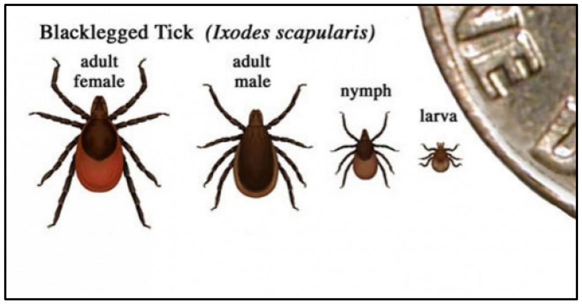
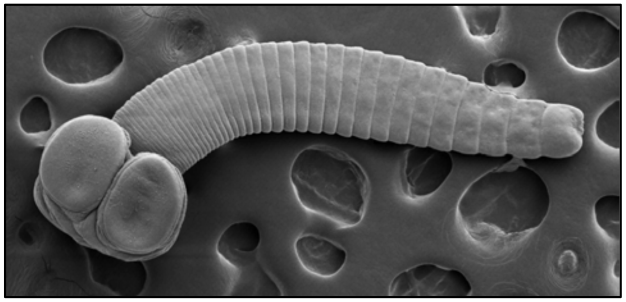
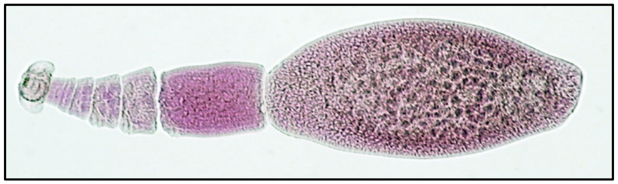
Small mammal contact zones and evolutionary cascades: Methods for genomic investigations within complex host-parasite systems

Andrew Hope (Kansas State)
Vasyl Tkach (University of North Dakota)
Stephen Greiman (Georgia Southern)
Sandra Talbot (USGS, Alaska Science Center)
Joseph A Cook (University of New Mexico)







Regarding myself…

- Fundamentally: Biogeographer
    - Processes that influence distributions
        - Individuals → populations → lineages → species
- Functionally: Molecular Ecologist
    - Interpret evolutionary history to understand ecology
        - Phylogenetic trees
- Purpose: Conservation of functional wild systems
    - Inform policy and management
    - Reveal connections
        - Hosts and parasites

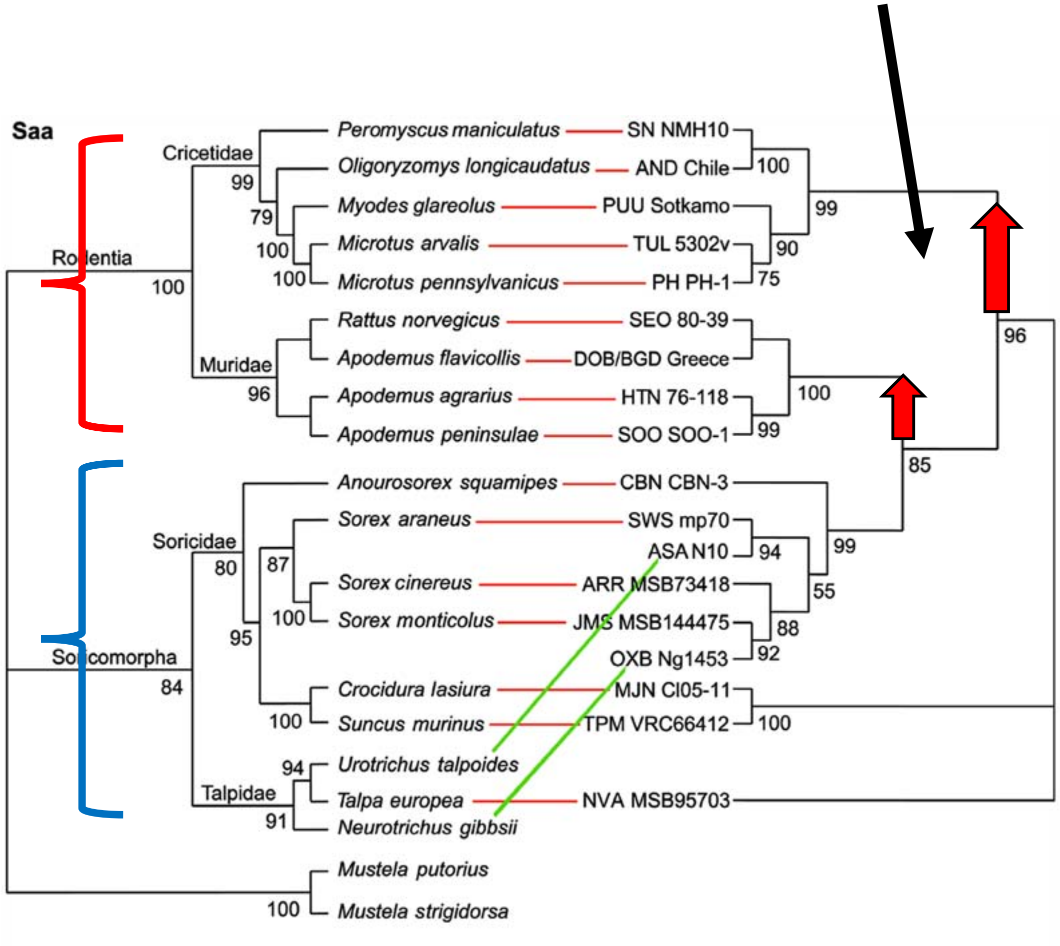
Hantavirus Mammal Crew
New Mexico
Ecology of Emerging Disease

"Satellite imagery characterizes local animal reservoir populations of Sin Nombre virus in the southwestern United States" – Glass et al. 2002, *PNAS*

Host-virus co-phylogeny

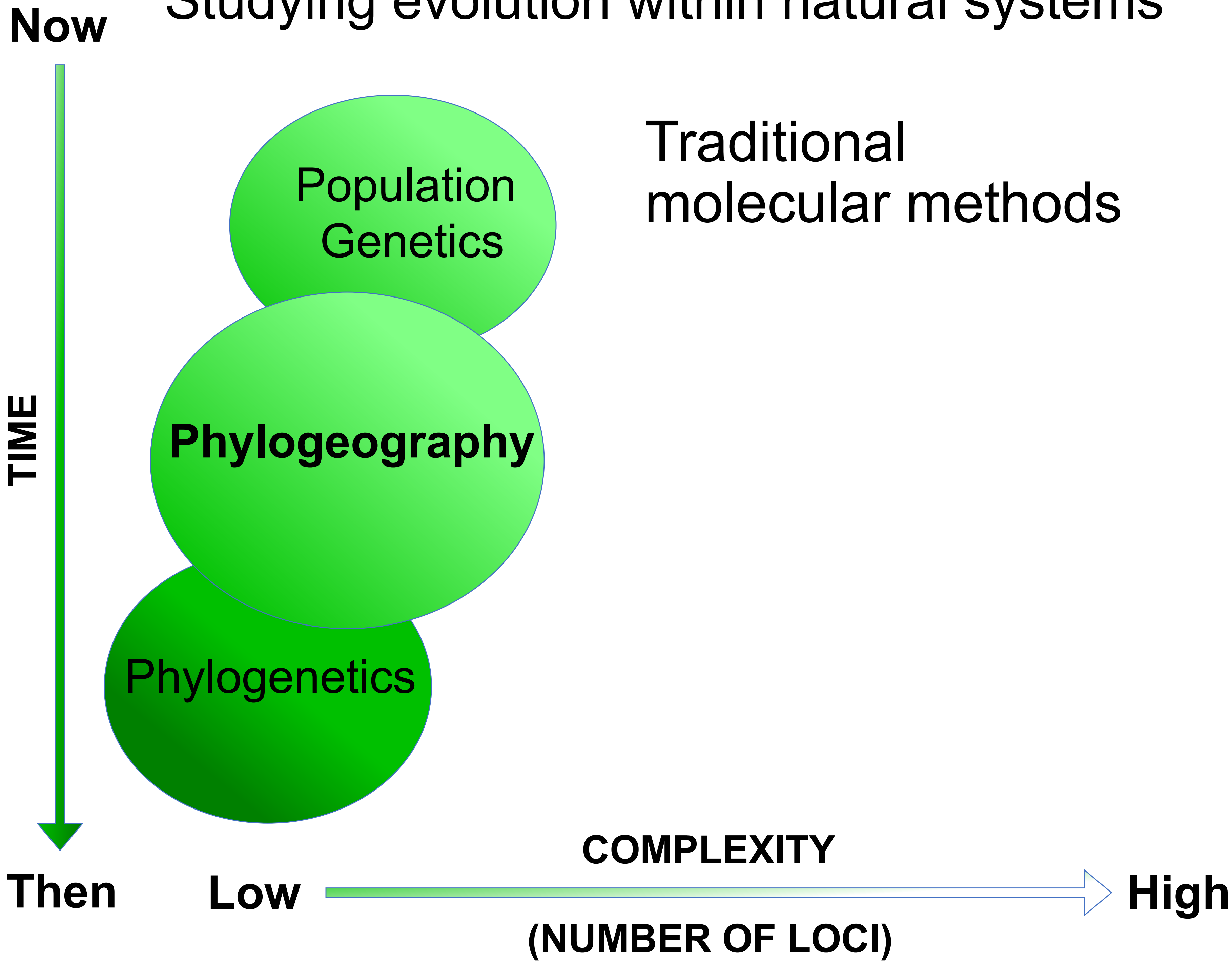
Major Host Switching Events

- Paradigm lost..
- Shrews, **not** rodents, as primordial reservoirs for hantavirus
- Tree-based methods reveal relationships
- Specimen archives are critical



Arai et al. 2008, *AmJTropMedHyg*; Kang et al. 2009, *PLoSOne*, 2009 *VirolJ*; Kang et al. 2010, *VectorBorneZoonoticDis*; Kang et al. 2011, *JVirol.*

Studying evolution within natural systems



Now

TIME

Then

Population Genetics

Phylogeography

Phylogenetics

Traditional molecular methods

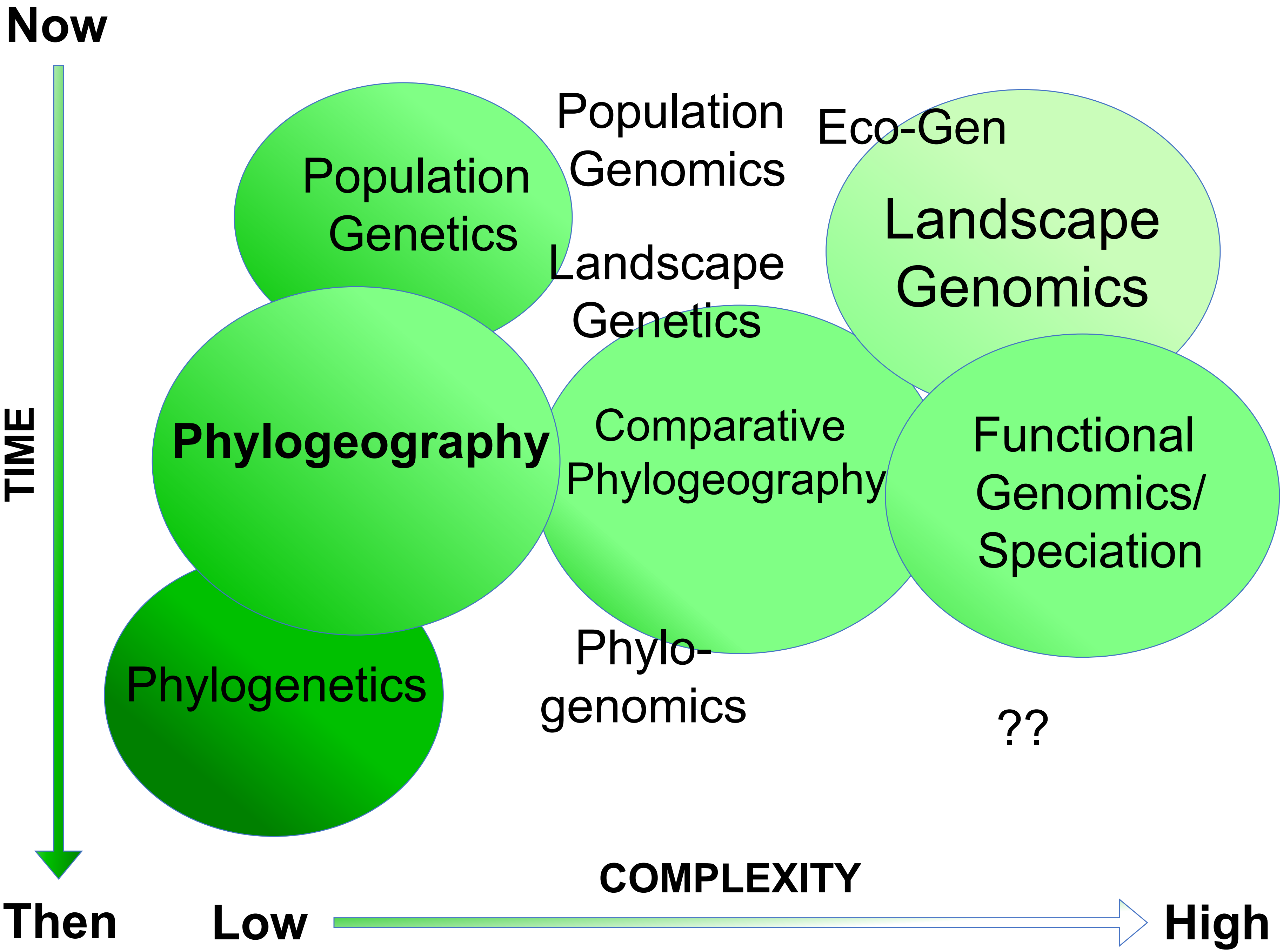
Low

COMPLEXITY

(NUMBER OF LOCI)

High

Modern Methods…



Now

TIME

Then

Population Genetics

Population Genomics

Eco-Gen

Landscape Genomics

Landscape Genetics

Phylogeography

Comparative Phylogeography

Functional Genomics/ Speciation

Phylogenetics

Phylo-genomics

??

COMPLEXITY

Low

High

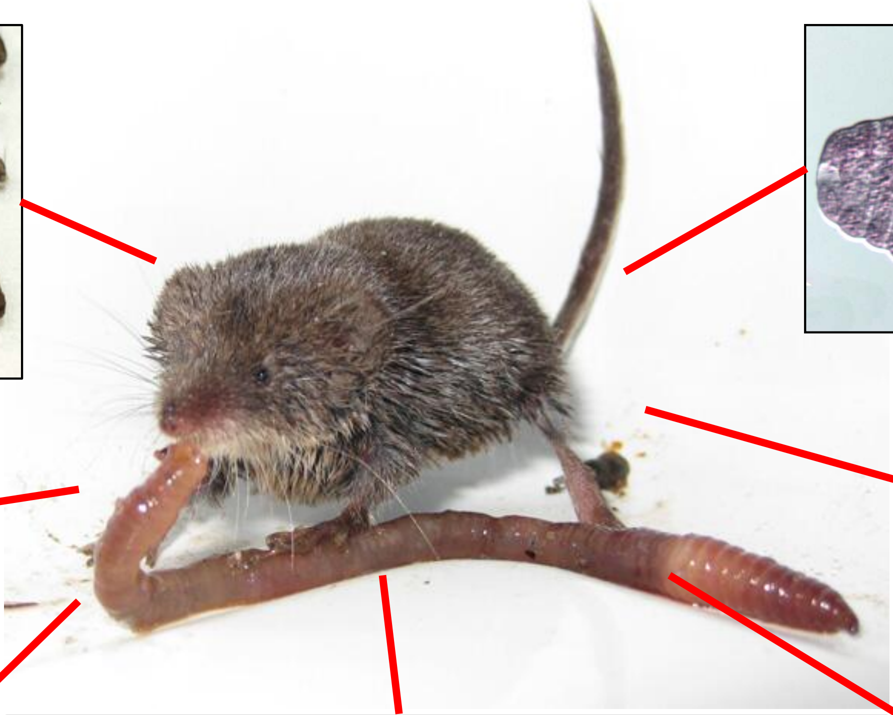
Impacts of environmental change

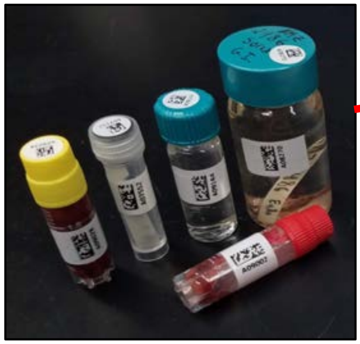
- Anthropocene trends
    - Increased rates of warming/development
    - More extreme events/disturbances
    - Elevated risk of extinction
    - Increased intensity of biological interactions

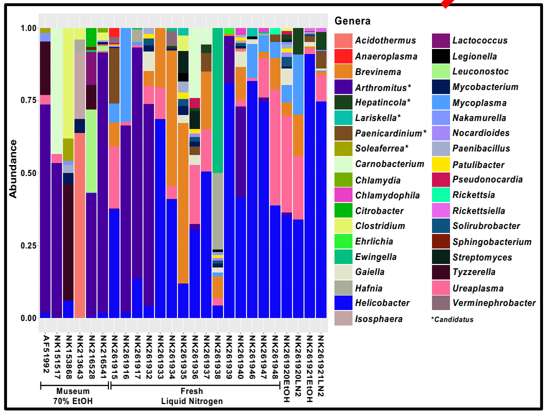
- Mitigation (from a phylogeographic perspective)
    - Resolve evolutionary complexity
        - Across landscapes
        - Across layers of biodiversity

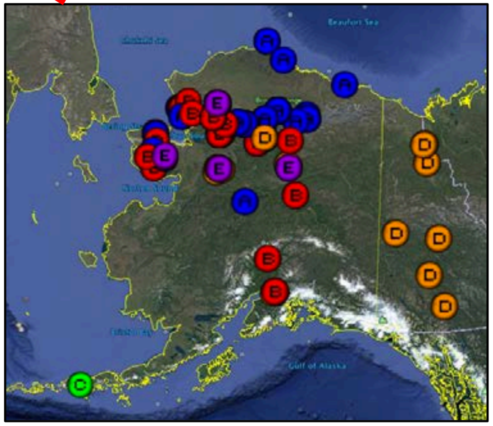
Hope Lab Questions:

How are functional biodiversity connections influenced by changes in: 1) Ecological associations within local communities; and 2) Genetic backgrounds of species, across space and through time?









