MOLECULAR DIVERGENCE AND PHYLOGENETIC RELATIONSHIPS OF CHINCHILLIDS (RODENTIA: CHINCHILLIDAE)

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Molecular phylogenetic relationships were investigated in 6 species of Chinchillidae (Chinchilla lanigera, C. brevicaudata, Lagidium peruanum, L. viscacia, L. wolffsohni, and Lagostomus maximus), 1 species of Dinomyidae (Dinomys branickii), 1 of Abrocomidae (Abrocoma cinerea), and 1 of Octodontidae (Octodon degus) using the first 548 base pairs of the mitochondrial cytochrome-b gene. Maximum-parsimony and maximum-likelihood analyses consistently showed Chinchillidae as a robust clade and confirmed a close relationship with Dinomyidae. Both Chinchilla species differed at 22 sites, and 3 were nonsilent; average genetic distances were approximately 6%. Sequences from domestic C. lanigera and wild C. brevicaudata showed low levels of variation. Although all topologies obtained were congruent with current taxonomy, Lagidium exhibited large genetic distances (range 5.9–8.9%), suggesting the existence of more than the 3 species currently recognized.

Key words: Abrocoma, Argentina, Chinchilla, Chile, cytochrome b, Dinomys, Lagidium, Lagostomus, mtDNA

Chinchillids are endemic South American rodents (Rodentia, Hystricognathi, Chinchillidae) and occur along the Andes. They include chinchillas (Chinchilla), mountain viscachas (Lagidium), and pampas viscachas (Lagostomus).

Chinchillas are medium-sized rodents whose wild populations, once thought to be extinct, still remain. The small-bodied, large-eared, and long-tailed form, Chinchilla lanigera, from central Chile, was domesticated in the United States (Parker 1975) and is now raised in numerous chinchilla ranches. The large-bodied, small-eared, and short-tailed form, Chinchilla brevicaudata, from the Altiplano (the highlands of Peru, Bolivia, northern Chile, and Argentina), was domesticated in Chile around 1931 (Grau 1986). A few reports suggest some crosses between C. lanigera and C. brevicaudata have occurred as a result of captive breeding (Grau 1986).

Most species limits and taxonomic relationships of chinchillas remain unstudied with molecular methods despite the need for it because of an unstable taxonomy (Anderson 1997; Woods 1993). Moreover, familial relationships have been controversial (Glanz and Anderson 1990), with an unexpected molecular relationship with Dinomyidae (pacarana) from northern South America (Adkins et al. 2001; Huchon and Douzery 2001). Despite being popular mammals, chinchillas have been poorly studied (Anderson 1997). The major question is the number of species to be recognized: 1 (Bennett 1829; Osgood 1941), 2 (Cabrera 1961; Corbet and Hill 1980; Woods 1993), or 3 (Bidlingmaier 1937; Prell 1934). No convincing case, based on adequately documented specimens of known provenance, has been made for recognition of more than 1 species (Anderson 1997). Both C. lanigera and C. brevicaudata are considered endangered (Cofré and Marquet 1999; Miller et al. 1983; Reca et al. 1996). Given such status and the persisting trafficking of skins at the local level, a molecular phylogeny of chinchilla species and geographic populations would benefit conservation efforts.

Viscachas are large sized (up to 9 kg), living on both sides of the Andes. The number of species of mountain viscachas is considered to be 3 by most authors: Lagidium peruanum (northern or montane viscacha), L. viscacia (southern or mountain viscacha), and L. wolffsohni (Wolffsohn’s viscacha, chinchillón anaranjado). However, taxonomy usually differs by author (e.g., L. peruanum is included within L. viscacia according to Anderson 1997).

Our goal was to document the extent of molecular divergence among nominal taxa and the phylogenetic relation-
ships within the family. Specifically, we analyzed the cytochrome-\(b\) gene (\(\text{Cytb}\)) sequences from 22 wild specimens of chinchillas and viscachas from Chile (Spotorno et al. 1998) and Argentina as well as from 7 domestic specimens of both \textit{Chinchilla} species. Our sample covered all nominal species of the family (Woods 1993).

**Materials and Methods**

**Specimens.**—All wild-caught animals (Appendix I) were collected from natural populations; nonlethal sampling of hair, blood, feces, or ear tissue were taken for most specimens. In some cases, individuals or ear returned alive to original sites. Skulls and skins, whenever available, were prepared as voucher specimens, and most were deposited in the collection of the Laboratorio de Citogenética Evolutiva, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile, or the Colección de Vertebrados from the Departamento de Biología, Facultad de Ciencias, Universidad de La Serena, Chile.

