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Phylogenetic and biogeographic relationships of the mouse opossum *Thylamys* (Didelphimorphia, Didelphidae) in southern South America

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Abstract

Nucleotide sequence data from the mitochondrial cytochrome *b* gene were used to evaluate the phylogenetic relationships among mouse opossum species of the genus *Thylamys*. Based on approximately 1000 bp in five of the six species of the genus and including different localities for some of the species, we concluded that *T. macrura* from the subtropical forests of eastern Paraguay is the most primitive taxon. Subsequent radiation of the genus is explained mainly via founder effect speciation. This evolutionary scenario would account for the speciation of *T. pusilla*, *T. venusta*, *T. pallidior*, and *T. elegans* in the Chaco, southern Bolivia and northern Argentina, the Andean Altiplano, the Coastal Desert of Chile, and coastal Perú, respectively. Calibration of a molecular clock set the Pleistocene as the period for the differentiation of *Thylamys* species. The molecular results confirm the strong genetic connection between populations that inhabit the “pre-cordillera” of northern Chile (*T. pallidior*) and the canyons that run through the Atacama Desert to the lowlands in northern Chile. Our results confirm the occurrence of two *Thylamys* species in Chile, *T. pallidior* and *T. elegans*, within and south to the Atacama Desert, respectively.

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1. Introduction

Contrasting with the distribution of most mouse opossums, the genus *Thylamys* (Gray, 1843) ranges in the central and southern portions of South America inhabiting open dry-type habitats in contrast to a preference for more mesic environments of other genera. Tate (1933) originally divided mouse opossums into five species assemblages under one genus, *Marmosa*. Today, after further systematic revisions that included morphological, chromosomal, and biochemical analyses (Creighton, 1984; Gardner and Creighton, 1989; Reig et al., 1985, 1987), Tate's groups *murina*, *cinerea*, *noctivaga*, *microtarsus*, and *elegans* gained generic status and they are currently recognized as the genera *Marmosa*, *Micoureus*, *Marmosops*, *Gracilinanus*, and *Thylamys*, respectively.

Marmosine opossums are mouse-like marsupials, whose activity is mainly arboreal and crepuscular, feeding preferentially on insects and small vertebrates (Mann, 1978; Redford and Eisenberg, 1992). *Thylamys* are also characterized by their tail incrustation during winter, particularly those taxa restricted to more extreme climates (e.g., *T. pallidior*, *T. elegans*). Six species are currently recognized in central and southern South America, each of them being more or less restricted to a particular biome (Gardner, 1993; Palma, 1995a, 1997; Fig. 1): *T. velutinus* occurs in the Cerrado and Caatinga of Brazil; *T. pusilla* in the Chaco and Monte region; *T. venusta* in the oriental flank of the Andes in southern Bolivia and northern Argentina; *T. pallidior* in the Andean highlands of southern Peru, northern Chile, Argentina, and western Bolivia at elevations over 3500 m (Palma, 1995a); *T. elegans* ranges along the coastal and central areas of Peru and Chile, southward to the Bío-Bío river. However, Handley (1956) recognized the Peruvian *Thylamys* from the Lima area as *T. tatei*, which

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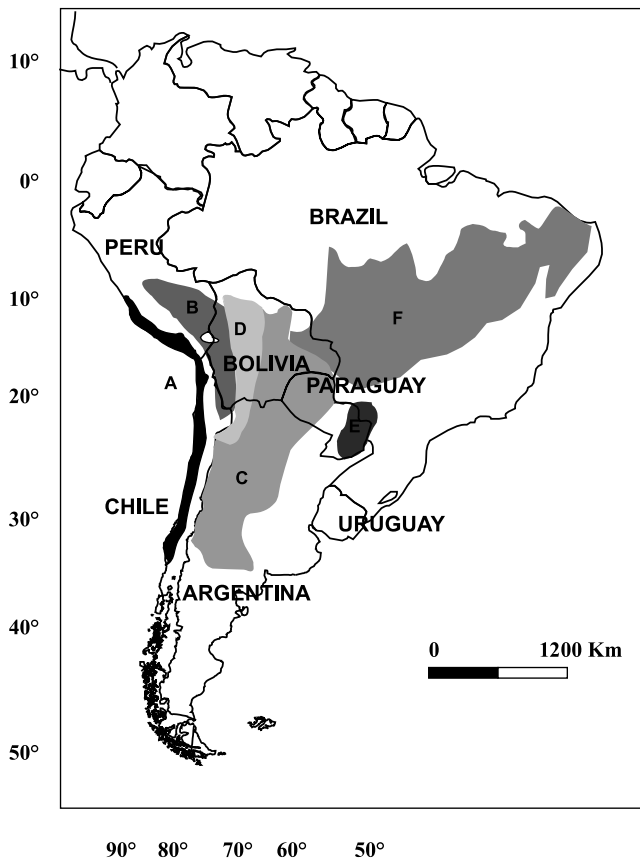


