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Phylogeography of the red-tailed chipmunk (*Tamias ruficaudus*), a northern Rocky Mountain endemic

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Abstract

The northern Rocky Mountains have experienced a complex history of geological events and environmental fluctuation, including Pleistocene glaciation. To provide an initial assessment of the genetic impact of this history on the regional biota we estimated phylogenetic relationships within *Tamias ruficaudus*, a regional endemic, from cytochrome *b* sequence variation using parsimony, maximum likelihood, and nested clade analysis. Analyses of sequence variation in 187 individuals from 43 localities across the distribution of *T. ruficaudus* indicate a history of vicariance events and range fluctuation consistent with successive periods of extensive Pleistocene glaciation in the northern Rocky Mountains. Intraspecific divergence levels (*c.* 4.7% uncorrected) and phylogenetic structure are consistent with a genealogical vicariance initiated prior to the Late Pleistocene, whereas nested clade analyses indicate more recent population history structured by both fragmentation and range expansion. A comparison of sequence variation with bacular morphology indicates that the two genetically and morphologically differentiated entities exhibit a zone of differential character introgression. Sequence data support a multiple refugia hypothesis and provide a phylogeographical case study for the ongoing synthesis of regional biogeography for northern Rocky Mountain endemics.

Keywords: cytochrome *b*, likelihood, nested clade analysis, northern Rocky Mountains, phylogeography, *Tamias ruficaudus*

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Introduction

The northern Rocky Mountains of British Columbia, western Montana, and northern Idaho have experienced a complex history of geological events and environmental fluctuation, including rain-shadow effects incurred by Pliocene coastal orogeny (Wolfe 1969) and successive Pleistocene glacial–interglacial cycles (Richmond 1965; Graham 1993). Knowledge of these geological and climatological events has led to several biogeographical hypotheses to explain contemporary distributions of regional biota (Brunsfeld *et al*. 2001), yet the historical biogeography of northern Rocky Mountain endemics remains under-examined. Much of what has been postulated relies on the interpretation of contemporary species' ranges and fossil records of vascular plants (Daubenmire 1975), including indirect inference of select faunal history (Johnson 1988). However, few studies have quantified genetic patterns of variation and tested the hypotheses under a phylogeographical framework (but see Nielson *et al*. 2001).

Geographical structure of intraspecific genetic variation is often associated with the widespread disruption of biotic communities during Pleistocene glacial cycles (e.g. Graham & Mead 1987; Hewitt 1993; Bush 1994; Avise *et al*. 1998; but see Riddle 1996, 1998; Zink 1996). The northern Rocky Mountains experienced episodic glaciation throughout the Pleistocene (Richmond 1965), with much of the region covered with Cordilleran and alpine ice during glacial maxima (Delcourt & Delcourt 1993). Consequently, it is likely that endemic taxa were subjected to range shifts during glacial cycles (FAUNMAP 1996), which may have influenced the geographical structure of genetic variation (Hewitt 1993). Regional geographical heterogeneity provides a basis for the erection of Pleistocene models of northern Rocky Mountain biogeography. Specifically, altitude relief along the Bitterroot Range (generally corresponding

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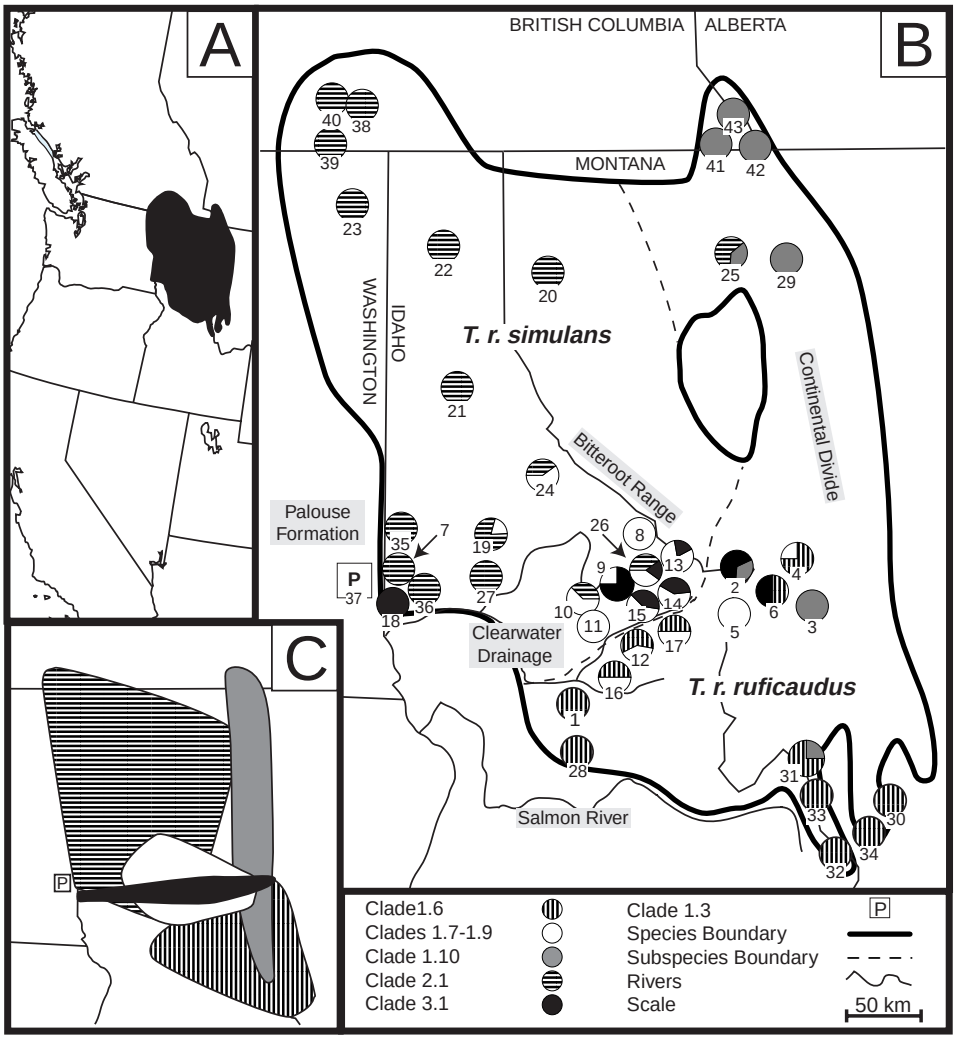

Fig. 1 (A) Distribution of *Tamias ruficaudus* (modified from Hall 1981). (B) Collection localities and corresponding nested clade frequencies. Numbers correspond to population numbers indicated in Appendix I, species and subspecies boundaries modified from Hall (1981) and Patterson & Heaney (1987). The subspecific boundary in the Clearwater Drainage was inferred directly from observed bacular variation. (C) Generalized overlay of nested clades on geography. All clade distributions were drawn using minimum convex polygons.

to the northcentral Idaho/Montana border) and thermal retention within major river canyons suggests the possibility of fragmentation of the expansive mesic forest communities that currently dominate the region. Brunsfeld *et al*. (2001) identified a number of alternative hypotheses and outlined phylogeographical predictions expected under various scenarios of glacial refugia. These predictions include: (i) a general east–west genetic partition associated with the Bitterroot Range; (ii) north–south structure associated with multiple valley refugia and; (iii) a lack of geographical structure due to recent (postglacial) colonization.

