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Coevolving histories inside and out: phylogenetics, comparative parasitology, and host affinities of chipmunk sucking lice and pinworms

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COEVOLVING HISTORIES INSIDE AND OUT: PHYLOGENETICS, COMPARATIVE PARASITOLOGY, AND HOST AFFINITIES OF CHIPMUNK SUCKING LICE AND PINWORMS

by

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DISSERTATION

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ABSTRACT

Diversification of parasite species, in light of their host association, is an area ripe for testing hypotheses of evolution when one species requires another for survival. The 23 species of western North American chipmunks (genus *Tamias*) host two species of ectoparasitic sucking lice (Anoplura) and two species of endoparasitic pinworms (Nematoda). I used a phylogenetic approach to investigate the evolutionary histories of the parasites in light of the hosts and the landscape. In comparing the parasites, I found that the two pinworm species have similar diversification patterns, linked to hosts, but those processes occurred on different time scales. As another paired investigation, the chipmunk sucking lice revealed some lineages that correspond to host relationships, but the lice have different histories from the hosts, as well as each other. Overall, this system demonstrates that parasite diversification cannot be explained as a simple process of codivergence and that parasite evolution, even when comparing parasites from the same hosts and ecological roles, is complex and the history is unique to each species. While I found a role for hosts, host demographic history, and landscape in shaping genetic structure in all four parasites, these processes impacted each parasite species differently.

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INTRODUCTION

The evolution of interacting species and their selective influence on one another has interested biologists for some time; even Darwin (1859) described it in *On the Origin of Species*. In the past, coevolutionary investigations generally expected cospeciation when the interaction is sufficiently tightly linked for one species to require the other. However, biologists are discovering that the evolution of interacting organisms is more nuanced than simplistic assumptions about cospeciation. In fact, some have even questioned if classic examples of host-parasite codivergence, such as gophers and chewing lice (Hafner and Nadler 1988, 1990), were robustly assessed (Brooks et al. 2015). While this overturns a paradigm of coevolutionary thought, it leaves many open questions of how interacting organisms impact each other's evolution. In particular, we should develop a deeper understanding of how specificity and abiotic factors shape interspecies interactions. The evolution of parasite species, in light of their host association, is an area ripe for testing hypotheses related to fundamental evolutionary processes when one species requires another for survival.

Parasites are often considered a neglected group of organisms, systematically and ecologically, but generally thought to be rich with undescribed diversity. Research is continually revealing their important roles in ecosystem function, community dynamics, and persistence of host species (Poulin 1999, Thompson et al. 2005, Hudson et al. 2008, Stringer and Linklater 2014). There is also a need to understand evolutionary and transmission dynamics of parasites in the context of changing climates. By revealing how past climate change impacted parasite evolution, we can begin to develop models focused

on how contemporary climate change will impact wild populations (Brooks and Hoberg 2007, Kutz et al. 2009).

My dissertation explores questions and tests hypotheses regarding parasite diversification in the context their rodent hosts distributed across western North America. The 23 species of western North American chipmunks (genus *Tamias*, subgenus *Neotamias*) inhabit a variety of habitats and commonly host two species of ectoparasitic sucking lice (Anoplura), *Hoplopleura arboricola* (Hoplopleuridae) and *Neohaematopinus pacificus* (Polyplacidae), and two species of endoparasitic pinworms (Nematoda; Oxyurida), *Heteroxynema cucullatum* (Heteronematidae) and *Rauschtineria eutamii* (Oxyuridae). The broad distribution of these species, relatively high host diversity, and paired sets of ecto- and endoparasites make chipmunks and their parasites an ideal system to investigate the roles of host history, climate, and geography in parasite diversification. While there is a range of approaches to investigating coevolving systems, a useful starting point is to build phylogenies based on broad geographic sampling, multiple independent genomic loci, and comprehensive taxonomic diversity. Phylogenies are valuable tools for revealing cryptic diversity, the structure of populations, and evolutionary histories. Robust intraspecific phylogenies are critical for understanding variation across the landscape and determining the roles of historical events in lineage diversity.

The first chapter of my dissertation, *Expanded Host Range of Sucking Lice and Pinworms of Western North American Chipmunks*, emphasizes the importance of an integrated approach to building the broad geographic and taxonomic sampling needed for phylogenetic analyses. This chapter summarizes the findings from years of fieldwork in

western North America and the sucking lice discoveries made using museum study skins. Through these examinations of field-collected specimens and museum specimens, I found the ectoparasitic sucking lice (*Hoplopleura arboricola* and *Neohaematopinus pacificus*) and endoparsitic pinworms (*Heteroxynema cucullatum* and *Rauschtineria eutamii*) of chipmunks have much broader host and geographic distributions than previously described. Determining this host and geographic breadth was a requisite first step to understanding the evolutionary history of these parasite taxa.

Chapter Two, *Temporal and Spatial Mosaics: Deep Host Association and Shallow Geographic Drivers Shape Genetic Structure in a Widespread Pinworm,* Rauschtineria eutamii *(Nematoda; Oxyuridae),* employs a phylogenetic and phylogeographic approach to delimit the diversity and the distribution of that diversity with a species of chipmunk pinworm. I found that the deepest genetic divergences within this species generally correspond to associations with a single chipmunk species or a single chipmunk species group. Genetic structuring within these lineages, at the shallow time scale, was largely structured by geography. I also uncovered evidence of host switching among these lineages, which corresponds to geographic localities of host species sympatry. While host switching is possible, the lack of evidence of host switches at a deep time scale suggest that these switches are unstable or ephemeral. Overall, the phylogeographic pattern of *R. eutamii* reflects a history of primary association with a host and secondary divergence driven by taxon pulses and ecological fitting when closely related species are in contact.

For my third chapter, *Multiple Parasites Show Deep, but Asynchronous, Concordance in Diversification that Contrasts with Shallow Phylogeographic Structure*, I

tested hypotheses of the roles of similar host associations and biogeographic histories in shaping the diversity and divergences of two pinworm species parasitizing the same physical space of chipmunks. Comparing the genetic structure of these two species reveals that they both have deep divergences associated with hosts, however these events happened on different timescales. Although the process that shaped these divergences were likely similar in both pinworm species, they were more recent in *R. eutamii* than in *H. cucullatum.* Additionally, the fine scale genetic structuring among populations of each species is different, the events of host population fluctuations and repeated contact of chipmunk species likely due to cyclic climatic events has differentially impacted the distribution of pinworm diversity across the landscape.

The concluding chapter, *Disentangling Lousey Relationships: a Phylogenomic Perspective on Host and Parasite Diversification*, uses molecular data sampled across the genome to understand chipmunk and lice evolutionary histories. I used over 800 exons assembled from whole genome sequencing to generate species trees for each sucking louse, *Hoplopleura arboricola* and *Neohaematopinus pacificus*. Chipmunk phylogenetic relationships were reconstructed using over 3,000 ultraconserved element loci. The species tree for chipmunks is the first molecular phylogeny that has complete taxon sampling for the genus (25 species) and was able fully resolve the relationships among most chipmunk species. This volume of data revealed lineages within both lice species that generally correspond to host species or species groups, but these parasite relationships did not mirror the relationships among hosts, rejecting the primary hypothesis of codiversification. I also found that *H. arboricola* was not monophyletic with respect to their presumed closest relative, the *Tamias striatus* sucking louse (*H.*

erratica). The genetic structure within *H. arboricola* indicated cryptic diversity and a need for taxonomic revision. Genetic structure in the *N. pacificus* phylogeny exhibited a different history of association with the hosts than *H. arboricola*, as monophyletic host groups did not host monophyletic louse lineages. There was also evidence of higher rates of host switching among *N. pacificus* lineages when divergent host species were sympatric. This phylogenetic investigation lays the groundwork for future investigations into the timing of diversification events in both the hosts and parasites, which will reveal synchronous events and how they correspond to past climatic cycling.

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CHAPTER 1

EXPANDED HOST RANGE OF SUCKING LICE AND PINWORMS OF WESTERN NORTH AMERICAN CHIPMUNKS (GENUS *TAMIAS*)

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ABSTRACT

Biological inventories often miss parasites, a critical component of biodiversity, and even well studied host species generally have a paucity of parasite records. Efforts to document the host diversity and distribution of parasites can utilize newly collected specimens as well as museum specimens. We focus on a group of widespread, well-documented hosts, western North American chipmunks (Rodentia: genus *Tamias*). Field-collected and museum specimens of chipmunks from across western North America were examined externally for sucking lice (Anoplura) and gastro-intestinal tracts were examined for pinworms (Oxyuriodea). We documented new hosts and expanded the geographic distribution for four parasite taxa under investigation, *Hoplopleura arboricola*, *Neohaematopinus pacificus*, *Heteroxynema cucullatum*, and *Rauschtineria*

eutamii. This effort demonstrates the utility of museum collections as well as the pressing need for continued field collection to characterize global biodiversity.

KEY WORDS: chipmunks, *Tamias*, sucking lice, Anoplura, *Hoplopleura arboricola*, *Neohaematopinus pacificus*, pinworms, Oxyuroidea, *Heteroxynema cucullatum*, *Rauschtineria eutamii*, new records, museum collections, North America

Parasites as a group are largely neglected in discussions of changes in global diversity (Kutz et al., 2009). This failure to recognize a significant component of biodiversity is due in part to both the lack of sampling effort and high cryptic diversity in some taxa (Makarikov, et al. 2013). Recent emphasis has shifted research focus from documenting descriptive patterns to assessing more universal processes of parasite evolution (Poulin and Morand, 2000), however, a prerequisite for nearly all parasitological investigations is a thorough understanding of both the host and geographic distributions of parasites. Field inventories, besides providing baseline distributional data, may also contribute to an improved understanding of ecosystem health (Marcogliese, 2005; Hudson et al., 2006) and emerging infectious diseases (Brooks and Hoberg, 2000; Brooks and Hoberg, 2007).

Sucking lice (Insecta: Phthiraptera: Anoplura) and pinworms (Secernentea: Oxyurida: Oxyuroidea) are among the most common parasites of rodents. As with other rodents, chipmunks of western North America (genus *Tamias* Illiger, 1811, subgenus *Neotamias* Howell, 1929) are frequently parasitized by parasites belonging to both of these groups. Differences in ectoparasite assemblages were used as support for dividing

the genus *Tamias* into 3 genera (currently recognized as subgenera), *Eutamias* (*T. sibiricus* in Eurasia), *Neotamias* (the 23 species in western North America), and *Tamias* (*T. striatus* in eastern North America) (Jameson, 1999). However, there are no records for ectoparasites from several western chipmunk species. With 23 described species of *Neotamias* chipmunks spread across western North America (Wilson and Reeder, 2005), this clade has experienced a dynamic and complex evolutionary history (Patterson, 1982; Good and Sullivan, 2001; Piaggio and Spicer, 2001; Demboski and Sullivan, 2003; Sullivan et al., 2014). These chipmunks and their parasites are an excellent system to investigate the evolution of specificity and potential adaptive responses in a widely distributed and rapidly diverging host-parasite community. Chipmunks also provide multiple opportunities to explore host switching, as many species are sympatric and some hybridize (Good et al., 2003; Reid et al., 2012; Sullivan et al., 2014).

Two species of ectoparasitic sucking lice (Anoplura), *Hoplopleura arboricola* Kellogg and Ferris, 1915 (Hoplopleuridae) and *Neohaematopinus pacificus* Kellogg and Ferris, 1915 (Polyplacidae) infest 10 species of *Neotamias* (Table 1; Kim et al., 1986; Durden and Musser, 1994; Kucera et al., 2007). Three species of pinworms (Oxyuriodea), *Heteroxynema cucullatum* Hall, 1916, *Rauschtineria eutamii* (Tiner, 1948) Hugot, 1980, and *Dentostomella grundmannii* Chitwood, 1963, are known to infect *Neotamias*. Pinworms of the former 2 species have been reported only from 6 host species (Table 1; Hall, 1916; Tiner, 1948; Frandsen and Grundmann, 1961; McBee and Hendricks, 1973; Kennedy, 1986; Archie et al., 1988), while *D. grundmannii* has been reported only in *T. umbrinus* in Utah (Table 1; Chitwood, 1963). Our objective is to determine the host and geographic range of the sucking lice and pinworms that infest and

infect *Neotamias*. To that end, we conducted extensive field sampling and also examined collections of hosts from 3 museums, the Museum of Southwestern Biology, Albuquerque, New Mexico, the Moore Laboratory of Zoology, Los Angeles, California, and the United States National Museum of Natural History, Washington D.C.

Chipmunk specimens (n = 842) were field collected from 2004 through 2013 representing 21 host species (all *Neotamias* species were studied except *T. bulleri* and *T. durangae*). All field collecting followed approved institutional animal care and use mammal handling and collecting protocols (Sikes et al., 2011). Following euthanization, chipmunks were examined externally for parasites by parting the fur with a probe under a dissecting microscope. All arthropods were collected and placed in either 70% or 95% ethanol or were frozen in liquid nitrogen and later transferred to either -86C or -20C freezers. Following standard museum protocols for collection of host tissues, the entire gastrointestinal (GI) tract was placed in a petri dish with water. The GI tract was cut into sections (stomach, small intestine, cecum, and large intestine) and then opened longitudinally. Cestodes and acanthocephalans were relaxed in water prior to preservation. All helminths were collected and preserved in either 70% or 95% ethanol or frozen in liquid nitrogen and later transferred to either -86C or -20C freezers. Nematodes were placed directly into ethanol or frozen. All chipmunk specimens were cataloged at the Denver Museum of Nature & Science or Museum of Southwestern Biology and are searchable on the Arctos museum database (http://arctos.database.museum/; Supplement 1). Researchers from other institutions (J. L. Patton, D. S. Rogers, and E. A. Rickart) also combed recently collected specimens for ectoparasites and preserved GI tracts in ethanol or frozen and deposited them with the Museum of Southwestern Biology.

In addition to field sampling, we screened 2226 museum study skins representing 21 species (all *Neotamias* species except *T. ochrogenys* and *T. panamintinus*) collected between 1897 and 2007 to recover dried arthropods that were still attached to host specimens. That effort significantly expanded sampling of host diversity and spatial and temporal variation, including endemic host species from Mexico. Specimens were combed over white paper and then examined under a dissecting microscope. Recovered arthropods were preserved in 70% ethanol. Lice were identified under 150X magnification and sorted using the two pairs of enlarged setae on the sternal plate as characters for *H. arboricola* and spiniform seta on the base of the antennae as characters for *N. pacificus* (Kim et al., 1986). Nymphs were not identified to species, however specimens can be sorted into the two candidate species using the characters described for *H. arboricola* by Cook and Beer (1959). So that they can be used in molecular studies, pinworms were not cleared for identification; instead they were examined in ethanol under 20X magnification. Pinworms were identified and sorted using characters from the original descriptions of each of the candidate species (Hall, 1916; Tiner, 1948). In particular, the oval shape of the esophageal bulb, the cervical alae and blunt posterior ends of *H. cucullatum* and the circular esophageal bulb and tapering, filamentous posterior ends of *R. eutamii*, were used to distinguish between species. No *D. grundmannii* were recovered. Parasites recovered from Museum of Southwestern Biology, Museum of Vertebrate Zoology, Monte L. Bean Life Science Museum, and Natural History Museum of Utah host specimens are cataloged in the Division of Parasites at the Museum of Southwestern Biology (Accessions 2014.029.Para (lice) and

2014.021.Para (pinworms)), while other parasites will be returned to the institution where the host is permanently cataloged (Supplement 1).

Our sampling resulted in new records for lice and pinworms and the recovery of parasites from all 23 host species (Table 2) across western North America (Figure 1). For field-collected hosts, overall sucking louse prevalence was 67.1%. Museum study skins had a lower recovery rate, with a louse prevalence of 36.5%. We recovered *H. arboricola* from all species of western chipmunks and *N. pacificus* from 19 host species. We identified *H. arboricola* from 212 field-collected specimens and 575 museum study skins and *N. pacificus* from 72 field-collected hosts and 106 museum study skins. Co-infestations occurred in 37 field-collected specimens and 33 museum study skins.

Overall pinworm prevalence was 59.9% and we recovered and identified pinworms from 17 of 19 species examined. One pinworm, *H. cucullatum*, was recovered from 16 host species, while *R. eutamii* was present in 10 host species, and *D. grundmannii* was not detected. We identified *H. cucullatum* from 349 host individuals and *R. eutamii* from 127 individuals, with 61 co-infected individuals. Pinworms were recovered from *T. ochrogenys* hosts that are not *H. cucullatum* or *R. eutamii*, but have not been identified.

Although our sampling was extensive, further field collection is needed, particularly for the 9 host species with fewer than 10 individuals examined for both ecto- and endoparasites. While we are not the first to take advantage of museum collections to recover lice (see Hellenthal and Price, 1991; Clayton and Walther, 1997), this investigation further supports the overarching importance of natural history collections and their role in documenting biodiversity, integrating across taxonomic groups, and

addressing unanticipated questions. Using these archives, we expanded host and geographic sampling and documented sucking lice occurrence as early as 1897 from *T. bulleri* in the United States National Museum of Natural History. We anticipate sequencing DNA from some of the lice collected from museum skins for phylogenetic investigations. Existing museum specimens are valuable, but do not obviate the need for expanded collecting efforts. Extensive fieldwork is necessary to rigorously survey parasite diversity for a variety of hosts from across the globe. Documenting changes through time can only be accomplished if we continually collect and archive specimens in museums.

Biodiversity inventories too often miss parasites, one of the primary integrating components of ecosystems (Brooks and Hoberg, 2000). Contemporary climate change is driving range shifts for many taxa (Parmesan and Yohe, 2003; Mortiz et al., 2008) and is likely impacting host-parasite assemblages now, as it has in the past (Kutz et al., 2005; Parmesan, 2006; Brooks and Hoberg, 2007; Hoberg and Brooks, 2008; Lafferty, 2009; Ostfeld, 2009). To understand these dynamics, we must determine the current and, if possible, historic distributions of parasites. The chipmunk-louse-pinworm system is ideal for tracking host-parasite dynamics because the hosts are widespread in western North America, parasite prevalence is high, chipmunk specimens are relatively well-represented in museum collections, and some chipmunk populations and ranges appear to be responding to climate change (Moritz et al., 2008; Myers et al., 2009; Rubidge et al., 2011). Documenting distributions with specimens is necessary to understand the biological shifts on our changing planet and, more importantly, provides critical

background knowledge and sample availability for future investigations of phylogenetics, phylogeography, and evolution.

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Table 1. Previous records of chipmunk sucking lice (*Hoplopleura arboricola*, *Neohaematopinus pacificus*) and pinworms (*Heteroxynema cucullatum*, *Rauschtineria eutamii*, *Dentostomella grundmannii*). Only host species with records are represented. Numbers in the columns refer to the publications listed below the table where the parasite occurrence was documented.

Tamias host species	*H. arboricola* reported	*N. pacificus* reported	*H. cucullatum* reported	*R. eutamii* reported	*D. grundmannii* reported
T. alpinus	1, 2	1, 2			
T. amoenus	1, 2	1, 2	4, 8	6, 8, 9	
T. dorsalis	1, 2	1, 2		6	
T. merriami	1, 2	1, 2		42	
T. minimus	1, 2, 3	1, 2, 3	9	5, 6	
T. ochrogenys	1, 2				
T. palmeri			10	10	
T. panamintinus			10	10	
T. quadrivittatus	1, 2	1, 2			
T. speciosus	1, 2	1, 2			
T. townsendii	1, 2	1, 2			
T. umbrinus		3			7

[1] Kim et al., 1986; [2]Durden and Musser, 1994; [3]Kucera et al., 2007; [4] Hall, 1916; [5]Tiner, 1948; [6]Frandsen and Grundmann, 1961; [7]Chitwood, 1963; [8]McBee and Hendricks, 1973; [9]Kennedy, 1986; [10]Archie et al., 1988

Table 2. Number of *Tamias* specimens of each species examined and the species of sucking lice (*Hoplopleura arboricola*, *Neohaematopinus pacificus*) and pinworms (*Heteroxynema cucullatum*, *Rauschtineria eutamii*) detected.

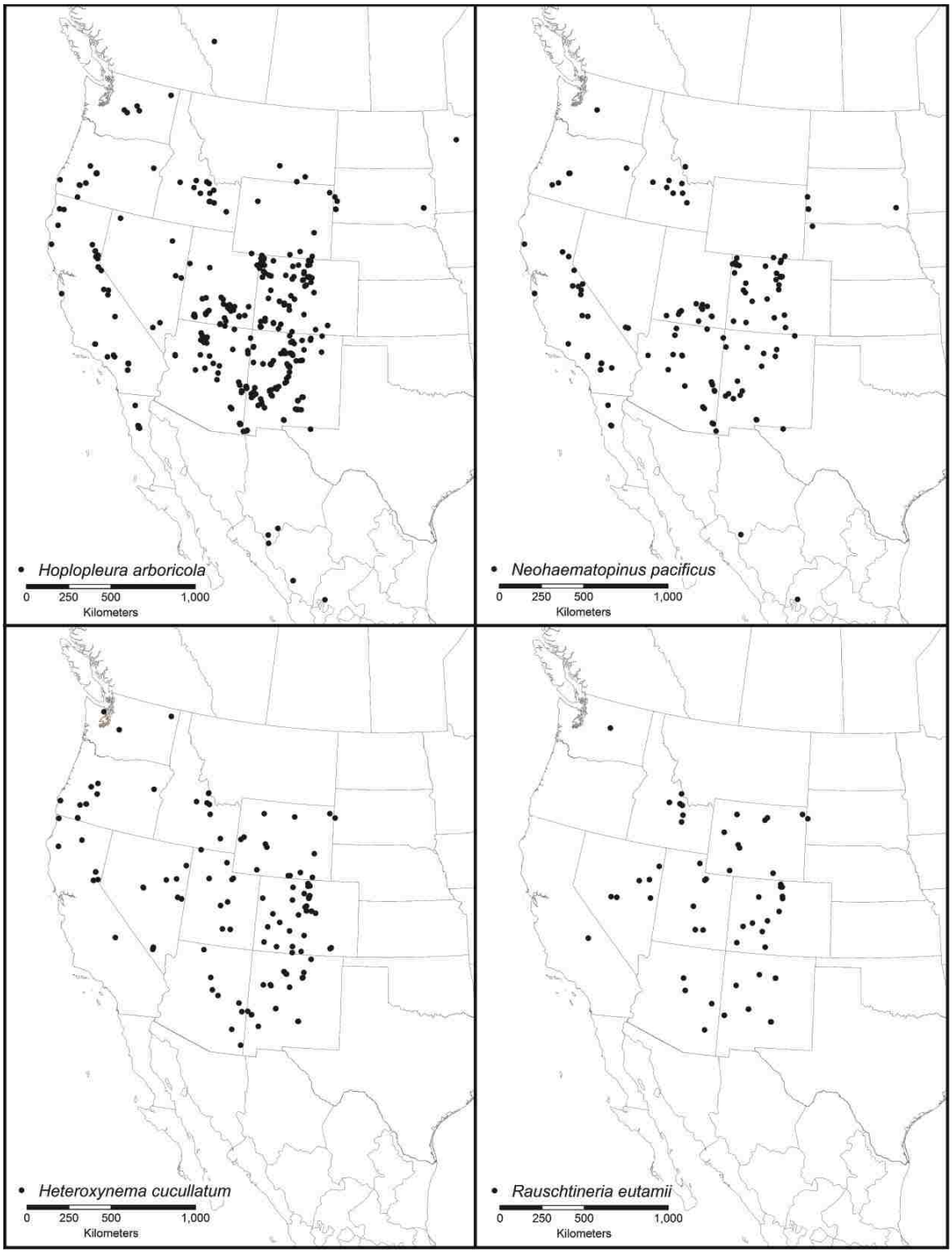
Tamias host species	No. field collected	No. museum study skins	Lice detected	Pinworms detected
T. alpinus	6	8	*H. arboricola** *N. pacificus*	*R. eutamii*
T. amoenus	127	26	*H. arboricola** *N. pacificus*	*H. cucullatum** *R. eutamii*
T. bulleri	0	17	*H. arboricola* *N. pacificus*	not examined
T. canipes	17	80	*H. arboricola* *N. pacificus*	*H. cucullatum** *R. eutamii*
T. cinereicollis	37	205	*H. arboricola** *N. pacificus*	*H. cucullatum** *R. eutamii*
T. dorsalis	55	404	*H. arboricola** *N. pacificus*	*H. cucullatum** *R. eutamii*
T. durangae	0	12	*H. arboricola* *N. pacificus*	not examined
T. merriami	1	42	*H. arboricola* *N. pacificus*	not examined
T. minimus	295	401	*H. arboricola**	*H. cucullatum**

			N. pacificus	*R. eutamii*
T. obscurus	6	65	*H. arboricola**	not examined
			N. pacificus	
T. ochrogenys	10	0	*H. arboricola**	none detected
			N. pacificus	
T. palmeri	10	7	*H. arboricola**	*H. cucullatum*
			N. pacificus	
T. panamintinus	7	0	*H. arboricola*	*H. cucullatum*
T. quadrimaculatus	1	11	*H. arboricola*	none detected
			N. pacificus	
T. quadrivittatus	66	417	*H. arboricola**	*H. cucullatum**
			N. pacificus	*R. eutamii*
T. ruficaudus	7	3	*H. arboricola*	*H. cucullatum*
T. rufus	12	168	*H. arboricola*	*H. cucullatum**
			N. pacificus	*R. eutamii*
T. senex	10	5	*H. arboricola*	*H. cucullatum*
T. siskiyou	34	8	*H. arboricola**	*H. cucullatum*
			N. pacificus	
T. sonomae	5	2	*H. arboricola*	*H. cucullatum*
T. speciosus	27	38	*H. arboricola**	*H. cucullatum**
			N. pacificus	*R. eutamii*
T. townsendii	35	20	*H. arboricola**	*H. cucullatum*
			N. pacificus	

T. umbrinus	74	286	*H. arboricola**	*H. cucullatum**
			N. pacificus	*R. eutamii*

*Indicates at least one co-infestation or co-infection was detected in that host species.

Figure 1. Point localities for each of the four focal parasite species. Sucking lice are top panels *Hoplopleura arboricola* (left) and *Neohaematopinus pacificus* (right) and pinworms are bottom panels, *Heteroxynema cucullatum* (left) and *Rauschtineria eutamii* (right).


• Hoplopleura arboricola
0 250 500 1,000
Kilometers
• Neohaematopinus pacificus
0 250 500 1,000
Kilometers
• Heteroxynema cucullatum
0 250 500 1,000
Kilometers
• Rauschtineria eutamii
0 250 500 1,000
Kilometers

Supplement 1

Lists of the catalog numbers for the hosts that each parasite species was recovered from. Institution catalog abbreviations are designated in parentheses.

Anoplura, unidentified nymphs

Denver Museum of Nature & Science Mammal host catalog numbers (ZM)

11108, 11109, 11110, 11116, 11117, 11120, 11124, 11126, 11127, 11128, 11129, 11130, 11131, 11132, 11133, 11136, 11137, 11139, 11143, 11144, 11145, 11146, 11147, 11148, 11149, 11150, 11151, 11152, 11153, 11154, 11155, 11156, 11157, 11158, 11159, 11160, 11161, 11162, 11163, 11165, 11166, 11167, 11168, 11169, 11170, 11171, 11172, 11173, 11174, 11180, 11181, 11182, 11183, 11184, 11188, 11189, 11190, 11394, 11404, 11414, 11421, 11427, 11428, 11433, 11541, 11542, 11544, 11548, 11596, 11626, 11652, 11656, 11668, 11669, 11670, 11671, 11673, 11674, 11678, 11681, 11682, 11684, 11685, 11686, 11687, 11689, 11691, 11692, 11694, 11695, 11696, 11697, 11698, 11699, 11701, 11702, 11792, 11794, 11795, 11798, 11800, 11801, 11802, 11804, 11806, 11808, 11814, 11817, 11822, 11823, 11825, 11827, 11828, 11829, 11830, 11831, 11838, 11839, 11840, 11841, 11842, 11843, 11844, 11845, 11846, 11848, 11849, 11852, 11853, 11854, 11867, 11872, 11873, 11874, 11875, 11877, 11878, 11879, 11880, 11881, 11913, 11914, 11916, 11917, 11921, 11924, 11925, 11926, 11929, 11930, 11931, 11933, 11934, 11941, 11956, 11969, 11981, 11982, 11989, 12026, 12039, 12041, 12042, 12067, 12068, 12086, 12087, 12088, 12090, 12091, 12093, 12113, 12115, 12120, 12121, 12125, 12129, 12130, 12131, 12133, 12137, 12139, 12140, 12142, 12143, 12144, 12145, 12151, 12153, 12154, 12156, 12157, 12158, 12159, 12160, 12161, 12162, 12163, 12165, 12168, 12171, 12172, 12173, 12174, 12175, 12176, 12177, 12178, 12179, 12180, 12181, 12182, 12183, 12184, 12185, 12186,

12187, 12188, 12189, 12190, 12191, 12192, 12193, 12194, 12195, 12196, 12197, 12198, 12200, 12201, 12202, 12203, 12204, 12205, 12206, 12207, 12208, 12209, 12210, 12211, 12212, 12213, 12214, 12215, 12216, 12217, 12218, 12220, 12221, 12222, 12223, 12224, 12229, 12233, 12235, 12281, 12284, 12285, 12286, 12288, 12289, 12306, 12334, 12335, 12355, 12452, 12478, 12957, 13094, 13105, 13125, 13694, 13714, 13741, 11208, 12232

Moore Laboratory of Zoology Mammal host catalog numbers (MLZ)

535, 980

Museum of Southwestern Biology Mammal host catalog numbers (MSB)

744, 2973, 2979, 2999, 3049, 3195, 3287, 3391, 9311, 10066, 11598, 11603, 18506, 32976, 35073, 40097, 40353, 43023, 43070, 43254, 47363, 47372, 47402, 47404, 47413, 53291, 56776, 56779, 57919, 59045, 60578, 60580, 65685, 69531, 69539, 69543, 69557, 73507, 73717, 73722, 81242, 86118, 89544, 91559, 100179, 100206, 100243, 100250, 100260, 101711, 101739, 102277, 102328, 102329, 102924, 103030, 103170, 103605, 104129, 104260, 104487, 105757, 106116, 107400, 107711, 107714, 107941, 108826, 108827, 109113, 109487, 109489, 110335, 112082, 112926, 113014, 113601, 113635, 113699, 113748, 113943, 114022, 114543, 115688, 115770, 115776, 115779, 115787, 115819, 115829, 116279, 116412, 116707, 118472, 119048, 119998, 120386, 120424, 120486, 120497, 120502, 120548, 121130, 121155, 121187, 121422, 121446, 122298, 122437, 122445, 122446, 123421, 123603, 123607, 123609, 138480, 138739, 140219, 140220, 144875, 155402, 191954, 191957, 191962, 191979, 191987, 191995, 192006, 192009, 192028, 192060, 192064, 192074, 192077, 192079, 192080, 192081, 192089, 192091, 192111, 192114, 192121, 192122, 192126, 192129, 192130, 192131, 192134,

192142, 192146, 192152, 192154, 192156, 192170, 192197, 192212, 192213, 192217, 192243, 192245, 192248

United States National Museum Mammal host catalog numbers (USNM)

115939, 398282

Hoplopleura arboricola

Denver Museum of Nature & Science Mammal host catalog numbers (ZM)

11026, 11027, 11032, 11038, 11096, 11097, 11098, 11099, 11100, 11101, 11102, 11113, 11115, 11123, 11134, 11135, 11202, 11203, 11204, 11205, 11206, 11378, 11393, 11395, 11398, 11401, 11405, 11406, 11407, 11408, 11409, 11413, 11415, 11417, 11418, 11419, 11420, 11422, 11424, 11425, 11426, 11430, 11431, 11436, 11437, 11498, 11499, 11578, 11649, 11653, 11654, 11693, 11805, 11816, 11819, 11821, 11824, 12069, 12070, 12089, 12108, 12122, 12134, 12150, 12155, 12164, 12167, 12169, 12170, 12351, 12359, 12363, 12367, 12395, 12396, 12397, 12398, 12399, 12404, 12406, 12408, 12415, 12428, 12429, 12430, 12436, 12437, 12443, 12444, 12445, 12446, 12960, 12968, 12971, 12979, 12983, 12989, 13011, 13012, 13024, 13026, 13060, 13061, 13088, 13089, 13090, 13096, 13098, 13099, 13113, 13116, 13122, 13136, 13693, 13695, 13718, 13720, 13722, 13739, 13754, 13953, 13955, 13956, 13963, 13964, 13998

Moore Laboratory of Zoology Mammal host catalog numbers (MLZ)

532, 536, 537, 538, 539, 541, 542, 545, 549, 981, 1034, 1035, 1041, 1044, 1902

Museum of Southwestern Biology Mammal host catalog numbers (MSB)

741, 743, 1502, 1506, 1674, 1675, 1685, 1866, 2088, 2235, 2236, 2237, 2245, 2317, 2914, 2961, 2971, 2972, 2975, 2982, 2986, 3041, 3065, 3066, 3067, 3068, 3124, 3125, 3127, 3129, 3133, 3138, 3142, 3174, 3175, 3177, 3178, 3179, 3180, 3181, 3183, 3185,

