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RESOLVING THE WESTERN CHIPMUNK PHYLOGENY

By

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Undergraduate Thesis presented in partial fulfillment of the requirements  
for the University Scholar distinction

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## **ABSTRACT**

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Resolving the Western Chipmunk Phylogeny

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Speciation is the biological process by which new species arise. Hybridization occurs in nature when two distinct lineages produce hybrid offspring and exchange genes. Understanding these events is key to understanding the process of evolution and the origin of biodiversity. Western chipmunks are an example of a widely distributed group with possible hybridization and gene flow between presently diverging species. Past studies examining this system suggest that there has been some hybridization and gene flow during the recent, rapid radiation of western chipmunk species. However, the overall importance and frequency of hybridization between chipmunk species remains unclear. Previously, the evolutionary relationships within the chipmunk group have been reconstructed using sequences from mitochondrial DNA and four nuclear genes. The full resolution of the chipmunk phylogeny rests on additional sequencing of DNA to provide a more complete picture of the relationships between the species, and the frequency and extent of hybridization. This project begins to address this by generating genome-wide sequencing data using samples from 40 chipmunks of 15 species and various localities. DNA extracts from each sample were prepared for next-generation genetic sequencing. A custom exon capture experiment was then used to target nine million base pairs of the chipmunk genome for Illumina sequencing. These sequencing efforts generated data spanning thousands of genes in all 40 samples, which was used to construct an overall phylogeny for the group. These data provide the foundation for ongoing studies to resolve the chipmunk phylogeny and the history for hybridization in this system.

## Resolving the Western Chipmunk Phylogeny

### **Introduction**

Western chipmunks are one of the most diverse groups of small mammals found in forest ecosystems across western North America. The 23 described species of western chipmunks are widely distributed in a variety of habitats (Hall, 1981), and in some regions up to four species may co-occur. One of the intriguing ecological features of chipmunks is the strong niche partitioning observed among broadly co-distributed species, typically resulting in altitudinal zonation and narrow zones of contact between some species (Heller, 1970). These narrow areas of overlap provide the potential for hybridization and gene flow between species. Classic models of speciation involve the isolation of diverging lineages and exclude the concept of gene flow between the diverging lineages (Mayr, 1942). However in sympatric populations (e.g. Western chipmunks), it is important to understand how gene flow impacts their evolutionary relationships and the process of divergence. This type of divergence with gene flow (DGF) has recently been further researched and modeled to illustrate the importance of this type of divergence to biological diversity in numerous systems (Pinho and Hey, 2010).

Past studies suggest that there has been some hybridization and gene flow during the recent, rapid radiation of Western chipmunk species. Gene flow and introgression are observed in mitochondrial data, but four nuclear loci show little evidence of gene flow. Additionally, introgression is seen between some species, while complete reproductive isolation is observed between others in this group (Good et al., 2003, Reid et al., 2010). To better estimate the evolutionary relationships in this group, introns from nuclear reproductive protein genes were sequenced due to the expectation that they resist introgression. These results yielded some resolution, but also showed nonmonophyletic relationships among closely distributed taxa, indicating that whole-genome data will be required to

uncover the extent of hybridization and fully resolve the evolutionary relationships in this group (Reid et al.,2012).

## **Methods**

### Sample Preparation:

First, next-generation sequencing libraries were created using previously extracted DNA from 40 chipmunk individuals representing 15 of the 23 described species. This was completed as described in the Meyer and Kircher protocol (2010). A transcriptome-based exon capture was designed as described in Bi et al. (2012), and the exon capture was completed after the library preparation also as specified in the Meyer and Kircher protocol. Next-generation sequencing of approximately nine million base pairs of DNA was then completed on an Illumina MiSeq at the University of Montana Genomics Core.

### Data Analysis:

Single nucleotide polymorphisms across the targeted exome were called using GATK (McKenna et al., 2010), and all gene sequences were concatenated. Following a GTR+Gamma model, an approximate-likelihood tree was constructed with these data using RAxML (Stamatakis, 2014).

## Results

	Mean	Median	Minimum	Maximum
Sequenced Reads	549,441	488,692	82,802	1,769,058
Mapped Reads	467,121	384,788	67,764	1,656,166

Table 1. Summary of the sequencing results. The average number of bases sequenced per read was 549,000, and 467,000 of those mapped to the reference genome. The minimum and maximum read length values show a very large spread, ranging from 82,000 to 1.8 million.

