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ORIGINAL ARTICLE

Inter- and intraspecific phylogeography of small mammals in the Atacama Desert and adjacent areas of northern Chile

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ABSTRACT

Aim We evaluated the phylogeography of sigmodontine taxa of the genera *Phyllotis* and *Abrothrix* at the intra and interspecific level, in the Atacama desert and adjacent Andean and Puna regions of northern Chile. The major goal was to test the hypothesis that sigmodontine mice differentiated in the lowlands, most likely via peripatric speciation, dispersing from highland to lowland areas across the desert vegetated canyons, thus reaching the Pacific coast. Dispersing individuals may have found favourable habitats along these valleys, in northern Chile, which connect the high altitude Puna region with the lowlands.

Location The study was conducted in northern Chile (18–22° S), in coastal pre-Puna and Puna regions.

Methods For phylogeographic analyses we analysed cytochrome *b* mitochondrial sequences for 29 specimens of the genera *Abrothrix* and *Phyllotis*, from the region of study. All results were analysed phylogenetically using maximum-likelihood, Bayesian, and uncorrected median-joining network methodology.

Results In *Phyllotis* we recognized two major clusters of taxa: one restricted to the Puna region identified as *Phyllotis xanthopygus chilensis*, in close association to a pre-Punean and lowland clade constituted by *Phyllotis limatus*, on the western slopes of the Andes. A similar pattern was distinguished for *Abrothrix*, where *Abrothrix andinus* was recognized in the Andean Altiplano-pre-Puna region and *Abrothrix olivaceus* in the lowlands of northern Chile.

Main conclusions We found that the radiation of sigmodontine mice in the central Andes may have been facilitated by the historical events that affected high Andean elevations during Pleistocene times, as well as changes in the vegetation composition and climate that started to prevail during that time. Our results also support previous hypotheses that the major mode of evolution for small mammals in the Andes region has been based on the founder effect or the peripheral isolates model, from a central range located in the Andes.

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Keywords

***Abrothrix*, Andean clade, Atacama Desert, cytochrome *b*, *Phyllotis*, phylogeography, Puna, sigmodontine mice.**

INTRODUCTION

The South American continent is characterized by a series of historical events that have affected the evolution and distribution of its biota (Potts & Behrensmeyer, 1992). The relative isolation of the continent during most of the Tertiary period (Darlington, 1965) allowed the differentiation and radiation of several groups of mammals, such as marsupials and edentates. The later re-connection of the continent to North America via the Panamanian Land Bridge allowed the entrance and radiation of several taxa such as carnivores, ungulates and sigmodontine rodents into South America (Marshall *et al.*, 1979, 1982). On the other hand, the great uplift of the 'Cordillera de los Andes' (by Plio-Pleistocene times) isolated a

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variety of lowland taxa, and created a corridor for dispersal of temperate and arid-adapted northern forms to reach southern latitudes (Vuilleumier & Monasterio, 1986; Webb, 1991). Furthermore, the orogeny of the Andes region and the climatic changes associated with Pleistocene glacial cycles allowed the formation of dry, sparsely vegetated areas such as the Andean Altiplano (= Puna), the Atacama and Coastal Desert, the Monte, the Chaco, and the Patagonia, thus providing new environments for the differentiation of local biota.

The small mammal fauna of northern Chile is mainly composed of sigmodontine mice, which are highly diverse and abundant and are distributed from the lowlands of northern Chile through the canyons that cross the Atacama Desert, the pre-Puna, up to high elevations of the Andes (Mann, 1978; Redford & Eisenberg, 1992). These rodents have been traditionally recognized as members of the Akodontini and Phyllotini tribes. The evolutionary history of these rodents in South America has long been debated with respect to the timing of the entry of this group onto the continent. Contending hypotheses postulate a tropical origin in North America, before spreading to South America by overland dispersal by Plio-Pleistocene times after the establishment of the Panamanian Land Bridge (Patterson & Pascual, 1972; Baskin, 1978; Simpson, 1980; Webb, 1991). Alternatively, recent calibrations based on molecular phylogeny suggested an origin prior to the formation of that Panamanian Land Bridge, *c.* 10 Myr BP (Spotorno, 1986), 5–9 Myr BP (Engel *et al.*, 1998), and 10 Myr BP (Smith & Patton, 1999). Despite these uncertainties, it is clear that by Pleistocene times, sigmodontine rodents were already widespread in South America, occupying most available biomes, particularly in the Andean area where major centres for diversification have been postulated (Reig, 1986). As indicated by Marshall *et al.* (1979), the Andes provided a major route for the dispersal of these taxa, via which they reached the southern cone of South America. However, it is not clear how these mice colonized the different biomes in this area in view of the fluctuating climatic conditions that characterized Pleistocene times. This becomes especially important for those biomes that are highly isolated within the continent such as the Atacama and the Coastal Desert of Peru.