Fig. 1. Distribution of *Thylamys* spp. in the Neotropical region: (A) *T. elegans*; (B) *T. pallidior*; (C) *T. pusilla*; (D) *T. venusta*; (E) *T. macrura*; (F) *T. velutinus*.

was later considered to be a synonym of *T. elegans* (Gardner, 1993). *T. macrura* is the only representative of the genus inhabiting subtropical humid forests in the oriental of Paraguay (east to the Paraguay river), also see Anderson (1997).

Until now, systematic studies in *Thylamys* have involved morphological, chromosomal, allozyme, and DNA hybridization analyses (Kirsch and Palma, 1995; Palma, 1995b; Palma and Yates, 1998; Reig et al., 1987). Reig et al. (1987), based on selected taxa, confirmed one of their former hypotheses recognizing *Thylamys* at the generic level and as the sister taxon of the Patagonian didelphimorph *Lestodelphys halli*. DNA hybridization analyses also supported this hypothesis, placing *T. macrura* as closely related to *T. pallidior* and *T. venusta* (Kirsch and Palma, 1995). Palma and Yates (1998) using protein electrophoresis confirmed the monophyly of the genus and, in contraposition to Cabrera (1958), split *T. elegans* on both sides of the Andes into *T. venusta* in the east and *T. elegans* in the west. In the same biochemical study, parsimony analyses did not resolve the phylogenetic relationships in all included thylamyines, recovering several equally parsimonious trees and showing several polytomies in the branching reconstructions

(Palma and Yates, 1998). The latter authors concluded that the differentiation of thylamyines may have been triggered by the great uplift of the Andes during Plio-Pleistocene times and suggested that dispersal was the major cause of speciation for thylamyines of the eastern side of the Andes (e.g., *T. pusilla* from the Chaco region).

Because protein electrophoresis did not fully resolve the phylogeny of *Thylamys* (Palma and Yates, 1998), the present paper evaluated the phylogenetic relationships of the composite species using sequences of the cytochrome *b* mitochondrial gene. We included all currently recognized species, with the exception of *T. velutinus* from Brazil due to the unavailability of samples. Because of the extensive distribution proposed for *T. elegans* (Gardner, 1993; Palma, 1997), we included additional sequences of specimens distributed on the coastal areas of Perú and Chile to evaluate the taxonomic status of this species along part of its extension range. On the other hand, for some thylamyine taxa and due to the difficulty in obtaining field samples, we included a single specimen as is the case for *T. macrura* from Paraguay. In fact, the specimen of the latter taxon here sequenced represents the sixth published record for the species (Palma, 1995b). Therefore, the main objective of this study is to reconstruct the phylogenetic history of *Thylamys* spp. and test the biogeographic hypotheses outlined in Palma and Yates (1998) regarding differentiation of the taxa in southern South American biomes.

2. Materials and methods

2.1. DNA sequencing

DNA was extracted from frozen tissue (mainly liver) according to the techniques outlined in Longmire et al. (1988) and Laird et al. (1991) or from museum skins using Chelex (Walsh et al., 1991). As shown in Appendix A, we sequenced a single specimen per species, except for *T. venusta*, *T. pallidior*, and *T. elegans*, for which we sequenced two, three, and four specimens, respectively. We amplified the cytochrome *b* mitochondrial gene via the polymerase chain reaction for 12 specimens (PCR; Saiki et al., 1988) using Taq DNA Polymerase (Gibco-BRL) and primers L 14724 (Kocher et al., 1989), L 15162 (Irwin et al., 1991), MVZ 35 (Smith and Patton, 1993), and H 15767 (Edwards et al., 1991). The names of the oligonucleotides indicate the light (L) and the heavy (H) strands and the position of the 3' end of the oligonucleotide according to the mouse mtDNA sequence (Bibb et al., 1981). The PCRs were performed using the following thermal profile: 94 °C denaturation (1 min 10 s), 52 °C annealing (45 s), and 72 °C extension (1 min 30 s) for 35 cycles. Double-stranded PCR products were

