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Phylogeny and Evolution of the Neotropical Rodent Genus *Calomys*: Inferences from Mitochondrial DNA Sequence Data

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Field mice of the genus *Calomys* are small, mostly granivorous rodents common to several habitats in South America. To date, phylogenies for the genus have been proposed on the basis of morphological, chromosomal, and biochemical data, often with contradictory results due to incomplete species sampling or methodological shortcomings. In this paper, we propose relationships among 10 species of *Calomys* based on the complete cytochrome *b* gene sequence. Our analyses show that *Calomys* is constituted by two major clades, one mostly associated with mountain habitats with subsequent invasions to lowland habitats and another with species restricted to lowland habitats both north and south of the Amazon basin. The evolution of the genus was likely accompanied by a reduction of chromosome diploid numbers that occurred independently in each of the two evolutionary lineages. A "clock" calibrated on the split between *Auliscomys* and *Loxodontomys* suggests that the almost nonexistent fossil record for the genus greatly underestimates divergence times among its species. © 2001 Academic Press

INTRODUCTION

The murid genus *Calomys* Waterhouse 1837 (Rodentia: Sigmodontinae) is one of the most widespread genera of the Neotropical rodent fauna. Members of this genus are small and mostly granivorous rodents that have received the colloquial name of "vesper mice" or "lauchas." The genus ranges from the Llanos of northern South America, including Colombia, Venezuela, and islands off the Venezuelan coast (Aruba, Curacao, and Trinidad), to the grasslands, savannas, and forest fringes of Brazil, Bolivia, Peru, Argentina, Paraguay, Uruguay, and portions of northern Chile (Fig. 1).

Phylogenies for *Calomys* based on morphological characters have been equivocal and even the monophyly of the genus has been questioned (Steppan, 1995). Species limits within the genus remain uncertain: for instance, Hershkovitz (1962), recognized 4 species based on body size and skull morphology, whereas Olds (1988) recognized 10 species. However, as Corti *et al.* (1987) pointed out, whether these groupings occur due to morphological convergence or cladistic divergence is yet to be determined. Thus, there is little agreement on the identity and number of species included.

In sharp contrast to their morphological uniformity, species of *Calomys* exhibit extensive interspecific chromosomal variation. Chromosomal and fundamental numbers were used to suggest three karyological groups of species: the laucha–hummelincki group with $2n = 60$ to 64, the venustus–callidus group with $2n = 48$ to 56, and the callosus–lepidus group with $2n = 36$ to 44 (Vitullo *et al.*, 1990; Espinosa *et al.*, 1997). Based on postulated chromosmal rearrangements Espinosa *et al.* (1997) proposed the phylogenetic hypothesis depicted in Fig 2a. Protein electrophoretic analyses have been undertaken for species of *Calomys* (e.g., Gardenal *et al.*, 1980, 1990; Garcia *et al.*, 1990, 1999; de Souza *et al.*, 1996). Most of these included only a few members of the genus, with strong bias toward central Argentina, and in most cases were designed to analyze population genetics and gene flow in particular species rather than to elucidate evolutionary relationships. Olds' (1988) inferred phylogeny based on morphological data is depicted in Fig. 2b.

Three published reports of studies using mitochondrial DNA (mtDNA) are pertinent to the relationships of *Calomys* (Smith and Patton, 1993, 1999; Engel *et al.*, 1998). Smith and Patton (1993, 1999) included two species of *Calomys* in their phylogenetic analysis of sequences of the cytochrome *b* gene (cyt *b*) of several taxa of South American sigmodontines, finding that these consistently grouped together. Engel *et al.* (1998) studied phylogenetic relationships among 33 genera of rodents using mtDNA sequence data from ND3, ND4L, arginine tRNA, and ND4 genes. They found *Calomys* to be polyphyletic, with *Calomys callosus* grouping within the cluster of *Oryzomys*. However, J. Salazar-Bravo *et al.* (unpublished) have, in contrast, found *Calomys* to be monophyletic based on the analysis of the cytochrome *b* gene sequence.

The origin of the genus has been thought to be

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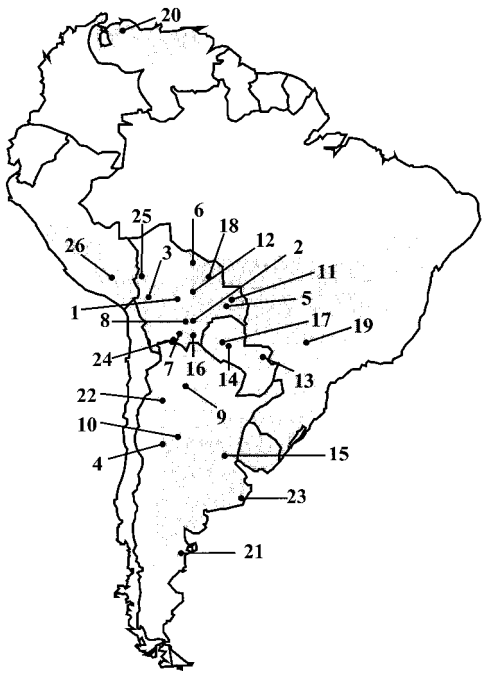

FIG. 1. Distribution of *Calomys,* with collecting localities marked. Numbers correspond to the localities listed in the Appendix.

closely related to the origin of the Andes and corresponding climatic and environmental changes (Reig, 1986). However, alternative biogeographic hypotheses that have been presented differ with respect to when and where the ancestral stock gave rise to the various groups within *Calomys*. Reig (1986) considered the ancestral stock to have originated in an early Altiplano (pre-Pliocene protopuna) where the genus speciated as it dispersed to the eastern lowlands [a scenario that has received some support from Steppan (1995, p.71)]. Alternatively, although no specific area of origin for *Calomys* was explicitly stated, Braun (1993) proposed a slightly modified version of Marshall's (1979) hypothesis in that a primitive phyllotine (probably related to *Calomys*) dispersed south from the Llanos of Venezuela and Colombia to the grasslands of central and eastern South America. Voss (1991) has presented evidence that gives little support to Marshall's hypothesis.

Sequences of the cyt *b* gene in sigmodontine rodents have been used to resolve species-, tribal-, and subfamilial-level questions (Smith and Patton, 1991, 1993, 1999). The gene has offered valuable information on phylogenetic relationships and bioegographic events that have occurred within a few million years (Hillis *et al.*, 1996b). Additionally, it is becoming apparent that current taxonomic species often agree reasonably well in number and composition with biotic entities registered in mtDNA genealogies (Avise and Walker, 1999).