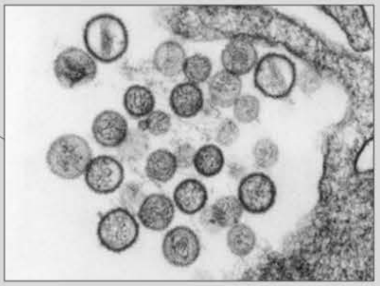
Most genomic assessments of wild organisms are specimen-based!

e.g., Dunnum et al. 2017, PLoS negl.trop.dis.

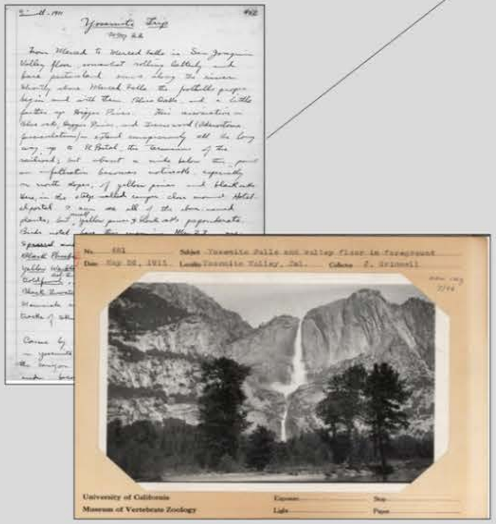
Geo-referenced locality

Cataloged Host Voucher

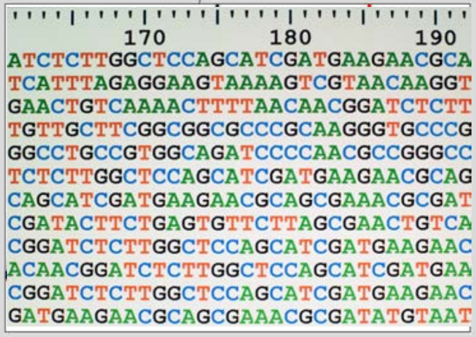
Ecto & endo parasites



Virus isolates



Field notes, ecological data



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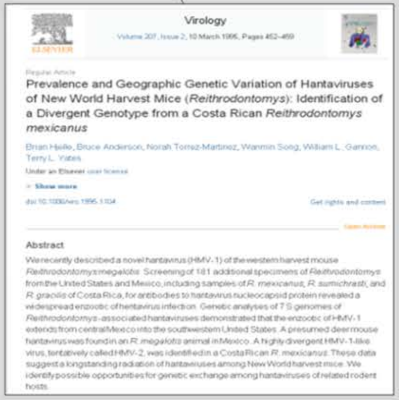
Genomic data



Virology

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Publications

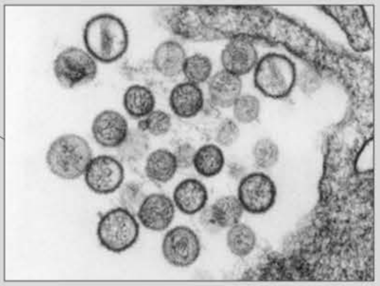
Ultrafrozen tissues

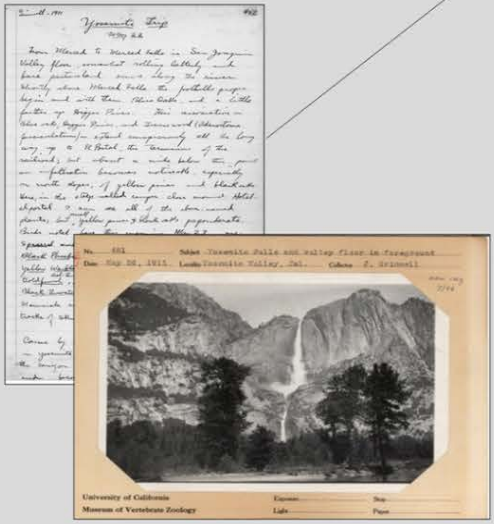
Genomic Contributions

Geo-referenced locality


Cataloged Host Voucher


Ecto & endo parasites

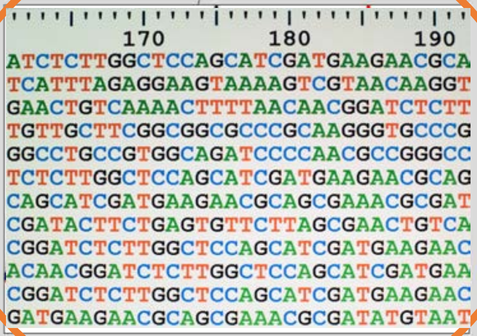

Virus isolates


Yosemite Trip

No. 481 Subject Yosemite Falls and valley floor in foreground
Date Sep 26, 1915 Locality Yosemite Valley, Cal. Collector J. Grinnell

University of California Exposure Stop
Museum of Vertebrate Zoology Light Paper

Field notes, ecological data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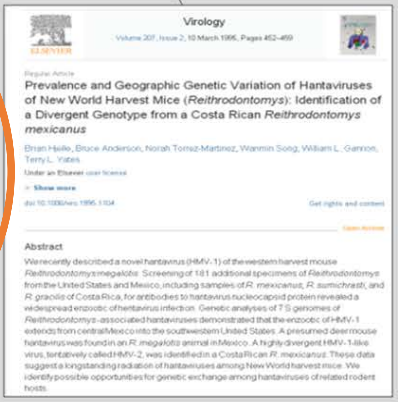
Genomic data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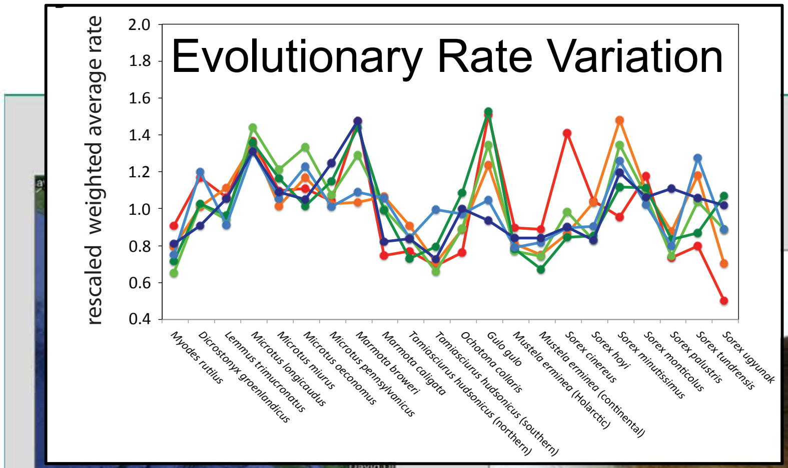

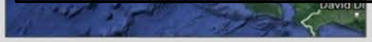

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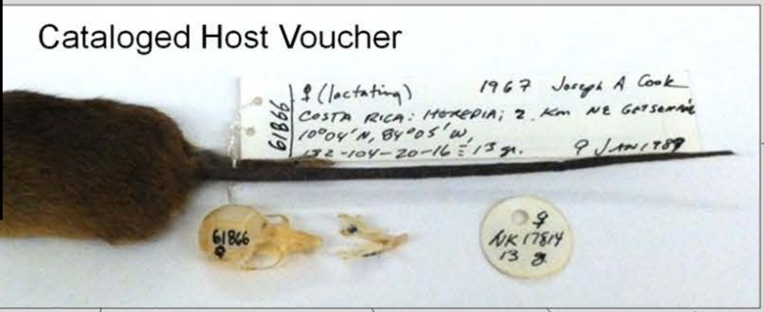
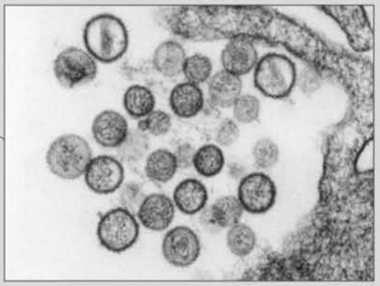


Ecto & endo parasites



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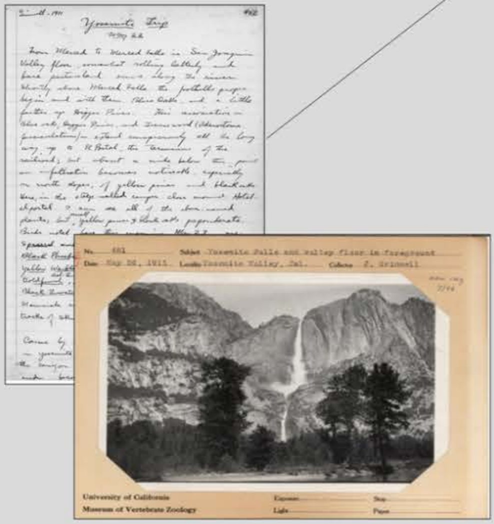

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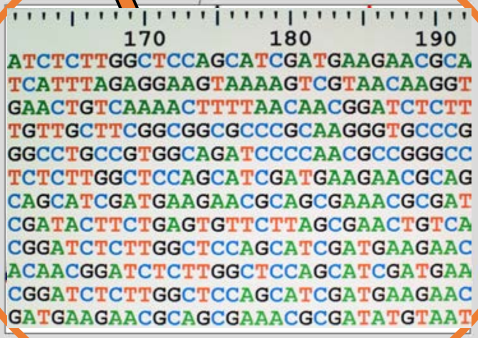

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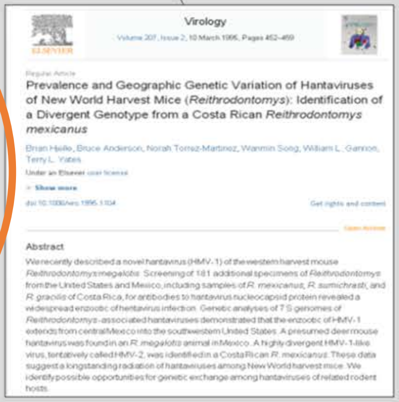

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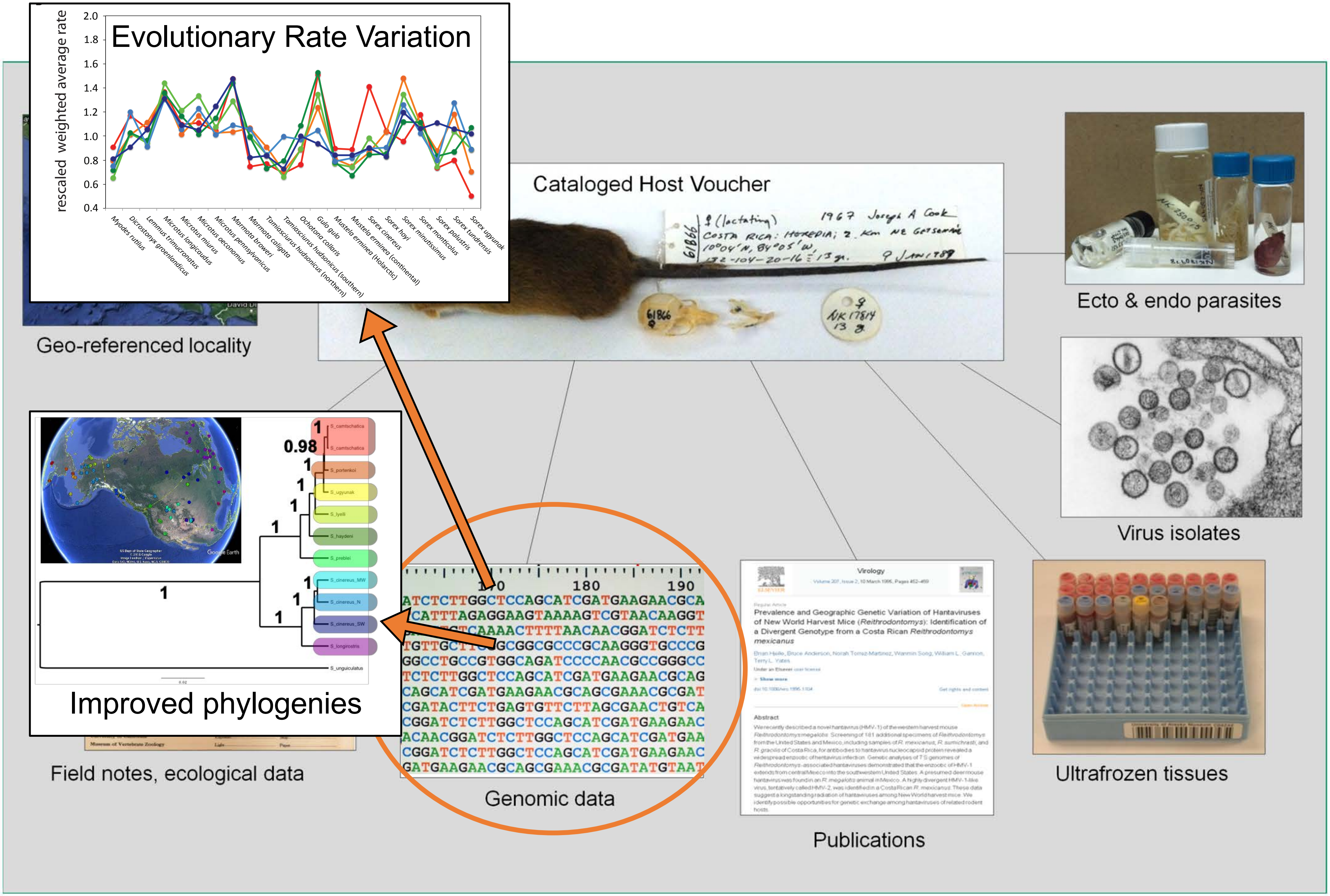

Evolutionary Rate Variation

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Virus isolates

Improved phylogenies

Field notes, ecological data

Genomic data

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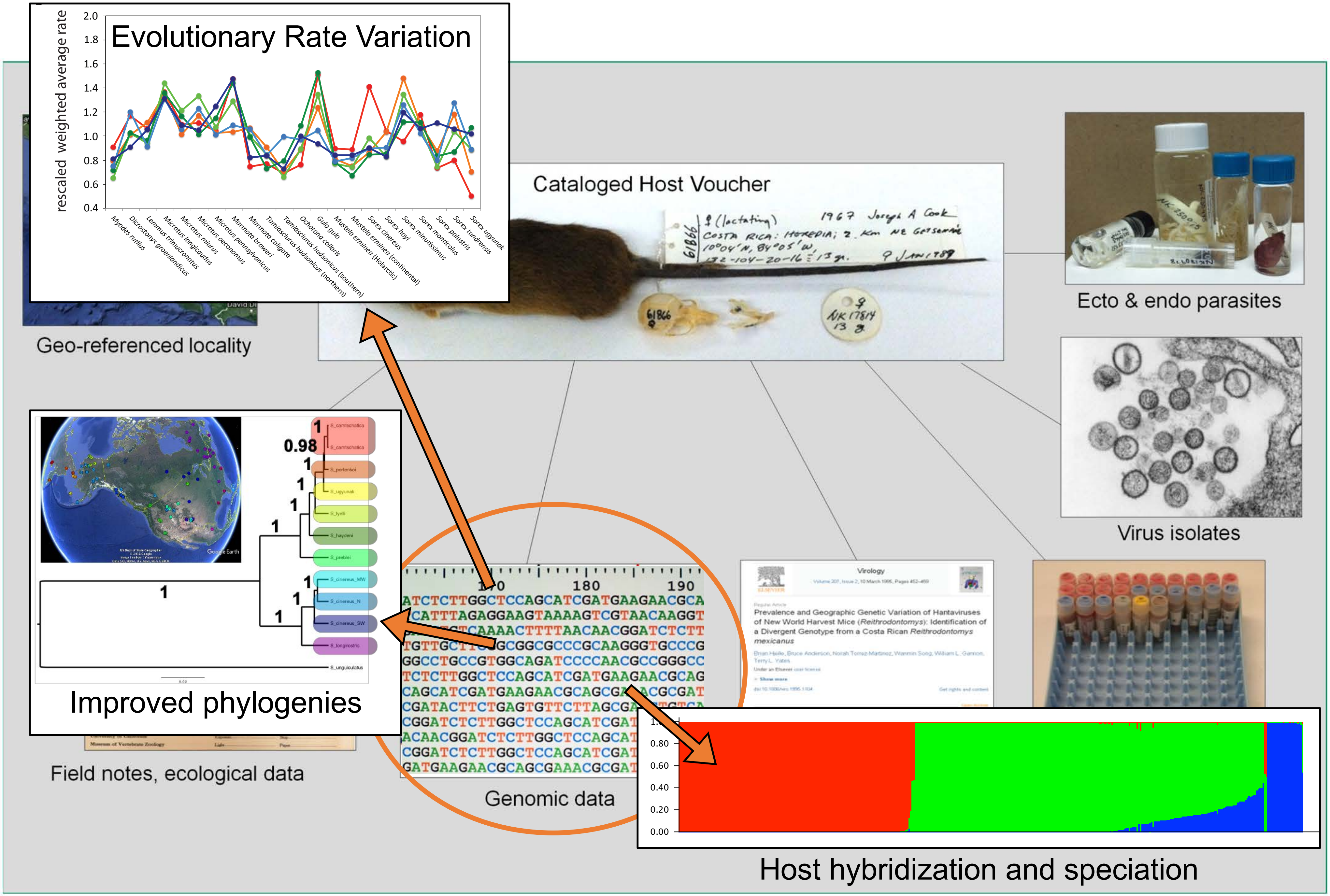
Metagenomics



