DIVERSIFICATION OF THE GENUS *SUAEDA* (AMARANTHACEAE):
USE OF GENOME SKIMMING TO EVALUATE PUTATIVE SPECIES RADIATION IN NORTHWESTERN MEXICO

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INTRODUCTION

Fig. 1 a) *Suaeda esteroa* inflorescence blooming in San Diego, CA
b, c) *Suaeda* blooming in San Ignacio, Baja California Sur

There are over 100 estuaries along the northwestern coast of Mexico. These estuaries are relatively isolated, which may promote diversification of the flora they support. *Suaeda* sect. Brezia (Amaranthaceae) is one of the few sexually reproductive halophytes that grows in these estuaries (Fig 1). Members of this genus are generally confined to saline or alkaline soils and have thick, succulent leaves. The seeds can be dimorphic, with one seed type dispersed by water while the other type falls to the ground next to the maternal plant (Wang *et. al* 2008). Over the course of 20 years (1980-2000), nearly 350 specimens of *Suaeda* were collected by Wayne Ferren from many localities (Fig 2a). Based on his field and herbarium work, Ferren and Roberts (2009) suggested that as many as nine unrecognized taxa may occur in these estuaries (Fig 2b). However, all of these putative taxa are currently within the circumscription of *S. esteroa*.

Collectively, these putative taxa may represent an important example of diversification, resulting in what may be the densest concentration of sect. Brezia among any group of estuaries in the world. Early phylogenetic work by Brandt *et al.* (2015) shows that samples from populations within this putative radiation form a clade. With increasing development in northwestern Mexico, it is important to understand whether these populations merit taxonomic recognition as species may be lost without ever being described.

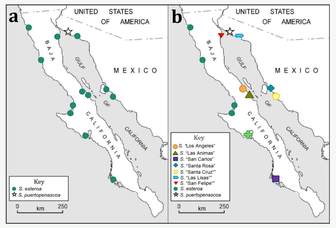


Fig. 2 a) Map of current, accepted distribution of *Suaeda esteroa* and *S. puertopenascoa* **b)** Map of proposed new *Suaeda* species, represented by different colored shapes

PROJECT GOAL

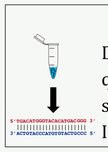
To use phylogenetic inference of *de novo* assembled chloroplasts and reference guided cistron assemblies to evaluate evidence for circumscription of new taxa in *Suaeda*.

METHODS

TAXON SAMPLING

Herbarium specimens from UC Santa Barbara Herbarium, collected and identified by Wayne Ferren were used for this project. We included four specimens for each of the putative new species, along with an additional single specimen of outgroup the species, *Suaeda taxifolia*, *S. calceoliformis*, *S. puertopenascoa*.

DNA EXTRACTION AND SEQUENCING



DNA was extracted using Thermo Scientific GeneJET Extraction Kit and quantified using a Qubit fluorometer. Global Biologics LLC prepared genome skimming libraries, with all samples pooled and paired-end sequenced on an Illumina HiSeq 2500 for 100 rapid cycles.

BIOINFORMATICS AND PHYLOGENETIC INFERENCE

An initial *de novo* assembly for one nuclear ribosomal cistron (nrDNA) contig was performed in Geneious (version 11.1.5), with reference guided assemblies for all subsequent samples. Chloroplast (cp) data was *de novo* assembled in the SPAdes pipeline (Nurk, Bankevich et al., 2013.)

All sequences were aligned using MAFFT (Katoh and Standley 2013) in Geneious under default settings. Maximum likelihood phylogenetic inference was performed in RAxML 7.2.7 (Stamatakis 2014), and Bayesian reconstructions in MrBayes 3.2.1 (Ronquist and Huelsenbeck 2003).

RESULTS

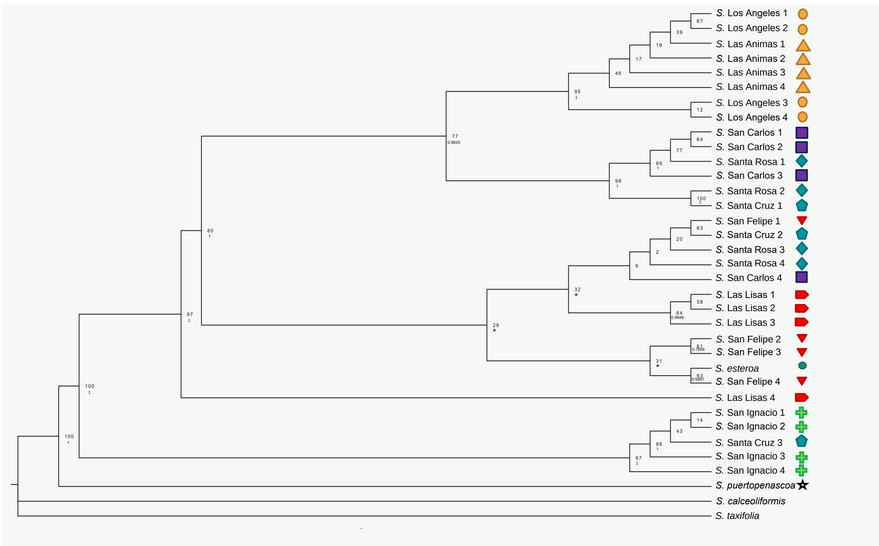


Fig. 3 Phylogenetic tree from maximum likelihood inference in RAxML of the nrDNA data matrix. Upper values are are ML bootstrap proportions, lower values are Bayesian posterior probabilities. * symbol indicates where the branching pattern between the two analyses conflict. Each locality or species is associated with the shape next to it. The color of these shapes correspond to geographic distribution (Fig. 5).

