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Temporal and spatial mosaics: deep host association and shallow geographic drivers shape genetic structure in a widespread pinworm, *Rauschtineria eutamii* (Nematoda: Oxyuridae)

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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 ten 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 with 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*. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **119**, 397–413.

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. Timm, 1983; Lyal, 1986; Hafner *et al.*, 1994,

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[Correction added on 20 June 2016, after first online publication: In the initial publication, the mitochondrial 12S locus was erroneously described as a nuclear locus; the appropriate changes have now been made to the text].

2003; Johnson & Clayton, 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 (Araujo *et al.*, 2015; Brooks *et al.*, 2015; Hoberg & Brooks, 2015; Hoberg *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 *et al.*, 2012; Hoberg & Brooks, 2013). 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; Waltari *et al.*, 2007; Koehler *et al.*, 2009; 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, 2003; Haukisalmi *et al.*, 2016) 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, 2016, for proposed reclassification) are broadly distributed across > 40° 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 ten 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 (Tiner & Rausch, 1950; Hugot, 1980). 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 faeces, 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.

MATERIAL AND METHODS

Chipmunks were collected from across the western United States and examined for endoparasites following approved mammal handling and collecting protocols (Sikes & Gannon, 2011). Recovered pinworms were preserved in 70% or 95% ethanol, frozen in liquid nitrogen, and later transferred to a −20 °C 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 (Fig. 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 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 55 °C for 5 min. Final elution was 30–60 μL 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 (accession numbers COI: KT875241–KT875323; 12S: KU668406–KU668432; 28S: KU668379–KU668405; Appendix 1).

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 10 000 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

