Journal of Mammalogy, 88(3):769–776, 2007

PHYLOGENETIC RELATIONSHIPS AMONG SPECIES OF THE GENUS *CALOMYS* WITH EMPHASIS ON SOUTH AMERICAN LOWLAND TAXA

TAIANA HAAG, VALÉRIA C. MUSCHNER, LORETA B. FREITAS, LUIZ FLAMARION B. OLIVEIRA, ALFREDO R. LANGGUTH, AND MARGARETE S. MATTEVI*

Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Rio Grande do Sul, Caixa Postal 15053, 91501-970, Porto Alegre, Rio Grande do Sul, Brazil (TH, VCM, LBF, MSM)
Museu Nacional, Setor de Mastozoologia, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, 20940-040, Rio de Janeiro, Rio de Janeiro, Brazil (LFBO)
Departamento de Sistemática e Ecologia, Universidade Federal da Paraíba, Campus Universitário, 58059-900, João Pessoa, Paraíba, Brazil (ARL)
Programa de Pós-Graduação em Genética e Toxicologia Aplicada, Universidade Luterana do Brasil, Avenida Farroupilha, 8001, 92450-900, Canoas, Rio Grande do Sul, Brazil (MSM)

Calomys Waterhouse, 1837, is one of the most speciose genera of the Phyllotini tribe of the South American sigmodontine rodents. Distributed predominately in southern South America, the genus has been proposed to have originated in the central Andes with further differentiation as subsequent occupations of the lowlands of the continent occurred. In this study, 30 newly obtained sequences of the cytochrome-*b* gene from specimens collected in Brazil were analyzed in conjunction with data available in GenBank in an attempt to discern the dispersion patterns of this genus in the South American lowlands. The analyses support a scenario where a phyllotine lineage appeared in the Andes and later separated into 2 larger clades. Members of 1 clade remained in the highlands (*C. musculinus*, *C. lepidus*, and *C. sorellus*), experiencing some local differentiation. Members of the 2nd clade invaded the lowlands of South America, especially nonforested biomes, where they underwent intense differentiation resulting in species with wide distributions in the continent. In the lowland clade, the "*callosus–venustus*" group is more derived, is characterized by a larger body size, and has a broad distribution; differentiation of this group was probably accompanied by some reduction in chromosomal diploid numbers.

Key words: Amazon domain, *Calomys*, Cerrado domain, cytochrome *b*, Pampas domain, phylogenetic analysis, South American lowlands

Calomys Waterhouse, 1837, is one of the most speciose genera in the Phyllotini tribe of the subfamily Sigmodontinae, the South American representatives of the rodent family Cricetidae (Musser and Carleton 2005). This genus is considered the most generalized of all within the Phyllotini and may closely resemble the ancestral condition of this taxon (Braun 1993; Reig 1986; Steppan 1995). The genus was revised by Hershkovitz (1962) who reduced the 10–15 species previously identified (Cabrera 1961; Ellerman 1941) to only 4, 2 of which—*C. laucha* and *C. callosus*—later proved to be species complexes (Corti et al. 1987; Pearson and Patton 1976; Reig 1986; Williams and Mares 1978). Musser and Carleton (2005) listed 12 species, but called attention to the fact that both the distributions and also the names of their synonyms must be observed with caution because the genus needs considerable alpha taxonomic work. The taxa recognized by these authors are *C. boliviae* (= *fecundus*), *C. callidus*, *C. callosus*, *C. expulsus*, *C. hummelincki*, *C. laucha*, *C. lepidus*, *C. musculinus*, *C. sorellus*, *C. tener*, *C. tocantinsi*, and *C. venustus*.

The karyotypes of several species of *Calomys* have been determined; the genus presents high chromosomal variation, with diploid numbers ranging from 36 to 66 (Mattevi et al. 2005). This large chromosomal differentiation resulted in a number of described karyotypes that is much higher than the number of nominal species attributed to the genus. Studies on the phylogenetic relationships of *Calomys* and its position within the Phyllotini have produced contradictory results as even the monophyly of the genus has been questioned. Some

* *Correspondent: mattevi@terra.com.br*

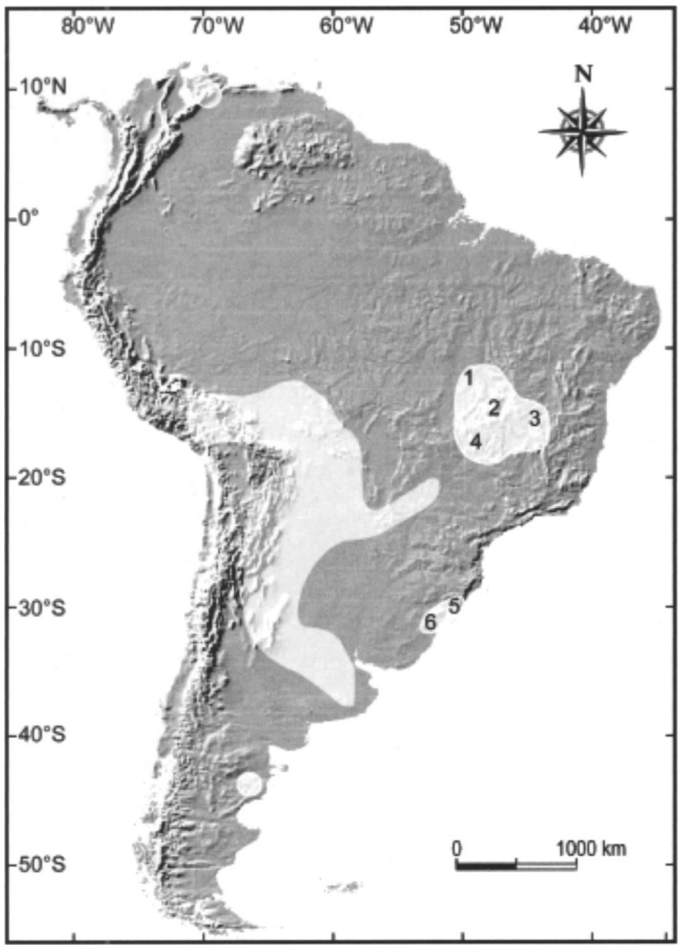

FIG. 1.—Collecting localities for samples analyzed in this report: 1, Aliança do Tocantins; 2, Serra da Mesa; 3, Mambai; 4, Ipameri; 5, Quintão; 6, Taim (coordinates are given in Table 1). Shaded regions at left represent general collection areas for specimens reported by Salazar-Bravo et al. (2001).