In this study, the relationships of 10 nominal species of the genus *Calomys* were investigated on the basis of an analysis of 1140 bp of the mtDNA cyt *b* gene. Taxa examined herein cover most of the distribution and range of the genus and represent all major lineages as defined on karyological grounds. Because estimates of the timing of cladogenetic events in some groups of the Muridae have been assessed in the past with this gene (for example, Conroy and Cook, 1999; Smith and Patton, 1993), we calibrate a "clock" to estimate the times of diversification for this genus. The ultimate objective of this work was to establish a molecular phylogeny of the genus *Calomys* and use it in the context of changing environments in South America to generate a better understanding of the evolutionary patterns behind this radiation.

MATERIALS AND METHODS

Specimens

Tissue samples (liver, heart, kidney) were collected from 35 specimens representing 10 nominal species of

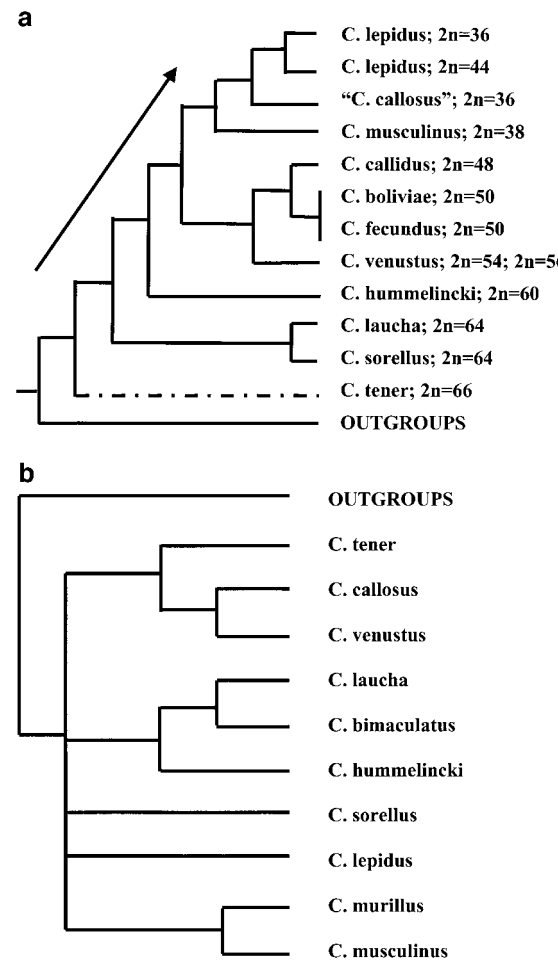

FIG. 2. Hypotheses previously presented on the evolution of *Calomys*. (a) The relationships among species of *Calomys* proposed by Espinosa *et al.* (1997). The arrow represents the postulated direction and reduction in chromosomal numbers, which in turn defined groups of species. (b) The groups of species in *Calomys,* postulated by Olds (1988) based on general morphological characters. Steppan (1995) gave support to some of these groups based on distribution of rib counts.

Calomys of 23 localities (see the Appendix) from throughout the range of the genus (Fig. 1) and five outgroups (*Eligmodontia puerulus, Andalgalomys pearsoni, Graomys griseoflavus, G. domorum,* and *Salinomys delicatus*). When available, at least two individuals of each species were sequenced and, when possible, from different localities. The only exceptions were *Calomys hummelincki* and *C. sorellus* for which only single specimens were available. In the case of *C. sorellus,* however, the sequence was matched and compared with a partial sequence obtained from GenBank (Accession No. UO3543). In addition, we attempted to obtain specimens from localities that were at or near type localities.

DNA extraction, PCR Amplification, and Sequencing

Total genomic DNA was extracted from either frozen or alcohol-preserved tissues with either the proteinase K–phenol–chloroform protocol (Hillis *et al.,* 1996b) or the Qiagen tissue extraction kit. Double-stranded symmetrical amplification and sequencing of the cyt *b* gene was obtained via the polymerse chain reaction (PCR) with several combinations of primers, as outlined in Anderson and Yates (2000). Sequences were aligned by eye and have been deposited on GenBank under Accession Nos. AF159285 to AF159292, AF385592 to AF385608, and AY033153 to AY033190.

Phylogenetic Analyses

Assessment of compositional bias was accomplished by the use of MEGA (Kumar *et al.,* 1993). Saturation plots and pairwise distance estimations were obtained with PAUP*. Relationships among species of *Calomys* and several outgroups were assessed by the use of the maximum-parsimony (MP) and maximum-likelihood (ML) methods in PAUP*.

Two different MP analyses were performed, both with the heuristic search algorithm, TBR branch swapping, and 100 random addition replicates. In the first analysis, only third-base position transversions (Tv) were included because third-base position transitions (Ts) appeared to be saturated at a distance of ca. 12% (data not shown). In the second analysis, transversions were weighted seven times as much as transitions, based on the transition/transversion rate ratio, κ, of 7.04 found in the ML tree obtained below. In both cases these trees were compared with random trees (Hillis and Huelsenback, 1992) to verify the existance of nonrandom information in the data set. Bremer decay indices (Erickson, 1998) and bootstrap values were calculated as surrogates for branch support.

In the ML analysis, sequences (no base weighting, all unordered) were analyzed on the basis of the model HKY + I + Γ, which optimizes base composition and estimates the proportion of invariable sites, the shape of the gamma distribution (α) for those sites that vary, and the ratio of transitions to transversions based on empirical data. This model was chosen because it was shown to maximize the likelihood following the procedure implemented by Sullivan and Swofford (1997).

Additionally, ML analyses were run with the same parameters set to those of the best ML tree, but with the tree topology constrained to match two previous hypotheses of relationships among species of the genus based on chromosomal (Fig. 2a) and morphological (Fig. 2b) data. The maximum-likelihoods of constrained vs unconstrained trees were compared with the likelihood ratio test of Kishino and Hasegawa (1989) as implemented in PAUP*. Because strong arguments have been raised against the use of this test in this manner (Goldman *et al.,* 2000) the Shimodaira and Hasegawa (1999) test as implemented in SHTests v 1.0 by A. Rambaut (http://evolve.zoo.ox.ac.uk/) was employed to further compare these alternative hypotheses.

