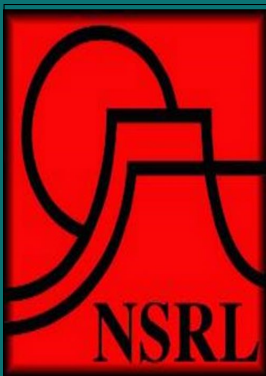


# Examination of Prion Disease in the Suborder Caniformia and Order Chiroptera Reveals a Genetic Basis for both Susceptibility and Resistance

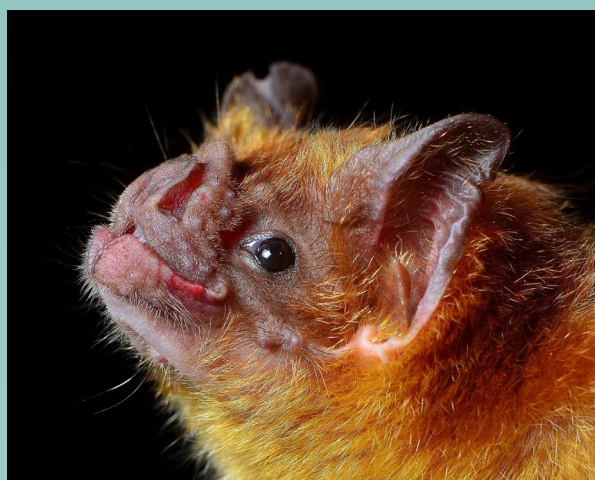


Vivienne A. Lacy<sup>1,2</sup>, Emily A. Wright<sup>2</sup>, Emma K. Roberts<sup>3</sup>, Daniel M. Hardy<sup>4</sup>, and Robert D. Bradley<sup>2,5</sup>

Pi Squared<sup>1</sup>, Department of Biological Sciences<sup>2</sup>, Climate Sciences Center<sup>3</sup>, Department of Cell Biology and Biochemistry<sup>4</sup>, Natural Science Research Laboratory<sup>5</sup>

## Introduction

- Spongiform encephalopathies (SE) are fatal neurodegenerative diseases caused by the alternative folding of the prion protein.
- There are three categories of SE's
  - familial, sporadic, and transmissible
- Investigations of protein misfolding cyclic amplification indicated that Canids and some Chiropterans display low susceptibility to prion diseases.
- Lowered susceptibility could be attributed to a specific nonsynonymous substitution (N191D/E) in exon 3 of the prion protein gene (*PRNP*).



*Myotis velifer*

*Desmodus rotundus*

*Urocyon cinereoargenteus*

## Objectives

1. Sequence entire prion protein (*PRNP*) exon 3 region of Chiropterans and Canids from different families.
2. Compare the distribution of the N1D/E191 substitution to determine if correlation between presence of substitution and susceptibility.
3. Find pattern of examined amino acid changes.

## 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 entire *PRNP* exon 3 (~771 bp) and sequencing of this region
  - i) *PRNP\_F* and *PRNP\_R*
  - ii) Annealing temperature is 54°C
  - iii) Cytiva PuReTaq PCR Beads
  - iv) Gel Electrophoresis
- 3) ExoSAP-IT
- 4) Cycle Sequencing
- 5) Sephadex
- 6) Analysis using Sequencher

## Preliminary Results Chiroptera

Myotis_davidii_chiro	F	G	N	E	Y	E	D	R	Y	Y	R	E	N	M	N	R	F	F	N	D	V	Y	Y	K	P	V
Myotis_lucifugus_chiro	F	G	N	E	Y	E	D	R	Y	Y	R	E	N	M	N	R	F	F	N	D	V	Y	Y	K	P	V
Eptesicus_fuscus_chiro	F	G	N	E	Y	E	D	R	Y	Y	R	E	N	M	N	R	F	F	N	D	V	Y	Y	K	P	V

Figure 1. Prion protein sequence data, focusing on the amino acid substitution asparagine to either aspartic acid or glutamic acid at codon 191. If the AA is "N" the taxon is susceptible, and if the AA is "D" or "E" the taxon is resistant.

