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PHYLOGEOGRAPHY OF *OLIGORYZOMYS LONGICAUDATUS* (RODENTIA: SIGMODONTINAE) IN TEMPERATE SOUTH AMERICA

R. Eduardo Palma,* Eric Rivera-Milla, Jorge Salazar-Bravo, Fernando Torres-Pérez, Ulyses F. J. Pardiñas, Pablo A. Marquet, Angel E. Spotorno, Andrés P. Meynard, and Terry L. Yates

Centro de Estudios Avanzados en Ecología y Biodiversidad and Departamento de Ecología, Pontificia Universidad Católica de Chile, Casilla 114–D, Santiago 6513677, Chile (REP, FTP, PAM, APM)
Developmental Neurobiology, University of Konstanz 78467, Konstanz, Germany (ERM)
Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409, USA (JSB)
Centro Nacional Patagónico (CENPAT–CONICET), Bvd. G. Brown s/n Casilla de Correo 128, 9120 Puerto Madryn, Chubut, Argentina (UFJP)
Programa de Genética Humana, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Casilla 70061, Santiago, Chile 6530499 (AES)
Department of Biology and Museum of Southwestern Biology, The University of New Mexico, Albuquerque, NM 87131, USA (TLY)

Phylogeographic relationships were evaluated at the intraspecific level using nucleotide sequence data from the mitochondrial cytochrome *b* gene of representative specimens of ''colilargo'' (*Oligoryzomys longicaudatus*) from 31 localities, along its distributional range over a large part of the western Andes and southern Argentina. Based on approximately 1,000 base pairs (bp), we recognized a single species on both the Chilean and the Argentinean side as far as at least latitude 51°S, rejecting the subspecific distinctiveness of *longicaudatus* and *philippi*. We thus placed the latter in full synonymy with *O. longicaudatus* as earlier studies proposed, and enlarged its range as far as Torres del Paine, about 51°S. The occurrence of subspecies in this range is doubtful given the low sequence divergence values and the absence of significant associations between haplotypes and their geography. Additionally, we hypothesized that the entrance of this species into the Chilean side of the Andes mountains occurred through the Patagonian forests of southern Argentina, with further dispersal to the north from the south.

Key words: Argentina, Chile, cytochrome *b* gene, *Oligoryzomys longicaudatus*, phylogeography

Oligoryzomys Bangs, 1900 is a genus of small mice recognized in the New World as part of the Tribe Oryzomyini (Muridae: Sigmodontinae). Until recently, this taxon was recognized as a subgenus of *Oryzomys*, but further morphological revisions based on external, cranial, tooth, and stomach morphology raised its taxonomic status to the generic level (Carleton and Musser 1989). Using these morphological data researchers also concluded that *Oligoryzomys* is a monophyletic lineage. This was later corroborated by protein electrophoresis and partial sequences of the cytochrome *b* gene (Dickerman and Yates 1995; Myers et al. 1995). Currently, 15 species are recognized in the genus (Musser and Carleton 1993) distributed throughout the Neotropics from Mexico (e.g., *Oligoryzomys fulvescens*) southward to Argentina and Chile (e.g., *O. longicaudatus*).

Osgood (1943) classified all Chilean *Oryzomys* as *O. longicaudatus* although he differentiated it into 3 subspecies based on subtle morphological features (Mann 1978; Osgood 1943). The ranges of the originally recognized subspecies are roughly congruent with 3 of Chile's major ecogeographic regions: *O. l. longicaudatus* from the Mediterranean region (Copiapó Valley south to the northern parts of the Province of Concepción); *O. l. philippi* in the temperate forest (northern Concepción Province south to ca. 50°S), and *O. l. magellanicus* in the Patagonian and Fuegian forests south of approximately 50°. In Argentina *O. longicaudatus* occurs from approximately 32°S (SW of Mendoza) to approximately 48°S (N of Santa Cruz Province). However, a further comprehensive phenetic revision of the species, including external, cranial, bacular, chromosomal, and allozyme characters led to the separation of the southernmost subspecies as *O. magellanicus* (Gallardo and Palma 1990;

* *Correspondent: epalma@genes.bio.puc.cl*

Palma 1987). In southern Chile *O. longicaudatus* is mainly associated with *Nothofagus* forests, the ecotone between these forests and the Patagonian steppe (Monjeau et al. 1998; Pearson 1983, 1987), whereas in central Chile it occurs in bushy areas with mesic conditions (Mann 1978). Some *Oligoryzomys* collected in the vicinity of San Blas (Buenos Aires, Argentina) have also been asigned to *longicaudatus*, as the subspecies *O. l. pampanus* (Massoia 1973).

In Chile *O. longicaudatus* is best known as ''colilargo'' (long-tailed mouse), although its traditional name has been ''long-tailed pygmy rice rat.'' Earlier studies that considered external, cranial, and bacular morphology of this species, and that included representative specimens of several populations throughout its range, failed to disclose evidence of subspecific differentiation that would distinguish between *longicaudatus* and *philippi* (Gallardo and Palma 1990). However, populations ascribed to *O. magellanicus* exhibited strong patterns of differentiation when compared with northern populations for almost all morphological features analyzed. For example, Gallardo and Palma (1990) showed differences in bacular morphology between *longicaudatus-philippi* and *magellanicus*, with the baculum of the latter being significantly larger than that of the northern taxon. Chromosomally, the same study showed that the diploid number between the karyotypes of *O. l. longicaudatus* and *O. l. philippi* were identical (2n = 56); that of *O. l. magellanicus* exhibited 2n = 54 (Gallardo and Patterson 1985; Palma 1987). Finally, phenetic analyses of allozyme data comparing 15 loci from 60 specimens among 10 populations of *O. l. longicaudatus* and *O. l. philippi* in Chile (between Coquimbo in Region IV and Aysén in Region XI; data are not available for *O. l. magellanicus*) exhibited high levels of genetic similarity (Palma 1987). Thus, based on the strong morphologic and genetic uniformity detected among populations along the range of the 2 northern subspecies, Gallardo and Palma (1990) recognized a single species between 28° and 50°S, *O. longicaudatus*, with *philippi* being a full synonym of *longicaudatus*. The same study concluded that Patagonian populations of the southernmost subspecies (*magellanicus*) constituted a valid species, ranging from 50°S, southward to the Patagonian forests and adjacent islands in Magallanes, Chile. The morphologic and genetic uniformity found in the range of *O. longicaudatus*, coupled with the ecological evidence that showed high vagility of this species (Murúa et al. 1986), led to the inference of high gene flow among *Oligoryzomys* populations along the latitudinal gradient (Palma 1987). This is of great importance because this species is a major reservoir for 1 of the several hantavirus strains, the Andes strain, which produces hantavirus pulmonary syndrome (HPS) in humans (Padula et al. 2000; Toro et al. 1998).