authors suggest that the genus represents a primitive branch in the group (Anderson and Yates 2000; Chiappero et al. 2002; Engel et al. 1998; Hershkovitz 1962; Pearson and Patton 1976; Spotorno et al. 2001; Steppan 1995; Steppan and Sullivan 2000).

Calomys is found predominately is southern South America, occupying a variety of habitats in Argentina, Chile, Bolivia, southeastern Brazil, Uruguay, Paraguay, and Peru, and in isolated sites in Venezuela and Colombia (Eisenberg and Redford 1999; Musser and Carleton 2005). Reig (1986) suggested that the distribution of *C. sorellus* may lie close to the location of the original area of differentiation of the genus—the Andes of southern Peru and Bolivia and of northern Chile and Argentina. According to Reig (1986), an ancestral form to the remainder of the phyllotines, possibly closely related to *Calomys*, must have arrived in the area of the Puna in the middle or late Miocene; this timing coincides with an important acclivity phase of the South American highlands. This ancestral phyllotine lineage could have diversified towards an increasingly herbivore/grazing morphotype, reaching the status of generalist that now characterizes *Calomys* (Reig 1986; Spotorno et al. 2001).

From this model, we would predict that species of *Calomys* inhabiting the Andean highlands would present more ancestral morphological characters than those species living outside this area. Indeed, phylogenetic analysis based on the cytochrome-*b* gene show that the genus comprises 2 major clades, 1 with species mostly associated with mountainous habitats and a 2nd mostly restricted to lowland habitats both north and south of the Amazon Basin (Salazar-Bravo et al. 2001).

Recent reports on the existence of at least 5 species of *Calomys* (*C. callosus*, *C. expulsus*, *C. laucha*, *C. tener*, and *C. tocantinsi*) with preferential or exclusive occurrence in Brazil clearly indicate that an important segment of its diversification must have occurred in the lowlands of South America. Considering that the analysis by Salazar-Bravo et al. (2001) included only 2 representatives from 1 locality in Brazil (Fig. 1), we analyzed the sequences of the cytochrome-*b* mitochondrial gene of 3 taxa of *Calomys* that were collected in Brazilian territory in an attempt to better understand the dispersion patterns of this genus in the South American lowlands.

MATERIALS AND METHODS

Specimens.—Samples of DNA were obtained from 30 animals of 3 taxa of *Calomys* (*C. tener*, *C. expulsus*, and *C. laucha*) collected in different Brazilian habitats: Cerrado, Cerrado–Amazon transition (Aliança do Tocantins), and Pampas (Table 1; Fig. 1). All sequenced animals were karyotyped to confirm morphological identifications (see Mattevi et al. 2005) and the skins and skulls of these specimens are deposited in the Mammal Collection of the Museu Nacional, Rio de Janeiro, and of University of Paraíba, João Pessoa (Appendix I). In the analyses below we also included 30 specimens of 10 species of *Calomys* from 21 South American localities reported by Salazar-Bravo et al. (2001). The sequences of 5 phyllotine taxa (*Eligmodontia puerulus*, *Andalgalomys pearsoni*, *Graomys griseoflavus*, *Graomys domorum*, and *Salinomys delicatus*) were included as outgroups (Table 1).

DNA amplification and sequencing.—The DNA was extracted from kidney, liver, heart, or muscle using the standard protocol described in Medrano et al. (1990). The mitochondrial cytochrome-*b* gene sequences were amplified via polymerase chain reaction (PCR) using the primers suggested by Anderson and Yates (2000) for other phyllotine taxa: *MUS* 14095 (light strand), 5′-GAC ATG AAA AAT CAT CGT TGT AAT TC-3′; and *MUS* 15398 (heavy strand), 5′-GAA TAT CAG CTT TGG GTG TTG RTG-3′.

Polymerase chain reaction products were purified with exonuclease I and shrimp alkaline phosphatase (Amersham Biosciences, Piscataway, New Jersey). The 2 strands were sequenced directly from purified polymerase chain reaction products employing the primers mentioned above and using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Boston, Massachusetts). Sequencing was done on an ABI Prism 3100 (Applied Biosystems,

TABLE 1.—*Calomys* taxa from which cytochrome-*b* DNA data were used for phylogenetic analyses. 2n, diploid number; FN, autosomal fundamental number.

