Title: Estimating Pathogen-Spillover Risk Using Host-Ectoparasite Interactions

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Abstract

1. Pathogen spillover corresponds to the transmission of a pathogen or parasite from an original host species to a novel host species, preluding disease emergence. Understanding the interacting factors that lead to pathogen transmission in a zoonotic cycle could help identify novel hosts of pathogens and the patterns that lead to disease emergence.
2. We hypothesize that ecological and biogeographic factors drive host encounters, infection susceptibility, and cross-species spillover transmission. Using a rodent-ectoparasite system in the Neotropics, with shared ectoparasite associations as a proxy for ecological interaction between rodent species, we assessed relationships between rodents using

geographic range, phylogenetic relatedness, and ectoparasite associations to determine the roles of generalist and specialist hosts in the transmission cycle of hantavirus.

3. A total of 50 rodent species were ranked on their centrality in a network model based on ectoparasites sharing (i.e., 91 fleas, 18 mites, 17 lice, and 5 tick species). Geographic proximity and phylogenetic relatedness were predictors for rodents to share ectoparasite species and were associated with shorter network path distance between rodents through shared ectoparasites.
4. The rodent-ectoparasite network model successfully predicted independent data of seven known hantavirus hosts. The model predicted five novel rodent species as potential, unrecognized hantavirus hosts in South America. Findings suggest that ectoparasite data, geographic range, and phylogenetic relatedness of wildlife species could help predict novel hosts susceptible to infection and possible transmission of zoonotic pathogens.
5. *Synthesis and Applications*: Hantavirus is a high-consequence zoonotic pathogen with documented animal-to-animal, animal-to-human, and human-to-human transmission. Predictions of new rodent hosts can guide active epidemiological surveillance in specific areas and wildlife species to mitigate hantavirus spillover transmission risk from rodents to humans. This study supports the idea that ectoparasite relationships among rodents are a proxy of host species interactions and can inform transmission cycles of diverse pathogens circulating in wildlife disease systems, including wildlife viruses with epidemic potential, such as hantavirus.

Keywords: ectoparasites, hantavirus, network, rodent, spillover, wildlife, zoonotic

Introduction

Zoonotic diseases originate in animals and infect humans, posing significant threats to public health worldwide (Cutler et al., 2010; Holmes, 2022). Cross-species spillover transmission, where a pathogen or parasite is transmitted from its reservoir host species (i.e., original or donor host) to a novel species (i.e., recipient host), is a critical precursor to zoonotic disease emergence (Kreuder Johnson et al., 2015; Olival et al., 2017). The possibility of a pathogen spilling over into novel species depends on many factors, some of which are directly associated with the reservoir host, including host distribution, density, and interactions with other potential hosts (Plowright et al., 2017). Understanding the underlying factors and mechanisms that drive pathogen transmission cycles in wildlife is crucial for identifying novel hosts and uncovering the ecological patterns that contribute to the emergence of diseases in humans (Alexander et al., 2018; Sánchez et al., 2021).

One group of zoonotic pathogens with global concern is hantaviruses. Hantaviruses are members of the family Hantaviridae and are primarily transmitted to humans through contact with infected rodents or their excreta (Laenen et al., 2019; Vial et al., 2023). Human infections can result in severe illnesses, including hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HCPS), which can be fatal (Jiang et al., 2017; Vial et al., 2023). Hantaviruses have a worldwide distribution and are prevalent in Asia, Europe, and the Americas, causing approximately 20,000-100,000 cases annually (Avšič-Županc et al., 2019; Jiang et al., 2017; Zhang et al., 2010). In the Americas, hantaviruses cause approximately 300 cases of HCPS annually (Vial et al., 2023). Although the seroprevalence and case rates of hantaviruses in the Americas are lower than in other regions, the case mortality and disease severity are higher (Vial et al., 2023). Andes orthohantavirus (ANDV), present in Argentina and Chile, is the only hantavirus with documented human-to-human transmission and has comparatively high case mortality rates (Hjelle & Torres-Pérez, 2010; Vial et al., 2023). In Chile, there is one known

reservoir rodent species for ANDV, *Oligoryzomys longicaudatus*, and six other known rodent hosts (*Abrothrix olivaceus*, *Phyllotis darwini*, *Abrothrix longipilis*, *Rattus rattus*, *Loxodontomys micropus*, and *Rattus norvegicus*) that have shown evidence of infection or exposure (Llanos-Soto & González-Acuña, 2019). Cross-species transmission of hantavirus from the primary reservoir host to secondary hosts has been documented in various hantaviruses (Delfraro et al., 2008; Medina et al., 2009; Vapalahti et al., 1999; Weidmann et al., 2005) and may play a role in human disease emergence (Kreuder Johnson et al., 2015; Olival et al., 2017).

For hantaviruses to spread from one rodent species to another, there must be ecological interaction between individuals of different rodent species, which can constitute aggressions, sharing of resources, or co-habitation (Palma et al., 2012). In Chile, the primary transmission mode for ANDV between rodent species is through saliva, but it could also include urine and feces, which are shared while rodents interact (Padula et al., 2004). In the case of ANDV, uncovering the ecological factors underlying cross-species viral transmission could be particularly informative because the mode by which hantaviruses have diversified in South America is still unclear (Kuhn & Schmaljohn, 2023; Rivera et al., 2015). The limited understanding of hantavirus evolution in South America restricts our capacities to anticipate zoonotic and cross-species spillover transmission.

Understanding the factors driving pathogen transmission and identifying potential hosts in the hantavirus transmission cycle in wildlife is essential for mitigating the risk of zoonotic spillover (Cleaveland et al., 2007; Hjelle & Torres-Pérez, 2010). Network analysis has emerged as a valuable tool in ecology, offering insights into the complex species interactions within ecosystems (Poulin, 2010; Proulx et al., 2005). Network analysis provides a framework for studying ecological systems by representing the relationships among different components and can be a valuable tool

for studying wildlife disease ecology and pathogen transmission (Craft & Caillaud, 2011; Silk et al., 2017). Ecological networks depict communities by representing parts of the community as nodes (individuals or groups) that are connected by edges (an interaction or shared characteristic linking nodes) (Craft & Caillaud, 2011). Factors contributing to pathogen transmission and spillover can be explored by reconstructing host networks (Bordes et al., 2017; Craft & Caillaud, 2011).

One challenge in constructing ecological networks is determining how nodes, in this case, potential hosts, should be connected to best represent species or individual interactions (Craft & Caillaud, 2011). Sharing of ectoparasites can serve as an informative link between two hosts in a network (Poulin, 2010) because it serves as a proxy for ecological interaction between hosts (Nieberding & Olivieri, 2007). Ectoparasites can be generalists (i.e., parasitizing many host species) or specialists (i.e., parasitizing one or very few host species) (Poulin, 2007). Host biology is an essential factor in whether an ectoparasite can successfully parasitize the host (Poulin, 2007). When hosts have an ectoparasite association in common, it indicates interaction or similarity between hosts (Poulin et al., 2011). Phylogenetic relatedness, habitat or range overlap, morphology, and phenology are some similarities that hosts may share when they share ectoparasites (MacDonald & Brisson, 2022; Poulin et al., 2011; Runghen et al., 2021; Sun et al., 2023). Ectoparasite sharing between hosts differs based on the parasite species. However, it can be broadly categorized into species that require direct contact to be shared, such as lice, nidicolous ticks, and some mites, and species that can be shared indirectly through shared environments, such as non-nidicolous ticks, some mites, and fleas (Di Giovanni et al., 2021). Regardless of how ectoparasites are transmitted between host species, the sharing or trait of being parasitized by two of the same ectoparasite species can indicate ecological similarity between hosts.