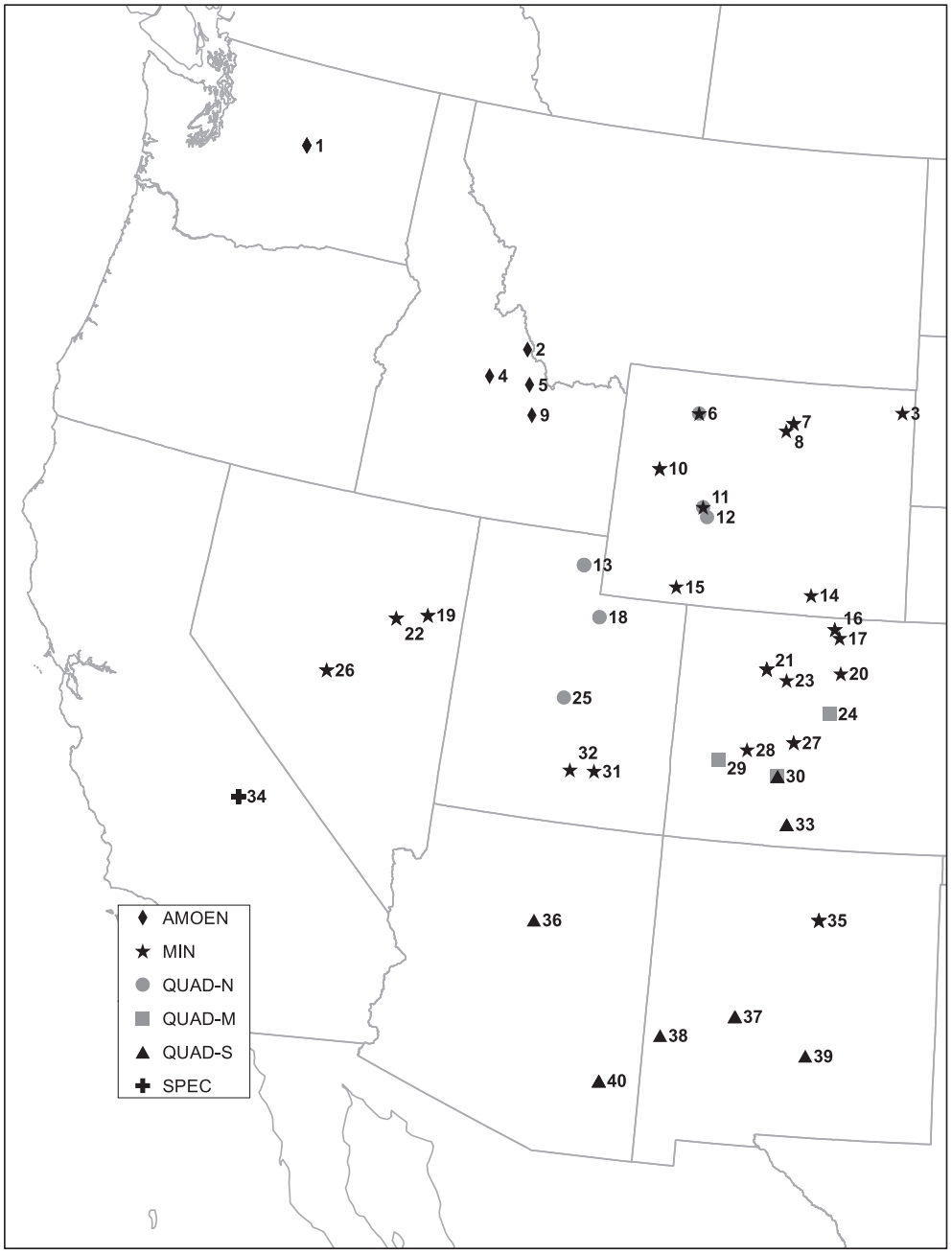

Figure 1. Map of sample localities in western North America. Symbols correspond to clade labels in Figures 2–4. Numbers correspond to tip labels on trees and localities in Appendix 1.

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 PJ, 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 2). 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 1; Supporting Information, Table S3).

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 F_{st} of 0.705 and pairwise F_{st} values ranging from 0.572 to 0.969.

The mitochondrial 12S gene tree (Supporting Information, Fig. S1) 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 (Supporting Information, Fig. S2). The Bayesian concordance analyses (BUCKy) did not have high support (concordance factor ≥ 50) for all six 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 (Fig. 3).

Within three of the six clades (QUAD-M, MIN, SPEC; Fig. 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 (Supporting Information, Table S1).

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 (Fig. 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 1). 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

Table 1. Locations with multiple host species

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

Host genetic distance is mean distance between species based on available cytochrome *b* sequences on GenBank (Appendix 2).