purified with the methods of Wizard-PCR Preps (Promega) and QIAquick (Qiagen). Sequencing was conducted through cycle sequencing (Murray, 1989) using primers L 14724, L 15162, MVZ 35, and H 15767 labeled with the Big Dye Terminator Cycle Sequencing Ready Reaction kit of Applied Biosystems and the sequencing reactions were analyzed in an ABI Prism 310 automated sequencer. The PCR products were sequenced at least two times to ensure sequence fidelity. Sequences were aligned by eye to maintain amino acid sequence and we used MacClade (Maddison and Maddison, 1992) to translate nucleotide codons into amino acids. In addition, the published sequence of *Marmosops impavidus* (Patton et al., 1996) was used as further guideline for the cytochrome *b* alignment. The following marsupial taxa were entered into GenBank (accession numbers in parenthesis): *T. elegans* (Perú, Lima; AF434179), *T. elegans* (Chile, Parque Nacional Fray Jorge; AF431929), *T. elegans* (Maipú, Quebrada de la Plata; AF431925), *T. macrura* (Concepción, Paraguay; AF431926), *T. pallidior* (Bolivia, Tarija; AF431924), *T. pallidior* (Chile, Enquelga; AF431930), *T. pallidior* (Chile, Quebrada de Camarones; AF431923), *T. pusilla* (Bolivia, Chuquisaca; AF431927), *T. venusta* (Bolivia, Chuquisaca; AF431922), *Gracilinanus agilis* (La Paz, Bolivia; AF431928).

2.2. Phylogenetic analyses

Phylogenetic reconstructions were performed through maximum parsimony using the test version of PAUP* 4.0b8 (David L. Swofford). All characters were analyzed as unordered with four possible states (A, C, G, and T), excluding phylogenetically uninformative characters. Equally parsimonious trees were found through an exhaustive search. Phylogenetic analyses were also accomplished using the neighbor-joining and maximum-likelihood algorithms available in PAUP. The neighbor-joining tree was generated from a distance matrix using the Kimura-two-parameter (K2P) option, while the maximum-likelihood topology was obtained

through a heuristic search with the two-parameter model variant for unequal base frequencies following the Hasegawa–Kishino–Yano model available in PAUP. Nodes in the maximum parsimony were evaluated via bootstrap (Felsenstein, 1985) performing 10,000 replicates, while for neighbor-joining and maximum-likelihood we conducted 1000 iterations. Additionally, for maximum parsimony we calculated the decay index (Bremer, 1988) obtained through the AutoDecay program (Eriksson, 1999). Trees were rooted with the outgroup criterion using the published sequence of the Andean slender mouse opossum *Marmosops impavidus* (Patton et al., 1996) and the agile gracile mouse opossum *Gracilinanus agilis*. Finally, by using the MEGA program (Molecular Evolutionary Genetic Analysis, version 1.02; Kumar et al., 1993), we obtained the frequencies of nucleotide bases, the number of transitions and transversions between every pair of taxa, and the transition/transversion rate.

2.3. Molecular clock

We calibrated a molecular clock to estimate the time of divergence of thylamyines. In calibrating the clock, we followed Patton et al. (1996), who based on the level of transversion evolution at third codon position for the cytochrome *b* gene proposed a rate of divergence of 1.09% per million years according to the mouse opossum-*Monodelphis* estimate (Patton et al., 1996). This rate is based on assumptions of divergence dates from the fossil record *sensu* Reig et al. (1987). The percentage of divergence by node was estimated through the MEGA program, using the K2P distance matrix considering transversions at third codon position, as well as considering the overall variation.