Estimations of Divergence Times

Sequence divergence based on third-position Tv only was calculated with MEGA (Kumar *et al.,* 1993). Irwin *et al.* (1991) have shown that Tv at third position tend to accumulate near linearly with time and are less prone to saturation than Ts over the scale of time inferred for the group under study [5 to 15 million years (Myr) based on Pardinas and Tonni (1998) and Smith and Patton (1999)].

To calibrate the molecular clock we used three estimates: one for the Muridae based on the *Mus–Rattus* split at 12 million years (Jacobs and Downs, 1994; Ruedas and Kirsch, 1997), one for the Sigmodontinae based on the *Akodon/Thaptomys–Bolomys* split at 3.7 million years (Smith and Patton, 1999), and one for the Phyllotini based on the *Auliscomys–Loxodontomys* split at 4.5 million years (Smith and Patton, 1999; Pardinas and Tonni, 1998). To test for the constancy of the molecular clock, a relative-rate test was performed with the number of third-position Tv rates (Li and Graur, 1991). Statistical estimation of the validity of the molecular clock hypothesis was performed with the χ^2 test (Fitch, 1976).

RESULTS

We obtained 1140 bp of the cyt *b* sequence—representing 380 codons—in all specimens analyzed. Strong biases are typically found in base composition for the mitochondrial DNA (Irwin *et al.,* 1991), and our data are concordant with this observation (Table 1). Significant composition bias exists at both the second and especially the third position; however, the values are close to those found in other rodent studies both in the old world (Barome *et al.,* 1998; Ducroz *et al.,* 1998) and in the Neotropics (e.g., Smith and Patton, 1993). On average, percentage nucleotide composition for all taxa and 1140 bases showed a deficiency of guanine (12.87%) and a slight deficiency of cytosine (25.52%), whereas the other two bases were more balanced

TABLE 1

Nucleotide Frequencies for the Taxa under Study

	First	Second	Third	Total
A	31.0 (0.06)	20.9 (0.04)	39.9 (0.15)	30.61 (0.71)
T	26.5 (0.01)	42.3 (0.17)	24.3 (0.01)	31.02 (0.70)
C	21.4 (0.03)	24.3 (0.01)	30.9 (0.06)	25.52 (0.61)
G	21.2 (0.04)	12.5 (0.12)	4.9 (0.20)	12.87 (0.43)
CB index	0.100	0.230	0.280	

Note. Mean frequency and standard deviation (in parentheses) are presented for 14 taxa and 1140 bp of cytochrome *b* gene sequence. CB, compositional bias.

(30.61% adenine and 30.02% thymine). The frequency of guanine differs greatly among the three base positions. The first and third positions are richer in adenines and the second position shows an excess of thymine. Again, these patterns are similar to those presented elsewhere for rodents of the family Muridae. They also conform closely to values reported for other mammals (Johns and Avise, 1999), especially in the composition at third position.

Phylogenetic Analyses

Of the 1140 bp obtained only 383 bp were parsimony informative in the Tv only at third position MP analysis. For the transition-to-transversion weighted MP analysis 401 bp were parsimony informative. In both cases nonrandom information was found in the data set. The MP analyses resolved two similar trees (Fig. 3a) that were qualitatively similar to the ML tree, with one exception: in both MP analyses a sister taxa relationship between *C. laucha* and *C. tener* was resolved. This relationship was not present in the ML analysis. The tree in Fig. 3a represents the strict consensus tree of six most parsimonious trees with scores of 4367 steps.

The maximum-likelihood (Fig. 3b) model includes variation in base composition, proportion of invariable sites (I), with gamma-distributed rates at variable sites (Γ), and the ratio of transition-to-transversion. This

