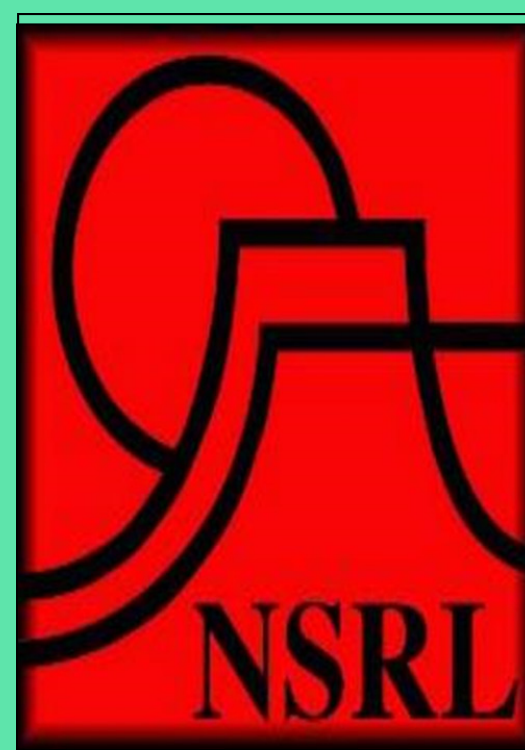
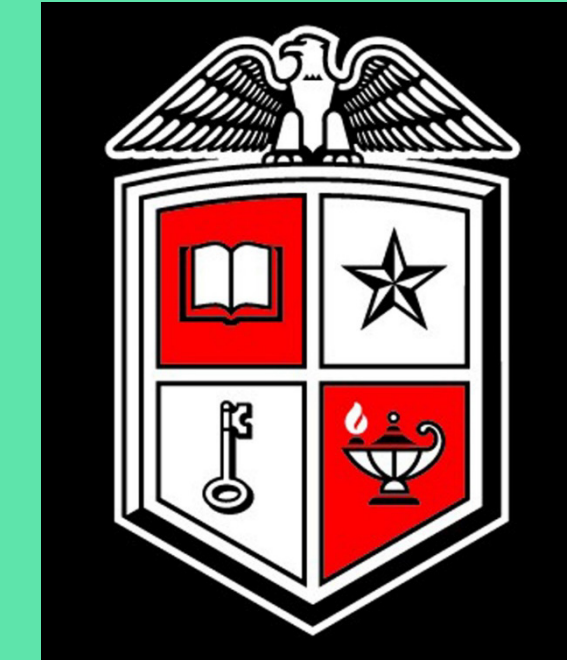




# Zonadhesin (*ZAN*): A Speciation Gene for Identifying Species of *Peromyscus*



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## Introduction

The Zonadhesin gene (*ZAN*) is of particular interest due to its involvement as a sperm-egg fusion protein (Roberts et al. 2022, 2023), leading to the hypothesis that it may have evolved in parallel with species divergences, potentially playing a crucial role in ensuring species boundary and gene pool integrity. If this hypothesis is true, the identification of a “speciation gene” would prove to be of an enormous value to mammalian systematics and studies of biodiversity where taxon specific markers are often used to confirm or reject speciation events.

To test this hypothesis, we sequenced a portion of the *ZAN* gene in three species in the genus *Peromyscus* (*P. leucopus*, *P. maniculatus*, and *P. attwateri*). These data were compared to sequences of the cytochrome b gene, a mitochondrial marker commonly used to evaluate speciation, in the same four species. These species were selected given that they are species representing two cases of closely related species (*leucopus* and *maniculatus*, and *attwateri*) as well as distantly related taxa (*leucopus* to *attwateri* and *truei*; *maniculatus* to *attwateri* and *truei*). These relationships provide the opportunity to examine molecular divergence under the scenarios of recent versus ancient diversification events.

## Objectives

Within *Peromyscus*, this gene is approximately 104,650 bases long, divided into 4 von Willibrand domains (VWD), which likely refers to a specific structural domain within the gene's protein product. The VWD3 domain contains a series of duplicated TIL + E cassette exons referred to as partials. Given the large size of *ZAN*, we developed primers to capture intron and exon sequences representing the gene region D3p24 TIL and D3p24 E.