Taxon	*n*	2n/FN	Locality	GenBank accession no.
C. expulsus[a]	10	66/68	Serra da Mesa, Brazil (13°45′S, 47°50′W)	AY964031, AY964032, AY964034, AY964035, AY964036, AY964037, AY964038, AY964039, AY964040, AY964050[a]
	10		Mambai, Brazil (14°29′S, 46°06′W)	AY964028, AY964029, AY964033, AY964043, AY964044, AY964045, AY964046, AY964047, AY964048, AY964051[a]
	5		Ipameri, Brazil (17°44′S, 48°37′W)	AY964026, AY964027, AY964041, AY964042, AY964049[a]
C. expulsus variant 1[a]	1	64/66	Ipameri, Brazil (17°44′S, 48°37′W)	AY964030[a]
C. laucha[a]	1	64/68	Taim, Brazil (31°10′S, 51°31′W)	AY964052[a]
C. tener[a]	1	66/66	Serra da Mesa, Brazil	AY964053[a]
	1		Aliança do Tocantins, Brazil (11°18′S, 48°56′W)	AY964055[a]
	1		Quintão, Brazil (29°40′S, 50°12′W)	AY964054[a]
C. callosus[b]	4	50/66	Santiago de Chiquitos, Bolivia	AY033177[b]
			San Miguel Rincon, Bolivia	AY033183[b]
			Parque Nacional Cerro Corá, Paraguay	AY033185[b]
			Monte Palma, Paraguay	AY033187[b]
C. boliviae[b]	5	54/66	Tucumilla, Bolivia	AY033164, AY033166[b]
			Monteagudo, Bolivia	AY033160, AY033161[b]
			Biological Reserve at Horco Molle, Argentina	AY033173[b]
C. hummelincki[b]	1	60/64	Isiro, Venezuela	AF385598[b]
C. innom.[b]	2	50/66	Beni Department, Bolivia	AY033153, AY033156[b]
C. laucha[b]	3	62–64/68	Maximo Paz, Argentina	AF385593[b]
			Estancia Bolivar, Bolivia	AF385594[b]
			Filadelfia, Paraguay	AY033190[b]
C. lepidus[b]	3	36–44/68	Iscayachi, Bolivia	AF385605, AF385606[b]
			Reserva de Fauna Ulla-Ulla, Bolivia	AF385607[b]
C. sorellus[b]	1	62–64/68	Caylloma, Peru	AF385608[b]
C. tener[b]	3	66/66–70	Santa Rosa de la Roca, Bolivia	AF385595[b]
			Tupi Paulista, Brazil	AF385596, AF385597[b]
C. venustus[b]	2	56/66	Espinillo, Argentina	AY033174, AY033175[b]
C. musculinus[b]	6	36–38/48–56	Puerto Madryn, Argentina	AF385599, AF385600[b]
			Monte Palma, Paraguay	AF385601[b]
			Andalgala, Argentina	AF385602[b]
			Mar del Plata, Argentina	AF385603[b]
			Tucumilla, Bolivia	AF385604[b]
Andalgalomys pearsoni[b]	1		Roboré, Bolívia	AF159285[b]
Eligmodontia puerulus[b]	1		Andrés de Machaca, Bolívia	AF159289[b]
Graomys domorum[b]	1		Boyuibe, Bolivia	AF159291[b]
G. griseoflavus[b]	1		Comarapa, Bolivia	AF159290[b]
Salinomys delicatus[b]	1		Salinas del Bebedero, Argentina	AF159292[b]

[a] Specimens sequenced in this work.
[b] Specimens sequenced in Salazar-Bravo et al. (2001).

Foster City, California) in accordance to the manufacturer's instructions. All sequences are available in GenBank (Table 1).

Data analysis.—The sequences obtained by us were read employing the program Chromas 1.45 (http://www.technelysium.com.au/chromas.html), aligned with the program Clustal X 1.81 (Thompson et al. 1997) and revised manually with the aid of the program BioEdit (Hall 1999). The composition of bases was determined using the program MEGA, version 3.1 (Kumar et al. 2004). Saturation curves were obtained with DAMBE 4.2.9 (Xia and Xie 2001) and phylogenetic analyses were performed in PAUP, version 4.0b10 (Swofford 1998).

Maximum-parsimony analyses were performed using heuristic searches with tree-bisection-reconnection branch swapping, the MULPARS option, and 100 random-addition replicates. Bootstrap statistical support (Felsenstein 1985) was based on 10,000 replications of heuristic search with simple taxon addition, with the ALL TREES SAVED option.

The appropriate model of nucleotide substitution for maximum-likelihood analysis was determined using MODELTEST 3.06 (Posada and Crandall 1998). For maximum-likelihood tree estimation, heuristic searches with as-is, tree-bisection-reconnection branch swapping, and MULPARS option were conducted with PAUP. Because obtaining support estimates for the branches of the maximum-likelihood trees by bootstrap analysis is computationally too intensive with a data set of this size, we conducted a bootstrap analysis (100 replications) using the neighbor-joining method under a maximum-likelihood model, with parameters settings estimated by MODELTEST, as described by Xiang et al. (2002) and Muschner et al. (2003).

TABLE 2.—The pairwise *p* distances among the 11 species of genus *Calomys* and between them and 1 phyllotine outgroup. CLE, *C. expulsus*; CLB, *C. boliviae*; CLI, *C.* innom; CLO, *C. callosus*; CLV, *C. venustus*; CLL, *C. laucha*; CLT, *C. tener*; CLH, *C. hummelincki*; CLS, *C. sorellus;* CLP, *C. lepidus*; CLM, *C. musculinus*; ELP, *Eligmodontia puerulus*. NC, distances not computed.[a]

	CLE	CLB	CLI	CLO	CLV	CLL	CLT	CLH	CLS	CLP	CLM	ELP
CLE	0.004											
CLB	0.118	0.009										
CLI	0.119	0.013	0.003									
CLO	0.118	0.026	0.024	0.007								
CLV	0.129	0.033	0.031	0.041	0.013							
CLL	0.126	0.136	0.135	0.134	0.145	0.035						
CLT	0.141	0.137	0.135	0.137	0.142	0.136	0.024					
CLH	0.149	0.147	0.144	0.145	0.155	0.152	0.148	NC				
CLS	0.163	0.170	0.169	0.171	0.181	0.170	0.157	0.158	NC			
CLP	0.160	0.165	0.163	0.161	0.173	0.166	0.152	0.161	0.178	0.025		
CLM	0.149	0.161	0.159	0.154	0.168	0.150	0.138	0.147	0.107	0.176	0.020	
ELP	0.184	0.184	0.184	0.180	0.190	0.181	0.183	0.183	0.175	0.220	0.175	NC

[a] ——— Lowland × lowland species; ——— Lowland × highland species; ═══ Highland × highland species.