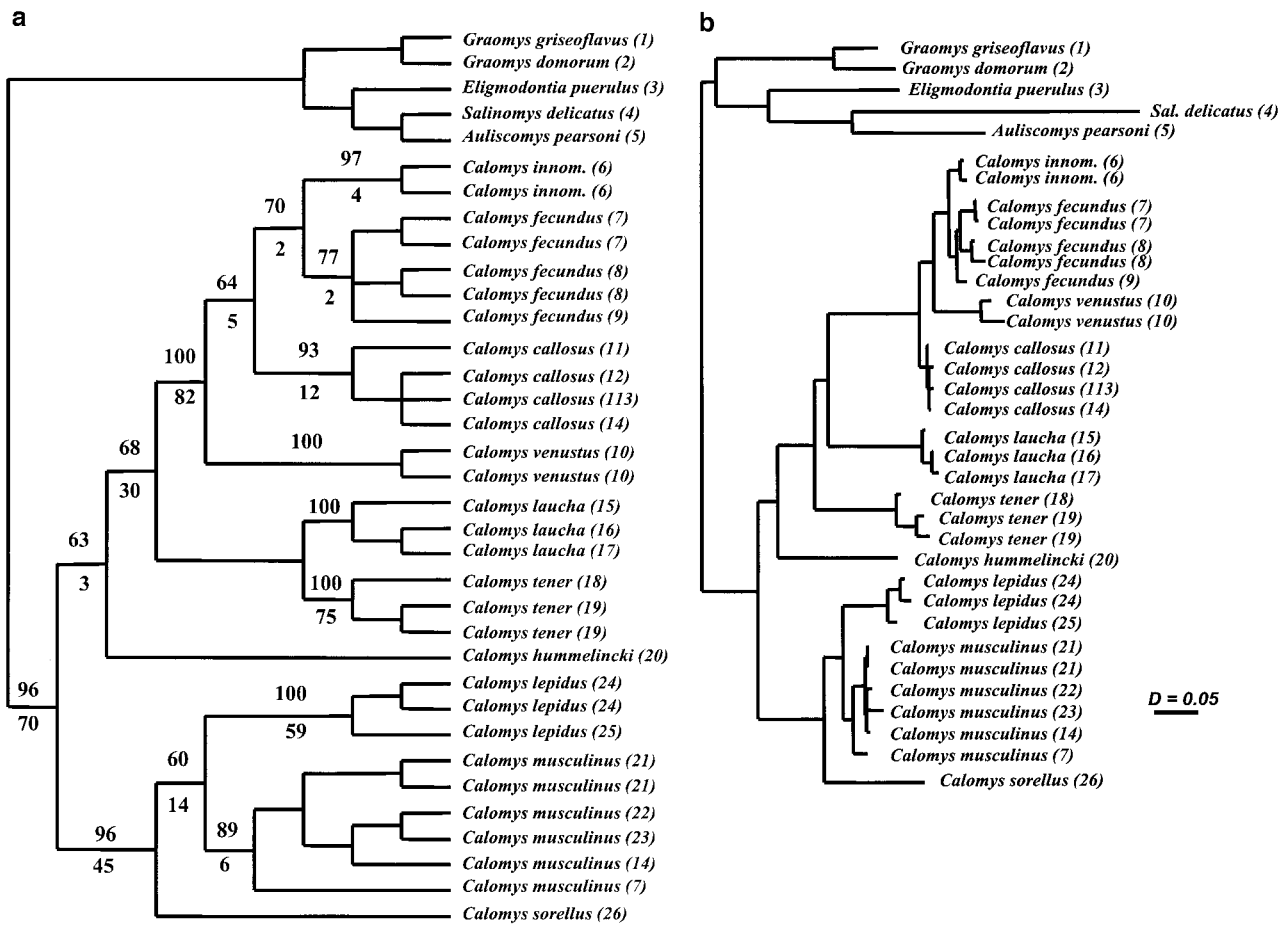

FIG. 3. Phylogenetic relationships resolved among the species and specimens of *Calomys* and outgroups included in the analyses. (a) Consensus tree of six most parsimonious trees (transition:transversion = 1:7) with a score of 4367 steps. Bootstrap values (1000 replicates, 10 random orders) are included above the branches and decay values under the branches. (b) The maximum-likelihood. The values of the estimated parameters are -log likelihood = 8074.57203, estimated base frequencies A = 0.311818, C = 0.295845, G = 0.082619, T = 0.309718, estimated Ti/Tv ratio = 3.461368 (κ = 7.042895), estimated value of proportion on invariable sites (I) = 0.441868, and the estimated value of gamma shape parameter (α) = 1.135253.

TABLE 2

Results of the Kishino–Hasegawa (KH) and Shimodaira–Hasegawa (SH) Tests for the Comparison of the Resolved Phylogenies Presented in This Paper and Alternative Topologies[a] for the Systematics of *Calomys*

	KH		SH	
Tree	Δ-lnL	*P*	Δ-lnL	*P* (Δ)
Our hypothesis (Fig. 3b)	0.0000	1.0000	0.0000	1.0000
Espinosa *et al.* (1997)	333.72593	<0.0001	452.5599	<0.0001
Olds (1988)	143.19356	<0.0001	26.670	0.0050

Note. The log-likelihood estimates of each tree (-ln L) are based on 1140 bp of cytochrome *b*. The KH test was calculated with PAUP* and the SH with SHtest.

[a] Espinosa *et al.* (1997) is depicted in Fig. 2a; Olds (1988) hypothesis is depicted in Fig. 2b.

model was chosen because it was shown that it maximized the estimated likelihood. The In-likelihood value under the HKY + I + Γ model for the tree in Fig. 3b is −8074.57203, with estimated base frequencies (A = 0.311818, C = 0.295845, G = 0.082619, T = 0.309718), a transition-to-transversion ratio of 3.461368 (κ = 7.042895), estimated proportion of invariable sites of 0.441868, and the value of the gamma shape parameter, α, of 1.1352539.

Calomys is characterized by two clades: one composed of species mostly distributed in the lowlands (below 1000 m) and another that includes the highland species (*C. lepidus* and *C. sorellus*) and *C. musculinus*. *C. hummelincki* is situated in the middle of the two clades, with closer relationships to the "lowlands" clade.

The likelihood scores of different tree topologies were compared with the best and unconstrained tree with the Kishino–Hasegawa (HK) and the Shimodaira–Hasegawa (SH) tests. Under the constraints of each of the alternative topologies from Figs. 2a and 2b, we generated likelihoods that were compared using the HK and SH tests and these results are summarized in Table 2.

The results of the relative rate test show that for multiple comparisons of lineages there are no significant differences at the 95% level of substitution rates, except in two cases. In these two cases, *Calomys venustus* appears to be accumulating substitutions at a higher rate.

Times of Divergence

We have identified three sets of possible divergence times for the major nodes recovered in the phylogenetic hypothesis given in Fig. 3. One uses a rate of about 2.3% Myr based on the *Akodon/Thaptomys* and *Bolomys* split (e.g., Smith and Patton, 1993). The second estimate is based on the split between *Loxodontomys* and *Auliscomys* dated at 4 to 5 Myr (average 4.5 million years) rooted on the presence of *Auliscomys (= Loxodontomys) formosus* (Steppan and Pardinas, 1998) in the Montehermosense of Buenos Aires (Cione and Tonni, 1995) and the sister taxa relationship between *Auliscomys pictus* and *Loxodontomys micropus* (Smith and Patton, 1999; J. Salazar-Bravo *et al.*, unpublished). There are 26 third-base-position Tvs between *Auliscomys* and *Loxodontomys* in the 1140 bp (380 codons) analyzed (J. Salazar-Bravo *et al.*, unpublished), yielding a divergence of 6.84%. The rate of third-position Tv change would then be 1.52% Myr (i.e., 6.84%/4.5 Myr). For the date of the third estimation (the *Mus–Rattus* split) we decided to use 12 Myr instead of the 10 Myr estimate used by Smith and Patton (1993, 1999), based on recent arguments (Ruedas and Kirsch, 1997; Ducroz *et al.*, 1998). The divergence rate estimated from these two taxa would, therefore, be on the order of 1.53%/Myr (Ducroz *et al.*, 1998).

The rates estimated from the *Auliscomys–Loxodontomys* and the *Mus–Rattus* splits are practically identical and are 30 to 60% slower than the estimated value for the Akodontini. We present the estimations for divergence times among and between some clades of *Calomys* in Table 3. In general, the divergence estimations based on the rate in Phyllotini place the different cladogenetic events some 2.5 times earlier than the estimations based on the *Akodon/Thaptomys–Bolomys* split.

Our data show that the split between the two main clades of *Calomys* occurred some 9 Myr ago and the split between *C. sorellus* and the other two species in that clade (C. musculinus and *C. lopidus*) occurred about 4 Myr ago. Other informative dates are the differentiation of *C. hummelincki* from the rest of the lowland clade at about 7 Myr, which predates the divergence time between *C. tener* and *C. laucha* (at ca. 5.7 Myr), and the divergence of *C. lepidus* from *C. musculinus* at ca. 2.0 Myr ago. The youngest clade of this radiation is composed of species that diverged some 1.0 Myr ago (e.g., *C. callidus, C. venustus, C. callosus,* etc.)

