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Andes virus infections in the rodent reservoir and in humans vary across contrasting landscapes in Chile

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Abstract

Hantavirus cardiopulmonary syndrome (HCPS) is an emerging infectious disease first reported in Chile in 1995. Andes hantavirus (ANDV) is responsible for the more than 500 cases of HCPS reported in Chile. Previous work showed that ANDV is genetically differentiated in Chile across contrasting landscapes. To determine whether the reservoir rodent (*Oligoryzomys longicaudatus*) populations are also geographically segregated, we conducted range-wide spatial genetic analyses of *O. longicaudatus* in Chile using the mitochondrial DNA cytochrome *b* gene. Given that landscape structure influences the incidence of hantavirus infections, we also tested 772 *O. longicaudatus* specimens for antibodies to ANDV captured during the period 2000 – 2006. Population genetic analyses of *O. longicaudatus* are largely congruent with those reported for ANDV, with the host primarily differentiated according to three defined ecoregions, Mediterranean, Valdivian rain forest and North Patagonian rain forest. Significant differences in the relative prevalence of anti-ANDV antibodies in rodent samples also were found across the three ecoregions. We relate these results to the number of reported human HCPS cases in Chile, and discuss the importance of landscape differences in light of ANDV transmission to humans and among rodent populations.

Keywords

Andes virus; Chile; Ecoregion; Landscape structure; HCPS; *Oligoryzomys longicaudatus*; Seropositivity; Spatial Genetics

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1. Introduction

Hantavirus cardiopulmonary syndrome (HCPS) is an emerging infectious disease mainly known in North America from the outbreak of Sin Nombre virus (SNV) in the Four Corners region of the United States in 1993 (Nichol et al., 1993; Hjelle et al., 1994). The disease produces a variable number of human cases every year, with a case-fatality ratio between 30–50%. Rodents (Muridae and Cricetidae) are considered the reservoirs of hantavirus; however, recent discoveries have also documented a number of species of shrews (Soricomorpha) from at least three continents as hosts of viruses of unknown pathogenicity (Arai et al., 2007; Klempa et al., 2007; Song et al., 2009). Hantaviruses are transmitted to humans through rodent excreta and secretions (Botten et al., 2002; Padula et al., 2004). In Chile, the related Andes virus (ANDV) is responsible for all cases of HCPS, and the long-tailed pygmy rice rat (*Oligoryzomys longicaudatus*) appears to be the main reservoir (Toro et al., 1998; Medina et al., 2009). ANDV epidemiology is complicated slightly because person-to-person transmission has been well documented in Argentina and Chile although this is unique among hantaviruses (Martinez et al., 2005; Ferres et al., 2007). Since the first documented outbreak in Chile in 1995, serological surveys of hantavirus have confirmed the presence of ANDV from 30° 56'S to 53° 37'S (Toro et al., 1998; Torres-Pérez et al., 2004; Belmar-Lucero et al., 2009). This wide latitudinal range spans contrasting geographic features and landscapes ranging from a Mediterranean heterogeneous vegetation mosaic (Mediterranean ecoregion) to mixed evergreen-deciduous Temperate Forests (Valdivian and North Patagonian rain forest ecoregions) (Armesto et al., 2007; Veblen, 2007). Across these diverse ecotypes, strong differences in population structure and density have been documented for several species of small mammals (Murua et al., 1986; Simonetti and Aguero, 1990; Cofre et al., 2007). In a previous study, we reported that ANDV in Chile is segregated into distinct lineages that correspond to the limits of ecoregions (Medina et al., 2009). Populations of *O. longicaudatus*, however, seem to show a relatively homogeneous genetic structure (Palma et al., 2005), although ecogeographic subdivision has not been fully assessed. The observation that there exist areas wherein genetic lineages are subdivided according to different ecotypes may be the result of complex geographical, historical, and/or ecological processes with differential selective regimes or random genetic drift acting on populations (Wright, 1931; Hartl and Clark, 2007).

