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RESEARCH ARTICLE

Range expansion of *Oligoryzomys longicaudatus* (Rodentia, Sigmodontinae) in Patagonian Chile, and first record of Hantavirus in the region

Ampliación del rango de distribución de *Oligoryzomys longicaudatus* (Rodentia, Sigmodontinae) en la Patagonia de Chile y primer registro de Hantavirus en la región

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RESUMEN

Actualmente se reconocen 20 especies de *Oligoryzomys* (Rodentia, Sigmodontinae) en la región Neotropical, la mayoría de ellas distinguidas por sus cariotipos, los que fluctúan entre 46-70 cromosomas. En Chile se reconocen dos especies, *Oligoryzomys longicaudatus* (Bennet, 1832; "colilargo"; 2n = 56), desde los 27º S hasta aproximadamente 51º S, y *Oligoryzomys magellanicus* (Bennet, 1836; 2n = 54), al sur de los 51 º S en la región Patagónica de Chile y Argentina. Como parte de una investigación en curso en la Patagonia sur de Chile, reportamos los resultados de muestreos de pequeños mamíferos en seis localidades. Cariotipamos 28 especímenes y secuenciamos la región hipervariable I del mtDNA en 22 individuos, alineando estas secuencias con una filogenia de *O. longicaudatus* en desarrollo. Adicionalmente evaluamos la serología y la presencia de carga viral en todos los especímenes capturados con el objeto de detectar la presencia de anticuerpos anti-Andesvirus (ANDV) mediante Strip Immunoblot Assay (SIA), y de genoma viral mediante RT-PCR. Los resultados muestran consistentemente que el cariotipo de los especímenes de la Patagonia sur de Chile es 2n = 56, similar al de *O. longicaudatus*, y que los individuos de esta región no se diferencian filogenéticamente de aquellos del norte del rango de distribución de esta especie. Adicionalmente, los análisis serológicos demostraron la presencia de anticuerpos IgG anti-ANDV y de genoma viral en corazón, riñón, bazo y pulmones de un especimen de *Oligoryzomys* de la localidad de Fuerte Bulnes en la Región de Magallanes. Concluimos que todos los especímenes capturados al sur de los 51º S corresponden a *Oligoryzomys longicaudatus,* expandiendo así el rango de distribución de esta especie hasta al menos 55º S. Los resultados también extienden la distribución de la cepa Andes de Hantavirus al extremo sur de la Patagonia.

Palabras clave: "colilargo", Hantavirus, *Oligoryzomys longicaudatus*, *O. magellanicus*, Patagonia.

ABSTRACT

At present, 20 species of *Oligoryzomys* (Rodentia, Sigmodontinae) are recognized in the Neotropical region, most of them distinguished by their karyotypes, which fluctuates between 46-70 chromosomes. Two species are currently recognized in Chile, *Oligoryzomys longicaudatus* (Bennet, 1832; "colilargo" or the long-tailed pygmy rice rat; 2n = 56), which ranges from 27° to approximately 51° S, and *O. magellanicus* (Bennet, 1836; Magellanic pygmy rice rat; 2n = 54), south of 51° S in the Patagonian region of Chile and Argentina. As part of an ongoing research on the southern Patagonia of Chile, we report the results of small mammal samplings in six localities. We karyotyped 28 specimens and we also sequenced the hypervariable mtDNA region I in 22 individuals, aligning these sequences with an under development phylogeny of *O. longicaudatus*. We also evaluated the serology and viral charge in all captured specimens to detect the presence of antibodies to Andes virus (ANDV) through Strip Immunoblot Assay (SIA), and of viral genome by RT-PCR. The results consistently showed that the karyotype of southern Patagonia specimens was 2n = 56, equal to that of *O.*

longicaudatus, and that individuals from this area do not differentiate phylogenetically from those of the northern range of distribution. In addition, the serology showed the presence of antibodies IgG anti-ANDV and of viral genome in heart, kidney, spleen, and lungs of a single specimen of *Oligoryzomys* from the locality of Fuerte Bulnes in the Magallanes region. We conclude that all specimens trapped south of 51° S correspond to *Oligoryzomys longicaudatus*, thus expanding the distribution of this specieì from 51° to at least 55° S. The results also extended the disîribution of the Andes strain of Hantavirus to southernmost Patagonia.

Key words: "colilargo", Hantavirus, *Oligoryzomys longicaudatus*, *O. magellanicus*, Patagonia.

