Western National Parks Association (WNPA) grant
FINAL REPORT. Grant reference #20-07. Due December 2022.
Title: *Unveiling a potential new species of California Jewelflower endemic to Pinnacles National Park*
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Park: Pinnacles National Park

SUMMARY

The goal of this project was to elucidate the status of what appeared to be a new species of jewelflower (common name given to plants belonging to the genera *Streptanthus, Caulanthus, Guillenia*) recently discovered at Pinnacles National Park. The occurrence of individuals of jewelflowers has been recorded in Pinnacles for some time. Populations from the Park seemed "unique" but had not been formally studied from either a morphological or a molecular standpoint, so the species they belonged to had remained unclear. Until now.

Throughout this project, we conducted botanical surveys in the field and herbaria (collections of dried plant specimens), consolidated morphological and geographic occurrence data, and performed state of the art genomic analyses based on sequences of DNA. Based on our work we can now assert that multiple lines of evidence point to populations at Pinnacles National Park being in fact, a distinct species of jewelflower that is new to science!

An initial assessment based on morphological similarity and species distributions, suggested that individuals from this mysterious taxon at Pinnacles National Park seemed to be intermediate between two species, *Streptanthus glandulosus* and *Streptanthus insignis*, with some resemblance to a third one, *Caulanthus coulteri*. However, our phylogenomic analyses of the evolutionary relationships of the Pinnacles Jewelflower show quite conclusively that it belongs to the genus *Caulanthus* and closely related to *Caulanthus coulteri*.

Taken together, our data on morphology, geography and evolutionary relationships show that populations of the Pinnacles Jewelflower conform in fact a new species of jewelflower, in the genus *Caulanthus*. We are in the process of formally naming this species, for which a taxonomic description must be published in a scientific paper. We are evaluating naming this species to honor the Chalon people whose homeland included the Chalone Creek watershed, where most of the populations of the Pinnacles Jewelflower occur.

In summary, as part of our multi-faceted research program we have: (1) Conducted a series of botanical surveys to locate and document populations of the Pinnacles Jewelflower as well as other jewelflowers in the area; (2) Generated state of the art genomic data for representatives of the Pinnacles Jewelflower as well as individuals of other species of *Streptanthus, Caulanthus* and *Guillenia*, and other Brassicaceae to serve as references for our analyses; (3) Performed analyses of genomic data to assess how the Pinnacles Jewelflower is related to other jewelflowers; (4) Consolidated morphological and ecological information on jewelflowers of San Benito County, California, that because of their geography or morphology were candidates to be closely related to the Pinnacles Jewelflower; (5) Shared our work and results in two outreach forums, and; (6) Produced a prototype of materials highlighting our research on the Pinnacles Jewelflower to be used by the park for visual interpretation.

ALL PRODUCTS DERIVED FROM THIS PROJECT

1) *Botanical surveys*– As part of this project, we conducted a series of botanical surveys in Pinnacles National Park to locate populations of this focal taxon as well as other jewelflowers in the area, including collections to document their morphology and occurrence.
2) *Genomic data*– Generated state of the art genomic data for a total of 94 samples that include representatives of the Pinnacles Jewelflower as well as individuals of other species of *Streptanthus, Caulanthus* and *Guillenia*, as well as other Brassicaceae to serve as references for our analyses (see Table 1).
3) *Morphological and ecological information*– We have consolidated morphological and ecological information on the Pinnacles Jewelflower as well as on other selected taxa of jewelflowers of San Benito County, California (see Table 2). We focused on those species that were candidates to be closely related to the Pinnacles Jewelflower based on geography or morphology. We thus included species of *Streptanthus*, *Caulanthus* and *Guillenia* that occur in San Benito County and that show some resemblance to the Pinnacles

Jewelflower in their leaves, flowers, or other morphological attributes. We generated plates illustrating such species (see Figure 1), and maps that show where species occur in San Benito County and in relation to the Pinnacles Jewelflower (see Figure 2). We are in the process of generating final versions of illustrations and maps, for publication.

