A morphological and genetic analysis of *Suaeda* from Mexican estuaries.

Carina Motta[1] , Matt Guilliams[2], Wayne Ferren[3], Susan Mazer [1], Katja Seltmann[1]

[1]University of California, Santa Barbara, Santa Barbara, CA 93106

[2]The Santa Barbara Botanic Garden, 1212 Mission Canyon Road, Santa Barbara, CA 93105

[3]Channel Islands Restoration, 928 Carpinteria Street Ste. 3 Santa Barbara, CA, 93103

Introduction

Fig. 1 Blooming Estuary Seablite (*Suaeda esteroa*) San Dieguito Lagoon, San Diego County, CA

The northwestern coast of Mexico is comprised of the coast of the Baja California peninsula along the Pacific Ocean and the Gulf of California. Included in these regions are a little over 100 estuaries that have evolved as a result of sea level and sedimentation rate[1,2]. These estuaries are isolated, creating a series of unique habitats for [illegible] tion may be facilitated due to the relative isolation of wetlands. Over the course of 20 years (1980-2000), nearly 350 specimens of *Suaeda* were collected by Wayne Ferren from these Mexican estuaries [2,3]. Plants found in nine regions, San Ignacio, San Gregorio, San Carlos, Las Animas, Los Angeles, San Felipe, Las Lisas, Santa Rosa, and Santa Cruz, were thought to represent new taxa[4]. With increased [illegible] be lost without ever being studied and with them, our understanding of how these unique wetlands may lead to speciation will also be lost.

There are 110 species of *Suaeda* (Chenopodiaceae) worldwide, with seven species described from Mexico. *Suaeda* are generally confined to saline or alkaline soils and have thick, succulent leaves. These morphological characteristics are seen in various halophytes, or those plants that thrive in saline habitats. *Suaeda* has dimorphic seeds, a life history strategy in which individuals of a species produce two kinds of seeds per plant. One type of seed is dispersed by water the other typically falls to the ground next to the maternal plant[6]. Unlike many halophytes, *Suaeda* species reproduce sexually rather than through rhizomatous growth.

Project Goal: To use morphological and genetic data to assess the validity of nine putative species of *Suaeda* from Baja California.

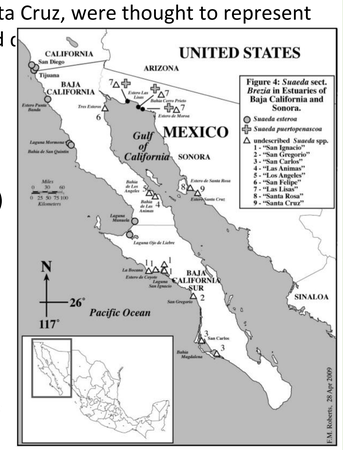


Fig. 2 Map of Baja California Mexico showing the distribution of putative species of Suaeda
Ferren, W. R., and F. Roberts. "The genus Suaeda (Chenopodiaceae) and conservation of estuaries in the Baja California peninsula and Sonora, Mexico." *Proc CNPS Conservation Conference. Sacramento*. 2011.

Leaf Morphology Methods

Specimens collected from 1980-2000 from Baja California and Sonora, Mexico are currently unmounted and housed at the Cheadle Center for Biodiversity and Ecological Restoration (CCBER) at the University of California, Santa Barbara.

Leaf Selection and Preparation

Three specimens from each locality (with the exception of San Gregorio due to lack of sufficient material) were randomly selected. The largest branch leaves were identified on each specimen and five of these largest leaves were randomly selected, totaling in 15 leaves from each locality. The leaves were rehydrated by soaking in water for 30 minutes to increase flexibility, and mounted in a grid on paper (Fig. 2). The sheet on which the leaves were mounted was then scanned with a ruler in view for reference. Leaf measurements of *S. esteroa* and *S. puertopenascoa* used in our analyses come from the description of *S. puertopenascoa* as a new species[7].

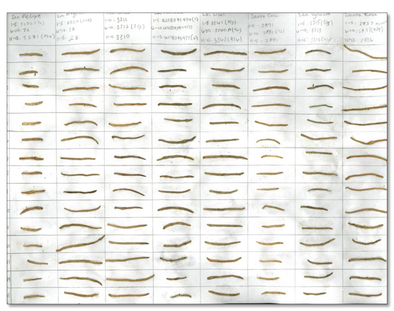


Fig. 2: Five leaves from each specimen and three specimens from each locality, totaling in fifteen leaves from each locality

Fig. 3: Leaf length measured using Segment Line

Fig. 4: Blue dots each mark one third of the leaf

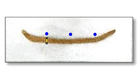


Fig. 5: Width measured at each of the three points

Measuring Using ImageJ

The number of pixels equivalent to one millimeter was set using the ruler in view. Each leaf was measured using the "Segmented Line" feature, allowing the curvature of the leaves to be taken into account. From this measurement the midpoint of the leaf and the midpoints of the leaf halves were found (Fig. 3 and 4). The width was measured at each of these three points (Fig. 5).