Marquet (1989, 1994), Meserve & Kelt (1990), and Moreno *et al.* (1994) postulated that once sigmodontine rodents reached the central Andean Puna highlands or Altiplano, they colonized the Argentinean biomes as well as the lowland Atacama and Coastal Peruvian desert and migrated south to central and southern Chile, across the Pacific slope of the Andes. This hypothesis is supported by the existence of sigmodontines physiologically adapted to the harsh conditions imposed by the Atacama and Peruvian coastal desert (Bozinovic & Marquet, 1991), by palaeobiogeographical patterns in Pleistocene fossil mammals, by the widespread colonization of desert habitats by these rodents (Marquet, 1994; Marquet *et al.*, 1998) and by the palaeoclimatic evidence for the existence of Pleistocene as well as Holocene pluvial phases in the Atacama desert (Sylvestre *et al.*, 1999; Betancourt *et al.*, 2000; Latorre *et al.*, 2003). More recently, Steppan (1998) analysed the phylogenetic relationships and species limits within the genus *Phyllotis* (which is a member of the Phyllotine tribe within the Sigmodontinae) present in the lowland Atacama and Peruvian coastal desert, as well as in the high Andean area or Altiplano. He suggested that lowland taxa along the Pacific slope of the Andes were derived, via peripatric speciation, from the widespread high altitude taxon *Phyllotis xanthopygus* (Waterhouse). However, as Steppan (1998) acknowledged, more detailed geographical sampling would be required to test this model.

In this paper, we attempt to provide an assessment of the phylogeographic history of *Phyllotis* and *Abrothrix* rodents in the central Andes of Chile. Specifically, we evaluated the phylogeographic relationships and the degree of differentiation of representative species of *Phyllotis* and *Abrothrix* distributed in northern Chile and southern Perú. We tested the hypothesis that sigmodontine mice became differentiated in the lowlands, most likely via peripatric speciation. This would have occurred, for example, if mice originally dispersed from highland to lowland areas, across the desert vegetated canyons, thus reaching the Pacific coast (Marquet, 1994; Moreno *et al.*, 1994; Palma, 1995). Dispersing individuals may have found favourable habitats along these valleys in northern Chile, that connect the high altitude Puna and pre-Puna with the lowlands (e.g. Quebrada de Camarones and Quebrada de Tarapacá; Marquet, 1989; Meserve & Kelt, 1990). The differentiation of small mammals may have occurred due to the cyclic episodes of wet and dry periods in northern Chile that occurred during Pleistocene times, which may have induced the formation of local refuges and the restriction of distributional ranges. Within this scenario, small mammals may have speciated or differentiated in local refuges, constituting sister taxa accross the altitudinal range (lowlands vs. Puna highlands). Furthermore, we would also expect that populations distributed across the lowlands, or across the Puna, would be more phylogenetically related across the same elevations than between highland (Puna) and lowland areas. Thus, by sequencing the cytochrome *b* mitochondrial gene we should be able to recognize phylogeographical haplogroups representing differentiated taxa across the altitudinal gradient. However, a lack of differentiation of populations between the highlands and the lowlands would suggest that climate cycles were not strong enough to restrict the gene flow across populations, along the altitudinal gradient.

MATERIALS AND METHODS

Study area

The Puna region is located between 15 and 27° S latitude, at an elevation that fluctuates between 3800 and 4500 m in the central Cordillera de los Andes of South America. This biome is characterized by a mean annual precipitation of 150–230 mm and vegetation composed of steppe grasses such as *Festuca* and *Stipa*, as well as cushion plants such as *Azorella*

(Negrete-Córdova, 1997). Between 3200 and 3800 m the precipitation regime decreases to between 70 and 150 mm and tolar shrubs (e.g. *Parastrephia* spp. and *Chuquiraga* spp.), some columnar cacti, and summer annual plants in the Poaceae and Asteraceae are characteristic (Kalin-Arroyo *et al.*, 1997). The pre-Puna region is located between 2600 to 3200 m and the mean annual precipitation ranges from 20 to 70 mm, supporting salt tolerant shrubs (*Atriplex*), cushion cacti (*Opuntia*) and a few annual plants (Negrete-Córdova, 1997). At lower elevation, the pre-Puna region gives way to the Atacama Desert, a barren landscape that lacks vegetation due to decades without any precipitation.

Specimens examined

We analysed the cytochrome *b* mitochondrial gene in 29 sigmodontine mice of the genera *Abrothrix* and *Phyllotis*. The specimens examined and sequenced per locality are listed in Appendix S1, including the GenBank accession number for each sequence. Figure 1 shows the localities sampled in northern Chile. Taxonomy to recognize *Abrothrix* and *Phyllotis* in the area of study followed methods by Mann (1978); Steppan (1998) and Smith & Patton (1993, 1999). We recognized the following species and subspecies: *A. olivaceus* (Waterhouse), *A. andinus* (Philippi), *Phyllotis xanthopygus rupestris* (Gervais), *P. x. chilensis* Mann and *P. limatus* Thomas. In order to identify the clades recovered in our analyses, we included the published GenBank sequences of *P. limatus* (Arequipa, Perú, AF484208), *P. x. rupestris* (Toconao, Chile, AF484211; Talabre, Chile, AF484210); *A. olivaceus* (La Serena, Chile, AF027305), *A. andinus* (Arequipa, Perú, AF108671, M35713). For outgroup comparisons we used the published GenBank sequences of *Abrothrix longipilis* (Waterhouse) (U03530) and *Chroeomys jelskii* (Thomas) (AY275114) for *Abrothrix*, and *Phyllotis magister* Thomas (Tacna, Perú, U86824) for *Phyllotis*.

For the goals of this paper, we identified three major altitudinal regions that constituted collecting sites for small mammals in southern Peru and northern Chile (the exact locations and geographical coordinates are give in Appendix S1): (1) lowlands (populations located from sea level to 2400 m) – Tacna (Perú), Camarones, Tarapacá, Tamarugal, Río Loa, La Serena; (2) pre-Puna (populations between 2400 and 3500 m) – Arequipa (Perú), Zapahuira, Socoroma, Talabre, Tignamar, Alto Belén, Toconao; (3) Puna (over 3500 m) – Putre, Chungará, Parinacota, Suricayo, Enquelga and Arroyo Coya.

