

SYSTEMATICS OF *NECROMYS* (RODENTIA, CRICETIDAE, SIGMODONTINAE): SPECIES LIMITS AND GROUPS, WITH COMMENTS ON HISTORICAL BIOGEOGRAPHY

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We present the most comprehensive systematic study to date of *Necomys*, a rodent genus distributed in open areas north and south of Amazonia and in Andean grasslands. The study is based on sequences of the cytochrome-*b* gene that were analyzed by parsimony and Bayesian approaches. The analyses include sequences of 62 specimens from 51 localities from Argentina, Bolivia, Brazil, Colombia, Paraguay, Peru, Uruguay, and Venezuela, representing all but 1 of the species currently recognized in the genus. *Necomys* was recovered as a monophyletic group and we found a large polytomy at its base that involves 3 lineages. One, represented by the Andean *N. lactens*, shows a marked phylogeographic pattern. The 2nd clade is formed by *N. urichi* from the northern grasslands of South America and *N. amoenus* from the central Andes. Results suggest that each of these taxa may represent more than 1 biological species. The 3rd clade is formed by lowland species found south of Amazonia. Within this clade *N. obscurus* is sister to the remaining species. Haplotypes recovered from specimens assigned to *N. benefactus*, *N. temchuki*, and *N. lasiurus* form a clade, but these taxa do not form reciprocally monophyletic groups, nor does this large clade possess geographic structure. These genealogical results, discussed in the context of genetic variation, are the basis of taxonomic (e.g., *N. benefactus* and *N. temchuki* are regarded as junior synonyms of *N. lasiurus*) and biogeographic considerations.

Key words: *Bolomys*, *Cabreramys*, Cricetidae, gene tree, Muroidea, *Necomys*, South America, systematics, taxonomy, *Zygodontomys*

The diversity of living sigmodontines is impressive; at last count recent species numbered 380 in about 84 genera (D'Elía et al. 2007; Musser and Carleton 2005; see also D'Elía and Pardiñas 2007). These numbers make Sigmodontinae the most diverse subfamily of New World mammals and on a global scale 2nd only to the Old World Murinae. Diversity of species is unevenly distributed among sigmodontine genera, with *Akodon* and *Thomasomys* having well over 40 species, whereas many genera (e.g., *Deltamys*, *Lundomys*, and *Sooretamys*) are monotypic, presenting the well-known (although not well-understood) hollow-curve distribution of species richness (Reig 1986). Among the most diverse genera in the akodontine tribe,

Necomys includes 9 recognized species (Musser and Carleton 2005).

Necomys departs from other sigmodontine genera of equivalent diversity (e.g., *Abrothrix*, *Neacomys*, and *Brucepattersonius*) because of its distribution. It occurs in open areas from Venezuela and Trinidad and Tobago in the northern part of South America to central Argentina in the south, and from the Atlantic in Brazil, Uruguay, and Argentina to Andean elevations of about 5,000 m above sea level. Moreover, *Necomys* is the 2nd oldest known South American sigmodontine genus (2nd only to *Auliscomys*), because *N. bonapartei*† is known from sediments of about 4–3.5 million years of age, corresponding to the Chapadmalalan of the late Pliocene (Pardiñas and Tonni 1998). Therefore, strong hypotheses about relationships among species in the genus and the position of *Necomys* within Akodontini are of fundamental importance to understanding sigmodontine systematics and biogeography.

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TABLE 1.—Abridged historic account of living taxonomic forms assigned to *Necomys*.

Gyldenstolpe (1932)	Cabrera (1961)	Reig (1987) ^a	Musser and Carleton (2005)	Present study
<i>Bolomys amoenus</i> <i>B. lactens</i>	<i>Akodon (B. amoenus)</i> <i>A. (B.) lactens</i> (includes <i>negrito orbus</i>)	<i>B. amoenus</i> <i>B. lactens</i> (includes <i>negrito orbus</i>)	<i>Necomys amoenus</i> <i>N. lactens</i> (includes <i>negrito orbus</i>)	<i>N. amoenus</i> (Thomas, 1900) <i>N. lactens</i> (Thomas, 1918) (includes <i>negrito</i> (Thomas, 1926), <i>orbus</i> (Thomas, 1919))
<i>B. negrito</i> <i>B. orbus</i>				
<i>Zygodontomys lasiurus</i>	<i>Z. lasiurus</i> (includes <i>fuscinus</i> , <i>brachyurus</i> , <i>pixuna</i>)	<i>B. lasiurus</i> (includes <i>arviculoides</i> , <i>brachyurus</i> , <i>fuscinus</i> , <i>lasiotis</i> , <i>pixuna</i>)	<i>N. lasiurus</i> (includes <i>arviculoides</i> , <i>brachyurus</i> , <i>fuscinus</i> , <i>lasiotis</i> , <i>orobinus</i> , <i>pixuna</i> , <i>renggeri</i>)	<i>N. lasiurus</i> (Lund, 1840) (includes <i>arviculoides</i> (Wagner, 1842), <i>benefactus</i> (Thomas, 1919), <i>brachyurus</i> (Wagner, 1845), <i>elioi</i> (Contreras, 1982), <i>fuscinus</i> (Thomas, 1897), <i>lasiotis</i> (Lund, 1839), <i>liciae</i> (Contreras, 1982), <i>orobinus</i> (Wagner, 1842), <i>pixuna</i> (Moojen, 1943), <i>renggeri</i> (Pictet, 1844), <i>temchuki</i> (Massoia, 1982))
<i>A. arviculoides</i> (includes <i>orobinus</i>) <i>A. benefactus</i>	<i>A. arviculoides</i> (includes <i>orobinus</i> , <i>renggeri</i>) Included in <i>A. obscurus</i>	Included in <i>B. obscurus</i> <i>B. temchuki</i> (includes <i>elioi</i> , <i>liciae</i>)	<i>N. benefactus</i> <i>N. temchuki</i> (includes <i>elioi</i> , <i>liciae</i>)	
<i>Hesperomys brachyurus</i> <i>Z. fuscinus</i> <i>A. lenguarum</i>	Included in <i>A. obscurus</i>	<i>B. lenguarum</i> (includes <i>tapirapoanus</i>)	<i>N. lenguarum</i> (includes <i>tapirapoanus</i>)	<i>N. lenguarum</i> (Thomas, 1898) (includes <i>tapirapoanus</i> (Allen, 1916))
<i>Z. tapirapoanus</i> <i>A. obscurus</i>	<i>A. tapirapoanus</i> <i>A. obscurus</i>	<i>B. obscurus</i> (includes <i>benefactus</i>) <i>B. innom.</i> sp. (would correspond to the later <i>scagliarum</i>)	<i>N. obscurus</i> (includes <i>scagliarum</i>)	<i>N. obscurus</i> (Watherhouse, 1837) (includes <i>scagliarum</i> Galliari and Pardiñas, 2000)
<i>Z. punctulatus</i> <i>A. (Chalcomys) urichi</i>	<i>Z. punctulatus</i> <i>A. urichi</i> (includes <i>chapmani</i> , <i>meridensis</i> , <i>saturatus</i> , <i>venezuelensis</i>)	Not considered <i>A. urichi</i> (includes <i>saturatus</i> , <i>venezuelensis</i>)	<i>N. punctulatus</i> <i>N. urichi</i> (includes <i>chapmani</i> , <i>meridensis</i> , <i>saturatus</i> , <i>tobagensis</i> , <i>venezuelensis</i>)	<i>N. punctulatus</i> (Thomas, 1894) <i>N. urichi</i> (Allen and Chapman, 1897) (includes <i>chapmani</i> (Allen, 1913), <i>meridensis</i> (Allen, 1904), <i>saturatus</i> (Tate, 1939), <i>tobagensis</i> (Goodwin, 1962), <i>venezuelensis</i> (Allen, 1899))
<i>A. (Ch.) chapmani</i> <i>A. (Ch.) meridensis</i> <i>A. (Ch.) venezuelensis</i>				

^a Some taxa are missing from Reig's (1987) scheme because he only dealt with taxa he considered to be Akodontini; moreover, because his work was not a taxonomic catalogue he did not mention all akodonts.

The phylogenetic position of *Necomys* within the sigmodontine radiation is well established; it has been addressed by the analysis of both mitochondrial and nuclear gene sequences (D'Elía 2003; Smith and Patton 1999). *Necomys* forms part of the *Akodon* division of Akodontini, and is sister to *Thalpomys* (D'Elía 2003).

Necomys is different from most other genera of sigmodontines because it was originally described from a fossil form (Ameghino 1889). Species currently recognized in *Necomys* have been placed in *Akodon*, *Bolomys*, *Calomys*, *Cabreramys*, *Chalcomys*, or *Zygodontomys* (Table 1; for a complete review

of the taxonomic history of *Necomys* see Anderson and Olds [1989], Reig [1987], and Tate [1932]). The current contents of *Necomys* were established 30 years ago, when Reig (1978) resurrected *Bolomys* to encompass a group of species morphologically similar to *amoenus*, type species of the genus (Thomas 1916). Some of the species that Reig (1978, 1987) placed in *Bolomys* had been, until then, considered part of *Akodon* and *Zygodontomys* (e.g., *amoenus*, *lactens*, and *lasiurus*), whereas others (e.g., *benefactus*, *obscurus*, and *temchuki*) were in *Cabreramys* erected by Massoia and Fornes (1967). Like Reig (1978), Massoia and Fornes (1967)

recognized that some species placed in *Akodon* and *Zygodontomys* constituted a homogenous group distinct from the typical morphotypes of both genera. However, Massoia and Fornes (1967) failed to acknowledge that the species in consideration also were associated with *amoenus*, type species of *Bolomys*, which was available to contain those forms. Therefore, Reig (1987) placed *Cabreramys* under the synonymy of *Bolomys*. Massoia and Pardiñas (1993) recognized that *Necromys conifer*†, a fossil form described by Ameghino (1889) from the late Pleistocene of the Argentinean Buenos Aires Province and type species of *Necromys*, is a synonym of *B. benefactus*. Because *Necromys* has priority over *Bolomys* (and *Cabreramys*), this is the generic name currently used for the group of akodontine mice herein studied. Further, Smith and Patton (1993) expanded *Necromys* by showing that *Akodon urichi*, from northern South America, belonged to *Necromys*. This hypothesis was later corroborated with broader taxonomic (Smith and Patton 1999) and character (D'Elía 2003) sampling. Galliari and Pardiñas (2000) suggested that *Bolomys* may be retained to encompass the Andean species and *Necromys* restricted to lowland forms. D'Elía (2003) discussed this hypothesis together with other classificatory alternatives, favoring the broad definition of *Necromys* that is reflected in current classifications (e.g., Musser and Carleton 2005). Today, 9 species are placed in *Necromys* (Musser and Carleton 2005). Nevertheless, and despite the abundance of specimens in museums of natural history, the alpha taxonomy of the genus remains chaotic and species boundaries remain poorly understood. In part, this is because most revisionary works have been conducted at local scales or within a single species (Anderson and Olds 1989; Galliari and Pardiñas 2000; Macêdo and Mares 1987; Oliveira 1992).

