Molecular Phylogenetics and Evolution xxx (xxxx) xxx

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Disentangling lousy relationships: Comparative phylogenomics of two sucking louse lineages parasitizing chipmunks

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ARTICLE INFO

Keywords:
Phylogenomics
Lice
Anoplura
Hoplopleura arboricola
Neohaematopinus pacificus
Chipmunks
Tamias

ABSTRACT

The evolution of obligate parasites is often interpreted in light of their hosts' evolutionary history. An expanded approach is to examine the histories of multiple lineages of parasites that inhabit similar environments on a particular host lineage. Western North American chipmunks (genus *Tamias*) have a broad distribution, a history of divergence with gene flow, and host two species of sucking lice (Anoplura), *Hoplopleura arboricola* and *Neohaematopinus pacificus*. From total genomic sequencing, we obtained sequences of over 1100 loci sampled across the genomes of these lice to compare their evolutionary histories and examine the roles of host association in structuring louse relationships. Within each louse species, clades are largely associated with closely related chipmunk host species. Exceptions to this pattern appear to have a biogeographic component, but differ between the two louse species. Phylogenetic relationships among these major louse clades, in both species, are not congruent with chipmunk relationships. In the context of host associations, each louse lineage has a different evolutionary history, supporting the hypothesis that host-parasite assemblages vary both across the landscape and with the taxa under investigation. In addition, the louse *Hoplopleura erratica* (parasitizing the eastern *Tamias striatus*) is embedded within *H. arboricola*, rendering it paraphyletic. This phylogenetic result, together with comparable divergences within *H. arboricola*, indicate a need for taxonomic revision. Both host divergence and biogeographic components shape parasite diversification as demonstrated by the distinctive diversification patterns of these two independently evolving lineages that parasitize the same hosts.

1. Introduction

Comparative phylogenetic studies have great potential to reveal processes driving biological diversification, but they are dependent on the accuracy of underlying phylogenetic analyses. New approaches in phylogenomics not only improve our understanding of evolutionary history of individual clades, but also have the potential to advance our exploration of evolutionary interactions among organisms, especially the complex histories of hosts and their associated parasitic taxa. These advances now allow investigators to address diverse questions in greater detail across a broad array of organisms (da Fonseca et al., 2016).

In studies comparing host and parasite phylogenies, much of the focus has been on Fahrenholz's Rule, the hypothesis that parasite phylogenies should mirror host phylogenies. A classic example of Fahrenholz's Rule (strict cospeciation; Eichler, 1948), the chewing lice and pocket gophers (Geomyidae), have been held as a model of codivergence, exemplifying concurrent divergence events between hosts and parasites at multiple scales (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 would have limited dispersal abilities and few opportunities for switching to new host species. Support for codivergence or phylogenetic congruence has been demonstrated in other louse-host systems as well, such as ground doves and wing lice (Sweet and Johnson, 2016), ground doves and body lice (Sweet et al., 2017), and muroid rodents and sucking lice (Bothma et al., 2020). While the expectation of host-parasite codivergence can serve as a null hypothesis, numerous examples highlight incongruent host and parasite phylogenies (e.g., chipmunk

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https://doi.org/10.1016/j.ympev.2020.106998
Received 21 August 2020; Received in revised form 21 October 2020; Accepted 22 October 2020
Available online 29 October 2020

pinworms, Bell et al., 2018; marine mammal digeneans, Fraija-Fernández et al., 2016; rodent coccidia, Mácová et al., 2018; avian malaria, Ricklefs et al., 2004). Such incongruence also occurs in some groups of lice, such as raptor feather lice (Catanach and Johnson, 2015) and mammalian sucking lice (du Toit et al., 2013, Light et al., 2010). Indeed, the factors dictating both host and parasite divergence and evolution are complex (Brooks et al., 2019, Hoberg and Brooks, 2010) and likely to vary 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).

Beyond host-associated diversification (or codivergence), parasites may switch to parasitizing new host species. The phylogenetic distance effect (Engelstädter and Fortuna, 2019) predicts that parasites can more easily shift between closely related hosts. This prediction is intuitive if we assume that the resources or traits to which parasites are adapted have a phylogenetic signal, in which a parasite is more likely to be compatible with a closely related host than a distantly related host. A different prediction, ecological fitting (Janzen, 1985, Agosta et al., 2010), is that parasites are adapted to particular host resources or traits that are not phylogenetically correlated and parasites track resources, not specific hosts. We can test for the phylogenetic distance effect by examining the parasites of hosts where closely related and distantly related host species have overlapping geographic distributions. The expectation in these scenarios is that closely related host species are more likely to share parasites than the distantly related hosts, even when there should be similar opportunities for host shifts. However, either possibility relies on the parasite's ability to successfully disperse to a new host species. There is evidence that the lack of ability to disperse is a primary barrier for lice switching to new hosts (Clayton et al., 2004).

Despite the body of literature that exists regarding host and parasite evolution, it is difficult to generalize these processes across different host-parasite pairs. Some of this is simply due to lack of data, but the variation in interactions across the diversity of hosts and parasites provides groundwork for investigation. Several dynamics shape host-parasite coevolutionary patterns and the Stockholm Paradigm (Brooks et al., 2019) describes the interplay of these dynamics. If we assume that cycles of geographic expansion and contraction (e.g. taxon pulse hypothesis, Erwin, 1981) lead to ecological disruption and create opportunities for parasites to switch to hosts with the requisite resources (ecological fitting, Janzen, 1985), there will be alternating patterns of parasite generalization and specialization (oscillation hypothesis, Janz and Nylin, 2008). These dynamics can vary across space, as described in the geographic mosaic theory of coevolution (Thompson, 2005). While we can expect these processes to vary between individual host-parasite pairs, investigating pairs of parasites that utilize the same resource on the same hosts may allow us to observe patterns that shape parasite evolution across broader scales.

Here we employ a phylogenomic approach to compare the evolutionary relationships and host associations of two species of sucking lice that parasitize western North American chipmunks. 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 multiple bouts of mitochondrial introgression (Good et al., 2003, Reid et al., 2012, Sullivan et al., 2014). While introgression among the species has occurred across different time scales, some species appear to have fixed ancient introgressions, some appear very recent, and some are on-going, all introgressions have occurred among species that have some degree of overlapping distributions (Sullivan et al., 2014). Western chipmunk species are widely sympatric, with multiple co-occurring species in contact throughout western North America (e.g., Hall, 1981; summarized in Sullivan et al., 2014). They are parasitized by two species of sucking lice (Anoplura), *Hoplopleura arboricola* Kellogg and Ferris 1915 (Hoplopleuridae) and *Neohaematopinus pacificus* Kellogg and Ferris 1915 (Polyplacidae). Both species of lice have been reported from 19 of 23 western chipmunk species (Bell et al., 2015). The widespread host associations of these two lice may be due to three (not mutually exclusive) phenomena: (1) these lice are generalists within western chipmunks, (2) chipmunk divergences are sufficiently recent that in terms of parasite adaptations they are all essentially the same host species, or (3) there is cryptic diversity within the lice that corresponds to greater host specificity. Similar to gopher chewing lice, these wingless insects spend their entire life cycles on the hosts and may have limited opportunities for dispersal, potentially increasing the likelihood of host codiversification. As with all Anoplura (Kim et al., 1986), these lice have similar life histories (obligate permanent parasites) and the same transmission mechanisms (primarily disperse through host–host contact), providing an opportunity to test the role of host association and historical biogeography in louse diversification.