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Improved phylogenies

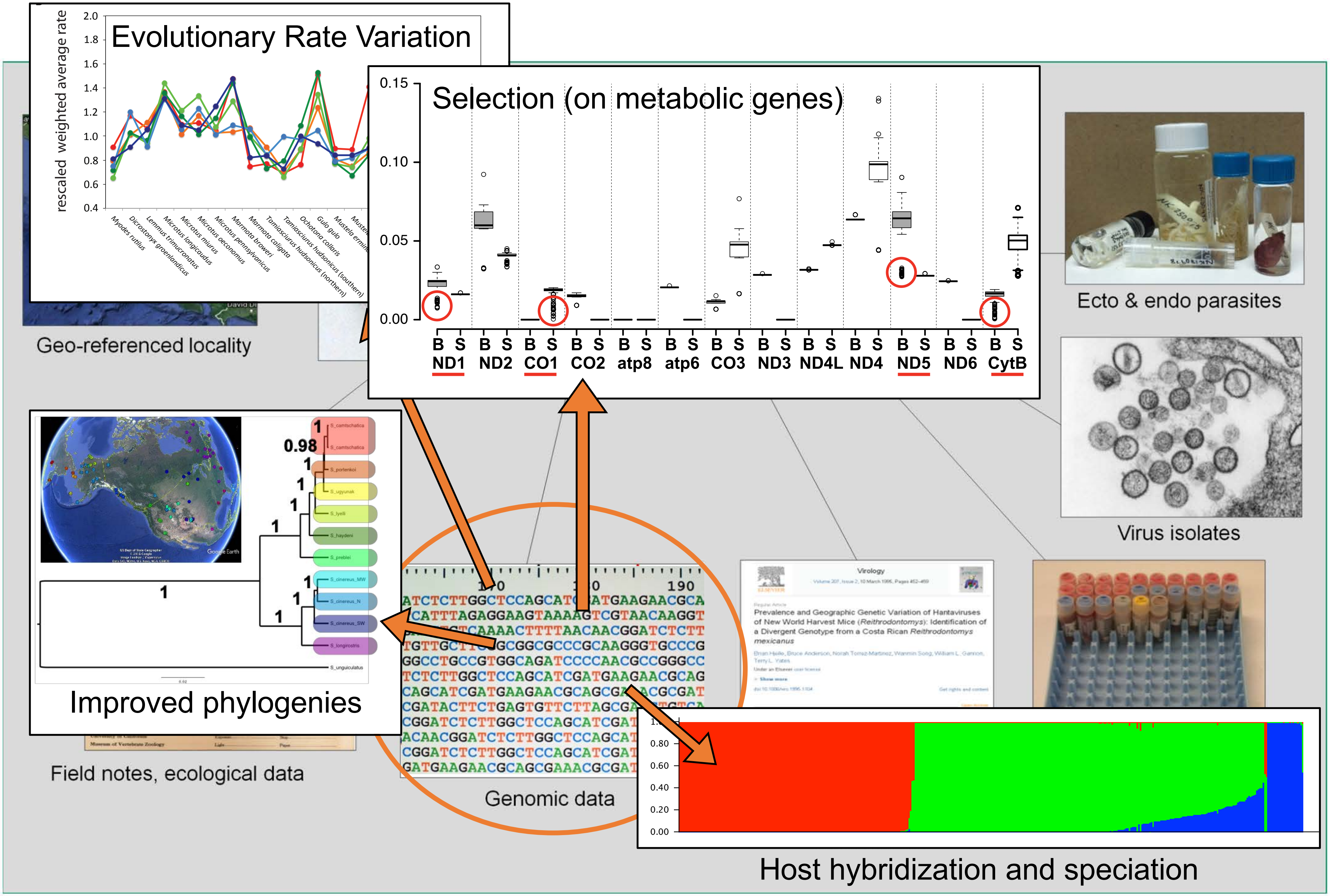
Field notes, ecological data

Genomic data

Host hybridization and speciation

Genomic Contributions

Metagenomics



Evolutionary Rate Variation

Geo-referenced locality

Selection (on metabolic genes)

Ecto & endo parasites

Virus isolates

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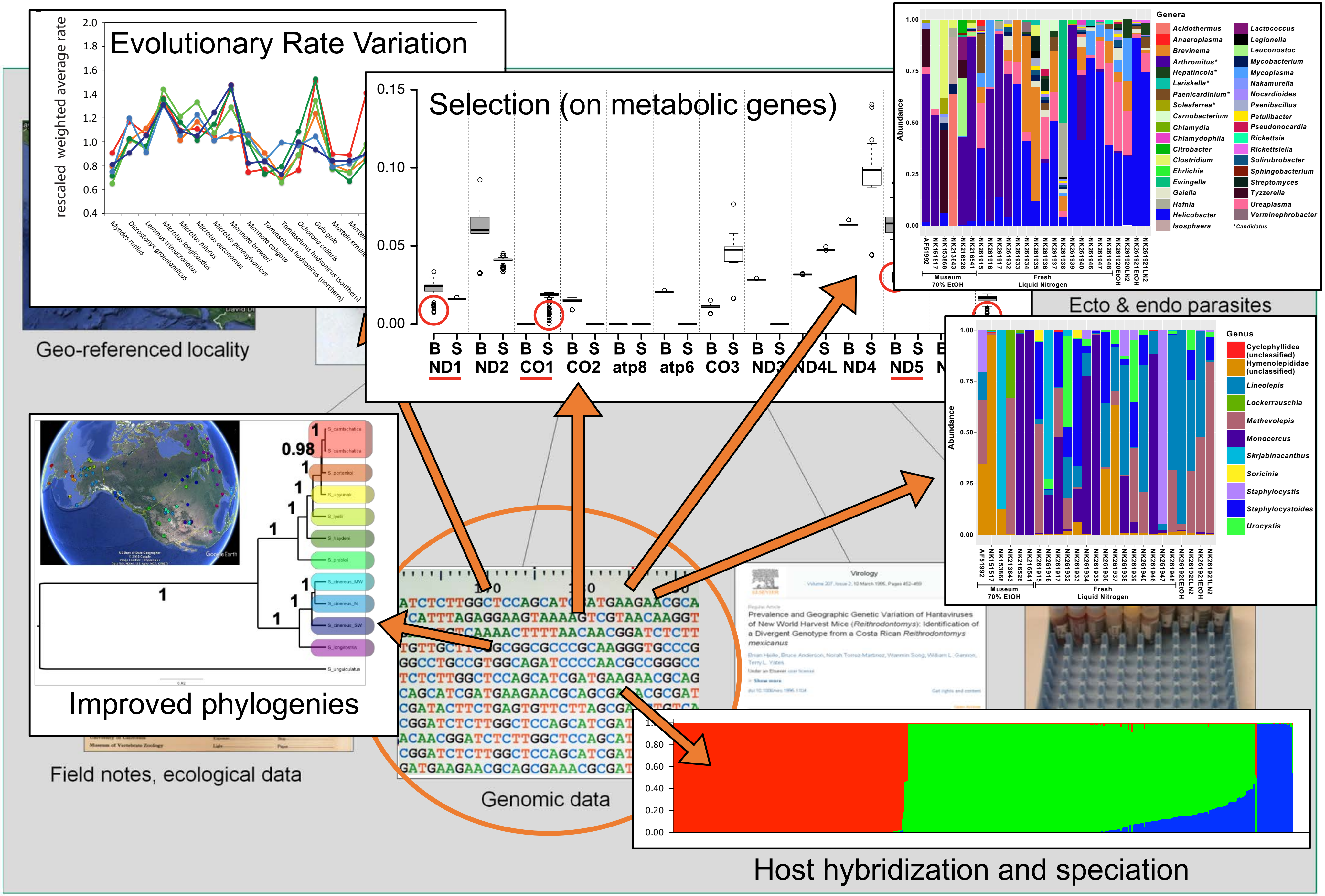
Host hybridization and speciation

Genomic Contributions

Parasite Metagenomics



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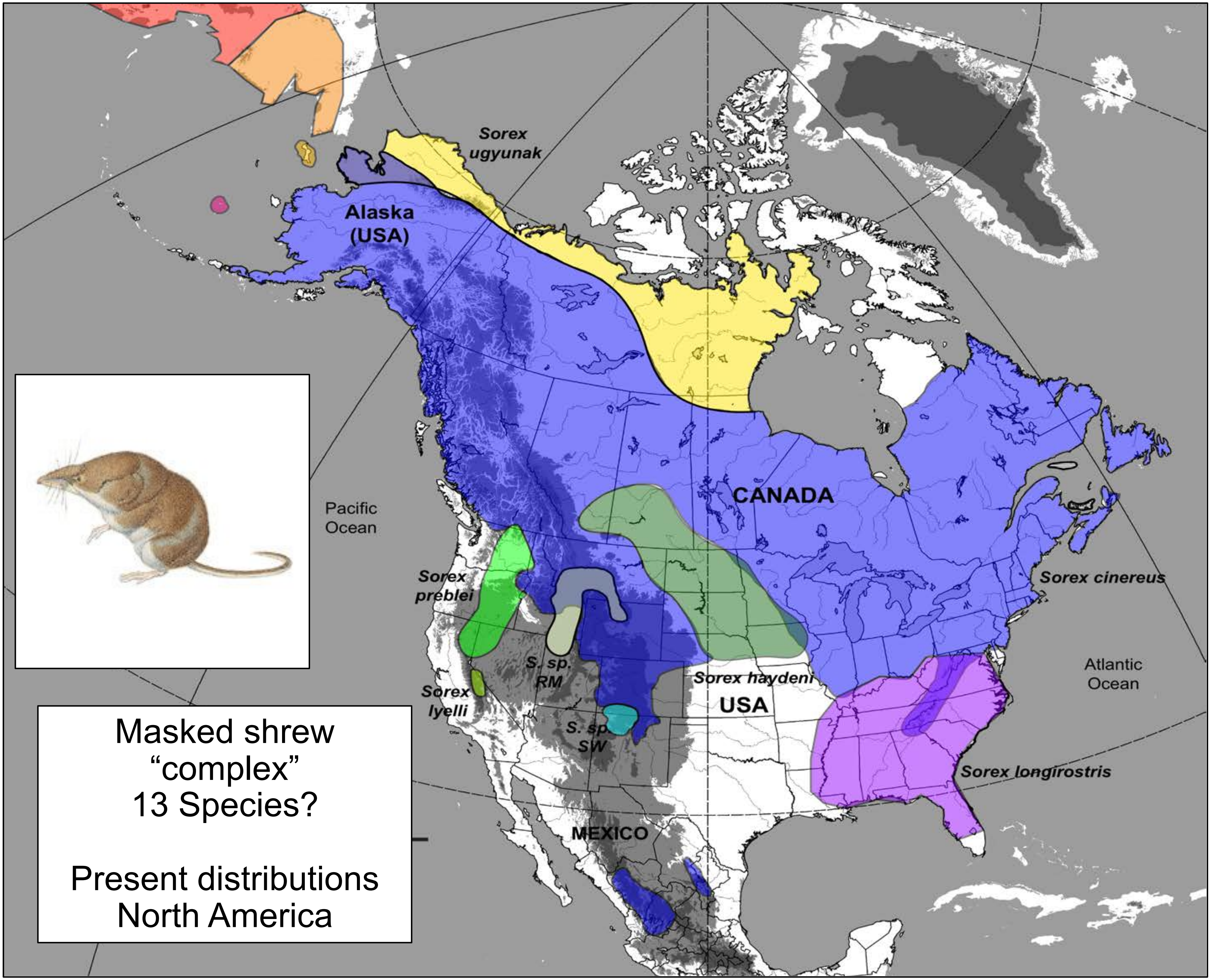
Resolving generalizable processes of change

Shrews, genus *Sorex* ~80 Holarctic species

- Tiny, abundant, ubiquitous
- High metabolism, turnover → rapid evolution
- Insectivorous → complex parasite life cycles

Masked shrew complex

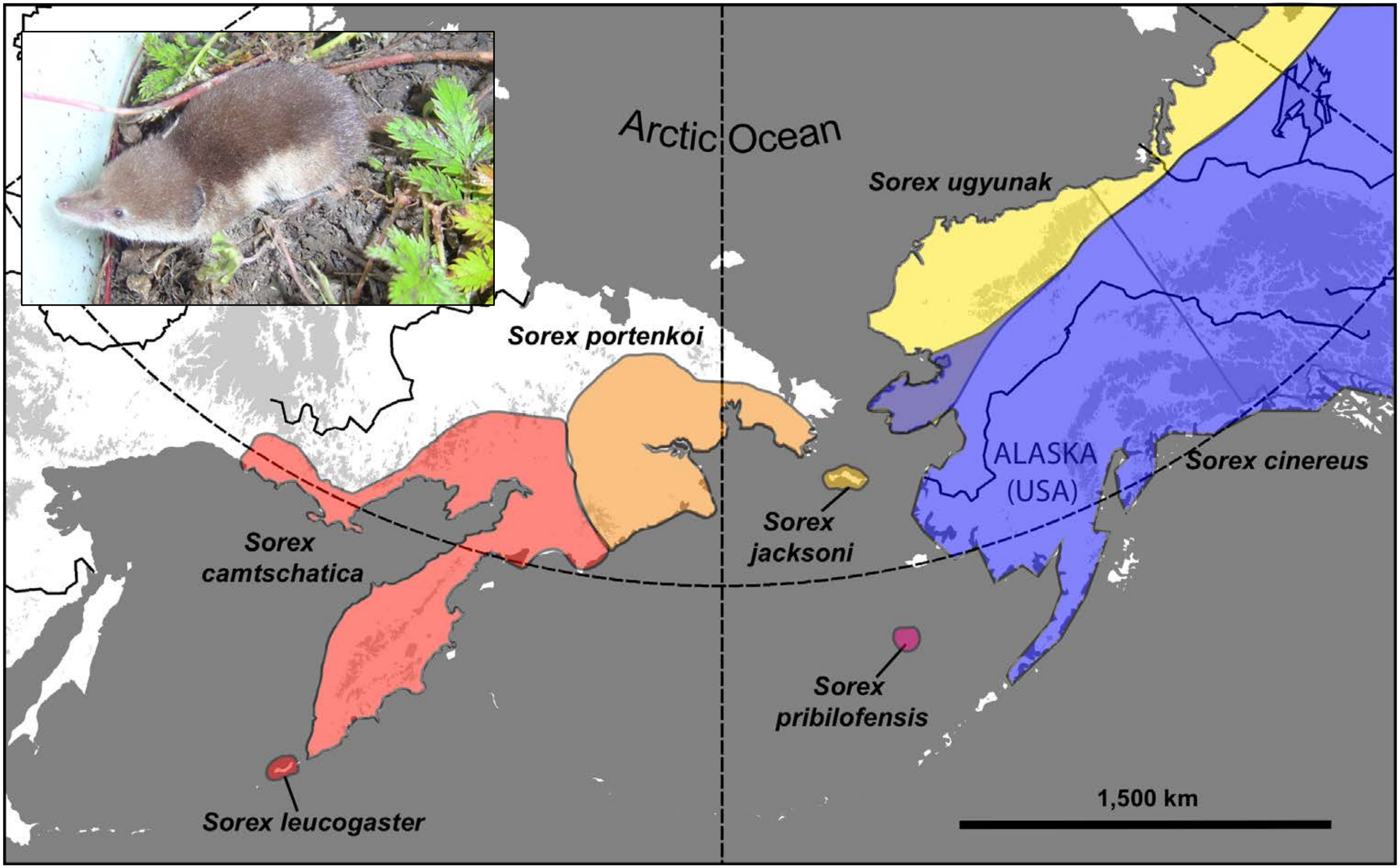
- 13 described species
  - Closely related
  - Complex geographic distribution
- Diverse macro- and micro-parasite faunas
  - Communities within communities
  - Cascading change
- Multiple contact zones
  - Hybridization, competition, speciation
  - Naturally replicated experiment:
    - **Evolutionary, geographic, ecological, temporal…**



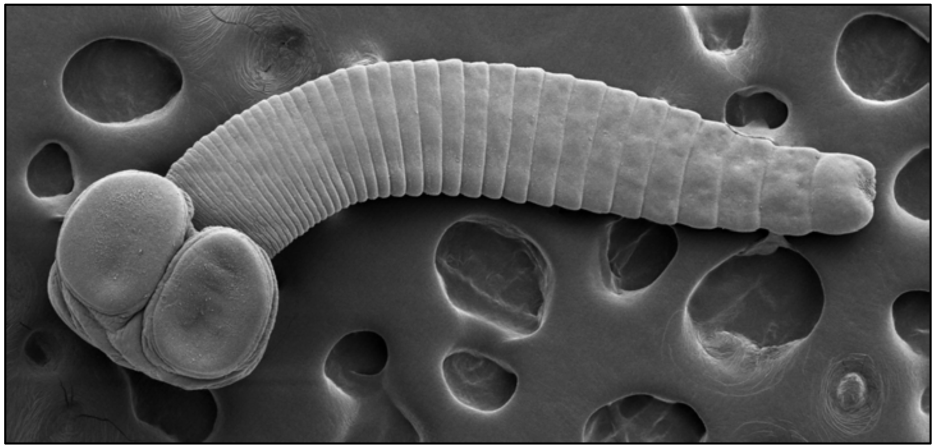
Masked shrew
“complex”
13 Species?

Present distributions
North America

Present distributions: Beringia



Interdependent communities

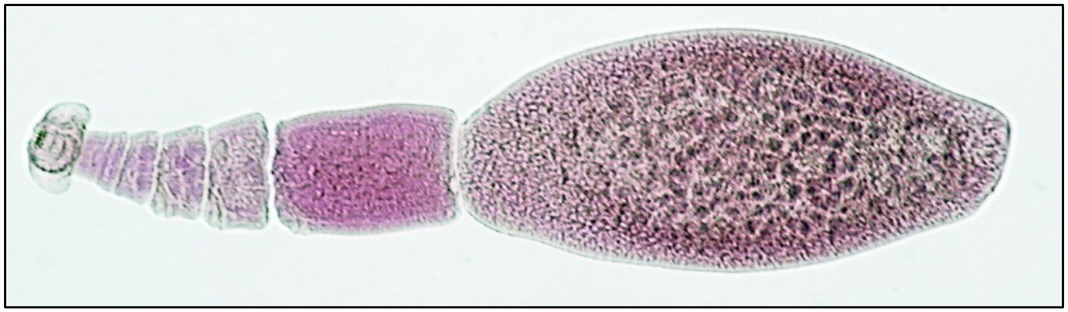


New Genus

Lineolepis sp.

Mathevolepis sp.

Urocystis sp. n.



Staphylocystoides asketus

Shrew endoparasites - helminths

- Highly diverse with complex life cycles
- Variable levels of host specificity
- Woefully underexplored!

