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Phylogeny of southern South American mouse opossums (*Thylamys*, Didelphidae) based on allozyme and chromosomal data

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Abstract

We evaluated the phylogeny of mouse opossums of the proposed genus *Thylamys* using 26 enzymatic loci and standard karyotypes in four of the five recognized species. Allozyme data were analyzed through parsimony, distance, and likelihood methods. Chromosome data showed a conservative diploid and fundamental number in all analyzed taxa (2 n = 14, FN = 20), although the FN differed with respect to other forms of small opossums by having a FN = 24. Parsimony, distance, and likelihood trees confirmed *Thylamys* as a monophyletic group when compared to other Neotropical mouse opossums. The recognition of *T. elegans* at the eastern and western side of the Andean Cordillera is not supported through allozyme analyses, validating Tate's (1933) contention that two species are present. Other reconstructions found *T. pallidior* to be phylogenetically related to *T. elegans* from the western Andes of Chile, while *T. elegans* from Bolivia appeared as the most basal thylamyine. Our data suggest that the latter should again be recognized as a full species, *T. venusta*.

Key words: Phylogeny, *Thylamys*, South America, allozymes, chromosomes

Introduction

Marsupials of the genus *Marmosa* Gray, 1821 have been under taxonomic revision since Tate (1933) recognized five species groups: *murina*, *cinerea*, *noctivaga*, *microtarsus*, and *elegans*. Tate (1933) based these groupings on morphological characters and suggested that they appeared to represent monophyletic lineages with the exception of *microtarsus*, which might be part of the *elegans* group. Based on morphological, serological, and chromosomal studies some authors have suggested that these species groups approximate the genera *Marmosa* (sensu stricto; Tate's *murina* group), *Micoureus* (Lesson, 1842, Tate's *cinerea* group), *Thylamys* (Gray, 1843, Tate's *elegans* group), *Marmosops* (Matschie, 1916, Tate's *noctivaga* group), and *Gracilinanus* (Gardner and Creighton, 1989, Tate's *microtarsus* group; Creighton 1984; Reig et al. 1987; Gardner and Creighton 1989). Morphologically, *Thylamys* spp. differentiate from the rest of mouse opossums by their three-colored pattern, their capacity to store fat in the tail (at least in temperate forms), the small size of the feet and claws in relation to the body, and the slender nasals that do not expand at the maxillo-frontal suture (Fig. 1; Creighton 1984; Tate 1933). The skull of *Thylamys* has a large tympanic bulla and a narrowed interorbital region in comparison to other marmosines (Tate 1933).

Marmosine opossums comprise a species-rich assemblage consisting of 33 currently recognized species (Gardner 1993), distributed from central Mexico (e. g., *Marmosa mexicana*, Hall 1979) southward to central Argentina and Chile (e. g., *Thylamys*, Tate 1933).

Altitudinally, marmosines range from the lowlands of the Amazon Basin (e. g., *Micoureus*, Emmons 1990) to elevations as high as 3 500 m in the Andes (*T. pallidior*, Tate 1933). Although the majority of mouse opossums inhabit humid tropical and semitropical forests of the Neotropical region, *Thylamys* seems to be restricted to open areas, and has the southernmost distribution in South America (Mann 1978, Fig. 2). *Thylamys elegans* (Waterhouse, 1839) occurs in the Coastal Desert of Chile and Peru, and the lowlands and middle altitudinal areas of southern Bolivia and northern Argentina. *Thylamys pallidior* (Thomas, 1902) inhabits elevations as high as 3 500 m in the rocky slopes of the Andean Altiplano. *Thylamys pusilla* (Desmarest, 1804) is restricted to the Chaco and Monte Desert regions. *Thylamys macrura* (Olfers, 1818) is restricted to the moist forests of eastern Paraguay, and *Thylamys velutinus* (Wagner, 1842) inhabits the Cerrado of Brazil.

Although the patterns of distribution of marmosines in general, and *Thylamys* in particular, are fairly well known, their systematic relationships are pooly understood. Reig et al. (1987), using morphologic, karyologic, and serologic data, proposed the recognition of *Thylamys* based on Tate's (1933) *elegans* and *microtarsus* groups. Although Reig et al. (1987) did not evaluate relationships within *Thylamys*, they placed this genus as the sister-taxon of the Patagonian opossum *Lestodelphys* and suggested that both were more closely related to the short-tailed opossum *Monodelphis* than to *Marmosa* (sensu stricto) and *Micoureus*. Hershkovitz (1992), using morphological data proposed the new family Marmosidae, comprised of all marmosine genera, as well as *Monodelphis*, *Lestodelphys*, and *Metachirus*. In this new family, Hershkovitz (1992) recognized the subfamilies (among others) Thylamyinae (for *Thylamys*), and Marmosinae (including *Marmosa*, *Marmosops*, *Micoureus*, and *Gracilinanus*). Recent phylogenetic hypotheses based on cytochrome b sequences did not include *Thylamys* (Patton et al. 1996). Kirsch and Palma (1995) using DNA-hybridization proposed *Thylamys* as a differentiated lineage in which *T. pusilla* and *T. macrura* appeared as the most divergent taxa.

