Species boundaries and biogeography of *Hyalella* spp. (Amphipoda: Hyalellidae), using ultraconserved elements and traditional genetic markers

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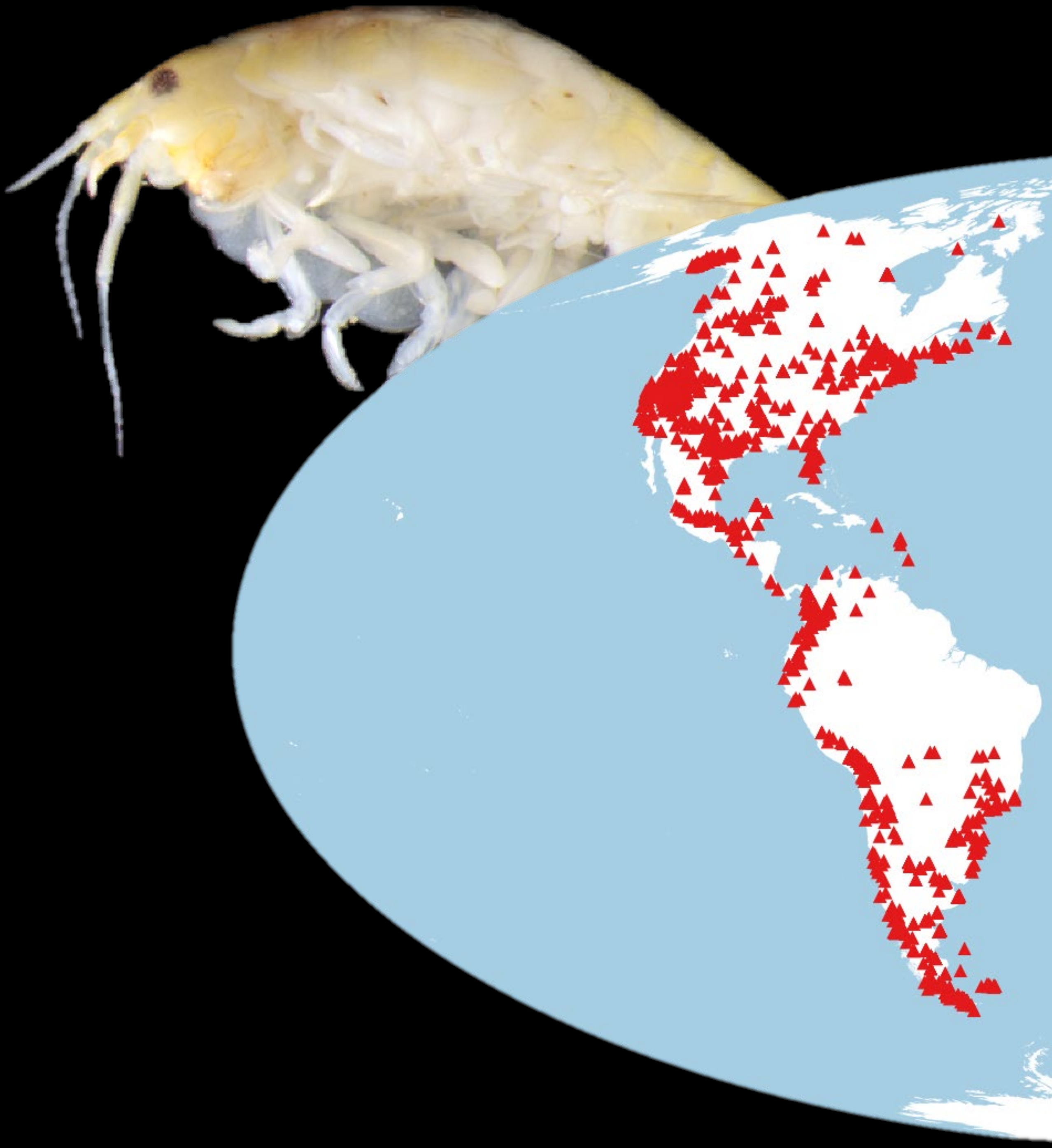
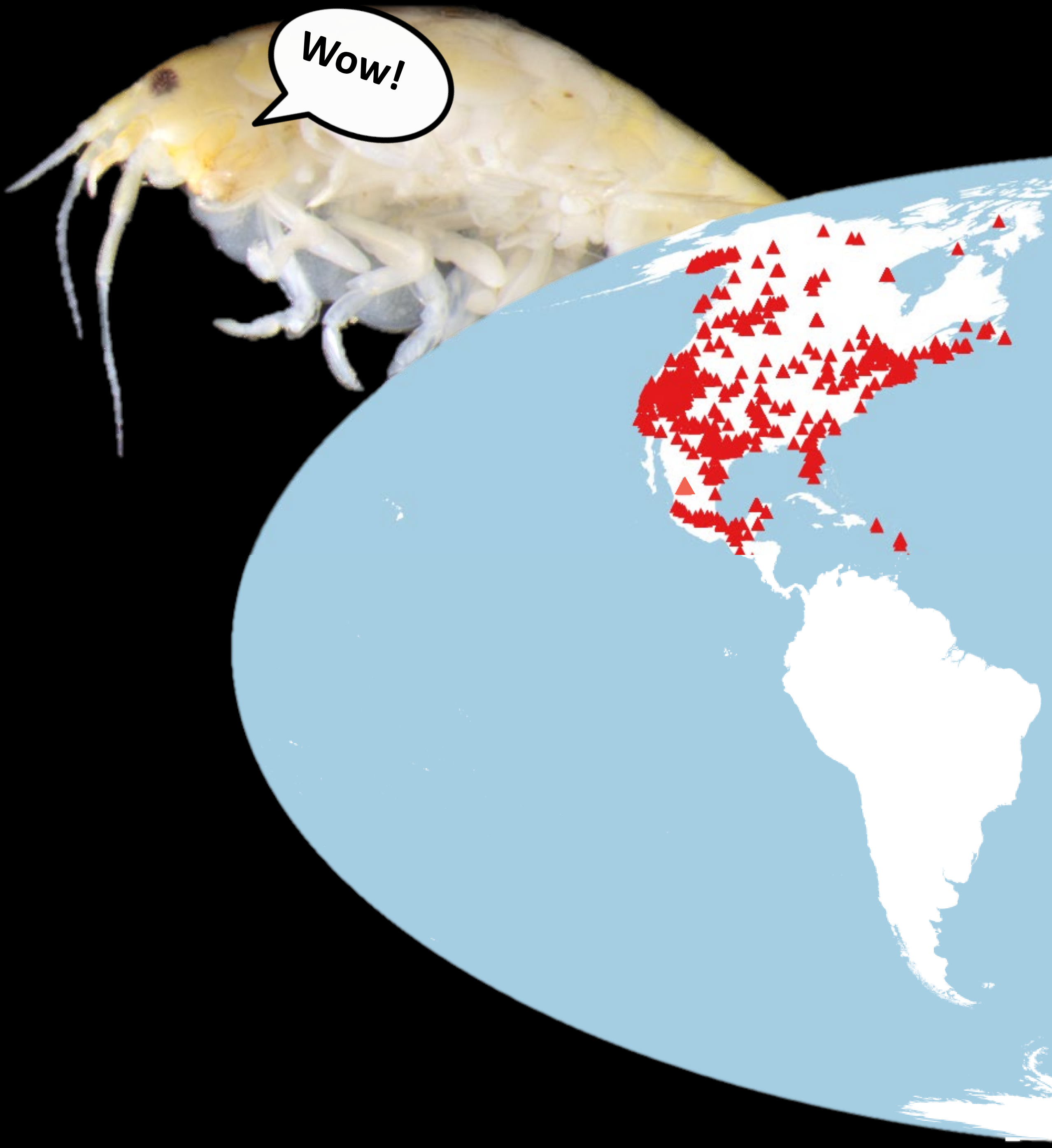
Hyalella

- Genus of freshwater amphipods distributed throughout North and South America.
- Most (described) diversity is present in South America.
- One of the most common and widely distributed freshwater invertebrate genera in North America.



