UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

FACULTAD DE CIENCIAS

LA ESTRUCTURA DE COMUNIDADES DE MACROPARÁSITOS Y SU INFLUENCIA EN LA PRESENCIA DE MICROPARÁSITOS EMERGENTES EN ROEDORES

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

PAULINA ALEJANDRA PONTIFES CORTÉS

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CIUDAD UNIVERSITARIA, CD. MX. , SEPTIEMBRE 2022

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COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS
FACULTAD DE CIENCIAS
DIVISIÓN ACADÉMICA DE INVESTIGACIÓN Y POSGRADO
OFICIO FCIE/DAIP/358/2022

ASUNTO: Oficio de Jurado

M. en C. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académica del Posgrado en Ciencias Biológicas, celebrada el día **30 de mayo de 2022** se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la estudiante **PONTIFES CORTÉS PAULINA ALEJANDRA** con número de cuenta **306183133** con la tesis titulada: **"La estructura de comunidades de macroparásitos y su influencia en la presencia de microparásitos emergentes en roedores",** realizada bajo la dirección del (la**) DR. GERARDO SUZÁN AZPIRI:**

Presidente:	**DR. VÍCTOR MANUEL GUILLERMO SÁNCHEZ-CORDERO DÁVILA**
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Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Ciudad Universitaria, Cd. Mx., a 09 de agosto de 2022

COORDINADOR DEL PROGRAMA

POSGRADO EN CIENCIAS BIOLOGICAS
COORDINACIÓN

DR. ADOLFO GERARDO NAVARRO SIGÜENZA

Agradecimientos institucionales

Agradezco al Posgrado en Ciencias Biológicas de la Universidad Nacional Autónoma de México (UNAM), máxima casa de estudios, por brindarme el espacio, los recursos y la formación que hicieron posible concluir esta etapa.

Al Consejo Nacional de Ciencia y Tecnología (CONACyT) por la beca otorgada para realizar mis estudios de Doctorado (CVU: 610342).

Al Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT) de la UNAM por el apoyo económico otorgado al proyecto IN225219.

A mi tutor principal, el Dr. Gerardo Suzán Azpiri, por la dirección de esta tesis y su confianza en mi trabajo.

Al Dr. Gabriel Ernesto García Peña y al Dr. Christopher Stephens, por el apoyo brindado como miembros de mi Comité Tutor.

Agradecimientos a título personal

A los miembros de mi sínodo: Dr. Víctor Sánchez-Cordero, Dr. Alfonso Valiente Banuet, Dra. Roxana Acosta Gutiérrez, Dra. Ella Vázquez Domínguez y Dr. Gabriel García Peña, por enriquecer mi tesis con sus comentarios, correcciones y sugerencias. Les agradezco el tiempo que brindaron a mi trabajo para pulirlo en su mejor versión.

A Gerardo, por darme la bienvenida a tu grupo y escuchar todas mis ideas con entusiasmo.

A los miembros y colaboradores del Laboratorio de Ecología de Enfermedades y Una Salud: Gabriel García, Rafa Ojeda, Óscar Rico, Claudia Muñoz y Benjamin Roche, por las enseñanzas y oportunidades que me brindaron en todas las ocasiones que me acerqué a ustedes buscando orientación científica y una dosis de sano escepticismo.

A David Roiz, por compartir el espacio y el entusiasmo por indagar nuevos horizontes científicos.

A los miembros del grupo "Candidaturas": Ana, Juan, Adriana, Diana e Ivette. Saber que contaba con ustedes me salvó más veces de las que pueden imaginar.

A mi familia, por siempre apoyarme en este camino de la ciencia de manera incondicional.

A Chimal. Porque todos los caminos lleven a Roma.

El físico Leo Szilard le anunció a su amigo Hans Bethe que estaba pensando en llevar un diario. "No tengo intención de publicarlo. Sólo voy a registrar los hechos para conocimiento de Dios". "¿No crees que Dios ya conoce los hechos?" le preguntó Bethe. "Sí," le respondió Slizard. "Conoce los hechos, pero no conoce *esta* versión de los hechos."

-Hans Christian von Bayer, *Domando Al Átomo*

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Resumen

Los últimos 40 años han sido testigos de un aumento global en brotes de enfermedades infecciosas, particularmente aquellas causadas por patógenos capaces de infectar a más de una especie (denominados parásitos multihospedero), que representan amenazas severas al bienestar humano y la conservación de la biodiversidad. La complejidad de estos sistemas ha requerido un cambio de paradigma, el cual radica en estudiar las interacciones parásito-hospedero en el contexto de la comunidad biológica en que persisten, y no de manera aislada. Esto es particularmente importante para el diseño de estrategias de control efectivas de parásitos multihospedero, ya que en estos sistemas no todas las especies son igualmente importantes para la transmisión del patógeno. Determinar si el control requiere intervenir una o más especies depende de identificar las especies de hospederos involucradas en su transmisión, así como evaluar la frecuencia de la transmisión inter-especie y las barreras que la acotan. Lo anterior supone un reto que requiere de evidencia empírica de alta resolución, que permita caracterizar los matices de las interacciones parásito-hospedero en estos sistemas. El presente trabajo parte precisamente de un enfoque empírico para evaluar el papel de la composición de la comunidad y la transmisión inter-especie en la dinámica de parásitos multihospedero. Como sistema de estudio analicé las infecciones por *Bartonella spp*, un género de bacterias de transmisión mediada por pulgas, en roedores silvestres del noroeste de México. Para determinar si la transmisión inter-especie ocurre de manera generalizada o está acotada por barreras de encuentro (vector-hospedero, dadas por la especificidad de las pulgas), o de compatibilidad (bacteria-hospedero o bacteria-vector, asociada a la especificidad de variantes), caractericé la comunidad de vectores y las interacciones roedor-vector-bacteria a partir de la identificación de variantes genéticas de *Bartonella* con el marcador *glt*A. Con base en análisis de redes y modelos estadísticos, identifiqué variables asociadas a diferencias en la carga parasitaria del vector de *Bartonella* en los roedores. Asimismo, identifiqué que la presencia de una especie clave de los pastizales norteamericanos, *Cynomys ludovicianus*, también afecta la estructura de los ensambles de pulgas, aumentando su abundancia y facilitando asociaciones positivas entre pulgas. Los análisis de redes destacaron que otras especies de roedores, como *Onychomys arenicola*, también actúan como puente para el intercambio de pulgas entre roedores. En cuanto a la infección con *Bartonella* en pulgas, detecté que las especies generalistas presentan la prevalencia más baja. La caracterización de los ensambles Bartonella-roedor-pulga reveló que ninguna variante está presente en todas las especies, sino que distintas variantes están asociadas a combinaciones específicas de roedores y pulgas; en el caso de los roedores, esta asociación está dada en parte por parentesco filogenético entre hospederos Estos resultados indican que, incluso con la presencia de especies que reducen las barreras de encuentro entre vectores, para la mayoría de las variantes detectadas se mantiene una estructura dada por su especificidad. Cabe mencionar que sí se detectaron casos de variantes compartidas entre especies de distintas familias de roedores, incluyendo una variante zoonótica (*Bartonella washoensis*). Los resultados de este trabajo resaltan que la transmisión inter-especie en la persistencia de parásitos mutihospedero es compleja, y enfatizan la importancia de estudiar las interacciones en los sistemas multihospedero en el contexto de la composición de la comunidad. Ello es relevante considerando los cambios acelerados en la composición de comunidades por eventos como la extinción de especies nativas o la introducción de especies

invasoras, que generan cambios en las interacciones que subyacen la aparición de nuevas enfermedades, o la reaparición de antiguos enemigos.

Abstract

The last 40 years have witnessed a global increase in outbreaks of infectious diseases, particularly those caused by pathogens capable of infecting more than one species (hence called multi-host parasites), which pose severe threats to human well-being and the conservation of biodiversity. The complexity of these systems has required shifting the paradigm from studying host-parasite interactions in isolation, to studying them in the context of the biological community in which they persist. This is particularly important for designing effective control strategies for multi-host parasites, as not all species contribute equally to pathogen transmission. Determining whether management requires targeting one or more species depends on identifying the host species involved, as well as evaluating the frequency of inter-species transmission and the barriers that limit it. This is a challenge that requires high-resolution empirical data to characterize the nuances of parasite-host interactions in these systems. This thesis takes an empirical approach to characterize the role of community composition and inter-species transmission in the dynamics of multihost parasites. Using *Bartonella spp* (a genus of bacteria transmitted by fleas) in wild rodents from northwestern Mexico as a study system, I investigated whether interspecies transmission occurs and whether it is limited by encounter barriers (vector-host, given the specificity of fleas), or compatibility barriers (bacteria-host or bacteria-vector, associated with the specificity of variants). To this end, I characterized the vector community and rodent-vector-bacteria assemblages, using the *gltA* marker to identify genetic variants of Bartonella in both hosts and vectors. Using network analysis and statistical models, I identified variables associated with differences in the burden of the Bartonella vector in rodents. I also found that the presence of a keystone species of North American grasslands, *Cynomys ludovicianus*, also affects the structure of flea assemblages, increasing their abundance and facilitating positive associations between fleas. Network analysis identified other species of rodents, such as *Onychomys arenicola*, which act as a bridge for the exchange of fleas between rodents. Regarding the infection with Bartonella in fleas, I found that generalist species had the lowest prevalence. The characterization of the Bartonella-rodent-flea assemblages revealed that no variant is present in all species, but that different variants are associated with specific combinations of rodents and fleas; in the case of rodents, this association is given in part by phylogenetic kinship between hosts. These results indicate that, even with the presence of species that reduce encounter barriers between hosts and vectors, most of the detected variants show structure associated with host specificity. It is worth mentioning that cases of variants shared between species of different families of rodents were detected, including a zoonotic variant (Bartonella washoensis). Taken together, these results highlight that interspecies transmission in multihost parasite persistence is complex and emphasize the importance of studying interactions in multihost systems in the context of community composition. This is relevant considering the accelerated changes in the composition of communities due to events such as the extinction of native species or the introduction of invasive species, which generate changes in the interactions that underlie the appearance of new diseases, or the re-emergence of old enemies.

Introducción general

Los últimos 40 años han sido testigos de un aumento global en brotes de enfermedades infecciosas que afectan no sólo la salud humana, sino también la de especies domésticas y silvestres, generando grandes pérdidas para la economía y la conservación de la biodiversidad (Jones et al., 2008; Smith et al., 2014). La creciente disrupción de los ecosistemas silvestres y la hiperconectividad de la sociedad actual aumenta el riesgo de exposición y dispersión rápida de enfermedades nuevas o re-emergentes de origen animal (zoonosis), particularmente aquellas causadas por parásitos capaces de infectar y cumplir su ciclo de vida en más de una especie (Jones et al., 2013; Allen et al., 2017). Estos parásitos, denominados multihospedero, son la norma más que la excepción en la naturaleza, y algunos de sus representantes se encuentran entre los causantes de pandemias y amenazas a la conservación de la biodiversidad más severas, como la peste bubónica, el SARS y la influenza (Cleaveland et al., 2001; Pedersen et al., 2007).

La reciente pandemia por SARS-COV2 ha dejado en claro las consecuencias de marginar la interdependencia entre el bienestar humano y la salud de los ecosistemas, y ha resaltado la importancia de adoptar el enfoque de Una Salud[1] para el estudio de enfermedades infecciosas emergentes (Banco Mundial, 2018; Worobey et al., 2022). Sin embargo, la adopción de este enfoque requiere desarrollos teóricos y empíricos que capturen adecuadamente la diversidad y matiz de las interacciones parásito-hospedero, y que permitan traducir este conocimiento a estrategias de control integrales y basadas en evidencia (Morse et al., 2012). Esto es particularmente relevante para los sistemas multihospedero, que involucran diversos agentes a menudo a lo largo de distintas escalas espaciales con interacciones complejas entre sus componentes (Buhnerkempe et al., 2015; Penczykowski et al., 2016). Dicho nivel de complejidad requiere integrar herramientas analíticas y marcos conceptuales que nos permitan identificar los factores que gobiernan la estructura y dinámica de las interacciones

[1] Enfoque que reconoce explícitamente que el bienestar humano está ligado a la salud de los ecosistemas. Este es el enfoque priorizado por organismos internacionales como la OMS, la PAHO y el Banco Mundial para abordar el reto que suponen las enfermedades infecciosas en el siglo XXI, particularmente las de origen zoonótico. Ver por ejemplo: https://www.who.int/news-room/questions-and-answers/item/one-health

parásito-hospedero. Por lo anterior, en años recientes se ha enfatizado la importancia de estudiar a los sistemas multihospedero desde un enfoque holístico en el contexto de la ecología de comunidades, en particular con respecto a su composición (Johnson et al., 2015; Seabloom et al., 2015).

Dado que los parásitos multihospedero son capaces de transmitirse tanto entre individuos de una misma especie como entre especies, la composición de la comunidad de hospederos es crucial: desde la perspectiva del parásito, la composición de la comunidad implica diferencias en la cantidad e idoneidad de los hospederos disponibles (Woolhouse, 2001; Dobson, 2004). Desde la perspectiva del hospedero, la composición de la comunidad supone diferencias en susceptibilidad y exposición a los parásitos (Fenton & Pedersen, 2005). Esta heterogeneidad y asimetrías tienen como consecuencia que no todas las especies dentro de la comunidad, e incluso no todos los individuos dentro de una misma especie, contribuyen por igual a la dispersión y persistencia de parásitos multihospedero (Perkins et al., 2003; Rudge et al., 2013); por ejemplo, en el Serengueti, las infecciones por rabia en carnívoros silvestres ocurren por eventos frecuentes de transmisión entre perros domésticos infectados y carnívoros, pero no entre carnívoros (Lembo et al., 2008). Estas diferencias en contribución tienen impactos importantes en la dinámica de transmisión del parásito, así como implicaciones para fenómenos como el efecto de dilución, hipótesis que postula que a mayor diversidad biológica en una comunidad la prevalencia de enfermedades infecciosas es menor (LoGiudice et al., 2008; Han et al., 2015a).

Identificar a los elementos o especies clave para la dispersión y persistencia de los parásitos multihospedero supone un reto tanto teórico como empírico (Haydon, 2008). Por ejemplo, el cambio de paradigma de los sistemas unihospedero (un parásito-un hospedero) aislados de su contexto ecológico, al marco de los sistemas multihospedero, ha requerido integrar a su estudio la identidad de los hospederos. Ésta define las características ecológicas y evolutivas que condicionan procesos relevantes para la transmisión y persistencia del parásito, como las tasas de encuentro intra e interespecies entre hospederos infectados y susceptibles, así como la probabilidad de dispersión del parásito entre especies de hospederos evolutivamente distantes (Fenton et al., 2015; Viana et al., 2014). Sin embargo, a pesar de la creciente

complejidad de los modelos de parásitos multihospedero (que inicialmente conceptualizaban los componentes clave para la persistencia del parásito únicamente en términos de densidades poblacionales, ver Haydon et al., 2002; Holt et al., 2003), éstos siguen ofreciendo una resolución limitada para distinguir mecanismos subyacentes entre patrones similares de prevalencia, restringiendo su capacidad para identificar componentes clave. Por ejemplo, una prevalencia alta a nivel comunidad de un parásito multihospedero puede ser el resultado de distintos procesos; puede ocurrir a partir de la transmisión frecuente del parásito entre múltiples especies de hospederos (Miguel et al., 2013), o bien a partir de una transmisión dominada por una sola especie que actúa como fuente (Lembo et al., 2008) o incluso una transmisión entre individuos de una misma especie de manera independiente a la inter-especie (Streicker et al., 2010). En este sentido, complementar los desarrollos teóricos con datos empíricos es crucial para esclarecer los mecanismos detrás de la prevalencia observada de un parásito multihospedero en las poblaciones de hospederos que infecta. Este tipo de información permitiría refinar las estrategias de control de enfermedades de relevancia para la salud pública y la economía.

La integración de métodos moleculares a la caracterización de las interacciones parásito-hospedero en sistemas multihospedero ha brindado nuevas perspectivas sobre la importancia de la composición de comunidades biológicas en la dinámica de transmisión de estos sistemas, así como resaltado los riesgos de desacoplar la teoría de datos empíricos de alta resolución (Archie et al., 2009; Forrester & Hall, 2014). Por ejemplo, estudios serológicos asociaban la presencia del Virus del Oeste del Nilo (VON)[2] en comunidades de aves urbanas en el noreste de Estados Unidos con la especie más abundante, el gorrión común (*Passer domesticus*) (Komar et al., 2001). Estos hallazgos ajustaban con modelos teóricos donde la abundancia era un factor asociado a especies centrales para la persistencia de parásitos multihospedero (Haydon et al., 2002). Sin embargo, estudios moleculares de los contenidos sanguíneos de mosquitos encontraron que la seroprevalencia alta del gorrión común era más un indicativo de su exposición al virus que de su capacidad de transmisión, y que una especie menos común, el petirrojo americano (*Turdus migratorius*) era central para la transmisión

[2] Zoonosis transmitida de aves silvestres a humanos y otros mamíferos como caballos a través de mosquitos infectados. Introducida en el hemisferio occidente a finales de los 90.

inter-especie (Kilpatrick et al., 2006). Otros trabajos han resaltado que la alta variación genética de microparásitos (virus y bacterias) a nivel especie genera que distintas variantes se comporten como unidades ecológica y epidemiológicamente distintas. Esto ha puesto en perspectiva los supuestos sobre la frecuencia de la transmisión entre especies en sistemas multihospedero, resaltando que podría ocurrir menos de lo supuesto por modelos teóricos o estudios basados en evidencia indirecta o correlacional (Telfer et al., 2007; Withenshaw et al., 2016).

La transmisión inter-especie en sistemas multihospedero está acotada por la especificidad de la interacción parásito-hospedero, que puede entenderse en términos de barreras de compatibilidad y barreras de encuentro (Combes et al., 2001). Las primeras tienen un origen evolutivo (por ejemplo, la especificidad de receptores celulares) y superarlas conlleva un proceso de adaptación biológica. Por su parte, las barreras de encuentro consisten en limitantes físicas o de comportamiento que evitan la transmisión entre especies, la cual podría ocurrir si éstas se eliminan. Caracterizar estas barreras en sistemas multihospedero permite anticipar cambios a la dinámica de dispersión o transmisión de parásitos en respuesta a modificaciones a la composición de la comunidad, por ejemplo por la introducción de especies invasoras (Juliano & Lounibos, 2005) o la extinción de especies clave (*sensu* Paine, 1966) (Collinge et al., 2010). Esta caracterización es de particular relevancia en el contexto de cambios globales rápidos que facilitan la coocurrencia entre hospederos y parásitos que de manera natural no se encuentran. Tal es el caso de la introducción del Zika y el chikungunya a las Américas, cuyas infecciones son mediadas por mosquitos del género *Aedes* (a su vez son especies introducidas) y cuya presencia resultó en importantes epidemias entre 2014-2017 en esta región (Paixao et al., 2018).

Mientras que la composición de la comunidad define la heterogeneidad en capacidad de transmisión, y la especificidad acota la posibilidad de una transmisión exitosa entre especies, la persistencia y dispersión de parásitos multihospedero también dependen en gran medida de la estructura de contactos en la comunidad (Godfrey, 2013; Webster et al., 2017). Las interacciones entre factores intrínsecos, como la biología de la especie, y factores extrínsecos como la distribución de recursos, influyen en los patrones y frecuencia de contacto y

movimiento de hospederos, por lo que la estructura representa una fuente de heterogeneidad que condiciona las oportunidades de transmisión intra e inter-especie (Altizer et al., 2003). Existen diversos métodos para inferir la estructura de contactos en una comunidad basándose en la co-ocurrencia entre especies o la observación directa de la interacción (Gotelli, 2000; Krause et al., 2013; Stephens et al., 2017). En años recientes, el acoplamiento de métodos moleculares con análisis de redes ha permitido caracterizar redes de transmisión de alta resolución en sistemas multihospedero mediante la comparación de la similitud genética de los parásitos aislados de individuos infectados (VanderWaal et al., 2014a, 2014b). Estos avances han expandido los límites de estudios correlacionales (Fenton et al., 2014) y han permitido poner a prueba hipótesis sobre temas como las características asociadas a individuos superpropagadores, o la importancia relativa de diferentes métodos de transmisión en la persistencia de parásitos (White et al., 2017; Portier et al., 2019).

A pesar del valor que tienen los estudios empíricos de alta resolución para refinar el estudio de los parásitos multihospederos, los retos logísticos para su ejecución requieren de sistemas de estudio con características que permitan discernir los efectos de la composición y estructura de la comunidad (Pedersen & Fenton, 2015). En este sentido, el estudio de interacciones parásito-hospedero tiene una trayectoria importante en roedores silvestres; por una parte, su tamaño, longevidad y rango hogareño típicamente restringidos facilitan la caracterización y seguimiento temporal a distintos niveles (individuo, población, comunidad) (Pedersen & Fenton, 2019). Por otra parte, son hospederos de una gran diversidad de parásitos, algunos de ellos zoonóticos (Han et al., 2015b).

Entre los múltiples parásitos que infectan a roedores silvestres, las bacterias del género *Bartonella* spp. representan un grupo de interés desde el punto de vista de sistemas multihospedero, pues son capaces de infectar especies simpátricas de roedores, así como a otros mamíferos. Las pulgas, ectoparásitos del orden Siphonaptera, son el vector principal de esta bacteria en roedores, que establece infecciones de largo plazo mediante una estrategia silenciosa que le permite transmitirse eficientemente al infectar y ocultarse del sistema inmune en eritrocitos y células endoteliales (Birtles, 2005). *Bartonella* presenta una alta prevalencia en poblaciones de roedores alrededor del mundo, pero la especificidad tanto de

los vectores como de especies e incluso las variantes de la bacteria es variable (Gutiérrez et al., 2015). Por dichas características, el sistema *Bartonella*-roedores es un sistema adecuado para investigar los factores que contribuyen a la persistencia y dispersión de parásitos multihospedero (Regier et al., 2016). Asimismo, representa un sistema de interés desde el punto de vista de la salud pública, ya que algunas especies y variantes asociadas a roedores son zoonóticas y pueden causar diversas afecciones que van de moderadas a severas (Gutiérrez et al., 2015).

Con el fin de contribuir al conocimiento de parásitos multihospedero desde un enfoque empírico, en el presente trabajo investigo los efectos de la composición y estructura de la comunidad en la dinámica de parásitos multihospedero, usando como sistema de estudio a *Bartonella* en roedores silvestres en praderas de la Reserva de la Biósfera de Janos (RBJ), en el noroeste de México. La tesis está dividida en tres capítulos, desarrollados en formato de artículos científicos. El primer capítulo aborda el efecto de la identidad y el contexto ambiental de los hospederos en la abundancia del vector principal de *Bartonella* en roedores: las pulgas. El objetivo del capítulo fue identificar variables asociadas a diferencias en carga parasitaria de estos ectoparásitos, con el fin de detectar especies, grupos de individuos y factores ambientales asociados a un mayor riesgo de transmisión, ya que la carga parasitaria es directamente proporcional a la probabilidad de transmisión de microparásitos (Eisen & Gage, 2012). Este análisis se realizó a distintas escalas espaciales, para determinar si las mismas o distintas variables son relevantes a diferentes escalas. Los resultados de este capítulo destacan la importancia de integrar la variación espacial en estudios de carga parasitaria.

El segundo capítulo es un estudio de la estructura de las comunidades de pulgas y las variables que influyen en la compartición de estos ectoparásitos entre roedores simpátricos en la RBJ, específicamente considerando el efecto de la presencia del perrito de la pradera de cola negra (*Cynomys ludovicianus*), especie clave de los pastizales de Norteamérica (Kotliar et al., 1999). La presencia de especies clave (*sensu* Paine 1969) modifica significativamente la composición e interacciones entre especies en una comunidad (Mills & Doak, 1993), aunque su efecto en las interacciones entre parásitos ha sido poco explorada.

La presencia de *C. ludovicianus* en el sistema de estudio supone un experimento natural que permitió explorar el efecto de especies clave en las comunidades de parásitos y evaluar si modifica las asociaciones entre pulgas y por ende las rutas potenciales de transmisión de *Bartonella*, con implicaciones para la transmisión de otros microparásitos de relevancia, como la peste bubónica (Gage & Kosoy, 2005).

Finalmente, el tercer capítulo es un análisis de las variables que influyen en la infección con *Bartonella* tanto en pulgas como en roedores de la RBJ, así como una caracterización de los ensambles vector-*Bartonella*-hospedero para evaluar la ocurrencia de transmisión entre especies a partir de la similitud genética de las Bartonellas encontradas tanto en roedores como en pulgas. Este diseño permitió analizar si la transmisión de *Bartonella* en la RBJ está acotada por barreras de encuentro o de compatibilidad a nivel hospedero o vector, y si diferencias en la composición de comunidades (representados por la presencia de *C. ludocivianus*) modifica la ocurrencia de transmisión intra e inter-especie.

