# Phylogenomic insights into the systematics of the Vitisarizonica complex in North America

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The Vitis arizonica, or canyon grape, species complex is found in the southwestern region of the United States of America. The species complex exhibits an allopatric geographic distribution and morphological variability, including but not limited to the lobing and pubescence of leaves, variations in the small clusters of fruits, and seed structure. The goal of the project is to construct a phylogenomic framework that allows us to analyze the clustering of the clades. The phylogeny will allow us to understand relationships within the *Vitis arizonica* complex and relate them to the observed morphological and geographic patterns.

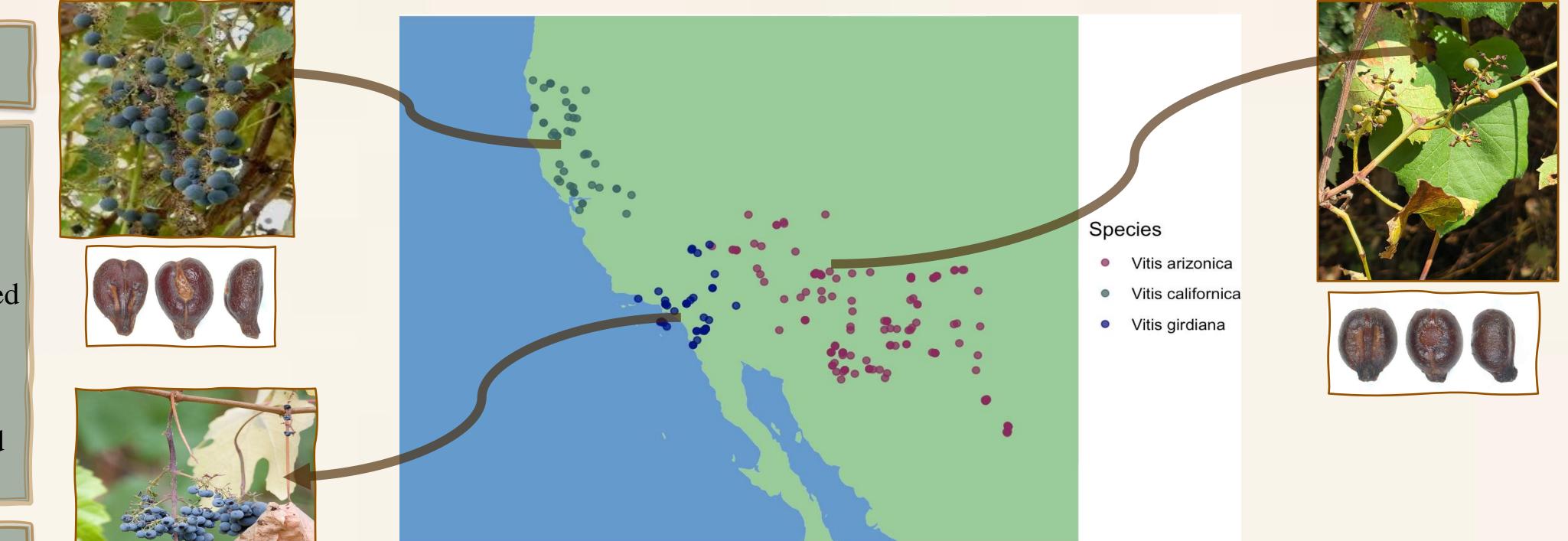
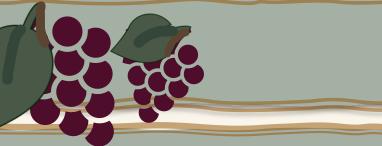


Figure 1: Map displaying distribution of Vitis girdiana, Vitis

arizonica, and Vitis californica National herbarium specimens.

Photos by Jun Wen and Brooklyn Swen.