115 Based on ecological, biogeographic, or evolutionary similarities, estimating ectoparasite
116 sharing between potential host species in a pathogen transmission network could be a powerful
117 tool to infer host interaction. In the case of ANDV cross-species spillover transmission,
118 ectoparasite sharing may be particularly relevant because ANDV transmission between rodents
119 requires direct host contact or sharing of resources which can be well represented by sharing
120 ectoparasite species. Beyond ANDV, this approach can help translate data from one study system
121 into another. This is specifically useful for pathogen transmission systems, especially those that
122 depend on ectoparasites, because there may be little data on how potential hosts interact due to the
123 difficulty of collecting this type of data. At the same time there can be an abundance of other data
124 (ie. ectoparasite associations for those hosts) that can help bridge the gap for understanding host
125 interactions. Using host-parasite interactions to create a network structure and study interactions
126 is not novel (Bordes et al., 2017; Dattilo et al., 2020; Runghen et al., 2021). However, previous
127 approaches tend to include ectoparasites in the network in the same way that hosts are included
128 (i.e. both are included as nodes; Dattilo et al., 2020, Runghen et al., 2021). Here, we demonstrate
129 how known ectoparasite associations can be utilized to infer host interactions, as connections
130 between nodes, to understand pathogen transmission. This provides a framework for viewing
131 ectoparasite sharing as a host trait independent of which specific species are being shared. In this
132 framework, we lose knowledge of which ectoparasite species are contributing to interactions, but
133 we gain the ability to more clearly visualize how hosts interact, how a species ensemble is
134 structured, and the strength of interactions between hosts.

135 In this study, we investigated the evolutionary and ecological factors that predict known
136 and potential novel hantavirus hosts using network analysis on a dataset of rodent-ectoparasite
137 records in Chile. To uncover interactions and possible transmission dynamics between hosts, we

hypothesize that shared ectoparasites are a proxy for the ecological interactions among host species which can be used to build a host interaction network. By examining the connections and centrality of rodent species within the ectoparasite network, we aim to uncover the ecological, phylogenetic, and biogeographic drivers of host centrality and cross-species transmission events. Ultimately, this research adds to a growing body of work showing the applications of network analysis in disease ecology which can inform targeted surveillance efforts to specific rodent species and regions and contribute to the mitigation of hantavirus emergence.

Materials and Methods

Host-Parasite Association Dataset

We constructed a dataset of published rodent-ectoparasite associations in Chile by updating the review by Landaeta-Aqueveque et al. (2021). We conducted a search of rodent-ectoparasite reports in Chile published from January 2015 to March 2023 in PubMed and Web of Science. We conducted our search on March 10, 2023. We used the same search terms as the original review: (("Acari" OR "Ixodida*" OR "Phthiraptera*" OR "Siphonaptera*" OR "tick*" OR "mite*" OR "lice" OR "louse") AND (Chile)) AND (Rodent* OR Rodentia). Our inclusion criteria required reports to be original (i.e. not a review), conducted in Chile, and include collection of rodents and ectoparasites. We used Covidence software (www.covidence.org) to streamline the filtering, eligibility, and data extraction process. Findings were used to update the Landaeta-Aqueveque et al. (2021) review of rodent-ectoparasite associations in Chile. We corrected rodent names according to the taxonomic standing in the Integrated Taxonomic Information System (ITIS; www.itis.gov) and removed any genus-level ectoparasite or rodent record.

Host Geographical Range

We obtained geographic range data of rodents of Chile using the International Union for Conservation of Nature (IUCN 2023) database with the search criteria to include results with taxonomy set to 'Rodentia' and land area to 'Chile'. We used the administrative boundary for Chile from DIVA-GIS (http://www.diva-gis.org/gdata) to restrict the IUCN ranges to Chile. For the seven rodent species with documented ectoparasite associations that did not have range data available in IUCN, we searched GBIF (GBIF.org, 2024) for occurrence data to build an approximate range.

We directly downloaded range data from IUCN for species available. For those from GBIF (datasets: Oyander et al., 2023; GBIF.ES, 2023), we used the R package *gbif.range* (Chauvier et al., 2022.) to generate approximate ranges. Occurrence data from GBIF was downloaded on March 6, 2024. Using spatial analysis packages *sf* (Pebesma E., 2023) and *raster* (Hijmans, 2023) in R version 2022.12.0 (R Core, 2023), we constructed species richness maps for the 67 rodent species recorded in Chile and the subset of 45 rodent species with ectoparasite associations. We then calculated the geographic overlap for rodent species using Jaccard's similarity index (Real & Vargas, 1996) with the *sf* package in R where we used the function, 'st intersection', to obtain the area of overlap between the two species. Following the equation for Jaccard's similarity, we divided the intersection by the total of both ranges minus the intersection. We also used the *sf* package in R to calculate geographic distance using range centroids between rodent species.

Host Phylogenetic Relatedness

To calculate pairwise phylogenetic distance for the rodent species in our network, we accessed the mammalian phylogeny from Upham et al. 2019 through the VertLife project (https://vertlife.org/).

We used the subsetting tool to request 1,000 trees for only the rodent species in our rodent-ectoparasite network (50 rodents in the network, 48 available through VertLife). Using the *ape* package in R, we built the most probable tree based on the 1,000 trees from for the species available in VertLife. Using *ape*, we also calculated the pairwise phylogenetic distance (with the cophenetic function) for the rodents in our network for downstream analysis.

Ectoparasite Sharing

We assessed how geographic and phylogenetic factors influence rodent connections using two methods: (1) probability as a function of geographic and genetic relatedness to share ectoparasites using logistic regressions and (2) correlation of phylogenetic and geographic distances with network path distances using Mantel tests (see *Network Analysis*). We used R software version 2022.12.0 (R Core, 2023) for all statistical analyses and considered results with $p< 0.05$ to be significant. First, we calculated the probability of rodent species to share an ectoparasite based on pairwise phylogenetic distance, geographic overlap, and geographic distance between rodent species using the *geotax* package in R version 2022.12.0 (Robles, 2023; Robles-Fernández & Lira-Noriega, 2017). We excluded ectoparasites associated with less than three rodent species from the probability analysis to mitigate uncertainty related to low sample size, similar to Dáttilo et al., 2020 which excluded those with less than one association. Then, we constructed pairwise interaction matrices representing the phylogenetic distance, geographic overlap, and geographic distance between rodent species. We then constructed binary interaction matrices between ectoparasites and rodent species with "1" representing a documented association between a rodent and an ectoparasite and "0" representing no interaction. Using these interaction matrices, we calculated logistic regression coefficients drawn from a distribution of 1000 permutations with

phylogenetic distance, geographic overlap, or geographic distance as the predictor variable for the sharing of ectoparasites between two rodent species. We calculated logistic regression coefficients for each ectoparasite species and for the overall species pooled for each predictor. With the logistic regression coefficients, we calculated the probability of rodent species to share ectoparasites based on each predictor. We used the *geotax* package in R version 2022.12.0 (Robles, 2023; Robles-Fernández & Lira-Noriega, 2017) to create the binary interaction matrices and to calculate logistic regression coefficients and probability vectors.