Flower Morphology Methods

Different specimens from the same collection collected over a 20 year period in Baja California and Sonora, Mexico which are currently unmounted and housed at the Cheadle Center for Biodiversity and Ecological Restoration (CCBER) at the University of California, Santa Barbara, were used for flower sampling.

Flower Selection and Preparation

Three specimens from each locality bearing flowers (with the exception of San Gregorio due to lack of sufficient material) were randomly selected, in addition to three specimens each of *S. esteroa* and *S. puertopenascoa*. From each of these specimens, three flowers were randomly selected, totaling in nine flowers from each locality or species. These flowers were rehydrated by soaking overnight in a refrigerator in a mix of water and dish soap (to disrupt cohesion).

Brightfield Microscopy and ImageJ

For each flower, a front view and side view was imaged using brightfield microscopy. The number of pixels equivalent to one millimeter was set using the ruler in view. Using the side view image, each flower was measured from the two most distant points, what we call the "diameter" of the flower. From this measurement the midpoint of the flower and the midpoints of the flower-halves were found (Fig. 6 and 7). The width was measured at each of these three points (Fig. 8).

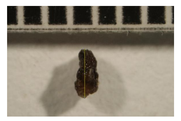


Fig. 6 Flower diameter being measured

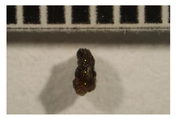


Fig.7 Three points at which width was measured

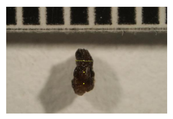


Fig. 8 Flower width being measured

Morphological Analysis Methods

In our preliminary morphological analyses, states for four quantitative characters derived from leaf and flower morphology were recorded. These characters were selected because they are an important difference between *S. esteroa* and *S. puertopenascoa*, as referenced in the *S. puertopenascoa* species description [7].

Exploratory graphs were constructed using R[9] package ggplot2 to visualize trends between flower diameter and locality. Measurements of both S. esteroa and S. *puertopenascoa* were included in these graphs. For the analysis, we calculated the pairwise dissimilarities (distance) between leaf and flower observations in the data using euclidean[10] coefficient. Dendograms were then constructed using the unweighted pair-group method using arithmetic averages (UPGMA; Sneath and Sokal 1973; function hclust in the R package vegan; Oksanen et al. 2013) on the distance matrix. A dendrogram was also constructed via a UPGMA hierarchical cluster analysis, using "average" agglomeration method. Measurements of *S. puertopenascoa* and *S. esteroa* were not included in this final analysis due to missing data (leaf width).

Genetic Analysis Methods

Over the course of 20 years (1980-2000), nearly 350 specimens were collected by Wayne Ferren from Mexican estuaries. Species found in nine regions, San Ignacio, San Gregorio, San Carlos, Las Animas, Los Angeles, San Felipe, Las Lisas, Santa Rosa, and Santa Cruz, were proposed to be novel. Four specimens from each region (with the exception of San Gregorio due to limited material) were selected based on quality of preservation to be used for DNA extraction. In addition, one specimen each of *S. taxifolia*, *S. puertopenascoa*, *S. calceoliformis*, and *S. esteroa* were selected as outgroups in genetic analysis. DNA was extracted from each specimen using a modified Short Version Small Scale CTAB procedure adapted from Doyle/ Doyle/Marcos/Baldwin procedure[8]. Modifications were made due to the fact that 20-30 year old herbarium specimens were being using and included a longer incubation in the water bath as well as longer centrifuge times. DNA RAD-Seq library prep was performed at Santa Barbara Botanic Garden and the resulting samples were sent to UC Riverside for sequencing. This method was chosen due to the large volume of SNPs typically produced, straight-forward bioinformatics via existing pipelines, and low cost.