## Methods

#### Genetic Analysis

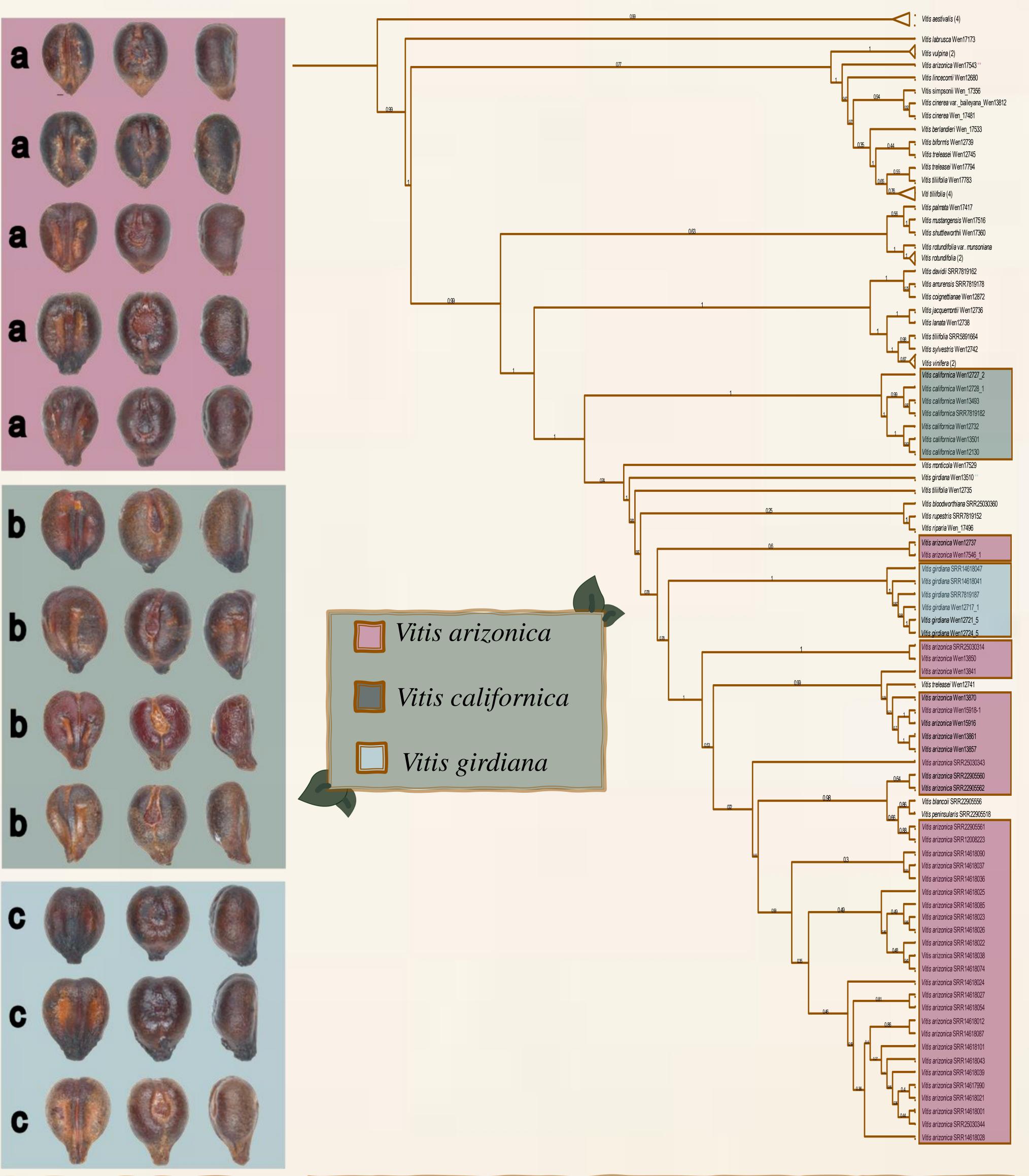
- Within this study, we utilized a total of 24 *Vitis arizonica* samples, that covered a broad distribution of locations and differing morphologies.
- DNA was extracted from silica-dried samples and then sheared to an average size of about 500 bp.
- Genomic libraries were constructed and enriched for 1013 nuclear genes, utilizing a Vitaceae-specific bait set.
- Following enrichment, libraries were then pooled and sequenced with Illumina NovaSeq.
- Utilizing Hybpiper, raw reads were trimmed and assembled, aligned utilizing Mafft, and constructed with RAxML (Talavera et al., 2023).
- ✤ A nuclear tree was then constructed utilizing ASTRAL.











Morphological seed analysis

- Seeds from preserved specimens of *Vitis arizonica*, *Vitis californica*, and Vitis girdiana were sampled to allow for analysis of the seed morphology.
- Dorsal, ventral, and lateral perspectives of the seeds were imaged utilizing a Leica microscope imaging system.
- Seeds images were organized into a seed plate utilizing Inkscape.

# Results & Discussion

Through geographic analysis of the National herbarium specimens of Vitis girdiana, Vitis arizonica, and Vitis californica, we can observe a comprehensive display of their distribution. The map shown in **Figure 1** highlights the allopatric nature of these species. All three cluster in distinct, adjacent regions with no overlap. The distinct groupings found in geography persist when comparing seed morphological differences unique to the Vitaceae family. Analysis of the seeds found *Vitis arizonica* seeds have a distinct band present on the apical notch, differing from *Vitis californica* seeds that possess a more grooved apical notch. *Vitis girdiana* seeds display both banding and grooving in the apical notch, but to a far lesser extent then both *Vitis arizonica and Vitis girdiana*. This intermediate character makes sense, as Vitis girdiana can be found in a region directly between *Vitis californica* and *Vitis arizonica*. The phylogenetic tree constructed utilizing 70 samples, presents Vitis californica as sister to both Vitis arizonica and Vitis girdiana. Additionally, *Vitis arizonica* is polyphyletic, appearing in four distinct clades on the phylogeny, with *Vitis girdiana* being nested within the *Vitis* arizonica complex. The polyphyletic nature of Vitis arizonica supports a novel hypothesis that the species may be comprised of multiple distinct genetic lineages. For this study, the next steps would be to quantify the observed morphological traits utilizing ImageJ and conduct statistical tests. This would all us to confirm whether the differences in morphology truly correspond to geographic distribution. Additionally, to enhance the accuracy of the phylogeny, we plan on adding the 24 newly sequenced samples to the existing tree.

# Acknowledgments

I would like to thank Jun Wen, Gabe Johnson, Luke Sparreo, and Alicia Talavera for their incredible laboratory instruction, support, and guidance throughout this project. Another thank you to Jessica Johnston, Vannesa Gonzalez, Ioan Lascu, and Virginia Power for their administrative support and guidance, I truly appreciate all the opportunities they have provided. Lastly, a special thank you to the Smithsonian for funding.

Figure 3: Dorsal, lateral, and ventral perspectives of Vitis girdiana, Vitis arizonica, and Vitis californica seeds. Photos by Brooklyn Swen.

Figure 2: Phylogenetic tree exhibiting the evolutionary relationships amongst specimens of Vitis girdiana, Vitis arizonica, Vitis californica, and relevant outgroups. Phylogeny by Brooklyn Swen



Talavera, A., Nie, Z. L., Ma, Z. Y., Johnson, G., Ickert-Bond, S. M., Zimmer, E. A., & Wen, J. (2023). Phylogenomic analyses using a new 1013-gene Vitaceae bait-set support major groups of North American Vitis. *Molecular phylogenetics and evolution*, 186, 107866. https://doi.org/10.1016/j.ympev.2023.107866