Biogeographically, the occurrence of *Oligoryzomys* in Chile has been hypothesized to be the result of dispersal from Argentina, particularly through the lower Andean elevations where the *Nothofagus* forests are continuous throughout the Andes (Gallardo and Palma 1990; Palma 1987). Further displacement of the species, particularly northward expansion in Chile, might have been facilitated by the series of glacial cycles that severely affected southern forests during the Pleistocene glaciations. During these glaciations, there was a northward shift in vegetation and associated fauna along the coastal mountain range and the central valley of Chile (Villagrán and Hinojosa 1997). This hypothesis has been preferred to an alternative northern scenario of entrance to the Chilean side, mainly because of the occurrence of the Atacama Desert in the north (since Miocene times) that certainly has constituted a major barrier for the dispersal of this taxon. In fact, some ecological studies have established the affinity of the ''colilargo'' for forest and mesic areas that are not present in the north (Kelt et al. 1994; Meserve et al. 2003; Murúa et al. 1986).

The current study was designed to evaluate the hypothesis of genetic and morphological uniformity along the latitudinal gradient of *Oligoryzomys longicaudatus* in Chile, and to re-evaluate the existence of subspecies in its range. We additionally included specimens from 2 localities that lie in the range of *O. magellanicus* according to Gallardo and Palma (1990). Furthermore, we tested whether southern forms of *O. longicaudatus* constitute the basal populations because the Temperate Chilean Forests have been hypothesized to be the area of entrance from Argentina to Chile (Gallardo and Palma 1990; Palma 1987). To achieve these goals we sequenced the cytochrome *b* mitochondrial gene from 33 specimens of *O. longicaudatus* and *O. magellanicus*, encompassing the entire latitudinal range of the former taxon, with specimens from 27 localities in Chile and 4 in Argentina.

Materials and Methods

Tissues and specimens analyzed.—Voucher specimens for the individuals sequenced in this study were deposited in the Colección de Flora y Fauna Profesor Patricio Sánchez Reyes (SSUC), Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago, Chile; Colección de Mamíferos del Centro Nacional Patagónico (CENPAT), Puerto Madryn, Argentina (field numbers UP and LB); the Museum of Southwestern Biology (MSB), Department of Biology, University of New Mexico; and the Instituto de la Patagonia (CZIP), Universidad de Magallanes, Chile. Tissues and other data associated with each specimen are cross-referenced directly to each voucher specimen and stored in the collection using a special field catalog number, the NK number used by the SSUC and the MSB. GD is the field catalog of Guillermo D'Elia (specimens not yet cataloged and stored at Museo Nacional de Historia Natural from Paraguay). A detailed list of the specimens sequenced per locality is given in Appendix I. We followed the ASM Guidelines during the collection and care of the animals used in this work (Animal Care and Use Committee, 1998).

Nucleotide sequence analyses.—DNA was extracted from frozen liver and blood samples according to the technique described by Laird et al. (1991). The mitochondrial cytochrome *b* gene (approximately 1,000 bp) was amplified for 33 individuals representing 31 localities (33 haplotypes) throughout the range of *O. longicaudatus*. Given the low rate of variation observed for the cytochrome *b* gene when analyzed at the intraspecific level (see results), in the majority of cases we sequenced a single specimen per locality. This low variability has also been shown for the same molecular marker when sequenced in related sigmodontine taxa (Smith et al. 2001; Smith and Patton 1999; Steppan 1998). The first half of the gene was amplified using primers MVZ 07 and MVZ 26 (Smith and Patton 1993) with the following thermal cycle (35 cycles): denaturation for 1 min 30 s at 94°C, annealing for 30 s at 54°C, and extension for 1 min 10 s at 72°C. The

2nd half of the gene was amplified with primers LBE 05 (5′ CTA CAC GAA ACA GGC TC 3′) and H 15767 (Edwards et al. 1991) using the same protocol as above, except that annealing was modified to 59°C for 25 s, with a total of 30 cycles. Double-stranded PCR products were purified with Wizard PCR Preps (Promega) and Qiaquik (Qiagen). Cycle sequencing was performed (Murray 1989) using primers MVZ 07, LBE 05, and H 15767 labeled with the Big Dye Terminator kit (Perkin Elmer, Norwalk, Connecticut). The sequencing reactions were analyzed on an Applied Biosystems Prism 310 (Foster City, California) automated sequencer. Sequences were aligned using the ClustalW program (Higgins et al. 1996), and by eye, allocating the proper codon position from the beginning of the cytochrome *b* sequence using the *Mus musculus* sequence as a reference (Bibb et al. 1981). We used MEGA software (Molecular Evolutionary Genetic Analysis, version 1.02—Kumar et al. 1993) to obtain the frequencies of nucleotide bases in all ''colilargos,'' the number of transitions and transversions between every pair of taxa, and the transition/transversion rates.