Hyalella “azteca”

- Type species of genus, described from Veracruz. Once though to be widely distributed throughout New World.
- Now understood to be a species complex.
- Probably doesn’t even occur in the US.



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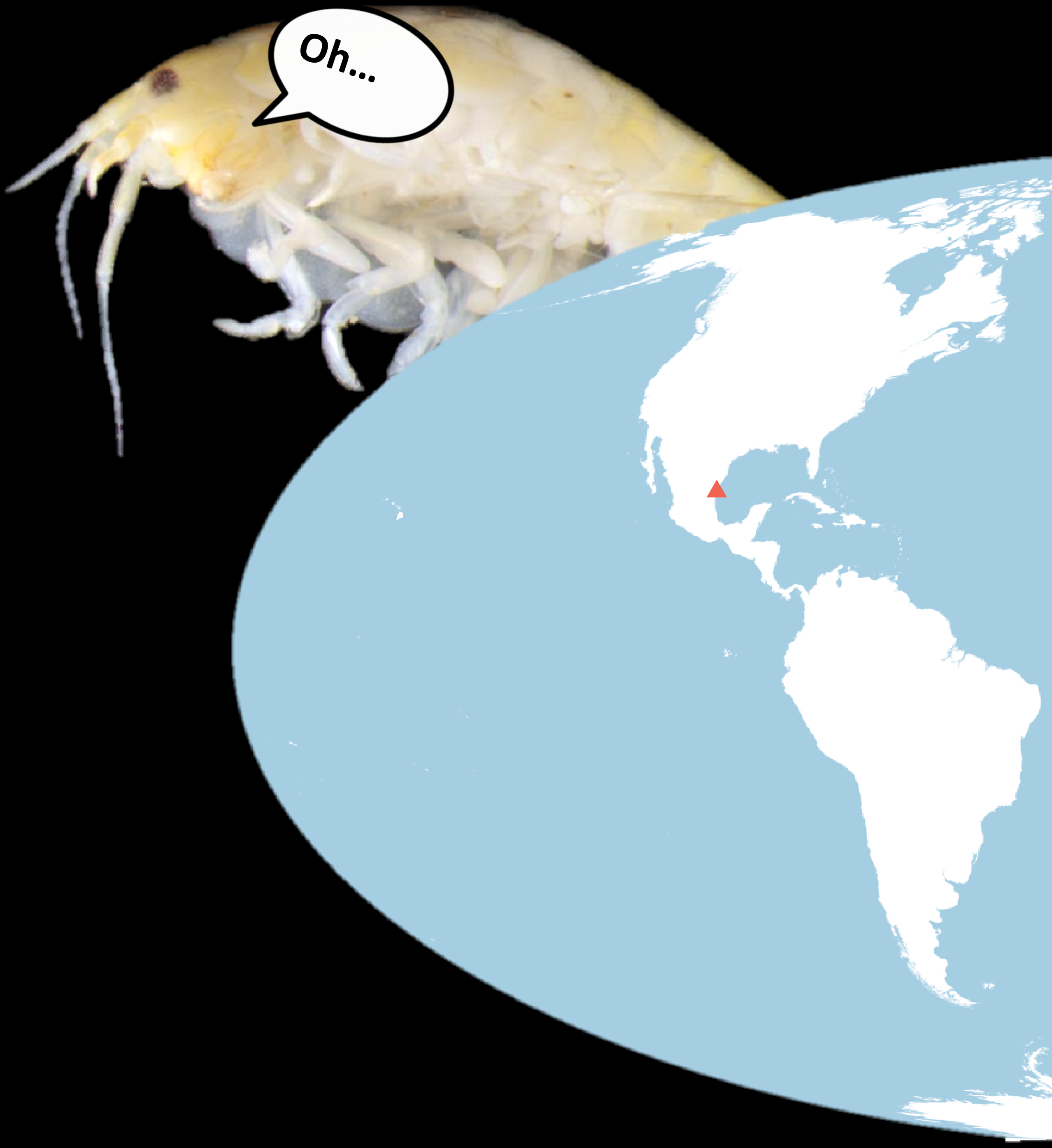
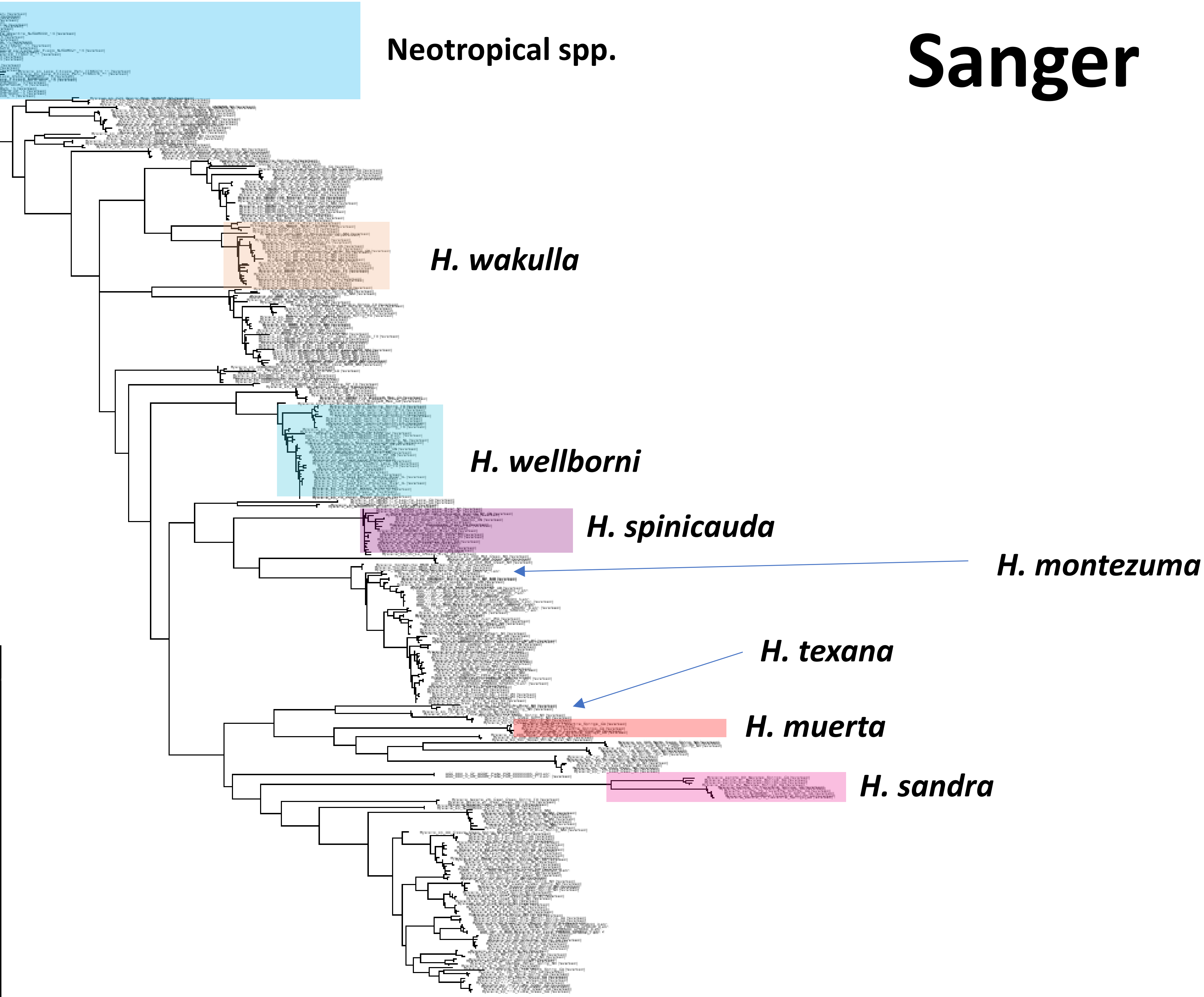