En conjunto, los resultados de este trabajo resaltan que identificar a las especies importantes para la persistencia y dispersión de parásitos multihospedero requiere integrar metodologías que permitan caracterizar con detalle el papel de la transmisión intra e interespecie. Asimismo, destacan la importancia de estudiar estos sistemas en el contexto de la comunidad de los hospederos. Contar con el conocimiento y herramientas que nos permitan identificar componentes e interacciones clave en estos sistemas complejos puede representar la diferencia entre prevenir el surgimiento o resurgimiento de enfermedades zoonóticas, o lidiar con las consecuencias de ellas.

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Capítulo I. Drivers of flea abundance in wild rodents across local and regional scales in the Chihuahuan Desert, northwestern Mexico

Artículo requisito publicado en

Ecosphere

https://doi.org/10.1002/ecs2.4013

Received: 17 May 2021 | Revised: 17 August 2021 | Accepted: 27 September 2021

DOI: 10.1002/ecs2.4013

ECOSPHERE
AN ESA OPEN ACCESS JOURNAL

ARTICLE
Disease Ecology

Drivers of flea abundance in wild rodents across local and regional scales in the Chihuahuan Desert, northwestern Mexico

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Funding information
Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT), Grant/Award Number: IN225219; National Autonomous University of Mexico; Consejo Nacional de Ciencia y Tecnología

Handling Editor: Andrea Swei

Abstract

The broad distribution of macroparasites and their thriving populations are matters of health and economic concern. Macroparasites cause damage both directly through their feeding habits, which impact host fitness, and indirectly through the transmission of various infectious diseases of relevance to human and domestic animal health and wildlife conservation. Because the impacts of macroparasites on host health and the risk of disease transmission are directly related to their abundance, understanding the drivers of macroparasite burden is of relevance. Various host traits and environmental factors have been associated with differences in macroparasite abundance. In addition to these variables, spatial scale is increasingly incorporated to understand how these drivers vary across space. However, variation in the relative importance of host traits and environmental factors as predictors of abundance at different scales is not well understood. To further clarify the relationship between scale and drivers of macroparasite abundance, we investigated the effects of host traits and environmental factors on flea abundance in rodents of the Chihuahuan Desert in northwestern Mexico on three levels: within a single site, between sampling sites with different vegetation types, and across the region. This partitioning allowed us to compare drivers at both local and regional scales. Fleas provide a natural model to assess the interplay between host and environmental variables across scales because their life cycles alternate between on-host and off-host environments and their hosts have varying ranges of distribution. We sampled 1311 fleas from 674 rodent individuals of 14 different species across 40 sampling plots between 2012 and 2013. Using generalized linear mixed models, we found that flea abundance was associated with different combinations of host traits such as size and sex. The specific combination of predictive variables differed across species, while the effects on flea abundance showed context and scale dependency, although this could only be tested at the full level of analysis on the most abundant species, *Dipodomys merriami*.

Sampling season was the only variable consistently significant across scales, reflecting the far-reaching effects of large-scale, interannual environmental fluctuations. These results emphasize that integrating spatial scale can strengthen study design for monitoring macroparasite burden.

KEYWORDS
Chihuahuan Desert, flea abundance, macroparasites, mixed models, rodents, spatial scale

INTRODUCTION

Macroparasites can reduce host fitness directly through their feeding habits and indirectly through the transmission of various infectious diseases of relevance to human and domestic animal' health and wildlife conservation (Wall & Shearer, 1997). The negative impact of macroparasites on their hosts is related to their abundance: Higher burdens result in more consumption of host resources and increased risk of disease transmission in the case of vector macroparasites (Bethony et al., 2006; Eisen & Gage, 2012). Therefore, understanding the drivers behind macroparasite abundance has important epidemiological implications.

Various environmental factors and host traits have been extensively studied as drivers of macroparasite abundance in vertebrates, with a few patterns reported across different systems. For example, temperature and humidity can modify macroparasite development and mortality (Wilson et al., 2002). Host sex influences macroparasite abundance through differences in host behavior, size, and immune-modulating effects of sex hormones (Zuk & McKean, 1996), with males typically being more parasitized than females (Skorping & Jensen, 2004). However, even widely observed patterns are not universal and many studies report variation in the role of host traits and environmental variables between different host–parasite systems and even within the same systems depending on location (Kiffner et al., 2013, 2014). Furthermore, other studies have found that the effects and relative importance of host traits and environmental variables on macroparasite abundance might be inconsistent across space (Cardon et al., 2011; Young & Maccoll, 2016). Thus, while extensive research has assessed the role of host traits and environmental variables on macroparasite abundance, less is understood about how the effect and relative importance of these drivers change when considered across spatial scales.

Studies performed at a local scale capture small-scale variability in the host's biotic and abiotic environment. However, hosts are subjected to spatiotemporal variation in environmental conditions across their distribution range (Penczykowski et al., 2016). This variation may influence macroparasite abundance directly through differences in environmental conditions such as soil humidity or temperature across sites (Krasnov et al., 2001, 2002a) or indirectly through effects on resource availability or host behavior (Khokhlova, 2004; Ostfeld et al., 2006). Furthermore, spatiotemporal variation in individual-level (sex, age, size, and reproductive status) and population-level (density, age distribution, and sex ratio) host traits will also differ across the host's landscape. Thus, drivers of macroparasite abundance show dependence on the host's spatiotemporal context.

Incorporating spatial scale into the analysis of drivers of macroparasite abundance can help clarify their effects across scales and assess their relative importance across levels of ecological organization. For example, Young et al. (2015) found that environmental variables and host traits driving flea abundance in small mammals of the East African savanna had a higher predictive power across species but were not significant at the individual level. Linardi and Krasnov (2013) found that at lower hierarchical levels (between individuals), flea and mite abundance was affected by host and parasite traits and environmental factors (although effects differed between flea and mites), whereas at the higher levels (communities across a landscape), host traits and environmental variables drove variation in ectoparasite abundance.

In this study, we assess whether host traits and environmental variables have the same relative importance and effect on macroparasite abundance across spatial scales. We use fleas in rodent communities in a natural reserve within the Chihuahuan Desert in northwestern Mexico as a study system. Fleas (order Siphonaptera) provide a natural model to assess the interplay between host and environmental factors at different scales, as their life cycle alternates between on-host and off-host environment, requiring them to cope with the host's immune and behavioral responses, as well as with varying degrees of environmental exposure (dependent on the host's burrowing/nesting habits and range) (Krasnov, 2008). The broad distribution of some rodent species of the Chihuahuan Desert will allow us to assess how variation in host traits and environmental characteristics contributes to flea abundance at a local and a regional scale,

across different levels of host traits: individuals within a single site, between sites, and across host species within a region. Furthermore, by comparing flea abundance in sympatric rodents with a range of burrowing habits (fossorial, semi-fossorial, or shallow), we can explore the role of variation in microclimate conditions on flea abundance within and between host species. These results will enhance our understanding of host and environmental effects on flea abundance across spatial scales and highlight the importance of incorporating spatial context to accurately assess the effects of drivers of macroparasite abundance.

METHODS

Study region

The study was conducted in northwestern Chihuahua, Mexico, in the Janos Biosphere Reserve (JBR), a nature reserve located in the Chihuahuan Desert. The reserve covers an extent of 5264.9 km^2, with mosaics of grassland and shrubland vegetation interspersed with patches of agricultural land and human settlements (CONANP, 2013). The dominant climate in this region is temperate and semi-arid, with an annual average of 381 mm of rain, 77% of which falls between April and August (CONANP, 2013). Temperature varies seasonally, with annual fluctuations of over 14°C.

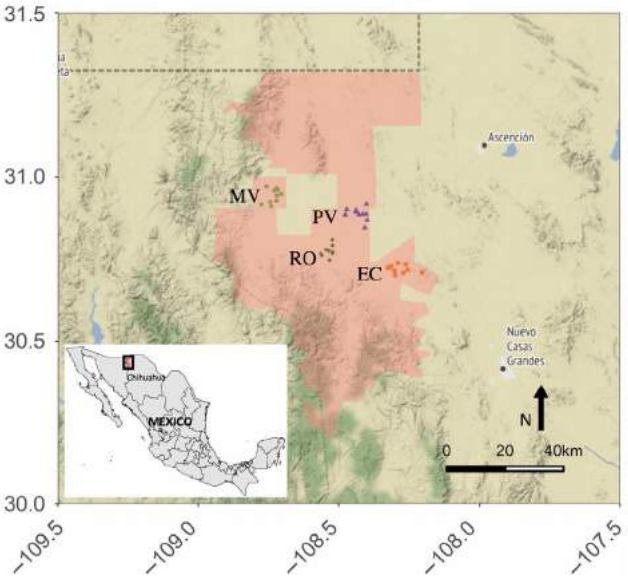

FIGURE 1 Map of the study sites within the Janos Biosphere Reserve (red polygon outlines the study region) in the Chihuahuan Desert. Sampling was conducted across a 1000-km^2 area, considering a total of 40 sampling locations distributed across four sampling areas, to represent the local and regional ranges of ecological conditions of the study region. Sampling areas correspond as follows: Monte Verde (MV), Rancho Ojitos (RO), Pancho Villa (PV), and El Cuervo (EC)

Rodent sampling

We sampled nocturnal rodents between 2012 and 2013 over three sampling sessions (May and October 2012, and May 2013), using Sherman traps (H.B. Sherman 8 × 8 × 23 cm, Tallahassee, FL) at four sampling areas (either MV, RO, EC, or PV; see Figure 1). At each sampling area, ten 7 × 7 grids with a 10-m spacing between traps were set. Sampling plots were located approximately 700–900 m apart from each other. Rodent movement between grids (assessed by the presence of individual rodents in multiple grids within a sampling area) was monitored to ensure sampling plot independence. Traps were baited with a mixture of oats and vanilla extract and set for three consecutive nights at each trapping grid, yielding 147 trap nights per site. After use, each trap was cleaned with hospital-grade detergent.

Captured rodents were identified to species level using taxonomic keys (Anderson, 1972; Reid, 2006). Body mass, length, and sex of host were recorded. Reproductive status was established upon observation of descended testes for males and perforated vagina or pregnancy (determined by abdominal palpation) or lactation signs for females (Gurnell & Flowerdew, 2006). Sampled rodents were ear-tagged to avoid resampling. Most animals were released at the point of capture, although some were euthanized for morphological voucher specimens to verify identification. Procedures for trapping and handling were approved by the Animal Care Committee of the National Autonomous University of Mexico (UNAM) and by the Secretariat of Environment and Natural Resources (license number FAUT-0250) and followed the standards set by the American Society of Mammologists (Sikes & Gannon, 2011).

Flea sampling

Each captured rodent was placed in a plastic chamber with a cotton ball dosed with isoflurane. This method anesthetizes both the host and its fleas, which dislodge from their hosts (Himsworth et al., 2020). Animals in the chamber were monitored to remove the lid and the cotton ball as soon as motor activity nearly ceased, in order to reduce the risk of death following anesthesia. Each anesthetized animal was held stretched and thoroughly combed within the chamber for 2 min with a standardized number of passes to collect fleas that had not fallen

off during anesthesia. Fleas were collected from the container by hand and stored separately in microtubes containing 70% ethanol and kept at −80°C until identification. The plastic chamber was cleaned with water and detergent after each use. Fleas were identified to species level using a dissecting scope (SZx12 Olympus, Melville, NY) and taxonomic keys (Acosta & Morrone, 2003; Hubbard, 1974). Given the purposes of the research, all analyses were conducted using total flea burden.

Environmental characteristics and sampling season

Microclimate conditions in different vegetation types, even within the same region, can lead to differences in soil humidity and air temperature, parameters that have been shown to affect flea development and mortality (Krasnov et al., 2001, 2002a, 2002b). To explore the potential role of differences in microclimate on flea abundance, we recorded vegetation type at each sampling plot as either grassland or shrubland (considering the classification of previous analyses at the same sampling plots as part of ongoing research in the study area [see Rubio et al., 2015 for details]). Sampling session and year were combined as a categorical variable with three levels, corresponding to spring (May 2012 or May 2013) or autumn (October 2012) fieldwork sessions, to account for temporal variation between sampling sessions.

Statistical analysis

We examined three levels of analysis: across individuals within a single site, between sites, and across species within the region (see Figure 1 for a map of the study region and see Appendix S1: Figure S1 for a visual representation of the analysis at each scale). Flea abundance was considered as the total number of individual fleas per host, expressed as either the absolute number of fleas or the mean number at the corresponding level (site or species). We considered the number of fleas per individual to represent the success of the fleas on the host once established (rationale discussed in Appendix S1). Although we report flea abundance for all host species (Appendix S1: Tables S1 and S2), statistical analyses were only conducted for host species where sample size $n > 30$, with at least three individuals in each of the levels of the factor variables. In addition to the previous criteria, only species that were present in at least half of the sampling locations in each sampling area were considered for analysis between sites.

All analyses described in the following sections were conducted using R v. 4.0.0 (R Core Team, 2020). Mixed models were implemented with the package glmmTMB (Brooks et al., 2017). All statistical analyses were restricted to nonpregnant adults to avoid confounding effects of weight gain and loss associated with pregnancy and growth (Appendix S1: Table S3). Separate models were run for each species at the local and between-site levels. Fixed effects for each level are described in Appendix S1: Table S4. Collinearity between explanatory variables in the final models was assessed using a variance inflation factor test (VIF < 2). Continuous fixed effects were mean-centered prior to analysis. The intraclass correlation coefficient (ICC) was calculated as an indicator of variation in flea abundance due to differences within and between sampling plots, as a proxy to assess the role of unspecified environmental variation associated with local conditions of the sampling plots, either within a single site or across the host's landscape.

We used Nakagawa's and collaborators' R^2 as an estimator of the proportion of variance accounted for by the final models (Nakagawa et al., 2017), implemented in the package "MuMIn" (Barton, 2013). This metric distinguishes between variance due to fixed effects (R^2_{marginal}) and variance conditional both on fixed and on random effects ($R^2_{\text{conditional}}$). To estimate the relative importance of the different variables on flea parasitism across scales, we used Akaike's information criterion weights (AICw), considering only the subset of models with $\Delta AIC_c < 2$ for model averaging and standardized predictor variables (Schielzeth, 2010). Model averaging to obtain the relative importance of predictors was performed using the MuMIn package. Note that model inference is not based on averaged coefficients but rather on a single competitive model (as determined by model selection) (Cade, 2015). The full set of models for each level of analysis is presented in Appendix S1: Tables S5–S7.

Across individuals within a locality

The number of fleas per host was modeled with either a negative binomial or a Poisson error structure (Appendix S1: Table S6). Only sampling locations from a single area (RO in Figure 1) with the same habitat type across sampling plots (shrubland) were considered for analysis at this level. Fixed effects included sex, reproductive status, body size, weight, and sampling season, with sampling plot as a random effect. Previous to statistical modeling, we assessed the correlation between morphometric variables (weight and body length) (Appendix S1: Figure S2). Model comparison was performed by backward stepwise elimination of nonsignificant terms ($p < 0.05$) from a maximal model that considered all terms and plausible biological interactions (Appendix S1: Table S5). The significance of the variables

and their interactions was evaluated using likelihood ratio tests (LRTs). Further support for variables in the final model was provided by inspecting their relative importance according to model averaging results (Appendix S1), although model coefficients in result tables are presented for a single competitive model (lowest AIC value). Model diagnostics and checks for overdispersion and zero inflation were conducted using the package DHARMa (Hartig, 2016) to ensure final models did not violate any assumptions (Zuur et al., 2010).

Across sites

The response variable at this level of analysis was the mean abundance of fleas per sampling location. Fixed effects included habitat type at location (either shrubland or grassland) and the morphometric variables explored at the previous level, scaled appropriately. Note that data from the two sampling sessions in 2012 were pooled after checking for differences in mean abundance of fleas (see Appendix S1 for details), so the categorical variable sampling season variable has two levels. Finally, terms to represent conspecific host abundance or abundance of demographic subgroups (males, females, and reproductive or nonreproductive individuals) were also included. While host abundance has been associated with effects of macroparasite abundance on a theoretical and empirical level in some systems (Anderson & May, 1978; Stanko et al., 2002), we wanted to assess the role of specific subgroups on flea abundance, as demographic structure might be key to identifying drivers of abundance within a population (see, e.g., Perkins et al., 2003). Separate sets of models were run for each demographic subgroup. Additional methods, results, and further description of the rationale for analysis at this level are included in Appendix S1. We included sampling location as a random effect to assess the contribution of within-location and between-location variation. Model comparison for each set was conducted as described in the previous section. It is important to mention that although we only considered first capture individuals for our analysis, we monitored recaptures to assess the movement of individuals between sampling plots.

Across host species within region

To assess drivers of flea abundance across species within the region, we used average flea abundance as a response variable. This response variable was modeled with a Gaussian distribution and identity link function. Fixed effects included sampling season, body size, and mass, while sampling area was considered a random factor. Only results for the best-fit model are shown. Additionally, we assessed the role of host identity on flea abundance to evaluate whether certain host species were associated with higher flea abundance. For these models, we used total flea counts on individuals as a response variable, modeled with negative binomial distribution. Only species with at least $n > 30$ individuals were considered for analysis at this level. Fixed effects were included to control for variation associated with sampling season, vegetation type, and sex-related biases. Morphometric variables were excluded due to high collinearity (VIF > 2) with host identity. Additionally, sampling area and location were considered random effects to assess the proportion of variance explained by spatial differences within and between sampling sites within areas.

RESULTS

Fieldwork

We captured and sampled a total of 674 rodents belonging to 14 species across three families (Cricetidae, Heteromyidae, and Sciuridae) (Appendix S1: Table S1). A total of 1311 fleas were collected from sampled rodents (spring 2012: 98 fleas; autumn 2012: 400 fleas; and spring 2013: 813 fleas). Summary information and details of the fleas found are presented in Appendix S1: Table S2. Three host species, *Dipodomys merriami*, *D. spectabilis*, and *Onychomys arenicola*, represented 66% of total individuals sampled, 80% of individuals with at least one flea, and between 37% and 92% of total individuals per sampling locations. We did not trap any same rodents on different sampling grids throughout our fieldwork, indicating that the separation between our sampling plots was an adequate representation of the maximum movement distance of the sampled species.

Across individuals within a locality

Three species met the criteria for analysis at this level: *D. merriami*, *D. spectabilis*, and *O. arenicola*. The negative binomial distribution offered the best fit for *D. merriami* and *D. spectabilis* flea abundance data, while *O. arenicola* was best modeled by a Poisson distribution (Appendix S1: Table S6). Sampling season was the only significant and important variable across the three species, with the strongest effects observed during the sampling season corresponding to May 2013 in two of the three species analyzed (Table 1). Different effects of host traits on flea abundance were observed in *D. merriami* and *O. arenicola*; in the latter, flea abundance was higher in

individuals of smaller body size (−0.02 [−0.04, −0.001 log units]). An interaction between sex and size was detected in *D. merriami*, with larger-than-average females presenting higher flea abundance than their male counterparts (Figure 2). This interaction was significant, as assessed by a LRT ($\chi^2 = 7.75$, df = 1, $p = 0.005$), with a relative importance greater than 0.5 (Table 1). However, the effect size on the response variable was small (0.03 [0.01, 0.05] log units). Interestingly, while the ICC value for *D. merriami* and *O. arenicola* was low (<0.001), indicating larger within-grid variance than between-grid variance, ICC for *D. spectabilis* had a value of 0.57, representing a moderate correlation between observations in the same grid. The relevance of the random effects in *D. spectabilis* is further supported by the total variance explained by the models, where R^2_c accounted for 46% of the variation in flea abundance, in contrast to the proportion accounted for by R^2_m (27%). The marginal variance explained by the models for *D. merriami* (36%) or *O. arenicola* (65%) did not change for R^2_c.

Across sites

Analysis at this level was only conducted for one species, *D. merriami*, as it met the selection criteria outlined in the Methods section. Mean flea abundance in this species across sites was negatively associated with male abundance, while the third sampling season had a markedly positive effect (Table 2). An interaction between vegetation and body size, where shrubland sites with larger-than-average individuals were associated with a higher mean flea abundance (compared to grassland sites with larger-than-average individuals), was significant (LRT: $\chi^2 = 11.89$, df = 1, $p = 0.001$). All fixed effects had high relative importance. The among-grid variance was larger than within-grid variance (Table 2). Fixed effects, according to the best-fit model, accounted for 79% of variation in the response variable, which increased when considering the conditional R^2 (86%).

Across host species within region

Sampling season was identified as the single most significant and important variable associated with variation in mean flea abundance across species. Specifically, the last sampling season increased mean flea abundance across species by 2.79 (1.01, 4.58) (Table 3). Sampling season accounted for 19% of variation in mean flea abundance at this level of analysis, a moderate result that did not increase when considering the effect of the sampling site. However, the within-site variance was very high ($\sigma^2 = 6.7$). Models assessing the role of host identity on flea abundance showed that specific host species were associated with higher abundance (Appendix S1: Table S8). Sampling season had a marked effect on flea abundance across species (Figure 3).

TABLE 1 Variables explaining flea abundance across individuals of *Dipodomys merriami*, *D. spectabilis*, and *Onychomys arenicola* at a local scale, according to generalized linear mixed model results

Host species	Fixed effect	Estimate	SE	*p*	RI	σ^2	τ_{00}	ICC	R^2_m	R^2_c
D. merriami	Intercept	−1.97	0.46	**<0.001**	1.00	0.82	<0.001	<0.001	0.36	0.36
	Season 2	0.22	0.59	0.71	1.00					
	Season 3	3.05	0.48	**<0.001**	1.00					
	Size	−0.02	0.01	**0.03**	0.83					
	Sex (F)	−0.07	0.19	0.71	0.83					
	Size : Sex (F)	0.03	0.01	**0.005**	0.83					
D. spectabilis	Intercept	−0.70	0.59	0.23	1.00	0.51	0.67	0.57	0.27	0.46
	Season 2	1.72	0.51	**0.001**	1.00					
	Season 3	2.64	0.52	**<0.001**	1.00					
O. arenicola	Intercept	1.21	0.27	**<0.001**	1.00	0.31	<0.001	<0.001	0.65	0.65
	Season 2	−0.82	0.36	**0.02**	1.00					
	Season 3	0.40	0.33	0.49	1.00					
	Size	−0.02	0.01	**0.03**	1.00					

Note: Parameter estimates and standard errors (SE) shown correspond to the single best fixed-effects model as measured by the lowest AIC and likelihood ratio test comparison. Other models within 2 ΔAIC are reported in Appendix S1: Table S7.

Abbreviations: σ^2, within-group variance; τ_{00}, between-group variance; ICC, intraclass correlation coefficient; R^2_c, conditional R^2; R^2_m = marginal R^2; RI, the relative importance of the variable across models.

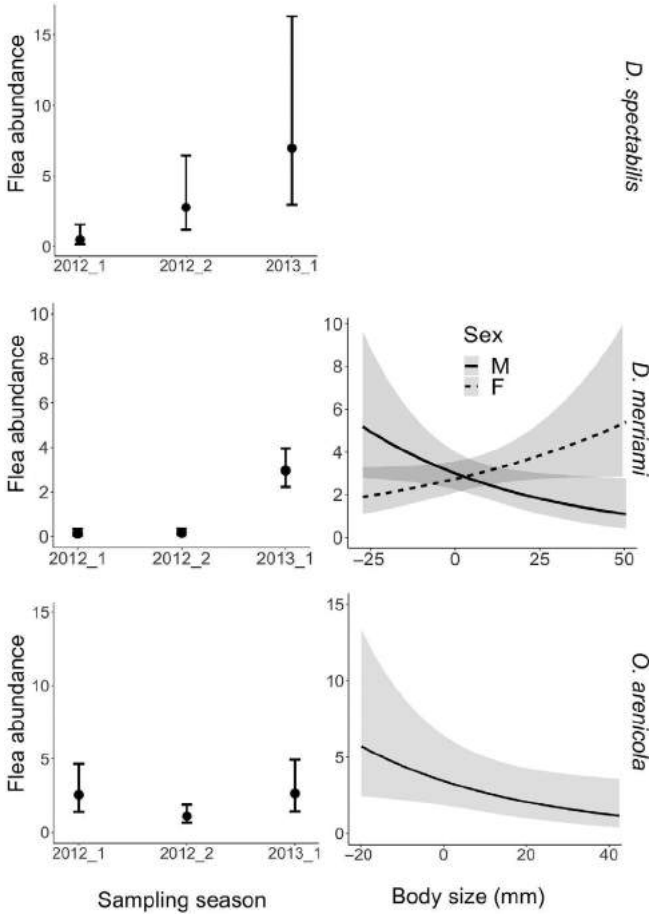

FIGURE 2 Plots showing model predictions for flea abundance in individuals of *Dipodomys merriami*, *D. spectabilis*, and *Onychomys arenicola* at a local scale (single site). Variables shown correspond to those in the top model for each species, as determined by model comparison. Each plot shows the marginal effects with the nonfocal variables held constant. Plots were made using the package ggeffects (v1.1.1; Lüdecke, 2018). *Note*: The plot showing the interaction between size and sex in *D. merriami* (M for male and F for female) shows predicted results only for the third sampling season

DISCUSSION

In this study, we assessed how spatial scale affects the role of host and environmental factors as drivers of flea abundance. We found that the effect and relevance of host traits differed across species and across scales, highlighting a dependence on the host's environmental context (Tables 1 and 2). We also found evidence for consistent effects of large-scale factors (sampling season) on flea abundance across species (Table 3) and across scales. While specific host species were associated with higher flea abundance (Appendix S1: Table S8), variation within and between sampling sites indicates an important role of local-scale variability, although their contribution toward accounting for flea abundance depended on the host species and the scale of analysis (Tables 1 and 2). Overall, these results underline that drivers of flea abundance, particularly those associated with host traits, exhibit variation across scales.