Sucking louse populations may exhibit some level of phylogenetic structure, which in turn can be associated with hosts, geography, or both. If there is host-associated phylogenetic structure in the lice, we can further explore whether louse lineages are able to parasitize multiple chipmunk species. The large amount of sympatry and multiple bouts of interspecies introgression in chipmunks offer opportunities for lice to switch hosts, either currently or in the past. Louse lineages parasitizing hosts with disjunct distributions will suggest that lice were able to move among chipmunk species historically, early in chipmunk evolutionary history. We use loci from across the genomes of these sucking lice to address three fundamental questions: (1) Are louse phylogenies consistent with chipmunk phylogenetic relationships, or are there other factors influencing louse diversification? (2) Are there parallel evolutionary histories for these two obligate parasites that share the same hosts? (3) Is there evidence of similar host-switching events in both species of lice (i.e., do both lice have lineages that have switched among the same hosts)?

2. Methods

2.1. Specimen collection

Chipmunks were field collected following appropriate animal care and use guidelines (Sikes, 2016). All chipmunk specimens are archived at either the Denver Museum of Nature & Science (DMNS) or the Museum of Southwestern Biology (MSB) (Appendix A). Chipmunk species identifications were determined using the male genital bone, size, pelage, skeletal traits, geography, and some individual identifications confirmed by DNA sequencing in other studies (Reid et al., 2012, Sullivan et al., 2014). Additionally, we have decades of experience working with chipmunks and we are confident in our ability to correctly identify chipmunk species. Individual chipmunks were examined under 20X magnification for sucking lice adhered to hairs. All lice, including nymphs, were collected into 70% or 95% ethanol and frozen in liquid nitrogen or −20 °C. Adult sucking lice were identified to species using characters from Kim et al. (1986). Additionally, sucking lice were collected from museum study skins and fluid-preserved specimens dating to 1957 from DMNS and MSB by carefully combing dried specimen skins over white paper, then examining under 20X magnification, and preserving all arthropods in 95% ethanol and −20 °C. 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 (Appendix A). We used 34 *Hoplopleura arboricola* collected from 19 host species and 21 *Neohaematopinus pacificus* individuals collected from 16 host species. Louse samples were selected by prioritizing host individuals with both species of lice. We intended to use one *Hoplopleura erratica* from a *Tamias striatus* as an outgroup for *H. arboricola*, however it appears to be an in-group relative to *H. arboricola* (see results). To ensure we had a sample that was not part of the ingroup, we generated phylogenies for *Hoplopleura* with one

N. pacificus as an outgroup and for *N. pacificus* with one *H. arboricola* serving as an outgroup.

2.2. Sequencing

Sucking lice have small genomes (~108 megabases; Kirkness et al., 2010), making whole genome sequencing for many individuals methodologically and economically feasible. Whole genome sequencing allowed us 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. These loci and methods have been used to build robust phylogenetic trees across a number of louse clades, including Anoplura, making them ideal markers for this study (Allen et al., 2017, Johnson et al., 2018, de Moya et al., 2019, Virrueta Herrera et al., 2020).

Whole genomic DNA was extracted from sucking lice by grinding one individual in extraction buffer. Samples DZTM0377N and NK217095H were each sequenced using 10 individual lice collected from one host, respectively, for a different project (Allen et al., 2017). 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. Louse DNA was prepared for whole genome sequencing with KAPA Hyper Prep Kit (Kapa Biosystems, Wilmington, MA). Libraries for 9 or 10 samples were pooled and 150 bp paired-end reads were run on six Illumina HiSeq 2500 lanes at the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana-Champaign.

2.3. Data processing

Sequencing reads were first examined using FastQC v0.10.1 (Babraham Bioinformatics, Andrews, 2010) to screen for sequencing anomalies. We 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) by removing the first 5 bases with consistently lower scores 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 1 nt. Finally, trimmed reads with fewer than 75 nt were removed from the dataset.

A curated set of 1,107 1:1 orthologous insect genes from the human louse, *Pediculus humanus*, has been previously identified as good targets for target restricted assembly in aTRAM (Allen et al., 2015, 2017). These loci were assembled in aTRAM v1.0 (Allen et al., 2015) using the ABySS v1.5 assembler (Simpson et al., 2009) and 3 iterations, using the protein sequence from *Pediculus humanus* as the target for *tblastn* searches of quality trimmed reads. Following assembly of loci, the exons of each locus were assembled together using the exon_stitching program in aTRAM as described in Allen et al. (2017). In this exon-stitching step, we used the program Exonerate v2.2 (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 aligned with a translated alignment in PRANK v.170427 (Löytynoja, 2014) and back translated to DNA. Following that alignment, we removed sites with over 90% missing data or gaps in trimAL v1.2 (Capella-Gutierrez et al., 2009). Each locus was then aligned with MAFFT v7 (Katoh, 2002; Katoh and Standley, 2013). We visually inspected 20 randomly chosen alignments for each group to verify the pipelines were functioning properly.

For a traditional comparison of genetic distances, we also assembled cytochrome *c* oxidase I (COI). COI was assembled in aTRAM v2.0 (Allen et al., 2018) using ABySS v2.0.2 (Simpson et al., 2009), with the COI protein sequence from *Hoplopleura kitti* (GenBank accession KJ648943) as the target for *tblastn* searches of quality trimmed reads. Sequence assemblies were trimmed to just COI and aligned with MAFFT v7. Four of the samples would not assemble using ABySS in aTRAM, so we did those manually, by running aTRAM without an assembler, taking the *tblastn* hits and then mapping those to the target read in Geneious Prime 2020.2 (https://www.geneious.com) and extracting the consensus sequence. COI sequences were aligned in MAFFT v7 and trimmed to the open reading frame corresponding to the target sequence. All nuclear and COI alignments are available in Dryad (https://doi.org/10.5061/dryad.59zw3r25s).

2.4. Phylogenetic reconstructions

We conducted three phylogenetic reconstructions for each taxon, *H. arboricola* and *N. pacificus*, using the assembled nuclear ortholog loci. We estimated species trees using ASTRAL-III v5.7.3 (Zhang et al., 2018), with local posterior probabilities (Sayyari and Mirarab, 2016) to gauge support. We also used SVDQuartets (Chifman and Kubatko, 2014, 2015) as implemented in PAUP* (v4.0a build167; Swofford, 2002), evaluating all possible quartets and inferring a tree under the multispecies coalescent with 1000 bootstrap replicates. We reconstructed trees with concatenated nuclear sequences using ModelFinder (Kalyaanamoorthy et al., 2017) to determine the best-fit model in IQ-TREE v2.0 (Minh et al., 2020a) and assessed support with 1000 bootstraps (Hoang et al., 2018). All trees were visualized in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). We calculated net between clade p-distances for all concatenated nuclear genes and COI separately in MEGA v7.0.26 (Kumar et al., 2016) for each species.