Objectives

- (I) Investigate molecular diversity of the genus *Hyalella.* Using both genetic and genomic tools.
- (II) Identify species richness in the Great Basin and beyond.
- (III) Correlate species distributions to geology/hydrology.

Sanger

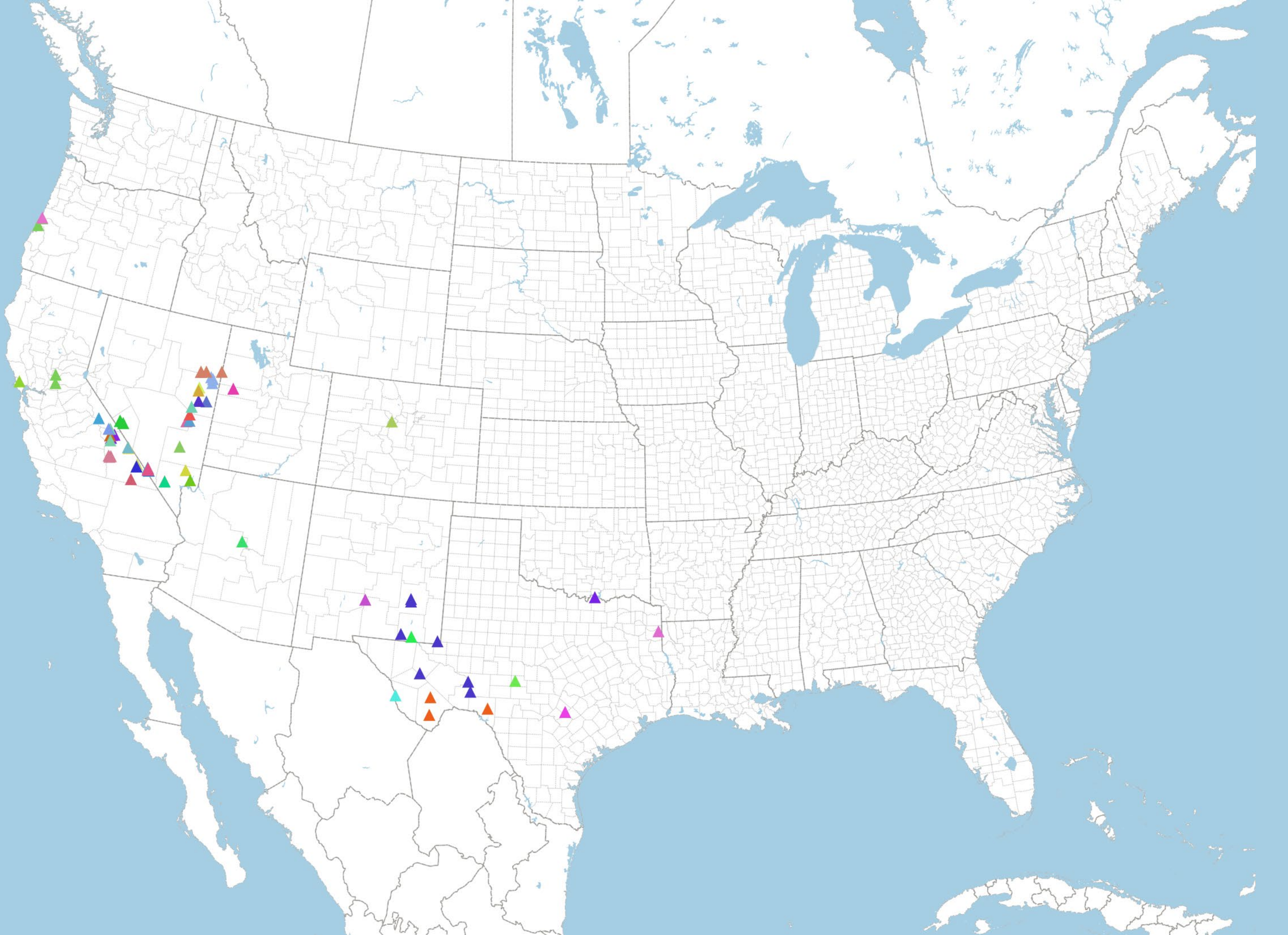
- Maximum-likelihood phylogeny, reconstructed using RAxML.
- Based on ~530 bp segment of COI.



