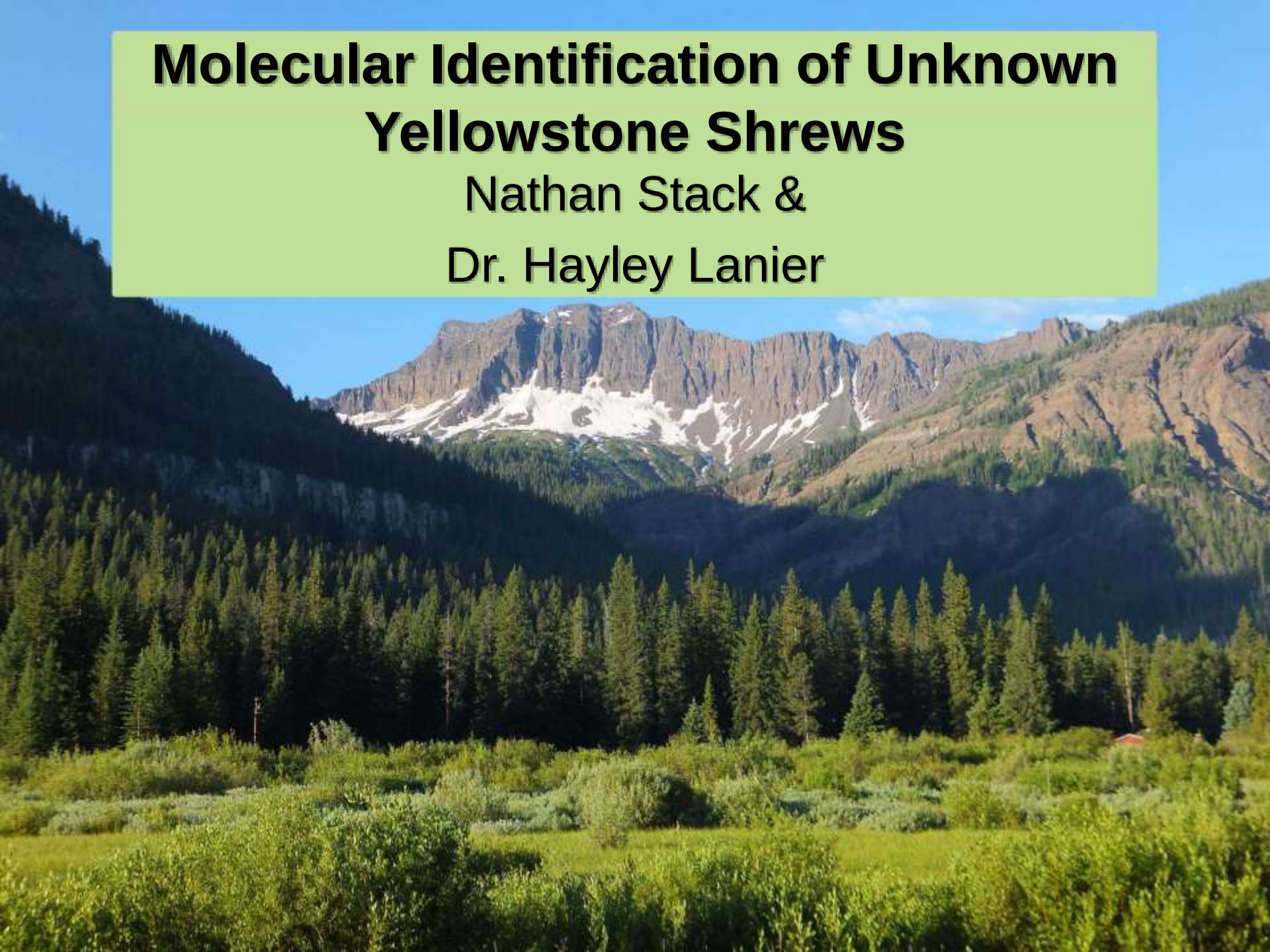
Molecular Identification of Unknown Yellowstone Shrews

Nathan Stack &

Dr. Hayley Lanier



Acknowledgements

- Wyoming INBRE
- Field collection
  - Dr. Scott Seville
  - Dr. Zac Roehrs
  - Meredith Roehrs
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  - Dr. Motriuk-Smith
- UW Core facility
  - UW Macromolecular Analysis Core

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WYOMING INBRE

Introduction

- Ecological & biodiversity studies

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  - Challenges
    - Difficult to see
    - Hard to collect

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    - Types of traps

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  - Identification of cryptic species
    - External characters

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      - Different methods
      - Types of traps
    - Identification of cryptic species
      - External characters
  - Shrews
    - Hard to determine species in the field
    - Look very similar

