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

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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 fluctuation and varied sedimentation rate[1,2]. These estuaries are relatively isolated, creating a series of unique habitats where diversification 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 proposed to represent new species[4]. With increased development [5], such species may be lost without ever being studied and with them, our understanding of how these unique wetlands may lead to speciation will also be lost.

Fig. 2 Map of Baja California Mexico showing 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.

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 (Lenticular/L) the other typically falls to the ground next to the maternal plant (Flat/F)[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.

Seed Morphology Methods

Seeds were collected from 1996-1998 by Wayne Ferren from estuaries within Baja California. This collection includes *S. esteroa* (N= 3F; N=7L) and *S. taxifolia* (N=9L) seeds as well as seeds from the following localities: Las Animas (N=31F), Los Angeles (N=7F; N=18L), San Ignacio (N=30F), and San Felipe (N=12F).

Brightfield Microscopy Imaging and Measurement

Seeds from the UCSB herbarium were mounted on glass microscope slides using double stick tape and labeled accordingly. Each seed was then imaged using a brightfield microscope with a ruler in view. Once imaged, the seeds were measured using ImageJ software and the area and perimeter of each seed was calculated. Each measurement was exported from ImageJ to Excel and it was used to calculate a mean and standard deviation for each locality and seed type.

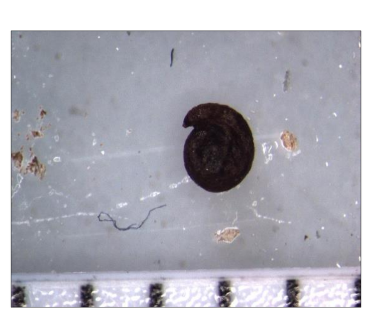

Fig. 3 Brightfield microscopy image of flat seed type from Los Angeles locality

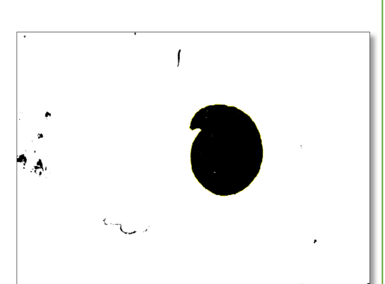

Fig. 4 Brightfield microscopy image of flat seed type from Los Angeles locality in ImageJ with conversion to 8-bit and altered threshold

Scanning Electron Microscopy (SEM)

One of each seed type from Las Animas, Los Angeles, San Ignacio, and San Felipe were imaged using SEM along with *S. esteroa* and *S. taxifolia*. These seeds were chosen because they were well preserved and exemplified common characteristics of their type and locality . These images were used to study surface sculpture.

Seed Morphology

Suaeda Species	Locality	Seed Type	Average Seed Area (mm, mean ± SD)	Average Seed Perimeter (mm, mean ± SD)
S. esteroa	California and Baja California	Dimorphic *Flat, Lenticular*	F: 1.90 ± 0.15 L: 1.43 ± 0.11	F: 5.92 ± 0.78 L: 4.79 ± 0.63
S. taxifolia	California and Baja California	Monomorphic *Lenticular*	L: 1.77 ± 0.26	L: 5.34 ± 0.26
S. sp_LA	Las Animas	Monomorphic *Flat*	F: 1.71 ± 0.24	F: 5.71 ± 0.73
S. sp_LANG	Los Angeles	Dimorphic *Flat, Lenticular*	F: 1.05 ± 0.08 L: 0.93 ± 0.10	F: 4.74 ± 0.68 L: 3.77 ± 0.67
S. sp_SI	San Ignacio	Monomorphic *Flat*	F: 1.27 ± 0.23	F: 5.21 ± 0.18
S. sp_SF	San Felipe	Monomorphic *Flat*	F: 1.54 ± 0.31	F: 5.25 ± 0.41

Table 1 Each *Suaeda* species or locality with seed type, average seed area, and perimeter