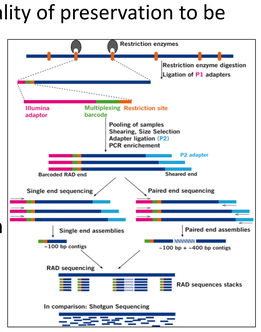
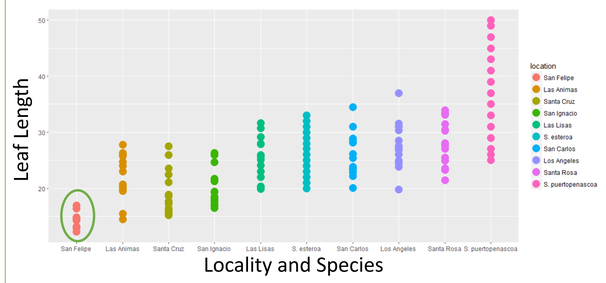
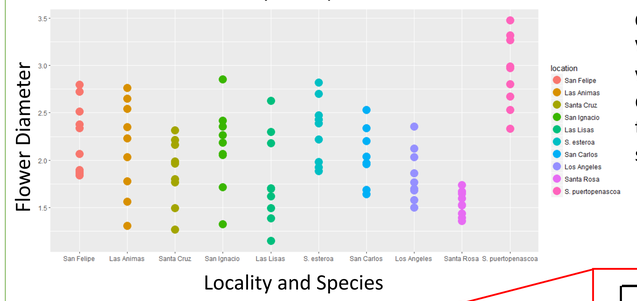


Fig. 14 Visual representation of ddRADseq method "RAD-Seq." *Floragenex | Your partner from DNA to data*. N.p., n.d. Web. 07 Apr. 2017.

Morphological Analyses



Graph 1 Visualization of variation in leaf length according to locality and species



Graph 2 Visualization of variation in flower diameter according to locality and species

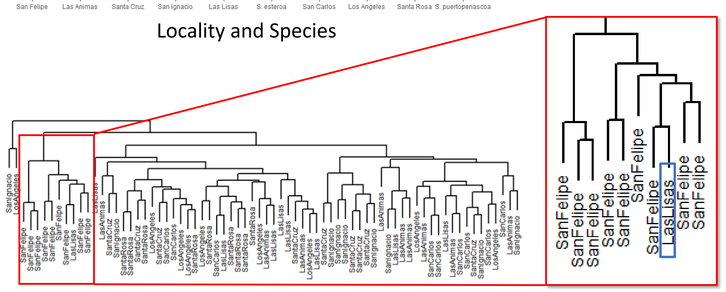


Fig. 9 UPGMA hierarchical cluster analysis using flower diameter, flower width, leaf length and leaf width.

Conclusions

In observing variation in leaf length, it was noted that leaves from the locality San Felipe were shorter than those of other localities, suggesting it to be unique. This was reaffirmed by out UPGMA cluster analysis in which most of the other localities did not form distinct clusters, while San Felipe, for the most part, did. Interestingly, Las Lisas also appeared in this cluster. While San Felipe and Las Lisas are not in the same hypothesized floristic regions, they are close geographically in relation to the other localities [4]. It was also noted from the variation in flower diameter that the flower diameters of Santa Rosa specimens are more conserved in their morphology than the other puntative species. From these observations, we plan to conduct further morphological study on specimens from San Felipe and Santa Rosa, and integrate these results with our molecular analysis.

Future Work

- Expand study of morphological characteristics to include more specimens and characters
- Complete genetic analysis and estimate hypothesis of phylogenetic relationships
- Visit estuaries to attain fresh material and observe habit, preferred growth location, etc.

References

[1] Isla, F. I. (1989). Holocene sea-level fluctuation in the southern hemisphere. *Quaternary Science Reviews*, *8*(4), 359-368.

[2] Lankford, R. R. (1977). Coastal lagoons of Mexico: their origin and classification. *Estuarine processes*, *2*, 182-215.

[3] Ganz, H. H., & Burton, R. S. (1995). Genetic differentiation and reproductive incompatibility among Baja California populations of the copepod T. californicus. *Marine Biology*, *123*(4), 821-827.

[4] Ferren, W. R., Jr., & Roberts, F. (n.d.). Three new species of Suaeda (Amaranthaceae) from estuaries in Baja California, Mexico.

[5]Ibarra-Obando, S. E., & Escofet, A. (1987). Industrial development effects on the ecology of a Pacific Mexican estuary. *Environmental Conservation*, *14*(02), 135-141.

[6] Wang, L., Huang, Z., Baskin, C. C., Baskin, J. M., & Dong, M. (2008). Germination of dimorphic seeds of the desert annual halophyte Suaeda aralocaspica (Chenopodiaceae), a C4 plant without Kranz anatomy. *Annals of Botany*, *102*(5), 757-769.

[7] Watson, M. Carolyn, and Wayne R. Ferren Jr. "A new species of Suaeda (Chenopodiaceae) from coastal Northwestern Sonora, Mexico." Madroño (1991): 30-36.

[8] Doyle, J., and J. L. Doyle. "Genomic plant DNA preparation from fresh tissue-CTAB method." Phytochem Bull 19.11 (1987): 11-15.

[9] R Core Team 2017

[10] Sneath, P. H. A. "BASIC program for a significance test for clusters in UPGMA dendrograms obtained from squared Euclidean distances." Computers & Geosciences 5.1 (1979).

Contact Information: carina_motta@umail.ucsb.edu