Introduction

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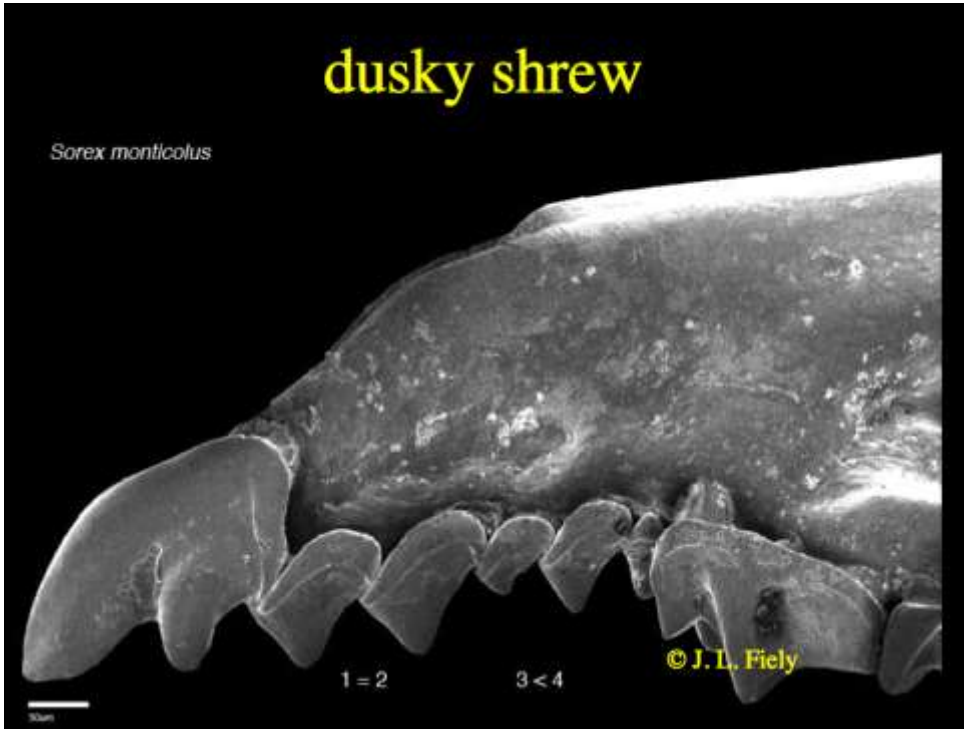
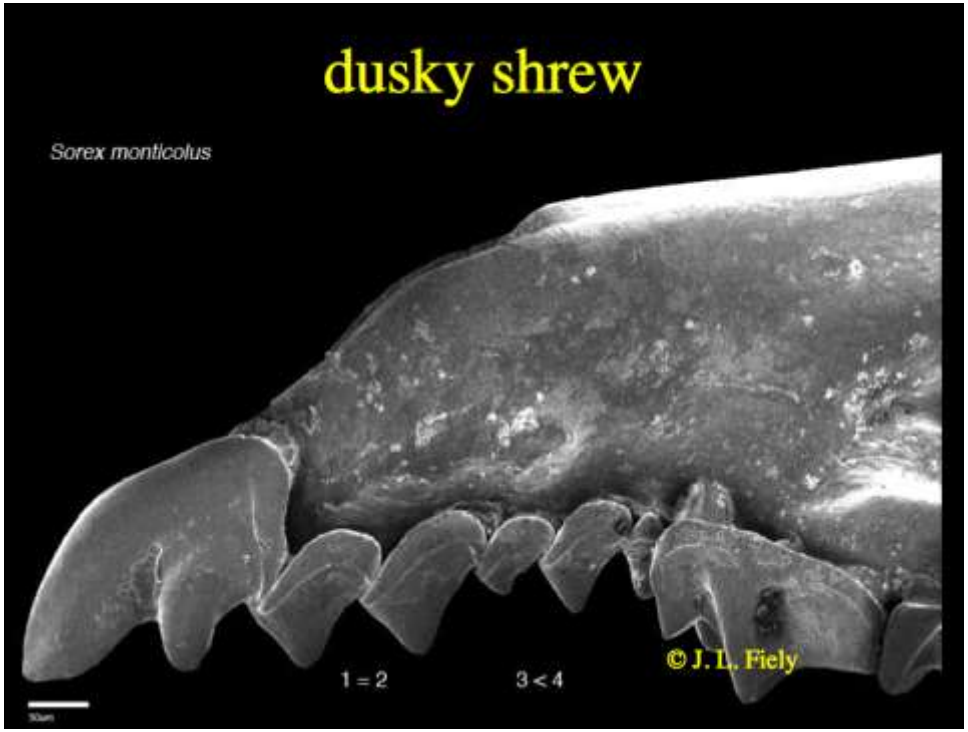
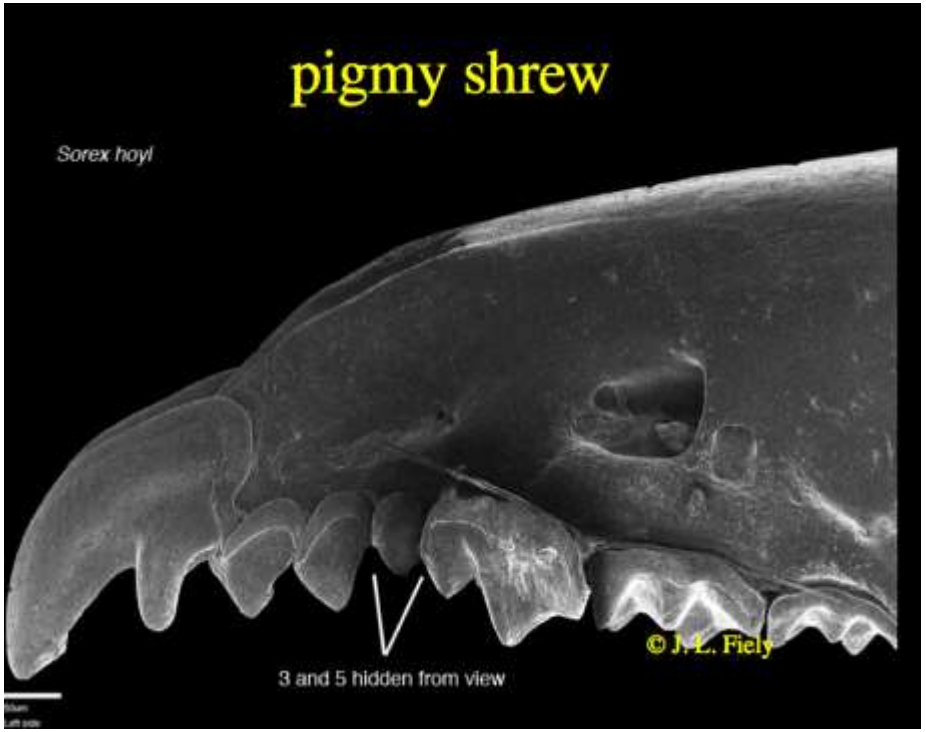
Sorex Unicuspids



