

A Comparative Analysis of the Genetic Diversity of *Amsonia kearneyana*



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Introduction

Endemic species with small population sizes are at risk of extinction. Conservation genetics can be used to assess the genetic health of threatened populations. *Amsonia kearneyana* is endemic to a single mountain side in southern Arizona and has been listed under the Endangered Species Act since 1989. This study aims at informing the conservation management of *A. kearneyana* by 1. understanding its population genetics and 2. evaluating its relatedness to other *Amsonia*.

Objectives

- Assess the genetic diversity and inbreeding for *Amsonia kearneyana*
- Determine if there is gene flow across subpopulations
- Construct a phylogeny of *A. kearneyana* and determine if it is monophyletic



Methods

Sample Collection

- Leaves were dried on silica or collected from herbarium vouchers
- *Amsonia palmeri* (30 ind), *A. kearneyana* (50 ind)
 - UBC - 30, LBC - 10, ST - 10, Historical - 6

DNA Extractions

- DNA extractions were done using a modified CTAB procedure and quantified using a Qubit

DNA Library Prep & Sequencing

- Library prep was done following a modified ddRADseq protocol. Libraries were sequenced at the NUSEq Core facility at Northwestern University

Analyses

- SNPs were called using the STACKS. VCFtools was used to filter SNPs for population genetic analyses. We used the R programs Hierfstat and DartR to estimate observed heterozygosity (H_o) and inbreeding coefficients (F_{is}). ADMIXTURE was used to detect if gene flow was occurring across *A. kearneyana* populations. The best K was chosen based on the lowest cross-validation value

References: Fish and Wildlife Service. 2020. Kearney's Blue Star (*Amsonia kearneyana*) Final Recovery Plan, First Revision. Southwest Region, Tucson, Arizona, USA. 75 pp.

Results

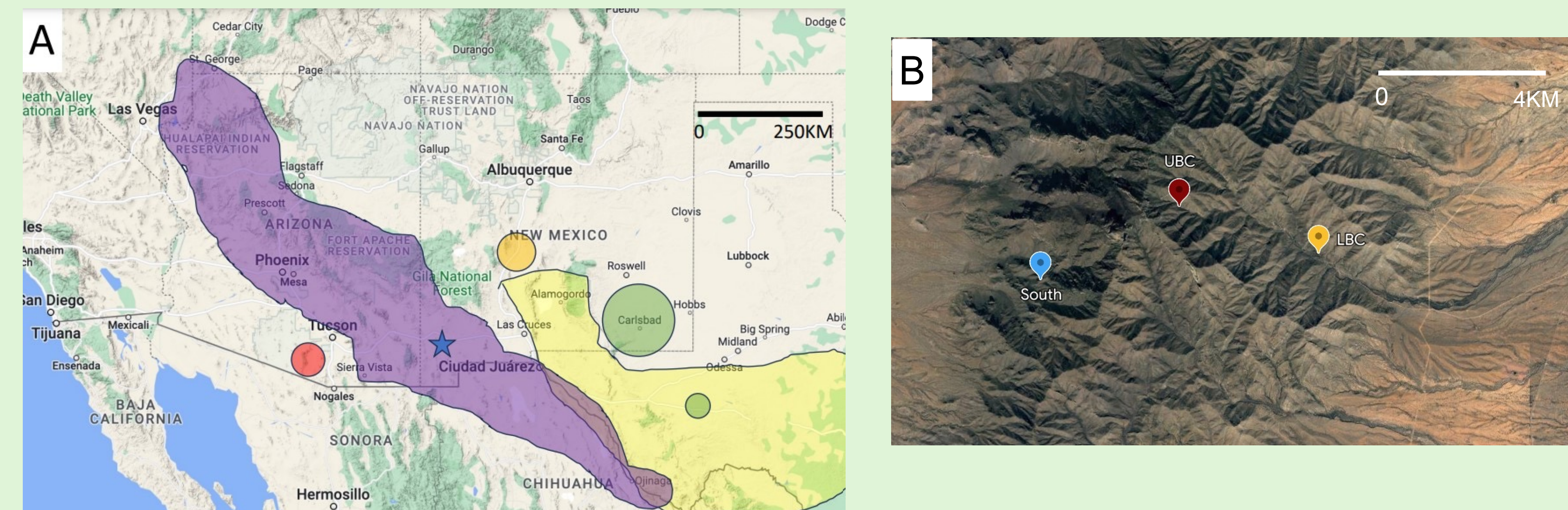


Figure 1. (A) Distribution map of *Amsonia kearneyana* (red), *A. palmeri* (purple), *A. fugatei* (orange), *A. tharpaii* (green), and *A. longiflora* (yellow). Star indicates the population of *A. palmeri* used in this study. (B) Map of the three *A. kearneyana* sub-populations included in study (south (ST), Upper Brown Canyon (UBC), and Lower Brown Canyon (LBC)).