4) *Phylogenetic and genetic analyses*– We have performed phylogenetic analyses that offer convincing evidence that the Pinnacles Jewelflower is a species, and that it belongs to the *Caulanthus* clade (see Figure 3). We are in the process of analyzing an expanded dataset that will leverage the data we produced from this project with data we had on previous projects. This expanded dataset will also be the basis for genetic analyses.

5) *Outreach and communication activities*– So far, we have shared our work and results through the following presentations:

   a) Talk aimed at the public (free event), given twice to chapters of the California Native Plant Society. Title: *A Preview of the "Pinnacles" jewelflower: a potential undescribed species*. Presenter A. Ryan. Milo Baker Chapter, Santa Rosa, CA, March 16, 2021; Monterey Chapter, Pacific Grove, CA, January 13, 2022.

   b) Talk aimed at the public at the Fort Worth Botanical Garden in Fort Worth, Texas. Title*: Jewelflowers: A story of diversity, ecology, and evolution*. Presenter: NI Cacho. Research Lecture Series. Fort Worth, TX, USA. March 29, 2022.

   c) Poster presentation at the annual meeting of the California Native Plants Society 2022, a scientific conference where botanists discuss recent advances and perspectives related to native flora of California. Title: *The Pinnacle of Jewelflowers–An undescribed potential species of Streptanthus at Pinnacles National Park*. Authors: Amelia Ryan, Alejandra Vasco, Charlotte Okraska, Bryan T. Drew & NI Cacho. Presenter: Amelia Ryan. October 20, 2022.

   d) We are also working on a piece for a general audience on the discovery of the Pinnacles Jewelflower. Our goal is to show the importance of botanical collections and exploration as keystones of discovery and conservation, and how National Parks can play a central role in these endeavors.

6) *Scientific research articles*– We are in the process of preparing for submission two research articles communicating the results derived from this project:

   a) A first paper focuses on the taxonomic formal description and naming of the Pinnacles Jewelflower. Tentative title: *A new species of Jewelflower from Pinnacles National Park, supported by morphological, geographic, and genomic data*. Target Journal: Systematic Botany.

   b) A second paper will present the most up to date evolutionary relationships (phylogeny) of Jewelflowers and include the phylogenetic placement of a few recently described species, including the Pinnacles Jewelflower. We are currently completing analyses (both phylogenetic and genetic) on an expanded dataset built by combining the data generated from this project with previous data. Tentative title: *A phylogenomic perspective on an iconic plant radiation highlights the importance of ongoing exploration*. Target Journal: American Journal of Botany.

7) *Visual interpretation materials*– We have produced a prototype of visual interpretation materials to be used by the Park. See below for more detail (see Figure 4).

PLAN FOR AN INTERPRETATION-RELATED PRODUCT

Our plan for interpretation-related products involves two products aimed at the public visiting Pinnacles National Park: a visual banner for display at the park and a flyer or handout for visitors to the park.

We have produced a prototype of visual interpretation materials to be used by the park, see Figure 4. This banner can directly be printed for display and can also be the basis for additional materials, including a flyer or fact sheets for visitors to the Pinnacles National Park. We also have additional visual materials that can be used to generate material for display at the park (for example, a self-explanatory presentation for display on a monitor at the Park headquarters) or at the NPS park and/or WNPA websites.

Finally, we will incorporate our data and analyses in class activities currently under development to promote critical thinking and analyses of biological data aimed at undergraduate and graduate students. In a first phase, will test and implement these materials at classes at the University of Nebraska at Kearney, and at

the National Autonomous University, in Mexico City. Later, we will make them available widely through social media and our network of friends and collaborators.

DESCRIPTION OF RESEARCH PERFORMED AND GOALS ACHIEVED

Goals– We aimed at providing a solid framework for a taxonomic assessment and description (including a formal name) for the Pinnacles Jewelflower, a potential new species of jewelflower that grows in Pinnacles National Park by integrating morphology, evolutionary history, genetic diversity, and systematics.

