# Phylogeny of *Thylamys* (Didelphimorphia, Didelphidae) species, with special reference to *Thylamys karimii*

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**ABSTRACT.** The genus *Thylamys* Gray, 1843 lives in the central and southern portions of South America inhabiting open and shrub-like vegetation, from prairies to dry forest habitats in contrast to the preference of other Didelphidae genera for more mesic environments. *Thylamys* is a speciose genus including *T. elegans* (Waterhouse, 1839), *T. macrurus* (Olfers, 1818), *T. pallidior* (Thomas, 1902), *T. pusillus* (Desmarest, 1804), *T. venustus* (Thomas, 1902), *T. sponsorius* (Thomas, 1921), *T. cinderella* (Thomas, 1902), *T. tatei* (Handley, 1957), *T. karimii* (Petter, 1968), and *T. velutinus* (Wagner, 1842) species. Previous phylogenetic analyses in this genus did not include the Brazilian species *T. karimii*, which is widely distributed in this country. In this study, phylogenetic analyses were performed to establish the relationships among the Brazilian *T. karimii* and all other previously analyzed species. We used 402-bp fragments of the mitochondrial cytochrome *b* gene, and the phylogeny estimates were conducted employing maximum parsimony (MP), maximum likelihood (ML), Bayesian (BY), and neighbor-joining (NJ). The topologies of the trees obtained in the different analyses were all similar and pointed out that *T. karimii* is the sister taxon of a group constituted of taxa from dry and arid environments named the dryland species. The dryland species consists of *T. pusillus, T. pallidior, T. tatei*, and *T. elegans*. The results of this work suggest five species groups in *Thylamys.* In one of them, *T. velutinus* and *T. kariimi* could constitute a sister group forming one *Thylamys* clade that colonized Brazil.

**KEYWORDS.** Cytochrome *b*, Didelphidae, Didelphimorphia, South America, *Thylamys* phylogeny.

**RESUMO. Filogenia das espécies de *Thylamys* (Didelphimorphia, Didelphidae), com ênfase a *Thylamys karimii.*** O gênero *Thylamys* Gray, 1843 ocorre na região central e ao sul da América do Sul, habitando vegetações abertas e arbustivas, desde pradarias até florestas de ambientes secos, em contraste à preferência por habitats mais úmidos dos outros gêneros de Didelphidae. O gênero inclui *T. elegans* (Waterhouse,1839), *T. macrurus* (Olfers, 1818), *T. pallidior* (Thomas, 1902), *T. pusillus* (Desmarest, 1804), *T. venustus* (Thomas, 1902), *T. sponsorius* (Thomas, 1921), *T. cinderella* (Thomas, 1902), *T. tatei* (Handley, 1957), *T. karimii* (Petter, 1968) e *T. velutinus* (Wagner, 1842). Análises filogenéticas anteriores não incluíram a espécie brasileira *T. karimii*, que apresenta uma ampla distribuição no país. Neste estudo foram feitas análises filogenéticas visando estabelecer a relação entre a espécie brasileira *T. karimii* e as demais espécies incluídas em outras análises. Foram utilizados fragmentos de 402pb do gene mitocondrial citocromo *b*. As filogenias foram estimadas pelos métodos de máxima parcimônia (MP), máxima verossimilhança (ML), Análise Bayesiana (BY) e Neighbor-Joining (NJ). As topologias das árvores obtidas nas diferentes análises mostraram-se semelhantes e evidenciaram que *T. karimii* agrupa-se com as espécies *T. pusillus, T. pallidior, T. tatei*, and *T. elegans*, de ambientes secos e áridos. Os resultados obtidos neste trabalho sugerem cinco grupos de espécies em *Thylamys*, dos quais um poderia ser composto pelo grupo-irmão *T. velutinus* e *T. kariimi*, o qual seria o clado que colonizou o Brasil.

**PALAVRAS-CHAVE.** Citocromo b, Didelphidae, Didelphimorphia, América do Sul, filogenia de *Thylamys.*

Considering mainly the morphology, TATE (1933) grouped the small American marsupials (mouse opossums) into five informally named units within the *Marmosa* genus: *murina*, *cinerea*, *noctivaga*, *microtarsus*, and *elegans*. Later, based on systematic revisions that included morphological and chromosomal characters and biochemical studies, the groups defined by TATE (1933) gained generic status and are currently recognized as the genera *Chacodelphys* (Voss, Gardner & Jansa, 2004), *Cryptonanus* (Voss, Lunde & Jansa, 2005), *Marmosa* (Gray, 1821), *Micoureus* (Lesson, 1842), *Marmosops* (Matschie, 1916), *Gracilinanus* (Gardner & Creighton, 1989) and *Thylamys* (Gray, 1843), respectively (CREIGHTON, 1985; REIG *et al.*, 1985; GARDNER & CREIGHTON, 1989; VOSS *et al*., 2004; VOSS *et al*., 2005).