DISCUSSION

The Contribution of Molecular Analysis to Calomys Systematics

Two major clades can be identified within *Calomys*. The first clade is composed of species mostly associated with lowland biomes (<1000 m, except for *C. fecundus*) and includes *C. hummelincki, C. tener, C. laucha, C. callosus, C. venustus, C. fecundus,* and an unnamed species from Beni, with *C. hummelincki, C. tener,* and *C. laucha,* as basal members. The second clade is composed of three species, *C. musculinus, C. lepidus,* and *C. sorellus,* the last species appearing as the most basal member of this clade. Geographically, two of the three

TABLE 3

Estimates of Divergence Times for Various Clades in the Genus *Calomys* and Outgroups

	Number of 3rd position transversion differences (mean)	Divergence time estimates (Myr)	
		Phyllotini at 1.52% per Myr	Akodontini (graphical estimation)
Among genera			
Calomys to *Eligmodontia*	66.8	11.6	NA
Calomys to *Salinomys*	76.6	13.3	NA
Calomys to *Andalgalomys*	74.5	12.9	NA
Calomys to *Graomys*	66.0	11.4	NA
Within *Calomys*			
C. sorellus/(C. lepidus + C. musculinus)	23.8	4.1	≈1.8
C. sorellus/C. hummelincki	51.0	8.8	NA
C. hummelincki/(C. laucha + C. tener)	45.2	8.2	NA
C. tener/C. laucha	33.3	5.7	≈2
C. musculinus/C. lepidus	13.3	2.3	≈1.2
C. laucha/(C. callosus + C. fecundus + C. sp)	39.6	6.8	NA
C. tener/(C. callosus + C. fecundus + C. sp)	34.4	6.0	NA

Note. Because the "calibration" between *Mus/Rattus* is similar to *Auliscomys/Loxodontomys,* we use only the latter. For comparison, the "dates" estimated from a graph similar to Fig. 10 in Smith and Patton (1999) are also included. NA, not estimated.

species members of this clade (*C. sorellus* and *C. lepidus*) are associated with high-altitude grasslands (altiplano) in the Andes of western South America. The third species (*C. musculinus*) is a widespread taxon that ranges at middle elevations (from south central Bolivia to the foothills of central Argentina) and may even range through Paraguay well into central Brazil.

Olds (1988) recognized five species groups based on tail length, diploid number (2*n*), and size and shape of skulls. Her groups included group 1, *C. callosus, C. venustus,* and *C. tener;* group 2, *C. laucha, C. bimaculatus,* and *C. hummelincki*; group 3, *C. murillus* and *C. musculinus*; group 4, *C. lepidus*; and group 5, *C. sorellus*.

Steppan (1995) gave some support to these groups from an analysis of the distribution of the number of ribs, as members of group 1 shared the derived condition of 12 ribs and seven lumbar vertebrae, and at least some members of group 2 (e.g., *C. laucha*) and *C. lepidus* (group 4) shared the primitive condition of 13 ribs and six lumbar vertebrae. *C. sorellus, C. musculinus,* and *C. lepidus* share the primitive condition, whereas species in the "lowlands" clade (except *C. laucha*) share the derived condition (Steppan, 1995). We postulate that rib 13 was lost twice in the evolution of this genus: once in *C. hummelincki* and again in the clade that includes all the *Calomys* except *C. laucha*. In all our analyses, the positions of *C. laucha* and *C. tener* are variable. The alternative hypothesis is that *C. laucha* regained rib 13. Therefore, some of the groupings proposed by Olds (1988) seem to be compatible with our results, although the statistical tests in Table 2 rejected her overall hypothesis.

Vitullo *et al.* (1990) and Espinosa *et al.* (1997) suggested three groups of species in *Calomys* based on chromosomal data. These groups were defined by chromosome numbers: group I, species with high chromosomal numbers, from $2n = 64$ (*C. sorellus* and *C. laucha*) to $2n = 60$ (*C. hummelincki*); *C. tener,* with $2n = 66$, should be part of this group also; group II, species of *Calomys* with asymmetric karyotypes and intermediate chromosomal numbers, ranging from $2n = 36$ and $2n = 44$ (*C. lepidus*) to $2n=56$ (*C. venustus*); they also included *C. callosus, C. venustus,* and "*C. boliviae = C. fecundus*" in this group; and group III, species of *Calomys* with low diploid numbers that are highly divergent from the purported ancestral condition, *C. musculinus* ($2n = 38$) and "*C. callosus*" ($2n = 36$).

Our phylogeny does not correspond to this chromosome scheme, especially if a unidirectional change in chromosomal number and morphology is invoked, as suggested by these authors. In fact the relationships proposed by Vitullo and colleagues (1990) was rejected in our test of the tree topologies (Table 2).

Recently, Garcia *et al.* (1999), analyzing allozyme variation in *C. hummelincki,* suggested that this species was more closely related to *C. venustus* than to *C. laucha* or *C. musculinus*. These results, based on genetic distances, are not supported by our analyses, since the topology that we would have expected would be (*C. musculinus (C. hummelincki (C. laucha–C. venustus)*)). Garcia *et al.* (1999) obtained the data for *C. hummelincki* independent from those of the other species, the assigned alleles (and allele frequencies) in this study were based on approximate distances from origin, not on a side-by-side comparison, and thus were not amenable to phylogenetic analysis.

Corach (1990) reported on sequence homology in repetitive DNA in five species of South American ro-

dents, including *C. musculinus, C. laucha,* and *C. callidus*. *C. musculinus and C. laucha* showed higher repetitive DNA sequence homology than either one to *C. callidus*. Corach and Semorile (1989) demonstrated that *C. laucha* and *C. musculinus* showed species-specific organization of repetitive DNA sequence (identified as restriction patterns when digested with 12 different restriction endonucleases). We interpret these results as consistent with our phylogenetic hypothesis, in which *C. laucha* is a basal member of the lowlands clade. Thus, the higher repetitive DNA homology that *C. laucha* and *C. musculinus* (members of different clades) share may be due to the presence of ancient families of repetitive sequences in the ancestors of both species that were either lost or rearranged in *C. callidus*. The analysis of more species of *Calomys* with the techniques that Corach (1990) used would shed light on the molecular evolution of this genus under the phylogenetic hypothesis proposed herein.

The close relationship among *C. musculinus, C. lepidus,* and *C. sorellus* has not been previously proposed, probably due to the lack of a comprehensive treatment of the genus. Cabrera (1961) considered *C. sorellus* a subspecies of *C. lepidus,* but Hershkovitz (1962) considered them two different species. *C. musculinus* was considered a subspecies of *C. laucha* (Hershkovitz, 1962; and references therein) until Massoia *et al.* (1968) distinguished these species both morphologically and chromosomally.