Sequences were analyzed using Bayesian Inference and other phylogenetic methods. Additionally, genetic divergences (Kimura 2-parameter, Kimura 1980) were estimated to distinguish rates of evolution among regions and taxa.

We also analyzed the cytochrome b gene which encodes a protein component of the mitochondrial electron transport chain. This gene is widely used in evolutionary biology and population genetics studies due to its relatively conserved nature across species and its utility in determining phylogenetic relationships (Bradley et al. 2006) and provides a benchmark of the genetic divergence of each species relative to the evolution *ZAN*.



*Peromyscus maniculatus*



*Peromyscus leucopus*



*Peromyscus attwateri*



*Peromyscus truei*

## Materials and Methods

- 1) Isolate DNA from muscle tissue sample provided by NSRL using the Qiagen DNeasy blood and tissue kit
- 2) Perform PCR on D3p24 (~606 bp) and sequencing of the following regions:
  - i) D3p24 TIL\_sense and D3p24 TIL\_Asense (annealing temperature is 53 °C)
  - ii) D3p24 E\_sense and D3p24 E\_Asense (annealing temperature is 50 °C)
  - iii) Cytiva PuReTaq PCR Beads
  - iv) Gel Electrophoresis
- 3) ExoSAP-IT and/or Qiagen QIAquick Gel Extraction Kit
- 4) Cycle Sequencing
- 5) Sephadex
- 6) Analysis using Sequencher

## Preliminary Results

We obtained ~700 bp from the region of *ZAN* corresponding to the D3p24 TIL partial.

Sequences presented in Figure 1 contain species of interest *P. leucopus* and *P. maniculatus* alongside closely and distantly related species within the rodent suborder Myomorpha.

Similarly, 1143 bp were obtained from the cytb gene and used to construct a phylogenetic tree depicted in Figure 2.

Additionally, genetic distances were estimated for both genes (*ZAN* and cytb) independently and are presented in tables 1 and 2, respectively.

For the Zonadhesin gene, genetic distances ranged from 1.03% (*P. leucopus* compared to *P. maniculatus*) to 2.92% (*P. leucopus* and *P. maniculatus* compared to *P. californicus*) (Table 1).

For the cytochrome b gene, genetic distances ranged from 10.4% (*P. maniculatus* compared to *P. leucopus*) to 16.2% (*P. truei* compared to *P. leucopus*) (Table 2).

Fig 1.

