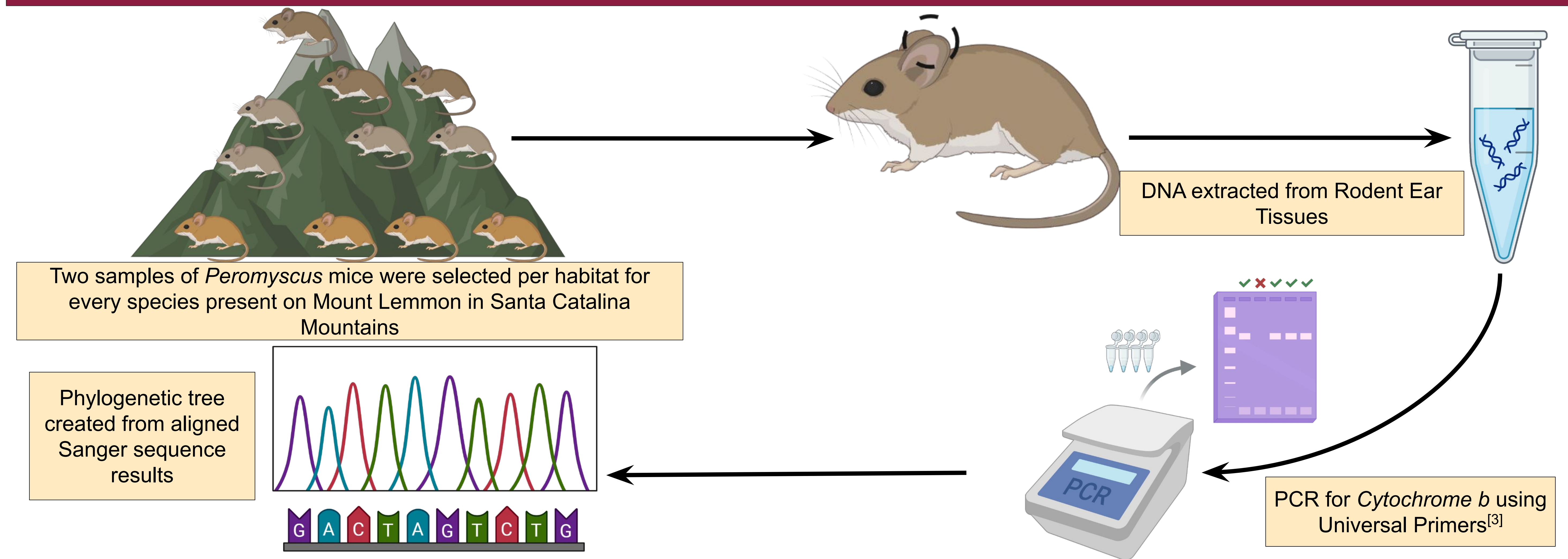


Background

The Santa Catalina mountains of the Madrean Sky Islands in Arizona provide an ideal environment for studying the effects of geographic isolation on the evolution of animals due to the gradual separation and isolation. Particularly, the *Peromyscus* genus (Deer Mouse) occupy diverse habitats across a sharp elevational gradient. To properly understand the evolutionary implications of these geographical changes, we need to identify closely related mice species, a difficult process when relying on morphological characteristics alone. One method of identifying different species is through the amplification of cytochrome *b*, a mitochondrial gene^[1]. Due to its presence in all tissue samples and species-specific mutation sites within the gene sequence, cytochrome *b* provides a simpler method of identification of *Peromyscus* species^[2].

This project aims to characterize *P. eremicus*, *P. boylii*, and *P. melanotis* via cytochrome *b* gene amplification to understand the diversification and ecology of the *Peromyscus* species present within Mount Lemmon.