The second group of species is a much more problematic group. A close relationship of *C. tener, C. hummelincki,* and *C. laucha* has been suggested by Hershkovitz (1962), who considered both *C. tener* and *C. hummelincki* subspecies of *C. laucha* based on morphological similarity. These three species are successive basal members of the "lowlands" clade (Fig. 3), and their resemblance is based on plesiomorphic characters. Nested within this clade and terminal to this group, there exists a group of nominal species with poorly understood species limits. Those species are widely distributed in the lowlands and middle-elevations of the eastern flanks of the Andes, from the Department of El Beni (Bolivia), south to the Paraguayan–Argentinean Chaco, and east to the Brazilian Caatinga, and are well characterized on morphological and chromosomal grounds (J. Salazar-Bravo *et al.*, unpublished). The close relationships of members of this species complex are supported by this analysis.

Trends in the Chromosomal Evolution of Calomys

Because sigmodontine rodents show high levels of diversity in their karyotypes, chromosomal variation has been used to establish phylogenies at different taxonomic levels. Karyotypes have been described for most species of *Calomys,* although some widespread and/or polytypic species have not been properly sampled. Some species (e.g., *C. laucha* from Uruguay and Argentina; Brum-Zorrilla *et al.*, 1990) show apparently so high levels of chromosomal polymorphism [in diploid number (2*n*), but mainly in fundamental number (FN)] that we wonder whether misidentified animals were included in the analysis. Nevertheless, karyotypes have been described for most species of *Calomys* that occur in Argentina, Bolivia, Brazil, Paraguay, and Peru and less so for Uruguay. Furthermore, banding techniques (mostly G and C bands) that allow specific identification of chromosomes have been used in five species of the genus with a strong bias toward species present in Argentina. Pearson and Patton (1976), Vitullo *et al.* (1990), and Espinosa *et al.* (1997) have attempted to use general chromosomal morphology to establish phylogenies, but none of their phylogenies are supported by the results presented herein.

Within the species analyzed in our study, 2*n* varies from 36 to 66, and FN ranges from 48 to 68. When the chromosomal information concerning 2*n* and FN is plotted on the phylogenetic tree (Fig. 4), several patterns are evident. A tendency to reduce the number of chromosomes is apparent, but this reduction occurred independently in each group of species. This is in contradiction to Pearson and Patton (1976) and Vitullo and colleagues (Vitullo *et al.,* 1990; Espinosa *et al.*, 1997), who correctly argued for a reduction in diploid number, but incorrectly suggested a unidirectional across-all-species process which defined species groups based on chromosomal numbers. Also, except in *C. musculinus,* the tendency appears to have been toward a reduction of the chromosomal 2*n* without significantly affecting the FN, which has been conserved in rather high numbers (median = 68) across the genus. The fact that both lineages in this radiation demonstrated a reduction in the diploid number is in agreement with earlier claims (Gardner and Patton, 1976; Pearson and Patton, 1976) that suggested this as a primary model of chromosomal evolution in sigmodonts. This pattern, also found in South American marsupials (Svartman and Vianna-Morgante 1998), suggests that the causative processes may have profound evolutionary importance. Whether these are the same processes that Wyttenbach *et al.* (1998) have found in shrews would require in-depth analysis and experimentation. Nonetheless, the pattern of independent reduction of chromosomal numbers in this genus appears to be well substantiated.

In the case of the *C. sorellus–C. lepidus–C. musculinus* clade a major chromosomal rearrangement appears to have taken place previous to the split of the last two species. This rearrangement involved some 10 chromosomal Robertsonian fusion events. Moreover, once these last two species had diverged, *C. musculinus* suffered a drastic reorganization of its genome that involved at least three additional Robertsonian fusion events and some five pericentric inversions (the number of pericentric inversions must at present be considered cautiously, as several values of FN have been cited in the literature for this species). Whether these

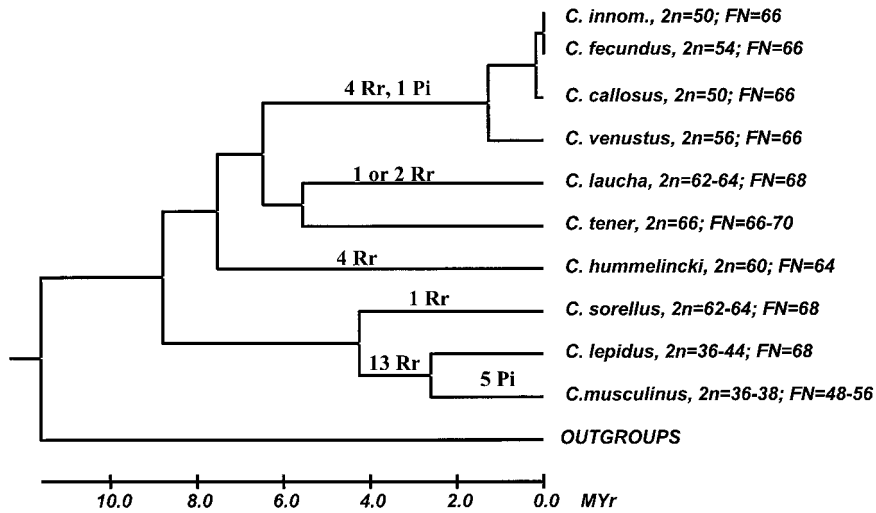

FIG. 4. A synthesis of phylogenetic relationships among *Calomys* species inferred from the sequence data used in this work. Chromosomal characterization of each taxon is included. Postulated chromosomal changes are marked on the tree with Pi denoting pericentric inversions and Rr denoting Robertsonian events. The time scale at the base of the figure (in Myr) is based on the *Loxodontomys/Auliscomys* calibrated clock at 1.52%/Myr.

chromosomal repatternings are the reason for a smaller DNA content per cell (compared to that of *C. laucha*) as presented in Ciccioli and Poggio (1993) is not known at this time, and comparisons should be made with *C. lepidus* and *C. sorellus* to incorporate the phylogenetic component in the analysis.