Surveys and sampling– We first went through all collections at the Pinnacles Herbarium (PINN) and based on that information and previous records from staff at the Park, we surveyed field sites and documented the status of the Pinnacles Jewelflower in the Park. In total, we have documented a total of nine sites where we have corroborated individuals of the Pinnacles Jewelflower grow.

Additionally, we compiled data on geographic occurrence for all species of jewelflowers that grow in San Benito County, California, consulting databases (such as GBIF and Calflora), our own records, and consultation with experts from local offices at the Bureau of Land Management. These species are: *Streptanthus glandulosus, S. insignis, S. breweri, Caulanthus coulteri* var. *lemmonii*, and *Caulanthus anceps.* Based on the data we compiled we built occurrence maps for all these species, including the Pinnacles Jewelflower.

Morphologically, individuals collected in the Park seemed to be intermediate between the species *Streptanthus glandulosus* and *Streptanthus insignis*; this last species was originally described in the Jepson Manual as "intermediate between *S. glandulosus* an *Caulanthus coulteri*" (Hickman, 1993). Our current understanding of evolutionary relationships within jewelflowers, based on phylogenetic analyses of DNA sequence data of eight molecular markers (Cacho & Strauss, 2013; Cacho et al, 2014), indicates that *S. insignis* is in fact a close relative of *S. glandulosus*, and rather distant of *C. coulteri*. However, the relationships of *S. glandulosus* to *S. insignis* and other taxa remain currently unresolved, and this group is referred to as the 'Glandulosus Complex'. Thus, to identify the closest relatives of *Streptanthus* occurring at PINN, and infer its geographic provenance (i.e., where was Pinnacle colonized from), it is necessary to resolve the evolutionary relationships among the six species that conform Glandulosus Complex.

Integrating data from our fieldwork, herbarium work, and collections from previous projects, we assembled a first set of target samples for DNA extraction (prior to be sent for sequencing), prioritizing individuals that would represent as many populations as possible of the Pinnacles Jewelflower, and species that because of their geography (grow in close proximity) or their morphology (resemble in some aspect the Pinnacles Jewelflower) could be candidates to be close relatives of this mysterious taxon.

Lab work and sequencing– Lab work consisted of DNA extraction, purification, and quantification to guarantee yields and quality needed to generate high quality genomic data. Lab work was performed at the University of Nebraska at Kearney by student collaborator Charlotte Okrasaka under supervision of Dr. Bryan Drew, and at the Sumner Molecular Laboratory at the Botanical Research Institute of Texas (BRIT– https://fwbg.org/research/), by PIs Cacho and Vasco. We processed as many as 230 samples, of which only the best 94 (number of samples admitted per round of sequencing) were chosen to send for sequencing. Our final sampling consisted of samples from 36 species, with variable numbers of individuals per species, and 14 individuals from the Pinnacles Jewelflower (see Table 1). We extracted genomic DNA with the Plant DNeasy Kit (Qiagen, Valencia, CA), and performed quality check and quantification with a combination of agarose gel electrophoresis and Qbit. We normalized samples to a final concentration of ~50ng/µL, prior to shipping to the sequencing facility.

RADseq data generated by the company Floragenex (https://www.floragenex.com), where barcoding, library construction, and Illumina sequencing were performed following standard protocols. RADseq data (Etter & Johnson, 2012) has been used successfully in studies of phylogeography (Emerson et al, 2010), phylogenetics (Eaton & Ree, 2013; Hipp et al, 2014) and population structure (Zellmer et al, 2012).

Data processing–We did our quality check and processing of reads using the bioinformatic pipelines implemented in *ipyrad* (Eaton 2014). We did two assemblies, one without a reference genome, and one using a draft from the genome sequence of *Caulanthus amplexicaulis*, available at: https://phytozome-next.jgi.doe.gov/info/Camplexicaulis_v1_1. RADLoci and SNPs were generated using default values implemented in *ipyrad*.