INTRODUCTION

The genus *Oligoryzomys* Bangs, 1900 includes small mice in the tribe Oryzomyini (Muridae: Sigmodontinae) and both morphological and molecular data support its recognition as a monophyletic lineage (Carleton & Musser 1989, Dickerman & Yates 1995, Myers et al. 1995, Weksler 2003, Palma et al. submitted). Until recently, 18 species were recognized in this genus (Musser & Carleton 2005), distributed throughout the Neotropics, from Mexico (e.g., *Oligoryzomys fulvescens* [Saussre, 1860]) to Patagonian Chile and Argentina (e.g., *O. longicaudatus* [Bennet, 1832]). One remarkable feature of *Oligoryzomys* is the variation in the diploid number (2n) across species, ranging from 46 to 70 chromosomes (Weksler & Bonvicino 2005). Most species within the genus differ in their diploid number; thus, karyotypes constitute a useful taxonomic tool at the species level for this genus. In fact, two new species, *Oligoryzomys moojeni* (Weksler & Bonvicino, 2005) and *Oligoryzomys rupestris* (Weksler & Bonvicino, 2005), have recently been described based on morphology and karyotypes extending the number of species up to 20.

In Chile, Osgood (1943) and Mann (1978) recognized a single species of *Oligoryzomys*, *Oligoryzomys longicaudatus* (Bennet, 1832; "colilargo" or long-tailed pygmy rice rat) with three morphologically-based subspecies whose ranges are more or less restricted to the three major ecogeographic regions of this country: *O. longicaudatus longicaudatus*, in the Mediterranean region from the Copiapó Valley (27° S) southward to the north of Concepción Province (37° S); *O. longicaudatus philippi*, in the temperate forests, from north of Concepción Province to approximately 50° S; and *O. longicaudatus magellanicus* in the Patagonian and Fuegian forests south of 50° S. However, cytogenetic analyses demonstrated the existence of two different karyotypes, 2n = 56 for *O. l. longicaudatus* and *O. l. philippi*, and 2n = 54 for *O. l. magellanicus* (Gallardo & Patterson 1985, Palma 1987). On this basis, Gallardo and Patterson suggested the occurrence of cytological incompatibility between *O. l. magellanicus* and the northern forms, leading to the interruption of gene flow between them. Later, Gallardo & Palma (1990), based on a further revision of the external, cranial, and bacular morphology, showed the existence of high morphological uniformity between *O. l. longicaudatus* and *O. l. philippi* and, simultaneously, a marked differentiation with individuals of populations in the range of *O. l. magellanicus*. On the other hand, phenetic allozyme analysis, comparing 15 loci in 60 specimens from 10 populations of *O. l. longicaudatus* and *O. l. philippi*, showed high levels of genetic uniformity (Palma 1987). Based on these data Gallardo & Palma (1990) reject the subspecific differentiation between the latter two nominal subspecies, recognizing a single species between 28 and 50° S, thus uniting *O. l. phillippi* and *O. l. longicaudatus*, as full synonyms of *O.longicaudatus*. They also concluded that *O. l. magellanicus* constituted a valid species distributed in the Patagonian and Fuegian forests south of 50° S. A recent phylogeographic study along the range of *O. longicaudatus* based on cytochrome b mitochondrial sequences (Palma et al. 2005), concluded a high degree of molecular homogeneity between populations of *O. longicaudatus*, suggesting high levels of gene flow along the species range. This homogeneous pattern agreed with previous data based on morphology, chromosomes, and isozymes (Palma 1987, Gallardo & Palma 1990), and encompassed localities as far south as Torres del Paine National Park (51° S; Palma et al. 2005). The latter study thus confirmed the existence of a single species of *Oligoryzomys* from the southern portion of the Atacama Desert to as far

south as 51° S in Chile (Fig. 1). The authors also suggested that *Oligoryzomys magellanicus* (Bennet, 1836) should be restricted to populations south of 51° S and/or the type locality (Harrison Island, 54° S).

Oligoryzomys longicaudatus is characterized by its long tail (almost twice the body length), large hind limbs, reduced ears, a general yellowish dorsal color pattern without a particular pattern, and whitish ventral coloration (Osgood 1943, Mann 1978). The species inhabits mainly mesic areas of the Temperate and Fuegian forests of southern Chile and Argentina. The ecology of *O. longicaudatus* is characterized by its high vagility and large home range, in contrast to coexisting species (e.g., *Abrothrix olivaceus*