Sorex unicuspids





Yellowstone Project

- 1988 fire in Greater Yellowstone Area (GYA)
  - ~570,000 ha

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- Shrews
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  - Must eat large quantities of invertebrates
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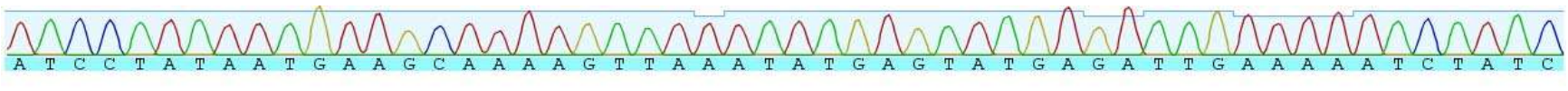
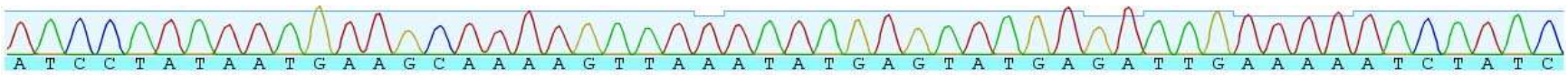
- Species identification
  - Field
  - Lab
    - Molecular identification methods
    - DNA barcoding