Network Analysis

We built a network estimating how rodents were connected through the ectoparasites they share using Gephi software with (Bastian M., 2009). We first built a weighted interaction matrix to show the number of ectoparasites shared by each pair of rodents. With this, we built a weighted network in Gephi using *Fruchterman Reingold* followed by *Force Atlas 2* visualization algorithms with rodents as nodes and shared ectoparasites as edges (Bastian M., 2009). The bipartite network structure we chose to use displays the species of rodents as nodes and the connection between them the ectoparasites they share. With this network structure we lose the information of which ectoparasite species are being shared and all species are treated equally in their contribution to the relationship between two rodents. We chose this structure because we are primarily focused on the rodent hosts, using the ectoparasites as a tool by which to connect them.

From the rodent-ectoparasite network model, we extracted the closeness centrality of each rodent species in Gephi. The closeness centrality measures the average distance from one node to all other nodes in the network and provides a concept for how central or key a species is in the community (Brandes, 2001). Closeness centrality is an appropriate metric in this context because

we are using a diverse set of ectoparasite species as connections, meaning that connection may not have to be direct for rodents to share ectoparasites, but could happen through the environment or shared habitat. Additionally, our pathogen of interest, the Andes virus, may be transmitted between rodent species through habitat or resource sharing and not necessarily through direct contact. This justified the use of closeness centrality in both cases because closeness centrality goes beyond traditional network metrics and considers spread through a network in a mode that is not entirely reliant immediate interactions (Bloch et al., 2023).

We also generated a path distance matrix for the network using the package *igraph* in R which calculates the shortest path between each pair of nodes (Nepusz, 2006). The path distance matrix is a pairwise matrix with rodents as column and row headers and the interaction between each representing the path distance (i.e. number of rodents through ectoparasites) between each pair. Using the pairwise path matrix, we tested for correlation between path length with both phylogenetic and geographic distance. We tested for correlation using a Mantel test in the R package *vegan* (Oksanen J et al., 2022) using pairwise matrices of path length vs phylogenetic distance, path length vs. geographic distance, and finally geographic and phylogenetic distance.

Spillover Potential

We tested how closeness centrality of our network model predicted hantavirus hosts using a phylogenetic logistic regression for binary dependent variables with closeness centrality as a continuous predictor and hantavirus-host status as the binary dependent variable (Ives & Garland, 2010). This model accounts for the non-independence of the host species and evaluates how a binary trait, in this case being a hantavirus host, evolves and is correlated according to both the phylogeny and the continuous variable, closeness centrality. We implemented this model using the

package 'phylolm' in R with the algorithm from *Ho & Ané, 2014*. To explain the model summary, we follow the interpretation of α from the example in *Ives & Garland, 2010* as well as that suggested by *Cooper et al., 2016* using the phylogenetic half-life. We also applied a quantile threshold to closeness centrality which split rodents into four groups based on how central they are in the network model. Based on these groups, we predicted that the most central quantile group would represent likely hantavirus hosts and tested this using cumulative binomial probability. For the cumulative binomial probability test (Loader, 2000), the number of species in the highest quantile was considered the number of trials, the rodent species known to be hantavirus host were the successes, and the proportion of species in the highest quantile from the entire number of species studied was considered the probability of success.

Hantavirus in the Network

We examined the relationship between rodent hantavirus sharing with geographic overlap and distance, and phylogenetic distance using the same methodology as described for ectoparasite sharing. For this, we added the hantavirus associations between each rodent host to the rodent-ectoparasite dataset. From this, we obtained logistic regression coefficients and probability vectors for the relationships between hantavirus host status with phylogenetic relatedness, geographic distance, and geographic overlap. We compared how hantavirus sharing and ectoparasite sharing are related to the geographic overlap and distance and phylogenetic relatedness of their rodent hosts.

Results

Our search resulted in six new reports of ectoparasites on rodents in Chile, with a total of 33 papers between the original review (Landaeta-Aqueveque et al., 2021) and our update (Table S1). This created a dataset with 50 rodent species parasitized by 131 ectoparasites and 376 unique interactions between them (Table S1). From the review we updated seven species names according to valid ITIS names (Table S2). Of the 50 rodent species documented with ectoparasites, data were available to construct a species richness map for 45 rodents (43 from IUCN and two from GBIF; Figure 1A) and a phylogenetic tree for 48 rodents (Figure 1B). We found that the highest rodent species richness in Chile covered latitudes of 30°S to 55°S (Figure 1A, Figure S1). Our phylogenetic tree included 26 genera representing 48 species of rodents.

Ectoparasite Sharing

We found that phylogenetic distance, geographic overlap, and geographic distance were predictors for rodents to share ectoparasite species (Table 1). More closely related rodent species were overall more likely to share ectoparasite species and each individual ectoparasite also followed this trend with negative slopes for each regression (Table 1: overall models, All ectoparasites: Table S4). Sharing of ectoparasites was also strongly predicted by geographic overlap and geographic distance between rodent species (Table S4). The overall trends followed for each individual ectoparasite with positive slopes for geographic overlap and negative slopes for geographic and phylogenetic distance (Figure 2). For the phylogenetic regression, 37 species of ectoparasites and 48 species of rodents were included and for the geographic regressions 37 species of ectoparasites and 45 species of rodents were included based on available data and our limiting the analysis to ectoparasites with 3 or more rodent host associations. Based on the slopes of the regression model, phylogenetic distance was the strongest predictor for sharing ectoparasites, followed by

298 geographic overlap and geographic distance. Individual ectoparasites followed the trends to
299 different degrees, indicating strong or weak relationships with geographic and genetic factors.
300
301 **Rodent-ectoparasite Network**
302 We included all 50 rodent and 131 ectoparasite species, with 376 unique interactions between
303 rodents through ectoparasites, in our network model (Figure 3). In the rodent-ectoparasite network,
304 we found that individual rodent species were parasitized by up to 46 ectoparasite species with a
305 maximum of 186 unique relationships to other rodents through ectoparasites. The strongest
306 connection in our model was between *A. olivaceus* and *A. longipilis* which shared 24 ectoparasite
307 species (Figure 4). The overall dataset indicated a median ectoparasite to relationship ratio of 4.3,
308 meaning that on average, each ectoparasite species connects its host to about four other hosts
309 (Figure 5). Quantiles of network closeness centrality (CC) varied from CC=0 (*Ctenomys osgoodi*,
310 one ectoparasite, no relationships) to CC= 0.78 (*A. olivaceus,* 46 ectoparasites, 184 relationships).
311 Similarly, for matrix correlations, we found that path length in the rodent-ectoparasite network
312 was correlated with both geographic distance (Mantel test, n= 44 rodent species, p=0.001 r=0.363)
313 and phylogenetic distance (Mantel test, n= 47 rodent species, p=0.025 r= 0.115). We found no
314 association between geographic and phylogenetic distance (Mantel test, n= 44 rodent species
315 p=0.945 r=-0.071). Although we had geographic data for 45 species and phylogenetic for 48
316 species, we did not include *Ctenomys osgoodi* in the mantel tests because it had no relationships
317 to other rodent species, making the total species included one less than that which we had available
318 data.
319
320 **Spillover Prediction**