Environmental features influence the geographic distribution of diseases by affecting ecological properties of host and vectors (Linard et al., 2007). Landscape structure influences the incidence of hantavirus infections (Langlois et al., 2001; Glass et al., 2007; Heyman et al., 2009) and when combined with other environmental factors, may prove to be a strong determinant in the transmission of the virus to humans (Linard et al., 2007; Zhang et al., 2009). Variable host population structure has implications for viral population demographics and transmission (Adler et al., 2008). Given differences in ecology of *O. longicaudatus* populations across their latitudinal range in Chile, transmission rates of ANDV may also differ, resulting in differences in potential rates of infection of rodent hosts. Consequently, humans may be differentially exposed to viral infection.

In this study, we used molecular data from the rodent (*O. longicaudatus*) to explore genetic discontinuities across Chile, and discuss the importance of host population structure in the transmission of virus among rodents and to humans. We also compare the seroprevalence of ANDV in *O. longicaudatus* across the latitudinal gradient, and provide a quantitative assessment of the distribution of incident and fatal cases of HCPS during the period 2000–2006 in Chile. We predict differences in *O. longicaudatus* population structure across the ecogeographic regions in southcentral Chile (congruent with ANDV genetic structure), and that prevalence of ANDV-seropositive *O. longicaudatus* differs across those ecoregions. Our study highlights the value of combining information from host population structure,

epidemiology, viral phylogeography, and geography to gain insights into the transmission and persistence of infectious diseases.

2. Materials and methods

2.1. Mitochondrial DNA Sequences and Spatial Genetic Analyses

A total of 108 *O. longicaudatus* from 11 localities in Chile ranging from 30°S to 46°S (Fig. 1A) were used for mitochondrial DNA cytochrome *b* (*cyt-b*) amplification and sequencing following procedures explained previously (Palma et al., 2005). Sequences were edited using BioEdit Sequence Alignment Editor (Hall, 1999), and aligned using Clustal W implemented in BioEdit. Sequences are deposited in GenBank (Accession numbers GQ282502-GQ282603, AF346566, AF346568, AY275692, AY275693, AY275698, AY275699).

A spatial analysis of molecular variance was performed in SAMOVA v.1.0 (Dupanloup et al., 2002). This method uses a simulated annealing approach to identify groups of populations (*K*), which are geographically homogeneous and maximally differentiated by maximizing *F*_{ct} (the proportion of the total genetic variance due to differences among groups of populations). *F*_{ct} values were calculated by running the program sequentially (100 random initial conditions), and forcing the data into *k* groups (where *k* = 2 to 9). Population subdivision was estimated using the fixation index (*F*_{st}) between all population pairs generated in Arlequin 3.1 (Excoffier et al., 2005). For detecting dissimilar patterns among *O. longicaudatus* groups (ecoregions), population pairwise *F*_{st} values were used to perform a non-metric multidimensional scaling (MDS) analysis using Systat v.12 (SYSTAT Software, Inc); the analysis provides a visual representation of the pattern of genetic distances (Kruskal and Wish, 1978).

We used the Median Joining method (Bandelt et al., 1999) to perform a phylogenetic network analysis with *O. longicaudatus* haplotypes as implemented in Network 4.2.0.1 software (<http://www.fluxus-engineering.com/sharenet.htm>) to assess intraspecific relationships.

2.2. Serology, human hantavirus cases and data analysis

We sampled 76 sites throughout the distribution of *O. longicaudatus* in Chile from 28°S to 47° S between 2000 and 2006 (Fig. 1B). A total of 772 *O. longicaudatus* serum samples were subjected to screening for antibodies against the ANDV N protein using a strip immunoblot assay (SIA) as previously described (Yee et al., 2003). The relative anti-ANDV antibody prevalence *O. longicaudatus* was standardized using the ratio between the number of seropositive *O. longicaudatus* at each sampled locality and the trapping success (number of captures by the number of total trap-nights) of *O. longicaudatus* captured at that locality (Torres-Pérez et al., 2004).