Methods



Results

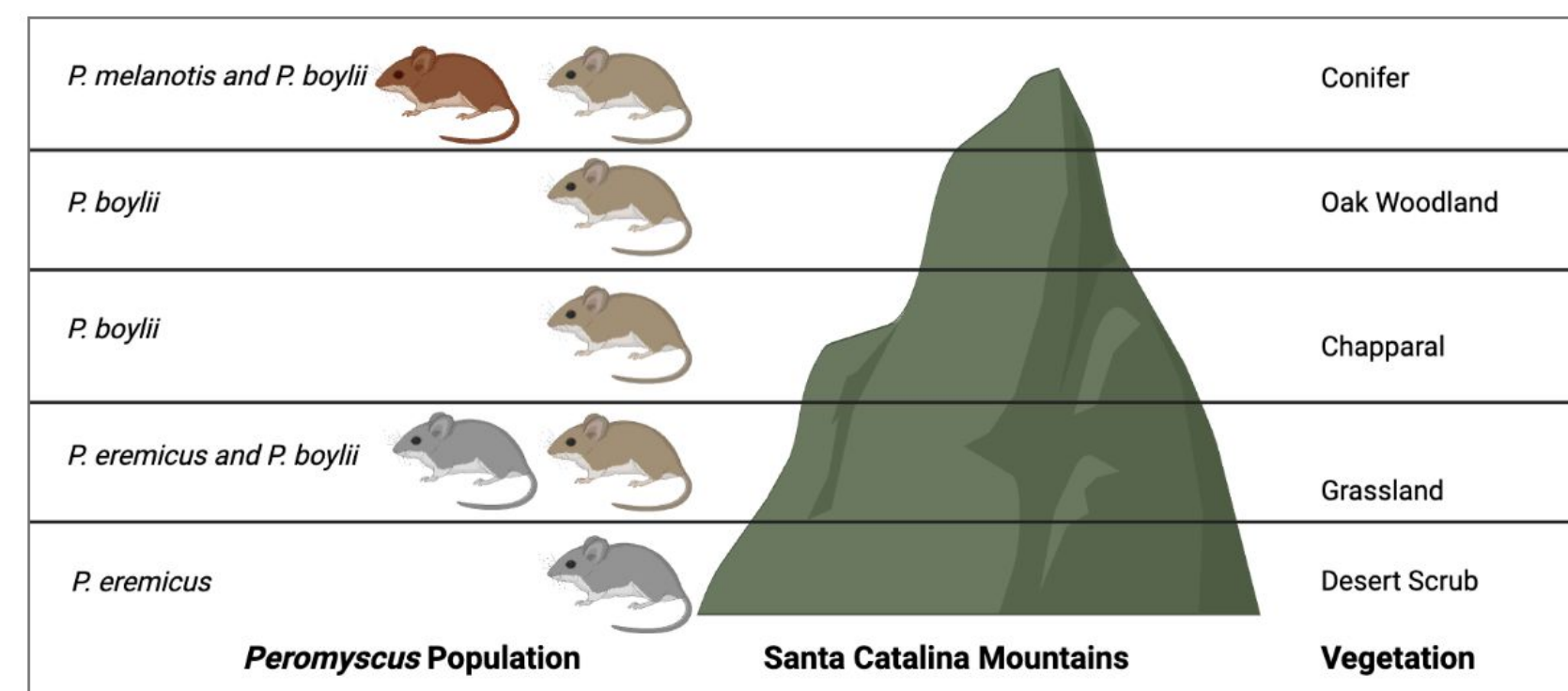


Figure 1. *Peromyscus* population and vegetation across Mount Lemmon of Santa Catalina Mountains.

Habitat Cluster	Sample ID	Extraction DNA Conc. (ng/μL)	Amplification DNA Conc. (ng/μL)
SC 1	NSU0131	30.3	26.9
SC 5	DMR0222	169.1	73.2
SC 7	NSU0167	106.2	47.6
SC 8	GSR007	73.1	50
SC 8	GSR011	39.9	28.8
SC 9	SUZ044	45.3	42.3
SC 10	SUZ042	37.1	24.6
SC 10	SUZ046	98.9	33.7

Table 1. Samples used, corresponding habitat clusters, and DNA yield before and after PCR amplification.