Across individuals within a locality

Host traits at a local scale (across individuals within a locality) were predictive of flea abundance in two of the three species analyzed. Specifically, in *D. merriami*, larger-than-average females presented higher flea abundance than larger-than-average males (Figure 2). Interestingly, larger size and male hosts are more frequently associated with higher parasite burdens in rodents and other vertebrates (Eads et al., 2020; Johnson & Hoverman, 2014). However, given the lack of size dimorphism in *D. merriami* and no effects of reproductive status, the interaction between sex and size in this species might be related to behavioral differences between males and females. Indeed, patterns of movement in this species, which are wider in males, have been proposed as a

TABLE 2 Variables explaining mean flea abundance in *Dipodomys merriami* across sites, according to generalized linear mixed model results

Host species	Fixed effect	Estimate	SE	*p*	RI	σ^2	τ_{00}	ICC	R^2_m	R^2_c
D. merriami	Intercept	1.53	0.38	**<0.001**	1.00	0.04	0.13	0.79	0.79	0.86
	Season 3	2.49	0.25	**<0.001**	1.00					
	Size	−0.06	0.04	0.07	1.00					
	Vegetation (shrubland)	−0.54	0.33	0.10	1.00					
	Male abundance	−0.22	0.05	**<0.001**	1.00					
	Size : vegetation (shrubland)	0.16	0.04	**<0.001**	1.00					

Note: Parameter estimates and standard errors (SE) shown correspond to the single best fixed-effects model as measured by the lowest AIC and likelihood ratio test comparison. Other models within 2 ΔAIC are reported in Appendix S1: Table S7.
Abbreviations: σ^2, within-group variance; τ_{00}, between-group variance; ICC, intraclass correlation coefficient; RI, the relative importance of the variable across models.

TABLE 3 Variables explaining mean flea abundance across species, according to generalized linear mixed model results

Host species	Fixed effect	Estimate	SE	*p*	RI	σ^2	τ_{00}	R^2_m	R^2_c
Across species	Intercept	1.03	0.64	0.11	1.00	6.7	<0.001	0.19	0.19
	Season 2	0.50	0.91	0.59	1.00				
	Season 3	2.79	0.91	**0.002**	1.00				

Note: Parameter estimates and standard errors (SE) shown correspond to the single best fixed-effects model as measured by the lowest AIC. Other models within 2 ΔAIC are reported in Appendix S1: Table S7.
Abbreviation: σ^2, within-group variance; τ_{00}, between-group variance; ICC, intraclass correlation coefficient; RI, the relative importance of the variable across models.

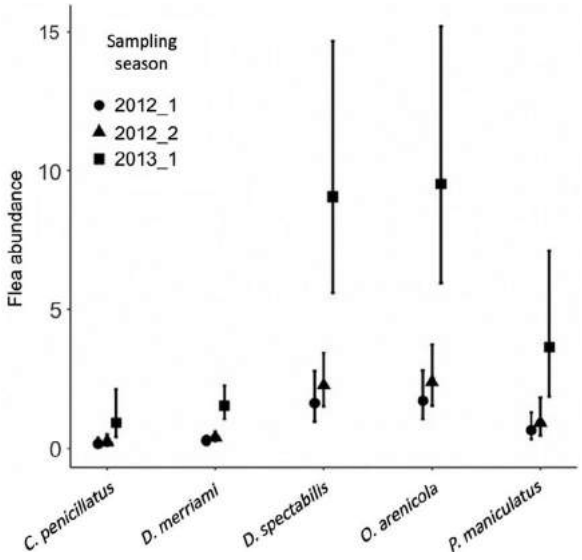

FIGURE 3 Model predictions for flea abundance across sampling seasons in host species with $n > 30$ individuals. The third sampling season was associated with higher flea abundance across all of the analyzed species. Between-species comparison also shows that model predictions indicate a lower overall flea abundance for species such as *Chaetodipus penicillatus* and *Dipodomys merriami*, with more variable burdens for *D. spectabilis*, *Onychomys arenicola*, and *Peromyscus maniculatus*. 2012_1 = spring 2012; 2012_2 = autumn 2012; and 2013_1 = spring 2013. Each plot shows the marginal effects with the nonfocal variables held constant. The predicted effects were estimated using the ggpredict and the ggplot functions in the ggeffects package (Lüdecke, 2018)

mechanism to escape parasitism pressure (Behrends et al., 1986). The size was also associated with variation in flea abundance in *O. arenicola*, with larger individuals harboring fewer fleas than smaller ones, a pattern hypothesized to correspond to better defenses against parasites in larger individuals (Kiffner et al., 2013; Sheldon & Verhulst, 1996).

Sampling season was the only variable associated with flea abundance in *D. spectabilis*. Indeed, sampling season had a large effect on individual-level flea abundance across all three species analyzed (Table 1), with the third sampling season, corresponding to spring 2013, showing the most positive effect, whereas spring 2012 had an overall lower mean flea abundance (Figure 3), indicating a strong effect of annual variation over seasonal variation. The first period of our fieldwork coincided with the end of the most severe drought on record in northwestern Mexico (Murray-Tortarolo & Jaramillo, 2019), generating large-scale conditions of low humidity and high temperatures, which have been observed to decrease larval survival significantly and induce desiccation in adult fleas (Krasnov et al., 2002a, 2002b). Thus, under drought conditions, we would expect to see lower flea abundance, as observed in this study, particularly in rodent species with shallow burrows, which are more exposed to aboveground conditions.

Variation between sampling plots encompasses differences in variables such as substrate type or vegetation cover, which in turn affect parameters such as soil humidity and temperature that are relevant to flea development and survival (Krasnov, 2008). Although our study did not measure these directly, our models did indicate a role for variation between sampling plots at a local scale in *D. spectabilis* (Table 1) whose ICC shows a moderate correlation between observations from the same sampling plot. This implies that conditions within plots are more similar than conditions in other plots, even within the same sampling area. Such small-scale effects in plot-to-plot variation might be more relevant for flea abundance of fossorial or semi-fossorial species such as *D. spectabilis*, as small variation in these conditions could influence the burrow's microenvironment (Kay & Whitford, 1978).

Across sites

The interaction between size and vegetation type observed at this level indicates that, while body size is a consistent predictor of flea abundance in *D. merriami* at both local and across-site levels, its effect is context-dependent: Mean flea abundance was higher at sites with larger-than-average specimens only at sites with shrubland, with the reverse pattern at grassland sites (Table 2). Increased near-surface air temperature at nighttime

has been reported in shrubland as compared to grassland vegetation in the Chihuahuan Desert (D'Odorico et al., 2010), resulting in local warming effects that could increase flea growth or reproductive parameters (Krasnov, 2008). This effect could be particularly marked on hosts with shallow burrows, such as *D. merriami*, where there are no significant differences between burrow and ambient atmosphere (Burda et al., 2007). Interestingly, while between-site variance was not relevant in *D. merriami* at a local scale (which included only sites with shrubland vegetation) (Table 1), across-site models for *D. merriami* (considering multiple locations with either shrubland or grassland vegetation) show that flea abundance at this level is affected by variation between sites (Table 2). Sampling season was also associated with flea abundance, showing the same effects as at the local scale. Finally, mean flea abundance in *D. merriami* across sites was negatively associated with male host abundance in this species (Table 2). While host abundance and macroparasite abundance have long been known to correlate (Anderson & May, 1978), the demographic structure of host species populations is seldom considered, despite a potential role as drivers of parasite abundance; for example, large and sexually mature males of *Apodemus flavicollis* were found to drive *Ixodes ricinus* tick abundance (Perkins et al., 2003). Although longitudinal data would be required to understand the dynamics of rodent populations, our results suggest that higher abundance of male *D. merriami* could be associated with lower flea abundance, in line with other results that have found that specific demographic subgroups can drive ectoparasite abundance.

Across host species within the region

No host traits were found to be associated with mean flea abundance across species within the study region (Table 3). Our results at this scale differ from those obtained from a similar study with rodents in Africa (Young et al., 2015), where the authors found that body mass accounted for a large proportion of the variation in mean flea burden across species. Additionally, the small amount of variation explained by models at this scale contrasts with the moderate-to-high variation accounted for by models at the previous scales (Tables 1 and 2). Furthermore, model results indicate high within-sampling-area variance (Table 3), suggesting that heterogeneities in the environmental context of the host, or in individual or across-site host traits, are important determinants of flea abundance, which are not represented by a high-level pooling of data at a regional scale.

In terms of host identity effects, three species were associated with higher flea abundance: *D. spectabilis*, *O. arenicola*, and *Peromyscus maniculatus* (Appendix S1: Table S8). Different host species differ in behavioral characteristics such as territoriality, which modifies movement and contact patterns, and burrowing habits, which fundamentally define flea exposure to the environment, which is, in turn, affected by variation in the microenvironmental conditions of the host's surroundings (Lareschi & Krasnov, 2010). Despite the diversity of life-history traits, sampling season had significant and consistent effects across all species studied. Interestingly, although there is a marked effect of sampling season on flea abundance across species, abundance in rodent species with shallow or simple burrows such as *D. merriami* and *P. maniculatus* reaches lower values during the sampling season associated with direct and post-drought effects (sampling seasons 1 and 2) according to model predictions (Figure 3). It is also interesting to note that, despite reports of higher flea abundance in spring and summer months as compared to fall or winter months in the ecoregion (López-Pérez et al., 2018), flea abundance during the second sampling season (autumn 2012) was four times higher than abundance in the first sampling season (spring 2012). Interannual season comparison shows that flea abundance during the third sampling season (corresponding to spring 2013) was eight times higher than sampling during spring 2012. While we note that the extraordinary climatic conditions associated with drought might not be representative of typical year-to-year seasonal variation in the study system, the significance of sampling season effects highlights that temporal variation, particularly if driven by large-scale interannual fluctuations, has far-reaching effects on flea abundance across all species included in the analysis.

CONCLUSIONS

In agreement with current knowledge of drivers of flea abundance, we found that both host and environmental variables, with specific combinations differing among host species, drive flea abundance. However, we found that drivers of flea abundance also varied across space, highlighting the context dependency of host traits at local spatial scales, and the far-reaching effects of large-scale annual fluctuations. Indeed, although significant, the effect size of host traits on flea abundance was small, particularly at the local scale, and most noticeable on the third sampling season for some variable combinations (Figure 2). Thus, through its strong influence on flea abundance, environmental variation (associated with drought conditions in the case of our study) could be

modifying the effect of host traits on flea abundance. Long-term empirical data would be essential to determine whether the effects we observed vary in magnitude across years, particularly because our data were collected during nonstandard conditions. However, this does not preclude interest in the result that large-scale environmental fluctuations can have significant effects on flea abundance regardless of the scale of analysis and the species (with the important caveat that none of the species we analyzed have deep burrows that could buffer against drastic changes in aboveground conditions). Indeed, changes in weather patterns and in the frequency of extreme weather events worldwide due to climate change are expected to modify both macroparasite abundance and disease transmission (see, e.g., Eads et al., 2016).

Although the generality of our findings to other macroparasite systems would require further empirical research accounting for differences in transmission mode, dispersal capabilities, and a more comprehensive range of habitats, our results indicate that investigations of macroparasite abundance need to consider both large-scale fluctuations in environmental conditions and context dependence of effects of host traits across scales to accurately assess the relative importance of the factors that affect flea abundance and even disease dynamics. In this regard, a study by Ben Ari et al. (2011) analyzed the limitations of assuming scale independence and linearity in drivers of plague dynamics at large scales and found that effects at a given scale cannot be accurately extrapolated from effects observed at smaller scales, further highlighting the importance of understanding drivers across scales.

While our results might overlook specific host–flea interactions, focusing on total burden can help to elucidate the most significant drivers of abundance within and between spatial scales, and is of relevance not only for fleas, given the presence of macroparasites with varying degrees of host specificity in any given host assemblage. Indeed, generalist fleas represented 20% of the sampled fleas in our system, while fleas that not only show family-level specificity but can also parasitize sympatric rodents represented 44% of samples (Appendix S1: Table S2).

Our results highlight potential lines of research, but further exploration considering longitudinal study design, broader parasite groups, and geographic areas would be desirable to continue advancing our understanding of the effect of scale on the drivers of macroparasite abundance. Understanding these links will further expand our capacity to monitor the abundance of macroparasites and mitigate their ecosystem and health impacts.

ACKNOWLEDGMENTS

This work was supported by a PhD scholarship from the CONACyT (Consejo Nacional de Ciencia y Tecnología) to Paulina A. Pontifes. The authors also acknowledge the National Autonomous University of Mexico (UNAM) for the support provided through a grant of the PAPIIT Program (Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica UNAM-PAPIIT), project IN225219. Paulina A. Pontifes would like to thank the Biological Sciences Graduate Program at the UNAM (Posgrado en Ciencias Biológicas) and acknowledge that this manuscript fulfills the publication requirement for obtaining a PhD degree in accordance with this program.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Adriana Fernández-González and Gerardo Suzán designed the field studies and were involved in the collection of field data. Adriana Fernández-González conducted flea identification and sample processing. Gabriel E. García Peña, Benjamin Roche, and Paulina A. Pontifes designed the statistical approach. Paulina A. Pontifes led the writing of the manuscript. Benjamin Roche and Gerardo Suzán contributed equally. All coauthors contributed comments to the manuscript.

DATA AVAILABILITY STATEMENT

Data (Peña et al., 2021) are available from Figshare: https://doi.org/10.6084/m9.figshare.17303933.

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SUPPORTING INFORMATION

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How to cite this article: Pontifes, Paulina A., Adriana Fernández-González, Gabriel E. García-Peña, Benjamin Roche, and Gerardo Suzán. 2022. "Drivers of Flea Abundance in Wild Rodents across Local and Regional Scales in the Chihuahuan Desert, Northwestern Mexico." *Ecosphere* 13(4): e4013. https://doi.org/10.1002/ecs2.4013

Capítulo II. Effects of black-tailed prairie dog presence on flea community structure in rodents of the Chihuahuan desert, Mexico.

Manuscrito por ser enviado a la revista *OIKOS*

Effects of black-tailed prairie dog presence on flea community structure in rodents of the Chihuahuan desert, Mexico.

Abstract

Parasites can alter host populations through their effects on host fitness and survival. Likewise, parasites are also affected by their hosts, particularly by those whose presence modifies parasite community structure through altering host availability and micro-environmental conditions relevant to parasite reproduction and survival, thus behaving as keystone species for both the hosts and parasites. However, the effect of such keystone species on parasite communities remains largely unexplored. To address this gap, we studied the effect of keystone species on parasite community structure using the black-tailed prairie dog (BTPD) and flea assemblages of sympatric rodents in north-western Mexico as a study system. We collected 218 rodents and quantified flea occurrence at grassland sites with and without BTPD across three localities within the study region and found a positive effect of BTPD presence on flea prevalence, abundance and diversity, as well as higher occurrence of flea coinfections in sites where this species was present. We further assessed the role of BTPD on flea sharing between sympatric species using a network approach, by analysing metrics of structural heterogeneity in host-parasite bipartite and unipartite networks, the latter based on the similarity of flea assemblages. We found lower network modularity indicating enhanced flea sharing at BTPD sites, albeit bounded by host specificity. We further identified nodes relevant to parasite sharing, by assessing the centrality of individual nodes with three complementary metrics: eigen value centrality, betweenness and closeness centrality. While BTPD scored high centrality values in all three metrics, *Onychomys arenicola* was associated with higher values in betweenness, albeit with intraspecific variability, suggesting that individuals of this species may act as a bridge for parasite sharing between hosts. In sites where BTPD was absent, *Dipodomys spectabilis* occupied the highest centrality values in all three metrics, highlighting the potential of burrows as sites for flea exchange. These results highlight the effect of keystone species on parasite community structure and the importance of considering the community context of host-parasite interactions, particularly in the light of emerging vector-borne diseases and rapid changes to community structure due to human-driven global change.

Introduction

Fleas (order Siphonaptera) are obligate hematophagous arthropod ectoparasites whose feeding habits position them as ideal vectors to various pathogens (Bitam et al. 2010). Flea-transmitted zoonotic diseases, such as murine typhus and the bubonic plague, have re-emerged in some countries (Blanton et al. 2015, Alderson et al. 2020) and remain a source of concern both from the public health and the biological conservation standpoint, due to the sensitivity of some species to these diseases (Russell et al. 2019).

While flea abundance is associated with disease transmission risk (Eisen and Gage 2012), flea identity affects how efficient their transmission is (Fenton et al. 2015). In addition, although some flea species are specific to some host species, many fleas can exploit several host species opportunistically, which may or may not be related at higher taxonomical levels (Krasnov et al. 2011). Thus, flea community structure is relevant to flea-transmitted disease dynamics (Friggens and Beier 2010).

Flea community structure is the net result of the interactions between species in the community and how these interactions are modulated by extrinsic and intrinsic factors (Krasnov, 2008). Thus, variables that modify the strength and direction of flea-flea interactions, for example, through altering micro-environmental conditions that modify host availability or flea abundance, are central to understanding flea community structure. One of these largely unexplored variables is the presence of keystone species in the host community. Keystone species (Paine, 1966) play a pivotal role within their communities or ecosystems, as they modify environmental characteristics and the structure and composition of ecosystems (Mills and Doak 1993). Because flea survival throughout their life cycle is highly dependent on heat and humidity conditions of the host's nest or burrow (Krasnov et al. 2001, 2002), keystone species that affect flea exposure to these variables have the potential to significantly modify flea abundance and diversity, in addition to their effects on host abundance and distribution (Hammond et al. 2019).

Prairie dogs are one such keystone species. Their presence in North American grasslands has been associated with changes in vegetation and soil characteristics, as well as differences

in the distribution and abundance of small mammals, birds and reptiles (Kotliar et al. 1999). Prairie dog burrows have been proposed to act as sites that enhance flea exchange and survival (Thiagarajan et al. 2008, Friggens et al. 2010) by providing stable micro-environments - especially during harsh aboveground conditions such as droughts (Eads et al. 2016) - and through acting as attractors to hosts and fleas alike (Brinkerhoff et al. 2008). Furthermore, their presence modifies the abundance of rodents and flea species that have been associated as reservoirs and vectors of pathogens such as the bubonic plague (Kraft and Stapp 2013).

Although these studies have provided evidence that prairie dog presence is associated with enhanced flea diversity, prevalence and abundance, few have assessed if their presence influences flea community structure (but see Bangert and Slobodchikoff 2006). To address this gap in knowledge of how prairie dog presence affects fleas, we investigated diversity and patterns of flea assemblages in rodents in grasslands in north-western Mexico at sites with and without black-tailed prairie dog (*Cynomys ludovicianus,* hereafter BTPD). To this end, we analysed patterns of flea species co-occurrence and compared the frequency of flea coinfections (more than one flea species on a host) in grasslands with and without BTPD. Because most studies have been conducted on prairie dog colonies in the US (but see Zapata-Valdés et al. 2018), we also evaluated the effect of BTPD on flea abundance and prevalence, by comparing results on grassland with and without BTPD presence. Praire dog presence has been associated with enhanced flea presence; thus, we expect a higher prevalence, diversity and abundance of fleas at BTPD sites, as well as a higher occurrence of flea coinfections and predominantly positive flea-flea associations, indicative of increased host-switching and facilitative mechanisms, potentially mediated by BTPD presence (Pedersen and Fenton 2007).

To further address the potential role of BTPD as a keystone species for flea sharing between sympatric rodents, we used an individual-based network approach, which places emphasis on the transmission process and reduces the loss of information that occurs when aggregating data into species-level averages (Tompkins et al. 2011, Godfrey 2013). Following the hybrid strategy proposed by Pilosof et al. (2015), we analysed network structural heterogeneity in

host-parasite bipartite and unipartite networks (the latter based on linking nodes by the similarity of parasite assemblages) to maximise information on the structure and underlying mechanisms of flea sharing. Specifically, we assessed metrics of modularity and centrality in networks of sites with and without BTPD presence. Metrics of centrality have been used to identify host species or individuals that are relevant for parasite sharing (Dallas et al. 2019), while modularity can help to delimit host subgroups that interact with similar parasites and identify the traits associated with specific module affiliation, such as phylogenetic relatedness (Krasnov et al. 2012). Because we hypothesise BTPD presence to enhance flea exchange through facilitating sympatric rodent encounters within their burrows, we expect the modularity of flea-rodent assemblages to be lower at sites with BTPD, indicating a higher degree of flea-sharing, albeit restricted by flea-host specificity. Likewise, we expect host identity to be associated with centrality, and specifically, for BTPD to be associated with higher values of centrality.

While the effect of prairie dogs on fleas has been more widely researched, to our knowledge this is the first study that attempts to provide a network-based insight into the role of this keystone species on flea assemblages. Indeed, while the effect of disease on keystone species and ecosystem structure is an active area of research (for a review see Collinge et al. 2010), the effect of keystone species on parasite communities is underexplored. Our results highlight the importance of studying host-parasite interactions within the community context, particularly in light of emerging vector-borne pathogens and rapid changes to community structure induced by species extinction due to human-driven global change.

Methods

Rodent and flea sampling

We sampled rodents and fleas in grassland sites with and without active BTPD colonies in the Janos Biosphere Reserve (JBR), located in north-western Mexico, during two sampling sessions (October 2012 and May 2013). JBR is home to the largest remaining BTPD colonies in Mexico (Ceballos 2014). We sampled rodents using Sherman traps (H.B Sherman 8x8x23 cm Tallahassee FL) at three localities across the JBR (MV, EC or PCV, see Fig. S2). At each locality, we set seven 7x7 sampling grids with a 10 m spacing between traps. Grids were set

on grassland sites with active prairie dog colonies or grassland sites with no BTPD presence (i.e., no presence of BTPD or burrows), the latter located 800-900 m apart from sites with an active colony. Traps were baited with a mixture of oats and vanilla extract, set in the evening and checked the following morning during three consecutive nights.

Each captured rodent was placed in an anesthetizing chamber containing a cotton ball doused with isoflurane. This method facilitates handling and collection of ectoparasites (Brinkerhoff et al. 2008). The lid of the chamber and the cotton ball were removed as soon as motor activity nearly ceased, to minimize the risk of death by anaesthesia. The plastic chamber was cleaned after each use. Rodents were identified to species level using taxonomic keys (Anderson 1972, Reid 2006), and ear-tagged to avoid re-sampling. Anaesthetized rodents were held stretched and combed thoroughly within the chamber for 2 minutes with a standardized number of passes. Fleas obtained from each rodent were collected by hand from the container and stored in microtubes containing 70% ethanol and kept at -80ºC until identification. Flea species were identified using a dissecting scope (SZx12 Olympus, Melville, NY) and taxonomic keys (Hubbard, 1974; Acosta and Morrone, 2003). Flea abundance was calculated as the number of fleas recovered from each individual, while flea presence was coded as a binary value. BTPD and their fleas were also sampled, following the methods outlined in Zapata-Valdés et al. (2018). Procedures for trapping and handling were approved by the animal care committee of the National Autonomous University of Mexico (UNAM) and by the Secretariat of Environment and Natural Resources (license number FAUT-0250), and followed the standards set by the American Society of Mammalogists (Sikes and Gannon 2011).

Description of flea and host assemblages

We summarised the relative abundance of flea species by pooling sampling site level data by BTPD presence of absence, after assessing the similarity in flea assemblages across sampling sites, using the abundance based Morisita-Horn index (Chao et al. 2006). Additionally, because host species composition and sampling adequacy can affect flea community composition, we also assessed variation in host composition across the region, and verified

sample efficiency across localities (Chao and Chiu 2016), see Supplementary material for further details on these analyses.

Statistical analysis

Effect of BTPD presence on flea abundance and presence

All the analyses described in the following sections were conducted using R V.4.0.0 (R Core Development Team, 2020). To test for effects of BTPD on flea abundance and presence, we used generalised mixed models with negative binomial and binomial error structure, respectively. Models were run excluding and including BTPD data, but only results excluding BTPD are presented, as this species has high flea loads that could bias our analysis, and our main interest was the effect of their presence on flea occurrence in sympatric hosts. Only rodent species with n >5 individuals were included for analysis. Rodent species where less that 5 individuals had fleas were also excluded. Mixed models were implemented with the packages glmmTMB (Brooks et al. 2017), and followed the data exploration protocol recommended by Zuur et al. (2010). Model diagnostics were conducted using the package DHARMa (Hartig, 2017).