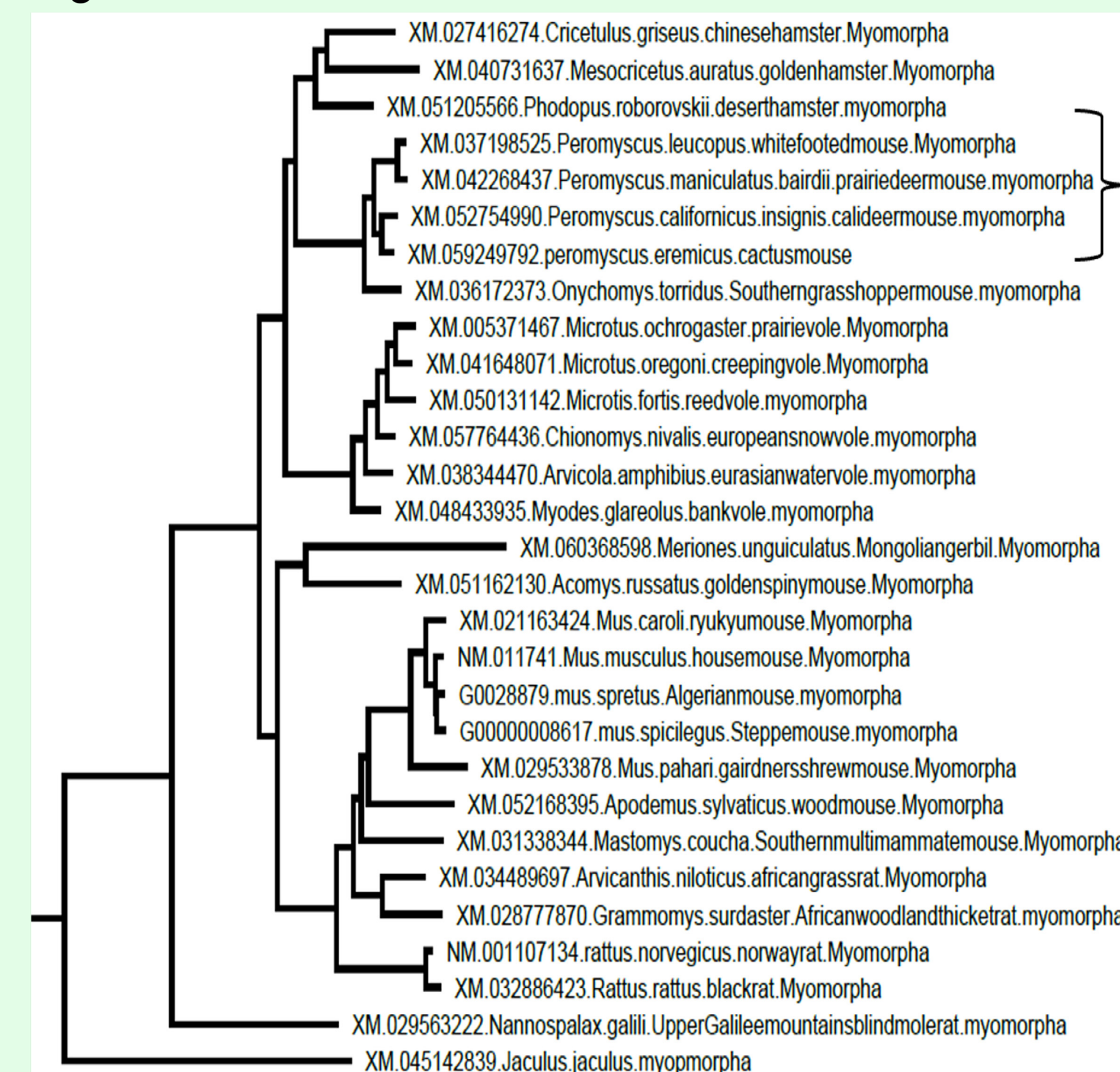


Fig 1. Phylogenetic tree obtained from the Kimura 2-parameter model of evolution for Zonadhesin (*ZAN*). Bracket indicates species group of interest.

Table 1.

Species	<i>P. leucopus</i>	<i>P. maniculatus</i>	<i>P. californicus</i>	<i>P. eremicus</i>
<i>P. leucopus</i>	-	1.03%	2.92%	2.82%
<i>P. maniculatus</i>	-	-	2.92%	2.80%
<i>P. californicus</i>	-	-	-	1.49%
<i>P. eremicus</i>	-	-	-	-

Table 1. Genetic distance matrix obtained from the Kimura 2-parameter model of evolution for Zonadhesin (*ZAN*).

Fig 2.

