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# Spatial but not Temporal Co-divergence of a Virus and its Mammalian Host

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## Abstract

Co-divergence between host and parasites suggests that evolutionary processes act across similar spatial and temporal scales. Although there has been considerable work on the extent and correlates of co-divergence of RNA viruses and their mammalian hosts, relatively little is known about the extent to which virus evolution is determined by the phylogeographic history of host species. To test hypotheses related to co-divergence across a variety of spatial and temporal scales, we explored phylogenetic signatures in Andes virus (ANDV) sampled from Chile and its host rodent, *Oligoryzomys longicaudatus*. ANDV showed strong spatial subdivision, a phylogeographic pattern also recovered in the host using both spatial and genealogical approaches, and despite incomplete lineage sorting. Lineage structure in the virus seemed to be a response to current population dynamics in the host at the spatial scale of ecoregions. However, finer scale analyses revealed contrasting patterns of genetic structure across a latitudinal gradient. As predicted by their higher substitution rates, ANDV showed greater genealogical resolution than the rodent, with topological congruence influenced by the degree of lineage sorting within the host. However, despite these major differences in evolutionary dynamics, the geographic structure of host and virus converged across large spatial scales.

### Keywords

Chile; co-divergence; hantavirus; *O. longicaudatus*; phylogeography; spatial genetics

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**Data accessibility** DNA sequences: GenBank accessions GQ282502-GQ282603; JN034440-JN034536; AF 346566; AY275693; AY275692; AY275694; AY275696; AY275699; AY452197; AY452198; AF346568; AY275690. (For an individual-by-individual listing, please see the online supplemental material.)
Final DNA sequence assembly uploaded as online supplemental material

## Introduction

Host-parasite interactions occur across several levels of organization (Mideo *et al*. 2008), and are influenced by processes that act at various temporal scales (Archie *et al*. 2009; Pybus& Rambaut 2009). Changes in host ecology, population dynamics, and evolutionary processes are all associated with changes in parasite population structure. Among parasites, RNA viruses represent an important group of emerging human pathogens. High mutation rates, large population sizes, and short generation times mean that RNA viruses evolve extremely rapidly (Holmes 2009b), with viral phylogenies often providing information about temporal and spatial dynamics (Holmes 2004). Therefore, RNA viruses typically exhibit far greater phylogenetic resolution than that of their hosts. For example, feline immunodeficiency virus (FIV) provided a more detailed view of demographic expansion in cougars (*Puma concolor*), particularly in recent time, than could be inferred from host data alone (Biek *et al*. 2006).

Distinct evolutionary processes act to shape the pattern of host-parasite congruence at different hierarchical scales. Intimate interactions between the two organisms, combined with vertical transmission of the parasite through host generations, are key features that should increase the likelihood of shared common histories (Nieberding& Olivieri 2007). At the phylogeographic scale, founder, divergence and migration events act as major determinants of congruence/incongruence in host-parasite genealogies (Nieberding& Olivieri 2007), and generally lead to limited congruence between the phylogenetic trees of RNA viruses and their hosts (Rannala& Michalakis 2003). Indeed, thus far, co-divergence of RNA viruses and their hosts has been observed infrequently (Liu *et al*. 2008; Switzer *et al*. 2005).

Landscape features may also strongly structure populations of reservoir species involved in zoonotic emergences (Archie *et al*. 2009; Biek& Real 2010; Reisen 2009), thereby influencing the dynamics of host-parasite systems (Hirzel *et al*. 2007; Su *et al*. 2009; Webb *et al*. 2007) and ultimately the complexity of epidemiological patterns (Barton *et al*. 2010). The physical environment affects host-parasite interactions by influencing gene flow among host populations, and by acting as a selective force in the form variable environmental conditions (Biek& Real 2010). Indeed, the genetic structure of parasites depends heavily on the overall dispersal of the host (especially in obligatory parasites like RNA viruses) and responds to the spatial heterogeneity affecting the population dynamics of the species (Biek& Real 2010; Nadin-Davis *et al*. 2010). For RNA viruses, geographic discontinuities in the host may lead to strong phylogenetic signatures in the virus that can be revealed using a suite of phylogeographic methods (Biek *et al*. 2007; Holmes 2004; Real *et al*. 2005).

Globally, Hantavirus (Bunyaviridae: *Hantavirus*) includes several strains recognized as human pathogens (Hjelle& Torres-Pérez 2010; Jonsson *et al*. 2010; Schmaljohn& Hjelle 1997). Rodents of the families Muridae and Cricetidae were described as the primary zoonotic reservoirs of these viruses, but distinct hantaviruses also have been discovered in several species of shrews and moles (Soricomorpha) (Arai *et al*. 2007; Kang *et al*. 2009; Klempa *et al*. 2007; Yadav *et al*. 2007). Hantaviruses are segmented negative-strand RNA viruses, and contain small (S), medium (M), and large (L) genomic segments, encoding nucleocapsid (N), glycoproteins Gn and Gc, and RNA-dependent RNA polymerase, respectively (Jonsson& Schmaljohn 2001). Horizontal transmission by rodent-to-rodent contact is the main mechanism for hantavirus maintenance and spread (Hjelle& Yates 2001). Human infection by hantaviruses is thought to follow accidental exposure to secretions or excretions produced by infected rodents. To date, person-to-person transmission has only been documented in the case of the South American Andes virus (Enria *et al*. 1996; Ferres *et*