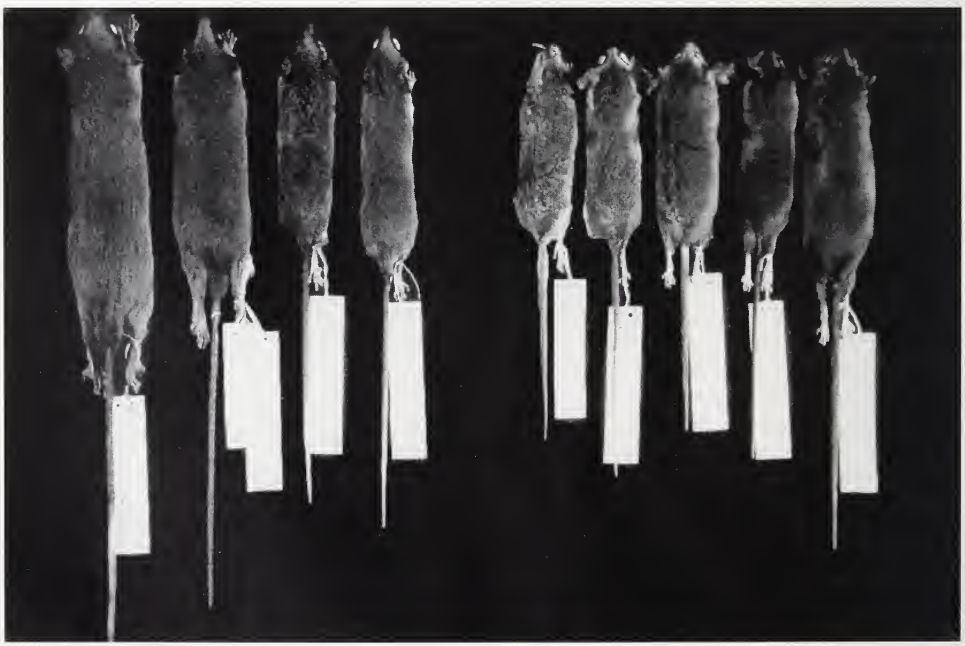


Fig. 1. Dorsal view of skins of mouse opossums (from left to right): *Micoureus cinereus*, *Marmosa robinsoni*, *Marmosops dorothea*, *Gracilinanus agilis*, *Thylamys pallidior*, *T. pusilla*, *T. elegans*, *T. venusta*, and *T. macrura* (total length *T. macrura* = 284 mm).

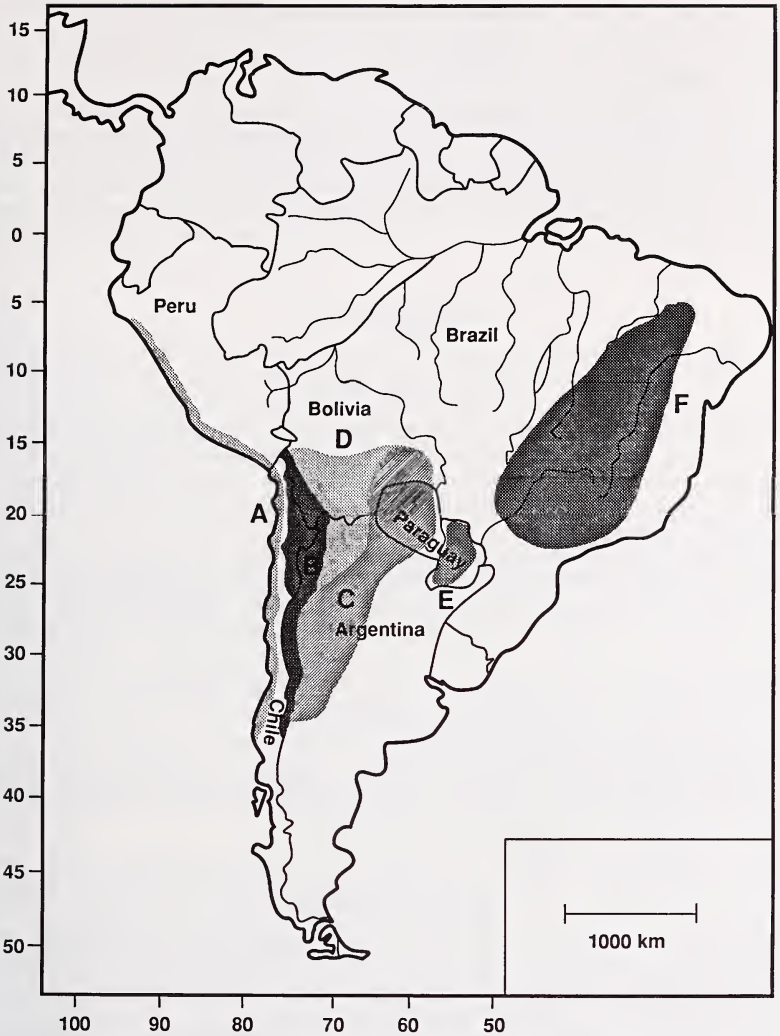


Fig. 2. Ranges of distribution of *Thylamys* in South America. The range of *T. elegans* and *T. venusta* is shown as hypothesized by Tate (1933), and confirmed with the results of this study. A = *Thylamys elegans*, B = *T. pallidior*, C = *T. pusilla*, D = *T. venusta*, E = *T. macrura*, F = *T. velutinus*.

The present study was designed to test the monophyly of *Thylamys* spp., to assess the phylogenetic relationships among *T. elegans*, *T. pallidior*, *T. pusilla*, and *T. macrura*, and to evaluate the relatedness between populations of *T. elegans* across the Andes. The latter objective was focused on Tate's (1933) contention that two species of *Thylamys* were present across the Andean Cordillera, *T. elegans* in the western flank and *T. venusta* in the eastern side, in contrast to Cabrera (1958) who recognized both as *T. elegans*. The systematic relatedness among all taxa was inferred from chromosomes and allozymes. Outgroups included specimens of *Gracilinanus agilis*, *Micoureus cinereus*, *Marmosops dorothea*, and *Monodelphis domestica*.

Material and methods

Chromosomal analyses

Karyotypes were obtained from bone marrow following the conventional Velban technique described by Anderson et al. (1987). Twelve individuals were examined in *Thylamys*, and six from *Gracilinanus*, *Marmosops*, and *Micoureus*. A minimum of 10 metaphase spreads were counted for each specimen. Nomenclature for chromosome morphology and autosomal fundamental number (FN) followed Patton (1967). Chromosomes were arranged sequentially in order of decreasing size, with bi-armed elements preceding single-armed elements.