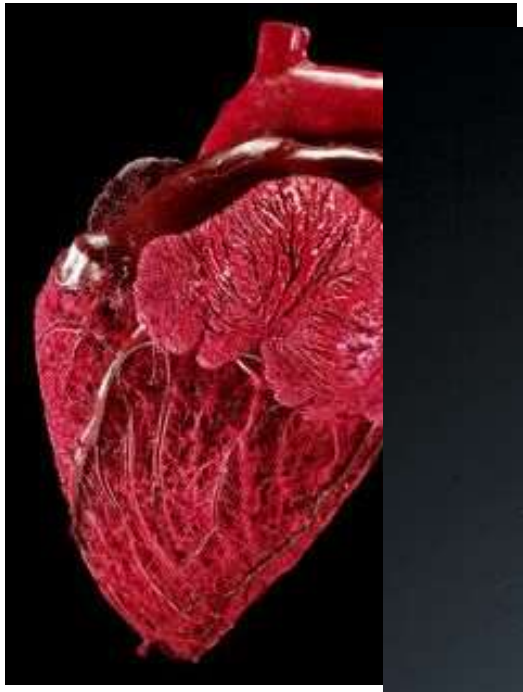
DNA Barcoding

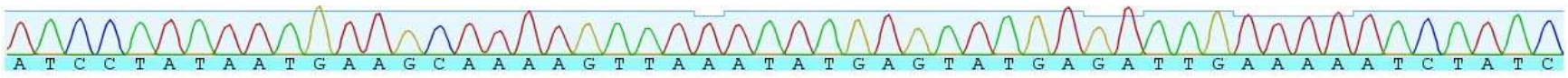


DNA Barcoding



DNA Barcoding





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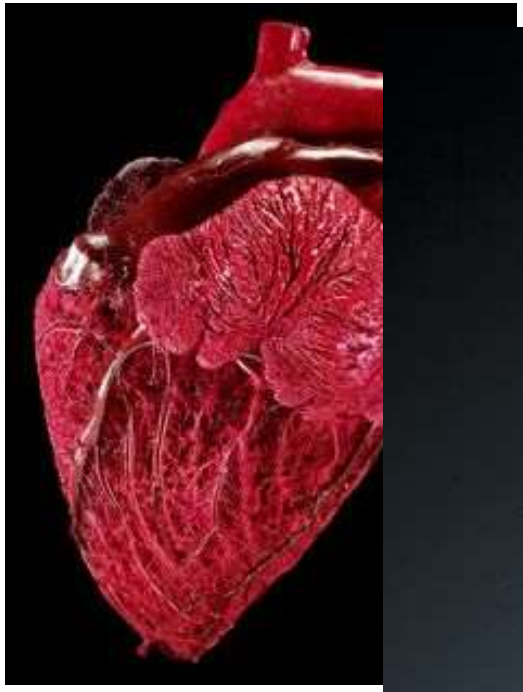
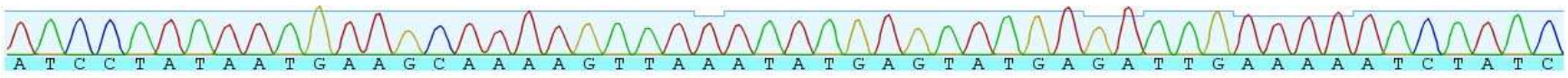
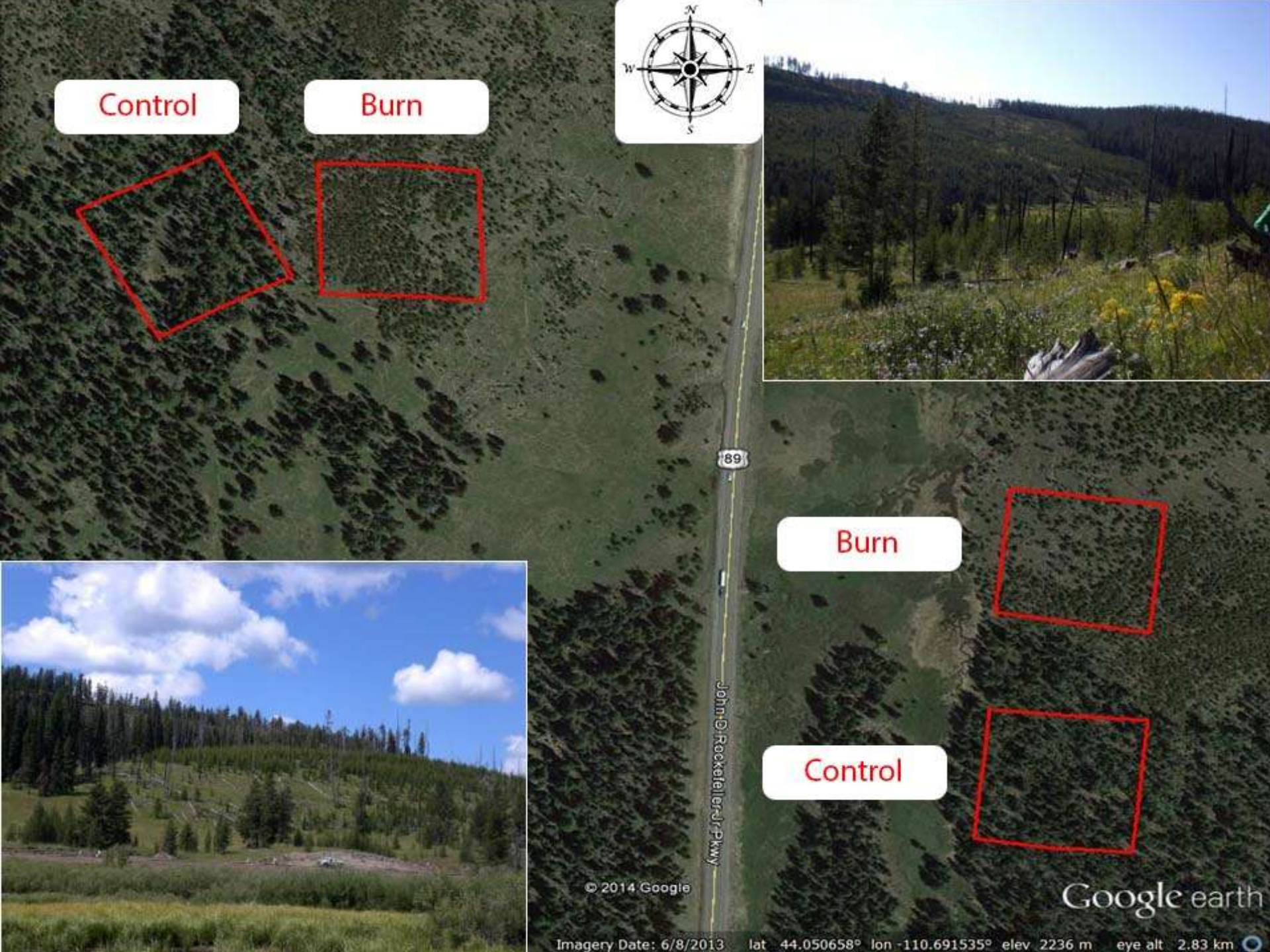