3187, 3188, 3190, 3191, 3192, 3194, 3279, 3286, 3351, 3357, 3392, 3393, 3394, 3395, 3396, 3397, 3398, 3399, 3401, 3409, 3419, 3442, 3573, 4036, 4037, 4039, 4993, 5009, 5011, 5187, 5193, 5395, 5528, 5532, 5533, 5970, 5971, 6886, 7585, 7586, 7914, 9141, 9142, 9868, 10051, 10077, 10148, 11022, 11890, 14303, 15603, 15841, 16536, 16716, 16717, 18200, 18503, 18505, 18671, 21016, 21043, 22314, 22425, 23407, 23408, 23436, 25232, 27767, 29533, 30415, 32975, 35643, 37615, 37636, 37640, 38003, 38008, 40101, 40351, 40356, 40359, 40433, 43024, 43026, 43068, 43176, 43178, 43183, 43241, 43242, 43252, 43253, 43256, 43257, 43296, 43327, 43328, 43430, 43677, 43678, 47352, 47360, 47365, 47371, 47373, 47403, 47418, 47422, 47424, 47432, 47433, 47437, 49767, 54680, 54757, 54859, 55473, 55759, 56901, 57927, 57931, 57937, 57942, 57944, 57953, 57954, 57955, 57956, 57957, 57982, 58028, 58029, 58031, 58032, 58033, 58041, 58042, 58047, 58048, 58049, 58050, 58051, 59003, 59004, 59005, 59006, 59007, 59046, 59047, 59362, 61634, 61635, 63639, 63640, 63642, 64556, 64557, 64558, 64562, 65689, 66861, 66864, 69530, 69533, 69534, 69535, 69542, 69555, 73716, 77852, 79071, 82127, 82128, 82129, 82130, 82131, 84978, 86620, 89260, 89547, 89548, 89550, 90852, 90983, 99235, 100178, 100209, 100210, 100211, 100212, 100214, 100238, 100239, 100242, 100263, 100265, 101720, 101723, 101732, 101735, 101741, 101744, 101761, 101968, 102326, 102327, 102330, 102333, 102688, 102751, 102923, 102925, 102926, 102927, 103608, 103616, 103995, 104245, 104247, 104270, 104485, 104491, 104658, 104878, 104879, 104881, 105511, 105761, 105762, 105763, 105906, 106108, 106109, 106111, 106113, 106114, 106681, 107395, 107396, 107397, 107712, 107785, 107786, 107936, 107937, 107938, 108490, 108824, 108825, 108882, 108884, 108885, 108886, 109267, 109486, 109488, 109516, 109517, 109554, 109962, 110608, 110659, 110660, 111069, 111070,

111071, 111139, 111252, 111253, 111329, 111531, 111567, 111575, 111628, 111635, 111742, 111743, 111778, 112020, 112130, 112131, 112168, 112202, 112203, 112388, 112389, 112390, 112391, 112392, 112395, 112396, 112397, 112501, 112502, 112638, 112639, 112640, 112643, 112644, 112645, 112646, 112648, 112650, 112732, 112791, 113252, 113253, 113256, 113301, 113302, 113303, 113537, 113538, 113604, 113628, 113698, 113700, 113703, 113724, 113725, 113726, 113818, 113935, 113937, 114024, 114082, 114544, 114545, 114664, 114912, 114932, 115089, 115684, 115692, 115769, 115774, 115775, 115778, 115782, 115783, 115789, 115810, 115812, 115818, 115820, 115825, 116234, 116236, 116237, 116333, 116334, 116335, 116337, 116338, 116379, 116380, 116381, 116390, 116391, 116410, 116411, 116707, 118409, 118473, 118512, 118557, 118558, 118560, 119000, 120018, 120375, 120377, 120381, 120383, 120385, 120413, 120423, 120443, 120469, 120499, 120500, 120504, 120506, 120507, 120605, 120610, 120975, 120976, 120977, 121021, 121025, 121156, 121157, 121159, 121160, 121165, 121173, 121185, 121208, 121238, 121262, 121263, 121344, 121403, 121410, 121421, 121424, 121665, 121729, 122418, 122432, 122436, 122437, 122447, 122448, 122545, 123289, 123600, 123605, 123606, 123607, 123608, 123609, 123914, 123935, 123949, 123959, 123991, 123992, 123998, 123999, 140212, 140213, 140867, 155404, 156919, 157152, 191920, 191921, 191922, 191923, 191927, 191928, 191952, 191956, 191963, 191964, 191966, 191972, 191978, 191981, 191986, 191992, 191994, 191999, 192000, 192004, 192024, 192026, 192033, 192041, 192046, 192055, 192059, 192063, 192066, 192082, 192083, 192113, 192119, 192124, 192132, 192133, 192141, 192144, 192151, 192155, 192165, 192169, 192171, 192172, 192175, 192177, 192191, 192192, 192193, 192203, 192207, 192211, 192214, 192215, 192216, 192218, 192220, 192221,

192222, 192224, 192225, 192228, 192229, 192230, 192231, 192232, 192234, 192236, 192240, 192249, 192250, 192251, 196598, 196601, 196607, 196617, 198461, 199686, 225676, 227184, 227219, 230567, 230608, 233557, 233567, 233570, 233587, 233631, 233634, 233636, 233645, 233654, 248151, 248964, 248977, 248982, 248984, 248986, 248999, 249000, 249012, 249014, 249969, 249972, 249973, 249979, 249981, 249984, 249985, 249986, 249988, 249989, 249991, 250115, 259302, 259308, 259329, 259341, 259349, 259354, 262521, 262529, 262537, 262540, 264105, 264107, 264114, 264300, 265604, 265608, 265609, 265906, 265937, 265940, 265940, 269057, 269057, 269638, 269638, 269654, 269654, 269656, 269656, 269658, 269658, 269678, 269678, 269680, 269680, 269855, 269855, 269856, 269856, 269858, 269858, 269869, 269869, 269870, 269870, 269871, 269871, 270057, 270057, 270059, 270059, 270060, 270060

Museum of Vertebrate Zoology Mammal host catalog Numbers (MVZ)

225305, 225306, 225307, 225309, 225310, 225311, 225312, 225313, 225315, 225316, 225318, 225323, 225326

United States National Museum Mammal host catalog numbers (USNM)

90850, 90851, 90853, 90855, 90856, 91967, 91968, 91970, 91975, 91976, 91977, 91978, 101066, 101144, 101253, 193140

Neohaematopinus pacificus

Denver Museum of Nature & Science Mammal host catalog numbers (ZM)

11026, 11027, 11111, 11112, 11115, 11379, 11380, 11381, 11395, 11413, 11417, 11420, 11422, 11430, 11432, 11499, 11534, 11585, 11657, 11980, 12025, 12043, 12089, 12094, 12108, 12138, 12155, 12363, 12428, 12960, 12963, 13012, 13088, 13095, 13112, 13114, 13720, 13722, 13739

Moore Laboratory of Zoology Mammal host catalog numbers (MLZ)

540, 1043

Museum of Southwestern Biology Mammal host catalog numbers (MSB)

1608, 2245, 3127, 3350, 3358, 5011, 5351, 6886, 7014, 7586, 11997, 15047, 18507, 18755, 20507, 21043, 40363, 43069, 43180, 43259, 43430, 47364, 47378, 47386, 47410, 47411, 47417, 47428, 47429, 47430, 56771, 56775, 56899, 57942, 57973, 58041, 58044, 58048, 58049, 58051, 58996, 58998, 59001, 59007, 61392, 62087, 66861, 66862, 82098, 84515, 101732, 101742, 101764, 101765, 102275, 102325, 102862, 104488, 107713, 107716, 108665, 108763, 108764, 109552, 109553, 110339, 112021, 112150, 112642, 113729, 113820, 115689, 115821, 115825, 116358, 118542, 120465, 120500, 120507, 120540, 120976, 121173, 121238, 121275, 121347, 122297, 122418, 123608, 140213, 191914, 191918, 191931, 191959, 192001, 192019, 192022, 192041, 192057, 192066, 192070, 192084, 192089, 192115, 192116, 192132, 192133, 192172, 192194, 192206, 192247, 224838, 225555, 233540, 233636, 248964, 249001, 249974, 249979, 249980, 250116, 264027, 265609, 265945, 265945, 269174, 269174, 269656, 269656, 269857, 269857, 270042, 270042, 270049, 270049, 270057, 270057, 270059, 270059

Museum of Vertebrate Zoology Mammal host catalog numbers (MVZ)

225304, 225305, 225306, 225309, 225310, 225311, 225312, 225314, 225319, 225323, 225324, 225326

United States National Museum Mammal host catalog numbers (USNM)

118916, 100640, 91973

Heteroxynema cucullatum

Denver Museum of Nature & Science Mammal host catalog numbers (ZM)

11099, 11100, 11101, 11109, 11113, 11115, 11116, 11118, 11123, 11125, 11129, 11132, 11135, 11136, 11137, 11138, 11139, 11140, 11142, 11147, 11148, 11149, 11160, 11161, 11162, 11163, 11166, 11167, 11168, 11169, 11171, 11172, 11173, 11174, 11184, 11205, 11207, 11208, 11380, 11381, 11400, 11401, 11407, 11409, 11413, 11420, 11426, 11428, 11430, 11431, 11433, 11453, 11498, 11583, 11596, 11605, 11611, 11625, 11627, 11649, 11650, 11651, 11654, 11668, 11669, 11670, 11671, 11672, 11675, 11686, 11687, 11689, 11694, 11701, 11792, 11793, 11794, 11796, 11798, 11799, 11800, 11807, 11808, 11817, 11818, 11819, 11820, 11822, 11826, 11830, 11837, 11841, 11842, 11843, 11844, 11846, 11847, 11849, 11850, 11852, 11853, 11854, 11867, 11872, 11873, 11874, 11875, 11881, 11914, 11919, 11921, 11924, 11929, 11930, 11931, 11932, 11934, 11941, 11969, 11979, 11980, 11981, 11982, 11983, 11989, 12026, 12028, 12041, 12044, 12067, 12086, 12087, 12088, 12089, 12090, 12091, 12092, 12108, 12115, 12121, 12122, 12130, 12132, 12154, 12155, 12157, 12158, 12163, 12164, 12165, 12166, 12167, 12168, 12169, 12171, 12172, 12173, 12174, 12178, 12179, 12180, 12182, 12183, 12185, 12188, 12189, 12190, 12191, 12193, 12198, 12199, 12207, 12208, 12209, 12210, 12211, 12212, 12220, 12224, 12230, 12231, 12289, 12345, 12347, 12348, 12355, 12359, 12361, 12363, 12365, 12370, 12375, 12377, 12378, 12390, 12420, 12428, 12430, 12443, 12979, 12983, 12987, 12989, 12991, 12993, 12995, 12997, 12999, 13011, 13024, 13098, 13112, 13113, 13114, 13115, 13122, 13124, 13125, 13716, 13722, 13741

Monte L. Bean Life Sciences Museum Mammal host catalog numbers (BYU)

35042, 35044, 35697, 35698, 35699, 35739, 35740, 35741, 35742, 35749, 35750, 35767, 36371, 36372

Museum of Southwestern Biology Mammal host catalog numbers (MSB)

230569, 230578, 233540, 233581, 233585, 233586, 233587, 233589, 233595, 233599, 233606, 233607, 233616, 233627, 233630, 233631, 233632, 233636, 233651, 233816, 248963, 248964, 248977, 248978, 248979, 248980, 248983, 248984, 248999, 249007, 249009, 249014, 249014, 249969, 249971, 249972, 249973, 249989, 249991, 259315, 259318, 259325, 259327, 259331, 259341, 259343, 259345, 259347, 259351, 262520, 262522, 262529, 262530, 262534, 262538, 262539, 262560, 262567, 262568, 262585, 263662, 263858, 263872, 264026, 264105, 264113, 264125, 264132, 264136, 264177, 264178, 264187, 264188, 264207, 264208, 264209, 265587, 265594, 265604, 265609, 266025, 267285, 269654, 269656, 269658, 269695, 269867, 269870, 269871, 269882, 270042, 270044, 270044, 270045, 270046, 270048, 270049, 270052, 270054, 270055, 270056

Museum of Vertebrate Zoology Mammal host catalog numbers (MVZ)

225310, 225311, 225314, 225317, 225321, 225324

Utah Museum of Natural History Mammal host catalog numbers (UMNH)

34471, 34474, 34488

Rauschtineria eutamii

Denver Museum of Nature & Science Mammal host catalog numbers (ZM)

11115, 11142, 11147, 11153, 11158, 11160, 11161, 11162, 11163, 11164, 11166, 11183, 11420, 11426, 11428, 11429, 11545, 11546, 11548, 11578, 11600, 11649, 11672, 11673, 11675, 11679, 11681, 11682, 11687, 11690, 11699, 11701, 11792, 11793, 11794, 11795, 11822, 11827, 11838, 11841, 11843, 11848, 11853, 11875, 11876, 11881, 11916, 11921, 11925, 11982, 12040, 12044, 12068, 12132, 12134, 12137, 12138, 12160, 12163, 12164, 12166, 12167, 12168, 12169, 12171, 12172, 12173, 12183, 12184, 12185, 12186, 12188,

12203, 12208, 12211, 12212, 12217, 12219, 12234, 12235, 12396, 12397, 12444, 12975, 13094, 13095

Monte L. Bean Life Sciences Museum Mammal host catalog numbers (BYU)

35698, 35699, 35739, 35741, 35749, 35751, 35754, 35767

Museum of Southwestern Biology Mammal host catalog numbers (MSB)

127121, 127124, 230567, 230578, 233623, 233628, 233634, 233646, 248978, 249014, 249991, 262520, 262535, 262537, 262538, 262540, 262585, 264113, 265940, 269855, 269856, 269857, 269858, 269868, 270041, 270042, 270045

Museum of Vertebrate Zoology Mammal host catalog numbers (MVZ)

225305, 225308, 225311, 225312, 225314, 225315, 225316, 225317, 225318, 225320, 225325

CHAPTER 2

TEMPORAL AND SPATIAL MOSAICS: DEEP HOST ASSOCIATION AND SHALLOW GEOGRAPHIC DRIVERS SHAPE GENETIC STRUCTURE IN A WIDESPREAD PINWORM, *RAUSCHTINERIA EUTAMII*

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ABSTRACT

Climate and host demographic cycling often shape both parasite genetic diversity and host distributions, processes that transcend a history of strict host-parasite association. We explored host associations and histories based on an evaluation of mitochondrial and nuclear sequences to reveal the underlying history and genetic structure of a pinworm, *Rauschtineria eutamii*, infecting 10 species of western North American chipmunks (Rodentia:*Tamias*, subgenus *Neotamias*). *Rauschtineria eutamii* contains divergent lineages influenced by the diversity of hosts and variation across the complex topography of western North America. We recovered six reciprocally monophyletic *R. eutamii* mitochondrial clades, largely supported by a multilocus concordance tree, exhibiting divergence levels comparable to intraspecific variation reported for other nematodes. Phylogenetic relationships among pinworm clades suggest that *R. eutamii* colonized an ancestral lineage of western chipmunks and lineages persisted during historical isolation in diverging *Neotamias* species or species groups. Pinworm diversification, however, is incongruent and asynchronous relative to host diversification. Secondarily, patterns of shallow divergence were shaped by geography through events of episodic colonization reflecting an interaction of taxon pulses and ecological fitting among assemblages in recurrent sympatry. Pinworms occasionally infect geographically proximal host species; however, host switching may be unstable or ephemeral, as there is no signal of host switching in the deeper history of *R. eutamii*.

KEYWORDS: chipmunk, ecological fitting, parasite, phylogeography, *Tamias*, taxon pulse, western North America

INTRODUCTION

Parasites have high, often cryptic, species diversity (Pérez-Ponce de León & Nadler, 2010), yet our understanding of the processes that drive high diversification is still developing. Historically, cospeciation, or association by descent with hosts (Fahrenholz's Rule), was assumed to be a major driver of parasite diversity (Eichler, 1948; Brooks, 1979; reviewed in Klassen, 1992). Codiversification has been proposed as a defining phenomenon and considered especially evident among obligate ectoparasites (e.g. Lyal, 1986; Johnson & Clayton, 2003; Timm 1983; Hafner *et al*., 1994; Hafner *et al*., 2003) and there is evidence consistent with codivergence in endoparasitic pinworms (e.g. Hugot, 1999, 2003). Apparent congruence in host and parasite phylogenies (a primary prerequisite for recognition of cospeciation sensu Brooks, 1979) outlined in these and other studies may suggest that taxa are associated by descent. Detailed investigation across a diverse assemblage of host-parasite systems, however, indicates that diversification in coassociated lineages is mechanistically complex. Coaccommodation, the microevolutionary counterpart to cospeciation, and colonization processes are strongly interactive across events in evolutionary and ecological time (e.g., Brooks, 1979; Hoberg & Brooks, 2008; Brooks, Hoberg, & Boeger, 2015). Observations and an expanding network of empirical data emphasize complexity in diversification with faunal assembly driven by hosts, parasites, biogeography, ecology, and history (e.g., Hoberg *et al*., 2012).

An emerging synthesis for parasite diversification and faunal assembly, the Stockholm Paradigm (Brooks *et al*., 2015; Hoberg & Brooks, 2015; Hoberg *et al*., 2015; Araujo *et al*., 2015), integrates geography, ecology, and evolution as drivers of the

dynamic origins and persistence of biodiverse systems across evolutionary and ecological time (Brooks & McLennan, 2002; Brooks *et al*., 2006; Agosta, Janz, & Brooks, 2010; Hoberg & Brooks, 2010). Episodes of biotic expansion and isolation lead to complex faunal assembly involving mosaics that vary across evolutionary, ecological, and geographic space and these processes are not strictly dictated by parasite specificity (Hoberg & Brooks, 2013; Hoberg *et al*., 2012). Consequently, parasites may be restricted to a particular host or spectrum of hosts during periods of climatological (and ecological) stability, possibly leading to specialization, although perturbation is predicted to alter these dynamics providing opportunity for parasite expansion into new hosts.

Host switching as a driver for diversification requires successful establishment within the new host and subsequent persistence of parasite lineages over space and time. Accordingly, modern investigations of parasite diversification should test potential roles of landscape and climate in structuring parasite diversity (see Hoberg, 1997, 2005; Hoberg & Klassen 2002, Koehler *et al*., 2009; Waltari *et al*., 2007; Galbreath & Hoberg, 2012, 2015). Those processes generally have been investigated in either one host and one parasite or multiple host taxa and their corresponding parasites. Relatively few studies (e.g., Wickström *et al*., 2001; Wickström *et al*., 2003; Haukisalmi, *et al*., 2015) have explored phylogenetic structuring of a single parasite across a widespread, diverse host group, yet such investigations provide exceptional opportunities to determine the relative roles of host association and geography in driving parasite diversity and distributions. Western North America is a topographically complex region where both biotic and abiotic landscapes were strongly shaped by climatic cycling during the Quaternary Period (Hewitt 2000; Brunsfeld *et al*., 2001; Swenson & Howard, 2005). The interplay of

climate cycling and geographic features has led to high morphological and genetic diversity of mammals and many taxa in western North America (Simpson, 1964; Riddle, 1996).

Western North American chipmunks (genus *Tamias* Illiger 1811, subgenus *Neotamias* Howell 1929; see Patterson & Norris, 2015 for proposed reclassification) are broadly distributed across > 40 degrees of latitude (Hall, 1981) and inhabit a variety of biomes, including desert scrub, boreal forest, temperate rain forest, alpine tundra, and isolated sky islands in the Southwest. The 23 species of *Neotamias* diverged relatively recently (~2.75 Myr), and are characterized by multiple episodes of hybridization and introgression (summarized in Sullivan *et al*., 2014). Parasites that infect this diverse clade of chipmunks offer an opportunity to investigate the roles of host association, host-parasite biogeographic history, and ecological perturbation in parasite evolution.

One species of pinworm (Oxyuroidea; Oxyuridae), *Rauschtineria eutamii* (Tiner, 1948; Hugot, 1980), was found to infect 10 chipmunk species (Bell *et al*., 2015). We have recovered *R. eutamii* from three of the five *Tamias* species groups (as defined with mitochondrial DNA in Piaggio & Spicer, 2001): *T. amoenus*, *T. minimus*, and *T. quadrivittatus*. Our investigations have not recovered *R. eutamii* among any of the five species constituting the *T. townsendii* group, suggesting that it may not infect species in that complex (Bell *et al*., 2015). Representatives from the remaining species group, *T. merriami*, were not examined in this study.

Initial observations suggest that occurrence of *R. eutamii* is the result of a colonization event of an ancestral lineage of western chipmunks (subgenus *Neotamias*) because there are no known pinworms associated with the other two species of *Tamias*

distributed in either Asia (*T. sibiricus*; Pisanu *et al*., 2007) or eastern North America (*T. striatus*; Snyder, 1982; Kennedy, 1986; Grear, Luong, & Hudson, 2013). *Rauschtineria* appears to be restricted to North America, as the only other known species of *Rauschtineria* infects other species of Nearctic ground squirrels (Hugot, 1980; Tiner & Rausch, 1950). Pinworms have a direct life cycle and a large portion of the transmission is likely vertical, with host offspring infected in the natal burrow. Because eggs are shed with host feces, there may be opportunities for pinworms to infect syntopic hosts and host switching has probably played a role in the evolution of some oxyurids (Okamoto *et al*., 2007; Okamoto, Urushima, & Hasegawa, 2009).

We hypothesize that the history of chipmunks across the topographically diverse landscape in western North America has led to pinworm diversification structured by biogeographic history and host associations, per the Stockholm Paradigm (Araujo *et al*., 2015; Hoberg & Brooks, 2015). Due to this complex history, we anticipate that the molecular phylogeny of *R. eutamii* will reveal multiple evolutionary and demographic processes and our predictions regarding the hypotheses are not mutually exclusive. Hypothesis 1**:** Parasite codiversification occurred during periods of host isolation and climate stability. This process will be supported if the *R. eutamii* phylogeny is structured by host (chipmunk) species associations deep in the tree, but does not preclude host switching of pinworm lineages. A pinworm phylogeny largely incongruent with the host phylogeny is inconsistent with the process of codiversification. Hypothesis 2: When host species expand resulting in secondary contact, distinct host-associated parasite lineages may switch to new host species. This process will be supported if the *R. eutamii* phylogeny has divergent, host-associated lineages nested within clades of *R. eutamii*

associated with another host species or species group (past host switching). Contemporary host switching will be evident if closely related or identical *R. eutamii* lineages from one host species are found to infect other, sympatric host species.

Hypothesis 3**:** As with many free-living organisms, we predict that biogeographic history is also structuring parasite diversity. This process will be supported if the *R. eutamii* phylogeny has geographic structure, either within host-associated structure or irrespective of hosts.

MATERIALS AND METHODS

Chipmunks were collected from across the western United States and examined for endoparasites following approved mammal handling and collecting protocols (Sikes *et al*., 2011). Recovered pinworms were preserved in 70% or 95% ethanol, frozen in liquid nitrogen, and later transferred to a -20C freezer. Specimens were numbered according to the host tissue number (e.g. NK or DZTM) and then sequentially for multiple pinworms examined from the same host (e.g. Re1, Re2). We generated partial mitochondrial cytochrome oxidase c subunit I (COI) sequences (767 bp) for 83 sexually mature pinworms from 73 host individuals (10 species) from 40 localities (Figure 1). We did not include more than two *R. eutamii* individuals from the same host individual for phylogenetic analyses and the COI gene tree was generated with sequences from 79 samples. Ribosomal RNA loci, 12S (487 bp) and 28S (763 bp), were also sequenced for a subset of individuals (27 and 25, respectively). Although rooting phylogenies with an outgroup is ideal, we were unable to locate a sample (e.g., *R. tineri*) with suitable DNA for sequencing, so phylogenetic trees were midpoint rooted, which is appropriate when there is not a suitable outgroup (Hess & de Moraes Russo, 2007). DNA extractions

consisted of excising the midportion of a worm and preserving both anterior and posterior ends as vouchers for archival deposition in museum collections. A few extractions used partial pinworms, leaving only an anterior or posterior voucher. All vouchers are deposited at either the Museum of Southwestern Biology or the Denver Museum of Nature & Science (Appendix I). The midportion was cut into at least three smaller pieces and extractions followed the protocols in the QIAamp DNA Mini extraction kit (Qiagen, Hilden, Germany), using carrier RNA at the AL Buffer step. Manufacturer's protocols were modified by heating and incubating the elution buffer on the membrane at 55C for five minutes. Final elution was 30-60uL per sample. All loci were PCR-amplified (primers COI: SyphaCOIF, SyphaCOIR (Okamoto *et al*., 2007); 12S: 12Sf, 12Sr (Casiraghi *et al*., 2004); 28S: C1, D2 (Gouÿ de Bellocq *et al*., 2001)), purified with polyethylene glycol precipitation, and cycle sequenced in both the forward and reverse direction with the same primers. Sequenced products were read on an ABI 3100 in the Molecular Biology Facility in the Department of Biology at the University of New Mexico. Sequence chromatograms were assembled, edited, and aligned using Sequencher version 5.1 (Gene Codes Corporation, Ann Arbor, MI USA). All sequences are available on GenBank (accessions COI: KT875241-KT875323; 12S: KU668406-KU668432; 28S: KU668379-KU668405; Appendix I).

We generated gene trees and a multi-locus concordance tree annotated with host species to test Hypotheses 1 and 2. We conducted Maximum Likelihood gene tree estimation in RAxML v.8 (Stamatakis, 2014) using a GTRCAT model and 10000 bootstrap replicates to assess support. Bayesian gene trees were generated using the reverse-jump search in MrBayes 3.2 (Ronquist *et al*., 2012), with four chains and two

runs for 20 million generations, sampling the trees and parameters every 500 generations. The first 20% of sampled trees were discarded as burn-in. Bayesian gene trees were combined using default settings in BUCKy (Ané *et al*., 2007; Larget *et al*., 2010) to assess concordance of clades across loci. All trees were visualized with midpoint rooting in FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

In addition to mapping clades from the phylogeny, we calculated diversity and population metrics to assess geographic structuring of diversity for Hypothesis 3. Uncorrected pairwise genetic distances were calculated in MEGA 6.06 (Tamura *et al*., 2013). Pairwise geographic distances were calculated from decimal latitude and longitude points in Geographic Distance Matrix Generator v1.2.3 (Ersts, http://biodiversityinformatics.amnh.org/open_source/gdmg/). Mantel tests of correlation between genetic and geographic distances (Mantel, 1967; Legendre & Legendre, 2012) were conducted using the vegan package (Oksanen *et al*., 2013) in R (R Core Team, 2014). These measures were used to determine if the level of genetic diversity is comparable to the host-level diversity and if there are genetic signals of geographic structure.

For a range of the host genetic divergences that the pinworm lineages are able infect, we estimated raw genetic distances between host *Tamias* species. These estimates are based on randomly selected cytochrome *b* sequences from seven individuals from GenBank for each of the seven species involved in host switches (Appendix II). There are not equal numbers of sequences available for each species (e.g., *T. rufus* only has seven available), so we randomly selected seven cytochrome *b* sequences for each species. We

used MEGA 6.06 to estimate raw distances between host species (Table 2 & Supplemental Table 3).

RESULTS

Methods for COI tree estimation resulted in similar topologies with six major clades (bootstrap support ≥ 70 or posterior probability support ≥ 0.9) for *R. eutamii* (QUAD-N, QUAD-M, QUAD-S, MIN, AMOEN, SPEC; Fig. 2). These clades largely support our first prediction of host-associated structure. Three *R. eutamii* clades were recovered from hosts in the Rocky Mountain region, primarily from the *T. quadrivittatus* species group (*T. canipes*, *T. cinereicollis*, *T. dorsalis*, *T. quadrivittatus*, *T. rufus*, *T. umbrinus*; Howell, 1929) (QUAD-N, n=10; QUAD-M, n=3; QUAD-S, n=13). A fourth clade is composed primarily of *R. eutamii* recovered from *T. minimus* (MIN, n=32). The fifth clade consists of *R. eutamii* from *T. amoenus* (AMOEN, n=10) and the sixth is from California composed of pinworms recovered from *T. speciosus* and *T. alpinus* (SPEC, n=10). Diversity and demographic analyses in Arlequin used these six clades as "populations". We recovered 45 unique haplotypes for *R. eutamii*. Average uncorrected pairwise sequence divergence between clades is 4.02% (1.80 - 4.93%). All clades are highly differentiated, with an overall Fst of 0.705 and pairwise Fst values ranging from 0.572 to 0.969.

The 12S gene tree (Supplemental Figure 1) supports most of the clades recovered in the COI gene tree, with the exception of the QUAD-N clade. The nuclear 28S gene tree did not recover any clades concordant with the COI gene (Supplemental Figure 2). The Bayesian concordance analyses (BUCKy) did not have high support (concordance factor ≥ 50) for all 6 of the COI clades, however the most common topology yielded five

of the same monophyletic clades as COI, again with QUAD-N as the exception (Figure 3).

Within three of the six clades (QUAD-M, MIN, SPEC; Figure 2) we recovered individual *R. eutamii* with COI sequences associated with a different host group, supporting our second prediction of recent host switching. All Mantel tests found significant ($p < 0.01$) correlation between geographic and genetic distance (prediction 3), however the coefficient (r) increased when the tests were conducted using the clade samples as subsets (Supplemental Table 1).

In several instances, geographic location and host sympatry may explain the distribution of pinworm lineages. Most of the hosts of the QUAD clades are the six species of the closely related *T. quadrivittatus* species group (Figure 2 inset; Reid, Demboski, & Sullivan, 2012), so we considered these lineages capable of infecting all species of *T. quadrivittatus* group without classifying it as a host switch. The QUAD-N and MIN clades appear to be in contact in western Wyoming (Table 2). Both clades were recovered from *T. umbrinus* hosts at a locality in Wyoming where no *T. minimus* were trapped (locality 10), although *T. minimus* occurs in the Wind River Range (Hall, 1981). The only host switch in any of the QUAD clades is into a *T. minimus* in Colorado (locality 29, DZTM529_Re1). There is one instance of geographically overlapping pinworm clades recovered from non-sister host species in Wyoming (DZTM273_Re1-MIN from *T. minimus* and DZTM267_Re2-QUAD-N from *T. umbrinus*). In Nevada, there are three instances of *R. eutamii* from the MIN clade being recovered from *T. umbrinus* and *T. minimus* hosts at the same locality (locality 19: DZTM599, DZTM603; locality 22: DZTM594, DZTM595; locality 26: DZTM587, DZTM588). Three additional

examples of pinworms in the MIN clade were recovered from other host species (DZTM187_Re1 from *T. rufus*; DSR11372_Re1 and DSR11363_Re1 from *T. dorsalis*) at localities where no pinworms were recovered from *T. minimus* or no *T. minimus* were collected (Supplemental Table 3), however both localities are within the range of *T. minimus* (Verts & Carraway, 2001). All pinworms recovered from *T. amoenus* formed a well-supported clade (AMOEN). The SPEC clade is composed of individuals from both *T. speciosus* and *T. alpinus*. The pinworms recovered from *T. alpinus* form a subclade within the SPEC clade, suggesting a recent host switch. All specimens in this clade were collected from the same locality.

DISCUSSION

The evolutionary history of *R. eutamii* reveals deep divergence events that appear to be due to host association, followed by a series of shallower divergence events and host switching episodes reflected in geographic genetic structure. Based on average pairwise sequence divergence between clades, deep divergence values (1.8-4.9%; Table 1) in *R. eutamii* may not reflect multiple cryptic species, as these values are well below the average pairwise sequence divergence (11% ± 2.9) reported for congeneric species of other nematodes (Herbert, Ratnasingham, & de Waard, 2003). Nonetheless, these lineages of *R. eutamii* have maintained independent evolutionary trajectories and formed long-term host associations. Relationships among clades do not mirror the relationships among the hosts (Figure 4), rejecting our first hypothesis that *R. eutamii* lineages codiversified with chipmunk species. Gene trees and species trees for western chipmunks often yield different topologies, however, deep relationships among *R. eutamii* clades are not congruent with relationships among the host species in available molecular

phylogenies of *Tamias* (Piaggio & Spicer, 2001; Reid, *et al*., 2012; Sullivan *et al*., 2014). With the exception of a single worm from *T. minimus*, three clades (QUAD-N, QUAD-M, QUAD-S) are composed of pinworms recovered from closely related host species (*T. quadrivittatus* species group) with divergence of those hosts estimated at 1.78 mya (Sullivan *et al*., 2014). The other three pinworm clades (MIN, AMOEN, SPEC) each were recovered primarily from a single host species, however, there is evidence of contemporary host switching of pinworm lineages into other sympatric host species.

In partial support of our second hypothesis, we detected contemporary host switching events in clades SPEC, MIN, and QUAD-M. The pinworms in QUAD-N, QUAD-M, and QUAD-S clades appear to be divided primarily by geography, not by affiliation with a single host species, although all QUAD-N hosts are *T. umbrinus*. It is possible that the pinworms in these three clades were not isolated with a single host species and have continuously infected these hosts since the three clades first diverged. As such, we did not consider the diversity of hosts within these clades as examples of host switching, instead, it is likely that the QUAD pinworm lineages have a host breadth that allows them to infect these closely related species (Choudry & Dick, 2001). Eight instances of pinworm host switching (from 73 examined host individuals) appear to be contemporary because the lineages are not divergent and the hosts are sympatric. We uncovered no contemporary evidence for past host switching in the form of lineages associated with one host species nested within clades associated with a different host.

