Investigating the origins of the American Amphitropical Disjuncts
Larrea tridentata and *Larrea divaricata*

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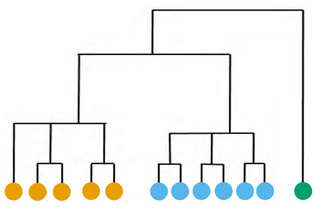
BIG PICTURE

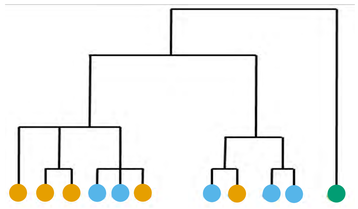
Introduction

- **Amphitropical disjunctions :** >>100 closely related genera or species occur in deserts of North and South America (Simpson et al. 2017)
- Sometimes result in speciation, but can be recent or happen repeatedly = obscure genetic differentiation
- **Creosotebush** is a classic **amphitropical disjunct** comprising two extant sister species, both widespread and ecologically important desert shrubs in N and S America (Fig. 1):
  North America = *Larrea tridentata*
  South America = *Larrea divaricata*
- *L. divaricata* and *L. tridentata* are **closely related**, but **differ morphologically** (Barbour 1969; **Fig. 1**) and **genetically** in limited chloroplast DNA (cpDNA) analyses (Laport et al. 2012)
- Timing and mechanism of dispersal from S America to N America estimated to be ~1mya (Laport et al. 2012) via birds, but remains unclear

1. Do *L. divaricata* and *L. tridentata* form distinct phylogenetic clades, consistent with being unique species? (**Hypothesis 1 vs. 2**)
2. Did the ancestor of North American *L. tridentata* disperse from the northern portion of South American range? **(Fig. 1)**



Hypothesis 1: *L. tridentata* (orange) and *L. divaricata* (blue) are reciprocally monophyletic.



Hypothesis 2: *L. tridentata* (orange) and *L. divaricata* (blue) are not reciprocally monophyletic.

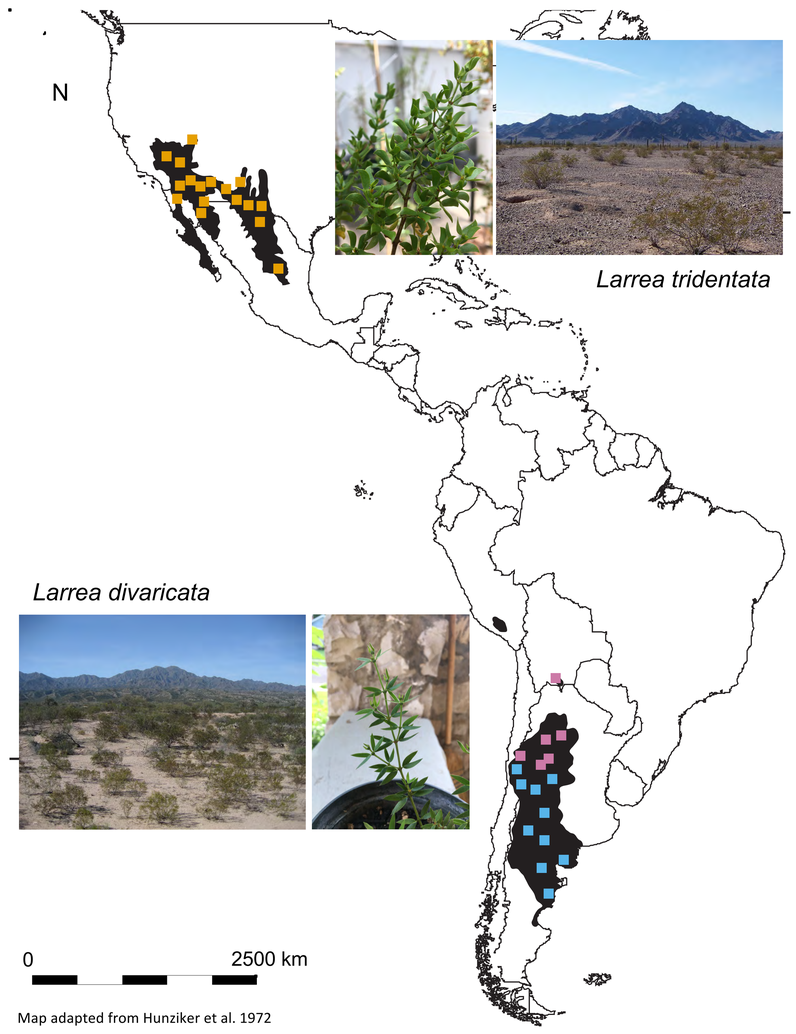


Figure 1: Collected *L. tridentata* (orange) and *L. divaricata* (blue) for haplotype and phylogenetic analyses span the North and South American range. North American populations are hypothesized to have originated via long distance dispersal from the northern portion of the South American range (lavender).

