geographic distribution of the genus. Samples of *Bucepattersonius*, *Necomys* and *Thalpomys* were used as outgroups. Sequences from GenBank and their accession numbers are listed in parentheses in the cladograms.

Genomic DNA was extracted from liver or muscle samples preserved in alcohol or at $-80\text{ }^{\circ}\text{C}$, following the protocol described by Fetzner (1999). Fragments were am-

plified using primers MVZ 05 or MVZ 127 for the light-strand, and MVZ 16 or MVZ 14 for the heavy-strand (Smith and Patton, 1993, 1999). Polymerase chain reaction

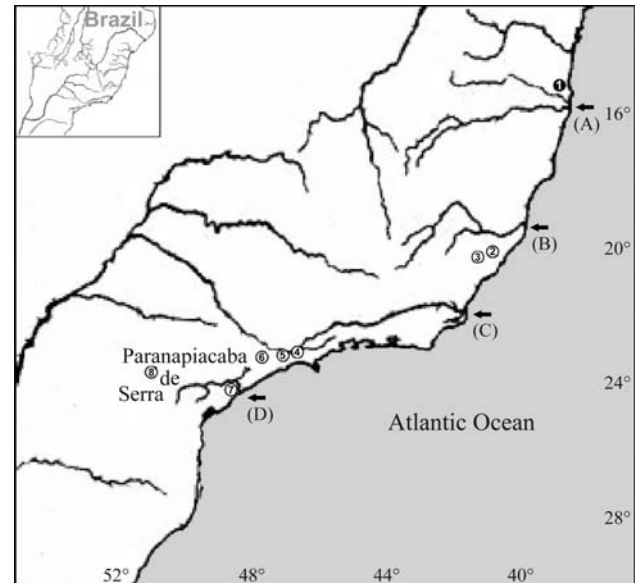


Figure 1 - Map of localities of *Thaptomys* specimens with $2n = 50$, $FN = 48$, and $2n = 52$, $FN = 52$, used in the present study. Arrows indicate from north to south the (A) Pardo and Jequitinhonha River system, (B) Doce River, (C) Paraíba do Sul River, and (D) Ribeira de Iguape River. Localities: ❶ Una, BA; ❷ Santa Teresa, ES; ❸ Domingos Martins, ES; ❹ Biritiba Mirim, SP; ❺ São Bernardo do Campo, SP; ❻ Pilar do Sul, SP; ❼ Iguape, SP; ❽ Ortigueira, PR.

Table 1 - *Thaptomys* samples used in the present study, with their respective identification numbers, localities, karyotypes and haplotype numbers.

Genus	Species	Identification number	Locality	UF ¹	LN ²	Geographic coordinates	Karyotype	mtDNA haplotype
<i>Thaptomys</i>	sp.	CIT 904	Una	BA	❶	15°29' S; 39°07' W	2n = 50, FN = 48	H1
		CIT 913					2n = 50, FN = 48	H2
		CIT 935					2n = 50, FN = 48	H3
<i>Thaptomys</i>	<i>nigrita</i>	LGA 116	Santa Teresa	ES	❷	19°56' S; 40°36' W	2n = 52, FN = 52	H4
		LGA 117					2n = 52, FN = 52	H5
		LGA 959	Domingos Martins	ES	❸	20°21' S; 40°39' W	2n = 52, FN = 52	H6
		LGA 974					2n = 52, FN = 52	H7
		LGA 977					2n = 52, FN = 52	H8
		UNIBAN 2031	Biritiba Mirim	SP	❹	23°57' S; 46°03' W	2n = 52, FN = 52	H9
		UNIBAN 2132					2n = 52, FN = 52	H9
		CIT 1667	São Bernardo do Campo	SP	❺	23°69' S; 46°56' W	2n = 52, FN = 52	H9
		CIT 1034	Pilar do Sul	SP	❻	24°42' S; 47°33' W	2n = 52, FN = 52	H10
		CIT 1133					2n = 52, FN = 52	H11
		CIT 175					2n = 52, FN = 52	H12
		CIT 323	Iguape	SP	❼	24°42' S; 47°33' W	2n = 52, FN = 52	H12
		MII 077					2n = 52, FN = 52	H13
		MII 078	Ortigueira	PR	❽	24°12' S; 50°56' W	2n = 52, FN = 52	H14
		MII 082					2n = 52, FN = 52	H15

¹Abbreviations of Brazilian states: BA = Bahia; ES = Espírito Santo; PR = Paraná; SP = São Paulo. ²Locality numbers as represented on the map (Figure 1) and cladograms (Figure 2).