Each set of models included BTPD presence as a fixed effect, expressed in terms of a factor (prairie dogs absent or present). In addition, to test for potential effects of seasonal variation on BTPD influence on fleas, we assessed an interaction term between BTPD presence and sampling season, corresponding either to spring or autumn (May and October sampling sessions, respectively). To control for differences in flea abundance or presence due to host species effects, we included rodent species as a random effect. We also considered sampling year as a random effect, to control for potential interannual variation, and sampling site to account for site-specific differences in flea occurrence.

Effect of BTPD on flea-flea associations

To test if BTPD presence facilitates flea exchange between sympatric rodents, we tested for differences in the distribution of coinfected hosts in grassland sites with and without their presence, considering only the hosts with at least one flea present.

We analysed the effect of BTPD presence on flea associations in grasslands with and without BTPD using the probabilistic approach developed by Stephens et al. (2009) and detailed in

Stephens et al. (2020). This method applies a Bayesian inference framework to calculate the test statistic ε, which quantifies the sign and strength of interactions between pairs of species, classifying pairwise associations as positive or negative, depending on whether they happen more or less frequently than expected by chance. Briefly, interactions are identified through deviations of the co-occurrence of pairs of flea species on individual hosts, relative to a benchmark (no interactions) based on a statistical ensemble of all the individuals captured at a specific type of grassland (BTPD or non BTPD).

Network analysis

To assess differences in network structure in the presence or absence of BTPD, we used the hybrid approach proposed by Pilosof et al. (2015), constructing host-parasite bipartite networks and unipartite networks based on parasite sharing. For each locality, we built one bipartite network and identified modules composed of host individuals that interacted with similar parasites using the function computeModules in the package bipartite (v.2.16) (Dormann and Strauss 2014, Beckett 2016), and tested the significance of the modularity values obtained by comparing with the results obtained from 5000 random networks constructed with a probabilistic null model (further details in supplementary material).

To identify host individuals associated with high levels of parasite sharing, we constructed unipartite networks for rodent assemblages at each locality, where nodes represented host individuals and edges depicted shared parasites. Briefly, edge weights were calculated using the Jaccard index as a measure of the similarity in parasites infecting a pair of individuals (Koleff et al. 2003). Edges of pairs of individuals parasitized by the exact same flea species had a maximum value of 1, while 0 indicated no fleas were shared. For further details on their construction see Pilosof et al. 2015 and Dallas et al. 2019. We used three measures of centrality to quantify a node's importance in terms of promoting parasite sharing: closeness, betweenness, and eigenvalue centrality. These metrics were chosen based on the complementary aspects of node importance that each capture. Closeness centrality is a measure of host parasite sharing, with higher values indicating the hosts that share many parasites with many other hosts. Betweenness centrality can be understood as a measure of the extent to which a node plays a bridging role in the network (i.e., the extent to which a

node falls on the shortest path between other pairs of nodes) (Dale and Fortin, 2021). Thus, hosts with high betweenness represent potential pathways for parasite sharing between groups of hosts. Finally, eigenvalue centrality indicates nodes that have connections to other nodes that are themselves highly connected. This measure has been associated with hosts that are likely to play a key role in network dynamics (Allesina and Pascual 2009). Specifically, in host-parasite networks this metric has been linked to a measure of the transmission potential of the node (Canright and Engø-Monsen 2006). All centrality metrics were estimated with the package igraph (v.1.2.5).

Results

Description of host and flea assemblages

Similar numbers of sites with and without BTPD were sampled in total (grassland = 10, BTPD = 11), with adequate sampling in all sites (Table S1). Excluding BTPDs (n = 58), we sampled 218 individual rodents, representing rodent species from three families (Cricetidae, Heteromyidae and Sciuridae), of which 100 were collected in grasslands and 118 in BTPD grasslands. Counting BTPD, host species richness was higher in BTPD grasslands (n = 9) than grasslands (n = 8). Host diversity was not significantly different between sampling seasons (t =-0.03, p = df = 12, p = 0.974) (Table S2-S3).

Dipodomys spectabilis and *Perognathus flavus* were the most abundant host species in grasslands with no BTPD, representing over 75% of the individuals captured at these sites (Fig.S1). Excluding BTPDs, three rodent species in BTPD grasslands comprised over 50% of individuals (*D. merriami*, *D. spectabilis* and *Onychomys arenicola*). Dissimilarity analysis grouped hosts sampled at sites with BTPD present within a same cluster, while sites where BTPD were absent were grouped together. Dissimilarity within groups ranged between 13-36%, whereas dissimilarity between groups approached 60% (see Supplementary material for details).

We collected a total of 924 fleas, belonging to 9 flea species. Flea species richness was higher in BTPD grasslands (n = 7) than grasslands where this species was absent (n = 6).

Two flea species, *Jellisonia ironsi* and *Orchopeas leucopus*, were not found in BTPD habitat, while *Pulex simulans*, *Meringis altipecten* and *Oropsylla hirsuta* were only found in BTPD grasslands. Both grasslands with and without BTPD shared four flea species (Fig.1A).

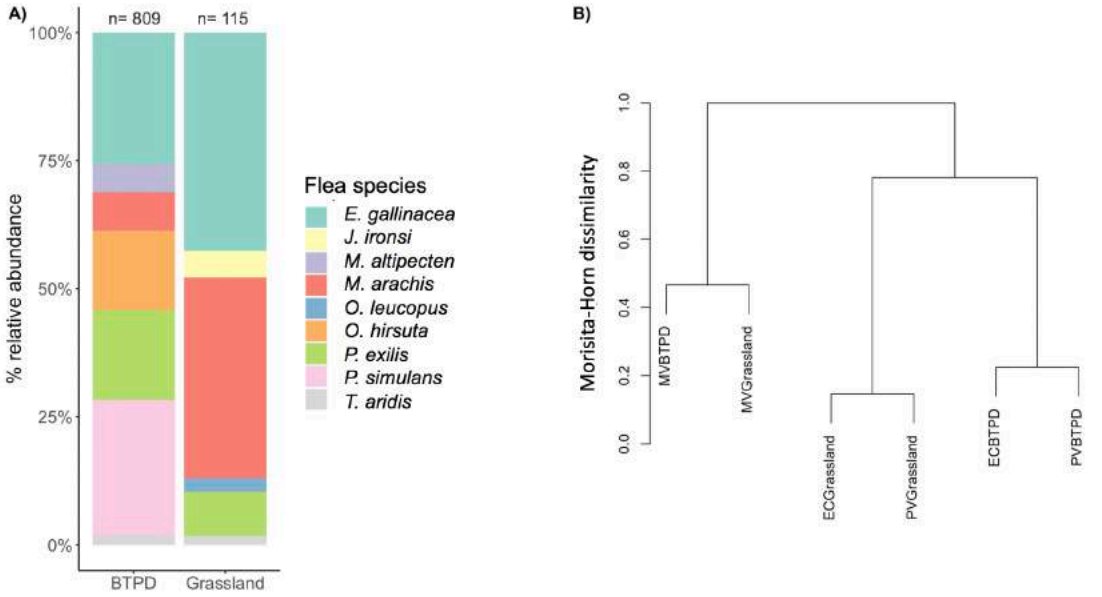

Fig.1. A) Relative abundance of fleas collected at grassland sites with and without BTPD presence at each of the three sampling sites (EC, MV or PV). Each sampling site had grassland habitat, but BTPD were either present or absent; the subscript following each sampling site abbreviation denotes whether this species was present (BTPD) or absent (grassland). Flea diversity was lower at sites with no BTPD, with six flea species described. *E. gallinacea*, *M. arachis*, *P. exilis* and *T. aridis* were collected at both habitat types. B) Dissimilarity was estimated with the Morisita-Horn index, where 1 is completely dissimilar and 0 is identical.

Two flea species represented over 80% of fleas sampled in grasslands with no BTPD: *Echidnophaga gallinacea*, a cosmopolitan flea that parasitizes poultry and various mammals, and *Meringis arachis* that parasitizes mainly rodents of the Heteromyidae family. The remaining percentage of fleas collected in grasslands with no BTPD were comprised of species specialists, such as *J. ironsi*, which has been associated exclusively with the northern pygmy mouse (*Baiomys taylori*), or fleas with genre-level preferences like *O. leucopus* (primarily a flea of the *Peromyscus* genre), or *Pleochaetis exilis* (primarily a flea of the *Onychomys* genre).

In grasslands with BTPD, 2 flea species represented over 50% of sampled fleas: *E. gallinacea* and *P. simulans*. Both fleas are generalists, but *P. simulans* is typically associated with larger rodents such as prairie dogs and squirrels. Indeed, all *P. simulans* were collected

on BTPD. *O. hirsuta*, and *P. exilis,* fleas respectively found in close association with prairie dogs and grasshopper mice (Onychomys genre) followed in terms of relative abundance. Rarer fleas, which comprised <15% of flea species sampled, included *M. arachis* and *M. altipecten*, both fleas of heteromyid rodents (the latter more closely associated with the *Dipodomys* genre), and *Thrassis aridis*, another flea considered primarily a parasite of *Dipodomys* sp.

An assessment of the overall dissimilarity of flea assemblages between habitat types by locality grouped EC and PV grasslands by the presence or absence of BTPD with a moderate dissimilarity between groups (range 15-22%) (Fig.1B). The analysis grouped together MV sites, however, they only shared one flea species *P. exilis*, whereas MV_{BTPD} site shared 5 flea species with BTPD sites at EC and PV (see Table S4). Non-specific flea-host associations were more frequent in BTPD (Table S5-S6).

Effect of BTPD on flea abundance and presence

Fleas were twice as likely to occur in individuals in grasslands with BTPD, irrespective of host species or sampling site (odds ratio: 1.06-5.83, see Table S7 for details). The sampling season corresponding to spring also had a positive effect on flea presence. Flea abundance was also affected by BTPD presence; however, this effect was dependent on the season, with spring on BTPD sites resulting in higher abundances (Table S8). Variance in flea abundance between host species was relevant (LRT: $X^2 = 15.38$, df = 1, p = <0.001).

Effect of BTPD on flea-flea associations

In grasslands with BTPD, rodents were more likely to be coinfected with different flea species than in grasslands without BTPD ($X^2 = 9.73$, df = 1, p = 0.002), with coinfected rodents occurring six times more frequently in BTPD grasslands (Table S9). All coinfected individuals belonged to one of four species: *D. merriami*, *D. spectabilis*, *O. arenicola* or *C. ludovicianus,* with coinfected individuals of the two latter harbouring up to three different flea species. Positive pairings represented 24% of the 21 pairings analysed in BTPD, whereas in grasslands without this species, we only detected 1 positive pairing (7%). The highest ε

value occurred between pairings of *O. hirsuta* and *P. simulans*, as well as *E. gallinacea* with both these species (see Table S10).

Network modularity and centrality analysis of flea assemblages

For each habitat type at each locality, we identified modules composed of host species that interacted with similar parasites (Fig. S2). This analysis detected different number of modules within each locality (see Table 1). Interestingly, modularity values were consistently lower at sites where BTPD were present (compared at a locality level). A closer inspection showed that only in PV_{BTPD} and MV_{BTPD} networks, modules contained multiple host species, whereas in EC_{BTPD} site, modules consisted of individuals of the same species but some shared multiple flea species (Fig. S2). Modules at sites where BTPD were absent were almost entirely composed of individuals of a single species, with the exception of a single individual of *P. flavus*.

Table 1. Modularity analysis results for host-parasite bipartite networks for each habitat type by locality. All modularity values obtained were significant. Differences in modularity between networks were not a result of differences in the size or connectance of networks (Supplementary materials). Prevalence was calculated as the proportion of hosts with fleas (including BTPD) from the total number of hosts sampled. Standardised modularity scores are provided in Table S11.

Site	N hosts	Flea richness	Prevalence	Modularity (# modules)	p-value
EC_{BTPD}	30	5	80%	0.38 (5)	<0.001
EC_{grass}	40	2	30%	0.49 (2)	0.023
PV_{BTPD}	39	5	69%	0.45 (5)	0.005
PV_{grass}	26	6	42%	0.60 (5)	0.009
MV_{BTPD}	107	7	64%	0.62 (6)	<0.001
MV_{grass}	34	2	15%	0.69 (3)	0.046

Following Pilosof et al., 2015, edges in the unipartite networks represent individuals that form part of a transmission chain due to either ecological or physiological characteristics that promote parasite sharing. In BTPD networks at PV and EC localities, individuals with the highest eigen values were consistently *C. ludovicianus*. This species also scored high values in metrics of betweenness and closeness, along with the grasshopper mouse (*O. arenicola*), except at EC where fleas were not shared between species (Fig. S3, panel 3). Interestingly, at MV_{BTPD}, individuals of *D. merriami* along with *O. arenicola* displayed the highest eigen value centrality, while a few individuals of *D. spectabilis* had high closeness values (Fig. S3 panel 5). The highest ranking for centrality metrics in networks where BTPD was absent were scored by *D. spectabilis*, with high variation in the value of these metrics depending on the individual (Fig. S3, panels 2 and 4). Figure 2 exemplifies the main results of centrality analysis in the MV_{BTPD} network.

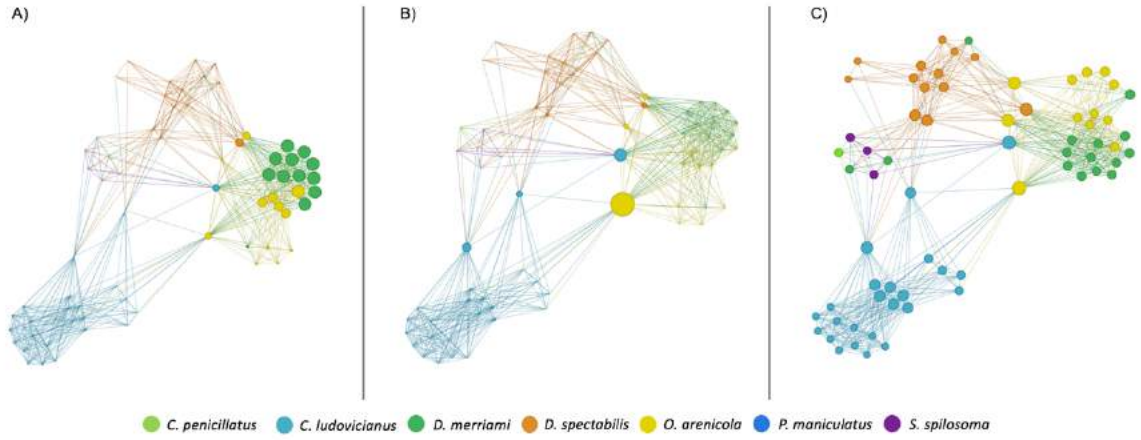

Fig.2. Transmission potential networks of hosts in MV_{BTPD}. The size of the nodes is relative to the position of the individuals in the network, as estimated by three different centrality metrics, where larger nodes indicates higher centrality for the particular metric. Weight of edges is not included in the network visualization, for clarity. A) Eigen value centrality; B) Betweenness centrality; C) Closeness centrality.

Discussion

The influence of prairie dogs as keystone species in North American grasslands is well-established, with positive effects on small mammal abundance and diversity (albeit with spatial variation, see Cully et al. 2010). However, their role as potential keystone species to fleas is not well explored, despite the relevance of this system to prairie dog conservation and public health due to the threat of flea-transmitted diseases like the bubonic plague (Gage and Kosoy, 2005). In this study, we found that fleas were more diverse, abundant

and prevalent in grasslands with prairie dogs than without prairie dogs. These effects have been reported in other prairie-dog /flea studies in the US and Sonora, Mexico, and thus our results provide further support that prairie-dogs have significant ecological influence on fleas. We also found higher occurrence of coinfected rodents and lower network modularity in grasslands with prairie dogs, suggesting that this species facilitate flea exchange between sympatric species. Furthermore, our study also found evidence that other rodent species are of significance to flea sharing dynamics, highlighting the importance of considering the community context of flea-host interactions.

Flea assemblages were markedly different in sites with and without BTPD; in the latter, *E. gallinacea* and *M. arachis* dominated in terms of relative abundance and although they were also present in sites with BTPD, they did not represent the majority of the fleas collected (Fig. 1). It is interesting to note that *E. gallinacea* has a broad range of hosts including domestic birds and large mammals, but wild rodents are considered accidental hosts (Stewart, 1932). Although it is unknown if they are indicative of ecosystem disturbance, this species is often associated with a peridomestic or urban context (Otiang et al. 2021). Differences in host assemblage composition account for the absence or presence of species-specific fleas; for example, *J. ironsi* was only found in association to its host (*B. taylori*). Previous studies have found that some host species are positively associated with prairie dog colonies, while others are more associated to grasslands without this species (Pruett et al. 2010), and although we did observe differences in abundance of some rodents between habitats (Table S1), low numbers of the species present at only either of the habitats precluded statistical analysis.

Besides differences in the composition of flea assemblages, our results also indicate that BTPD influence flea occurrence. Although the number of hosts sampled in each habitat type was similar (n = 100 in grasslands and n = 118 in BTPD grasslands), fleas were twice as likely to occur in rodents when BTPD was present (Table S7), as well as 2.3 times more abundant in their presence (7 times more, if BTPD data is included). BTPD could be influencing flea occurrence and interactions through a double mechanism: on one hand, their deep and complex burrows could be providing favourable microclimatic conditions for flea development and survival that prevent desiccation under variable temperature and rainfall, thus positively influencing flea abundance (Van Vuren and Ordeñana 2012, Eads et al. 2016).

Our results in terms of flea abundance and prevalence are consistent with this mechanism; fleas were less abundant and less likely to occur in sites with no BTPD, and we observed a seasonal-dependent effect on abundance when BTPD was present (Table S8). On the other hand, BTPD could facilitate intra and interspecific flea exchange through enhancing contact rates between hosts, either through increased encounters within their burrows, or through attracting and concentrating hosts and fleas alike, increasing abundance (Agnew et al., 1986). In ecological theory, a higher diversity and abundance of resources (hosts) has been proposed to enhance the number of coexisting consumers (parasites) (Pimm, 1979). In this regard, we found higher diversity and overall higher rodent abundance in BTPD sites (Table S1-S3), and increased interspecific flea sharing at BTPD sites in two of our sampled localities (MV and PV): flea-host modules in these sites were comprised of multiple host species and had lower modularity values than grassland sites with no BTPD (Table 1).

This higher degree of flea sharing in BTPD sites suggests that some flea species can parasitize sympatric rodents when the ecological opportunity arises, and that BTPD presence may thus reduce encounter barriers (Combes et al., 2001). This is further evidenced by the higher occurrence of fleas parasitizing non-specific hosts in these sites (Table S5-S6). This was also observed by Kraft and Stapp (2013) in *Onychomys leucogaster* in BTPD colonies in Colorado, whose flea assemblages consisted of 10% of fleas with other host preferences. We also observed fleas parasitizing non-specific hosts in BTPD sites, (12% for *O. arenicola,* a close relative of *O. leucogaster*, and 6% overall total) and higher frequency of coinfections (Table S9). However, note that although modularity values in BTPD networks were lower in comparison to grasslands without this species (Table 1 and Table S10), they did indicate some degree of structure. This could be the result of two non-exclusive mechanisms: fleas are not as strictly phylogenetically constrained as other ectoparasites, but they do show some degree of host preference based on host relatedness (Krasnov et al. 2012). On the other hand, while BTPD presence can reduce encounter barriers, some rodents will be more closely associated with exploiting BTPD burrows than others (Pruett et al. 2010). Thus, some degree of encounter filters will remain. These results highlight two things: that while host-flea interactions are limited by the flea's specificity or encounter barriers (hence the presence of structure), multi-host interactions are possible and are enhanced by the presence of BTPD.

Whether this enhanced host switching is beneficial to flea fitness is a matter that needs further research.

Interestingly, increased abundance in BTPD did not translate into overall positive associations, as suggested by a majority of negative interactions detected by the co-occurrence analysis. Flea communities have been widely established to tend towards aggregative patterns, although competitive exclusion has also been observed (Krasnov et al. 2006, López-Pérez et al. 2018) due to fleas essentially competing for the same resource. However, host body area specificity might reduce some of this competition and could explain some of the positive associations observed in our study between various flea species and the generalist *E. gallinacea* (Table S10), which has strong preferences for attaching to the head area of the host (Suter, 1964), unlike other fleas that get on the host to feed but otherwise spend time off host.

In terms of its role on flea sharing, while BTPD tended towards higher values in all measures of centrality as expected, we found that other species were also relevant to distinct roles, albeit with variation between localities. In most BTPD sites, *O. arenicola* had higher betweenness values, suggesting that this species may act as a bridge for parasite sharing between hosts. Indeed, the frequent use of prairie dog burrows and predatory behaviour of this species has implicated its relative *O. leucogaster*, as a potential reservoir and spreader of the plague bacteria, *Yersinia pestis* because of increased encounter rates with other hosts (Kraft and Stapp, 2013). Consistent with this behaviour, this species also scored high in EC and closeness, indicating a high degree of flea sharing both with other hosts and with highly connected hosts. Interestingly, in one of the sites (MV_{BTPD} see Fig. 2), *D. merriami* also scored high eigen value centrality, possibly due to a high degree of parasite sharing with *O. arenicola* (Fig. S3 panel 5), through predation (Hope and Parmenter, 2007), although the nature of their interaction is unclear. In sites with no BTPD, flea sharing occurred mostly between individuals of the same species. In most of these sites, *D. spectabilis* occupied the highest centrality values in all three metrics, a result potentially associated with the shallow networks of tunnels that this species builds, which might be playing a similar role to BTPD burrows (Kay and Whitford, 1978). These results highlight the potential of burrows as sites that not only enhance flea survival and sustain flea populations, but also enhance flea

exchange through chance or directed (e.g., through predation) host encounters, as many flea species tend to spend most time off-host when they're not feeding.

Although we did not analyse traits potentially accounting for specific node centrality, our individual level approach to network analysis highlights that both interspecific and intraspecific variability are relevant to understand flea sharing, as evidenced by the high centrality values of a few individuals from a few species in each assemblage (Fig. S3). Indeed, while flea sharing between species was more frequent when BTPD was present, there was also site-level variation. In EC_{BTPD}, for instance, fleas were only shared between individuals of the same species that are also their typical hosts (Fig. S2). This result is likely indicative of site-level variation, perhaps due to a lower abundance of bridging individuals in that site (a role associated with *O. arenicola* in other BTPD localities, of which only 3 individuals with fleas were found in EC_{BTPD}), and not due to incomplete sampling of hosts (Table S1) or fleas, as the method employed has a high detection success (Eads et al. 2013). However, further fieldwork would be required to determine the causes of site-level differences. It should also be noted that, although clearly delimiting the extent of a community is a complex matter, we did not record movement of rodents between our sampling sites during the sessions.

Our results provide evidence of significant differences in flea diversity, abundance and prevalence under the presence of BTPD. It also highlights the potential role of this species to function as a keystone species to fleas by enhancing host switching and facilitative associations within their burrows, and also detects other host species of relevance to flea sharing. However, our study represents a snapshot of how BTPD influences flea community composition. Long term studies, ideally across the range of prairie dog distribution, would provide further knowledge on the effect of this species on fleas within a community context.

Acknowledgements

This work was supported by a PhD scholarship from the CONACyT (Consejo Nacional de Ciencia y Tecnología) to PAP. The authors also acknowledge the National Autonomous University of Mexico (UNAM) for the support provided through a grant of the PAPIIT Program, project IN225219.

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Capítulo III. Bartonella-rodent-flea assemblages in sympatric rodents of northwestern Mexico highlight the nuances of inter-species transmission in multihost systems.

Manuscrito por ser enviado a la revista *Parasites & Vectors*

Bartonella-rodent-flea assemblages in sympatric rodents of northwestern Mexico highlight the nuances of inter-species transmission in multihost systems.

Abstract

Pathogens that can circulate within multiple hosts represent a challenge for public health and disease control, as their management requires identifying host species and host population subgroups that are crucial to their survival and reproduction. The extent to which these pathogen species rely on inter-species transmission for their persistence is unclear for many multihost systems but is generally assumed to be common occurrence, based on indirect evidence and correlates of pathogen species prevalence and host abundance. However, genetic variants of the same pathogen species can behave in ecologically and epidemiologically distinct manners, in terms of both their host associations and their pathogenicity. High-resolution empirical studies in natural communities are therefore critical to provide an evidence-based understanding of the nuances of inter-species transmission and identifying key hosts that harbor and transmit variants of interest. In this study, we characterized the variants of a flea-transmitted bacterial parasite, *Bartonella sp.,* present in sympatric rodents and their fleas in two sites in northwestern Mexico to investigate the occurrence of inter-species transmission and variables associated with a higher risk of infection with specific variants. Using a network analysis approach, we determined that host-Bartonella associations were significantly modular, with phylogenetic relatedness of rodents determining module affiliation. However, we also found that these limits appear variant-specific, and in some instances Bartonella variants were present in more distantly related rodent species. The flea vectors ranged in host specificity, but generalist fleas had a low Bartonella prevalence. Rather, fleas with family level preferences had the highest prevalence and diversity of hosted Bartonella variants. Our study system differs from previous characterizations of *Bartonella sp.* in sympatric rodents in that the presence of burrows of fossorial species can enhance contact between different host species and their fleas. Indeed, we found that links in the Bartonella transmission network were more likely to occur between individuals linked by flea sharing, although the imperfect overlay between networks shows that this is restricted to some host-flea combinations. Thus, the Bartonella-host-flea assemblages we detected were consistently conformed by specific combinations. Overall,

these findings highlight the complexity of inter-species transmission in multihost systems, and how fine-scale characterization is crucial for targeted disease management.