For a precise comparison of infraspecific relationships between the two louse species, we constructed phylogenies using one louse of each species from the same host individual. We sampled 16 individual lice of each louse species from the same host individual, respectively, for 14 host species. We used IQ-TREE to generate individual gene trees for each louse species using only the samples in the reduced sample set. We then used ASTRAL to generate a phylogeny from those gene trees. To measure congruence between the two species using the samples from the same host individuals, we generated concordance factors in IQ-TREE (Minh et al., 2020b) for the reduced sample gene trees of each species with the reduced sample ASTRAL species trees of each species. This analysis calculates the percentage of gene trees that have a node that is present on the given species tree, allowing for a comparison of topologies. We calculated the concordance factors for: *H. arboricola* gene trees with the *H. arboricola* species tree, the *N. pacificus* gene trees with the *N. pacificus* species tree, the *H. arboricola* gene trees with the *N. pacificus* species tree, and the *N. pacificus* gene trees with the *H. arboricola* species tree. We included the congruence factors of the same species gene trees and species trees (e.g. *H. arboricola* gene trees and *H. arboricola* species trees) for context to compare to the congruence for the opposite species gene trees and species trees (e.g. *N. pacificus* gene trees and *H. arboricola* species tree).

3. Results

Depending on the sample, aTRAM successfully assembled between 451 and 1053 genes (mean 993) that had at least 50% of the gene sequence. The lowest number of genes was assembled from a small nymph (DZTM2740Ha) for which DNA quantity may have been limiting. The three louse samples collected from museum study skins (*H. arboricola*: MSB2245 from 1957 and ZM.10492 from 2001; *N. pacificus*: MSB 84515 from 1995) and one from a fluid preserved host (*H. arboricola* NK195685 from 2010) assembled a number of genes comparable to the freshly collected specimens (905–1020 genes with 50% or more of the gene sequence). In total there were 1,660,328 bp (305,235 bp with no gaps or missing data) for *H. arboricola* and 1,637,670 bp (483,425 bp with no gaps or missing data) for *N. pacificus*.

All methods used to reconstruct the *H. arboricola* phylogeny resulted in similar topologies, with slight differences that were well-supported in

different trees (Fig. 1 and Supplemental Fig. 1). The trees consisted of eight well-supported clades with two or more individuals within each clade that are primarily associated with closely related hosts (Fig. 1). We named the clades based on the primary host species or host species group the lice were associated with (Table 1). Most of the differences among the different tree topologies were either associated with short internal branches or reflected relationships among taxa within clades, which were largely well-supported overall. The species trees and concatenated trees differed in the support values for the AMOEN clade of lice parasitizing *T. amoenus* and *T. alpinus* and the support and placement of NK215133Ha with respect to the other *T. speciosus* louse and the *T. merriami* and *T. obscurus* lice (MERR clade, Fig. 1 and Supplemental Fig. 1). The eastern chipmunk louse, *H. erratica*, fell out sister to the MIN, AMOEN, TOWN-N, and TOWN-S clades. Excluding distances to the *H. erratica* sample, the concatenated nuclear sequence divergences between clades ranged from 0.5% (QUAD-W and QUAD-S) to 4% (SPEC and MIN), with an average of 2.4% between clades (Table 2). The COI sequence divergences between clades was substantially larger and ranged from 4% (QUAD-W and QUAD-S, and AMOEN and MIN) to 20.2% (SPEC and TOWN-S), with an average of 12.4% between clades (Table 2). There were multiple examples of *H. arboricola* lice exhibiting geographic structure, but not host-associated structure (Fig. 1). For example, the lice sampled from *T. panamintinus* (DZTM584Ha and DZTM2798Ha) are in the QUAD-W clade with lice sampled from *T. umbrinus* and *T. palmeri*, which are distantly related to *T. panamintinus*.

The methods for reconstructing *N. pacificus* phylogenies resulted in well-supported clades similar to the *H. arboricola* clades; however, the host associations (Table 1) and placement of those clades varied among the three methods (Fig. 2). The biggest difference is the placement of the QUAD lice clades that are associated with the *T. quadrivittatus* host species group (*T. cinereicollis*, *T. dorsalis*, *T. palmeri*, *T. panamintinus*, *T. quadrivittatus*, *T. umbrinus*). In the SVDQuartets tree, *N. pacificus* from the *T. quadrivittatus* group form two sister clades, QUAD-E and QUAD-W (Supplemental Fig. 1). However, in the ASTRAL tree and concatenated tree, QUAD-E is sister to the lice collected from *T. minimus*, the MIN clade (Fig. 2). As with *H. arboricola*, the *N. pacificus* clades generally correspond to host species or species groups (Fig. 2). There were also

Table 1
Clades and host associations for each louse species.

Louse clade	*Tamias* host species
Hoplopleura arboricola	
AMOEN	*T. alpinus*, *T. amoenus*
MERR	*T. merriami*, *T. obscurus*, *T. speciosus*
MIN	*T. minimus*
QUAD-NE	*T. dorsalis*, *T. quadrivittatus*, *T. rufus*
QUAD-S	*T. canipes*, *T. cinereicollis*, *T. dorsalis*, *T. quadrivittatus*
QUAD-W	*T. palmeri*, *T. panamintinus*, *T. umbrinus*
SPEC	*T. speciosus*
TOWN-N	*T. siskiyou*, *T. townsendii*
TOWN-S	*T. ochrogenys*, *T. sonomae*
Hoplopleura erratica	*T. striatus*
Neohaematopinus pacificus	
AMOEN	*T. amoenus*, *T. ruficaudus*
MERR	*T. merriami*, *T. obscurus*
MIN	*T. minimus*
QUAD-E	*T. cinereicollis*, *T. dorsalis*, *T. quadrivittatus*
QUAD-W	*T. palmeri*, *T. panamintinus*, *T. umbrinus*
SPEC	*T. alpinus*, *T. speciosus*
TOWN	*T. ochrogenys*, *T. siskiyou*, *T. townsendii*

some differences among methods of the placement of taxa within clades and short internal branches (Fig. 2 and Supplemental Fig. 1). The concatenated nuclear sequence divergences between clades ranged from 0.6% (AMOEN and QUAD-W) to 3.3% (TOWN and MERR), with an average of 1.9% between clades (Table 3). The COI sequence divergences between clades again were much larger and ranged from 4% (QUAD-W and QUAD-S, and AMOEN and MIN) to 13.9% (TOWN and SPEC), with an average of 9.7% between clades (Table 3). There were two instances of a single louse clade recovered from distantly related hosts: QUAD-W clade (*T. umbrinus*, *T. palmeri*, and *T. panamintinus*) in one case, and SPEC (*T. speciosus* and *T. alpinus*) in the other. This finding is consistent with episodes of host switching of *N. pacificus* lineages between phylogenetically distant, but geographically proximate, host species.

