

## Introduction:

- The milkweed genus *Asclepias* (Apocynaceae) has been a model system in studies of plant reproduction, plant defense, plant-animal interactions, and increasingly plant genomics (Fishbein et al. 2018).
- Asclepias* species are the sole host plant for the larvae of monarch butterflies, a species of high conservation concern.
- There are over 130 species of *Asclepias* in the Americas; there are 75 in the United States and Canada, 75 in Mexico and Central America, and 8 in South America.
- The phylogeny (evolutionary relationships) of *Asclepias* has been used as a basis to study the evolution of adaptations to insect herbivory and drying climates (Agrawal et al. 2009, Fishbein et al. 2018).
- The Sonoran Desert Clade consists of nine species in the southwestern United States and northern Mexico and is the earliest diverging group of *Asclepias* species (Fig 1., Table 1).
- The unusual traits of the Sonoran Desert Clade make it an important group for studying adaptations to aridity; the phylogenetic relationships of this group are still not well understood.
- Whole chloroplast DNA sequences (plastomes) are a useful source of data for determining phylogenies.

## Sonoran Desert Clade

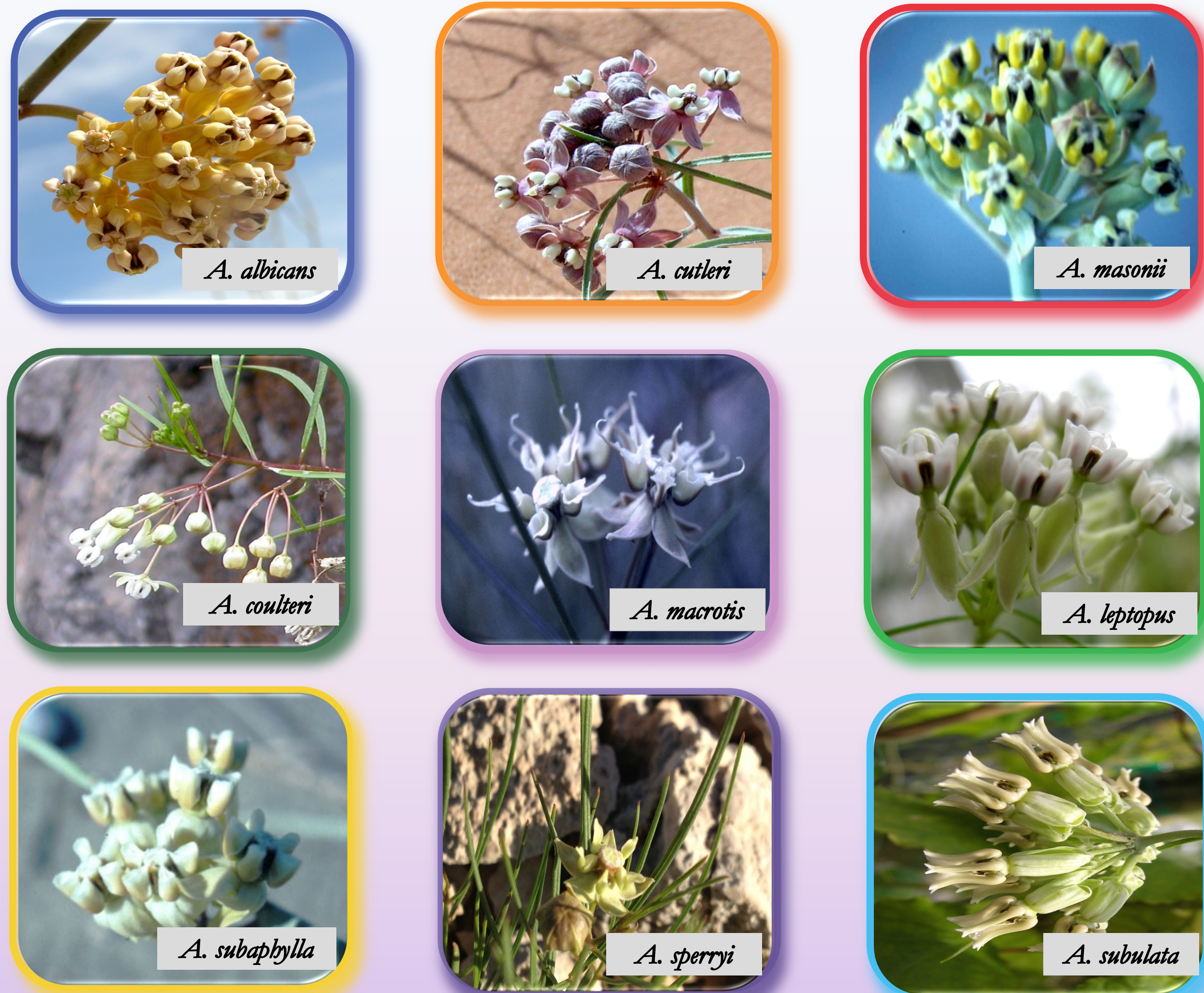


Fig 1. These are pictures of the 9 species of the Sonoran Desert Clade

## Methods:

- 1-9 samples of each Sonoran Desert Clade species were obtained and sequenced through "genome skimming" by Fishbein and Straub lab members (Straub et al. 2011; Weitemier et al. 2014). 17 new plastomes were assembled for this part of the project following the steps of Fishbein et al. (2018).
- Sequence reads were filtered and trimmed to remove low quality data in Geneious.
- Reads were then assembled into contiguous sequences (contigs) using either Geneious or IOGA.
- In Geneious, portions of contigs matching a reference plastome (*A.nivea\_022431*) were found using BLAST and then aligned to the reference to produce a draft plastome.
- To finish the assembly of the plastome sequence the trimmed reads were mapped to the draft to fill in gaps between contigs.
- Multiple sequence alignments were produced using the online implementation of MAFFT, with other representatives of American and African milkweeds used as outgroups.
- Character exclusion sets were produced using GBLOCKS executing in Mesquite to omit ambiguously aligned regions.
- IQTREE was used to find the best fit model of sequence evolution and to estimate the maximum likelihood (ML) phylogeny with clade support estimated by fast bootstrapping.

## Results:

- 17 new plastomes were assembled:
  - Range of sequence reads assembled per sample: 39,914 to 411,110
  - Range of depth of coverage of the assembled plastomes: 26.8 to 360.6
  - Range length of assembled plastomes: 129,899 to 136,946
- The Sonoran Desert Clade doesn't have multiple clades; all nine species form a single lineage. (Fig 2.)
- For five of the nine species all of the sampled individuals were each other's closest relatives: *A. cutleri*, *A. macrotis*, *A. sperryi*, *A. leptopus*, and *A. masonii*; there was only one individual of *A. subaphylla*.
- For the remaining three species at least one sample was grouped with a different species. *A. coulteri* sample 1790 was as closely related to *A. sperryi* and *A. macrotis* as to the other *A. coulteri* samples. *A. albicans* and *A. subulata* were intermixed in two different clades. (Fig 2.)
- The original divergence of the Sonoran Desert clade was into eastern (*A. coulteri*, *A. sperryi*, *A. macrotis*) and western (*A. cutleri*, *A. leptopus*, *A. subulata*, *A. subaphylla*, *A. masonii*, and *A. albicans*) groups. (Fig 2.)
- Individuals within a species that came from relatively close geographical locations seem to be more closely related on the phylogenetic tree. (Table 1, Fig 2.)
- The hybrid individuals (*A. albicans* x *subulata*) are placed in two separate clades indicating they had separate origins (Fig 2.)

## Conclusion/Discussion:

- The phylogeny from plastomes sequences is well resolved with bootstrap values mostly between 90 and 100%.
- The results demonstrate that the original step in diversification was the formation of eastern and western clades.
- A. cutleri* was found on a very long branch compared to other species. This has to deal with how the species experienced more evolutionary changes than the others. The reason for this is unknown and needs further study. (Fig 2.)
- An interesting relationship that was found was the relationship between *A. masonii* and *A. subaphylla*. These two species are very rare and grow in restricted geographical locations (*A. masonii* on a single island off the shore of Baja California Sur and *A. subaphylla* on the coast of Sonora and Sinaloa), however they are each other's closest relatives. (Table 1., Fig 2.)
- For the species in which the individuals don't form clades it is possible that there is hybridization among species (Fig 2), because there are known hybrids between *A. albicans* and *A. subulata*. That makes their relationships difficult to determine.
- These results are found useful in furthering the knowledge on the relationships among the species in the Sonoran Desert Clade.