Phylogenetic analysis.—Phylogenetic analysis was conducted using the maximum-likelihood algorithm available in PAUP* 4.0 (Swofford 2002). To choose the best fitting model of sequence evolution we used Modeltest 3.06 (Posada and Crandall 1998). The Akaike information criterion (AIC—Akaike 1974) identified the transversion + gamma model (TVM+G) as optimal (-lnL = 2105.6758, AIC 4227.3516), with base frequencies A = 0.3011, C = 0.2751, G = 0.1338, T = 0.2900; and substitution models A–C = 2.9512, A–G = 7.6073, A–T = 1.4982, C–G = 1.2048, C–T = 7.6073, G–T = 1. The optimal base composition, substitution rate matrix, and among site substitution rate heterogeneity parameters were simultaneously estimated during the maximum-likelihood heuristic search. The proportion of invariable sites (I) was 0. Reliability of nodes was estimated by maximum-likelihood bootstrap percentages (Felsenstein 1985) obtained after 100 pseudoreplications using the previously estimated maximum-likelihood parameters with tree bisection reconnection branch swapping. We rooted the tree with the outgroup criteria, using *Oligoryzomys fornesi*. We used this species as the outgroup because an ongoing phylogeny of the genus based on the cytochrome *b* and the NADH1 mtDNA genes shows that *O. longicaudatus* is part of a clade that includes *O. fornesi* from the Chaco and Andean regions in the Neotropics (Palma et al. in litt.).

The amount of sequence divergence between groups (i.e., subspecies per ecogeographic region) was evaluated using MEGA software. We considered whole transitions (ts) and transversions (tv) for all codon positions using the Tamura-Nei and Kimura-2-parameter (K2P) models available in MEGA. Grouping of localities by subspecies and ecoregions was as follows (ecogeographic regions according to Armesto et al. in press; Fig. 1): a) *O. l. longicaudatus* (Mediterranean region): La Silla, Fray Jorge, Salamanca, Quebrada del Tigre, San Antonio, Rínconada Maipú, Apoquindo, San Fernando, Romeral, Bullileo, Quillón, Tucapel; b) *O. l. philippi* (Temperate forests region): Carahue, Temuco, Villarrica 048, Villarrica 083, Quetrupillán, Zapala, Chos Malal, Las Breñas, Panguipulli, Riñihue, Chiloé 656, Chiloé 659, Mininco 245, Mininco 268, Río Simpson, Alto Río Ibañez; c) *O. l. magellanicus* (Patagonian Forest region): Torres del Paine, Penitente; d) *O. l. pampanus*: San Blas UP 374, San Blas UP 377.

Phylogeographic and genetic structure analyses.—We conducted a Mantel test (Mantel 1967) to see if there was any correlation between the genetic distance obtained from a Tamura-Nei (TN) distance matrix using MEGA and the geographic distance among specimen localities measured in m. For this analysis we used the Mantel program 2.0 (Liedloff 1999) with 100 permutations. Additionally, we conducted an analysis of molecular variance (AMOVA—Excoffier et al., 1992) at 2 hierarchical levels (i.e., within subspecies and between subspecies)

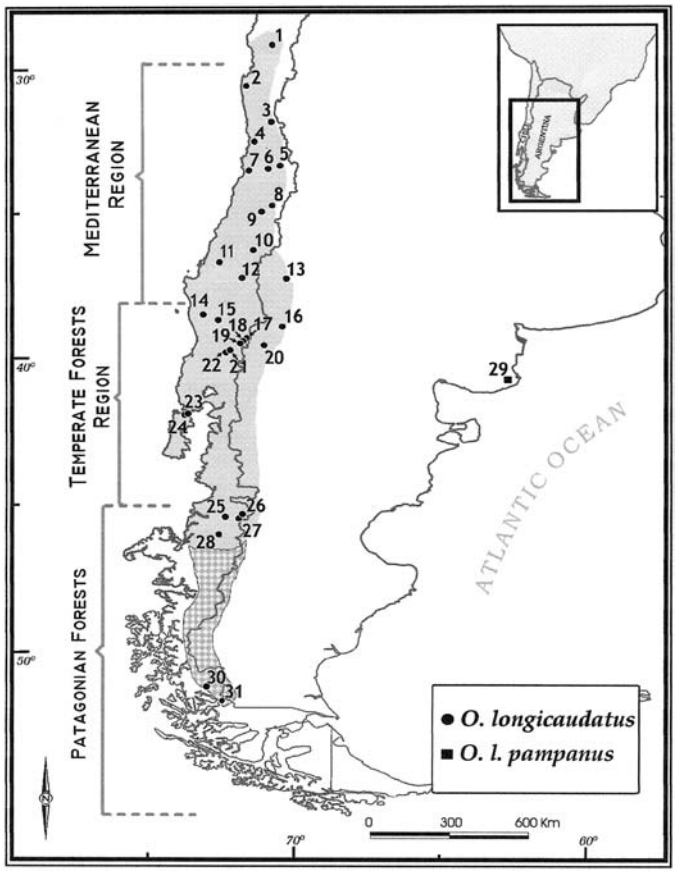

FIG. 1.—Approximate range of distribution of *Oligoryzomys longicaudatus* in Chile and Argentina (shaded area); crossed lines indicate species range extension; circles represent other localities where the species has been reported in Argentina. Numbers indicate localities sampled corresponding to four subspecies. *O. l. longicaudatus*: 1) La Silla, 2) Fray Jorge, 3) Salamanca, 4) Quebrada del Tigre, 5) Apoquindo, 6) Rinconada de Maipú, 7) San Antonio, 8) San Fernando, 9) Romeral, 10) Bullileo, 11) Quillón, 12) Tucapel, 13) Chos Malal; *O. l. philippi*: 14) Carahue, 15) Temuco, 16) Zapala, 17) Quetrupillán, 18) Villarrica 083, 19) Villarrica 048, 20) Las Breñas, 21) Panguipulli, 22) Riñihue, 23) Chiloé 659, 24) Chiloé 656, 25) Río Simpson, 26) Mininco 245, 27) Mininco 268, 28) Río Ibañez; *O. l. pampanus*: 29) San Blas; *O. l. magellanicus*: 30) Torres del Paine, 31) Río Penitente.

using ARLEQUIN version 2.000 which takes into account both haplotype frequency and molecular divergence (Schneider et al. 2000). We pooled localities for each subspecies according to the ecogeographic regions where they were described (see above). Finally, we conducted a mismatch-distribution analysis, using ARLEQUIN, for all sequences to detect whether or not different haplogroups occurred, and to evaluate if the frequency distribution of haplotypes in *Oligoryzomys* followed any particular modal distribution (Rogers and Harpending 1992).