Data analyses– We first processed our data with a phylogenetic perspective using a Maximum Likelihood framework and the program RAXML (Stamatakis, 2014) as implemented in *ipyrad* (Eaton 2014; Parameters: rapid Bootstrap analysis with molecular evolution model GTRGAMMA, and N=100) to assess whether: (a) the individuals of the Pinnacles Jewelflower belong to only one clade (group); (b) if so, whether this clade is distinct from others, and (3) the evolutionary relationships of such clade. **These were the core questions in our proposal, and we have answered them all**: The individuals of the Pinnacles Jewelflower do form a clade, one that is distinct from other species of Jewelflowers, and data suggest it is most closely related to *Caulanthus coulteri* var. *lemmonii* (see Figure 3, clade of the Pinnacles Jewelflower in red). We had to exclude some samples due to inaccessibility during the pandemic. To alleviate any issues arising from excluding some samples that could have critical importance to identify what species the Pinnacles Jewelflower is most closely related to, we are assembling a new dataset that incorporates previous data that includes representatives of unsampled species of the Glandulosus Complex (e.g., *S. callistus*), and representatives of *Caulanthus coulteri* var. *coulteri*. We will conduct population structure (using STRUCTURE; Pritchard et al, 2000), a quantitative morphological assessment, and additional phylogenetic analyses based on this larger dataset. With these analyses we would be fully completing the goals we originally proposed. We nevertheless want to stress that our current results show quite convincingly that the Pinnacles Jewelflower conforms a separate entity, that is genetically cohesive, phylogenetically independent, and morphologically distinct from *S. glandulosus, S. insignis S. breweri, C. coulteri, C. anceps,* and all other species of jewelflowers currently described.

Wrap-up and publication of scientific results– Two scientific articles communicating our research will be published. We are analyzing an expanded dataset and completing our morphological dataset. We anticipate we will have completed such analyses and have a final draft of our first paper in the next few months, and after that, we will focus on our second, broader, paper on Jewelflower evolution. Once the first paper is completed, the park has indicated they plan to share these results widely through handout materials as well as social media outreach and events to celebrate the new species.

HOW OBJECTIVES AND RESULTS CONFORM TO THOSE ORIGINALLY PROPOSED

Our objectives and results conform quite nicely to those originally proposed. We have compiled morphological, geographic and genomic data that altogether support quite convincingly that the Pinnacles Jewelflower is in fact a new species for Pinnacles National Park, and new to science. Logistic limitations during the pandemic prevented us from access to all samples we originally anticipated, and certainly delayed our progress. We had to compromise in a couple of instances and move forward, but we alleviated such shortcomings by integrating the data we generated in this project to previous genomic data we already had. Thus, analyses were computationally demanding and time consuming. Another aspect of our project affected by delays imposed by the pandemic was the ability to work at herbaria to collect our quantitative morphological characters, and which we are now in the process of completing. Thus, aside from delays that impacted our timing, our objectives and results are aligned with those originally proposed.

LITERATURE

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TABLE1. Species represented in our final set of 94 samples, that were sent for RADseq sequencing at Floragenex.

Species	n individuals	In San Benito County, CA	morphologically similar	Glandulosus complex
Caulanthus cooperi	2			
Caulanthus coulteri lemmonii	**5**	yes	yes	
Caulanthus crassicaulis	1			
Caulanthus heterophyllus	2			
Caulanthus inflatus	1			
Caulanthus simulans	2			
Guillenia anceps	**5**	yes		
Guillenia flavescens	10			
Guillenia lasiophylla	1			
Hesperidanthus linearifolius	1			
Neuontobotrys tarapacana	1			
PINNACLES JEWELFLOWER	**14**			
Romanschulzia arabiformis	1			
Sibara macrostachya	1			
Stanleya albescens	1			
Streptanthus albidus	2			yes
Streptanthus barbiger	4			
Streptanthus batrachopus	1			
Streptanthus bracteatus	1			
Streptanthus breweri	**3**	yes		
Streptanthus carinatus	2			
Streptanthus fenestratus	2			
Streptanthus glandulosus	**13**	**yes**	**yes**	**yes**
Streptanthus hispidus	1			yes
Streptanthus hyacinthoides	1			
Streptanthus insignis	**5**	**yes**	**yes**	**yes**
Streptanthus lilacinus	2			
Streptanthus morrisonii	2			
Thelypodiopsis linearifolia	1			
Thelypodium ambigua	1			
Thelypodium flexuosum	1			
Thelypodium howellii	2			
Thelypodium laciniatum	1			
Thelypodium wrightii	1			