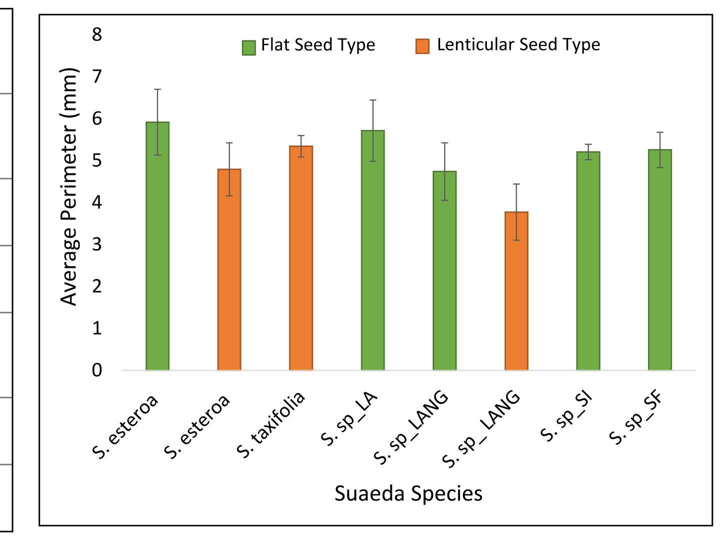

Fig. 5 Average perimeter of each seed type from each species or locality

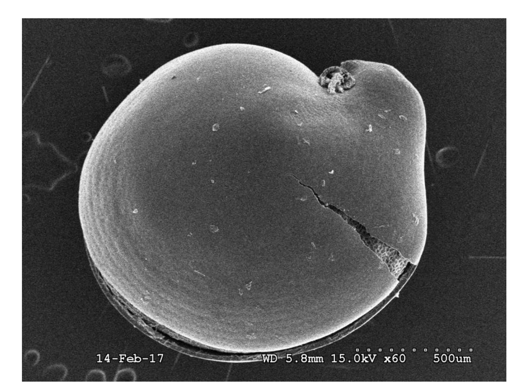

Fig. 6 *S. esteroa*: Lenticular seed type, mostly smooth with a linear reticulate pattern along the posterior side, discrete and rounded radicle, shiny and black.

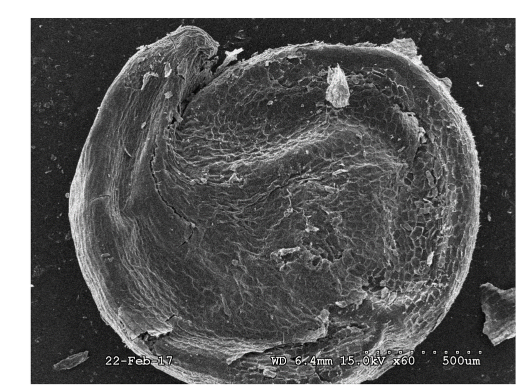

Fig. 7 *S. esteroa*: Flat seed type, coiled, coarse and fairly regular reticulate pattern, pronounced and pointed radicle, dull and brown.

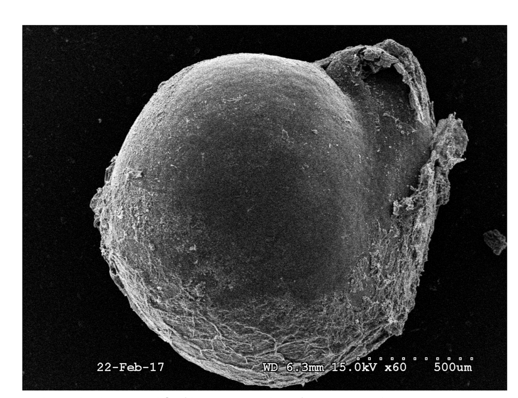

Fig. 8 *S. taxifolia*: Lenticular seed type, mostly smooth with reticulate pattern along posterior side, discrete and rounded radicle, shiny and black.