GARDNER & CREIGHTON (1989) recognized five valid species of *Thylamys*: *T. elegans* (Waterhouse, 1839), *T. macrurus* (Olfers, 1818), *T. pallidior* (Thomas, 1902), *T. pusillus* (Desmarest, 1804), and *T. velutinus* (Wagner, 1842). Additional species have been recognized as valid (JULIEN-LAFERRIÈRE, 1994; PALMA, 1995; FLORES *et al*., 2000; SOLARI, 2003): *T. cinderella* (Thomas, 1902), *T. karimii* (Petter, 1968), *T. tatei* (Handley, 1957), *T. venustus* (Thomas, 1902), and *T. sponsorius* (Thomas, 1921). This latter species, which was proposed previously by FLORES *et al.* (2000), was not supported as a valid species by the phylogenetic results of BRAUN *et al.* (2005).

Opossums of the genus *Thylamys* are small in size and have long, soft, hairy coats with a brown-gray three-color pattern on the shoulders. They are pouchless, have exposed teats in the abdominal region, and a prehensile tail with storage capacity for a reserve substance at the base (GARDNER & CREIGHTON, 1989; HERSHKOVITZ, 1992; EISENBERG & REDFORD, 1999). Specimens of this genus are exclusive to South America and are found in Brazil, Peru, Bolivia, Chile, Paraguay, Uruguay, and Argentina in open, dry, semi-arid habitats mainly from sea level to heights of over 3,500 meters (CREIGHTON, 1985; PALMA, 1995).

GARDNER (2005) lists three *Thylamys* species in Brazil: *T. karimii*, found only in the states of Pernambuco and Mato Grosso; *T. macrurus*, found in the southern region; and *T. velutinus*, found in the southeastern region.

However, ecological studies conducted in Central Brazil pointed to the presence of *T. velutinus* in the central portions of the Cerrado biome, therefore extending the geographic distribution of this species (PALMA & VIEIRA, 2006).

In the review of the genus conducted by CARMIGNOTTO & MONFORT (2006), the geographic distribution of *T. karimii* is considerably extended, including the glades in the ''Cerrado'' and ''Caatinga'' biomes in northeastern, southeastern, and central Brazil. The genus is currently known to live in the Brazilian states of Rondônia, Mato Grosso, Tocantins, Piauí, Pernambuco, Bahia, Goiás, and Minas Gerais. In central Brazil, *T. karimii* lives sympatrically with *T. velutinus*.

CARMIGNOTTO & MONFORT (2006) established that the three Brazilian species of *Thylamys* are different from one another and from the other members of the genus in terms of the combination of physical and craniodental characteristics. *Thylamys karimii* presents morphological traits adapted to terrestrial habits with an inconspicuous three-color dorsal pattern. The tail length is shorter than the sum of the head and the body length. The tail end is not prehensile and the paws and fingers are very small, with dermatoglyph-bearing plantar pads either too small or absent. The species that most resembles *T. kariimi* is *T. velutinus*, though in the former the dorsal region is brown and the sides are whitish, while the latter presents a reddish-brown dorsal region and grayish sides. Like the other species of the genus, *T. karimii* presents a chromosome diploid number of $2n = 14$ with fundamental number variation: FN = 20 or 24 (CARMIGNOTTO & MONFORT, 2006; CARVALHO *et al*., 2002, respectively).

Molecular data published by PALMA & YATES (1998) and PALMA *et al*. (2002) suggest that there is a close relationship between *T. macrurus* and *T. pusillus*, which makes these species a separate group from the other species of the genus. These proposals led SOLARI (2003) to propose three species groups in *Thylamys*: the Andean group (*T. elegans*, *T. pallidior*, *T. tatei*, and *T. venustus*), the Paraguayan group (*T. macrurus* and *T. pusillus*), and the Brazilian group (*T. karimii* and *T. velutinus*). SOLARI (2003) and CARMIGNOTTO & MONFORT (2006) considered that the Brazilian group derived from the Paraguayan group, spreading across the Brazilian glades. Nevertheless, BRAUN *et al*. (2005) obtained arrangements that support four different groups: the Paraguayan (*T. macrurus*), Yungas (*T. venustus* and related taxa), Chacoan (*T. pusillus*), and Andean (*T. elegans* and related taxa and *T. pallidior*). In this proposal, the "forest species", represented by the Yungas and Paraguayan groups, are the most basal and the "dryland species", which are highly adapted to arid and semi-arid environments (Chacoan and Andean groups), are the most derived assemblage.