Allozyme analyses

Horizontal protein electrophoresis was conducted on frozen tissue preparations from 95 specimens of *Thylamys*, of which 77 corresponded to *T. elegans*. Voucher specimens (skins and skeletons) are deposited in the Museum of Southwestern Biology (MSB), Department of Biology, The University of New Mexico, Albuquerque, New Mexico; the American Museum of Natural History (AMNH), New York; Museo Nacional de Historia Natural, La Paz, Bolivia; Museo Noel Keempf Mercado, Santa Cruz, Bolivia; and Museo Nacional de Historia Natural, Asunción, Paraguay. Tissues and cell suspensions are deposited in the Division of Biological Materials of the MSB. Specimens examined were (numbers in parentheses indicate the sample size):

Thylamys elegans, Bolivia: Department of Chuquisaca: Monteagudo (7), Padilla (7), Porvenir (24), Tarabuco (5); Department of Tarija, Tarija (10); Department of Santa Cruz: Vallegrande (8), Quiñe (7).

T. elegans, Chile: Province of Limarí: Fray Jorge (4); Province of Valparaíso: La Campana (5).

T. pusilla, Bolivia: Department of Tarija, Department of Chuquisaca, and Department of Santa Cruz (9).

T. pallidior, Bolivia: Department of Chuquisaca and Department of Tarija (8).

T. macrura, Paraguay: Department of Concepción (1).

Micoureus cinereus, Paraguay: Department of Amambay (1).

M. constantiae, Bolivia: Department of Pando (1).

Monodelphis domestica, Bolivia: Department of Tarija (1); Paraguay: Department of Amambay (1).

Marmosops dorothea: Bolivia: Department of Santa Cruz (1).

Gracilinanus agilis, Bolivia: Department of Santa Cruz (1).

Twenty-one enzymes constituting 26 presumptive loci were examined. These corresponded to: [1] glucose dehydrogenase (GDH), [2] glucose-6-phosphate dehydrogenase (G-6-PDH), [3] hexose-6-phosphate dehydrogenase (H-6-PDH), [4–5] isocytrate dehydrogenase (IDH-2 and IDH-1), [6–7] L-lactate dehydrogenase (LDH-1 and LDH-2), [8–9] malate dehydrogenase (MDH-3 and MDH-1), [10] sorbitol dehydrogenase (SDH), [11] xanthine dehydrogenase (XDH), [12–13] esterases (ES-1 and ES-9), [14] fumarate hydratase (FUMH), [15] octanol dehydrogenase (ODH), [16–17] glutamate oxalate transaminase (GOT-1 and GOT-2), [18] glucose phosphate isomerase (GPI-1), [19] alfa-glycerophosphate dehydrogenase (alfaGPD-1), [20] hexokinase (HK), [21] malic enzyme (ME-1), [22] nucleoside phosphorilase (NP), [23–25] peptidases (PEP-A, PEP-B, and PEP-F), and [26] phosphoglucomutase (PGM-1). An additional protein, alcohol dehydrogenase (ADH), was also assayed, and although it showed polymorphism among populations it could not be scored consistently in all samples. Isozyme systems, stains, and electrophoretic procedures followed Selander et al. (1971), Murphy et al. (1990), and Yates and Greenbaum (1982). Genotypes at each locus were analyzed using the BIOSYS-1 computer program (Swofford and Selander 1981), which computed levels of polymorphism (P), heterozygosity (H), and the degree of population structure (computing Hardy-Weinberg equilibrium and Wright's hierarchical F-statistics (Wright 1965).

Phylogenetic analyses

Allozyme data were analyzed under the principle of maximum parsimony using the computer program PAUP*, version 4.0d52 (Phylogenetic Analysis Using Parsimony, written by David L. Swofford). The allozymic data were analyzed as unordered characters in two ways: first, using the locus as a character, and the allelic combination of the locus as the character-state (Buth 1984); and second, using the allele as a character, and its presence or absence as the state. All equally parsimonious trees were found

through an exhaustive search excluding uninformative characters, and strict and 50% majority-rule consensus trees are presented to summarize the shortest tree lengths. Branch-and-bound bootstrap analyses with 1000 replicates were performed on the data sets to estimate the confidence for each node. Phylogenetic analyses were also accomplished using the Neighbor-Joining algorithm available in PAUP 4.0, and the nodes in the tree were evaluated by a Neighbor-Joining search bootstrap with 1 000 replicates using PAUP. Additionally, the maximum-likelihood technique was implemented on the gene frequencies using the CONTML program in PHYLIP 3.5 c (Felsenstein 1993). Since the allele frequencies among populations of *Thylamys* spp. were highly similar, they were pooled by species in the maximum-likelihood analysis. The same procedure was followed among the outgroup taxa, and is why we refer to these taxa collectively as "marmosines" through this paper.

Results

Chromosomal variation

The autosomal complements among *T. elegans*, *T. macrura*, *T. pallidior*, and *T. pusilla* appeared to be identical in number and morphology with $2n = 14$ and $FN = 20$ (Fig. 3, Tab. 1; Palma and Yates 1996). The autosomes in these four species consist of three large submetacentric chromosomes (pairs 1–3), a medium-sized metacentric complement (pair 4), and two small acrocentric elements (pairs 5–6). No variation in this pattern was found in any specimen analyzed. The X chromosome is a small acrocentric in *T. elegans* and *T. pallidior*, and a small submetacentric in *T. macrura* and *T. pusilla*. The Y chromosome was absent in males of *T. elegans* (Bolivia) and *T. pallidior* (Figs. 3 b and 3 c).