A haplotype network was constructed using TCS program version 1.0 (Clement et al. 2000), and clades were nested according to rules outlined in Templeton et al. (1992). GEODIS version 2.0 (with 10,000 resampling events—Posada et al. 2000) was used to test for significant associations between haplotype and geography. When significant associations were detected, an inference key given by Templeton (2004) was used to determine the likely cause of associations.

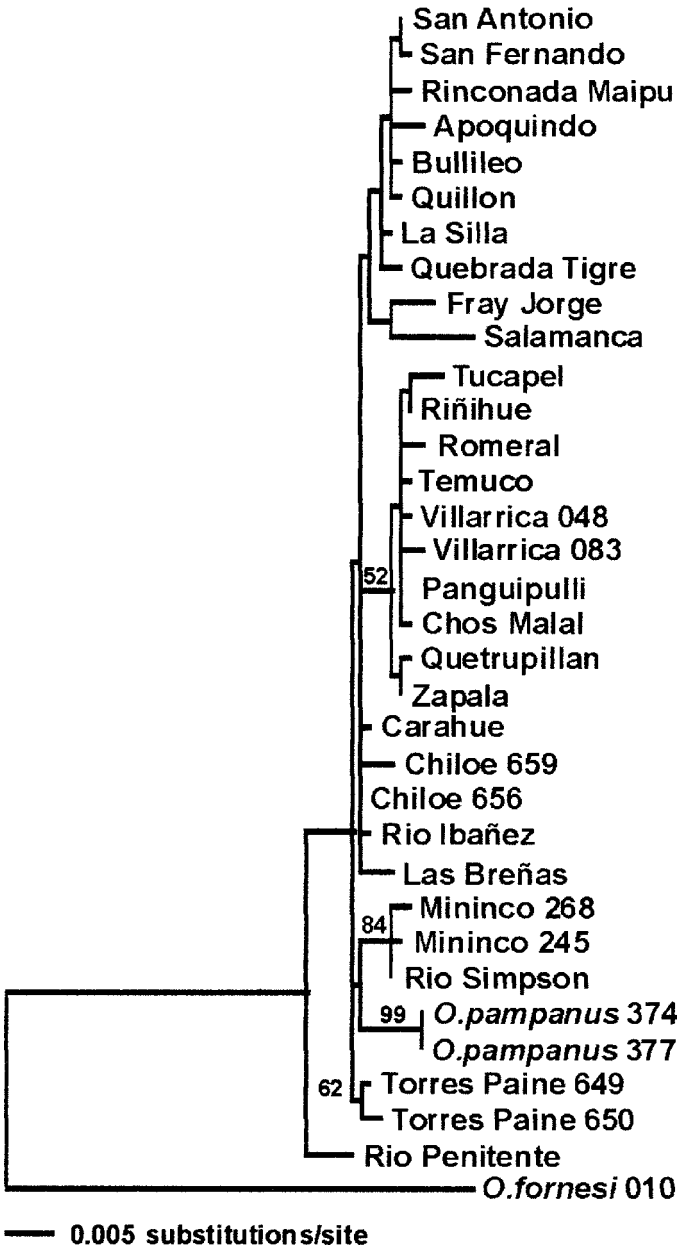

FIG. 2.—Maximum-likelihood tree obtained from the cytochrome *b* gene sequences of *O. longicaudatus*. Numbers on nodes represent 100 bootstrap replicates.

RESULTS

Phylogenetic analyses.—Figure 2 shows the tree obtained from maximum-likelihood, with Modeltest parameters as given above. The–lnL value for this tree was 2105.6758. Bootstrap values were low, and those shown in the tree represent values over 50%; while branch lengths were mostly short. The tree clearly shows recovery of a single clade (with the exclusion of Río Penitente) representing localities of southcentral Chile and Argentina between San Antonio and Torres del Paine and that should be recognized as *O. longicaudatus*. In this clade, we distinguished a southern-north latitudinal trend on which southern localities were more basal (e.g., Mininco, Río Simpson, Torres del Paine) than northern localities that were recovered as the most derived (e.g., San Antonio–Quebrada del Tigre, closely tied to Fray Jorge–Salamanca). This clade also included adjacent Argentinean localities, as well as *O. l. pampanus* from Buenos Aires Province, and specimens from Torres del Paine National Park (51°S) that according to Gallardo and Palma (1990) should be recognized as *O. magellanicus*. The representative from Río Penitente (52°S) constitutes the 1st outgroup to the *longicaudatus* clade.

Nucleotide sequence variation, structure analyses, and nested clade analyses.—We observed a ts/tv ratio of 4.15 and 4.17 using K2P and TN, respectively, with strong bias towards ts of the CT type. Whole sequence divergence using both K2P and TN models of sequence evolution was 0.9%. Divergence values between *O. longicaudatus* subspecies varied between 0.9 and 1.3% for all codon positions. The sequence divergence between *O. longicaudatus* (all pooled localities) and the outgroup *O. fornesi*, using both nucleotide variation criteria, was 12.9. The Mantel test did not show any significant association between the distance matrices ($P = 0.05$; critical value = 1.645: $G = 0.1296$, Mantel coefficient (Z) = 6608.22, $r = 0.0172$). However, the total F_{st} value obtained from the AMOVA analysis for the 4 nominal subspecies showed structuring of groups (i.e., the existence of subspecies; $F_{st} = 0.2556$, $P < 0.01$).

Haplotypes that differed by as many as 13 single mutational steps were parsimoniously connected at the 95% confidence level, resulting in the construction of one network with a total of 33 haplotypes (Fig. 3). The GEODIS analysis revealed no significant association in the geographic distance analysis; thus we failed to reject the null hypothesis of no geographical association in all comparisons, including the entire clade level. At level 4 we ended with 3 clades: 4-1 was constituted mostly by *O. l. philippi* haplotypes and haplotypes from Tucapel and Romeral (*O. l. longicaudatus*); 4-2 included *O. l. pampanus*, *O. l. magellanicus*, and the localities of Mininco and Río Simpson (*O. l. philippi*); the last clade, 4-3, contained most of the *O. l. longicaudatus* haplotypes. Finally, the mismatch distribution analysis showing the relationship between the number of differences between haplotypes and haplotype frequency exhibited a unimodal curve (Fig. 4).