Between 1995 and 2006, a total of 492 HCPS cases were reported in Chile (with three additional retrospective cases in 1975, 1993, and 1994; <http://epi.minsal.cl>). All HCPS cases were located between 30° 56'S and 46° 46'S. Of these, 391 cases occurred between 2000 and 2006. The number of HCPS cases in each group was expressed as the ratio of the number of cases and the rural population ($\times 1,000$) (information available in the Instituto Nacional de Estadísticas de Chile, <http://www.ine.cl>). The rural population (people inhabiting, working in, or visiting rural areas) is reported to be at highest risk of contracting HCPS (Castillo et al., 2002; Riquelme et al., 2003), due to closer contact with the habitat of the rodent host (*O. longicaudatus*). We acknowledge that an index based on rural population may be slightly underestimated due to a few HCPS cases that are not related to people inhabiting rural areas. Finally, we obtained data reflecting the number of fatal HCPS cases during the same period, which are reported standardized by the number of HCPS and the rural population. Seropositivity analyses and HCPS incidence and fatal cases are reported by grouping data according to the three ecoregions

Mediterranean, Valdivian rain forests and North Patagonian rain forest. These clusters follow the results reported by Medina et al. (2009) and spatial genetic analyses derived from SAMOVA (see below).

3. Results

Using mitochondrial *cyt-b* sequences, spatial analysis of molecular variance was performed to assess substructure within the *O. longicaudatus* populations in Chile. F_{CT} values ranged from 0.4703 to 0.4832, with the group structure maximized at $k = 3$. Collection sites from Fray Jorge to Los Ruiles (ca. 30° – 36° S) formed the first group, Tome to Puyehue (ca. 37° – 41° S) clustered into a second group, and three localities (ca. 45° – 46° S) clustered into a third group. These groups correspond to the major ecogeographic regions Mediterranean, Valdivian rain forests and North Patagonian rain forests respectively, that differ in climate, geology, and vegetation composition (Armesto et al., 2007; Veblen, 2007). F_{st} -values among *O. longicaudatus* populations in Chile (Table 1) ranged between 0.002 (Tome vs Nahuelbuta and P.N.Villarrica) to 0.670 (Fray Jorge vs Lago Atravesado), with the highest values within an ecogeographic region found between Mediterranean populations (range 0.063 – 0.234). Non-hierarchical analysis (MDS) using the fixation index (F_{st}) derived from *cyt-b* sequences (Fig. 2) showed a strong dispersal of *O. longicaudatus* populations into multidimensional space (Stress = 0.03), segregated in three groups that correspond to ecoregions. The unrooted phylogeographic network (Fig. 3) was concordant with SAMOVA and MDS analyses showing haplotypes primarily associated with ecogeographic distribution (Mediterranean, Valdivian and Patagonian), although some groups shared haplotypes across different ecoregions.

A total of 772 *O. longicaudatus* were captured during the years 2000 – 2006. Of these, 38 were positive to anti-ANDV antibodies, representing an overall seroprevalence of 4.9%. For the Mediterranean region, 171 *O. longicaudatus* were captured with 19 (11.1%) seropositive for ANDV. For the Valdivian and North Patagonian rain forests regions, 512 and 89 *O. longicaudatus* were collected with 14 (2.73%) and five (5.62%) individuals seropositive for ANDV, respectively. Using these values, the relative seropositivity was significantly higher in the Mediterranean (0.1490 ± 0.0782) compared to Valdivian (0.0339 ± 0.00496) and Patagonian regions (0.0506 ± 0.00509) (Fig. 4). The anti-ANDV antibody prevalence was significantly higher in the Patagonian than Valdivian region. During the same period, 98, 273, and 20 human HCPS cases were reported for the Mediterranean, Valdivian and Patagonian regions, respectively. Patagonia shows the highest number of HCPS cases relative to rural population compared to those from Mediterranean and Valdivian regions. The highest absolute number of fatal HCPS cases in the same period occurred in the Valdivian region (109 cases), followed by the Mediterranean (32 cases) and Patagonian (4 cases). However, standardizing fatal HCPS cases by the number of HCPS infections / rural population, the difference between Valdivian and Mediterranean regions decrease (0.279 and 0.380, respectively), with Patagonia remaining significantly lower (0.0036).

