Midpeninsula Regional Open Space District

R-26-45
Meeting 26-10
April 08, 2026

AGENDA ITEM 6

AGENDA ITEM

Santa Cruz Kangaroo Rat Genetic Analysis Presentation

GENERAL MANAGER'S RECOMMENDATION(S)

Receive a presentation on the outcomes of the Santa Cruz kangaroo rat genetics analysis funded by the Wildlife Conservation Board. No Board action required.

SUMMARY

The University of California, Davis (UC Davis) completed genetic analyses that determined that the Santa Cruz kangaroo rat (SCKR, *Dipodomys venustus venustus*) present in Sierra Azul Open Space Preserve (Sierra Azul) is a named subspecies of the narrow-faced kangaroo rat (NFKR, *Dipodomys venustus*). Results of the analyses will inform potential future species listings under the California Endangered Species Act (CESA) and the development of conservation actions within Sierra Azul to protect SCKR. The Midpeninsula Regional Open Space District (District) is working with Jodi McGraw Environmental Consulting to develop a site-specific Habitat and Population Management Plan (HPMP) to manage SCKR habitat and improve population resiliency.

DISCUSSION

A new population of the Critically Imperiled Santa Cruz kangaroo rat was discovered in Sierra Azul Open Space Preserve (Sierra Azul) in 2019. The Critically Imperiled state designation is a California Natural Diversity Database Conservation Status Ranking that indicates the subspecies is at a very high risk of extirpation/extinction. At present, SCKR is not listed as state or federally threatened or endangered. The process of ecological succession, coupled with historic fire suppression, is causing vegetation to encroach on limited habitat for the population within Sierra Azul. This encroachment limits mobility, burrowing, and foraging opportunities for SCKR, reducing long-term viability of both the local population and the subspecies. The broader species of NFKR are facing similar threats and have lost significant portions of their historic population range. Recognizing that the SCKR population at Sierra Azul is geographically isolated and is likely highly susceptible to extirpation or extinction, the District began work to assess habitat conditions to better protect this unique population.

On November 10, 2021, the District Board of Directors (Board) approved Resolution No. 21-40, allowing the District to apply for up to $295,000 of WCB grant funding towards SCKR research. The grant funding was approved by the Wildlife Conservation Board on April 24, 2022. Under Phase I, monitoring and population assessments are supporting the development of a Habitat and Population Management Plan that will inform future conservation actions for the species performed under Phase II. In addition to the Sierra Azul SCKR population assessment, habitat assessment, and rare plant surveys (R-22-144), these funds have been utilized to support a state-wide genetic species assessment by UC Davis of the NFKR (of which SCKR is a subspecies).

UC Davis's genetic analyses confirmed that the subspecies of NFKR found within Sierra Azul is the SCKR. Furthermore, the analysis of historic specimens indicate that the historic range of SCKR extended through large portions of the Santa Cruz Mountains. SCKR are now only confirmed to be present in Henry Cowell State Park and Sierra Azul. Prior to this study, kangaroo rats in the Mt. Hamilton area of the Diablo Range were considered to belong to the SCKR. However, analyses of museum specimens collected from 1892-1938 from Mt. Hamilton, as well as from a newly identified population at Henry Coe State Park, show that the NFKR in the Diablo Range may be a distinct subspecies (Attachment I). Results indicate that NFKR subspecies ranges have become significantly reduced and isolated when compared to historic distributions. Genetic results support the argument that several populations, including the SCKR found in Sierra Azul and Henry Cowell State Park, should be considered distinct Evolutionary Significant Units (ESU) and awarded threatened or endangered status under CESA. An ESU under CESA can encompass the entire range of a subspecies if that subspecies is geographically isolated within a specific region, as is the case with SCRK in the Santa Cruz Mountains.

The population assessment in Sierra Azul was completed in May 2024, detecting 24 individual SCKR spread across nine trapping sites. Population modeling estimated a total of 23-29 individual SCKR within the trapping areas. Trapping was not conducted in all potential habitat within Sierra Azul since some areas were inaccessible to researchers due to dense vegetation and steep terrain. The number of individuals at each site was considered very low (0-10 individuals per site) with little or no connectivity between habitat fragments.

On January 8, 2025, the Board approved an award of contract with Jodi McGraw Environmental Consulting for the development of the HPMP (R-25-05), which is currently in progress and will be completed in June of 2026. The HPMP will identify opportunities and constraints for implementable site-specific habitat enhancements to increase the population resiliency of SCKR within Sierra Azul.

FISCAL IMPACT

None

PRIOR BOARD AND COMMITTEE REVIEW

November 10, 2021: Board approved Resolution No. 21-40, allowing for the submittal of a grant application for the Santa Cruz Kangaroo Rat Habitat and Population Management Project. (R-21-154, Minutes)

December 14, 2022: Board authorized the General Manager to enter into an agreement with Nomad Ecology for related botanical surveys and habitat assessments (R-22-144, Minutes)

January 8, 2025: Board authorized the General Manger to enter into an agreement with Jodi McGraw Environmental Consulting to create a Habitat and Population Management Plan to increase the population resiliency of Santa Cruz kangaroo rat within Sierra Azul (R-25-05, Minutes)

PUBLIC NOTICE

Public notice was provided as required by the Brown Act.

CEQA COMPLIANCE

This item is not a project subject to the California Environmental Quality Act.

NEXT STEPS

The District will continue the development of the SCKR HPMP, which will be informed by the analyses by UC Davis. The District will consider recommendations from the plan for future land management decisions within known and potentially suitable SCKR habitat in Sierra Azul. In addition, the District will implement habitat and SCKR monitoring strategies where appropriate to assess the effectiveness of any management actions aimed at improving SCKR population resiliency.