Introduction

The ubiquity and economic impact of multi-host pathogens has resulted in a shift in focus in the study of the ecology of infectious diseases, from single-host systems to approaches that center on the community ecology context of disease (Johnson et al. 2015). This context is especially relevant in multi-host systems, because pathogen persistence and disease spreading are affected by intra and interspecific variability in host competence and transmission. Thus, some hosts are more important than others in terms of their contribution to multi-host pathogen dynamics (Streicker et al. 2013, Fenton et al. 2015).

Identifying key hosts in multi-host systems is a challenging task. Part of this complexity lies on determining variables affecting infection risk (i.e., the probability of an infection occurring given exposure to an infectious agent) across different levels of biological organization: at the individual level, age or gender-related differences in exposure and immunity are known to affect infection risk (Kiffner et al. 2013), while at the population level, specific demographic subgroups might be driving it (Perkins et al. 2003, Erazo et al. 2021). In turn, at the community level, interspecific differences in competence and interactions drive species-level heterogeneities in infection risk, resulting in certain species accounting for a larger proportion of infections due to a combination of higher competence and higher frequency of contacts (Behdenna et al. 2019).

In addition to identifying traits or hosts that are associated with higher risk of infection, establishing key hosts in multi-host systems also entails assessing the role of intra and inter-species transmission, as multi-host pathogens might rely on transmission between individuals of different host species, between conspecifics, or both. True multi-host pathogens are characterized by high within and between species transmission, which allows them to persist independently in separate host populations (Fenton and Pedersen 2005). However, establishing inter-species transmission is complicated, and is often assumed on the basis of indirect evidence, like the correlation between the prevalence of a pathogen in a

host species with the population density of other host within a community (Haydon et al. 2002, Haydon 2008), although this approach is being increasingly challenged, as different transmission processes can result in similar patterns of infection prevalence. Low resolution characterization of pathogen prevalence (for example, using serological studies) can also hinder a clear assessment of interspecies transmission (Viana et al. 2014, Fenton et al. 2014).

Incorporating parasite genetics can help to address the gap in assessing inter-species transmission, particularly in natural settings where sick individuals are rarely observed, and contact does not necessarily lead to an infection (Archie et al. 2009). By characterizing the genetic variants of pathogens in a host community, transmission can be inferred if two individuals of the same or different species share the same genetic variant (VanderWaal et al. 2014a, b). This strategy can reveal the hosts involved in inter-specific transmission (Rudge et al. 2013), and help determine if it occurs as frequently assumed by indirect evidence (Telfer et al. 2007, Withenshaw et al. 2016). These studies have highlighted that asymmetries in inter-specific transmission may be due to encounter (limits in contact or exposure) or compatibility (limits in physiological affinity) barriers (Combes, 2001). Although this has critical implications for effective disease control, empirical studies remain rare and have focused for the most part on directly transmitted viral diseases although other pathogen taxa such as bacteria cause more zoonoses (Han et al. 2015, Becker and Albery 2020).

Part of this limitation lies in the need for amenable study systems to investigate the underlying mechanisms barriers to inter-species transmission in multihost systems from an empirical approach. In this sense, vector-borne multihost pathogens in rodents present a dual advantage; rodent populations are easy to sample and track, while vector-borne parasites allows testing for barriers to transmission and its subjacent mechanisms on two levels: the pathogen and the vector itself. Highly specialized vector-pathogen assemblages show high host specificity, but limited interspecies transmission of generalist vectors can also occur if the pathogen cannot infect the host (Medeiros et al. 2013, Esser et al. 2016). On the other hand, if encounter barriers are limiting transmission, modifying or

eliminating them could result in higher vector-host encounters, which could potentially lead to widespread pathogen prevalence across species, if the pathogen itself is not limited by compatibility barriers (Simpson et al. 2012).

To address the question of inter-species transmission and key hosts in multi-host systems, we studied Bartonella infection in fleas of sympatric rodents in grasslands of northwestern Mexico. *Bartonella spp*. are vector-borne intracellular bacteria that are especially diverse in rodents (Gutiérrez et al. 2015). Fleas (order Siphonaptera) are considered the chief vector of this bacteria in rodents (Edvinsson et al. 2021), which establishes chronic infections and reaches high prevalence in sympatric rodents (Birtles et al. 2001). Although pathological manifestations of Bartonella infection are not apparent in rodents, some rodent-associated Bartonella species are zoonotic, causing mild to severe disease in humans (Krügel et al. 2022). Previous studies of Bartonella dynamics in rodents have found that variables that affect flea sharing through modifying contact between other hosts and their fleas, either by facilitating flea exchange or altering host abundance, are likely key to Bartonella dynamics (Telfer et al. 2007).

Empiric studies designed to modify vector sharing are rare (Pedersen and Fenton 2015). Indeed, while studies have highlighted the possibility of limited inter-species transmission in multi-host systems like Bartonella, thus countering the conventional assumption of widespread inter-species transmission in sympatric hosts (Withenshaw et al. 2016), it is unclear whether transmission limitations are due to parasite compatibility barriers, or vector encounter barriers, because of the use of study systems where sympatric species do not overlap in activity patterns or microhabitat usage. Here, we use the presence of fossorial keystone species (*Dipodomys spectabilis* and *Cynomys ludovicianus*) in our study sites as a natural experiment to overcome the limitations of previous empirical studies in assessing barriers to interspecies transmission in Bartonella in rodents. In particular, the black-tailed prairie dog, *C. ludovicianus* (herafter, BTPD) has been previously established as a keystone host to fleas in our study site by facilitating flea sharing among sympatric hosts within their burrows, effectively reducing encounter barriers (Pontifes et al. unpublished).

Through genetic characterization of Bartonella infections in sympatric rodents and their fleas, we aim to assess the structure of inter-species transmission and contribute to a better understanding of whether transmission in multi-host systems may be limited by encounter or compatibility barriers, mediated by the vector, the pathogen or both. Furthermore, we couple the characterization of Bartonella-flea-rodent assemblages with the identification of risk factors linked to Bartonella infection both in rodents, in order to identify potential key hosts associated with the persistence of Bartonella. Our study highlights how identifying limits to inter-species transmission can provide clearer targets for infectious disease management in multi-host systems.

Methods

Rodent and flea sampling

We live-trapped rodents in the Janos Biosphere Reserve (JBR) in northwestern Mexico during two sampling sessions in 2012 and 2013, corresponding to autumn and spring seasons (October and May, respectively) at two sites, Rancho Ojitos (RO) or Monte Verde (MV). At each of the two sites, we set ten 7x7 trapping grids with 10 m spacing between traps (H.B Sherman 8x8x23 cm Tallahassee FL), and baited traps with a mixture of oats and vanilla extract. Traps were set in the late afternoon and checked the following morning during three consecutive nights (Rubio et al. 2015).

At first capture, each rodent was ear-tagged and measured to obtain morphometric data (body size, weight, sex and reproductive state), and then placed in an anesthetizing chamber containing a cotton ball doused with isoflurane. As soon as motor activity ceased, the lid and cotton ball were removed from the chamber. Anaesthetized rodents were held stretched and combed thoroughly within the chamber for 2 minutes with a standardized number of passes. This method has reported a high sampling efficiency to collect ectoparasites (Eads et al. 2013). Ectoparasites obtained from each rodent were collected by hand from the container and stored in microtubes containing 70% ethanol and kept at -80ºC until identification, which we conducted using a dissecting scope (SZx12 Olympus, Melville, NY) and taxonomic keys (Hubbard, 1974; Acosta and Morrone, 2003). We recorded ectoparasite abundance as the numbers collected from each individual.

Procedures for trapping and handling were approved by the animal care committee of the National Autonomous University of Mexico (UNAM) and by the Secretariat of Environment and Natural Resources (license number FAUT-0250), and followed the standards set by the American Society of Mammalogists (Sikes and Gannon 2011).

PCR and sequence analyses

Bartonella variants in rodents and fleas were characterized as part of ongoing research in the area, and methods are detailed in the corresponding papers (Rubio et al. 2014, Fernández-González et al. 2016). Briefly, DNA was extracted from Bartonella blood cultures from rodents, identified as Bartonella based on colony morphology. Fleas were tested for Bartonella by PCR conducted on a triturated sample after verifying for amplifiable DNA. In both cases, PCR to identify variants used the citrate synthase (*glt*A) gene, which is considered to have greater power than other loci to differentiate between variants (Roux and Raoult 1995, Scola et al. 2003). Amplicons of the correct size were purified, sequenced and compared to known Bartonella species sequences on GenBank, using the species cut-off proposed by La Scola et al. 2003. Flea amplicons corresponded to those of Bartonella-positive fleas collected from rodent hosts where Bartonella was sequenced. At least one flea from each of these hosts was sequenced.

Network and statistical analysis

Flea-host specificity

To investigate inter-species transmission across all the components of our system, we first assessed whether opportunities for Bartonella transmission could be limited by flea-host preference by assessing the host specificity of fleas. For each of the study sites, we constructed flea-host bipartite networks and estimated the indices of specialization proposed by Blüthgen et al. (2008) denominated d' and H_2', which respectively measure structural specificity of the interactions at the species level (within-network variation in specialization), and the structural specificity of the entire network. Values for both indices range from 0 for the most generalist species, to 1 for the most specialist one, and correct for rare species bias (which would otherwise be classified as more specific simply because of their rarity). To evaluate the significance of our results, we compared estimated values

against the results obtained from 1000 random networks constructed under a null model following Vázquez et al. (2007) (further methods detailed in Supplementary Material).

Bartonella in rodents

After establishing flea specificity, we assessed between-species transmission of Bartonella in rodents by constructing individual-based host-parasite bipartite networks, depicting the association between Bartonella variants (top) and rodent species (bottom) for each of our sampling sites. For each network, we assessed modularity and identified modules composed of rodents that carried the same Bartonella variants, using the function 'computeModules' implemented in the package bipartite (v.2.16) (Dormann and Strauss 2014, Beckett 2016), and estimated the modularity (Q) of each network. Because modularity values are potentially influenced by network size, we corrected the value of Q by null model expectation, using null model replicates (n = 1000). We then conducted logistic multiple regression on distance matrices (MRM) following Lima et al. 2012 and Pilosof et al. 2015 to assess whether module affiliation could be predicted by traits associated with infection risk (sex and weight), or seasonal variation. Because closely related species are more similar in characteristics that determine compatibility between hosts and parasites, we also tested the role of phylogenetic distance on module affiliation (details of tree construction in Supplementary material). Note that while all of the Bartonella-rodent associations are depicted for visualization purposes, module affiliation was conducted excluding species where n<5. Due to issues with inferences of MRM results based on information criteria methods, (Franckowiak et al. 2017), we used coefficient values and statistical significance to interpret our results. Further details on this analysis are provided in the Supplementary material.

The role of flea sharing in Bartonella transmission

While assessing flea specificity indicates whether inter-species transmission could be limited by strong host preferences, it does not assess the possibility of fleas limiting inter-species transmission due to lack of encounters. Thus, we further assessed whether opportunities for Bartonella transmission were limited by encounter filters between fleas, following a two-step approach: firstly, we evaluated the overlay between the unipartite

host network based on flea sharing, and the unipartite host network based on Bartonella sharing by rodents, under the premise that, if flea sharing is crucial for transmission, the occurrence of links in the flea-sharing network should correlate with links in the Bartonella transmission network. This approach is based on the rationale developed by VanderWaal et al. (2014) where individuals with high similarity in their parasite communities or the parasite variants they share can be thought of as forming part of the same transmission chain; in the specific case of our study system, if two individuals share the same genetic variant of Bartonella, we infer that transmission has occurred through exposure to infected fleas. Thus, in the absence of compatibility barriers at the flea-rodent level (i.e., vector to host transmission) the Bartonella-rodent network should match the flea-host network. To assess this overlay, we used the multiple regression quadratic assignment procedure (MR-QAP) (Krackhardt 1988, Dekker et al. 2007). This method overcomes the limits of independence assumptions required for most statistical analysis that dyadic data used in networks do not comply with, by producing correlation coefficient estimates and p-values through matrix permutations. We used the 'netlogit' function implemented in the sna package (v2.6) and 1000 permutations to investigate the effect of flea assemblage similarity between hosts in the flea sharing network on the log-odds of a link occurring in the Bartonella-host network. Secondly, we identified instances of fleas carrying Bartonella variants not detected in their hosts, to assess the possibility that fleas can become infected with variants that cannot be transmitted to their hosts, suggesting the presence of host-Bartonella barriers. Methods for constructing unipartite parasite-sharing networks are provided in the Supplementary material.

Bartonella-flea-rodent assemblages and predictors of Bartonella infection in rodents

To facilitate the identification of potential key hosts involved in the transmission of specific variants, we characterized host-flea assemblages based on the similarity of their shared Bartonella variants, following the methods detailed in the Supplementary material for the construction of unipartite parasite sharing networks. Finally, we assessed whether specific host traits in host species associated with specific variants were linked to a higher risk of infection, representing potential key hosts to Bartonella transmission. While previous work in the study area has established that specific host and flea species are associated with

higher Bartonella prevalence (Rubio et al. 2014; Fernández-González et al. 2015), we explore whether specific host traits are linked to higher infection risk, as has been shown in other studies (Telfer et al. 2007). Generalized mixed models with binomial error and Bartonella infection as a response variable were implemented with the package glmmTMB (Brooks et al. 2017). Details on the explanatory variables, data exclusion criteria for statistical analysis and tests conducted to assess model compliance with assumptions are included in the Supplementary material.

Results

We collected a total of 393 rodents (shrubland = 186 BTPD = 207), representing 11 rodent species from three families (Cricetidae, Heteromyidae and Sciuridae). Of these individuals, 54.7% had fleas (n= 215), and we collected a total of 975 fleas from them (RO = 524, MV = 451). Flea richness was represented by 10 different species from three different families (Ceratophyllidae, Ctnenophtalmidae and Pulicidae). Summary information for rodents and fleas collected can be found in Tables S1 and S2.

Flea-host specificity

The overall specificity of flea assemblages, as quantified by the H_2 index, was high in both sites (BTPD =0.85, shrubland = 0.74). At the species level (d_i'), it ranged from 0.33 to 0.97 in BTPD and from 0.11 to 0.98 in shrubland habitat, with the majority of values showing a low to medium specificity in shrubland, and a medium to high specificity in BTPD sites (Fig. 1b). Higher d_i values corresponded to fleas with known marked host preferences, like *Orchopeas leucopus* and *Orchopeas sexdentatus*, whereas known generalist fleas had lower d_i values (*Echidnophaga gallinacea*), while others showed intermediate values and are associated with family level preferences (*Meringis altipecten*) (Table S3). Flea species present in both sites were consistent in their specificity values, i.e., no flea with low d_i values in MV had values at the other end of the range in RO and viceversa (Table S3).

The specificity profiles of flea assemblages in each rodent species shows that most rodents harbor fleas with a range of specificity as measured by d_i' (i.e., few rodent species host solely fleas with very high or very low values), although the relative composition varies by

species and site, with some rodent species hosting flea assemblages with predominantly specialized or generalist fleas (Fig. 1a, c).

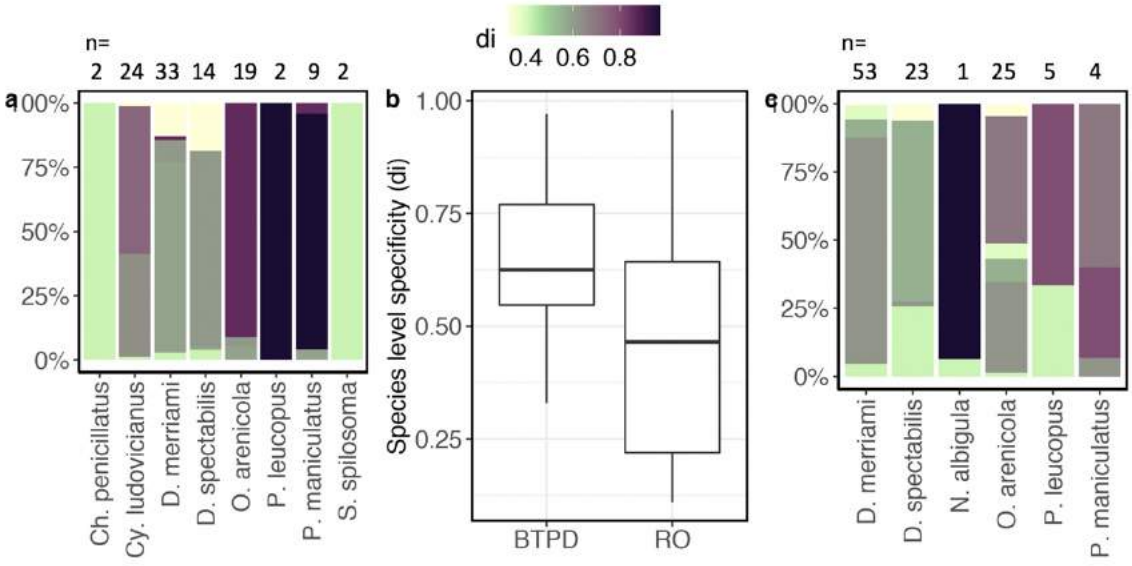

Figure 1. Specialization profile of flea assemblages of rodent species in a)MV and c)RO sites as measured by d_i'. Panel b) summarizes values of d_i' at each of the sites. Most host species harbor fleas with a range of structural specificity, indicating that they harbor both fleas that parasitize them more than they do other hosts (fleas with strong host preference), and fleas that parasitize a variety of host species (generalist fleas). The number above each bar plot indicates the total of individuals from each species that harbored fleas.

Bartonella in rodents

We obtained Bartonella cultures from blood samples of 205 individuals (52.1%) and sequenced a total of 84 randomly selected isolates from 80 individuals (41 from grassland and 39 from shrubland). Classification of the identified variables into phylogroups of these samples is reported in Rubio et al. 2014, but here we use variant shared within the same sampling site as the basis for Bartonella sharing analysis in rodents and fleas. In terms of general patterns of Bartonella infection, overall prevalence in rodents in MV was 48.8% (101/207) and 55.9% (104/186) in RO. Two of the variants detected (variants 2 and 3) are associated with zoonotic disease. Variants are numbered sequentially, and their accession number detailed in Table S4.

A total of 14 genetic Bartonella variants were identified in rodents, of which 7 were present in both sampling sites. No single Bartonella variant was shared among all host species collected. Rather, variants were highly specific, or were shared between individuals

belonging to a few different species, constrained by the species present at the sampling site; for instance, variant 3 was only found in *Peromyscus maniculatus* in RO, but in MV it was also present in *Peromyscus leucopus*, which wasn't present in RO. Coinfections were rare, with only 4 individuals of either *Onychomys arenicola* or *Dipodomys spectabilis* hosting more than one variant (Fig. S2). Two variants were detected in 3 or more rodent species (variant 14 and variant 10). Variants shared among species spanned up to two different rodent families (present in both Cricetid and Heteromyid rodents, and Heteromyid and Sciurids).

Rodent-Bartonella bipartite networks at each site were significantly modular (Table 1 $p < 0.05$), with little difference between their modularity values, although the MV network had a higher number of modules than the network in RO.

Table 1. Modularity of rodent-Bartonella networks in study sites. Note that modularity was calculated considering only species where n>5.

Site	# of individuals	Modularity (Q)	# of modules
Monte Verde	34	0.77* (0.046)	8
Rancho Ojitos	34	0.79* (0.022)	6

After determining the modular structure of the rodent-Bartonella network, we assessed the effect of individual-level traits, seasonal variation and phylogenetic distance on module composition (affiliation of individuals to specific modules). Phylogenetic distance was a significant predictor of module affiliation in both sites, with more closely related individuals occurring more likely in the same module (Fig. 2). Body mass and sampling season were also significant, but only in MV.

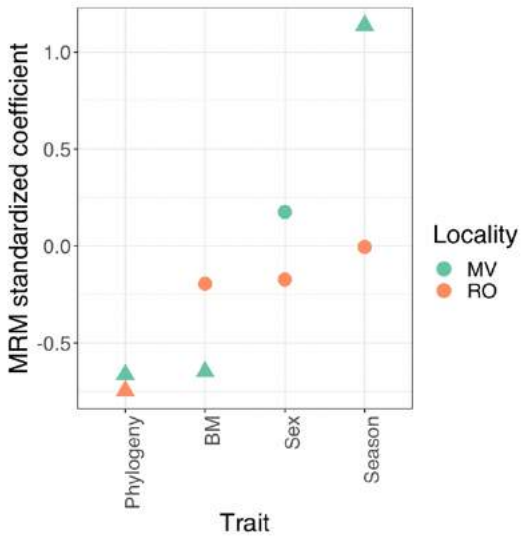

Figure 2. Traits used to assess module affiliation of rodents infected with Bartonella variants. The plot shows the z-score-standardized coefficients of an MRM analysis. Phylogeny refers to the taxonomic distance between two individuals. BM – body mass; Season – is a factor depicting the sampling season. Statistical significance is indicated by the shape of the point; triangles indicate the variable was significant ($p < 0.05$).

Role of flea sharing in Bartonella transmission between rodents

Bartonella positive fleas represented 40% of all collected fleas, but prevalence varied between species (Table S2). Within the sequenced subset, we detected seven instances of fleas which were negative to Bartonella but were collected in Bartonella-positive host, and one instance of a Bartonella-positive host with no fleas.

We tested the extent to which flea sharing predicted the presence of a transmission link (i.e., sharing a Bartonella variant). Individuals connected by flea sharing were more likely to be part of the same Bartonella transmission chain; in MV, being part of the same transmission chain was 8 times more likely in the presence of flea sharing, whereas in RO it was twice as likely (Table 2). However, while the likelihood was higher, the correlation of link correspondence between networks was moderate, indicating a partial overlay. This can be visually assessed in Fig. 3.

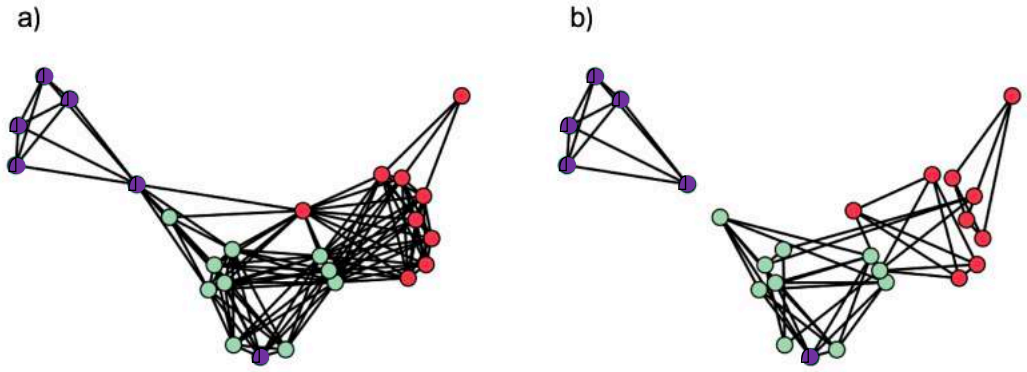

Figure 3. Comparison of the flea sharing network (a) and the Bartonella transmission network (b) for a subset of nodes in MV. A link in either of the networks represents that individuals share at least one flea, although the similarity of their assemblages may differ, but this is not shown here for clarity. For additional visualization purposes, only links between *D. spectabilis* (red), *O. arenicola* (purple) and *P. maniculatus* (mint green) are shown.

We found 2 instances of fleas carrying variants not found in the host species from which they were collected; variant 4 was detected in a *Pleochaetis exilis* flea parasitizing a *P. maniculatus* individual, while variant 11 was identified in a *M. altipecten* flea collected in an *O. arenicola* (otherwise these variants were found associated with *Dipodomys sp.*). An additional instance of variants in atypical host species was variant 2, found in an individual of *D. merriami*. However, no fleas were collected from this individual.

Table 2. Correlation between ties in flea-sharing and Bartonella transmission network, and effects of flea sharing on the probability (log-odds) that two individuals are linked in the transmission network. Regression coefficients were estimated using MR-QAP, whereas the correlation coefficient was obtained with a QAP correlation.

Site	Corr	p_{corr}	$Coef_{log\text{-}odds}$	P_{coef}
MV	0.44	<0.001	2.11	0.005
RO	0.25	<0.001	0.80	<0.001

Bartonella-flea-rodent assemblages

We identified distinct host-flea species combinations carrying specific variants (Fig. S3); for example, variant 5 was predominantly identified in association with *P. exilis* fleas, with occasional occurrences in other 2 flea species (*M. arachis* and *M. parkeri*) but was only

found in two host species *O. arenicola* and *P. maniculatus*, although *M. arachis* and *M. parkeri* can parasitize other rodents of the Dipodomys genus. Other variant combinations were more specialized; variant 3 was only detected in *O. leucopus* fleas which in turn were only found in rodents of the Peromyscus genre. Variants carried by the same flea species belonged to one of more phylogroups (Table S4), with the majority of hosted variants placed within the same phylogroup.