The goal of this study is to generate a phylogenetic hypothesis for *Necromys*. This genealogy allows us to evaluate the content of the genus and its species limits, and to pose preliminary comments on its historical biogeography.

MATERIALS AND METHODS

Taxonomic sampling and sequence acquisition.—Animal care and use procedures followed guidelines approved by the American Society of Mammalogists (Gannon et al. 2007). Genealogical analysis was based on the first 801 base pairs (bp) of the cytochrome-*b* gene of 62 specimens of *Necromys* belonging to 50 populations from Argentina, Bolivia, Brazil, Colombia, Paraguay, Peru, Uruguay, and Venezuela (Fig. 1; Appendix I). Specimens from which DNA sequences were gathered belong to 8 of the 9 currently recognized species of *Necromys*. We are missing representatives of *N. punctulatus*, a species with uncertain geographic distribution in Ecuador and Colombia. However, our taxonomic coverage is broad; it includes specimens assignable to several of the nominal forms (e.g., *Akodon fuscus*, *Bolomys temchuki liciae*, and *Necromys obscurus scagliarum*) currently considered synonyms of valid species, as well as specimens collected at or near some type localities of several forms (e.g., *Akodon chapmani*, *A. fuscus*, *A. lactens*, *Cabreramys temchuki*, *Mus lasiurus*, and *N. ob-*

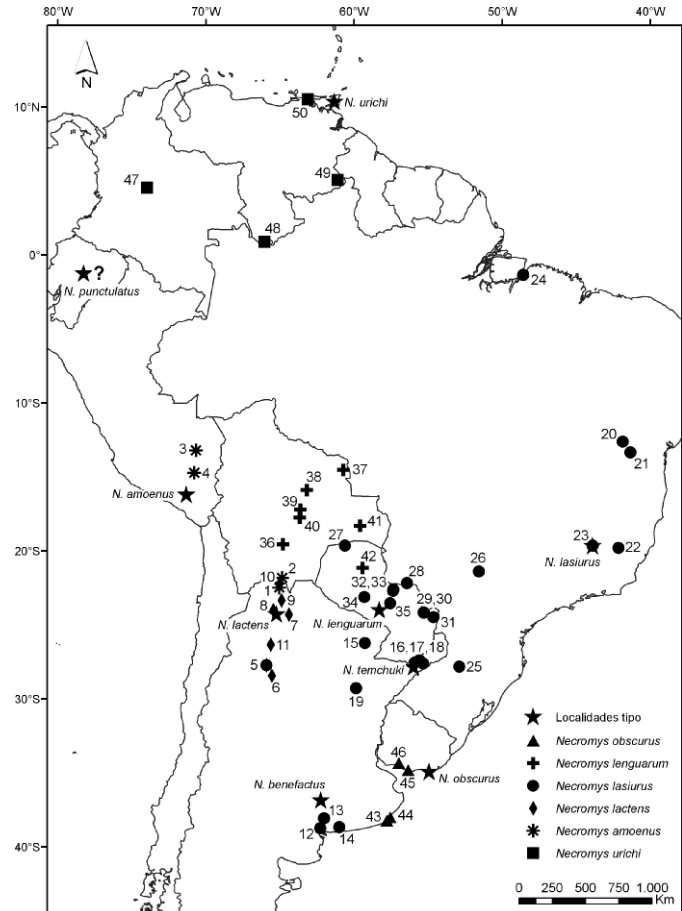


FIG. 1.—Map of collecting localities of the specimens of *Necromys* used in the present study. Locality numbers refer to Appendix I. The type localities of the *Necromys* species considered valid in Musser and Carleton (2005) also are shown. The exact type locality of *N. punctulatus* is not known and is here depicted with a question mark in Ecuador, the country from which it is supposed to have come.

scurus scagliarum). The phylogeny was rooted with haplotypes of specimens of *Akodon*, *Deltamys*, *Thalpomys*, and *Thaptomys*. *Thalpomys* is the sister genus of *Necromys* and all 5 genera constitute the *Akodon* division (sensu D'Elía 2003) of the tribe Akodontini. Sequences of 11 *Necromys* specimens and those of the outgroups were obtained from Smith and Patton (1991, 1999), Anderson and Yates (2000), D'Elía (2003), and D'Elía et al. (2003). Cytochrome-*b* gene sequences generated in this study were amplified and sequenced from a single fragment using primers MVZ 05–MVZ 16 (da Silva and Patton 1993) and the protocol detailed in D'Elía and Pardiñas (2004). In all cases, both heavy and light DNA strands were sequenced and compared. Sequences were visualized, reconciled, and translated to proteins to proof for stop codons using Sequence Navigator version 1.0.1 (Applied Biosystems, Inc., Foster City, California) and SeqMan (DNASTAR 2003). All sequences were deposited in GenBank (accession numbers EF531643–EF531693).

Analyses.—Sequences were aligned with Clustal X (Thompson et al. 1997) by using the default values for all

alignment parameters; no adjustment by eye was needed. Observed percentage of sequence divergence was calculated with PAUP* (Swofford 2000) ignoring those sites with missing data. Aligned sequences were subjected to maximum parsimony (Farris 1982; Kluge and Farris 1969) and Bayesian inference (reviewed in Huelsenbeck et al. 2001). Characters used in maximum-parsimony analysis were treated as unordered and equally weighted. PAUP* (Swofford 2000) was used to perform 200 replicates of heuristic searches with random addition of sequences and tree-bisection-reconnection branch swapping. Two measures of clade support were calculated. Because of computational time, Bremer support values (BS—Bremer 1994) were computed for selected nodes in PAUP* using command files written in TreeRot version 2 (Sorenson 1999) and with MAXTREE set to 10,000. One thousand parsimony jackknife (JK—Farris et al. 1996) replications with 1 addition sequence replicate each, the deletion of 33% of the character data, and MAXTREE set to 1,000 were performed using PAUP*. Branches with <50% of support were allowed to collapse. Bayesian analysis was conducted in MrBayes 3.1 (Ronquist and Huelsenbeck 2003). Ten analyses each consisting of 2 independent runs with 3 heated and 1 cold Markov chains each were performed. For each analysis, a model with 6 categories of base substitution, a gamma-distributed rate parameter, and a proportion of invariant sites was specified; all model parameters were estimated in MrBayes. Uniform interval priors were assumed for all parameters except base composition and GTR parameters, which assumed a Dirichlet process prior. Runs were allowed to proceed for 1, 1.5, 2, and 2.5 million generations; trees were sampled every 100 generations for each chain. To check that each run converged on a stable log-likelihood value, we plotted the log-likelihood values against generation time for each. The first 25% of the trees were discarded as burn-in and the remaining trees were used to compute a 50% majority rule consensus tree and obtain posterior probability (PP) estimates for each clade.

For *N. amoenus*, a hierarchical analysis of the distribution of genetic diversity was conducted in the form of analyses of molecular variance (AMOVA—Excoffier et al. 1992) using Arlequin version 2.000 (Schneider et al. 2000). Hierarchical levels were defined on the basis of sampling localities and major clades found in the phylogenetic analysis. Similarly, for the sample of *N. lasiurus* the neutrality tests of Tajima (1989) and Fu (1997) were implemented in Arlequin.

RESULTS

The data set contained 348 variable sites, 69 of which correspond to 1st codon positions, 45 to 2nd positions, and 234 to 3rd positions. Observed sequence divergence within and between localities of the same species ranges from 0% to 3.87% (based on specimens 9 localities of *N. amoenus*, *N. lasiurus*, *N. lenguarum*, and *N. obscurus*) and 0% to 10.86%, respectively. Values of comparisons between species pairs range from 5.2% to 18.26%.

Of the 348 variable characters of the whole data set, 263 are parsimony informative. The parsimony analysis of this data set

produced 22,107 shortest trees, each 982 steps long. Each tree had an ensemble consistency index (CI) of 0.473 and a retention index (RI) of 0.798. The strict consensus tree (Nelson 1979) defined 44 nodes belonging to *Necromys* (Fig. 2), 10 of which involved basal polytomies. At the base of the *Necromys* clade are 3 lineages: a clade composed of *N. urichi* and *N. amoenus* (JK = 54, BS = 1); another clade formed by *N. lactens* (JK = 100, BS = 20); and a clade formed by all lowland species south of Amazonia (JK = 72, BS = 1). Support for the clades recovered in the strict consensus is highly variable. In the jackknife analysis 3 nodes of the *Necromys* clade, involving haplotypes of *N. lasiurus*, collapse at the cutoff of 50%. Bremer support values for nodes of the *Necromys* clade range from 1 to 22.

The Bayesian analyses produced 3 different topologies, where the relationships among the groups of species varied. For example, *N. lactens* alternates from being the sister group to the remaining *Necromys* (PP for this later clade = 0.50) in 1- and 2-million generation runs, to being the sister to the “southern lowland” clade (PP for this relationship < 0.55) in 1-, 1.5-, and 2-million generation runs. In addition, in 2.5-million generation runs Bayesian analysis recovered the same basal polytomy found in the maximum-parsimony analysis. In light of these results, we have chosen to discuss only the Bayesian topology with the basal polytomy (Fig. 3). This tree shows a total of 12 intraspecific polytomies.