3. Results

The transition/transversion rate within all mouse opossums including the outgroups *Gracilinanus* and

Table 1
Nucleotide sequence distance matrix generated by the Kimura-2-parameter criterion of MEGA 1.01 among pairwise comparisons of *Thylamys* and the outgroups*, based on the cytochrome *b* mitochondrial gene

Taxa	1	2	3	4	5	6	7	8	9	10
1. Telegans Fjorge										
2. Telegans Qplata	0.05095									
3. Tpallidior Bolivia	0.10800	0.10998								
4. Tpallidior Colchane	0.11492	0.11739	0.01242							
5. Tpallidior Arica	0.12290	0.10659	0.06242	0.06818						
6. Tvenusta	0.17129	0.16459	0.17607	0.17460	0.16880					
7. Tpusilla	0.30671	0.31338	0.30549	0.30871	0.31684	0.31684				
8. Tmacrura	0.28237	0.26051	0.27415	0.27411	0.25563	0.25563	0.35348			
9. Gracilinanus*	0.22212	0.22029	0.24275	0.24259	0.23581	0.23581	0.28335	0.18492		
10. Marmosops*	0.24213	0.23776	0.23725	0.23630	0.22811	0.22811	0.29557	0.17699	0.06769	

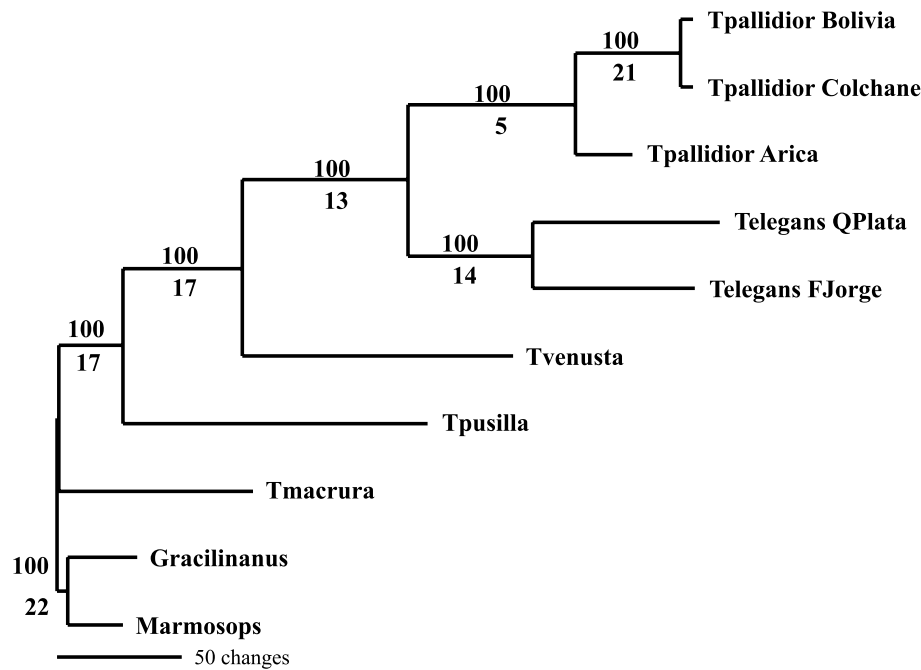


Fig. 2. Maximum-parsimony tree obtained from the aligned sequences of the cytochrome *b* mitochondrial gene for mouse opossums. Numbers above the nodes represent 10,000 replicates bootstrap values, while those below represent the decay values.

Marmosops was 1.97, transitions of the CT type being the most frequent nucleotide substitutions. Sequence divergence (Table 1) within localities of the same species had values below 7%; for example, between *T. elegans* south of the Atacama Desert (QPlata and FJorge) sequence divergence was about 5%. The same values within *T. pallidior* averaged 4.7% and between the highland (Bolivia and Colchane) and the lowland *T. pallidior*'s populations divergence averaged 6.5%. Sequence divergence between *T. pallidior* and *T. elegans* was about 11.5%, while between these and *T. venusta* was of about 17%, being even greater when considering the other basal mouse opossums if compared to *T. venusta* and the Andean forms (Table 1).

A single most parsimonious tree was obtained through the exhaustive search option of PAUP, 589 steps long, consistency index (CI)=0.6316, homoplasy index (HI)=0.3684, retention index (RI)=0.6431 (Fig. 2). Of the 992 bp cytochrome *b* sequences obtained, only 260 were phylogenetically informative characters. As observed in the maximum-parsimony tree (Fig. 2), *Thylamys* was recovered as a monophyletic group that included *T. elegans* and *T. pallidior* (the Andean-Pacific clade) as sister species with a bootstrap support of 100% and *T. venusta* from Bolivia as the most recent common ancestor. *T. pusilla* from the Chaco region is recovered as the sister taxon to the latter reconstruction, while *T. macrura* from the subtropical forests of eastern Paraguay constituted the most basal species of the genus. The bootstrap iterations gave 100% support

for the position of *T. macrura*, *T. pusilla*, and *T. venusta*, with decay indexes of 17, 17, and 13, respectively (Fig. 2).