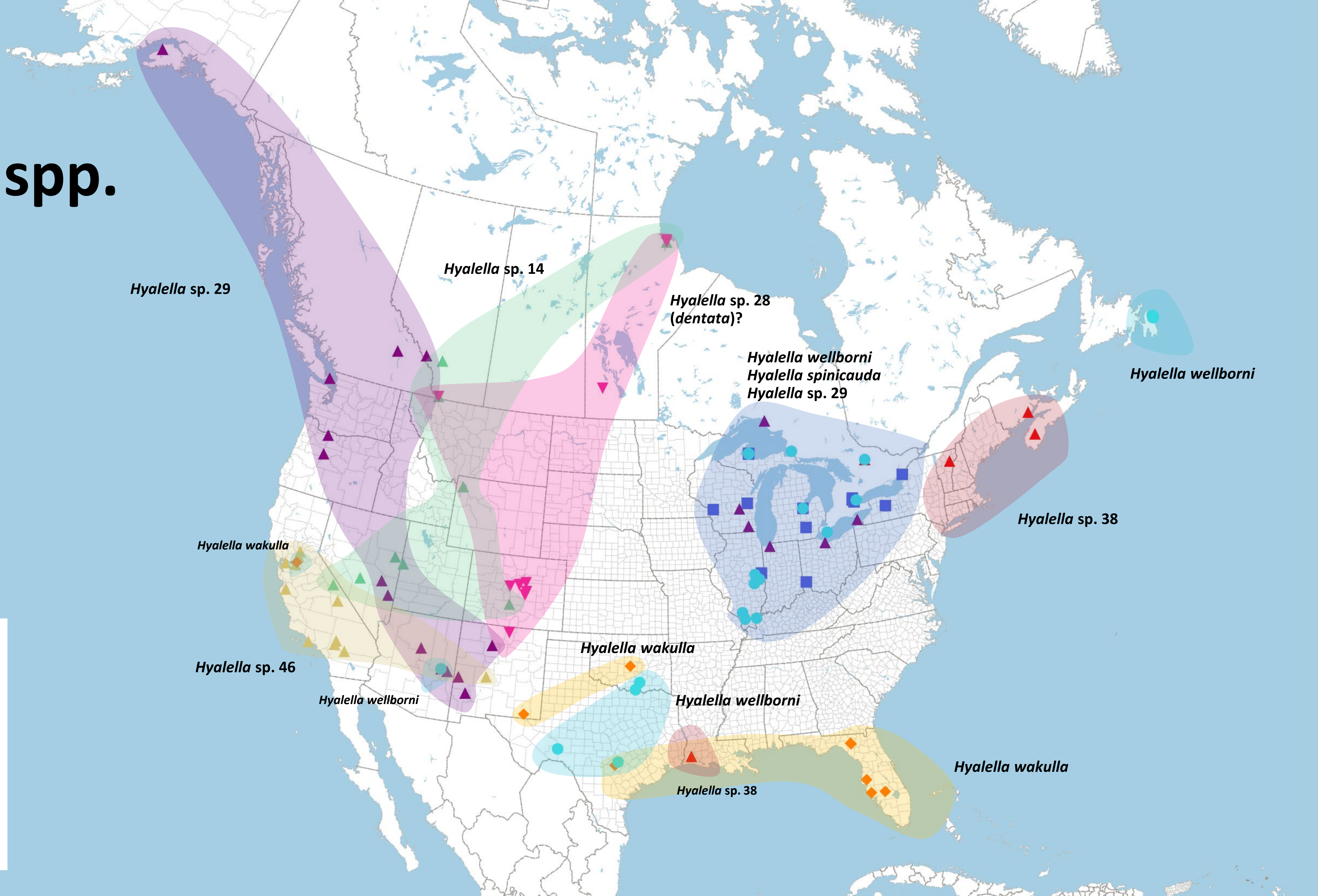

Hyalella muerta

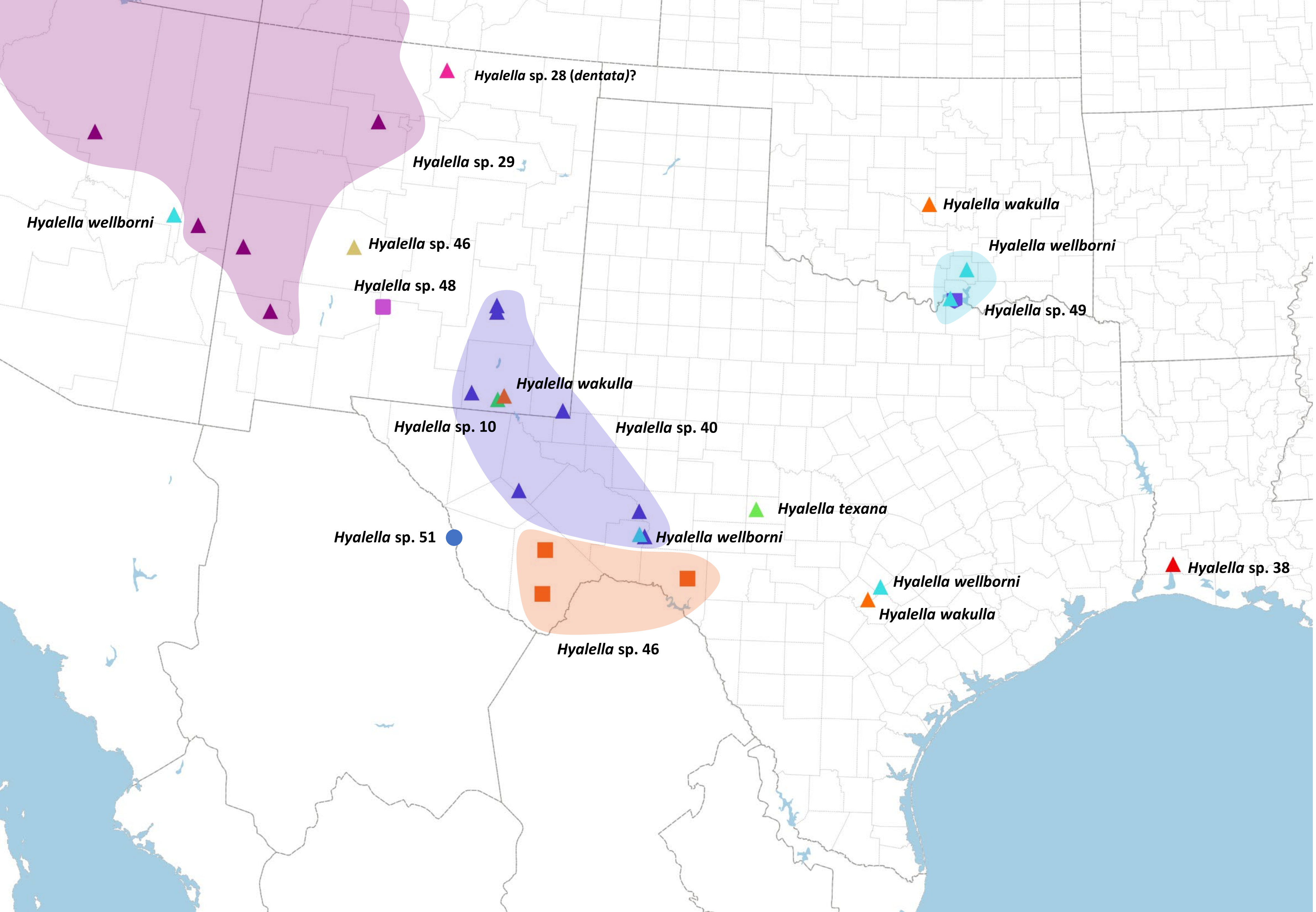
Locally endemic spp. (Sanger)

N= 53!!



Wide Ranging spp. (Sanger)