DISCUSSION

Necromys is a sigmodontine genus in great need of revision. Problems range from nomenclatural issues, to species limits, to the delimitation of the genus itself (Anderson and Olds 1989; Galliari and Pardiñas 2000; Reig 1987). Few recent studies have tackled these issues. Galliari and Pardiñas (2000) studied the southern subtropical lowland species based on qualitative and quantitative morphologic variation. Ventura et al. (2000) used quantitative morphologic variation to assess the status of different taxonomic forms of *N. urichi*. Provencal et al. (2005) assessed the genetic and morphometric variation of 4 populations of *N. benefactus*. Thus, the present study is the 1st one dealing with systematics of *Necromys* by analyzing most of the recognized species with a broad geographic coverage and an explicit genealogical approach based on variation of the cytochrome-*b* gene. Maximum-parsimony analysis retrieved 22,107 shortest trees of 982 steps. Shortest trees have relatively high consistency (0.473) and retention (0.798) indices. Their strict consensus is a well-resolved cladogram, although clades show a wide range of support (Fig. 2). Bayesian analysis (Fig. 3) retrieved a topology congruent for the most part with that of the maximum-parsimony analysis. A few clades appearing in the Bayesian consensus are collapsed in the parsimony consensus; the reverse is also true with regards to a few clades. Discrepancies between both topologies mostly involve intraespecific relationships with low support in one or other consensus tree. The following discussion is structured along some of the main problems of *Necromys* systematics and on clades recovered by our analyses.

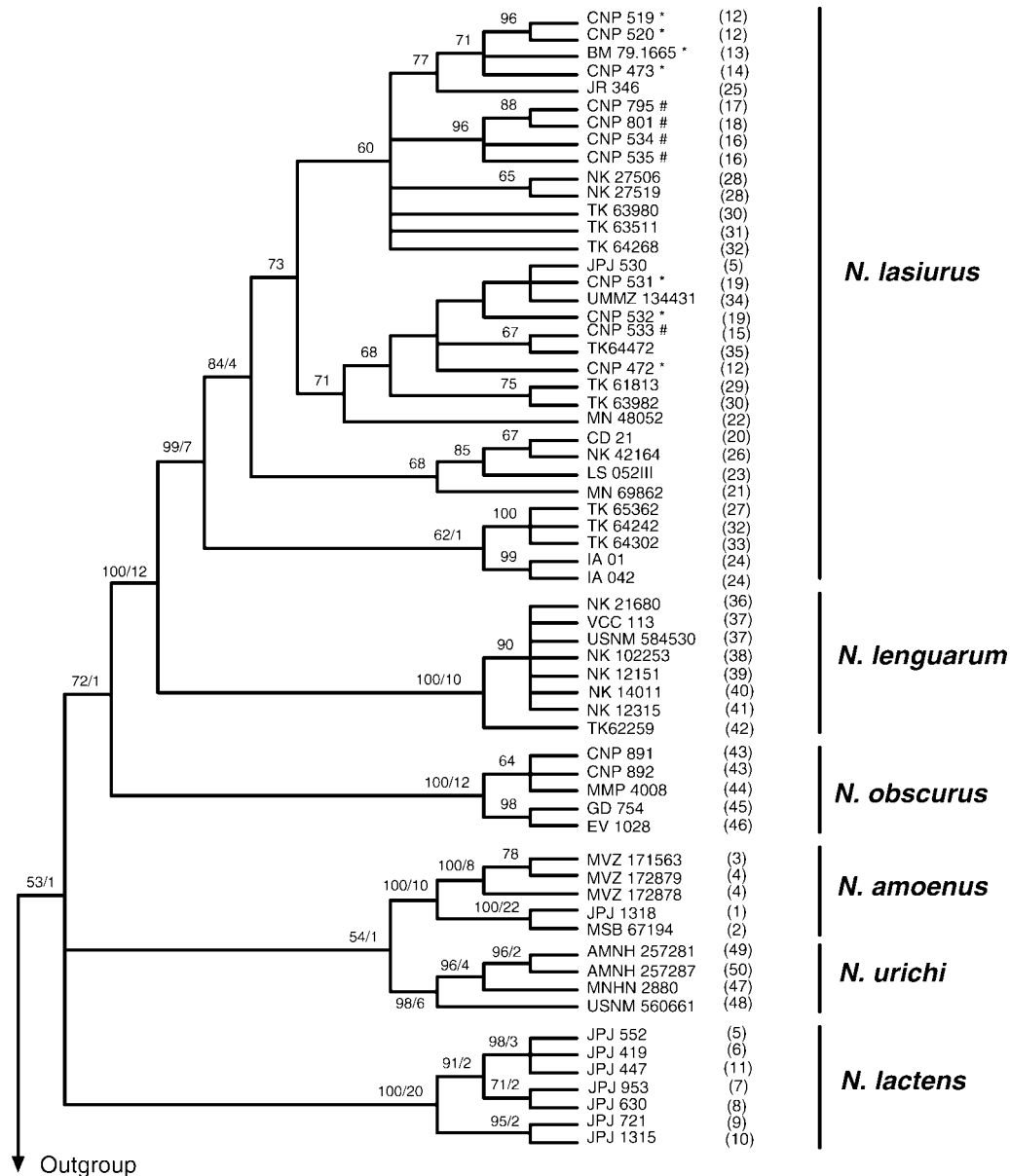


FIG. 2.—Strict consensus tree of the 22,107 most-parsimonious trees (length 982, CI = 0.473, RI = 0.798) obtained in the maximum-parsimony analysis of the cytochrome-*b* gene sequences. Numbers indicate parsimony jackknife (left of the diagonal; only values >50% are shown) and Bremer support (right of the diagonal) values of the nodes to their right. If only a number appears it corresponds to the jackknife value. A node without any number at its left implies that the node has less than 50% of jackknife support and that no Bremer support was calculated for it. Numbers next to specimen identification refer to the locality where they were collected (see Fig. 1 and Appendix I). An asterisk (*) or a pound symbol (#) indicates that before this study the specimen in question was assigned to *Necromys benefactus* or *N. temchuki*, respectively.

The basal radiation of Necromys.—*Necromys* is recovered as monophyletic in both analyses, although with deeply different levels of support. In the maximum-parsimony analysis, *Necromys* received 53% of JK support, and only 1 extra step is needed to collapse the clade and refute the hypothesis of monophyly; on the contrary, in the Bayesian analysis a monophyletic *Necromys* is strongly supported (PP = 0.96). This difference is notable, but not necessarily unexpected. Empirical studies by Simmons et al. (2004) have shown that JK tends to underestimate support values, that the Bayesian method consistently overestimates support, and that both methods perform

poorly when the taxon sample is large but the character sample is relatively limited. It is likely that the latter may explain our disparate results, because we sequenced more than 60 animals, but examined only a fragment of the cytochrome-*b* gene. In addition, theoretical and simulation studies lack consensus on the merit of using PP as a support measure in phylogenetics (reviewed in Alfaro and Holder [2006]). In a maximum-parsimony analysis of combined mitochondrial (complete cytochrome-*b*) and nuclear sequences (759-bp fragment of the 1st intron of the interphotoreceptor retinoid-binding protein [IRBP] gene) with a broad sampling of sigmodontines, D'Elia

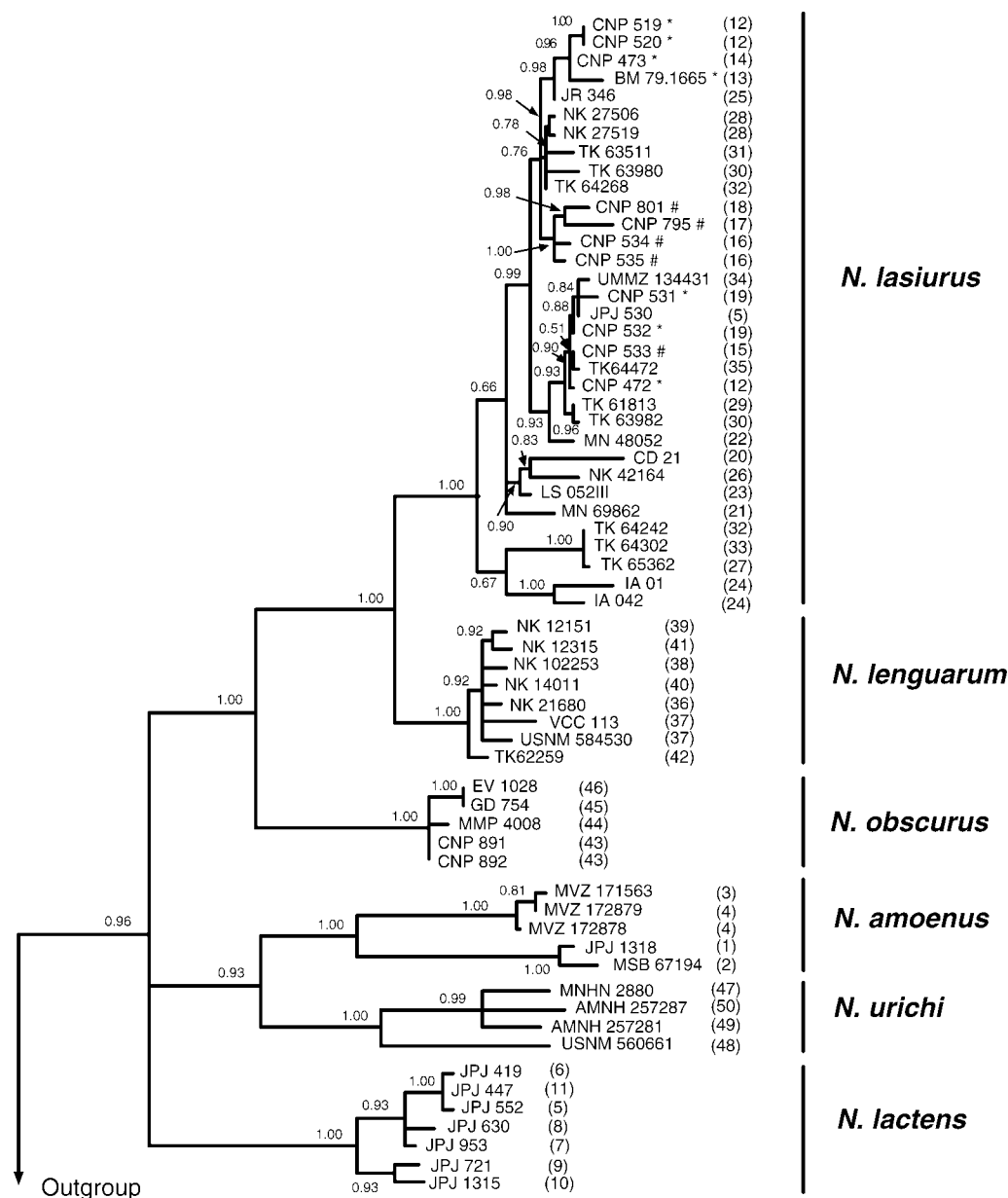


FIG. 3.—Majority-rule consensus resulting from the Bayesian analysis of the cytochrome-*b* gene sequences. Numbers indicate posterior probability values of the nodes at their right. Numbers next to specimen identification refer to the locality where they were collected (see Fig. 1 and Appendix I). An asterisk (*) or a pound symbol (#) indicates that before this study the specimen in question was assigned to *Necromys benefactus* or *N. temchuki*, respectively.