**Sequence analysis.**—DNA was extracted from liver samples fixed in 75\% alcohol using sodium dodecyl sulfate-proteinase K extraction and alcohol precipitation (Maniatis et al. 1992). The mitochondrial \textit{Cytb} was amplified via the polymerase chain reaction (PCR) using Taq DNA polymerase (Promega, Madison, Wisconsin). PCR was applied using the thermal profile of 95\°C denaturation (45 s), 54\°C annealing (30 s), and 72\°C extension (1 min) for 30 cycles. Double-stranded PCR products were purified by Wizard PCR Preps (Promega). The thermal protocol for cycle sequencing (using the Gibco-BRLs kit, obtained from Life Technologies, Rockville, Maryland) was 95\°C (30 s), 55\°C (1 min) for 30 cycles, using conserved primers L14724a, H15050, and H15400, as described (Anderson et al. 1981). Radiolabeled sequencing products were resolved by vertical acrylamide electrophoresis and visualized by autoradiography. However, most of the sequencing reactions were analyzed in an ABI Prism 310 automated sequencer, labeling primers with the Big Dye Terminator kit from Perkin Elmer (Applied Biosystems, Foster City, California). Sequences were entered into GenBank with the following accession numbers: AF122820 for domestic \textit{C. lanigera} and AF244378–AF244388 for the remaining taxa.

**Phylogenetic analysis.**—Samples from \textit{Dinomys branickii} (\textit{Dinomyidae}), \textit{Abrocoma cinerea} (\textit{Abrocomidae}), and \textit{Octodon degus} (\textit{Octodontidae}) sequences were obtained (see Appendix I) and included to assist in providing structure and resolution for in-group taxa. Sequences were aligned using the Clustal V software program (Higgins et al. 1992) and proofed by eye. Frequencies of nucleotide bases and compositional biases were estimated using computer programs MEGA2 (Kumar et al. 1993) and PAUP 4.0b8a (Swofford 1998). Homogeneity among taxa was evaluated using chi-square tests in the programs MEGA2 and PAUP 4.0b8a (Swofford 1998). The MODELTEST program (Posada and Crandall 1998) was used to select a model of DNA evolution that best fits the data for likelihood analysis. The HKY85+G model was identified as generating a significantly better likelihood score. This model was used to perform heuristic searches with the tree-bisection-reconnection branch swapping. Because of computational limitations, 200 bootstrap replicates were implemented in PAUP 4.0b8a using the fast stepwise-addition method.

**Results**

Sequence data included 562 base pairs for 27 specimens of Chinchillidae plus 3 related taxa. For \textit{Lagidium wolffsohni}, only the first 350 base pairs were obtained; therefore, it was excluded from maximum-likelihood analysis. All taxa were similar in base composition (chi-square test; \(P = 1.00, d.f. = 87\)). Average base compositions were \(T = 28.3\%\), \(C = 28\%\), \(A = 28.4\%\), and \(G = 15.3\%\), with significant compositional biases at the 2nd (\(G = 16.5\%\)) and 3rd (\(G = 5.8\%\)) codon positions.

Maximum-likelihood analysis (–lnL 2,175.6, \(t_i:t_v\) ratio = 3.8276, gamma shape = 0.3009) using the HKY+G model produced the topology shown in Fig. 1. Three clades were recognized within Chinchillidae, corresponding to the 3 genera. Minor clades were depicted within major clades and corresponded to currently recognized species, particularly in the case of the 2 \textit{Chinchilla} species. The clade containing \textit{Lagidium viscacia} received no significant statistical support.

Maximum-parsimony analyses produced 3 equally parsimonious trees (length = 355, CI = 0.682, \(RC = 0.558\)), with ingroup taxa topologies identical to each other and to that obtained in the maximum-likelihood analysis (Fig. 1). In all analysis, chinchillid sequences remained together.

Substantial molecular divergences in the \textit{Cytb} sequences existed between the 2 \textit{Chinchilla} species. Their molecular distances had a mean value of 5.9\% (range 4.9–6.2). Twenty-two sites consistently exhibited differences among sequences—most for 3rd codon positions, except for 1 for 1st position (site 28) and 1 for 2nd position (site 156). Among 3rd positions, 8 sites had character states that were exclusive and diagnostic for \textit{C. brevicaudata}: 15, 156, 171, 180, 300, 321, 399, and 525. By contrast, all \textit{C. lanigera} had 3 sites with exclusive character states at sites 11 (2nd position), 93, and 150.