Figure 1 Map of northern Chile (Tarapacá and Antofagasta Regions) showing the localities sampled for small mammals in the altitudinal gradient from the coast to the Puna.

DNA sequencing

DNA was extracted from frozen tissue (mainly the liver) according to the techniques outlined in Longmire *et al.* (1988) and Laird *et al.* (1991), or from museum skins using Chelex (Walsh *et al.*, 1991). We amplified the cytochrome *b* mitochondrial gene via the polymerase chain reaction (PCR; Saiki *et al.*, 1988) using Taq DNA polymerase (Gibco-BRL, Rockville, Maryland, USA) and the following combination of primers per taxon: *Abrothrix*: L 14724 (Kocher *et al.*, 1989), H 15915 (Irwin *et al.*, 1991), MVZ 35 (Smith & Patton, 1993), MVZ 04 (Smith & Patton, 1993); *Phyllotis*: L 14724 and H 15767 (Edwards *et al.*, 1991). The names of the oligonucleotides indicate the light (L) and the heavy (H) strands and the position of the 3′ end of the oligonucleotide according to the mouse mtDNA sequence (Bibb *et al.*, 1981). The PCR was performed using the following thermal profiles (*Abrothrix*): 94 °C denaturation (1 min), 56 °C annealing (23 s), 72 °C extension (1 min) for 23 cycles; (*Phyllotis*): 94 °C denaturation (1 min 30 s), 56 °C annealing (20 s), 72 °C extension (1 min 30 s) for 15 cycles. Double-stranded PCR products were purified with the method of QIAquik (Qiagen, Valencia, California, USA). Sequencing was conducted through cycle sequencing (Murray, 1989) using primers L 14724, MVZ 35, L 15162 (Irwin *et al.*, 1991), H 15767, and H 15915 labelled with the Big Dye Terminator Cycle Sequencing Ready Reaction kit of Applied Biosystems (Foster City, California, USA). The sequencing reactions were then analysed in an ABI Prism 310

automated sequencer (Applied Biosystems). The PCR products were in many cases sequenced twice to ensure sequence fidelity. Cytochrome b sequences were aligned by eye and with the codon position option available in MacClade (Maddison & Maddison, 1992). All sequences were entered into GenBank and accession numbers can be found in Appendix S1.

Phylogenetic analyses

We used the maximum-likelihood (ML) algorithm and the Bayesian Metropolis-coupled Markov Chain Monte Carlo simulation (MCMC) method to reconstruct the phylogenies for *Phyllotis* and *Abrothrix*. The ML algorithm was performed using PAUP 4.0 (Swofford, 2002) and the best fitting model of sequence evolution was selected using the Akaike Information Criterion (AIC; Akaike, 1974) in Modeltest 3.06 (Posada & Crandall, 1998). The AIC indicated that for *Phyllotis* the TRN + *I* was the best model to describe the evolutionary process using cytochrome *b* sequences, whereas GTR + *G* was the best model for *Abrothrix*. Values for *Phyllotis* were: $-\ln L = 2589.5054$, AIC = 5189.0107, with base frequencies A = 0.2934, C = 0.3040, G = 0.1262, T = 0.2763; substitution model A–C = 1, A–G = 14.4852, A–T = 1, C–G = 1, C–T = 13.1472, G–T = 1. For *Abrothrix* values were: $-\ln L = 2697.9373$, AIC = 5413.8745, base frequencies A = 0.2797, C = 0.2975, G = 0.1208, T = 0.3021; substitution models A–C = 0.7586, A–G = 14.6297, A–T = 1.9646, C–G = 0.1839, C–T = 10.6989, G–T = 1. The optimal base composition, substitution rate matrix and among site substitution rate heterogeneity parameters were simultaneously estimated during the ML heuristic search. The proportion of invariable sites (*I*) was 0.7058 for *Phyllotis* whereas the gamma distribution shape parameter (*G*) for *Abrothrix* was 0.2017. Reliability of nodes was estimated by ML bootstrap percentages (Felsenstein, 1985) obtained after 100 pseudo-replications using the previously estimated ML parameters with TBR branch swapping. Trees were rooted with the outgroup criterion. For *Phyllotis* we used *P. darwini* Waterhouse and *P. magister* as outgroups following Steppan's (1998) phylogeny of the genus, whereas for *Abrothrix* we used *C. jelskii* and *A. longipilis*, *sensu* Smith & Patton (1999) and D'Elía (2003).

The Bayesian analysis was performed using the program MrBayes 3.0 (Huelsenbeck & Ronquist, 2001). The TrN + *I* and the GTR + *G* models used were obtained from the AIC in ModelTest (Posada & Crandall, 1998) to infer the simplest best-fit model of evolution for *Phyllotis* and *Abrothrix*, respectively. To explore the parameter space more thoroughly, for both sets of data, we ran the model with four incrementally heated chains, using the default values. From a random starting tree we ran 300,000 generations with the resulting trees sampled at every tenth generation (saving 30,000 trees). We determined when stationarity was reached (to discard the burn-in samples) by plotting the log likelihood scores of sample points against generation time. The first 10,000 generations (1000 trees) were discarded as burn-in and only the results from the last 270,000 generations (29,000 trees) were used to compute a consensus tree. The percentage of samples that recover any particular clade on this tree represented that clades's posterior probability; these were the *P* values, and $P \geq 95\%$ was considered evidence of significant support for a clade (Huelsenbeck & Ronquist, 2001).