The third prediction was supported by geographic structure within the host-associated clades. The three QUAD clades exhibit geographic structure among the clades, but also in substructure within each clade. Sampling in the QUAD-S clade includes

populations from the sky islands of the Southwest with substructure within the clade corresponding to expectations of isolation (e.g., locality 40, Pinaleño Mountains). However, the sample from the geographically isolated host species *T. canipes* (locality 39, Sacramento Mountains) are not as divergent as might be expected given that the host is a distinct species isolated from other chipmunk populations (Sullivan *et al*., 2014). Geographic structure within the MIN clade is less pronounced than structure recovered among the three QUAD clades, but MIN geographic structure may reflect montane isolation in Nevada, Utah, and Colorado. The AMOEN clade is geographically partitioned between eastern Idaho and central Washington, which also corresponds to a deep divergence in the hosts (J and B clades in Demboski & Sullivan, 2003).

The SPEC clade represents a single locality (34) and two host species, suggesting a recent switch from *T. speciosus* to *T. alpinus*, species that are geographically and elevationally adjacent (Heller, 1971; Walsh *et al*., 2016). *Tamias alpinus* apparently diverged from *T. minimus* as a peripheral isolate in the Sierra Nevada during the Pleistocene (~522 kyr; Sullivan *et al*., 2014; Rubidge, Patton, & Moritz 2014), however, pinworms for *T. alpinus* were not inherited from ancestral *T. minimus* as they were not nested within the MIN clade. This illustrates that some *R. eutamii* lineages are capable of recent switches to infect deeply divergent hosts (from *T. speciosus* to *T. alpinus*; Sullivan *et al*., 2014).

The six major clades are reciprocally monophyletic (Figure 2), however, patterns of differentiation among and within these clades are not congruent with our understanding of the phylogenetic structure of hosts based on species tree methods (Figure 1a in Sullivan *et al*. 2014), suggesting that pinworm lineages have not

codiversified with host species. While molecular dating to establish a timeline for divergence events is valuable, it is problematic in this system because there are no closely related taxa with robust estimates of mutation rates and no pinworm fossil record. Further, it is inappropriate to use the divergence estimates for mitochondrial genes in the host as a proxy, as the pinworms have not diversified at the same rates as their hosts (6 pinworm lineages, 10 host species), and chipmunks have a history of mitochondrial introgression (Sullivan *et al*, 2014). Without robust dates for parasite divergence events, we are neither able to determine when *R. eutamii* colonized *Neotamias*, nor identify historical processes that led to the host-associated lineages. The presence of *R. eutamii* in four major host clades (*T. minimus*, *T. speciosus*, *T. amoenus*, and *T. quadrivittatus* species group), no known records in the *T. townsendii* host species group, and an absence of pinworm records in other chipmunk species (*T. sibiricus* and *T. striatus*), are consistent with the hypothesis that *R. eutamii* colonized chipmunks after the divergence of the *T. townsendii* species group but prior to the diversification of the rest of *Neotamias*. However, species in the *T. townsendii* species group may host *R. eutamii*, or did in the past, but our sampling (95 individuals) remains insufficient to detect their presence (Bell *et al*., 2015).

All of the sampled hosts infected with *R. eutamii* in Nevada hosted the MIN lineage. We do not know the evolutionary or biogeographic history of chipmunks in Nevada, but pinworm lineage(s) associated with the other host species (the QUAD lineages) possibly were not part of the colonization(s) of this region by the *T. quadrivittatus* species group (i.e., a "missing the boat" event).

Individuals in the QUAD-M and QUAD-S clades both seem to be able to infect multiple members of the *T. quadrivittatus* host species group and the structure among these pinworms appears to be primarily geographic, as has been demonstrated in other parasite taxa (Catanach & Johnson, 2015). Furthermore, all the QUAD-N and QUAD-M localities and several of the QUAD-S localities are found within the geographic distribution of *T. minimus*, yet we only captured one instance of a switch to a *T. minimus* host in the QUAD clades.

Observed genetic structure in *R. eutamii* could be due to host specificity (see Brooks & McLennan, 2002). Alternatively, vertical transmission may present limited opportunities to switch to other species, serving as an encounter filter (Combes & Théron, 2000). For example, the seven instances of MIN individuals infecting other host species and the presence of only one clade between both host species in California indicate that *R. eutamii* lineages are capable of infecting other, often deeply divergent, host species. These data also suggest variation in the ability of pinworm lineages to infect multiple host species, which is consistent with the Ecological Fitting (Janzen, 1985) and Geographic Mosaic (Thompson, 2005) aspects of the Stockholm Paradigm (Hoberg & Brooks, 2015; Araujo *et al.*, 2015). The diversity of hosts in the QUAD-S clade indicates that at least some pinworms have a wide range of hosts they are capable of infecting. If *R. eutamii* lineages are able to easily switch to a new host and maintain infections across generations, then we should detect historic switches in our phylogeny. Instead, the host switches we uncovered may simply be opportunistic and ephemeral, representing a window in ecological time, rather than persistence and establishment (see Araujo *et al.*, 2015).

Associations of the six clades with a host species or host species group (except *R. eutamii* from *T. alpinus*) likely arose via past geographic isolation in hosts and these associations were maintained by mother to offspring transmission. High levels of differentiation between clades deep within the *R. eutamii* phylogeny (Figure 2) and the pinworm's ability to infect different host species suggests that isolation with the hosts may have been the original driver of *R. eutamii* diversification, but this does not entirely preclude the lineages from infecting other species of potential hosts where these are in secondary or recurrent contact. This scenario is consistent with the Stockholm Paradigm, current host-associated lineages represent the stability phase of the Taxon Pulse Hypothesis, while the contemporary host switches are consistent with expansion and Ecological Fitting (Agosta et al., 2010; Hoberg & Brooks, 2008, 2015; Araujo *et al*., 2015). Given that these host species arose and persisted during the Pleistocene (Sullivan *et al*., 2014), host-associated *R. eutamii* lineages are likely relatively young and seem to have remained demographically stable during the Pleistocene glacial cycles during which the hosts diversified.

A rich body of literature on phylogeography in western North America has illustrated that complex topography and Pleistocene glacial cycling played a large role in structuring the distributions of many species (Hewitt, 2000; Swenson & Howard, 2005). As with other studies (e.g. Galbreath, Hafner, & Zamudio, 2009; Shafer, Cote, & Coltman, 2011; Malaney, Frey, & Cook, 2012), our findings support a role of montane isolation in genetic structuring, however this is largely within the host associated genetic structure. The genetic structure in *R. eutamii* from the Rocky Mountains and Great Basin does not correspond to common breaks found in some other taxa in these regions (e.g.,

Swenson & Howard, 2005), although some of the breaks among the QUAD clades are similar to those identified in other species (e.g., *Zapus* spp. Malaney, *et al*., 2012; Malaney *et al*., 2013). Still, relatively few taxa have been sampled that encompass our northernmost and southernmost sampling, so our findings may correspond to substructure in additional species that has yet to be documented. There have been few phylogeographic studies of parasites in western North America, but there are examples illustrating the value of understanding parasite phylogeography in addition to hosts (e.g., Koehler *et al*., 2009; Galbreath & Hoberg, 2012). It is clear that host responses to climatic fluctuations structure parasite populations in ways that are not clearly delineated by hosts (Koehler *et al.*, 2009; Hoberg *et al*., 2012; Galbreath & Hoberg, 2015).

Overall, our results suggest that diversification in *R. eutamii* is dynamic and driven by host associations and biogeographic history of the pinworm and the hosts, consistent with the diversification of pinworms via mechanisms in the Stockholm Paradigm. Integration of four hypotheses and theories constitute the synthesis at the core of the Stockholm Paradigm: Ecological Fitting (Janzen, 1985); the Oscillation Hypothesis (Janz & Nylin, 2008); the Geographic Mosaic Theory of Coevolution (Thompson, 2005); and the Taxon Pulse Hypothesis (Erwin, 1985). Central to this synthesis is recognition of the importance of ecological perturbation and host colonization in diversification and processes for faunal assembly over time, which involves the interaction of opportunity and capacity (e.g., Hoberg & Brooks, 2008; Araujo *et al*., 2015). Opportunity is linked to the Taxon Pulse and episodic ecological disruption accompanied by geographic colonization or expansion countered by isolation and stability. During expansion and breakdown in ecological isolation, Ecological Fitting provides the capacity for host

switches through resource tracking (hosts with ancestral resources) or through exploitation of new resources in what is termed sloppy fitness space (Agosta & Klemens, 2008; Agosta *et al*., 2010). Episodic pulse dynamics and ecological fitting broaden host range and are the foundation for alternating patterns of generalization and specialization described under Oscillation. Host range expansion followed by fragmentation, isolation, and relative stability may drive origins of new specialists through cospeciation and microevolutionary processes of coaccommodation that are described in the Geographic Mosaic of Coevolution.

Geographic distributions, landscape setting, and host ecologies of *Neotamias* provide an ideal system to test the multiple drivers of parasite diversification, the ability of parasites to reveal host histories, and the impact of host hybridization on parasite diversification. Western chipmunks are infected with another species of pinworm (*Heteroxynema cucullatum*) that has been recovered from 16 host species and is more common across the host species distribution than *R. eutamii* (Bell *et al*., 2015). Not only could the increased prevalence and denser sampling for *H. cucullatum* potentially provide a more detailed signal of past pinworm-chipmunk interactions, but a history of shared ecological affinities of host species may be uncovered if we detect host switching between chipmunk species that are not currently sympatric. Additionally, a history of hybridization and mitochondrial capture events in chipmunks has added an interesting layer of complexity towards resolving the phylogenetic history of *Neotamias* (Piaggio & Spicer, 2000, 2001; Good *et al*., 2003, 2008; Reid *et al*., 2012; Sullivan *et al*., 2014). Utilizing robust species trees from both species of pinworms could potentially resolve some of the outstanding questions about the evolutionary history of *Neotamias*. A

comparative phylogeographic approach to host-parasite dynamics that focuses on these two pinworms could explore how pinworms evolve in similar environments (e.g. host ceca), as well as respond to similar host demography and episodic climate events.

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FIGURE LEGENDS

Figure 1. Map of sample localities in western North America. Symbols correspond to clade labels in Figures 2-4. Numbers correspond to tip labels on trees and localities in Appendix I.

Figure 2. COI gene tree. Support values above branches are posterior probabilities, values below are maximum likelihood bootstraps. Labels on the right correspond to clade names and symbols correspond to localities on map in Figure 1. Tip labels in bold are host switches, numbers at end of tip labels correspond to locality numbers in Figure 1. Top left inset is host species tree modified from Sullivan *et al*., 2014, gray circles represent posterior probability support ≥ 0.95.

Figure 3. Concordance tree of COI, 12S, and 28S. Stars on branches indicate concordance factors ≥ 0.5. Symbols to correspond to COI clades and numbers refer to localities in Figure 1. Top left inset legend for mitochondrial clades.

Figure 4. Tanglegram connecting *R. eutamii* concordance tree tips (left) to corresponding hosts on *Tamias* species tree (right). *Tamias* species tree modified from Sullivan et al., 2014. Top left inset legend of mitochondrial clades.

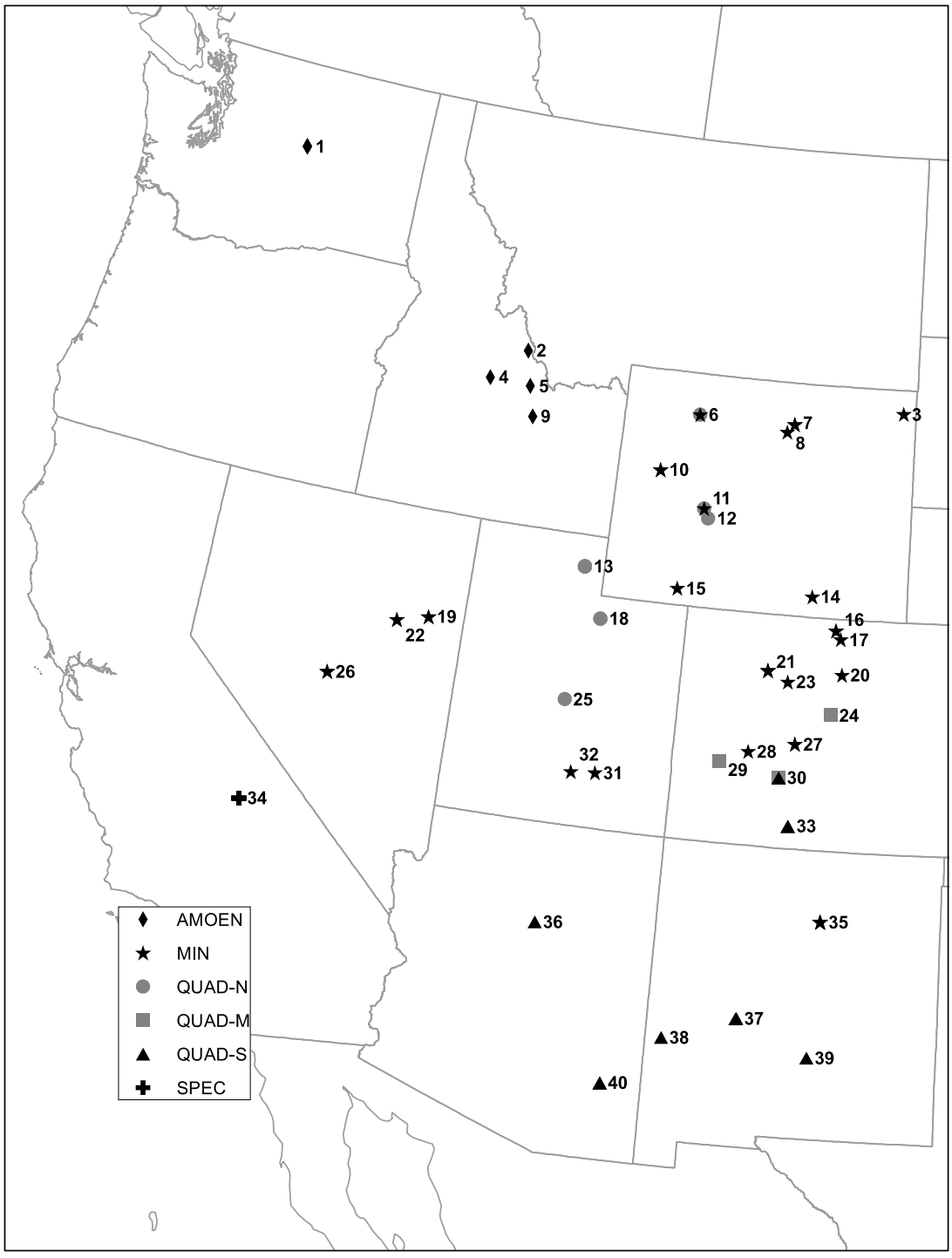
TABLES

Table 1. Pairwise raw genetic distance between mitochondrial clades.

	QUAD-N	QUAD-M	QUAD-S	MIN	AMOEN
QUAD-M	0.044				
QUAD-S	0.043	0.041			
MIN	0.049	0.049	0.043		
AMOEN	0.037	0.045	0.043	0.033	
SPEC	0.039	0.042	0.043	0.032	0.018

Table 2. Locations with multiple host species. Host genetic distance is mean distance between species based on available cytochrome *b* sequences on GenBank (Appendix II).

Locality	**Host species**	**Pinworm clade**	**Host switch**	**Host genetic distance**
11: WY, Park Co., Carter Mountain	*T. minimus* *T. umbrinus*	MIN QUAD-N	No	0.079
19: NV, Elko Co., Cherry Creek Mountains	*T. minimus* *T. umbrinus*	MIN MIN	Yes	0.079
34: CA, Mono Co., Cirque Lake	*T. alpinus* *T. speciosus*	CA CA	Yes	0.045
22: NV, White Pine Co., Ruby Mountains	*T. minimus* *T. umbrinus*	MIN MIN	Yes	0.079
26: NV, Nye Co., Toquima Range	*T. minimus* *T. umbrinus*	MIN MIN	Yes	0.079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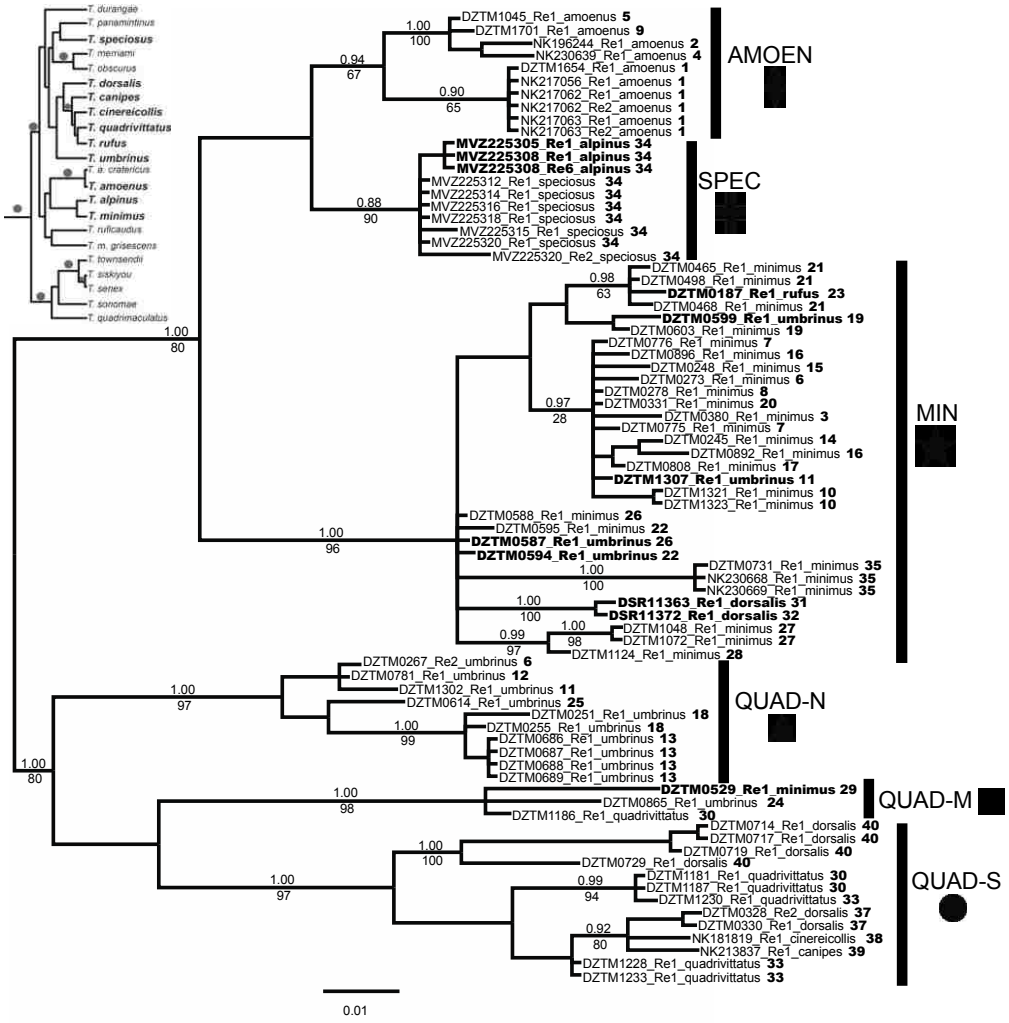
AMOEN
MIN
QUAD-N
QUAD-M
QUAD-S
SPEC

Figure 1


T. durangae
T. panamintinus
T. speciosus
T. meriami
T. obscurus
T. dorsalis
T. canipes
T. cinereicollis
T. quadrivittatus
T. rufus
T. umbrinus
T. u. cratericus
T. amoenus
T. alpinus
T. minimus
T. ruficaudus
T. m. grisescens
T. townsendi
T. siskiyou
T. senex
T. sonomae
T. quadrivittatus
DZTM1045_Re1_amoenus 5
DZTM1701_Re1_amoenus 9
NK196244_Re1_amoenus 2
NK230639_Re1_amoenus 4
DZTM1654_Re1_amoenus 1
NK217056_Re1_amoenus 1
NK217062_Re1_amoenus 1
NK217062_Re2_amoenus 1
NK217063_Re1_amoenus 1
NK217063_Re2_amoenus 1
AMOEN
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MVZ225320_Re2_speciosus 34
SPEC
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DZTM0187_Re1_rufus 23
DZTM0468_Re1_minimus 21
DZTM0599_Re1_umbrinus 19
DZTM0603_Re1_minimus 19
DZTM0776_Re1_minimus 7
DZTM0896_Re1_minimus 16
DZTM0248_Re1_minimus 15
DZTM0273_Re1_minimus 6
DZTM0278_Re1_minimus 8
DZTM0331_Re1_minimus 20
DZTM0380_Re1_minimus 3
DZTM0775_Re1_minimus 7
DZTM0245_Re1_minimus 14
DZTM0892_Re1_minimus 16
DZTM0808_Re1_minimus 17
DZTM1307_Re1_umbrinus 11
DZTM1321_Re1_minimus 10
DZTM1323_Re1_minimus 10
DZTM0588_Re1_minimus 26
DZTM0595_Re1_minimus 22
DZTM0587_Re1_umbrinus 26
DZTM0594_Re1_umbrinus 22
DZTM0731_Re1_minimus 35
NK230668_Re1_minimus 35
NK230669_Re1_minimus 35
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DSR11372_Re1_dorsalis 32
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DZTM1124_Re1_minimus 28
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DZTM1302_Re1_umbrinus 11
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DZTM0686_Re1_umbrinus 13
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DZTM0688_Re1_umbrinus 13
DZTM0689_Re1_umbrinus 13
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DZTM0865_Re1_umbrinus 24
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QUAD-S
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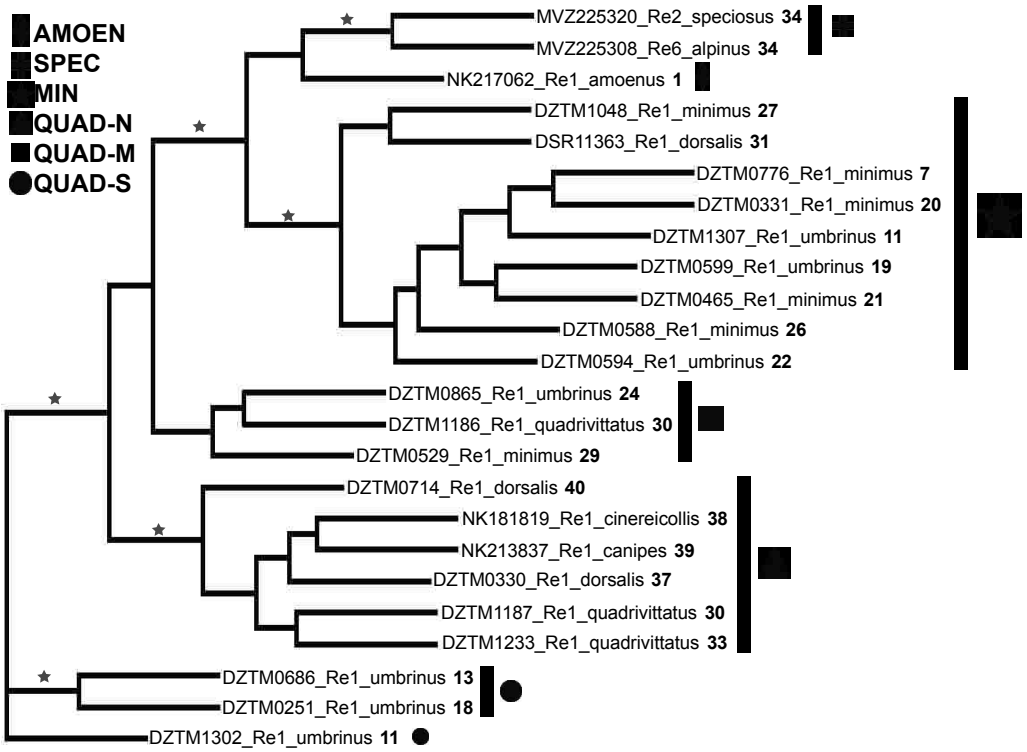
Figure 2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DSR11363_Re1_dorsalis 31
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DZTM1307_Re1_umbrinus 11
DZTM0599_Re1_umbrinus 19
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DZTM0529_Re1_minimus 29
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NK213837_Re1_canipes 39
DZTM0330_Re1_dorsalis 37
DZTM1187_Re1_quadrivittatus 30
DZTM1233_Re1_quadrivittatus 33
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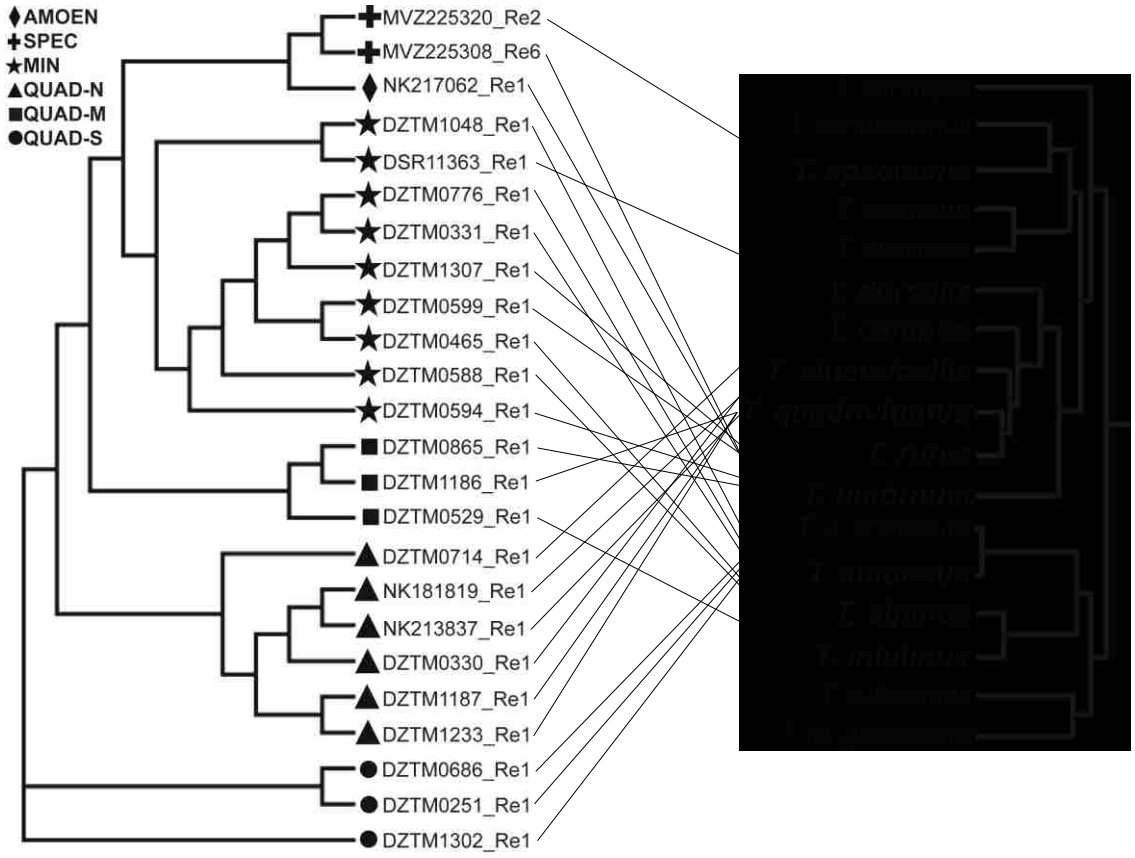
DZTM1302_Re1_umbrinus 11

Figure 3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DZTM1302_Re1

Figure 4

APPENDIX I

Host and parasite catalog numbers with host species, COI clade, and GenBank accession numbers for COI, 12S and 28S sequences.

Institutional Catalog Abbreviations are: BYU = Monte L. Bean Life Sciences Museum Mammal, ZM = Denver Museum of Nature and Science Mammal, MSB Para = Museum of Southwestern Biology Parasite, MSB Mamm = Museum of Southwestern Biology Mammal, MVZ = Museum of Vertebrate Zoology Mammal. Hosts are all genus *Tamias*.

Sample Name	Parasite Catalog	Host Catalog Number	Host species	Locality	Clade	COI Accessio	12S Accession	28S Accession
DSR11363_Re1	MSB Para 20745	BYU 35739	*T. dorsalis*	31	MIN	KT875269	KU668409	KU668379
DSR11372_Re1	MSB Para 20762	BYU 35751	*T. dorsalis*	32	MIN	KT875270		
DZTM0187_Re1		ZM 11205	*T. rufus*	23	MIN	KT875271	KU668408	
DZTM0245_Re1		ZM 11183	*T. minimus*	14	MIN	KT875272		
DZTM0248_Re1		ZM 11142	*T. minimus*	15	MIN	KT875254		
DZTM0251_Re1		ZM 11160	*T. umbrinus*	18	QUAD-	KT875241	KU668428	KU668380
DZTM0255_Re1		ZM 11164	*T. umbrinus*	18	QUAD-	KT875242		
DZTM0267_Re2		ZM 11147	*T. umbrinus*	6	QUAD-	KT875255		
DZTM0273_Re1		ZM 11153	*T. minimus*	6	MIN	KT875256		
DZTM0278_Re1		ZM 11158	*T. minimus*	8	MIN	KT875257		
DZTM0328_Re2		ZM 11426	*T. dorsalis*	37	QUAD-S	KT875258		
DZTM0330_Re1		ZM 11428	*T. dorsalis*	37	QUAD-S	KT875273	KU668423	KU668381
DZTM0331_Re1		ZM 11429	*T. minimus*	20	MIN	KT875259	KU668411	KU668382
DZTM0380_Re1		ZM 11649	*T. minimus*	3	MIN	KT875260		
DZTM0465_Re1		ZM 11545	*T. minimus*	21	MIN	KT875243	KU558415	KU668383
DZTM0468_Re1		ZM 11548	*T. minimus*	21	MIN	KT875274		
DZTM0498_Re1		ZM 11578	*T. minimus*	21	MIN	KT875261		

DZTM0529_Re1	ZM 11600	*T. minimus*	29	QUAD-	KT875244	KU668426	KU668399
DZTM0587_Re1	ZM 11681	*T. umbrinus*	26	MIN	KT875262	KU668416	KU668400
DZTM0588_Re1	ZM 11682	*T. minimus*	26	MIN	KT875245	KU668414	KU668384
DZTM0594_Re1	ZM 11672	*T. umbrinus*	22	MIN	KT875263	KU668412	KU668385
DZTM0595_Re1	ZM 11673	*T. minimus*	22	MIN	KT875246		
DZTM0599_Re1	ZM 11686	*T. umbrinus*	19	MIN	KT875247	KU668413	KU668386
DZTM0603_Re1	ZM 11690	*T. minimus*	19	MIN	KT875264		
DZTM0614_Re1	ZM 11701	*T. umbrinus*	25	QUAD-	KT875275		
DZTM0686_Re1	ZM 11792	*T. umbrinus*	13	QUAD-	KT875276	KU668427	KU668387
DZTM0687_Re1	ZM 11793	*T. umbrinus*	13	QUAD-	KT875277		
DZTM0688_Re1	ZM 11794	*T. umbrinus*	13	QUAD-	KT875278		
DZTM0689_Re1	ZM 11795	*T. umbrinus*	13	QUAD-	KT875279		
DZTM0714_Re1	ZM 11838	*T. dorsalis*	40	QUAD-S	KT875248	KU668418	KU668388
DZTM0717_Re1	ZM 11841	*T. dorsalis*	40	QUAD-S	KT875249		
DZTM0719_Re1	ZM 11843	*T. dorsalis*	40	QUAD-S	KT875280		
DZTM0729_Re1	ZM 11853	*T. cinereicollis*	36	QUAD-S	KT875265		
DZTM0731_Re1	ZM 11827	*T. minimus*	35	MIN	KT875266		
DZTM0775_Re1	ZM 11875	*T. minimus*	7	MIN	KT875267		
DZTM0776_Re1	ZM 11876	*T. minimus*	7	MIN	KT875250	KU668410	KU668389
DZTM0781_Re1	ZM 11881	*T. umbrinus*	12	QUAD-	KT875268		
DZTM0808_Re1	ZM 11925	*T. minimus*	17	MIN	KT875281		
DZTM0865_Re1	ZM 11982	*T. umbrinus*	24	QUAD-	KT875282	KU668424	KU668401
DZTM0892_Re1	ZM 12040	*T. minimus*	16	MIN	KT875283		
DZTM0896_Re1	ZM 12044	*T. minimus*	16	MIN	KT875251		
DZTM1045_Re1	ZM 12132	*T. amoenus*	5	AMOEN	KT875284		
DZTM1048_Re1	ZM 12134	*T. minimus*	27	MIN	KT875285	KU668407	KU668390
DZTM1072_Re1	ZM 12137	*T. minimus*	27	MIN	KT875286		
DZTM1124_Re1	ZM 12160	*T. minimus*	28	MIN	KT875287		
DZTM1181_Re1	ZM 12169	*T. quadrivittatus*	30	QUAD-S	KT875252		

DZTM1186_Re1		ZM 12172	*T. quadrivittatus*	30	QUAD-	KT875288	KU668425	KU668391
DZTM1187_Re1		ZM 12173	*T. quadrivittatus*	30	QUAD-S	KT875253	KU668421	KU668392
DZTM1228_Re1		ZM 12183	*T. quadrivittatus*	33	QUAD-S	KT875289		
DZTM1230_Re1		ZM 12185	*T. quadrivittatus*	33	QUAD-S	KT875291		
DZTM1233_Re1		ZM 12188	*T. quadrivittatus*	33	QUAD-S	KT875290	KU668422	KU668393
DZTM1302_Re1		ZM 12208	*T. umbrinus*	11	QUAD-	KT875292	KU668429	KU668394
DZTM1307_Re1		ZM 12211	*T. umbrinus*	11	MIN	KT875293	KU668417	KU668395
DZTM1321_Re1		ZM 12217	*T. minimus*	10	MIN	KT875294		
DZTM1323_Re1		ZM 12219	*T. minimus*	10	MIN	KT875295		
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DZTM1701_Re1		ZM 12444	*T. amoenus*	9	AMOEN	KT875302	KU668431	
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MVZ225308_Re4	MSB Para 20690	MVZ 225308	*T. alpinus*	34	SPEC	KT875306		
MVZ225308_Re5	MSB Para 20690	MVZ 225308	*T. alpinus*	34	SPEC	KT875307		
MVZ225308_Re6	MSB Para 20690	MVZ 225308	*T. alpinus*	34	SPEC	KT875309	KU668430	KU668396
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MVZ225315_Re1	MSB Para 20697	MVZ 225315	*T. speciosus*	34	SPEC	KT875315		
MVZ225316_Re1	MSB Para 20698	MVZ 225316	*T. speciosus*	34	SPEC	KT875312		
MVZ225318_Re1	MSB Para 20701	MVZ 225318	*T. speciosus*	34	SPEC	KT875313		
MVZ225320_Re1	MSB Para 20711	MVZ 225320	*T. speciosus*	34	SPEC	KT875316		
MVZ225320_Re2	MSB Para 20711	MVZ 225320	*T. speciosus*	34	SPEC	KT875317	KU668406	KU668397 KU668398
MVZ225320_Re3	MSB Para 20711	MVZ 225320	*T. speciosus*	34	SPEC	KT875314		
NK181819_Re1	MSB Para 20725	MSB Mamm 262538	*T. cinereicollis*	38	QUAD-S	KT875318	KU668419	KU668402 KU668403
NK196244_Re1	MSB Para 20751	MSB Mamm 230578	*T. amoenus*	2	AMOEN	KT875319		

NK213837_Re1	MSB Para 20771	MSB Mamm 249014	*T. canipes*	39	QUAD-S	KT875320	KU668420	KU668404
NK217056_Re1	MSB Para 20651	MSB Mamm 233623	*T. amoenus*	1	AMOEN	KT875297		
NK217062_Re1	MSB Para 20652	MSB Mamm 233628	*T. amoenus*	1	AMOEN	KT875298	KU668432	KU668405
NK217062_Re2	MSB Para 20652	MSB Mamm 233628	*T. amoenus*	1	AMOEN	KT875299		
NK217063_Re1	MSB Para 20653	MSB Mamm 233634	*T. amoenus*	1	AMOEN	KT875300		
NK217063_Re2	MSB Para 20653	MSB Mamm 233634	*T. amoenus*	1	AMOEN	KT875301		
NK230639_Re1	MSB Para 20662	MSB Mamm 269855	*T. amoenus*	4	AMOEN	KT875321		
NK230668_Re1	MSB Para 20671	MSB Mamm 270041	*T. minimus*	35	MIN	KT875322		
NK230669_Re1	MSB Para 20673	MSB Mamm 270042	*T. minimus*	35	MIN	KT875323		

APPENDIX II.