The pattern of chromosomal evolution in the "lowlands" clade does not involve the major changes that chromosomal inversions, unequal translocations, and whole-arm heterochromatin additions or deletions are thought to have in the structural morphology of the chromosome complement as evidenced by changes in FN. Instead it appears that the change was through a series of Robertsonian fusions which decreased the diploid number. Interestingly, some groups of species are defined by particular chromosomal rearrangements: for example *C. callosus, C. venustus, C. fecundus, C. callidus,* and *Calomys* sp. share a pericentric inversion (all of these species show the same FN), but vary in diploid number. As shown by Vitullo *et al.* (1990) some of these species could interbreed, but F_1 males are infertile, probably following Haldane's rule of "hybrid sterility" (cited by Dobzhansky, 1982).

Temporal and Spatial Aspects of the Evolution of Calomys

The issues of constancy in the rates of evolution and the prediction of times of divergence among taxa based on molecular data are clearly subjects of major controversy (e.g., Hillis *et al.,* 1996a; Wang *et al.,* 1999; Foote *et al.,* 1999). Notwithstanding, there appears to be a general consensus that molecular clocks can be used among closely related species that share a recent common evolutionary history.

We found that the rate of Tv changes at third position in *Mus/Rattus* and *Loxodontomys/Auliscomys* is, for all purposes, identical, although we note that several arguments could be raised against this observation. For example, it has recently been suggested on the basis of several molecular markers that the split between *Mus* and *Rattus* is much older than the traditional fossil-based estimate of 12 Myr (Kumar and Hedges, 1998). This argument would imply a very much longer evolutionary radiation than the group's first observed fossil record, something that has been disputed by Foote *et al.* (1999). A caveat that also needs to be addressed is the contention of Steppan (1995) and Steppan and Pardinas (1998) that there is a close relationship between *Loxodontomys* and the *Reithrodon* group of the Phyllotini, to the exclusion of *Auslicomys,* based on morphological characters. Analyses in our laboratory (J. Salazar-Bravo *et al.,* unpublished) and the most comprehensive molecular analyses of the Sigmodotinae to date (Smith and Patton, 1999) have failed to support this relationship. These analyses support a *Loxodontomys–Ausliscomys* sister taxa relationship most of the time and do not support a putative *Reithrodon* group. Therefore, we think it is possible to use the *Ausliscomys/Loxodontomys* relationship to "calibrate" a clock for the Phyllotini.

At the heart of the controversy in the temporal aspect of the evolution of the genus is the incomplete nature of the fossil record, which is at odds with the abundance and diversity of present-day species. The fossil rodent *Bensonomys* has been treated as the earliest representative of the genus (Baskin, 1978) and this has fueled a sometimes heated debate (cf., Reig, 1986; Baskin, 1986). To date, there is no agreement on the level of relationship between *Calomys* and *Benson-*

omys, and the "partisan" line appears to be drawn between paleontologists (who recognize *Bensonomys* and *Calomys* as no more distinct than subgenera of the same genus) and neontologists (who suggest that *Calomys* and *Bensonomys* are not only differentiated at the generic level, but that *Bensonomys* is in the direct line of descent from †*Copemys* to *Peromyscus*). However, as argued by Steppan (1995), *Bensonomys* cannot be a representative of *Calomys* as the genus is currently understood because ". . .[*Bensonomys*] possess mesolophs that are entirely absent among all extant *Calomys* and in the sister group to *Calomys,* the remaining phyllotines." (Steppan, 1995, p. 60). New fossil records from South America, both from traditionally rich fossiliferous localities in the southern part of the continent and from other areas (e.g., Fejfar *et al.,* 1996), are bound to widen our perspective and clarify the evolution of this and other groups of Neotropical murid rodents.

The data presented must be considered crude approximations; yet they point to times of divergence that are much older than the fossil record of South American murid rodents indicates. The oldest murid rodents known in South America are *Auliscomys formosus* from the Montehermosan of Argentina (5 to 4 Myr) and *Bolomys bonapartei* from the Chapadmalalan (4 to 3.5 Myr; Pardinas and Tonni, 1998). The oldest fossil record of *Calomys* comes from the Ensenadan (ca. 1.5 Mya) of Argentina (Pardinas, 1999). Our estimation of divergence times of *Calomys* from the outgroups included in the analysis indicates an average of 12 Myr of independent evolution for the genus. However, our data indicate that the major groups within *Calomys* diverged between 8 and 9 Myr ago (late Miocene–early Pliocene).

Smith and Patton (1999), using the same gene, estimated that the basal radiation of sigmodontines was reasonably dated at 10 to 14 Myr. This date accommodates the results of our analysis, especially if, as suggested by our results (see also Smith and Patton, 1999), *Calomys* is a basal member of the radiation. At odds with the temporal estimation based on cyt *b* are the data provided by DNA–DNA hybridization studies. Dickerman (1992) suggested that phyllotines and akodontines diverged between 3.5 and 4.3 Myr, which is in almost perfect accord with the fossil record (e.g., Pardinas and Tonni, 1998) and shows times of divergence almost 30% younger than those estimated from cyt *b*. This pattern of rather disparate estimations has been found also in other murids, for example by Ducroz *et al.* (1998), in which the time of divergence of *Arvicanthis* sp. from cyt *b* data is 5.3 Myr, whereas the same date is estimated to be 1.5 Myr with DNA–DNA hybridization data. Clearly, technical and theoretical studies are still needed to understand the reasons for these differences, which would in turn help to bring consensus to the application of molecular data to the dating of evolutionary events.

Phylogeny and Biogeography

The evolutionary history of *Calomys* has been multifaceted and is probably older than the fossil record shows. Where did the original radiation of this group occur? We do not know. Collins *et al.* (1996), using palaeoceanographic circulation models, isotopic evidence, and foraminiferan originations, suggested that the divergence between the Atlantic and the Pacific basins (and the formation of an incipient continental bridge) began as early as 8 Mya. Therefore, our dates suggest that the original diversification of the genus could have occurred on either side of the Panamanian Isthmus.