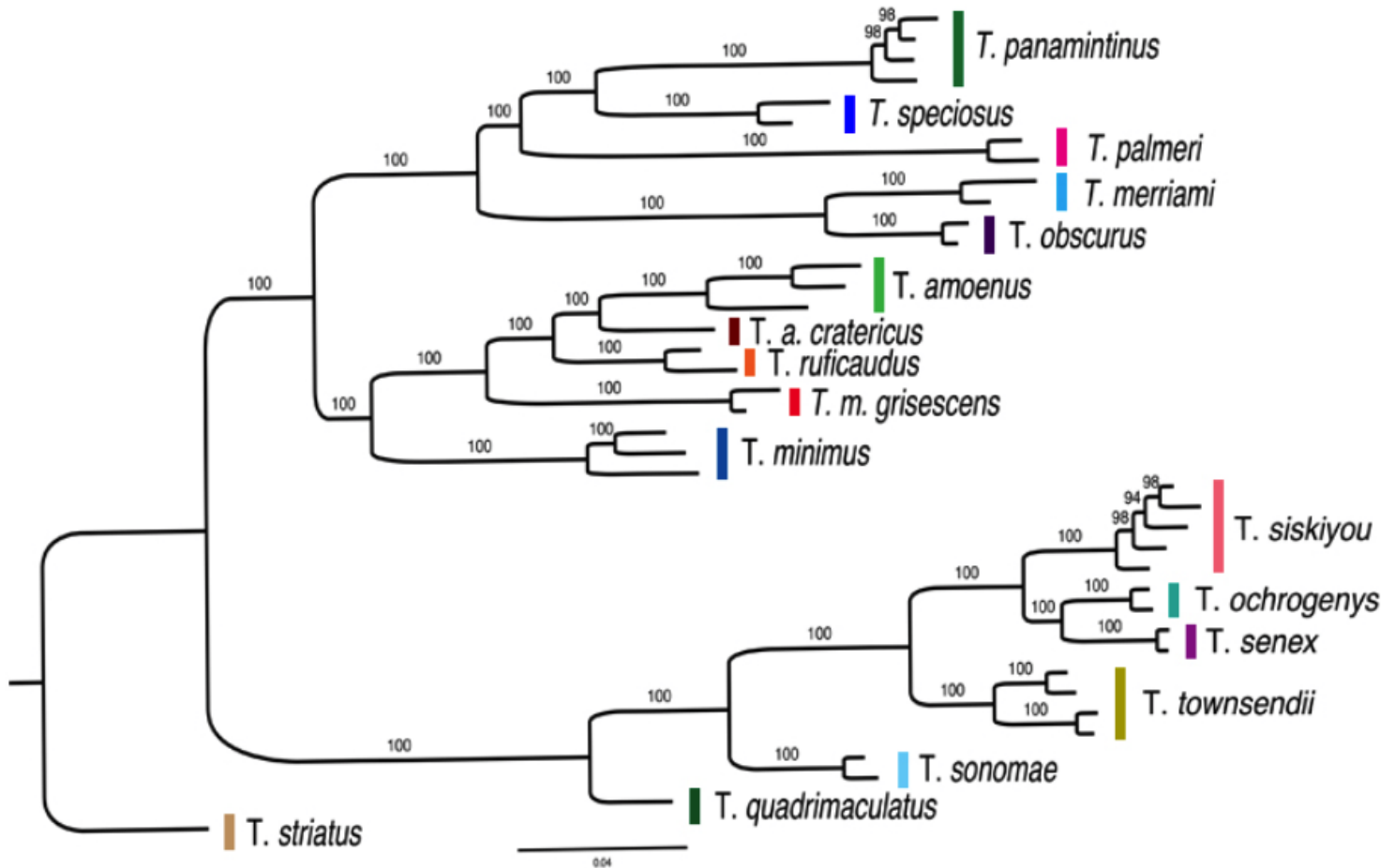


Figure 1. Resulting overall phylogeny created from the data.

## Discussion

As seen in figure 1, the overall phylogeny depicts monophyletic relationships among nearly all the species of this group. This pattern suggests that interbreeding and gene flow are not rampant between the species. Had gene flow been largely at play, a lack of bifurcations would have been observed in the tree. Although previous studies provide evidence for hybridization between some chipmunk species, this phylogeny presents evidence that hybridization does not greatly impact the evolutionary relationships of this group as a whole.

One previous hypothesis states that *T. a. cratericus*, a described subspecies of *T. amoenus*, is in fact a distinct lineage (Demboski and Sullivan, 2003). The phylogeny created in this study supports that hypothesis, as *T. a. cratericus* clearly branches separately from *T. amoenus*. Another notable finding of this tree occurs in the *T. minimus* clade. This species' branching pattern is paraphyletic due to the split of *T. m. grisescens* from the rest of the *T. minimus* individuals. This indicates that *T. minimus* is in fact diverged into multiple distinct lineages, which is not surprising due to the vast geographical range of this species (Sullivan et al, 2012).

To solidify the findings of this study and complete the resolution of the chipmunk phylogeny, deeper sequencing with additional taxa will be done. This additional data will allow for further phylogenetic analyses at the gene level in order to determine the history of hybridization in this system.

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