Within *T. pallidior*, the population located in the lowlands of Arica, Quebrada de Camarones, was associated to the "pre-cordillera" populations of Bolivia and Colchane with a bootstrap support of 100% and a decay value of 5. The two populations of *T. elegans*, on the other hand, were also recovered with 100% of support and a Bremer index of 21 (Fig. 2).

The neighbor-joining reconstruction represented in Fig. 3 recovered a tree comparable to that obtained with maximum parsimony and all nodes with almost 100% bootstrap support. The same topology was obtained with the maximum-likelihood algorithm (not shown). Sequence divergence represented in the branch lengths of the distance tree of Fig. 3 was obtained from the K2P distance matrix and is shown in Table 1. In the same topology, the percentages of sequence divergence estimated with the MEGA program are shown, based on a divergence rate of 1.09% per year (Patton et al., 1996). Time since divergence within *Thylamys* spp. varied between 42,000 and 247,000 years BP. For instance, the split between *T. macrura* and the other thylamyines was calibrated as 174,000 years ago, while that between the Chacoan *T. pusilla* is hypothesized as 247,000 years BP, and the split between *T. elegans* and *T. pallidior* is hypothesized as occurring about 110,000 years ago. The high value of divergence time obtained for *T. pusilla* (247,000 years,

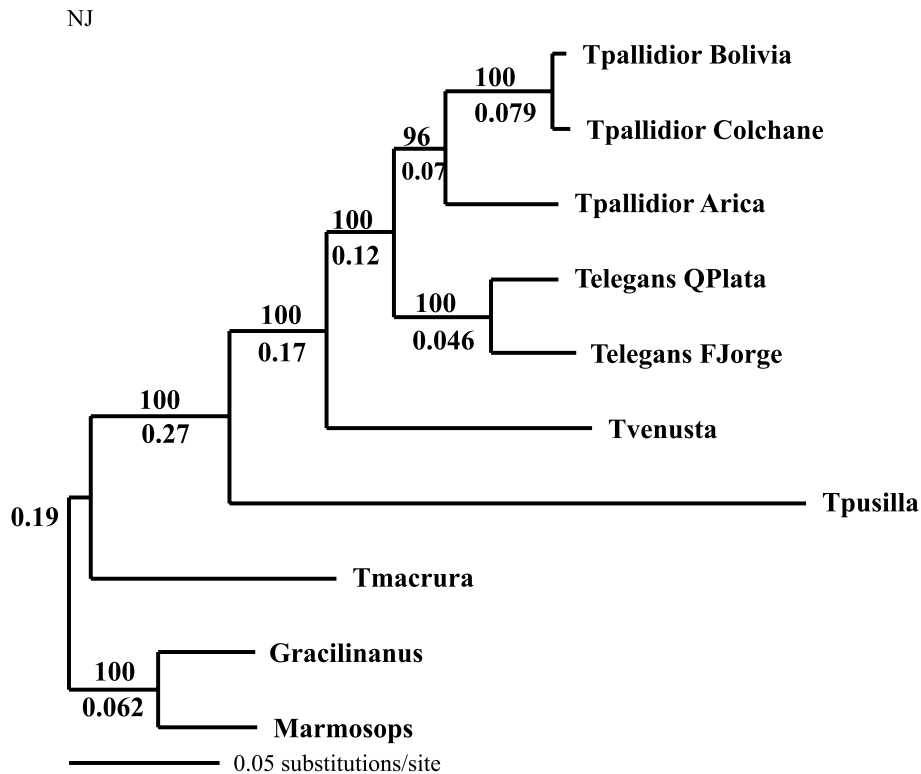


Fig. 3. Neighbor-joining tree obtained from the K2P distance matrix. Values above the nodes represent 1000 bootstrap replicates, while those below show the percentage of nucleotide divergence for each node (obtained from the 1.09% per million years rate, sensu Patton et al., 1996; see Section 2).

in contrast to that of 174,000 obtained for *T. macrura*), may be due to the high substitution rate per site along the *T. pusilla* branch, as a probable consequence of the passage of thylamyines from forest humid to more open and dry environments (see below). The values of sequence divergence did not change significantly when considering the total pairwise comparison among taxa. In fact, the percentage values in each node varied by about 2% with respect to values obtained when considering transversions at third codon position.