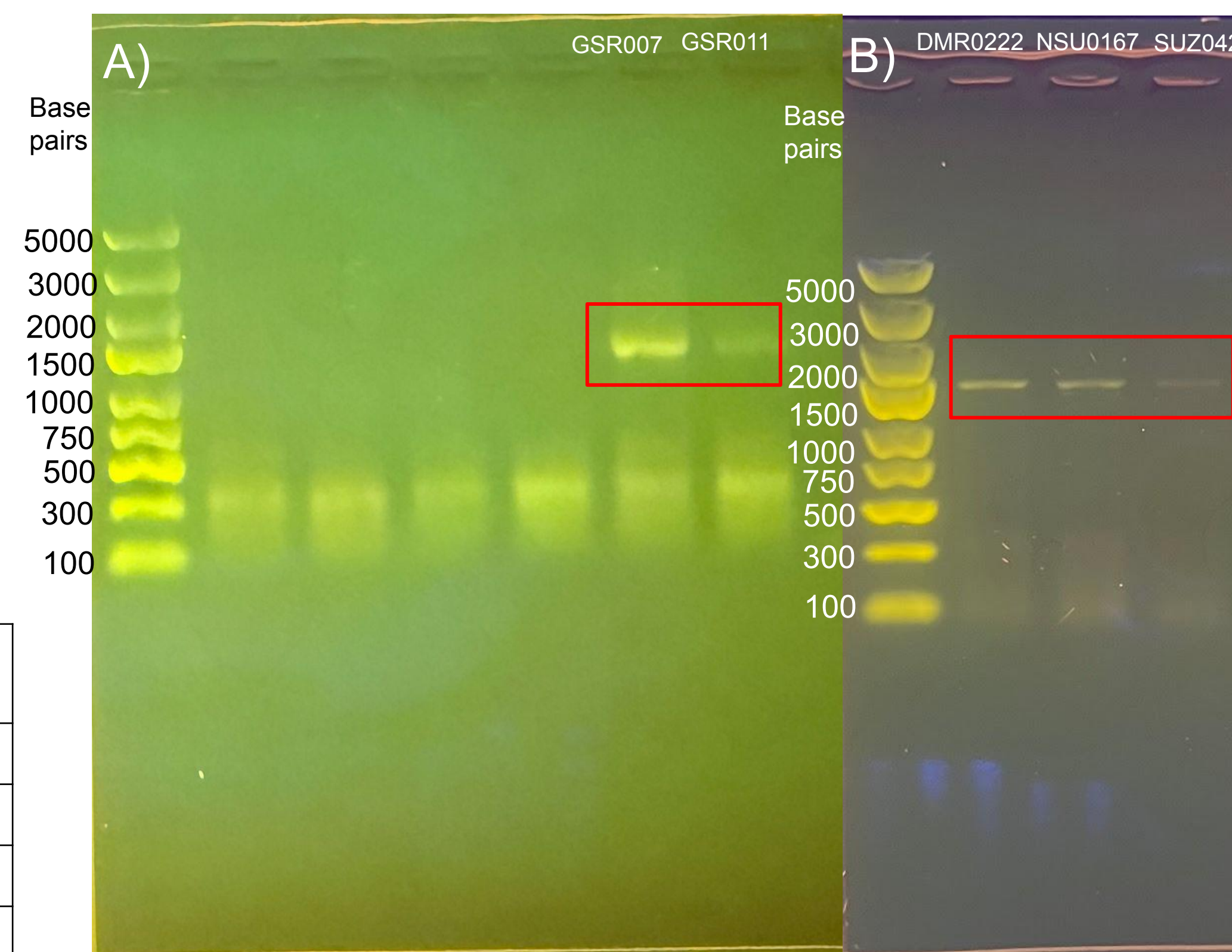


Figure 3. Gel electrophoresis results of A) the successful DNA extraction and PCR amplification of cytochrome *b* gene in samples GSR007 and GSR011 of SC 8 habitat cluster, and B) PCR purification of cytochrome *b* gene in samples DMR0222, NSU0167, and SUZ042.