The autosomes of *Micoureus cinereus* were identical to that of *Thylamys* ($2n = 14$, $FN = 20$). However, *Gracilinanus agilis* and *Marmosops noctivagus* while sharing the $2n = 14$ diploid number differed in fundamental number ($FN = 24$) from that found in *Thylamys* and *Micoureus*, due to pairs 5 and 6 being submetacentric instead of acrocentric. The X chromosome in *G. agilis* and *M. noctivagus* is a small submetacentric, while the Y in *G. agilis* is a small submetacentric and that in *M. noctivagus* is a tiny dot (Fig. 3 h).

Allozyme variation

Of the 26 analyzed loci, eight (MDH-1, MDH-3, GDH, H-6-PDH, LDH-1, FUM, PEP-F, and PGM-1) were monomorphic in all taxa examined, whereas the other 18 loci exhibited fixed allelic differences between marmosines and thylamyines. Within *Thylamys*, 10 loci were polymorphic (0.99 eriterion): H-6-PDH, IDH-2, LDH-1, ODH, ES-1, GOT-1, alpha-GPD, ME-1, PEP-A, and PEP-B. *Thylamys elegans* (Chile) and *T. pallidior* had fixed differences at four loci (IDH-2, PEP-A, PEP-B, ME-1) relative to *T. elegans* from Bolivia and other thylamyines from Bolivia and Paraguay. *Thylamys elegans* from Chile and *T. pallidior* shared the same alleles although at different frequencies at IDH-2, PEP-A, PEP-B, and ME-1.

The overall percentage of polymorphic loci (P) across all 12 populations of *Thylamys* was 2.88 (0.95 criterion), or 3.84 (0.99 criterion). Mean heterozygosity per locus per population varied between 0 and 0.01. Similar low values of heterozygosity were obtained in Australian dasyurid marsupials where up to 28 loci were examined (Baverstock et al. 1982; Sherwin and Murray 1990 and references therein). For *Thylamys*, values of chi-square goodness-of-fit showed a departure from Hardy-Weinberg expectation at each variable locus per population (1 D.F., $P < 0.05$), varying between 8.229 (ODH; Quiñe) and 47.022 (alpha-GPD, ME-1, ES-1; Porvenir). The mean value of Wright's F-statistic (F_{ST}, the fixation-index of population subdivision) for all variable loci within *Thylamys* was 0.845.

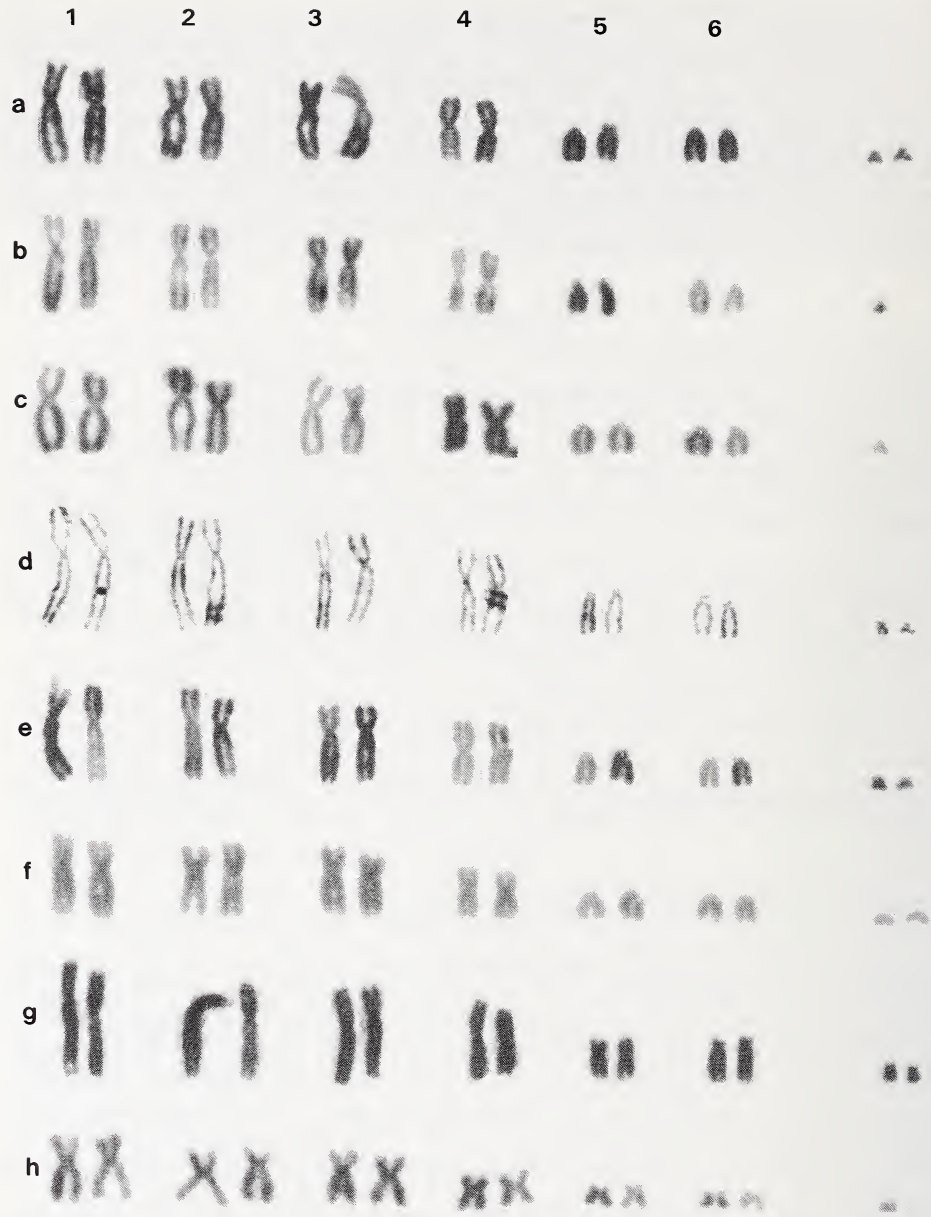


Fig. 3. Standard karyotypes of mouse opossums. The last two chromosomes on each row constitute the sexual chromosomes: a) female *Thylamys elegans* (Chile), b) male *T. elegans* (Bolivia), c) male *T. pallidior*, d) female *T. pusilla*, e) female *T. macrura*, f) female *Micoureus cinereus*, g) female *Gracilinanus agilis*, and h) male *Marmosops noctivagus*.