4. Discussion

The distribution of *O. longicaudatus* in Chile spans nearly 4,000 km of latitude and encompasses contrasting landscapes ranging from semi-desert thorn-scrub in the north to southern Magellanic rain forest. Population dynamics and rodent-to-rodent interactions necessary to transmit ANDV also vary considerably across this latitudinal span. The Mediterranean region is characterized by a highly heterogeneous vegetation mosaic (Armesto et al., 2007), with less suitable habitat for *O. longicaudatus* than the Temperate rain forest region (Murua, 1996). Differences in landscape structure and climate result in distinctive ecological parameters and differences in population genetic structure (Pilot et al., 2006). Close correspondence between population genetic structure and habitat subdivision (corresponding

to distinct ecoregions) was reported in coyotes (*Canis latrans*) in California (Sacks et al., 2008). These differences were attributable mainly to the effects of random genetic drift in ecotypes with greater population subdivision. Population genetic structure (based on mtDNA *cyt-b*) of *O. longicaudatus* differs across the latitudinal span of Chile. Genetic divergence within Mediterranean populations was higher than those within either Valdivian or Patagonian rain forests populations, reflecting higher genetic substructure in northern populations. This rodent shows a tight association with mesic habitats (Cortes et al., 1988; Kelt et al., 1994), so greater spatial heterogeneity in the Mediterranean region due to a more xeric environment is likely a major cause of interregional differences. However, geographic factors (such as natural barriers or spatial distance) and behavioral differences, as well as historical processes (such as population expansion, bottlenecks) may also contribute to difference in genetic substructure as they also influence gene flow among populations (Hartl and Clark, 2007). We have not evaluated all of these processes, but greater population subdivision in the Mediterranean region may allow both deterministic and stochastic genetic processes to be prominent drivers in differentiating *O. longicaudatus* populations within ecoregions (as in the coyote example). No evident geographic barriers or behavioral features are responsible for producing genetic discontinuity in *O. longicaudatus* at the boundaries of these ecoregions, although isolation-by-distance and postglacial colonization have been suggested as important factors determining the current spatial distribution of this species in Chile (Palma et al., 2005).

Given that ecological dynamics and genetic structure of *O. longicaudatus* differs across the ecoregions in Chile, we speculated that transmission rates of ANDV may also vary by region, resulting in differences in *O. longicaudatus* seroprevalences. Mean relative seropositivity, the standardized incident, and number of fatal HCPS cases differed across ecoregions in Chile. An unexpected result of our study is that the highest relative seroprevalence of *O. longicaudatus* is in the Mediterranean region. Hantavirus seroprevalence in rodent populations depends on many factors affecting ecological dynamics of the virus and the host. Variables like landscape structure (Glass et al., 2007), density-based and/or frequency-based dependence (Madhav et al., 2007; Adler et al., 2008; Lehmer et al., 2008), dilution effect (Keesing et al., 2006; Tersago et al., 2008; Suzan et al., 2009), viral recrudescence (Kuenzi et al., 2005), climatic variability (Hjelle and Glass, 2000), habitat loss and fragmentation (Suzan et al., 2008), may all contribute to complex interactions that make predicting relative seropositivity to ANDV difficult. Our study spans seven years of rodent sampling, therefore factors related to changes through time (viral recrudescence, climatic variability, changes in abundances through time) although not assessed, are implicitly accounted. Transmission rates depend on contact rates among individuals and the proportion of susceptible individuals (McCallum et al., 2001; Clay et al., 2009). These parameters scale with host population density which differs across ecoregions in Chile. We postulate that spatial features such as landscape structure and habitat fragmentation are major components in differences in serological rates of this rodent in Chile, but alternative models should be explored. Suzan *et al.* (2009) showed that high species richness in a rodent community influences both abundance of reservoir hosts and infection prevalence and leads to decreased encounter rates between infected and susceptible hosts. Species richness of mammalian communities varies in a non-linear pattern across ecoregions in Chile (Cofre et al., 2007) so this factor should be evaluated in the future.