*al*. 2007; Lazaro *et al*. 2007; Martinez *et al*. 2005; Padula *et al*. 1998; Wells *et al*. 1998; Wells *et al*. 1997). Previously, hantaviruses were hypothesized to co-diverge with their associated mammalian hosts (Plyusnin *et al*. 1996; Yates *et al*. 2002). However, studies comparing rates of nucleotide substitution and times to most recent common ancestry suggest that at least some hantaviruses have diverged far more recently than implied under the assumption of divergence such that much of their evolution is dominated by cross-species transmission (Ramsden *et al*. 2009; Ramsden *et al*. 2008). The latter strongly contrasts with the temporal scale of divergence of their mammalian hosts (Palma *et al*. 2010; Ramsden *et al*. 2009). In the southern cone of South America, the sigmodontine rodent, *Oligoryzomys longicaudatus*, constitutes the main reservoir for Andes virus (ANDV). In Chile, this rodent occurs in mesic habitats from 28°S to 55°S latitude (Belmar-Lucero *et al*. 2009; Palma *et al*. 2005), where > 65% of the human population resides. This area encompasses climates varying from warm semi-desert in northern Chile to cold subpolar in the extreme south. In southern Chile and Patagonia, biotic communities were structured by the dramatic glacial cycles of the Pleistocene (Harrison 2004; Lessa *et al*. 2010).

To date, the extent and pattern of co-divergence among RNA viruses and their hosts has generally been studied either at deeper phylogenetic (i.e. inter-specific) levels or on much shorter time-scales by way of comparisons of intra-host dynamics (Paterson& Piertney 2011). Far fewer studies have explored these processes simultaneously using both phylogeographic and population genetic approaches (Biek *et al*. 2006; Dekonenko *et al*. 2003; Nemirov *et al*. 2010). To determine the ecological and evolutionary processes that might be responsible for genetic diversity of hantaviruses, we undertook a detailed analysis of the spatial and temporal scale of co-divergence utilizing both contemporary spatial genetics and longer-term co-phylogeny. These data allow us to evaluate whether ANDV in Chile and the associated host follow similar genealogical patterns, and if ANDV might be thought of as a genealogical proxy that is tracking ecological processes in *O. longicaudatus*. The obligate parasitic nature of ANDV in *O. longicaudatus* (Medina *et al*. 2009), coupled with a broad latitudinal distribution in Chile, provides a useful model for examining processes acting to shape contemporary population structure in hosts and parasites and ultimately exploring the history and epidemiology of zoonotic diseases.

## Materials and Methods

### Sampling and rodents sequencing

A total of 197 *O. longicaudatus* were collected from 22 localities in Chile ranging from 28°S to 51°S (Fig. 1, Table S1, Supporting Information). Rodents were collected in live traps (H.B. Sherman Traps, Inc., Tallahassee, FL), following established safety guidelines for rodent captures and processing (Gannon& Sikes 2007; Mills *et al*. 1995). Genomic DNA from liver tissue was extracted using a modified salt extraction method (Fleming& Cook 2002). A fragment of 924 bp of the mitochondrial cytochrome *b* (cyt *b*) was amplified using the polymerase chain reaction (PCR), and primers MVZ 07, MVZ 26, LBE 05 and H 15767, following the procedures described previously (Palma *et al*. 2005). We also sequenced the mtDNA control region (1014-nt) for 12 selected individuals used in the co-divergence analyses using primers (DLO-L) CGGAGGCCAACCAGTAGA-3′ and (DLO-H) TAAGGCCAGGACCAAACC-3′. PCR products were purified using QIAquick PCR purification kit (Qiagen Inc., Valencia, CA, USA). Cycle sequencing used flanking primers labeled with the Big Dye terminator Kit (Perkin Elmer, Norwalk, Connecticut, USA). Sequencing reactions were analyzed on an ABI Prism 3100 (Applied Biosystems) automated sequencer. Sequences were edited using the BioEdit Sequence Alignment Editor (Hall 1999), and aligned using Clustal W implemented in BioEdit.

### ANDV Sequences, Recombination and Selection Analyses

Sequences from ANDV were obtained from human cases and seropositive rodents (Medina *et al*. 2009). We sequenced the S-segment (396-nt) in 38 samples representative of the known geographic range of the virus in Chile (Toro *et al*. 1998; Torres-Pérez *et al*. 2004) (GenBank Accession numbers EU241665-EU241702). We also included four ANDV samples taken from GenBank (Accession numbers AY228237, AF004660, AF291702, AF482712), for a total of 42 sequences. These samples were used to perform spatial genetic and molecular clock analyses. For the co-divergence analyses we used a longer S-segment (931-nt) from fewer ANDV samples (N = 12).

We screened for potential recombination in 42 S-segment sequences (396-nt) used in phylogeographic analyses (Medina *et al*. 2009), and the 12 S-segment (931-nt) sequences used in co-divergence analyses (see below). These analysis were performed using the Recombination Detection Program (RDP3, http://darwin.uvigo.es/rdp/rdp.html), which applies a suite of recombination detection methods (Martin *et al*. 2005). In all cases, analyses were performed using default detection thresholds. The Genetic Algorithm for Recombination Detection (GARD) method (Kosakovsky Pond *et al*. 2006) was also used to examine the occurrence of recombination events between ANDV sequences (as implemented in datamonkey.org resource). When potential recombination among sequences was detected, analyses were performed using only the regions in which recombination was not suspected.

Analyses of selection pressure were conducted on the Datamonkey server (Kosakovsky Pond& Frost 2005a) using the following methods: single-likelihood ancestor counting (SLAC), fixed effects likelihood (FEL), random effects likelihood (REL), and PARRIS (a PARtitioning approach for Robust Inference of Selection). All these methods allow positive selection to be detected at individual nucleotide sites (Kosakovsky Pond& Frost 2005b).We also tested for selection using alternative evolutionary models under a Bayesian approach implemented in the Selecton server (Stern *et al*. 2007).