Parsimony analysis

Locus as a character: Nine equally most-parsimonious trees were obtained through the exhaustive search option of PAUP, all of them 34 steps long, with a consistency index (CI) of 0.941 and a retention index (RI) of 0.933. All rival trees exhibited three polytomous

clades containing *Monodelphis*, *Micoureus*, and the ancestor of a clade that included (*Marmosops-Gracilinanus*)-*Thylamys*. All trees showed *Marmosops* and *Gracilinanus* as the first outgroups to *Thylamys*, and the latter appeared as monophyletic in all most parsimonious trees (Figs. 4 a, 4 b, and 4 c). The bootstrap value for the *Thylamys* clade was

Table 1. Diploid and fundamental number for ten thylamines and marmosines. Abbreviations are 2 N (diploid number), FN (number of autosomal arms), M (metacentric), SM (submetacentric), A (acrocentric), X (X chromosome), and Y (Y chromosome).

Species	2 N	FN	M	SM	A	X	Y
Thylamys elegans	14	20	2	6	4	A	–
Thylamys pallidior	14	20	2	6	4	A	–
Thylamys pusilla	14	20	2	6	4	SM	–
Thylamys macrura	14	20	2	6	4	SM	–
Micoureus cinereus	14	20	2	6	4	A	–
Gracilinanus agilis	14	24	2	10	–	SM	SM
Marmosops noctivagus	14	24	2	10	–	SM	–
Marmosops dorothea	14	24	2	10	–	M	A
Marmosa murina	14	24	2	10	–	M	A
Marmosa robinsoni	14	24	2	10	–	M	A

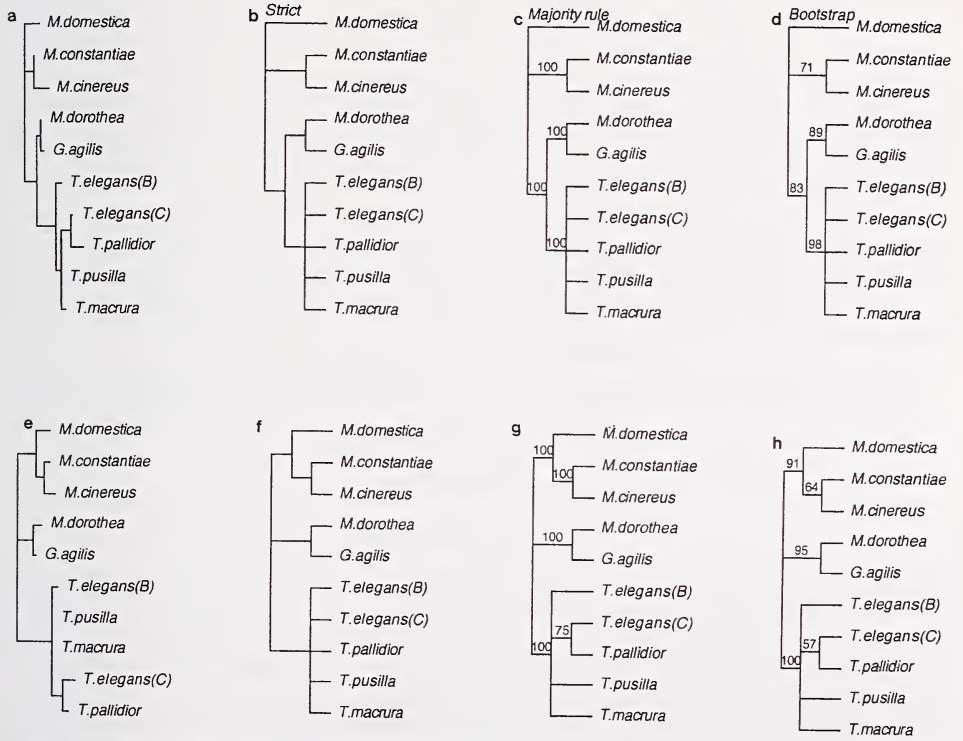


Fig. 4. Allozyme data using the locus as the character (above): a) one of the nine most parsimonious trees showing *T. elegans* from Bolivia as the most basal form, b) strict-consensus tree, c) majority-rule tree d) bootstrap tree. Allozyme data using the allele as a character (below): e) one of the four most-parsimonious trees depicting *T. elegans* from Bolivia as the basal taxon in *Thylamys*, f) strict-consensus tree, g) majority rule tree, h) bootstrap tree. (B = Bolivia, C = Chile).

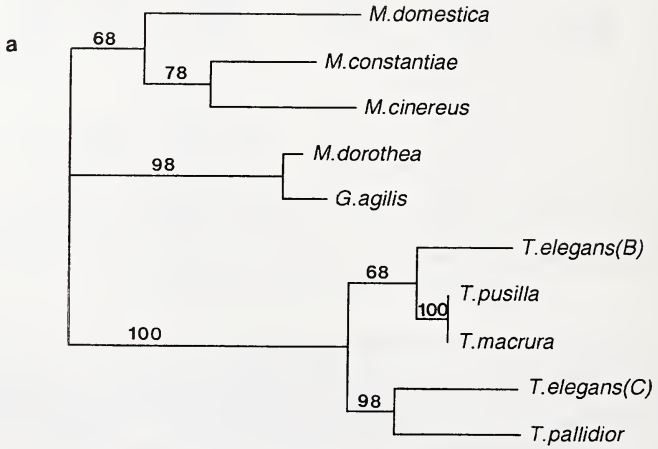
98% (Fig. 4 d). Most of the topologies did not resolve the sequence of phylogenetic relationships within *Thylamys*, particularly the relationships of *T. pusilla* and *T. macrura*. In three reconstructions, *T. pallidior* appeared as the sister-taxon of *T. elegans* from Chile, while the other six rival trees depicted *T. elegans* from Bolivia, *T. elegans* from Chile, and *T. pallidior*, as the most basal thylamyines.