4. Discussion

4.1. Phylogenetic analyses

Former phylogenetic studies based on allozyme and DNA hybridization methodology (Kirsch and Palma, 1995; Palma and Yates, 1998) recognized the monophyly of *Thylamys*, results that are also confirmed by this study. However, the phylogeny based on cytochrome *b* sequences more fully resolves the relationships among thylamyine taxa. In fact, the allozyme study, when using the locus and the allele as a character, recovered several equally parsimonious and

polytomous trees but did not resolve, for instance, the relationships between *T. pusilla* and *T. macrura*. Furthermore, the phylogeny obtained through allozyme data suggested that *T. venusta* was basal to *T. pusilla* and *T. macrura* (Palma and Yates, 1998), in contrast to the present study which suggested that the inhabitant of the subtropical forests of eastern Paraguay (*T. macrura*) was the most primitive species. The latter hypothesis is more plausible from a historical biogeographic perspective, since the forests of the Neotropical region are considered to be more primitive vegetational formations than the open xeric-like environments where the rest of thylamyines are distributed (Potts and Behrensmeyer, 1992; Solbrig, 1976). This is because dry open biomes currently present in southern South America are the result of a series of glacial cycles during the Pleistocene that triggered the formation of savanna-like and xeric habitats in most areas of the continent (Potts and Behrensmeyer, 1992; Romero, 1986; Villagrán and Hinojosa, 1997; Webb, 1991). Therefore, the recovery of the sylvan *T. macrura* as the most basal species within the genus agrees with the ancestrality of the environment where this species occurs and is reflected by some of the adaptations of this mouse opossum to a sylvan way of life (Palma, 1997).

4.2. Biogeographic scenarios for thylamyine differentiation

The radiation of thylamyines to the southern biomes as suggested by this study in the basal part of the phylogenetic tree can be hypothesized to be the result of founder effect speciation. We postulate the dispersal of peripheral isolates of *T. macrura* from the subtropical Paraguayan forests to the contiguous areas of the Chaco and Monte Desert. The invaders to the latter region may have subsequently given rise to *T. pusilla*. The dispersal hypothesis for the differentiation of *T. pusilla* from *T. macrura* is preferred over a vicariant scenario, because the Paraguay river that divides Paraguay into two biomes (Chaco and subtropical humid forests) does not seem to constitute an effective barrier to dispersal (see Myers, 1982 for discussion on the small mammal fauna at both sides of the Paraguay river in Paraguay). The colonization of *T. macrura*'s peripheral isolates into the Chaco region may have triggered the differentiation of survival invaders into the latter biome due to a progressively reduced gene flow with respect to parental populations, and to the effects of genetic drift, since just some individuals may have succeeded into the new habitat. This scenario may currently be reflected by the strong nucleotide substitution rate that was recovered in the tree's branch length (e.g., neighbor-joining) that represents *T. pusilla*. This form then, according to the phylogenetic reconstructions obtained, is hypothesized to be one of the first thylamyines that experienced the passage from a subtropical humid to a more open dry environment. Although *T. velutinus* was not included in this study, we could hypothesize its phylogenetic position in the tree as derived from *T. macrura*—probably sister to *T. pusilla*—since the Brazilian taxon may have also differentiated by dispersal to open semi-dry environments as constituting the present day's Brazilian Caatinga and Cerrado biomes.

The occurrence of *T. venusta* in southern Bolivia and northern Argentina may also be explained by founder-effect speciation derived from dispersalists from the neighboring Chaco region. Probably, the original range of *T. venusta* also encompassed the south-eastern Andes. According to Solbrig (1976), previous to the major uplift of the Andean Cordillera during Plio-Pleistocene times, the vegetational formation of the southern portion of South America constituted or was part of what is known as the "Chaco Dominion," with vegetation adapted to open semi-dry environments shared on both sides of the Andes (e.g., *Larrea*, *Prosopis*, *Acacia*). *T. pusilla* restricted to the Monte Desert and the Chacoan region and *T. venusta* from southern Bolivia and the adjacent Andean Cordillera might have been some of the earlier thylamyine mouse opossum marsupials inhabiting the Chaco Dominion. In fact, the oldest fossil record for *Thylamys* is from the Pliocene (Monte Hermoso), Bue-

nos Aires Province, Argentina (Mones, 1980; Reig et al., 1987). New populational isolates of what are now known as *T. venusta* in the eastern flank of the Andes appeared to have dispersed to the west, across lower elevations of the Andes (Pleistocene times), with subsequent colonization of the western lowlands. Further colonization of the lowlands might have been facilitated by the occurrence of several vegetational canyons or "quebradas" that run across the desert connecting the Andes mountains with the coasts of Perú and northern Chile (e.g., Quebrada de Camarones, Quebrada de Tarapacá in the northern Region I of Chile; Marquet, 1989).