Bayesian analyses of the data were performed using MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001) to generate a posterior probability distribution using Markov chain Monte Carlo methods. No a priori assumptions about the topology of the tree were made and all searches were provided with a uniform prior. The models of DNA substitution used were those estimated by MODELTEST and used in the standard maximum-likelihood analyses. The Markov chain Monte Carlo processes were set so that 4 chains were run simultaneously for 1 million generations, with trees being sampled every 100 generations for a total of 10,000 trees. We excluded the first 100,000 generations as the burn-in period. To calculate the posterior probability of each bipartition, a 50% majority-rule consensus tree was constructed from the remaining trees using PAUP.

The median-joining network method, version 4.1.0.0 (Bandelt et al. 1999; http://www.fluxus-engineering.com) also was used to analyze the phylogeographic pattern of *C. expulsus*. For distance analyses, trees were constructed using the neighbor-joining method (Saitou and Nei 1987) using proportional (*p*) and Kimura 2-parameter distances. Reliability of the trees was tested using 10,000 bootstrap replications (Hedges 1992).

RESULTS

The complete cytochrome-*b* gene (1,143 base pairs [bp]) was sequenced in the 30 samples of 3 species of the genus *Calomys* collected for this study. For all individuals, small portions (3 bp of the 5′ and 6 bp of 3′ ends, respectively) were excluded from the analyses because the sequence quality was poor in these regions. The average base composition of the cytochrome-*b* gene observed in these 30 specimens of *Calomys* showed a guanine deficiency (12.2%) and a slight cytosine deficiency (23.8%), whereas the other 2 bases (A = 32.1%; T = 31.9%) presented an overall even pattern.

Substitutions were scored in the 30 aligned sequences, and a total of 21 haplotypes was found. Each of the 3 individuals of *C. tener* showed a distinct haplotype, *C. laucha* showed a unique haplotype, and 17 different haplotypes were found in the 26 specimens of *C. expulsus*. The most frequent haplotype in *C. expulsus* occurred in 5 of 26 animals and was the only haplotype present in Ipameri and Mambai.

Saturation curves (not shown) that included the Brazilian species collected by us plus other *Calomys* analyzed by Salazar-Bravo et al. (2001) indicated no evidence of saturation in any codon position, thus there should be no loss of phylogenetic signal among the more divergent taxa (i.e., outgroup–ingroup) in these analyses.

The pairwise *p* distances among the 11 species of *Calomys* varied from 1.3% (*C. boliviae* × *C.* innom) to 18.1% (*C. sorellus* × *C. venustus*; Table 2). When geographic origin is taken into account, pairwise distances among species in the lowlands ($n = 7$) ranged from 1.3% between *C.* innom. versus *C. boliviae* to 14.5% between *C. laucha* versus *C. venustus*, whereas pairwise *p* distances among species typically found in highlands ($n = 3$) ranged from 10.7% to 17.8%. Between lowland and highland species, pairwise *p* distances ranged from 13.8% to 18.1%. *C. hummelincki* showed *p* distances intermediate to those of both lowland and highland taxa (Table 2).

Phylogenetic analysis.—Topologies of the phylogenetic analyses were very similar, regardless of the method of analyses employed (i.e., maximum-parsimony, neighbor-joining, maximum-likelihood, and Bayesian). In the maximum-parsimony analyses, 397 sites were parsimony informative. The heuristic search resulted in 213 most-parsimonious trees of 1,657 evolutionary steps with a consistency index of 0.457 and a retention index of 0.833. In the strict-consensus tree (not shown) the bootstrap values ranged between 51% and 100%, and several polytomies were found. The trees obtained by the neighbor-joining method with Kimura 2-parameter and *p* distances were very similar with some polytomies being better resolved. The selected model for the maximum-likelihood analysis using MODELTEST was TrN+I+G. Under this model the value of ln-likelihood was −4,813.15083, the estimated proportion of invariable sites was 0.4327, and the value gamma shape parameter (α) was 1.0645.

The topology generated by the maximum-likelihood analysis showed *Calomys* to be a monophyletic group (80% bootstrap