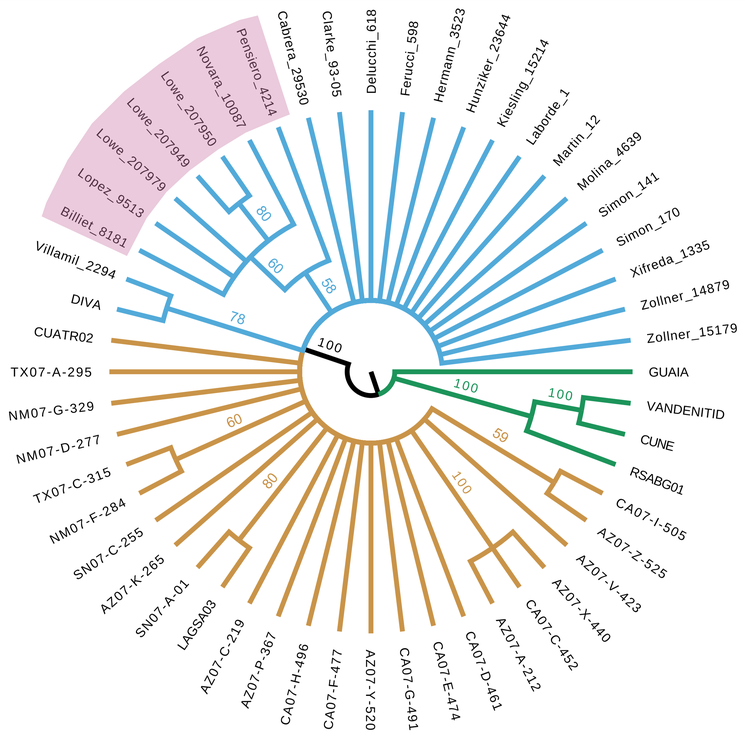


Figure 2: Phylogenetic relationships between *L. divaricata* (blue) and *L. tridentata* (orange) inferred from concatenated sequences of four cpDNA intergenic spacers do not support reciprocal monophyly. Lavender shading indicates N Argentina *L. divaricata* **(Fig. 1)**. Values above branches = bootstrap values; Green = outgroups

Takeaway

1. *L. tridentata* and *L. divaricata* **not reciprocally monophyletic** based on cpDNA (**Fig. 2**)
2. Populations in N Argentina genetically distinct from C & S Argentina (**Fig. 2 & 3**); suggests ***L. tridentata* shares MRCA with *L. divaricata* populations currently in C & S Argentina**
3. Sequence polymorphisms (**Fig. 3**) & conflict between individual intergenic spacers (**Fig. 4**) consistent with recent, rapid divergence = **need genomic level marker/sequence information**

A CLOSER LOOK

Collect tissue samples and extract DNA

- Herbarium tissues (NY, ARIZ, COLO, MO, MEXU) and field specimens
- Collections span North and South American range (**Fig. 1**)

Polymerase Chain Reaction

- Amplify cpDNA intergenic spacers
- cpDNA loci: rpoB/trnC, rpl16F/rpl16R, psbA/rpl2, rpl32/trnL

Sequence amplified DNA

- Amplified products Sanger sequenced (GeneWiz)
- Sequences manually quality checked and edited (Geneious)

Phylogenetic analysis

- Sequences manually aligned (Mesquite)
- Phylogenetic inference via maximum likelihood (RAxML; CIPRES Science Gateway) and parsimony (PAUP*); Bootstrap node support (100 replicates)
- Phylogenies visualized with FigTree