DISCUSSION

Phylogenetic analysis.—Recent molecular calibrations placed the differentiation of South American sigmodontines between 4.5 and 9.5 million years ago, with the origin of *Oligoryzomys* occurring around 8 million years ago (Smith and Patton 1999). Carleton and Musser (1989) suggested that the evolution of the genus *Oligoryzomys* proceeded in stages and probably involved the repeated invasion of this genus into several environments in the Neotropics, from the uplands of the Andes (e.g., *O. andinus*) to lowlands, forests, savannas, and shrublands.

Using maximum-likelihood with Modeltest parameters we obtained a tree that—with the exclusion of Río Penitente—

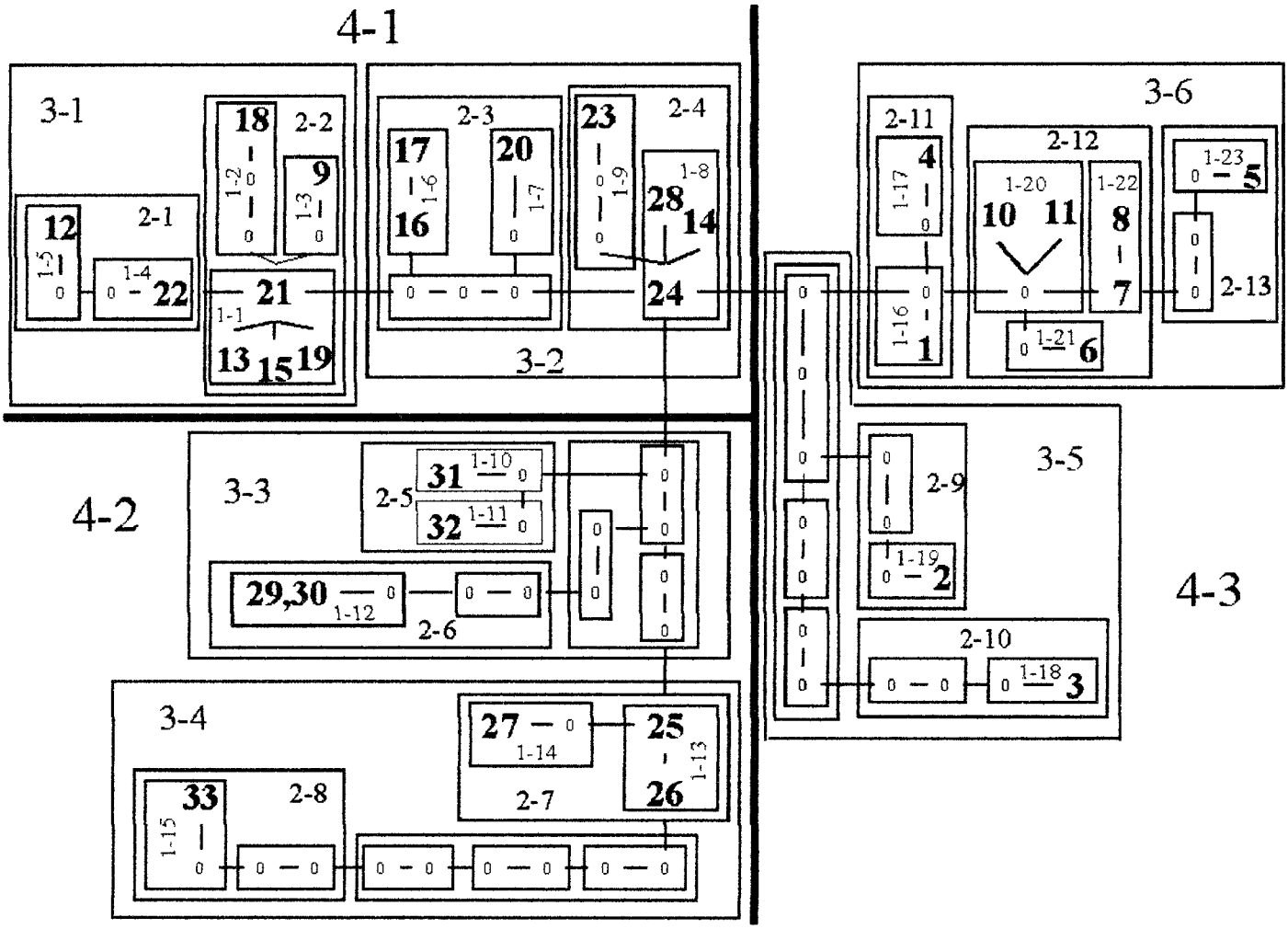

FIG. 3.—Unrooted cytochrome *b* haplotype network for *O. longicaudatus* using TCS (version 1.0—Clement et al. 2000), and their associated nested design according to rules outlined in Templeton et al. (1995). Different haplotypes are represented by numbers in bold (see Appendix I for cross-reference with localities). Each line segment corresponds to one mutational step, and intermediate missing haplotypes are represented by zeroes.

recovered a single clade in the southcentral gradient of Chile and adjacent areas in Argentina. This clade represents currently known *O. longicaudatus* in the latitudinal gradient where subspecies *longicaudatus* and *philippi* were traditionally recognized. However, the same clade also included representatives from the southern locality of Torres del Paine that, according to Gallardo and Palma (1990), should be recognized as *O. magellanicus*. Although the 2 southernmost populations of Torres del Paine National Park occur within the range of *magellanicus* as currently understood, we consider them as part of the same species, *O. longicaudatus*, and refer *magellanicus* to its synonym, thus enlarging the range of *O. longicaudatus* at least as far as latitude 51°S. Pardiñas et al. (2002) also documented *O. longicaudatus* at about latitude 51°S in Argentina. Interestingly, at the conclusion of this work we karyotyped some *Oligoryzomys* from Torres del Paine (located at 51°S), and all forms showed an identical karyotype to that of *O. longicaudatus* (2n = 56, NF = 70), in contrast to the 2n = 54 reported for *O. magellanicus* (Gallardo and Patterson 1985). However, the specimen from Río Penitente (52°S) might well be part of a different taxon (subspecies?) because it was recovered outside the major *longicaudatus* clade. Further specimens and the phylogeography of southern *magellanicus* will be part of a different study.