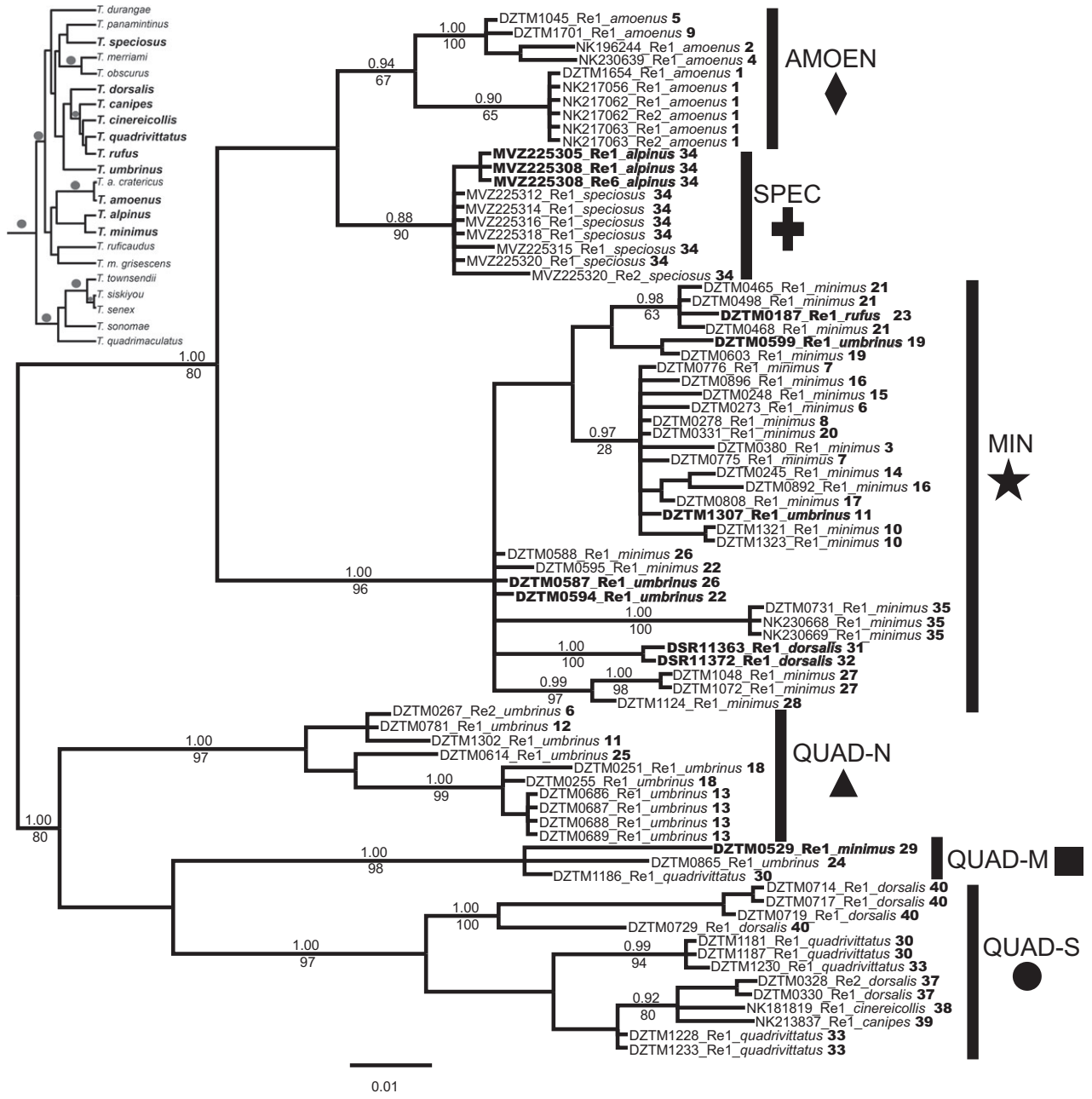

Figure 2. COI gene tree. Support values above branches are posterior probabilities, values below are maximum likelihood bootstraps. Labels on the right correspond to clade names and symbols correspond to localities on map in Figure 1. Tip labels in bold are host switches, numbers at end of tip labels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

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*). Additionally, at two localities different pinworm lineages were recovered from the same host species (Supporting Information, Table S2). 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*;

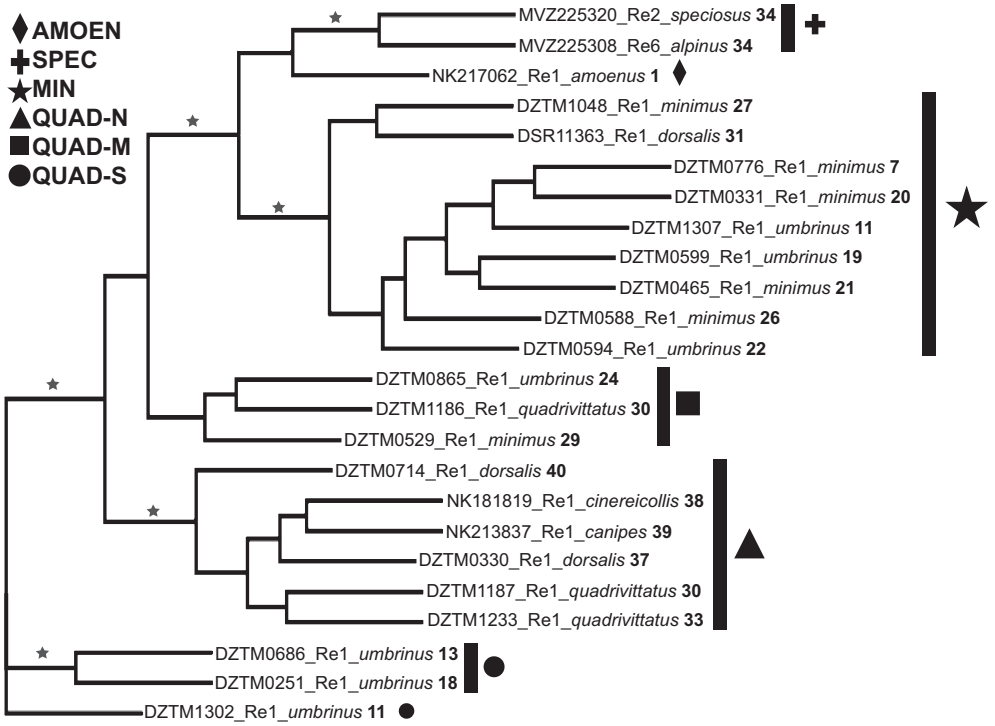

Figure 3. Concordance tree of COI, 12S, and 28S. Stars on branches indicate concordance factors ≥ 0.5. Symbols correspond to COI clades and numbers refer to localities in Figure 1. Top left inset legend for mitochondrial clades.