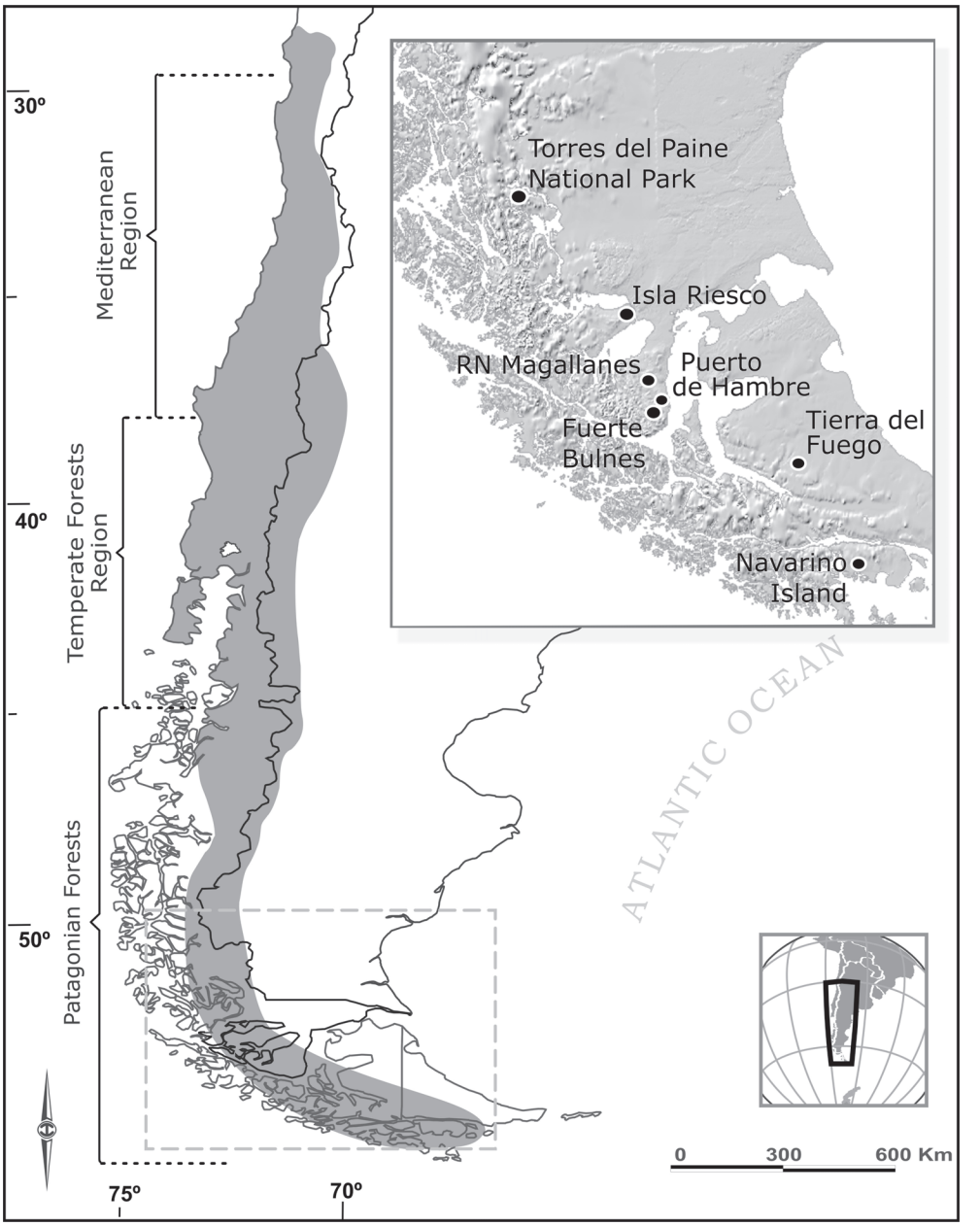

Fig. 1: Geographic distribution of *Oligoryzomys longicaudatus* and sampling sites in Patagonian Chile.

Distribución geográfica de *Oligoryzomys longicaudatus* y sitios de muestreo en Chile patagónico.

[Waterhouse, 1837]), particularly in the southern part of its range (Murúa 1986). *Oligoryzomys magellanicus*, on the other hand, is distinguished from *O. longicaudatus* by differences in its external, cranial and bacular morphology. Gallardo & Palma (1990) showed that specimens in the range of *O. magellanicus* varied according to ecogeographic rules, having darker coloration and shorter appendages (e.g., tail, feet) than *O. longicaudatus*. Also, the glans and baculum in *O. magellanicus* are significantly larger than in *O. longicaudatus*. However, no data are available concerning the ecology of the former species.

O. longicaudatus has been a focus of epidemiological research given this species is the main reservoir of the Andes strain of hantavirus (ANDV; Levis et al. 1998, Toro et al. 1998, Bohlman et al. 2002). The Andes virus is the etiologic agent of Hantavirus Pulmonary Syndrome (HPS), an emerging infectious disease first recorded in North America in 1993 (Nichol et al. 1993, Schmaljohn & Hjelle 1997), and later reported in Argentina and Chile (Levis et al. 1998, Toro et al. 1998). In the latter country, HPS cases have been reported throughout the range of *O. longicaudatus* since 1995, and the intraspecific seropositive rate of this species is about 4.5 % (Torres-Pérez et al. 2004). However, no cases of both HPS and seropositive rodents have been reported in southern Patagonia of Chile.