Sequence divergence, structure, and phylogeographic analyses.—In addition to the results of the maximum-likelihood tree that shows the occurrence of a single taxon in the range of *O. longicaudatus*, the sequence divergence between nominal subspecies *O. l. longicaudatus* and *O. l. philippi* was 0.9%. This value was obtained for Chilean and Argentinean localities and when using different nucleotide criteria for sequencing evolution. This low sequence divergence pattern was even maintained when we included the southernmost population samples of Torres del Paine, because their inclusion increased the value slightly to 1.2%. Other sequence divergence values for cytochrome *b* in oryzomyines are not available; in phyllotine and akodontine mice, however, sequence divergence values fluctuate around 2% and 3.5% for subspecies (Steppan 1998) and about 10% for species (Smith et al. 2001). F_{st} values obtained from AMOVA showed that the subspecies are

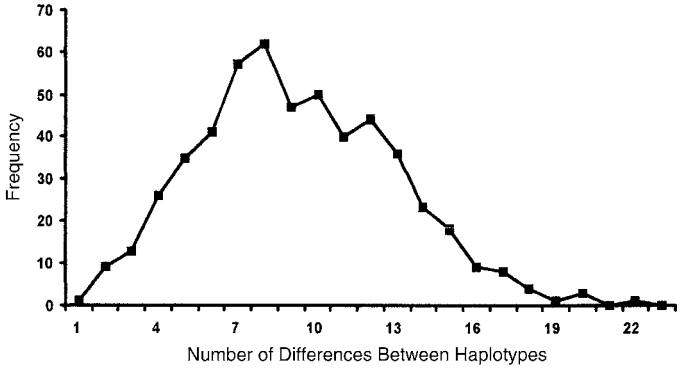

FIG. 4.—Observed pattern of mismatch distribution for an analysis that included all haplotypes of *O. longicaudatus* ($n = 33$).

structured ($F_{st} = 0.25506$, $P < 0.001$), although when we excluded *O. l. pampanus*, the value decreased to 0.16397; between *longicaudatus* and *philippi* it was 0.12663 ($P < 0.001$). In spite of the decrease in these parameters, the values were still significant. Nevertheless, we did not find any significant relationships between geographic distance and the degree of genetic variation among localities represented in the range of *O. longicaudatus*, as reflected by the Mantel test. Therefore, the results suggest that gene flow is likely an important source of homogeneity among them. Consequently, we reject the subspecific distinctiveness of *longicaudatus* and *philippi* and place the latter in full synonymy with *O. longicaudatus* as earlier proposed by Gallardo and Palma (1990), thus enlarging its range to Torres del Paine, about 51° S (we actually believe that *O. magellanicus* occurs south of Torres del Paine). In fact, the 2n = 54 karyotype was reported in the vicinities of Punta Arenas and southern islands (e.g., Harrison Island, 54°S), placing the occurrence of this taxon far south, in Patagonia (Gallardo and Patterson 1985). *O. l. pampanus*, on the other hand, is also part of the *longicaudatus* clade and might indeed constitute a subspecies, considering that the F_{st} value increased when these samples were included in the analysis. However, further sampling within the current distribution of *O. l. pampanus* (i.e., the Buenos Aires province) and the west central part in Argentina will be necessary to adequately assess the taxonomic status of this form (although it seems likely that *pampanus* is only known from its type locality in the Buenos Aires province).

The nested clade analysis did not exhibit significant associations between the haplotypes and their geographical positions. However, at level 4 the nominal subspecies were more or less recovered: clade 4-1 contained most of the *O. l. philippi* haplotypes, although it also included some from the northernmost subspecies (Tucapel and Romeral); clade 4-2 included the subspecies *pampanus* and *magellanicus*, and also some haplotypes in the range of *philippi*. Finally, clade 4-3 contained mostly haplotypes representing localities in the range of *O. l. longicaudatus*. Nevertheless, none of the 3 nested-clades at the 4-× level showed significant associations between geography and haplotypes, thus leading us to accept the null hypothesis of no geographical association. The lack of geographical association with haplotypes could be due to the occurrence of significant gene flow among populations, indicating that populations are panmictic (Templeton et al. 1995). In fact, when we performed the exact test of population differentiation under the null hypothesis of panmixia, there were no significant differences between the 4 groups (i.e., subspecies), corroborating our observation of a nested pattern. According to Templeton et al. (1995), the lack of geographical association with haplotypes could be due to a) panmixia of populations, b) inadequate sampling, or c) insufficient variability. We discard inadequate sampling because we sampled specimens from the entire distributional range of *O. longicaudatus*. Additionally, because network analysis showed 33 distinctive haplotypes, with every geographic locality represented by a haplotype (although with few mutational steps among haplotypes), we also discard a lack of variability among samples. In contrast to the F_{st} values obtained from AMOVA, all other results suggested that populations of *O. longicaudatus*, characterized by having high gene flow, might be homogenizing any possible differentiation between populations along its range. Previous geographic variation studies based on morphology and chromosomes showed a similar uniform pattern for the species. This high gene flow likely is facilitated by the high vagility and large home range reported for this species, mainly in southern Chile (Murúa et al. 1986) where it is more abundant due to its preference for mesic habitats, such as those found in the southern Temperate Forests. However, patterns of genetic homogeneity along the range of the species might also be the result of historical events, suggesting that patterns of molecular variation were shaped relatively recently in geologic time. Scenarios of expansions and retractions of biota during the last glaciation cycles of the Quaternary might have allowed multiple instances of contact between isolated populations, because several glacial cycles affected the biota of southern Chile during the Pleistocene (Villagrán 1990; Villagrán and Hinojosa 1997).