We also note that at about the time of the divergence between the basal members of the genus (late Miocene and Pliocene), an important global ecological change was underway: an abrupt and widespread increase in C_4 biomass that may be related to the decrease in atmospheric CO_2. This change has been associated with significant faunal turnover in several continents (Cerling *et al.,* 1997, 1998). It is tempting to suggest that the development of grasslands in South America (ca. 8 Myr ago; Flynn and Wyss, 1998) may have actually set the stage for the evolution of *Calomys*. Additionally, it has been postulated that the late Miocene was a time of major geological and biotic events in and around the Amazon basin (Hooghiemstra and Vanderhammen, 1998), which included the formation of the present-day Orinoco River due to the upheaval of the northern Andes (Hoorn, 1994; Hoorn *et al.,* 1995; Rasanen *et al.,* 1995). If the timing of these events is correct then we can postulate a proto-*Calomys,* distributed mostly south of what would eventually become the Amazon River, which at about 8.5 or 9 Myr diverged in two branches. One branch was mostly associated with the western pre-puna and one was mostly associated with the eastern lowlands. This eastern clade gave rise to the ancestors of *C. hummelincki,* as a result of long-range dispersal. At about 5 or 6 Myr, several other species (*C. laucha, C. tener,* and the ancestor to the *C. venustus–C. callosus* and allies) would have originated. At this time it is unknown what kind of factor(s) may have triggered this particular radiation. Most problematic to this scenario is the apparent recentness of the "*C. callosus–C. venustus*" clade, especially in light of the suggested age of the genus in general and the age of the rest of the species.

The radiation of the "mountain" group would have followed the "corridor uniting the biotas of the Andes and Argentina" that Marroig and Cerqueira (1997) suggest would be the signal of the great Fouaratian transgression when the La Plata–Paraguay depression suffered a marine invasion (ca. 2–3 Myr ago) reaching almost to the foothills of the Andes and the upriver above Asuncion. Taxa like *C. musculinus* would have reached the southern part of this corridor and once the

water receded would have colonized farther to the north and northeast.

These scenarios are supported by the proposed phylogenetic hypothesis and give support to the hypothesis proposed by Reig (1986). Interestingly, Steppan (1995) also conferred support to this hypothesis, based on the fact that *C. sorellus* possesses a large number of primitive characters.

CONCLUSION

The cyt *b* gene appears to be suitable for the reconstruction of intraspecific phylogenetic hypotheses for the genus *Calomys*. The controversial taxonomy of *Calomys* was clarified by examination of the specific status of several taxa. Two primary clades are evident but we suggest that three evolutionary events, led to the diversification of the genus, probably centered around a band of habitat south of what would become the Amazon basin: one event that ended in *C. hummelinckii* to the north of the South American continent, one event that led to the *C. sorellus–C. musculinus–C. lepidus* clade, and one event that led to the clade composed of *C. tener, C. fecundus, C. callosus, C. venustus, C. laucha, C. callidus,* and *Calomys* sp. The evolution of the genus was possibly triggered by the expansion of grasslands in South America which started about 8 Mya. Other climatic and biotic events may have triggered speciation within each clade.

APPENDIX

Species Sequenced in This Study, Geographic Origin, Identification Numbers, and Museum Sources of the Samples Studied

Species	Sample location[1]	Identification code	Source
Graomys griseoflavus	Bolivia, Santa Cruz, 5 km SE Comarapa (1)	NK 23331	MSB[2]
Graomys domorum	Bolivia, Santa Cruz, 53 km E Boyuibe (2)	NK 12123	MSB
Eligmodontia puerulus	Bolivia, La Paz, 8.5 km W Andrés de Machaca (3)	NK30652	MSB
Salinomys delicatus	Argentina, San Luis, 15 km E Salinas del Bebedero (4)	AK 13524	SNOMNH[3]
Andalgalomys pearsoni	Bolivia, Santa Cruz, 29.5 km W Roboré (5)	NK 12402	MSB
Calomys innom	Bolivia, Beni Department (6)	NK 27668, NK 37787	MSB
Calomys fecundus	Bolivia, Tarija, 1 km E Tucumilla (7)	NK 23650, NK23697	MSB
	Bolivia, Chuquisaca, 2 km SE Monteagudo (8)	NK21330, NK21355	MSB
	Argentina, Tucumán, Biological Reserve at Horco Molle (9)	AK15276	SNOMNH
Calomys venustus	Argentina, Córdoba, 2 km S Espinillo (10)	TK49115, TK49116	TTU[4]
Calomys callosus	Bolivia, Santa Cruz, Santiago de Chiquitos (11)	NK12308	MSB
	Bolivia, Santa Cruz, San Miguel Rincon (12)	NK11590	MSB
	Paraguay, Amambay, Parque Nacional Cerro Corá (13)	NK22532	MSB
	Paraguay, Boqueron, Monte Palma (14)	NK72344	MSB
Calomys laucha	Argentina, Santa Fe, Maximo Paz (15)	NK 15988	MSB
	Bolivia, Tarija Dept., Estancia Bolivar (16)	NK 25156	MSB
	Paraguay, Boqueron, Filadelfia (17)	NK 72376	MSB
Calomys tener	Bolivia, Santa Cruz, Santa Rosa de la Roca (18)	NK 21054	MSB
	Brazil, Sao Paulo, Tupi Paulista (19)	NK 42140, NK 42183	MSB
Calomys hummelincki	Venezuela, Falcón, Isiro (20)	AM V001 (am9)	CIEZA[5]
Calomys musculinus	Argentina, Chubut, Puerto Madryn (21)	RE 126, RE 127	cnpMaN[6]
	Paraguay, Boqueron, Monte Palma (14)	NK 72358	MSB
	Argentina, Catarmarca, 13 km NNW of Andalgala (22)	AK 15025	SNOMNH
	Argentina, Buenos Aires, Mar del Plata (23)	MMP 4009	MMMP[7]
	Bolivia, Tarija, 1 km E Tucumilla (7)	NK23706	MSB
Calomys lepidus	Bolivia, Tarija, 1 km E Iscayachi (24)	NK 14643, NK 14656	MSB
	Bolivia, La Paz, Reserva de Fauna Ulla-Ulla (25)	NK 31032	MSB
Calomys sorellus	Peru, Dept. Arequipa, Caylloma (26)	FMNH 107709	FMNH[8]

[1] Numbers in parentheses correspond to localities in Fig. 1.
[2] Museum of Southwestern Biology, University of New Mexico, Albuquerque, NM 87131.
[3] Division of Mammals, Sam Noble Oklahoma Museum of Natural History, University of Oklahoma, Norman, OK 73072.
[4] Museum of Texas Tech University, Lubbock, TX 74909-3191.
[5] Centro de Investigación en Ecologia y Zonas Aridas, Coro 4101-A, Venezuela.
[6] Colección de Mamiferos, Centro Nacional Patagónico, Universidad Nacional de la Patagonia, Argentina.
[7] Colección de Mamiferos, Museo Municipal de Mar del Plata, Argentina.
[8] Division of Mammals, Field Museum of Natural History, Chicago, IL 60605.

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