TABLE 2. Morphological and ecological traits of the Pinnacles Jewelflower in relation to similar species.

Trait/species		*PINN Jewelflower*	*S. insignis*	*S. glandulosus*	*C. coulteri*
Cauline leaf	shape	lobed (narrow)	lobed (broad)	lanceolate	lanceolate/ovate
	attachment	sessile	clasping	clasping	clasping
	indumentum	bristly	bristly	bristly	simple/sparse
Fruit	orientation	reflexed	ascending	spreading/reflexed	reflexed
	indumentum	sparse-glabrous	bristly hairy	sparse-glabrous	glabrous
Sterile terminal cluster		rudimentary	prominent	absent	present
Calyx	shape	cylindrical, spreading	inflated, bell	inflated, urn	cylindrical, spreading
	color	reddish purple	purple	dark purple	red/green
Habitat		sandy gravelly slopes	rocky slopes	rocky soil & outcrops	sandy gravelly slopes
	geology	volcanic/sedimentary	serpentine/Franciscan	various	sedimentary

FIGURE 1. Species that exhibit morphological similarity or geographical proximity (i.e., occur in San Benito County, CA) to the Pinnacles Jewelflower.

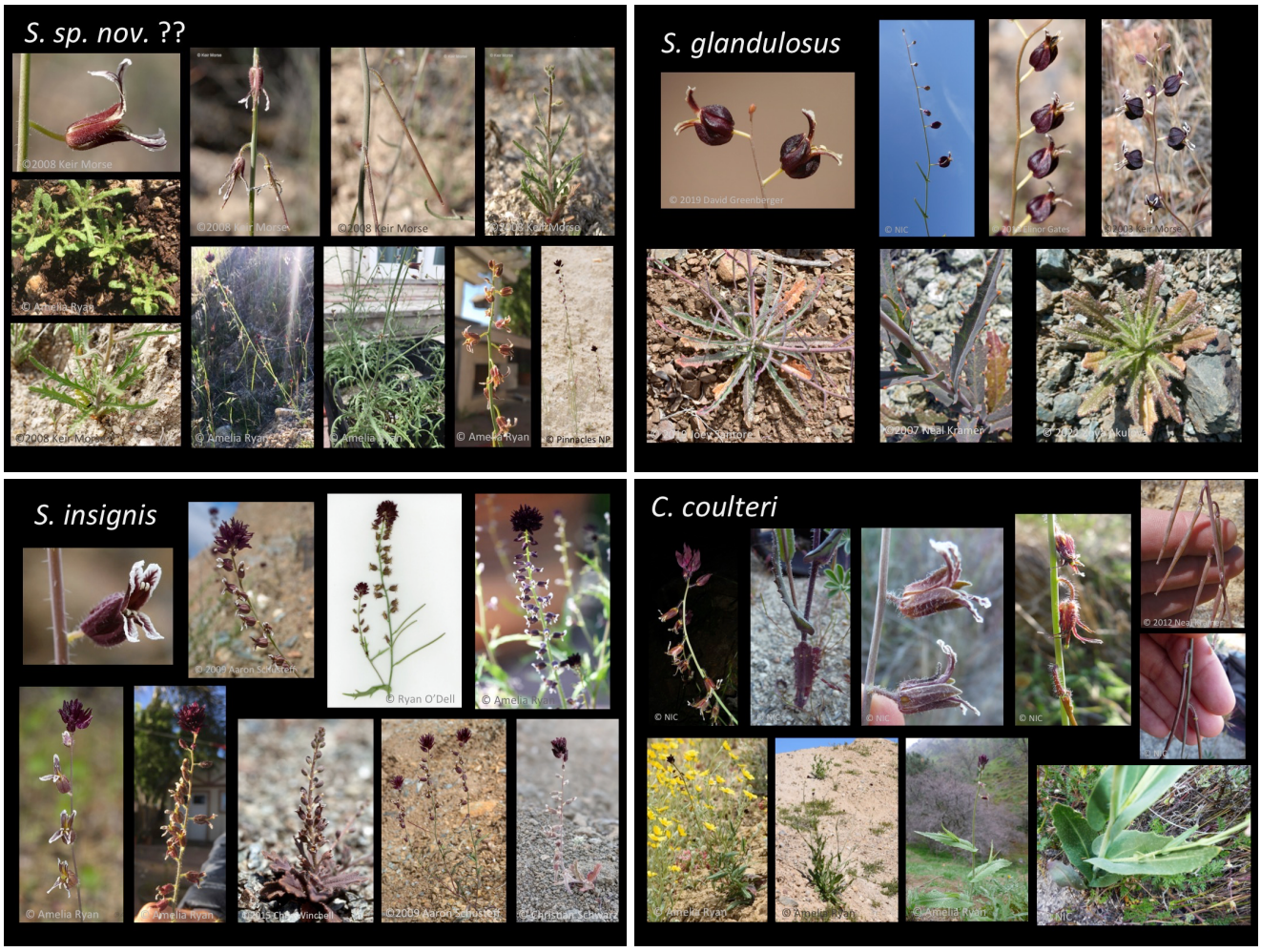
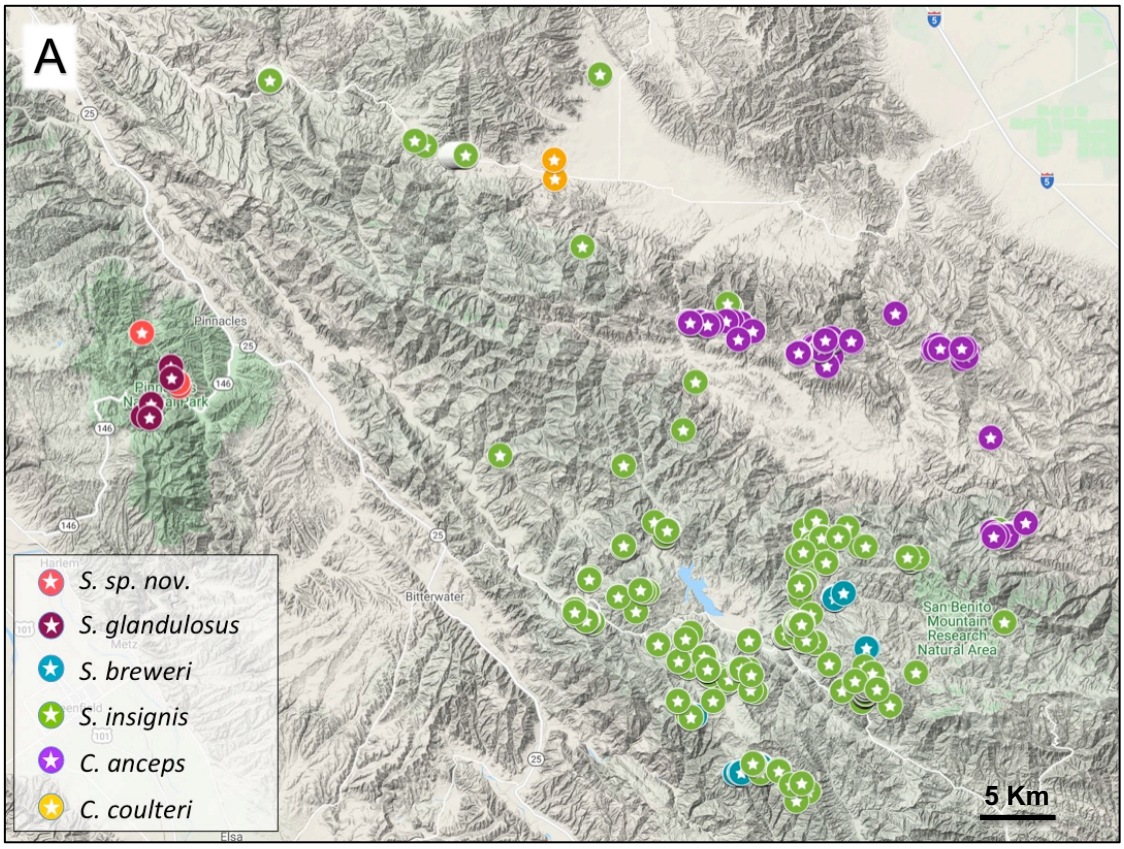