Differences in rodent seropositivity rates across ecoregions suggest that humans may be differentially exposed to infection with ANDV. People inhabiting the Mediterranean region are therefore potentially at higher risk of contracting HCPS. However, more HCPS cases (per rural population) have been reported in the southern regions (<http://:epi.minsal.cl>), and our study corroborates that finding. We can parsimoniously hypothesize that more HCPS cases in southern regions are related to a higher rural population. In the Temperate forest regions (Valdivian and Patagonia), despite a lower absolute number of people compared to the Mediterranean, there is a proportionally larger rural population. People associated with rural

environments (farmers, residents and also visitors) are at higher risk of contracting HCPS in Chile (Castillo et al., 2002; Torres-Pérez et al., 2004), as is also evidenced in other countries within South America (Nichol et al., 1993; Williams et al., 1997; Bayard et al., 2004). *O. longicaudatus* is found closer to human settlements in rural and periurban areas, increasing the likelihood of exposure to the virus (Ortiz et al., 2004; Torres-Pérez et al., 2004). Also, the higher host abundance of ANDV in the southern regions of Chile is a major factor contributing to disease transmission risk, due to increased contact with humans.

Our study assesses factors affecting rodent host dynamics that directly or indirectly impact the distribution of ANDV and ultimately the incidence of HCPS. Ecology and geography may significantly influence spatial and temporal dynamics of the ANDV host reservoir. The spatial epidemiology of *O. longicaudatus* requires further investigation to determine more accurately transmission rates of ANDV within/among rodent populations, and ultimately to humans.

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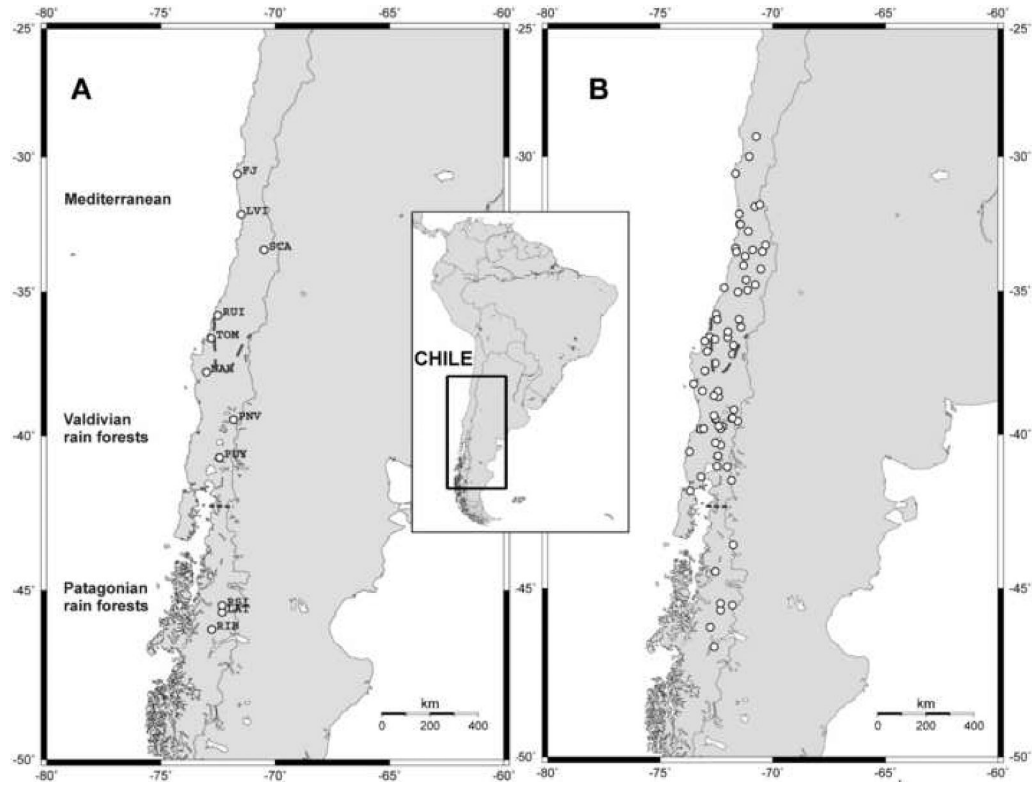


Figure 1.