### Rates of nucleotide substitution and time to the most recent common ancestor

We used the Bayesian Markov Chain Monte Carlo (MCMC) method available in BEAST v1.5.3 (Drummond& Rambaut 2007) to estimate the rate of nucleotide substitution and time to the most recent common ancestor (tMRCA) for ANDV in Chile. Because of the short length of the fragment analyzed and the relatively limited time span of sampling (nine years) we employed the simple HKY85 model of nucleotide substitution; more complex substitution models resulted in model over-parameterization and unreliable estimates (not shown). This analysis also employed the conservative Bayesian skyline coalescent prior and both strict and relaxed (uncorrelated lognormal) molecular clocks, and which produced similar results (see below). The MCMC chains were run for $1 \times 10^8$ steps with a discarded 10% burn-in and statistical uncertainty is depicted in values of the 95% Highest Probability Density (HPD). For *O. longicaudatus*, a strict molecular clock was found to be the best-fit to the data (employing the GTR model and the Bayesian skyline coalescent), and convergence of the MCMC was reached after running the BEAST program for $6 \times 10^8$ steps. The samples from two runs were combined, and convergence of the chain, sampling and mixing was confirmed by inspection of the MCMC samples using the program Tracer v.1.5 (Rambaut& Drummond 2009). All analyses were performed until parameter convergence was obtained. For our molecular clock analysis of rodent mtDNA, we assumed a sigmodontine-based rate of 2.3% substitutions per site per million years (Smith& Patton 1993). Finally, to check for temporal structure in ANDV, essential for the accurate estimation of evolutionary dynamics, the BEAST analysis described above was repeated using 20 data sets in which the year of sampling of each sequence had been randomized (and

assuming an uncorrelated lognormal relaxed molecular clock). Real data containing temporal structure would be expected to produce results that are qualitatively different to those seen in the random data sets.

## Spatial Genetic Analyses

Spatial analysis of molecular variance was performed in SAMOVA v.1.0 (Dupanloup *et al*. 2002). This analysis was previously shown to be useful in identifying latitudinal structure in *O. longicaudatus* in south-central Chile (Torres-Pérez *et al*. 2010), so we expanded the analysis by increasing sample size (195 sequences) across a larger geographic range. This approach uses a simulated annealing approach to identify partitions of geographically adjacent sampling areas (*K*) that are maximally differentiated by maximizing Fct (the proportion of the total genetic variance due to differences among groups of populations). The number of groups was estimated by running 500 random initial conditions and forcing the data into *k* groups (where $k = 2$ to 10).

We used an interpolation-based graphical method to generate a three-dimensional genetic landscape shape (GLS) within the program Alleles in Space (AIS) (Miller 2005). The procedure creates peaks in areas where genetic distances between individuals are high, and valleys or troughs where genetic distances between individuals are low, providing a visual perspective of the spatial distribution of genetic structure over landscapes (Miller *et al*. 2006). GLS analysis was performed to visually explore the congruence in the spatial pattern of genetic divergence in ANDV and *O. longicaudatus*. A location-based connectivity network (based on Delaunay triangulations) was created first among all sampling locations. Then, residual genetic distances between observations (or surface heights) are calculated and placed at the midpoints of each connection in the network. To infer surface heights, AIS incorporates an inverse distance weighted interpolation procedure across a uniform grid. We selected a distance weighting parameter (*a*) of 0.5 for ANDV and 1.0 for *O. longicaudatus* (we also tested $a = 0.2$, 0.5, 1.0, and 1.2), and a grid dimension of $50 \times 50$. The AIS program was also used for spatial autocorrelation analysis and calculates the statistic *Ay* for each of the $y = 1$ to *Z* distance classes. Analyses were performed using $Z = 10$ and 20 distance classes. A permutation procedure of 10,000 replicates was performed as a global test for the full data set to quantify the heterogeneity of *Ay* values among distance classes.

## Phylogenetic Analyses and Host-parasite Co-divergence

We used a selected number of sequences (pruned data set) to test the hypothesis of congruence (co-divergence) in the *O. longicaudatus* and ANDV tree topologies. ANDV may eventually be found in rodent species other than *O. longicaudatus* due to “spillover” (Toro *et al*. 1998; Torres-Pérez *et al*. 2004). However, ANDV prevalence in these non-reservoir species is always significantly lower than in *O. longicaudatus* (Medina *et al*. 2009; Toro *et al*. 1998). Also, ANDV segregates in Chile by geographic location of the sampling site, and does not segregate by host source (Medina *et al*. 2009). To prevent bias due to non-reservoir sources, co-phylogenetic analyses only used ANDV sequenced from *O. longicaudatus*, and both sets (mitochondrial and S-segment sequences) were obtained from the same *O. longicaudatus* specimens. Analyses of ANDV were performed using 931-nt of the S-segment, while analyses of *O. longicaudatus* were performed using concatenated cyt *b* (927-nt) and d-loop (1014-nt) sequences. In all cases maximum likelihood (ML) phylogenetic trees were inferred using the PAUP* package (Swofford 2002). jModeltest (Posada 2008) identified TVM + I and HKY + I as the best-fit models of sequence evolution for ANDV and *O. longicaudatus*, respectively. Node support was evaluated with 1,000 non-parametric bootstrap pseudoreplicates (Felsenstein 1985) using the settings obtained from jModeltest.