PALMA *et al.* (2002) and BRAUN *et al.* (2005) used cytochrome *b* sequences to estimate the phylogenetic relationships between the species of the *Thylamys* genus, but neither completely clarified the issue because in both investigations the Brazilian *T. velutinus* and *T. karimii* species (this one widely distributed in South American territories) were not included. The object of this study is, therefore, to re-analyze the relationships within the *Thylamys* genus using the same molecular markers, including *T. karimii*, to assess its position.

## MATERIAL AND METHODS

Specimens. DNA samples were obtained from three *Thylamys karimii* specimens collected in two localities in Brazilian ''Cerrado'' (Goiás State – 55 km N Niquelândia city: 14°28'S; 48°27'W and 20 km NW Colinas do Sul city: 14°09'S; 48°04'W). Skins and skulls of these specimens are stored in the Mammals Collection at the Museu Nacional, Rio de Janeiro (MN36926, MN36285, and MN36405). The analysis also included 17 individuals of seven *Thylamys* species from five countries in South America (Tab. I) reported by PALMA *et al.* (2002) and BRAUN *et al.* (2005) (Fig. 1).

Since the localities coordinates of the exemplars collected by PALMA *et al.* (2002) and BRAUN *et al.* (2005) were not referred, they were estimated using the Global Gazetteer Software Version 2.1. (available at: http://www.fallingrain.com/world/).

The sequences of *Gracilinanus agilis* (Burmeister, 1854) and *Marmosops impavidus* (Tschudi, 1845) were chosen as outgroups because these genera were recovered in the same clade as *Thylamys*, in a phylogenetic analysis of didelphids employing the nuclear interphotoreceptor retinoid binding protein (IRBP) gene sequence (JANSA & VOSS, 2000).

Nucleotide acid sequence analysis. DNA was extracted from the kidney, liver, heart, or muscle (stored at –20ºC or in ethanol 70 % purity) using the standard protocol described in MEDRANO *et al.* (1990). The partial mitochondrial cytochrome *b* gene sequences were isolated via polymerase chain reaction (PCR) using the primers MVZ 05 (light-strand) – CGA AGC TTG ATA TGA AAA ACC ATC GTT G with MVZ16 (heavy-strand) - TAG GAA RTA TCA YTC TGG TTT RAT, as suggested by SMITH & PATTON (1993), and the following primers were used to amplify the complete cytochrome *b* sequence MVZ 05 (as mentioned above) with *Mus* 15398 (heavy-strand) - GAA TAT CAG CTT TGG GTG TTG RTG in accordance with ANDERSON & YATES (2000). PCR products were purified with exonuclease I and shrimp alkaline phosphatase (Amersham Biosciences). The specimens were sequenced directly from purified PCR products using the primers cited above and the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) according to the manufacturer's instructions. Sequencing of both strands was done using an ABI Prism 3100 Genetic Analyser (Applied Biosystems). The complete (MN36926) and partial (MN36405 and MN36285) cytochrome *b* sequences of the voucher specimens are available in GenBank as shown in table I.

Data analysis. The sequences obtained were read using the Chromas 1.45 program, and aligned using the

Clustal X 1.81 program (THOMPSON *et al.*, 1997) under the default setting costs, and were manually refined with the aid of the GeneDoc program (NICHOLAS & NICHOLAS, 1997). The composition of bases and Kimura 2-parameter distance (KIMURA, 1980) were obtained with the Molecular Evolution Genetics Analysis software, Version 4 (MEGA 4; TAMURA *et al.*, 2007).

The phylogenetic analysis was performed using neighbor-joining (NJ), maximum likelihood (ML), and maximum parsimony (MP) algorithms using PAUP* v.4.0b10, (SWOFFORD, 2001). Prior to the analyses, the most appropriate model of DNA sequence evolution was evaluated using the ModelTest 3.7 (POSADA & CRANDALL, 1998). The ModelTest chose the HKY+I Ã model as the best fit for our data. For ML tree estimation, heuristic searches with as-is and tree bisection-reconnection (TBR) branch swapping were selected. The support estimates for the ML trees branches by bootstrap analysis were obtained as described in XIANG *et al.* (2002). MP analysis was performed using a heuristic search with TBR branch swapping with the MULPARS option in effect (this option requests the saving of all equally most parsimonious trees; without this option in effect, only one shortest tree is saved in each replicate) and 100 random-addition replicates. Bootstrap statistical support (FELSENSTEIN, 1985) was carried out with 10,000 replications of the heuristic search and simple taxon addition, with the "all trees saved" option.

Bayesian analyses of the data were performed using MrBayes 3.0b4 (HUELSENBECK & RONQUIST, 2001) to generate a posterior probability distribution using Markov Chain Monte Carlo (MCMC) methods. No *a priori* assumptions about the topology of the tree were made and all searches were provided with a uniform prior. The MCMC processes were set so that four chains were run simultaneously for one million generations, with trees being sampled every 100 generations for a total of 10,000 trees. We excluded the first 100,000 generations as the "burn-in" period.