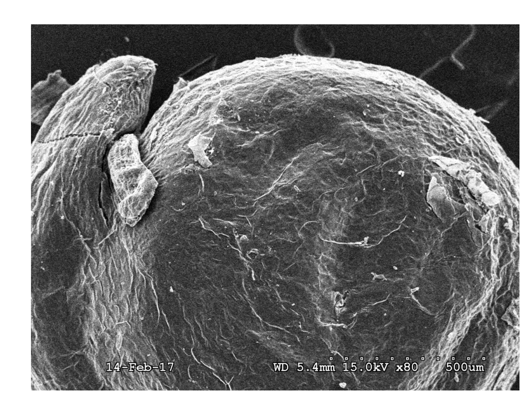

Fig.9 *S. sp_LA*: Flat seed type, coiled, lightly reticulate with irregular pattern, pronounced and pointed radicle, dull and brown.

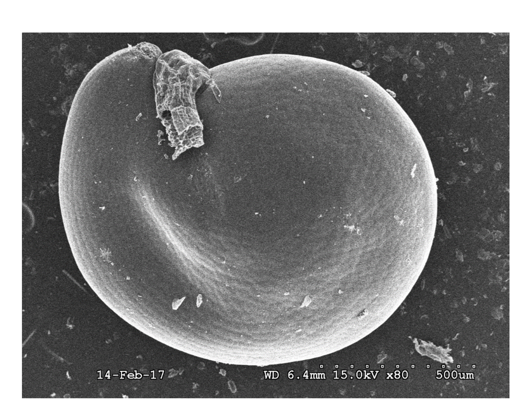

Fig. 10 *S. sp_LANG*: Lenticular seed type, lightly reticulate and mostly smooth, discrete and rounded radicle, shiny and black.

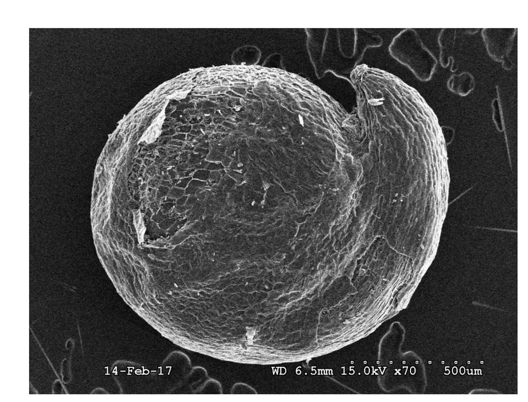

Fig .11 *S. s_LANG*: Flat seed type, coiled, reticulate pattern with striation along radicle, pronounced and pointed radicle, dull and brown.

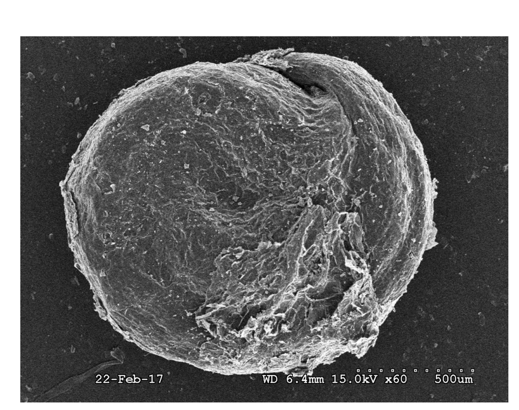

Fig. 12*: S. sp_SI*: Flat seed type, coiled, rough, irregular pattern, pointed radicle, dull and brown.

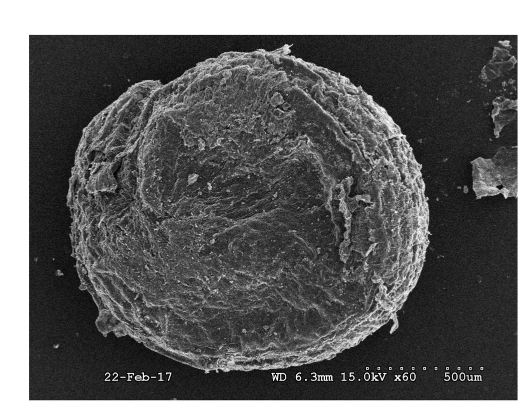

Fig. 13*: S. sp_SF*: Flat seed type, coiled, rough, irregular pattern, pointed radicle, dull and brown.