In addition, we employed a Bayesian MCMC procedure using BayesPhylogenies (Pagel& Meade 2004) to infer a posterior distribution of phylogenetic trees and assuming the general time-reversible (GTR) model of gene-sequence evolution with gamma distribution of rate variation among sites. From random starting trees, $5 \times 10^6$ generations were run with four Metropolis-coupled chains, with the resulting trees sampled every 1,000 generations. The first 500 trees of the sample were removed to avoid including trees sampled before convergence of the Markov Chain, and the last 4,500 trees were used to compute a 50% majority rule consensus tree. A posterior probability of $P \geq 95\%$ was considered as evidence for significant support for any individual node (Alfaro *et al*. 2003).

The genealogical sorting index (Cummings *et al*. 2008) was used to estimate the degree of exclusive ancestry of individuals in specific groups on a rooted tree. Here, a tree topology is used to quantify coalescent events uniting a group. Under the null hypothesis that the degree of exclusive ancestry of branch tips observed is that which might be observed at random, this index assesses the statistical significance by holding the tree constant with subsequent permutation of the group labels assigned to the tips of the tree, thus randomizing the common ancestry of members of the groups. Analyses were performed in *O. longicaudatus* using 82 samples (plus 2 outgroups) randomly selected, and assigning samples (Mediterranean N = 31, Valdivian N = 34, Patagonian N = 17) to groups as derived from SAMOVA analysis. Significance was evaluated under 10,000 permutations.

To test for co-divergence of *O. longicaudatus*-ANDV, we used both topology-based and distance-based approaches. The congruence index (Icong) is a topology-based approach based on the maximum agreement subtree (MAST) between two trees (de Vienne *et al*. 2007). Icong tests the null hypothesis that two trees are not more congruent topologically than expected by chance. Rejecting the null hypothesis indicates that evolution in one group is dependent to some extent on evolution in the other (de Vienne *et al*. 2009). For the distance-based approach, we used the CopyCat program (Meier-Kolthoff *et al*. 2007), which incorporates a wrapper for the program Parafit (Legendre *et al*. 2002). Parafit uses matrices of raw or patristic distances (summed branch lengths along a phylogenetic tree) transformed into principle coordinates. The program assesses the fit between host and parasite phylogenetic distances, through a matrix representing host-parasite associations. Statistical significance was evaluated by performing 9,999 permutations to test the null hypothesis of no co-divergence between ANDV and *O. longicaudatus* populations.

We compared the results of the above tests with those from two other programs commonly used in cophylogenetic studies. Specifically, TreeMap 1.0 and TreeMap 2.0β are topology-based approaches that reconcile two trees by introducing four types of events (cospeciation, duplication, lineage sorting, and host switching). TreeMap 1.0 incorporates multiple reconstructions that attempt to produce the fewest possible number of events and to maximize the number of co-divergence events. A randomization test (10,000 permutations) was performed on each reconstruction to assess if both phylogenies are more similar to each other than expected by chance. TreeMap 2.0β uses jungles (instead of parsimony), which is a directed graph of all possible mappings of one tree onto another. When resolved, all potentially optimal solutions are reported (POpt) (Charleston 1998). The optimal reconstruction is that which minimizes the global cost. To evaluate the statistical significance of any congruence observed, a null hypothesis that the extent of co-divergence between trees is no more than that expected by chance is tested. Significance was then tested by comparing the jungle with 100 randomized virus trees.

# Results

## Genetic diversity and analyses of selection pressures

Estimations of allele, haplotype and nucleotide diversity (Table 1) revealed similar levels of genetic diversity for *O. longicaudatus* mtDNA sequences sampled from the Mediterranean and Valdivian rain forests regions, but lower levels in Patagonia. For ANDV, allele diversity was similar across the three ecoregions, although higher nucleotide diversity was observed in those viruses sampled from the Mediterranean region. An analysis of selection pressures in ANDV using a variety maximum likelihood approaches revealed no evidence for positive selection. Similar results were obtained using a Bayesian approach, and in some revealed a dominance of purifying selection on the viral S segment.

## Evolutionary Rates and Times to Common Ancestry

We estimated the overall nucleotide substitution rate for the S segment of ANDV to be between $0.17 – 2.06 \times 10^{-3}$ nucleotide substitutions per site, per year (subs/site/year) under a relaxed molecular clock and $0.19 – 1.96 \times 10^{-3}$ subs/site/year assuming a strict clock. These rates are similar to those seen in a broad array of RNA viruses (Duffy *et al*. 2008; Holmes 2008) and may be > 5 orders of magnitude higher than those reported for the mitochondrial genome of rodents (Lessa& Cook 1998; Smith& Patton 1993). In addition, the mean evolutionary rate estimated under the relaxed molecular clock ($1.15 \times 10^{-3}$ subs/site/year) fell outside of the range of substitution rates estimated in 20 randomized data sets (range of 95% HPD values = $1.07 \times 10^{-3}$ to $2.28 \times 10^{-8}$ subs/site/year) indicating that they have been drawn from different distributions and that there is temporal structure in these data. In addition, the lower 95% HPD values in the randomized data tended toward a zero substitution rate (range $8.28 \times 10^{-5}$ to $2.28 \times 10^{-8}$ subs/site/year) as expected given a lack of temporal structure. However, that there is some overlap between the 95% HPD values of the real and randomized data indicates that more sequence data are required to obtain a precise estimate of evolutionary dynamics in ANDV. Similarly, the estimated tMRCA values for ANDV in Chile fell between 28 – 229 and 26 – 224 years before the most recent sample (2004) for the strict and relaxed clocks, respectively. In marked contrast, the tMRCA for *O. longicaudatus* in Chile was estimated to be between 134,000 – 346,300 years before the present (ybp) (95% HPD values; mean of 231,400 ybp). Overall, these results show that scale of evolutionary change in the genomes of ANDV and associated hosts differs dramatically.