(2003) showed that monophyly of *Necromys* was strongly supported (JK = 99, BS = 9). However, that study lacked several species in the genus, including representatives of *N. lactens*, 1 of the 3 main lineages recovered in our analyses above. Several studies (e.g., Galewski et al. 2006; Stepan et al. 2005) have shown that the utility of mitochondrial sequences declines rapidly with increasing phylogenetic depth. Thus, until nuclear sequences for more species of *Necromys* are available, it is difficult to reconcile the disparity between support values in this study and those presented elsewhere.

Analyses reported in Figs. 2 and 3 found a polytomy at the base of *Necromys*, composed of the following 3 lineages:

a clade composed of *N. urichi* and *N. amoenus* (JK = 54, BS = 1, PP = 0.93); another formed by *N. lactens* (JK = 100, BS = 20, PP = 1.00); and a clade formed by all lowland species south of Amazonia (JK = 72, BS = 1, PP = 1.00). Considering this polytomy, an alternative classification option is to recognize 3 genera: *Necromys* for the morphologically homogeneous lowland forms; *Bolomys* for *amoenus* and *urichi*; and a 3rd genus to encompass *lactens*. We do not agree with this alternative for 2 reasons: phenotypic patterns of morphological similarity show that *N. lasiurus* and *N. lenguarum* are more similar to *N. lactens* than to *N. obscurus* (Galliari and Pardiñas 2000), although in our analyses *obscurus* is shown to be a sister

to a clade formed by *lasiurus* and *lenguarum*; and the generic diagnosis of *Necromys* (Anderson and Olds 1989) accommodates the discrete levels of variation among the recognized species, including *amoenus*. Given the data currently available, we agree with the contents and limits of *Necromys* as currently accepted (i.e., Musser and Carleton 2005). Nevertheless, this hypothesis should be further tested with the inclusion of *N. punctulatus* and analysis of nuclear loci.

Necromys lactens as 1 of the 3 main lineages of *Necromys*.—*Necromys lactens* (JK = 100, BS = 20, PP = 1.00) represents a highly differentiated lineage within the radiation of *Necromys*. It ranges from northwestern Argentina to central Bolivia at elevations between 1,500 and 4,000 m. This is a region with a complex topography and associated biotic formations. In northern Argentina and southern Bolivia, *N. lactens* occurs on high-altitude grasslands that develop above the “sierras” (Jayat and Pacheco 2006). The sierras are a complex of parallel-running hills of different heights and extensions that replace themselves from east to west and north to south. The grasslands of the sierras are separated from each other by relatively large river valleys covered with arid or semiarid habitats (e.g., Chaco, Monte, and Prepuna) and the humid Yungas forest (Cabrera 1976).

We included haplotypes recovered from specimens collected at or near the type localities of *Akodon lactens* Thomas, 1918 (JPJ 630), *A. orbus* Thomas, 1919 (JPJ 419), and *Bolomys negrito* Thomas, 1926 (JPJ 447). Currently, the latter forms are considered synonyms of *N. lactens*. Our results support this taxonomy: these haplotypes appear closely related and the percentage of divergence among them is around 2.0%.

Our sampling of *N. lactens* was not large enough to constitute a phylogeographic analysis, but our results provide a preliminary view on the geographic structure of this species. We sequenced 7 specimens, each from a different locality in Argentina, including 1 close to the type locality. All have different haplotypes and genetic differences among them range from 0.1% to 3.8%. Genetic variation within *N. lactens* is geographically structured: a northern clade (JK = 95, BS = 2, PP = 0.93) is formed by 2 haplotypes from the north of Salta Province; and a central-southern clade (JK = 91, BS = 2, PP = 0.93) is represented by samples from Catamarca, Jujuy (south), and Tucumán. Genetic divergence between clades ranges from 3.0% to 3.8%. Specimens from the northern clade were collected on the western slopes of the Sierra de Santa Victoria, near the Bolivian border, and Sierra de Zenta. These systems are separated from the central and southern sierras by the Grande River that runs along the Quebrada de Humahuaca. Haplotypes within the central-southern clade also are geographically arranged. Those from the farthest south (Sierras de Ambato, Ancasti, and Cumbres Calchaquies systems) form a well-supported clade (JK = 98, BS = 3, PP = 1.00) that in the maximum-parsimony analysis is sister to a central clade (JK = 71, BS = 2; Chañi and Centinela systems).

Our results suggest that the phylogeography of *N. lactens* was shaped by the history of high-altitude grasslands in the central Andes and associated mountain systems. Today, glaciers occupy some of the mountain ridges in the area (e.g.,

Nevados del Aconquija, Nevados del Chañi, and Nevados de Cachi) above 5,000 m. During the ice ages, glaciers in these areas extended to 4,000 m, and even to 3,800 m in some places (Halloy 1978; Rohmeder 1942). This must have lowered the vegetation belts on mountain slopes and increased the extent of vegetative zones at higher elevations. Under these conditions, current patches of high-altitude grassland inhabited by *N. lactens* may have come into contact and allowed dispersal of the species along the different sierras. This hypothesis is supported by the discovery of a fossil community of Lujanian Age (late Pleistocene) in which high-altitude grassland species occur at a relatively low altitude (1,900 m). This reinforces the idea that at least some members of present-day communities were displaced to lower elevations in the Pleistocene (Ortiz et al. 1998; see also Ortiz et al. 2000). In light of these results, a broad phylogeographic analysis of *N. lactens* and other grassland specialists (e.g., *Phyllotis osilae*—Jayat and Pacheco 2006) would be of much interest.

The clade of N. amoenus and N. urichi.—*Necromys amoenus* and *N. urichi* form a natural group in both analyses but the group received strong support only in the Bayesian analysis (PP = 0.93). In a combined maximum-parsimony analysis of mitochondrial and nuclear sequences that included 4 species of *Necromys*, *N. amoenus* and *N. urichi* formed independent lineages in a polytomy at the base of the genus (D’Elía 2003). The *N. amoenus*-*N. urichi* clade has a large distribution in the central and northern Andes, extending from northwestern Argentina to Trinidad and Tobago, and including the highlands of Bolivia, Colombia, and Peru, and lowlands of Venezuela.

Based on geography, *N. punctulatus*—the only currently recognized species of *Necromys* not included in our analyses—may be part of or closely related to the *N. amoenus*-*N. urichi* clade, although we note that *N. punctulatus* is morphologically indistinguishable from *N. lasiurus* (Voss 1991). The assumed distribution of *N. punctulatus* is Ecuador and probably Colombia (Musser and Carleton 2005), although several attempts to obtain additional specimens of this species in the last 15 years in various habitats in Ecuador have failed.

The last expansion of content in *Necromys* was proposed by Smith and Patton (1999), who placed in it the form *urichi*. This species ranges throughout the islands of Trinidad and Tobago, but it also occurs in Colombia and Venezuela. The taxonomy of *N. urichi* is presently unsettled. One of the main conflicts centers on whether the form *saturatus* from Auyan-Tepui in southern Venezuela represents a subspecies of *N. urichi* (Ventura et al. 2000) or a separate species (Linares 1998). At least 2 other forms, *meridensis* and *venezuelensis*, are considered valid subspecies (Ventura et al. 2000).

In our analyses, we used 4 specimens assigned to *N. urichi*: 2 from Venezuelan localities south of the Orinoco (1 geographically proximate to the type locality of *saturatus*), 1 from the Caribbean coast of Venezuela (geographically proximate to the type locality of *venezuelensis*), and 1 from the Andes of Colombia (proximate to the type locality of *chapmani*). The 4 specimens have highly divergent haplotypes (genetic distances 3.6–9.0%) that formed a well-supported clade (JK = 98,

BS = 6, PP = 1.00). Relationships among the 4 haplotypes are fully resolved and highly supported in the maximum-parsimony analysis (Fig. 2), although 1 of the clades collapses in the Bayesian analysis (Fig. 3). Haplotypes from specimens collected south of the Orinoco, which can be ascribed to *saturatus* (Linares 1998; Ventura et al. 2000), do not form a monophyletic group. In the maximum-parsimony analysis, 1 of these haplotypes from nearby San Ignacio de Yuruani falls in a well-supported clade with a haplotype from the Caribbean coast that represents *venezuelensis* (JK = 96, BS = 2). Both localities are less than 1,000 m above sea level. Of the 4 haplotypes studied, these 2 are the most similar, showing 3.6% divergence. However, this clade collapses in the Bayesian analysis. The 2nd haplotype assignable to *saturatus* comes from southern Venezuela (Cerro de la Neblina, near the Brazilian border). This is the most divergent of all haplotypes (genetic distances 8.1–9.0%) and is recovered as sister to the remaining haplotypes of *N. urichi* (JK = 96, BS = 4, PP = 0.99). These results cast doubt on the genealogical distinctness of *saturatus*, at least as it is currently delimited (Ventura et al. 2000). The specimen from Cerro de la Neblina was collected at 2,100 m, an elevation similar to that of the type locality (Auyan-Tepui) of *saturatus*. Perhaps *venezuelensis* ranges at low elevations south and north of the Orinoco, with *saturatus* occurring at higher altitudes. If so, *venezuelensis*, *saturatus*, and *chapmani* (another highland form that appears sister to *venezuelensis*) should be given equivalent rank (i.e., species or subspecies). Regardless of the status of *saturatus*, our results imply that the current geographic delimitation of this taxon is erroneous and illustrate the need to reevaluate the status of forms currently assigned to *N. urichi*. As currently defined, this species may be a composite of >1 biological species. However, any taxonomic changes at this point are premature.