Allele as a character: Four equally most parsimonious trees were obtained using the exhaustive-search option, each 56 steps long with CI = 0.679 and RI = 0.788. Again, as observed in figure 4 e, three polytomous subsets were obtained: the *Monodelphis* clade, the *Micoureus* clade, and the ancestor of a (*Marmosops-Gracilinanus*)-*Thylamys* clade. *Marmosops* and *Gracilinanus* appeared as the sister-group to *Thylamys*, and this association was recovered 100% of the time in the consensus and bootstrap analyses (Figs. 4 f and 4 h). Three out of four trees exhibited *T. elegans* (Chile) as the sister-taxon of *T. pallidior*, as shown by the consensus tree in figure 4 e. The fourth topology showed *T. elegans* (Bolivia) as the sister-group of *T. pallidior*. As with the locus-as-character analysis, the sequence of phylogenetic relationships of *Thylamys* was not completely resolved for *T. macrura* and *T. pusilla*. *Thylamys elegans* (Bolivia) was found to be the most basal form in the majority of topologies.

Distance and likelihood analyses

The Neighbor-Joining tree differentiated mouse opossums in three major subsets: *Monodelphis-Micoureus*, *Marmosops-Gracilinanus*, and *Thylamys* spp. (Figs. 5 a and 5 b). Within *Thylamys*, the grouping between *T. pallidior* and *T. elegans* Chile was recovered 98 and 58 percent of the time in the locus and allele bootstrap analyses, respectively (Figs. 5 a and 5 b). The sister relationship of *T. elegans* from Bolivia and *T. pusilla–T. macrura* was supported 68 and 78 percent of the times in the locus and the bootstrap analyses, respectively. *Thylamys elegans* from Bolivia was the outgroup to the latter two species in both analyses (Figs. 5 a and 5 b).

The topology of the maximum-likelihood tree (28 trees examined, Ln Likelihood = 193.52829) also showed the three major subsets: marmosines, *T. pallidior–T. elegans* (Chile), and *Thylamys* spp. from the eastern Andes (Fig. 6). Furthermore, as recovered with the Neighbor-Joining tree, *T. elegans* from Bolivia appeared as the basal form related to the *T. macrura–T. pusilla* grouping.



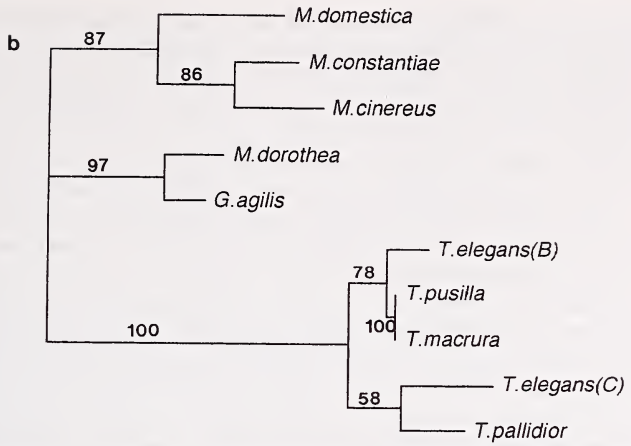


Fig. 5. Neighbor-joining trees based on a) the locus as the character, and b) the allele as the character. Numbers on the nodes represent the 1 000 bootstrap replicate values.

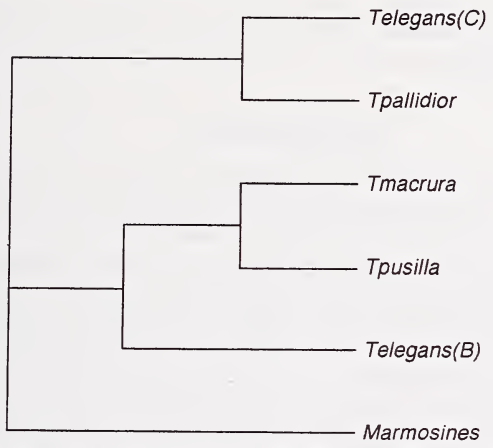


Fig. 6. Maximum-likelihood tree among *Thylamys* spp. (28 trees examined, Ln likelihood = 193.52).

Discussion

Chromosomal analyses

Although marmosine opossums are known for a lack of variation in the total number of chromosomes (2 n = 14; Hayman 1990, although see Engstrom and Gardner 1988), differences are apparent in fundamental number (Reig et al. 1977; Hayman 1990; Palma and Yates 1996). The FN = 20 reported here for *Thylamys* contrasts to the FN = 24 of *Marmosa*, *Marmosops*, and *Gracilinanus*, for example. The karyotype of *Micoureus* presented here, and those obtained from additional localities in Peru and Venezuela (Reig et al. 1977), appear identical to that of *Thylamys*, although these apparent chromosomal affinities should be examined using banding methods. The differences in the number of autosomal arms in mouse opossums are most likely the result of non-Robertsonian changes, such as pericentric inversions which involve changes in the number of autosomal arms but not in the number of chromosomes (Patton and Sherwood 1983).

The morphology of sex chromosomes was found to be highly variable within *Thylamys*. The X chromosomes varied from acrocentric (*T. elegans*) to submetacentric (*T. macrura*). This pattern has also been found in other marmosines (e. g., *Micoureus*, *Gracilinanus*; Palma and Yates 1996). However, in *T. elegans* (Chile and Bolivia) and *T. pallidior*, the Y chromosome was not observed (Palma 1995 a), whereas in *M. noctivagus* it was dot-like as detected in the aquatic opossum *Chironectes minimus* (Palma and Yates 1996).