Tamias cytochrome *b* sequences from GenBank used for estimating interspecific genetic distances. Numbers in parentheses refer to sample names on GenBank records.

T. alpinus: KJ452867 (ULCEMR33), KJ452874 (ULCEMR45), KJ452899 (VL207209), KJ452934 (MPT 197A), KJ452936 (MPT 196A), KJ452941 (MPT 175), KJ452953 (OL219998)

T. dorsalis: KJ139582 (DZTM582), KJ139581 (DZTM583), KJ139580 (DZTM203), KJ139578 (DZTM202), KJ139575 (UWBM.79671), KJ139569 (DZTM586), KJ139568 (DZTM711)

T. minimus: KJ453103 (Adobe221277), KJ453027 (BM222667), KJ453098 (Bishop221251), KJ453010 (Sonora224146), KJ453015 (PineC224150), KJ453038 (BMJAC405), JN042466 (DMNS:Mamm:11141)

T. quadrivittatus: KJ139480 (DZTM815), KJ139474 (DZTM222), KJ139529 (DZTM1230), JN042424 (DMNS:Mamm:11024), KJ139483 (DZTM071), KJ139522 (DZTM1178), KJ139481 (DZTM824)

T. rufus: KJ139469 (DZTM190), KJ139468 (DZTM189), KJ139467 (DZTM187), KJ139466 (DZTM186), KJ139463 (DZTM571), JN042433 (MVZ:Mamm:199281), JN042432 (DMNS:Mamm:11203)

T. speciosus: JN042484 (KWE013), JN042483 (JRD288), JN042482 (KWE003), JN042481 (MSB:Mamm:84515), JN042480 (MSB:Mamm:90785), JN042479 (K4216), EU259279 (MVZ:Mamm:207237)

T. umbrinus: KJ139616 (DZTM615), KJ139631 (DZTM268), KJ139609 (DZTM592), KJ139586 (DZTM164), KJ139626 (DZTM690), JN042404 (HSUVM:6239), KJ139617 (DZTM257)

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. 12S gene tree. Values above branches are posterior probabilities and values below branches are bootstraps. Symbols correspond to COI clades (inset) and sample localities.

Supplemental Figure 2. Nuclear 28S gene tree. Values above branches are posterior probabilities and values below branches are bootstraps. Symbols correspond to COI clades (inset) and sample localities.

SUPPLEMENTAL TABLES

Supplemental Table 1. Results of Mantel tests of correlation between geographic and genetic distance for all samples and within mitochondrial clades.

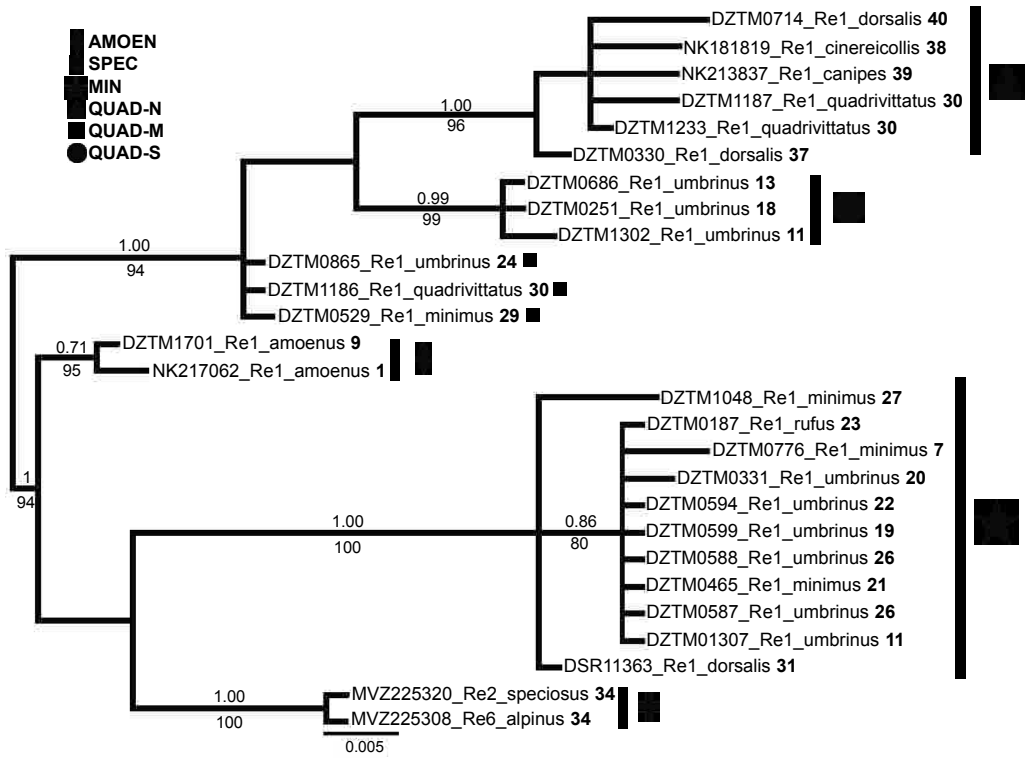
Clade	**r-value**	**p**
All samples	0.285	0.001
QUAD-N, QUAD-M, QUAD-S	0.695	0.001
MIN	0.389	0.001
AMOEN	0.969	0.008

Supplemental Table 2. Locations with multiple pinworm clades from the same host species

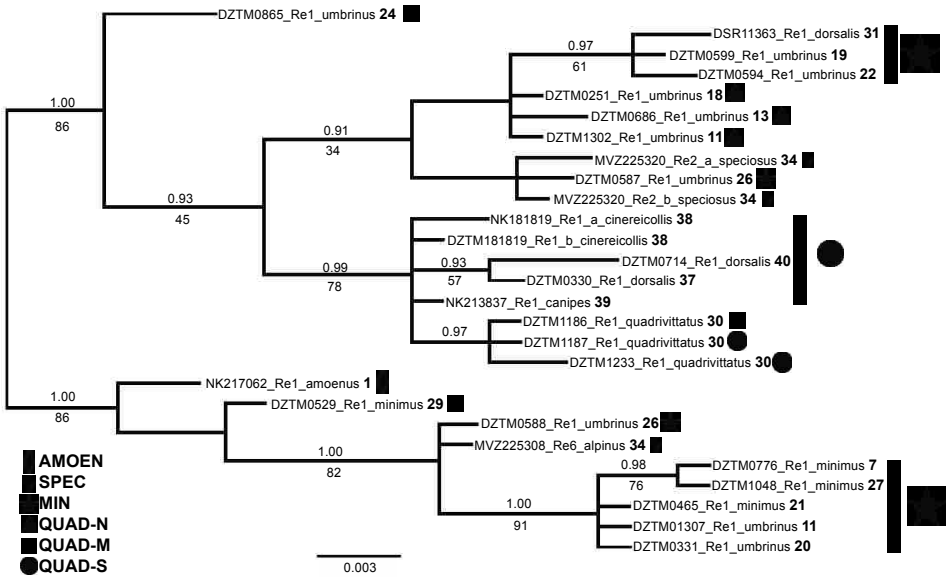
Locality	Host species	Pinworm clades
30: CO, Saguache Co.	*T. quadrivittatus*	QUAD-M (n=1) QUAD-S (n=2)
11: WY, Fremont Co.	*T. umbrinus*	MIN (n=1) QUAD-N (n=1)

Supplemental Table 3. Locations where the MIN pinworm clade is recovered from non-*T. minimus* hosts. No *T. minimus* were collected at the Utah locality but they should occur in the vicinity. One *T. minimus* was collected at the same locality as the *T. rufus* in Colorado, but no pinworms were recovered from it.

Locality	**Host species**	**Host species genetic distance from *T. minimus***
23: CO, Eagle Co.	*T. rufus*	0.078
31 & 32: UT, Garfield Co.	*T. dorsalis*	0.081



Supplemental Figure 1



Supplemental Figure 2

CHAPTER 3

MULTIPLE PARASITES SHOW DEEP, BUT ASYNCHRONOUS, CONCORDANCE IN DIVERSIFICATION THAT CONTRASTS WITH SHALLOW PHYLOGEOGRAPHIC STRUCTURE

Kayce C. Bell

Abstract

Parasitism is one of the most common symbiotic interactions, yet the processes that drive parasite diversification are not well understood. Both host evolution and factors external to the host-parasite interaction have contributed to parasite evolution in some systems. Comparing the phylogenies of two parasites inhabiting the same physical space within a host provides an opportunity to explore the relative roles of host evolution and other factors in parasite diversification. I tested for host-parasite and parasite-parasite phylogenetic concordance in a diverse host system in western North America, chipmunks (Rodentia; Sciuridae), and two species of pinworms (Nematoda; Oxyurida). The phylogenies recovered signals of host-associated divergences in both pinworm species, however the relationships among host-associated lineages were not congruent with host relationships. Phylogeographic structure in the two pinworms was discordant, suggesting that although pinworms experienced similar climate and landscape processes, these cyclical processes resulted in different diversification events. Contrasting parasite diversification events in these two pinworm species may therefore be primarily due to differences in timing of association with their chipmunk hosts.

Introduction

Because parasitism is so pervasive across the Tree of Life, deciphering the processes that shape parasite evolution is central to understanding biological

diversification. In addition to the influence of host association and history, parasites that move among closely related host lineages can be strongly influenced by geological history and geography (Wickström et al. 2003; Nieberding et al. 2004; Fallon et al. 2005; Gomez-Diaz et al. 2007; Koehler et al. 2009; Galbreath and Hoberg 2012). Therefore, parsing the contribution of host association, geological history, and geography to parasite occurrence and diversification is critical for understanding these symbiotic systems.

Comparative phylogeography in free-living systems has revealed the influence of geography, climate cycling, and geological history in biogeography and the cryptic processes driving geographic diversification (Arbogast and Kenagy 2001). Integrating parasite phylogeographic patterns and biogeographic history, in addition to comparing host and parasite phylogenies, can elucidate the contributions of each of these processes to shaping parasite evolution. Furthermore, examining multiple parasites of a single host can be a powerful approach to illustrate the nuanced nature of diversification by comparing parasite patterns. Although most host-parasite systems studied to date have focused on a single parasite, the few that have explored multiple parasites of the same host have revealed both congruent (Light and Hafner 2007) and discordant (Galbreath and Hoberg 2015; Sweet et al. 2016) genetic structure between parasites. A comparative approach with parasite species that share similar life histories, diets, infection sites, and host species can be especially powerful (Light and Hafner 2007) because such systems provide replicated evolutionary experiments for testing hypotheses regarding the roles of host association, dynamic climate, and complex landscapes in shaping parasite evolution.

Western North America is ecologically and topographically complex with elevations ranging from below sea level to over 4,000 meters. Within the western United

States, five major mountain ranges can be subdivided into many smaller ranges. These mountain ranges are generally characterized by boreal forest or alpine habitats at higher elevations and often separated by intermountain basins consisting of sagebrush steppe, grassy plains, and arid deserts (Merriam 1895). This complex landscape, along with Pleistocene glacial cycling, shaped biotic diversity in the region (Hewitt 2004). While the continental ice sheets of the Pleistocene did not fully extend into the western United States, the cooler glacial periods led to development of montane glaciers and massive pluvial lakes. During the warmer interglacial periods, flora and fauna likely expanded into previously unavailable regions, often moving northward or higher in elevation. When glaciers subsequently advanced, populations were extirpated or retreated to ice-free or more hospitable regions, often lower in elevation or further south. Pleistocene glacial cycling forced populations to repeatedly contract, often followed by expansion leading to repeated secondary contact between closely related and recently diverged lineages. During contact closely related lineages often hybridized with various long-term genetic consequences, ranging from mitochondrial capture to the formation of long-term hybrid zones (Dobeš et al. 2004; Edwards et al. 2006; Runck et al. 2009; Miller et al. 2012; Good et al. 2015). Following the last glacial maximum (11.7 kya), the West has aridified and mesic-adapted species have moved north and higher in elevation (Pielou 1991; Hewitt 2000; Lessa et al. 2003).

Western North American chipmunks (genus *Tamias*, subgenus *Neotamias*) are broadly distributed across a variety of biomes and are characterized by a recent radiation ($\leq$ 4 mya; Sullivan et al. 2014) leading to 23 recognized species. For western chipmunks, Piaggio and Spicer (2001) proposed five species groups based on mitochondrial DNA

(Table 1). Subsequent phylogenetic investigation, bolstered by nuclear loci (Reid et al. 2012), supported monophyly in two of the proposed species groups relevant to my investigations, the *quadrivittatus* group and the *townsendii* group, although three taxa proposed for these groups (*T. bulleri*, *T. palmeri*, and *T. ochrogenys*) were not sampled and *T. quadrimaculatus* was recovered as an additional member of the *townsendii* group (Table 1). Deciphering the evolutionary history of western chipmunks has been complicated by a history of repeated hybridization events between species across the subgenus (Sullivan et al. 2014). This history of rampant introgression in *Neotamias*, combined with rapid simultaneous divergence, has hampered resolution of interspecific relationships; however, analysis of nuclear DNA supports monophyly for most of the currently recognized species (Reid et al. 2012). The processes responsible for poorly resolved chipmunk relationships may have similarly impacted the evolutionary trajectories of their parasites.

Among species of western chipmunks (subgenus *Neotamias*), hybridization events that led to mitochondrial introgression likely occurred repeatedly, with some species currently sharing identical haplotypes, consistent with recent bouts of introgression (Sullivan et al. 2014). One line of evidence supporting mitochondrial introgression (as opposed to incomplete lineage sorting) in the *quadrivittatus* species group is the geographic structure of shared and introgressed haplotypes (Reid et al. 2012; Sullivan et al. 2014). While introgression seems to be rampant among at least four species in the *quadrivittatus* group, *T. canipes* and *T. rufus* do not show evidence of introgression, possibly due to persistent allopatry or less extensive sampling of those species. During periods of contact, regardless of whether or not hybridization occurred,

parasites also could be transferred between previously isolated and locally diverged populations. With an expectation that parasite lineages may be capable of cross infecting closely related host taxa, this system lends itself to tests of congruence between mitochondrial introgression and parasite transmission when hosts are sympatric. I can ask: are the processes that lead to introgression conducive to parasite transfer as well?

Western chipmunks host two species of endoparasitic pinworms (Nematoda: Oxyurida: Oxyuroidea) that inhabit both the cecum and large intestine. *Rauschtineria eutamii* Tiner 1948 (Oxyuridae) has been found in 10 of 19 host species examined and *Heteroxynema cucullatum* Hall 1916 (Heteronematidae) has been found in 16 host species (Bell et al. 2015). Both species of pinworms were recovered from nine host species, with approximately 14% of pinworm-infected host individuals harboring both species (Bell et al. 2015). Pinworms have a direct life cycle and transmission dynamics in wild populations are not fully understood (J-P. Hugot pers. comm.); however, transmission generally occurs via ingestion of eggs. These parasite species are only known to be associated with chipmunks, so patterns of occurrence and genetic diversity should not be influenced by transmission from non-chipmunk hosts. Previous work found *R. eutamii* to have deeply divergent lineages consistent with long-term association with host lineages, but geographic structuring within those host associated lineages and multiple instances of host switching among lineages in areas of host sympatry (Bell et al. in press).

Here I address the evolution and diversification of pinworm parasites from western North American chipmunks. The comparison of intraspecific phylogenies of two chipmunk pinworm species that infect the lower gastrointestinal tract allows exploration

of a series of expectations including: 1) these two species have similar histories with the hosts, 2) the ability to switch between hosts or infect new hosts is generalizable, 3) there is concordance in geographic genetic structuring, and 4) there is evidence for parasite transmission during episodes of contact between host species. My investigation of host association and dependence, variation in those associations, and evolutionary and biogeographic histories can inform hypotheses of parasite evolution and coevolution with hosts.

I test two major hypotheses in this system through analysis and comparison of phylogenetic structure between two pinworm species that parasitize 17 species western chipmunks. First, does each pinworm species have host-affiliated lineages (HALs) with similar relationships and levels of divergence? Based on differences in prevalence and distribution in hosts (*H. cucullatum* has a broader host distribution and higher prevalence; Bell et al. 2015), I hypothesize that each parasite has an independent history with the hosts but that the deep divergences in both species will have led to lineages primarily associated with one host species or species group. I predict that each pinworm species will have a distinctive history with the hosts that will be characterized by differing levels of diversity and divergence between and within HALs. Distinctive patterns will suggest that either different processes or different timing of similar processes have shaped the host-affiliated diversification of each species. Second, did the same processes and landscape features shape the diversity of both pinworm species? Consistent with a concerted response to external factors, I hypothesize that the shallow or recent histories of the two pinworm species will reveal similar genetic structure across the landscape due

to transmission and abiotic factors beyond host-association. I predict similar branching topology (particularly within HALs) and genetic structure among populations.

Methods

Fieldwork and specimen collection

I examined at least one member from four (of the five; excluding *merriami*) *Neotamias* mitochondrial species groups (per Piaggio and Spicer 2001; Table 1). I previously recovered *H. cucullatum* from the all four (*amoenus*, *minimus*, *quadrivittatus* and *townsendii*) groups and *R. eutamii* from three (not *townsendii*; Bell et al. 2015). Through extensive fieldwork, I collected pinworms from 17 chipmunk species (285 individual hosts) at 122 localities across 11 western states following approved mammal handling and collecting protocols (Sikes et al. 2011). Recovered pinworms were preserved in 70% or 95% ethanol, frozen in liquid nitrogen, and later stored at -20C. Specimens were numbered according to the institution specific host tissue number (e.g., NK or DZTM) and then sequentially for multiple pinworm individuals examined from the same host individual (e.g. Hc1, Hc2, Re1, Re2, etc.; Appendix I). Some pinworms were recovered from chipmunk gastrointestinal tracts collected by other researchers at the Museum of Vertebrate Zoology, Utah Museum of Natural History, and Monte L. Bean Museum of Natural History. In those cases, pinworm individuals were numbered with the host catalog number (Appendix I).

Molecular analyses

I generated partial mitochondrial cytochrome oxidase c subunit I (COI) sequences (*H. cucullatum*: 224 individuals, 705 bp; *R. eutamii*: 80 individuals, 767 bp; Appendix I) from 122 localities across the western United States (Supplemental Figure 1). Most *R.*

eutamii sequences are from Bell et al. (in press), but I supplemented with two additional sequences representing new localities. I included all samples for diversity and demographic analyses, but subsampled unique haplotypes by host species from each locality (119 *H. cucullatum*, 53 *R. eutamii*) for COI gene trees. I screened several additional loci for a subset of both species. For *R. eutamii*, I sequenced three loci, mitochondrial 12S (27 individuals, 487 bp), partial nuclear 28S (27 individuals, 794 bp), and partial nuclear 18S (SSU primers, 20 individuals, 914 bb; Supplemental Table 1). For *H. cucullatum*, I sequenced two nuclear loci, partial 28S (28 individuals, 750 bp) and ITS-1, 5.8S, and ITS-2 (ITS+; 62 individuals 1195 bp; Supplemental Table 1). Host mitochondrial sequences for the *quadrivittatus* group were obtained from an existing dataset (Reid et al. 2012; Sullivan et al. 2014) and cytochrome *b* fragments were generated (740 bp) for 52 *T. minimus* (L-14115/H-149963, Sullivan et al. 2000; Appendix II).

DNA extractions consisted of excising the midportion of a worm and preserving both anterior and posterior ends as vouchers for archival deposition in museum collections. A few extractions used partial pinworms, leaving only an anterior or posterior voucher. All vouchers, including hosts, are deposited at either the Museum of Southwestern Biology or the Denver Museum of Nature & Science (Appendix 1). The midportion was cut into at least three smaller pieces and extractions followed the protocols in the QIAamp DNA Mini extraction kit (Qiagen, Hilden, Germany), using carrier RNA at the AL Buffer step. Manufacturer's protocols were modified by heating and incubating the elution buffer on the membrane at 55C for five minutes. Final elution was 30-60uL per sample. All loci were PCR-amplified (Supplemental Table 1), purified

with polyethylene glycol precipitation or Exo-Sap-IT (Affymetrix, Santa Clara, California, USA), and cycle sequenced in both the forward and reverse directions. Sequenced products were read on an ABI 3100 in the Molecular Biology Facility in the Department of Biology at the University of New Mexico. Sequence chromatograms were assembled, edited, and aligned using Sequencher version 5.1 (Gene Codes Corporation, Ann Arbor, MI USA). All sequences for each locus for each species were aligned in MUSCLE (Edgar 2004).

I generated gene trees and a multilocus concordance tree annotated with host species. For gene tree estimation in both pinworm species, COI was partitioned by codon position and in *H. cucullatum* the ITS+ fragments were partitioned by region (ITS-1, 5.8S, ITS-2). I conducted Maximum Likelihood gene tree estimation in RAxML v.8 (Stamatakis 2014) using a GTRCAT model and 10000 bootstrap replicates to assess support. Bayesian gene trees were generated using the reversible-jump search in MrBayes 3.2 (Ronquist et al. 2012), with four chains and two runs for 20 million generations (10 million for nuclear loci), sampling the trees and parameters every 500 generations. The first 25% of sampled trees were discarded. I was unable to locate outgroup individuals with suitable DNA for sequencing, so all phylogenies were mid-point rooted. All trees were visualized with midpoint rooting in FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

My approach to multilocus species tree reconstruction for each pinworm species (63 *H. cucullatum* and 29 *R. eutamii*) used multiple methods to assess support for HALs. I filled in missing sequences at any locus for a taxon with ambiguous characters (Ns) so that sequence files had complete sampling at every locus. First, I concatenated loci and

ran MrBayes (partitioned by locus) for each pinworm species using the search parameters described above for 50 million generations. Additionally, I generated species trees for each pinworm species using all loci in *BEAST 1.8.2 (Drummond et al. 2012). All *BEAST estimations were run with a GTR model and a Yule process species tree prior. Because the rate of evolution of each locus was unknown, I ran each *BEAST analysis with both a relaxed clock and strict clock. Clock rates were estimated with normal distributions and run for 50 million generations. I assessed convergence and parameter estimation in Tracer 1.6.0 (Rambaut et al. 2014). For *H. cucullatum*, *BEAST was run with a strict clock after determining a right skewed standard deviation in the clock rate with a relaxed clock. The *R. eutamii* *BEAST analysis was run with a relaxed clock after determining a normally distributed standard deviation in the clock rate. Lastly, I constructed pinworm species trees in BUCKy (Larget et al. 2010) using the tree files from the MrBayes runs and default settings to generate concordance factors for nodes across loci for each species. The concordance factors give the level of concordance for relationships among the trees sampled in MrBayes.

For a direct comparison of genetic structure between the two pinworm species, I used individuals from each pinworm species that represent both species either recovered from the same host individual (N=14) or the same host species at the same locality (N=13; excluding individuals recovered from a host other than the one primarily associated with that HAL) for analyses in BUCKy. Recently developed methods have used BUCKy as a comparative phylogeography tool to quantify the phylogeographic concordance among different taxa across regions (Satler and Carstens 2016). I extended

that approach to determine levels of concordance among HALs between the two pinworm species.

Pinworm and host diversity and demography

Phylogeographic comparisons between the two pinworm species used the COI sequences generated for all specimens. I also compared diversity and demographic measures for two groups of chipmunk hosts (*T. minimus* and *quadrivittatus* group). Sequence data were not available for all chipmunk hosts, so I used previously published cytochrome *b* sequences from GenBank (Reid et al. 2012, Sullivan et al. 2014; Appendix II) and new sequences to estimate population demographic and structure signals for *T. minimus* (N=48) and the *quadrivittatus* group (*T. canipes*, *T. cinereicollis*, *T. dorsalis*, *T. palmeri*, *T. quadrivittatus*, *T. rufus*, *T. umbrinus*; N=76). I also generated four Bayesian gene trees for each chipmunk group that had a corresponding pinworm, two *quadrivittatus* group trees (N=66 *H. cucullatum* hosts, N=21 *R. eutamii* hosts) and two *T. minimus* trees (N=31 *H. cucullatum* hosts, N=24 *R. eutamii* hosts) in MrBayes using the reversible-jump search method with sequences partitioned by codon position in two runs with four chains for 10 million generations, sampling every 500 generations and discarding the first 25% of trees as burn-in. I conducted the same MrBayes approach as above with COI for the individual pinworms that were recovered from each of the hosts that I had sequence data for, so that the pinworm gene trees for comparison only include individuals with host sequence. I analyzed the *quadrivittatus* group and *T. minimus* samples separately for each pinworm species. I did not include pinworm individuals from hosts that were known to belong to a different HAL. To compare phylogeographic structure and concordance values for these relationships I generated concordance trees in

BUCKy with default settings. I then midpoint rooted each MrBayes tree in Figtree and manually created tanglegrams for each host-pinworm comparison.

To compare variation and divergences, I estimated population demographic and diversity measures using Arlequin 3.5 (Excoffier and Lischer 2010) and MEGA 6.06 (Tamura et al. 2013). I tested for demographic and spatially expanding populations in Arlequin via a mismatch distribution analysis (Schneider and Excoffier 1999; Excoffier 2004). For comparison of geographic structuring of populations, I estimated Fst values and calculated uncorrected average pairwise divergences among all samples, between lineages, and within lineages.

Results

Phylogeny

The COI gene tree for *H. cucullatum* revealed five major lineages that were primarily associated with a host species or host species group (Figure 1). There were two *H. cucullatum* lineages primarily associated with *quadrivittatus* group hosts, a northern clade (QUAD-N) and a southern clade (QUAD-S). A clade restricted to the Pacific Northwest (TOWN) was primarily associated with the *townsendii* group. The MIN clade was recovered from *T. minimus* and *T. ruficaudus* (both members of the *minimus* group), with a subclade nested within that was primarily associated with *T. amoenus* (MIN-AMOEN). The relationships among the HALs in *R. eutamii* (Figure 2) were very similar to *H. cucullatum* except for *T. amoenus* associated *R. eutamii* (AMOEN) clade was distinct from the MIN clade. The Maximum Likelihood gene trees yielded similar topologies as the MrBayes analysis, with high bootstrap support (Figures 1 and 2).

Nuclear loci were less informative than COI and 12S, however ITS+ in *H. cucullatum* exhibited structure that supported some aspects of the COI relationships (Supplemental Figure 2). All multilocus trees yielded topologies with the HALs, but had varying levels of support for the monophyly of the HALs (Figure 3). The concatenated Bayesian tree had high support for HALs (Figure 3, A, D) while the *BEAST trees support most of the HALs (Figure 3, B, E), and the BUCKy trees yielded low concordance values for most nodes (Figure 3, C, F), where a concordance value ≥ 0.5 signified the presence of that clade in at least half of the unique topologies for the sampled Bayesian gene trees.

Host switches were implicated when phylogenetic lineages primarily associated with one species or species group (most of the individual pinworms within a lineage were recovered from one species or species group) were recovered from different species. There was evidence of contemporary host switching between HALs in both pinworm species (Table 2). I found no additional instances of host switching in *R. eutamii* than previously reported (Bell et al. in press), which included switches: into a *T. minimus* host in the QUAD-M clade (1), into *T. alpinus* in the SPEC clade (2 host individuals at the same locality), in the MIN clade 4 into *T. umbrinus* (3 in Nevada in different localities, 1 in Wyoming), into *T. dorsalis* (1), and into *T. rufus* (1). Although I found seven host switches in *H. cucullatum* HALs, these were over a substantially larger sample size spanning a larger geographic area than in *R. eutamii*. Both the *H. cucullatum* TOWN and SPEC lineages appeared capable of infecting both *T. speciosus* and *T. senex* in areas where the two chipmunk species are sympatric. The *H. cucullatum* QUAD-N lineage was recovered from one *T. minimus* in Colorado and two *T. panamintinus* (member of the

minimus group) where *T. panamintinus* and *T. palmeri* are elevationally parapatric in the Spring Mountains of Nevada. There were two instances of the *H. cucullatum* QUAD-S lineage being recovered from *T. minimus* hosts in regions of sympatry with *T. quadrivittatus*. The *H. cucullatum* MIN-AMOEN lineage was completely nested within the MIN HAL (Figure 1), suggesting a past expansion from the MIN lineage into *T. amoenus* hosts.

Phylogenetic concordance between pinworms

For a strict comparison of the relationships among HALs between the two pinworm species, I compared pinworms recovered from the same host individual or the same host species at the same locality, not including likely host switches (see below). The BUCKy tree had concordance values ≥ 0.5 for most of the lineages found in both pinworm species (Figure 4). The MIN clade had nodal concordance of 0.52, with a highly concordant AMOEN clade (1.0) nested within. There was also high concordance for both the QUAD-S (0.97) and QUAD-N (1.0) clades, although less support for the monophyly of the QUAD lineages (0.29).