Billings
Bozeman
I-90
Livingston
Bighorn Canyon
National
Recreation
Area
Fort Smith
Beartooth Mtns
Yellowstone River
US 89
US 191
US 287
Madison River
Gallatin Range
Clark's Fork
MONTANA
WYOMING
Gardiner
Cooke City
US 212
Lovell
Bighorn Mtns
Yellowstone
National
Park
West
Yellowstone
Shoshone River
Cody
US 20
Absaroka Mts
Bighorn River
IDAHO
John D. Rockefeller Jr.
Memorial Parkway
Grand Teton
National Park
Teton Range
Wind River Ra
US 26
Jackson


Control
Burn
N
W
E
S
89
John D. Rockefeller Jr. Pkwy
Burn
Control
© 2014 Google
Google earth
Imagery Date: 6/8/2013 lat 44.050658° lon -110.691535° elev 2236 m eye alt 2.83 km

Methods & Materials

- Collection
  - 222 shrews trapped, 173 specimens (& tissues) kept
  - Sherman traps & pitfall traps

Methods & Materials

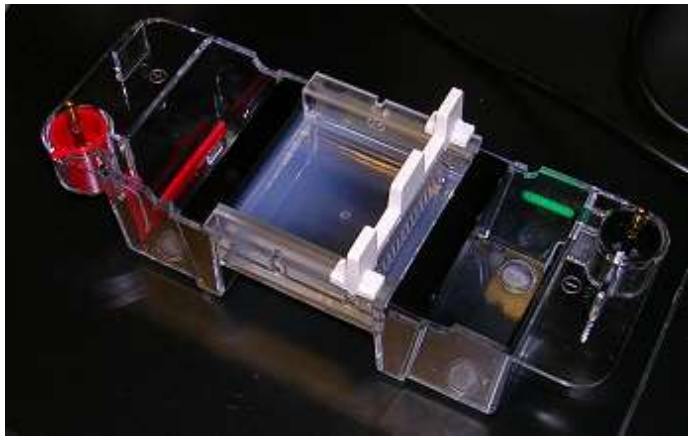
- Extraction
  - Genomic DNA
  - DNeasy Blood & Tissue Kit

Methods & Materials

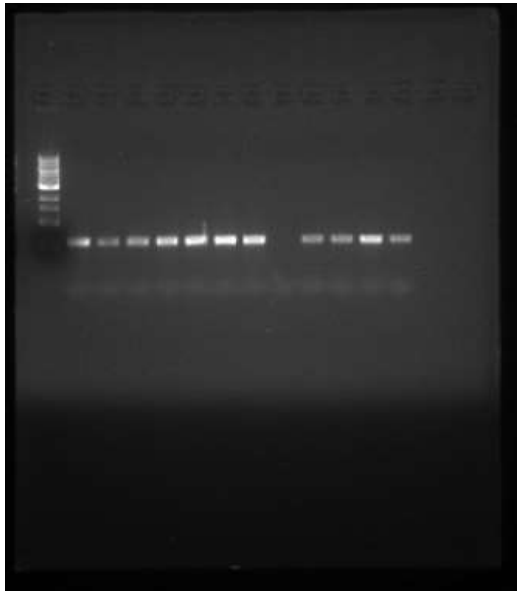
- PCR optimization
  - Custom cycles designed based on thermal gradients
- DNA amplification
  - Polymerase chain reaction (PCR) using thermocycler
- Primers
  - Cytochrome b
  - Breast Cancer Susceptibility
    1 Gene
  - Apoplipoprotein B gene

Methods & Materials

- Electrophoresis
  - After amplification, gels visualized



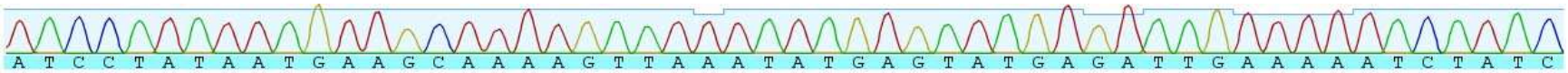
- DNA visualization



Methods & Materials

- DNA cleanup & sequencing preparation
  - Exosap

- Sequencing



Results

- Total of 50 samples sequenced

Actual Species

Originally designated species	Number Captured	*Sorex cinereus*	*Sorex monticolus*	*Sorex* spp.
Sorex cinereus				
Sorex monticolus				
***Sorex* spp.**				

Results

- Total of 50 samples sequenced

Actual Species

Originally designated species	Number Captured	*Sorex cinereus*	*Sorex monticolus*	*Sorex* spp.
Sorex cinereus	24			
Sorex monticolus				
***Sorex* spp.**				