Five specimens of *N. amoenus* were analyzed and a different haplotype was recovered from each; the monophyly of the species appears well supported in both analyses (JK = 100, BS = 10, PP = 1.00). Interestingly, *N. amoenus* shows a deep phylogeographical break between haplotypes recovered from specimens collected in Cuzco and Puno, in southern Peru (JK = 100, BS = 8, PP = 1.00) and those from Tarija in southern Bolivia and Salta in northwestern Argentina (JK = 100, BS = 22, PP = 1.00). Genetic divergence between these clades is large, ranging from 7.75 to 10.9%. Moreover, an AMOVA indicates that 88.1% of the genetic variation recovered in the sample of *N. amoenus* is due to differences among the 2 main clades. This suggests that *N. amoenus* as now understood may be a composite of >1 biological species. To test this hypothesis more individuals need to be analyzed, in particular from central Bolivia, and a comprehensive morphological study undertaken.

A clade of species from the open lowlands south of the Amazon.—A large clade formed by haplotypes from all species of *Necromys* from the lowlands south of Amazonia (i.e., *N. benefactus*, *N. lasiurus*, *N. lenguarum*, *N. obscurus*, and *N. temchuki*) was recovered with high support in both analyses (JK = 72, BS = 1, PP = 1.00). However, not all lowland species were recovered as monophyletic. The 1st split within the lowland clade separates individuals assigned to *N. obscurus*

from the remaining species (Figs. 2 and 3). This result corroborates previous analyses of morphological variation showing that *N. obscurus* is markedly different from the other lowland species (Galliari and Pardiñas 2000; Massoia and Fornes 1967). Support for the monophyly of *N. obscurus* is high (JK = 100, BS = 12, PP = 1.00). Two subspecies with disjunct distributions are currently recognized in *N. obscurus*. The nominal form ranges along a narrow band of habitat bordering the La Plata River and the Atlantic coast of Uruguay, whereas *N. o. scagliarum* is known from only a few localities centered on the Argentinean city of Mar del Plata (Galliari and Pardiñas 2000). In the maximum-parsimony analysis, *N. o. scagliarum* was recovered as monophyletic (JK = 64), but in the Bayesian analysis it was paraphyletic with respect to *N. o. obscurus*. Genetic distances among subspecies average 1.0%, comparable to the distance among haplotypes of *N. o. scagliarum* (0.9%). In contrast, both specimens of *N. o. obscurus*, collected in localities about 80 km apart, share the same haplotype. More specimens need to be analyzed to test whether *N. o. scagliarum* has more genetic variation than *N. o. obscurus* or whether the observed pattern is caused by our limited geographic sampling.

Our study also corroborates that at least 2 species of *Necromys* inhabit the Chaco, one of the most poorly studied ecoregions of South America. One of the species is *N. lasiurus* (JK = 99, BS = 7, PP = 1.00), whose distribution encompasses a myriad of ecological regions. For convenience, the 2nd Chacoan species is here referred to as *N. lenguarum* (JK = 100, BS = 10, PP = 1.00). This taxon is currently considered a valid species, but at times it has been regarded as a synonym of *N. lasiurus* (e.g., Macêdo and Mares 1987). On average, haplotypes of the 2 species differ by 5.4% (range 5.2–8.1%) and the monophyly of each species is strongly supported (Figs. 2 and 3). *N. lenguarum* occurs in the Chaco of Bolivia and Paraguay, and possibly in the Argentinean Chaco as well (Galliari et al. 1996; J. P. Jayat, in litt.). Our assignment of the eastern Bolivian and Paraguayan specimens (TK 62259) to *N. lenguarum* must be regarded with caution for the following reasons: the validity of *N. lenguarum* was not properly tested because we failed to analyze topotype specimens from Waikthlatingwaialwa; the validity of *tapirapoanus*, a taxon currently synonymized under *N. lenguarum*, was not tested, although the type locality of this taxon is less than 400 km from eastern Bolivia (localities 37 and 41); specimens of *Necromys* collected in the general area of Waikthlatingwaialwa (localities 34 and 35) are all referable to *N. lasiurus*; and our only specimen referable to *lenguarum* from Paraguay comes from Laguna Placenta and has yet to be compared with the holotype of *lenguarum*. Until these issues are resolved, the taxonomic status of *N. lenguarum* will remain elusive. However, our analyses confirm the identity of an additional species of *Necromys* in the Chaco region.

Necromys lasiurus as a senior synonym to N. benefactus and N. temchuki.—One of the most relevant results of our study is that *N. lasiurus*, a broadly distributed species, appears paraphyletic with respect to 2 southern species, *N. benefactus* and *N. temchuki*. Haplotypes of *N. benefactus* do not form

a monophyletic group, being related to different haplotypes of *N. lasiurus*. Haplotypes of *N. temchuki* sensu stricto, which come from 3 geographically proximate localities (including the type locality) in Misiones, Argentina, are very similar to each other and form a well-supported clade (JK = 96, PP = 1.00). The haplotype assignable to *N. t. liciae* is not sister to the *N. t. temchuki* clade.

The lack of monophyly for *N. benefactus*, *N. lasiurus*, and *N. temchuki* may simply reflect a disagreement between the inferred gene tree and the species tree. Random fixation of alternative haplotypes via lineage sorting may result in a pattern like the one presented here (Neigel and Avise 1986). This also may be a case of introgression via hybridization of the mitochondrial genome of 1 species into the nuclear background of a 2nd (e.g., Patton and Smith 1994). Currently, however, there is no reason to assume that the recovered gene tree does not reflect the species tree. We support this assertion on 2 lines of reasoning. First, there is no alternative data set amenable to comparison with the mitochondrial tree presented here; that is, no other *Necromys* data set has been analyzed with an explicit historical approach that suggests that the mitochondrial tree departs from the species tree. Therefore, the gene tree should be accepted as the best available hypothesis of taxonomic relationships (Brower et al. 1996). Second, the 3 species are phenotypically very similar and no discrete morphological groups can be delimited (Galliari and Pardiñas 2000; Macêdo and Mares 1987; G. D'Elía et al., in litt.); similarly, cytogenetic evidence shows that *N. lasiurus* and *N. temchuki* have the same chromosome number and morphology as well as the same C- and G-banding patterns (Vitullo et al. 1986).

The phenotypic similarity of *N. temchuki*, a poorly described species of northwestern Argentina, to *N. lasiurus* was already noted by several authors, some of which questioned its specific identity (e.g., Galliari et al. 1996). Taken at face value, our results suggest that *N. benefactus*, *N. lasiurus*, and *N. temchuki* represent a single biological species. Therefore, we formally synonymize *N. benefactus* and *N. temchuki* under *N. lasiurus*, the oldest available name for this clade. Thus, the type species of *Necromys*, the fossil form *N. conifer*, is hereby also relegated to a junior synonym of *N. lasiurus*.

As here understood, *N. lasiurus* has one of the largest distributions within the Sigmodontinae. Geographic limits are not totally clear, but we have specimens assigned to this species from the Ilha do Arapiranga in the Brazilian state of Para (approximately 1°19'S), from southern Buenos Aires Province (38.7°S), from close to the Brazilian Atlantic coast, and from the Sierra de Ambato on the Argentinean Andes. Although our sampling of *N. lasiurus* is extensive (33 specimens and 25 populations), large areas (especially central Brazil) within these limits are not represented in our analyses, and many others are represented by singletons. Nevertheless, a preliminary picture of geographic structure and demographic history of *N. lasiurus* can now be articulated.

Observed divergence ranges from 0% to 3.9% and from 1.3% to 6.3% at the intra- and interpopulation level, respectively. These are large values, and the last one is larger than some values corresponding to comparisons between haplotypes of *N. lenguarum* and *N. lasiurus* (range 5.2–8.1%); however,

these species' reciprocal monophyly is strongly supported (Figs. 2 and 3). Within *lasiurus* we note 2 clades, 1 composed of 4 specimens from localities as far from each other as the Ilha do Arapiranga (in the Brazilian state of Para) and 2 Paraguayan localities, and another that includes the remaining individuals sampled including 1 specimen from the type locality for *lasiurus* (Lagoa Santa, Minas Gerais, Brazil). Observed divergence between haplotypes from both clades ranges from 3.2% to 6.3%. Support for both clades is weak: JK = 62, BS = 1, PP = 0.67 and JK = 84, BS = 4, PP = 0.66, respectively.

Remarkably, despite its large geographic distribution, *N. lasiurus* does not show significant phylogeographic structure; for example, the 2 main clades of *N. lasiurus* overlap in Paraguay, whereas different haplotypes recovered in 1 collecting locality at Serranías de San Luis National Park (locality 32), fall into the 2 main clades of *N. lasiurus*. Several other examples of this lack of phylogeographic structuring are evident.

When all haplotypes of *N. lasiurus* are analyzed together, both Tajima's (1989) and Fu's (1997) tests of neutrality provide negative and significant values ($D = -1.59$, $P = 0.03$; $F_s = -12.20$, $P = 0.002$), indicating that *N. lasiurus* may have recently experienced an increase in population size. In combination with the lack of phylogeographic structure, these results appear to indicate that *N. lasiurus* has recently invaded several regions of the current distributional range. In addition, given the relatively large observed genetic divergence (up to 6.3%) between some pairs of haplotypes of *N. lasiurus*, it is tempting to suggest that the presumptive range expansion occurred either from a past population that was both large and stable or from >1 population. The fossil record of *N. lasiurus* shows that in the course of a few hundred years the distributional range of this species has shifted markedly (Galliari and Pardiñas 2000; Pardiñas 1999). This type of dynamic scenario may produce the lack of equilibrium between genetic drift and gene flow that we observe in *N. lasiurus*. Further tests of these hypotheses require the analyses of more individuals and of a locus not linked to the cytochrome-*b* gene. A better understanding of the history of tropical and subtropical open areas of South America is needed to compose a detailed scenario for the dynamic of *N. lasiurus*.