Tkach et al.
in prep (x3)

Goals

1. Investigate species limits within *cinereus* group
    - Phylogeographic history
    - Phylogenomics
    - Selection/Drift

2. Characterize **shrew** hybridization
    - Are dynamics repeatable across multiple hybrid zones?
    - Function of conserved vs. introgressed genome regions
    - Role in continued diversification

3. Understand changes in associated **parasite** diversity
    - **Evolutionary, geographic, ecological, temporal…**
    - Relative host specificity across hybrid zones
    - Cascading impacts of change
        - Shrew → helminths → microbiome

1. Shrew species limits

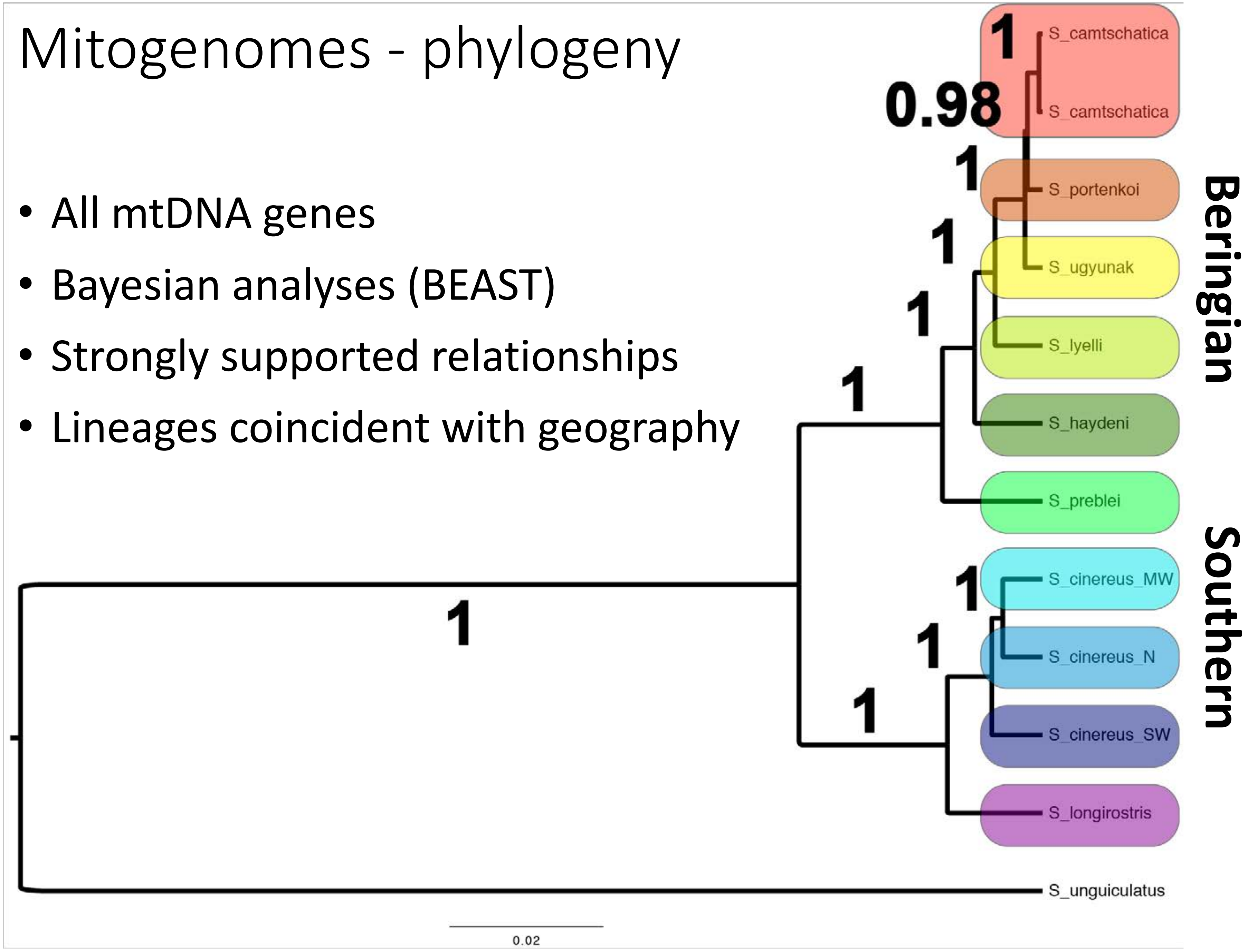
- Shotgun sequencing of mitogenomes
  - 12 shrews on Illumina MiSeq
  - High coverage (10-350x /bp)

- Expanded mitogenome sequencing (in progress)
  - Long-range PCR of 190 shrews
  - Selection on metabolic genes across environmental gradients

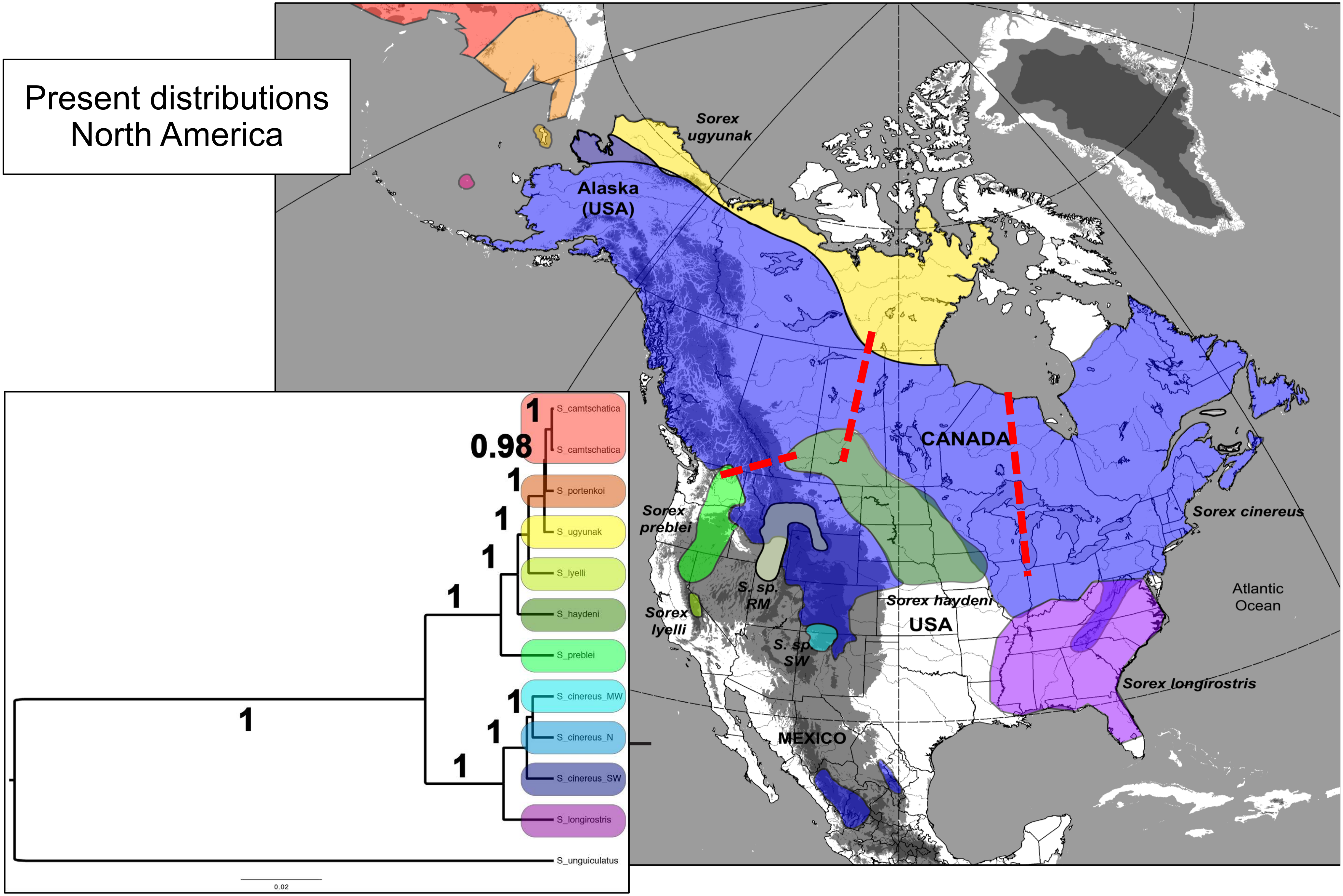
- Nuclear reduced representation sequencing (in development)
  - Selection on nuclear genes (e.g. immune function)
  - Genomics of hybridization

Mitogenomes - phylogeny

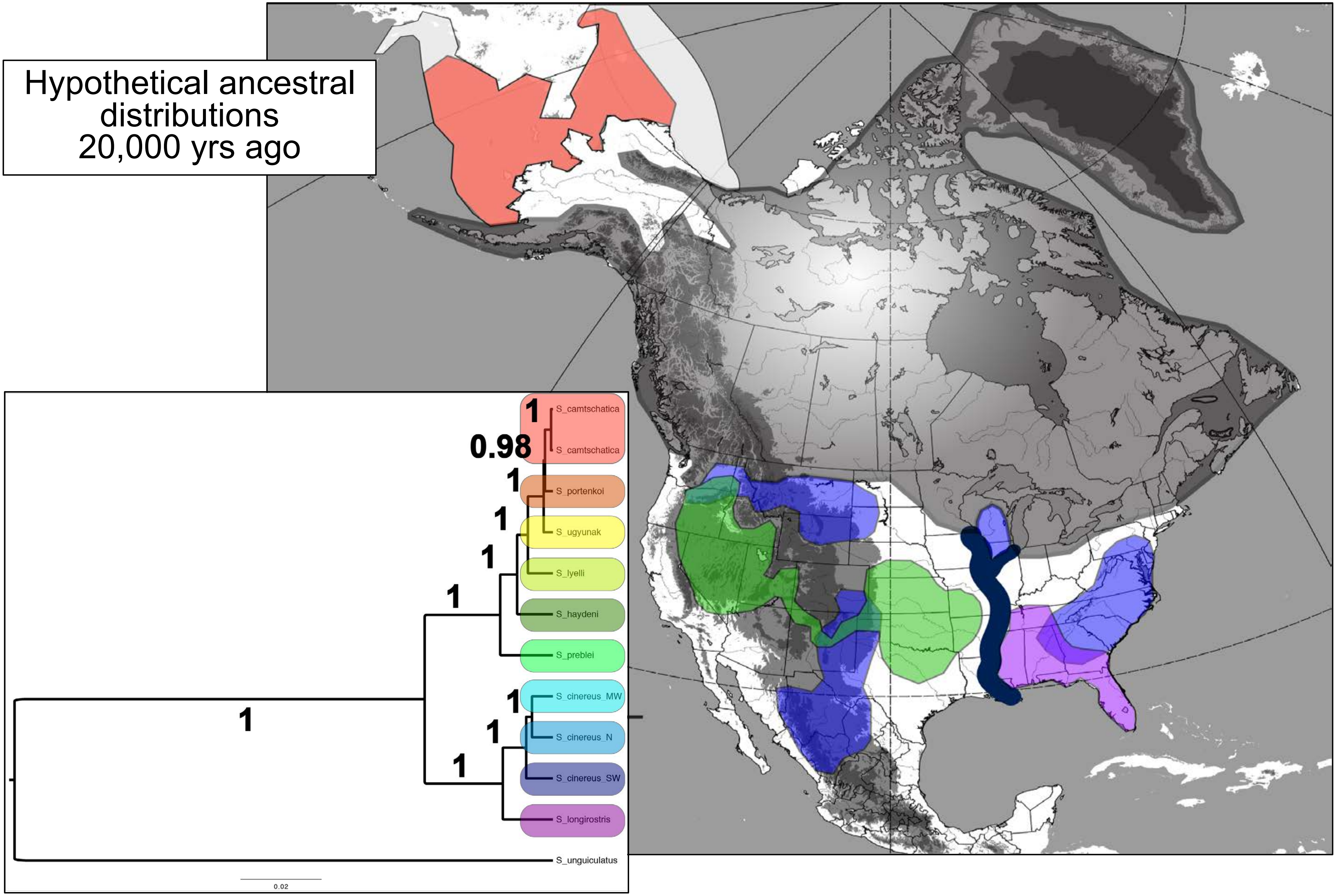
- All mtDNA genes
- Bayesian analyses (BEAST)
- Strongly supported relationships
- Lineages coincident with geography



Present distributions
North America

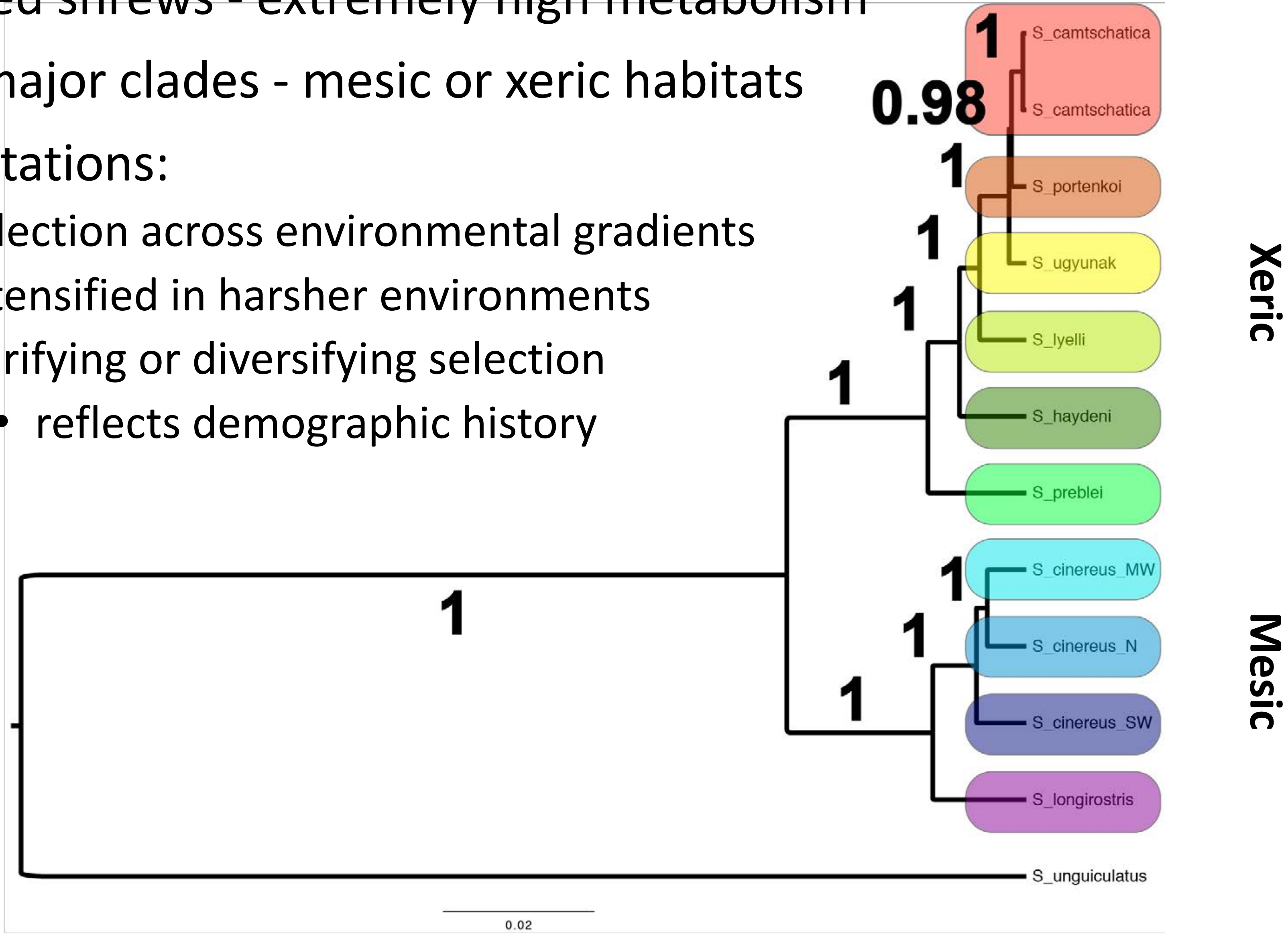


Hypothetical ancestral distributions 20,000 yrs ago



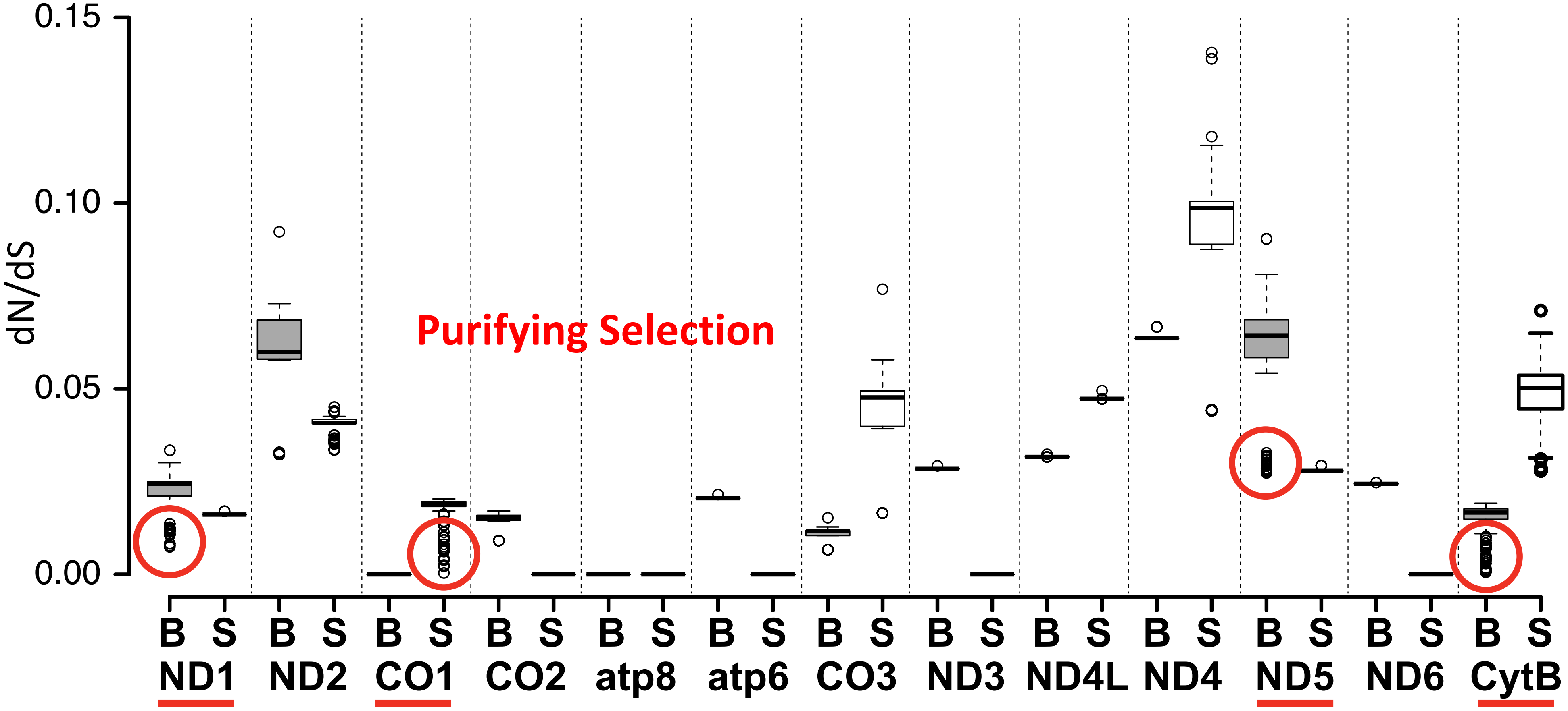
Mitogenomes – adaptation to local environments