Pairwise differences between haplotypes (i.e., mismatch distribution—Rogers and Harpending 1992) for all *O. longicaudatus* localities pooled into 1 single sample exhibited a single peak (Fig. 4). According to the mismatch distribution model of Rogers and Harpending (1992) this pattern of pairwise differences between haplotypes represents populations expanding from a central range of distribution, whereas samples drawn from populations at demographic equilibrium show a multimodal pattern with several peaks (e.g., isolated populations that secondarily reestablish contact). Our mismatch model suggests that *O. longicaudatus* populations are characterized by recent range expansions, making their populations tightly connected genealogically (Ibrahim et al. 1996). Similar patterns were obtained for *Abrothrix olivaceus* in southern Argentina and Chile (Smith et al. 2001). Northern displacement of *Oligoryzomys* might have been facilitated during glacial times in the Pleistocene when the climate of southern Chile was colder and drier (Mercer 1983). This scenario would have allowed northern displacement of biota to less harsh climates at lower latitudes, via non-glaciated routes through the western

slopes of the Andes and the coastal range of Chile. This fact, associated with the great vagility of the species, allowed *Oligoryzomys* to colonize different areas along the Chilean side of the Andes—on the coast, in the central valley, and in the foothills of the Andes—all areas where the species is found today. The distribution of *Oligoryzomys* has reached as far north as the Copiapó Valley in the Atacama region in Chile. As hypothesized in the maximum-likelihood topology of Fig. 2, *Oligoryzomys* might have reached the northernmost part of Chile more recently, after glacial recession, because a significant part of southern Patagonia was completely glaciated during the Last Glacial Maximum in Quaternary times (Holling and Schilling 1981; Moreno et al. 1999).

Biogeography.—In spite of the short branch lengths and low bootstrap values of the maximum-likelihood tree, it is possible to distinguish a south-to-north latitudinal pattern in the phylogeography of *O. longicaudatus*, suggesting that the species colonized from the south to the north of Chile. As proposed earlier by Reig (1986), it is reasonable to suggest that the southern Andes served as 1 of the areas of radiation for this group of oryzomyines. We propose the existence of lowland dispersal of *Oligoryzomys* forms from the highlands of the Andes to the eastern lowlands such as in northwest Argentina, a portion of the Yungas forests. In fact, *O. longicaudatus* has been reported to occur in that area (Mares et al. 1989; Redford and Eisenberg 1992), although recent molecular studies of *O. longicaudatus* that included populations from western Argentina did not recognize northern populations as belonging to this species (González-Ittig et al. 2002). Furthermore, these northern forms are the reservoir for a different hantavirus strain, the Oran strain (Padula et al. 2000). This northern Argentinean taxon has a disjunct distribution with respect to southern *O. longicaudatus* in Argentina and might well be related to this species. This hypothesis would sustain southern dispersal of peripheral isolates of *Oligoryzomys* to southern Patagonian forests, with subsequent speciation into *O. longicaudatus*. Further entrance to the Chilean side from the east might have followed the continuous *Nothofagus* forest vegetation and lower elevation of the Andes in the south, reaching the Chilean side. These lower elevation passes in the Andes have also been proposed to explain the evolution of another sigmodontine, *Abrothrix olivaceus* across the Andes (Smith et al. 2001). *Oligoryzomys* dispersal to central-northern Chile might have been facilitated by a northern dispersal of biota during the Pleistocene as explained above. *Oligoryzomys* from the south might have followed nonglaciated routes such as the Coastal Cordillera and the central valley in Chile, because glaciations to the north advanced mainly throughout the Andes (Holling and Schilling 1981; Mercer 1983; Moreno et al. 1999). This biogeographic scenario would explain the occurrence of *O. longicaudatus* in the north-central Mediterranean region whose position in the phylogenetic tree was recovered as the most derived clade. An alternative scenario would set the entrance of *O. longicaudatus* from the north, and a southward dispersal along the Chilean side might have followed, reaching temperate and Patagonian forests both in Argentina and Chile. However, this hypothesis is doubtful because the topology of the maximum-likelihood tree left southern forms as basal, supporting a southern advance from the south—thus an entrance from Argentina (about 39-40°S).

O. longicaudatus and hantavirus.—The genetic uniformity found among *O. longicaudatus* populations along its geographic range in Chile acquires much significance because this species is the major reservoir of Andes virus (Bohlman et al. 2002; Padula et al. 2000). Seropositive specimens have been confirmed all along its distributional range (Padula et al. 2000; in litt.). Recent studies on Andes virus genetics (Medina et al. in litt.) have shown low levels of sequence divergence, mirroring the major results shown here for its reservoir species. This in turn might help researchers to understand why ''colilargos'' from throughout the Chilean range show an equal probability of disease transmission. Finally, we did not find any bias towards cytochrome *b* sequence and antibody status (positive versus negative) in our sample, although several localities (e.g., Carahue and Villarrica) included both positive and negative individuals.

We concluded that the phylogeographic analyses of *Oligoryzomys longicaudatus* in Chile and adjacent areas in Argentina confirm the existence of this species as far as latitude 51°S in both Argentina and Chile. The occurrence of subspecies within this range is not supported by the results from cytochrome *b* sequences, thus supporting previous studies based on morphology and chromosomes. Southern populations from temperate forest regions were more basal than those from the northern range, thus supporting a southern entrance of this taxon from Argentina to the Chilean side, with further dispersal to northern latitudes in Chile. Our results confirm previous analyses in connection with the morphologic and genetic homogeneity that characterize the evolutionary history of this species.

Resumen

Se evaluaron las relaciones filogeográficas a nivel intraespecífico del roedor sigmodontino *Oligoryzomys longicaudatus* (''colilargo''), usando secuencias nucleotídicas del gen mitocondrial citocromo *b*. El estudio incluyó representantes de 31 localidades cubriendo así gran parte de su rango de distribución en Chile y sur de Argentina. En base a aproximadamente 1000 pb hipotetizamos la existencia de una única especie, *O. longicaudatus*, tanto en el lado chileno como argentino, descartando la existencia de las subespecies *longicaudatus* y *philippi* y dejando esta última en sinonimia como *O. longicaudatus* tal como lo habían propuesto estudios anteriores. El rango de *O. longicaudatus* se amplía hasta Torres del Paine ca. 51° de latitud sur. El descarte de subespecies es debido a los bajos valores de secuencia de divergencia nucleotídica y a la ausencia de asociaciones significativas entre los haplotipos y su geografía. Adicionalmente, reconocemos que la entrada de esta especie hacia el lado chileno ocurrió a través de los bosques Patagónicos del sur de Argentina, luego de lo cual la especie se dispersó hacia el norte de Chile, desde el sur.