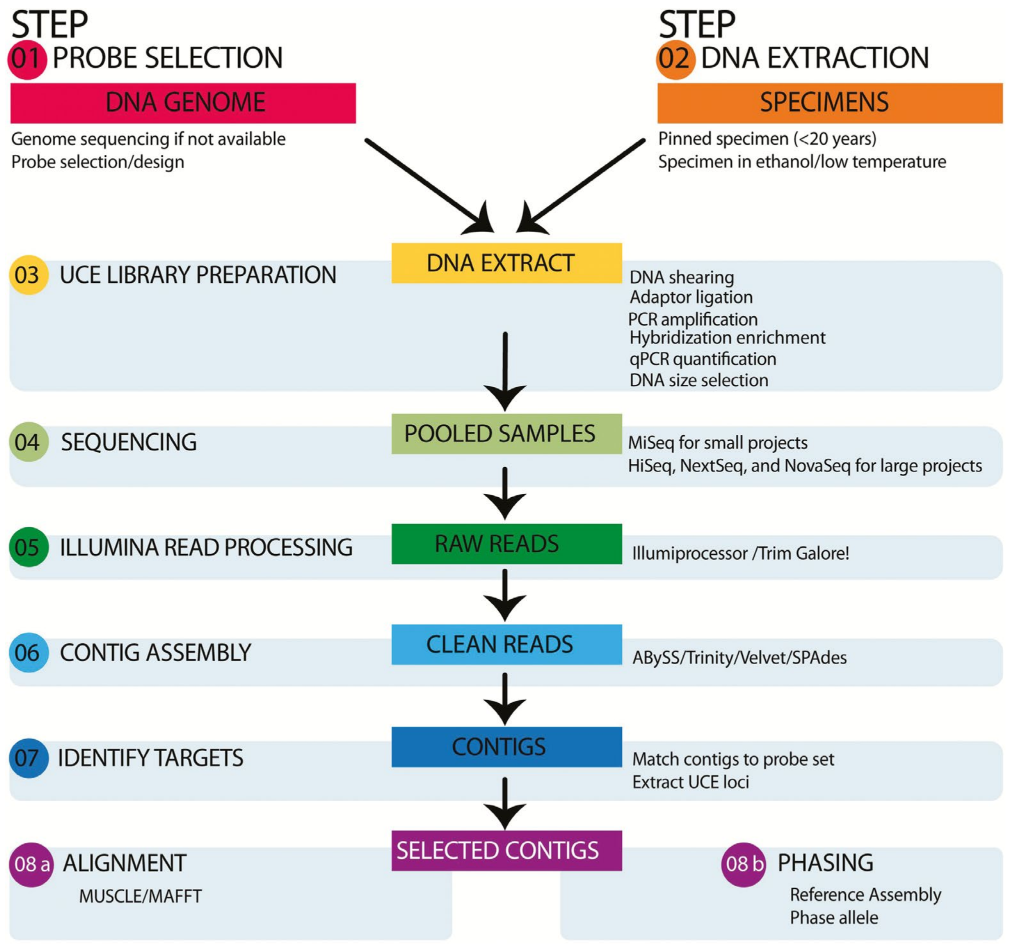


Texas!

And the surrounding region…

Ultraconserved Elements

- Highly conserved regions of a genome shared among evolutionary distant taxa.
- UCEs themselves useful in resolving difficult or deep phylogenetic relationships.
- Flanking regions show utility in resolving shallow or population-level relationships.



STEP 01 PROBE SELECTION

DNA GENOME

Genome sequencing if not available
Probe selection/design

STEP 02 DNA EXTRACTION

SPECIMENS

Pinned specimen (<20 years)
Specimen in ethanol/low temperature

03 UCE LIBRARY PREPARATION

DNA EXTRACT

DNA shearing
Adaptor ligation
PCR amplification
Hybridization enrichment
qPCR quantification
DNA size selection

04 SEQUENCING

POOLED SAMPLES

MiSeq for small projects
HiSeq, NextSeq, and NovaSeq for large projects

05 ILLUMINA READ PROCESSING

RAW READS

Illumiprocessor /Trim Galore!

06 CONTIG ASSEMBLY

CLEAN READS

ABySS/Trinity/Velvet/SPAdes

07 IDENTIFY TARGETS

CONTIGS

Match contigs to probe set
Extract UCE loci

SELECTED CONTIGS

08 a ALIGNMENT

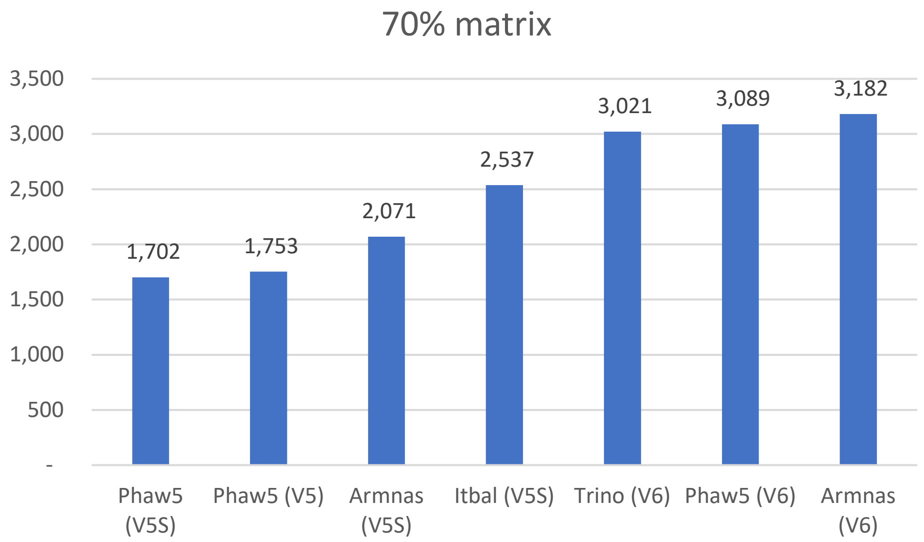
MUSCLE/MAFFT

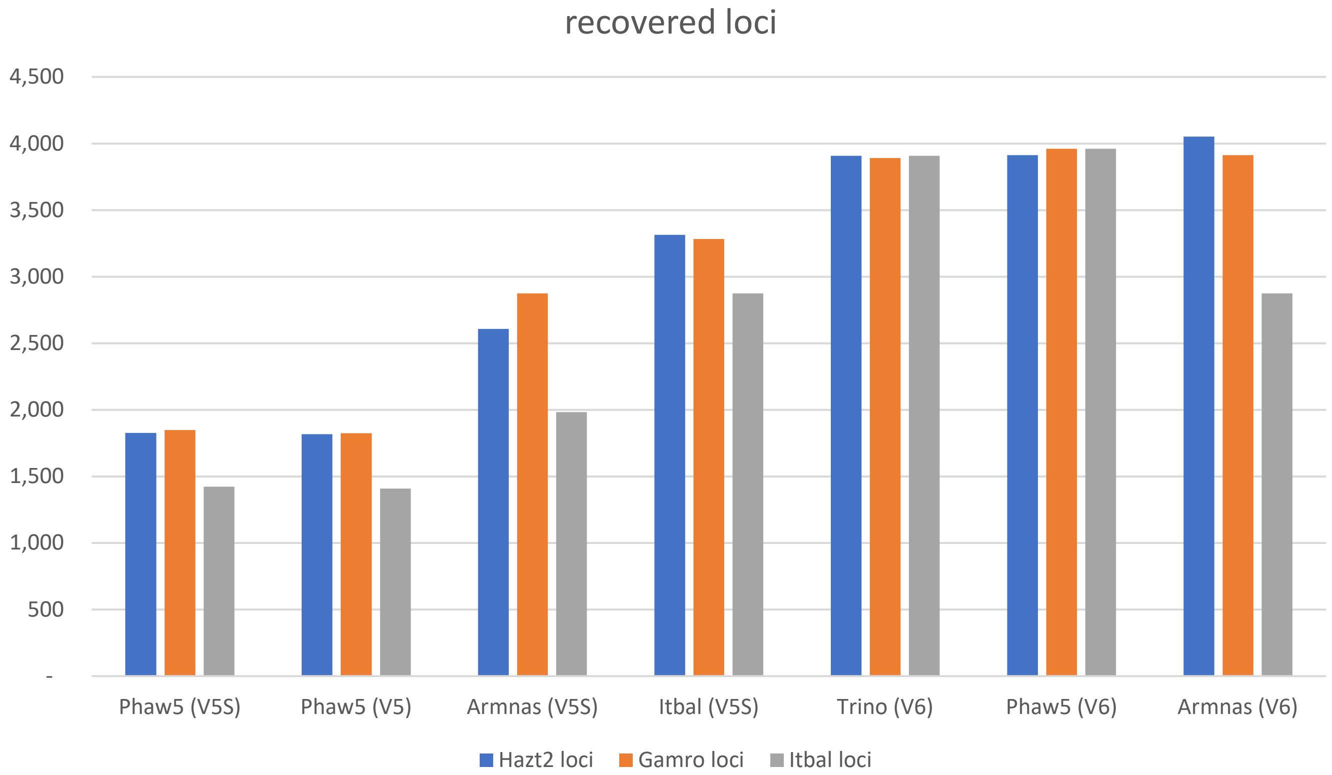
08 b PHASING

Reference Assembly
Phase allele

Insect Syst Divers, Volume 3, Issue 5, September 2019, 3, https://doi.org/10.1093/isd/ixz016