Despite its wide geographic distribution and its ecological and epidemiological importance, both the diversity of the genus *Oligoryzomys* and the taxonomic status of some of their species remain unclear. Thus, since populations of *Oligoryzomys* south of 51˚ S should correspond to *O. magellanicus* based on previous cytogenetic and morphologic studies (Gallardo & Patterson 1985, Gallardo & Palma 1990), and the fact that the range of *O. longicaudatus* has recently been extended as far south as Torres del Paine National Park (Palma et al. 2005), our study was designed to evaluate the taxonomy and systematics of populations of *Oligoryzomys* south of 51° S. We karyotyped specimens from five localities in southern Patagonian Chile in the vicinity of Punta Arenas, since Gallardo & Patterson (1985) reported a karyotype 2n = 54 for *Oligoryzomys* in this area. Additionally, we sequenced the hypervariable domain I of the mtDNA control region of specimens from populations between 51° and 55° S, aligning these sequences with those of an ongoing phylogeographic study in *O. longicaudatus*. We analyzed the latter results phylogenetically to evaluate if southern Patagonian populations constitute a different taxon with respect to *O. longicaudatus*. Simultaneously, as part of a long-term study on hantavirus and its reservoir rodents, we evaluated the presence of antibodies anti-ANDV and viral charge in all trapped specimens.

METHODS

Tissues and specimens analyzed

Small mammals were collected in January 2006 and 2007, in three localities in the vicinity of Punta Arenas, Magallanes region in the Patagonia of Chile (Fig. 1): Reserva Nacional Magallanes (53˚07'59" S, 71˚01'30" W); Puerto de Hambre (53˚36'09" S, 70˚56'26" W); Fuerte Bulnes (53˚37'42" S, 70˚55'19" W). In addition, we sampled three other localities to the north and south of Punta Arenas: Parque Nacional Torres del Paine (51˚21'33" S, 73˚05'37" W); Isla Riesco (52˚51'48" S, 71˚32'45" W); Tierra del Fuego, Reserva Karukinka (54˚04'06.1" S, 68˚42'57.1" W). For all of these samplings, Sherman traps were used. We also included two loaned specimens from Bahía Inútil (54˚59' S, 68˚13' W) and Parque Omora (54˚57' S, 67˚39' W), in Navarino Island, Magallanes region, and one from Bahía La Pataia, 10 km W from Ushuaia, Tierra del Fuego, Argentina, for nucleotide sequencing and phylogenetic analyses. A detailed list of specimens analyzed for chromosomes, DNA sequencing and serology is given in Appendix 1. Voucher specimens for both sequenced and karyotyped individuals were deposited in the Colección de Flora y Fauna Profesor Patricio Sánchez Reyes (SSUC), Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago, Chile, and the Museum of Southwestern Biology (MSB), Department of Biology, University of New Mexico. Tissues and other data were cross-referenced directly to each voucher specimen and stored in the collection using a special field number, the NK number used by the SSUC and the MSB. We followed ASM Guidelines during the collection and care of the animals used in this work (Animal Care and Use Committee 1998).

Diploid number determination

Chromosomal preparations were obtained from the bone marrow of 28 specimens from 5 localities, following Patton (1967). These preparations were later stained under the standard Giemsa-Phosphate Buffer method and ≥ 10 metaphasic spreads per specimen were counted for 2n determination. We excluded Torres del Paine of this analysis because Palma et al. (2005) already reported a 2n = 56 for *Oligoryzomys* in this locality. Chromosome morphology and fundamental numbers followed Patton (1967).

Nucleotide sequencing analysis

Genomic DNA was extracted from frozen liver of 22 specimens following the technique described by Longmire et al. (1988). We included specimens from the 6 sampled localities plus the 3 loan specimens from Navarino Island and Tierra del Fuego. The control region (approximately 1200 bp) of the mitochondrial DNA (mtDNA) was amplified using primers DLO-L (5' CGG AGG CCA ACC AGT AGA 3') and DLO-H (5' TAA GGC CAG GAC CAA ACC 3') with the following thermal profile (25 cycles): denaturation for 30 s at 94° C, annealing for 25 s at 57° C and extension for 1 min 30 s at 72° C. Double-stranded PCR products were purified with QIAquik (Qiagen). We sequenced only the hypervariable domain I of the mtDNA control region because previous studies have shown that the hypervariable domain II and the conserved central domain are less informative when analyzing intraspecific nucleotide variation (Avise 2000). Cycle sequencing (Murray 1989) was performed using primer DLO-L 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. These 22 Patagonian sequences were later aligned with sequences of 162 specimens as part of an ongoing phylogeographic study in *O. longicaudatus* using the ClustalW program (Higgins et al. 1996). A haplotypes matrix was generated using DnaSP 4.0 (Rozas et al. 2003), where the 184 sequences were grouped in 123 haplotypes, excluding outgroups.