Map of the sampled localities of *O. longicaudatus* used for (A) molecular analyses, and (B) seropositivity to ANDV in Chile. Locality names are explained in Table 1. Dashed lines represent approximate limits of ecoregions. Maps were generated using Online Map Creator (www.aquarius.ifm-geomar.de).

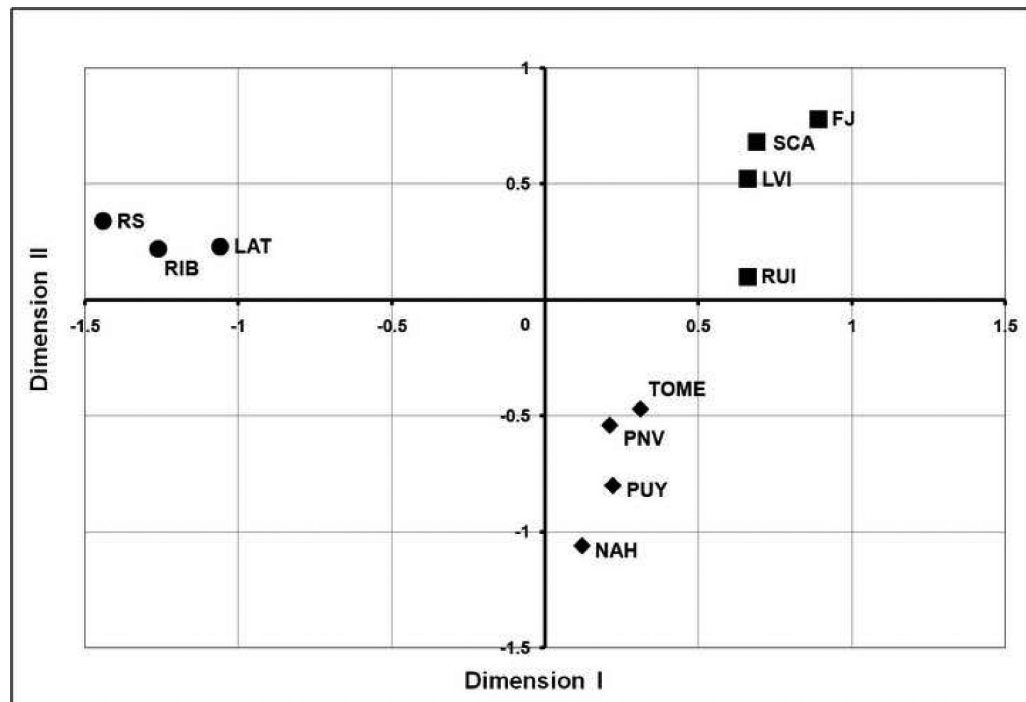


Figure 2. Bidimensional ordination of pairwise F_{st} values for *O. longicaudatus* populations using a non-metric multidimensional scaling method (Stress = 0.03). Squares represent Mediterranean localities: Fray Jorge (FJ), Los Vilos (LVI), San Carlos de Apoquindo (SCA), Los Ruiles (RUI); Diamonds are Valdivian rain forests localities: Tome (TOM), Parque Nacional Nahuelbuta (NAH), Parque Nacional Villarrica (PNV), Puyehue (PUY); Circles are North Patagonian rain forests localities: Reserva Nacional Río Simpson (RSI), Río Ibáñez (RIB), Lago Atravesado (LAT).