DSR11372_Re1 and DSR11363_Re1 from *T. dorsalis*) at localities where no pinworms were recovered from *T. minimus* or no *T. minimus* were collected (Supporting Information, Table S3), 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.

Table 2. 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

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 2) 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 (Fig. 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

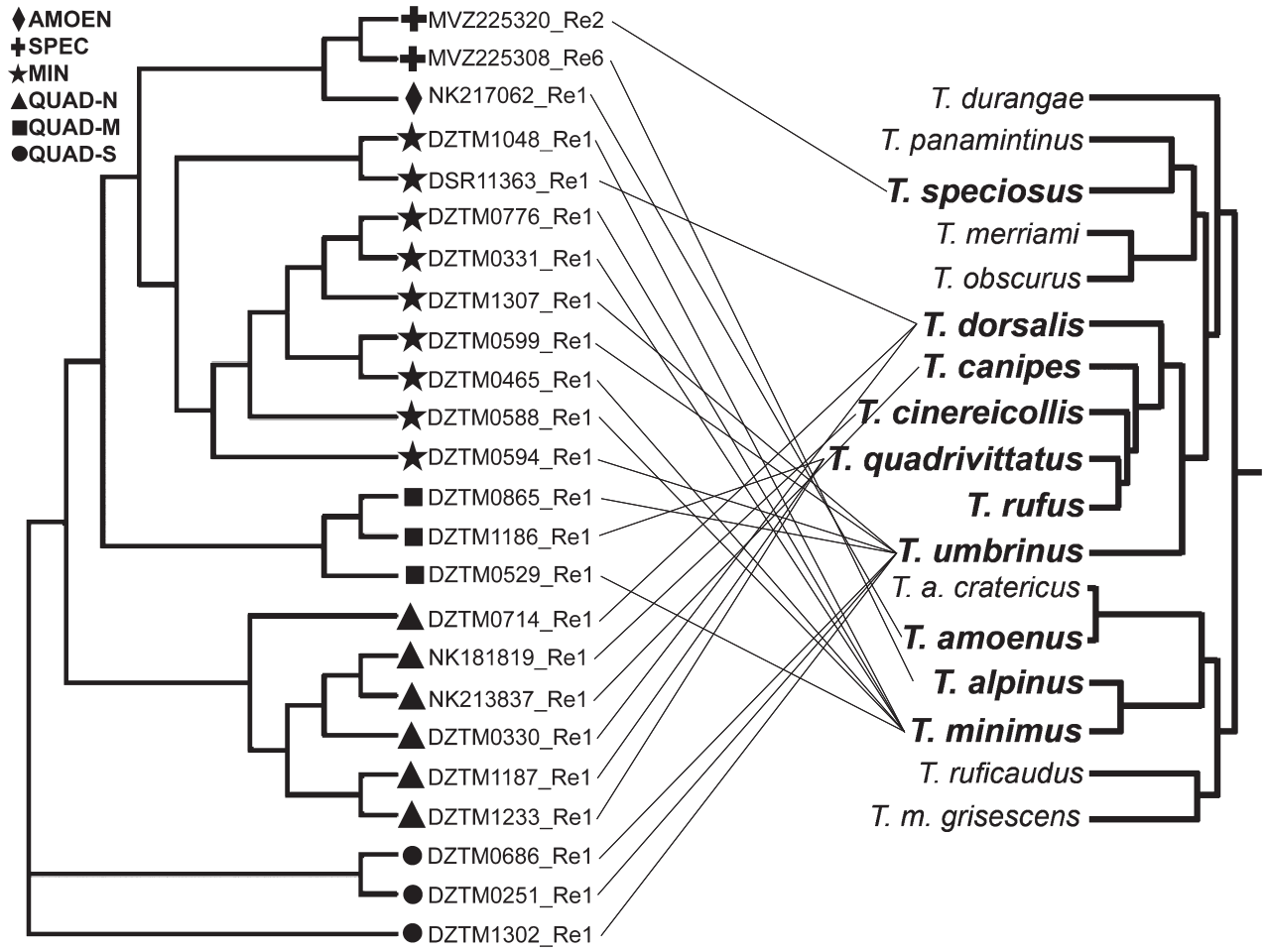

Figure 4. Tanglegram connecting *R. eutamii* concordance tree tips (left) to corresponding hosts on *Tamias* species tree (right). *Tamias* species tree modified from Sullivan *et al.*, 2014. Top left inset legend of mitochondrial clades.

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 (Choudhury & 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 (Fig. 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 (Fig. 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 (six pinworm lineages, ten 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*. Species in the *T. townsendii* species group, however, 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 (Araujo *et al.*, 2015; Hoberg & Brooks, 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 (Fig. 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 in which 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 (Hoberg & Brooks, 2008, 2015; Agosta *et al.*, 2010; 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, 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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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Mitochondrial rRNA 12S gene tree. Values above branches are posterior probabilities and values below branches are bootstraps. Symbols correspond to COI clades (inset) and sample localities.

Figure S2. 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.

Table S1. Results of Mantel tests of correlation between geographic and genetic distance for all samples and within mitochondrial clades.

Table S2. Locations with multiple pinworm clades from the same host species.

Table S3. 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.

APPENDIX 1

Table A1. HOST AND PARASITE CATALOGUE NUMBERS WITH HOST SPECIES, COI CLADE, AND GENBANK ACCESSION NUMBERS FOR COI, 12S AND 28S SEQUENCES