Tamias ruficaudus, the red-tailed chipmunk, is endemic to the coniferous forests of the northern Rocky Mountains (Hall 1981; Fig. 1A). It generally inhabits dense, mesic forests characterized by late successional dominance of western hemlock (*Tsuga heterophylla*), western redcedar (*Thuja plicata*), and Douglas-fir (*Pseudotsuga menziesii*), but can also be found in both xeric and subalpine habitats along the periphery of its distribution (Best 1993). Variation in pelage colouration and morphology has led to recognition of two subspecies, the western *T. r. simulans* and the eastern *T. r. ruficaudus* (Fig. 1B; Howell 1922; White 1953; Johnson & Ostenson 1959). Furthermore, some investigators have suggested that the two subspecies are specifically distinct based on the degree of divergence found in bacular (*os penis*) morphology (Fig. 2), a taxonomic character frequently used to infer reproductive isolation in *Tamias* (e.g. Callahan 1977; Patterson 1984; Sutton & Patterson 2000) and other mammals (Patterson & Thaeler 1982).

The purpose of this study is to use mitochondrial DNA (mtDNA) sequence variation to examine the phylogeography

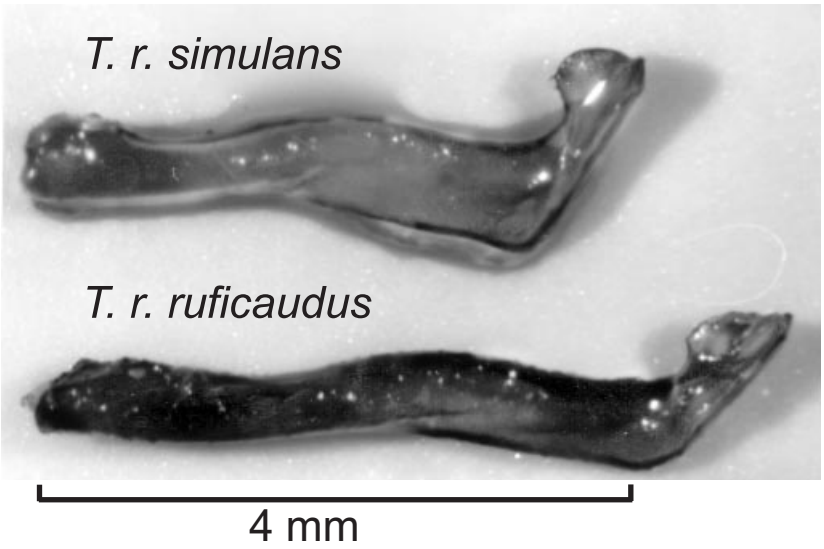

Fig. 2 Bacula of the red-tailed chipmunk, *Tamias ruficaudus*. The upper figure represents the typical form of *T. r. simulans*, sampled near Moscow, ID (Appendix I, Population 7). The lower figure represents the typical form of *T. r. ruficaudus*, sampled on the Lochsa River, ID (Appendix I, Population 16).

of *T. ruficaudus*. In addition, phylogenetic relationships inferred here provide the basis for examining concordance of mitochondrial cytochrome *b* (cyt *b*) sequence variation with previously described bacular morphotypes (e.g. Patterson & Heaney 1987). These analyses provide insight into the evolutionary history of this taxon, further the resolution of taxonomic relationships, and contribute to our expanding understanding of regional biogeography.

Methods

Sampling

One hundred and eighty-seven individuals were examined from 43 localities across the distribution of *Tamias ruficaudus* (Fig. 1B; Appendix I). Field collections were supplemented with specimens obtained through loan from the Royal British Columbia Museum, the Burke Museum of Natural History and Culture (University of Washington), the Connor Museum of Natural History (Washington State University), and the Field Museum of Natural History. Sampling efforts were intensified in the Clearwater Drainage to examine the possible contact of two bacular morphotypes, as hypothesized by Patterson & Heaney (1987). In addition, we included samples of *T. amoenus*, the *T. townsendii* species group (including *T. townsendii* and *T. senex*) and *T. minimus* as outgroups. Previous work on mtDNA sequence variation in *Tamias* has demonstrated that *T. minimus* represents the sister group to *T. ruficaudus* (Piaggio & Spicer 2000).

Data collection

DNA was extracted from approximately 10 mg of liver tissue using a CTAB/DTAB protocol (Gustincich *et al.* 1991). An approximately 820 base pair (bp) fragment of the cyt *b* gene was amplified; primers were as follows: L-14115: 5′-GATATGAAAAACCATCGTTG-3′; L-14553: 5′- CTACCATGAGGACAAATATC-3′ (Kocher *et al*. 1989; Sullivan *et al*. 1997) and H-14899: 5′-TCTGGGTCTCCA-AGGAGGT, specifically designed for this project. Primer numbers refer to positions on the *Mus* mitochondrial genome (Bibb *et al*. 1981). Polymerase chain reaction (PCR) products were purified using polyethylene glycol precipitation and sequenced using a BigDye Kit (Applied Biosystems, Inc.) with 15–45 ng of PCR product in 10 µL reactions. Sequencing reactions were filtered using CentriSep columns (Princeton Separations, Inc.) and run on an ABI 377 automated sequencer using 4% Long Ranger gels. Sequences are available on GenBank under accession numbers AF401759–AF401949.

We employed two complementary methods of data analysis: phylogenetic estimation, and nested clade analysis (NCA), to resolve genealogical relationships within *T. ruficaudus*. Under this framework phylogenetic estimation [e.g. maximum parsimony (MP) and maximum likelihood (ML)] was used to estimate deep structure, whereas the coalescent-based NCA (Templeton *et al.* 1995; Posada & Crandall 2001) was employed to examine shallow structure and infer recent population history.