For the distance analysis, trees were constructed using the NJ method (SAITOU & NEI, 1987) with Kimura two-parameter distances. Reliability of the trees was tested using 10.000 bootstrap replications (HEDGES, 1992).

## RESULTS

Two partial (727bp and 758bp) and one complete cyt *b* gene sequences (1149bp) were obtained for the *T. karimii* specimens analyzed in this study. However, as the majority of the taxa was studied using a 402-bp cyt *b* gene sequence fragment (BRAUN *et al.*, 2005), we performed the phylogenetic analyses using this fragment length to gather information about the majority of the *Thylamys* species.

In the analysis, 156 variable sites were observed, of which 30 (19.23 %) were in the first position, 13 (8.34 %) were in the second position, and 113 (72.43 %) were in the third codon position. The average transition/transversion rate observed among the taxa analyzed was 2.6 and the CT transition occurred most frequently. Generally, the tree topologies obtained by these different methods of analysis were similar in that all bootstrap values of the nodes generated were higher than 50 %, the majority being higher than 90 %.

The tree generated by the ML analysis is shown in figure 2, where *T. macrurus* is recovered as the most basal taxon sister group of clades with high support (91 %). In the first clade (grouping the "dryland species"), *T. karimii* exemplars are positioned as the sister group of the clade *T. pusillus* + (*T. pallidior* + *T. tatei* + *T. elegans*) with a bootstrap value of approximately 70 %. The second clade, named Yungas group, includes *T. cinderella* and *T. venustus* recovered as reciprocally monophyletic sister groups with bootstrap support of 54.

Table I. Localities and GenBank accession numbers of the analyzed specimens of the genus *Thylamys*.

| Species | Localities[a] | Approximate Coordinates | Accession numbers |
|---|---|---|---|
| *T. cinderella* | Argentina – Tucumán[1] | 26°49'S;65°13'W | AY803333; AY803332; AY803331 |
| *T. elegans* | Chile – Coquimbo[2] | 30°46'S;70°52'W | AF431929 |
| *T. elegans* | Chile – Metropolitana de Santiago[3] | 33°16'S;70°46'W | AF434178; AF431925 |
| *T. macrurus* | Paraguay – Concepción[4] | 22°19'S;57°32'W | AF431926 |
| *T. pallidior* | Argentina – San Luis[5] | 32°17'S;66°58'W | AY803315 |
| *T. pallidior* | Bolivia – Tarija[6] | 21°40'S;65°16'W | AF431924 |
| *T. pallidior* | Chile-Tarapacá[7] | 19°28'S;68°63'W | AF431930 |
| *T. pusillus* | Argentina – Santiago del Estero[8] | 28°46'S;65°40'W | AY803322; AY803323 |
| *T. pusillus* | Paraguay – Boquerón[9] | 20°26'S;62°08'W | AY803324 |
| *T. karimii* | Brazil – Goiás[10] | 14°28´S, 48°27´W | EF051700 |
| *T. karimii* | Brazil – Goiás[11] | 14°09´S, 48°04´W | EF114742; EF114743 |
| *T. venustus* | Argentina – Jujuy[12] | 24°22'S;65°16'W | AY803335 |
| *T. venustus* | Argentina – Tucumán | 26°49'S;65°13'W | AY803336 |
| *T. venustus* | Bolívia – Chuquisaca[13] | 20°85'S;63°17'W | AF431922 |
| *T. tatei* | Peru – Lima[14] | 11°33'S;77°16'W | AF434179 |
| | Outgroups | | |
| *Gracilinanus agilis* | Bolívia – La Paz | - | AF431928 |
| *Marmosops impavidus* | Peru – Cusco | - | U34670 |

The most parsimonious tree (not shown) presented 332 steps, a consistency index (CI) of 0.608, a retention index (RI) of 0.789, and the homoplasy index (HI) = 0.391. In the parsimony analysis, 130 sites were informative. The *T. macrurus* exemplar was recovered as basal to the other *Thylamys* taxa with a bootstrap value of 100. As seen in the ML tree, in this analysis (MP) the same two clades (dryland and Yungas) were observed, also with high support. In the clade that comprises the *T. karimii* specimens, it was also the most basal member but with a lower bootstrap value. The relationship among the other specimens of this clade (*T. pusillus, T. pallidior, T. tatei,* and *T. elegans*) was an unresolved politomy. The other clade comprised *T. cinderella* and *T. venustus* as sister groups.

In the NJ and BY analysis (not shown) the topologies generated in other analyses were in general maintained. In both analyses, *T. karimii* specimens positioned as the sister group of *T. pusillus, T. pallidior, T. tatei*, and *T. elegans* with 86% support (NJ) and 0.92 of probability (BY). *T. venustus* and *T. cinderella*, as observed in the ML tree, presented as the sister group of the other species of *Thylamys* with a bootstrap of 99 % (NJ) and a posterior probability of 1.0 (BY).