Results

- Total of 50 samples sequenced

Actual Species

Originally designated species	Number Captured	*Sorex cinereus*	*Sorex monticolus*	*Sorex* spp.
Sorex cinereus	24	2		
Sorex monticolus				
***Sorex* spp.**				

Results

- Total of 50 samples sequenced

Actual Species

Originally designated species	Number Captured	*Sorex cinereus*	*Sorex monticolus*	*Sorex* spp.
Sorex cinereus	24	2	4	
Sorex monticolus				
***Sorex* spp.**				

Results

- Total of 50 samples sequenced

Actual Species

Originally designated species	Number Captured	*Sorex cinereus*	*Sorex monticolus*	*Sorex* spp.
Sorex cinereus	24	2	4	-
Sorex monticolus	71			
***Sorex* spp.**				

Results

- Total of 50 samples sequenced

Actual Species

Originally designated species	Number Captured	*Sorex cinereus*	*Sorex monticolus*	*Sorex* spp.
Sorex cinereus	24	2	4	-
Sorex monticolus	71	-	3	
***Sorex* spp.**				

Results

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Actual Species

Originally designated species	Number Captured	*Sorex cinereus*	*Sorex monticolus*	*Sorex* spp.
Sorex cinereus	24	2	4	-
Sorex monticolus	71	-	3	-
***Sorex* spp.**	78			

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Actual Species

Originally designated species	Number Captured	*Sorex cinereus*	*Sorex monticolus*	*Sorex* spp.
Sorex cinereus	24	2	4	-
Sorex monticolus	71	-	3	-
***Sorex* spp.**	78	4		

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Actual Species

Originally designated species	Number Captured	*Sorex cinereus*	*Sorex monticolus*	*Sorex* spp.
Sorex cinereus	24	2	4	-
Sorex monticolus	71	-	3	-
***Sorex* spp.**	78	4	15	

Results

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Actual Species

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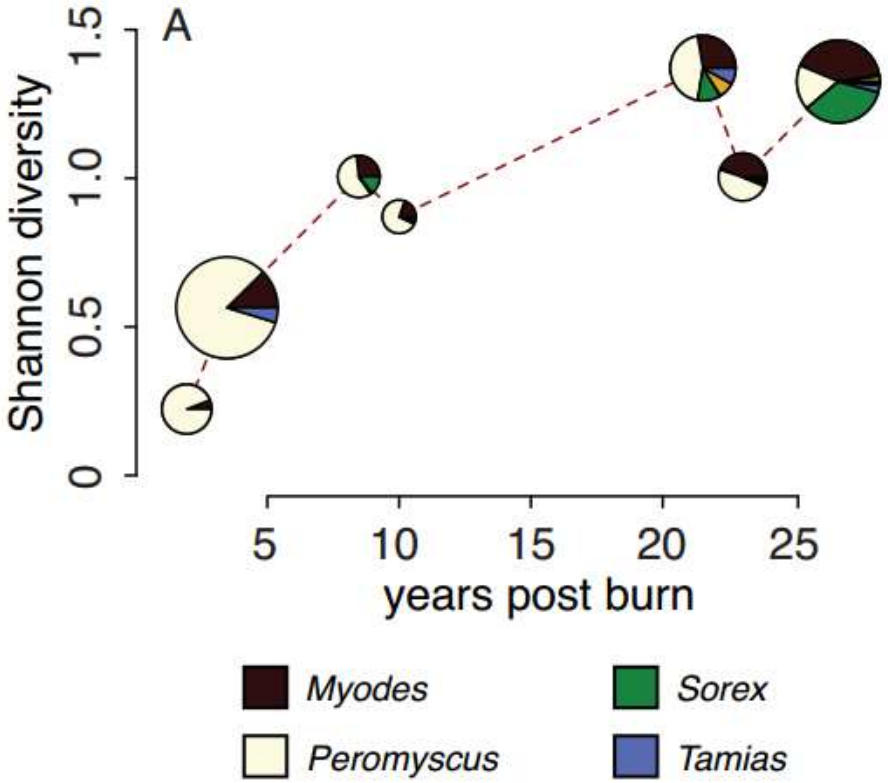
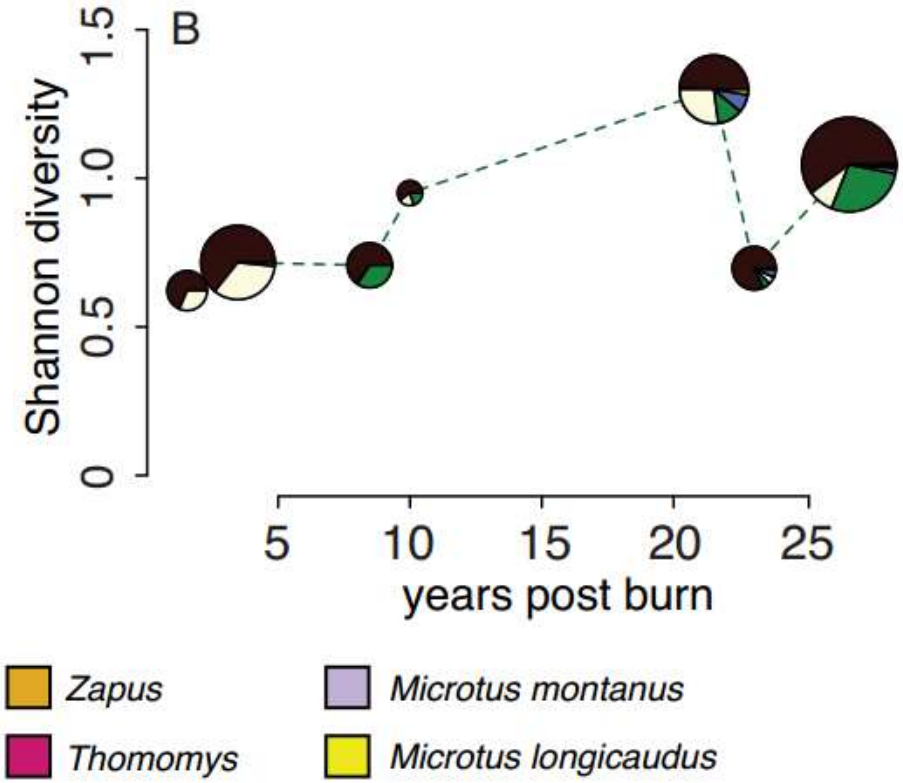
Sorex cinereus	24	2	4	-
Sorex monticolus	71	-	3	-
***Sorex* spp.**	78	4	15	1