- Masked shrews - extremely high metabolism
- Two major clades - mesic or xeric habitats
- Expectations:
  - selection across environmental gradients
  - intensified in harsher environments
  - purifying or diversifying selection
    - reflects demographic history



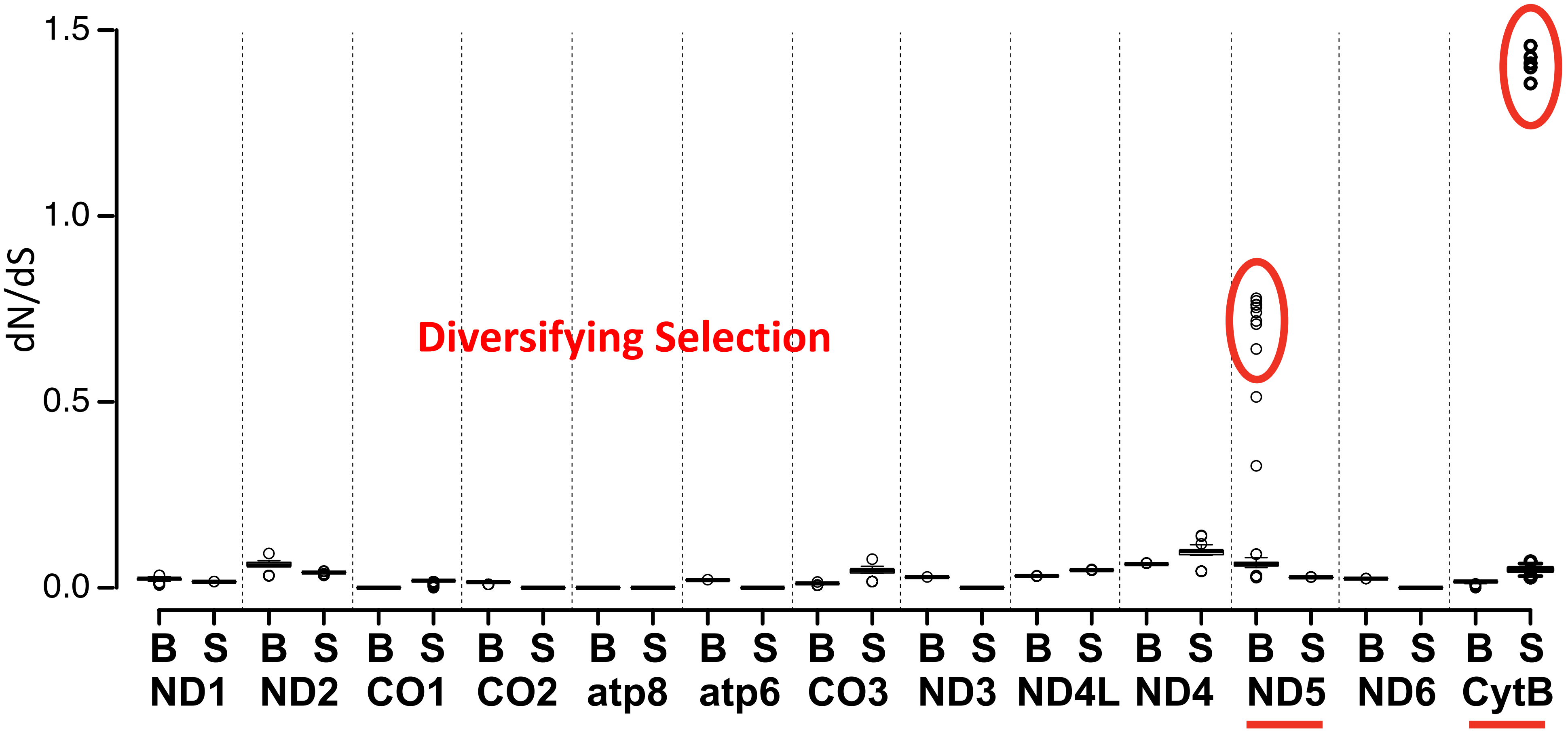
Mitogenomes - selection

- REL (random effects likelihood) tests - HyPhy
- dN/dS ratios
- By site, by branch, by clade
- Only individual sites significant

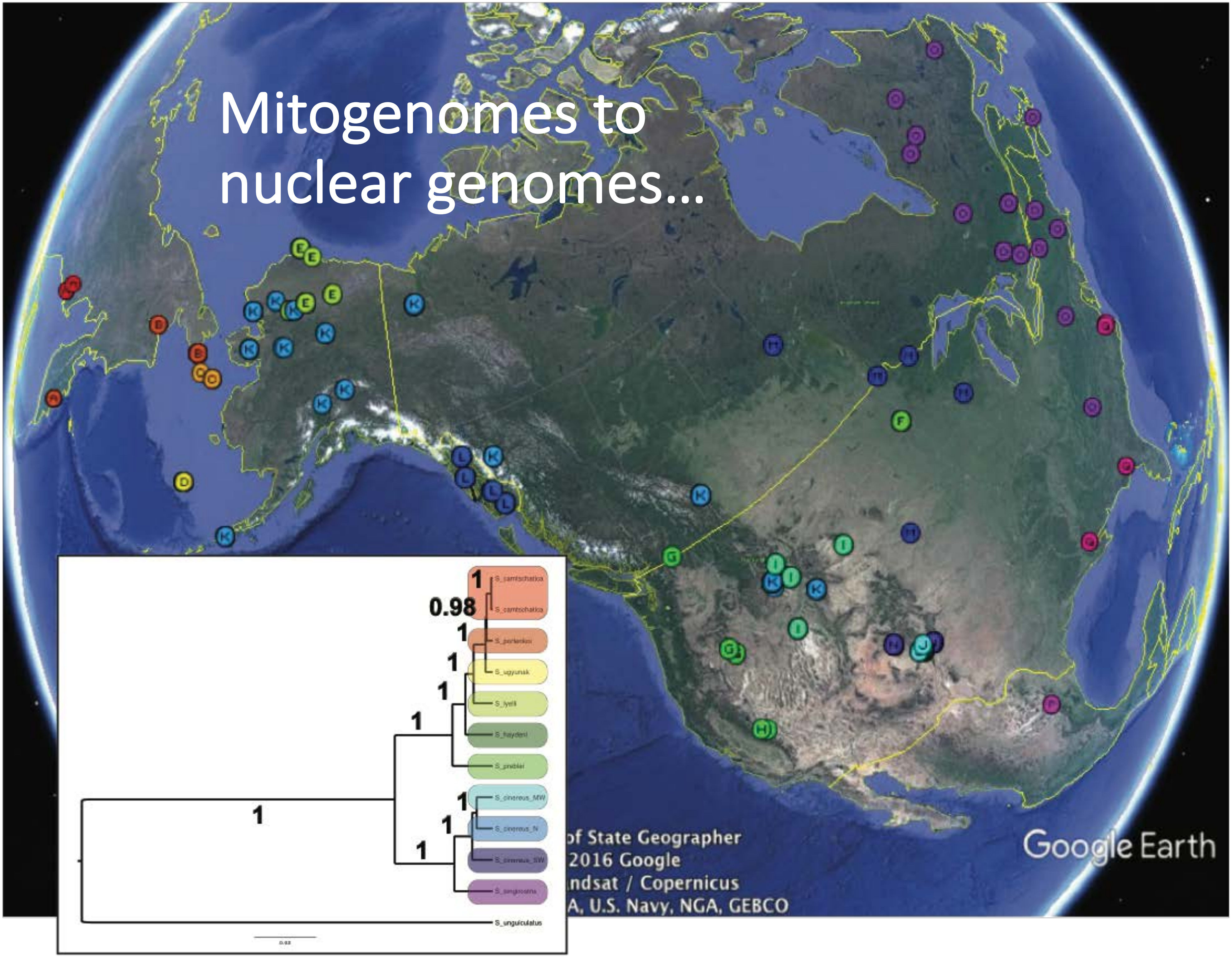


Mitogenomes - selection

- **Zoomed out…**
- Overall very low dN/dS ratios (very low sample size)
- Positive selection in two genes