Genus species	Sample Size	Diet	Amino acid	Family
<i>Eptesicus fuscus</i>	2	Insectivores	E	Vespertilionidae; NA
<i>Myotis davidii</i>	3	Omnivores	N	Vespertilionidae; China
<i>Myotis brandtii</i>	3	Insectivores	D	Vespertilionidae; Eurasia
<i>Myotis daubentonii</i>	2	Insectivores	N	Vespertilionidae; Eurasia
<i>Myotis lucifugus</i>	4	Insectivores	D	Vespertilionidae; US and Canada
<i>Myotis myotis</i>	5	Insectivores	N	Vespertilionidae
<i>Myotis velifer</i>	1	Insectivores	N	Vespertilionidae
<i>Parastrellus hesperus</i>	1	Insectivores	E	Vespertilionidae; NA
<i>Perimyotis flavus</i>	1	Insectivores	E	Vespertilionidae; NA
<i>Dasypterus xanthinus</i>	1	Insectivores*	E	Vespertilionidae; US and Mexico
<i>Lasiurus borealis</i>	1	Insectivores	E	Vespertilionidae; NA
<i>Corynorhinus rafinesquii</i>	1	Insectivores	E	Vespertilionidae; NA
<i>Corynorhinus townsendi</i>	1	Insectivores	E	Vespertilionidae; NA
<i>Pipistrellus kuhlii</i>	7	Insectivores	E	Vespertilionidae; NA
<i>Pteropus alecto</i>	3	Nectarivores (pollen)	N	Pteropodidae; Australia
<i>Pteropus giganteus</i>	2	Frugivorous	N	Pteropodidae; Australia
<i>Pteropus vampyrus</i>	4	Flowers, nectar, fruit	N	Pteropodidae; Malaysia
<i>Rousettus aegyptiacus</i>	5	Frugivorous or leaves	N	Pteropodidae; Africa
<i>Cynopterus sphinx</i>	6	Frugivorous	N	Pteropodidae; se asia
<i>Macrotus californicus</i>	2	Gleaning insectivore	N	Phyllostomidae; southwest US/northwest mexico
<i>Desmodus rotundus</i>	4	Sanguivores	N	Phyllostomidae; Latin America
<i>Artibeus jamaicensis</i>	4	Frugivorous	N	Phyllostomidae; Mexico, SA, Caribbean
<i>Phyllostomus discolor</i>	5	Nectarivores (pollen/flowers)	N	Phyllostomidae; Latin America
<i>Sturnira hondurensis</i>	4	Frugivorous	N	Phyllostomidae; Latin America
<i>Hipposideros armiger</i>	2	Insectivores	N	Hipposideridae; China, etc
<i>Rhinolophus ferrumequinum</i>	7	Insectivores	N	Rhinolophidae; Eurasia
<i>Miniopterus natalensis</i>	2	Insectivore, fish	N	Miniopteridae; Africa
<i>Molossus molossus</i>	5	Insectivores	N	Molossidae; NA, SA
<i>Tadarida brasiliensis</i>	1	Insectivores	N	Molossidae; NA
TOTAL		89		

Table 1. Samples obtained from GenBank and NSRL are listed with corresponding sample size, dietary preferences, amino acid substitution at codon 191 (Amino Acid), and taxonomic family.