Discussion

East facing burn



East facing control



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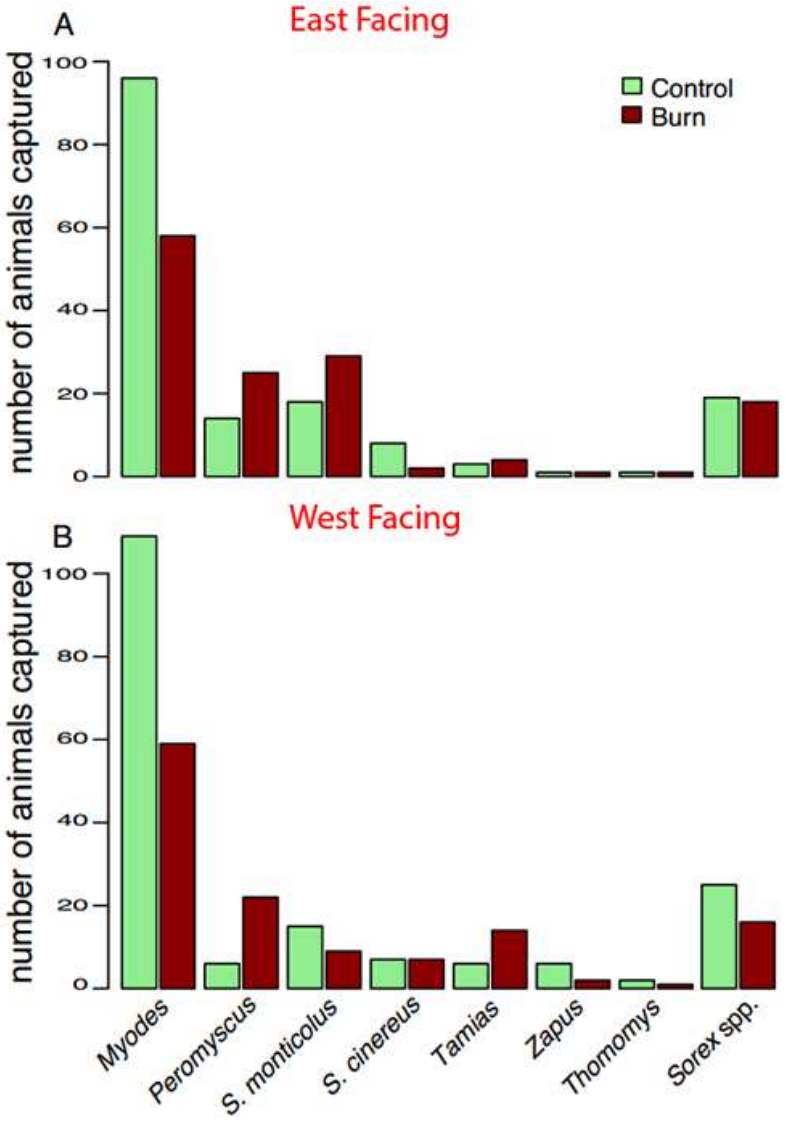
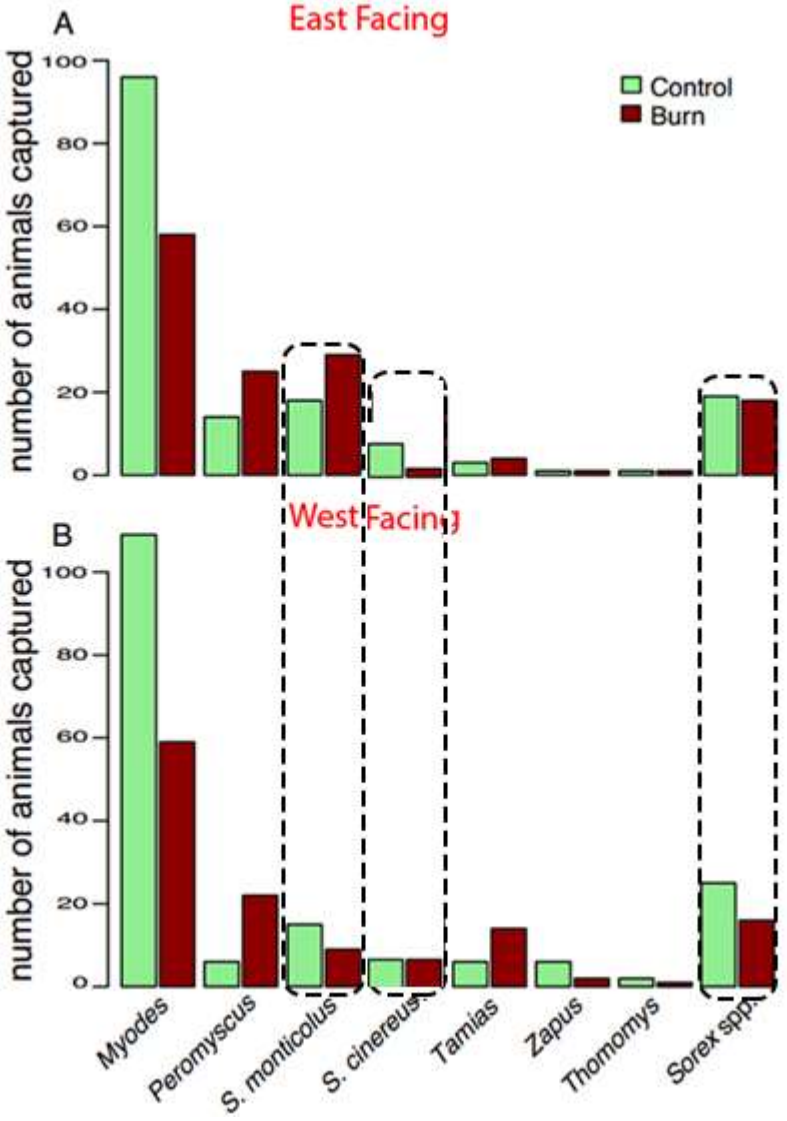