Bartonella infection in rodents

An assessment of risk factors for Bartonella presence in rodents (considering all host species) identified weight as a significant predictor of infection status, although the size effect was small (Table S5). Because we had previously detected that specific Bartonella variants were shared among specific host species, we assessed variables that could influence risk of infection separately for each Bartonella variant and their associated species (although this was only conducted for variant 14 due to small sample sizes of other variants). Controlling for significant variation in infection due to potential species-level effects by including it as a random factor, the best model according to AICc included an interaction between sex and eigenvalue centrality and site, with male individuals with higher centrality being less likely to be infected with this variant (Table S6) regardless of the species. However, an LRT indicated the interaction to be marginally significant (LRT: $X^2 = 2.983$, df = 1, p = 0.064). Rodents were less likely to be infected with this variant in RO (-1.81[-3.42, -0.19] log units).

Discussion

Determining the occurrence of inter-species transmission of multihost parasites is crucial to understand its role in parasite persistence and identify key hosts (Webster et al. 2017). While earlier studies assumed that between-species transmission was commonplace based on indirect or correlational evidence (Haydon et al. 2002), more recent frameworks have highlighted the complexity of the host-parasite interactions underlying patterns of prevalence (Viana et al. 2014, Fenton et al. 2014). Here, using Bartonella in sympatric rodents as a study system, we found empirical evidence for delimited Bartonella-rodent-vector assemblages, and restricted inter-species transmission potentially due to

incompatibility barriers, particularly at the flea-rodent level. However, we also found that these limits were variant-specific, and in some instances did not correspond to phylogenetic relatedness, thus highlighting the potential of some variants to be involved with several flea and rodent species.

Flea-host specificity

Flea assemblages of host species showed range in structural specificity, and although no host species had solely specialist species (except for *P. leucopus*), there were marked differences in whether the assemblage was dominated by fleas with strong or weak host preferences (Fig. 1). Although generalist fleas represented a small fraction of total fleas collected (Table S2), most flea species identified in the study sites are known to parasitize rodents of the same family (e.g., the Thrassis genus), but we also detected them on rodent species different from their typical host range (e.g., *Meringis spp*. are considered fleas of the Heteromyid family, but we found them on cricetids and sciurids, see Fig.S3). Fleas are not considered to be highly host specific, with some parasitizing multiple mammalian orders (Krasnov et al. 2008). Additionally, burrow settings are reported to increase the frequency of non-specific hosts encounters. Thus, strong host preferences are unlikely to be a generalized barrier to inter-species transmission of Bartonella in our study system.

Bartonella in rodents

Microparasites, a term which refers to bacteria and virus, show a high level of genetic variation (Archie et al. 2009, Vander Wal et al. 2014). Although classification of variants is commonly performed by clustering analysis of the genetic divergence between variants, recent advances in parasite genetics have evidenced that genetically distinct variants may represent distinct epidemiological and ecological units. Variant-host specificity in Bartonella has been reported in experimental infection studies (Chan and Kosoy 2010), and in field settings with wild rodents, where hosts were associated with a distinguishable assemblage of variants despite flea sharing (Withenshaw et al. 2016). In line with these results, we found significant modularity in Bartonella-rodent associations (Table 1) and a significant effect of phylogenetic distance on module composition (Fig. 2), indicating that closely related hosts shared the same variants. Studies on the molecular basis of host

specificity in Bartonella indicate that this is mediated by host-specific adhesion mechanisms of bacterial factors that intervene in erythrocyte colonization (Vayssier-Taussat et al. 2010). This may explain why phylogenetic relatedness was predictive of module affiliation, as closely related species are more similar in characteristics that determine host-parasite compatibility (Poulin et al. 2011, Shaw et al. 2020). However, relatedness was not the only predictor of module affiliation; rodents occurring in the same season were also more likely to share the same variant (i.e., occur in the same module), a result that also aligns with previous studies that highlight a seasonality of Bartonella infection (Telfer et al. 2007), although in our study system it is unclear whether different variants have distinct seasonality or same-season encounters were more likely to result in variant sharing.

While phylogenetic signal indicates a restriction to potential inter-species Bartonella-host associations, we detected instances of variants shared both between closely related hosts and more distantly related, spanning up to two different rodent families (Heteromyidae and Cricetidae, and Cricetidae and Sciuridae). While this result contrasts with previous studies that found little support for inter-species transmission of Bartonella variants between sympatric rodents even in the presence of overall generalist flea assemblages (Withenshaw et al. 2016), rodent species in those studies present differences in microhabitat use. This contrasts with our study system, where the presence of burrowing species, especially the keystone species BTPD modifies encounter and activity patterns of hosts, effectively breaking down potential encounter barriers, as fleas rarely jump host, but exchanges can occur in close contact under long periods within the burrow microhabitat as fleas climb up and down from their hosts after feeding. Indeed, acquisition of nonspecific Bartonella variants from more distantly related host species has also been reported in other species in grassland ecosystems of North America which typically harbor a variety of semi-fossorial and fossorial species (Bai et al. 2007).

This result highlights the importance of considering the community context of host-parasite interactions in multihost systems, as the same parasites may be present across a wide geographic rang but host interactions may change depending on community composition.

Role of flea sharing in Bartonella transmission between rodents

We found evidence of flea sharing (inferred from the similarity of flea assemblages between individuals) and evidence for a role of flea sharing on Bartonella transmission, as individuals with shared fleas were more likely to form part of the same Bartonella transmission chain (Table 2). However, the correlation between networks was partial. A closer look at the overlay between networks shows that some links found on the flea sharing network were not found in the Bartonella transmission network (Fig. 3), indicating that some individuals that share flea species do not share Bartonella variants. Thus, Bartonella transmission of different variants would seem restricted to some host-flea combinations. This is further supported by differences in the prevalence of Bartonella in flea species (Table S2), as generalist fleas which are shared among several species, have a considerably lower prevalence than fleas with more restricted preferences. This is potentially indicative of compatibility barriers at the flea-rodent level. Networks had a higher correspondence in MV than in RO (Table 2), and although further analysis would be required to determine a potential mechanism, it's interesting to note that BTPD was present at MV sampling plots.

Bartonella-flea-rodent assemblages and infection risk

Our results provide evidence that host specificity, both at the rodent and the vector level, is dependent on the variant. Our approach also allowed us to identify the specific combinations of hosts and vectors involved with distinct variants (Fig. S3). Interestingly, two variants have been previously associated with zoonotic disease (see Rubio et al. 2014): *Bartonella vinsonii sub. arupensis* (variant 3) and *Bartonella washoensis (*variant 9). These were found in association with well-defined host combinations; variant 3 was found in *P. maniculatus* and *O. leucopus*, while variant 9 was found in BTPD and *P. simulans*. Both flea vectors are highly associated with these specific hosts. These results highlight the importance of a variant-based approach to characterize host-parasite associations and assess the occurrence of inter-species transmission; the specificity of these zoonotic variants would indicate when a targeted management scenario would be more appropriate (Rigaud et al. 2010).

Interestingly, we also found evidence for several variants being able to parasitize different flea species (Fig. S3). It's interesting to note that these fleas are not considered broad generalists (a result that matches our findings using the structural specificity index d_i') but rather family level specialists with high abundance within our study system. While instances of fleas having variants never found in the host species in which they were collected were rare, our results show that a single flea species can be infected with several variants, some of them not phylogenetically close (Table S2). This highlights the role of flea sharing between sympatric rodents in inter-species transmission; indeed, other studies have highlighted that fleas may enhance Bartonella diversity by promoting lateral gene transfer between variants (Buffet et al. 2013, Québatte and Dehio 2019). Thus, high flea sharing could increase the opportunities for host shift despite host specificity of variants.

An assessment of predictors of overall infection status showed a significant and negative association with weight, but the effect was very small (Table S5). Studies with Bartonella variants in the UK have found that specific variants differ in their seasonal dynamics and risk predictors (Telfer et al. 2007; Withenshaw et al. 2016). Thus, an approach that considers overall prevalence might be masking specific traits and variables associated with infection with different variables. Although we were only able to assess variables associated with a higher probability of infection in one variant (Table S6), we found a potential effect of host traits across species that shared said variant, and a site-dependent effect of probability, potentially related to the presence of BTPD at the site with higher infection probability, although further assessment is needed. Indeed, validation of our results would require further sampling, as our current results are based on a small sample size, which reflects on the marginal significance of the variables identified. Indeed, given these results, a longitudinal study would be of interest to look at the transmission dynamics of distinct variants, and determine the role of specific demographic subgroups. As our current results stand, we identified some traits potentially related with the infection of a specific variant, with an interesting effect across the three species in which infection in which this variant was identified (*D. merriami*, *D. spectabilis* and *O. arenicola*). These

results can inform future study design, but more work is needed to identify key hosts involved in the persistence of specific variants.

Our approach to analyzing Bartonella-host-flea associations at a variant level provided us with several insights of interest on the nature of the occurrence of inter-species transmission, and the role of compatibility limitations in determining the assemblages of host and vectors involved. However, several aspects of our study design could be enhanced; namely, characterizing the population genetic structure of the different flea species involved to properly quantify the frequency of individual flea transfer between hosts, as our current inference of flea transfer based on assemblage similarity may be overestimating this.

In conclusion, our results show that transmission of a multihost parasite between sympatric rodents is structured, with specific host-flea combinations associated with distinct variants, and an effect of phylogenetic similarity restricting host-Bartonella associations. However, in contrast with results of other studies conducted in study systems where fleas are generalists but there is little overlap in host encounters due to absence of shared microhabitats, we found evidence for interspecies transmission involving family-level flea specialists (rather than generalists), and an important role of flea sharing in Bartonella transmission. Taken together, these results highlight the relevance of fine-scale characterization of host-parasite associations within their community context in multihost systems to assess the occurrence of inter-species transmission.

Acknowledgements

This work was supported by a PhD scholarship from the CONACyT (Consejo Nacional de Ciencia y Tecnología) to PAP. The authors also acknowledge the National Autonomous University of Mexico (UNAM) for the support provided through a grant of the PAPIIT Program, project IN225219.

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Discusión General

La ecología de comunidades provee un marco teórico para estudiar los factores que gobiernan la estructura y dinámica de las interacciones parásito-hospedero, el cual es especialmente relevante en el contexto de patógenos multihospedero ya que su persistencia depende tanto de la transmisión intra como interespecie, y por tanto de la composición de la comunidad. La transmisión de estos parásitos entre distintas especies de hospedero es central en estos sistemas. Sin embargo, la escasa evidencia empírica sobre este tema indica un escenario más complejo, donde la persistencia de estos parásitos no depende necesariamente de la transmisión generalizada o frecuente entre especies. Estos trabajos han enfatizado la complejidad de estos sistemas, así como la necesidad de integrar distintas herramientas que permitan caracterizar detalladamente las interacciones de los parásitos multihospedero.

El presente trabajo representa una contribución precisamente desde un enfoque empírico para evaluar el papel de la composición de la comunidad y la transmisión inter-especie en la dinámica de parásitos multihospedero, usando a *Bartonella spp* en roedores silvestres del noroeste de México como sistema de estudio. De manera general, los hallazgos de este trabajo destacan la importancia de integrar información de alta resolución para caracterizar la transmisión inter-especie, así como para determinar las variables asociadas a un mayor riesgo de infección y distinguir los hospederos e interacciones clave para la persistencia de parásitos multihospedero dentro de la comunidad en que circulan. Desde el punto de vista aplicado, estos resultados son relevantes para el diseño de estrategias de control focalizadas, ya que muestran que no todos los componentes en un sistema multihospedero están involucrados en la transmisión de variantes de relevancia para la salud humana o animal. Esto contrasta con lo supuesto por modelos teóricos que asumen una transmisión inter-especie generalizada en estos sistemas, basándose en evidencia indirecta y a menudo de baja resolución, como la correlación entre abundancia y seroprevalencia.

Resumen de hallazgos

Dado que *Bartonella spp* en roedores se transmite primordialmente por la picadura de pulgas infectadas, y que la carga de vectores macroparásitos (también denominados ectoparásitos) es proporcional a la probabilidad de transmisión de microparásitos, en el primer capítulo se

identificaron las variables asociadas a diferencias en la carga parasitaria de este vector en roedores, considerando la influencia de la escala espacial en la importancia relativa de características del hospedero y del ambiente a nivel local y regional. A pesar de que estas variables han sido ampliamente estudiadas en diversos sistemas de ectoparásitos y hospederos (Linardi y Krasnov, 2013; Cassin Sackett, 2018), el enfoque en la escala espacial permitió identificar que la variación interanual en condiciones ambientales fue la única variable con un efecto consistente en la carga parasitaria en distintas especies, tanto a nivel local como regional. Asimismo, los hallazgos destacaron la posibilidad de que la variación interanual asociada a eventos de gran escala (por ejemplo, una sequía) modifique el efecto de características de hospederos o la variación estacional en la carga parasitaria. Esto representa una posible explicación a resultados de otros estudios donde los efectos de distintas variables asociadas al hospedero o al ambiente (por ejemplo, sexo, peso o estado reproductivo) no son consistentes incluso entre poblaciones de una misma especie, dificultando la identificación de patrones universales en variables asociadas a diferencias en carga parasitaria (Kiffner et al. 2013; Sweeny et al. 2020), resaltando la importancia de integrar la variación espacial de las características del hospedero y del ambiente que influyen en la carga parasitaria, por ejemplo, mediante la inclusión de réplicas espaciales en el diseño de muestreo.

En el segundo capítulo, la comparación de la estructura de ensambles de pulgas en sitios con y sin perrito de las praderas de cola negra (*Cynomys ludovicianus*) permitió evaluar el efecto de la presencia de especies clave en las asociaciones entre estos ectoparásitos, así como su influencia en la estructura de sus interacciones con los roedores que parasitan. Los hallazgos de este capítulo resaltan la importancia de estudiar las interacciones en los sistemas multihospedero en el contexto de la composición de la comunidad, al enfatizar que distintas especies de hospederos ocupan roles distintos al compartir ectoparásitos; por ejemplo, se detectó que el ratón chapulinero, *Onychomys arenicola*, actúa como puente para la transferencia de pulgas entre especies. Por otra parte, estos resultados también contribuyen al estudio del efecto de las especies clave en comunidades parasitarias: a pesar de que la influencia de especies clave se ha investigado en múltiples comunidades de vertebrados, plantas e insectos, su efecto en los parásitos continúa siendo un área relativamente

inexplorada. Esto es una posible consecuencia de que por mucho tiempo los parásitos se consideraron componentes marginales de las comunidades biológicas (Poulin, 2021). Los hallazgos de este capítulo enfatizan también que ciertas diferencias en la composición de la comunidad de hospederos se traducen en cambios en la estructura de comunidades de macroparásitos, lo cual tiene implicaciones significativas para la dinámica de enfermedades emergentes transmitidas por vectores. Así, estudios como el presente que exploran las variables que gobiernan la estructura y dinámica de interacciones entre macroparásitos vectores son de alto interés para el desarrollo de estrategias de prevención.

Finalmente, los resultados del tercer capítulo permitieron identificar que la transmisión de *Bartonella spp*. está acotada primordialmente por barreras de compatibilidad que operan tanto a nivel de transmisión roedor-pulga como pulga-roedor. Esto implica que, incluso con la presencia de especies que modifican la estructura de contactos entre pulgas (como los perritos de la pradera de cola negra y las ratas canguro), y facilitan el intercambio de ectoparásitos entre especies (en otras palabras, que reducen la barrera de encuentro entre vectores), para la mayoría de las variantes detectadas se mantiene una estructura donde las variantes de Bartonella están acotadas a un grupo definido de especies. El resultado fue similar en pulgas: en los casos donde no se encontraban en hospederos no-específicos, su infección estaba dada primordialmente por Bartonellas asociadas a sus hospederos específicos. Sin embargo, a diferencia de otros estudios donde detectaron una separación completa entre variantes de Bartonella y roedores simpátricos (Withenshaw et al. 2016), en este trabajo se encontraron casos de variantes compartidas entre especies filogenéticamente cercanas, e incluso se detectaron variantes compartidas entre especies de distintas familias (Heteromyidae y Cricetidae, y Cricetidae y Sciuridae), aunque su baja representatividad en las muestras obtenidas no permitió incluir estos casos en los análisis. Sin embargo, estos resultados indican que algunas variantes sí pueden infectar múltiples especies de hospedero de distintas familias.

Resolución en estudios de parásitos multihospedero: la importancia de la variación genética

El estudio de los sistemas multihospederos ha requerido el desarrollo de modelos teóricos que permiten evaluar la contribución de distintos componentes de la comunidad a la

persistencia de estos parásitos. Sin embargo, la complejidad de estos sistemas vuelve esencial integrar los desarrollos teóricos con estudios empíricos, a fin de refinar los supuestos sobre los que basan sus inferencias.

La importancia de esta integración destaca con respecto a los supuestos sobre el papel de la transmisión inter-especie. El uso de evidencia indirecta, como la correlación de la prevalencia de parásitos multihospedero en las distintas especies que infectan, lleva a suponer que la transmisión entre especies en estos sistemas ocurre de manera generalizada entre hospederos que comparten la misma especie de parásito (Fenton et al. 2014). Sin embargo, la incorporación de métodos moleculares, que permiten estudiar eventos de transmisión entre individuos a partir de la similitud genética de las variantes de parásitos que comparten, ha esbozado una realidad más compleja. Esto es especialmente importante a la luz de hallazgos en la genética de virus y bacterias, que indican que poblaciones de estos microparásitos están compuestas por múltiples variantes, de las cuales no todas son de relevancia desde el punto de vista de la salud animal o humanan y están asociadas de manera específica a distintas especies de hospedero, por lo cual son tanto ecológica como epidemiológicamente distintas entre sí (Kosoy et al. 2012; Buffet et al. 2013).

En este sentido, la aproximación de este trabajo de caracterizar las interacciones parásito-hospedero a nivel variante y no a nivel de especie o filogrupo, permitió identificar a los hospederos y vectores involucrados en la transmisión de variantes específicas de Bartonella, incluyendo aquellas asociadas a zoonosis. Asimismo, permitió determinar que, en el sistema de estudio empleado, la transmisión inter-especie ocurre primordialmente entre especies filogenéticamente cercanas (aunque se detectaron algunos casos de variantes infectando individuos de distintas familias). Esto contrasta con trabajos realizados anteriormente, que basándose en la identificación a nivel de especie de Bartonella, concluyeron que estas podían infectar a múltiples especies de hospederos simpátricos en sitios de estudio en Reino Unido (Birtles et al. 2001; Telfer et al. 2007), aunque un trabajo posterior en la misma región concluyó que a nivel de variantes no había evidencia de transmisión inter-especie (Withenshaw et al. 2016). En conjunto, estos resultados aportan evidencia para sostener que

caracterizar microparásitos multihospedero a nivel de especie podría no ser suficiente para identificar su dinámica de transmisión o los hospederos relevantes para su persistencia.

Los estudios empíricos de alta resolución de parásitos multihospedero son escasos, y enfocados a unos cuantos grupos (Becker y Albery, 2020). Su aporte en términos de contribuir a desentramar los mecanismos que subyacen la transmisión en sistemas multihospedero es considerable y esencial para el desarrollo de estrategias de control efectivas, particularmente en sitios de alta biodiversidad donde el número de posibles interacciones es mayor y la caracterización a nivel variante es crucial para inferencias correctas sobre los hospederos relevantes para la transmisión, más aún porque muchos de estos sitios están asociados a cambios rápidos en la composición de sus comunidades y un riesgo alto de zoonosis (Allen et al. 2017).

Contexto ecológico y evolutivo de las barreras de compatibilidad

Identificar si la transmisión de parásitos multihospedero está acotada por limitantes al encuentro entre hospederos (o entre vectores y hospederos) o por barreras de compatibilidad entre el parásito y su hospedero (o con su vector) es crucial para evaluar la contribución de distintos hospederos a la persistencia de estos parásitos. En el caso del presente estudio, los datos indican límites en la compatibilidad tanto a nivel variante-hospedero como variante-pulga. Esto es consistente con el mecanismo de infección a nivel celular de Bartonella, que depende de un proceso de adhesión a las células que infecta (eritrocitos) mediado por un complejo de receptores que esencialmente determina el rango de hospederos que puede infectar una variante específica de Bartonella (Vayssier-Taussat et al. 2010). De esta manera, la especificidad de la asociación entre Bartonella y sus hospederos depende de la variante en cuestión; por ejemplo, *Bartonella washoensis* ha coespeciado con sciuridos del hemisferio norte y sus pulgas específicas. De hecho, esta variante fue detectada en perritos de la pradera de cola negra (*C. ludovicianus*) y sus pulgas específicas (*Oropsylla hirsuta*) en el presente trabajo, pero no en las pulgas generalistas que también están asociadas a esta especie, a pesar de que estas representaban una fracción importante del total de pulgas colectadas en esta especie. Esto explica también que se identificara la filogenia como un factor determinante para explicar la asociación entre variantes de Bartonella y hospederos, ya que una cercanía

evolutiva puede estar asociada a una mayor similitud a nivel de receptores celulares. Estudios realizados en otros sistemas multihospedero con transmisión por vectores, refuerzan que las barreras de encuentro entre vectores no limitan la transmisión, sino más bien es un efecto a nivel compatibilidad mediado por la cercanía evolutiva (Poulin et al. 2011; Medeiros et al. 2013).

Cabe recordar que los resultados del presente estudio representan una fotografía de un sistema dinámico, y por tanto es pertinente una breve discusión sobre la estabilidad de las barreras de compatibilidad y las barreras de encuentro entre parásito-hospedero y parásito vector, ya que estas son el resultado de presiones selectivas y procesos evolutivos que influyen en la facilidad con la que estas barreras pueden franquearse dado un evento de transmisión inter-especie. Por ejemplo, los parásitos de poblaciones de hospederos aisladas históricamente que entran en contacto entre ellas mediante la desaparición de las barreras físicas que limitaban su encuentro, pero cuyos parásitos no pueden infectarlas debido a la co-evolución con su hospedero, se presentarían como un caso de transmisión inter-especie limitada por barreras de compatibilidad (VanderWal et al. 2014). Sin embargo, si se hubiera estudiado este sistema previo a la desaparición de las barreras físicas, se clasificaría como un caso de barreras de encuentro. Esto indica que más que representar una dicotomía, el tipo de barreras que limitan la transmisión no son estáticas, sino que son el resultado del contexto evolutivo y los cambios en escala ecológica de la interacción parásito-hospedero.

Perspectivas

No obstante las contribuciones del presente trabajo al estudio de los parásitos multi-hospedero, cabe resaltar aspectos adicionales que sería recomendable considerar en investigaciones futuras sobre este tema. En particular, el desarrollo de estudios longitudinales que vayan más allá del monitoreo de uno o dos años, aunque logísticamente representan un reto, es deseable pues permiten distinguir fuentes de variación significativa en la dinámica de transmisión de estos parásitos (Sweeny et al. 2020). Este punto es especialmente importante considerando que la variación interanual puede enmascarar el efecto de ciertas variables en parámetros de interés como la carga parasitaria (como se detectó en este trabajo). Por otra parte, la confirmación de hospederos clave en la transmisión y

persistencia de parásitos multihospedero requiere también de un enfoque experimental, donde se manipulen parámetros como la tasa de contacto entre hospederos y vectores, con el fin de establecer la dirección de las interacciones parásito-hospedero, y determinar no sólo quiénes están involucrados en la cadena de transmisión, sino cómo inician. Esto es particularmente importante en el contexto de interacciones parásito-parásito que coinfectan al mismo hospedero, dada la ocurrencia de efectos de prioridad donde el orden de establecimiento de distintas variantes o especies de parásitos tiene consecuencias en facilitar o antagonizar el establecimiento de otras (Wilbur y Alford, 1985; Hoverman et al. 2013).

Conclusiones

Nunca como ahora, las enfermedades infecciosas causadas por parásitos multihospedero representan un reto para la salud humana y animal, acentuado en el marco de cambios globales sin precedentes en mobilidad, conectividad y uso de suelo, que modifican el riesgo de exposición y dispersión a estos patógenos (Baker et al. 2022). La complejidad de estos sistemas ha requerido un cambio de paradigma que considere las interacciones parásito-hospedero en su contexto ecológico y no de una manera aislada, dado que existe variación intra e interespecífica en la contribución de las distintas especies de hospedero a la dispersión y persistencia de estos parásitos. Los hallazgos del presente trabajo resaltan que los trabajos desde un enfoque empírico son cruciales para identificar estas diferencias en contribución, y por tanto determinar el efecto de la composición de la comunidad en la persistencia de parásitos multihospedero. Asimismo, también enfatizan que una caracterización adecuada de las interacciones parásito-hospedero en estos sistemas requiere la integración de distintas metodologías para obtener datos de alta resolución. Sin esta información, que además permite refinar los supuestos de modelos teóricos, anticipar cómo los cambios en la composición de la comunidad de hospederos se reflejarán en cambios en la dinámica de parásitos es imposible. Este desconocimiento representa un riesgo considerable para la salud humana y la de los ecosistemas, dado el contexto actual de cambios rápidos en la composición de comunidades por eventos cada vez más frecuentes, como la extinción de especies nativas y la introducción de especies invasoras, que generan cambios en las interacciones que subyacen la aparición de nuevas enfermedades, o la reaparición de antiguos enemigos. Profundizar en el estudio de parásitos multihospedero desde un enfoque integrado permitirá el desarrollo de estrategias focalizadas de prevención y control, basadas en evidencia y cimentadas en realismo ecológico.