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 Thermo Scientific GeneJET Plant GenomicDNA Purification Mini Kit. Once extracted, the DNA was stored in a freezer until assayed for concentration using a Qubit fluorometer. A gel was also run to assess DNA quality. The DNA samples will be sent to Global Biologics for ddRADseq library preparation and high throughput sequencing. This method was chosen due to the large volume of SNPs typically produced, straight-forward bioinformatics via existing pipelines, and low cost.

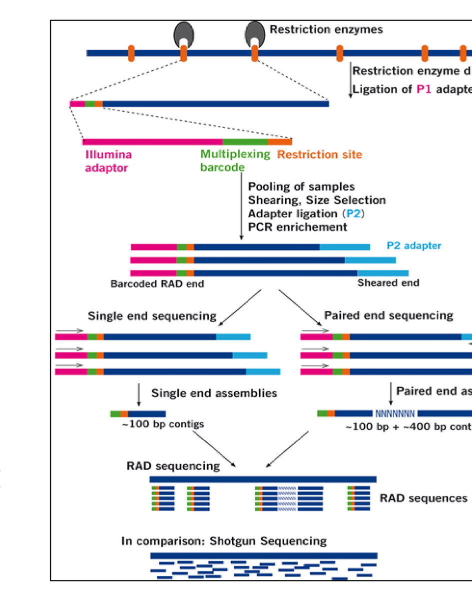

Fig. 14 Visual representation of ddRADseq method "RAD-Seq." *Floragenex | Your partner from DNA to data*. N.p., n.d. Web. 07 Apr. 2017.

Conclusions

Measurements of the seeds revealed *S. sp_LANG* had both the smallest seeds for flat and lenticular seed types. Meanwhile, *S. esteroa* had the largest flat seed type and *S. taxifolia* had the largest lenticular seed type. Seeds from *S. sp_SI* and *S. sp_SF* were both monomorphic, producing flat seeds similar in size. However, small sample size should be taken account and prompts further measurements.

The number of seed types produced by a species varies, with *S. esteroa* and *S. sp_LANG* being dimorphic while *S. taxifolia, S. sp_LA, S. sp_SI, and S. sp_FE* are monomorphic. *S. taxifolia* is monomorphic, producing lenticular seeds, as is representative of *Suaeda* sect. *Libogermen*[8]. Though monomorphic, unlike *S. taxifolia*, *S. sp_LA, S. sp_SI, and S. sp_FE* only produce flat type seeds. As of now, no example exists where it is characteristic for only flat seed type to be produced. *S. sp_LANG,* like *S. esteroa*, also produces both flat and lenticular seed types. The two different seed types serve different purposes and are intended to germinate at different times[6]. The lenticular seed type is covered with a hard, shiny seed coat and is dormant. This makes it difficult for the seed to imbibe water easily and delays germination. The flat seed type is only covered with a thin membranous seed coat and is not dormant. This allows it to germinate quickly, sometimes while still attached to the maternal plant, since the thin seed coat limits the dispersal ability of this seed type [7].

Future Work

- ❖ Expand study of morphological characteristics to include characters such as leaf scar shape
- ❖ Complete genetic analysis and estimate hypothesis of phylogenetic relationships
- ❖ Look for biogeographic and phylogenetic patterns corresponding to seed type and other morphological characters
- ❖ Explore why certain seed types are favored and whether this trait is caused by genetic or environmental factors. If caused by environmental factors, investigate which factor by studying differences in salinity, temperature, average rainfall, and other potential stressors.

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