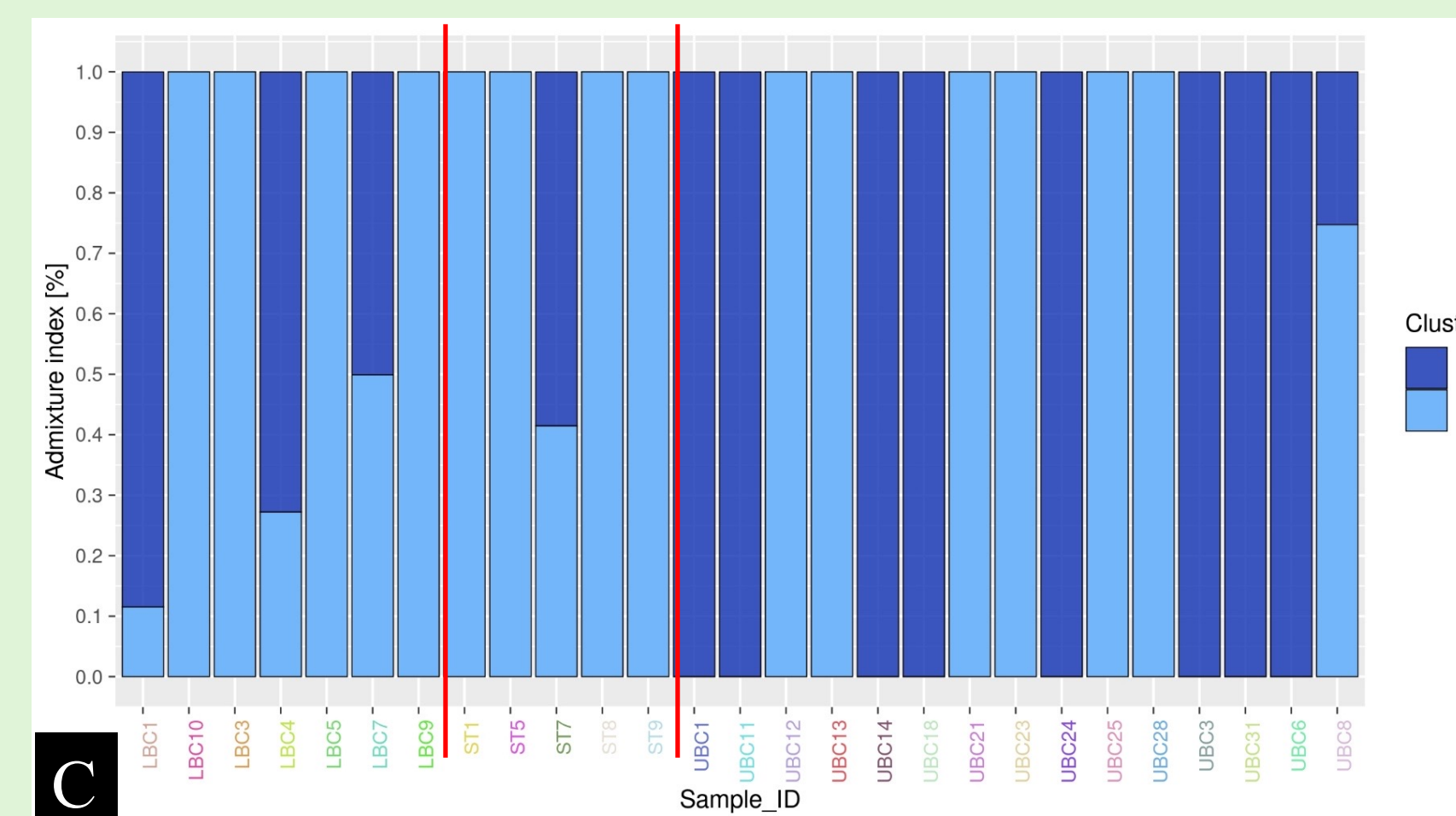
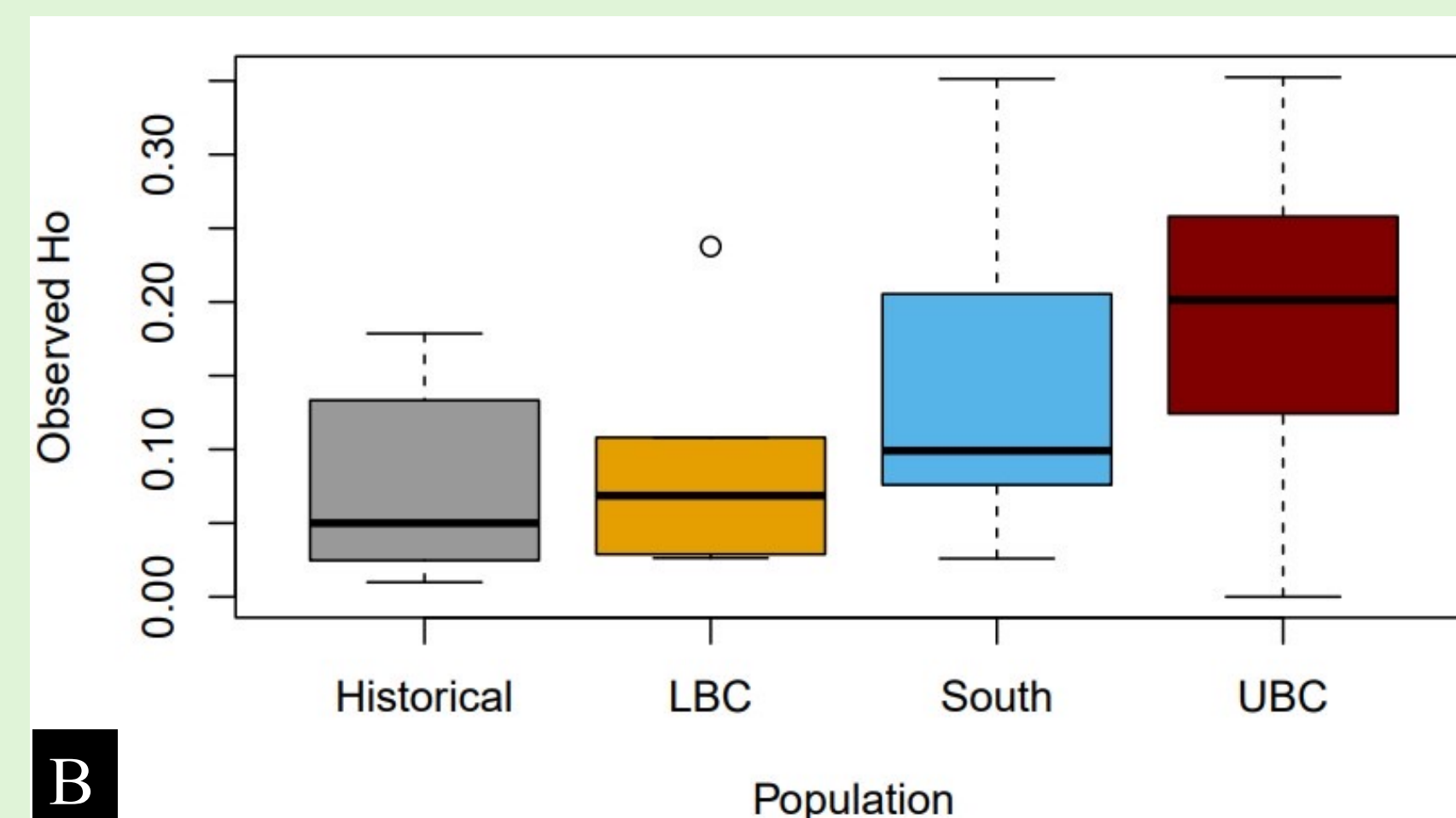