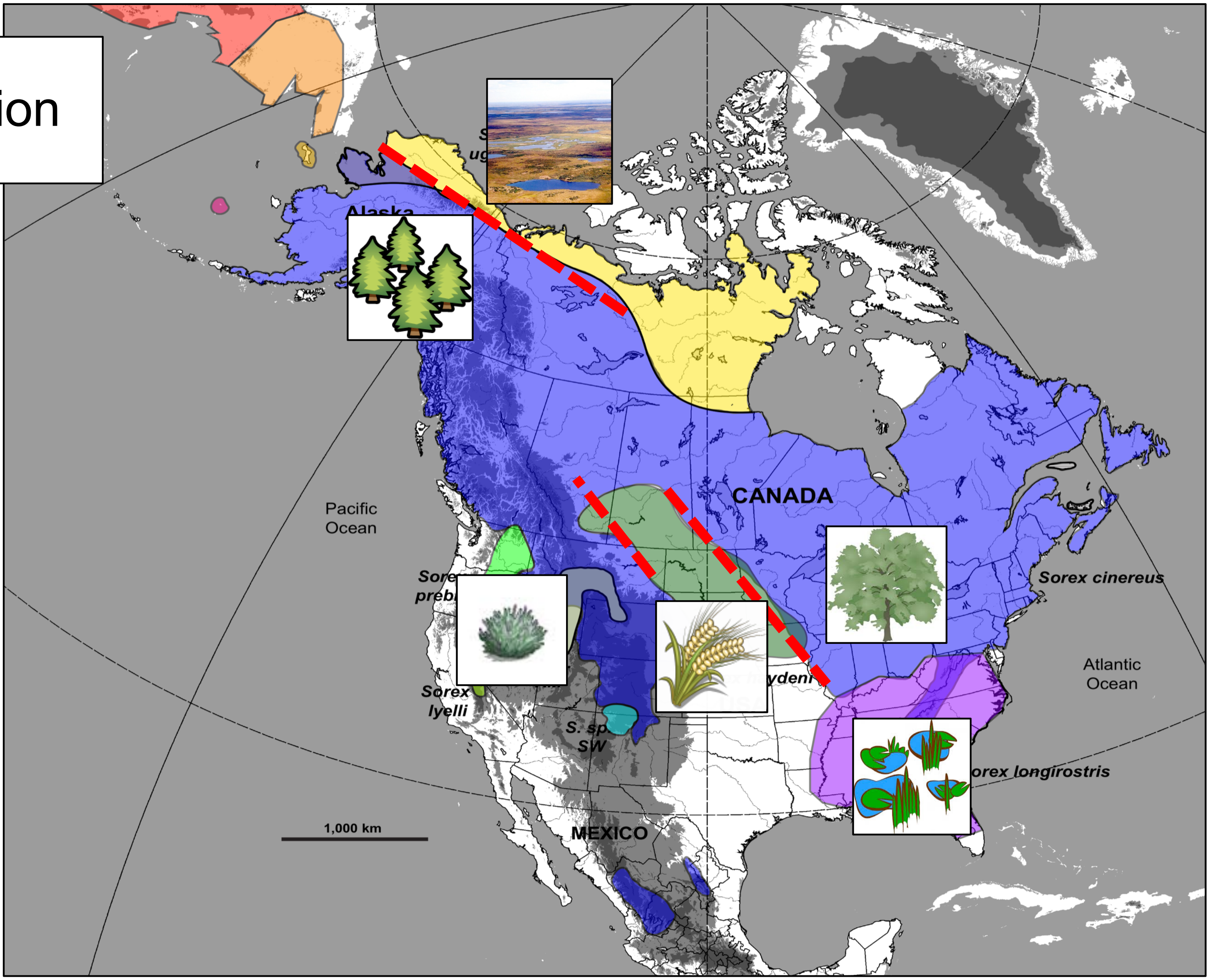


Mitogenomes to nuclear genomes…



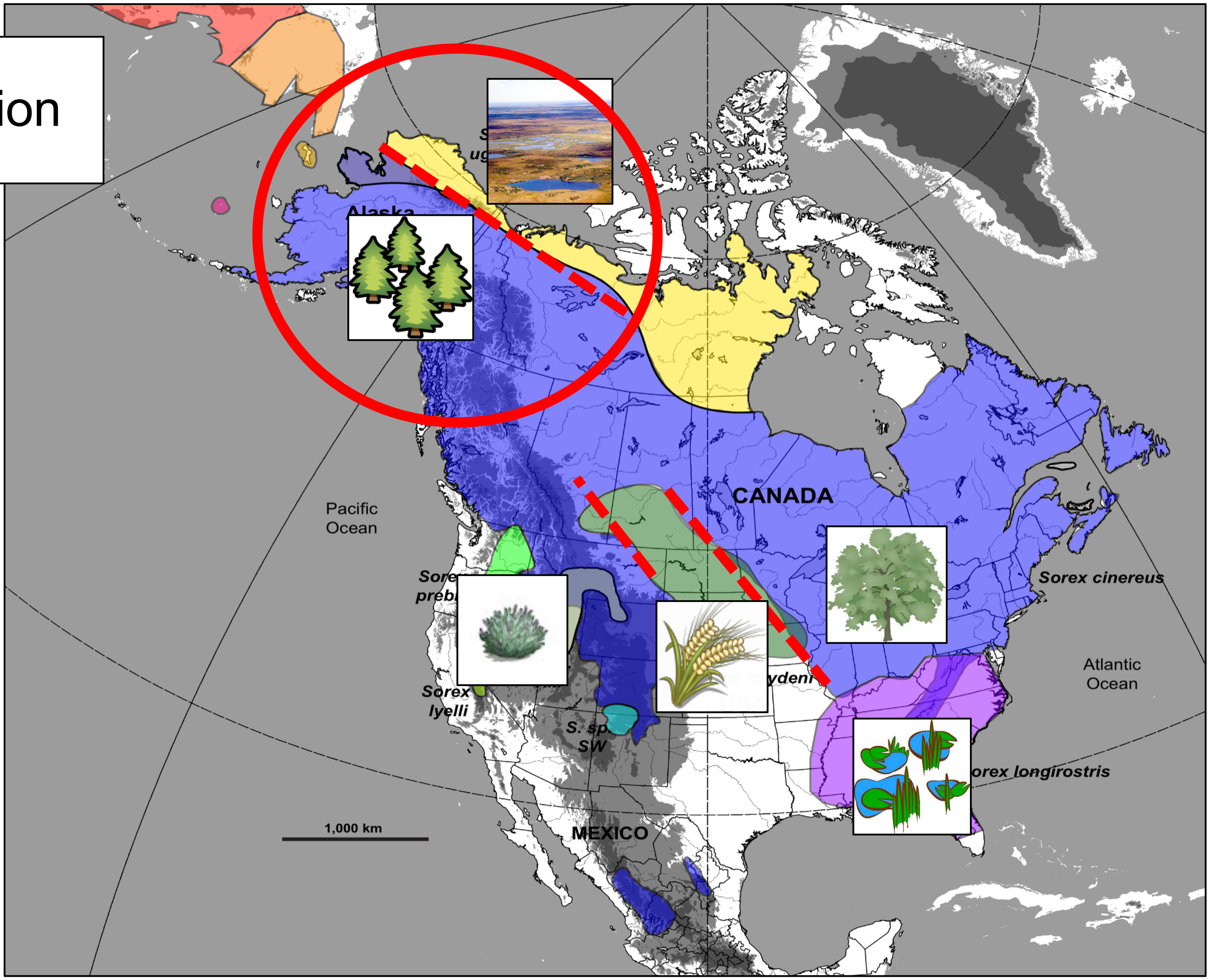
Shrew Hybridization

- Complexity:
  - Evolutionary
  - Geographic
  - Ecological
  - Temporal



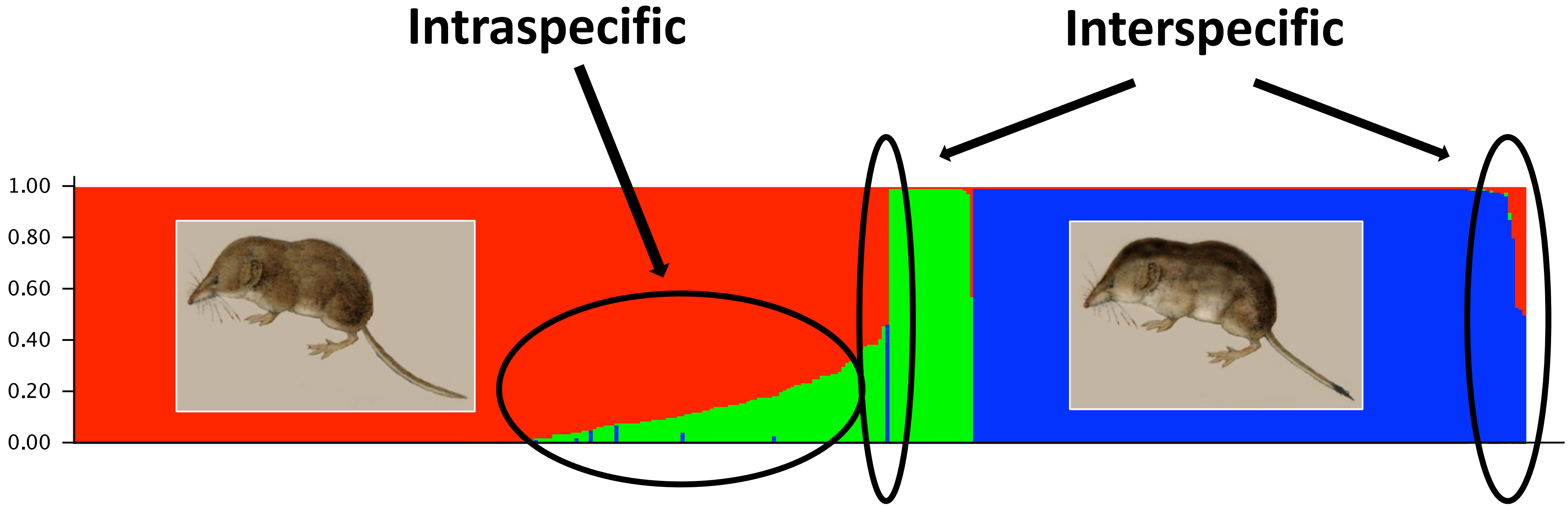
Shrew Hybridization

- Complexity:
  - Evolutionary
  - Geographic
  - Ecological
  - Temporal



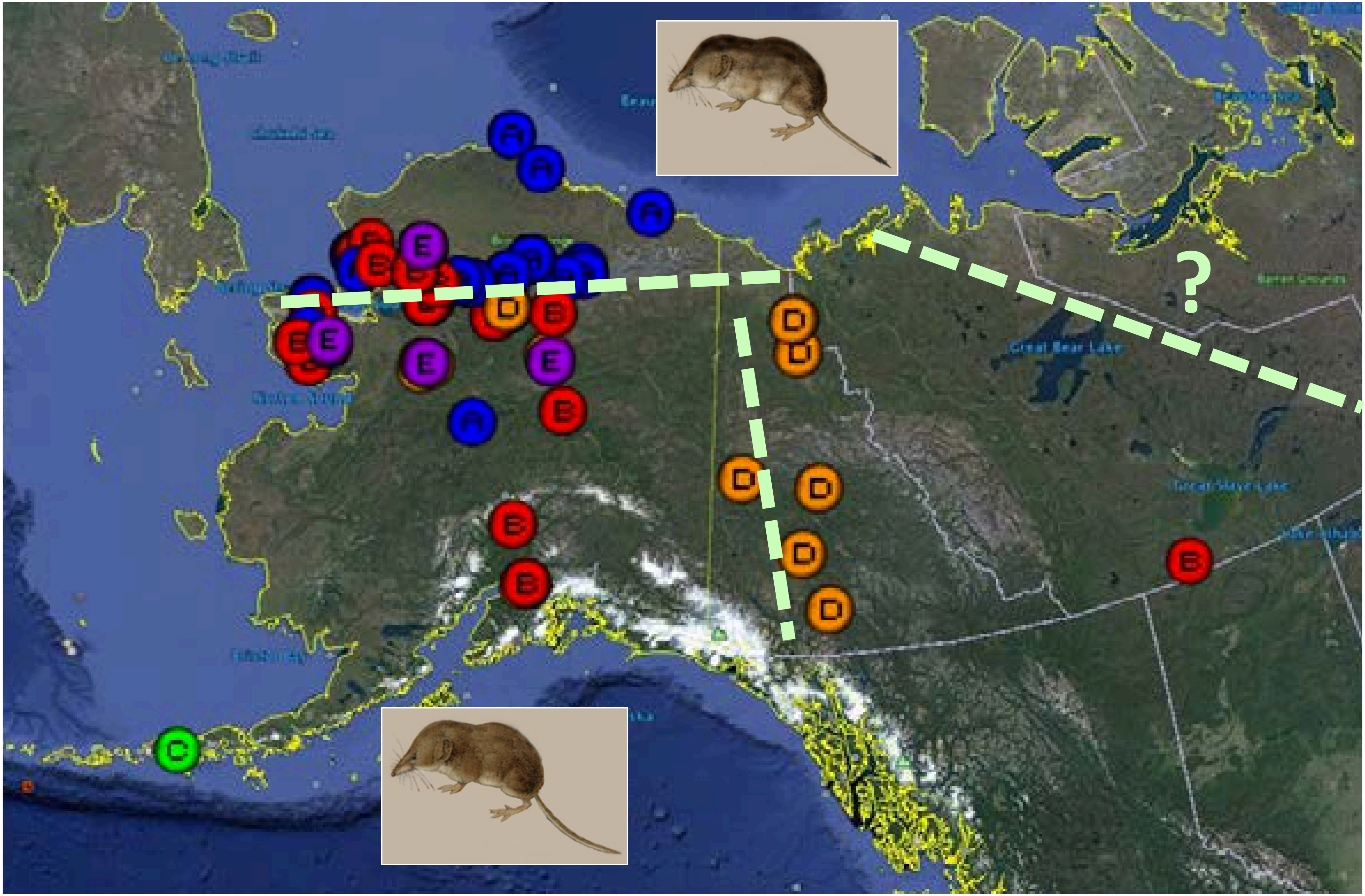
2. Shrew Hybridization

- Shotgun sequencing (MiSeq) to develop >20 microsatellites
- Rigorous sampling across boreal-tundra contact zone
  - *S. cinereus* vs. *S. ugyunak*
  - ~400 archived specimens



Intraspecific

Interspecific


A
B
C
D
E
?
Great Bear Lake
Great Slave Lake

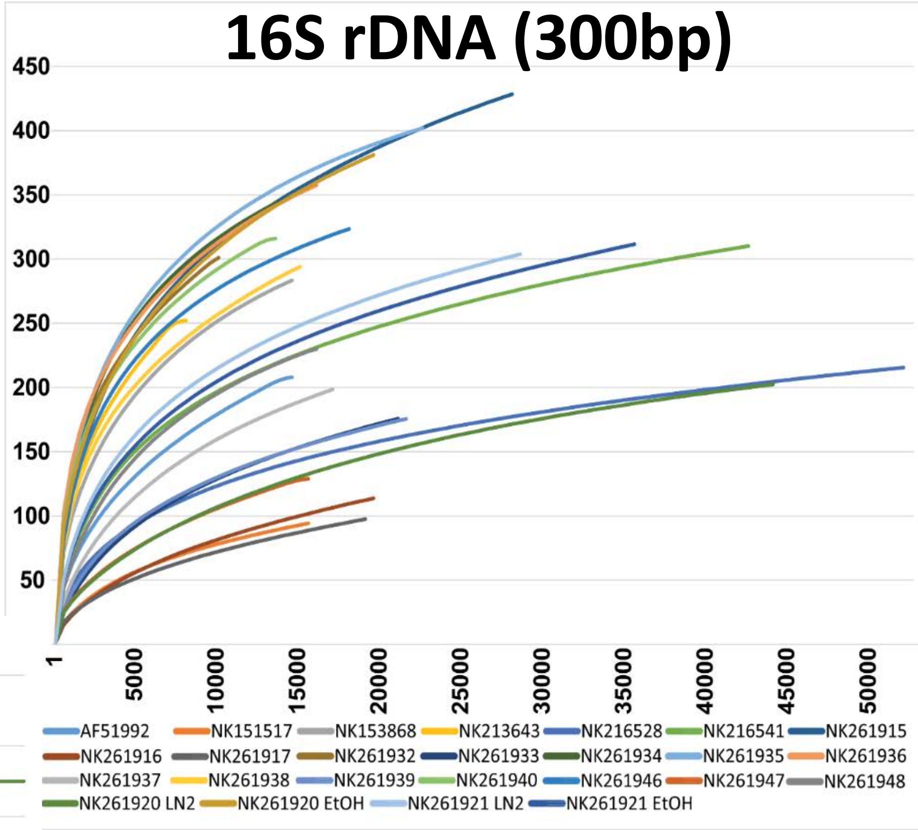
3. Variation in Intestinal Biodiversity

- Metagenomic methods – 12S, 16S, 28S; MiSeq
  - Spatial gradients across host contact zones
  - In-situ temporal change using museum specimens

- Parasite (helminths)
  - Biodiversity discovery
  - Host-parasite co-evolution and phylogeography
  - Parasite hybridization, host switching
- Microbiomes
  - Fluctuating pathogens, commensals, symbionts
    - Temporal, spatial, host variability

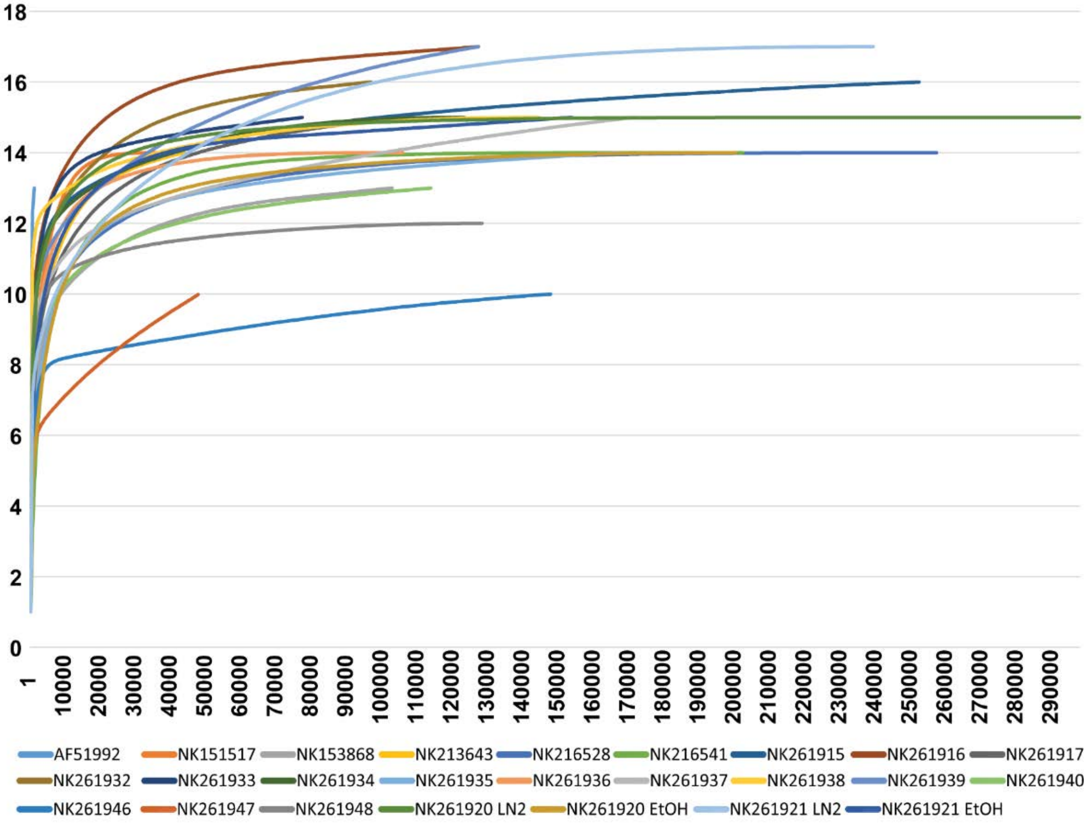
Rarefaction curves

- Parasite OTUs from 25 shrew specimens


16S rDNA (300bp)

Microbiome

16S mtDNA (120bp)