Phylogenetic concordance with hosts

I generated Bayesian gene trees with a subset of *H. cucullatum* and *R. eutamii* samples that had host sequence data and also for those corresponding hosts (for those with available sequences; Sullivan et al. 2014 or new sequences). This restricted me to comparisons of the QUAD clades and *T. canipes*, *T. cinereicollis*, *T. dorsalis*, *T. palmeri*, *T. quadrivittatus*, *T. rufus*, *T. umbrinus* (*quadrivittatus* group) and the MIN clades and *T. minimus*. I compared mitochondrial gene trees using the concordance approach in BUCKy and by generating tanglegrams (Figure 5). Pinworms and hosts were largely

discordant in all cases. Although Satler and Carstens (2016) suggested a concordance value of 0.71 signified ecological interaction, I know the chipmunks are obligate hosts for the pinworms, so considered majority concordance values (≥ 0.5) worthy of note for chipmunk and pinworm lineages. Overall, *H. cucullatum* had lower concordance with the hosts than *R. eutamii*. The lowest concordance was between *H. cucullatum* QUAD lineages and the *quadrivittatus* group, having 15 of 63 nodes with concordance values ≥ 0.5 and an average concordance of 0.25 across all nodes. The *H. cucullatum* MIN lineage and *T. minimus* BUCKy tree had 11 of 27 nodes with concordance values ≥ 0.5 and an average of 0.39. *Rauschtineria eutamii* QUAD lineages also had lower concordance with the hosts than the MIN clade, with concordance values ≥ 0.5 in 7 of 20 nodes and an average of 0.31. The highest concordance was between *R. eutamii* MIN and *T. minimus* with concordance values ≥ 0.5 on 10 of 22 nodes and an average of 0.51 across all nodes.

Geographic structure

Both pinworm species exhibited phylogeographic structure, both within and among HALs. Geographic structure among HALs was difficult to parse from host-associated structure, except for examples where chipmunk species were sympatric. Host switching in areas of chipmunk species sympatry further support the nature of geographic structure in pinworm lineages. Within the TOWN and QUAD groups, pinworms seem capable of infecting multiple chipmunk species, from the *townsendii* and *quadrivittatus* groups, respectively. For *H. cucullatum,* the TOWN clade was structured spatially and not by host, with well-supported subclades corresponding to geographic regions (e.g. the largest subclade consisted of all the samples from Oregon). In both *H. cucullatum* and *R. eutamii*, the QUAD lineage structure appeared to be primarily geographic, although there

was some overlap of QUAD-S and QUAD-M lineages in *R. eutamii*. The *R. eutamii* QUAD-M and QUAD-S clades were consistent with the distribution of *H. cucullatum* QUAD-S.

Concordance of substructure within the HALs (for the BUCKy tree comparing pinworms from the same host or locality) rarely met the threshold suggested by Satler and Carstens (2016) for phylogeographic concordance, 0.71. Within the MIN lineages the concordance values averaged 0.56 (range 0.4-1.0), with the highest level of concordance for the node uniting samples from the same locality. Concordance for the two nodes within the AMOEN lineage was below 0.5. Within the QUAD-N HALs there was minimal support for concordance (0.58 and 0.51). The QUAD-S HALs were all also less than 0.71, which was particularly striking since there were three nodes for samples from the same locality, yet they were not consistently concordant between the two pinworm species (0.3, 0.5, and 0.65).

Both pinworms were found in isolated mountain ranges in the Southwest and levels of divergence from other nearby populations varied. Average pairwise divergence for the COI gene within the *H. cucullatum* QUAD-S lineage was 2.2% and 1.9% in *R. eutamii* QUAD-S. *Tamias dorsalis* in the isolated Pinaleño Mountains of Arizona had pinworms in the QUAD-S lineages; however the pinworms were not distinctly more divergent than other populations, 1.9% in *H. cucullatum* and 2.2% in *R. eutamii*. Populations of *T. canipes* in the isolated Sacramento Mountains of New Mexico also hosted both pinworms, with *R. eutamii* 2.1% divergent from other QUAD-S populations, while *H. cucullatum* were noticeably more divergent (4 %).

Comparative diversity and demography

Average pairwise COI divergences between HALs were deeper in *H. cucullatum* (range 4.4-8.4%, mean 6.7%) than in *R. eutamii* (range 1.7-5.2%, mean 4.3%). Nucleotide diversity was also higher in *H. cucullatum* ($\pi = 0.054 +/- 0.026$) than in *R. eutamii* ($\pi = 0.032 +/- 0.016$); however, haplotype diversities were comparable in the two species (Supplemental Table 2). I could not reject a model of spatial expansion for any of the HALs. I rejected a model of demographic expansion for a single clade, *H. cucullatum* MIN-AMOEN, however this lineage had low diversity (and is technically a subclade of MIN). Three of the HALs had significantly negative Fu's Fs values, *H. cucullatum* QUAD-N and MIN and *R. eutamii* MIN. While these values may be indicative of selection, significantly negative Fs values often correspond to recently expanded populations.

Haplotype diversity was relatively high (> 0.93) among all examined pinworm and chipmunk lineages, with the exception of the *H. cucullatum* MIN-AMOEN lineage (0.77). Comparisons of hosts within the *quadrivittatus* group and *H. cucullatum* and *R. eutamii* QUAD lineages revealed marginally higher haplotype and nucleotide diversities in the pinworms than in the hosts (Supplemental Table 2). I was unable to reject demographic expansion for either of the host groups, but did reject a model of spatial expansion for the *T. minimus* hosts (p=0.04). My *T. minimus* samples were drawn from a limited southern portion of the broad distribution of this species, and these populations may have been historically stable, while northern (unsampled) populations expanded.

Discussion

Comparative phylogenetics and phylogeography are powerful, yet often independently applied, approaches for understanding community assembly across diverse

landscapes (Arbogast and Kenagy 2001; Brunsfeld et al. 2001; Soltis et al. 2006) and specialized coevolutionary relationships (Waltari et al. 2007; Roe et al. 2011; Galbreath and Hoberg 2015). Using these complementary approaches to investigate the processes that led to the genetic structure and host associations of two species of endoparasites that infect the same hosts, I found that host associations and landscape strongly shaped both parasites, but in asynchronous and discordant ways. I found support for the hypothesis that each parasite, although sharing the same host environment (i.e., cecum and large intestine), has an independent history that includes distinctive host-associated diversities and divergences. Nevertheless, both pinworm species had strongly supported lineages primarily associated with a single host or host species complex. Low concordance among COI gene trees for the two species was not consistent with the hypothesis that similar extrinsic factors shaped parasite divergences within host associated lineages (HALs). With regard to the history of this host-parasite system, these findings are broadly consistent with the taxon pulse, oscillation, ecological fitting, and geographic mosaic mechanisms proposed in the Stockholm Paradigm for host-parasite community assembly (Araujo et al. 2015; Hoberg et al. 2015).

Pinworm lineages with respect to hosts

Using the western chipmunk phylogeny to understand the history of the subgenus *Neotamias* (Sullivan et al. 2014), the existence of HALs support a scenario where *H. cucullatum* infected an ancestor of western chipmunks, subsequently diversified across at least four major chipmunk complexes, and then persisted in these host groups through climatic cycling, occasionally moving between closely related hosts when in contact. My results also suggest that *R. eutamii* became a parasite of western chipmunks at some point

after the *townsendii* group diverged (2.483 my; Sullivan et al. 2014), based on the absence of *R. eutamii* in these hosts; however, a similar process of isolation and divergence likely led to the evolution of HALs (exclusive of *townsendii*) similar to those found in *H. cucullatum*. Based on differing patterns and levels of divergence among HALs, the same processes apparently shaped deep divergences in each pinworm species but the timing of these events was idiosyncratic, supporting my first hypothesis. In *T. minimus* hosts, the *H. cucullatum* MIN lineage (primarily parasitizing *T. minimus*) appears to be restricted to the Rocky Mountains and the Black Hills; however, it also was found in *T. ruficaudus* hosts to the west. There was also evidence of a past host switch from the MIN lineage into *T. amoenus*, which may not have harbored their own lineage, either through extinction or a "missing the boat" event (Paterson and Gray 1997), whereby a host lineage diverges from the ancestral lineage but the parasites are not associated with that descendant lineage. One *T. minimus* in Idaho hosted the AMOEN lineage. Given that this lineage was derived from the MIN lineage, and *T. amoenus* and *T. ruficaudus* have a history of introgression (Good et al. 2003; Good et al. 2008; Reid et al. 2010; Sullivan et al. 2014), further sampling of *T. amoenus* and *T. ruficaudus* populations will yield insight into the geographic and host breadth of this pinworm lineage. QUAD-N and QUAD-S lineages were broadly distributed within members of the *quadrivittatus* group (Table 1).

There are several lines of evidence that support a longer history of parasitizing chipmunks for *H. cucullatum* than for *R. eutamii*. First, *H. cucullatum* has been recovered from all western chipmunk species groups examined, including the oldest diverging *townsendii* group (per divergence dating in Sullivan et al. 2014). In contrast, *R. eutamii* is

missing from this early branch of western chipmunks. Second, the magnitude of divergence among HALs, diversity within HALs, and overall diversity were all higher in *H. cucullatum* than in *R. eutamii*. Likewise, pinworms infecting the same isolated host populations had different levels of divergence potentially reflecting the cyclical nature of Pleistocene climate fluctuation on isolation, whereby pinworm lineages were transferred among host populations at different points in time. For example, based on levels of COI divergence, *H. cucullatum* appears to have been isolated with *T. canipes* longer than *R. eutamii*. Repeated contact between chipmunk species through the Late Pleistocene with expansion and contraction of forests across southern mountain ranges may have allowed an earlier transfer of *H. cucullatum* to *T. canipes*. Expansion and contraction of western *Tamias* distributions likely occurred repeatedly in response to changing climate and habitats, leading to different timing of pinworm transfers among populations. Third, I found evidence of higher rates of HAL host switching in *R. eutamii* than in *H. cucullatum* (Table 1). Although I had much higher sampling in *H. cucullatum* (N=224) than *R. eutamii* (N=80), I only documented 7 instances (3.1%) of host switching in *H. cucullatum*, whereas there are 9 instances (11.3%) among *R. eutamii*, suggesting the possibility that *H. cucullatum* HALs are more host specific and less capable of infecting multiple host species than *R. eutamii*. The longer that HALs are associated or isolated with a particular host species or species group, processes such as genetic drift and adaptation may lead to more specialized parasites that are less capable of infecting other, particularly distantly related, hosts. However, investigations into the evolution of host specificity have uncovered a tendency for parasites to evolve to be generalists (Poulin et

al. 2006) and found that generalists evolve from specialists (Johnson et al. 2011). Testing for host specificity and breadth will require further investigation.

Geographic structure in pinworms

In each pinworm species, phylogenies identify geographically structured lineages that correspond to host species or species groups. Chipmunks within the *quadrivittatus* and *townsendii* species groups are relatively closely related and recently diverged (1.635 mya in *townsendii* group and 1.781 mya in *quadrivittatus* group, Sullivan et al. 2014) and pinworm lineages exhibit substantial host breadth among these host groups (i.e., ecological fitting or sloppy fitness space, Janzen 1985; Agosta and Klemens 2008). The phylogeny for *H. cucullatum* clearly shows deep divergences among the HALs that mirror the host structuring found in *R. eutamii*, with the exception that no *H. cucullatum* pinworms were associated with the *townsendii* group chipmunks and the AMOEN clade nested within MIN. Within and among HALs there was a signature of genetic geographic structuring in pinworms, because pinworm lineages are capable of infecting closely related hosts (e.g. across members of a host group), leading to parasite geographic structure independent of species level host association. Genetic structure of QUAD lineages did not correspond to host mitochondrial structure (Figure 5 A, C) because although pinworms were transmitted among geographically proximate *quadrivittatus* group hosts, mitochondrial introgression does not appear to have occurred in all of these same geographically proximate chipmunk populations. Some host distributions are disjunct, such as *T. umbrinus* (Supplemental Figure 3), and while their pinworms were geographically partitioned, this structure was not clearly delineated along the distributional breaks for disjunct host populations. One *H. cucullatum* haplotype was

found in Fremont County, Wyoming and Summit County, Utah, crossing a break in the *T. umbrinus* host distribution (Supplemental Figure 3). The identical *H. cucullatum* haplotype in disjunct *T. umbrinus* hosts may be relictual from when *T. umbrinus* had a contiguous distribution, may be the result of long distance dispersal in *T. umbrinus*, or may be the result of pinworm gene flow through transmission via other host species. The QUAD lineages seem capable of infecting all *quadrivittatus* group hosts, but I also detected occasional *H. cucullatum* QUAD lineages in *T. minimus.* It is possible that *T. minimus* is mediating gene flow between disjunct QUAD populations. Indeed, most breaks in the *T. umbrinus* distribution are spanned by other members of the *T. quadrivittatus* clade or *T. minimus* (Supplemental Figure 3), which appears capable of serving as a different, possibly non-deal but suitable, host that essentially links otherwise disjunct populations of *T. umbrinus*. This aspect of host-parasite interactions may be important to consider when developing models and hypotheses of pathogen and zoonotic spread, particularly in light of shifting ranges in response to climate change.

In both *H. cucullatum* and *R. eutamii* the QUAD-N lineages were distributed in the Rocky Mountains and west into the Great Basin. A notable exception was the absence of the *R. eutamii* QUAD-N lineage in Nevada, while the *H. cucullatum* QUAD-N lineage occurred there. The populations, and in one instance the same individual, of *T. umbrinus* (a member of the *T. quadrivittatus* group) in Nevada hosted the *H. cucullatum* QUAD-N lineage and the *R. eutamii* MIN lineage. I have no records of *H. cucullatum* infecting *T. minimus* in Nevada, however more sampling may uncover infections. *Heteroxynema cucullatum* infecting *T. minimus* were largely in the MIN lineage and confined to the southern Rocky Mountains in Wyoming, Colorado, and New Mexico, with a few

exceptions (e.g. the Black Hills in Wyoming). One *T. minimus* from south of the Snake River Plain in Idaho was infected with *H. cucullatum* in the MIN-AMOEN lineage in a population where *T. amoenus* and *T. minimus* are sympatric, a finding that is not surprising because this pinworm lineage arose from within the MIN clade. However, the MIN clade may be more broadly distributed and my sampling did not capture it, or was more broadly distributed in the past, because the *T. ruficaudus* (member of the *minimus* group) in northeastern Washington were also infected with the MIN lineage.

The geographic split between QUAD-N and QUAD-S lineages was largely consistent with a suture zone proposed by Remington (1968). The north-south split along the Rocky Mountain chain seems at least partially coincident with the region where *T. quadrivittatus* shifts from elevational parapatry with *T. umbrinus* and parapatry with *T. rufus* to allopatry with respect to the other *quadrivittatus* group species (Supplemental Figure 3). This split may be a legacy of host colonizations of the area, where the QUAD-N pinworm lineages colonized the region with hosts from the north and west and the QUAD-S lineages colonized from the south and east, but have since spread among host species in contact in each region. High genetic divergence in *H. cucullatum* QUAD-S samples from *T. canipes* in the Sacramento Mountains of New Mexico is consistent with longer isolation with those hosts. Similarly, the red squirrel lineage (*Tamiasciurus fremonti*) from those mountains is highly distinctive (Hope et al. 2016). Taken together, these findings warrant expanded investigation of other taxa from this isolated range. Across the geographic ranges of both pinworm species the variation in host-associated genetic structure was consistent with variation in interspecific interactions predicted by the Geographic Mosaic Theory of Coevolution (Thompson 2005).

Comparing pinworm phylogeographic structure to host mitochondrial structure

Although direct comparisons of host and pinworm phylogenies were limited to a subset of samples, I was able to compare the parasite intraclade divergences (QUAD and MIN) to the intraclade divergences for both *T. minimus* and *quadrivittatus* group hosts. Fine scale examination allowed me to illuminate the impact of geography and past and ongoing host contact on parasite intraspecific diversity most closely in the QUAD HALs. Members of the *quadrivittatus* group have a history of mitochondrial introgression that generally corresponds to geography (Reid et al. 2012; Sullivan et al. 2014). Because both mitochondrial and pinworm transmission occurs when chipmunks are sympatric or parapatric, I hypothesized similar patterns of transmission. Relationships among pinworms, however, did not mirror relationships among chipmunk mitochondrial clades (Figure 5 A, C). There was substantial host breadth of pinworm QUAD lineages (i.e., capable of infecting all members of the *quadrivittatus* group), but species barriers among some hosts have apparently not been breached by mitochondrial introgression (e.g., *T. rufus*, *T. canipes*; Sullivan et al. 2014), so that pinworms are being transferred among hosts even when there is no signature of mitochondrial introgression. I also hypothesized that phylogeographic structure would be similar between *T. minimus* hosts and their pinworms, but discordant phylogenies (Figure 5 B, D) do not support the hypothesis that common abiotic factors and landscape features shaped genetic structure.

Comparing pinworm host associated and geographic structure

With *R. eutamii* showing deep host associated divergences and landscape scale phylogeographic structuring (Bell et al. in press), I hypothesized that comparisons of phylogenies of the two pinworms infecting western chipmunks would highlight

similarities and differences of coevolution in parasites inhabiting the same physical space in the hosts. Genetic signatures reveal similar deep history patterns (HALs), but the timing of divergence events and intraclade (phylogeographic) structure (based on different levels of COI divergence) seem to reflect different histories with chipmunks for each pinworm species. Bell et al. (in press) suggested that the broader distribution and higher prevalence of *H. cucullatum* may reveal more about the history of chipmunk population fluctuations and contact than the record of chipmunk hybridization. Here I found that the pinworms indeed appear to be transmitted even when there is no evidence of host introgression. The lack of population differentiation among widely disjunct pinworm populations within a clade (e.g. the Pinaleño Mountain samples within QUAD-S) suggest that host contact has occurred, or is occurring, on a temporal scale that is not reflected in all instances of host hybridization.

Phylogenetic structure among HALs was not concordant between the parasites; however, superficial comparisons of the major lineages appear to be similar. With one exception, there is no intraclade host-pinworm or pinworm-pinworm concordance of relationships among geographic regions, using the suggested standard of 0.71 for phylogeographic concordance (Satler and Carstens 2016). The *H. cucullatum* from *quadrivittatus* group hosts in central and northern Colorado are concordant as a monophyletic group with their hosts (0.99); however, substructure among those samples is discordant. This finding is contrary to the large-scale species-level codivergence patterns found in other coevolving systems, such as Andes virus and its rodent host (Torres-Perez et al. 2011). However, like the pinworms, the Andean system also has different fine-scale genetic structure between the host and virus. In both systems, the host

genetic structure reflects vertical transmission of genetic material, while the virus and pinworm are capable of being transmitted both vertically and horizontally. Horizontal transfer (e.g., host switching in the pinworms) generates phylogeographic structure that is not congruent with the hosts, particularly at fine scales. The most likely explanation for the lack of concordance between chipmunks and pinworms is that the parasite HALs are capable of infecting hosts other than the species or group they are primarily associated with and horizontal transfer then facilitates gene flow among pinworms that is incongruent with host gene flow. Pinworm eggs can be shed with feces and when multiple chipmunk species are active in the same environment (e.g. forage in the same areas) allowing opportunities for transfer of pinworm lineages among host species. Lack of phylogeographic concordance between the pinworms is likely due to different histories of association with the hosts, and possibly different abilities to spillover and infect other host species.

Pinworm taxonomic considerations

Distinctive lineages in each pinworm species raise the question of whether multiple species are represented by these clades and warrant further investigation using additional loci and morphological characters. Although, when an additional locus and more samples were analyzed concordance values dropped for *R. eutamii* HALs (Bell et al. in press) (Figure 3, F). The original species description of *H. cucullatum* was based on a specimen from a *T. minimus* (referred to as *Eutamias amoenus operarius*; Hall 1916) in Colorado and was likely a member of the MIN lineage, as that lineage is still found in that region. *Rauschtineria eutamii* was originally described from a *T. minimus* host in Minnesota (Tiner 1948). As no other *Neotamias* occur there, the type specimen likely

belonged to the *R. eutamii* MIN lineage, but I have no molecular data from any pinworm specimens that far north and east. My molecular study provides a foundation for assessing the potential presence of cryptic species, but a thorough taxonomic treatment of either *H. cucullatum* or *R. eutamii* will require further sampling. However, the host and parasite samples I have collected provide a reference for future taxonomic investigations and, importantly, these are archived in museum collections with host and parasite database records linked. Properly vouchering both hosts and parasites is crucial for accurate species identifications and descriptions of interactions, particularly when new species are described (Frey et al. 1992; McLean et al. 2016).

Conclusions

Chipmunks have been the subjects of diverse biological investigations, including behavior (Broadbooks 1970; Chapell 1978), physiology (Geiser et al. 1997; Kenagy and Place 2000; Hammond et al. 2015), niche partitioning (Heller 1971; Chappell 1978; Poffenroth and Matson 2007; Lowrey and Longshore 2013), hybridization and reproductive isolation (Patterson 1982; Patterson and Thaeler 1982; Reid et al. 2012; Sullivan et al. 2014), and responses to climate change (Rubidge et al. 2012; Rowe et al. 2015). Yet, until recently, relatively little was known about the identity and distribution of their common parasites (Bell et al. 2015). I show that host and geography have had additive – and sometimes confounding – effects on nematode diversification at both deep and shallow levels. HALs were recovered with high support in the mitochondrial trees, reflecting the deep associations within this host-parasite system. At a finer scale, geographic structure evident today in these two parasites, as well as their chipmunk hosts, appears to reflect population expansion into current distributions since the last glacial

maximum except in southern populations. In the south, their hosts likely retracted to higher elevations, often isolating populations on sky islands for millennia. However, impacts of postglacial host contact and sharing of parasite lineages are evident at shallow phylogeographic levels as well, highlighting the fact that cyclical host population contact and potentially other factors can shape parasite diversity at multiple hierarchical scales. Advances in genome sequencing technology and statistical tests are refining our ability to date historic events, such as hybridization (e.g., Miller et al. 2012). Applying those techniques to the western North American chipmunk-pinworm system, in concert with more intensive genomic investigations of the parasites themselves, will further our understanding of the dynamics of host hybridization and parasite transfer and evolution.

Two approaches, not mutually exclusive, have the potential to reveal fine-scale coevolutionary dynamics in the chipmunk-pinworm system. First, more intensive sampling of hosts and parasite populations in regions of host sympatry, both with and without evidence of introgression, will allow further exploration of the role of host hybridization in parasite transmission and evolution. A comparison of fine-scale phylogeographic structure between the two species will also enable a better understanding of the processes impacting divergence of these two species that rely on the same resource. Second, genomic data, particularly for the parasites, will reveal if there are thresholds of divergence between lineages that prevent host switching. There are currently few genomic resources for non-model invertebrates that do not infect humans or livestock. Advances in sequencing techniques and statistical approaches will open many opportunities to use these tools to understand coevolutionary dynamics in wild populations.

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Figure Legends

Figure 1. *Heteroxynema cucullatum* Bayesian COI gene tree, labeled by host associated lineage. Posterior probability support values above branches and maximum likelihood bootstrap values below branches. Bolded taxa labels indicate a host switch. Colored circles on map correspond to sampling localities for the lineages.

Figure 2. *Rauschtineria eutamii* Bayesian COI gene tree, labeled by host associated lineage. Posterior probability support values above branches and maximum likelihood bootstrap values below branches. Bolded taxa labels indicate a host switch. Colored circles on map correspond to sampling localities for the lineages.

Figure 3. Multi-locus trees for *Heteroxynema cucullatum* (top) and *Rauschtineria eutamii* (bottom). Circles represent posterior support values ≥ 0.95 (black) or ≥ 0.9 (gray) for Bayesian tree of concatenated loci (A, D) and for *BEAST multi-locus tree (B, E). Circles on BUCKy trees (C, F) represent concordance values ≥ 0.5. Lineage colors correspond to the designations in Figures 1 and 2.

Figure 4. BUCKy COI concordance tree for *Heteroxynema cucullatum* and *Rauschtineria eutamii* individuals infecting the same host individual (N=14) or the same host species at the same locality (N=13), excluding individuals known to have host switched. Values above branches indicate concordance for the relationships among the sampled trees.

Figure 5. Tanglegrams of Bayesian COI gene trees for pinworm individuals and corresponding host individuals. Posterior probability support values above the branches. Colors only correspond to each tanglegram. A) *Heteroxynema cucullatum* QUAD lineages (left) and the corresponding *quadrivittatus* species group host (right). B) *Heteroxynema cucullatum* MIN lineage (left) and the corresponding *Tamias minimus* host

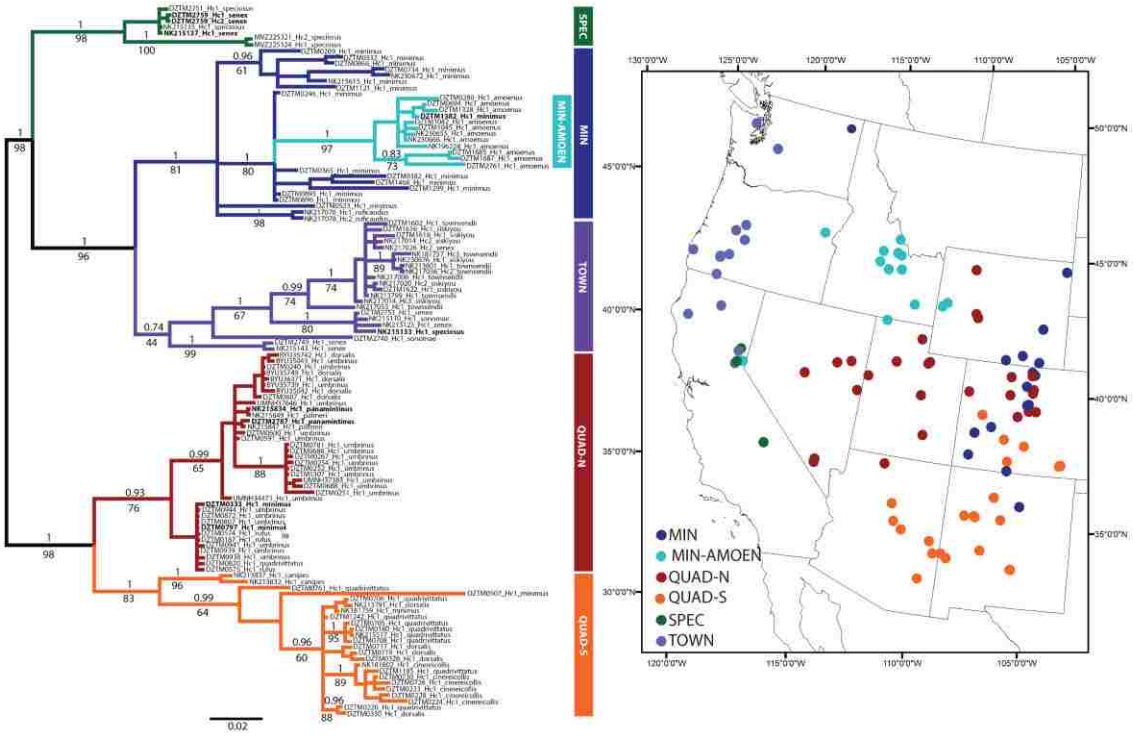
(right). C) *Rauschtineria eutamii* QUAD lineages (left) and the corresponding *quadrivittatus* species group host (right). D) *Rauschtineria eutamii* MIN lineage (left) and the corresponding *Tamias minimus* host (right).

Table 1. Mitochondrial species groups in western *Tamias*, as described in Piaggio and Spicer (2001). One species, *T. quadrimaculatus*, was moved from the *minimus* group, as proposed by Piaggio and Spicer, to the *townsendii* group, based on the findings of nuclear data (Reid et al. 2012). Reid et al. did not recover *T. durangae* as a member of the *quadrivittatus* group and *T. bulleri*, *T. palmeri*, and *T. ochrogenys* were not sampled.

Species group	Member species
amoenus	*T. amoenus*
merriami	*T. merriami*, *T. obscurus*
minimus	*T. minimus*, *T. panamintinus*, *T. ruficaudus*
quadrivittatus	*T. bulleri*, *T. canipes*, *T. cinereicollis*, *T. dorsalis*, *T. durangae*, *T. palmeri*, *T. quadrivittatus*, *T. rufus*, *T. umbrinus*
townsendii	*T. ochrogenys*, *T. quadrimaculatus*, *T. senex*, *T. siskiyou*, *T. sonomae*, *T. townsendii*

Table 2. Host switches among host associated lineages in *Heteroxynema cucullatum* and *Rauschtineria eutamii*. Locality number references map in Supplemental Figure 1.

Pinworm Clade	Locality	Host Species
H. cucullatum TOWN	71	*T. speciosus*
H. cucullatum SPEC	71	*T. senex*
H. cucullatum QUAD-N	62	*T. minimus*
H. cucullatum QUAD-N	104	*T. panamintinus*
H. cucullatum QUAD-M	67	*T. minimus*
H. cucullatum QUAD-S	85	*T. minimus*
H. cucullatum QUAD-S	110	*T. minimus*
R. eutamii SPEC	101	*T. alpinus*
R. eutamii MIN	93	*T. dorsalis*
R. eutamii MIN	94	*T. dorsalis*
R. eutamii MIN	86	*T. umbrinus*
R. eutamii MIN	66	*T. umbrinus*
R. eutamii MIN	61	*T. umbrinus*
R. eutamii MIN	68	*T. rufus*
R. eutamii MIN	34	*T. umbrinus*
R. eutamii QUAD-M	89	*T. minimus*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MIN
MIN-AMOEN
TOWN
QUAD-N
QUAD-S
0.02
130°0'0"W
125°0'0"W
120°0'0"W
115°0'0"W
110°0'0"W
105°0'0"W
50°0'0"N
45°0'0"N
40°0'0"N
35°0'0"N
30°0'0"N
MIN
MIN-AMOEN
QUAD-N
QUAD-S
SPEC
TOWN