Closeness centrality was a significant predictor for rodents to be hantavirus hosts and there was very little phylogenetic signal for this trait (Closeness centrality: p=0.00166, Phylogenetic signal: α=0.00046, a=-3.34, $t_{\frac{1}{2}}$ =1498, mean tip height= 40.367). Closeness centrality quantiles identified rodents prone to cross-species hantavirus transmission, including twelve highly connected rodents (Figure 6; *A. olivaceus*, *P. darwini*, *A. longipilis*, *O. longicaudatus*, *R. rattus*, *L. micropus*, *Reithrodon auritus*, *Aconaemys porteri*, *Phyllotis xanthopygus*, *Octodon degus*, *Chelemys macronyx*, and *R. norvegicus*). Independent host data revealed that known hantavirus hosts were successfully predicted better than by chance ($p(x=7)$=0.009). Mapping the predicted hantavirus hosts revealed hotspots of spillover transmission risk found between latitudes 45°S and 55°S in Chile (Figure S1A).

Considering known hantavirus-rodent relationships revealed that hantavirus infection among rodents follows the same trends as ectoparasite infestations. That is, the probability for a pair of rodent species to share hantavirus increased with geographic overlap and decreased with geographic and phylogenetic distance. Hantavirus sharing had stronger relationships to all three predictor variables than overall ectoparasite sharing (Table S4).

Discussion

Host networks have been used in wildlife disease ecology to understand pathogen transmission, but often rely on social interactions between hosts, which can be difficult to define or collect in many systems. We posit that ectoparasites can act as a proxy for ecological interaction between hosts, which has been proposed previously (Nieberding & Olivieri, 2007) and is based on literature that ectoparasites are indicative of host phenology (MacDonald & Brisson, 2022), host phylogenetics and geographic distribution (Poulin et al., 2011), morphology (Sun et al., 2023), and

other traits (Poulin et al., 2007; Runghen et al., 2021). We built a host network using this concept of ecological interaction through ectoparasites to understand how other parasites or pathogens may move through a host species assemblage. Using a well-understood rodent system in the Neotropics, we identified underlying predictors for rodents to share ectoparasites, built a weighted interaction network, and used network centrality to predict hantavirus hosts. We successfully predicted all known hosts of hantavirus and identified five rodent species as potential hantavirus reservoirs. Proximity in geographic range and phylogenetic relatedness are strong predictors for rodents to share ectoparasites and to be more connected in the network. Our findings revealed hotspot areas to inform surveillance of hantavirus in rodents in Chile.

A pair of rodent species were more likely to be parasitized by the same ectoparasite if they were closely phylogenetically related, overlapped more in range, and had a shorter distance between the center of their ranges. The trends between geographic and genetic factors with parasite sharing in the rodent-ectoparasite system are similar to trends in other systems, including viruses in bats (Wang et al., 2023), helminth and microparasites in rodents (Bordes et al., 2017), *Bartonella* in bats (McKee et al., 2019), avian malaria in bird communities (Clark & Clegg, 2017), plants and beetle species (Robles-Fernández & Lira-Noriega, 2017), and small mammals and ectoparasites (Dáttilo et al., 2020). We expand on the understanding of how evolutionary and ecological factors influence host interactions by exploring their role in a network of hosts versus in individual host relationships. Based on our regression analysis, we found that ectoparasite sharing between individual rodents had a stronger relationship with geographic distance and overlap than with phylogenetic distance. In agreement, shorter network path distance between rodents was more correlated with geographic distance than with phylogenetic distance. This contributes to both

general frameworks for understating ectoparasite-host communities and parasite sharing, and to the hantavirus system in Chile specifically.

From the rodent-ectoparasite network, we found that closeness centrality can infer hantavirus host status. We demonstrated that closeness centrality showed significant correlation with hantavirus host status when there was very little phylogenetic signal, implying that using the ectoparasite-network model is a significantly better tool than phylogenetic relationships alone for predicting hantavirus host status. We found that the twelve highly connected rodents included all seven known hantavirus hosts and five potential hosts. It has been suggested previously that highly connected rodents in a network may play a role in zoonotic disease spillover (Bordes et al., 2017). We support high network centrality as a driver of spillover in the rodent-hantavirus system. Using the twelve most central rodents, we identified the south-central region of Chile as having the greatest richness of known and potential hantavirus host and reservoir species. This area has previously been defined as a mammalian biodiversity hotspot (Hernández-Mazariegos et al., 2022), which could place it at a higher risk for emerging zoonotic diseases (Allen et al., 2017). The rodent species and areas identified can be used to inform surveillance programs aiming to identify novel hosts and regions where the virus could emerge. Identifying novel hosts and high-risk areas for hantavirus spillover in Chile is critical as the hantavirus, ANDV, associated circulating in this region has revealed a risk of human-to-human transmission (Martinez-Valdebenito et al., 2014) and is also associated with the second highest case mortality rate for all hantaviruses worldwide (Vial et al., 2023). Anticipating and assessing for cross-species transmission events of hantavirus in Chile can allow assessment of ANDV evolution in transmissivity and virulence, which is fundamental for early detection of enhanced pathogenicity and pandemic risk.

389 Pathogens and parasites can be specialists, exploiting one or few hosts, or generalists,
390 exploiting many hosts. Phylogenetic relatedness and geographic distance can influence the ability
391 of a pathogen or parasite to exploit multiple hosts (Poulin et al., 2011). Within the generalist-
392 specialist framework, some pathogens and parasites act as generalists regarding one factor while
393 they are specialists with regard to another. This means that a parasite or pathogen can be a
394 generalist in terms of host range but a specialist in terms of geographic range, which makes
395 classifying a parasite or pathogen into only one role reductive of its multiple interactions (Poulin
396 et al., 2011). For emerging diseases and vectors, this is relevant because the context of the parasite
397 or pathogen specificity is essential in identifying where or in what species a disease or vector has
398 the potential to expand. For example, in our rodent-ectoparasite network, the flea *Plocopsylla*
399 *crypta,* had a strong relationship with geographic proximity of hosts, but a weak relationship with
400 phylogenetic relatedness. This indicates that *P. crypta* is more of specialist in terms of parasitizing
401 hosts in a restricted geographic area, but within that area it can act as a generalist, parasitizing
402 distantly related host species. Although the majority of ectoparasites in our analysis were fleas, the
403 next largest group, mites, notably had stronger relationships with phylogenetic relatedness and
404 geographic proximity than fleas in general did. This may be because it is more beneficial for
405 directly transmitted parasites, like fleas, to parasitize many different host species while it can be
406 detrimental for indirectly transmitted parasites to do the same (Poulin et al, 2007). Using this
407 framework can help expand the definition of generalist and specialist parasites because it considers
408 multiple factors that influence the ability of parasites to exploit hosts.
409 Our findings may also be informative in deciphering how hantaviruses have diversified in
410 South America. There are over 28 hantaviruses that can cause human disease and others of
411 unknown zoonotic risk found in a wide range of wildlife species, including rodents, bats, moles,