The *Thylamys* from the lowland area of Quebrada de Camarones, Arica (region I of Chile), recovered as closely related to both populations of *T. pallidior* from the Andean "pre-cordillera," confirmed the genetic connection of small mammal populations along the canyons with those located in highland Andean areas (Marquet, 1989). In fact, sequence divergence between the lowland and the highland populations is similar to that found between both populations of *T. elegans* from the Mediterranean region of Chile. Therefore, we recognize as *T. pallidior* the taxon found in the lowland coastal areas of northern Chile, along the "quebradas," and in the pre-mountain areas of the Andes. The differentiation of *T. pallidior* can be postulated as populations that in the process of dispersal from the east (as former *T. venusta*'s off-shots) across the Andes during Pleistocene times speciated locally in middle-elevational areas of the Cordillera de los Andes with subsequent colonization throughout the canyons reaching coastal areas as reported for northern Chile. Recognition of *T. pallidior* in the northern lowlands of the latter country is consistent with previous hypotheses proposed by Palma (1995a) in the sense that these populations were not *T. elegans* as recognized by Pine et al. (1979), but *T. pallidior* (Palma, 1995a). The specimens collected by Pine et al. (1979) also included one of the localities sequenced here, the "Valle" or "Quebrada de Camarones." Ongoing phylogeographic studies in the northern region I of Chile including other taxonomic groups of mammals such as the sigmodontine mice *Phyllotis*, *Abrothrix*, and *Akodon* are evaluating the same routes of connection between the Andes and coastal areas through the "quebradas" (Palma and Marquet, in preparation).

Once mouse opossums dispersed from the highlands to lower elevations they may have started to colonize southward mainly among lower elevation Andean areas and along the coast. To the south, *Thylamys* populations reached the Mediterranean region of Chile, dispersal events that allowed these populations to become isolated from *T. pallidior*, speciating into *T. elegans*. The major geographic barrier that divides both taxa is the Atacama Desert. We recognize *T. elegans* (the "llaca")

as the mouse opossum occurring south of the Atacama Desert, inhabiting the north central coast, the central valley, and lower elevations in the foothills of the Chilean Andes (up to about 2000 m), being its southern limit of distribution the Bío–Bío river (region VIII of Chile).

The dispersal of *Thylamys* along coastal areas may have also reached middle central Peru. In fact, the occurrence of *Thylamys* in the lowlands of the latter country has been reported up to latitudes of 10° S, in the Department of Ancash (Handley, 1956). Handley, named this form as *Thylamys tatei*. However, Gardner (1993) re-assigned this taxon to *T. elegans* considering the Peruvian form to be a junior synonym of the latter. Ongoing morphological studies involving *Thylamys* spp. that have been contrasting specimens from the area of Lima, Peru, with *T. tatei*'s type specimen (*sensu* Handley) confirm the validity of the latter as a good species (Sergio Solari, personal communication). In this study, we were able to extract DNA from a small piece of museum skin identified as *Thylamys* from the area of Lima. We amplified and sequenced the cytochrome *b* for this specimen. However, these sequence data did not contribute any resolution to the relationship of the Peruvian form to the other *Thylamys* species, making it difficult to interpret its relationships to other thylamyine taxa. Nevertheless, we think that the *Thylamys* recognized as *elegans* in Peru is a different species (probably *T. tatei*), but that hypothesis will find additional support by having better samples from different localities and contrasted with other thylamyines (e.g., *T. pallidior*, *T. elegans* from Chile) probably as part of a future phylogeographic study.