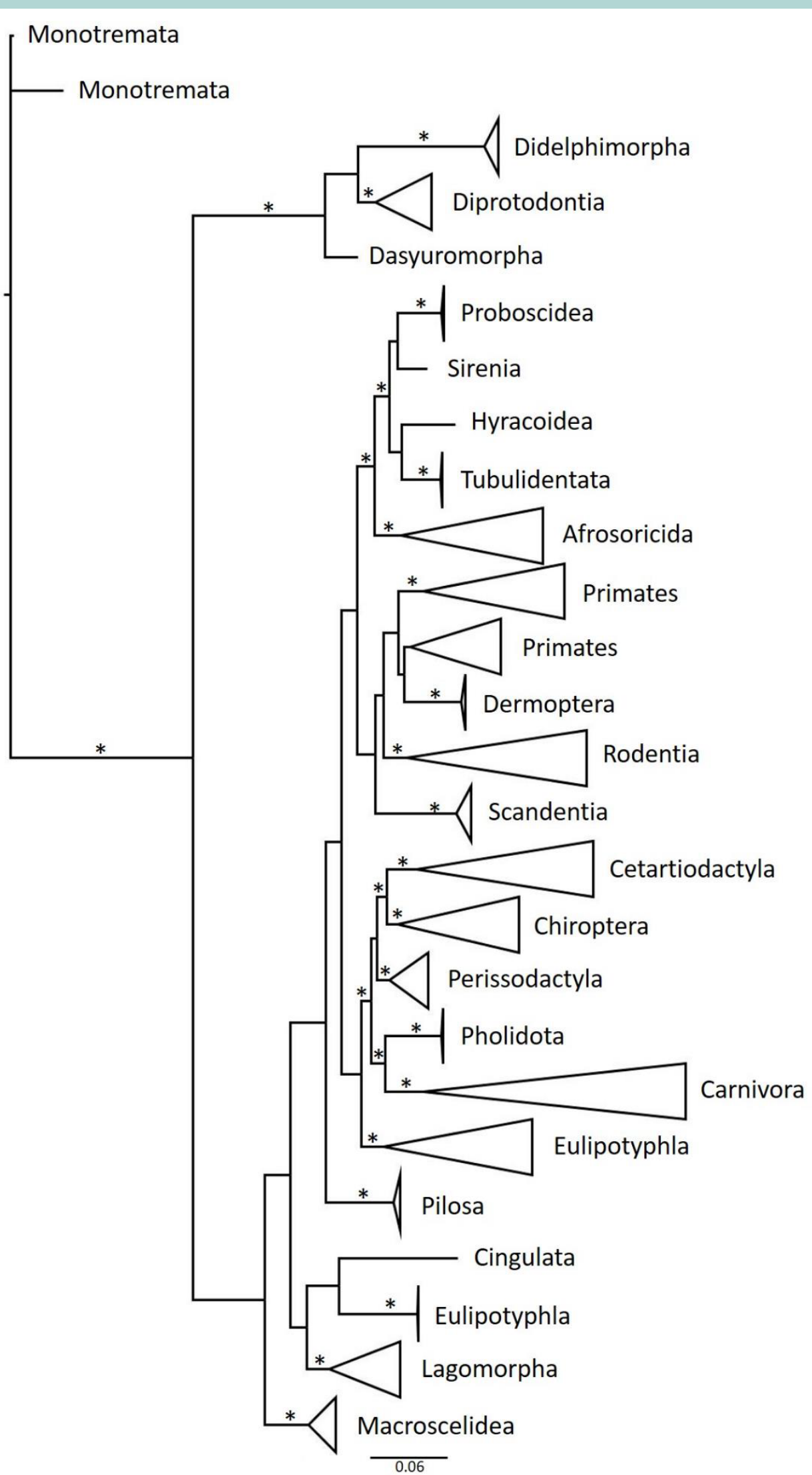


Figure 2. Bayesian phylogeny of *PRNP* exon 3 in mammalian species. Shown is the *PRNP* exon 3 tree, constructed by Bayesian analysis of 520 aligned, (1168 bp alignment, GTR+I+G nucleotide substitution model and 10,000,000 generations) from 23 of 27 mammalian orders, with the primitive mammalian species, duck-billed platypus (*Ornithorhynchus anatinus*), as outgroup. Asterisks denote nodes with Bayesian posterior probability support values,  $p \geq 0.95$ .

## Preliminary Results Canids

Genus Species	Sample Size	Amino Acid	Belgian Tervuren	1	E	Mustela putorius	11	N
<i>Ursus americanus</i>	4	N	White Shepherd	1	E	<i>Mustela erminea</i>	6	N
<i>Ursus arctos</i>	6	N	Dobberman Pinscher	1	E	<i>Meles meles</i>	2	N
<i>Ursus maritimus</i>	1	N	<i>Vulpes lagopus</i>	2	D	<i>Lutra lutra</i>	2	N
<i>Canis latrans</i>	7	D	<i>Vulpes corsae</i>	2	D	<i>European mink</i>	1	N
<i>Canis lupus dingo</i>	4	D	<i>Vulpes vulpes</i>	7	D	<i>Martes martes</i>	2	D
<i>Canis lupus familiaris</i>	44	D/E	Pyrenean Shepherd	1	D	<i>Mustela sp</i>	1	N
<i>Canis lupus</i>	7	D	<i>Nyctereutes procyonoides</i>	2	D	<i>Lontra canadensis</i>	2	N
<i>Australian shepherd</i>	1	D	<i>Vulpes velox</i>	2	D	<i>Procyon lotor</i>	3	N
<i>Belgian malinois</i>	1	D	<i>Canis lupus hallstromii</i>	3	D	<i>Mephitis mephitis</i>	4	N
<i>Cocker Spaniel</i>	1	D	<i>Urocyon cinereoargenteus</i>	1	D	<i>Gulo gulo</i>	6	D
<i>German wirehaired pointer</i>	1	D	<i>Chrysocyon brachyurus</i>	2	D	<i>Mustela frenata</i>	1	N
<i>Schipperke</i>	1	D	<i>Speothos venaticus</i>	2	D	<i>Taxidea taxus</i>	1	D
<i>Old english sheepdog</i>	1	D	<i>Neovison vison</i>	1	N	<i>Bassariscus astutus</i>	1	N
<i>Border collie</i>	1	D	<i>Ailuropoda melanoleuca</i>	9	N	TOTAL	164	
<i>Pointer</i>	1	D	<i>Ailurus fulgens</i>	2	N			
<i>Poodle</i>	1	D						

Table 2. Samples obtained from GenBank and NSRL are listed with corresponding sample size and amino acid substitution at codon 191.