## References:

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- Straub SCK, Fishbein M, Livshultz T, Foster Z, Parks M, Weitemier K, Cronn RC, & Liston A (2011) Building a model: developing genomic resources for common milkweed (*Asclepias syriaca*) with low coverage genome sequencing. *BMC Genomics* 12:211.
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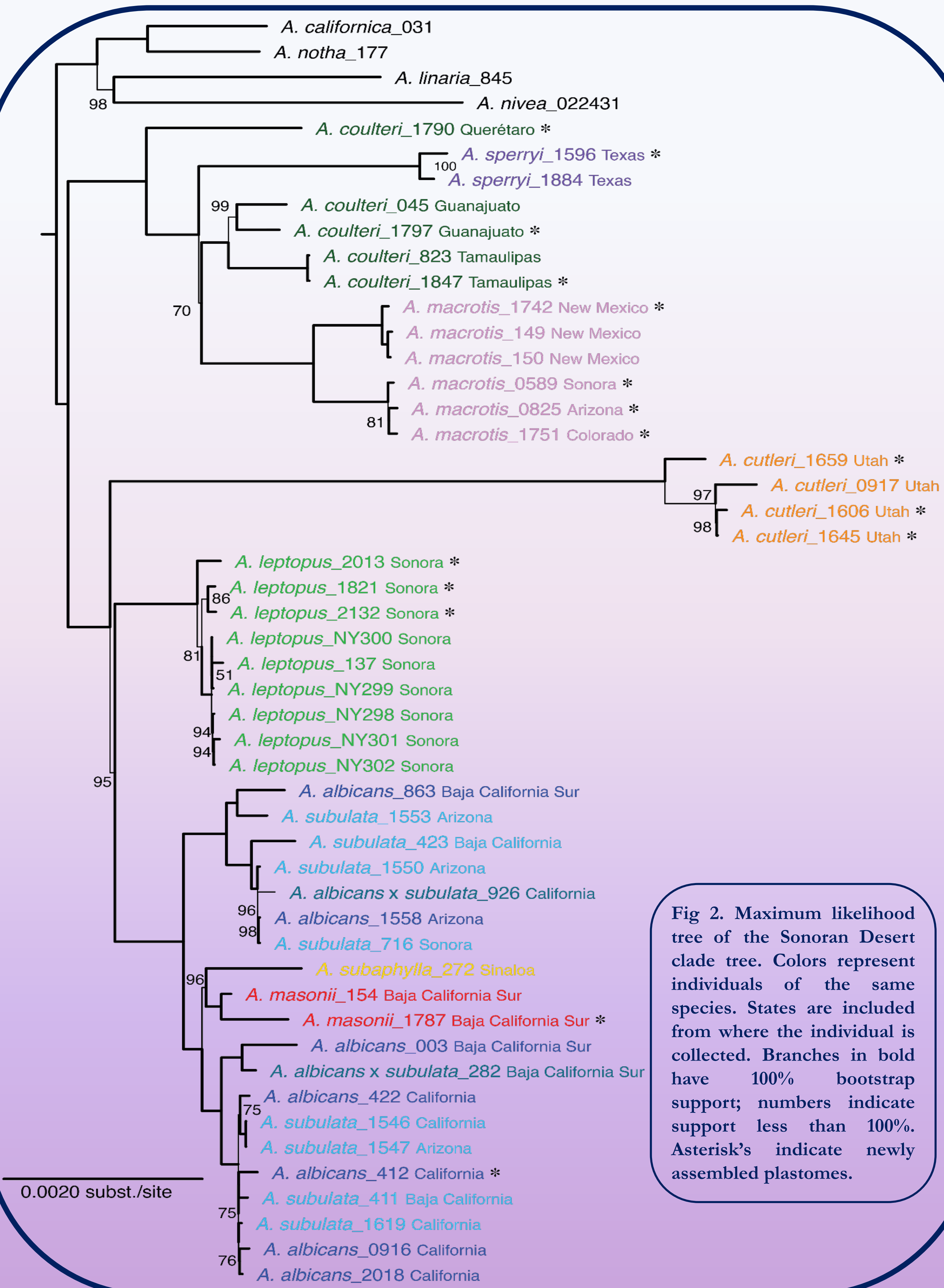


Fig 2. Maximum likelihood tree of the Sonoran Desert clade tree. Colors represent individuals of the same species. States are included from where the individual is collected. Branches in bold have 100% bootstrap support; numbers indicate support less than 100%. Asterisks indicate newly assembled plastomes.

Species	Location
<i>A. albicans</i>	Sonoran Desert in Arizona, California, Baja California, Baja California Sur, Sonora
<i>A. coulteri</i>	Sierra Madre Oriental in Guanajuato, Hidalgo, Querétaro, San Luis Potosí, Tamaulipas
<i>A. cutleri</i>	Colorado Plateau in Arizona, Utah
<i>A. leptopus</i>	Sierra Madre Occidental in Chihuahua, Sinaloa, Sonora
<i>A. macrotis</i>	Mountain slopes in desert and grassland in Arizona, Colorado, New Mexico, Oklahoma, Texas, Chihuahua, Coahuila, Sonora
<i>A. masonii</i>	Sonoran Desert on Isla Magdalena in Baja California Sur
<i>A. sperryi</i>	Mountain slopes in desert in Texas, Coahuila
<i>A. subulata</i>	Sonoran Desert in Arizona, California, Nevada, Baja California, Baja California Sur, Sonora
<i>A. subaphylla</i>	Coastal dunes in Sinaloa, Sonora

Table 1. Habitat and distribution of the Sonoran Desert Clade species.