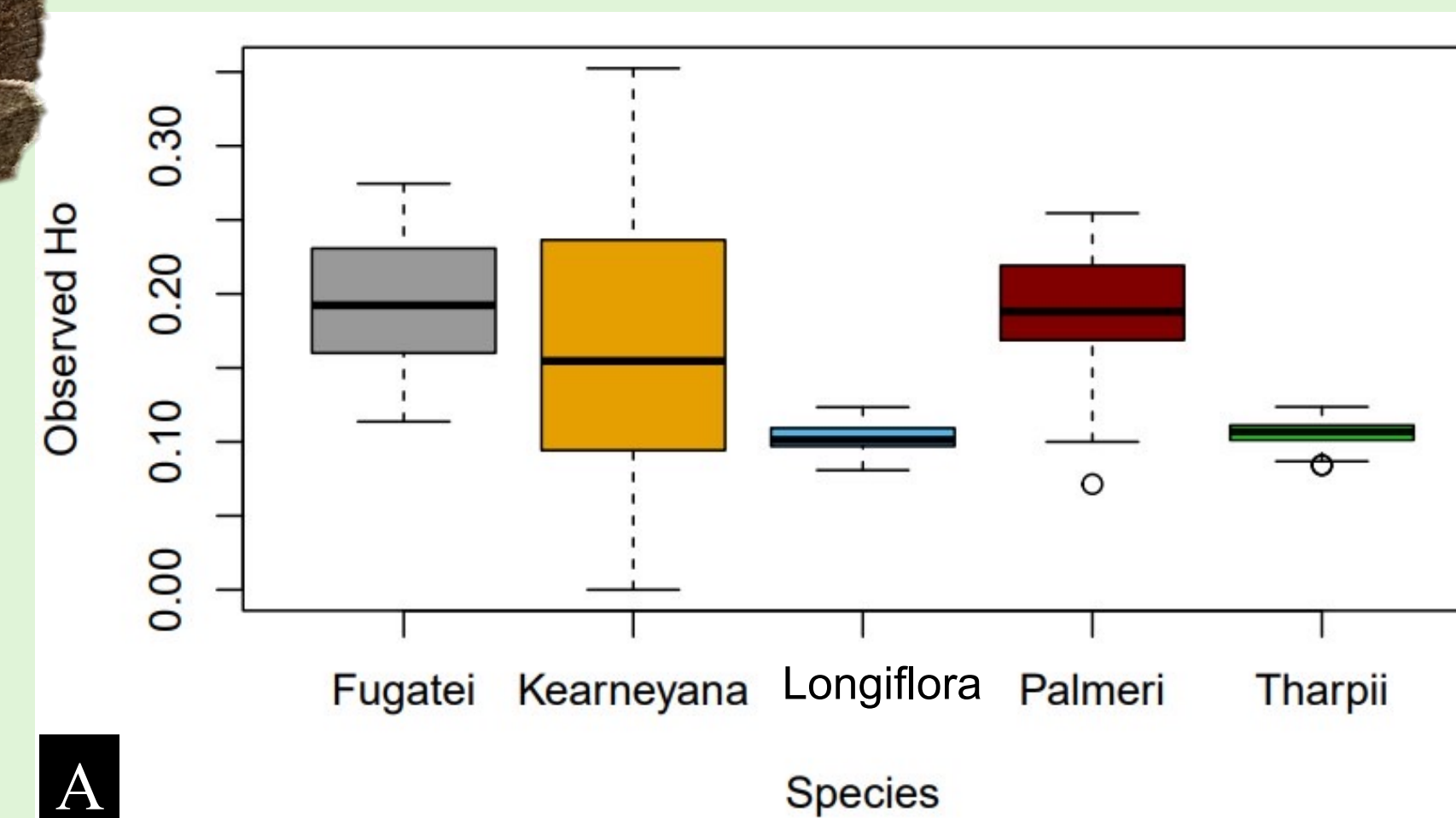