A
East Facing
number of animals captured
100
80
60
40
20
0
Control
Burn
B
West Facing
number of animals captured
100
80
60
40
20
0
Myodes
Peromyscus
S. monticolus
S. cinereus
Tamias
Zapus
Thomomys
Sorex spp.

Discussion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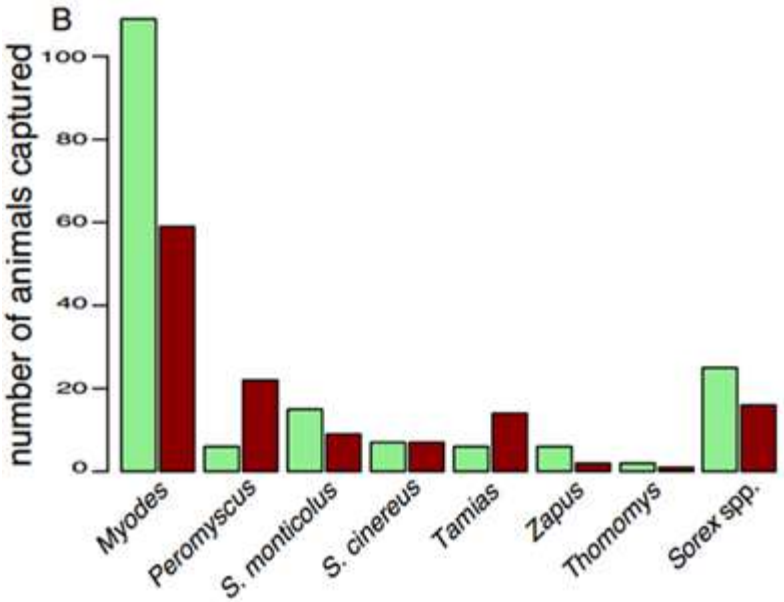
Sorex spp.

Discussion

- 28 sequences were clear enough to have nucleotide BLAST results on NCBI with a similar species
  - 5 identified correctly in the field
  - 4 misidentified in the field
  - 19 newly identified to species
- More species to be verified

or identified



Beyond shrews…

- Habitat change
  - Impacts biodiversity
- Molecular techniques have many uses
- Universal tool
  - Any species