The frequencies of nucleotide bases, and the transition/transversion rates were obtained using MEGA (Molecular Evolutionary Genetic Analysis, version 2.1; Kumar *et al.*, 2001). We considered whole transitions (ts) and transversions (tv) for all codon positions using the Tamura-Nei (TN) model of nucleotide evolution, available in MEGA. To portray relationships among *Phyllotis* and *Abrothrix* haplotypes, uncorrected median-joining networks were computed using the program NETWORK version 4.1 (Rohl, 2000).

Estimation of divergence times

We calculated a molecular clock for the composite species resulting in each of the taxonomic groups analysed with MEGA version 2.1 (Kumar *et al.*, 2001). We tested the selective neutrality of the sequences by estimating the sequences' *F* value (Fu & Li, 1993). Additionally, to check for deviations from neutral evolution of our sequences, significance values were calculated for Tajima's (1989) *D* parameter using the DnaSP program, version 4.1 (Rozas *et al.*, 2003). These tests did not reject neutral expectations for the total data set ($F = -0.6560$; $P > 0.1$ for *Phyllotis* and $F = -0.6945$; $P > 0.1$ for *Abrothrix*; $D = -0.1828$; $P > 0.1$ for *Phyllotis* and $D = -0.7018$; $P > 0.1$ for *Abrothrix*). For sequence divergence, we calculated the overall tv and ts in all codon positions (instead of just tv substitutions at the third position) as in most cases we were comparing representative forms of the same species or closely related species, implying a recent time of differentiation. For *Abrothrix* we calibrated a rate based on the *Akodon/Thaptomys-Bolomys* split at 3.5 Myr (Smith & Patton, 1999), and the rate obtained was 0.0465 per million years. For *Phyllotis* we calibrated the rate based on the *Auliscomys-Loxodontomys* split at 4.5 Myr (Pardiñas & Tonni, 1998; Smith & Patton, 1999), and the rate obtained per million years was 0.0345.

RESULTS

Phyllotis variation

By using Likelihood and Bayesian reconstructions (Fig. 2) we recovered identical topologies: a clade that included both lowland and pre-Punean localities of Tarapacá, Socoroma, Tamarugal, Tignamar, Alto Belén, Arequipa and Zapahuira. To the latter clustering, the representative forms from Toconao and Talabre joined. The other major association came from specimens representing localities from the Puna region: Arroyo Coya, Suricayo, Enquelga, Chungará and Parinacota. Basal nodes representing lowland/pre-Puna and Puna clades were

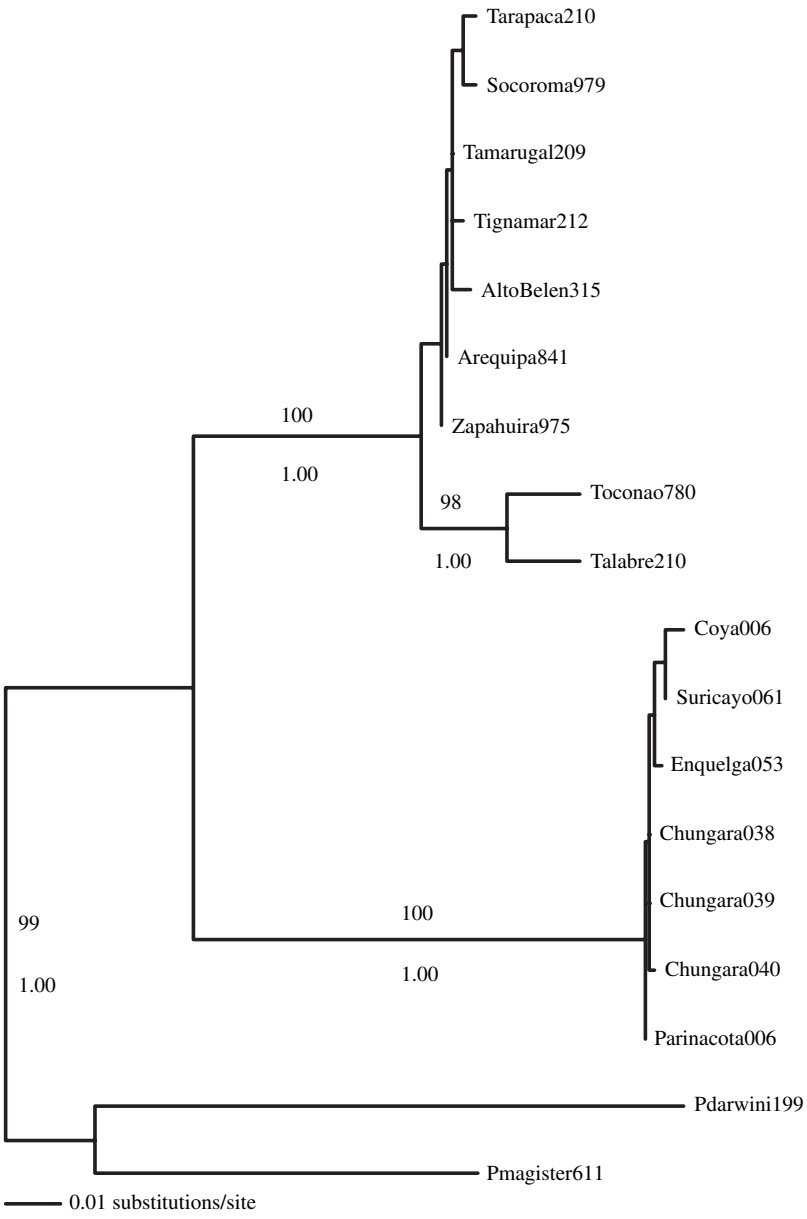

Figure 2 Maximum-likelihood and Bayesian tree based on the TrN + *I* model for the cytochrome *b* mitochondrial gene of *Phyllotis*. Numbers above the nodes represent 100 bootstrap replicates and below the posterior probability values.

characterized by presenting high bootstrap and posterior probability values, as shown by the values obtained for the split between the latter two clades (Fig. 2).