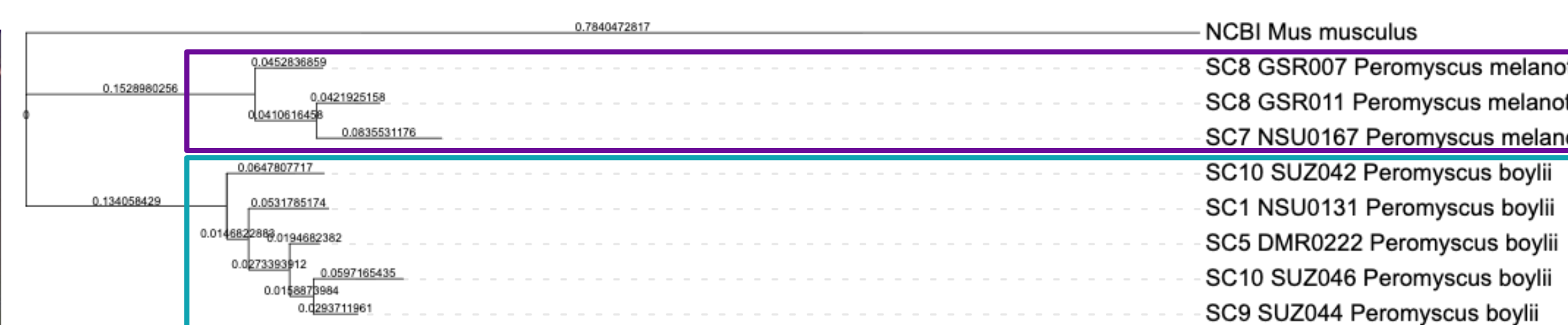


Figure 4. Phylogenetic tree created from aligned Sanger sequencing results. *Mus musculus* (house mouse) cytochrome *b* sequence was used as an outgroup to compare species sequences. Numbers on branches represents branch length. *P. melanotis* mice are highlighted in purple and *P. boylii* mice are highlighted in cyan.

Key Highlights

- Cytochrome *b* gene sequence was consistent with prior species identification based on morphology
- Individuals from the same location did not share the same genetic information → Alignment was closer to individuals from other habitat clusters
- Peromyscus boylii* is present at lower, middle and higher elevations which are abundant in desert and coniferous ecology containing cacti, seeds, shrubs, pines, oakwood, and conifers
- Peromyscus melanotis* is present at higher elevations which are abundant in coniferous forest ecology containing Pine trees and Conifers

Future Directions

- Incorporate cytochrome *b* sequences from public databases of *P. melanotis* and *P. boylii* into current phylogenetic tree
- Use multiple nuclear DNA markers to further confirm species identity
- Continue characterizing mice from both *Peromyscus* (Deer Mouse) and *Chaetodipus* (Pocket Mouse) genera to understand evolutionary relationships
 - Further understanding of genetic changes within each habitat cluster using a larger data set
 - Study the rodent phylogenies between Sky Islands in Arizona
- Evolutionary information from phylogenetic trees to guide research on host-specific gut microbial changes due to geographic isolation
 - Extract DNA from fecal samples and analyze gut microbes present to guide understanding
 - Observe similarities/differences in gut microbial communities present by habitat cluster

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References:

- A. Linacre and J. C.-I. Lee, "Species Determination: The Role and Use of the Cytochrome *b* Gene," *Methods in Molecular Biology*, pp. 287–296, 2016, doi: https://doi.org/10.1007/978-1-4939-3597-0_20.
- M. R. Farag et al., "Forensic Applications Of Mitochondrial Cytochrome B Gene In The Identification Of Domestic And Wild Animal Species," *Journal of Experimental Biology and Agricultural Sciences*, vol. 8, no. 1, pp. 1–8, Feb. 2020, doi: [https://doi.org/10.18006/2020.8\(1\).1.8](https://doi.org/10.18006/2020.8(1).1.8).
- A. Naidu, R. R. Fitak, A. Munguia-Vega, and M. Culver, "Novel primers for complete mitochondrial cytochrome *b* gene sequencing in mammals," *Molecular Ecology Resources*, vol. 12, no. 2, pp. 191–196, 2012, doi: [10.1111/j.1755-0998.2011.03078.x](https://doi.org/10.1111/j.1755-0998.2011.03078.x).