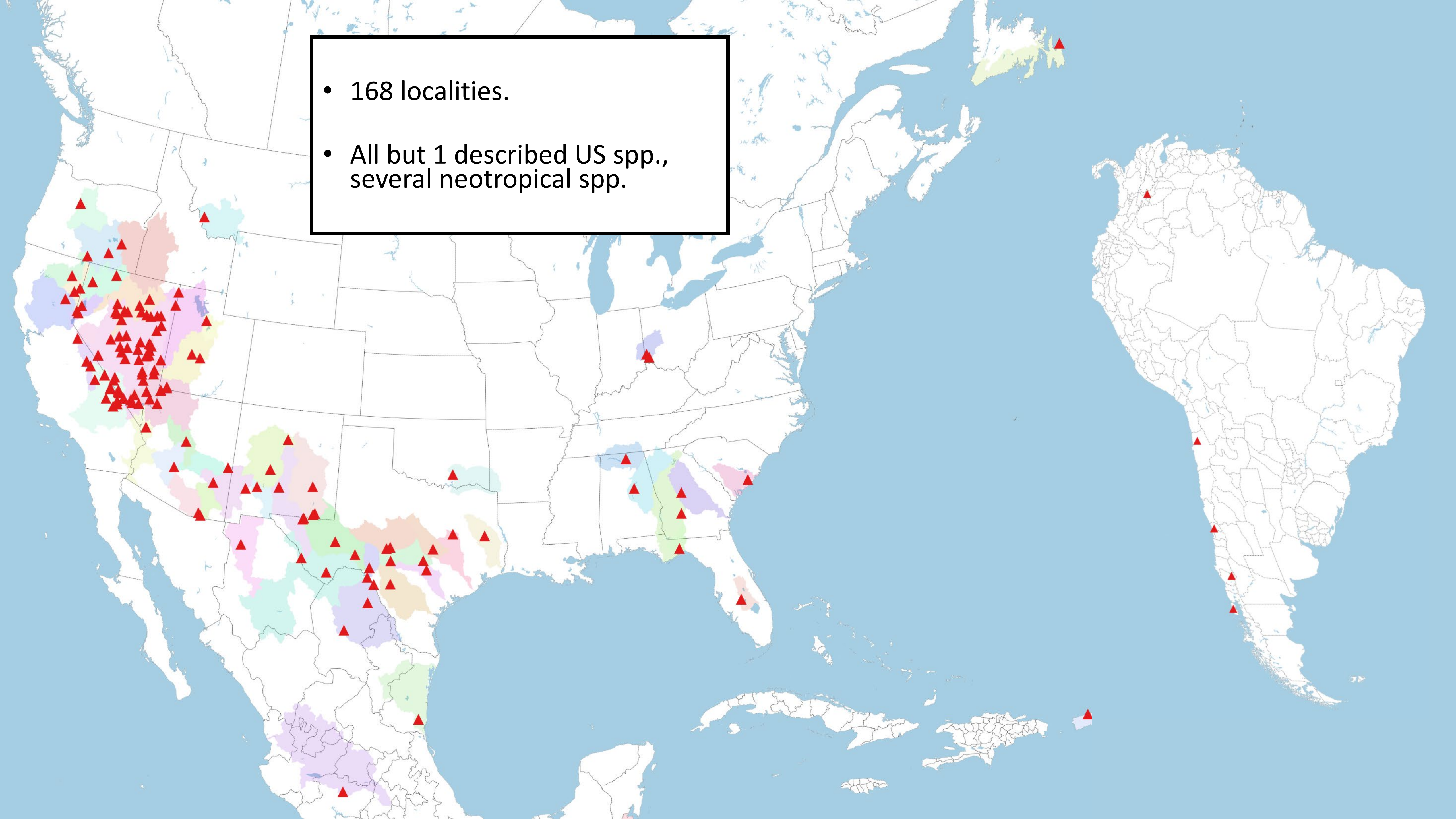
In-silico



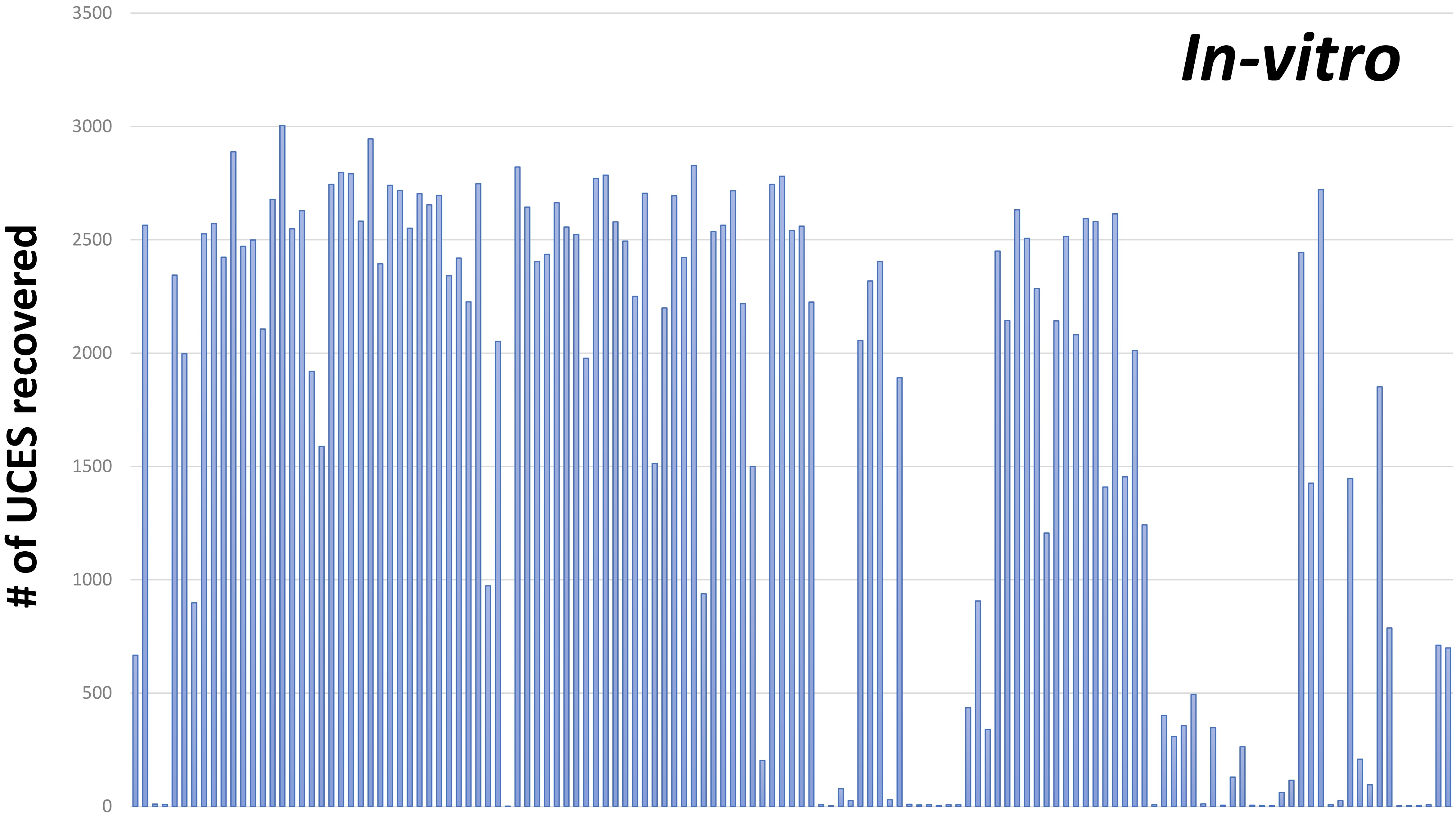


final probeset

Species	**# loci recovered**	**# loci retained**
Hyalella cf. *azteca*	5,171	3,913
Parhyale hawaiensis	5,233	4,207
Platorchestia sp.	5,205	4,221
Speziorchestia grillus	5,207	4,166
Trinorchestia longiramus	5,201	4,136
Gammarus roeselii	5,207	3,961