Sample name	Parasite catalogue number	Host catalogue number	Host species	Locality	Clade	COI Accession	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-N	KT875241	KU668428	KU668380
DZTM0255_Re1		ZM 11164	*T. umbrinus*	18	QUAD-N	KT875242		
DZTM0267_Re2		ZM 11147	*T. umbrinus*	6	QUAD-N	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-M	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-N	KT875275		

Table A1. *Continued*

Sample name	Parasite catalogue number	Host catalogue number	Host species	Locality	Clade	COI Accession	12S Accession	28S Accession
DZTM0686_Re1		ZM 11792	*T. umbrinus*	13	QUAD-N	KT875276	KU668427	KU668387
DZTM0687_Re1		ZM 11793	*T. umbrinus*	13	QUAD-N	KT875277		
DZTM0688_Re1		ZM 11794	*T. umbrinus*	13	QUAD-N	KT875278		
DZTM0689_Re1		ZM 11795	*T. umbrinus*	13	QUAD-N	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-N	KT875268		
DZTM0808_Re1		ZM 11925	*T. minimus*	17	MIN	KT875281		
DZTM0865_Re1		ZM 11982	*T. umbrinus*	24	QUAD-M	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-M	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-N	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		
DZTM1654_Re1		ZM 12397	*T. amoenus*	1	AMOEN	KT875296		
DZTM1701_Re1		ZM 12444	*T. amoenus*	9	AMOEN	KT875302	KU668431	
MVZ225305_Re1	MSB Para 20689	MVZ 225305	*T. alpinus*	34	SPEC	KT875303		
MVZ225308_Re1	MSB Para 20690	MVZ 225308	*T. alpinus*	34	SPEC	KT875308		
MVZ225308_Re2	MSB Para 20690	MVZ 225308	*T. alpinus*	34	SPEC	KT875304		
MVZ225308_Re3	MSB Para 20690	MVZ 225308	*T. alpinus*	34	SPEC	KT875305		
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
MVZ225312_Re1	MSB Para 20694	MVZ 225312	*T. speciosus*	34	SPEC	KT875310		

Table A1. *Continued*

Sample name	Parasite catalogue number	Host catalogue number	Host species	Locality	Clade	COI Accession	12S Accession	28S Accession
MVZ225314_Re1	MSB Para 20696	MVZ 225314	*T. speciosus*	34	SPEC	KT875311		
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	KU668397KU668398
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	KU668402KU668403
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		

Hosts are all genus *Tamias*.
Institutional Catalogue 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.

APPENDIX 2

*T*AMIAS 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).