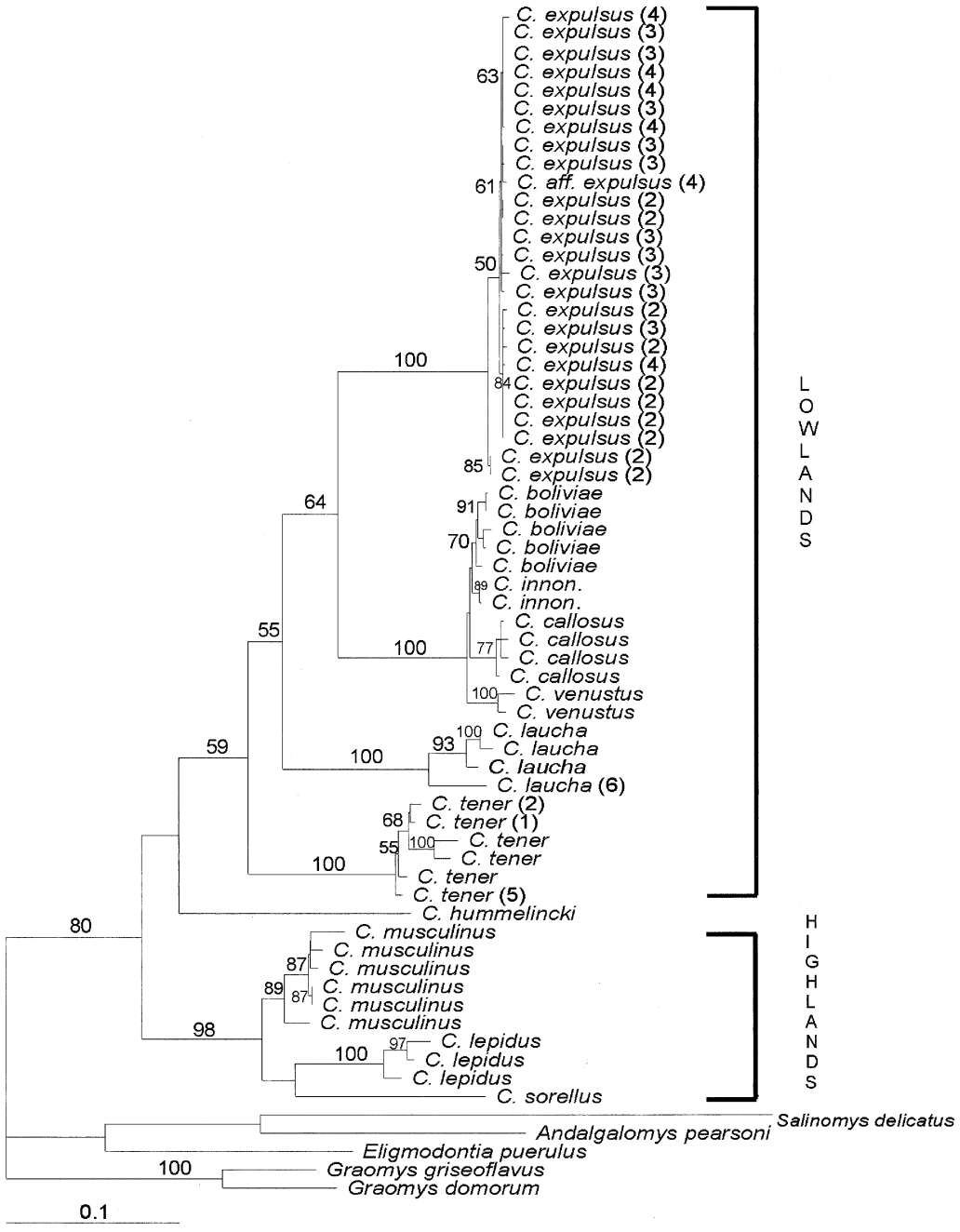

FIG. 2.—Maximum-likelihood tree for the representatives of the genus *Calomys* analyzed herein. Numbers above branches are bootstrap values. Numbers in brackets correspond to localities on map of Fig. 1. The values of the estimated parameters are: −log-likelihood = 4,813.15083, estimated proportion on invariable sites (I) = 0.4327, and the estimated value of the gamma shape parameter (α) = 1.0645.

value) characterized by 2 large clades (Fig. 2). One of these clades, named the "Lowlands Clade," is a large assemblage of taxa (59% bootstrap) that includes *C. expulsus*, followed by a well-supported group comprising *C. boliviae*, *C.* innom., *C. callosus*, and *C. venustus*, to which *C. laucha* and *C. tener* link. This clade comprises species that are associated primarily with lowland biomes (<1,000 m elevation, except for *C. boliviae*). The other clade, basal to the lowland clade, is the "Highlands Clade" (98% bootstrap), which includes the species *C. lepidus* and *C. sorellus*, associated with high elevations, and *C. musculinus*, associated with intermediate elevations. The species *C. hummelincki* was in an intermediate position between these 2 larger clades, but was more closely related to the cluster of species associated with the lowland biomes. The tree obtained by the Bayesian analysis (Fig. 3) was similar to the maximum-likelihood tree, showing posterior probability values of the principal nodes of 91 (Lowlands Clade) and 100 (Highlands Clade).

Phylogeographic analysis.—An unrooted haplotype tree based on a distance matrix of the number of the differences between haplotypes calculated from the entire cytochrome-*b* gene sequences of 26 *C. expulsus* from 3 localities of the Cerrado

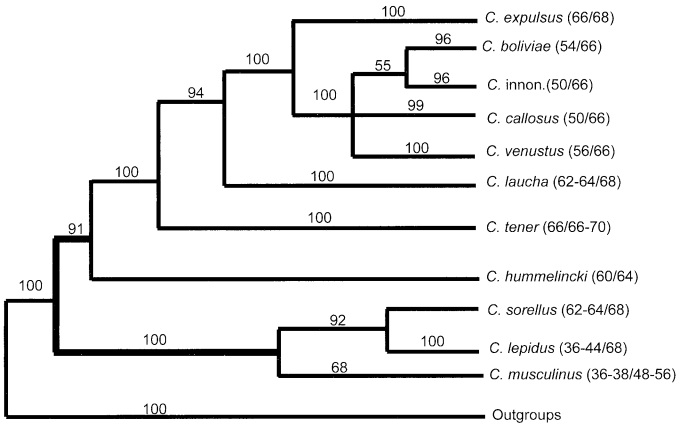

Fig. 3.—A synthesis of the topology generated by Bayesian analyses of genus *Calomys*. Diploid and autosomal arm numbers, respectively, are given in parentheses. Posterior probabilities values are included above the branches.

biome is shown in Fig. 4. Although some haplotypes were scattered throughout the tree, haplotypes from the same localities tended to cluster together.

Discussion

This is the 1st study to include a large sample of multiple Brazilian taxa and specimens in the genus *Calomys*. No evidence of substitution saturation appears to be present at any codon position, thus avoiding potential loss of phylogenetic information due to excessive multiple hits (Saitou 1989). The different methods of analyses employed (parsimony, likelihood, neighbor-joining, and Bayesian) recovered almost identical results with similar support values, thus generating a completely resolved phylogeny with several strongly supported clades.