Archaeomysis grebnitzkii	5,114	1,454
Trachelipus rathkii	4,762	2,622
Armadillidium nasatum	5,151	3,498
Ligia exotica	4,886	2,608
Idotea baltica	5,052	2,822
Bathynomus jamesi	5,206	3,265
Palaemon carinicauda	5,087	1,143

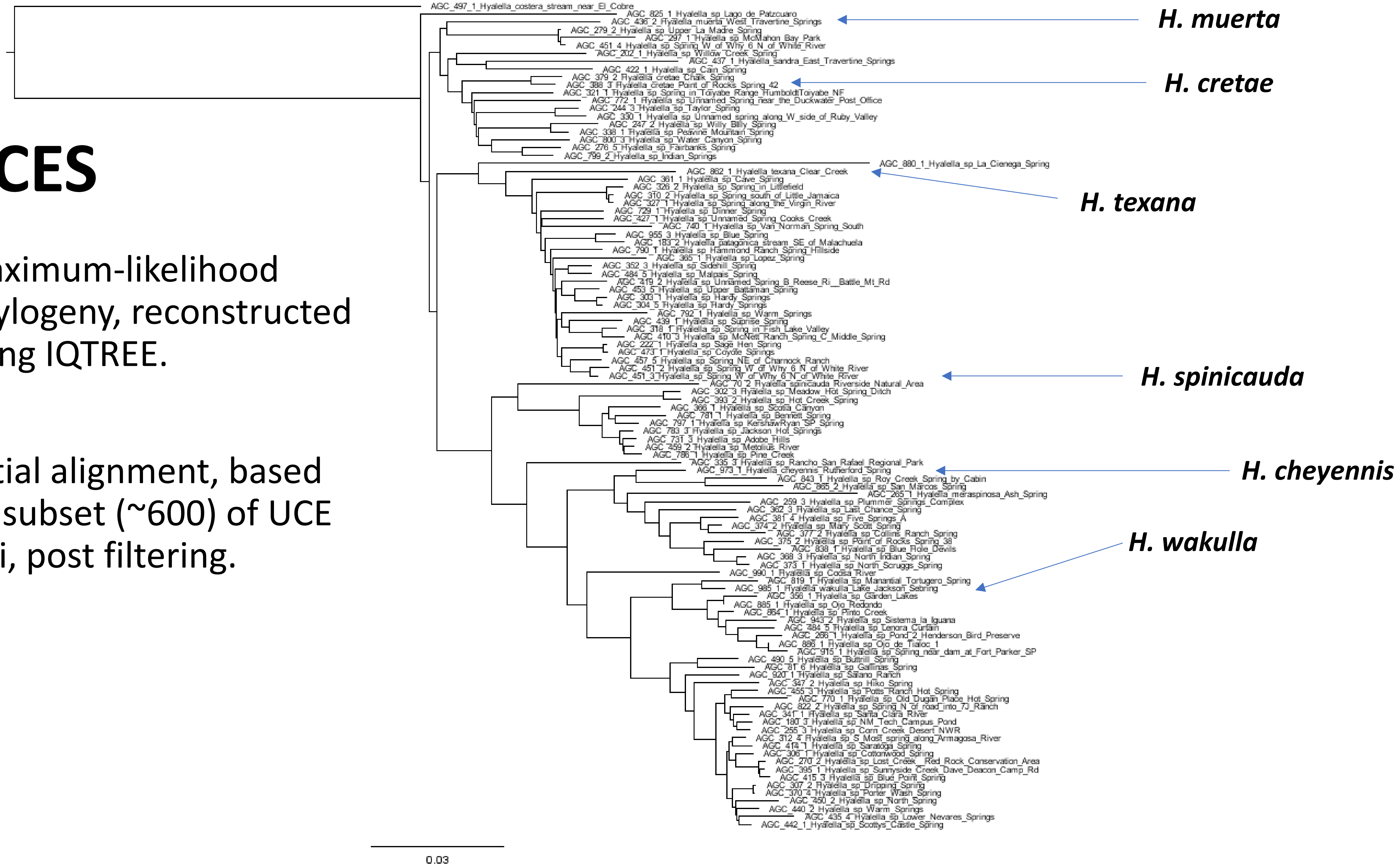


- 168 localities.
- All but 1 described US spp., several neotropical spp.