The geographic origin of Necromys.—*Necromys*, in spite of its moderate specific diversity, is among the most widely distributed genera within the sigmodontine radiation. Its geographic distribution encompasses large expanses of open environments both south and north of the Amazon basin. In terms of its general distribution, *Necromys* resembles the rodent genus *Calomys* (contra Almeida et al. 2007:460); the main difference between them is that the latter reaches Patagonia in southern South America, whereas *Necromys* has a larger Andean distribution.

Despite their similar geographic distributions, it appears that *Calomys* and *Necromys* have different biogeographic histories. The main difference involves the reciprocal monophyly of the groups of lowland and highland species of *Calomys* (although a lowland clade was not recovered in the maximum-parsimony analysis of Almeida et al. [2007]). This

implies a single split between lowland and Andean species, probably mediated by the uplift of the Andes. Lowland species of *Calomys* south and north of the Amazonia would have differentiated after the Amazonian forest expanded (Almeida et al. 2007; Salazar-Bravo et al. 2001). We can rule out a similar scenario for *Necromys* because *N. urichi* from the South American grasslands north of the Amazon is not sister to species from the southern lowlands (Figs. 2 and 3). In fact, *N. urichi* is most closely related to *N. amoenus*, and, therefore, we can rule out a single split between lowland and highland species in *Necromys*. Incorporation of *N. punctulatus* in future analyses may require a revision of this interpretation.

The oldest known *Necromys* corresponds to *N. bonapartei*† from late Pliocene sediments (approximately 4–3.5 million years ago) from southern Buenos Aires Province, Argentina (Pardiñas and Tonni 1998). From slightly younger beds of the same area Reig (1978) described *Dankomys*†, an extinct genus morphologically resembling *Necromys*. To date, there is no a formal phylogenetic analysis involving *Dankomys*†, but if *Dankomys*† and *Necromys* are closely related, it would imply a Pampean radiation of *Necromys*-like sigmodontines in the late Pliocene, which in turn may suggest a Pampean origin for *Necromys* (see Pardiñas 1995). However, we note that a clade of southern lowland species sister to the remaining species of *Necromys* was never recovered in our analyses. In addition, we note that none of our analyses recovered a southern clade that was paraphyletic with respect to the highland species, as the hypothesis of a southern origin for *Necromys* would predict.

Clearly, additional studies are needed to assess the historical biogeography of *Necromys*. The discoveries of new fossils that constitute conclusive evidence of the presence of a taxon in a specific geologic time and place would be key evidence. In addition, and as a 1st step toward the clarification of the place of the origin of *Necromys*, a fully resolved and well-supported topology at the base of the *Necromys* clade is much needed.

RESUMEN

Presentamos el estudio más amplio a la fecha sobre la sistemática de *Necromys*, un género de roedores distribuido en las áreas abiertas al norte y sur de la Amazonía y en los pastizales andinos. El estudio está basado en secuencias del gen que codifica para el citocromo *b*; las mismas fueron analizadas mediante análisis de máxima parsimonia y bayesiano. Los análisis se efectuaron sobre secuencias de 62 especímenes de 51 localidades de Argentina, Bolivia, Brasil, Colombia, Paraguay, Perú, Uruguay y Venezuela. Excepto una, las restantes especies actualmente consideradas como integrantes del género están representadas en el estudio. *Necromys* fue recuperado monofilético con una politomía basal que involucra a 3 linajes. Uno de estos linajes está formado por la especie andina *N. lactens*, que además presentó una marcada estructura filogeográfica. El segundo clado está formado por *N. urichi* de los pastizales del norte de América del Sur y *N. amoenus* de los Andes centrales. Los resultados sugieren que cada uno de estos taxones podría estar constituido por más de una especie biológica. El tercer clado está formado por las especies de las tierras bajas al sur de la Amazonía. Dentro

de este clado, *N. obscurus* es hermana de las restantes especies. Los haplotipos recuperados en especímenes asignados a *N. benefactus*, *N. temchuki* y *N. lasiurus* forman un clado, pero estos taxones no forman grupos recíprocamente monofiléticos ni el clado muestra estructura geográfica. Estos resultados genealógicos, discutidos en el contexto de la variación genética, son la base de consideraciones taxonómicas (e.g., *N. benefactus* y *N. temchuki* son considerados sinónimos subjetivos de *N. lasiurus*) y biogeográficas.

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LITERATURE CITED

- ALFARO, M. E., AND M. T. HOLDER. 2006. The posterior and the prior in Bayesian phylogenetics. *Annual Review of Ecology, Evolution, and Systematics* 37:19–42.
- ALMEIDA, F. C., C. R. BONVICINO, AND P. CORDEIRO-ESTRELA. 2007. Phylogeny and temporal diversification of *Calomys* (Rodentia, Sigmodontinae): implications for the biogeography of an endemic genus of the open/dry biomes of South America. *Molecular Phylogenetics and Evolution* 42:449–466.
- AMEGHINO, F. 1889. Contribución al conocimiento de los mamíferos fósiles de la República Argentina. *Actas de la Academia Nacional de Ciencias (Córdoba)* 6:1–1027, atlas, 98 pls.
- ANDERSON, S., AND N. OLDS. 1989. Notes on Bolivian mammals. 5. Taxonomy and distribution of *Bolomys* (Muridae, Rodentia). *American Museum Novitates* 2935:1–22.
- ANDERSON, S., AND T. L. YATES. 2000. A new genus and species of phyllotine rodent from Bolivia. *Journal of Mammalogy* 81:18–36.
- BREMER, K. 1994. Branch support and tree stability. *Cladistics* 10:295–304.
- BROWER, A. V. Z., R. DE SALLE, AND A. VOGLER. 1996. Gene trees, species trees and systematics: a cladistic perspective. *Annual Review of Ecology and Systematics* 27:423–450.
- CABRERA, A. 1961. Catálogo de los mamíferos de América del Sur. *Revista del Museo Argentino de Ciencias Naturales "Bernardino Rivadavia," Zoología* 4:xxii + 309–732.
- CABRERA, A. L. 1976. Regiones fitogeográficas argentinas. Pp. 1–85 in *Enciclopedia Argentina de agricultura y jardinería*, tomo II,