Our analyses corroborate the monophyly of the genus *Calomys* as a well-supported clade (78% of the maximum-parsimony bootstrap, 80% of the maximum-likelihood bootstrap, 93% of the neighbor-joining bootstrap, and 100% Bayesian posterior probability). All trees obtained generated the same topology with 11 species of *Calomys* grouping in 2 major clades, both with extensive branch lengths.

The clade containing *C. sorellus*, *C. lepidus*, and *C. musculinus* constitutes the Highlands Clade. *C. sorellus* is confined to the highlands (above 2,000 m elevation) of south-central Peru and was previously considered a subspecies of *C. lepidus* (Eisenberg and Redford 1999; Musser and Carleton 2005); *C. lepidus* is distributed in highland grasslands (3,300–5,000 m elevation) along the Andes of Peru, Bolivia, Chile, and northern Argentina; *C. musculinus* appears to favor arid habitats over a wide range of elevations in west-central Bolivia, western Paraguay, and Argentina (Musser and Carleton 2005). In the northern part of the Argentinean province of Mendoza and in eastern Paraguay, *C. musculinus* co-occurs with *C. laucha*; *C. musculinus* was previously considered a subspecies of *C. laucha*. All 3 species are distributed in a partially overlapping area (central to south Andes and adjacencies) of South America.

Calomys musculinus and *C. lepidus* also appeared as closely related species in 2 other phylogenetic analyses performed for the genus *Calomys* (allozymes—Chiappero et al. 2002; cytochrome-*b*—Salazar-Bravo et al. 2001) and share low diploid numbers. *C. sorellus* is morphologically similar to *C. lepidus* and displays a high diploid number, compatible with those seen in the majority of species of *Calomys*. It has a restricted distribution and could be a relictual species similar to the ancestral form from which this clade originated.

Calomys hummelincki is located at the base of the Lowlands Clade, which comprises *C. tener*, *C. laucha*, *C. expulsus*, and a cluster of 4 taxa (*C. venustus*, *C. callosus*, *C.* innom., and *C. boliviae*). These are species from the lowlands, and share several characteristics. *C. tener*, with type locality in Lagoa

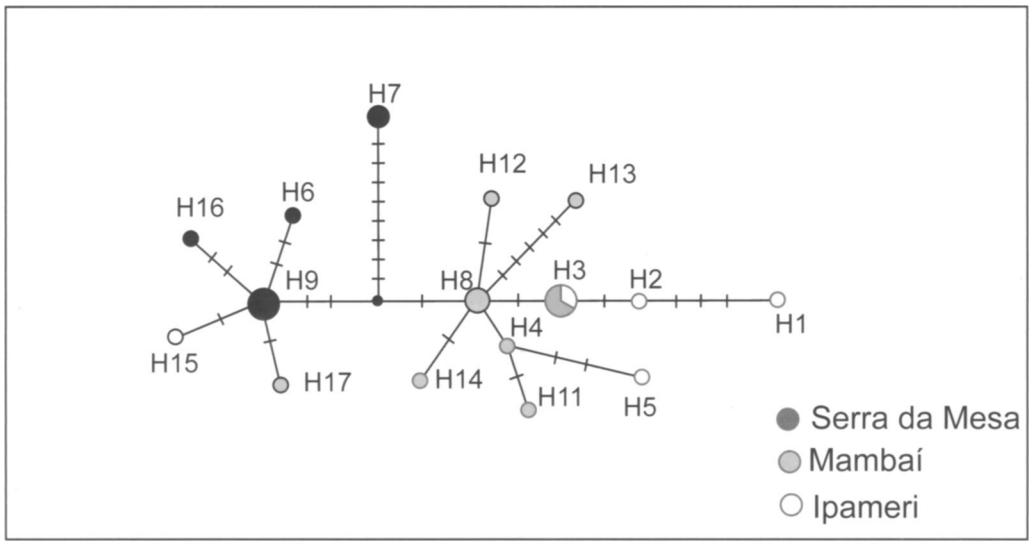

Fig. 4.—Median-joining network connecting the different cytochrome-*b* sequences from samples of *Calomys expulsus* collected at 3 localities in the Cerrado biome. The diameters of the circles indicate their frequencies, and the degree of shading their origin: white, Ipameri; dark shading, Mambai; dark, Serra da Mesa.

Santa (Minas Gerais, Brazil), is found in the Brazilian states of São Paulo, Minas Gerais, and Goiás, and was often considered a subspecies of *C. laucha* (Eisenberg and Redford 1999; Hershkovitz 1962). *C. laucha*, with its type locality near Asunción (Paraguay), ranges from northern Argentina and Uruguay to southwestern Bolivia, western Paraguay, and west-central Brazil (Musser and Carleton 2005). We trapped 1 individual of *C. laucha* near the Brazil–Uruguay border (parallel 32°S). *C. tener* and *C. laucha* share a high diploid number (66 and 64) and small body sizes. *C. expulsus*, with type locality in Lagoa Santa, was previously considered a synonym of *C. callosus* (type locality Neembucú, Paraguay—see Musser and Carleton 2005). However, based on karyological and morphometric analyses, Bonvicino and Almeida (2000) distinguished both species, proposing specific status for *C. expulsus*. This species also shows a high diploid number (66), but has a large body size and occurs in the Brazilian states of Pernambuco, Bahia, Goiás, and Minas Gerais; it is sympatric with *C. tener* in Minas Gerais and Goiás.