While there are similar host-associated clades and many similarities in the composition of these clades between the two louse species (Fig. 3), the relationships among those clades are different. There were two

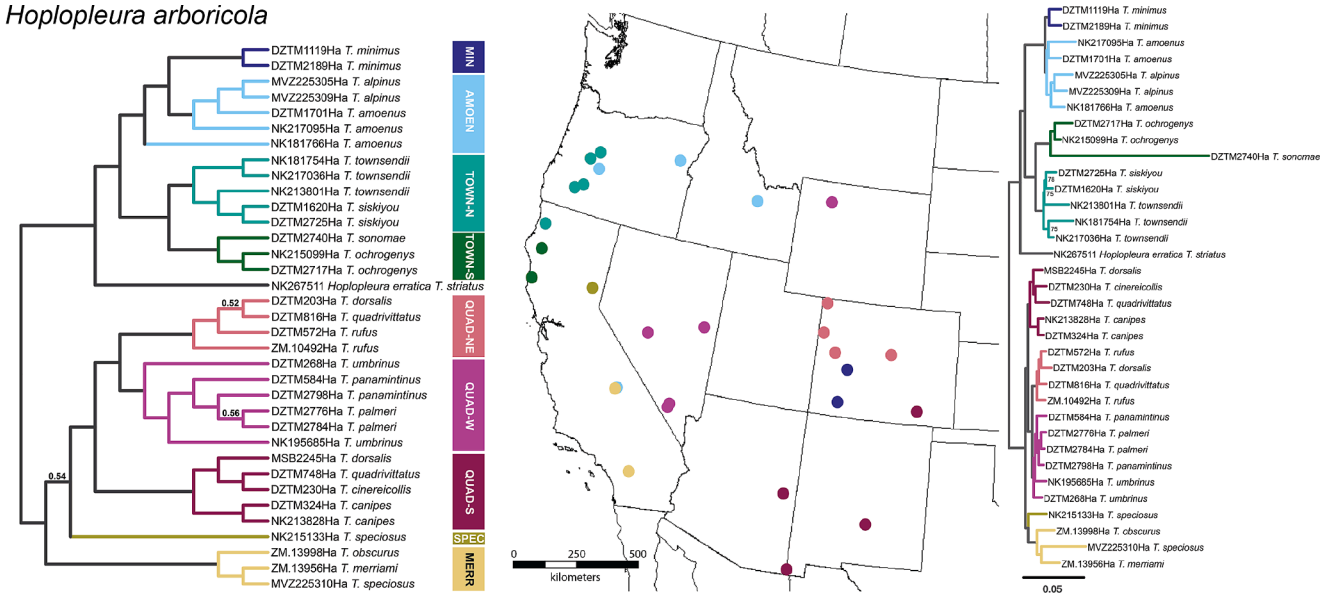

Fig. 1. Astral species tree (left) of *Hoplopleura arboricola*. All branches have local posterior probability support of 1 unless otherwise noted. Color coded clades are labeled according to host-associated lineage and correspond to sample points on map of western United States (middle). Concatenated maximum likelihood tree (IQ-TREE) for *Hoplopleura arboricola* (right), branches are color coded to match the Astral tree and the map. All nodes have bootstrap support of 100 unless otherwise noted. Tips of both trees are labeled with specimen number and host (*Tamias*) species. Both trees were rooted with *Neohaematopinus pacificus* and the sample was pruned from the tree.

Table 2
Net pairwise sequence divergences between clades of *Hoplopleura arboricola* and one *H. erratica*, displayed as percentages. Above the diagonal are COI sequences and below the diagonal are concatenated nuclear divergences.

	MIN	AMOEN	TOWN-N	TOWN-S	QUAD-NE	QUAD-W	QUAD-S	SPEC	MERR	*H. erratica*
MIN	–	4%	9.3%	13.3%	15%	11.2%	11.2%	17.1%	11.2%	13.7%
AMOEN	0.5%	–	10.5%	13.4%	14.4%	10%	9.9%	17.2%	10.6%	14.4%
TOWN-N	2.2%	2.1%	–	10.4%	15%	11.4%	10.1%	15.7%	11.6%	13.9%
TOWN-S	2.5%	2.1%	0.1%	–	18.4%	14.5%	13%	20.2%	13.9%	15.6%
QUAD-NE	3.4%	3.1%	3.1%	3.2%	–	6.3%	6.6%	15.4%	9.4%	18.8%
QUAD-W	3.4%	3.1%	0.3%	3.2%	0.4%	–	4%	11.6%	5.7%	13.8%
QUAD-S	3.1%	2.8%	2.8%	2.9%	0.7%	0.5%	–	11.1%	5%	13.6%
SPEC	0.4%	3.8%	3.7%	3.7%	1.7%	1.6%	1.5%	–	10.6%	20.3%
MERR	3.1%	2.8%	2.7%	2.5%	1.1%	1.1%	0.8%	1.6%	–	14.3%
H. erratica	3.5%	3.3%	3.1%	3.3%	3.9%	3.8%	3.6%	4.7%	3.6%	–

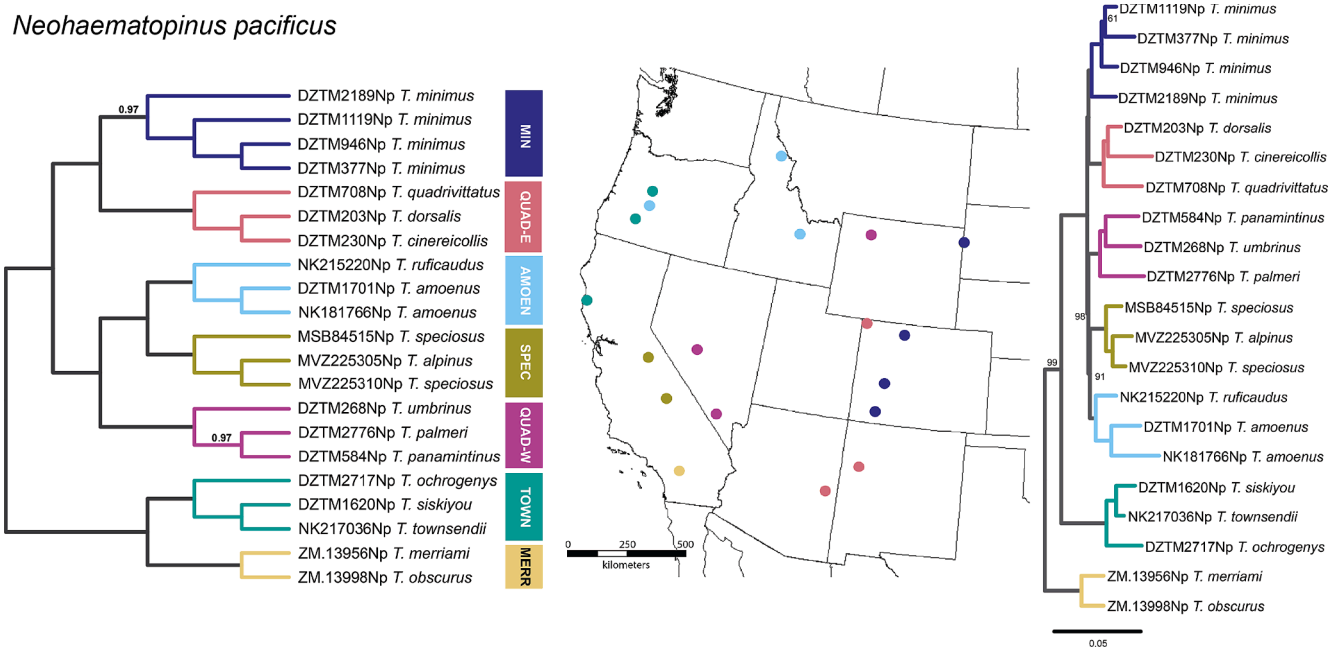

Fig. 2. Astral species tree (left) of *Neohaematopinus pacificus*. All branches have local posterior probability support of 1 unless otherwise noted. Color coded clades are labeled according to host-associated lineage and correspond to sample points on map of western United States (middle). Concatenated maximum likelihood tree (IQ-TREE) for *Neohaematopinus pacificus* (right), branches are color coded to match the Astral tree and the map. All nodes have bootstrap support of 100 unless otherwise noted. Tips of both trees are labeled with specimen number and host (*Tamias*) species. Both trees were rooted with *Hoplopleura arboricola* and the sample was pruned from the tree.