Phylogenetic analysis

Haplotype sequences were phylogenetically analyzed using the maximum-likelihood optimality criterion (ML) available in PAUP* 4.0 (Swofford 2002) with the nearest neighbor interchange (NNI) branch swapping option and a heuristic search with 10 random replicates. To choose the best fitting model of sequence evolution we used Modeltest 3.7 (Posada & Crandall 1998). The corrected Akaike information criterion (AICc; Akaike 1974) identified the Kimura 81 unequal base frequencies + gamma model (K81uf+ G) as optimal (-lnL = 1251.2770, AICc = 2515.7539, G = 1.5780), with base frequencies A = 0.2868, C = 0.3132, G = 0.0670, T = 0.3329. Reliability of nodes in the ML tree was estimated by bootstrap analysis (Felsenstein 1985) obtained after 100 pseudo-replicates. We rooted the tree with the outgroup criterion, using *Oligoryzomys fornesi* (Massoia, 1973) and *Oligoryzomys andinus* (Osgood, 1914) since an ongoing phylogeny of the genus recovered these two taxa as part of the same clade that includes *O. longicaudatus* (Palma et al. unpublished data). The mean genetic distances between Patagonian and Temperate forests and Mediterranean sequences were evaluated using the maximum-likelihood distance option available in PAUP* 4.0, including the model obtained through Modeltest.

Virological analyses

We evaluated the presence of antibodies anti-ANDV in 69 trapped rodents from the 6 localities. Blood was extracted and analyzed with the Strip Immunoblot Assay technique (SIA; Yee 2003). For samples testing positive, the presence of viral RNA was assessed through RNA extraction from frozen rodent tissues and amplification by RT-PCR of an specific 234 bp fragment of the S genomic segment of the virus.

RESULTS

The overall composition of the small mammal samples varied remarkably from 2006 to 2007. During 2006, the mean trapping success was 9.3 %, whereas in 2007 this value was 6.4 %.

In both years, the most abundant species were *Abrothrix olivaceus* (Waterhouse, 1837) and *Abrothrix longipilis* (Waterhouse, 1837), whereas *Oligoryzomys* represented about one-third of all captures. Other trapped species were *Rattus norvegicus* (Berkenhout, 1769) and *Loxodontomys micropus* (Waterhouse, 1837), although they only represented a small percentage of all captures.

The karyotypic analysis of 28 individuals from Isla Riesco, Reserva Nacional Magallanes, Fuerte Bulnes, Puerto de Hambre and Tierra del Fuego showed identical diploid number with 2n = 56 and fundamental number (FN) = 70 (Fig. 2). The karyotype consists of 27 pairs of autosomes, 21 acrocentric and 6 metacentric pairs. When ordered by size, acrocentric pair one is approximately one third larger than the next largest pair. Sex chromosomes are subtelocentric (Fig. 2). Diploid number and chromosome morphology in all analyzed specimens from southern Patagonian localities were identical to those described by Gallardo & González (1977) and Gallardo & Patterson (1985) for *O. longicaudatus* north of 50° S, and those reported in Palma et al. (2005) for Torres del Paine. The karyotype typical of *O. magellanicus* (2n = 54) was not observed.

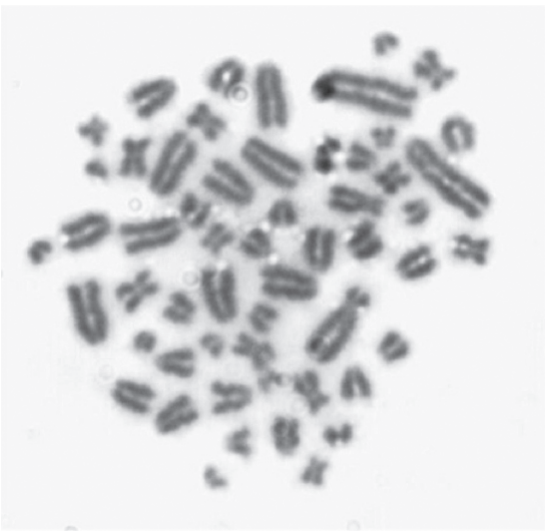

Fig. 2: Metaphase spreads of a male *Oligoryzomys* from Patagonian Chile.

Placas metafásicas de un macho *Oligoryzomys* de Chile patagónico.