FIGURE 2. Maps showing the distribution of those species that exhibit morphological similarity or geographical proximity (i.e., occur in San Benito County, CA) in relation to the Pinnacles Jewelflower. (**A**) Map showing where the five species of Jewelflowers have been collected in San Benito, Co. (**B**) Map highlighting the populations of the Pinnacles Jewelflower and the populations of *Streptanthus glandulosus,* the only other species of jewelflower that has been reported at Pinnacles National Park.



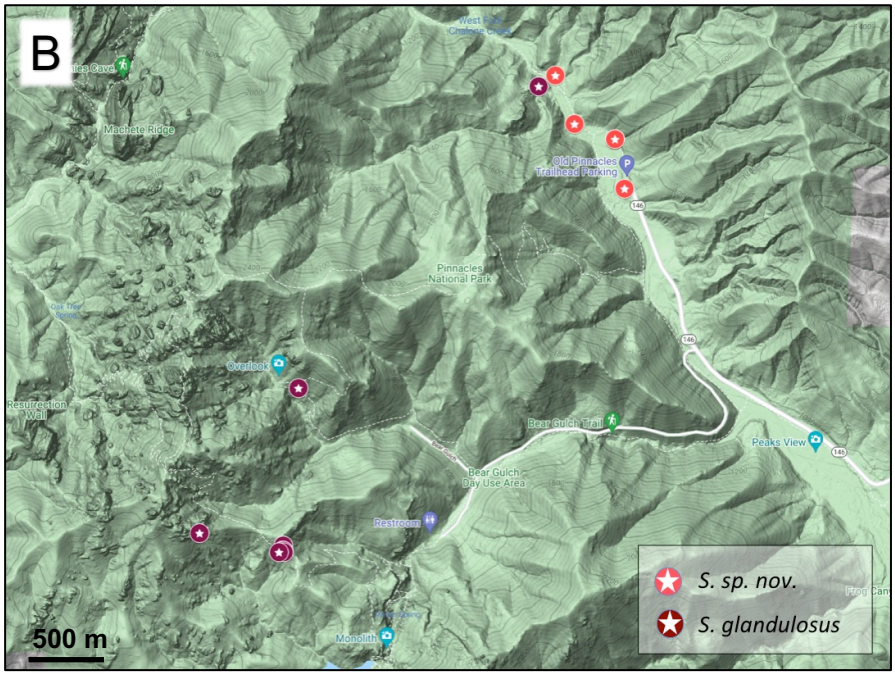


FIGURE 3. Phylogenetic tree based on Maximum Likelihood analyses of RADseq data depicting evolutionary relationships of samples included in this project. In red is highlighted the clade (group) that contains all and only samples of the Pinnacles Jewelflower. Species that exhibit morphological similarity or geographical proximity (i.e., occur in San Benito County, CA) to the Pinnacles Jewelflower are highlighted in different colors. This tree was inferred using the program RAXML (Stamatakis, 2014), as implemented in *ipyrad* Eaton, 2014).