Table 3
Net pairwise sequence divergences between clades of *Neohaematopinus pacificus*, displayed as percentages. Above the diagonal are COI sequences and below the diagonal are concatenated nuclear divergences.

	MIN	QUAD-E	AMOEN	SPEC	QUAD-W	TOWN	MERR
MIN	–	5.8%	4%	8.1%	6.5%	10.5%	10.6%
QUAD-E	0.8%	–	5.3%	8.5%	8.1%	11.9%	13.2%
AMOEN	0.7%	0.8%	–	7.1%	7.3%	10.7%	11.4%
SPEC	1%	1.2%	0.9%	–	9.7%	13.9%	13.3%
QUAD-W	0.7%	0.9%	0.6%	1%	–	12.1%	13%
TOWN	2.8%	2.9%	2.7%	3.1%	2.8%	–	13.5%
MERR	2.9%	3.2%	2.9%	3.2%	1%	3.3%	–

notable comparisons regarding host associations among lineages between the two louse phylogenies. The *H. arboricola* AMOEN lineage is associated with *T. alpinus* and *T. amoenus* hosts (Fig. 1), which is sister to the MIN clade. However, the *N. pacificus* sampled from *T. alpinus* was in the SPEC clade (Fig. 2) and distantly related to the *N. pacificus* MIN clade. Both louse species were collected from the same *T. alpinus* and *T. speciosus* host individuals at the same locality (MVZ225305 and MVZ225310). Both the *H. arboricola* and the *N. pacificus* collected from *T. panamintinus* are each, respectively, in the same clade as the lice collected from *T. umbrinus* and *T. palmeri* (Figs. 1–4). This does not appear to be only occurring at one geographic locality, as the *H. arboricola* samples collected from *T. panamintinus* are from different localities (DZTM584Ha and DZTM2798Ha) and both are in the QUAD-W clade.

Comparisons of samples of the two species of lice collected from the same host individuals (or same host species at the same locality) revealed very few host-associated divergence patterns that are consistent between *H. arboricola* and *N. pacificus* (Fig. 4). There are no shared deep divergences, and only five examples of nodes that are similar between the two louse species, all of which occur at shallow levels. Gene tree-species tree congruence factors calculated in IQ-TREE for both species suggest discordance in both louse species, but overall comparisons of the gene trees to the species trees of the same species had much higher congruence factors than the comparisons of gene trees to species trees between species (values are displayed on the branches in Fig. 4).

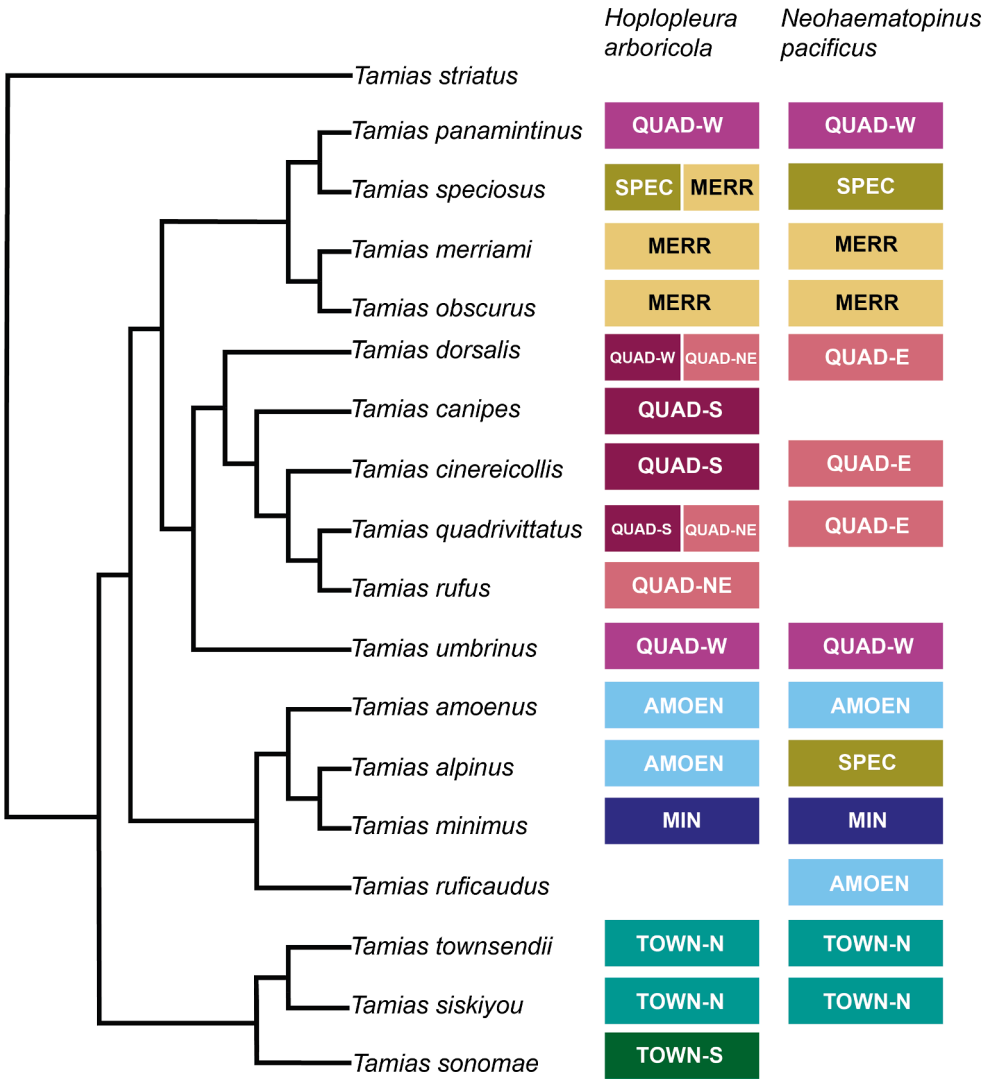

Fig. 3. *Tamias* cladogram modified from chronogram in Sullivan et al. (2014). Clades for each louse species are displayed with host-associated lineage names and colors that correspond to Fig. 1 (*Hoplopleura arboricola*) and Fig. 2 (*Neohaematopinus pacificus*).

4. Discussion

Genome-scale data for non-model parasites significantly advances coevolutionary investigations and understanding of species interactions across the Tree of Life. With the high resolution facilitated by genomic data, we determined that neither louse species appears to be tightly codiverging with the chipmunks at the host species level. In addition, although each louse has lineages that appear to be primarily associated with a host species or species group, relationships among lice lineages differ between the two louse species. We only found one instance of host-switching among distantly related hosts (or a generalist louse clade) that was mirrored in the two louse species, the QUAD-W clade parasitizing *T. panamintinus*. We uncovered one instance of a louse clade parasitizing hosts with disjunct distributions, the *H. arboricola* AMOEN clade parasitizing *T. alpinus* and *T. amoenus* (Fig. 1). This finding may be the result of the ancestor of the MIN and AMOEN clades parasitizing the ancestor of *T. minimus*, *T. alpinus*, and *T. amoenus*.