The uncorrected median-joining network analysis depicted a similar pattern as shown by likelihood and Bayesian trees in northern Chilean (and southern Peruvian) Andes (Fig. 3). The network tree recovered a pre-Puna lowland group of haplotypes representing *P. limatus* (black circles) separated by more than 70 mutational steps from the Puna haplotypes represented by *P. x. chilensis* (grey circles). Mutational changes within *P. limatus* were as high as 6 changes, and within *P. x. chilensis* 8 changes. In Fig. 3, *P. x. rupestris* (in black circles represented by Toconao and Talabre localities) was found to be closely related to *P. limatus* with only 27 nucleotide changes. Haplotypes representing *P. magister* and *P. darwini* (white circles) were also recovered more than 70 mutational steps away.

The transition–transversion rate within *Phyllotis* was 4.3, also showing a strong bias towards AG-type changes. Sequence divergence between both major clades of *Phyllotis*, that constitute the lowlands and pre-Puna populations vs. that of the Puna, was found to be 10%; between *P. x. rupestris* and *P. x. chilensis* that value was 10.7% and between *P. limatus* and *P. x. rupestris* (Toconao and Talabre) it was found to be 3.3%. Within clades, variation was found to be 1.61% for the lowland/pre-Puna clade and 0.3% for the Puna clade. The divergence time calibrated between the lowland/pre-Puna-Toconao/Talabre clades was found to be 478,000 yr BP, and between the lowland/pre-Puna and the Puna clades it was found to be *c.* 1.5 Myr BP.

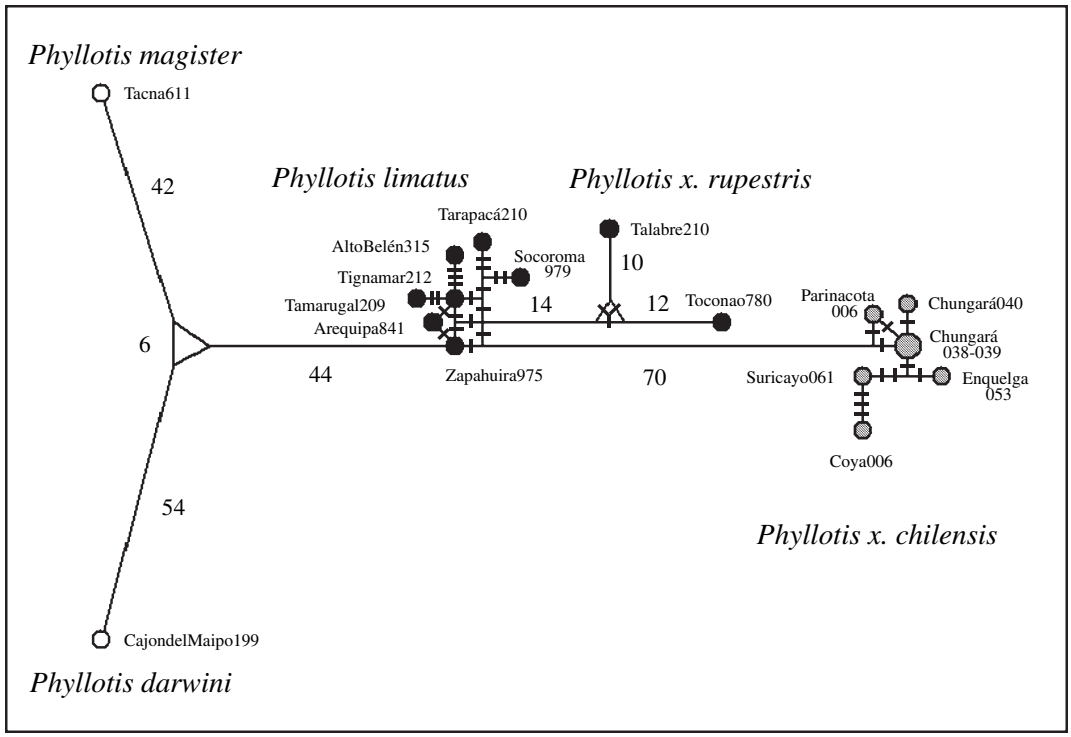

Figure 3 Uncorrected median-joining network showing phylogenetic relationships between haplotypes of the *Phyllotis*. Circled areas are approximately proportional to the number of individuals bearing a particular haplotype. Branch lengths are proportional to the number of mutations involved between haplotypes. Mutations are indicated as ticks with the longest branches represented by numbers. Seventy steps is the maximum number of mutations shown by the uncorrected median-joining network analysis.