(PCR) was performed in a final volume of 25 μ L for each sample, as follows: 4.0 μ L of dNTPs (5 mM); 2.5 μ L of 10x buffer; 2.5 μ L of $MgCl_2$ (25 mM); 2.0 μ L of each primer; 9.5 μ L of milli-Q water; 0.5 μ L of Recombinant *Taq* DNA Polymerase (5 U/ μ L), and 2.0 μ L of DNA template. The PCR thermocycling protocol consisted of an initial denaturation step of 94 $^{\circ}C$ for 5 min, followed by 40 cycles at 94 $^{\circ}C$ for 30 s, 48 $^{\circ}C$ for 45 s, 72 $^{\circ}C$ for 1 min, and a final extension step at 72 $^{\circ}C$ for 10 min.

The PCR products were directly purified with ExoSAP-IT kit (USB Corporation Biosciences), following the manufacturer's instructions. The sequencing reactions were performed using the ABI PRISM[®] Big Dye Terminator v. 3.0 kit (Applied Biosystems). Samples were sequenced with the automatic ABI PRISM[®] 3700 DNA Analyzer (PE Applied Biosystems Foster City, CA, USA).

Light and heavy strands were edited, manually aligned, and compared using Sequence Navigator (Applied Biosystems). Multiple alignments were done with Clustal X. The reading frame of the obtained sequences was inferred using MacClade.

Phylogenetic analyses were carried out by means of the maximum parsimony (MP) and maximum likelihood

(ML) methods, using PAUP* 4.0b10 (Swofford, 2001). The MP analysis was performed by a heuristic search using stepwise addition with rearrangement algorithm TBR (tree bisection and reconnection) and 10,000 replicates. The ML analysis was made using the appropriate nucleotide substitution model, selected by Modeltest (version 3.06, Posada and Crandall, 1998); the parameters of this model were used to reconstruct the phylogeny through a heuristic search by stepwise addition with TBR and 1,000 replicates.

The robustness of nodes was assessed by nonparametric bootstrap percentages (Felsenstein, 1985) after 1,000 (MP) and 500 (ML) pseudoreplicates. Bremer branch support (Bremer, 1994) was implemented in the MP analysis.

Fifteen haplotypes were recognized in the sample; specimens from the State of São Paulo UNIBAN2132, UNIBAN2031 and CIT1667 shared haplotype H9, and CIT175 and CIT323 shared haplotype H12 (Table 1).

The matrix generated from all the sequences presented in Table 1 has 1077 bp, with 783 bp constant, 87 bp uninformative and 207 bp informative sites in the parsimony analysis. The MP analysis recovered five most parsimonious trees with a length of 416 steps, a consistency index (CI) of 0.82, and a retention index (RI) of 0.83. Fig-