Phylogenetic analysis

Sequence data were edited and aligned using Sequencher (GeneCodes); alignments were unambiguous with no length polymorphism detected. Phylogenetic analyses were conducted using PAUP* (4.0b4a, Swofford 2000). We employed an iterative search strategy designed to utilize the statistical power of explicitly model-based methods and reduce the computational demands of simultaneous

optimization of all parameters on each tree during a ML tree search (Swofford *et al*. 1996; Sullivan & Swofford 1997). This utilizes parsimony to identify an initial topology on which alternative models of sequence evolution and their associated parameters are evaluated to determine a fully defined model of sequence evolution for subsequent tree searches under the ML criterion. All model-based analyses were performed with a pruned data matrix containing all nonredundant haplotypes. MP and ML searches were conducted using stepwise addition (10 random addition sequences) and tree bisection–reconnection (TBR) branch swapping. MP analyses were performed using equal weights. All ML searches were conducted under the GTR + I model of sequence evolution; model selection was based on likelihood-ratio tests of goodness of fit of 16 alternative models (see Sullivan *et al*. 1997) using a χ^2-approximation of the null distribution (Yang *et al*. 1995). Nodal support was estimated via bootstrap analysis (Felsenstein 1985) under both MP (500 replicates, stepwise addition, TBR branch swapping) and ML (100 replicates, MAXTREES = 1, NNI branch swapping) optimality criteria. In addition, posterior nodal probabilities were estimated with a Bayesian analysis using uniform priors (MRBAYES 1.1; Huelsenbeck 2000), which uses a Markov chain Monte Carlo approach to estimate posterior probabilities. This method has the advantage of integrating across uncertainty in model parameters when estimating nodal probabilities, whereas ML bootstrap values are usually estimated using parameters derived from the real data and that fit each pseudoreplicate poorly.

Nested clade analysis

Phylogeographical inferences have often relied on a simple inspection of the association between a haplotype tree and geography (Avise 1994 for a review). While this approach can suggest phylogeographical hypotheses, it assesses neither the limitations of sampling design nor the statistical significance of haplotype associations with geography (Templeton 1998). Under such a framework, it is difficult to distinguish objectively between alternative hypotheses explaining contemporary genetic patterns of geographical variation. NCA tests the null hypothesis of no association between sample locality and haplotype variation and provides an interpretation of statistically significant patterns (Templeton *et al*. 1995).

Levels of divergence for which connections have a 0.95 or greater probability of being parsimonious (Templeton *et al*. 1992) were calculated using TCS (version 1.06, Clement *et al*. 2000). The data were then converted into a nested minimum-spanning network in which haplotypes were grouped into hierarchical associations (i.e. 'clades') following the procedure of Templeton *et al*. (1987). Nesting ambiguities were addressed as recommended by Templeton & Sing (1993) and Crandall & Templeton (1993). When ambiguous relationships could not be objectively determined, clades were left unresolved and included in the next nesting level. We performed a two-part NCA using GEODIS (2.0, Posada *et al*. 2000). First, a simple contingency test of the geographical association of haplotypes was conducted. A χ^2 statistic was calculated from a contingency table generated by treating haplotype composition of a given clade and corresponding sampling localities as categorical variables. Statistical significance was determined by comparing the observed statistic to the null generated with 10 000 permutations of randomly assigning haplotypes to localities. Geographical distances within and among networks were not considered in this analysis, therefore we conducted a geographical distance-based analysis as follows. The geographical centre of each clade was specified as a weighted average (latitude and longitude) of all the sampling localities within that clade. From this, the average geographical distance of the individuals from the geographical centre of the clade (D_c) and the average distance of the individuals from the geographical centre of the hierarchically next most inclusive clade (D_n) were determined. Clade distance (D_c) measures the geographical spread of a clade and the nested-clade distance (D_n) measures the geographical association of a clade relative to other clades with which it is nested (Templeton *et al*. 1995). In addition, D_c and D_n values were calculated for interior vs. tip clades (I–T). Under the assumptions of coalescent theory, this contrast represents an approximation of the distribution of young vs. old haplotypes (Crandall & Templeton 1993; Castelloe & Templeton 1994). The joint analysis of these two statistics provides an indication of historical population-level processes by comparing observed statistical patterns to expectations of various models of population structure and historical events (for a review of expected patterns see Templeton *et al*. 1995; Templeton 1998).

Results

Phylogenetic analyses

Forty-five unique haplotypes were identified (excluding outgroups) among the 187 individuals examined (Appendix I). Uncorrected sequence divergence ranged up to 4.7%. Within *Tamias ruficaudus*, 11 (5%) first codon positions and 62 (26%) third codon positions were observed to vary; no substitutions were detected at second codon positions. Across the entire data set, including outgroups, 137 (19%) of the 709 sites examined varied, 86 (12%) of which were parsimony informative. MP analysis found 298 most parsimonious trees (103 steps, consistency index = 0.743, retention index = 0.894, rescaled consistency index = 0.665). The most general and parameter-rich model (GTR + I + Γ) had the best likelihood score (ln L =