Abrothrix variation

Both the Bayesian and the likelihood trees distinguished two major clusters in the topology (Fig. 4): one representing specimens from lowland coastal regions in northernmost Chile, such as Quebrada de Camarones, Quebrada de Tarapacá and Río Loa and the representative locality of La Serena. Sister to that grouping, three representatives from Arequipa (Perú) and Putre (Chile) were recovered. The network analysis also agreed with likelihood and Bayesian trees in representing two major groups of haplotypes (Fig. 5): one representing *Abrothrix olivaceus* from the lowlands (black circles) and the other represented by *A. andinus* (grey circles). *Abrothrix andinus* was differentiated by 43 mutational steps from *A. olivaceus*, whereas within *A. olivaceus* and *A. andinus* the mutational steps were as high as 24 and 23 mutational steps, respectively. Haplotypes representing *C. jelskii* and *A. longipilis* (white circles) were also recovered more than 70 mutational steps away.

The transition–transversion rate for *Abrothrix* was 4.8, with AG transitions the most frequent type of change. Sequence divergence between *A. andinus* and the lowland forms reached 9.0%. Intraspecific variation was found to be 1.7% for the lowland clade and 2.4% for the *A. andinus* clade (see Fig. 4). Divergence between *A. andinus* (represented by Putre and Arequipa) and the lowland clade was estimated to have occurred *c.* 1.0 Myr BP.

DISCUSSION

Phylogenetic analyses

Phyllotis

Phylogenetic analyses found two well resolved groupings for *Phyllotis* in northern Chile: one that reunited populations from the coastal-western slopes of the Andes, up to middle elevations in the mountains or pre-Puna, and the other restricted to Puna areas. When collecting *Phyllotis* in the canyons, lowlands, and middle elevational areas, up to *c.* 3000 m in northern Chile, these specimens were initially identified as *P. x. rupestris* based on the pelage coloration (lighter than *P. x. chilensis*), teeth morphology (deep and narrow incisors when compared to other *Phyllotis* spp. from northern Chilean elevations) and the geographical distribution ascribed to this taxon by Steppan (1995). On the other hand, the forms that we collected in the Puna, at elevations over 4000 m, were characterized by shallow and wide incisors and had darker dorsal pelage in contrast to *P. x. rupestris*, resembling *P. x. chilensis* (Steppan, 1998). Steppan (1998) found that using cytochrome *b* data there was a sequence divergence value of 8.5% between *P. x. rupestris* from the western slopes of the Andes and *P. x. chilensis*, with bootstrap scores that varied between 87% and 100% for the nodes that differentiate both taxa. That sequence divergence was similar

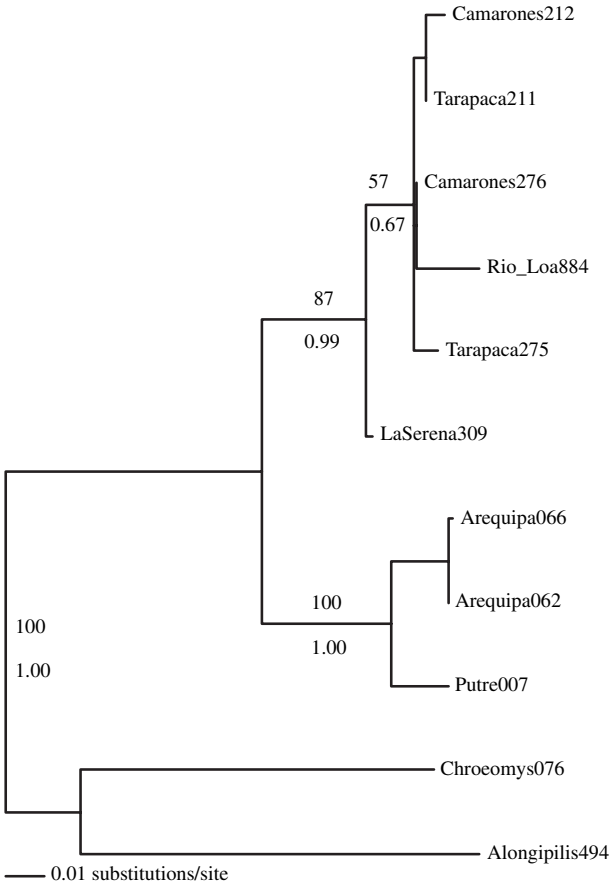

Figure 4 Maximum-likelihood and Bayesian tree based on the GTR + G model for the cytochrome *b* mitochondrial gene of *Abrothrix*. Numbers above the nodes represent 100 bootstrap replicates and below the posterior probability values.

to the mean value found for other species of the Phyllotini. This allowed Steppan (1998) to raise the taxonomic status of western-slope *rupestris* along with *P. darwini limatus* (from the southwest of Peru) to the specific level, *limatus* being the oldest available name. In this study, we report a sequence divergence value for the *limatus-chilensis* dichotomy of 10%, which agrees with the distance values reported by Steppan (1998), for different species within the Phyllotini tribe. Therefore, our results support the occurrence of *P. limatus* inhabiting the western slope of the Andes, the pre-Puna region, down to lowland and coastal localities of northern Chile and southern Peru. In fact, when we included in our analyses the sequence identified as *P. limatus* from Arequipa, Peru (Kuch *et al.*, 2002), this joined with the pre-Punean western slope clade. The representatives from Toconao and Talabre that joined beside the *P. limatus* clade, could well represent a subspecies of the latter taxon given that the mean sequence divergence of that clade falls within that taxonomic category (Steppan, 1998). In our analysis the average sequence divergence of the latter taxon was found to be 3.3% with respect to the *P. limatus* clade.