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Apéndice

Material suplementario capítulo II
Effects of black-tailed prairie dog presence on flea community structure in rodents of the Chihuahuan desert, Mexico

Effects of black-tailed prairie dog presence on flea community structure in rodents of the Chihuahuan desert, Mexico. Supplementary material

Host community analysis

Adequacy of sampling regime by locality

We verified the representativeness of our sampling regime by conducting abundance-based rarefaction and extrapolation, based on species richness (q = 0) and diversity (q = 1) and estimated by sample coverage with the INEXT package (v.2.0.20; Hsieh et al. 2016). This analysis suggested that host species richness was well represented across localities at both of the habitat types sampled (grasslands with or without BTPD presence), with a sample coverage (SC) above 95% for species richness (except in PVBTPD sites, where SC = 90%) and above 90% for host diversity (Table S1).

Host diversity comparisons

TableS1. Sample size and number of observed rodent species at each habitat type by locality. SC0 indicates the sample coverage based on species richness, while SC1 is based on diversity.

	PVgrass	PVBTPD	MVgrass	MVBTPD	ECgrass	ECBTPD
N	26	39	34	107	40	30
Richness	7	7	6	8	4	5
SC_0	0.90	0.98	0.95	0.99	0.98	1.00
SC_1	0.94	1.00	0.99	1.00	0.99	1.00

Note that the sampling effort was the same at each locality (i.e. 7 grids at each).

Overall, BTPD sites at each locality had a higher diversity than grassland sites where this species was absent (Table S2). Statistical analysis detected significant differences in diversity in BTPD sites at the MV locality (see TableS3), but no effect in the shift from autumn to spring. Thus, the differences detected by the model correspond to differences on locality-level diversity. In terms of species richness by locality, EC had 7 species, while PV had 8 and MV had 12.

TableS2. Host diversity at each site, habitat and season combination, as estimated by the Shannon index.

	PVgrass	PVBTPD	MVgrass	MVBTPD	ECgrass	ECBTPD
Autumn	1.119	1.458	1.342	1.855	0.823	1.168
Spring	1.413	1.435	1.517	1.517	1.012	0.849

TableS3. GLM results of variables associated with host diversity across sites and grassland types (BTPD and grasslands where this species isn't present). Significant p-values indicated in bold.

	Estimate	Standard error	t-value	p-value
Intercept	1.01	0.146	6.882	**<0.001**
$Season_{spring}$	-0.004	0.111	-0.033	0.975
$EC_{grassland}$	-0.09	0.192	-0.471	0.657
$MV_{grassland}$	0.421	0.172	2.193	0.080
MV_{BTPD}	0.678	0.192	3.528	**0.017**
$PV_{grassland}$	0.258	0.183	1.341	0.238
PV_{BTPD}	0.439	0.143	2.282	0.071

Relative abundance of host species and dissimilarity

Although our main aim was to assess differences in flea prevalence and abundance between grassland habitat with and without BTPD presence, we assessed the dissimilarity of host species across the sampling localities to justify the pooling of data at a habitat level. We used the SpadeR package (Chao et al., 2016; v.0.1.1) to compute the abundance-based Morisita-Horn index of dissimilarity between different habitat types (BTPD or grasslands with no BTPD) at each sampling locality. We also employed the SimilarityMult function to compute the same index but using a bootstrap approach of 500 simulations to measure compositional similarity. The Morisita-Horn index was deemed appropriate to our data, as it is relatively unsensitive to sample size and species richness (Chao et al. 2006), both of which were variable in our data (see Table S1).

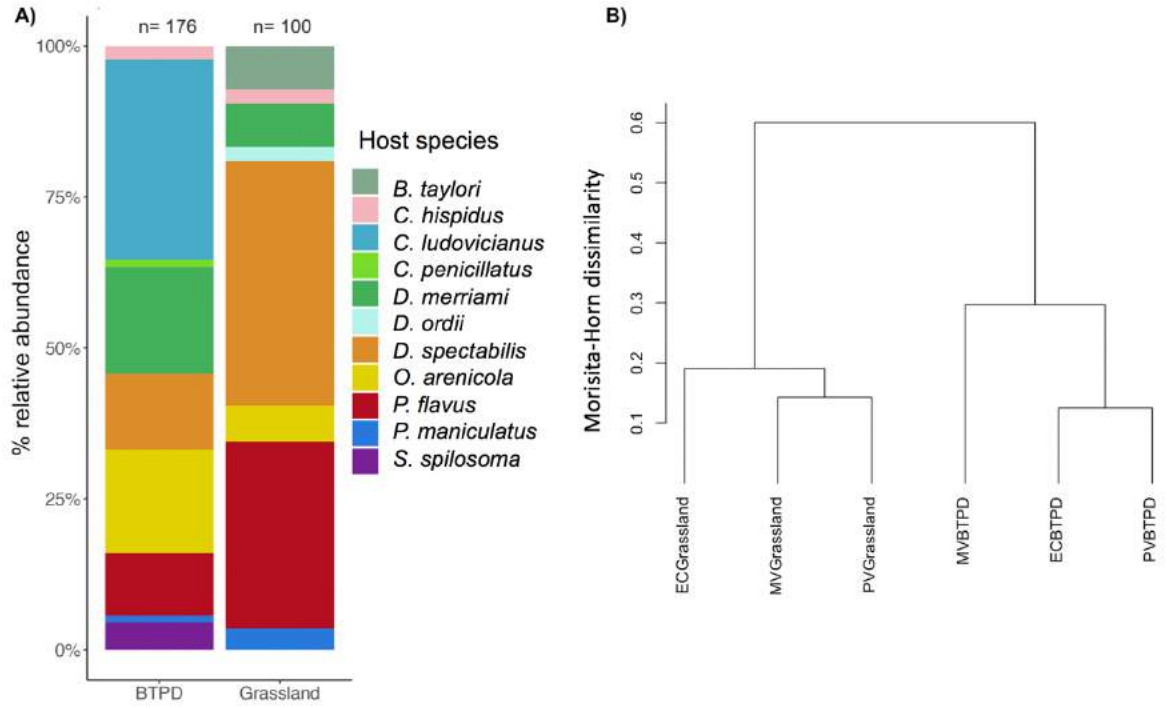


Fig. S1 A) Proportion of hosts sampled at each habitat type. The number of hosts sampled at each habitat is included above each bar. Both habitats shared 6 rodent species; two species were only recorded in grasslands (*Baiomys taylori* and *Dipodomys ordii*), while three were only found in BTPD grassland (*Chaetodipus penicillatus*, *Cynomys ludovicianus*, *Spermophilus spilosoma*). B) Cluster dendogram of the dissimilarity between hosts at each sampling locality by habitat, according to the estimated Morisita-Horn index, where 0 is identical composition and 1 is completely dissimilar.

According to results, localities where sites with BTPD were sampled belong to the same cluster, with dissimilarity ranging from 13-36%, while localities where grassland sites where BTPD was not present, were grouped in another cluster with ranges of dissimilarity between 14-23%. These results are in line with previous studies that have characterized the rodent communities at the study region as stable in terms of their composition (both across altitudinal regions and across the same vegetation type (Rivera Reyes et al., 2017)

Flea diversity

The following section describes the flea species detected at each locality, as well as the proportion of fleas found parasitizing non-specific hosts at sites with presence or absence of BTPD.

Table S4. Flea species present by habitat by locality.

	Echg	Jeli	Meral	Merar	Orcl	Oroh	Plee	Puls	Thra
EC_{BTPD}	1	0	0	1	0	1	1	1	0
EC_{grass}	1	0	0	1	0	0	0	0	0
MV_{BTPD}	1	0	1	1	0	1	1	1	1
MV_{grass}	0	0	0	1	1	0	1	0	0
PV_{BTPD}	1	0	0	1	0	1	1	1	0
PV_{grass}	1	1	0	1	1	0	1	0	1

Table S5. Percentage of fleas found parasitizing non-specific hosts, by host species. This proportion was calculated as the number of non-specific fleas found from the total of fleas found on each host species on either type of site (BTPD or grassland). Only host species where at least one non-specific flea was found are shown.

Host species	BTPD	Grassland
C. ludovicianus	5%	0
C. hispidus	0	50%
D. merriami	3%	0
P. flavus	0	100%
O. arenicola	12%	0
P. maniculatus	100%	33%

Note: non-specificity was determined by a literature review, summarised in Table S6. While the percentage of individuals with non-specific fleas by species might appear higher on grasslands, note that only 2 individuals of *C. hispidus* were collected in these sites, whereas of all *P. flavus* individuals collected (n = 51), a single one had a flea. Overall, from the total fleas collected at each site type, 6% corresponded to non-specific flea-host associations in BTPD, and 3% in grasslands with no BTPD.

Table S6. Host preference of flea species identified in this study. The column "Observed" refers to the host species in which the specific flea was detected, while "Literature" indicates the preferences reported in the literature. Fleas are ordered according to their families (Pulicidae, Ctenophtalmidae or Ceratophyllidae).

Flea species	Preference	Observed	Literature	References
Echidnophaga gallinacea	Generalist	*C. hispidus, C. penicillatus, C. ludovicianus, D. merriami, D. spectabilis, O. arenicola, S. spilosoma*	Generalist, observed also in carnivores and rabbits	Ritzi, 2014)
Pulex simulans	Generalist	*C. ludovicianus*	Carnivores, deer, larger rodents	Ritzi, 2014
Meringis altipecten	Family specialist	*C. ludovicianus, D. merriami, D. spectabilis, O. arenicola, P. maniculatus*	Flea of heteromyid rodents	Eads et al. 1987
Meringis arachis	Family specialist	*D. merriami, D. spectabilis, O. arenicola*	Flea of heteromyid rodents	Eads et al. 1987
Jellsonia ironsi	Species specialist	*B. taylori*	*B. taylori*	Eads, 1951
Orchopeas leucopus	Genre specialist	*P. maniculatus*	Primarily associated with *Peromyscus sp.*	Ritzi, 2014
Oropsylla hirsuta	Species specialist	*C. ludovicianus, O. arenicola, P. maniculatus*	Prairie dogs	Ritzi, 2014
Pleochaetis exilis	Genre specialist	*C. hispidus, C. ludovicianus, D. merriami, O. arenicola, P. maniculatus*	Primarily a flea of *Onychomys sp.*	Ritzi, 2014
Thrassis aridis	Genre specialist	*C. ludovicianus, D. spectabilis, O. arenicola, P. flavus*	Primarily a flea of *Dipodomys sp.*	Stark, 1957

Flea presence and abundance

Flea presence was 2 times more likely (odds ratio: 1.06-5.83) in individuals in grasslands where BTPD was present and more than twice as likely (2.5, odds ratio: 1.24 – 5.22) to occur during spring season, independently of BTPD presence (Table S7). We found no statistical support for an interaction between season and BTPD presence (LRT: $X^2 = 0.077$, df = 1, p = 0.781). Variance in flea presence between host species was marginally significant (LRT: $X^2 = 3.65$, df = 1, p = 0.056), while variance due to the other random effects included was not significant. However, all were retained in the model following a conservative approach.

Table S7. Generalized linear mixed model results of the effects of BTPD presence and season on the presence of fleas in rodents. Significant p-values are highlighted in bold. Variance due to random effects is included, where σ^2 indicates within-group variance, and τ_{00} represents between group variance due to either year, sampling site or host species. Note that this analysis was conducted excluding BTPD data, as this species typically harbours large numbers of fleas which could bias results.

				Random effect variance			
Fixed effect	Estimate	SE	p	σ^2	τ_{00Year}	τ_{00Site}	τ_{00sp}
				3.29	<0.001	<0.001	0.37
Intercept	-0.70	0.44	**0.034**				
BTPD presence	0.91	0.44	**0.037**				
Season(spring)	0.93	0.37	**0.011**				

In contrast with models for flea presence, the effect of BTPD presence on flea abundance depended on the season (LRT for interaction term: $X^2 = 4.56$, df = 1, p = 0.032), with higher flea abundances attained in grasslands with BTPD presence during spring (Table S8). Variance in flea abundance between host species was relevant (LRT: $X^2 = 15.38$, df = 1, p = <0.001).

Table S8. Generalized linear mixed model results of the effects of BTPD presence and season on the abundance of fleas. Significant p-values are highlighted in bold. Variance due to random effects is included, where σ^2 indicates within-group variance, and τ_{00} represents between group variance due to either year, sampling site or host species. Note that this analysis was conducted excluding BTPD data, as this species typically harbours large numbers of fleas which could bias results.

				Random effect variance			
Fixed effect	Estimate	SE	p	σ^2	τ_{00Year}	τ_{00Site}	τ_{00sp}
				1.22	<0.001	0.18	0.62
Intercept	0.25	0.55	0.645				
BTPD presence	-0.24	0.51	0.646				
Season(spring)	-0.56	0.46	0.223				
BTPD presence:Season(spring)	1.28	0.59	**0.031**				

Flea-flea associations

A X^2 contingency analysis showed that the distribution of coinfected individuals differed significantly between BTPD and grassland sites without this species, with coinfections occurring 6 times more frequently in the latter than the former ($X^2 = 9.73$, df = 1, p = 0.002) (Table S9). This difference increased if BTPD fleas were included in the analysis (15 times more, results not shown) ($X^2 = 21.82$, df = 1, $p < 0.001$).

Table S9. Number of coinfected and single infected (only one flea species present) individuals in grassland sites where BTPD was absent or present.

	coinfected	single
BPTD	20	98
Grass	3	97

Bayesian inference framework to characterise flea-flea interactions

Inferring interactions between species from co-occurrence data is sustained by a large body of theory, and multiple methodologies exist. The method developed by Stephens et al. (2009) and applied in this paper uses a Bayesian inference framework, where interactions are identified through deviations in the distribution of the co-occurrences of pairs of species relative to a benchmark that assumes no interactions. The original method divides up a geographic region of interest into spatial cells and counts the occurrence of species A and species B in each cell, as well as the co-occurrences of both species in each cell. Because we were interested in the co-occurrence of fleas at the individual host level, to calculate ε, we quantified the number of instances in which pairs of flea species co-occurred in individual hosts, as well as the number of individual flea species occurrences on each host, considering all individuals captured at a specific type of grassland (BTPD or non BTPD). Negative values of ε correspond to overlaps that are less than what is expected under the null hypothesis, i.e., the pairs of species have a negative association. Positive values of ε that are above 2 indicate an inconsistency between the data and the null hypothesis of no association at the 95% confidence level. The higher the value of ε, the stronger the positive association between pairs of species. The formula and steps to determine ε are detailed in Stephens et al. 2009.

Table S10. List of flea pairings collected in grassland sites with and without BTPD, ranked by ε. Positive associations for each flea pair (spA, spB) are highlighted in bold.

BTPD		
spA	spB	ε
E. gallinacea	*O. hirsuta*	**5.12047729**
	P. simulans	**4.3715449**
	M. arachis	1.28465949
	P. exilis	1.1687368
	T. aridis	-0.0494166
	M. altipecten	-0.078992
M. altipecten	*P. exilis*	**3.2107615**
	M. arachis	1.35058175
	O. hirsuta	0.50560765
	T. aridis	-0.0519524
	P. simulans	-0.1346071
M. arachis	*T. aridis*	**5.27317198**
	P. exilis	1.22871049
	P. simulans	0.39100154
	O. hirsuta	-0.1040957
O. hirsuta	*P. simulans*	**7.68262107**
	P. exilis	1.01215576
	T. aridis	0.83452296
P. exilis	*P. simulans*	0.83310081

	T. aridis	-0.0472192
P. simulans	*T. aridis*	-0.0349428
No BTPD		
E. gallinacea	*T. aridis*	**2.59807621**
	M. arachis	0.99303127
	O. leucopus	-0.0450835
	P. exilis	-0.0790569
	J. ironsi	-0.0790569
M. arachis	*O. leucopus*	-0.0318788
	T. aridis	-0.0453609
	P. exilis	-0.0559017
	J. ironsi	-0.0559017
P. exilis	*O. leucopus*	-0.0637577
	T. aridis	-0.0907218
	J. ironsi	-0.1118034
T. aridis	*O. leucopus*	-0.0780869
	J. ironsi	-0.1111111
J. ironsi	*O. leucopus*	-0.0637577

Network analysis

The algorithm employed to compute modules performs well on small networks (Beckett 2016). However, because values of modularity are potentially influenced by network size, we corrected the value of Q by null model expectation, using null model replicates (n = 5000) with the swap.web algorithm (Dormann et al. 2009). This procedure allowed us to assess whether the modularity values obtained are actually significant. Standardised values, obtained for a clearer comparison of modularity results between sites, were obtained as detailed in Dormann and Strauss (2014).

Table S11. Modularity values (Q_{obs}) obtained for bipartite host-flea networks, and standardized results of the modularity index ($Q_{standardised}$), estimated with the swap.web algorithm. Standard deviation (σ) and confidence intervals (C.I.) correspond to the 5,000 randomly generated networks.

	Q_{obs}	σ	$Q_{standardised}$	C.I.
EC_{BPTD}	0.423	0.033	2.02	1.679 – 2.380
EC_{grass}	0.485	0.067	3.81	3.424 - 4.196
PV_{BTPD}	0.448	0.034	2.59	2.234 - 2.952
PV_{grass}	0.602	0.105	3.62	3.194 – 4.055
MV_{BTPD}	0.622	0.021	4.13	3.751 - 4.519
MV_{grass}	0.685	0.111	4.35	3.759 - 4 .950

Because z-scores are assumed to be normally distributed, values that are approximately above 2 indicate significant modularity. Standardised Q values were congruent with initial non-standardised results (higher modularity values in grassland sites where BTPD was absent), although there was overlap in the estimates of MV sites.

Bipartite flea-rodent networks

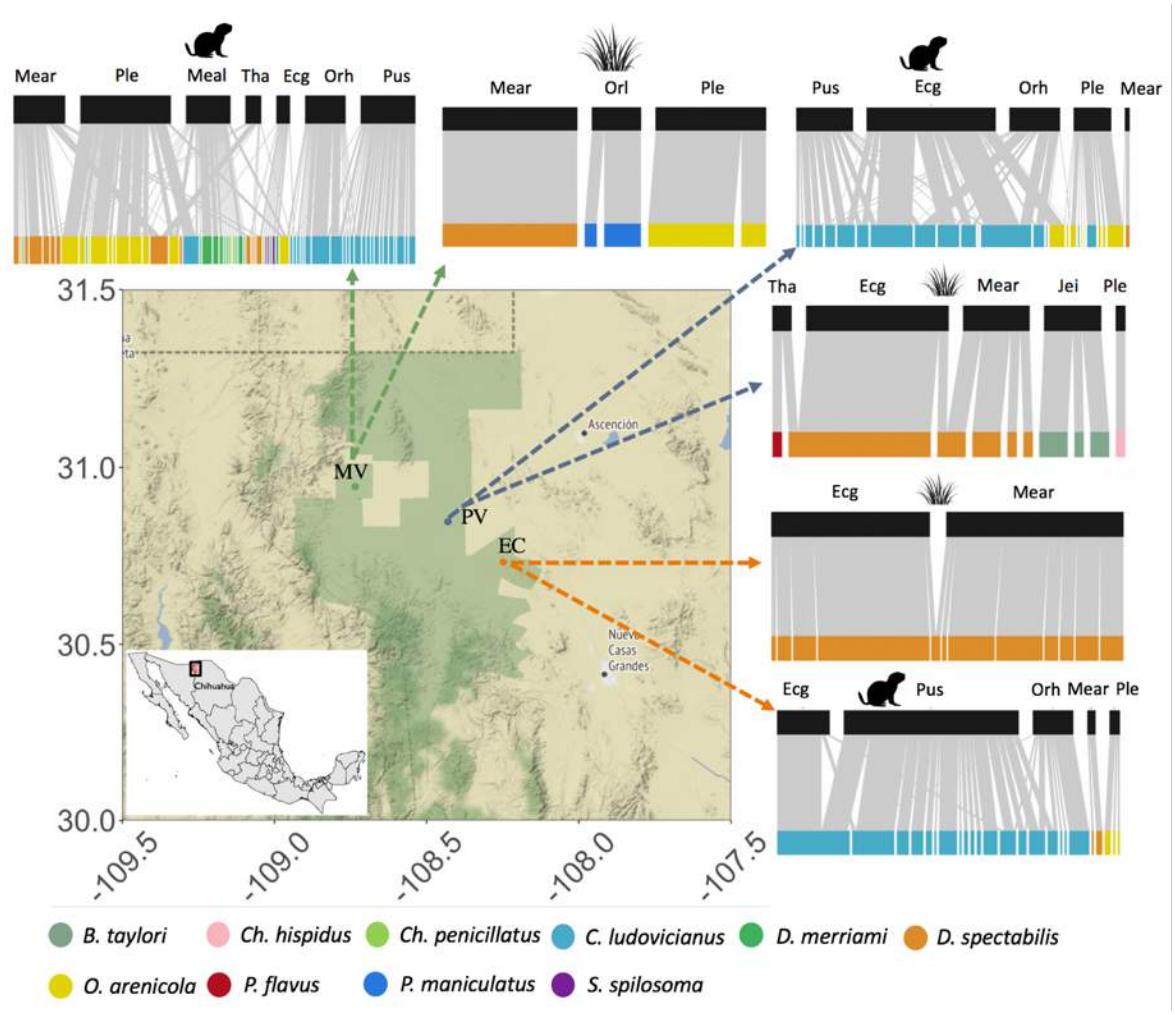
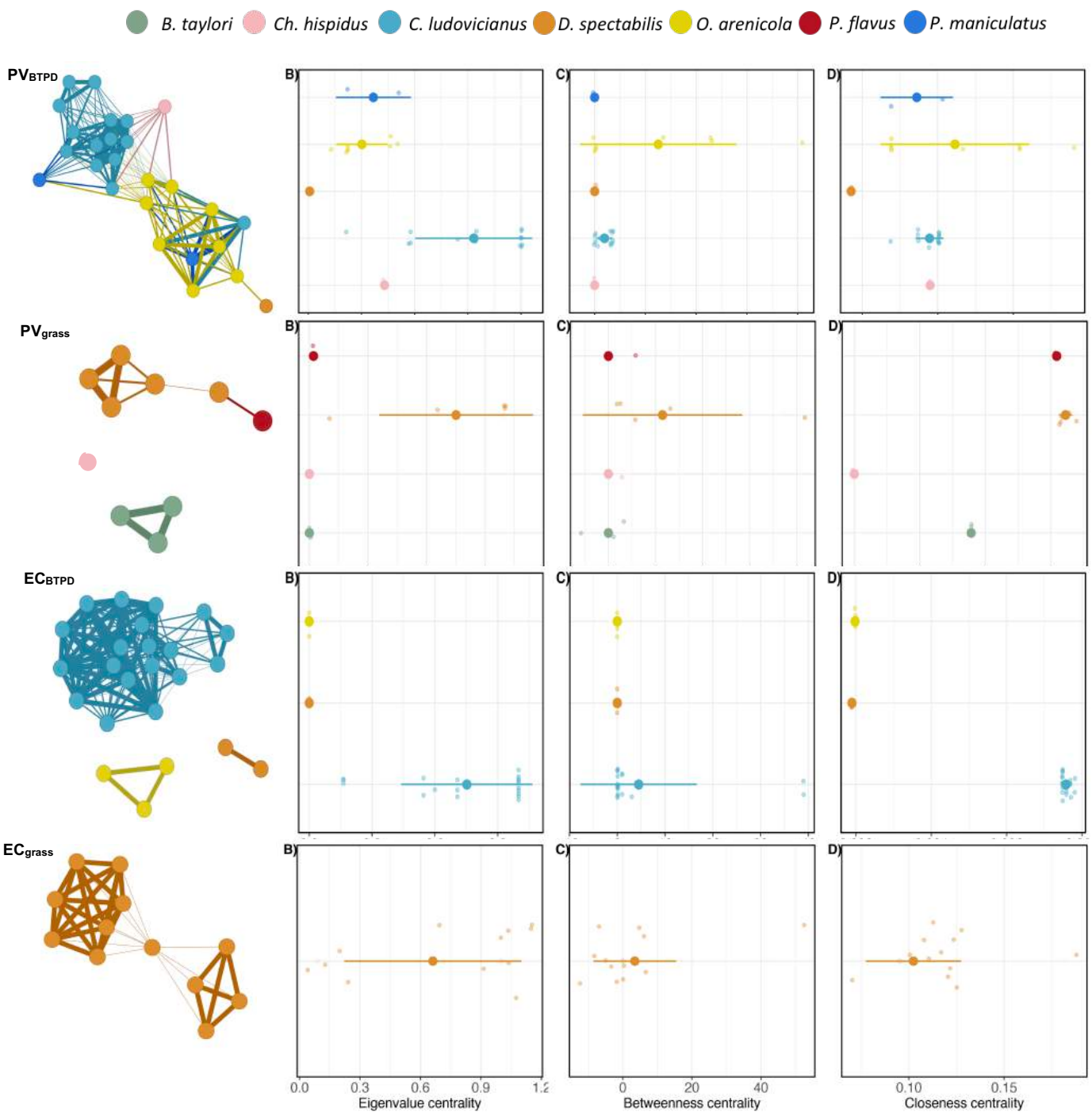


Fig. S2. Host-parasite networks at each of the sampled localities, grouped by BTPD presence or absence as indicated by icons. Upper nodes correspond to flea species, while lower nodes are individual rodents, with the colour representing the species. The width of the higher rectangles is proportional to the number of individuals harbouring a specific flea. The inset map corresponds to the study region, with each sampling locality depicted as a circle. See Methods for a description of the sampling design. Ecg = *E. gallinacea*, Jei = *J. ironsi*, Mear = *M. arachis*, Meal = *M. altipecten*, Orh = *O. hirsuta*, Orl = *O. leucopus*, Ple = *P. exilis*, Pus = *P. simulans*, Tha = *T. aridis*.