Using the species trees, we found that neither louse phylogeny is congruent with host chipmunk phylogeny (Reid et al., 2012, Sullivan et al., 2014), rejecting a scenario of strict host-parasite codiversification. Although this approach cannot identify the processes impacting louse diversification, the biogeographic histories of the hosts have most likely played a large role; for which there is evidence in other louse-host systems (du Toit et al., 2013). Climatic cycling during the Quaternary would have caused chipmunk populations to geographically contract, expand, and periodically come into contact in response to habitat shifts. These fluctuations in host populations apparently allowed multiple instances of contact among multiple lineages of louse species. While both groups of lice have lineages that are primarily, if not exclusively, associated with a single host species or a closely related group of host species, the relationships among those lineages are not mirrored between the two louse species. Because *H. arboricola* is non-monophyletic with respect to *H. erratica*, coupled with subtle morphological characters that distinguish *H. arboricola* and *H. erratica* (Kim et al., 1986), further resolution of this group is needed. We did not notice any morphological characters that correspond to different *H. arboricola* clades, but further examination may reveal traits that distinguish the clades. The *H. erratica* sampled from *T. striatus* in Maryland is too geographically distant for an incidental transfer or host switch of *H. arboricola* from a western chipmunk species hosting *H. arboricola*. However, *T. striatus* contacts *T. minimus* (likely the *H. arboricola* clade MIN) in the Great Lakes region of Canada and the United States, but those populations of *T. minimus* and *T. striatus* were not sampled for this study. It is possible that *H. erratica* and *H. arboricola* were originally described as distinct species primarily based on host associations, which has proven to be a poor guide for louse taxonomy in other groups (Johnson et al., 2002). Overall, the magnitude

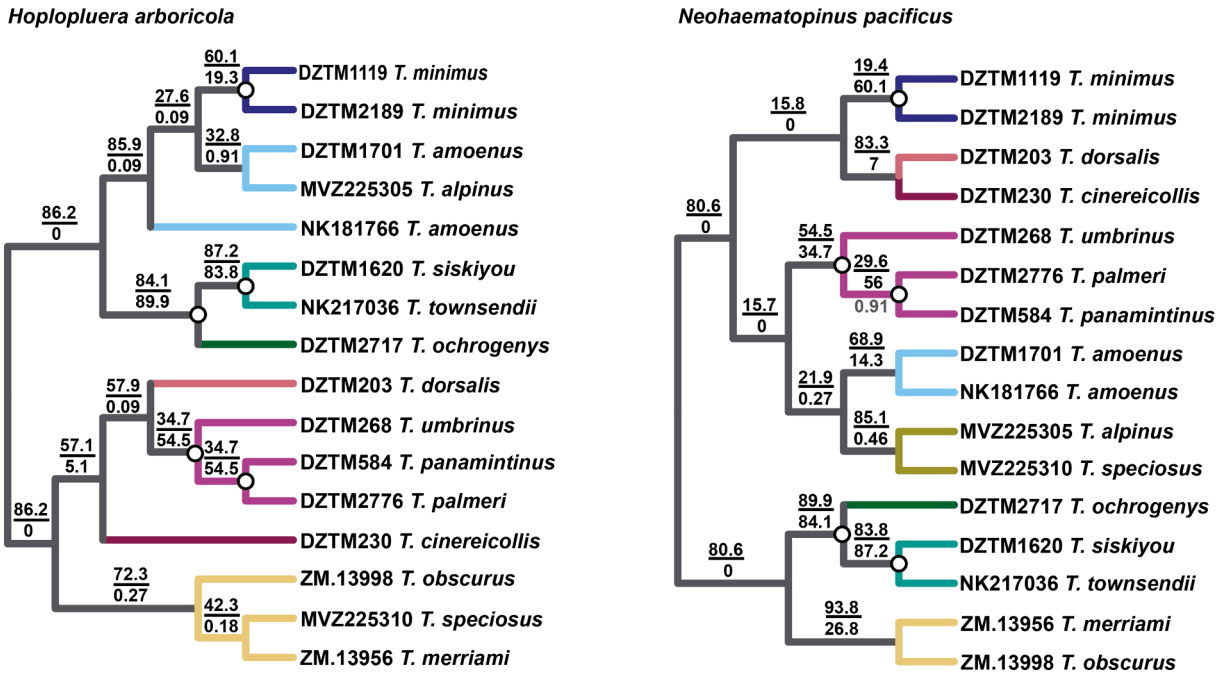

Fig. 4. ASTRAL species trees for *Hoplopleura arboricola* (left) and *Neohaematopinus pacificus* (right) of samples collected from the same host individuals. Ratios above the branches are the congruence factors for the gene trees (generated in IQ-TREE), the numerator is the congruence factor for the same species gene trees with the species tree and the denominator is the congruence factor for the other species gene trees. All branches of the species trees have local posterior probability support of 1, unless otherwise noted below the branch. White circles on nodes show clades that are the same in both species. Branch colors on both trees correspond to the colors assigned to the *H. arboricola* clades (Figs. 1 and 4).

of sequence divergences among clades, some level of host-specificity in louse lineages, and *H. arboricola* non-monophyly support the existence of cryptic species. Additional sampling, morphological examinations, and sequencing will be necessary to establish updated taxonomy.

Our sampling included some instances of multiple lice from the same host species at different geographic localities. In several instances, such as *H. arboricola* and *N. pacificus* sampled from *T. minimus*, different louse populations form a single clade united by their host. However, the *H. arboricola* sampled from *T. dorsalis* appear to be closely related, but in non-sister clades (QUAD-NE and QUAD-S) and the two *H. arboricola* from *T. speciosus* are not monophyletic. These patterns suggest a process whereby host-associations are shaping some level of louse diversification; however, geography is also impacting population structure. The *H. arboricola* MIN clade is also divergent in COI from the geographically closest clades, QUAD-S (11.2%) and QUAD-NE (15%), suggesting there is no gene flow among these populations of lice, even though their host species are in contact in some areas. The levels of COI divergence among the *H. arboricola* QUAD clades are comparatively low (4%–6.6%), indicating potential gene flow among these clades. This is noteworthy given that this clade of hosts (with the exception of *T. panamintinus*) has a history of hybridization and mitochondrial relationships are geographically structured among many of these chipmunks (Reid et al., 2012, Sullivan et al., 2014). A different dynamic is evident in the *N. pacificus* sampled from the *T. quadrivittatus* species group hosts (clades QUAD-E and QUAD-W); these have relatively higher levels of COI divergence (Table 3) and exhibit geographically structured clades that are non-sister in the ASTRAL and IQ-TREE analyses (Fig. 2). However, these two clades are sister in the SVDQuartets tree (Supplemental Fig. 1). Additional, population-level sampling within each louse clade for both species will allow us to further explore the geographic and host distributions of these clades.