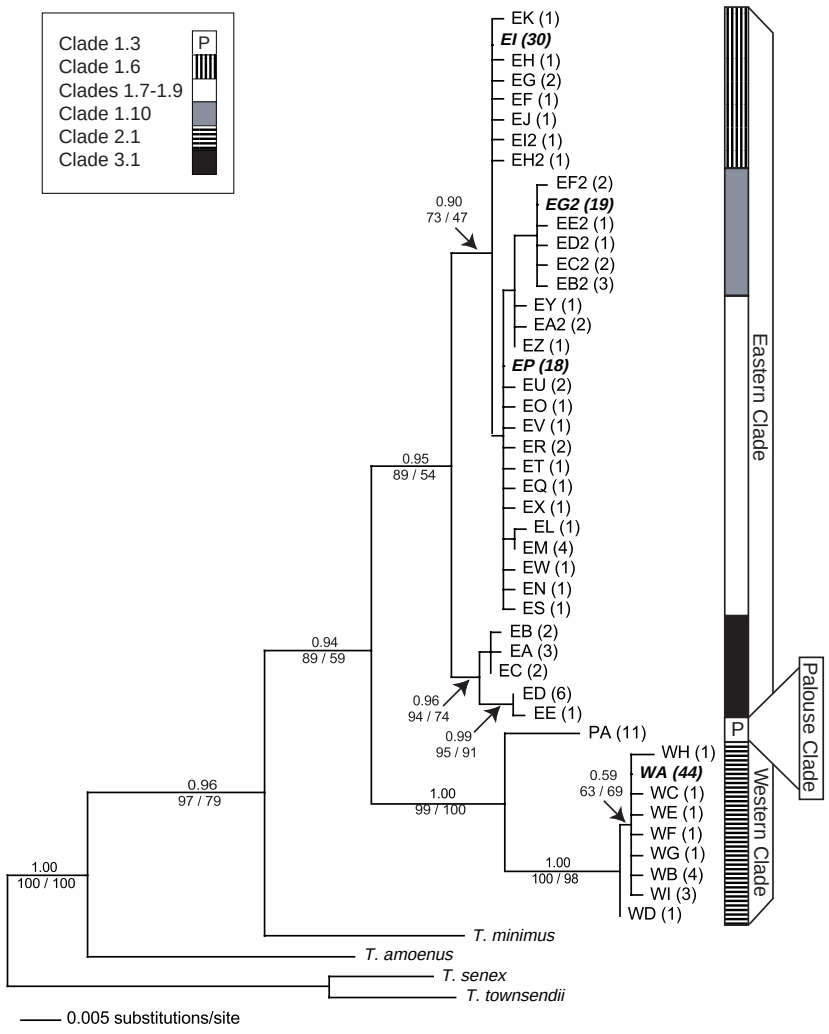

Fig. 3 One of two maximum likelihood phylogenies (–ln L = 1985.62), estimated under the GTR + I model of sequence evolution. The trees differed only in alternative placements of haplotypes EL and EM. Letter designations indicate haplotypes corresponding to Appendix I and Fig. 4. Numbers in parentheses indicate number of individuals in which each haplotype was found. Numbers above nodes indicate Bayesian posterior probabilities estimated with a Markov chain Monte Carlo approach using uniform priors. Values below nodes represent MP (500 replicates) and ML (100 replicates) bootstrap support, respectively. Key indicates nested clade designations inferred from the minimum-spanning haplotype network (Fig. 4).

–1985.47), however, both GTR + I and GTR + Γ had nearly identical scores that were not significantly worse than GTR + I + Γ (lnL = –1985.58 and –1985.60; $\chi^2_1 = 0.22, 0.26$; $P > 0.8$, respectively). We selected GTR + I because it produced a slightly higher likelihood score than GTR + Γ and all other simpler models were rejected as significantly worse than GTR + I + Γ. Subsequent ML searches under GTR + I produced two equally likely trees (lnL = –1984.62), which differed only by the placement of haplotypes EL and EM (Fig. 3). Intraspecific structure was characterized by three major clades. The first bifurcation divided the tree into an Eastern and a Western clade with geographical distributions generally corresponding to previously described subspecies (Figs 1B and 3; Hall 1981). In addition, there was a secondary partition of the Western clade into a geographically widespread Western clade and a restricted Palouse clade (Fig. 3). We could not reject the molecular clock for the two most likely trees using a likelihood-ratio test (Felsenstein 1988; $\chi^2_{45} = 35.73$; $P > 0.9$ and $\chi^2_{45} = 34.60$; $P > 0.8$).

Nested clade analyses

For 709 nucleotides, haplotypes connected by ≤ 11 substitutions have at least a 0.95 probability of being parsimoniously connected; this criterion subdivided the data set into three networks (Fig. 4). Of these subdivisions, clade 1.3 (Palouse clade) was excluded from NCA because it contained a single haplotype, PA, sampled in 11 individuals from a single location (Population 37; Fig. 1B; Appendix I). NCA of clade 2.1 (Western clade) indicates haplotype distribution within this subset is not significantly associated with geography. Within clade 4.1 (Eastern clade) there are numerous significant associations indicated by frequency and nesting location. Haplotype EI of clade 1.6 was determined to be the oldest haplotype observed from frequency and nesting structure (Crandall & Templeton 1993; Fig. 3). The nested contingency test of the geographical associations of haplotypes indicated clades 1.7, 1.10, 2.4, 3.2, and 4.1 are all significantly associated with geography ($P = 0.046$; $P = 0.021$; $P < 0.001$;

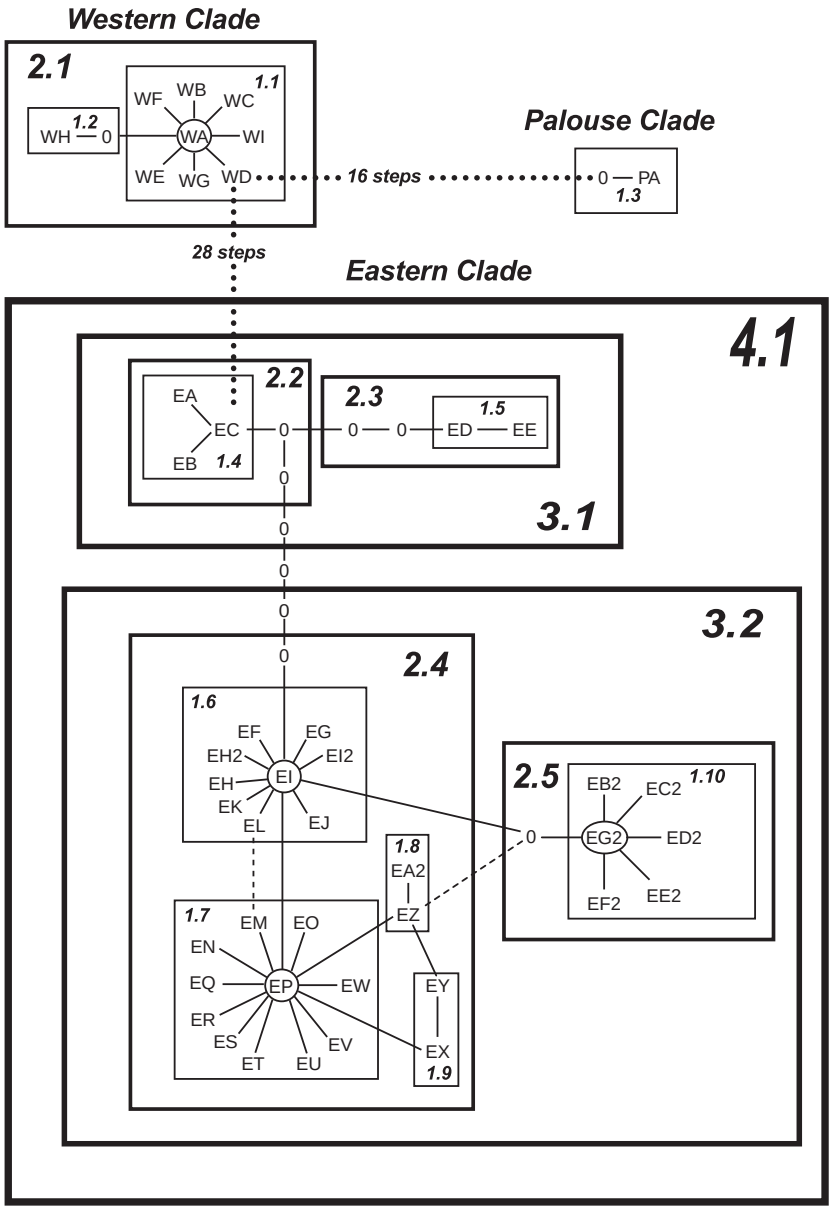

Fig. 4 Minimum-spanning haplotype network nested following the procedures of Templeton *et al*. (1987). Connections ≤ 11 substitutions have a 0.95 probability of being parsimonious. Sampled haplotypes are indicated by letter designations, corresponding to Appendix I and Fig. 3. Substitutions are represented by dashes, and unsampled haplotypes are represented by a '0'. Bold lines indicate the partitioning of the network into three parsimonious groups, geographically corresponding to Eastern, Western, and Palouse clades. Broken lines indicate alternative connections. Hierarchical nesting design specified is by boxes and numbered clade designations.