Figure 1

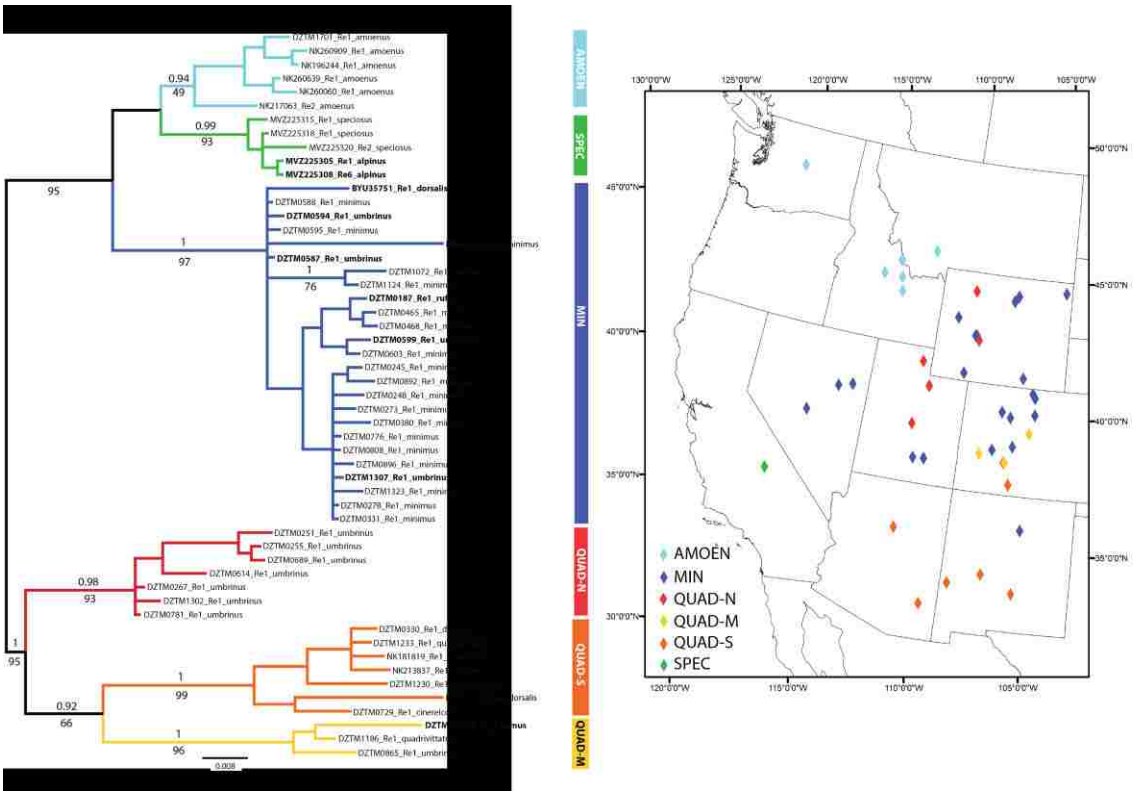

AMOEN
SPEC
MIN
QUAD-N
QUAD-S
QUAD-M
AMOEN
MIN
QUAD-N
QUAD-M
QUAD-S
SPEC

Figure 2

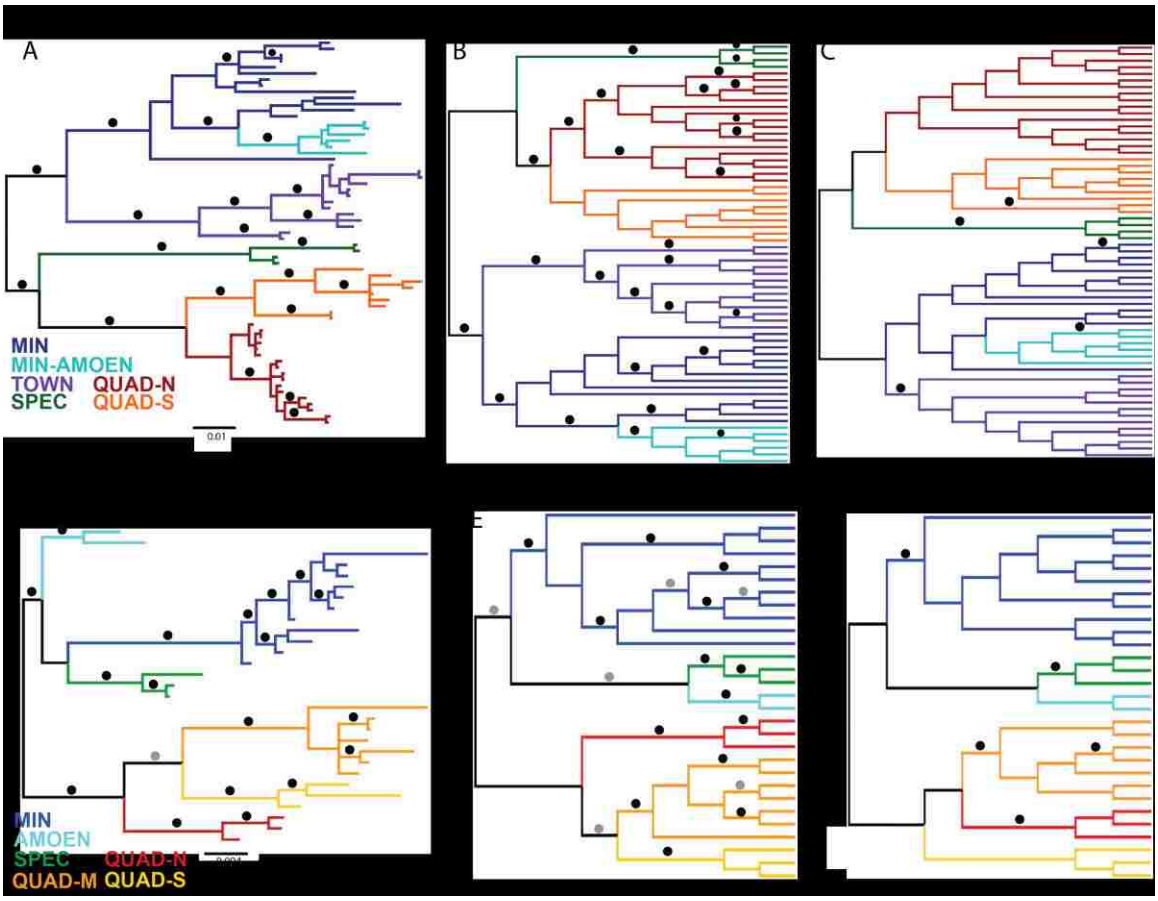

A
B
C
MIN
MIN-AMOEN
TOWN
QUAD-N
SPEC
QUAD-S
0.01
MIN
AMOEN
SPEC
QUAD-N
QUAD-M
QUAD-S

Figure 3

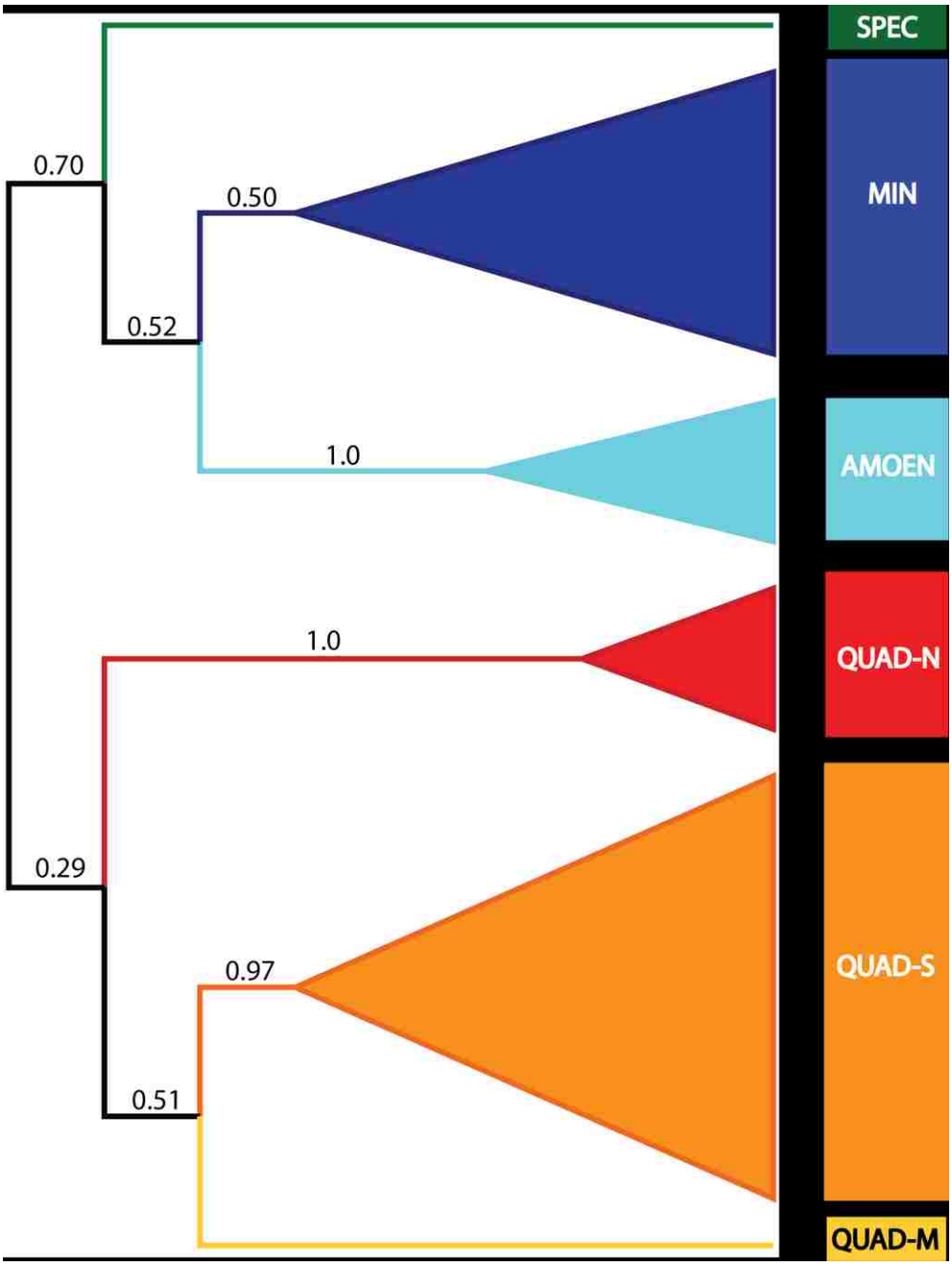


Figure 4

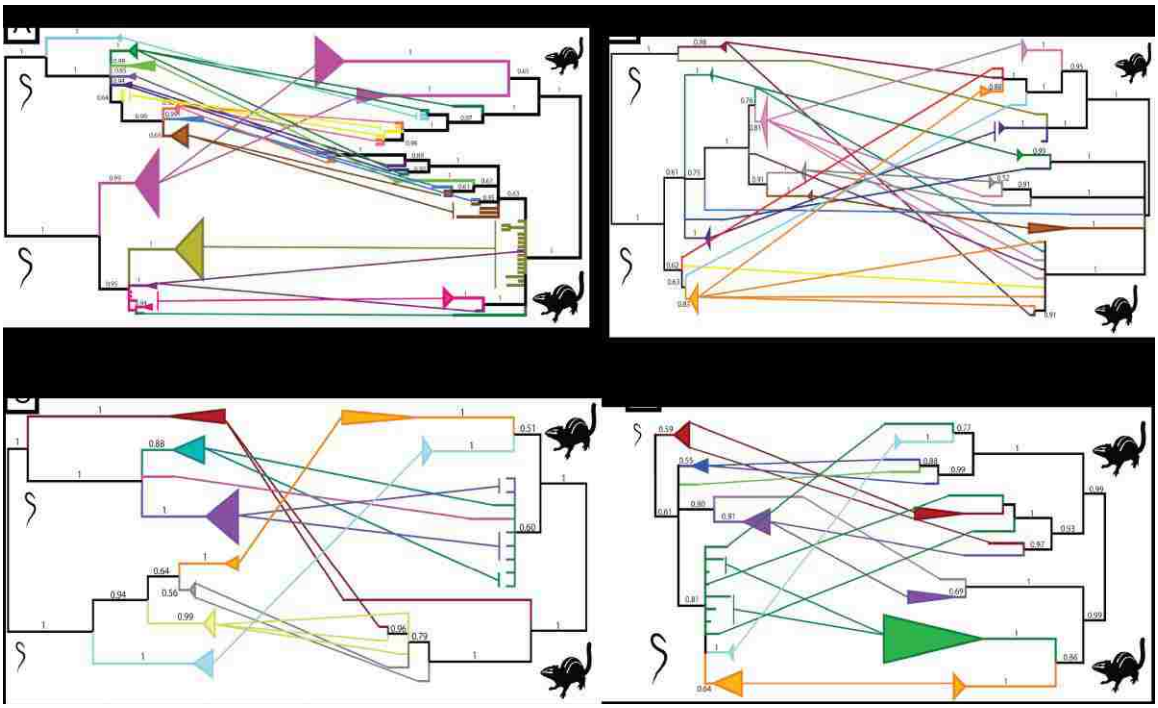


Figure 5

Supplemental Figure 1. Sampling localities for *Heteroxynema cucullatum* and *Rauschtineria eutamii* in the western United States; numbers correspond to localities in Appendix I.

Supplemental Figure 2. Bayesian gene trees for *Heteroxynema cucullatum* (A) 28S, (B) NC5 and *Rauschtineria eutamii* (C) 28S, (D) 12S, (E) SSU. Colors correspond to COI host-associated lineages. Posterior probability support values above branches.

Supplemental Figure 3. Distribution of members of the *quadrivittatus* species group and *T. minimus* in the western United States

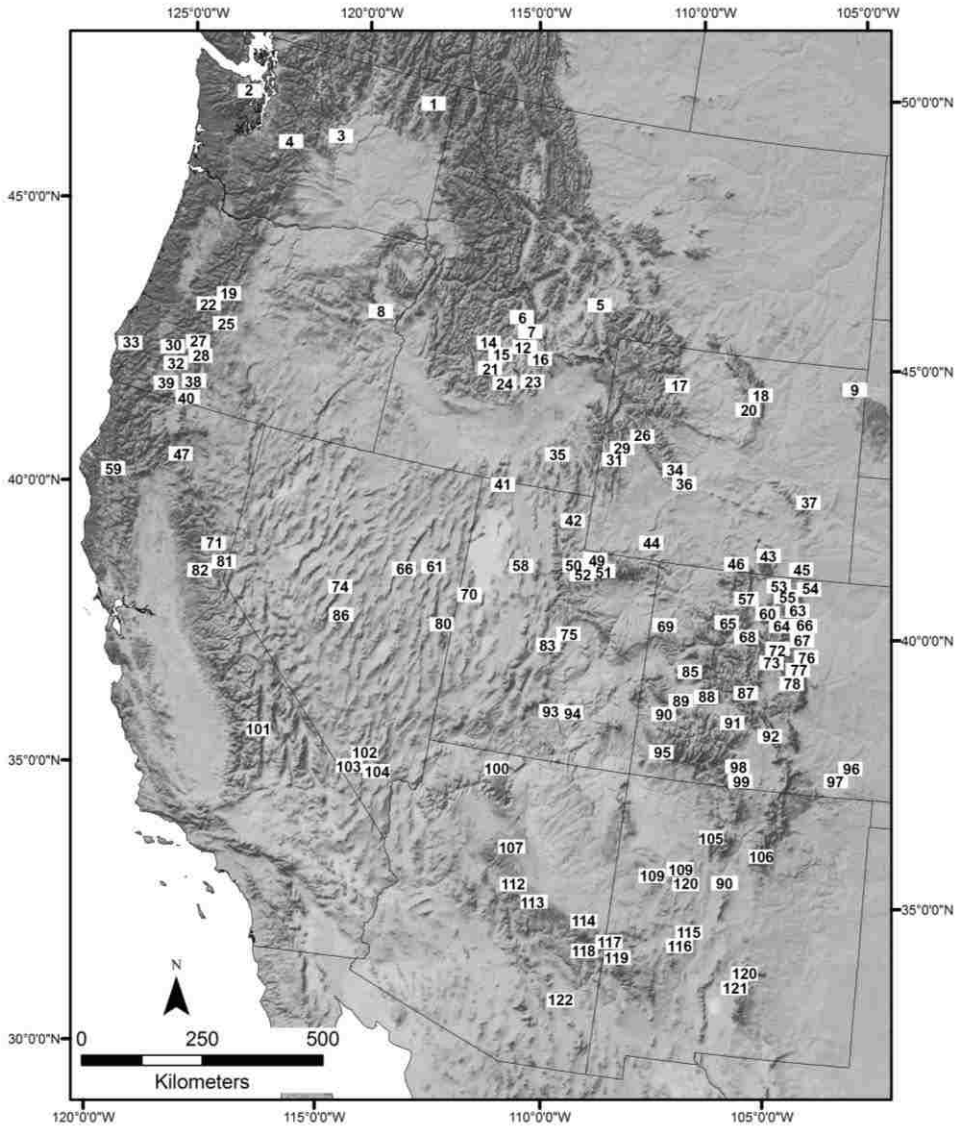
Supplemental Table 1. Primers and annealing temperatures that were sequenced for *H. cucullatum* and *R. eutamii*.

Primers	Species	Locus	Annealing (°C)	Reference
SyphaCOIf/SyphaCOIr	Both	COI	45	Okamoto et al. 2007
12Sf/12Sr	*R. eutamii*	12S	56	Casiraghi et al. 2004
28S-C1/28S-D2	Both	28S	55	Gouÿ de Bellocq et al. 2001
NC5f/NC5r	*H. cucullatum*	ITS-1, 5.8S, ITS-2	60	Newton et al. 1998
SSUA/NC2	*R. eutamii*	18S	53	Dorris et al. 2002 Gasser et al. 1993

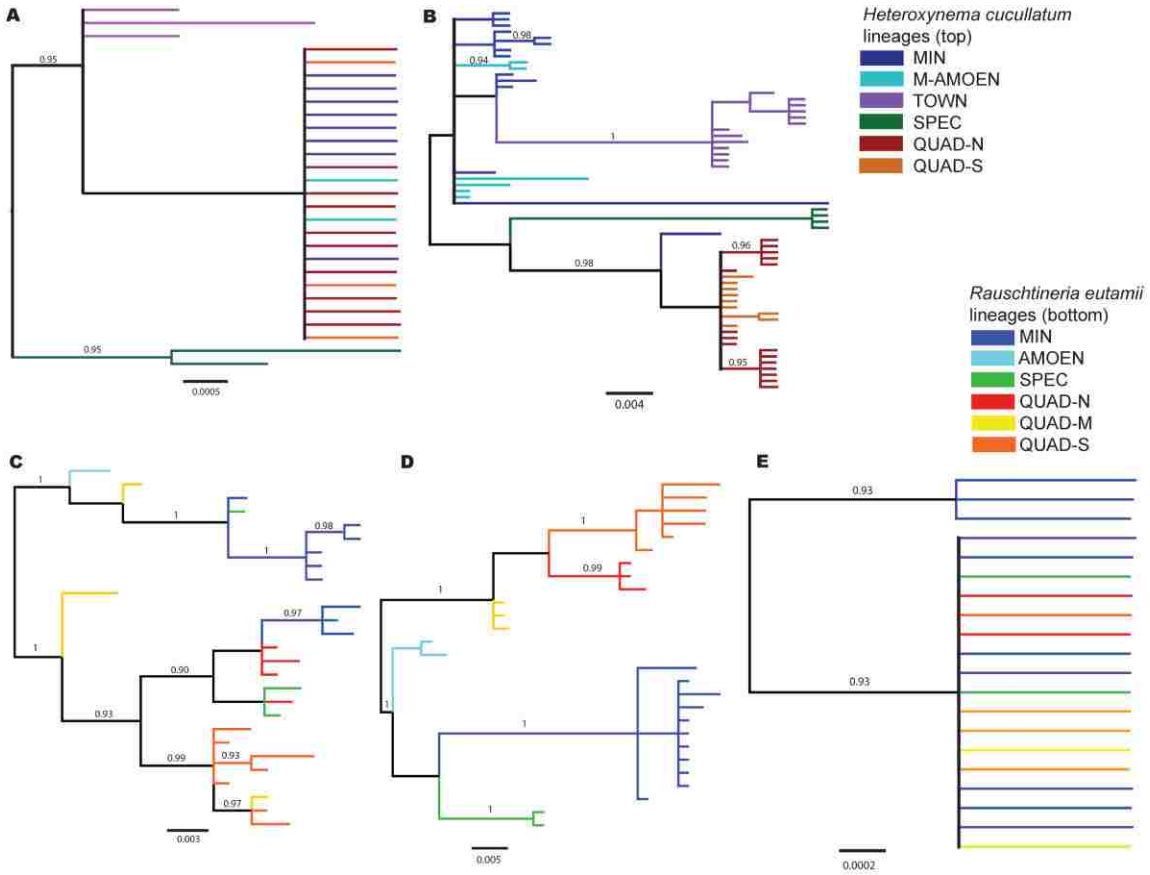
Supplemental Table 2. Diversity and demographic measures for *Heteroxynema cucullatum* COI (705 bp) and *Rauschtineria eutamii* COI (767 bp) lineages and two sets of hosts, *Tamias minimus* cyt*b* (732 bp) and the *quadrivittatus* species group cyt*b* (769 bp). N=sample size, S=number of segregating sites, h=number of haplotypes, Hd=haplotype (gene) diversity (and standard deviation), π=nucleotide diversity (and standard deviation), D=Tajima's D (significance), Fs=Fu's Fs (significance), MM-D=p-value of mismatch test of demographic expansion, MM-S=p-value of mismatch test of spatial expansion (not significant p-values indicate inability to reject a model of expansion). Non-significant p-values (n.s.) are above 0.05. Hc_MIN includes the *H. cucullatum* MIN-AMOEN subclade, however MIN-AMOEN clade is also assessed independently. The *H. cucullatum* same and *R. eutamii* same are measures for pinworms collected from the same host individual (N=14) or the same host species at the same locality (N=13).

Lineage	**N**	**S**	**h**	**Hd**	**π**	**D**	**Fs**	**MM-D**	**MM-S**
Heteroxynema cucullatum	224	174	179	0.998 (0.001)	0.054 (0.026)	NA	NA	NA	NA
QUAD	97	113	73	0.992 (0.003)	0.033 (0.016)	NA	NA	NA	NA
QUAD-N	60	58	46	0.987 (0.007)	0.016 (0.008)	-0.310 (n.s.)	-14.78 (0.003)	n.s.	n.s.
QUAD-S	37	82	27	0.981 (0.011)	0.023 (0.011)	-0.605 (n.s.)	-2.124 (n.s.)	n.s.	n.s.
MIN	62	114	51	0.993 (0.004)	0.038 (0.019)	0.362 (n.s.)	-7.759 (0.045)	n.s.	n.s.
SPEC	14	28	11	0.967 (0.037)	0.019 (0.010)	2.090 (n.s.)	2.428 (n.s.)	n.s.	n.s.
MIN-AMOEN	12	18	7	0.773 (0.128)	0.010 (0.006)	1.092 (n.s.)	1.024 (n.s.)	0.014	n.s.
TOWN	51	82	44	0.992 (0.007)	0.022 (0.011)	-0.491 (n.s.)	-1.757 (n.s.)	n.s.	n.s.
Rauschtineria eutamii	80	136	71	0.994 (0.005)	0.032 (0.016)	NA	NA	NA	NA
QUAD	26	89	24	0.994 (0.013)	0.031 (0.016)	NA	NA	NA	NA
QUAD-N	10	24	9	0.978 (0.054)	0.011 (0.006)	-0.047 (n.s.)	0.470 (n.s.)	n.s.	n.s.
QUAD-M	3	18	3	1.00 (0.272)	0.016 (0.012)	NA	NA	NA	NA
QUAD-S	13	35	12	0.987 (0.035)	0.017 (0.009)	0.644 (n.s.)	-0.202 (n.s.)	n.s.	n.s.
MIN	32	55	32	1.00 (0.008)	0.014 (0.007)	-0.801 (n.s.)	-8.812 (0.007)	n.s.	n.s.

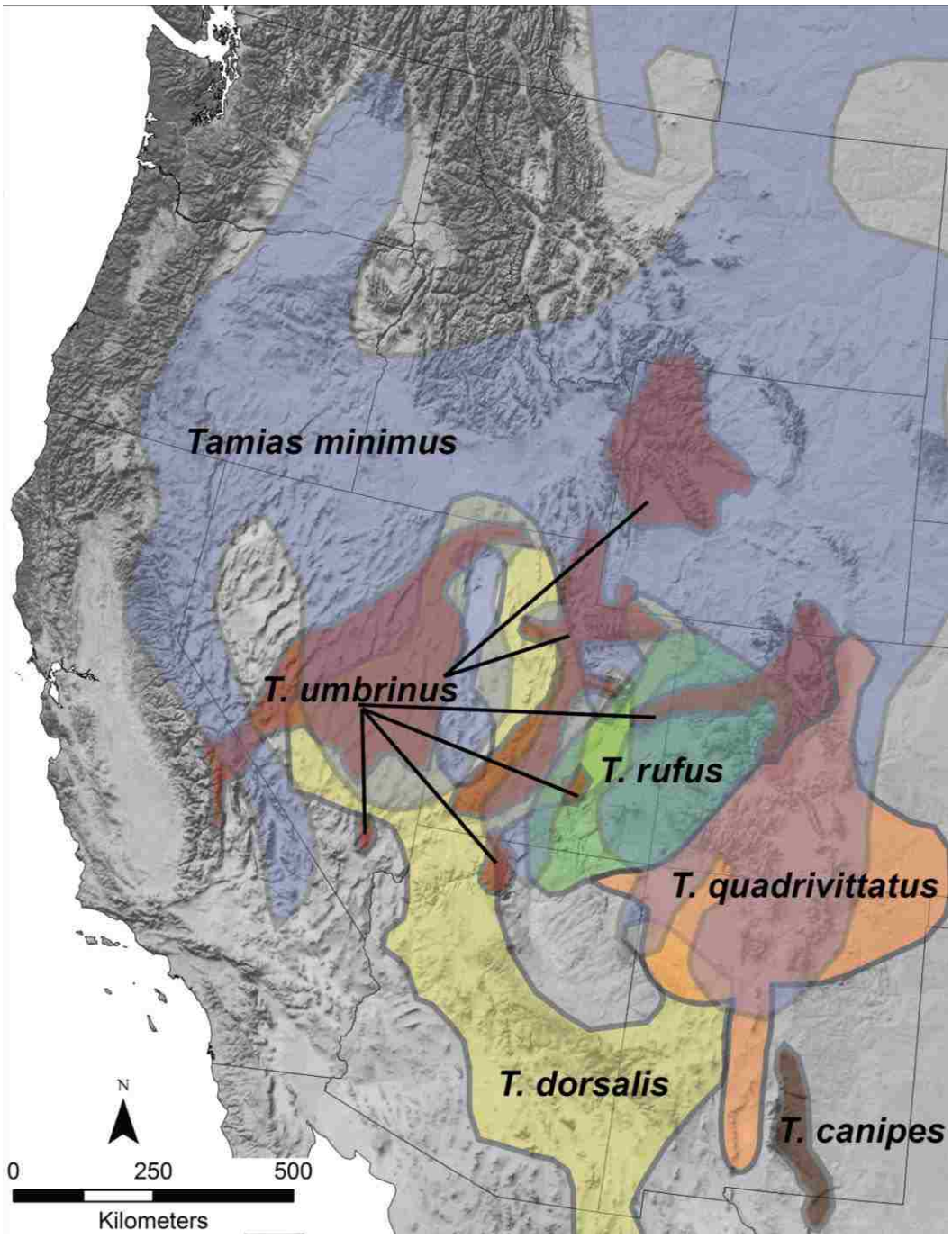
SPEC	10	7	8	0.933 (0.078)	0.002 (0.002)	-1.421 (n.s.)	0.166 (n.s.)	n.s.	n.s.
AMOEN	23	50	18	0.972 (0.022)	0.015 (0.008)	-0.855 (n.s.)	-3.289 (n.s.)	n.s.	n.s.
H. cucullatum same	33	125	30	0.992 (0.010)	0.051 (0.026)	NA	NA	NA	NA
R. eutamii same	33	124	33	1.00 (0.008)	0.034 (0.017)	NA	NA	NA	NA
T. minimus hosts	48	131	38	0.982 (0.011)	0.038 (0.019)	NA	NA	n.s.	0.04
quadrivittatus species group hosts	76	76	39	0.966 (0.009)	0.019 (0.009)	NA	NA	n.s	n.s.



Supplemental Figure 1.



Supplemental Figure 2



Supplemental Figure 3

Appendix I.

Heteroxynema cucullatum (Hc) and *Rauschtineria eutamii* (Re) specimen number, catalog number (if cataloged independently from host), mitochondrial clade, host catalog number, host species, and locality number. All hosts are genus *Tamias*. Parasite catalog numbers are all Museum of Southwestern Biology (MSB). Host catalog numbers are Monte L. Bean Museum of Natural History Mammal Collection (BYU), Denver Museum of Nature & Science Mammal Collection (ZM), Museum of Southwestern Biology Division of Mammals (MSB), Utah Museum of Natural History Mammal Collection (UMNH). Locality numbers are illustrated in Supplemental Figure 1.

Sample	Catalog Number	COI Clade	Host Catalog	Host species	Locality
BYU35042_Hc1	MSB 20718	QUAD-N	BYU 35042	*T. umbrinus*	58
BYU35043_Hc1	MSB 20777	QUAD-N	BYU 35043	*T. umbrinus*	58
BYU35739_Hc1	MSB 20744	QUAD-N	BYU 35739	*T. dorsalis*	93
BYU35740_Hc1	MSB 20746	QUAD-N	BYU 35740	*T. dorsalis*	93
BYU35742_Hc1	MSB 20749	QUAD-N	BYU 35742	*T. dorsalis*	93
BYU35749_Hc1	MSB 20763	QUAD-N	BYU 35749	*T. dorsalis*	93
BYU36371_Hc1	MSB 20776	QUAD-N	BYU 36371	*T. dorsalis*	75
DZTM0126_Hc1		MIN	ZM.11498	*T. minimus*	72
DZTM0135_Hc1		MIN	ZM.11453	*T. minimus*	60
DZTM0180_Hc1		QUAD-S	ZM.11100	*T. quadrivittatus*	92
DZTM0187_Hc1		QUAD-N	ZM.11205	*T. rufus*	68
DZTM0189_Hc1		QUAD-N	ZM.11207	*T. rufus*	68
DZTM0190_Hc1		QUAD-N	ZM.11208	*T. rufus*	68
DZTM0207_Hc1		MIN	ZM.11123	*T. minimus*	99
DZTM0209_Hc1		MIN	ZM.11125	*T. minimus*	99
DZTM0220_Hc1		QUAD-S	ZM.11136	*T. quadrivittatus*	111
DZTM0221_Hc1		QUAD-S	ZM.11137	*T. quadrivittatus*	111
DZTM0224_Hc1		QUAD-S	ZM.11109	*T. cinereicollis*	117
DZTM0228_Hc1		QUAD-S	ZM.11113	*T. cinereicollis*	118
DZTM0230_Hc1		QUAD-S	ZM.11115	*T. cinereicollis*	114
DZTM0233_Hc1		QUAD-S	ZM.11118	*T. cinereicollis*	113
DZTM0240_Hc1		QUAD-N	ZM.11381	*T. umbrinus*	100
DZTM0246_Hc1		MIN	ZM.11184	*T. minimus*	43
DZTM0251_Hc1		QUAD-N	ZM.11160	*T. umbrinus*	56
DZTM0252_Hc1		QUAD-N	ZM.11161	*T. umbrinus*	56
DZTM0253_Hc1		QUAD-N	ZM.11162	*T. umbrinus*	56
DZTM0254_Hc1		QUAD-N	ZM.11163	*T. umbrinus*	56
DZTM0257_Hc1		QUAD-N	ZM.11166	*T. umbrinus*	51
DZTM0259_Hc1		QUAD-N	ZM.11168	*T. umbrinus*	51
DZTM0262_Hc1		MIN-AMOEN	ZM. 11171	*T. amoenus*	41
DZTM0263_Hc1		MIN-AMOEN	ZM.11172	*T. amoenus*	41

DZTM0264_Hc1	MIN-AMOEN	ZM.11173	*T. amoenus*	41
DZTM0267_Hc1	QUAD-N	ZM.11147	*T. umbrinus*	17
DZTM0268_Hc1	QUAD-N	ZM.11148	*T. umbrinus*	17
DZTM0269_Hc1	QUAD-N	ZM.11149	*T. umbrinus*	17
DZTM0280_Hc1	MIN-AMOEN	ZM.11174	*T. amoenus*	41
DZTM0328_Hc1	QUAD-S	ZM.11426	*T. dorsalis*	116
DZTM0330_Hc1	QUAD-S	ZM.11428	*T. dorsalis*	115
DZTM0332_Hc1	MIN	ZM.11430	*T. minimus*	67
DZTM0333_Hc1	QUAD-N	ZM.11431	*T. minimus*	67
DZTM0335_Hc1	QUAD-N	ZM.11433	*T. umbrinus*	67
DZTM0355_Hc1	MIN	ZM.11400	*T. minimus*	50
DZTM0356_Hc1	MIN	ZM.11401	*T. minimus*	50
DZTM0363_Hc1	MIN	ZM.11407	*T. minimus*	37
DZTM0365_Hc1	MIN	ZM.11409	*T. minimus*	37
DZTM0370_Hc1	MIN	ZM.11413	*T. minimus*	45
DZTM0380_Hc1	MIN	ZM.11649	*T. minimus*	9
DZTM0381_Hc1	MIN	ZM.11650	*T. minimus*	9
DZTM0382_Hc1	MIN	ZM.11651	*T. minimus*	9
DZTM0507_Hc1	QUAD-S	ZM.11583	*T. minimus*	85
DZTM0523_Hc1	MIN	ZM.11596	*T. minimus*	90
DZTM0563_Hc1	QUAD-N	ZM.11625	*T. umbrinus*	84
DZTM0565_Hc1	MIN	ZM.11627	*T. minimus*	84
DZTM0574_Hc1	QUAD-N	ZM.11807	*T. rufus*	69
DZTM0575_Hc1	QUAD-N	ZM.11808	*T. rufus*	69
DZTM0590_Hc1	QUAD-N	ZM.11668	*T. umbrinus*	66
DZTM0591_Hc1	QUAD-N	ZM.11669	*T. umbrinus*	66
DZTM0592_Hc1	QUAD-N	ZM.11670	*T. umbrinus*	66
DZTM0599_Hc1	QUAD-N	ZM.11686	*T. umbrinus*	61
DZTM0600_Hc1	QUAD-N	ZM.11687	*T. umbrinus*	61
DZTM0607_Hc1	QUAD-N	ZM.11694	*T. umbrinus*	80
DZTM0686_Hc1	QUAD-N	ZM.11792	*T. umbrinus*	42
DZTM0687_Hc1	QUAD-N	ZM.11793	*T. umbrinus*	42
DZTM0688_Hc1	QUAD-N	ZM.11794	*T. umbrinus*	42
DZTM0690_Hc1	QUAD-N	ZM.11796	*T. umbrinus*	42
DZTM0692_Hc1	MIN-AMOEN	ZM.11798	*T. amoenus*	31
DZTM0693_Hc1	MIN-AMOEN	ZM.11799	*T. amoenus*	31
DZTM0694_Hc1	MIN-AMOEN	ZM.11800	*T. amoenus*	31
DZTM0704_Hc1	QUAD-S	ZM.11818	*T. quadrivittatus*	109
DZTM0705_Hc1	QUAD-S	ZM.11819	*T. quadrivittatus*	109
DZTM0706_Hc1	QUAD-S	ZM.11820	*T. quadrivittatus*	109
DZTM0708_Hc1	QUAD-S	ZM.11822	*T. quadrivittatus*	109
DZTM0717_Hc1	QUAD-S	ZM.11841	*T. dorsalis*	122
DZTM0719_Hc1	QUAD-S	ZM.11843	*T. dorsalis*	122
DZTM0722_Hc1	QUAD-S	ZM.11846	*T. cinereicollis*	112
DZTM0726_Hc1	QUAD-S	ZM.11850	*T. cinereicollis*	112
DZTM0728_HC1	QUAD-S	ZM.11852	*T. cinereicollis*	106
DZTM0730_Hc1	QUAD-S	ZM.11854	*T. cinereicollis*	106
DZTM0734_Hc1	MIN	ZM.11830	*T. minimus*	106
DZTM0748_Hc1	QUAD-S	ZM.11867	*T. quadrivittatus*	96
DZTM0761_Hc1	QUAD-S	ZM.11872	*T. quadrivittatus*	97
DZTM0781_Hc1	QUAD-N	ZM.11881	*T. umbrinus*	36
DZTM0797_Hc1	QUAD-N	ZM.11914	*T. minimus*	62
DZTM0804_Hc1	MIN	ZM.11921	*T. minimus*	53
DZTM0807_Hc1	QUAD-N	ZM.11924	*T. umbrinus*	54
DZTM0820_Hc1	QUAD-N	ZM.11605	*T. quadrivittatus*	77
DZTM0825_Hc1	QUAD-N	ZM.11941	*T. quadrivittatus*	78