The evolutionary histories for these two species of lice differ in several ways. At a very coarse scale, the *H. arboricola* species tree is more similar to the phylogeny of chipmunks in the sense that some of the louse lineages correspond to groups of closely related hosts (e.g. QUAD, MIN, AMOEN, and TOWN clades), with the striking exception of *H. erratica*, the *T. striatus* louse. In contrast, *N. pacificus* lineages form similar host-associated clades, but the host composition of those clades differ and, importantly, the relationships among *N. pacificus* clades are very different from the relationships among *H. arboricola* clades. One of these comparisons, the lice collected from *T. alpinus* and *T. speciosus*, suggests that distantly related chipmunk hosts living in sympatry may share the same lineage of one louse species (*N. pacificus*), but not the other (*H. arboricola*).

There is no existing genus-level phylogeny for either louse genus, so it is not clear what the host species are for the most closely related species of lice to those sampled here. The genus *Hoplopleura* is diverse and found worldwide, primarily on murid and cricetid hosts, but some species also parasitize Asian and North American tree squirrels (Durden and Musser, 1994). An ancestor of *H. arboricola* (including *H. erratica*) may have parasitized the ancestor of all chipmunks and been lost on the only extant Eurasian chipmunk (*Tamias sibiricus*), which harbors a family and genus of louse (Enderleinellidae: *Enderleinellus*) not found on North American chipmunks. Alternatively, the ancestor of *T. striatus* or the western chipmunks may have acquired *H. arboricola* (or its ancestor), which has since switched to the other group of chipmunks. Many species of the other genus, *Neohaematopinus*, are found across species of Sciuridae in Asia and the Americas, suggesting the potential of a switch to chipmunks from another sciurid. Given that *N. pacificus* is only found on western chipmunks, we hypothesize that it switched to parasitizing western chipmunks following phylogenetic divergence from the other two *Tamias* species. Additionally, the genetic divergences among the *N. pacificus* clades are shallower than *H. arboricola*, which may be due to a shorter time of association with these hosts. Questions regarding the biogeographic and host history of these sucking lice can only be answered by comprehensive phylogenetic investigations that sample much more broadly the diversity of species in each genus. The sole large-scale phylogeny for Anoplura (Light et al., 2010) sampled 8 of the 16 families and found that the families of chipmunk lice, Hoplopleuridae (*Hoplopleura*) and Polyplacidae (*Neohaematopinus*), are not

monophyletic. As such, extensive sampling across species of both genera will be required to confidently resolve relationships within each of those families.

Comparing the phylogenies of chipmunk sucking lice to other chipmunk parasites provides a broader picture of host-parasite relationships in this system. Our findings share some similarities with previous work on western chipmunks and two species of their endoparasitic pinworms, but there are differences (Bell et al., 2016, 2018). All four parasite species have lineages that primarily correspond to host lineages, either species or species groups. However, the relationships among those lineages vary and suggest that while host diversification impacts parasite evolution, it is impacting each parasite differently, either asynchronously (pinworms), or with 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 phylogenies of parasite lineages with phylogenies of chipmunk species. While chipmunk lice exhibit clades primarily associated with a single host or host species group, deeper divergences vary between the louse species. Contrary to patterns of louse divergence, chipmunk pinworms have deep divergences that primarily correspond to host species or species groups and those divergences are largely congruent between two pinworm species (Bell et al., 2016, 2018). The chipmunk pinworm investigations, however, were based on relatively few loci and genome level data will presumably refine, and potentially uncover different patterns in, the pinworm phylogenies.

While there have been several phylogenies for chipmunks (Piaggio and Spicer, 2001, Reid et al., 2012, Sullivan et al., 2014), none has sampled all 25 species. This makes it difficult to resolve the relationships among the hosts and fully characterize the chipmunk and parasite coevolutionary histories. Additionally, what we do know about western chipmunk relationships suggests that they have a relatively recent history of diversification (approximately 2.75 my) and closely related species are often codistributed and may hybridize (mitochondrial introgression; Sullivan et al., 2014). These factors complicate our efforts to disentangle host-associated divergences and geographic structure in louse lineages. The next steps to characterize this host-parasite system is a comprehensive phylogeny for *Tamias* and estimating divergence times among host species and louse clades. Such analyses will allow us to place lineage diversification within the context of when host species diverged.

The phylogenetic distance effect predicts that closely related hosts will be parasitized by closely related parasites. A few examples allow us to reject the phylogenetic distance effect as the primary process driving chipmunk sucking lice divergences. Members of the *Tamias quadrivittatus* species group (*T. cinereicollis*, *T. dorsalis*, *T. palmeri*, *T. quadrivittatus*, *T. umbrinus*) are parasitized by divergent *N. pacificus* lineages, despite close geographic distributions and close phylogenetic relationships of the hosts. In some instances, louse clades parasitize hosts that span deep splits in the chipmunk phylogeny, such as *N. pacificus* parasitizing *T. speciosus* and *T. alpinus* (~2 my; Sullivan et al., 2014). The placement of *H. erratica* within *H. arboricola* is a notable example of louse lineages not reflecting host relationships, as *T. striatus* is sufficiently distant from the western chipmunks that they have been proposed to be classified as different genera (Patterson and Norris, 2016). In addition to this evidence for non-codivergence, no previous chipmunk phylogeny, even accounting for mitochondrial and nuclear discordance (e.g., Piaggio and Spicer, 2001, Good et al., 2008, Reid et al., 2012, Sullivan et al., 2014), has recovered relationships that mirror any of the louse topologies we found. While it is possible that we are sampling the early stages of host-associated louse diversification, the level of support for the topologies we recovered suggest that further time and divergence will not support louse-chipmunk codivergence across the louse lineages. As mentioned above, a complete host phylogeny will be necessary to identify points in evolutionary history where lice and chipmunks potentially codiverged, however the relationships as we understand them now do not support overall sucking louse divergences driven by host phylogeny.

The dynamics of host-parasite interactions and parasite biogeographic history are likely driven by a complex suite of forces that include taxon pulses, ecological fitting, oscillation, and the geographic mosaic of evolution (Stockholm Paradigm; Brooks et al., 2019). Chipmunk sucking louse lineages are able to parasitize both closely and distantly related chipmunk hosts by tracking shared host resources (ecological fitting). Additionally, the demographic and biogeographic history of chipmunks appear to have shaped different codiversification dynamics with each species of louse (taxon pulse). While some patterns of louse lineage associations with hosts are geographically generalizable, there is variation across the landscape between the two lice species and the hosts they parasitize (geographic mosaic of coevolution, Thompson, 2005). Chipmunk sucking lice diversification has been driven by these forces and demonstrates that the contemporary host associations were not always generated by the same processes across different louse species. Population level investigations in other parasites have also uncovered varying, complex evolutionary patterns in parasites of hosts of the same genus, shaped by the interplay of host-parasite interactions and responses to abiotic conditions (Engelbrecth et al., 2016, Martinů et al., 2018, Nieberding et al., 2008).

Phylogenomic tools for non-model organisms are permitting unprecedented insights into evolutionary history (e.g., Blaimer et al., 2015, Kawahara et al., 2019). Applying these tools, in conjunction with previous findings in chipmunk pinworms (Bell et al., 2016, 2018) to the chipmunk-louse system has revealed that there are few processes of diversification that can be generalized for parasites, even when considering the same hosts. 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, biogeographic, 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. The chipmunk-parasite system demonstrates that parasite diversification cannot be explained as a simple process of codivergence. It is these systems with two or more parasite species in the same ecological roles, such as lice, that will allow us to decipher parasite genomic, morphological, or ecological traits that correspond to the ability (or lack of) to switch among hosts. Our results point to similar host associations evolving in distantly related lice, however the paths to those associations differ. We look forward to exploring the underlying mechanisms facilitating these host associations.