$P < 0.001$; $P < 0.001$, respectively). NCA of clade 4.1, incorporating geographical distance data, is summarized in Fig. 5. Significance was determined using $\alpha = 0.05$. Significantly large clade distances (D_c^L) indicate geographical displacement of the haplotype/clade from its geographical centre, whereas significantly small clade distances (D_c^S) indicate subsets that are geographically restricted. Significant nested clade distances (D_n^L and D_n^S) indicate significant displacement or restriction of the haplotype/clade from the geographical centre of all other subsets with which it is nested. Likewise, significant interior-tip contrasts [(I–T) D_c's and (I–T) D_n's] suggest the relative restriction or displacement of young vs. old haplotypes. Inferences into historical population-level processes were made, in part, from the standard NCA inference key (Templeton *et al*. 1995; Templeton 1998) and are indicated in Fig. 5. Inferences are also provided at two nesting levels (Fig. 5, Clades 2.4 and 4.1) where we find informative patterns, yet a strict interpretation of the inference key provides an inconclusive or biologically inconsistent result.

Discussion

The integration of phylogeographical patterns of codistributed species with historical biogeography, geology, and climatology has provided a powerful tool in the resolution of regional biogeographical hypotheses (Hewitt 1999; Avise 2000). The complex geological and environmental history of the northern Rocky Mountains

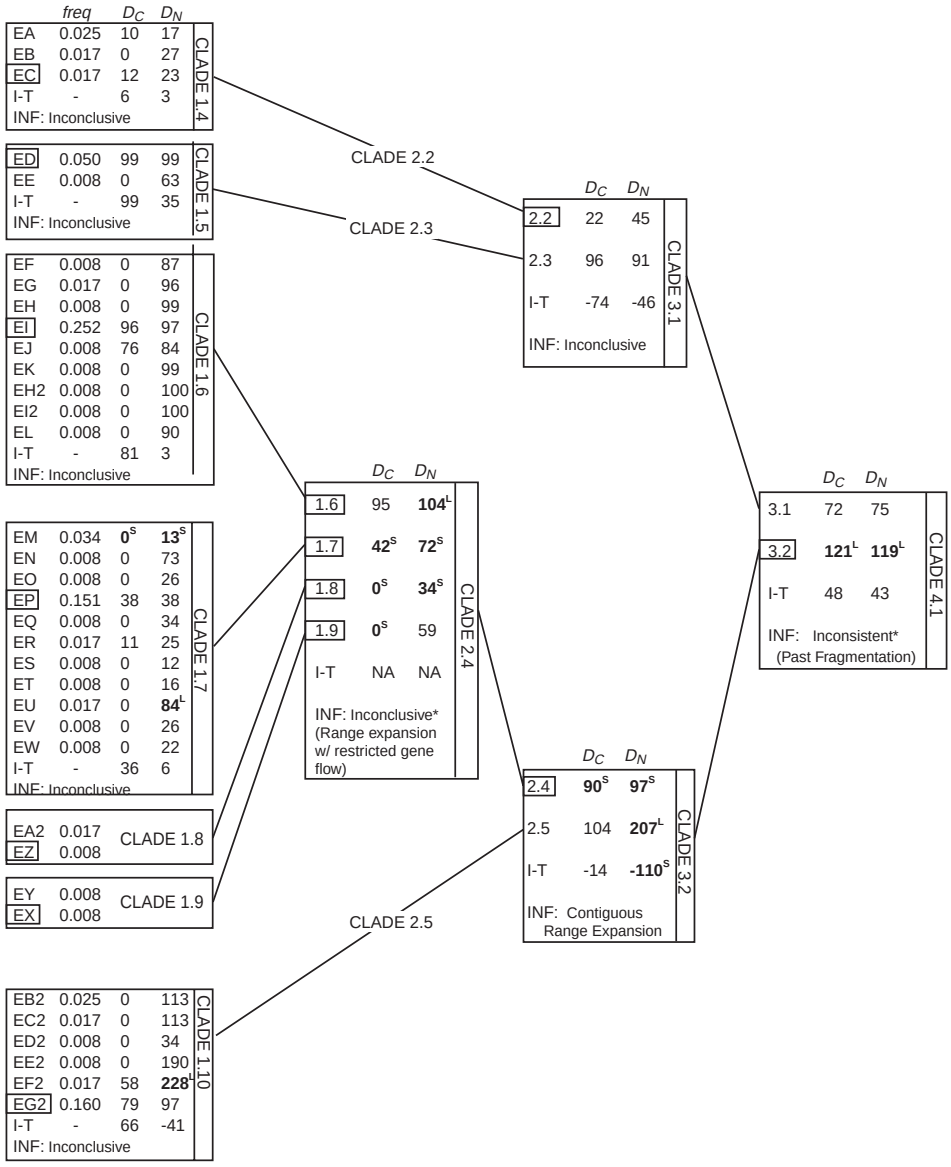

Fig. 5 Results of the nested clade analysis of the Eastern clade (clade 4.1, Fig. 3). Nesting hierarchy proceeds from left to right, initiating with the one-step nesting of individual haplotypes and cumulating with the all-inclusive clade 4.1. A box designates each nesting level; within each box, columns indicate haplotype/clade membership and corresponding frequency (*freq*), clade distance (D_c), and nested clade distance (D_N) in kilometers. Private boxes indicate interior haplotypes/clades. If applicable, interior vs. tip contrasts (I–T) are given for both D_c and D_n. Both significantly small and large statistics are in bold with S or L superscripts, respectively. Inferences into historical population-level processes were derived from the standard NCA inference key (Templeton *et al*. 1995; Templeton 1998). Inferences are provided at two nesting levels (clades 2.4 and 4.1) where we find informative patterns yet a strict interpretation of the inference key provides an inconclusive or biologically inconsistent result.

provides a model system for the application of this approach; however, there are few examples of phylogeographical studies of regional endemics in the literature (Brunsfeld *et al*. 2001). The cyt *b* genealogy of *Tamias ruficaudus* allows new insight into the evolution and phylogeographical history of this taxon and provides a starting point for comparative phylogeography of northern Rocky Mountain endemics. These data provide further resolution of taxonomic hypotheses and are consistent with a complex history of vicariance, range fluctuation, and introgression associated with secondary contact.