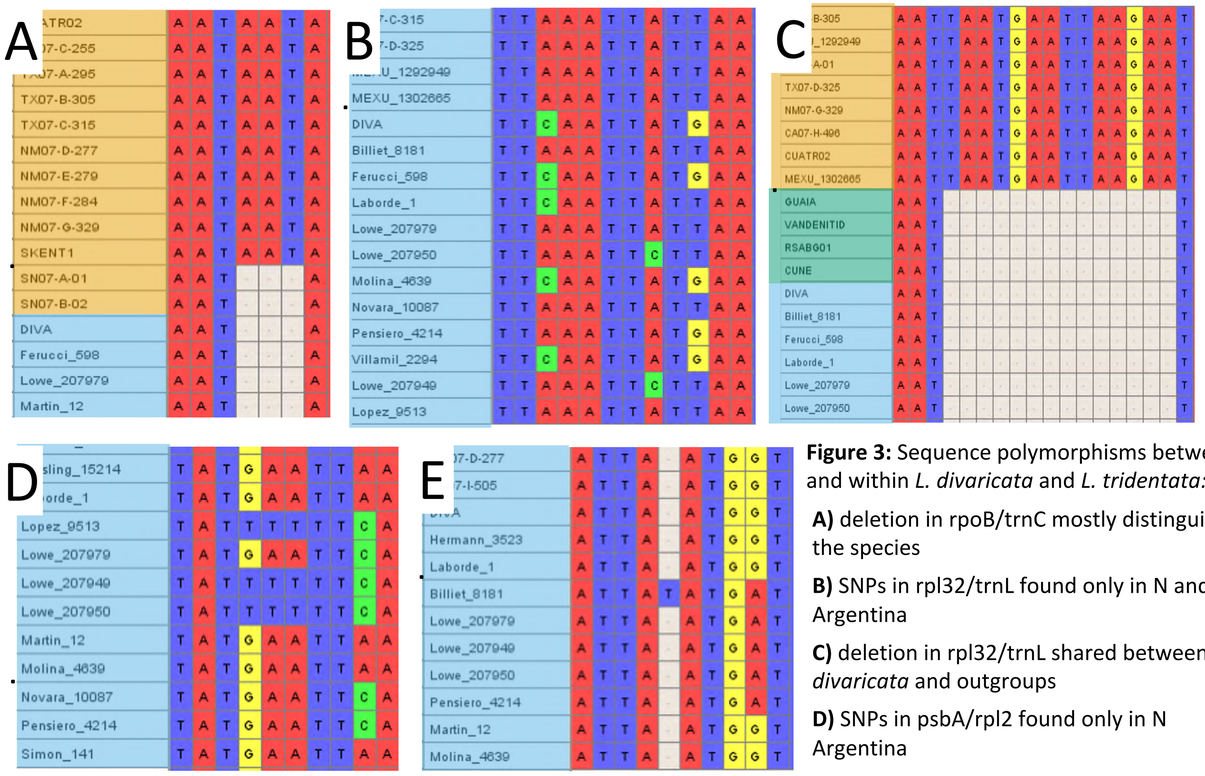


Figure 3: Sequence polymorphisms between and within *L. divaricata* and *L. tridentata:*

A) deletion in rpoB/trnC mostly distinguishes the species

B) SNPs in rpl32/trnL found only in N and E Argentina

C) deletion in rpl32/trnL shared between *L. divaricata* and outgroups

D) SNPs in psbA/rpl2 found only in N Argentina

E) SNP in rpl32/trnL found only in N Argentina

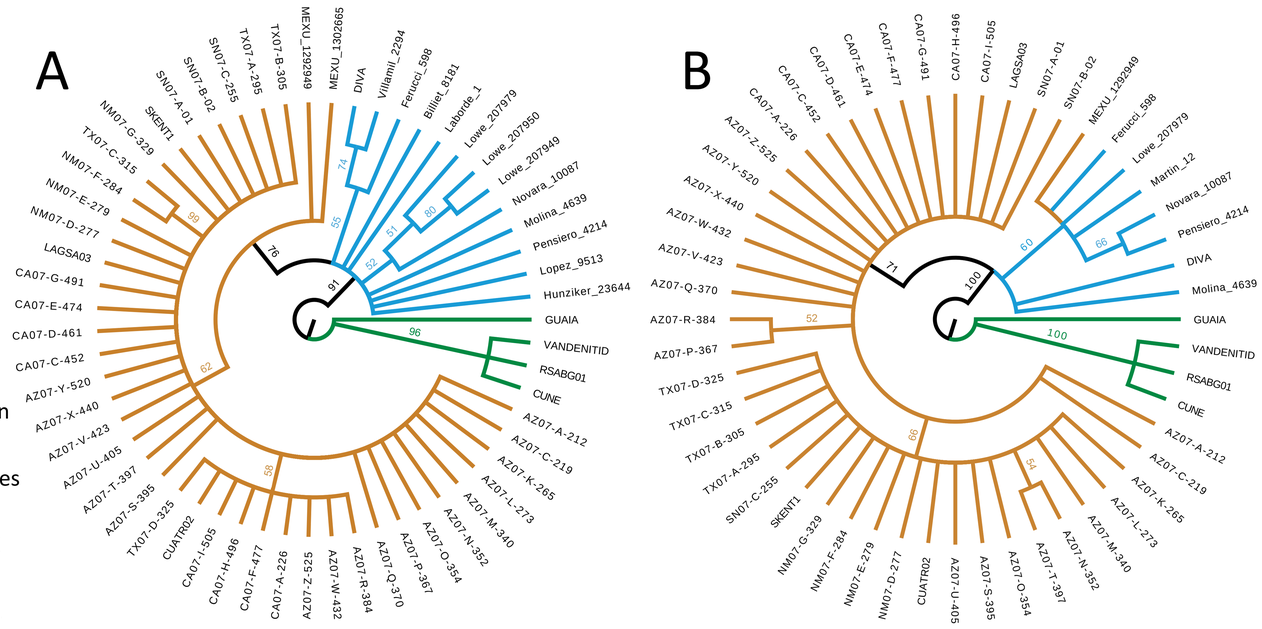


Figure 4: Phylogenetic relationships inferred from cpDNA intergenic spacers (A) rpl32/trnL and (B) rpoB/trnC suggest **conflict among informative characters** (Fig. 3) **contributes to unresolved concatenated phylogeny**, and argues for using low coverage genomic markers/sequences to help resolve relationships. Values above branches = bootstrap values; Green = outgroups.

Acknowledgements

W. Weaver- Univ. of Michigan
S. Smith and the Smith Lab – Univ. of Colorado Boulder
J. Ng - Univ. of Colorado Boulder

BSI Biological Sciences Initiative — CIPRES Cyber Infrastructure for Phylogenetic Research — NSF

NSF-DEB-1556371

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