CRediT authorship contribution statement

Kayce C. Bell: Conceptualization, Formal analysis, Investigation, Writing - original draft, Visualization, Funding acquisition. **Julie M. Allen:** Software, Formal analysis, Writing - review & editing. **Kevin P. Johnson:** Resources, Writing - review & editing, Funding acquisition. **John R. Demboski:** Resources, Writing - review & editing, Funding acquisition. **Joseph A. Cook:** Resources, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Funding: This work was supported by the National Science Foundation DEB [1311076 to KCB, 1925312 to JMA, 1239788, 1342604,

1926919, and 1925487 to KPJ, 0716200 to JRD, 0731350, 0956129, and 1258010 to JAC], the University of New Mexico Department of Biology and Biology Graduate Student Association, the American Society of Mammalogists, the Society of Systematic Biologists, and the Lloyd David and Carlye Cannon Wattis Foundation. This work would not have been possible without the generosity of the Denver Museum of Nature & Science, the Museum of Southwestern Biology, and the Museum of Vertebrate Zoology allowing us to destructively sample lice. Field collection was aided by many, including R. D. McCain, B. S. McLean, S. W. Liphardt, and L. F. Alexander. J. L. Patton combed recently collected specimens and provided lice samples, while historic museum study skins were combed by D. Matek. J. E. Light, S. W. Liphardt, and K. E. Galbreath provided guidance and helpful feedback on this manuscript. We thank two anonymous reviewers for providing comments that helped improve the manuscript.

Appendix A

Specimen catalog, NCBI Sequence Read Archive (SRA), and host data. DZTM and ZM lice samples are archived at the Denver Museum of Nature & Science as part of the host. Lice with catalog numbers are part of the Parasitology Collection at the Museum of Southwestern Biology (MSB). Hosts are cataloged at the Denver Museum of Nature & Science (ZM), the Museum of Vertebrate Zoology (MVZ), or the Museum of Southwestern Biology (MSB).

Sample	MSB parasite catalog number	SRA sample number	*Tamias* host species	Host catalog number
Hoplopleura arboricola				
DZTM203Ha		SAMN15866164	*T. dorsalis*	ZM.11395
DZTM230Ha		SAMN15866166	*T. cinereicollis*	ZM.11115
DZTM268Ha		SAMN15866167	*T. umbrinus*	ZM.11148
DZTM324Ha		SAMN15866174	*T. canipes*	ZM.11424
DZTM572Ha		SAMN15866175	*T. rufus*	ZM.11805
DZTM584Ha		SAMN15866176	*T. panamintinus*	ZM.11678
DZTM748Ha		SAMN15866177	*T. quadrivittatus*	ZM.11867
DZTM816Ha		SAMN15866178	*T. quadrivittatus*	ZM.11933
DZTM1119Ha		SAMN15866160	*T. minimus*	ZM.12155
DZTM1620Ha		SAMN15866161	*T. siskiyou*	ZM.12363
DZTM1701Ha		SAMN15866163	*T. amoenus*	ZM.12444
DZTM2189Ha		SAMN15866165	*T. minimus*	ZM.13012
DZTM2717Ha		SAMN15866168	*T. ochrogenys*	ZM.12960
DZTM2725Ha		SAMN15866169	*T. siskiyou*	ZM.12968
DZTM2740Ha		SAMN15866170	*T. sonomae*	ZM.12979
DZTM2776Ha		SAMN15866171	*T. palmeri*	ZM.13114
DZTM2784Ha		SAMN15866172	*T. palmeri*	ZM.13122
DZTM2798Ha		SAMN15866173	*T. panamintinus*	ZM.13136
ZM.10492Ha		SAMN15866193	*T. rufus*	ZM.10492
ZM.13956Ha		SAMN15866180	*T. merriami*	ZM.13956
ZM.13998Ha		SAMN15866179	*T. obscurus*	ZM.13998
MVZ225305Ha	20577	SAMN15866182	*T. alpinus*	MVZ225305
MVZ225309Ha	20582	SAMN15866183	*T. alpinus*	MVZ225309
MVZ225310Ha	20583	SAMN15866184	*T. speciosus*	MVZ225310
MSB2245Ha	19726	SAMN15866181	*T. dorsalis*	MSB2245
NK181754Ha	20381	SAMN15866185	*T. townsendii*	MSB249969
NK181766Ha	20386	SAMN15866186	*T. amoenus*	MSB249979
NK195685Ha	27035	SAMN15866187	*T. umbrinus*	MSB227184
NK213801Ha	20362	SAMN15866188	*T. townsendii*	MSB248964
NK213828Ha	20363	SAMN15866189	*T. canipes*	MSB248977
NK215099Ha	20418	SAMN15866190	*T. ochrogenys*	MSB259308
NK215133Ha	20437	SAMN15866191	*T. speciosus*	MSB259341
NK217036Ha	20348	SAMN15866162	*T. townsendii*	MSB233636
NK217095Ha	20350	SAMN05930902	*T. amoenus*	MSB233654
Hoplopleura erratica				
NK267511He	31462	SAMN15866192	*T. striatus*	MSB275309
Neohaematopinus pacificus				
DZTM203Np		SAMN15866197	*T. dorsalis*	ZM.11395
DZTM230Np		SAMN15866199	*T. minimus*	ZM.13012
DZTM268Np		SAMN15866200	*T. umbrinus*	ZM.11148
DZTM377Np		SAMN05930903	*T. minimus*	ZM.11420
DZTM584Np		SAMN15866203	*T. panamintinus*	ZM.11678
DZTM708Np		SAMN15866204	*T. quadrivittatus*	ZM.11822
DZTM946Np		SAMN15866205	*T. minimus*	ZM.12094
DZTM1119Np		SAMN15866194	*T. minimus*	ZM.12155
DZTM1620Np		SAMN15866195	*T. siskiyou*	ZM.12363
DZTM1701Np		SAMN15866196	*T. amoenus*	ZM.12444
DZTM2189Np		SAMN15866198	*T. minimus*	ZM.13012
DZTM2717Np		SAMN15866201	*T. ochrogenys*	ZM.12960
DZTM2776Np		SAMN15866202	*T. palmeri*	ZM.13114
ZM.13956Np		SAMN15866207	*T. merriami*	ZM.13956
ZM.13998Np		SAMN15866206	*T. obscurus*	ZM.13998
MVZ225305Np	20578	SAMN15866208	*T. alpinus*	MVZ225305
MVZ225310Np	20584	SAMN15866209	*T. speciosus*	MVZ225310
MSB84515Np	20020	SAMN15866210	*T. speciosus*	MSB84515

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Sample	MSB parasite catalog number	SRA sample number	*Tamias* host species	Host catalog number
NK181766Np	20385	SAMN15866211	*T. amoenus*	MSB249979
NK215220Np	20444	SAMN15866212	*T. ruficaudus*	MSB264027
NK217036Np	20347	SAMN15866213	*T. townsendii*	MSB233636

Appendix B. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2020.106998.

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