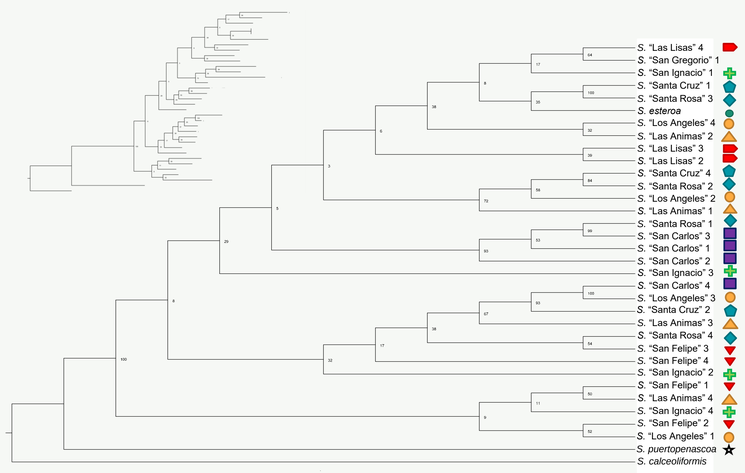


Fig. 4 Phylogenetic tree from maximum likelihood inference in RAxML of the chloroplast data matrix. Phylogram in upper left corner shows branch length. Each locality or species is associated with the shape next to it. The color of these shapes correspond to geographic distribution (Fig. 5).

CONCLUSIONS

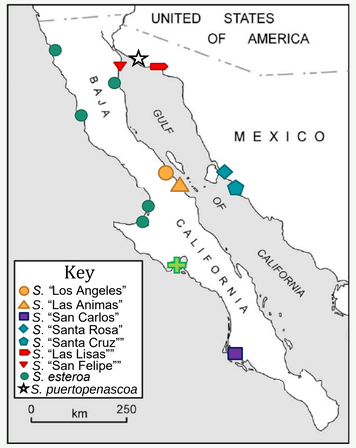


Fig. 5 Map of sampled localities. Symbols correspond to those in Fig. 3 and Fig. 4. Each locality is represented by a different shape while localities that occur near each other are the same color. The distribution of *Suaeda puertopenascoa* and *S. esteroa* are also shown on the map. The nrDNA shows geographic struture, while the cpDNA does not.

Our inferences using the nrDNA matrix reveals some interesting, well supported phylogenetic patterns both at deeper levels in the tree and towards the tips that correspond to geographic distribution of the samples (Fig. 5). We root the tree with *S. taxifolia* as it is from sect. Libogermen. *S. calceoliformis* and *S. puertopenascoa*, both members of sect. Brezia, are well outside our clade of interest. The entire putative radiation forms a well-supported clade with *S. esteroa* well-embedded among our samples from San Felipe, congruent with current taxonomy. Samples from Las Animas and Las Angeles are recovered as a clade and are found in estuaries proximally close together (Fig. 3). Samples from Las Lisas as well as San Ignacio also form clades, supporting Ferren's putative species for these locations. However, samples from Santa Cruz, San Carlos, and Santa Rosa are scattered amongst well supported lineages, and need further focused study because they are non monophyletic and no apparent patterns emerge with our current dataset that reflect morphology or geography.

Our chloroplast data yields several unexpected results (Fig. 4). First, in the *de novo* assemblies, ambiguities are recovered within samples. In several variable regions of the chloroplast, roughly half the reads that assemble to a site will be one of two nucleotides, resulting in ambiguities in our assemblies. Further bioinformatic investigation is needed to understand these results. Second, despite over 150kb of sequence data, the phylogenies from our cpDNA analyses lack detectable geographic and phylogenetic patterns.

TAKE HOME MESSAGE

While the nrDNA reconstruction shows patterns that appear to correlate with geographic distribution, the chloroplast data does not. The well-supported clades need further investigation for morphological patterns that might provide evidence for taxonomic recognition.

FUTURE DIRECTIONS

- Further field study is needed to:
  - Gather fresh tissue for genomic approaches that utilize nuclear data
  - Observe habitat and distribution within the estuaries
  - Collect and photograph fresh material for morphological study
- Conduct morphological analyses on fresh material that might be used to circumscribe any new taxa within the genus.
- Investigate biogeographic patterns of *Suaeda* in these estuaries

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