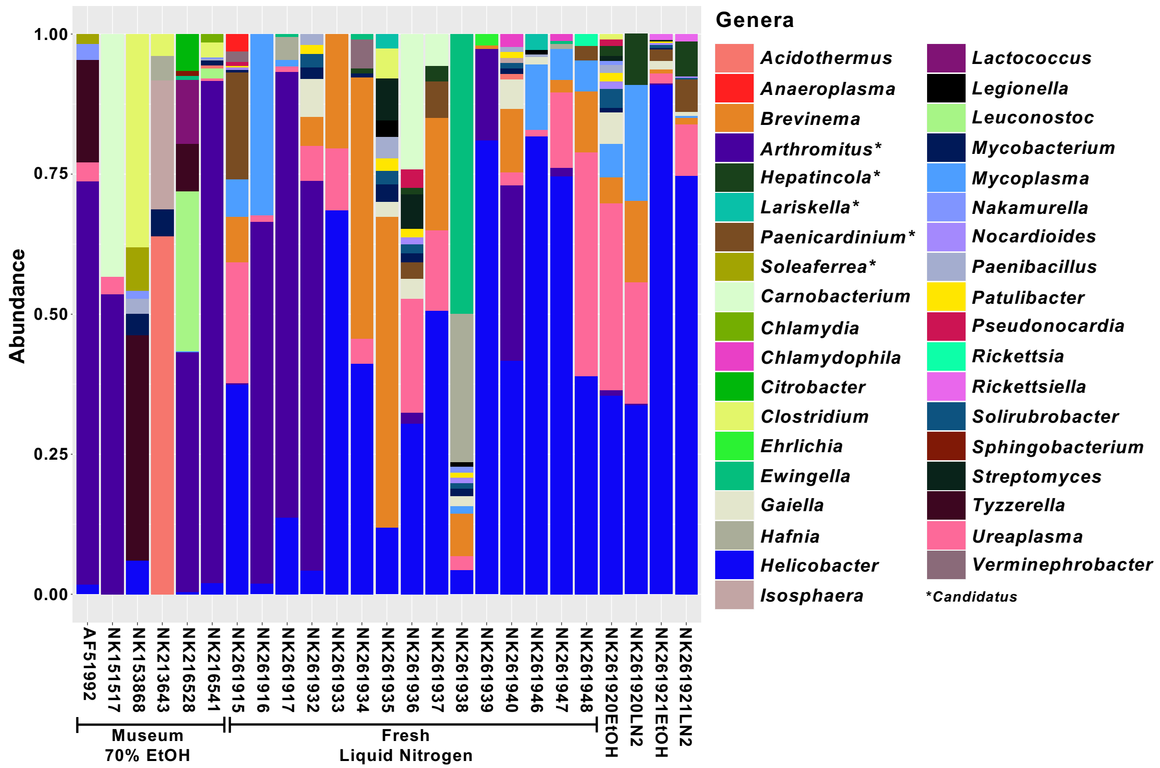


Helminths

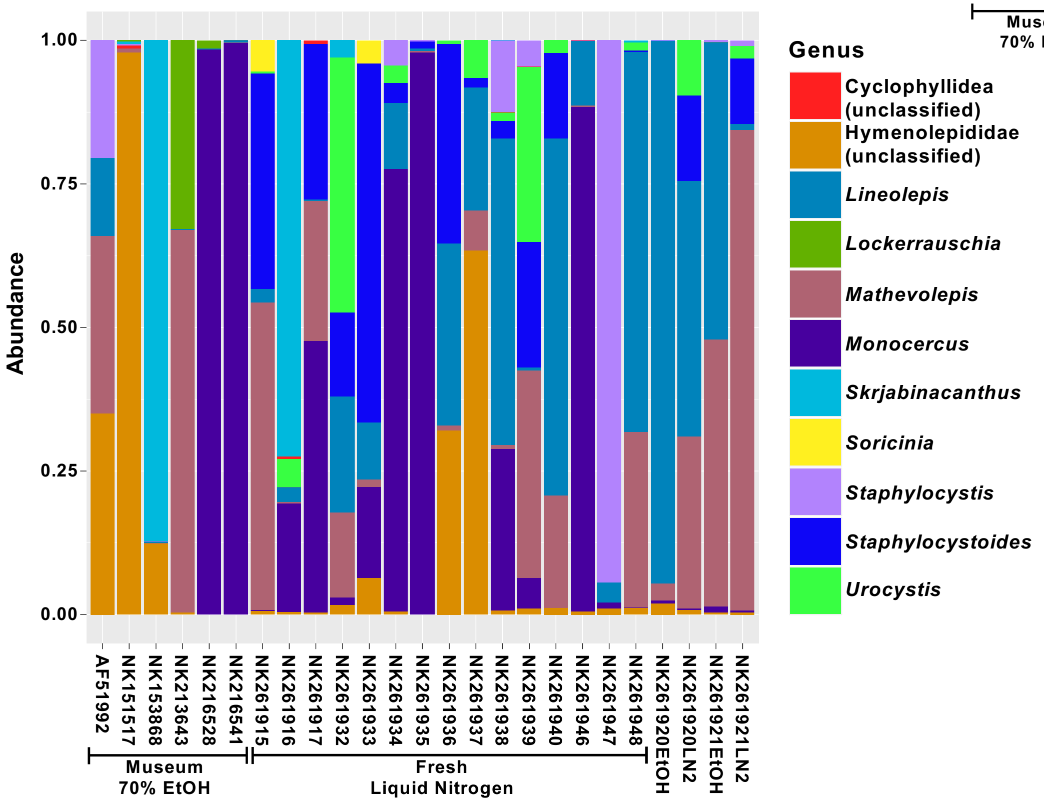
Greiman et al., *in prep.*

Major genera

- Parasites from old and new shrews

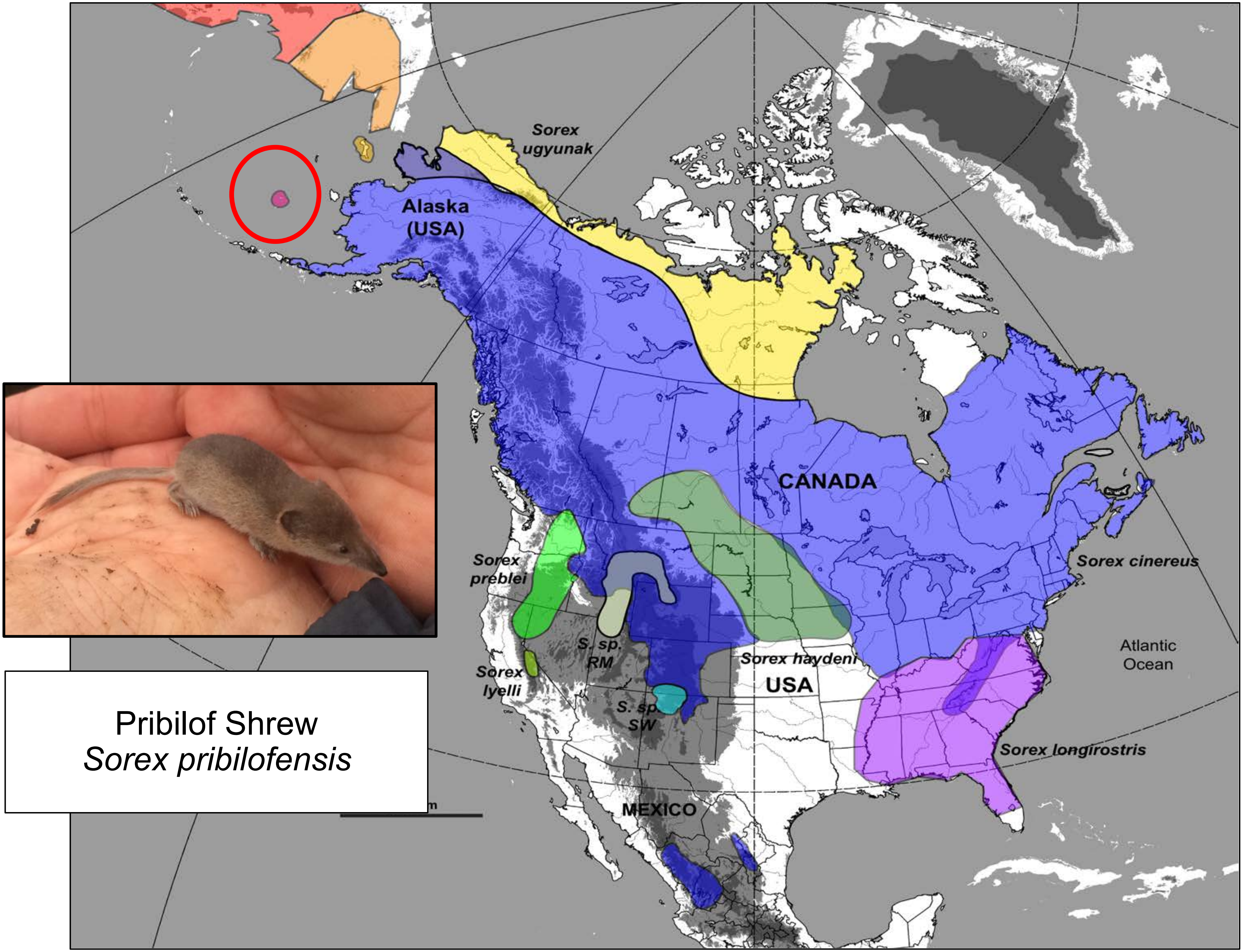


Microbiome



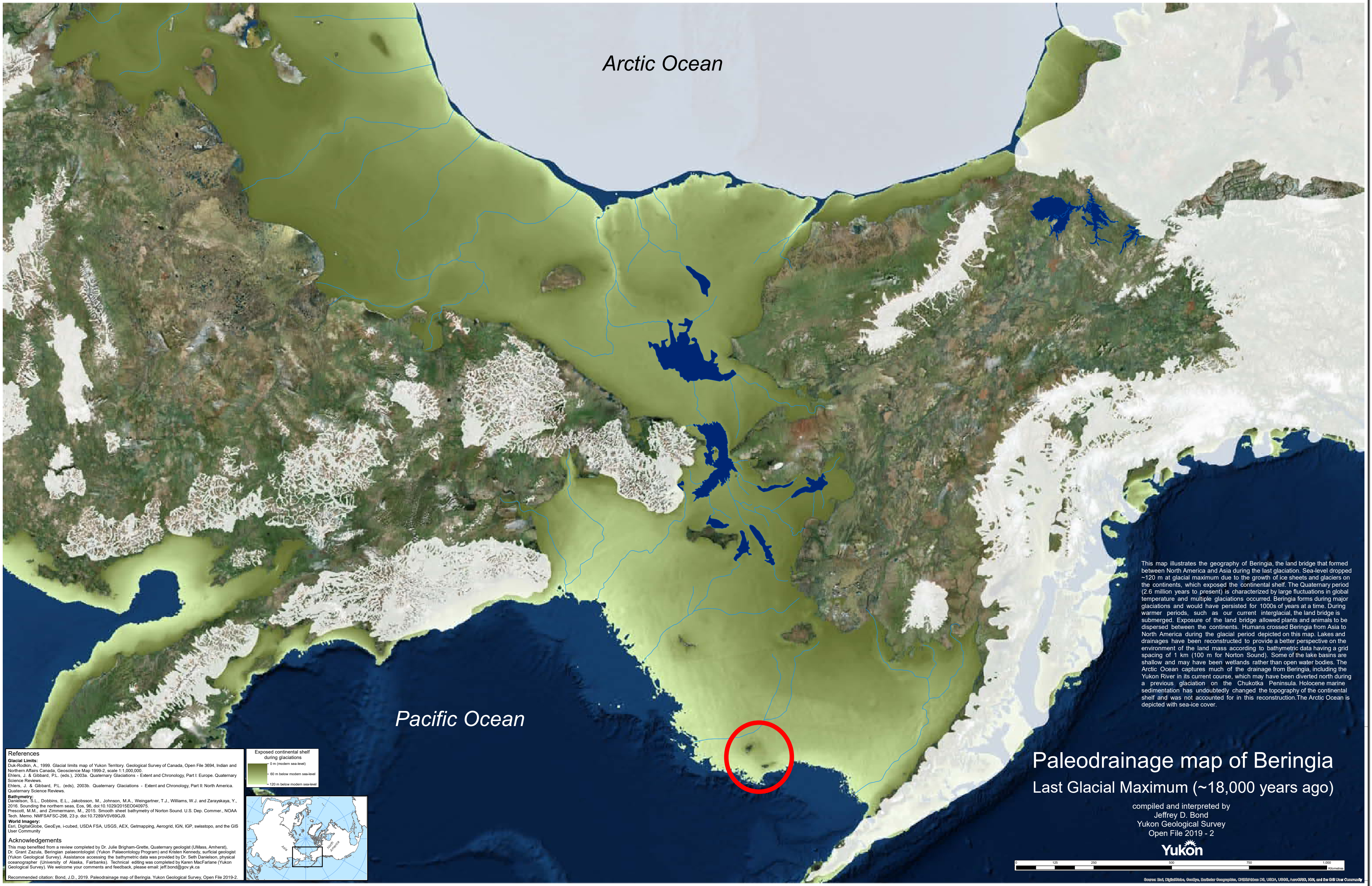
Helminths

Greiman et al., *2018. J.Parasitol.*



Pribilof Shrew
Sorex pribilofensis

St. Paul Island, Alaska.



Paleodrainage map of Beringia

Last Glacial Maximum (~18,000 years ago)

compiled and interpreted by
Jeffrey D. Bond
Yukon Geological Survey
Open File 2019 - 2

This map illustrates the geography of Beringia, the land bridge that formed between North America and Asia during the last glaciation. Sea-level dropped ~120 m at glacial maximum due to the growth of ice sheets and glaciers on the continents, which exposed the continental shelf. The Quaternary period (2.6 million years to present) is characterized by large fluctuations in global temperature and multiple glaciations occurred. Beringia forms during major glaciations and would have persisted for 1000s of years at a time. During warmer periods, such as our current interglacial, the land bridge is submerged. Exposure of the land bridge allowed plants and animals to be dispersed between the continents. Humans crossed Beringia from Asia to North America during the glacial period depicted on this map. Lakes and drainages have been reconstructed to provide a better perspective on the environment of the land mass according to bathymetric data having a grid spacing of 1 km (100 m for Norton Sound). Some of the lake basins are shallow and may have been wetlands rather than open water bodies. The Arctic Ocean captures much of the drainage from Beringia, including the Yukon River in its current course, which may have been diverted north during a previous glaciation on the Chukotka Peninsula. Holocene marine sedimentation has undoubtedly changed the topography of the continental shelf and was not accounted for in this reconstruction.The Arctic Ocean is depicted with sea-ice cover.

Exposed continental shelf during glaciations

0 m (modern sea-level)
60 m below modern sea-level
120 m below modern sea-level

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Danielson, S.L., Dobbins, E.L., Jakobsson, M., Johnson, M.A., Weingartner, T.J., Williams, W.J. and Zarayskaya, Y., 2016. Sounding the northern seas, Eos, 96, doi:10.1029/2015EO040975.

Prescott, M.M., and Zimmermann, M., 2015. Smooth sheet bathymetry of Norton Sound. U.S. Dep. Commer., NOAA Tech. Memo. NMFSAFSC-298, 23 p. doi:10.7289/V5V69GJ9.

World Imagery:

Esri, DigitalGlobe, GeoEye, i-cubed, USDA FSA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

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Recommended citation: Bond, J.D., 2019. Paleodrainage map of Beringia. Yukon Geological Survey, Open File 2019-2.

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Predictions for island biodiversity

1. Low species richness – faunal relaxation
    - Species interdependence → cascades (more extreme in north)
    - Low host richness → low parasite richness
2. Low genetic diversity
    - Small populations → periodic bottlenecks
    - Loss of diversity through rapid fixation of alleles (purging)
    - Lowered fitness?
3. High endemism
    - Divergence following fragmentation (high drift)
4. Host parasite evolutionary concordance?

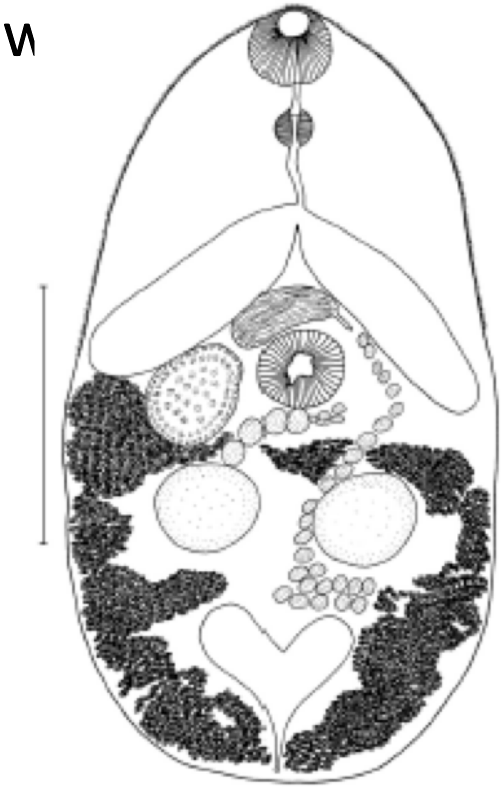
Mammals of St. Paul Island

Mammals of St. Paul Island

Obtained 22 frozen shrews from Aleut Community on St. Paul

Results – parasite diversity

- Very low Richness - 2 species of endoparasite
  - Cestode – *Lineolepis pribilofensis*
  - Trematode – *Maritrema* sp. → **Normally in birds!!**
- Very high parasite loads
  - Multiple hundreds of worms per shrew



Karpenko and Dokuchaev 2012

Results – shrew genetic diversity

- Cytochrome b gene (1140bp) for 22 shrews
- Virtually no genetic variability on St. Paul.

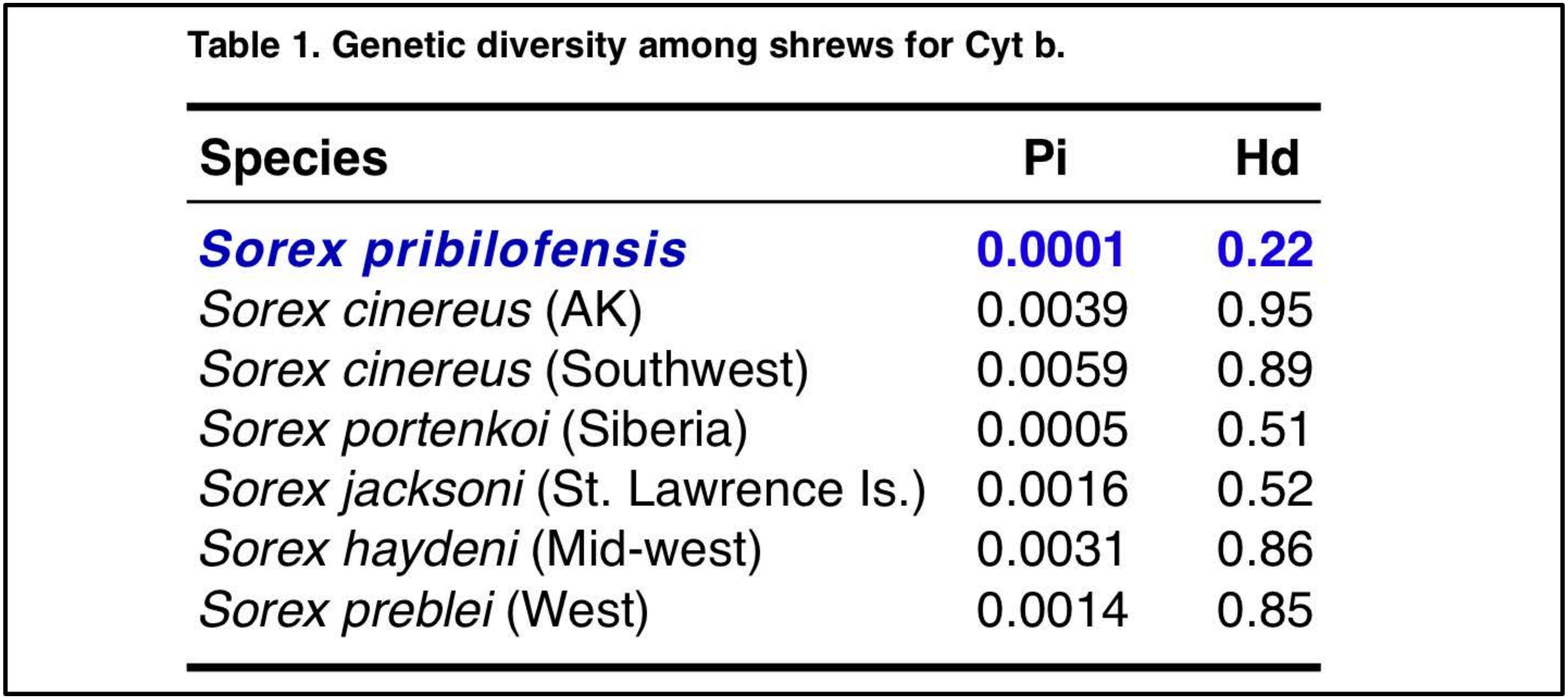
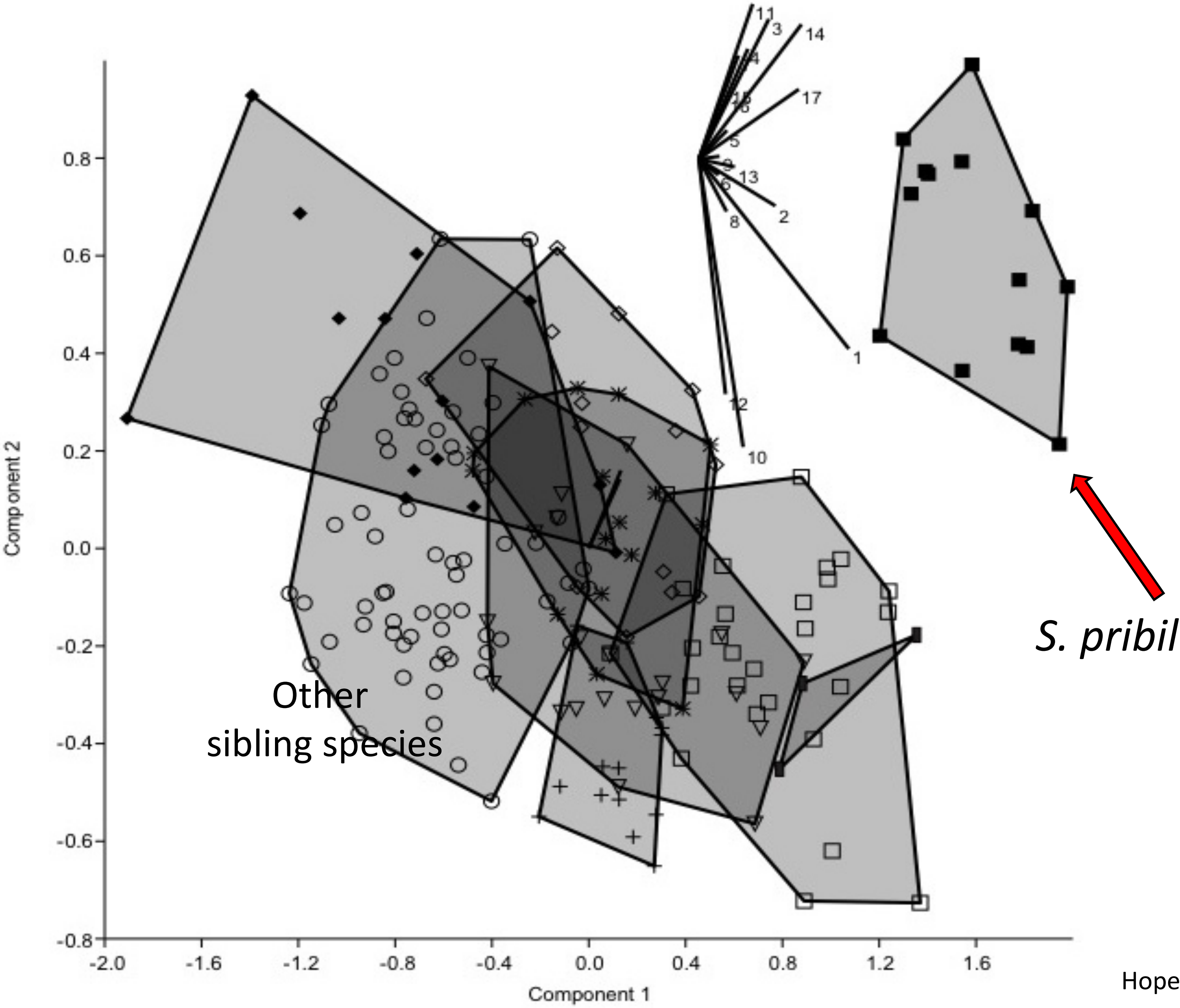


Table 1. Genetic diversity among shrews for Cyt b.