412 shrews, reptiles, and fish (Avšič-Županc et al., 2019; Vial et al., 2023). Hantaviruses were
413 previously thought to have co-evolved with their hosts. However, recent discoveries on new hosts,
414 new viral strains, and the evolution rate of hantaviruses do not entirely support this idea (Kuhn &
415 Schmaljohn, 2023). The possibility of preferential host-switching has been proposed but is not
416 supported in hantaviruses in South America, for which geographic proximity is a potential
417 explanation of hantavirus diversification (Rivera et al., 2015). Using the known hantavirus hosts
418 in Chile, we found that geographic proximity, phylogenetic relatedness, and centrality in the
419 rodent-ectoparasite network were predictors for rodents being known hantavirus hosts.
420 Hantavirus-host status was more dependent on geographic proximity and phylogenetic relatedness
421 than the average for ectoparasites, indicating that it is more of a specialist than most ectoparasite
422 species. We found that hantavirus had a stronger relationship with geographic overlap and
423 geographic distance between rodents than with phylogenetic distance. Relationships between
424 geography and phylogeny to explain infection provide support for exploring the hypothesis that
425 the geographic proximity of hosts has influenced the diversification of hantaviruses in South
426 America. Host sympatry may suggest a host interaction leading to cross-species spillover and
427 diversification. Of the five species predicted to be unknown ANDV hosts, two are non-Myomorph
428 species (*O. degus* and *A. porteri*). Both species are in close geographic proximity and share
429 considerable numbers of ectoparasites with known ANDV hosts, suggesting the potential for direct
430 interaction necessary for ANDV transmission between rodents. If found infected with ANDV,
431 these two rodent species would represent spillover into phylogenetically distinct hosts and could
432 provide significant context to how hantaviruses have diversified.

433 The interpretation of our results must be considered in the context of data availability and
434 geographic extent. Rodent-ectoparasite associations were based on documented associations and

435 may be biased toward rodent species of known health concern and not fully represent all
436 ectoparasite associations in Chile. Geographic range and genetic data did not fully represent the
437 50-rodent dataset, with five species missing geographic data and two species missing genetic data.
438 Notably, IUCN failed to include *R. rattus* or *R. norvegicus* distributions in Chile. However, both
439 species have documented hantavirus infections in Chile (Lobos et al., 2005). The rodent species
440 names and taxonomic standing that we included are according to the ITIS and may not represent
441 recent advances in Chilean rodent taxonomy (D'Elía, 2020). A comprehensive dataset geographic
442 ranges would improve our understanding of hantavirus transmission dynamics. Additionally, we
443 limited our study area to the geographic extent of Chile. We chose this limited extent to facilitate
444 clear boundaries for our literature review and to account for differences in sampling efforts
445 country-country in South America. Also, although many administrative country boundaries may
446 not be ecologically relevant and the boundaries of Chile correspond to biogeographic regions due
447 to the Andean cordillera (Morrone, 2018). Hantaviruses, however, are distributed globally and our
448 approach may not scale to a global extent or in every system where hantavirus is present.
449 We demonstrated that ecological networks based on shared ectoparasites can elucidate how
450 wildlife host species interact in the transmission of parasites and pathogens. Host interactions can
451 be challenging to estimate due to labor, financial, or biologically imposed constraints. Our findings
452 reveal that cross-species transmission dynamics are influenced by host phylogeny and geographic
453 range, which together culminate in ectoparasite sharing. To validate the predictive power of our
454 approach we suggest expanding the species tested for hantavirus in Chile, and even targeting
455 capture and sampling of potential hosts identified in our study. Although shared ectoparasites are
456 not a direct measure of interaction, they offer a less laborious method for connecting hosts while
457 still being ecologically relevant. Further studies focused on multiple pathogens or parasites could

use similar methods and include network communities to understand how more specialized pathogens or parasites cluster within specific hosts. Ectoparasite associations are indicative of host biology and using known interactions can help disease ecologists understand how hosts interact in ways that may otherwise be overlooked.

Contributions

LEE conceived the study concept. RNB designed the methodology, performed data analysis, and preparation of the first draft. All authors performed the data collection, co-wrote final version of the article, and approved the final version of the publication.

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Data Availability

Data and code available from the Github repository https://github.com/reillybren/ChileRodents. Supporting Information will be found in the online version of the article at the publisher's website.

Conflict of Interest

We declare no competing interests.

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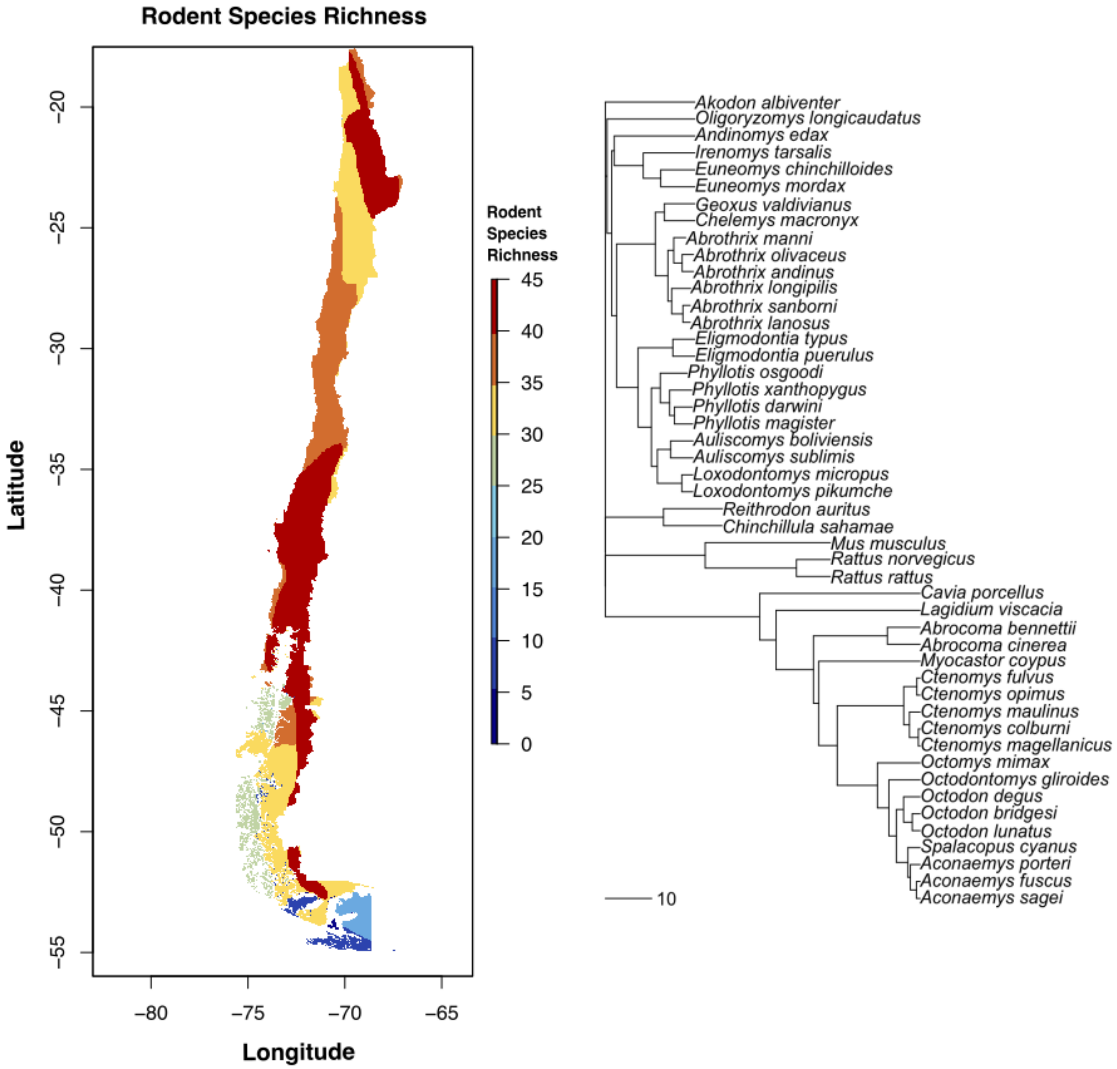
Tables