Taxonomic considerations

Intraspecific variation in *T. ruficaudus* was first examined by Howell (1922, 1929) who assessed pelage colouration

and delineated two subspecies; *T. r. simulans* and *T. r. ruficaudus*. The subsequent demonstration that these two forms displayed distinct bacular morphology (White 1953), thought to be a polygenic character which varies continuously with gene flow (Patterson & Thaeler 1982), supported the hypothesis that *T. ruficaudus* is a composite of two reproductively isolated species. This treatment was bolstered by Patterson & Heaney (1987), who found no indication of intermediate bacula in comparisons of 10 *T. r. simulans* specimens from Clearwater County (42 km NE Pierce, generally corresponding to our Population 9) with 14 *T. r. ruficaudus* specimens from adjacent Idaho County (Populations 1 and 2, Fig. 1B and Appendix I). Our data support the existence of two genetically and morphologically differentiated entities, yet refutes the hypothesis of complete reproductive isolation in the Clearwater Drainage. The phylogeny is characterized by a bifurcation into two clades with high nodal support (Fig. 3) and the geographical distribution of the haplotypes comprising these clades are generally concordant with the proposed subspecific distributions (Fig. 1B). All of the samples we examined from Idaho County (nine localities, 37 individuals, including 10 specimens examined by Patterson & Heaney 1987; Populations 1 and 2, Fig. 1B and Appendix I) have *T. r. ruficaudus* haplotypes, concordant with bacular variation (Patterson & Heaney 1987). Extensive sampling in Clearwater County (seven localities, 34 individuals; Fig. 1B and Appendix I) revealed contact between the two forms, however, the distribution of *T. r. ruficaudus* haplotypes extends a considerable distance north and east of the reported *T. r. simulans* bacular locality (i.e. 42 km NE Pierce, Patterson & Heaney 1987). In addition, *T. r. ruficaudus* haplotypes have the highest frequency in this general area (five localities, 25 individuals, 80% *T. r. ruficaudus* haplotypes; Appendix I). Furthermore, we have sampled multiple males with *T. r. ruficaudus* haplotypes that appear to retain characteristic *T. r. simulans* bacular morphology, suggesting extensive differential character introgression. We are currently conducting detailed morphometric analyses to assess the extent of hybridization and the nature of bacular variation in this region. Given our current data, we recommend the continued use of subspecific designations to recognize the intraspecific variation within *T. ruficaudus*. To avoid confusion in regions of character discord we use genetic designations (i.e. named haplotypes and clades in Fig. 4) to represent the intraspecific variation for the following phylogeography discussion.

Phylogeography

During the Pleistocene, the Cordilleran ice sheet expanded south through the Rocky Mountain trenches of eastern Washington, northern Idaho, and western Montana (Richmond *et al*. 1965; Waitt & Thorson 1983), occupying the northern third of the current distribution of *T. ruficaudus*. In addition, alpine glaciation extended across much of the Continental Divide and the Bittterroot Range (Delcourt & Delcourt 1993), while glacial dams created low elevation lakes, including expansive Lake Missoula of the Flathead Basin (Allen *et al*. 1986). Although the vegetative composition south of the Cordilleran ice sheet during glacial maxima remains speculative, climatic fluctuations probably resulted in both elevational and latitudinal range shifts throughout the region (Daubenmire 1975). Pollen records indicate Late Wisconsin postglacial ice recession led to regional expansion of cedar-hemlock coniferous forests, reaching contemporary vegetation assemblage *c*. 3000 years BP (Mack *et al*. 1978a,b; Baker 1983; Mehringer 1985). Given this regional history, it is apparent a priori that the evolutionary history of *T. ruficaudus* is layered with repeated cyclic disruption of population structure and that the current distribution of *T. ruficaudus* represents a fairly recent northward range expansion. Our data allow us to infer more precisely the phylogeographical history of this taxon.

Phylogeographical patterns in *T. ruficaudus* are consistent with a complex history of multiple vicariance events associated with successive glacial advances across the northern Rocky Mountain landscape during Pleistocene glaciation. Although we could not reject a molecular clock for this data set, an overall lack of fossil data for this genus makes calibration of a *Tamias*-specific clock problematic. However, it is unlikely that the deepest divergence observed (*c*. 4.7% uncorrected sequence divergence) could be explained by the Late Pleistocene events that have been postulated as driving speciation in *Tamias* and other North American mammals (e.g. Hoffman 1981). The ML estimate of phylogeny and the minimum-spanning network splits the mtDNA phylogeny into three genetic subsets: a deep east–west partition generally concordant with the *T. r. ruficaudus*; *T. r. simulans* taxonomic split; and a subsequent partition in the Western clade (Figs 3 and 4). NCA of the Eastern clade provides inference into population history structured by more recent processes (clade 4.1, Fig. 4), suggesting a complex pattern of northward range expansion coupled with past fragmentation and secondary contact. The majority of haplotype diversity and geographical spread found in the Eastern clade is represented by clade 3.2. Coalescent theory predicts that, in a given sample, ancient alleles will be in the highest frequency (Watterson 1976; Donnelly & Tavaré 1986) and be located in the interior (multiple connections) rather than at a tip (one connection) of a minimum-spanning network (Crandall & Templeton 1993). Haplotype EI of clade 1.6 fits these predictions (Figs 4 and 5) and is inferred to be the oldest haplotype of the Eastern clade. Analyses indicate northward postglacial range expansion of haplotypes from this

southern ancestral assemblage, presumably through the coniferous forests of the Bitterroot Range (predominately clade 1.7) and the Continental Divide (clade 1.10). Genetic structure typical of contiguous range expansion (Templeton *et al*. 1995) is most evident in the NCA of clade 3.2 (Fig. 5). Under this scenario we would predict a similar pattern within clade 2.4, however, homoplasy in the minimum-spanning network prevents us from unambiguously defining the nesting pattern and thus inhibits objective statistical inference at this level. Interestingly, when the network is rooted with clade 1.6 as interior and clades 1.7–1.9 as tips, the observed patterns within clade 2.4 deviate from those predicted by a general model of contiguous range expansion. Under this rooting scheme, the more ancient clade 1.6 is displaced from the geographical centre, while the remaining clades are composed of younger haplotypes and are significantly restricted in range, suggesting restricted gene flow with isolation by distance. A number of factors could be contributing to this result, including bias in our data collection introduced by intense sampling of the southern bacular contact zone. We believe this highlights the importance of understanding the relationship between sampling design and statistical inference with this method. Although fairly robust to sampling deviation, the reliance of NCA on frequency-weighted distance statistics suggests that failure to recognize extreme nonuniformity in sampling may result in misleading inferences. In addition, biological factors such as hybrid zone dynamics and corresponding dispersal consequences (e.g. Hewitt 1993, 1996) could affect the distribution of haplotypes through time and space, contributing to restricted gene flow and geographical restriction of clades 1.7–1.9. Clearly, understanding the evolutionary dynamics of this region will require a far more rigorous assessment of character distribution and gene flow than can be afforded by a single genetic marker.