Maximum likelihood bootstrap values were low, particularly at basal nodes, while branch lengths were mostly short (Fig. 3). The tree shows the recovery of a nearly basal clade in the phylogenetic tree, where most of the southern Patagonian haplotypes were located. The mean genetic distances between Patagonian, Mediterranean, and Temperate forest populations, under the K81uf + G model of sequence evolution, varied only slightly, between 2.2 and 3.2 %.

Serological analysis of 69 Patagonian specimens revealed the existence of a single seropositive rodent from the locality of Fuerte Bulnes, Punta Arenas (NK 129291). This finding represents an intraspecific seropositive rate of 1.4 % for southern Patagonia. Furthermore, the RT-PCR methodology revealed Hantavirus nucleic acids in heart, kidney, lungs, and spleen of that specimen (Fig. 4).

DISCUSSION

Most species of *Oligoryzomys* have been defined based on differences in their diploid numbers (Weksler & Bonvicino 2005). These differences have been suggested as one mechanism of species differentiation due to cytological incompatibility, which promotes reproductive isolation (Templeton 1989). As described above, the karyotypes of all specimens in this study consistently showed the occurrence of a unique karyotypic form of 2n = 56 in 5 of the localities sampled in the Patagonia of Chile, identical to that of *O. longicaudatus* from northern populations, and in agreement with Palma et al. (2005) for specimens from Torres del Paine. Although Gallardo & Patterson (1985) reported 2n = 54 both for Harrison Island and the vicinity of Punta Arenas, we did not observe this karyomorph, particularly since we sampled localities close to Punta Arenas, such as Reserva Nacional Magallanes and Fuerte Bulnes. However, we are not able to discard the occurrence of the 2n = 54 form (*O. magellanicus*) since it is highly probable that this taxon may be restricted to the type locality (Harrison Island, 54° S).

When we included sequences from the 6 localities analyzed in this work into the intraspecific phylogeny available for *O.*

longicaudatus, we did not observed any phylogenetic differentiation with respect to other localities analyzed for the species. In fact, the maximum-likelihood tree did not support phylogenetic differentiation among the populations we sampled suggesting that specimens of *Oligoryzomys* from the localities included correspond to the same species. Although most southern Patagonian haplotypes were recovered at the base of the tree, they did not constitute a supported clade given the low bootstrap values obtained, as is the general pattern for the rest of the grouping haplotypes representing the entire range of *O. longicaudatus*. In fact, haplotypes of both the Mediterranean and Temperate forest ecoregions tend to cluster together (Fig. 3). In addition, branch lengths in the whole phylogenetic tree