## Spatial Genetics

We assessed spatial genetic substructure within the host (*O. longicaudatus*, 195 samples) populations using SAMOVA (Spatial Analysis of Molecular Variance). $F_{CT}$ values ranged from 0.427 to 0.478, with the group structure maximized at $k = 5$. Collection sites from Fray Jorge to Los Ruiles (localities 1 to 7 in Fig. 1) formed the first group, Tome to Puyehue (localities 8 to 14) clustered into a second group, four localities (16 to 19) clustered into a third group, and Torres del Paine (locality 20) was separated as the fourth group (Fig. 1; Table S1). Four groups correspond to previously identified ecological regions in Chile: Mediterranean, Valdivian rain forests, North Patagonian rain forests and Magellanic subpolar forests (Armesto *et al*. 2007; Olson *et al*. 2001; Veblen 2007) (Fig. 1). Chiloé (locality 15), an isolated island, was separated as the fifth group but it is closely allied to the Valdivian rain forest ecoregion (Veblen 2007). Unlike *O. longicaudatus*, SAMOVA was unable to segregate discrete groups with higher FCT maximized values obtained as more groups (*k*) of ANDV sequences were added.

When determining the spatial distribution of alleles/haplotypes in the North – South latitudinal gradient, the genetic landscape shape interpolation analysis (Fig. 2) differed

between ANDV and its host. The highest levels of pairwise genetic distance were observed between 34° – 37° S (the limit between Mediterranean and Valdivian rain forests regions) for ANDV, while the highest values for the host were observed in the south (ca. 41°- 43°S). However, ANDV and *O. longicaudatus* were congruent in showing higher genetic heterogeneity within the Mediterranean ecoregion, with both higher and lower peaks observed especially between virus samples (Fig. S1). Peaks and valleys (areas of high and low genetic distance between individuals) occurred both along the coast (western edge) and in the Andes. Grid size and distance weighting parameters did not affect landscape shape.

Spatial autocorrelation analyses can be used to detect geographic regions with different (high or low) genetic similarity between samples (Diniz-Filho& De Campos Telles 2002). Our analyses indicated significant geographic structure in ANDV and *O. longicaudatus* ($P < 0.0001$). For ANDV, spatial autocorrelation analyses performed using Z = 10 (10 distance classes) and Z = 20 (Fig. 3) were similar with significant autocorrelation ending about 500 km. For *O. longicaudatus*, autocorrelation ceased between the distance classes 3 to 4 (for Z=10) and 6 to 7 (for Z = 20) (Fig. 3). These analyses suggest that spatial phylogeographic structure in ANDV can be detected at around 400-600 km, while for *O. longicaudatus* structure does not appear until about 600-800 km.

## Phylogenetic Relationships and Virus-Host Co-divergence

Phylogenetic analyses for ANDV (S-segment, 931-nt) and *O. longicaudatus* (concatenated cyt *b* and control region, 1942-nt) sequences were based on a reduced subset of 12 samples (Fig. 4) with both mitochondrial and S-segment sequences obtained from the same *O. longicaudatus* specimens. Topologies based on maximum likelihood (ML) and Bayesian MCMC did not show significant differences using the Shimodaira-Hasegawa (SH) test for either ANDV ($-\ln L_{ML} = 2969.668$, $-\ln L_{BMCMC} = 2965.829$, $P = 0.275$) or *O. longicaudatus* ($-\ln L_{ML} = 3263.962$, $-\ln L_{BMCMC} = 3263.962$, $P = 0.523$).

For ANDV, the ML (midpoint rooted) topology identified two primary clades. All samples in the Mediterranean ecoregion (Fray Jorge to Los Ruiles; Fig. 1) clustered into a well-supported clade (maximum likelihood bootstrap values = 100 and posterior probabilities = 1.0 using BMCMC). Within the second clade, two subclades were recovered, a first group with all samples belonging to the Valdivian rain forest region (MLB = 94, PP = 0.99), and a second group with the two samples from Patagonia (MLB = 89, PP = 0.96). For ANDV host, the clades identified had a few samples intermingled among ecoregions. Most Mediterranean samples (except Duao) were in a well-supported clade (MLB = 89, PP = 1.0). Samples from Valdivian rain forest region grouped together with either Duao (Mediterranean) or Rio Simpson (Patagonian) samples. Tucapel (a Valdivian rain forest sample) and Chile Chico (Patagonian sample) were in a highly supported clade (MLB = 99, PP = 1.0). Overall, and as expected given the disparity in evolutionary rates, ANDV sequences provide greater genealogical resolution than the associated host data. Incomplete lineage sorting was observed in populations of *O. longicaudatus* in the Mediterranean (Duao), Valdivian Temperate Forests (Tucapel) and Patagonian (Rio Simpson) regions. Based on the assumption of no lineage sorting in the *O. longicaudatus* samples, we estimated the degree of exclusive ancestry of individuals in specific groups using the genealogical sorting index (GSI) (Cummings *et al*. 2008). For the three groups tested, the index showed highly significant values ($GSI_{Mediterranean} = 0.2729$, $P < 0.001$; $GSI_{Valdivian} = 0.3011$, $P < 0.001$; $GSI_{Patagonia} = 0.5871$, $P < 0.001$), suggesting that placement of *O. longicaudatus* samples into the three respective groups is greater than expected by chance. Incomplete lineage sorting was not observed for ANDV.