Figure 2. (A) Observed heterozygosity (H_o) by *Amsonia* species: *A. fugatei* (gray), *A. kearneyana* (orange), *A. longiflora* (blue), *A. palmeri* (red), and *A. tharpaii* (green). (B) Observed heterozygosity (H_o) by subpopulations: Historical (gray), LBC (orange), South (blue), and UBC (red). (C) ADMIXTURE structure plot for LBC, ST, and UBC where $K=2$. Red bars separate results by subpopulations

A		B	
Population	Fis	Species	Fis
LBC	0.135	<i>A. kearneyana</i>	0.128
UBC	0.014	<i>A. palmeri</i>	0.135
South	0.202	<i>A. tharpaii</i>	0.062
Historical	-0.031	<i>A. longiflora</i>	0.075
		<i>A. fugatei</i>	0.088

Table 1. (A) subpopulations of *A. kearneyana* and their inbreeding coefficient. (B) sister species of *A. kearneyana* and their inbreeding coefficient.

Discussion and Conclusion

Phylogeny

- *Amsonia kearneyana* is monophyletic and sister species to *A. palmeri*. Together they are sister to *A. grandiflora* and *A. longiflora*.

Genetic diversity

- Genetic diversity (H_o) of *A. kearneyana* is not significantly lower than that of other larger *Amsonia* species populations (fig.2A). This suggests that genetic diversity is not a representative metric for measuring genetic health or extinction risk.
- Intraspecific assessment of genetic diversity (H_o) revealed that larger subpopulations are correlated with higher genetic diversity (fig 2.B).
 - Historical and LBC subpopulations have lower genetic diversity due to their very small population size
- Larger populations of *Amsonia* in general have a lower F_{is} value, suggesting higher rates of outbreeding

Gene flow

- ADMIXTURE suggested $K = 2$ for the three subpopulations (UBC, LBC, ST). Pollinators appear to be moving pollen across the entire distribution of *A. kearneyana*.