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APPENDIX I

List of *Oligoryzomys* taxa sequenced for the cytochrome *b* gene. Tissue number is collection number, Accession number is GenBank accession number, source is Museum or Collection where vouchers were deposited. Locality numbers are shown on map (Fig. 1); haplotype numbers were obtained using the TCS program (Fig. 3; Clement et al. 2000).

Taxon	Tissue number	Locality on the map	Haplotype number	Locality	Region	Country	Latitude	Longitude	GenBank Accession number	Source[a]
longicaudatus	96860	1	1	La Silla	Coquimbo	Chile	29°13′58″S	70°44′14″W	AY 275682	SSUC/MSB
	16.4	2	2	Fray Jorge	Coquimbo	Chile	30°38′58″S	71°41′14″W	AF 346572	SSUC
	95268	3	3	Salamanca	Coquimbo	Chile	31°53′12″S	70°47′47″W	AF 346570	SSUC/MSB
	96758	4	4	Quebrada del Tigre	Valparaíso	Chile	32°33′36″S	71°26′19″W	AY 275683	SSUC/MSB
	95299	5	5	Apoquindo	Metropolitana	Chile	33°24′13″S	70°29′01″W	AF 346568	SSUC/MSB
	96789	6	6	Rinconada de Maipú	Metropolitana	Chile	33°29′44″S	70°53′39″W	AY 275685	SSUC/MSB
	105860	7	7	San Antonio	Valparaíso	Chile	33°34′07″S	71°37′16″W	AY 275684	SSUC/MSB
	105929	8	8	San Fernando	O′Higgins	Chile	34°45′59″S	70°46′34″W	AY 275686	SSUC/MSB
	105931	9	9	Romeral	Maule	Chile	34°58′05″S	71°07′34″W	AY 275687	SSUC/MSB
	105885	10	10	Bullileo	Maule	Chile	36°17′20″S	71°24′47″W	AY 275688	SSUC/MSB
	105827	11	11	Quillón	Bío-Bío	Chile	36°42′46″S	72°34′18″W	AY 275689	SSUC/MSB
	105977	12	12	Tucapel	Bío-Bío	Chile	37°14′28″S	71°47′38″W	AY 275690	SSUC/MSB
	95614	14	14	Carahue	Araucanía	Chile	38°30′55″S	73°06′59″W	AF 346571	SSUC/MSB
	95573	15	15	Temuco	Araucanía	Chile	38°41′20″S	72°36′48″W	AY 275691	SSUC/MSB
	95370	17	17	Quetrupillán	Araucanía	Chile	39°25′38″S	71°47′16″W	AY 275693	SSUC/MSB
	95083	18	18	Villarrica	Araucanía	Chile	39°27′28″S	71°49′33″W	AF 346566	SSUC/MSB
	95048	19	19	Villarrica	Araucanía	Chile	39°27′19″S	71°49′11″W	AY 275692	SSUC/MSB
	96972	21	21	Panguipulli	Los Lagos	Chile	39°44′12″S	72°13′39″W	AY 275694	SSUC/MSB
	104559	22	22	Riñihue	Los Lagos	Chile	39°48′15″S	72°19′15″W	AY 275695	SSUC/MSB
	95659	23	23	Chiloé	Los Lagos	Chile	41°52′57″S	73°40′08″W	AF 346573	SSUC/MSB
	95656	24	24	Chiloé	Los Lagos	Chile	41°52′59″S	73°40′20″W	AY 275696	SSUC/MSB
	96562	25	25	Río Simpson	Aysén	Chile	45°27′42″S	72°19′21″W	AY 275698	SSUC/MSB
	95245	26	26	Mininco	Aysén	Chile	45°31′03″S	71°51′49″W	AF 346567	SSUC/MSB
	95268	27	27	Mininco	Aysén	Chile	45°30′44″S	71°47′25″W	AY 275697	SSUC/MSB
	95957	28	28	Río Ibañez	Aysén	Chile	46°05′09″S	72°34′52″W	AY 275699	SSUC/MSB
	105649	30	31	Torres del Paine	Magallanes	Chile	51°07′24″S	73°07′47″W	AY 452197	SSUC/MSB
	105650	30	32	Torres del Paine	Magallanes	Chile	51°07′24″S	73°07′47″W	AY 452198	SSUC/MSB
	1025	31	33	Río Penitente	Magallanes	Chile	52°06′45″S	71°32′10″W	AY 275706	CZIP
	UP 449	13	13	Chos Malal	Neuquén	Argentina	37°23′15″S	70°16′40″W	AY 275701	CENPAT
	UP 435	16	16	Zapala	Neuquén	Argentina	38°50′S	70°30′W	AY 275700	CENPAT
	LB 012	20	20	Las Breñas	Neuquén	Argentina	39°23′S	71°12′W	AY 275702	CENPAT
	UP 374	29	29	Bahía San Blas	Buenos Aires	Argentina	40°33′S	62°13′W	AY 275703	CENPAT
	UP 377	29	30	Bahía San Blas	Buenos Aires	Argentina	40°33′S	62°13′W	AY 275704	CENPAT
fornesi	GD010			620 m S Hotel Centu Cue	Misiones	Paraguay	26°15′06″S	57°1′35″W	AY 452199	GD

[a] SSUC: Colección de Flora y Fauna Profesor Patricio Sánchez Reyes, Pontificia Universidad Católica de Chile, Chile; MSB: Museum of Southwestern Biology, University of New Mexico; CZIP: Instituto de la Patagonia, Universidad de Magallanes, Chile; CENPAT: Colección de Mamíferos del Centro Nacional Patagónico, Puerto Madryn, Argentina; GD: Guillermo Delía catalog (Uruguay).