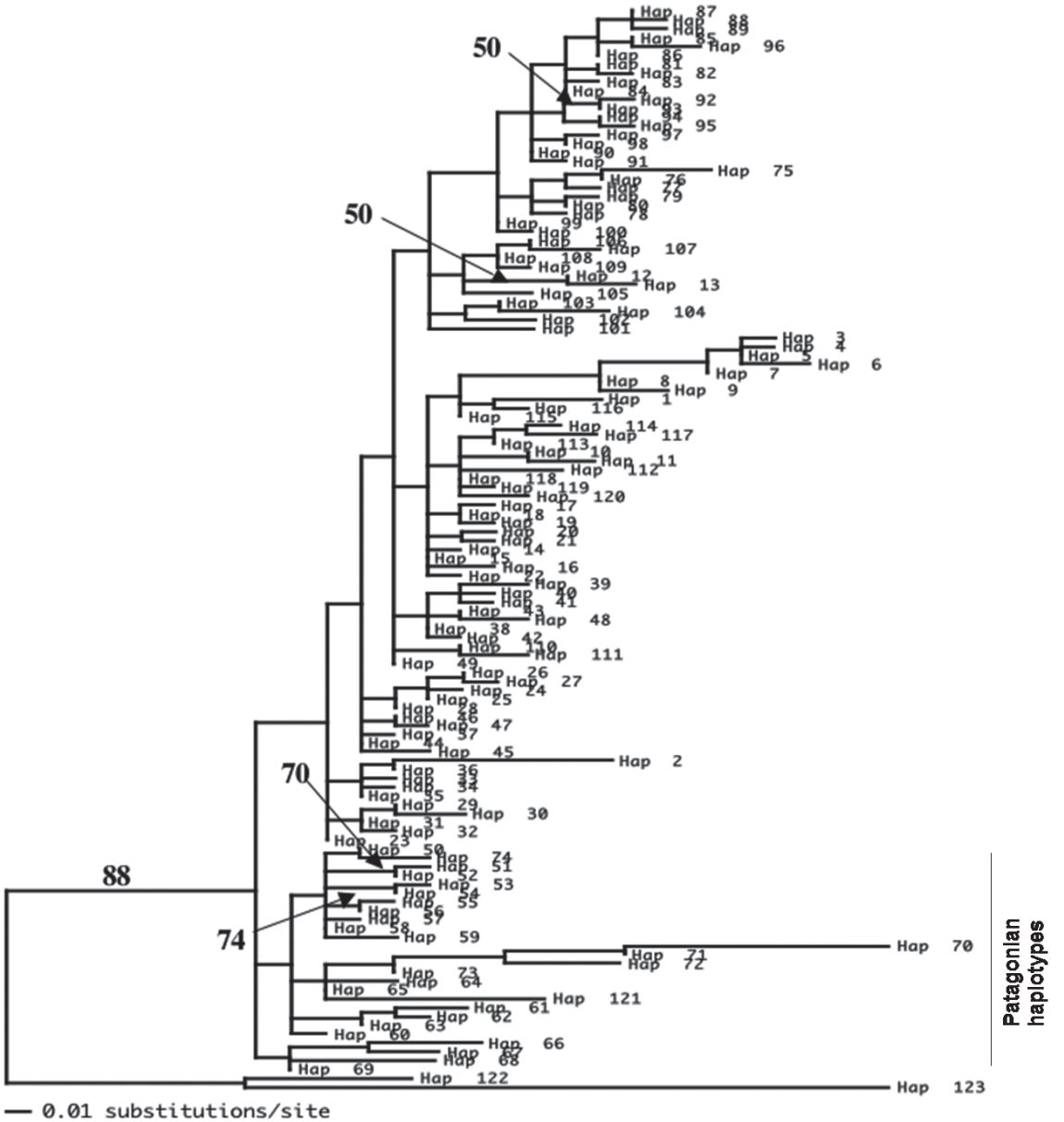

Fig. 3: Maximum-likelihood tree obtained from the hypervariable region I of the control region of mtDNA sequences of *O. longicaudatus*. Numbers on nodes represent 100 bootstrap replicates.

Árbol de Maximum-likelihood obtenido a partir de secuencias de la región hipervariable I de la región control del mtDNA de *O. longicaudatus*. Los números sobre los nodos representan 100 réplicas de bootstrap.

are mostly short, showing low differentiation among haplotypes representing different populations along the species range. We also observed the occurrence of shared haplotypes from different localities in southern Patagonia as characterizes the species in the entire range (Fig. 3; Palma et al. unpublished data). These results are in agreement with those observed by Palma et al. (2005) using sequences of the cytochrome b gene. Thus, specimens in the range of *O. magellanicus* included in this study are not genetically different from *O. longicaudatus*. In addition, mean genetic distances between Patagonian and northern populations are similar to those between the Mediterranean and Temperate forest ecoregions. These findings suggests that *O. longicaudatus* is distributed as far south as Tierra del Fuego and Navarino Island (55° S).

The pattern of genetic uniformity that characterizes *O. longicaudatus* along its geographical range has been previously demonstrated by several studies based on chromosomes, allozymes and DNA sequences of the cytochrome b gene (Palma 1987, Gallardo & Palma 1990, Palma et al. 2005). This may be predicted by its ecological features (e.g., high vagility and large home range), and the biogeographic history of southern Patagonia and Chile. Palma et al. (2005) suggested that *Oligoryzomys longicaudatus* may have entered Chile from Argentina through lower Andean elevations where the *Nothofagus* forests are continuous (about 40° S). Further displacement southward to Patagonia may have occurred along both the Chilean and Argentinean sides of the Andes. Furthermore, there may have been more than one recolonization from refuge areas since most of Patagonia as well as the Temperate forests were repeatedly glaciated during the Pleistocene (Holling & Schilling 1981, Mercer 1983). In fact, several refugia have been described in southern Chile, particularly in lowland and coastal areas (Villagrán & Hinojosa 1997, Premoli et al. 2000). Given this scenario, in addition to the results obtained from karyotypic analysis and DNA sequences, specimens sampled in this study south of 51° S should be recognized as *O. longicaudatus*, instead of *O. magellanicus*, thus extending the range of the former species as far south as 55° S.

The genetic uniformity of *O. longicaudatus* populations carries special importance since this species is the main reservoir of the Andes strain of Hantavirus (Bohlman et al. 2002, Padula et al. 2000). Seropositive specimens have been confirmed all along its distributional range (Torres-Pérez et al. 2004, Padula et al. 2000) and the molecular homogeneity of the species throughout its range suggests that the probability of human infection with Hantavirus is equally likely throughout the species' distribution. However, no case of seropositive rodents have been confirmed in the southern Patagonian Chile; the southernmost seropositive specimen was in the region of Aysen (48° S) in Chile, and Río Negro Province in Argentina (42° S). The seropositive mouse found at the locality of Fuerte Bulnes, Chile, constitutes the first record of a seropositive and infected rodent in southern Patagonia of this country. The absence of human infections to Hantavirus in southernmost Patagonian Chile might be explained by the low intraspecific rate of seroprevalence for the species in the region (1.4 %), which contrasts with the 4.5 % for the species northwards to Aisén.