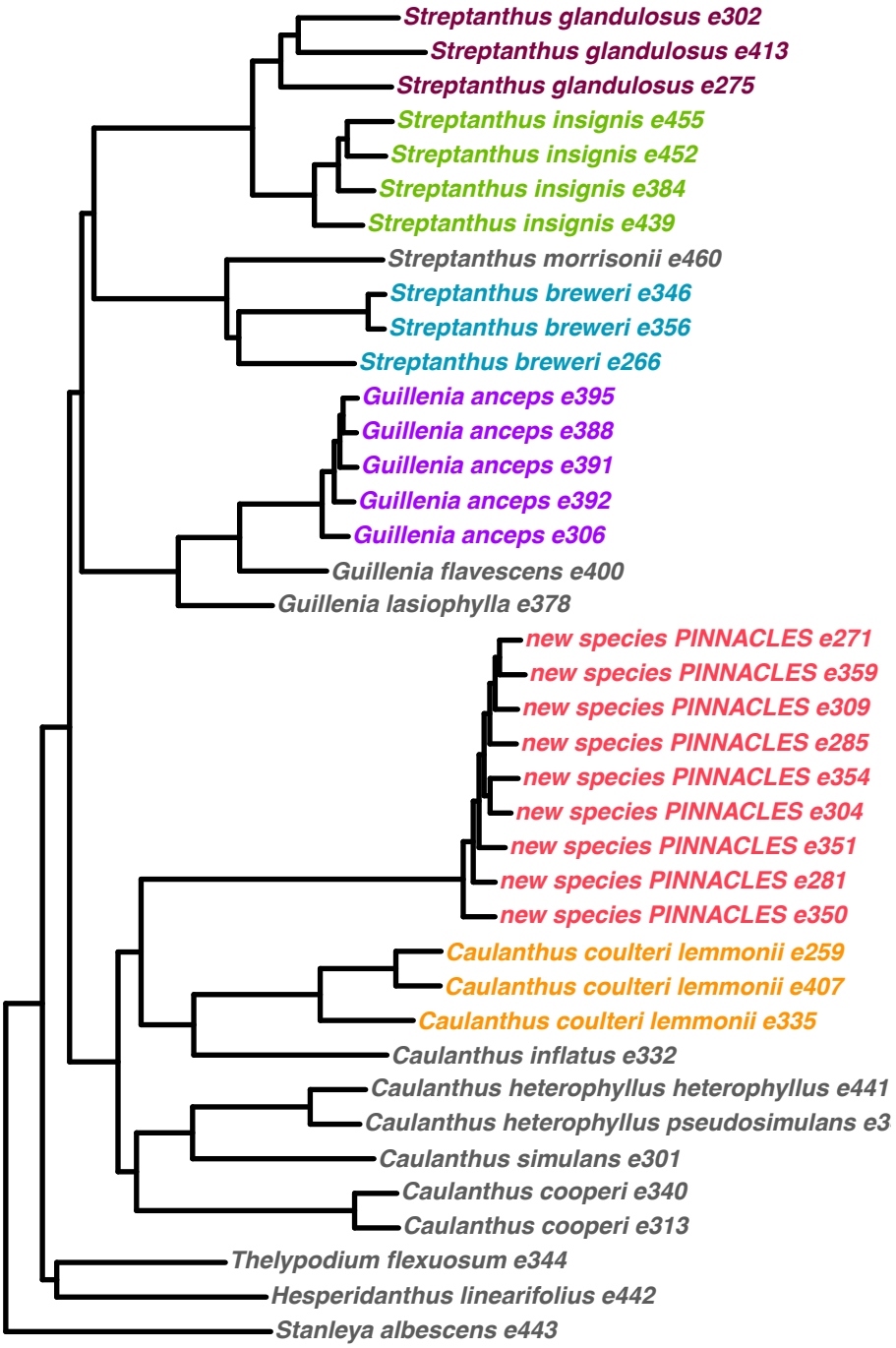


FIGURE 4. Prototype of visual interpretation materials to be used by the Park.