DZTM0864_Hc1	MIN	ZM.11981	*T. minimus*	76
DZTM0865_Hc1	QUAD-N	ZM.11982	*T. umbrinus*	76
DZTM0866_Hc1	MIN	ZM.11983	*T. minimus*	76
DZTM0872_Hc1	QUAD-N	ZM.11989	*T. umbrinus*	63
DZTM0880_Hc1	MIN	ZM.12028	*T. minimus*	48
DZTM0893_Hc1	MIN	ZM.12041	*T. minimus*	48
DZTM0896_Hc2	MIN	ZM.12044	*T. minimus*	48
DZTM0919_Hc1	QUAD-N	ZM.12067	*T. umbrinus*	55
DZTM0938_Hc1	QUAD-N	ZM.12086	*T. umbrinus*	57
DZTM0939_Hc1	QUAD-N	ZM.12087	*T. umbrinus*	57
DZTM0941_Hc1	QUAD-N	ZM.12089	*T. umbrinus*	57
DZTM0944_Hc1	QUAD-N	ZM.12092	*T. umbrinus*	57
DZTM0964_Hc1	QUAD-N	ZM.12108	*T. umbrinus*	57
DZTM1000_Hc1	MIN	ZM.12122	*T. minimus*	73
DZTM1042_Hc1	MIN-AMOEN	ZM.12130	*T. amoenus*	16
DZTM1045_Hc1	MIN-AMOEN	ZM.12132	*T. amoenus*	16
DZTM1118_Hc1	MIN	ZM.12154	*T. minimus*	88
DZTM1119_Hc1	MIN	ZM.12155	*T. minimus*	88
DZTM1121_Hc1	MIN	ZM.12157	*T. minimus*	88
DZTM1175_Hc1	QUAD-S	ZM.12163	*T. quadrivittatus*	91
DZTM1177_Hc1	QUAD-S	ZM.12166	*T. quadrivittatus*	91
DZTM1181_Hc1	QUAD-S	ZM.12169	*T. quadrivittatus*	91
DZTM1185_Hc1	QUAD-S	ZM.12171	*T. quadrivittatus*	91
DZTM1187_Hc1	QUAD-S	ZM.12173	*T. quadrivittatus*	91
DZTM1242_Hc1	QUAD-S	ZM.12193	*T. quadrivittatus*	98
DZTM1291_Hc1	MIN	ZM.12199	*T. minimus*	95
DZTM1299_Hc1	MIN	ZM.12207	*T. minimus*	95
DZTM1307_Hc1	QUAD-N	ZM.12211	*T. umbrinus*	34
DZTM1324_Hc1	MIN-AMOEN	ZM.12220	*T. amoenus*	29
DZTM1328_Hc1	MIN-AMOEN	ZM.12224	*T. amoenus*	29
DZTM1382_Hc1	MIN-AMOEN	ZM.12230	*T. minimus*	35
DZTM1468_Hc1	MIN	ZM.12289	*T. minimus*	46
DZTM1602_Hc1	TOWN	ZM.12345	*T. townsendii*	33
DZTM1612_Hc1	TOWN	ZM.12355	*T. siskiyou*	38
DZTM1616_Hc1	TOWN	ZM.12359	*T. siskiyou*	40
DZTM1616_Hc2	TOWN	ZM.12359	*T. siskiyou*	40
DZTM1616_Hc3	TOWN	ZM.12359	*T. siskiyou*	40
DZTM1618_Hc1	TOWN	ZM.12361	*T. siskiyou*	28
DZTM1622_Hc1	TOWN	ZM.12365	*T. siskiyou*	27
DZTM1627_Hc1	TOWN	ZM.12370	*T. siskiyou*	19
DZTM1634_Hc1	TOWN	ZM.12377	*T. siskiyou*	19
DZTM1635_Hc1	TOWN	ZM.12378	*T. siskiyou*	19
DZTM1647_Hc1	TOWN	ZM.12390	*T. townsendii*	2
DZTM1647_Hc4	TOWN	ZM.12390	*T. townsendii*	2
DZTM1677_Hc1	MIN	ZM.12420	*T. ruficaudus*	1
DZTM1677_Hc2	MIN	ZM.12420	*T. ruficaudus*	1
DZTM1685_Hc1	MIN-AMOEN	ZM.12428	*T. amoenus*	8
DZTM1687_Hc1	MIN-AMOEN	ZM.12430	*T. amoenus*	8
DZTM1700_Hc1	MIN-AMOEN	ZM.12443	*T. amoenus*	23
DZTM2463_Hc1	MIN-AMOEN	ZM.13741	*T. amoenus*	21
DZTM2740_Hc1	TOWN	ZM.12979	*T. sonomae*	59
DZTM2740_Hc2	TOWN	ZM.12979	*T. sonomae*	59
DZTM2740_Hc3	TOWN	ZM.12979	*T. sonomae*	59
DZTM2745_Hc1	SPEC	ZM.12983	*T. speciosus*	71
DZTM2745_Hc2	SPEC	ZM.12983	*T. speciosus*	71
DZTM2749_Hc2	TOWN	ZM.12987	*T. senex*	71

DZTM2749_Hc3		TOWN	ZM.12987	*T. senex*	71
DZTM2751_Hc1		SPEC	ZM.12989	*T. speciosus*	71
DZTM2753_Hc1		TOWN	ZM.12991	*T. senex*	71
DZTM2759_Hc1		SPEC	ZM.12997	*T. senex*	82
DZTM2759_Hc2		SPEC	ZM.12997	*T. senex*	82
DZTM2761_Hc1		MIN-AMOEN	ZM.12999	*T. amoenus*	79
DZTM2776_Hc1		QUAD-N	ZM.13114	*T. palmeri*	103
DZTM2777_Hc1		QUAD-N	ZM.13115	*T. palmeri*	103
DZTM2787_Hc1		QUAD-N	ZM.13125	*T. panamintinus*	103
MVZ225311_Hc1	MSB 20692	SPEC	MVZ 225311	*T. speciosus*	101
MVZ225311_Hc2	MSB 20692	SPEC	MVZ 225311	*T. speciosus*	101
MVZ225311_Hc3	MSB 20692	SPEC	MVZ 225311	*T. speciosus*	101
MVZ225317_Hc1	MSB 20699	SPEC	MVZ 225317	*T. speciosus*	101
MVZ225321_Hc1	MSB 20712	SPEC	MVZ 225321	*T. speciosus*	101
MVZ225321_Hc2	MSB 20712	SPEC	MVZ 225321	*T. speciosus*	101
MVZ225324_Hc1	MSB 20713	SPEC	MVZ 225324	*T. speciosus*	101
NK181757_Hc1	MSB 20703	TOWN	MSB 249971	*T. townsendii*	22
NK181757_Hc2	MSB 20703	TOWN	MSB 249971	*T. townsendii*	22
NK181757_Hc3	MSB 20703	TOWN	MSB 249971	*T. townsendii*	22
NK181759_Hc1	MSB 20705	QUAD-S	MSB 249973	*T. minimus*	110
NK181802_Hc1	MSB 20709	QUAD-S	MSB 262520	*T. cinereicollis*	119
NK196228_Hc1	MSB 20742	MIN-AMOEN	MSB 230569	*T. amoenus*	7
NK213791_Hc1	MSB 20752	QUAD-S	MSB 248955	*T. dorsalis*	108
NK213791_Hc2	MSB 20752	QUAD-S	MSB 248955	*T. dorsalis*	108
NK213799_Hc1	MSB 20753	TOWN	MSB 249007	*T. townsendii*	30
NK213800_Hc1	MSB 20754	TOWN	MSB 248963	*T. townsendii*	32
NK213801_Hc1	MSB 20755	TOWN	MSB 248964	*T. townsendii*	32
NK213816_Hc1	MSB 20756	QUAD-S	MSB 248965	*T. canipes*	120
NK213832_Hc1	MSB 20767	QUAD-S	MSB 248981	*T. canipes*	121
NK213832_Hc2	MSB 20767	QUAD-S	MSB 248981	*T. canipes*	121
NK213837_Hc1	MSB 20770	QUAD-S	MSB 249009	*T. canipes*	121
NK215110_Hc1	MSB 20605	TOWN	MSB 259318	*T. sonomae*	47
NK215119_Hc1	MSB 20608	TOWN	MSB 259327	*T. sonomae*	59
NK215123_Hc1	MSB 20609	TOWN	MSB 259331	*T. senex*	71
NK215133_Hc1	MSB 20610	TOWN	MSB 259341	*T. speciosus*	71
NK215135_Hc1	MSB 20611	SPEC	MSB 259343	*T. speciosus*	71
NK215137_Hc1	MSB 20612	SPEC	MSB 259345	*T. senex*	71
NK215143_Hc1	MSB 20614	TOWN	MSB 259351	*T. senex*	81
NK215143_Hc2	MSB 20614	TOWN	MSB 259351	*T. senex*	81
NK215511_Hc1	MSB 20616	MIN	MSB 264105	*T. minimus*	105
NK215517_Hc1	MSB 20617	QUAD-S	MSB 264113	*T. quadrivittatus*	105
NK215535_Hc1	MSB 20619	MIN	MSB 264125	*T. minimus*	105
NK215541_Hc1	MSB 20620	MIN	MSB 267285	*T. minimus*	105
NK215615_Hc1	MSB 20627	MIN	MSB 264208	*T. minimus*	105
NK215834_Hc1	MSB 20631	QUAD-N	MSB 265594	*T. panamintinus*	104
NK215847_Hc1	MSB 20633	QUAD-N	MSB 265609	*T. palmeri*	103
NK215849_Hc1	MSB 20634	QUAD-N	MSB 265604	*T. palmeri*	103
NK217004_Hc1	MSB 20636	TOWN	MSB 233616	*T. townsendii*	33
NK217006_Hc1	MSB 20637	TOWN	MSB 233606	*T. townsendii*	33
NK217006_Hc2	MSB 20637	TOWN	MSB 233606	*T. townsendii*	33
NK217012_Hc1	MSB 20639	TOWN	MSB 233816	*T. siskiyou*	39
NK217014_Hc1	MSB 20640	TOWN	MSB 233599	*T. siskiyou*	40
NK217014_Hc2	MSB 20640	TOWN	MSB 233599	*T. siskiyou*	40
NK217014_Hc3	MSB 20640	TOWN	MSB 233599	*T. siskiyou*	40
NK217016_Hc1	MSB 20641	TOWN	MSB 233627	*T. siskiyou*	38
NK217020_Hc1	MSB 20643	TOWN	MSB 233607	*T. siskiyou*	27

NK217020_Hc2	MSB 20643	TOWN	MSB 233607	*T. siskiyou*	27
NK217022_Hc1	MSB 20644	TOWN	MSB 233581	*T. siskiyou*	27
NK217026_Hc1	MSB 20646	TOWN	MSB 233586	*T. siskiyou*	27
NK217028_Hc1	MSB 20647	TOWN	MSB 233595	*T. siskiyou*	19
NK217028_Hc2	MSB 20647	TOWN	MSB 233595	*T. siskiyou*	19
NK217029_Hc1	MSB 20648	TOWN	MSB 233632	*T. siskiyou*	19
NK217029_Hc2	MSB 20648	TOWN	MSB 233632	*T. siskiyou*	19
NK217036_Hc1	MSB 20649	TOWN	MSB 233636	*T. townsendii*	19
NK217036_Hc2	MSB 20649	TOWN	MSB 233636	*T. townsendii*	19
NK217036_Hc3	MSB 20649	TOWN	MSB 233636	*T. townsendii*	19
NK217053_Hc1	MSB 20650	TOWN	MSB 233589	*T. townsendii*	4
NK217078_Hc1	MSB 20655	MIN	MSB 233585	*T. ruficaudus*	1
NK217078_Hc2	MSB 20655	MIN	MSB 233585	*T. ruficaudus*	1
NK230598_Hc1		MIN-AMOEN	MSB 269653	*T. amoenus*	24
NK230651_Hc1	MSB 20666	MIN-AMOEN	MSB 269867	*T. amoenus*	10
NK230655_Hc1	MSB 20669	MIN-AMOEN	MSB 269871	*T. amoenus*	11
NK230666_Hc1	MSB 20670	MIN-AMOEN	MSB 269882	*T. amoenus*	12
NK230672_Hc1	MSB 20676	MIN	MSB 270045	*T. minimus*	106
NK230676_Hc1	MSB 20680	TOWN	MSB 270052	*T. siskiyou*	25
NK260040_Hc1	MSB 24559	MIN-AMOEN	MSB 274470	*T. amoenus*	14
UMNH37383_Hc1		QUAD-N	UMNH 37383	*T. umbrinus*	123
UMNH34471_Hc1	MSB 20715	QUAD-N	UMNH 34471	*T. umbrinus*	74
UMNH34474_Hc1	MSB 20716	QUAD-N	UMNH 34474	*T. umbrinus*	74
UMNH37637_Hc1		QUAD-N	UMNH 37637	*T. umbrinus*	124
UMNH37646_Hc1		QUAD-N	UMNH 37646	*T. umbrinus*	125
BYU35739_Re1	MSB 20745	MIN	BYU 35739	*T. dorsalis*	93
BYU35751_Re1	MSB 20762	MIN	BYU 35751	*T. dorsalis*	94
DZTM0187_Re1		MIN	ZM.11205	*T. rufus*	68
DZTM0245_Re1		MIN	ZM.11183	*T. minimus*	43
DZTM0248_Re1		MIN	ZM.11142	*T. minimus*	44
DZTM0251_Re1		QUAD-N	ZM.11160	*T. umbrinus*	56
DZTM0255_Re1		QUAD-N	ZM.11164	*T. umbrinus*	56
DZTM0267_Re2		QUAD-N	ZM.11147	*T. umbrinus*	17
DZTM0273_Re1		MIN	ZM.11153	*T. minimus*	17
DZTM0278_Re1		MIN	ZM.11158	*T. minimus*	20
DZTM0328_Re2		QUAD-S	ZM.11426	*T. dorsalis*	116
DZTM0330_Re1		QUAD-S	ZM.11428	*T. dorsalis*	115
DZTM0331_Re1		MIN	ZM.11429	*T. minimus*	64
DZTM0380_Re1		MIN	ZM.11649	*T. minimus*	9
DZTM0465_Re1		MIN	ZM.11545	*T. minimus*	65
DZTM0468_Re1		MIN	ZM.11548	*T. minimus*	65
DZTM0498_Re1		MIN	ZM.11578	*T. minimus*	65
DZTM0529_Re1		QUAD-M	ZM.11600	*T. minimus*	89
DZTM0587_Re1		MIN	ZM.11681	*T. umbrinus*	86
DZTM0588_Re1		MIN	ZM.11682	*T. minimus*	86
DZTM0594_Re1		MIN	ZM.11672	*T. umbrinus*	66
DZTM0595_Re1		MIN	ZM.11673	*T. minimus*	66
DZTM0599_Re1		MIN	ZM.11686	*T. umbrinus*	61
DZTM0603_Re1		MIN	ZM.11690	*T. minimus*	61
DZTM0614_Re1		QUAD-N	ZM.11701	*T. umbrinus*	83
DZTM0686_Re1		QUAD-N	ZM.11792	*T. umbrinus*	42
DZTM0687_Re1		QUAD-N	ZM.11793	*T. umbrinus*	42
DZTM0688_Re1		QUAD-N	ZM.11794	*T. umbrinus*	42
DZTM0689_Re1		QUAD-N	ZM.11795	*T. umbrinus*	42
DZTM0714_Re1		QUAD-S	ZM.11838	*T. dorsalis*	122
DZTM0717_Re1		QUAD-S	ZM.11841	*T. dorsalis*	122

DZTM0719_Re1		QUAD-S	ZM.11843	*T. dorsalis*	122
DZTM0729_Re1		QUAD-S	ZM.11853	*T. cinereicollis*	107
DZTM0731_Re1		MIN	ZM.11827	*T. minimus*	106
DZTM0775_Re1		MIN	ZM.11875	*T. minimus*	18
DZTM0776_Re1		MIN	ZM.11876	*T. minimus*	18
DZTM0781_Re1		QUAD-N	ZM.11881	*T. umbrinus*	36
DZTM0808_Re1		MIN	ZM.11925	*T. minimus*	55
DZTM0865_Re1		QUAD-M	ZM.11982	*T. umbrinus*	76
DZTM0892_Re1		MIN	ZM.12040	*T. minimus*	48
DZTM0896_Re1		MIN	ZM.12044	*T. minimus*	48
DZTM1045_Re1		AMOEN	ZM.12132	*T. amoenus*	16
DZTM1048_Re1		MIN	ZM.12134	*T. minimus*	87
DZTM1072_Re1		MIN	ZM.12137	*T. minimus*	87
DZTM1124_Re1		MIN	ZM.12160	*T. minimus*	88
DZTM1181_Re1		QUAD-S	ZM.12169	*T. quadrivittatus*	91
DZTM1186_Re1		QUAD-M	ZM.12172	*T. quadrivittatus*	91
DZTM1187_Re1		QUAD-S	ZM.12173	*T. quadrivittatus*	91
DZTM1228_Re1		QUAD-S	ZM.12183	*T. quadrivittatus*	98
DZTM1230_Re1		QUAD-S	ZM.12185	*T. quadrivittatus*	98
DZTM1233_Re1		QUAD-S	ZM.12188	*T. quadrivittatus*	98
DZTM1302_Re1		QUAD-N	ZM.12208	*T. umbrinus*	34
DZTM1307_Re1		MIN	ZM.12211	*T. umbrinus*	34
DZTM1321_Re1		MIN	ZM.12217	*T. minimus*	26
DZTM1323_Re1		MIN	ZM.12219	*T. minimus*	26
DZTM1654_Re1		AMOEN	ZM.12397	*T. amoenus*	3
DZTM1701_Re1		AMOEN	ZM.12444	*T. amoenus*	23
MVZ225305_Re1	MSB 20689	SPEC	MVZ 225305	*T. alpinus*	101
MVZ225308_Re1	MSB 20690	SPEC	MVZ 225308	*T. alpinus*	101
MVZ225308_Re6	MSB 20690	SPEC	MVZ 225308	*T. alpinus*	101
MVZ225312_Re1	MSB 20694	SPEC	MVZ 225312	*T. speciosus*	101
MVZ225314_Re1	MSB 20696	SPEC	MVZ 225314	*T. speciosus*	101
MVZ225315_Re1	MSB 20697	SPEC	MVZ 225315	*T. speciosus*	101
MVZ225316_Re1	MSB 20698	SPEC	MVZ 225316	*T. speciosus*	101
MVZ225318_Re1	MSB 20701	SPEC	MVZ 225318	*T. speciosus*	101
MVZ225320_Re1	MSB 20711	SPEC	MVZ 225320	*T. speciosus*	101
MVZ225320_Re2	MSB 20711	SPEC	MVZ 225320	*T. speciosus*	101
NK181819_Re1	MSB 20725	QUAD-S	MSB 262538	*T. cinereicollis*	119
NK196244_Re1	MSB 20751	AMOEN	MSB 230578	*T. amoenus*	6
NK213837_Re1	MSB 20771	QUAD-S	MSB 249014	*T. canipes*	121
NK217056_Re1	MSB 20651	AMOEN	MSB 233623	*T. amoenus*	3
NK217062_Re1	MSB 20652	AMOEN	MSB 233628	*T. amoenus*	3
NK217062_Re2	MSB 20652	AMOEN	MSB 233628	*T. amoenus*	3
NK217063_Re1	MSB 20653	AMOEN	MSB 233634	*T. amoenus*	3
NK217063_Re2	MSB 20653	AMOEN	MSB 233634	*T. amoenus*	3
NK230639_Re1	MSB 20662	AMOEN	MSB 269855	*T. amoenus*	15
NK230668_Re1	MSB 20671	MIN	MSB 270041	*T. minimus*	106
NK230669_Re1	MSB 20673	MIN	MSB 270042	*T. minimus*	106
NK260060_Re1	MSB 24560	AMOEN	MSB 274490	*T. amoenus*	13
NK260909_Re1	MSB 24550	AMOEN	MSB 278003	*T. amoenus*	5

Appendix II.

Tamias catalog numbers (ZM) and tissue numbers (DZTM), used for host analyses. If sequences were downloaded from GenBank the accession number is denoted in parentheses after the tissue number. All specimens are cataloged in the Denver Museum of Nature & Science Mammal Collection. Tissue numbers correspond to sample numbers for the pinworms.

Tamias minimus: ZM.11123, DZTM207; ZM.11125, DZTM209; ZM.11142, DZTM248; ZM.11153, DZTM273; ZM.11158, DZTM278; ZM.11183, DZTM245; ZM.11184, DZTM246; ZM.11400, DZTM355; ZM.11401, DZTM356; ZM.11407, DZTM363; ZM.11413, DZTM370; ZM.11429, DZTM331; ZM.11430, DZTM332; ZM.11431, DZTM333; ZM.11498, DZTM126; ZM.11545, DZTM465; ZM.11548, DZTM468; ZM.11578, DZTM498; ZM.11583, DZTM507; ZM.11596, DZTM523; ZM.11600, DZTM529; ZM.11627, DZTM565; ZM.11649, DZTM380; ZM.11650, DZTM381; ZM.11651, DZTM382; ZM.11673, DZTM595; ZM.11682, DZTM588; ZM.11690, DZTM603; ZM.11827, DZTM731; ZM.11830, DZTM734; ZM.11875, DZTM775; ZM.11876, DZTM776; ZM.11914, DZTM797; ZM.11921, DZTM804; ZM.11925, DZTM808; ZM.11981, DZTM864; ZM.11983, DZTM866; ZM.12028, DZTM880; ZM.12040, DZTM892; ZM.12041, DZTM893; ZM.12044, DZTM896; ZM.12122, DZTM1000; ZM.12134, DZTM1048; ZM.12137, DZTM1072; ZM.12154, DZTM1118; ZM.12155, DZTM1119; ZM.12157, DZTM1121; ZM.12160, DZTM1124; ZM.12199, DZTM1291; ZM.12207, DZTM1299; ZM.12217, DZTM1321; ZM.12219, DZTM1323;

quadrivittatus species group

Tamias cinereicollis: ZM11109, DZTM224 (KJ139537); ZM.11113, DZTM228 (KJ139539); ZM.11115, DZTM230 (JN042414); ZM.11118, DZTM233 (KJ139533); ZM.11846, DZTM722 (KJ139536); ZM.11850, DZTM726 (KJ139544); ZM.11852, DZTM728 (KJ139540); ZM.11854, DZTM730 (KJ139532)

Tamias dorsalis: ZM.11426, DZTM328 (KJ139559); ZM.11428, DZTM330 (KJ139562); ZM.11694, DZTM607, (KJ139573); ZM.11838, DZTM714 (KJ139552); ZM.11841, DZTM717 (KJ139555); ZM.11843, DZTM719 (KJ139556)

Tamias palmeri: ZM.13114, DZTM2776

Tamias quadrivittatus: ZM.11100, DZTM180 (KJ139499)); ZM.11136, DZTM220 (KJ139471); ZM.11137, DZTM221 (KJ139473); ZM.11605, DZTM820 (KJ139487); ZM.11818, DZTM704 (KJ139475); ZM.11819, DZTM705 (KJ139492); ZM.11820, DZTM706 (KJ139476); ZM.11822, DZTM708 (KJ139478); ZM.11867, DZTM748 (KJ139502); ZM.11872, DZTM761 (KJ139503); ZM.11941, DZTM825 (KJ139488); ZM.12163, DZTM1175 (KJ139513); ZM.12166, DZTM1177 (KJ139528); ZM.12169, DZTM1181 (KJ139524); ZM.12171, DZTM1185 (KJ139515); ZM.12172, DZTM1186 (KJ139516); ZM.12173, DZTM1187 (KJ139517); ZM.12183, DZTM1228 (KJ139518); ZM.12185, DZTM1230 (KJ139529); ZM.12188, DZTM1233 (KJ139519); ZM.12193, DZTM1242 (KJ139520)

Tamias rufus: ZM.11205, DZTM187 (KJ139467); ZM.11207, DZTM189 (KJ139468); ZM.11208, DZTM190 (KJ139469); ZM.11808, DZTM575 (KJ139465)

Tamias umbrinus: ZM.11147, DZTM267 (JN042397); ZM.11148, DZTM268 (KJ139631); ZM.11149, DZTM269 (KJ139640); ZM.11160, DZTM251 (JN042394);

ZM.11161, DZTM252 (KJ139633); ZM.11162, DZTM253 (KJ139634); ZM.11163, DZTM254 (KJ139635); ZM.11164, DZTM255 (KJ13963); ZM.11166, DZTM257 (KJ139617); ZM.11168, DZTM259 (KJ139636); ZM.11381, DZTM240 (KJ139639); ZM.11433, DZTM335 (KJ139595); ZM.11625, DZTM563 (KJ139596); ZM.11668, DZTM590 (KJ139606); ZM.11669, DZTM591 (KJ139607); ZM.11670, DZTM592 (KJ139609); ZM.11686, DZTM599 (KJ139610); ZM.11687, DZTM600 (KJ139611); ZM.11701, DZTM614 (KJ139615); ZM.11792, DZTM686 (KJ139629); ZM.11793, DZTM687 (KJ139628); ZM.11794, DZTM688 (KJ139627); ZM.11796, DZTM690 (KJ139626); ZM.11881, DZTM781 (KJ139624); ZM.11982, DZTM865 (KJ139598); ZM.11989, DZTM872 (KJ139599); ZM.12067, DZTM919 (KJ139600); ZM.12086, DZTM938 (KJ139592); ZM.12087, DZTM939 (KJ139601); ZM.12089, DZTM941 (KJ139588); ZM.12092, DZTM944 (KJ139587); ZM.12108, DZTM964 (KJ139593); ZM.12208, DZTM1302 (KJ139623); ZM.12211, DZTM1307 (KJ139620)

CHAPTER 4

DISENTANGLING LOUSEY RELATIONSHIPS: A PHYLOGENOMIC PERSPECTIVE ON HOST AND PARASITE DIVERSIFICATION

Kayce C. Bell

Abstract

Investigations of host-parasite codiversification can reveal processes that shape parasite evolution. Incorporating phylogenomic techniques into comparative phylogenetic studies, such as host-parasite codiversification, allows for rigorous tests of codiversification and provides the necessary baseline for future investigations of coevolution. Western North American chipmunks (genus *Tamias*) have a broad distribution, history of divergence with gene flow, and host two species of sucking lice (Anoplura), *Hoplopleura arboricola* and *Neohaematopinus pacificus*. I used loci sampled across the genomes for the chipmunk hosts and lice parasites to investigate codiversification in this system. The first molecular phylogeny with complete taxon sampling for *Tamias* revealed support for previously suggested taxonomic changes and points to lineages that need further investigation. Louse phylogenies revealed lineages that correspond to host groups, with varying levels of host switching. Additionally, relationships of *H. arboricola* with respect to the *T. striatus* louse (*H. erratica*) were not monophyletic; thus, the species is in need of taxonomic revision. The phylogenetic relationships among louse lineages, in both species, were not congruent with the host relationships uncovered here. Neither louse shares a history of strict codivergence with the *Tamias* hosts, with genetic structure within host associated lineages instead often corresponding to geographic structure. The louse-chipmunk system is consistent with

previous work showing that parasite diversification is heavily shaped by host biogeographic histories.

Introduction

Comparative phylogenetic studies have the potential to reveal processes that drive biological diversification, but this potential is dependent on the rigor of underlying phylogenetic analyses. Limited information and poor resolution from a few loci can impair phylogenetic reconstruction and weaken subsequent inferences. Phylogenomic investigations of non-model organisms are not common, but are becoming more frequent as new methods open evolutionary genomics to a diverse set of questions across a broad array of organisms (da Fonseca et al. in press). While new tools can improve our understanding of evolutionary history of individual clades, they also should advance our ability to explore evolutionary interactions among organisms, especially the rich but poorly understood histories of hosts and their associated parasitic taxa.

Coevolutionary investigations aim to reveal the role of interspecific interactions in shaping species evolution. While coevolution has a strict definition (i.e., interacting organisms must reciprocally exert selection on each other; Janzen 1980), there is a range of comparative approaches to test hypotheses about coevolution. Many studies begin by exploring phylogenetic histories of the interacting species to test whether they have codiverged over time. Importantly, codiversification tests rely on accurate phylogenetic reconstruction to enable the comparison of the evolutionary histories of two (or more) species.

As an illustration of Fahrenholz's Rule (strict cospeciation; Eichler 1948), chewing lice and pocket gophers (Geomyidae) have been held as a model of

codivergence by exemplifying concurrent divergence events between hosts and parasites at multiple scales (Timm 1983, Hafner and Page 1995, Hafner et al. 2003, Light and Hafner 2007). The basis for an expectation of codivergence in lice is that flightless insects that spend their entire life cycle on the host should have limited dispersal abilities and few opportunities for expansion (i.e., switching) to new hosts. While the standard expectation of host-parasite codivergence serves as a useful starting point, numerous examples highlight incongruous host and parasite divergences (e.g., avian malaria, Ricklefs et al. 2004; rodent *Eimeria,* Kvičerová and Hypša 2013; marine mammal digeneans, Fraija-Fernández et al. 2016; raptor feather lice, Catanach and Johnson 2015; chipmunk pinworms, Bell et al. in press). Indeed, factors dictating both host and parasite divergence and evolution are complex and likely to vary geographically due to historical biogeography of hosts (e.g., expansions and retractions, taxon pulses; Erwin 1981), host breadth of the parasite (sloppy fitness space, ecological fitting; Janzen 1985), factors external to the biotic interactions (e.g., climate), and population variation in the specificity and strength of the host-parasite interaction (Thompson 2005).

I employ a phylogenomic approach to test for codivergence in diverse mammalian hosts (chipmunks) and their two species of sucking lice. The 23 species of western North American chipmunks (genus *Tamias*, subgenus *Neotamias*) are broadly distributed across a variety of habitats and have a complex evolutionary history characterized by introgression and divergence with gene flow (Sullivan et al. 2014). To date, the rapid radiation of the subgenus *Neotamias* (~4 my) followed by a history of extensive interspecific gene flow have hindered attempts to produce a well-resolved species tree (Reid et al. 2012, Sullivan et al. 2014). Chipmunks are parasitized by two species of

ectoparasitic sucking lice (Anoplura), *Hoplopleura arboricola* Kellogg and Ferris 1915 (Hoplopleuridae) and *Neohaematopinus pacificus* Kellogg and Ferris 1915 (Polyplacidae). As with gopher chewing lice, these wingless insects spend their entire life cycles on the hosts, likely leading to some level of codiversification. These two parasites share similar life histories and transmission mechanisms, providing an opportunity for paired tests of codiversification across this widespread and diverse host-parasite system.

I use loci from across the genomes of 25 species of *Tamias* and two species of sucking lice to address three fundamental questions: 1) Are genomic data capable of resolving previously recalcitrant relationships within the *Neotamias* subgenus? 2) Are louse phylogenies congruent with the chipmunk phylogeny, supporting a scenario of strict host-parasite codiversification, or are there also impacts outside of the interaction on louse diversification? 3) Are there parallel evolutionary histories for these two obligate parasites that are sharing the same hosts? By comparing the two lice phylogenies to the chipmunk phylogeny, I begin to identify points in the evolution of chipmunks that correspond to diversification events in the parasites.

Methods

Specimen collection

Chipmunks were field collected following appropriate animal care and use guidelines (Sikes et al. 2011). All chipmunk specimens are archived at either the Denver Museum of Nature & Science (DMNS) or the Museum of Southwestern Biology (MSB). Additional chipmunk samples were obtained from tissue loans from other institutions or from museum study skin clips or toe pads. Individual chipmunks were examined under 20X magnification for sucking lice adhered to hairs. All lice, including nymphs, were

collected and put in 70% or 95% ethanol and frozen in liquid nitrogen or -20C. Adult sucking lice were later identified to species using characters from Kim et al. (1986). Additionally, sucking lice were collected from museum study skins from the Moore Laboratory of Zoology, DMNS, and MSB dating back to 1937 by carefully combing dried specimen skins over a white piece of paper, examining it under 20X magnification, and preserving all arthropods in 95% ethanol and -20C. Researchers at the Museum of Vertebrate Zoology also collected lice from recent specimens by combing them over paper and preserving the contents in ethanol, which were later sorted and identified. All collected lice were deposited at either MSB or DMNS. Chipmunks were examined externally for parasites, prepared as museum voucher specimens, and tissues were frozen and then archived at -80C. I used 35 *H. arboricola* and 22 *N. pacificus* individuals and their corresponding hosts, and selected louse samples by prioritizing host individuals with both species of louse. I sampled *H. arboricola* from 19 host species and *N. pacificus* from 16 host species. I used one *Hoplopleura erratica* from a *Tamias striatus* as an outgroup for *H. arboricola*. I did not have another *Neohaematopinus* outgroup sample for *N. pacificus*, so trees were generated with one *H. arboricola* serving as an outgroup. I sampled all but two of the hosts with lice samples (43), plus 20 additional samples, including the other two subgenera (2 *T. sibiricus,* 3 *T. striatus*), to achieve complete taxon sampling for *Tamias*, leading to a total of 63 individuals.