The apparent lack of an obvious Y chromosome is difficult to verify with the methods followed in this study. It is possible that the Y has been translocated to another chromosome, or this condition may be another example of chromosome mosaicism, i. e. a difference in sex-chromosome composition between the germ line and cells of the somatic tissues (Hayman 1990). This phenomenon has also been detected in other marsupial taxa, such as in the Chilean microbiotheriid *Dromiciops gliroides* (Gallardo and Patterson 1987), and in some taxa of the Australasian families Pseudocheridae and Peramelidae (Hayman 1990). The missing Y chromosome in *Dromiciops* eased Gallardo and Patterson (1987) to hypothesize that *Dromiciops* may be more closely related to Australian than to American marsupials, supporting Szalay's (1982) contention that *Dromiciops* and Australian metatherians constitute the cohort Australidelphia. Data from this study and from Palma and Yates (1996) suggest that sex chromosome mosaicism may be found not only in *Dromiciops* and Australian metatherians, but in other American marsupials as well. If true, this evidence provides a typical case of parallelism in the evolution of metatherian sexual chromosomes.

Allozyme analyses

Allozyme data using parsimony, distance, and likelihood analyses consistently supported a monophyletic association among *Thylamys* spp. distinct from other opossums. These results agree with those obtained through chromosomal and DNA-hybridization methods (Kirsch and Palma 1995) that recognized *Thylamys* as a differentiated lineage with respect to other small didelphids. Consequently, *Thylamys* should be recognized as a monophyletic assemblage, and as a valid genus. Earlier morphologic studies also recognized thylamyines at the generic level (Reig et al. 1987), although these authors included Tate's *microtarsus* group in *Thylamys* instead of in the genus *Gracilinanus* (Gardner and Creighton 1989). Therefore, the hypothesis that *Gracilinanus* is a member of a lineage distinct from *Thylamys* (Gardner and Creighton 1989) is supported by the results of this and from DNA-hybridization studies (Kirsch and Palma 1995).

Allozyme parsimony using the locus as the character showed that *Marmosops* and *Gracilinanus* constitute the sister-group to *Thylamys*, concurring with DNA-hybridization studies (Kirsch and Palma 1995). These and other results allowed Kirsch and Palma (1995) to propose the subfamilies Thylamyinae (with the tribes Thylamyini and Marmosopsini; the latter including *Marmosops* and *Gracilinanus*), the Marmosinae (consisting of Marmosini; for *Marmosa* and *Micoureus*), and Monodelphini (for *Monodelphis*; Kirsch, and Palma 1995). This classification proved to be consistent with the three clades obtained with allozyme parsimony, although the analysis did not include the genus *Marmosa*. Patton et al. (1996) also found, that *Marmosops* was excluded from a clade containing *Marmosa*, *Micoureus*, and *Monodelphis* based on phylogenetic analyses of cytochrome b sequences. Although we did not evaluate the relationships between small-bodied versus large marsupials, the recognition of mouse opossums as a family including *Metachirus* (Marmosidae; Hershkovitz 1992) has found no support through molecular analyses using DNA-hybridization and cytochrome b methodologies (Kirsch and Palma 1995; Patton et al. 1996).

Allozyme distance and likelihood analyses suggests that a major historical biogeographic event (e. g., vicariance) may have triggered the differentiation of *Thylamys* across

the Andes, giving rise to western and eastern forms. This historical scenario may have allowed the speciation of *T. pallidior* in the Prepuna and Puna areas of the Andes, and of *T. elegans* in the western lowlands and Coastal Cordillera of Chile and Perú, leaving *T. elegans* (Bolivia), *T. pusilla*, and *T. macrura* at the eastern side of the Andes. The populations of *T. elegans* from Chile differed by fixed differences at four enzymatic loci with respect to eastern populations of *Thylamys* spp. These fixed allele differences were also shared by populations of *T. pallidior*, although at different frequencies. The high F_{ST} value obtained in this study suggests that strong genetic differentiation has occurred within *Thylamys* as evidenced by the fixed loci that differentiated populations across the Andes, and the low values of heterozygosity on either side of the Cordillera. The F_{ST} value also shows that thylamyine populations as a whole are not in Hardy-Weinberg equilibrium, indicating that populations are either not randomly mating, and/or some evolutionary force may be acting within populations. The low values of polymorphism and heterozygosity suggest that gene flow might be maintaining the genetic homogeneity among populations on either side of the mountains. Alternatively, the extremely low values of genetic heterozygosity and deviations from the Hardy-Weinberg expectations within *Thylamys*, would imply that some populations of the most basal species may have experienced one or more recent severe bottlenecks. The latter may have been triggered by geological events during the Plio-Pleistocene in the Andes and surrounding areas, during the great uplift of the Cordillera and/or the glacial periods (Potts and Behrensmeyer 1992). Although we cannot infer the most basal form from distance and likelihood analyses, allozyme parsimony suggests that *T. elegans* from Bolivia might be that form, and it should exhibit the higher levels of genetic variability than all of their descendants (Barton 1989; Avise 1993). Although this has not been found to be the case, the complete range of *T. elegans* in the eastern Andes has not been sampled in term of genetic variation, since the species is also distributed southward to the Argentinean Patagonia (where the oldest fossil records of *Thylamys* have been obtained; Reig et al. 1987). Concurring with allozyme parsimony, preliminary results based on cytochrome b sequences support *T. venusta* as the most basal form (Palma 1994), contrary to what DNA-hybridization studies might suggest (that the basal stock of *Thylamys* would be constituted by *T. macrura* along with *T. pusilla*). We think that the completion of sequencing analyses on the genus will help to clarify this disagreement.