Percentage sequence divergences based on Kimura 2-parameters corrected distances were calculated among the specimens (Tab. II). An average genetic distance of 16.29 % was observed (ingroup only). With the exception of *T. macrurus*, which presented considerable genetic distances (the highest observed was 29.0 % when compared with *T. pusillus* from Argentina), the remaining species of the genus had smaller average distances between them (ranging from a minimum of 9.7 % between *T. pallidior* and *T. pusillus* to a maximum of 20.4 % between *T. venustus* and *T. karimii*). The identification of the *T. macrurus* specimen sequenced by PALMA *et al.* (2002) could not be verified by us, so we relied on the authors voucher identification. The three *T. karimii* specimens we sequenced presented intraspecific variations of 0.53 % and interspecific divergences from 15.8 % to 26.0 % when compared with the sequences of *T. cinderella* and *T. macrurus*, respectively.

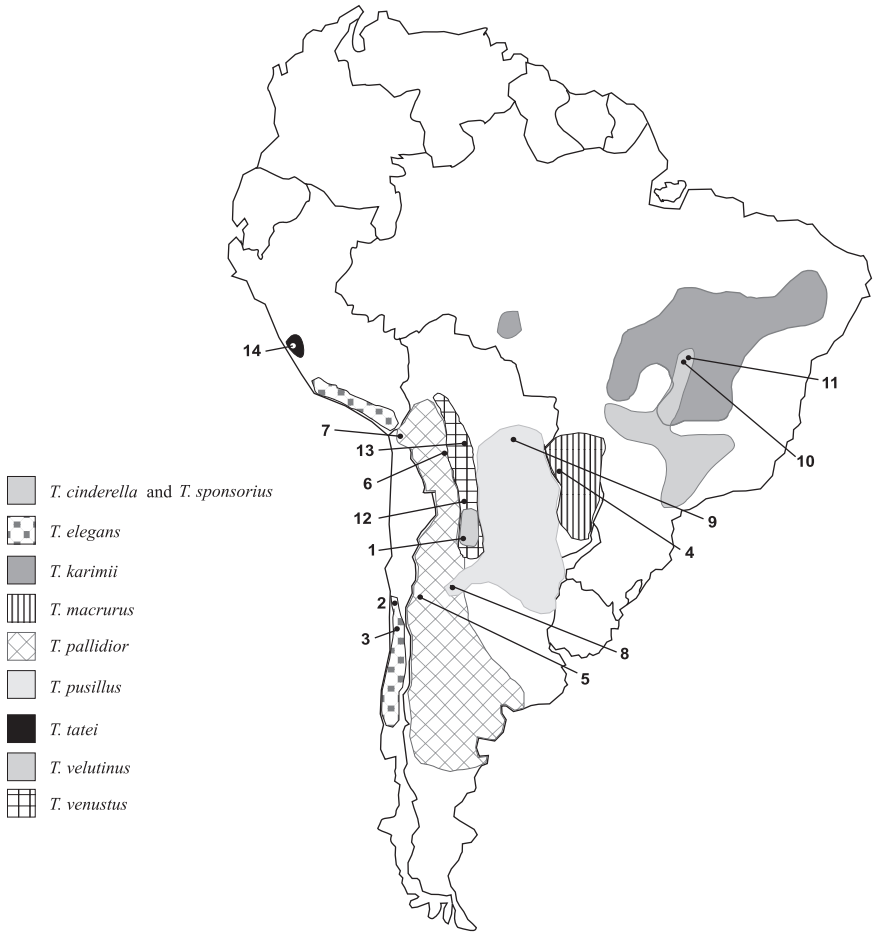


Figure 1. Map of South America showing the distribution of the ten *Thylamys* species based on BRAUN *et al.* (2005), GARDNER (2005), and CARMIGNOTTO & MONFORT (2006) with modifications. Numbers indicate the collect sites (aproximate coordinates in table I): 1. Argentina, Tucumán (AY803332 and AY803333-Yerba Buena; AY803331-Trancas; AY803336-Burruyacu); 2. Chile, Coquimbo, Fray Jorge National Park; 3. Chile, Metropolitana de Santiago, (AF434178-Colina Huechún; AF431925-Quebrada de la Plata); 4. Paraguay, Concepción, Escuela Agropecuaria; 5. Argentina, San Luis, Belgrano; 6. Bolivia, Tarija, Serranía Sama; 7. Chile, Tarapacá, Iquique Colchane; 8. Argentina, Santiago del Estero, (AY803323-Choya and AY803323-Guasayan); 9. Paraguay, Boquerón, P. N. Teniente Enciso; 10. 55 km N Niquelandia, Goiás State, Brazil; 11. 20 km NW Colinas do Sul, Goiás State, Brazil; 12. Argentina, Jujuy, Santa Bárbara, Laguna la Brea; 13. Bolivia, Chuquisaca, Porvenir; 14. Peru, Lima, Chancay, Lachay National Reserve.