In addition to the general pattern of northern range expansion from a southern assemblage, we find genetic evidence of additional population fragmentation, characterized by clade 3.1. Strict adherence to the standard NCA inference key suggests restricted gene flow with isolation-by-distance between clades 3.1 and 3.2, a biologically inconsistent result since 3.1 is almost completely geographically nested within the range of 3.2 (Fig. 1B,C). The geographical restriction and level of divergence of clade 3.1 from clade 3.2 is most consistent with a past population fragmentation (Templeton *et al*. 1995). Although the nesting of one subset within the other is a deviation from general patterns of past fragmentation where complete or mostly nonoverlapping distributions are expected (Templeton *et al*. 1995), it is certainly a feasible scenario given subsequent extensive range expansion. Several authors have suggested the Clearwater Drainage of northcentral Idaho formed an important refugium during Pleistocene glaciation (e.g. Daubenmire 1952). This hypothesis is based both on the topography of the drainage, which forms the first canyon south of glacial maxima deep enough to provide substantial thermal retention, and the existence of multiple, presumably relictual, plant and invertebrate endemics and coastal disjuncts (Daubenmire 1975; Johnson 1988). It is possible that the haplotypes of clade 3.1 represent a relictual Clearwater assemblage, although this inference is obscured by the expansion and secondary contact of both the Western clade and clade 3.2 of the Eastern clade in this region.

Little geographical structure exists in the Western clade; genetic diversity is dominated by one geographically widespread haplotype (WA), providing little resolution with NCA. Progressive allele loss culminating in genetic homogeneity is a process commonly associated with rapid leading edge dispersal from refugial populations, especially when population history includes repetitive cycles of restriction and expansion (Hewitt 1993, 1996; Ibrahin *et al*. 1996). Verification of this inference will require additional support from independent genetic loci. Although the lack of genetic structure in the Western clade inhibits historical population inference with NCA, it is apparent that post-glacial range expansion led to secondary contact with the Eastern clade in both the Clearwater Drainage (Fig. 1B and Appendix I; Populations 10, 19, and 26), the St. Joe Drainage (Population 24), and northwestern Montana (Population 25).

The Palouse clade is represented by 11 individuals from an isolated population located on a small timbered slope characterized by open xeric Ponderosa pine forest, *Pinus ponderosa*, surrounded by the Palouse prairie (Fig. 1B; Appendix I, Population 37). This locality lies just outside the previously described distribution of *T. ruficaudus* (Hall 1981). Two of the individuals from Population 37 are museum specimens (Connor Museum of Natural History, Washington State University; Appendix I) identified as a subspecies of the xeric forest species yellow-pine chipmunk, *T. amoenus canicaudus*. In addition, preliminary data suggest that these individuals are morphologically distinct from other *T. ruficaudus* examined (Good *et al*. unpublished data). We are currently investigating the geographical spread, ecological association, and interspecific association with *T. amoenus* of this assemblage.

Regional biogeography

Our sequence data present strong evidence for the multiple refugia hypothesis. Contemporary intraspecific structure within *T. ruficaudus* is dominated by an east–west partition, supporting population vicariance during episodic glaciation, possibly associated with the Bitterroot Divide. Initiation of this process almost certainly predates Late Pleistocene climatic events. Inference into recent population structure

also suggests additional fragmentation, possibly corresponding to river valley refugia. These findings support the temporal and spatial nesting of the general multiple refugia hypotheses identified by Brunsfeld *et al*. (2001). Other studies of northern Rocky Mountain taxa, although limited, also support a generalized multiple refugia biogeographical model. Mitochondrial sequence variation within *Ascaphus montanus*, the Rocky Mountain tailed frog, suggests a complex regional history of historic fragmentation associated with Pliocene and Pleistocene climatic events, including indirect evidence for regional restriction to river drainages (Nielson *et al*. 2001). S. J. Brunsfeld and J. Sullivan (unpublished data) have identified three geographically structured cpDNA lineages in Constance's bittercress (*Cardamine constancei*), an endemic herbaceous plant, suggesting recurrent isolation in separate river valley refugia. In contrast, allozyme variation in two species of conifers, western larch (*Larix occidentalis*; Fins & Seeb 1986) and western white pine (*Pinus monticola*; Steinhoff *et al*. 1983) show little regional differentiation, suggesting a recent colonization of the northern Rocky Mountains. Clearly the development of a multitaxon understanding of biogeography for the northern Rocky Mountains requires a synthesis of geological and climatological history and palaeo- and contemporary ecology, with phylogeographies of multiple elements of these communities. The accumulation of intraspecific genetic data of regional endemics, such as *T. ruficaudus*, in conjunction with the continued development of comparative analytical techniques, is vital to our understanding of the dynamic interaction between spatial genetic patterns and evolutionary processes in the northern Rocky Mountains.

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This paper represents a portion of Jeff Good's MS thesis conducted in the laboratory of Jack Sullivan. Good is interested in systematics, hybridization, and intraspecific and regional phylogeography. The Sullivan laboratory is interested in the synthesis of regional phylogeography through the comparison genetic patterns of codistributed taxa. Sullivan also conducts research in theoretical molecular systematics, especially in assessing the performance of heuristic strategies for model-based estimation of phylogeny from DNA sequence data.

Appendix I

Sampling localities for *Tamias ruficaudus*. Population numbers correspond to designations in Fig. 1(B). For each locality sample number and corresponding museum specimen-voucher numbers are given. Haplotype designations correspond to those used in Figs 3 and 4, bracketed values indicate frequency of redundant haplotypes