	Geographic Overlap	Geographic distance	Phylogenetic Distance
β_0	-2.82881254	-0.6467294	-0.769268852
Std. Error	0.11842025	0.10890083	0.133686998
z value	-23.8897436	-5.9419812	-5.75342839
Pr(>|z|)	1.66E-124	7.21E-07	3.41E-06
2.50%	-3.06091196	-0.8601711	-1.031290552
97.50%	-2.59671312	-0.4332877	-0.507247152
β_1	3.07615777	-0.1541742	-0.021322972
Std. Error	0.20441326	0.01446215	0.002463259
z value	15.0478327	-10.63058	-8.637090339
Pr(>|z|)	8.95E-44	3.77E-18	1.06E-10
2.50%	2.67551514	-0.1825195	-0.026150871
97.50%	3.47680041	-0.1258289	-0.016495074

Table 1. **Logistic regression coefficients and summary for ectoparasite sharing.** The overall logistic regression model coefficients for each of the predictor variables (phylogenetic relatedness, geographic distance, and geographic overlap) are reported with their summary statistics.

Figures



718

719 **Figure 1. Rodent species diversity in Chile. Left.** Rodent species richness for species with
720 documented ectoparasite associations. **Right.** Rodent phylogeny for species with documented
721 ectoparasite associations with maximum likelihood bootstrap percentages.

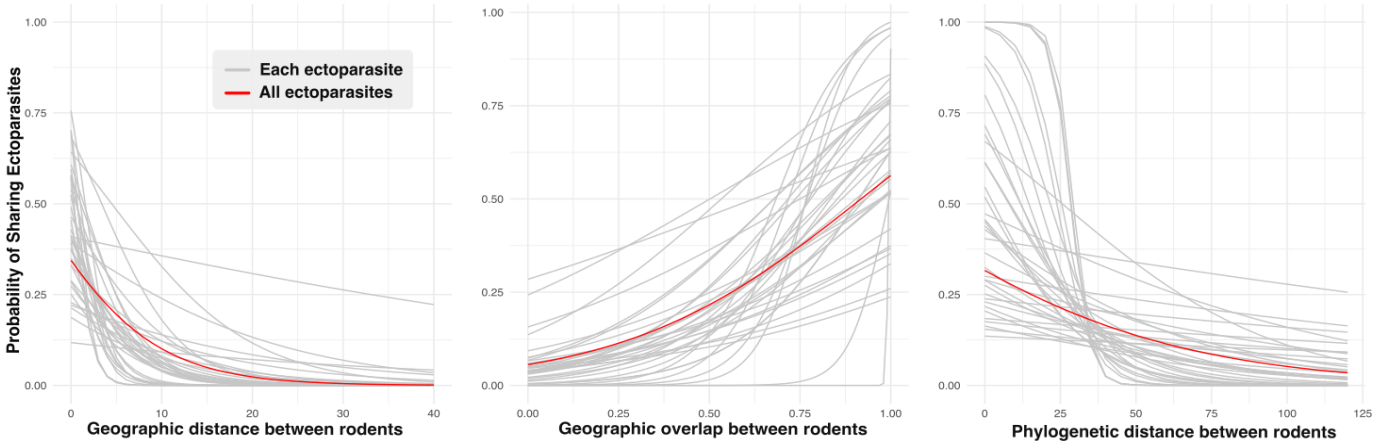


Figure 2. Association between sharing of ectoparasites and potential transmission drivers. Each panel shows the logistic regression curves for each ectoparasite (gray) and the overall model (red). We considered ectoparasites with three or more associations, using phylogenetic distance, geographic distance, or geographic overlap as the predictor variable. **A.** Ectoparasites sharing based on phylogenetic distance between rodents. **B.** Ectoparasites sharing based on geographic distance between the centroid of two rodent species. **C.** Ectoparasites sharing based on geographic overlap of rodent distributions.