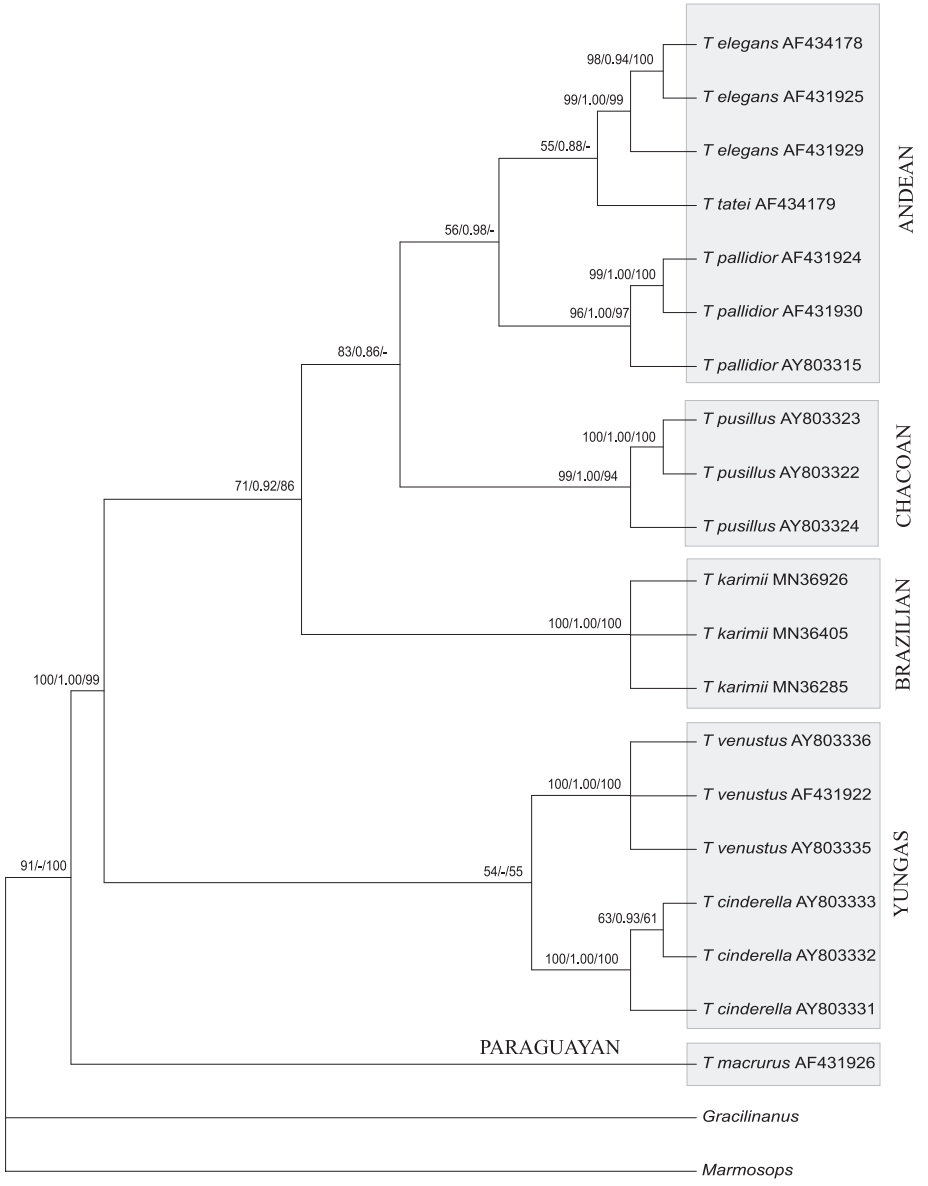


Figure 2. Maximum likelihood (ML) consensus bootstrap tree obtained from the partial sequences of the cytochrome *b* mitochondrial gene for *Thylamys* species. (maximum-likelihood/Bayesian/maximum-parsimony). Hyphens indicate bootstrap support below 50 %.

## DISCUSSION

Several hypotheses were suggested regarding the relationships of the three Brazilian *Thylamys* species with the other taxa of the genus. However, they were inconclusive because they did not include the species *T. karimii*, which is largely distributed in Brazilian territory. These relationships could be better clarified in this study with the inclusion, in a molecular analysis, of the Brazilian species *T. karimii*, which inhabits dry regions.