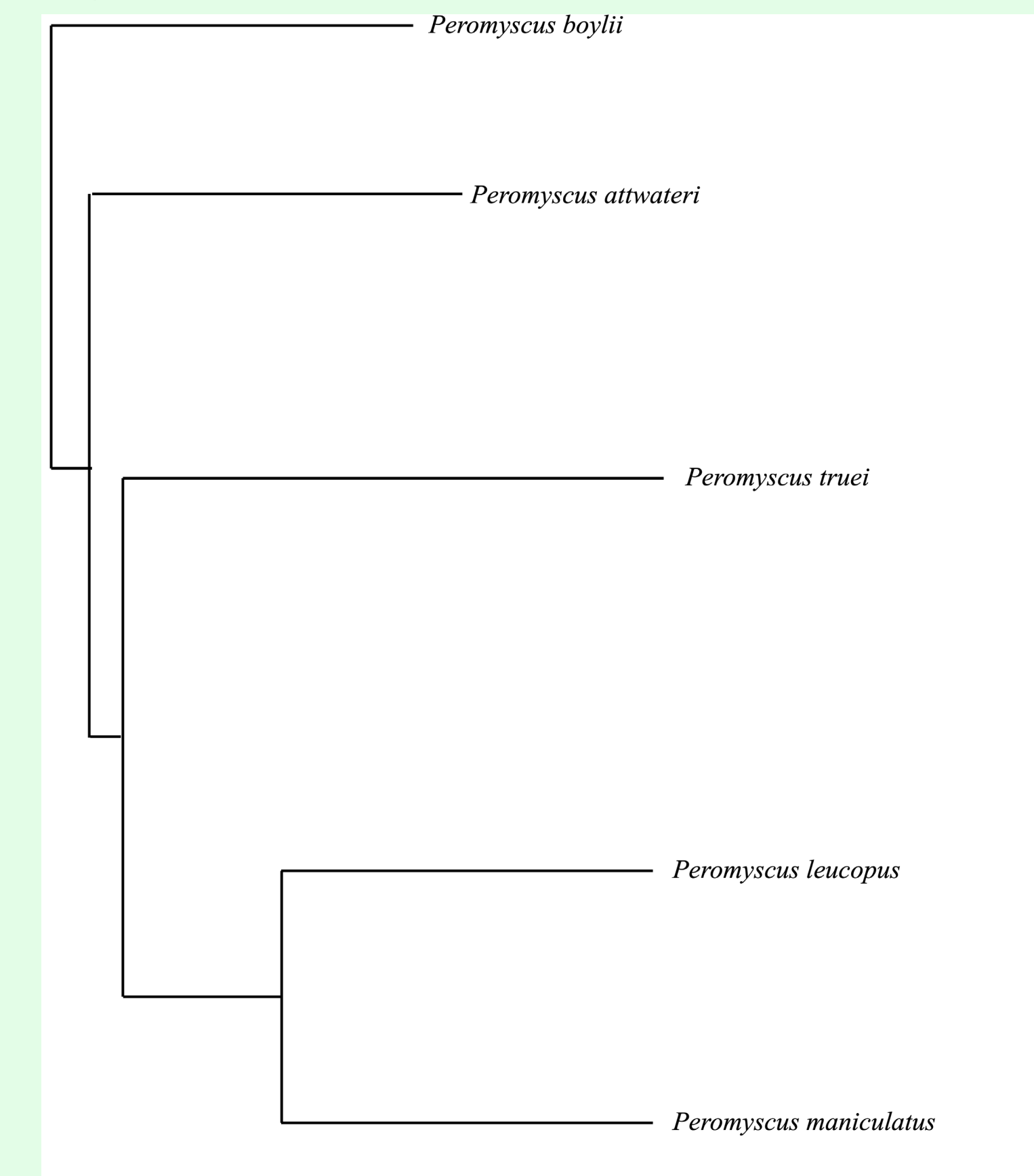


Fig 2. Phylogenetic tree obtained from the Kimura 2-parameter model of evolution for cytochrome b (cytb).

Table 2.

Species	<i>P. attwateri</i>	<i>P. truei</i>	<i>P. maniculatus</i>	<i>P. leucopus</i>
<i>P. attwateri</i>	-	14.1%	14.2%	13.8%
<i>P. truei</i>	-	-	15.3%	16.2%
<i>P. maniculatus</i>	-	-	-	10.4%
<i>P. leucopus</i>	-	-	-	-

Table 2. Genetic distance matrix obtained from the Kimura 2-parameter model of evolution for cytochrome b (cytb).

## Discussion

Our findings suggest *ZAN* shows significant sequence divergence between species, supporting the hypothesis of a potential role as a speciation gene. Moreover, the presence of species-specific markers within the gene further strengthens this hypothesis. Our study provides valuable insights into the evolutionary role of *ZAN* in mammals, highlighting its potential significance in maintaining species boundaries and gene pool integrity.

## Future Directions

- 1) Sequence additional *Peromyscus* species
- 2) Sequence additional genera within family Cricetidae (e.g. *Onychomys*, *Neotoma*, *Reithrodontomys*)
- 3) Sequence genera within family Heteromyidae (e.g. *Perognathus*, *Chaetodipus*, *Dipodomys*)
- 4) Expand dataset to include D3p24 E primer

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