Data from our study are consistent with Tate's (1933) suggestion that *T. elegans* on either side of the Andes mountains represent two distinct species. Thomas (1902) considered the Bolivian form as a subspecies of *elegans* (*Marmosa elegans venusta*). Later, Tate (1933) assigned it specific status naming it *Marmosa venusta* with the subspecies *venusta*, *sponsoria*, and *cinderella*. However, Cabrera (1958) recognized all these forms as subspecies of *T. elegans* from Chile. Therefore, our results support the recognition of two species of *Thylamys* across the Andean Cordillera, *T. elegans* (Waterhouse, 1839) on the western side, and *T. venusta* (Thomas, 1902) on the eastern side. Whether the differentiation of *Thylamys* across the Andes has been due to vicariance, dispersal, or some other historical biogeographic event, cannot be determined until the sequence of phylogenetic relationships among all thylamyines is understood.

Hypothesized relationships through parsimony place the Andean *T. pallidior* with *T. elegans* from Chile as sister taxa when using the allele as the character. Even, when data were analyzed using the locus-as-character, this reconstruction was obtained in three out of nine rival trees. The neighbor-joining and maximum-likelihood analyses also recovered the *T. pallidior–T. elegans* association, and the bootstrap analysis gave high support to this grouping. The occurrence of a recent common ancestor in the evolution of *T. elegans* from Chile and *T. pallidior* is evidenced by the four alleles that both forms share and that differentiate them from other thylamyine taxa. The phylogenetic relationships between the latter two taxa may be interpreted in light of the biogeographic distri-

bution of *T. elegans* and *T. pallidior*. Recent studies have shown *T. pallidior* occurring not only over the western flank of the northern Chilean Andes, but also over the western flank of the Cordillera in northern Chile, and nearby Coastal areas of the latter country where *T. elegans* is found (Palma 1995 b). Alternatively, other topologies in the locus analysis showed *T. pallidior* to be more phylogenetically related to *T. elegans* from Bolivia, as did studies using DNA-hybridization (Kirsch and Palma 1995). This relatedness is consistent with the biogeographic history between the Andes and the Chaco (Potts and Behrensmeyer 1992). Older biogeographic scenarios involve the expansion and contractions of areas during the multiple Pleistocenic glaciations of the Andes, which may have allowed contact between the Andean and eastern Chacoan biota, as hypothesized for the evolution of sigmodontine rodents inhabiting both regions (Mares et al. 1985; Braun 1993).

The parsimony analyses were unable to resolve the phylogenetic relationships within *Thylamys* with respect the placement of *T. macrura* and *T. pusilla* within the clade. This result was obtained when using the locus and the allele as a character, and whether including or excluding *Monodelphis* and *Micoureus* as outgroups. However, distance and likelihood analyses showed *T. pusilla* along with *T. macrura* as sister taxa, while *T. venusta* appeared as the first outgroup to this association. This genetic differentiation may be based on the distributional pattern of the former two taxa, since *T. pusilla* is a form mainly restricted to the Chaco region, while *T. macrura* occurs in the subtropical humid forests, east to the Chaco, suggesting that vicariance would account for the evolution of these forms in both vegetational zones. However, although the main geographic barrier that divides both regions is the Paraguay River, this has not been considered an effective barrier to gene flow, since the river is moderately broad and slow moving in some areas (Myers 1982). Former studies comparing several species of sigmodontine rodents of the Chaco and eastern Paraguay have concluded that the fauna of these regions have diverged due to the abrupt habitat, soil, and topography changes between these areas (Myers 1982). These factors, coupled with dispersal of faunal elements into the Chaco and the Eastern Paraguayan Forests may explain the differentiation of taxa in both vegetational zones (Myers 1982).

At the time of this research it was still believed that *T. velutinus* was "of rare occurrence" (restricted to the Atlantic Forests of Brazil), and this is the reason why this species was not included in the analyses. However, recent studies have shown that *T. velutinus* might not be as "rare" as previously believed, being now also known from the Brazilian Cerrado (Palma 1995 b). Consequently, the results of this study support the recognition of *Thylamys pusilla* (Desmarest, 1804), *Thylamys macrura* (Olfers, 1818), *Thylamys elegans* (Waterhouse, 1839), *Thylamys pallidior* (Thomas, 1902), and *Thylamys venusta* (Thomas, 1902), but is unable to address the status of *T. velutinus*.

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Zusammenfassung

Phylogenie von südamerikanischen Beutelmäusen (Thylamys, Didelphidae) auf der Grundlage von Allozym- und Chromosomendaten

Die phylogenetischen Beziehungen zwischen vier der fünf anerkannten Arten der Gattung *Thylamys* wurden mittels Allozymelektrophorese (26 loci) und Standardkaryotypen untersucht. Die Allozymdaten wurden mittels Parsimonie-, Distanz- und Maximum-Likelihood-Methoden ausgewertet. Bei allen untersuchten Taxa waren die Diploidieverhältnisse und die Chromosomenzahl konstant (2 n = 14, FN = 20). Die FN unterschied sich jedoch von jener anderer kleiner Opossumformen durch FN = 24. Die verschiedenen konstruierten Stammbäume bestätigten die Abgrenzung von *Thylamys* gegenüber anderen neotropischen Mausopossums als eine monophyletische Gruppe. Das Vorkommen von *T. elegans* sowohl auf der westlichen als auch auf der östlichen Seite der Anden wurde durch unsere Allozymdaten nicht bestätigt. Dies stützt die Behauptung von Tate (1933), daß hier zwei unterschiedliche Arten vorhanden sind. Andere Ergebnisse der Stammbaumanalysen ergaben eine gewisse Verwandtschaft von *T. pallidior* mit *T. elegans* von der chilenischen Westseite der Anden, während *T. elegans* aus Bolivien als die ursprünglichste Form der Thylamyinen zu interpretieren war. Nach unseren Befunden sollte die letztere Form vielleicht als eigene Art, *T. venusta*, anerkannt werden.

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