We conclude that our results are not in agreement with previous studies based on chromosomal and morphological characters that suggested the existence of only *O. magellanicus* south of 50° S. Thus, populations of *Oligoryzomys* as far south as 55° S should be considered as *O. longicaudatus* and associated to the Andes strain of Hantavirus. A definitive evaluation of the presence of *O. magellanicus* in Patagonian Chile may require further field work sampling in the type locality and other islands.

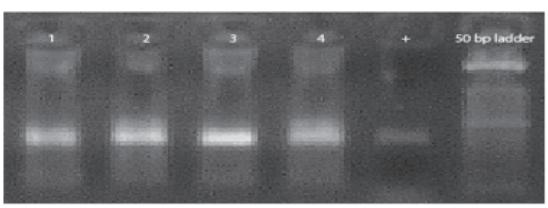

Fig. 4: Electrophoresis of RT-PCR products obtained from (1) Spleen; (2) Heart; (3) Lungs; (4) Kidney. (+): positive control.

Electroforesis de productos de RT-PCR obtenidos a partir de (1) Bazo; (2) Corazón; (3) Pulmones; (4) Riñón. (+): control positivo.

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APPENDIX 1

List of specimens analyzed for chromosomes, DNA sequencing and serology for each locality. *: specimens karyotyped by Palma et al. (2005). n/a: loan specimens not used for karyotype determination and not analyzed for serology.

Listado de especímenes analizados para cromosomas, secuenciación de DNA y serología para cada localidad. *: especímenes cariotipados por Palma et al. (2005). n/a: especímenes de préstamo no utilizados para determinación de cariotipos ni analizados serológicamente.

Locality	Catalog number	2n	Sequenced	Haplotype number	Genbank Accession number
Torres del Paine	NK105649	-	+	74	EU593090
	NK105650	-	+	121	EU593006
	NK105653	-	+	50	EU592967
	NK105654	-	-		-
	NK105659	-	+	52	EU592970
	NK105667	-	+	53	EU592971
	NK105668	-	-		-
	NK105670	-	-		-
	NK105675	-	-		-
	NK105680	-	-		-
	NK105681	56*	-		-
	NK105682	-	-		-
	NK105683	56*	-		-
	NK105684	-	-		-
	NK105696	-	-		-
	NK105703	-	-		-
	NK105704	-	-		-
	NK105706	-	-		-

APPENDIX 1 (continued)

Locality	Catalog number	2n	Sequenced	Haplotype number	Genbank Accession number
	NK105707	-	-		-
	NK105718	-	-		-
	NK105730	56*	-		-
	NK105731	-	-		-
	NK105740	-	-		-
	NK105743	-	-		-
	NK105745	-	-		-
	NK105746	-	-		-
	NK105747	-	-		-
	NK105749	-	-		-
	NK105750	-	-		-
	NK105757	-	-		-
	NK105758	-	-		-
	NK105759	-	-		-
	NK104942	-	-		-
	NK104943	-	-		-
	NK104944	-	-		-
	NK104945	-	-		-
	NK104975	-	-		-
	NK104976	-	-		-
	NK104977	-	-		-
	NK104980	-	-		-
	NK104994	-	-		-
R.N. Magallanes	NK129229	56	+	56	EU592974
	NK129230	56	+	54	EU592972
	NK129234	56	-		-
	NK129245	56	+	51	EU592969
Fuerte Bulnes	NK129291	56	+	60	EU592979
	NK129313	56	-		-
	NK129337	56	-		-
	NK129338	56	-		-
	NK129339	56	-		-
	NK129340	56	-		-
	NK129341	56	-		-
	NK129342	56	+	57	EU592975
	NK129345	56	-		-
	NK129349	56	-		-
Puerto de Hambre	NK129284	56	+	58	EU592976
	NK129288	56	-		-
	NK129289	56	-		-
	NK129290	56	+	55	EU592973
	NK129292	56	+	58	EU592978
Isla Riesco	NK142506	56	+	62	EU592981
	NK142507	56	+	50	EU592968
	NK142512	56	-		-
	NK142513	56	+	63	EU592982
	NK142514	56	+	59	EU592977
	NK142528	56	-		-
	NK142529	56	+	65	EU592983
	NK142530	56	+	61	EU592980
Tierra del Fuego	NK142550	56	-		-
	SSUC-Ma00406	n/a	+	72	EU592984
Bahía Inútil	JCT1960	n/a	+	65	EU593150
Parque Omora	JCT1950	n/a	+	64	EU593022