Using ML topologies, we performed co-divergence tests with 12 data sets. The index of congruence (Icong) indicates that evolution of ANDV is likely independent of that *O.*

*longicaudatus* ($P > 0.05$). The ParaFit Global test of cospeciation revealed a significant association between ANDV and *O. longicaudatus* samples ($P < 0.05$), although only two out of twelve host-parasite links were significant. Importantly, both TreeMap 1.0 and TreeMap 2.0β randomization tests suggested that the observed number of co-divergence events in the parasite tree was not significantly different from that observed in random associations. Therefore, with the exception of the ParaFit Global test, all analyses revealed that the phylogenetic trees of ANDV and their *O. longicaudatus* rodent hosts are no more congruent than expected by chance alone.

## Discussion

The extent of evolutionary congruence between host and parasite depends on processes that may not only act at different spatial and temporal scales, but also at different hierarchical scales; genes, individuals, populations, or species (Pybus& Rambaut 2009; Rannala& Michalakis 2003). For example, if viral evolutionary dynamics are coupled with ecological processes acting in female-host populations, congruence in spatial pattern may be revealed using a mitochondrial gene. An example is provided by rabies viruses sampled from British Columbia and which show a spatial correlation with cytochrome oxidase I gene from their bat host, suggesting a sex-biased pattern of viral transmission (Nadin-Davis *et al*. 2010). We evaluated current and historical population structure using spatial genetics and co-phylogeographic approaches in a virus – rodent system.

Previous work showed that nucleotide substitution rates in some rodent hantaviruses are significantly higher than their host, which leads to substantial differences in divergence times that appear incompatible with a history of codivergence (Ramsden *et al*. 2009). Indeed, estimates of the tMRCA of hantaviruses and their rodent hosts are similarly discordant (Ramsden *et al*. 2009; Steppan *et al*. 2004). At the phylogeographic scale of our study, the evolutionary rates and tMRCA estimated for ANDV and *O. longicaudatus* also differed substantially, although with a very large variance in the case of the virus. Specifically, the tMRCA of ANDV in Chile was under 250 ybp, while that of *O. longicaudatus* was about 223,000 ybp. Although inferences of tMRCA for RNA viruses using a Bayesian MCMC approach may be strongly biased (i.e. too recent) due to unsampled extant or extinct basal viral lineages or an inability to correct infer the number of mutational changes (Holmes 2009a), our results indicate that the evolution of ANDV and its rodent host is independent at the phylogenetic scale.

Both host and virus segregated across a latitudinal gradient corresponding to three major ecoregions, Mediterranean, Temperate Forests (Valdivian and Patagonian rain forests) and Magellanic Subpolar Forest (Armesto *et al*. 2007; Veblen 2007). Spatial genetic autocorrelation disappears at the boundaries of these ecoregions, with more rapidly evolving ANDV lineages more strongly segregated than *O. longicaudatus*. Some clades of the host share haplotypes across ecoregion borders (Palma *et al*. 2005; Torres-Pérez *et al*. 2010). Nonetheless, both SAMOVA and the genealogical sorting index recovered three groups that correspond to ecoregions despite incomplete lineage sorting in the host (Maddison 1997; Pamilo& Nei 1988), also congruent with three previously proposed *O. longicaudatus* subspecies (Osgood 1943). Ecoregions are defined by environmental characteristics of the landscape in aggregating areas with similar properties (relative homogeneity of ecosystems) (Loveland& Merchant 2004; Wilson *et al*. 2007), which may reflect differences in vegetation composition, soil type, climate, and geology (Bailey 1998; Olson *et al*. 2001). Complex geographical, historical, and/or contemporary evolutionary processes (e.g., differential selective regimes) acting on populations may result in geographic lineages that are partitioned across ecoregions. In this particular virus/mammal system there are no obvious geographic barriers that would reduce gene flow between ecoregions, nor was

positive selection detected in ANDV (Table S2). Alternatively, partitioning of viral variants across ecoregions may be explained by an isolation-by-distance model (Real *et al*. 2005). These and other models should be fully explored in the future when evaluating divergence of ANDV lineages. Populations that are widely separated or that have arisen through recent colonization (as those occurring in previously glaciated areas within the Valdivian region), may bias gene flow estimation as an equilibrium between genetic drift and gene flow has not been reached (Crispo& Hendry 2005). ANDV follows a phylogeographic pattern of strong spatial subdivision (Holmes 2008) that was nearly recovered in *O. longicaudatus*, suggesting that ANDV may be a genealogical proxy of its host.

Landscape structure is likely an important determinant of the genetic structure of ANDV. Transmission in obligate parasites depends on host dispersal and population dynamics, which may be strongly affected by spatial heterogeneity (Biek& Real 2010). For example, *O. longicaudatus* shows a heterogeneous population genetic structure across the latitudinal span of Chile. Within the Mediterranean ecoregion, populations of *O. longicaudatus* have higher genetic divergence than either Valdivian or Patagonian rain forest populations (Torres-Pérez *et al*. 2010). This finding is congruent with a pattern of higher genetic distances among northern ANDV samples as revealed by the genetic landscape shape analysis. The Mediterranean region is characterized by a heterogeneous vegetation mosaic that transitions between desert and mixed deciduous evergreen forests (Amigo& Ramirez 1998; Armesto *et al*. 2007; Veblen 2007). This highly fragmented area supports a large number of threatened endemics (Wilson *et al*. 2007) and 65% of the Chilean human population. For mesic species such as *O. longicaudatus*, this dry, fragmented environment should lead to greater population subdivision, reduced gene flow and strong effects on genetic diversity due to drift (Hartl& Clark 2007; Pilot *et al*. 2006; Sacks *et al*. 2008; Wright 1931). Hence, differences in processes such as fragmentation and drift in host populations likely impacted genetic structure of ANDV, with rapid evolutionary rates in the virus resulting in higher genealogical resolution and structuring than the host.