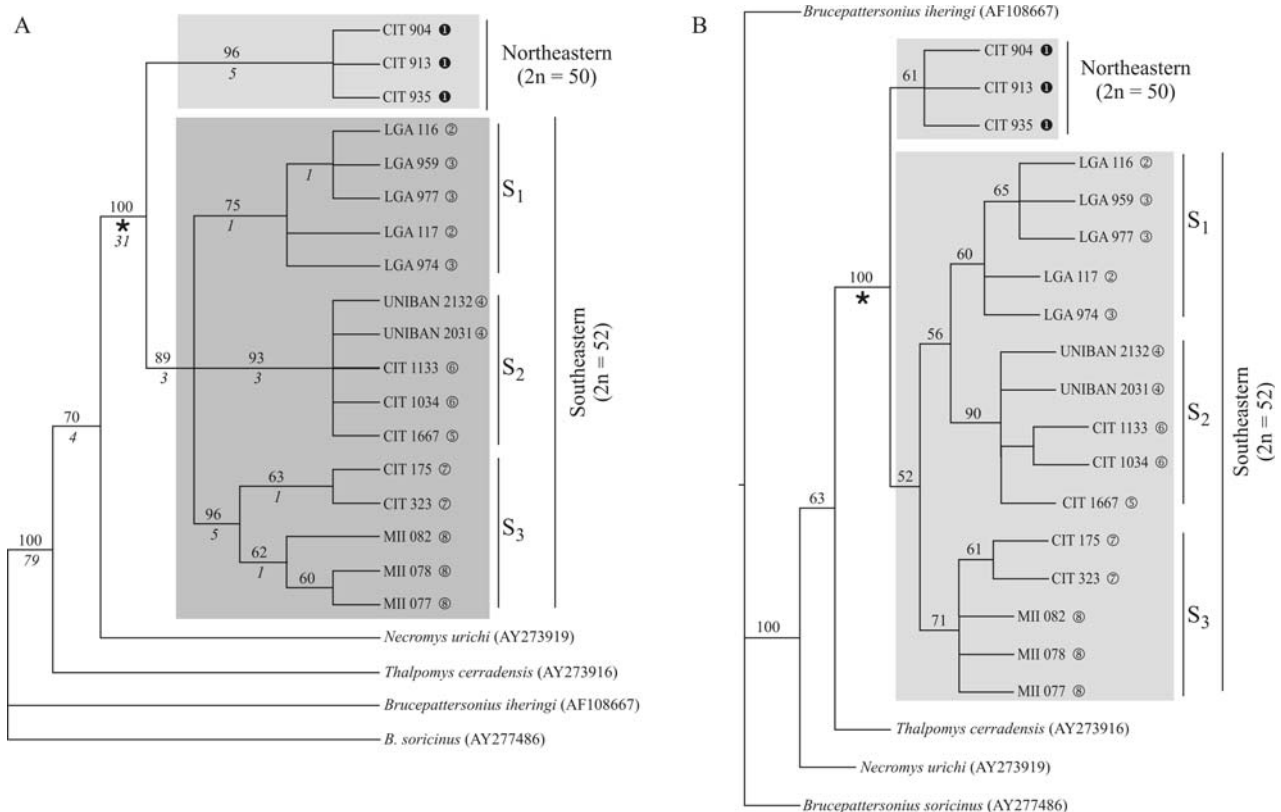


Figure 2 - Phylogenetic reconstructions for 18 *Thaptomys* specimens, besides outgroups. A) Strict consensus obtained from five most parsimonious trees (length = 416 steps, CI = 0.82 and RI = 0.83) resulting from 10000 replicates by the MP method. Numbers above nodes represent bootstrap values obtained with 1000 replicates; numbers under nodes are Bremer support values. B) Most likely tree obtained with 1000 replicates by the ML method. Numbers on clades represent bootstrap values obtained with 500 replicates. Access codes of sequences from GenBank are indicated between parentheses. ● to ○ represent localities of each specimen on the map (Figure 1).

ure 2A presents the strict consensus tree with the respective branch support values.

For the ML analysis, the most adequate model as revealed by Modeltest 3.06 (Posada and Crandall, 1998) was the GTR+I+G model (General Time Reversible, Rodríguez *et al.* 1990). The likelihood tree obtained and the respective bootstrap values are depicted in Figure 2B.

Both methods of tree reconstruction resulted in a clade containing the *Thaptomys* lineages (clade ☆) with bootstrap supports of 100% (Figures 2A and B), indicating monophyly of the genus. A basal dichotomy splits *Thaptomys* in two: lineages with 2n = 50 (northeastern), restricted to Una, BA, and with 2n = 52 (southeastern), distributed over the eastern and southern Atlantic Rainforest.