Although Steppan (1998) recognized *limatus* at the specific level, he did not find strong arguments to differentiate between *P. x. chilensis* and *P. limatus* at the specific level, concluding that additional morphological and molecular data are needed to clarify the dichotomy of both taxa. Interestingly, based on our topologies, which clearly separate populations from the Puna region (*P. x. chilensis*) and those of the western slopes of the northern Andes (*P. limatus*), as well as our findings of the strong bootstrap, posterior probabilities, mutational steps and level of sequence divergence values that also add evidence to support this split (10%), we believe that *chilensis* should be raised to the species level, restricted to higher elevations

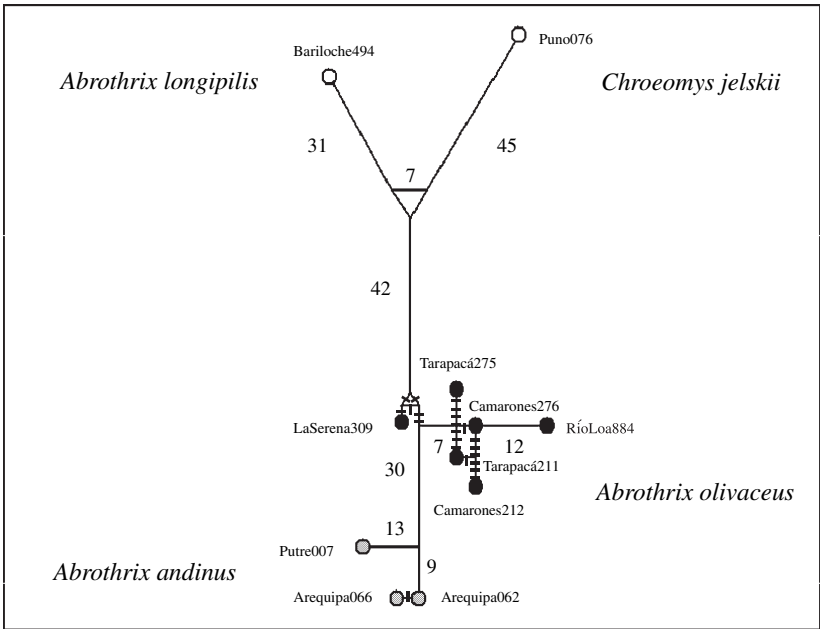

Figure 5 Uncorrected median-joining network showing phylogenetic relationships between haplotypes of the *Abrothrix*. Circled areas are approximately proportional to the number of individuals bearing a particular haplotype. Branch lengths are proportional to the number of mutations involved between haplotypes. Mutations are indicated as ticks with the longest branches represented by numbers. Seventy steps is the maximum number of mutations shown by the uncorrected median-joining network analysis.

(4000 m) in the Altiplano, and different from *P. limatus*, from the western Andean slopes.

Abrothrix

Following the pattern found for *Phyllotis* using cytochrome *b* sequences, two well-supported clades were also obtained for *Abrothrix*. One of them reunited specimens representing populations from the lowlands (which included the GenBank sequence of *Abrothrix olivaceus* from La Serena, Chile), showing that this species is also represented in the lowland regions of northern Chile. This topology was sister to a group representing *A. andinus* from Arequipa (Perú) and Putre (Chile) with 100% bootstrap support. Our results have shown that the two *Abrothrix* species are present in the lowlands, the canyons (*A. olivaceus*) and the pre-Puna and Puna regions of northern Chile (*A. andinus*). The TN sequence divergence obtained for both *Abrothrix* taxa representing that clade was found to be 9.0%, which is similar to that reported for other species (10%). Smith & Patton (1999) recognized this as the 'Andean clade', mostly constituted by *Abrothrix* taxa, and that it forms an apparent monophyletic group (D'Elía, 2003).

Biogeography and speciation

Reig (1986) found that the area of radiation for phyllotines was the southern Altiplano or the central Andes (Steppan, 1995). To date, of the 46 currently recognized species in the tribe, the majority of them are distributed in the 'Altiplano'. Some features, such as the karyotype number and morphology support the ancestral position of several phyllotine taxa (Spotorno *et al.*, 2001). In fact, the occurrence of telocentric karyotypes and the elevated number of chromosomes for selected forms have been traditionally considered ancestral characters in phyllotine evolution, and most of these taxa are restricted to the Andean Altiplano region of South America (Pearson & Patton, 1976; Spotorno *et al.*, 2001). On the other hand, the radiation of 'abrothrichines' (consisting of six genera) has also been hypothesized to have originally occurred in the central and southern Andes of Peru, Bolivia, Chile and Argentina (Reig, 1986; Smith & Patton, 1999).

The differentiation of *Phyllotis* spp. in the central Andes, has been hypothesized to have occurred via peripatric speciation from a widely distributed central phyllotine taxon. Peripheral isolates then dispersed to the Chilean and Peruvian Coastal deserts, with further dispersal to the north and south, to colonize semi-arid and temperate latitudes both in Peru and Chile. It is reasonable to propose a similar scenario of coastal dispersal through peripheral isolates for the Andean clade, constituted by *Abrothrix* ('Abrothrichini'). The latter taxon is only found in the southwest of the Andes and the species treated in our study as *A. olivaceus* and *A. andinus* constitute sister taxa (Smith & Patton, 1999). The same is true for *Phyllotis*, a genus only found in the western Andes, where *P. x. chilensis* is the sister taxon to *P. limatus*. Smith *et al.* (2001), in a study of the southernmost *Abrothrix olivaceus/xanthorhinus* complex in Chile and Argentina, found that northern populations of *A. olivaceus* were in an ancestral position with respect to southern forms and suggested that they might have remained isolated before migrating south, thus creating the differentiation in southern latitudes. These facts, together with physiological and palaeoecological evidence (see Introduction), coupled with our results, support the hypothesis that sigmodontines colonized the lowland Atacama and the Peruvian desert from the Puna region (Marquet, 1989, 1994; Meserve & Kelt, 1990; Moreno *et al.*, 1994; Steppan, 1998). It is possible that dispersal event may have occurred across the canyons that traverse the desert and reach down to the coastal areas. This hypothesis is preferred to the alternative, which suggested that sigmodontines from the Altiplano dispersed to the Monte in Argentina, entering central Chile across valleys, before reaching north up to Atacama (Caviedes & Iriarte, 1989).