- fascículo 1 (L. R. Parodi, ed.). Editorial Acmé, Buenos Aires, Argentina.
- D'ELÍ, G. 2003. Phylogenetics of Sigmodontinae (Rodentia, Muroidea, Cricetidae), with special reference to the akodont group, and with additional comments on historical biogeography. *Cladistics* 19:307–323.
- D'ELÍ, G., E. M. GONZÁLEZ, AND U. F. J. PARDIÑAS. 2003. Phylogenetic analysis of sigmodontine rodents (Muroidea), with special reference to the akodont genus *Deltamys*. *Mammalian Biology* 68:351–364.
- D'ELÍ, G., AND U. F. J. PARDIÑAS. 2004. Systematics of Argentinean, Paraguayan, and Uruguayan swamp rats of the genus *Scapteromys* (Rodentia, Cricetidae, Sigmodontinae). *Journal of Mammalogy* 85:897–910.
- D'ELÍ, G., AND U. F. J. PARDIÑAS. 2007. Putting names to the phylogenetic diversity of neotropical sigmodontine rodents: new genera for known species. *Mammalia* 71:146–149.
- D'ELÍ, G., U. F. J. PARDIÑAS, P. TETA, AND J. L. PATTON. 2007. Definition and diagnosis of a new tribe of sigmodontine rodents (Cricetidae: Sigmodontinae), and a revised classification of the subfamily. *Gayana* 71:187–194.
- DA SILVA, M. N., AND J. L. PATTON. 1993. Amazonian phylogeography: mtDNA sequence variation in arboreal echimyid rodents (Caviomorpha). *Molecular Phylogenetics and Evolution* 2:243–255.
- DNASTAR. 2003. Lasergene: expert sequence analysis software. Version 5. DNASTAR, Madison, Wisconsin.
- EXCOFFIER, L., P. E. SMOUSE, AND J. M. QUATTRO. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes—application to human mitochondrial-DNA restriction data. *Genetics* 131:479–491.
- FARRIS, J. S. 1982. The logical basis of phylogenetic analysis. Pp. 7–36 in *Advances in cladistics: proceedings of the second meeting of the Willi Hennig Society* (N. Planick and V. Funk, eds.). Columbia University Press, New York.
- FARRIS, J. S., V. A. ALBERT, M. KÄLLERSJÖ, D. LIPSCOMB, AND A. G. KLUGE. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12:99–124.
- FU, Y. X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147:915–925.
- GALEWSKI, T., M. TILAK, S. SANCHEZ, P. CHEVRET, E. PARADIS, AND E. J. P. DOUZERY. 2006. The evolutionary radiation of Arvicolinae rodents (voles and lemmings): relative contribution of nuclear and mitochondrial DNA phylogenies. *BMC Evolutionary Biology* 6: article 80.
- GALLIARI, C. A., AND U. F. J. PARDIÑAS. 2000. Taxonomy and distribution of the sigmodontine rodents of genus *Necomys* in central Argentina and Uruguay. *Acta Theriologica* 45:211–232.
- GALLIARI, C. A., U. F. J. PARDIÑAS, AND F. GOIN. 1996. Lista comentada de los mamíferos argentinos. *Mastozoología Neotropical* 3:39–61.
- GANNON, W. L., R. S. SIKES, AND THE ANIMAL CARE AND USE COMMITTEE OF THE AMERICAN SOCIETY OF MAMMALOGISTS. 2007. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *Journal of Mammalogy* 88:809–823.
- GYLDENSTOLPE, N. 1932. A manual of neotropical sigmodont rodents. *Kunglia Svenska Vetenskapsakademiens Handlingar*, Stockholm. Series 3, 11:1–164.
- HALLOY, S. 1978. Contribución al estudio de la zona de Huaca-Huasi, Cumbres Calchaquies, (Tucumán, Argentina) I: la biota actual en relación con la geología histórica. Seminario de Licenciatura, Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán, Tucumán, Argentina.
- HUELSENBECK, J. P., F. RONQUIST, R. NIELSEN, AND J. P. BOLIBACK. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* 294:2310–2314.
- JAYAT, J. P., AND S. PACHECO. 2006. Distribución de *Necomys lactens* y *Phyllotis osilae* (Rodentia: Cricetidae: Sigmodontinae) en el noroeste argentino: modelos predictivos basados en el concepto de nicho ecológico. *Mastozoología Neotropical* 13:69–88.
- KLUGE, A. J., AND J. S. FARRIS. 1969. Quantitative phyletics and the evolution of anurans. *Systematic Zoology* 18:1–32.
- LINARES, O. J. 1998. Mamíferos de Venezuela. Sociedad Conservacionista Audobon de Venezuela, Caracas, Venezuela.
- MACÊDO, R. H., AND M. A. MARES. 1987. Geographic variation in the South American cricetine rodent *Bolomys lasiurus*. *Journal of Mammalogy* 68:578–594.
- MASSOIA, E., AND A. FORNES. 1967. El estado sistemático, distribución geográfica y datos etoecológicos de algunos mamíferos neotropicales (Marsupialia y Rodentia) con la descripción de *Cabreramys*, género nuevo (Cricetidae). *Acta Zoológica Lilloana* 23:407–430.
- MASSOIA, E., AND U. F. J. PARDIÑAS. 1993. El estado sistemático de algunos muroideos estudiados por Ameghino en 1889. Revalidación del género *Necomys* (Mammalia, Rodentia, Cricetidae). *Ameghiniana* 30:407–418.
- 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.
- NEIGEL, J. E., AND J. C. AVISE. 1986. Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. Pp. 515–534 in *Evolutionary processes and theory* (S. Karlin and E. Nevo, eds.). Academic Press, San Diego, California.
- NELSON, G. 1979. Cladistic analysis and synthesis: principles and definitions, with a historical note on *Adanson's Familles des Plantes (1763–1764)*. *Systematic Zoology* 28:1–21.
- OLIVEIRA, J. A. 1992. Estrutura da variação craniana em populações de *Bolomys lasiurus* (Lund, 1841) (Rodentia: Cricetidae) do nordeste do Brasil. *Coordenação de Pós-Graduação em Zoologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil*.
- ORTIZ, P. E., U. F. J. PARDIÑAS, AND J. E. POWELL. 1998. Roedores del Pleistoceno–Holoceno del Valle de Tafí (Tucumán, Argentina). Aspectos sistematicos y paleoambientales. *Acta Geológica Lilloana* 18:173–174.
- ORTIZ, P., U. F. J. PARDIÑAS, AND S. STEPPAN. 2000. A new fossil phyllotine (Rodentia: Muridae) from northwestern Argentina and the relationships of *Reithrodon* group. *Journal of Mammalogy* 81:37–51.
- PARDIÑAS, U. F. J. 1995. Sobre las vicisitudes de los géneros *Bothriomys* Ameghino, 1889, *Euneomys* Coues, 1874 y *Graomys* Thomas, 1916 (Mammalia, Rodentia, Cricetidae). *Ameghiniana* 32:173–180.
- PARDIÑAS, U. F. J. 1999. Fossil murids: taxonomy, paleoecology, and paleoenvironments. *Quaternary of South America and Antarctic Peninsula* 12:225–254.
- PARDIÑAS, U. F. J., AND E. TONNI. 1998. Procedencia estratigráfica y edad de los más antiguos muroideos (Mammalia, Rodentia) de América del Sur. *Ameghiniana* 35:473–475.
- PATTON, J. L., AND M. F. SMITH. 1994. Paraphyly, polyphyly and the nature of species boundaries in pocket gophers (genus *Thomomys*). *Systematic Biology* 43:11–26.
- PROVENSAL, M. C., G. E. CALDERÓN, M. B. CHIAPPERO, C. N. GARDENAL, J. J. POLOP, AND M. S. SABATTINI. 2005. Morphometric and allozymic characterization of *Necomys benefactus* populations in central Argentina. *Mastozoología Neotropical* 12:261–268.

- REIG, O. A. 1978. Roedores cricétidos del Plioceno superior de la provincia de Buenos Aires (Argentina). Publicaciones del Museo Municipal de Ciencias Naturales de Mar del Plata, "Lorenzo Scaglia" 2:164–190.
- REIG, O. A. 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.
- REIG, O. A. 1987. An assessment of the systematics and evolution of the Akodontini, with the description of new fossil species of *Akodon* (Cricetidae, Sigmodontinae). Pp. 347–399 in Studies in neotropical mammalogy: essays in honor of Philip Hershkovitz (B. D. Patterson and R. M. Timm, eds.). Fieldiana: Zoology (New Series) 39:viii + 1–506.
- ROHMEDER, G. 1942. La glaciación diluvial de los Nevados del Aconquija (parte austral). Monografías del Instituto de Estudios Geográficos 2. Universidad Nacional de Tucumán, Tucumán, Argentina.
- RONQUIST, F., AND J. P. HUELSENBECK. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574.
- 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.
- SCHNEIDER, S., D. ROESSLI, AND L. EXCOFFIER. 2000. Arlequin, version 2.000: a software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.
- SIMMONS, M. P., K. M. PICKETT, AND M. MIYA. 2004. How meaningful are Bayesian support values? *Molecular Biology and Evolution* 21:188–199.
- SMITH, M. F., AND J. L. PATTON. 1991. Variation in mitochondrial cytochrome *b* sequence in natural populations of South American akodontine rodents (Muridae: Sigmodontinae). *Molecular Biology and Evolution* 8:85–103.
- SMITH, M. F., AND J. L. PATTON. 1993. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. *Biological Journal of the Linnean Society* 50:149–177.
- SMITH, M. F., AND J. L. PATTON. 1999. Phylogenetic relationships and the radiation of sigmodontine rodents in South America: evidence from cytochrome *b*. *Journal of Mammalian Evolution* 6:89–128.
- SORENSEN, M. D. 1999. TreeRot, version 2. Boston University, Boston, Massachusetts.
- STEPHAN, S. J., R. M. ADKINS, P. Q. SPINKS, AND C. HALE. 2005. Multigene phylogeny of the Old World mice, Murinae, reveals distinct geographic lineages and the declining utility of mitochondrial genes compared to nuclear genes. *Molecular Phylogenetics and Evolution* 37:370–388.
- Swofford, D. 2000. PAUP*: phylogenetic analysis using parsimony (* and other methods), 4.0. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.
- TAJIMA, F. 1989. The effect of change in population size on DNA polymorphism. *Genetics* 123:597–601.
- TATE, G. H. H. 1932. The taxonomic history of the South and Central American akodont rodent genera: *Thalpomys*, *Deltamys*, *Thaptomys*, *Hypsimys*, *Bolomys*, *Chroeomys*, *Abrothrix*, *Scotinomys*, *Akodon* (*Chalcomys* and *Akodon*), *Microxus*, *Podoxymys*, *Lenoxus*, *Oxymycterus*, *Notiomys*, and *Blarinomys*. *American Museum Novitates* 582:1–32.
- THOMAS, O. 1916. The grouping of the South-American Muridae commonly referred to *Akodon*. *Annals and Magazine of Natural History*, Series 8, 18:336–340.
- THOMPSON, J. D., T. J. GIBSON, F. PLEWNIAC, 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.
- VENTURA, J., M. J. LOPEZ-FUSTER, M. SALAZAR, AND R. PEREZ-HERNANDEZ. 2000. Morphometric analysis of some Venezuelan akodontine rodents. *Netherlands Journal of Zoology* 50:487–501.
- VITULLO, A. D., ET AL. 1986. Cytogenetics of South American akodont rodents (Cricetidae): new karyotypes and chromosomal banding patterns of Argentinean and Uruguayan forms. *Journal of Mammalogy* 67:69–80.
- VOSS, R. S. 1991. An introduction to neotropical genus *Zygodontomys*. *Bulletin of the American Museum of Natural History* 210:1–113.

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APPENDIX I

The specimens of *Necromys*, *Akodon*, *Deltamys*, *Thalpomys*, and *Thaptomys* used in the present study are listed here. Accession numbers are indicated for those specimens whose sequences were retrieved from GenBank. See Fig. 1 for locality numbers and locations of sites. Museum and collection acronyms and personal field numbers are as follows. Argentina: Colección de Mamíferos del Centro Nacional Patagónico, Puerto Madryn (CNP); Museo de Ciencias Naturales y Tradicional de Mar del Plata, Mar del Plata (MMP); field number of J. Pablo Jayat (JPJ, vouchers will be deposited in Colección Mamíferos Lillo, Tucumán). Bolivia: VCC field number of Verónica Chavez (VCC, vouchers will be deposited in Museo de Historia Natural Noel Kempff Mercado, Santa Cruz). Brazil: Museu Nacional, Rio do Janeiro (MN); field number of Lena Geise and field crew (CD, voucher will be deposited at the Museu de Zoologia Universidade de São Paulo, Brazil), MZUSP; field and laboratory numbers obtained from Erika Hingst-Zaher (BlasLS052III, IA to be deposited at the Museu Nacional, Brazil); field number of Jorge Marinho (JR, voucher will be deposited at Coleção Zoológica da Universidade Regional de Blumenau, Brazil). France: Muséum National d'Histoire Naturelle, Paris (MNHN). United Kingdom: The Natural History Museum, London (BMNH). United States: American Museum of Natural History, New York (AMNH); Museum of Southwestern Biology, Albuquerque (MSB and NK: tissue accession prefix); Museum of Vertebrate Zoology, University of California, Berkeley (MVZ); The Museum, Texas Tech University, Lubbock (TK: tissue accession prefix); University of Michigan Museum of Zoology, Ann Arbor (UMMZ); National Museum of Natural History, Washington, D.C. (USNM). Uruguay: field number of Guillermo D'Elía (GD, vouchers will be deposited at Museo Nacional de Historia Natural y Antropología, Montevideo); field numbers of the Sección Evolución, Facultad de Ciencias, Universidad de la República (EV, vouchers will be deposited at Nacional de Historia Natural y Antropología, Montevideo). An asterisk (*) or a pound symbol (#) indicates that before this study the specimen in question was assigned to *N. benefactus* or *N. temchuki*, respectively; both taxa are here regarded as junior synonyms of *N. lasiurus*. gb indicates that the sequence was retrieved from GenBank; all others were generated in this study.