Species	**Pi**	**Hd**
Sorex pribilofensis	**0.0001**	**0.22**
Sorex cinereus (AK)	0.0039	0.95
Sorex cinereus (Southwest)	0.0059	0.89
Sorex portenkoi (Siberia)	0.0005	0.51
Sorex jacksoni (St. Lawrence Is.)	0.0016	0.52
Sorex haydeni (Mid-west)	0.0031	0.86
Sorex preblei (West)	0.0014	0.85

Shrew morphology – PCA



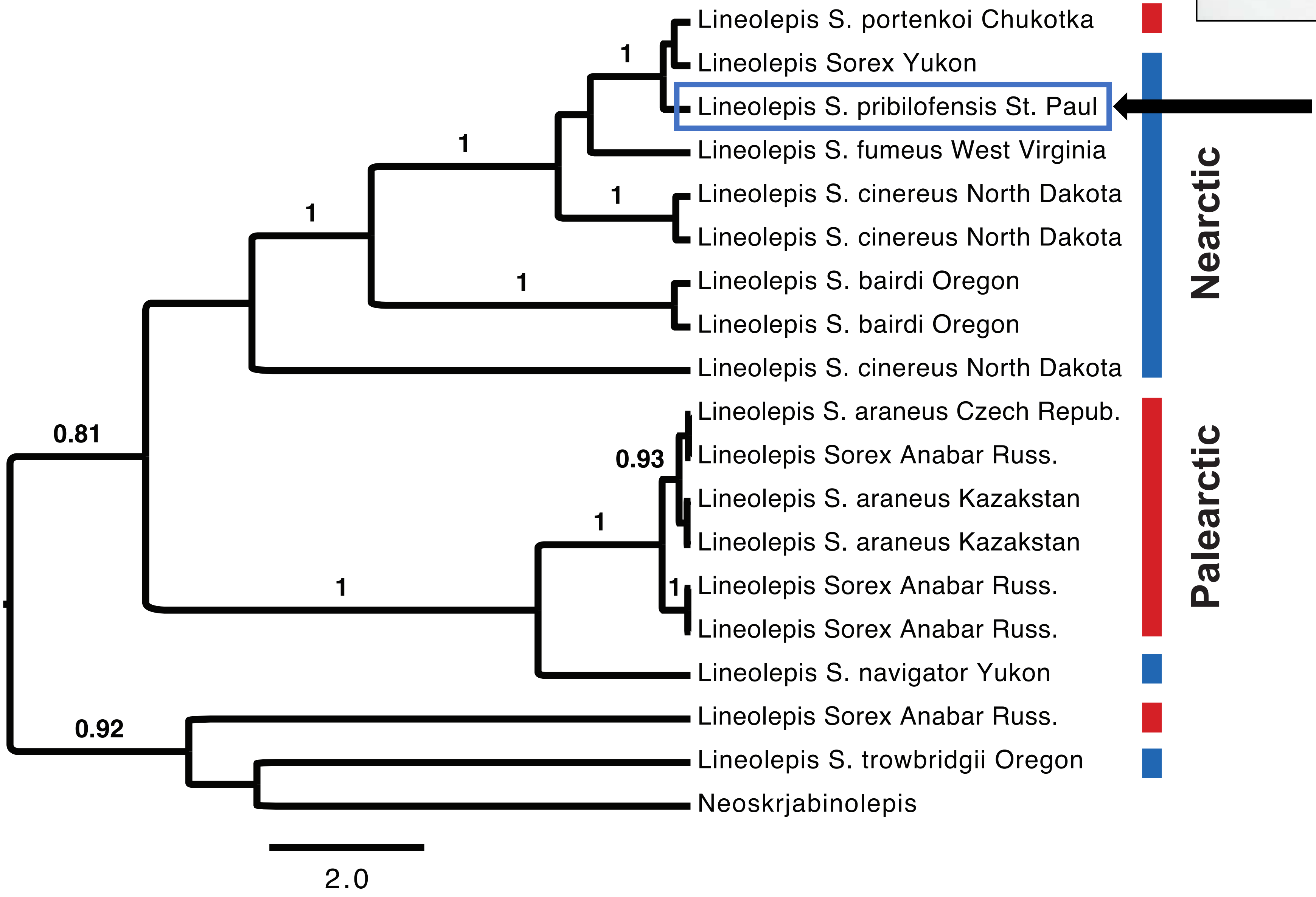
Other sibling species

S. pribilofensis

Hope et al. *in prep*

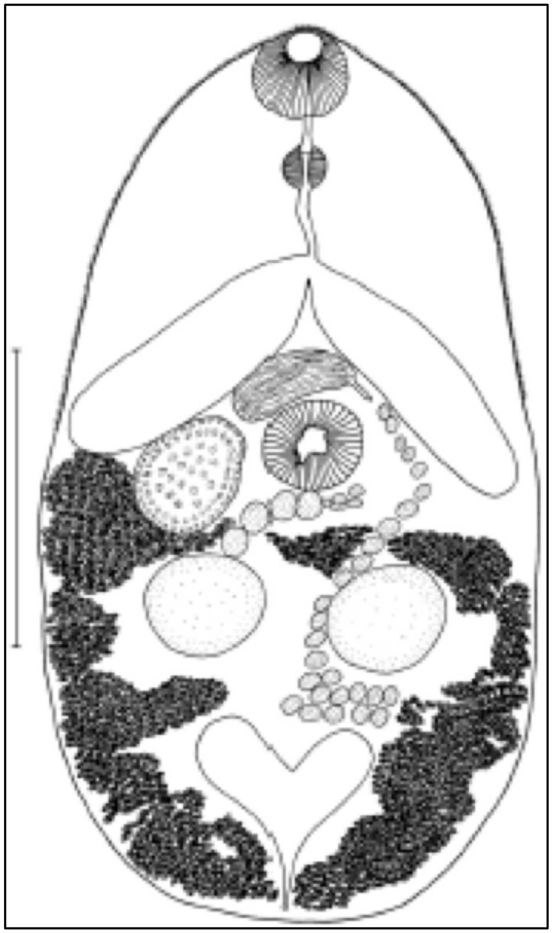
Results – co-evolution?

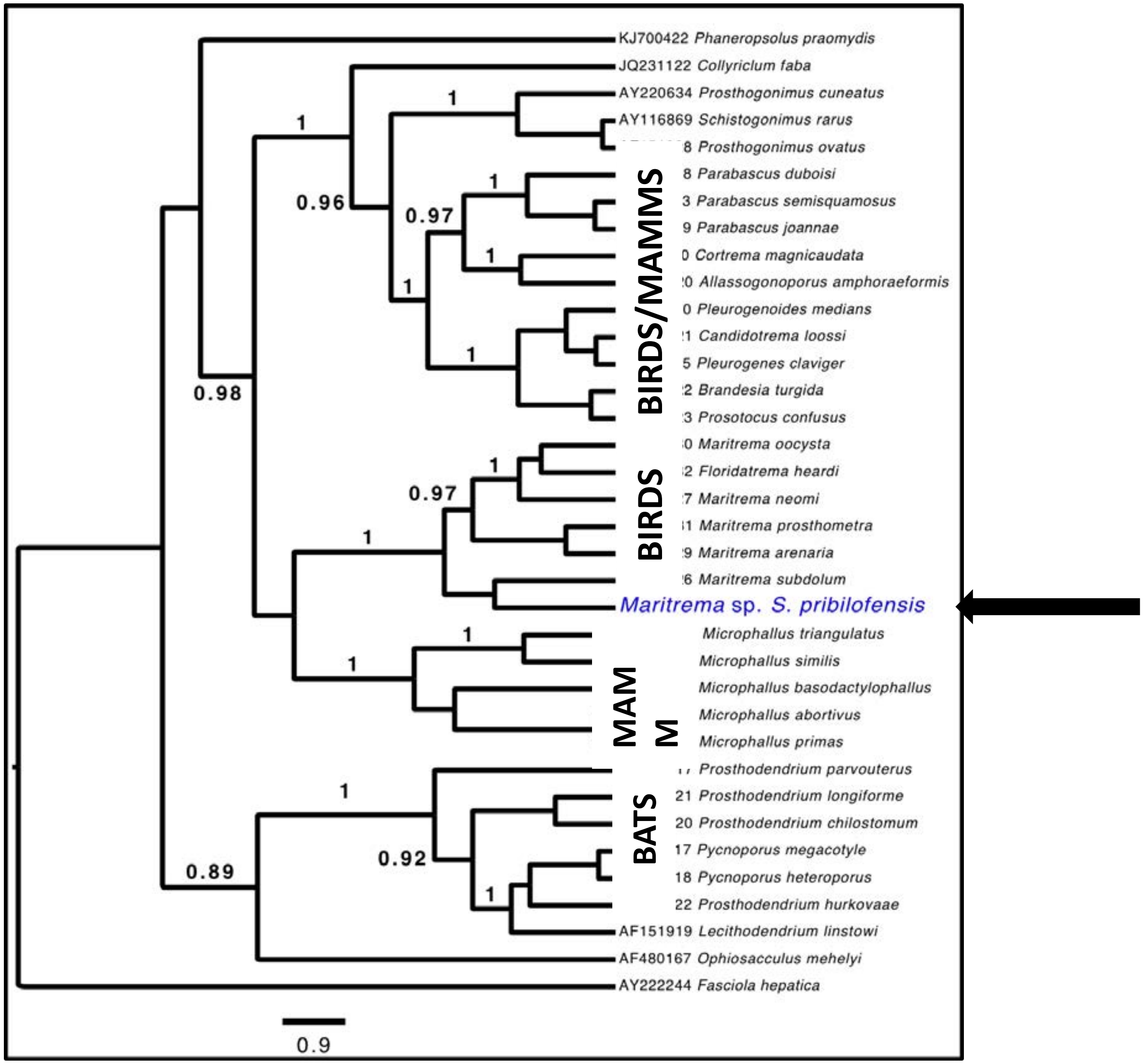
- Shrew cestode with North American origin.



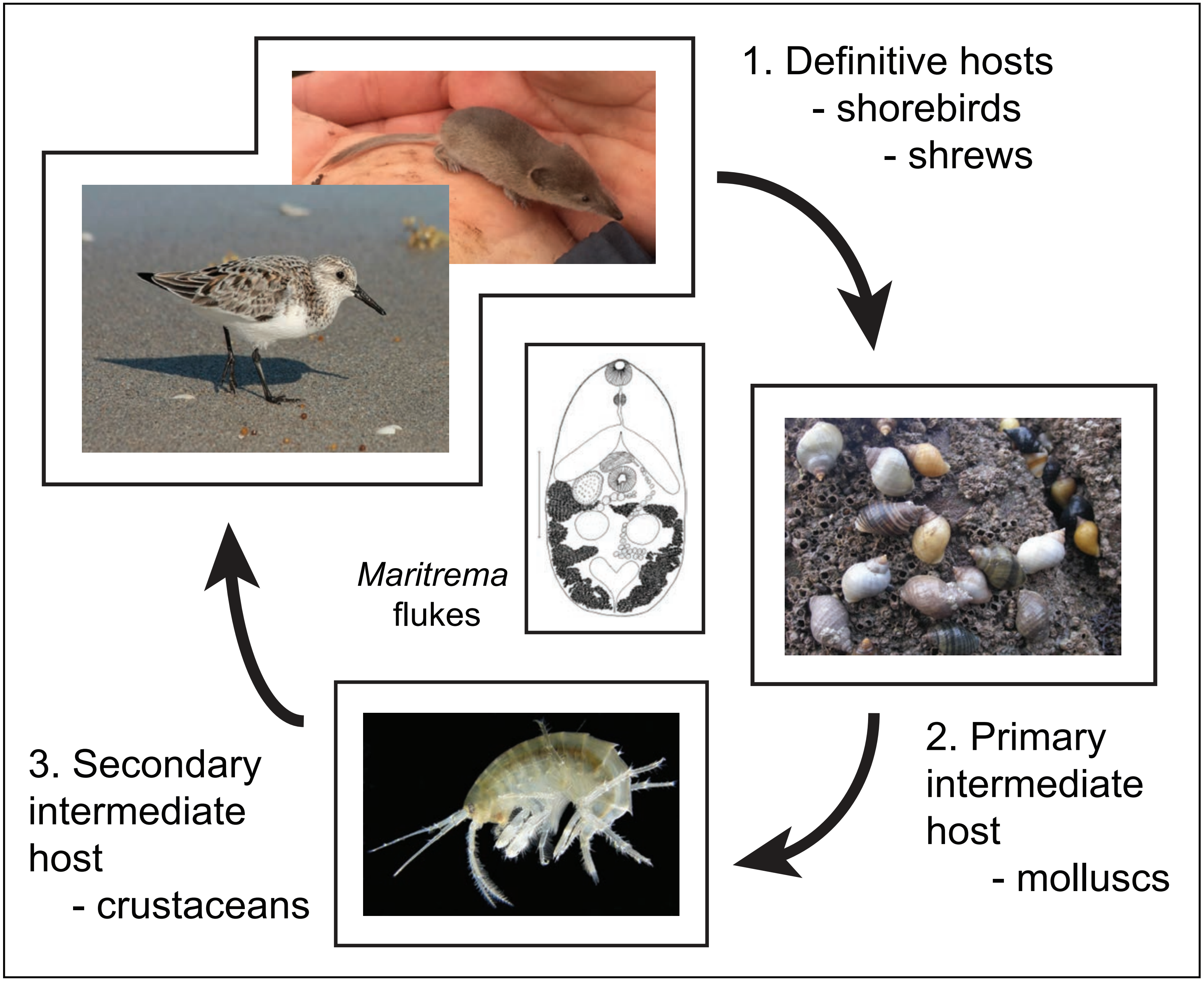
Results – co-evolution?

- Shrew trematode normally parasitizes birds!





Natural History



1. Definitive hosts
   - shorebirds
   - shrews

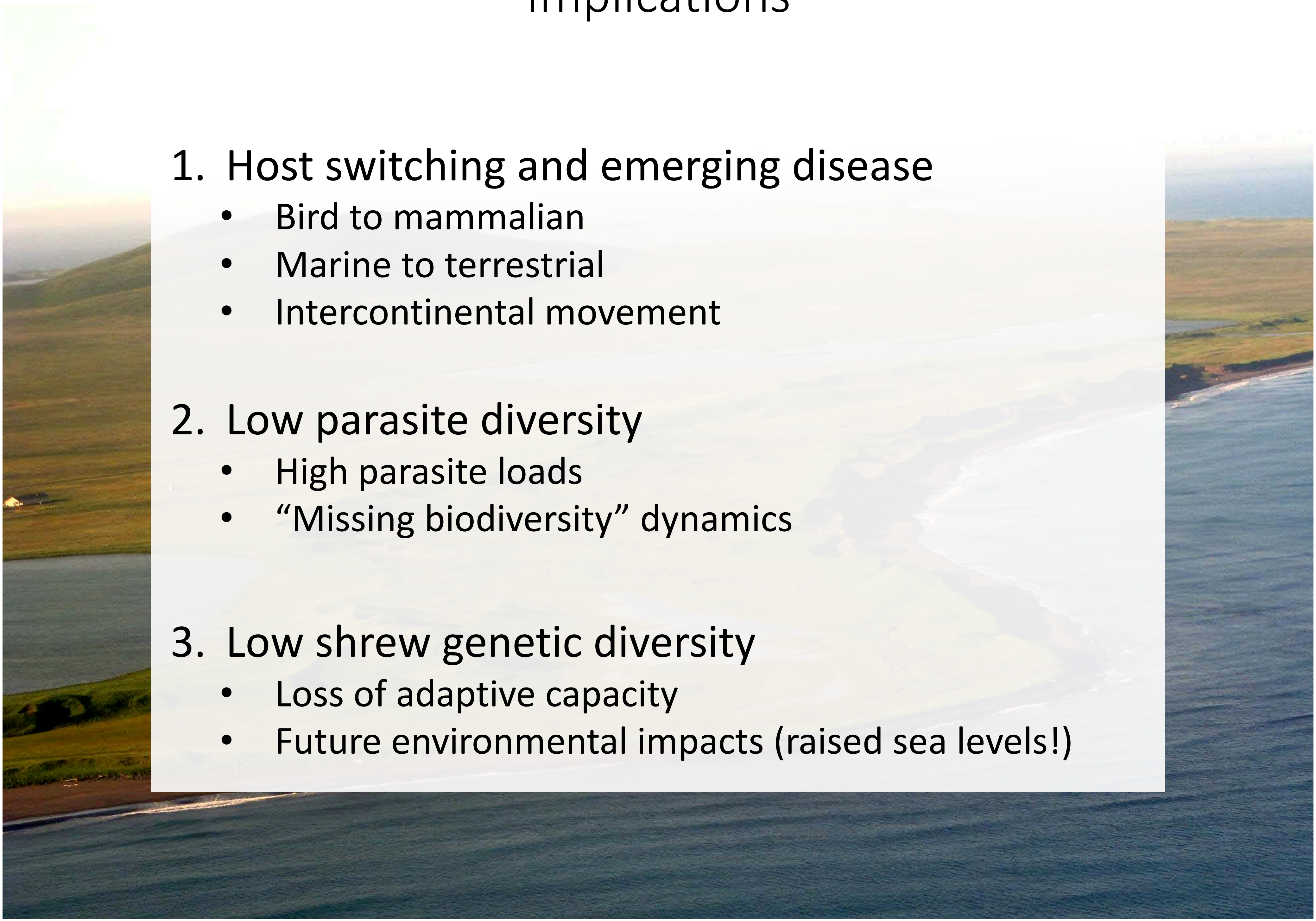
2. Primary intermediate host
   - molluscs

3. Secondary intermediate host
   - crustaceans

Maritrema flukes

Hope et al. 2016,
NOAA Arctic Report Card

Implications



1. Host switching and emerging disease
    - Bird to mammalian
    - Marine to terrestrial
    - Intercontinental movement

2. Low parasite diversity
    - High parasite loads
    - “Missing biodiversity” dynamics

3. Low shrew genetic diversity
    - Loss of adaptive capacity
    - Future environmental impacts (raised sea levels!)

Summary

- Genomic methods allow for highly integrated eco-evolutionary framework
  - Hybridization, speciation, co-evolution, community assembly
- Combined host-parasite analyses provide greater resolution of generalizable landscape processes
- Understanding interdependency among species…
  - Requires extensive diagnosis of existing biodiversity
  - Requires temporally deep, spatially broad, site intensive, and taxonomically comprehensive sampling

Thanks!

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NSF

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USGS
science for a changing world

NATIONAL PARK SERVICE

U.S. FISH & WILDLIFE SERVICE
DEPARTMENT OF THE INTERIOR

Museum of Southwestern Biology

The Beringian Coevolution Project

GEORGIA SOUTHERN UNIVERSITY

Arctos
Collaborative Collection Management Solution

KANSAS STATE UNIVERSITY

American Society of Mammalogists

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