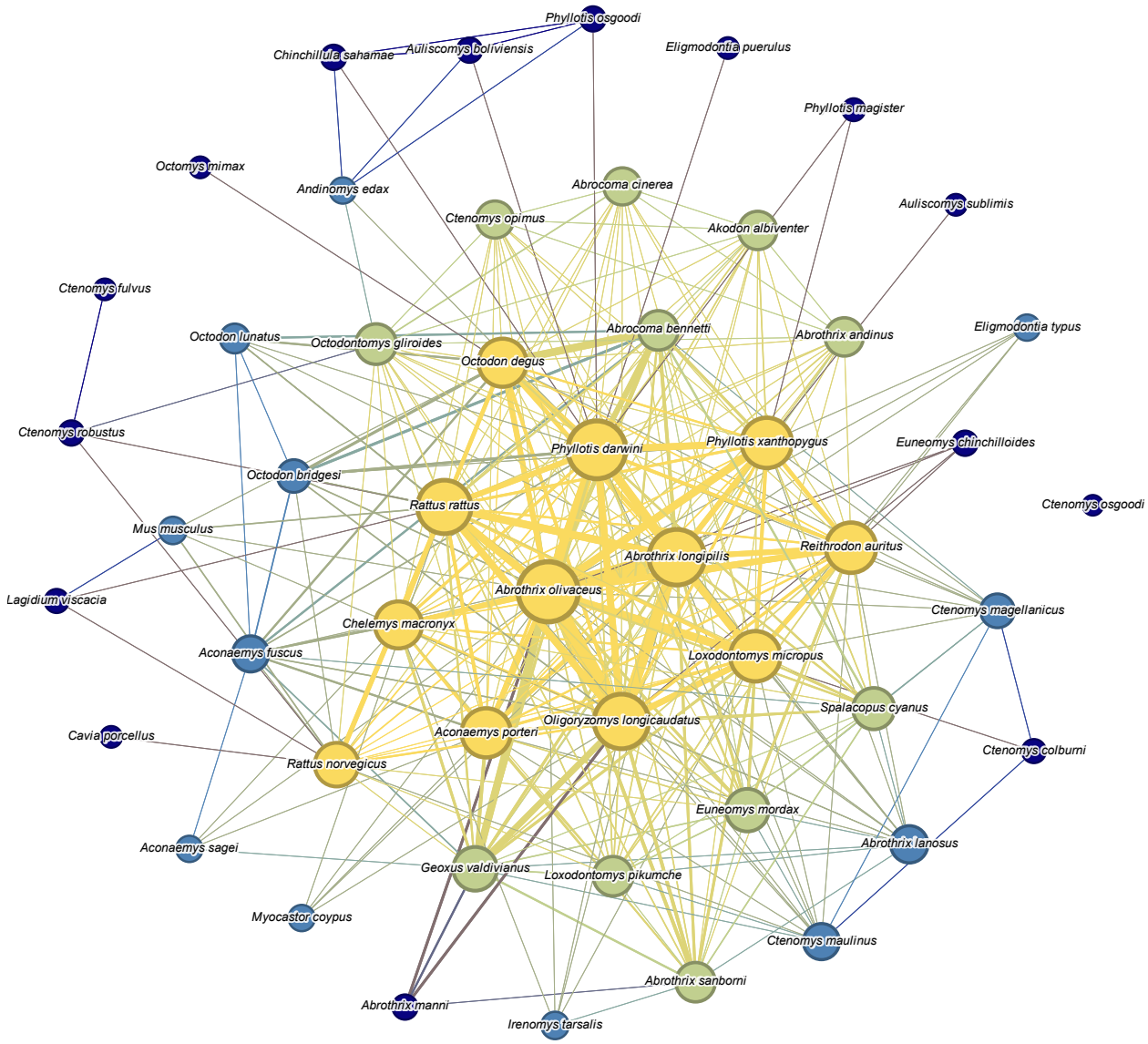


Figure 3. Interaction network model. The network model depicts the 376 unique interactions (edges) between 50 rodent hosts (nodes) through shared ectoparasites where interactions are weighted on the number of shared ectoparasites between rodents. **Node size:** Closeness centrality of each rodent **Edge size:** Number of shared ectoparasites between two rodent species. Yellow-Green-Blue-Dark Blue: Decreasing closeness centrality quantiles based on levels of rodent-parasite sharing.

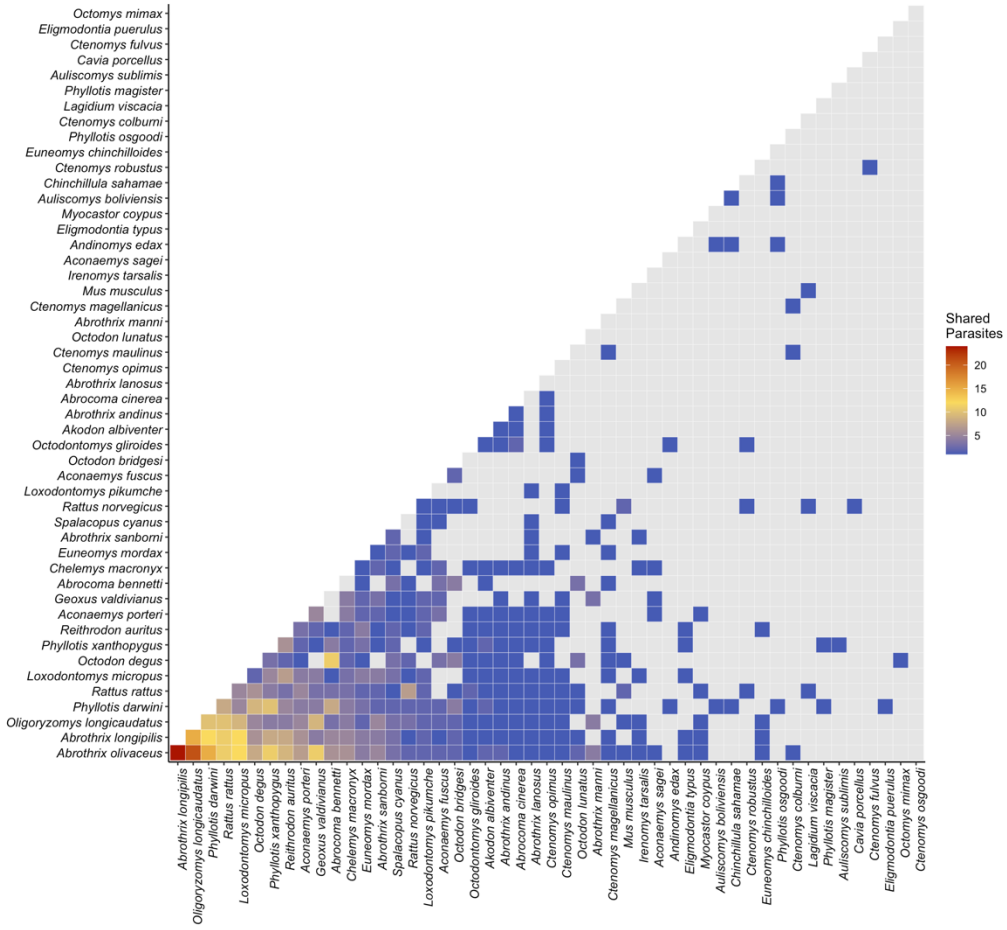


Figure 4. Interaction matrix among rodent species. The number of shared ectoparasites between each pair of rodents in Chile are ordered from those sharing the most ectoparasites to those sharing the least. Color denotes the number of shared ectoparasites between each pair of rodents with gray indicating no sharing.