Sequencing approaches

Because of differences in genome sizes and pre-existing resources, I used two different approaches to sample genomic loci for chipmunks and lice. There are no established target capture approaches for lice and general reduced representation

techniques require input volumes of DNA orders of magnitude higher than a single louse extraction yields. Sucking lice have very small genomes (~110 megabases; Kirkness et al. 2010), making whole genome sequencing for many individuals methodologically and economically feasible. Whole genome sequencing allowed me to use previously identified and curated loci (1,107 genes, Allen et al. 2015) for phylogenetic estimation, with the added benefit of generating genomic data for future investigations. This louse system contrasts with the relatively large genomes and readily available resources for mammalian systems. The ultraconserved elements approach (UCEs) used for *Tamias* phylogeny reconstruction generated thousands of loci (Faircloth et al. 2012) and has proven useful for resolving a diverse set of evolutionary relationships that were previously problematic (McCormack et al. 2012).

Sequencing preparation

Whole genomic DNA was extracted from sucking lice by grinding one individual louse (with 2 exceptions, DZTM0377N and NK217095H used 10 individual lice each) in extraction buffer. Extractions used the Qiagen QIAmp Micro kit (Qiagen, Hilden, Germany) following manufacturer's protocols with the following exceptions: samples digested for 48 h at 72°C and final elution buffer was heated to 55°C and incubated on the column membrane for 5 min at 55°C. Chipmunk DNA was extracted from frozen tissue with a standard salt extraction protocol. Toe pads or skin clips were soaked in 70% ethanol overnight, with at least 3 changes of ethanol. Samples were then cut into small pieces and soaked in STE buffer overnight at 4C, with at least 3 changes of buffer. Extractions then proceeded following manufacturer's protocols for Qiagen QIAamp Micro kit with the same exceptions as above. Aliquots of 0.5-4 micrograms of chipmunk

DNA were submitted to RapidGenomics, LLC (Gainesville, Florida) for UCE targeted enrichment and sequencing. Louse DNA was prepared for whole genome sequencing with KAPA Hyper Prep Kit (Kapa Biosystems, Wilmington, Massachusetts). Libraries for 9 or 10 samples were pooled and 160 bp paired-end reads were run on six Illumina HiSeq 2500 lanes at the High-Throughput Sequencing and Genotyping Unit, University of Illinois.

Data processing

Sequencing reads were first examined using FastQC v0.10.1 (Babraham Bioinformatics) to screen for sequencing anomalies. I removed duplicated sequence read pairs using the fastqSplitDups.py script available from the Mcscript Github package (https://github.com/McIntyre-Lab/mcscript). The de-duplicated reads were then quality trimmed in the FASTX Toolkit v0.0.14 (Hannon Lab). To do this the first 3 bases with consistently lower scores were removed from the 5' end of the sequence. All reads were then quality trimmed from the 3' end to remove bases with a phred score less than 28 using a sliding window of 1nt. Finally, any trimmed reads with fewer than 75 nt were removed from the dataset.

A curated set of 1,107 1:1 orthologous insect genes from *Pediculus humanus* has been previously identified as good targets for restricted target assembly in aTRAM (Allen et al. 2015). These loci were assembled in aTRAM using the ABySS assembler (Simpson et al. 2009) and 3 iterations, using the protein sequence from *Pediculus humanus* as the reference. Following assembly of the loci, the exons of each locus were assembled together (Allen et al. in review). In this exon-stitching step I used the program Exonerate (Slater and Birney 2005) to identify the exonic regions in each of the aTRAM assemblies

and then stitched them together into one contig that contained all the exons per gene. These loci were then aligned using MAFFT version 7 (Katoh et al. 2002; Katoh and Standley 2013).

Chipmunk UCE data were pre-processed in a manner similar to the lice data, except that duplicate reads were not removed. Due to concerns about assembler accuracy, I conducted aTRAM assembly tests on 5 samples at 10 UCE loci using Velvet (Zerbino et al. 2008), ABySS, and Trinity (Grabherr et al. 2011); Velvet and Trinity consistently assembled the same contigs and Velvet was faster. ABySS failed to assemble all 10 of the test loci and those that did assemble were shorter fragments. The UCE probe set (downloaded from ultraconserved.org) was used as the targets for aTRAM assembly of 5041 loci, using 5 iterations and Velvet as the assembler. Following assembly of loci, custom scripts (available at https://github.com/juliema/aTRAM_UCE_pipeline) were used to retrieve the longest contig from the aTRAM Best files, generate a consensus sequence for the UCE loci with multiple probes, combine sequences for all chipmunk samples by UCE locus, align the samples with MAFFT, and trim both the 5' and 3' alignments to the median length of sequence.

Phylogenetic reconstructions

I used a Maximum Likelihood approach for reconstructing the phylogenetic relationships within each taxon. For all three sets of taxa (*Tamias*, *H. arboricola*, and *N. pacificus*), I ran RAxML version 8 (Stamatakis 2014) with a GTRCAT model and 100 bootstraps for each locus. All best trees for each taxon were concatenated into a single file for species tree estimation in ASTRAL 4.7.12 (Mirarab et al. 2014) with 100 bootstraps and the bootstrap files from the RAxML runs. The ASTRAL analyses for

Tamias only used loci with at least half of the taxa represented (n ≥ 33), so 3,267 RAxML trees were used as input. ASTRAL explicitly assumes unrooted trees, so I pruned the *H. arboricola* sample from the *N. pacificus* species tree and the *N. pacificus* sample from the *H. arboricola* tree and then used mid-point rooting for all three, *Tamias*, *H. arboricola*, and *N. pacificus* species trees in FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). To compare patterns of codivergence, I manually drew tanglegrams between the *Tamias* tree and each of the lice species trees.

Results

My approach with aTRAM assembled UCE loci for all host taxa and louse genes for all louse samples. I successfully assembled the same 808 genes for all 22 *N. pacificus* samples, 432 genes for all *Hoplopleura* samples, and 975 genes for 35 of 36 *Hoplopleura* samples. I had noticeably lower success at assembling genes for the oldest louse sample (*H. arboricola* MLZ 541 from a study skin collected in 1937), with only 432 genes recovered compared to 975 for the other 35 *Hoplopleura*. However, the other three louse samples collected from museum study skins (*H. arboricola*: MSB2245 from 1957 and ZM.10492 from 2001; *N. pacificus*: MSB 84515 from 1995), assembled genes comparable to the freshly collected specimens (all over 800). The proportional success with the UCE loci was much lower, 160 loci with all 63 taxa and 3,267 loci with 33 of the taxa, the missing taxa varied across loci. All loci with at least 33 taxa (3,267 UCEs) were used for the *Tamias* species tree. I visually inspected 20 randomly chosen alignments for each group to verify that the pipelines were functioning properly.

The *Tamias* species tree generated by ASTRAL yielded clades with monophyletic resolution for most species (Figure 1), with a few notable exceptions. The *townsendii*

group was not resolved at the species level and *T. palmeri* and *T. umbrinus* were not reciprocally monophyletic. The subspecies *T. minimus grisescens* was previously identified as a unique lineage (Reid et al. 2012), and the two *T. m. grisescens* I sampled were recovered as independent from the other *T. minimus*. The UCE species tree also identified a *T. amoenus* individual as different from all other *T. amoenus*. While collected in the vicinity of the previously described unique *T. amoenus cratericus* subspecies (Reid et al. 2012), this individual does not have bacular morphology consistent with *T. a. cratericus*. Thus, although the UCE species tree yielded well-supported interspecific relationships and supported monophyly for most species, I was unable to fully resolve all relationships within the *Neotamias* subgenus.

The *H. arboricola* species tree resulted in seven well-supported clades that were primarily structured by closely related hosts (Figure 2). The largest average pairwise divergence (0.58%) in the tree for the entire exonic sequence length (2,462,077 bp) was between a large clade consisting of lice parasitizing *T. amoenus*, *T. alpinus*, *T. amoenus*, and *townsendii* group hosts and another large clade of lice collected from the other host species. With midpoint rooting, the *H. erratica* sample, chosen as an outgroup, was sister to one *H. arboricola* clade (raw average pairwise divergence 0.48%), and those together were sister to the other *H. arboricola* clade (0.52% divergent from *H. erratica*). The lack of reciprocal monophyly for *H. arboricola* and *H. erratica* suggests that *H. arboricola* needs a taxonomic revision. There was also evidence for lice infesting host species other than their primary host. For example, specimens of *H. arboricola* collected from two *T. panamintinus* in Nevada were in the clade with lice collected from *T. umbrinus* and *T. palmeri* (Figure 2).

As with *H. arboricola*, relationships among *N. pacificus* lineages were largely congruent with host species or species groups (Figure 3); however, there was no evidence of the deep divergences between clades from the same hosts as in *H. arboricola*. There were two instances of a single louse clade recovered from distantly related hosts, *T. umbrinus*, *T. palmeri*, and *T. panamintinus* in one case and *T. speciosus* and *T. alpinus* in the other. This indicates *N. pacificus* lineages are capable of host switches between distantly related host species that are geographically proximate. The *N. pacificus* samples from hosts in the *quadrivittatus* species group (*T. cinereicollis*, *T. dorsalis*, *T. quadrivittatus*, *T. umbrinus*, *T. palmeri*) did not form a monophyletic clade. The clade consisting of lice collected from the closely related *T. palmeri* and *T. umbrinus* also included a louse collected from a *T. panamintinus*. There were two notable comparisons regarding host switching among lineages between the two louse phylogenies. The *H. arboricola* lineage associated with the *T. alpinus* hosts was in the same clade as the *T. minimus* hosts (as would be expected if lice are codiverging), however, the *N. pacificus* lineage from *T. alpinus* was in the same clade as *T. speciosus* (non-sister host species), and the chipmunk species are sympatric at the collection locality for the *T. alpinus*. Additionally, both the *H. arboricola* and the *N. pacificus* collected from *T. panamintinus* were each, respectively, closely related to the lice collected from *T. umbrinus* and *T. palmeri*. This may be one of the few documented examples of multiple parasite species making similar switches among hosts.

The tanglegrams (Figures 4-5) suggest that some of the louse lineages are diverging into lineages that are primarily associated with host species or species groups. Although there was a possible signal of codiversification at shallower levels, the

diversification patterns at the deeper levels in the lice phylogenies do not mirror the deep divergences in the *Tamias* phylogeny.

Discussion

Because the resolution of phylogenetic history provides the primary foundation for understanding species interactions across the Tree of Life, the new ability to obtain genome-scale data for non-model hosts and parasites significantly advances coevolutionary investigations. This chipmunk-louse example is one of the first to use genome scale data to assess coevolutionary history. New perspectives derived from increased resolution include: 1) Monophyletic support for most chipmunk species and for the *townsendii* species group, although some interspecific relationships remain unresolved. 2) The species tree supports elevation of *T. minimus grisescens* to species status, as previously suggested (Reid et al. 2012). 3) The louse species trees provide the first genomic perspective on intraspecific relationships for parasites. 4) Notably, neither louse species appears to be codiverging with their hosts at the species level, in direct violation of Fahrenholz's Rule. 5) Although each louse has lineages that appear to be primarily associated with a host species or species group, the relationships among those lineages differ.

In addressing my first question related to whether genomic data were capable of resolving previously recalcitrant relationships within the subgenus *Neotamias*, I found that UCEs do not contain sufficient information to resolve all species relationships; however, for the 22 species that I had more than one individual, I did recover support for monophyly for 17 of those species. The *Tamias* species tree also resulted in relationships inconsistent with current taxonomy. First, one of the *T. amoenus* samples was not in the

clade containing the rest of the *T. amoenus* samples. Previous work recovered a distinct lineage of *T. amoenus* supported by genital bone morphology (White 1953, Sutton 1982) and molecular sequence (Demboski and Sullivan 2003; Reid et al. 2012). Of the five *T. amoenus* samples included in the UCE species tree, four were monophyletic and one, NK215801, was outside of, not even sister to, the remaining *T. amoenus* samples. However, the bacular morphology of this sample is not consistent with *T. amoenus cratericus*. Therefore, further morphological and molecular investigations for this group are needed. Second, the two *T. minimus grisescens* samples were not in the same clade as the other six *T. minimus* samples. This supports previous findings that *T. minimus grisescens* may be in need of elevation (Reid et al. 2012). Finally, *T. umbrinus* and *T. palmeri* were not reciprocally monophyletic, but instead together form a single clade. Other investigators have suggested that *T. palmeri* is not distinct, but instead an isolated subspecies of *T. umbrinus* (White 1953, Sutton 1982, Stanley 1991); my results support these previous findings. The last set of relationships that does not result in species monophyly is in the *townsendii* group, where I did not recover reciprocally monophyletic lineages of *T. senex*, *T. siskiyou*, or *T. sonomae*. Notably, this work is not the first to recover a lack of monophyly in this lineages, Reid et al. (2012) recovered a monophyletic lineage for *T. sonomae*, but similarly was unable to resolve relationships for *T. senex* and *T. siskiyou*. Sullivan et al. (2014) found different placement for *T. sonomae* with species tree and concatenated analyses, where *T. sonomae* was not included in the *townsendii* group in a phylogeny generated with four concatenated nuclear loci.

Using the species trees and tanglegrams, I found that neither louse phylogeny was congruent with the host chipmunk phylogeny, rejecting a scenario of strict host-parasite

codiversification. Although this approach cannot identify the processes impacting parasite diversification, the biogeographic histories of the hosts have likely played a role. The climatic cycling of the past should have led to fluctuating chipmunk populations that expanded, contracted, and periodically came into contact, in response to habitat shifts, at which time lice could have been exchanged among host lineages. The louse species trees recovered phylogenies that were discordant with each other, in terms of the relationships among host associated lineages. While both lice have lineages that were primarily, if not exclusively, associated with a single host or a closely related group of hosts, the relationships among those lineages were not the same when the two louse species were compared. Since *H. arboricola* did not form a monophyletic group with respect to *H. erratica*, further investigation is needed to try to identify morphological characters that correspond to the genetic relationships and *H. arboricola* needs to be taxonomically revised. This outcome for the lice suggests that the species do not have parallel evolutionary histories with the hosts.

My findings of louse diversification with respect to the hosts have both similarities and dissimilarities with previous findings regarding chipmunk pinworm diversification (Bell et al. in press; chapter 3). All four parasite species had lineages that correspond to host species or species groups. However, the relationships among those lineages varied and suggest that while host diversification impacts parasite evolution, it has impacted each parasite differently, either asynchronously (pinworms), or with completely different diversification patterns (lice). The dynamics of host range expansions and contractions, periodic host contact, and close evolutionary relationships among chipmunks have likely led to the moderate congruence of parasite lineages with

chipmunk species. As I demonstrate here with the lice, chipmunk pinworms also have deep divergences that largely corresponded to host species or species groups (Bell et al. in press, chapter 3). The evolutionary and biogeographic history of chipmunks has made it possible for parasites to move among closely related chipmunk species when they were in contact, but maintain a deep signal of association with a host lineage.

Phylogenomic tools for non-model organisms are permitting unprecedented insight into evolutionary history. Applying these tools to the chipmunk-louse system has revealed that there are few processes of parasite diversification that can be generalized across host-parasite systems. Investigating chipmunk parasites has consistently uncovered parasite lineages associated with hosts and shallow patterns of parasite genetic structuring across the landscape with varying correspondence of deep evolutionary histories with the hosts. Host evolutionary and demographic history is likely the largest unaccounted factor when investigating parasite diversification. Each point of contact among host populations presents a potential parasite transfer opportunity, whether that host contact is still evident today, or was historic and ephemeral. Transference and establishment of parasite lineages into new hosts is likely variable through time and across the landscape, however my findings in lice and pinworms (Bell et al. in press, chapter 3) suggest that a longer history of association with a host may limit the establishment of parasite lineages in new host species. The chipmunk-parasite system demonstrates that parasite diversification cannot be explained as a simple process of codivergence and that parasite evolution, even when comparing parasites from the same hosts and ecological roles, is complex and the history is unique to each species.

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Figure Legends

Figure 1. Maximum Likelihood (ASTRAL) cladogram species tree for all 25 *Tamias* species. Values above branches represent species tree bootstrap report.

Figure 2. Maximum Likelihood (ASTRAL) species tree for *Hoplopleura arboricola* (left) and geographic locality of the clades (right). Points on the map correspond to colors on the phylogeny.

Figure 3. Maximum Likelihood (ASTRAL) species tree for *Neohaematopinus pacificus* (left) and geographic locality of the clades (right). Points on the map correspond to colors on the phylogeny.

Figure 4. Tanglegram connecting chipmunk species tree (left) with their corresponding louse on the *Hoplopleura arboricola* species tree (right).

Figure 5. Tanglegram connecting chipmunk species tree (left) with their corresponding louse on the *Neohaematopinus pacificus* species tree (right).

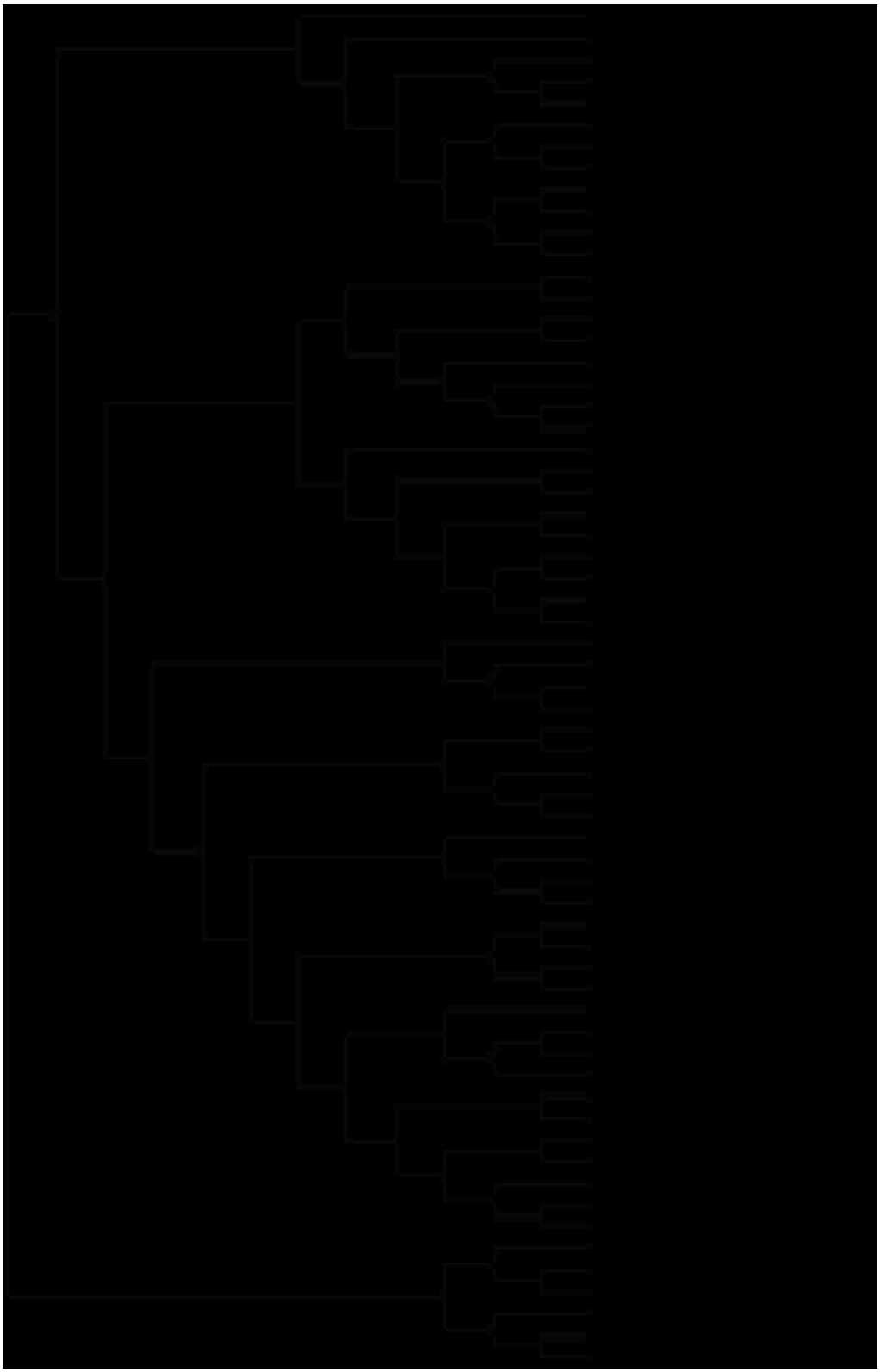
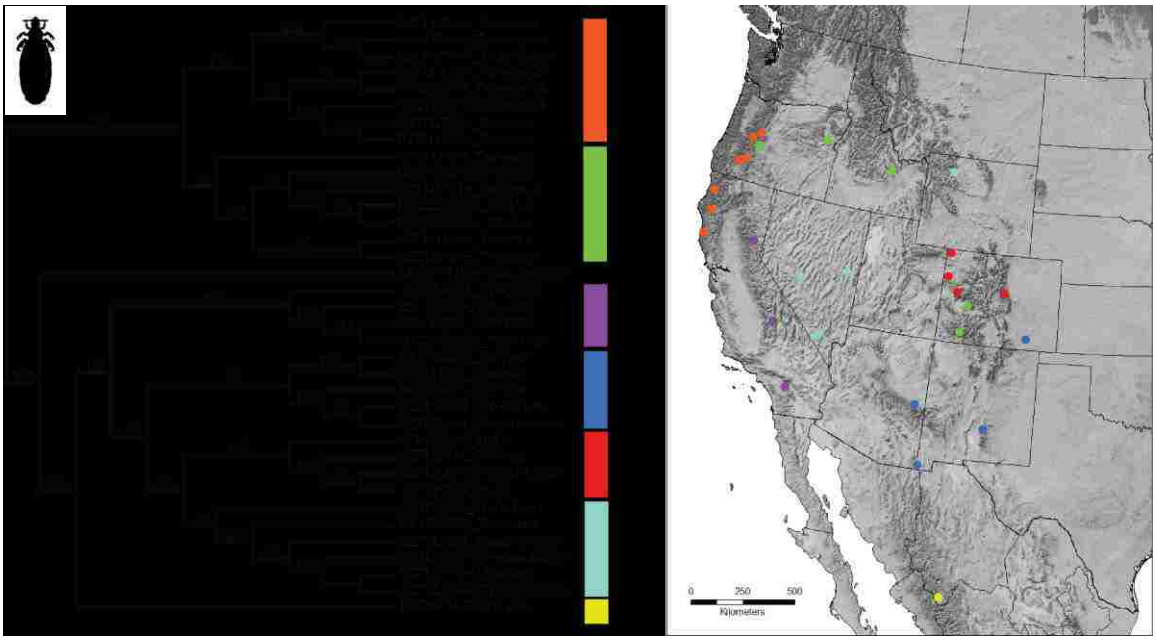


Figure 1



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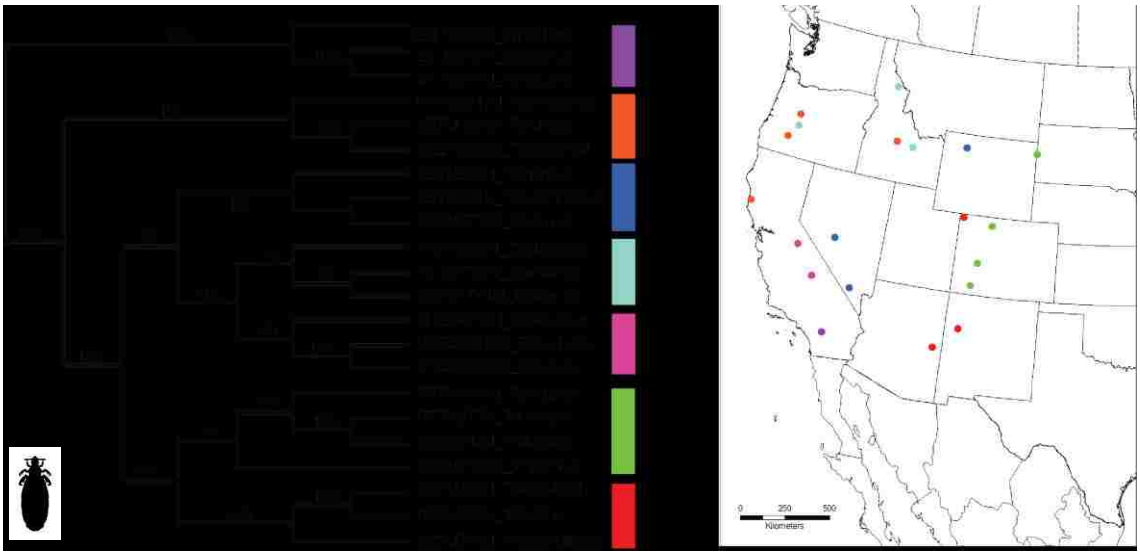

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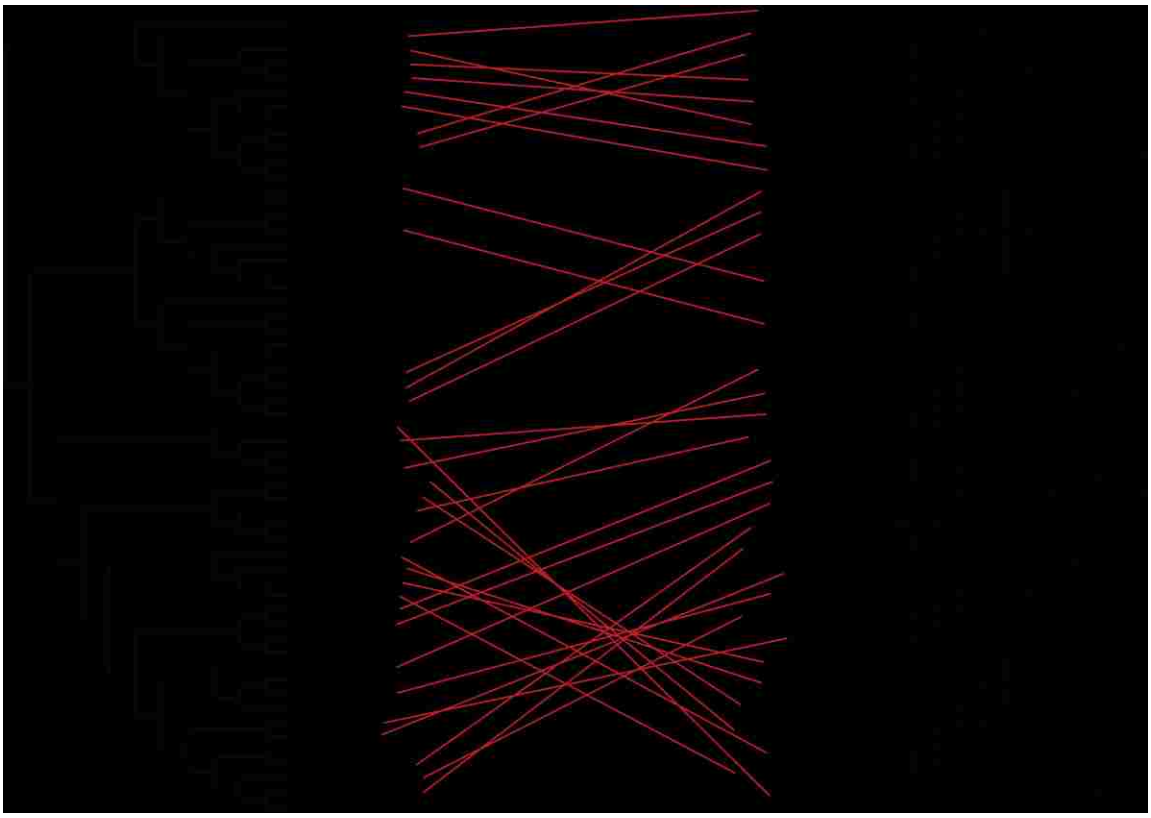

Figure 4

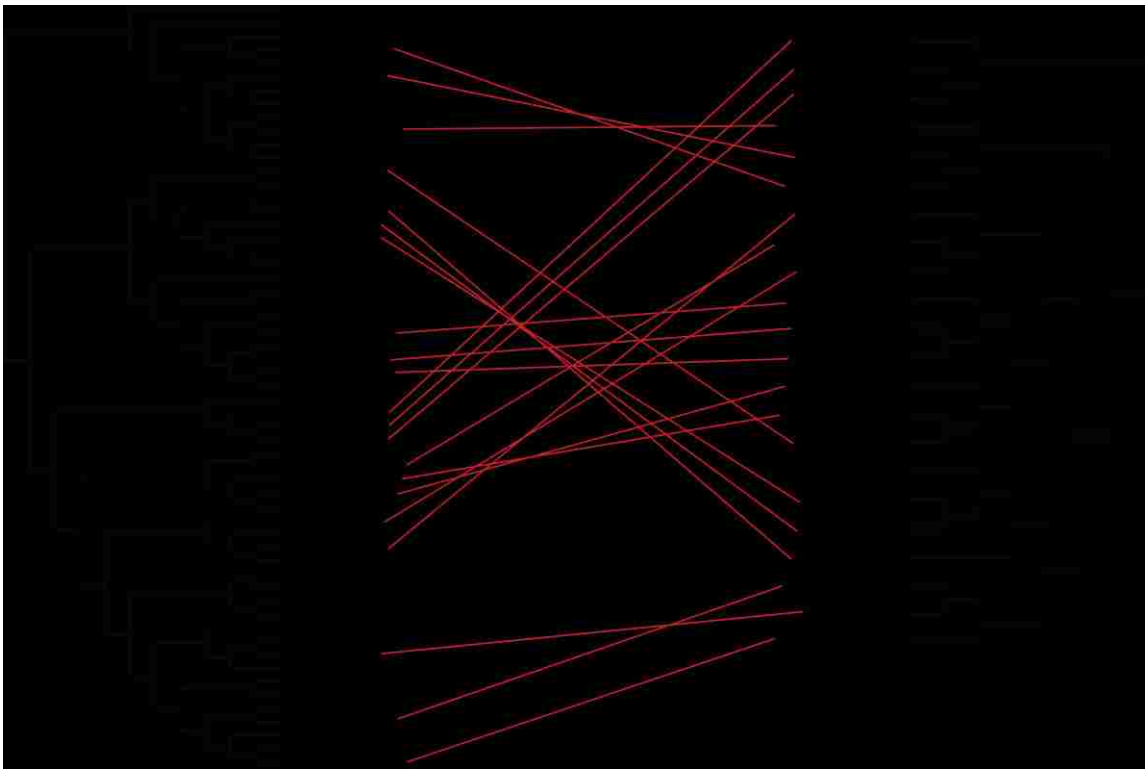

Figure 5

CONCLUSION

Parasites have been proposed to play a crucial role in many processes, from sexual reproduction (Lively and Morran 2014) to driving immune diversity (Froeschke and Sommer 2005, Westerdahl 2007) and maintaining healthy wildlife populations by contributing to functioning immune systems (Stringer and Linklater 2014). Yet, despite this outsized role in the biosphere, relatively little is known about the processes that drive parasite diversification. While strict codiversification has been the paradigm, increasing evidence illustrates that both host evolution and factors external to the host-parasite interaction contribute to parasite diversification. Elucidating the history of host-parasite interactions and the processes shaping parasite evolution will shed light on how parasites respond to changes in host demography and episodic climate events.

The research conducted for my dissertation has involved working in the field, museum, and laboratory. This variety of approaches also has presented many opportunities for training peers and undergraduate students, as well as engaging middle school students. The portions of my dissertation that relied on museum specimens demonstrate ways to incorporate museum data into educational opportunities, which could be extended to undergraduate courses (Cook et al. 2014). Research on a diverse set of taxa (rodents, lice, and pinworms) also has led to new collaborations. The lice genome dataset was used in developing software for targeted genome assembly and validating that approach (Allen et al., in review). In addition to primary research, this project has contributed to training underrepresented minorities in science, outreach to the lay community, and methods development.

Western chipmunks have a broad distribution, inhabit diverse landscapes, and have a history of divergence with gene flow. Paired with their sucking lice and pinworms, this system provides many opportunities to test the multiple drivers of parasite diversification, the ability of parasites to reveal host histories, and the impact of host hybridization on parasite diversification. I have taken a broad approach to understanding the host and geographic ranges of chipmunk parasites, which has served as a basis and provided the samples for phylogenetic investigations of geographic and host associated structure in these parasites. In comparing the parasites, I found that the two pinworm species have similar diversification patterns that were linked to their hosts, but those processes occurred on different time scales. As another paired investigation, the chipmunk sucking lice revealed some lineages that correspond to host relationships, but the lice have different histories from the hosts, as well as each other. Overall, this system demonstrates that parasite diversification cannot be explained as a simple process of strict codivergence and that parasite evolution, even when comparing parasites from the same hosts and ecological roles, is complex with each species having largely idiosyncratic histories. While I found a role for hosts, host history, and landscape in shaping genetic structure in all four parasites, these processes impacted each parasite species differently.

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