Fahrenholz's rule assumes that host and associated parasite trees will be congruent due to a shared history (Fahrenholz 1913). However, a growing number of molecular phylogenetic studies have challenged this idea (Jackson& Charleston 2004; Koehler *et al*. 2009; Ramsden *et al*. 2009). At the phylogeographic level, several factors likely contribute to decreased host-parasite congruence between *O. longicaudatus* and ANDV: i) Higher nucleotide substitution rates in ANDV suggest that evolution occurs at different temporal scales; ii) Hantaviruses are reported to be horizontally transmitted in the wild, although host-parasite co-divergence is most likely to occur under vertical transmission (see Nieberding & Olivieri 2007, and references therein); iii) Weak genealogical structure in *O. longicaudatus* contrasts with the increased sorting of the parasite; iv) Genealogical reconstructions of *O. longicaudatus* based on a female-inherited molecular marker such as mtDNA may not accurately reflect the history of ANDV transmission, because ANDV is thought to be primarily transmitted by males (Padula *et al*. 2004; Torres-Pérez *et al*. 2004). However, there is no evidence of differences in dispersal patterns between males and females of *O. longicaudatus* (Murúa *et al*. 1986), suggesting that our genealogical reconstructions likely reflect the history of the species.

Both current and historical processes have acted to produce major differences in the genetic structure of ANDV and *O. longicaudatus* populations. Globally, our results suggest that despite contrasting evolutionary rates, geographic structure of both the host and RNA virus may converge across large spatial scales. Host structure is related to historic (e.g. effects of glacial cycles during Pleistocene; Palma *et al*. submitted) and contemporary (e.g. reduced gene flow among ecoregions) dynamics of its populations. Although ANDV does not co-

diverge with *O. longicaudatus*, strong lineage structure in the virus seems to be a response to current population dynamics in the host at the scale of ecoregions.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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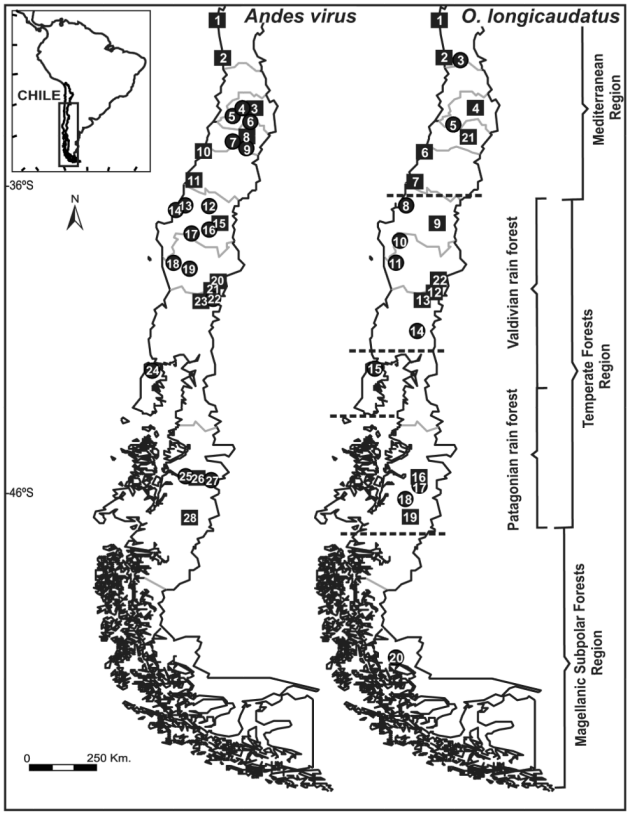


**Figure 1.**

Map of the sampled localities of ANDV in Chile (left) and *O. longicaudatus* (right). Squares represent localities used for co-divergence analyses. Dashed lines separate groups indicated from SAMOVA. Numbers are explained in Table S1 of the supplementary data.

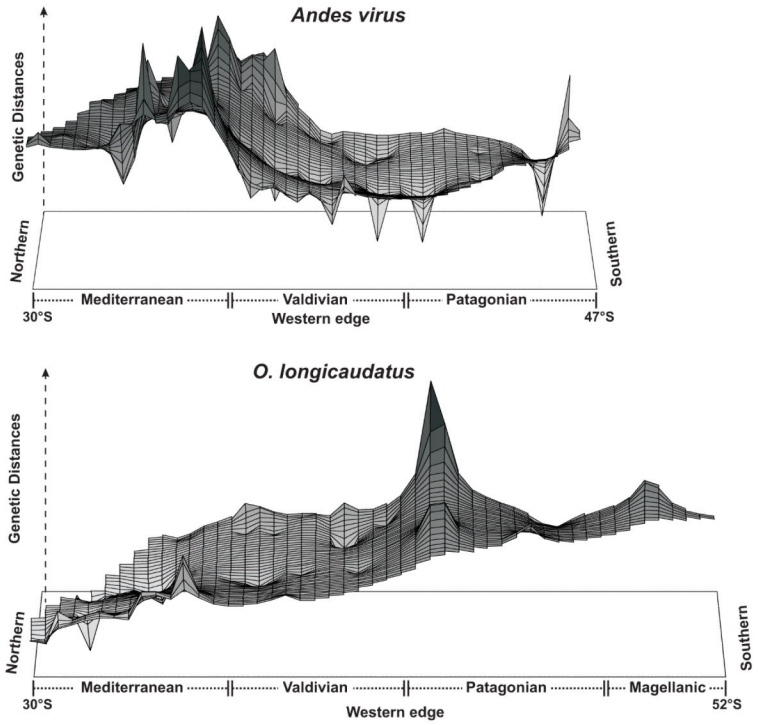


**Figure 2.**

A graphical interpolation-based representation of genetic structure made using a 50 × 50 grid and a distance weighting parameter of 0.5 for ANDV and 1.0 for *O. longicaudatus*. X- and y- axes represent geographic coordinates. Surface heights along the *Z*-axis indicate genetic distances. Gray scales are indicative of areas with high (black) or low (white) pairwise genetic distance between individuals. All samples (195 sequences) were included for *O. longicaudautus*. Scale at the bottom represents latitude in degrees and minutes.