In-vitro
# of UCES recovered
3500
3000
2500
2000
1500
1000
500
0

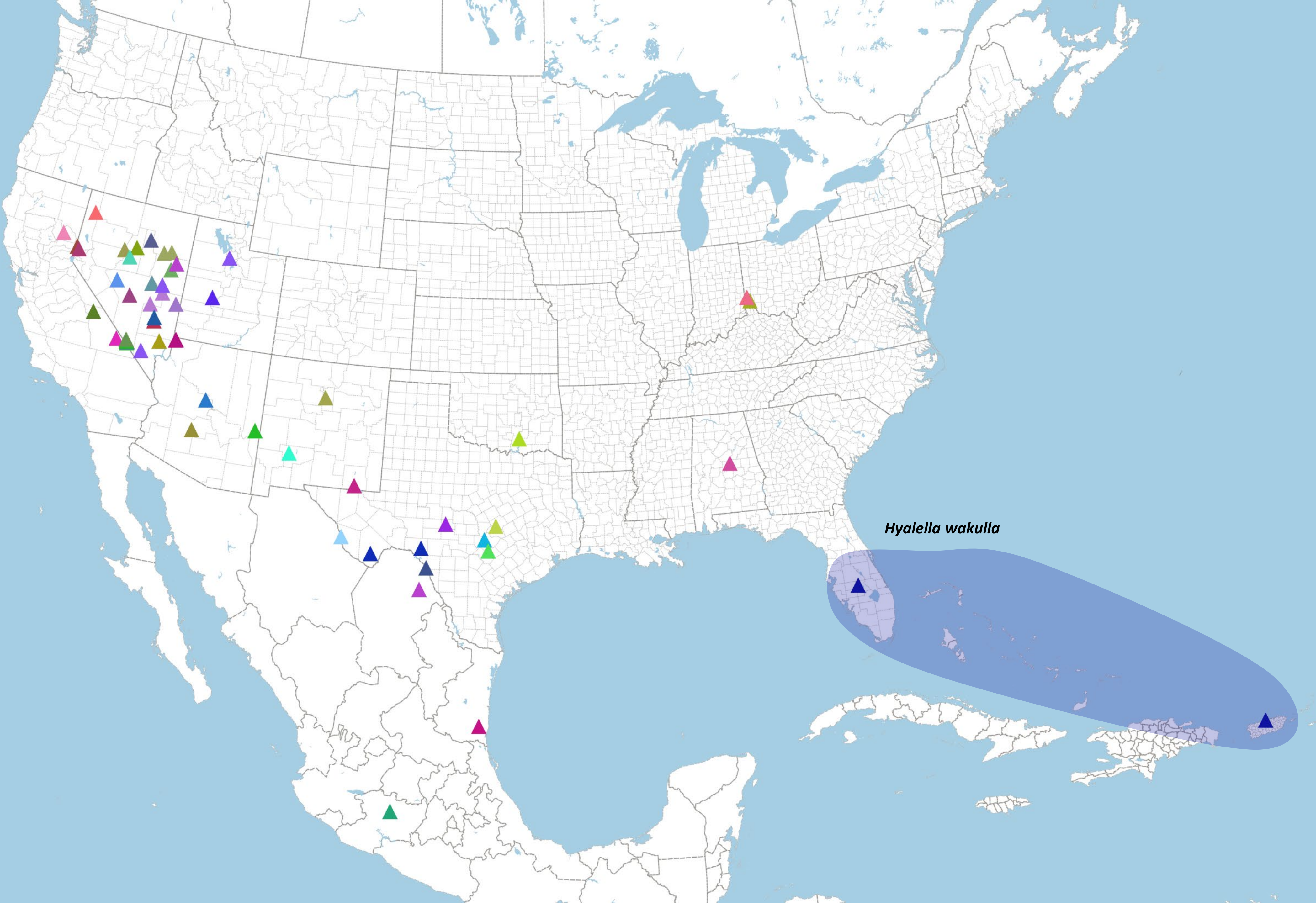
UCES

- Maximum-likelihood phylogeny, reconstructed using IQTREE.
- Initial alignment, based on subset (~600) of UCE loci, post filtering.

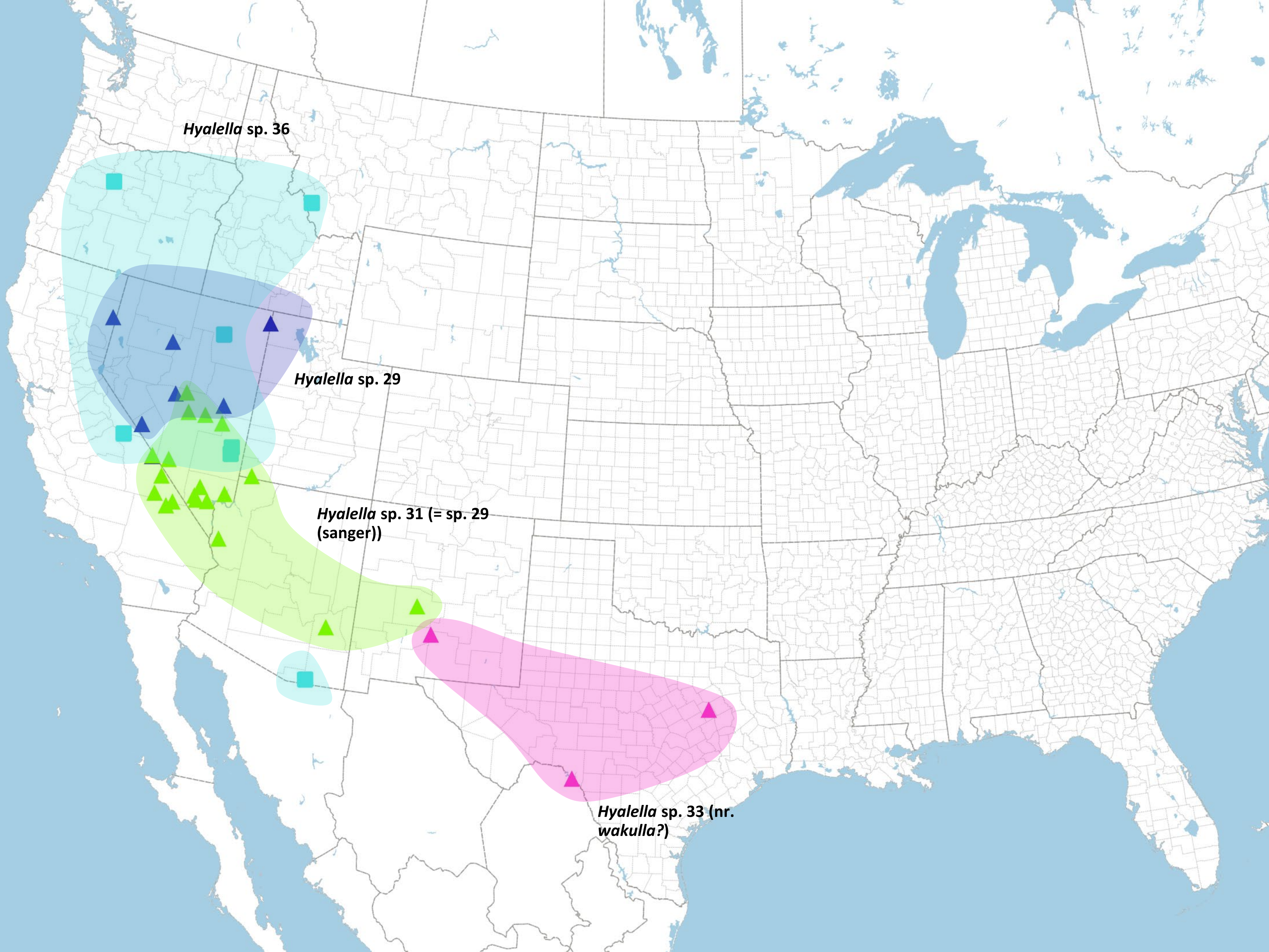


Locally endemic spp. (UCES)

N= 54!!



Wide Ranging spp. (UCES)



What's Next?

- Finish UCE assemblies, and mine "legacy loci"
- Build final, fully incorporated, phylogenies including time-calibrated trees.
- Analyses!
- Taxonomy!

Acknowledgments

- Randy Gibson, Pete Diaz, Ben Hutchins, Ben Schwartz, William Shepard, Gary Wellborn, Mardon Erbland, Thomas Sawicki , and others for help obtaining specimens.
- Kayla Childs, Zach Samsa, & Emma Girolami for help with sanger sequencing.
- Bureau of Land Management

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BUREAU OF LAND MANAGEMENT

Thank you!
Questions?