The next clade, strongly supported as monophyletic, contains a group of species (*C. venustus*, *C. callosus*, *C.* innom., and *C. boliviae*) all, in 1 way or another, related to *C. callosus*. *C. callosus*, a species of large body size and habitats with open vegetation, is broadly distributed, being found in eastern and southwestern Brazil, Bolivia, Paraguay, and northern Argentina (Eisenberg and Redford 1999). In southern South America it is found in the southern and western portions of the Paraguayan Chaco and across the northern provinces in Argentina. This distribution includes *C. venustus*, separated from *C. callosus* by Anderson (1997). *C. boliviae*, restricted to western Bolivia, is conventionally arranged as a subspecies or a synonym of *C. callosus* (Musser and Carleton 2005). The last taxon (*C.* innom.), not yet recognized formally, presented a unique karyotype and occurs in Bolivia, in Beni Province (Salazar-Bravo et al. 2002). Members of the "*callosus–venustus*" species group are characterized by having intermediate diploid numbers (50, 54, and 56).

In conclusion, the analyses presented herein consistently showed that the relationships found among the species of the genus *Calomys* are strongly supported, and that these species show high levels of genetic differentiation that suggest they diverged a long time ago. The likely scenario is of a phyllotine lineage with high diploid number (66–70) originating in the Andes that later separated into 2 large clades. One clade remained in the highlands, experiencing some local differentiation accompanied by a strong reduction in number of chromosomes in some cases (*C. musculinus*, *C. lepidus*, and *C. sorellus*). The other clade invaded the nonforested biomes and the forest fringes of eastern South America; this clade underwent extreme differentiation resulting in species with high diploid numbers, wide distributions, and body sizes ranging from the small *C. tener* and *C. laucha* to the large *C. expulsus*. In the Lowlands Clade, the "*callosus–venustus*" taxa appear as a more derived group, characterized by large body size and broad distributions, and their differentiation was probably accompanied by some reduction in diploid numbers (50–56). In this scenario, the position of *C. hummelincki* is intriguing. This species presents a disjunct distribution (northern border of the continent) compared to the other species of *Calomys* but its cytochrome-*b* sequences and diploid number (60) indicate that this taxon occupies an intermediate position between the Highland and Lowland clades.

Resumen

Calomys Waterhouse, 1837, es uno de los géneros mas especiosos de la tribu Phyllotini de los roedores sigmodontinos, siendo encontrado predominantemente en la parte sur de América del Sur y ha sido sugerido que los Andes centrales serian su área original de diferenciación con la subsiguiente ocupación de las tierras bajas del continente. En este estudio fue secuenciado el gen completo de citocromo *b* en 30 especimenes de 3 especies del género *Calomys* los cuales fueron analizados junto con otras especies de *Calomys* (GenBank) más los grupos externos. Los diferentes métodos de análisis empleados mostraron como probable escenario que un linaje phyllotino surgió en los Andes y subsecuentemente se dividió en dos clados mayores. Uno de ellos permaneció en las tierras altas (*C. musculinus*, *C. lepidus*, y *C. sorellus*), sufriendo alguna diferenciación local. El otro clado invadió las tierras bajas de América del Sur, especialmente los biomas no forestados, en los cuales sufrió intensa diferenciación resultando en especies que presentan una amplia distribución en el continente (2 especies pequeñas, *C. tener* y *C. laucha*, y 1 grande, *C. expulsus*). En el clado de tierras bajas, el grupo "*callosus–venustus*" surge como más derivado, mostrando mayor tamaño y una distribución más amplia y cuya diferenciación fue, probablemente, acompañada por alguna reducción en el número cromosomal diploide.

Acknowledgments

Conselho Nacional de Desenvolvimento Científico e Tecnológico, Financiadora de Estudos e Projetos, Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul, and the Organization of the American States have supported this study. We are grateful to Dr. A. P. Nunes and to Dr. J. L. P. Cordeiro for fieldwork and to L. S. Silva and F. Z. C. Marques for technical help.

Literature Cited

Anderson, S. 1997. Mammals of Bolivia, taxonomy and distribution. Bulletin of the American Museum of Natural History 231:1–652.

Anderson, S., and T. L. Yates. 2000. A new genus and species of phyllotine rodent from Bolivia. Journal of Mammalogy 8:18–36.

Bandelt, H.-J., P. Foster, and A. Röhl. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16:37–48.

Bonvicino, C., and F. C. Almeida. 2000. Karyotype, morphology and taxonomic status of *Calomys expulsus* (Rodentia: Sigmodontinae). Mammalia 64:339–351.

Braun, J. K. 1993. Systematic relationships of the tribe Phyllotini (Muridae: Sigmodontinae) of South America. Special Publication, Oklahoma Museum of Natural History, Norman.

Cabrera, A. 1961. Catálogo de los mamíferos de América del Sur. Revista del Museo Argentino Ciencias Naturales "Bernardino Rivadavia" 4:309–732.

CHIAPPERO, M. B., G. B. DE SOUSA, A. BLANCO, AND C. N. GARDENAL. 2002. Evolutionary relationships among eight species of South American phyllotine rodents (Rodentia: Muridae) based on allozymic data. Journal of Zoological Systematics and Evolutionary Research 40:1–7.

CORTI, M., M. S. MERANI, AND G. VILLAFANE. 1987. Multivariate morphometrics of vesper mice (*Calomys*): preliminary assessment of species, population, and strain divergence. Zeitschrift für Säugetierkunde 52:236–242.

EISENBERG, J., AND K. REDFORD. 1999. Mammals of the Neotropics. The central Neotropics. Ecuador, Peru, Bolivia and Brazil. University of Chicago Press, Chicago, Illinois.

ELLERMAN, J. R. 1941. The families and orders of living rodents. Vol. 2. Family Muridae. British Museum of Natural History, London, United Kingdom.

ENGEL, S. R., K. M. HOGAN, J. F. TAYLOR, AND S. K. DAVIS. 1998. Molecular systematics and paleobiogeography of the South American Sigmodontinae rodents. Molecular Biology and Evolution 15:35–49.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

HALL, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95–98.