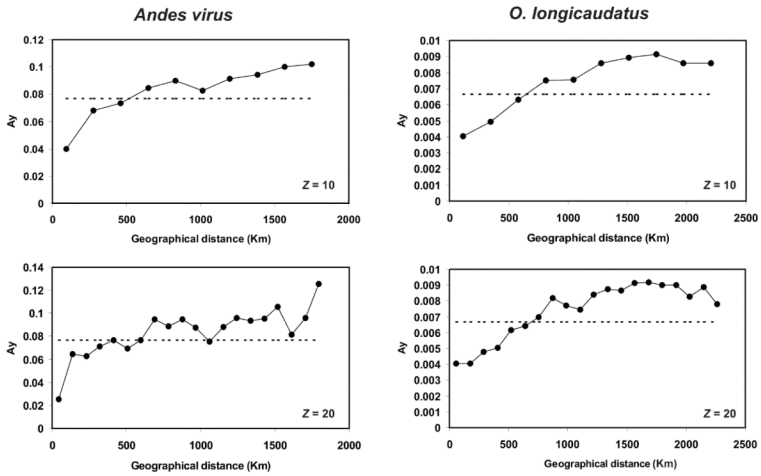


**Figure 3.**

Results of spatial autocorrelation analyses of ANDV (S segment) and *O. longicaudatus* in Chile. Analyses were performed using *Z* = 10 and 20 distinct distance classes. Ay: Average pairwise genetic distances of haplotypes that fall within the boundaries specified for distance class y. Horizontal dotted lines indicate the average pairwise genetic distance from full data set.

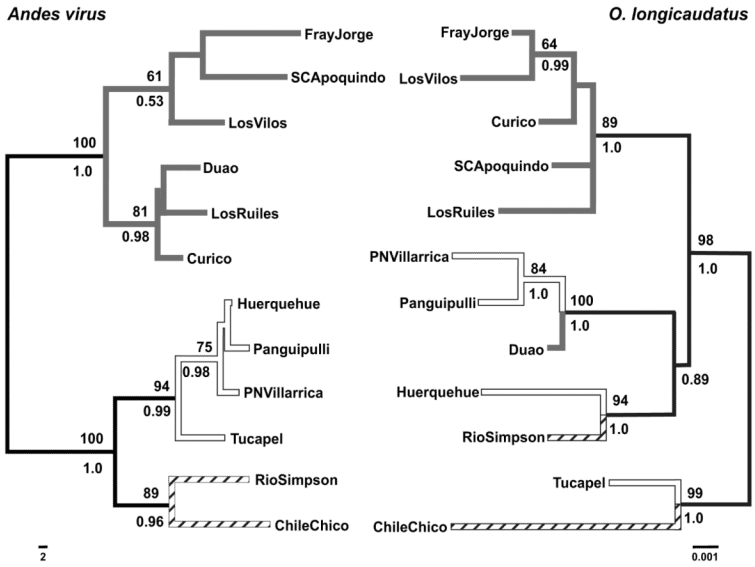


**Figure 4.**

Maximum Likelihood phylogenetic trees from pruned data sets of 12 samples using S-segment sequences (943-nt) for ANDV and concatenated cytochrome *b* and control region for *O. longicaudatus* (1,942-nt). Numbers above and below branches indicate bootstrap values obtained with maximum likelihood and posterior probabilities, respectively. Gray, white and dashed branches represent samples from Mediterranean, Valdivian rain forests and Patagonian rain forests regions, respectively. Bars indicate the number of nucleotide substitutions per site.

**Table 1**

Descriptive statistics of genetic variation of ANDV S segment (397-nt) and *O. longicaudatus* cyt b (927-nt) sequences in Chile. N*:* Number of individuals; Nh: Number of alleles/haplotypes; S: Number of segregating sites; Hd: Allele/haplotype diversity; π: Nucleotide diversity; SD: Standard deviation.

| | Eco-region | N | Nh | S | Hd ± SD | Π ± SD |
|---|---|---|---|---|---|---|
| *An* | 1.Mediterranean | 17 | 14 | 69 | 0.971 ± 0.032 | 0.056 ± 0.003 |
| | 2.Valdivian rain forests | 16 | 14 | 51 | 0.983 ± 0.028 | 0.032 ± 0.004 |
| | 3.Patagonian rain forests | 9 | 8 | 42 | 0.972 ± 0.064 | 0.030 ± 0.006 |
| | Total | 42 | 36 | 107 | 0.992 ± 0.007 | 0.076 ± 0.003 |
| *O. longicaudatus* | 1.Mediterranean | 69 | 34 | 49 | 0.941 ± 0.017 | 0.004 ± 0.0004 |
| | 2. Valdivian rain forests | 76 | 35 | 40 | 0.955 ± 0.011 | 0.004 ± 0.0002 |
| | 3. Patagonian rain forests | 38 | 11 | 16 | 0.839 ± 0.036 | 0.036 ± 0.0005 |
| | Total* | 183 | | 83 | 0.973 ± 0.004 | 0.006 ± 0.0002 |

(*)Does not include Magellanic Subpolar forests region (N = 12)