Future Research

This research can be taken a step further by comparing the effective population size (N_e) of all of the *A. kearneyana* subpopulations to sister species. Genotyping the offspring generation can also be used to understand how much genetic diversity has been lost over time.

Acknowledgments

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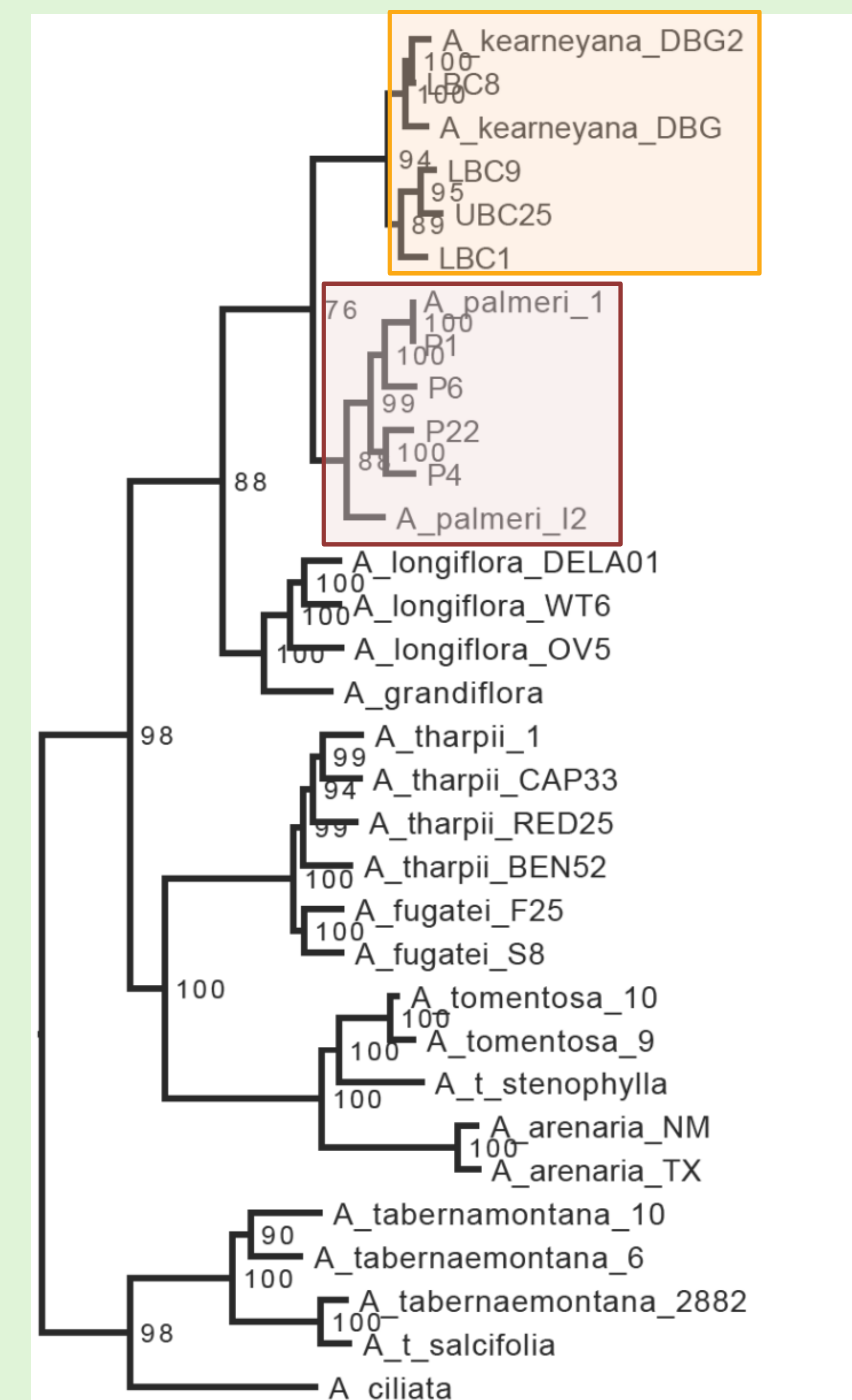


Figure 3. Phylogenetic reconstruction for western *Amsonia* species. Numbers at nodes indicate bootstrap support and values > 95 indicate strongly supported relationships. Several eastern *Amsonia* were used to root the phylogeny. (*A. kearneyana* (orange) and *A. palmeri* (red))