The southeastern clade presented three subclades by both reconstruction methods (Figure 2). Clade S₁ groups specimens from Santa Teresa and Domingos Martins in the State of Espírito Santo; S₂ includes specimens from Biritiba-Mirim, São Bernardo do Campo and Pilar do Sul, in the State of São Paulo; S₃ groups specimens from Iguape and Ortigueira, in the States of São Paulo and Paraná, respectively. By the MP method, the relationship among these three lineages was unresolved, while ML analysis placed S₃ as the sister group of the clade formed by S₁ and S₂.

Sequence divergence between individuals from the northeastern and southeastern clades ranged from 1.9% to 3.5%. Within the clades, sequence divergence varied from 0.2% to 1.1% in the northeastern clade, and from 0.1% to

2.1% in the southeastern clade. Variation found among specimens from the same geographic region was lower than 1% (Table 2).

The cladogram obtained by molecular phylogenetic analyses shows that cladogenesis events in *Thaptomys* are coincident with the presence of rivers (A) Pardo and Jequitinhonha, (B) Doce, (C) Paraíba do Sul and (D) Ribeira de Iguape, and the Serra de Paranapiacaba mountain chain (Figure 1).

The intra-generic relationships recovered by the maximum parsimony (MP) and maximum likelihood (ML) analyses corroborated the distinct diploid numbers, since the 2n = 50 and 2n = 52 karyotypes appeared as distinct monophyletic lineages, sister-group to each other, with the Pardo and Jequitinhonha river systems and Doce River basin as putative geographic barriers involved in their cladogenesis, and also isolated by the tandem chromosomal rearrangement and by the distance determined by their own geographic distribution (Figures 1 and 2; Table 2), which may represent reproductive barriers for the two forms.

Molecular analyses showed two geographically distinct lineages in the Atlantic Rainforest, allowing the identification of two broad regions in this area. Previous studies involving different groups of vertebrates also recognized two broad regions in the Atlantic Rainforest, separating the fauna into northeastern and southeastern components, as demonstrated for lizards (Vanzolini, 1988; Pellegrino *et al.*, 2005), birds (Bates *et al.*, 1998; Cabanne *et al.*, 2007), and

Table 2 - Upper diagonal: matrix of corrected genetic distance between *Thaptomys* specimens.

	①	①	①	②	②	③	③	③	④	④	⑤	⑥	⑥	⑦	⑦	⑧	⑧	⑧
①	0	0.002	0.008	0.027	0.027	0.029	0.028	0.028	0.029	0.025	0.024	0.028	0.031	0.025	0.022	0.030	0.028	0.030
①		0	0.011	0.025	0.025	0.027	0.026	0.026	0.027	0.023	0.023	0.026	0.029	0.022	0.020	0.028	0.027	0.029
①			0	0.029	0.029	0.031	0.030	0.030	0.027	0.019	0.019	0.019	0.027	0.028	0.025	0.034	0.033	0.035
②				0	0.002	0.002	0.003	0.001	0.007	0.006	0.006	0.009	0.009	0.015	0.012	0.015	0.013	0.014
②					0	0.004	0.003	0.003	0.007	0.005	0.005	0.008	0.009	0.014	0.011	0.014	0.013	0.014
③						0	0.004	0.003	0.009	0.008	0.008	0.011	0.011	0.016	0.013	0.017	0.015	0.016
③							0	0.004	0.008	0.006	0.006	0.009	0.010	0.015	0.012	0.015	0.014	0.015
③								0	0.008	0.006	0.006	0.009	0.010	0.015	0.012	0.015	0.014	0.015
④									0	0.000	0.000	0.003	0.002	0.018	0.016	0.017	0.018	0.018
④										0	0.000	0.003	0.001	0.016	0.016	0.018	0.016	0.018
⑤											0	0.003	0.001	0.016	0.016	0.017	0.016	0.017
⑥												0	0.001	0.019	0.019	0.021	0.019	0.021
⑥													0	0.019	0.017	0.019	0.020	0.020
⑦														0	0.000	0.004	0.002	0.004
⑦															0	0.004	0.003	0.004
⑧																0	0.002	0.003
⑧																	0	0.001
⑧																		0

Note: numbers on upper row and left column represent the same localities as on the map (Figure 1).

non-flying small mammals (Costa *et al.*, 2000; Costa, 2003).