BRITISH COLUMBIA: **38.** Km 4.9 Gold Creek Road (+49.439, –117.284), *n* = 3 (RBCM19654-55, 20036), Haps [WA(2)]; **39.** Church Creek Road (+49.009, –117.440), *n* = 4 (RBCM19656, 66–68), Haps [WA, WI(3)]; **40.** Giveout Creek (+49.466, –117.336), *n* = 6 (RBCM19658-62, 20038), Haps [WA(6)]; **41.** Sage Creek Road (+49.104, –114.450), *n* = 3 (RBCM19664-65, 82), Haps [EG2(3)]; **42.** Akamina Creek (+49.017, –114.073), *n* = 1 (RBCM19683), Haps (EG2); **43.** Middlepass Creek, Rainy Ridge (+49.257, –114.402), *n* = 14 (RBCM, 19875, 80, 84, 85, 87, 19906, 07, 14–20), Haps [EB2(3), EC2(2), EG2(9)]; IDAHO: Boundary County, **22.** 17 mi N Sandpoint, Jeru Creek (+48.513, –116.583), *n* = 2 (UI, JMS203, JMG1), Haps (WH, WA); Benewah County, **35.** 10 mi N Potlatch, Base Mineral Mt. (+47.053, –116.869), *n* = 1 (UI, JMG2), Hap (WO); Clearwater County, **8.** 19 mi N Kelly Forks, Rawhide Creek (+46.912, –115.065), *n* = 3(UI, JMS144-46), Haps (EO, EP, EV); **9.** 1.4 mi E Kelly Forks, Junction Creek (+46.719, –115.233), *n* = 4 (UI, JMS147-50), Haps (EA, EC, ED, EP); **10.** 0.7 mi E Mouth Weitas Creek (+46.642, –115.424), *n* = 8 (UI, JMS151-58), Haps [EP(3), ER, ET, WA(3)]; **13.** 38 mi NE Pierce, Cayuse Landing (+46.668, –115.067), *n* = 5 (UI, JMS166-70), Haps [EA, EM(4)]; **19.** 4 mi N Elk River (+46.823, –116.177), *n* = 5 (UI, JMS191-95), Haps [EN, WF, WA(3)]; **26.** 9 mi NW Kelly Forks, Mush Saddle (+46.753, –115.369), *n* = 5 (UI, JMS238-42), Haps [ED, EP, ES, WA(2)]; **27.** 4.5 mi E Teakean, Freeman Creek (+46.567, –116.267), *n* = 4 (UI, JMS257-60), Haps [WB(4)]; Idaho County, **1.** 13 mi S. Confluence Lochsa & Selway Rivers (+45.968, –115.593), *n* = 7 (FMNH, 126113–15, 20, 22–24), Haps [EH, EI(5), EK]; **2.** Lolo Pass (+46.635, –114.578) *n* = 3 (FMNH, 126126–28) Haps [EB(2), EF2]; **11.** 18 mi E Pierce, Rocky Ridge Lake (+46.439, –115.489) *n* = 5 (UI-JMS159-63), Haps (EP, EQ, ER, EX, EY); **12.** 30 mi NE Lowell, Eagle Mt. Pack Bridge (+46.430, –115.129) *n* = 5 (UI-JMS164-65, 252–54), Haps [EI(3), EP, EL]; **14.** 40 mi E Pierce, Indian Post Office (+46.540, –114.989), *n* = 5 (UI, JMS171-75), Haps [EA, EE, EZ, EA2(2)]; **15.** 30 mi E Pierce, Twelve-Mile Saddle (+46.510, –115.154), *n* = 5 (UI, JMS176-80), Haps [EC, ED, EP(2), EW]; **16.** 11 mi NE Lowell, Split Creek (+46.231, –115.412), *n* = 4 (UI, JMS181-82, 255–56), Haps [EI(2), EP(2)]; **17.** 41 mi NE Lowell, Warm Springs (+46.461, –115.018), *n* = 4 (UI, JMS183-86), Haps [EF, EI, EP(2)]; **28.** 10.5 mi SE Grangeville, Cougar Mt. (+45.835, –115.8353), *n* = 3 (UI, JRD18, 20, 22), Haps [EI(3)]; Kootenai County, **21.** 12.5 mi N Enaville (+47.708, –116.377), *n* = 3 (UI, JMS200-02), Haps [WG, WA(2)]; Latah County, **7.** 8 mi NE Moscow, Moscow Mt. (+46.808, –116.900), *n* = 11 (UI, JMS122-31, 33), Haps [WA(9), WC, WE]; **18.** 4 mi SE Moscow, Paradize Ridge (+46.690, –116.971), *n* = 2 (UI, JMS189-90), Haps [ED(2)]; **36.** 6 mi E Moscow, Seversen, Wallen Road (+46.723, –116.909), *n* = 1 (UI, JMG3), Hap (WA); Shoshone Coounty, **24.** 8.2 mi E Avery (+47.225, –115.606), *n* = 5 (UI, JMS211-15), Haps [EP(3), WA(2)]; MONTANA: Beaverhead County, **30.** 18 km NW Dillon, Saddleback Mt., Birch Creek (+45.417, –112.900), *n* = 2 (UI, JRD53-54), Haps [EI(2)]; **31.** 3 km NE Lost Trail Pass (+45.700, –113.850), *n* = 4 (UI, JRD55-5), Haps [EI(2), EJ, EF2]; **32.** Bloody Dick Creek (+45.150, –113.50), *n* = 5 (UI, JRD???), Haps [EI(3), EH2, EI2]; **33.** Lower Miner Lake (+45.317, –113.583), *n* = 1 (UI, JRD144), Hap (EI); **34.** 4 mi NW Comet Mt, FR 484 (+44.450, –113.0836), *n* = 6 (UI, JRD145, JMG12-16), Haps [EI(6)]; Flathead County, **25.** 5 mi NNE Whitefish (+48.506, –114.248) *n* = 8 (UI, JMS227-34), Haps [EG2(3), WA(5)]; **29.** Essex, along HWY 2 (+48.283, –113.605), *n* = 1 (UI, JRD26), Haps (ED2); Granite County, **33.** Slide Rock Mt. (+46.586, –113.554), *n* = 4 (FMNH, 126129–31), Haps [EE2, EG2(3)]; Lincoln County, **20.** 4 mi S Libby, Flower Creek (+48.343, –115.601), *n* = 2 (UI, JMS216, 18), Haps [WA(2)]; Missoula County, **4.** 5 mi S Lolo (+46.686, –114.077), *n* = 4 (FMNH126142-45), Haps [EI, EP, EG(2)]; Ravalli County, **5.** Bass Creek Campground (+46.576, –114.138), *n* = 2 (FMNH126146-47), Haps [EU(2)]; **6.** 4 mi W Florence (+46.620, –114.150), *n* = 2 (FMNH126148-49), Haps (ED, EI); WASHINGTON: Pend Orielle County, **23.** 3.7 mi NE Sullivan Lake (+48.850, –117.181), *n* = 4 (UI, JMS205-06, 08–09), Haps [WA(4)]; Whitman County, **37.** 5 mi N Albion, Smoot Hill Ecological Reserve, Washington State University (+46.820, –117.210), *n* = 11 (UI, JMG17-26, CMNH971301, 03), Haps [PA(11)]. OUTGROUPS; *Tamias amoenus*, Washington, Pend Orielle County, *n* = 1 (UI, JMS207); *Tamias minimus*, British Columbia (RBCM19565); *Tamias senex*, Oregon, Deschutes Co., *n* = 1 (UI, JRD75); *Tamias townsendii*, Washington, Pierce Co., *n* = 1 (BMNHC, SNV125).

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