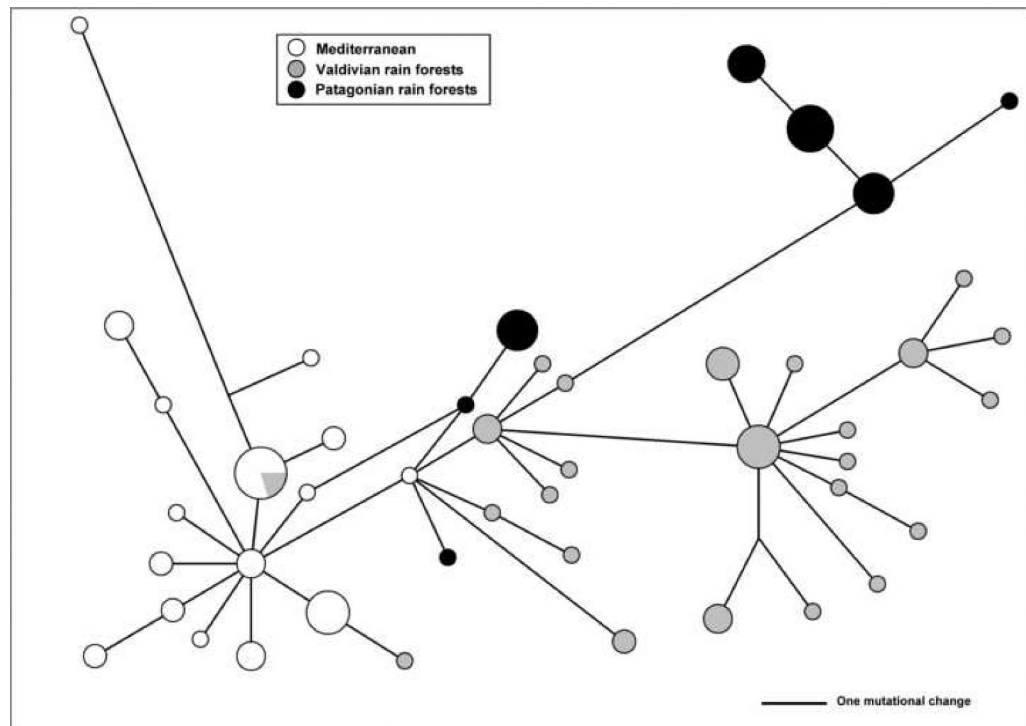


Figure 3. Unrooted network of *O. longicaudatus* using cytochrome b sequences with haplotypes depicted according to ecoregions. Size of circles represents the number of individuals per haplotypes.