The herein inferred sister-group relationship between the northeast and southeast components was found in three of these examples: the marsupial *Metachirus nudicaudatus* (Costa, 2003), the lizard *Gymnodactylus darwini* (Pellegrino *et al.*, 2005), and the bird *Xiphorhynchus fuscus* (Cabanne *et al.*, 2007). Pellegrino *et al.* (2005) attributed this disjunct pattern to the presence of the Doce River acting in a process of vicariance.

The hypothesis that the karyotype with $2n = 50$ might belong to a new species, cryptic of *Thaptomys nigrita* with $2n = 52$, has already been proposed, based on cytogenetic data and sampled localities with two kinds of cytotypes (Ventura *et al.*, 2004). The lineages recovered with molecular data seem to present a disjunct distribution, with no overlapping areas, since the northernmost recorded karyotyped sample of *Thaptomys nigrita* ($2n = 52$) is from Santa Teresa, ES, and *Thaptomys* sp. ($2n = 50$) is endemic of Una, BA, further north, which corroborates the hypothesis of Ventura *et al.* (2004).

Cytogenetics along with molecular phylogenetics and geographic distribution data suggest that *Thaptomys* sp. $2n = 50$ is a species distinct from *Thaptomys nigrita*, $2n = 52$, awaiting proper taxonomic review. The present data show that *Thaptomys* is a more diverse than previously assumed and highlight the idea that the Atlantic Forest has been an important geographic region in the diversification of the sigmodontines, as it harbors unique Sigmodontinae lineages (*e.g.*, *Thaptomys*, *Blarinomys* and *Brucepattersonius*), as pointed out by Smith and Patton (1999).

The multidisciplinary approach taken herein reinforces the importance of chromosome description in the discovery and characterization of new taxonomic entities. The molecular analyses corroborated chromosomal surveys, indicating that cytochrome *b* is a good marker for characterizing the diversity at the species level within this group of rodents.

Acknowledgments

We thank George Mendes Taliaferro Mattox for the English review, and Valéria Fagundes, Alexandre U. Christoff, Renata Cecília Amaro and Renata Pardini for collecting the specimens. Financial support was provided by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

Bates JM, Hackett SJ and Cracraft J (1998) Area-relationships in the Neotropical lowlands: An hypothesis based on raw distributions of passerine birds. *J Biogeogr* 25:783-793.
 Bremer K (1994) Branch support and tree stability. *Cladistics* 10:295-304.