- Analyzed *PRNP* exon 3 region amino acids for mutation in highly conserved region that confers resistance
- Chiropteran in Family Vespertilionidae were positive for amino acid change, not all bats in this family displayed change
- Only some *Myotis* species display AA change
- *Urocyon cinereoargenteus* has substitution while other canids (*Taxidea taxus*, *Mustela frenata*) do not have substitution
- No apparent pattern relating to geography detected
- Canidae and Mustelidae has substitution for Canids

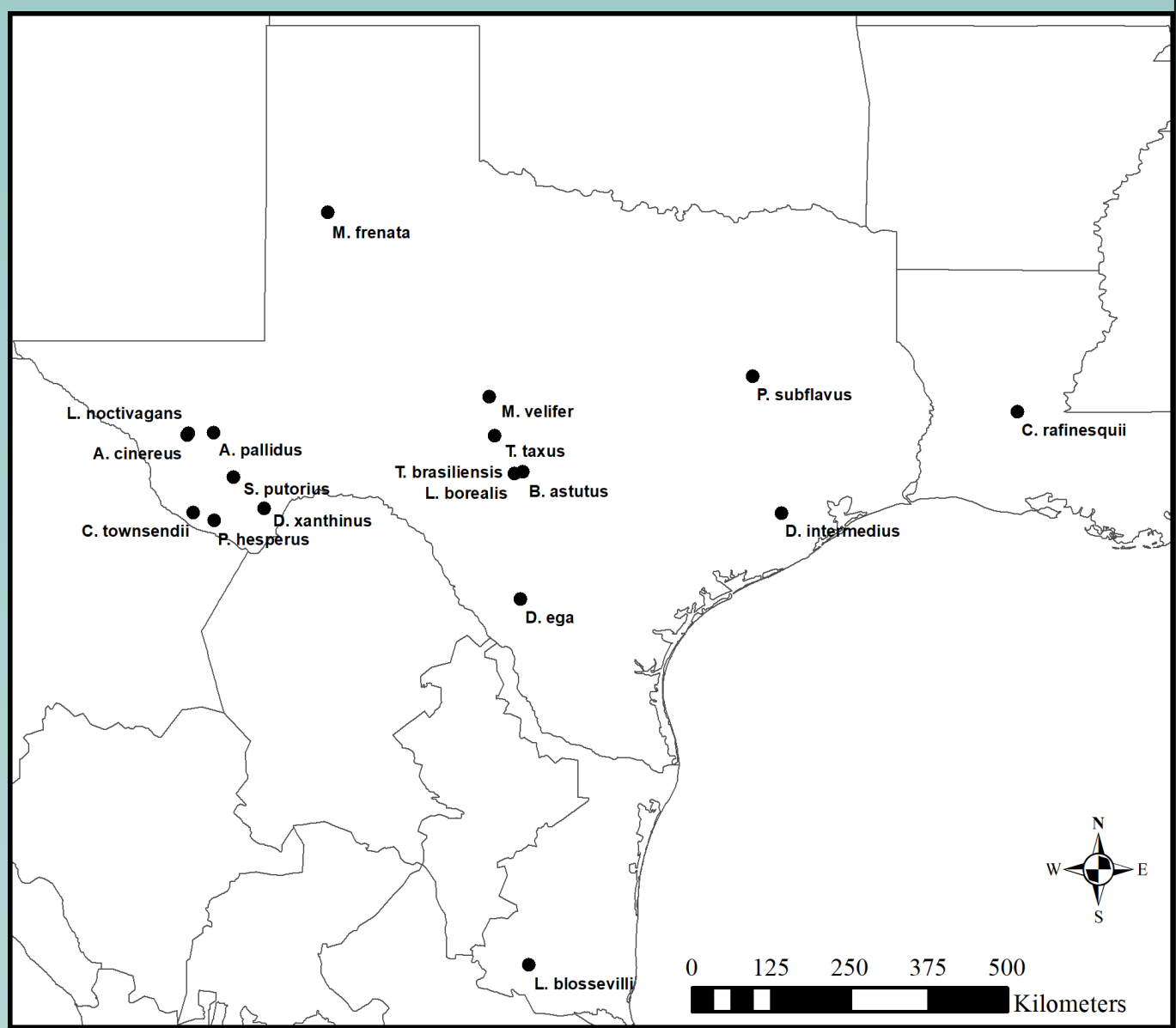


Figure 3. Geographic sampling of Canids and Chiropterans

## Future Directions

- Sequence Chiropterans and Canids from other families to understand the potential pattern between phylogenies
- Sequence *Lycalopex griseus* (False Fox) from SA
- Develop specific primers for Chiropterans

## Acknowledgments

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