A molecular phylogenetic study using mtDNA cytochrome *b* sequences that considered most of the Neotropical didelphids proposed that the timing of divergence between most marsupial clades was characterized by a rapid and nearly coincidental period of diversification (Patton et al., 1996). These results have also been supported by DNA–DNA hybridization studies (Kirsch and Palma, 1995). Based on the level of transversion evolution at third codon position for the cytochrome *b* gene, the former authors proposed a rate of divergence of 1.09% per million years according to the mouse opossum–*Monodelphis* estimate (Patton et al., 1996). This rate was based on assumptions of divergence dates from the fossil record estimations from Reig et al. (1987). Calibrating a molecular clock using cytochrome *b* third position transversions from our study and the rate of divergence of 1.09% per million years proposed by Patton et al. (1996) allows us to estimate a date of the differentiation for *Thylamys* back to Pleistocene times, particularly during the Quaternary period. In fact, in calibrating a molecular clock, the differentiation of *Thylamys* species examined here ran-

ged between 247,000 (for *T. pusilla*) and 110,000 years BP (*T. elegans*–*T. pallidior*). In this respect, the Quaternary characterized with about 20 cycles of glacial periods became an important cause for expansion and contraction of habitats in South America, allowing the differentiation of a vast array of fauna associated to both forests and savanna-like environments (Potts and Behrensmeier, 1992; Van der Hammen, 1982). In fact, by Quaternary times and during glacial maxima, cooler and drier climates in the southern latitudes led to expansion of grasslands and open-like environments, opening corridors for the savanna-adapted species in South America (Marshall, 1988). Scenarios like the latter may have favored the dispersal and further differentiation of forms like *T. pusilla* and *T. venusta*, while the series of glacial cycles in the Andes may have allowed the differentiation of forms like *T. pallidior*. Therefore, the calibration of a clock in the speciation process of *Thylamys* spp. does not support previous Palma and Yates's (1998) hypothesis of vicariance to explain the differentiation of *T. elegans* and *T. venusta*. Neither our results of the phylogenetic study do, since they are not sister taxa. The molecular clock set the differentiation of the latter two taxa after the great uplift of the Andes in Plio-Pleistocene times. As we hypothesized earlier, *T. elegans* is sister to *T. pallidior* and the differentiation of the former taxon may be due to isolation by distance from the latter.

We, thus, confirm the occurrence of two *Thylamys* species in Chile: *T. elegans* and *T. pallidior*, and what was earlier recognized for southern Bolivia and northern Argentina as *T. venusta* (Kirsch and Palma, 1995, Palma and Yates, 1998), strongly different from what was believed to be a subspecies of *T. elegans* (Cabrera, 1958). *Thylamys elegans* is here recognized as being distributed from the region of Coquimbo, Chile (30° S; IV region) southward to the Bío–Bío river (37° S).

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Appendix A

Species sequenced, locality of origin, and museum/collection source

Scientific name	Locality	Identification code	Source
<i>Gracilinanus agilis</i>	Bolivia, La Paz, Chijchijpa ^c	NK 25278	MSB ^a
<i>Thylamys elegans</i>	Chile, Coquimbo, Fray Jorge National Park ^c	NK 27583	MSB
	Chile, R. Metropolitana, Quebrada de la Plata ^c	NK 95436	MSB
	Chile, R. Metropolitana, Colina, Huechún	NK 95963	MSB
	Perú, Lima, Chancay, Lachay National Reserve ^c	10738	MUSM ^b
<i>Thylamys macrura</i>	Paraguay, Concepción, Escuela Agropecuaria ^c	NK 27536	MSB
<i>Thylamys pallidior</i>	Bolivia, Tarija, Serranía Sama, 3200 m ^c	NK 23533	MSB
	Chile, Tarapacá, Iquique, Colchane, 3900 m ^c	NK 96057	MSB
	Chile, Tarapacá, Arica, Quebrada Camarones ^c	EP 440 ^c	SSUC ^d
<i>Thylamys pusilla</i>	Bolivia, Santa Cruz, 53 km E Boyuibé ^c	NK 25139	MSB
<i>Thylamys venusta</i>	Bolivia, Chuquisaca, Porvenir, 675 m ^c	NK 12671	MSB
	Bolivia, Cochabamba, Tinkusiri	NK 23952	MSB

^a Museum of Southwestern Biology, The University of New Mexico, Albuquerque, NM 87131, USA.^b Museo Nacional de Historia Natural de Lima, Perú.^c R. Eduardo Palma's field catalog.^d Colección de Flora y Fauna Patricio Sánchez Reyes, P. Universidad Católica de Chile, Santiago, Chile.^e Sequences included in the analyses and deposited in the GenBank.

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