Necromys amoenus.—ARGENTINA: 1) Salta, Santa Victoria, 1 km ENE de Rodeo Pampa, km 59 de Ruta Provincial No. 7, 3,080 m, 22°14'47.7"S, 65°3'4.3"W (JPJ 1318: EF531643). BOLIVIA: 2)

Tarija, Serranía del Sama, 3,200 m, 21°27'S, 64°52'W (MSB 67194: AF159283 gb). PERU: 3) Cuzco, 20 km N (by road) Paucartambo, km 100, 3,580 m, 13°12'9.7"S, 70°40'0.0"W (MVZ 171563: AY273911 gb). 4) Puno, 12 km S Santa Rosa, 3,960 m, 14°43'9.4"S, 70°47'8.9"W (MVZ 172878: M35711 gb; MVZ 172879: M35712 gb).

Necomys lactens.—ARGENTINA: 5) Catamarca, Ambato, Las Chacritas, aprox. 28 km al NNW de Singuil, sobre Ruta Provincial No. 1, 1,888 m, 27°42'24.2"S, 65°54'40.6"W (JPJ 552: EF531646); 6) Catamarca, Valle Viejo, aprox. 2 km al SE de Huaico Hondo, sobre Ruta Provincial No. 42, al E del Portezuelo, 1,992 m, 28°25'10.9"S, 65°32'40.5"W (JPJ 419: EF531644); 7) Jujuy, Santa Bárbara, La Antena, Sierra del Centinela, al S de El Fuerte, 2,350 m, 24°17'56.46"S, 64°23'9.3"W (JPJ 953: EF531649); 8) Jujuy, Tumbaya, Bárcena, aprox. 3 km al S, sobre Ruta Nacional No. 9, 1,808 m, 24°0'2"S, 65°26'51.6"W (JPJ 630: EF531647); 9) Salta, Orán, Abra de Ciénaga Negra, aprox. 3 km al SE, 3,090 m, 23°19'49"S, 64°53'32"W (JPJ 721: EF531648); 10) Salta, Santa Victoria, 1 km ENE de Rodeo Pampa, km 59 de Ruta Provincial No. 7, 3,080 m, 22°14'47.7"S, 65°3'4.3"W (JPJ 1315: EF531650); 11) Tucumán, Trancas, aprox. 10 km al S de Hualinchay, sobre el camino a Lara, 2,300 m, 26°19'20.2"S, 65°36'45.5"W (JPJ 447: EF531645).

Necomys lasiurus.—ARGENTINA: 12) Buenos Aires, Bahía Blanca, Bahía Blanca, 38°43'S, 62°16'W (CNP 519*: EF531654; CNP 520*: EF531655); 13) Buenos Aires, Campamento Base del Cerro Ventana, 38°04'S, 62°01'W (BM 79.1665*: EF531651; CNP 472*: EF531652); 14) Buenos Aires, San Mateo, 38°39'34"S, 60°59'30.6"W (CNP 473*: EF531653); 5) Catamarca, Ambato, Las Chacritas, aprox. 28 km al NNW de Singuil, sobre Ruta Provincial No. 1, 1,888 m, 27°42'24.2"S, 65°54'40.6"W (JPJ 530: EF531661); 15) Formosa, 17 km W-SW de Colonia Mayor Villafañe, 26°12'57"S, 59°15'38"W (CNP 533#: AY273913 gb); 16) Misiones, Estancia Santa Inés, 27°31'32"S, 55°52'19"W (CNP 534#: EF531658; CNP 535#: AY273914 gb); 17) Misiones, Leandro N. Alem, 27°36'S, 55°19'W (CNP 795#: EF531659); 18) Misiones, Santa Ana, 27°23'S, 55°35'W (CNP 801#: EF531660); 19) Santa Fe, Berna, 29°16'S, 59°52'W (CNP 531*: EF531656; CNP 532*: EF531657). BRASIL: 20) Bahia, Lençóis, Chapada Diamantina, Remanso, 509 m, 12°36'41.5"S, 41°21'50.9"W (CD 21: EF531689); 21) Bahia, Mucugê, Parque Nacional da Chapada Diamantina, Rio Cumbuca, 1,002 m, 13°2'41"S, 41°20'58"W (MN 69862: EF531693); 22) Minas Gerais, Caratinga, Fazenda Montes Claros, 19°50'S, 41°50'W (MN 48052: EF531690); 23) Minas Gerais, Lagoa Santa, 19°39'S, 43°54'W (LS052III: EF531688); 24) Para, Ilha de Arapiranga, 0 m, 1°20'S, 48°34'W (IA01: EF531691; IA042: EF531692); 25) Rio Grande do Sul, Rondinha, Parque Estadual Florestal de Rondinha, 28°44'19.74"S, 50°16'40.57"W (JR 346: EF531662); 26) Sao Paulo, Tupi Paulista, 21°22'S, 51°36'W (NK 42164: EF531663). PARAGUAY: 27) Alto Paraguay, Palmar de Las Islas, 19°37'47.3"S, 60°36'45"W (TK 65362: EF531673); 28) Amambay: Bella Vista, Colonia Sargento Duré, 3 km E by road of Rio Apa, 22°10'S, 56°25'15.24"W (NK 27506: EF531664;

NK 27519: EF531665); 29) Canindeyu, Reserva Natural del Bosque Mbaracayu, 24°08.93'S, 55°18'W (TK 61813: EF531666); 30) Canindeyu, Reserva Natural del Bosque Mbaracayu, 24°09.61'S, 55°16.99'W (TK 63980: EF531668; TK 63982: EF531669); 31) Canindeyu, Reserva Natural Privada Itabo, 24°27.68'S, 54°38.33'W (TK 63511: EF531667); 32) Concepción, Parque Nacional Serranía de San Luis, 22°35.95'S, 57°21.28'W (TK 64242: EF531670; TK 64268: EF531671); 33) Concepción, Parque Nacional Serranía de San Luis, 22°40.96'S, 57°21.52'W (TK 64302: AY273912 gb); 34) Presidente Hayes, 8 km NE Juan de Zalazar, 23°6'0"S, 59°18'0"W (UMMZ 134431: U03528 gb); 35) Presidente Hayes, Estancia Loma Pora, 23°32'5.3"S, 57°33'38.3"W (TK 64472: EF531672).

Necomys languarum.—BOLIVIA: 36) Chuquisaca, Río Limón, 1,300 m, 19°32'51"S, 64°47'58.5"W (NK 21680: EF531677); 37) Santa Cruz, Velasco, Parque Nacional Noel Kempff Mercado, Campamento Los Fierros, 215 m, 14°33'40"S, 60°55'40"W (VCC 113: EF531680; USNM 584530: EF531679); 38) Santa Cruz, Estancia San Marcos, 6 km W Ascencion, approx. 400 m, 15°53'11.4"S, 63°11'10.39"W (NK 102253: EF531678); 39) Santa Cruz, 6 km N Buen Retiro, 300 m, 17°12'50.9"S, 63°37'58.4"W (NK 12151: EF531674); 40) Santa Cruz, 4.5 km N, 1.5 km E Cerro Amboro, Rio Pitisama, 620 m, 17°44'S, 63°40'W (NK 14011: EF531676); 41) Santa Cruz, 4 km N, 1 km W of Santiago de Chiquitos, 700 m, 18°17'51"S, 59°35'58.13"W (NK12315: EF531675). PARAGUAY: 42) Alto Paraguay, Laguna Placenta, 70 m, 21°8'37.1"S, 59°24'51.5"W (TK 62259: EF531681).

Necomys obscurus.—ARGENTINA: 43) Buenos Aires, 1 km aguas arriba puente sobre RP 11, arroyo de las Brusquitas, 38°14'08"S, 57°46'52"W (CNP 891: EF531684; CNP 892: EF531685); 44) Buenos Aires, Mar del Plata, 0 m, 38°00'S, 57°33'W (MMP 4008: EF531686). URUGUAY: 45) Montevideo, Parque Lecoq, 34°48.121'S, W 56°20.460'W (GD 754: EF531683); 46) San José, Estancia El Relincho, 34°20'S, 56°59'W (EV 1028: EF531682).

Necomys urichi.—COLOMBIA: 47) Paramo de Choachi, alrededores de Bogotá, 4°33'N, 73°40'W (MNHN 2880: EF531687). VENEZUELA: 48) Amazonas, Cerro Neblina, 0°51'24.17"N, 65°57'13.57"W (USNM 560661: U03549 gb); 49) Bolivar, 5.2 km NE San Ignacio Yuruaní (AMNH 257281: AY273918 gb); 50) Sucre, Finca Vuelta Larga, 9.7 km (by road) SE Guaraunos, 10°27'59.78"N, 63°55'51"W (AMNH 257287: AY273919 gb).

Akodon boliviensis.—PERU: Puno, 12 km S Santa Rosa [De Ayavirij]; elevation 3,950 m (MVZ 171607: M35691 gb).

Deltamys kempi.—ARGENTINA: Buenos Aires, La Balandra, 34°54'07"S, 57°45'56"W (CNP 893: AY 195861 gb).

Thalpomys cerradensis.—BRAZIL: Piauí, Estação Ecológica de Uruçui-Una (MZUSP 30400: AY273915 gb).

Thaptomys nigrita.—BRAZIL: São Paulo, Salesópolis; Estacao Biologica de Boraceia, 3 km E, 28 km SE Biritiba-Mirim, 850 m (MVZ 1830 44: AF108666 gb).