Cabanne GS, Santos FR and Miyaki CY (2007) Phylogeography of *Xiphorhynchus fuscus* (Passeriformes, Dendrocolaptidae): Vicariance and recent demographic expansion in southern Atlantic forest. *Biol J Linn Soc* 91:73-84.
 Cabrera A (1961) Catalogo de los mamíferos de America del Sur. *Rev Mus Arg Cien Nat Bernardino Rivadavia* 4:309-732.
 Costa LP (2003) The historical bridge between the Amazon and the Atlantic Forest of Brazil: A study of molecular phylogeography with small mammals. *J Biogeogr* 30:71-86.
 Costa LP, Leite YLR, Fonseca GABD and Fonseca MTD (2000) Biogeography of South American forest mammals: Endemism and diversity in the Atlantic Forest. *Biotropica* 32:872-881.
 D'Elía G (2003) Phylogenetics of Sigmodontinae (Rodentia, Muroidea, Cricetidae), with special reference to the akodont group, and with additional comments on historical biogeography. *Cladistics* 19:307-323.
 D'Elía G, Gonzales EM and Pardiñas UFJ (2003) Phylogenetic analyses of sigmodontine rodents (Muroidea), with special reference to the akodont genus *Deltamys*. *Mamm Biol* 68:351-364.
 Ellerman JR (1941) The Families and Genera of Living Rodents. V. II. Family Muridae. British Museum Natural History, London, 690 pp.
 Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783-791.
 Fetzner JW (1999) Extracting high-quality DNA from shed reptile skins: A simplified method. *Bio Techniques* 26:1052-1054.
 Hershkovitz P (1990) Mice of the *Akodon boliviensis* size class (Sigmodontinae, Cricetidae) with the description of two new species from Brazil. *Field Zool* 57:1-35.
 Hershkovitz P (1998) Report on some sigmodontine rodents collected in southeastern Brazil with descriptions of a new genus and six new species. *Bonner Zool Beitr* 47:193-256.
 Massoia E (1963) Sobre la posición sistemática y distribución geográfica de *Akodon (Thaptomys) nigrita* (Rodentia, Cricetidae). *Physis* 24:73-80.
 Musser GG and Carleton MD (2005) Superfamily Muroidea. In: Wilson DE and Reeder DM (eds) *Mammal Species of the World: A Taxonomic and Geographic Reference*. 3rd edition. Johns Hopkins University Press, Baltimore, pp 894-1531.
 Pellegrino KCM, Rodrigues MUT, Waite NA, Morando M, Yonenaga-Yassuda Y and Sites JWJR (2005) Phylogeography and species limits in the *Gymnodactylus darwini* complex (Gekkonidae, Squamata): Genetic structure coincides with river systems in the Brazilian Atlantic Forest. *Biol J Linn Soc* 85:13-26.
 Posada D and Crandall KA (1998) MODELTEST: Testing the model of DNA substitution. *Bioinformatics* 14:817-818.
 Reig OA (1987) An assessment of the systematics and evolution of the Akodontini, with description of new fossil species of *Akodon* (Cricetidae, Sigmodontinae). In: Patterson BD and Tim RM (eds). *Studies in Neotropical Mammalogy. Essays in Honor of Philip Hershkovitz*. Fieldiana Zoology, Chicago, pp 347-399.
 Rodriguez F, Oliver JF, Marin A and Medina JR (1990) The general stochastic model of nucleotide substitutions. *J Theor Biol* 142:485-501.
 Smith MF and Patton JL (1991) Variation in mitochondrial cytochrome b sequence in natural populations of South Ameri-

- can akodontine rodents (Muridae, Sigmodontinae). *Mol Biol Evol* 8:85-103.
- Smith MF and Patton JL (1993) The diversification of South American murid rodents: Evidence from mitochondrial DNA sequence data for the Akodontini tribe. *Biol J Linn Soc* 50:149-177.
- Smith MF and Patton JL (1999) Phylogenetic relationships and the radiation of sigmodontine rodents in South America: Evidence from cytochrome b. *J Mamm Evol* 6:89-128.
- Smith MF and Patton JL (2007) Molecular phylogenetics and diversification of South American grass mice, genus *Akodon*. In: Kelt DA, Lessa EP, Salazar-Bravo J and Patton JL (eds) *The Quintessential Naturalist: Honoring the Life and Legacy of Oliver P. Pearson*. University of California Publications in Zoology, Berkeley, pp 827-858.
- Swofford DL (2001) *PAUP* 4.0 - Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sinauer Associates, Sunderland.
- Vanzolini PE (1988) Distributional patterns of South American lizards. In: Vanzolini PE and Heyer WR (eds) *Proceedings of a Workshop on Neotropical Distribution Patterns*. Academia Brasileira de Ciências, Rio de Janeiro, pp 317-342.
- Ventura K, Silva MJJ, Fagundes V, Pardini R and Yonenaga-Yassuda Y (2004) An undescribed karyotype for *Thaptomys* ($2n = 50$) and the mechanism of differentiation from *Thaptomys nigrita* ($2n = 52$) evidenced by FISH and Ag-NORs. *Caryologia* 57:89-97.

Associate Editor: Louis Bernard Klaczko

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.