Transmission potential networks



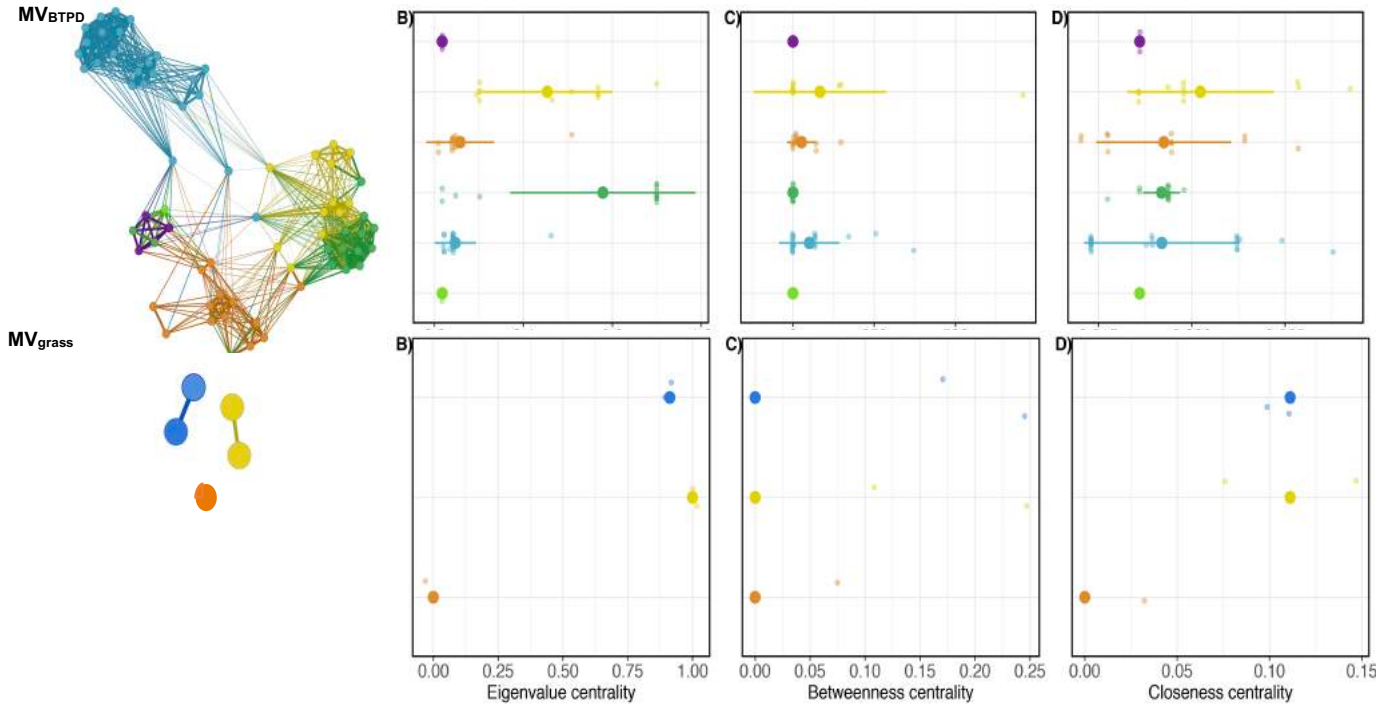


Fig. S3. Unipartite network and centrality metrics of rodent species found at sampling localities by habitat (grasslands with or without BTPD presence) within the RBJ in the Chihuahuan desert, Mexico. Networks were constructed by connecting nodes if they shared at least one parasite, using the Jaccard index as a measure of the similarity of shared flea communities. Thicker edges between nodes represent higher similarity. Each plot row is labelled with the site and the presence or absence is indicated by the subindex BTPD or grass, respectively. Thus, PV_{BTPD} is the network generated in PV localities where BTPD was present. Panels B), C) and D) each show the variation (mean and standard deviation) of each of the centrality metrics analysed, either eigenvalue, betweenness or closeness centrality, for each site by rodent species. Although species with less than 5 individuals were discarded for analysis purposes, in some instances only one or two individuals of that species had fleas and are shown as dots in the centrality plots. Raw data is plotted with a jitter parameter for visualization purposes.

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Material suplementario capítulo III
Bartonella-rodent-flea assemblages in sympatric rodents of northwestern Mexico highlight the nuances of inter-species transmission in multihost systems.

Bartonella-rodent-flea assemblages in sympatric rodents of northwestern Mexico highlight the nuances of inter-species transmission in multihost systems. Supplementary material

Additional information on methods

Estimating the significance of the structural specificity index (di')

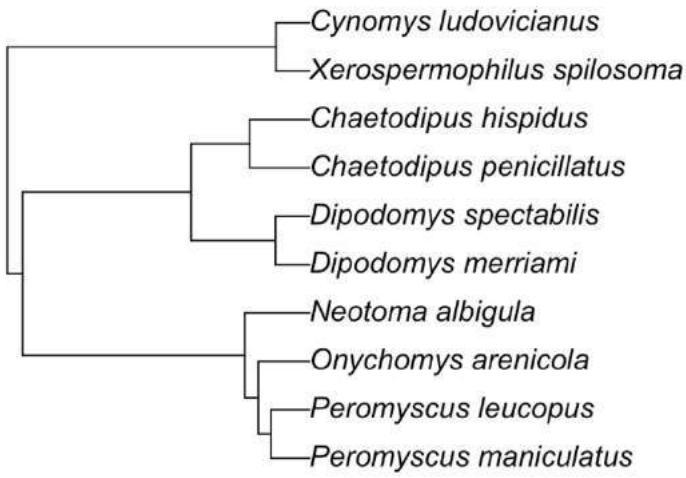
We used the structural specificity indices developed by Blüthgen et al. 2006 which were developed for analyzing bipartite networks, to assess the ecological specialization of fleas in our study sites. Both metrics, d_i' and H2', were calculated using weighted links (the relative frequencies with which flea-host associations occurred) and the respective functions implemented in the R package 'bipartite' v.2.16 (Dormann et al., 2009). Because values of modularity are potentially influenced by network size, we evaluated our estimated values against null model expectations using a null model based on the algorithm developed by Vázquez et al. 2007 and 1000 replicates. This algorithm was chosen because it accounts for unrealized connections between flea and host species in the original network, which might represent forbidden links (Olesen et al. 2011); i.e., restrictions in terms of which fleas can parasitize which hosts (for example, due to strong host-specific preferences or incompatibility.

Multiple regression on distance matrices (MRM)

MRM method involves performing a multiple regression of a response matrix using explanatory matrices, where each of these contain distances or similarities between all sampling units. In our case, rows and columns of each of the distance matrices represented host individuals. Each of the explanatory matrices describes pairwise differences between individuals in terms of a certain characteristic; for categorical variables, such as sex or season, cell values in the response matrix were 1 if two individuals shared the same characteristic (e.g., both females) or 0 if they didn't. For continuous variables, absolute differences in the values of the continuous characteristic between pairs of individuals were obtained. Note that continuous variables were standardized previous to analysis by converting them to z-scores, to avoid effects due to differences in scale. Cell values in the response matrix indicate whether two individuals are in the same module (1) or not (0). The output of this analysis returns a list of coefficient values and an estimate of their significance. Details on how statistical significance is calculated in this method can be found in Lichstein, 2007.

Phylogenetic distance between pairs of rodents was obtained by using the patristic distances (sum of phylogenetic branch lengths) using the packaged 'ape' (v.5.6-2) and the rodent tree constructed from the smoothed mammal time tree of life obtained by Hedges et al. 2015, and available for download in http://www.biodiversitycenter.org/ttol. Figure S1 shows the phylogenetic tree obtained considering the species involved in our study.

Fig. S1. Phylogenetic tree of the rodent species collected in our study sites.



Parasite sharing networks

Unipartite projections of bipartite host-parasite networks, or parasite sharing networks, depict hosts as nodes and links between nodes instances of shared parasitism (Dallas et al., 2019). Flea sharing networks were constructed following the methods described in Pilosof et al. 2015. Briefly, bipartite networks were projected by connecting two individual hosts if they shared at least one parasite in the bipartite network, with edges weighted by the similarity in flea assemblage composition between pairs of hosts as estimated by the Jaccard index. This was calculated as $a/(a+b+c)$, where a is the number of parasites infecting both host individuals, while b and c are the number of parasites infecting either host individuals. Maximum edge value is 1, when flea assemblages between pairs of individuals are identical, and 0 when they don't share any fleas.

Interpretation of edges in our network also follow that presented by Vander Waal et al. 2014, Dallas et al. 2019, and Pilosof et al. 2015, among other authors, in the sense that edges are assumed to show the potential for parasite transmission between pairs of individuals based on traits that promote parasite sharing, and thus connected individuals can be thought of forming part of the same transmission chain. However, it should be noted that we cannot infer actual directionality, so this network does not represent a transmission chain in the epidemiological sense.

Statistical models of risk of infection

Data used for statistical modelling excluded juvenile or pregnant individuals, to avoid confounding effects on weight, and was limited to host species where $n > 30$ and at least three individuals in each level of the categorical variables. We constructed separate sets of models to assess predictors of overall Bartonella infection as determined from testing blood samples, and for assessing those associated with specific Bartonella variants. For the latter, we used the subset of the individuals where Bartonella variants were sequenced. Using the

results of the Bartonella-flea-assemblage analysis, we determined host and flea individuals associated with specific variants, and set the Bartonella infection status to 1, if they presented the variant assessed, or 0 if they presented other variant. Host traits included sex, weight and reproductive status as fixed effects. Flea burden was also included, as it has been associated with infection risk (Eisen and Gage, 2012). We also tested whether flea sharing was a predictor of infection status, by estimating the eigenvalue centrality (EC) from the flea sharing network using the corresponding function in the package igraph (v.1.2.5). This centrality metric indicates indicates nodes that have connections to other nodes that are themselves highly connected. In host-parasite networks, this measure has been associated with hosts that are likely to play a key role in network dynamics (Allesina and Pascual 2009). Specifically, it has been linked to a measure of the transmission potential of the node (Canright and Engø-Monsen 2006).

Non-host variables included in the models were sampling season, to account for potential seasonal variation in infection risk, and sampling site as a proxy to assess the effects of habitat, as MV and RO had different vegetation types (MV sites had grassland vegetation with extensive BTPD presence, whereas RO sites had shrubland vegetation). Sampling plot and host species were included as random effects, to control for local and species level variation. Continuous fixed effects were mean-centered prior to analysis. The intraclass correlation coefficient (ICC) was calculated to estimate differences in variation within and between sampling grids, as a proxy to assess the role of unspecified environmental variation associated with local conditions of the sampling plot on infection status.

Significance of variables was evaluated using likelihood ratios tests (LRTs), from backward stepwise elimination of nonsignificant terms ($p < 0.05$) from a maximal model. All models were tested for collinearity between predictive variables (VIF < 2), with model diagnostics conducted with the package DHARMa (Hartig, 2016), to ensure that final models complied with assumptions (Zuur et al., 2010).

Raw data summaries

Table S1. Description of sampled hosts and Bartonella prevalence in rodent species. The columns represent n = the number of total hosts sampled from each rodent species in both sampling sites, + = the number of Bartonella positive individuals of the total as detected by testing blood samples, and % = overall Bartonella sp. infection prevalence in each host species.

	Sampling site		
Flea species	**n**	**+**	**%**
Chaetodipus hispidus	3	1	33.3
Chaetodipus penicillatus	31	3	9.7
Cynomys ludovicianus	25	3	12
Dipodomys merriami	177	100	56.5
Dipodomys spectabilis	50	27	54
Neotoma albigula	3	3	100
Onychomys arenicola	56	46	82.1
Perognathus flavus	4	0	0
Peromyscus leucopus	14	7	50
Peromyscus maniculatus	24	13	54.2
Spermophilus spilosoma	6	2	33.3
Total	**393**	**205**	**52.1**

Table S2. Description of sampled flea species and their Bartonella prevalence. The columns represent n = the number of total fleas collected in both sampling sites, + = the number of Bartonella positive individuals of the total as detected by PCR of flea triturates, and % = overall Bartonella sp. infection prevalence in each flea species.

	Sampling site		
Flea species	**n**	**+**	**%**
Echidnophaga gallinacea	97	2	2.1
Pulex simulans	43	2	4.6
Meringis altipecten	251	108	43.0
Meringis arachis	202	77	38.1
Meringis parkeri	16	6	37.5
Oropsylla hirsuta	37	0	0
Orchopeas leucopus	37	9	24.3
Orchopeas sexdentatus	15	7	46.7
Pleochaetis exilis	240	153	63.8
Thrassis aridis	37	25	67.6
Total	**975**	**389**	**39.9**

Additional results

Table S3. Structural specificity of each flea species as measured by d_i'. NP = Not Present at the site. Significance as assessed by null model comparison is indicated in parenthesis where: * $p < 0.05$, ** $p < 0.01$ and ns = not significant.

	Sampling site	
Flea species	**MV(p-val)**	**RO(p-val)**
Echidnophaga gallinacea	0.42(*)	0.23(*)
Meringis altipecten	0.59(*)	0.51(*)
Meringis arachis	0.61(*)	0.42(*)
Meringis parkeri	NP	0.19(*)
Orchopeas leucopus	0.97(**)	0.74(**)
Oropsylla hirsuta	0.64(**)	NP
Orchopeas sexdentatus	NP	0.98(*)
Pleochaetis exilis	0.86(*)	0.61(*)
Pulex simulans	0.74(**)	NP
Thrassis aridis	0.33(*)	0.11(*)

Table S4. Bartonella variants identified in rodent hosts in the study. The variant identifier is used throughout the text. Genbank accession number and phylogroup are based on the findings of Rubio et al. 2014, where these variants were originally identified.

Variant identifier	Genbank Accession Number	Phylogroup
var1	AF214557.1	II
var2	AF440275	III
var3	AF489539.1	II
var4	DQ357612.1	IV
var5	DQ357613.1	I
var6	KJ719284.1	I
var7	KJ719287.1	I
var8	KJ719288.1	III
var9	KJ719289.1	III
var10	KJ719293.1	IV
var11	KJ719295.1	IV
var12	KJ719296.1	V
var13	KJ719297.1	V
var14	KJ719298.1	V

Fig. S2 Bipartite plots of Bartonella-rodent, showing the association of host individuals with variants detected by sequencing in the two sampling sites involved in the study. The top plot corresponds to results in MV, whereas the bottom shows results for RO. The map shows the sampling sites in relation with their position within the JBR (green polygon), whereas the inset map highlights the localization of the reserve within Mexico. MV = Monte Verde, RO = Rancho Ojitos.

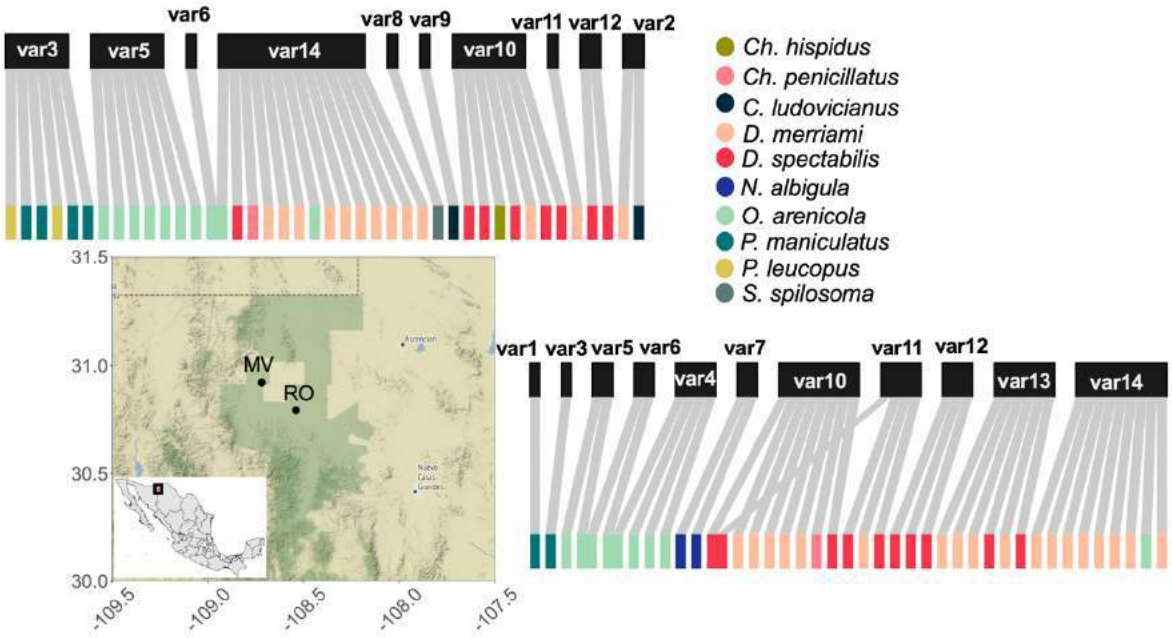
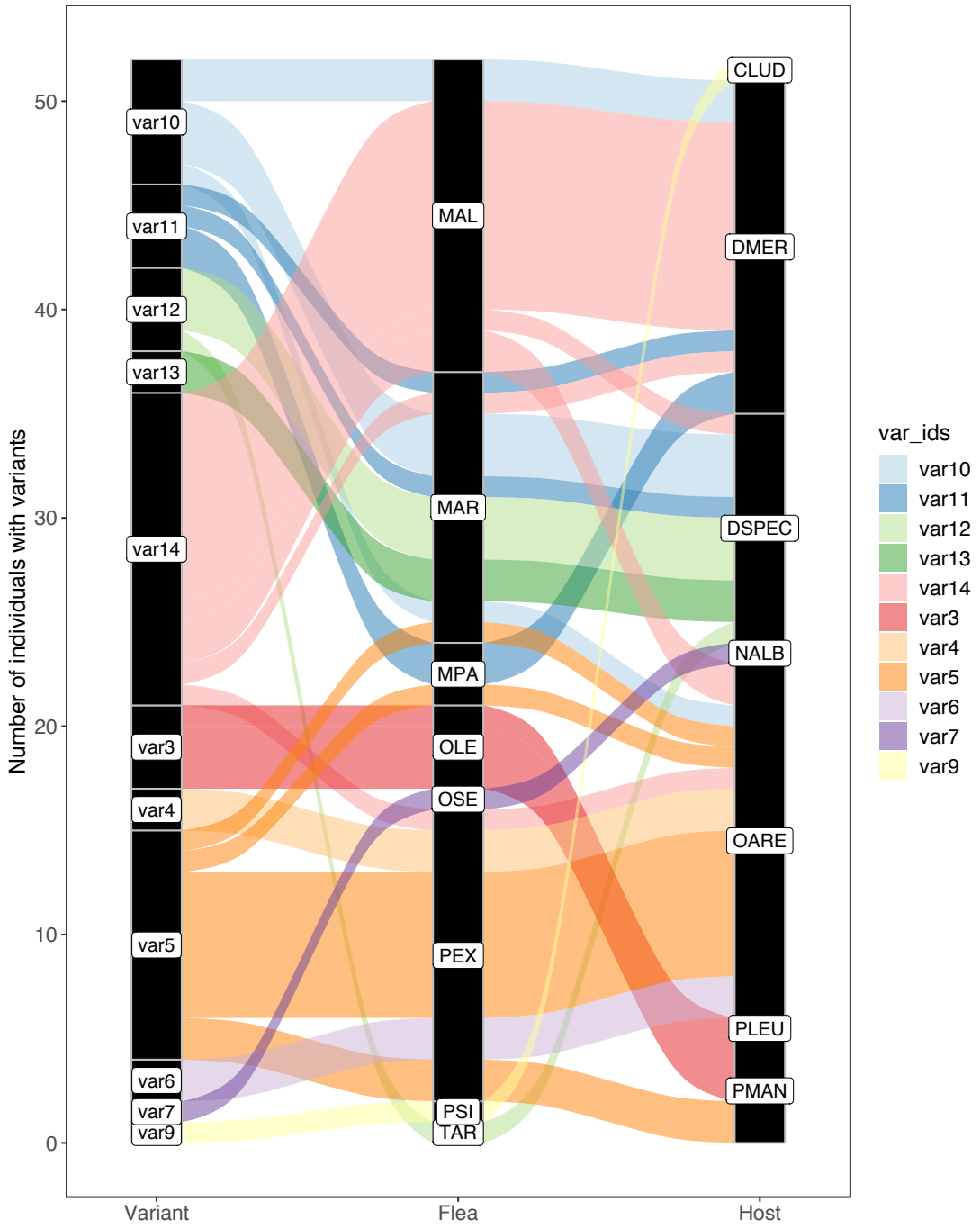


Fig. S3. Bartonella-flea-rodent assemblages. Note the plot does not show instances where variants in fleas were never found in the host species in which they were collected; i.e., the variant was never detected in the host even though it was collected on fleas that parasitize it. However, we only detected two instances of this happening.



Variants follow the variant identifier key (Table S4). Flea species are abbreviated as follows: MAL = *Meringis altipecten*, MAR = *Meringis arachis*, MPA = *Merinigs parkeri*, OLE = *Orchopeas leucopus*, OSE = *Orchopeas sexdentatus*, PEX = *Pleochaetis exilis*, PSI

= *Pulex simulans*, TAR = *Thrassis aridis*. Host species are as follows: CLUD = *Cynomys ludovicianus*, DMER = *Dipodomys merriami*, DSPEC = *Dipodomys spectabilis*, NALB = *Neotoma albigula*, OARE = *Onychomys arenicola*, PLEU = *Peromyscu leucopus*, PMAN = *Peromyscus maniculatus*.

Table S5. Predictors of Bartonella infection across rodent hosts, according to generalized mixed model results. Coefficient values and standard errors (SE) shown correspond to the single best fixed-effects model as measured by the lowest AIC and likelihood ratio test comparison. σ^2 = within-group variance; $\tau 00$ = between-group variance; ICC = intraclass correlation coefficient.

				Random effect variance			
Fixed effect	Estimate	SE	p	σ^2	$\tau_{00Species}$	τ_{00Grid}	ICC
				3.29	1.86	0.01	0.21
Intercept	0.09	0.46	0.844				
Site(RO)	-0.04	0.24	0.871				
Season(spring)	0.41	0.23	0.064				
Weight	-0.003	0.001	**0.024**				

Table S6. Predictors of infection with a specific Bartonella variant (variant 15, corresponding to Accession Number KJ719298.1 in Genbank) across rodent hosts, according to generalized mixed model results. Coefficient values and standard errors (SE) shown correspond to the single best fixed-effects model as measured by the lowest AIC and likelihood ratio test comparison. σ^2 = within-group variance; $\tau 00$ = between-group variance; ICC = intraclass correlation coefficient.

				Random effect variance			
Fixed effect	Estimate	SE	p	σ^2	$\tau_{00Species}$	τ_{00Grid}	ICC
				3.29	1.83	0.13	0.37
Intercept	-2.05	1.09	0.060				
Site(RO)	-1.81	0.82	0.871				
Sex(M)	2.32	1.10	0.035				
EC	2.73	1.30	0.035				
EC:Sex(M)	-2.72	1.63	0.054				

Model results suggest that there is a significant albeit small effect of weight on reducing the probability of being infected with Bartonella across all rodent host species involved in the study. A positive effect of spring was inconclusive. A moderately low value of ICC indicates larger within grid variance than between grid variance. Between species variant

in infection status had a large effect. This was previously reported in Rubio et al., 2014, who found specific host species to be more associated with Bartonella infection than others.

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