The taxonomic differentiation between *P. limatus* and *P. x. chilensis* obtained in the phylogenetic analyses is congruent with the distributional limit of both taxa (lowlands/pre-Puna and Puna, respectively), as well as with the climate and vegetational composition of the pre-Puna and Puna areas in the central Andes. The occurrence of both forms could also be the result of a peripheral isolate speciation model (as proposed by Steppan, 1998) by which an ancestral species gave rise to *P. x. chilensis* in the Puna region, and to *P. limatus* in the western slopes. The latter hypothesis of speciation (calibrated at *c.* 1.5 Myr BP) in both Andean habitats was confirmed by our data; the phylogenetic and the median-joining network analyses distinguished between two major clusters representing two clusters of haplotypes for the split between *P. limatus* and *P. x. chilensis*. Our results have shown that *chilensis* could be found as far south as the locality of Arroyo Coya, in the Puna of the II Region of Chile, at an altitude of 3700 m (see Fig. 1). Pizzimenti & De Salle (1980) found that *P. limatus* differed from *P. x. chilensis* in that the former had a diet richer in insects and seeds, whereas *P. x. chilensis* had a diet richer in abrasive grasses and a higher diversity of forbs.

The molecular variation detected in the major splits of *Phyllotis* and *Abrothrix* took a 'clock-like' mode. In fact, whereas the sequence divergence time between *A. andinus* and *A. olivaceus* was calibrated as 1.0 Myr BP involving *c.* 40 mutational steps between both haplogroups, the sequence divergence time between *P. limatus* and *P. x. chilensis* was set *c.* 1.5 Myr BP, with more than 70 mutational steps between the latter two taxa. Thus, this implied that the observed molecular variation detected between each pair of haplogroup taxa was a function of the divergence time between clades, suggesting that at least for the phyllotines analysed, they have had more time to accumulate mutations than *Abrothrix*. Thus, the molecular time estimated for the split between the upland/lowland divergence falls within a Pleistocene timeframe for the differentiation of *Phyllotis* and *Abrothrix*, in the west central Andes.

Recent fossil midden studies and wetland deposits seem to suggest the occurrence of strong habitat contractions of local

biota, during the late Holocene when current hyperaridity conditions became prevalent in what is now the central Atacama Desert (22–24° S; Betancourt *et al.*, 2000; Latorre *et al.*, 2003). Higher elevation shrubs and summer-flowering grasses expanded downslope from the Andes, in northern Chile, across what is now the Atacama Desert. In a recent study, Kuch *et al.* (2002) sequenced different fractions of mitochondrial DNA genes (cytochrome *b* and the 12S rRNA) from rodent and other mammal middens in the Atacama Desert (e.g. Lomas de Quilvar, Salar de Atacama). They identified the occurrence of a camelid, *Vicugna vicugna*, and the caviomorph rodent *Abrocoma cinerea*, species that inhabit the high Andean grasslands (Marquet *et al.*, 1998; Palma *et al.*, 2001). Furthermore, Kuch *et al.* (2002) reported the occurrence of *P. limatus*, which is not currently present in the midden study area, but *c.* 100 km north, suggesting that this taxon experienced a strong range contraction. This sub-fossil evidence supports the hypothesis of a former wide distribution of a mammal fauna which today is restricted to more mesic conditions in the 'quebradas' or canyons, the pre-Puna and Puna areas of the central Andes.

Our results confirm the occurrence of part of the Andean clade in northern Chile, with two representative taxa: *A. olivaceus* and *A. andinus*. The first species is characterized by having one of the largest ranges in Chile, encompassing most of the north, the central valley and the southern tip of the country, whereas *A. andinus* is restricted to high elevations in the pre-Puna and Altiplano regions of the Andes.

CONCLUSIONS

We conclude that there has been a three-way radiation of small mammals in northern Chile: one that restricted populations to high Andean elevations as is the case of '*Phyllotis x. chilensis*' in the Puna (which should be recognized at the specific level), another that resulted in a downward connection of the latter area with pre-Puna habitats (e.g. *Abrothrix andinus*), and a third that reached the lowlands, e.g. *Phyllotis limatus*. Puna and pre-Puna radiation of small mammals may be the result of habitat change during the major uplift of the central Andes during Plio-Pleistocene times, through the glaciation cycles of the Quaternary period, when the vegetation and climate shifts occurred differentiating pre-Puna and Puna environments. The route to reach lower elevation areas for the latter two taxa would have been facilitated by the occurrence of the transverse vegetated areas, which run from upper to lower elevations in northern Chile and that cross the Atacama Desert. Gene flow for these populations may have been facilitated by the fact that the canyons connect lowland and highland areas.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article from http://www.Blackwell-Synergy.com:

Appendix S1 Specimens examined.

BIOSKETCHES

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