All 6 wild \textit{C. lanigera} sequences were loosely affiliated (Fig. 1), with a characteristic A base at site 366. Nevertheless, the single specimen from La Higuera was clearly divergent (Fig. 1: \textit{C. lanigera}, specimen 2082Hig), with a genetic distance of 2\% and 5 distinct autopomorphies at sites 147, 264, 265, 266, and 429. In contrast, the 5 wild samples from Aucó were very similar and were defined by 3 unique variants (at sites 47, 234, and 495).

All 5 sequences of domestic \textit{C. lanigera} usually formed a branch (Fig. 1) defined by sites 33 (3rd position) and 85 and 574 (1st positions). But all of them also shared a characteristic \(G\) base at site 63 with the wild-caught specimen from La Higuera.

No unique variants associated with \textit{C. brevicaudata} were detected in any of the 5 specimens of domestic \textit{lanigera}, although all shared a C at site 243; however, this base was shared with most specimens of \textit{Lagidium}. Domestic \textit{C. brevicaudata} sequences were similar to those of their wild counterparts, except at site 339. The latter exhibited the lowest diversity within our sample, with genetic distances of approximately 0.1\%.
Samples of *Lagidium* constituted a robust clade (Fig. 1), with 10 defining variants identified. But there were clear molecular divergences between the 5 different geographic samples along the Andes Mountains, to the point that no consistent groups were resolved. The 2 northernmost samples of *L. peruanum* were 5.9% different from the nearest southern subspecies, *L. v. perlutea*, and 5.8% different from *L. v. viscacia*. The latter was 7.9% different from *L. v. boxi*. The largest genetic distance between samples of *Lagidium* was 8.9% (*L. v. boxi* and *L. peruanum*). In addition, maximum-parsimony analysis (not shown) grouped the short sequence of *Lagidium wolffsohni* with samples of *L. v. boxi* (bootstrap 86, Bremer support index 18).

**DISCUSSION**

Cytochrome-*b* sequences indicate that the family Chinchillidae is probably a monophyletic clade. This result is consistent with the unexpected molecular finding of a near relationship to the geographically distant Dinomyidae (Huchon and Douzery 2001), a monospecific family from northern South America. Both taxa formed a well-supported clade in all analyses (Fig. 1).

**FIG. 1.**—Topology obtained from the maximum-likelihood analysis of Chinchillidae samples based on cytochrome-*b* sequences. Numbers after taxonomic names are collection numbers (Appendix I); d = domestic specimen; Llu = Llullaillaco; Hig = La Higuera; Cuy = Cuyano, Aucó; Cur = Curico, Aucó. Bootstrap values (200 replicates) and Bremer support indices obtained from equally weighted parsimony analysis are to the left and right of the slash, respectively. Bootstrap values from likelihood analysis in italics below.
Chinchilla lanigera and C. brevicaudata emerge as distinct and divergent species, which agrees with previous morphological studies (Redford and Eisenberg 1992) and with the reported male sterility of their hybrids (Grau 1986). The genetic distance (5.9%) is larger than the 4–5% found for species pairs in the related hystricognath echimyid rodents from South America (Lara et al. 1996) and much larger than the 2% divergence level indicative of intraspecific variation for this gene (Bradley and Baker 2001). Moreover, such genetic divergence between Chinchilla species is probably an underestimation since it is based on sequences of the 1st part of the Cytb, which seems to evolve at a slower rate than the 2nd part of the gene in related rodent taxa (Lara et al. 1996). The defining molecular variants, in addition to providing information for unequivocal molecular identification of any wild or domestic chinchilla material, document a history of geographic disjunction and adaptation along the Andes (Spotorno et al. 1998) and, by inference, of probable genetic distinctiveness.

The close relationship of wild and domestic Chinchilla lanigera was expected, as samples representing this clade (in California) were derived from 12 wild individuals collected in Chile during the 20th century (Parker 1975). The molecular variability among the present domestic populations suggests individual differences in the 3 known founder females. The large amount of autoapomorphic variants in 2 of the 5 domestic individuals is consistent with this observation.

The divergence detected within the Cytb of wild chinchilla populations, particularly the uniquely derived bases, suggests a genetic diversity that is higher than those in the present populations of domestic chinchilla. Those might represent a potential source of well-integrated genetic variability eventually available for the large but seemingly homogeneous populations of present domestic chinchillas. Nevertheless, more variation eventually might be detected in other domestic populations. Further studies from other stocks also are required to elucidate this issue.

The molecularly divergent northern population of wild C. lanigera at La Higuera appears to deserve urgent and active conservation efforts. This apparently small population occurs along the Andes as implied by Anderson (1993). The molecular distances between the samples from any of these 4 Lagidium subspecies usually were larger than those observed between other Chinchilla species (Fig. 1), with distinctive differences involving not only silent variants at 3rd positions but also nonsynonymous sites: 3 in the case of L. v. boxi, 4 in L. v. cuvieri, 2 in L. v. viscacia, and 1 in L. v. perlutea. Because we have not studied all currently recognized subspecies and our samples are scattered along a wide geographic range, more extensive sampling and revisionary work is required for a more accurate taxonomic assessment within this group of gracile rodents.