Previously, SOLARI (2003) and CARMIGNOTTO & MONFORT (2006), proposed three species groups in *Thylamys* as the first approach to natural groups: the Andean (*T. elegans*, *T. pallidior*, *T. tatei*, and *T. venustus*), the Paraguayan (*T. macrurus* and *T. pusillus*), and the Brazilian groups (*T. karimii* and *T. velutinus*). PALMA *et al*. (2002), and SOLARI (2003) suggested that *T. karimii* would present a basal position, possibly derived from *T. macrurus* and related to *T. pusillus*. BRAUN *et al.* (2005), through molecular data, obtained arrangements that were incongruent with those suggested by the authors mentioned earlier. The results obtained by BRAUN *et al.* (2005) supported four different groups: Paraguayan (*T. macrurus*), Yungas (*T. venustus* and related taxa), Chacoan (*T. pusillus*), and Andean (*T. elegans* and related taxa and *T. pallidior*). Our study showed that *T. karimii* is the sister taxon of the Andean species-group of BRAUN *et al.* (2005). In all topologies generated by the different analyses methods, we observed *T. karimii* positioned in the base of the Andean group (*T. pusillus*, *T. pallidior*, *T. tatei*, and *T. elegans*, *sensu* BRAUN *et al*., 2005), constituting a clade that lives in dry habitats (the dryland species). PALMA *et al.* (2002) suggest that this preference for dry environments may be the result of past dispersion

Table II. Kimura-2-parameter corrected percentage sequence divergence among pairwise comparisons of *Thylamys* specimens, based on 402pb of cytochrome b mitochondrial gene. [1 ], [2] and [3] – *T. karimii* (Brazil); [4] and [5]- *T. venustus* (Argentina); [6]- *T. venustus* (Bolivia); [7], [8] and [9]- *T. Cinderella* (Argentina); [10], [11] and [12]- *T. elegans* (Chile); [13] *T. tatei* (Peru); [14] and [15]- *T. pusillus* (Argentina); [16]- *T. pusillus* (Paraguay); [17]- *T. pallidior* (Bolivia); [18]- *T. pallidior* (Chile); [19]- *T. pallidior* (Argentina); [20]- *Gracilinanus agilis*; [21]- *Marmosops impavidus*; [22]- *T. macrurus* (Paraguay).

| | [1] | [2] | [3] | [4] | [5] | [6] | [7] | [8] | [9] | [10] | [11] | [12] | [13] | [14] | [15] | [16] | [17] | [18] | [19] | [20] | [21] | [22] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| [1] | | | | | | | | | | | | | | | | | | | | | | |
| [2] | 0.8 | | | | | | | | | | | | | | | | | | | | | |
| [3] | 0.8 | 0.0 | | | | | | | | | | | | | | | | | | | | |
| [4] | 20.4 | 19.2 | 19.2 | | | | | | | | | | | | | | | | | | | |
| [5] | 20.4 | 19.2 | 19.2 | 0.0 | | | | | | | | | | | | | | | | | | |
| [6] | 20.4 | 19.2 | 19.2 | 0.0 | 0.0 | | | | | | | | | | | | | | | | | |
| [7] | 16.3 | 15.2 | 15.2 | 13.0 | 13.0 | 13.0 | | | | | | | | | | | | | | | | |
| [8] | 16.6 | 15.6 | 15.6 | 13.4 | 13.4 | 13.4 | 0.3 | | | | | | | | | | | | | | | |
| [9] | 16.7 | 15.6 | 15.6 | 13.1 | 13.1 | 13.1 | 0.5 | 0.8 | | | | | | | | | | | | | | |
| [10] | 17.0 | 16.0 | 16.0 | 17.1 | 17.1 | 17.1 | 15.5 | 15.1 | 15.4 | | | | | | | | | | | | | |
| [11] | 17.0 | 16.0 | 16.0 | 17.1 | 17.1 | 17.1 | 15.5 | 15.1 | 15.4 | 0.0 | | | | | | | | | | | | |
| [12] | 16.5 | 15.5 | 15.5 | 17.1 | 17.1 | 17.1 | 16.1 | 16.5 | 16.8 | 3.5 | 3.5 | | | | | | | | | | | |
| [13] | 16.8 | 16.1 | 16.1 | 18.0 | 18.0 | 18.0 | 16.9 | 17.3 | 17.0 | 12.7 | 12.7 | 12.2 | | | | | | | | | | |
| [14] | 17.7 | 16.6 | 16.6 | 18.8 | 18.8 | 18.8 | 18.2 | 18.5 | 17.5 | 13.3 | 13.3 | 13.2 | 13.5 | | | | | | | | | |
| [15] | 17.7 | 16.7 | 16.7 | 19.3 | 19.3 | 19.3 | 18.5 | 18.8 | 17.8 | 14.7 | 14.7 | 14.5 | 13.5 | 1.1 | | | | | | | | |
| [16] | 16.0 | 14.9 | 14.9 | 17.1 | 17.1 | 17.1 | 14.3 | 14.6 | 14.3 | 12.3 | 12.3 | 12.2 | 13.9 | 5.9 | 7.1 | | | | | | | |
| [17] | 18.9 | 17.8 | 17.8 | 20.5 | 20.5 | 20.5 | 17.6 | 18.8 | 16.9 | 10.8 | 10.8 | 12.0 | 13.6 | 11.0 | 12.0 | 10.1 | | | | | | |
| [18] | 17.8 | 16.7 | 16.7 | 19.4 | 19.4 | 19.4 | 16.5 | 16.9 | 15.9 | 11.1 | 11.1 | 11.7 | 13.3 | 11.3 | 12.3 | 10.4 | 0.8 | | | | | |
| [19] | 16.4 | 15.4 | 15.4 | 17.5 | 17.5 | 17.5 | 17.6 | 18.0 | 16.9 | 9.5 | 9.5 | 10.4 | 12.9 | 10.7 | 11.9 | 9.7 | 5.6 | 5.3 | | | | |
| [20] | 20.9 | 20.9 | 20.9 | 21.8 | 21.8 | 21.8 | 19.6 | 19.3 | 19.3 | 18.8 | 18.8 | 20.2 | 20.8 | 20.9 | 22.3 | 20.2 | 22.2 | 22.6 | 21.1 | | | |
| [21] | 22.4 | 22.4 | 22.4 | 21.4 | 21.4 | 21.4 | 20.7 | 20.3 | 19.9 | 20.9 | 20.9 | 22.7 | 21.5 | 21.2 | 21.9 | 20.4 | 21.7 | 22.1 | 20.6 | 4.7 | | |
| [22] | 26.6 | 25.8 | 25.8 | 20.4 | 20.4 | 20.4 | 24.0 | 23.6 | 24.4 | 25.8 | 25.8 | 26.2 | 27.1 | 28.6 | 29.0 | 26.6 | 27.5 | 27.1 | 25.5 | 14.5 | 15.6 | |