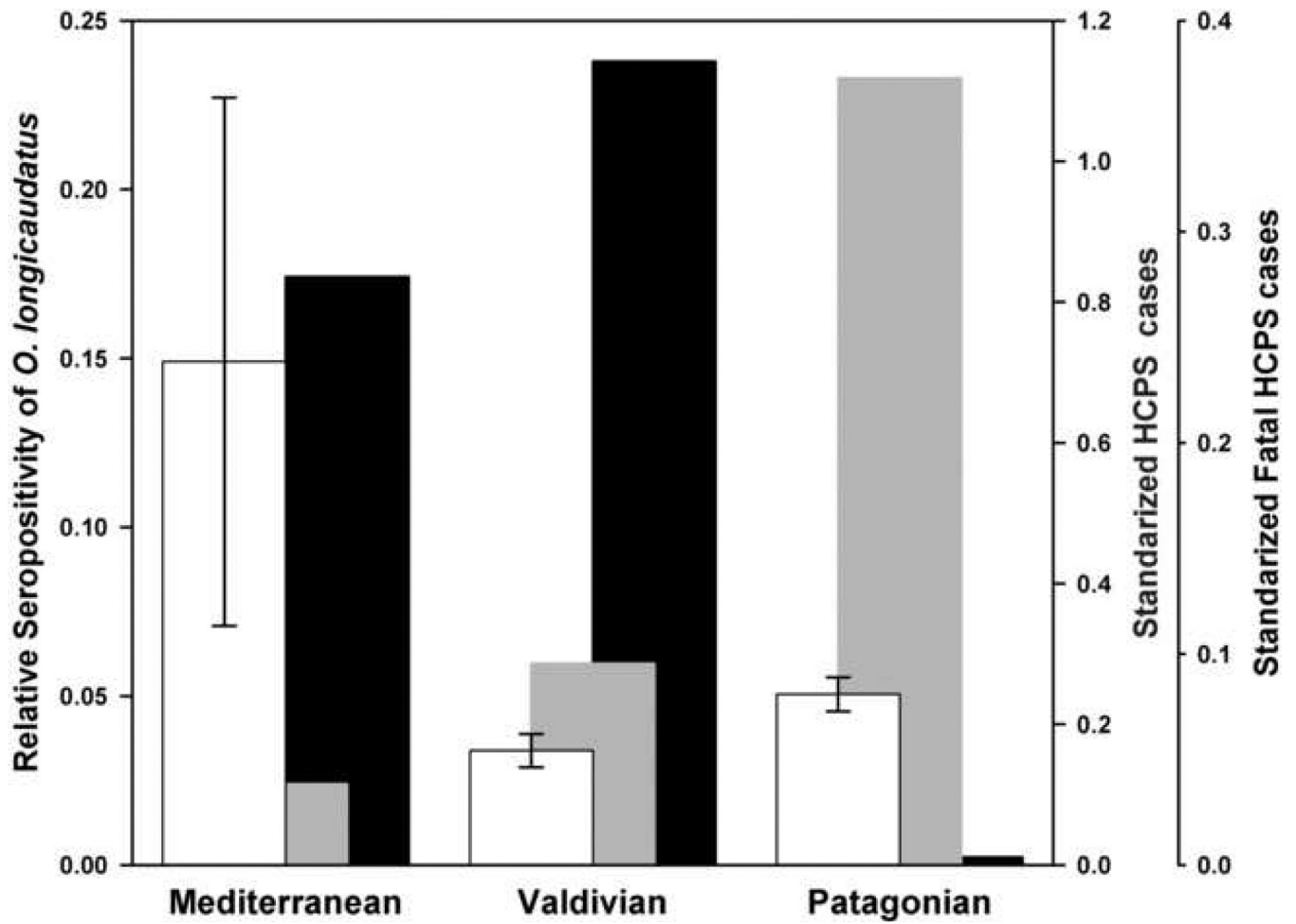


Figure 4. *O. longicaudatus* relative seropositivity (white bars), and standardized incidence (grey bars) and fatal (black bars) HCPS cases in three Chilean ecoregions during the period 2000– 2006.

Table 1

Fixation index (Fst) among *O. longicaudatus* sampling localities using mitochondrial DNA cytochrome *b*. (N = sample size). Numbers in bold represent significant differences ($P \leq 0.05$) after 10,000 permutations.

Ecoregion	Map number	Locality	FJ	LVI	SCA	RUI	TOM	NAH	PNV	PUY	RSI	RIB
Mediterranean	1	FJ (N=10)	---									
	2	LVI (N=10)	0.123	---								
	3	SCA (N=11)	0.234	0.157	---							
	4	RUI (N=10)	0.214	0.154	0.063	---						
Valdivian	5	TOM (N=10)	0.357	0.283	0.306	0.119	---					
	6	NAH (N=7)	0.540	0.426	0.493	0.334	-0.002	---				
	7	PNV (N=12)	0.507	0.399	0.467	0.306	-0.002	-0.057	---			
	8	PUY (N=10)	0.448	0.359	0.405	0.266	0.021	0.020	0.016	---		
Patagonian	9	RSI (N=9)	0.623	0.524	0.544	0.530	0.440	0.522	0.525	0.481	---	
	10	RIB (N=10)	0.646	0.545	0.567	0.554	0.466	0.547	0.549	0.505	-0.081	---
	11	LAT (N=9)	0.670	0.571	0.593	0.588	0.516	0.592	0.590	0.546	-0.049	-0.081

Localities: Fray Jorge (FJ), Los Vilos (LVI), San Carlos de Apoquindo (SCA), Los Riles (RUI), Tome (TOM), Parque Nacional Nahuelbuta (NAH), Parque Nacional Villarrica (PNV), Puyehue (PUY), Reserva Nacional Río Simpson (RSI), Río Ibáñez (RIB), Lago Atravesado (LAT).