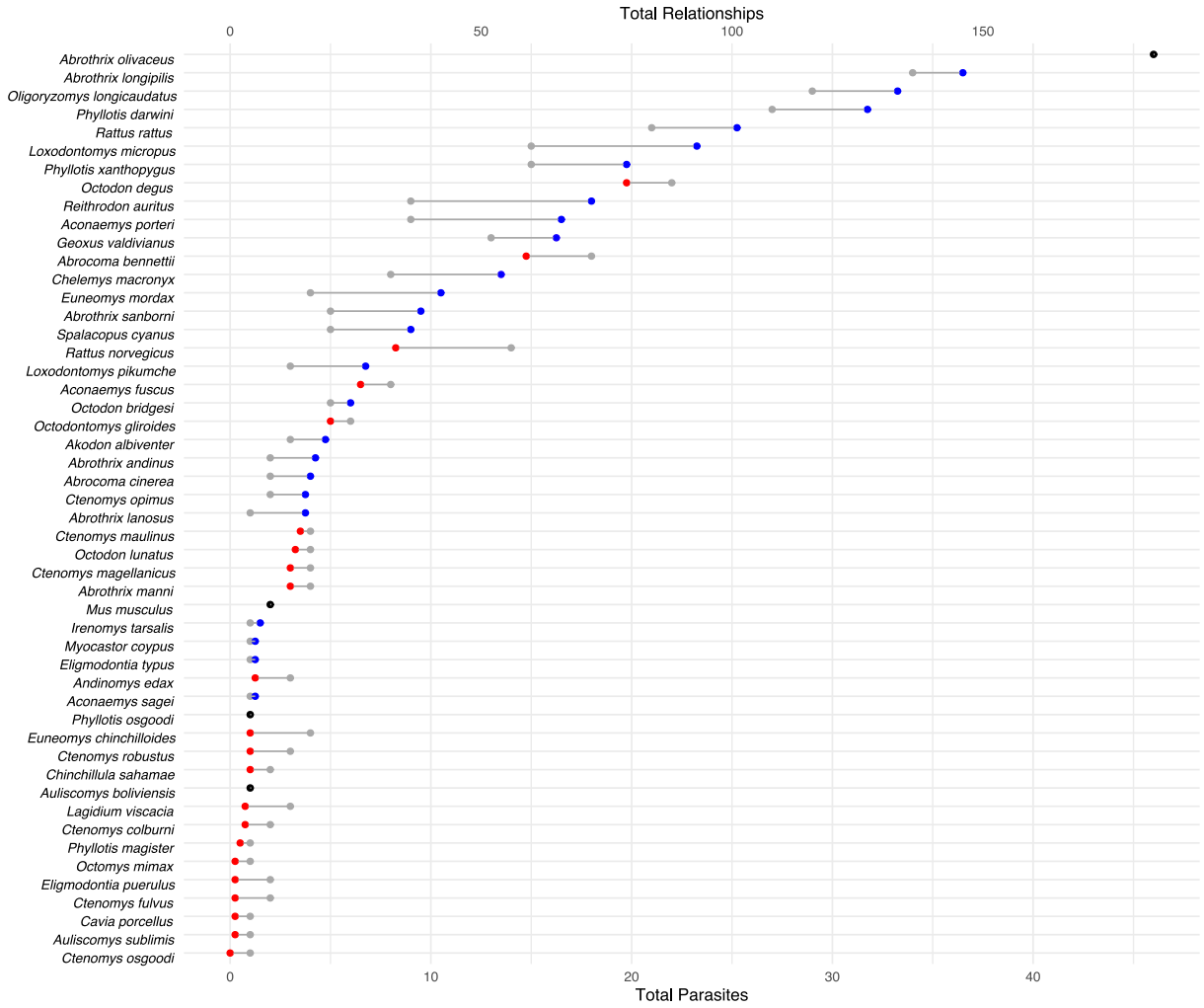


Figure 5. Rodent-ectoparasite association. Left axis: rodent species **Bottom axis:** Total number of parasite associations represented by the gray points for each rodent **Top axis:** Total number of connections to other rodents through ectoparasites. The gray point shows the number of ectoparasite associations each rodent has individually (bottom) and the colored point represents the total number of relationships to other rodents through shared ectoparasites (top). Blue points indicate that the rodent is connected to more than average (~4 connections per ectoparasite), red indicated less than average, and black is average.

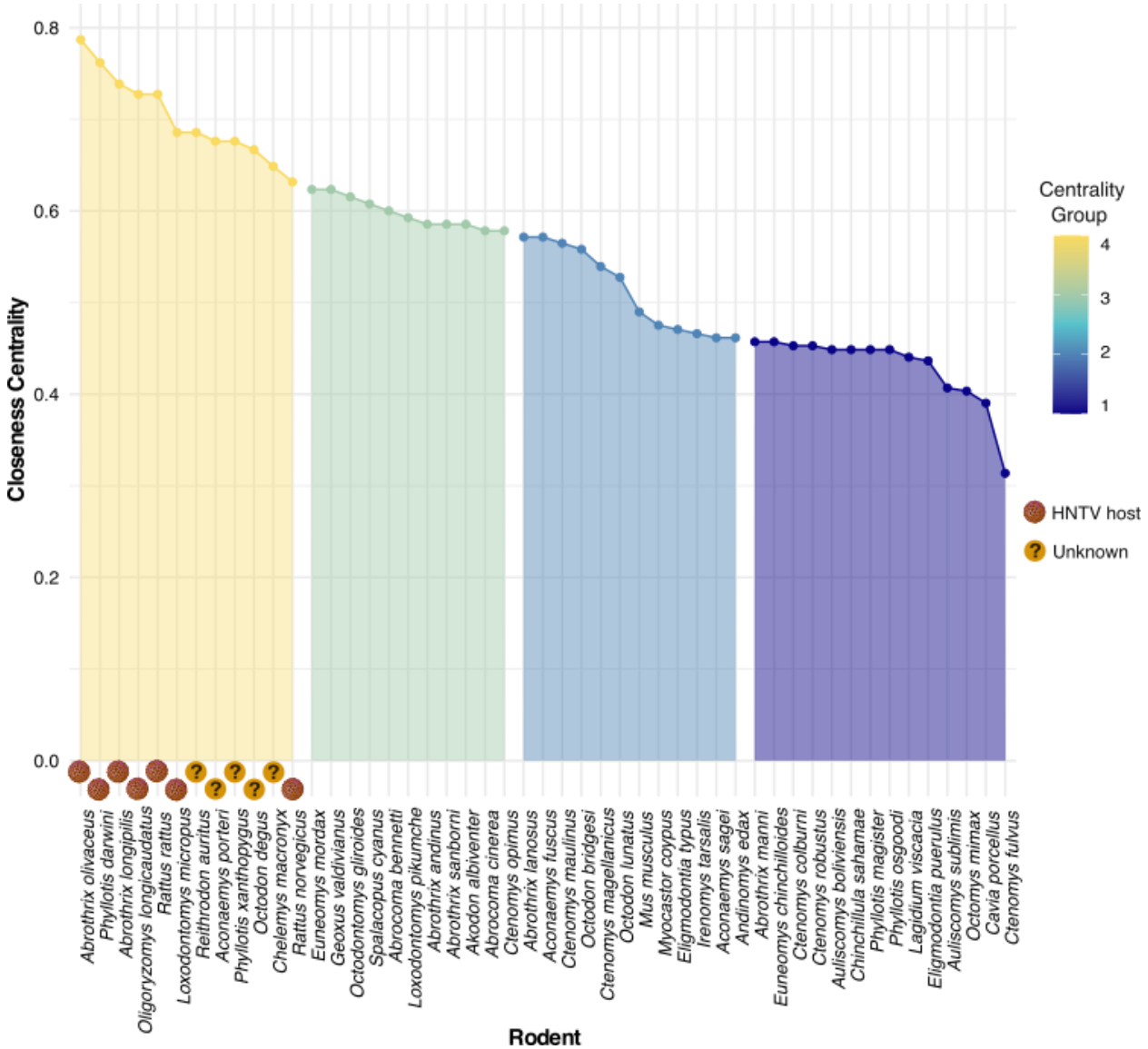


Figure 6. Spillover transmission risk categories based on the centrality of hosts in the rodent-ectoparasite network. Rodent host species are ranked based on their closeness centrality in the ectoparasite network, and this is used to predict cross-species transmission of hantavirus. Brown dots: Known hantavirus hosts. Question: Predicted potential hantavirus hosts.