HEDGES, S. B. 1992. The number of replications needed for accurate estimation of the bootstrap *p* value in phylogenetic studies. Molecular Biology and Evolution 9:366–369.

HERSHKOVITZ, P. 1962. Evolution of neotropical cricetine rodents (Muridae) with special reference to the phyllotine group. Fieldiana: Zoology 46:1–524.

HUELSENBECK, J. P., AND F. RONQUIST. 2001. MrBayes: Bayesian inference of phylogeny. Department of Biology, University of Rochester, Rochester, New York.

KUMAR, S., K. TAMURA, AND M. NEI. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings in Bioinformatics 5:150–163.

MATTEVI, M. S., T. HAAG, L. F. B. OLIVEIRA, AND A. R. LANGGUTH. 2005. Chromosome characterization of Brazilian species of *Calomys* Waterhouse, 1837 from Amazon, Cerrado and Pampas domains (Rodentia, Sigmodontinae). Arquivos do Museu Nacional, Rio de Janeiro 63:175–181.

MEDRANO, J. F., E. AASEN, AND L. SHARROW. 1990. DNA extraction of nucleated red blood cells. Biotechniques 8:1–43.

MUSCHNER, V. C., ET AL. 2003. A first molecular phylogenetic analysis of *Passiflora* (Passifloraceae). American Journal of Botany 90:1229–1238.

MUSSER, G. G., AND M. D. CARLETON. 2005. Superfamily Muroidea. Pp. 894–1531 in Mammal species of the world: a taxonomic and geographic reference (D. E. Wilson and D. M. Reeder, eds.). 3rd ed. Johns Hopkins University Press, Baltimore, Maryland.

PEARSON, O. P., AND J. L. PATTON. 1976. Relationships among South American phyllotine rodents based on chromosome analysis. Journal of Mammalogy 57:339–350.

POSADA, D., AND K. A. CRANDALL. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14:817–818.

REIG, A. O. 1986. Diversity patterns and differentiation of high Andean rodents. Pp. 404–439 in High altitude tropical biogeography (F. Vuilleumier and M. Monasterio, eds.). Oxford University Press, New York.

SAITOU, N. 1989. A theoretical study of the underestimation of branch lengths by the maximum parsimony principle. Systematic Zoology 38:1–6.

SAITOU, N., AND M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 9:945–967.

SALAZAR-BRAVO, J., J. W. DRAGOO, M. D. BOWEN, C. J. PETERS, T. G. KSIAZEK, AND T. L. YATES. 2002. Natural nidality in Bolivian hemorrhagic fever and the systematics of the reservoir species. Infection, Genetics and Evolution 1:191–199.

SALAZAR-BRAVO, J., J. W. DRAGOO, D. S. TINNIN, AND T. L. YATES. 2001. Phylogeny and evolution of the neotropical rodent genus *Calomys*: inferences from mitochondrial DNA sequence data. Molecular Phylogenetics and Evolution 20:173–184.

SPOTORNO, A. E., S. V. F. WALKER, M. YEVENES, J. C. MARÍN, AND C. ZULETA. 2001. Evolución de los filotinos (Rodentia, Muridae) en los Andes del Sur. Revista Chilena de História Natural 74:151–166.

STEPPAN, S. 1995. Revision of the tribe Phyllotini (Rodentia: Sigmodontinae) with a phylogenetic hypothesis for the Sigmodontinae. Fieldiana: Zoology (New Series) 80:1–112.

STEPPAN, S. J., AND J. SULLIVAN. 2000. The emerging statistical perspective in systematic biology: A reply to Mares and Braun on the status of *Andalgalomys* (Rodentia: Sigmodontinae). Journal of Mammalogy 81:260–270.

SWOFFORD, D. L. 1998. PAUP*: phylogenetic analysis using parsimony (* and other methods). Version 4.0b10. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

THOMPSON, J. D., T. J. GIBSON, F. PLEWNIAK, F. JEANMOUGIN, AND D. G. HIGGINS. 1997. The Clustal X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24:4876–4882.

WILLIAMS, D. F., AND M. A. MARES. 1978. A new genus and species of phyllotine rodent (Mammalia: Muridae) from northwestern Argentina. Annals of Carnegie Museum 47:193–221.

XIA, X., AND Z. XIE. 2001. DAMBE: software package for data analysis in molecular biology and evolution. Journal of Heredity 92:371–373.

XIANG, Q.-Y., M. L. MOODY, D. E. SOLTIS, C. FAN, AND P. S. SOLTIS. 2002. Relationships within Cornales and circumscription of Cornaceae—matK and rbcL sequence data and effects of outgroups and long branches. Molecular Phylogenetics and Evolution 24:35–57.

Submitted 23 September 2005. Accepted 2 January 2007.

Associate Editor was Jesús E. Maldonado.

APPENDIX I

Voucher specimens of *Calomys* taxa sequenced for the cytochrome-*b* gene with locality names in parentheses.

Calomys expulsus.—MN36230, MN36255, MN36270, MN36275, MN36276, MN36289, MN36360, MN36447, MN36508, MN37281 (Serra da Mesa, Brazil); UFPB3053, UFPB3054, UFPB3055, UFPB3059, UFPB3060, UFPB3062, UFPB3063, UFPB3066, UFPB3069, UFPB3071 (Mambai, Brazil); MN37860, MN3786, MN37862, MN37863, MN37864, MN37865 (Ipameri, Brazil).

Calomys laucha.—MN37866 (Taim Ecological Station, Brazil).

Calomys tener.—MN36437 (Serra da Mesa, Brazil); AN2557 (Aliança do Tocantins, Brazil); AFV02 (Quintão, Brazil).