RESUMEN

La diversidad molecular y las relaciones filogenéticas fueron investigadas en 6 especies de Chinchillidae (Chinchilla lanigera, C. brevicaudata, Lagidium peruanum, L. viscacia, L. wolffsohni, y Lagostomus maximus), 1 Dinomyinae (Dymnomys branickii), 1 Abrocomidae (Abrocoma cinerea), y 1 Octodontidae (Octodon degus) usando los 1os 548 pb del gen mitocondrial para citocromo-b. Análisis de máxima parsimonia y máxima verosimilitud consistentemente mostraron a Chinchilla como un clado robusto, y confirmaron su relación cercana con Dinomyinae. Las secuencias de DNA para ambas especies de Chinchilla dieren en 22 sitios, 3 de los cuales eran no silenciosos; las distancias genéticas promedio fueron aproximadamente de 6%. Las secuencias de C. lanigera doméstica y las de C. brevicaudata silvestres mostraron bajos niveles de variación genética. Aunque todas las topologías obtenidas fueran congruentes con la taxonomía actual del grupo, las muestras de Lagidium mostraron grandes distancias genéticas (intervalo 5.9–8.9%), sugiriendo la posible existencia de más de las 3 especies hasta ahora reconocidas.

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LITERATURE CITED


APENDIX I

Taxa (taxonomic names follow Woods 1993 in general), original localities, sex, and data of examined specimens (LCM numbers; acronym LCM refers to the Laboratorio de Citogenética de Mamíferos Collection, Santiago, Chile, in parentheses; m = male, f = female, d = domestic; GenBank accession numbers given if available) were as follows:

**Chinchilla lanigera**.—CHILE: Region IV Coquimbo; 15 km N of Aucó, Íllapel, near Reserva Nacional Las Chinchillas, Chile (LCM 2078f, ear, AF325014; LCM 2079, ear, AF244378; LCM 2080m, ear, AF325007; LCM 2310f, ear, AF325016); La Higuera (LCM 2082m, skin, AF325009); 6 domestic specimens from the Cardonal Chinchilla Ranch, Santiago, Chile (LCM 2002md, liver, AF283980; LCM 2003md, liver, AF464760; LCM 2004fd, liver, AF249314; LCM 2304fd, AF464761; LCM 2316d, liver, AF474762; and LCM 2011md, liver, AF283981).

**Chinchilla brevicaudata**.—CHILE: Region II Antofagasta; El Laco, 56 km southeast Socaire (1898f, liver, AF325022; LCM 1915m, liver, AF283977); Llulluilaico, LCM 2428m, ear, AF464759; LCM 2426m, ear, AF464758; LCM 2425f, ear, AF464757); 2 domestic specimens from Criadero Abaroa, Calama (2338md, liver, AF283981).

**Lagidium peruanum**.—CHILE: Region I Tarapaca; 2 km west of Parinacota (LCM 2027m, liver, AF244384; LCM 2330, liver, AF254885).

**Lagidium viscacia perulae**.—CHILE: Region II Antofagasta; Talabre (LCM 1968, liver, AY254886). ARGENTINA: Jujuy (LCM 2342, liver, AY254887).

**Lagidium viscacia viscacia**.—CHILE: Region III Atacama, Santa Rosa (LCM 2359, blood, AY254888), Region IV Coquimbo, Vicuña (LCM 2310.1m, blood, AF244385).

**Lagidium viscacia boxi**.—ARGENTINA: Neuquen (LCM 2343, skin, AY254889); LCM 2344, skin, AY254890.

**Lagidium wolffsohnii**.—CHILE: Region XII, Sierra Baguales, Ultima Esperanza (LCM 2389, foot, AF244386; from specimen CZIP 0231, Instituto de la Patagonia, Punta Arenas).

**Lagostomus maximus**.—ARGENTINA: 15 km North of San Luis (LCM 2332, liver, skin, AF245485).

Out-group sequences used for analysis: Dinomys branickii (1).—PERU: Depto. Amazonas, Kagka [Aguaruna Village], Rio Kagka of Rio Comaina (Museum of Vertebrate Zoology, University of California, Berkeley, loan, MVZ 153574) AY254884; Abrocoma cinerea (1).—CHILE: Region II Antofagasta, Talabre (LCM 1856, liver) AF244388.