events of *T. macrurus* (which we found occupying the most basal position in the genus) from a more humid environment to drier and more arid habitats, such as those found in the Brazilian Cerrado. This event would have promoted the differentiation of the Brazilian species of *Thylamys*, such as *T. karimii*. This is a small mouse opossum currently known to inhabit open vegetations, not living in fully semi-deciduous forests, from Paraguay to the southern Brazilian ''Cerrado'' (CÁCERES *et al*., 2007).

The Andean group is, in turn, formed by two sister groups: the *T. pusillus* specimens from Argentina and Paraguay (FLORES *et al*., 2000), and *T. pallidior*, *T. tatei*, and *T. elegans*. *Thylamys tatei*, an endemic species from Peru, positioned the sequenced specimens as the sister taxon of *T. elegans*, which inhabits Chile. These are in turn the sister groups of *T. pallidior*, a species widely distributed in western South America (west and south of Peru, north of Chile, and the south of Bolivia to the south of the Valdez peninsula in Argentina; GARDNER, 2005).

The second main clade is composed by *T. cinderella* and *T. venustus*, two taxa previously included in *T. elegans*, but in their revision FLORES *et al.* (2000) suggested that *T. cinderella* is a valid species. These authors also proposed that specimens from northern Argentina and Bolivia belonged to *T. venustus*, while those from southern Argentina were classified as *T. cinderella*. Afterwards, in a phylogenetic analysis BRAUN *et al.* (2005) examined the geographic relationships among these taxa by using molecular data and found that the specimens of the *T. venustus* (from Bolivia and Argentina) formed two well-defined clades, namely *T. venustus* and *T. cinderella.*

BAKER & BRADLEY (2006) suggest that the Kimura 2-parameter corrected distances to describe intraspecific variations for marsupials are on average 1.1 %, and the value to describe sister species variations are on average 10.4 %. Our low within-clade sequence divergence values are comparable with those reported by BAKER & BRADLEY (2006). The extensive sequence divergence within non-sister species observed in our study (more than 15 %) are also in the same level of the values described by these authors (18.1 % on average). As mentioned by PATTON & COSTA (2003), the high sequence divergence observed within other didelphidae genera (*Philander, Marmosa,* and *Monodelphis*, for example) suggests substantial depth to the ages of these taxa.

The relationship of *T. velutinus* to the other *Thylamys* species remains unknown at this time. We suggest five species groups in *Thylamys:* the four groups mentioned by BRAUN *et al*. (2005) and the Brazilian group observed in this work. It is possible that *T. velutinus* and *T. karimii* may constitute a sister group, forming one *Thylamys* clade that colonized Brazil.

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