The District will continue to foster relationships with partner organizations working towards the conservation of SCKR to improve population health for SCKR throughout the known range in the Santa Cruz Mountains.

Attachments:

1. WCB Grant Progress Report

Responsible Department Head:
Kirk Lenington, Natural Resources

Prepared by:
Matt Sharp Chaney, Wildlife Ecologist III, Natural Resources

Contact person:
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Annual Performance Report

1. **State:** California

   Grant Number: F22AF02259 (G2298082)

   Grant Name: Assessment of subspecies status, population substructure, and genetic diversity in *Dipodomys venustus* and development of a range-wide monitoring plan

2. **Report Period:** July 1, 2024 - June 30, 2025

3. **Location of work:** San Luis Obispo, Santa Cruz, San Benito, and Monterey Counties

4. **Purpose and Objectives**:

Research, Survey Data Collection and Analysis

1. **Conduct surveys to determine occupancy status of *D. venustus* across its range.**
   - Conduct 1 Investigation by 12/31/2024
   - Activity Tag 1: Fish and wildlife species data acquisition and analysis
   - Unit of Measurement: 1 investigation
   - Species: The narrow-faced kangaroo rat (*Dipodomys venustus*), including the Santa Cruz kangaroo rat (*D. v. venustus*), big-eared kangaroo rat (*D. v. elephantinus*) and Santa Lucia kangaroo rat (*D. v. sanctiluciae*).

2. **Develop habitat suitability models**
   - Conduct 4 Investigations by 12/31/2024
   - Activity Tag 1: Habitat data acquisition and analysis
   - Unit of Measurement: 4 investigations
   - Species: The narrow-faced kangaroo rat (*Dipodomys venustus*), including the Santa Cruz kangaroo rat (*D. v. venustus*), big-eared kangaroo rat (*D. v. elephantinus*) and Santa Lucia kangaroo rat (*D. v. sanctiluciae*).

3. **Design robust monitoring framework for detecting occupancy trends**
   - Develop 1 Technique by 6/30/2025
   - Activity Tag 1: Conservation techniques development
   - Unit of Measurement: 1 technique
   - Species: The narrow-faced kangaroo rat (*Dipodomys venustus*), including the Santa Cruz kangaroo rat (*D. v. venustus*), big-eared kangaroo rat (*D. v. elephantinus*) and Santa Lucia kangaroo rat (*D. v. sanctiluciae*).

4. **Resolve the taxonomic status of named subspecies**
   - Conduct 2 Investigations by 6/30/2025
   - Activity Tag 1: Fish and wildlife species data acquisition and analysis
   - Unit of Measurement: 2 investigations
   - Species: The narrow-faced kangaroo rat (*Dipodomys venustus*), including the Santa Cruz kangaroo rat (*D. v. venustus*), big-eared kangaroo rat (*D. v. elephantinus*) and Santa Lucia kangaroo rat (*D. v. sanctiluciae*)

- Objective (1): To resolve the modern taxonomy and population structure within *D. venustus*.
- Objective (2): To resolve the historical range of species/subspecies and historical diversity within *D. venustus.*
- Purpose (1): All three named subspecies are listed with varying degrees of conservation concern. There is uncertainty regarding the subspecific taxonomy with *D. venustus*. We will use genetics to resolve the evolutionary significant units within *D. venustus* and will use this information to recommend for consideration as distinct population segments or revised taxa.
- Purpose (2): The current range of *D. venustus* has been substantially reduced, especially in the range of *D. v. venustus*. The historical range of the species/subspecies is based on historical specimens. There is concern regarding the species/subspecies identification of historical specimens, particularly from peripheral parts of the range where *D. venustus* overlaps with other *Dipodomys* species. We will use genetic analysis of historical specimens to examine the historical range and genetic diversity of the species and subspecies. The identification of misidentified historical specimens would clarify the historical range and preferred habit of *D. venustus*.

Stakeholder Involvement

5. **Engage Midpeninsula Regional Open Space District and California State Parks**
   - Engage 2 Organizations by 12/31/2022
   - Activity Tag 1: Organizational engagement
   - Unit of Measurement: 2 organizations

5. Part of a Larger project: No

6. Describe how the objectives were met. (TRACS Questionnaire Q1)

This report summarizes the work so far on Objectives 1, 4 and 5.

<u>Objective 1 Determine occupancy status of the species across its range and model habitat suitability:</u>

We built the preliminary model of habitat suitability for *Dipodomys venustus*. This model was developed to devise a sampling scheme, whereby 80% of sites to trap will fall within "high suitability" areas and 20% of sites in "low suitability." The model itself was not an objective, but is being used to fulfill Objective 1.

We used standard methods to model habitat suitability using existing occurrence data from publicly available sources, a suite of environmental predictors, and MaxEnt to model the distribution. All analyses were performed in R. We acquired 523 *D. venustus* locations from the Global Biodiversity Information Facility ("GBIF") using package "dismo" (Hijmans et al. 2022). We had initially planned to model their distribution using contemporary occurrence data, but restricting the locations to those collected since 1980 reduced the sample to 132, from only a few locations across their range. Species of conservation concern often undergo "niche reduction" (Scheele et al. 2017; Rutrough et al. 2019), i.e. they are extirpated disproportionately from some portions of their environmental niche. We therefore decided to model their distribution using all of the historical records to better estimate their potential distribution and maximize sampling across their range. Four additional locations were added based on recent investigations by collaborator Ken Hickman. Two locations were removed based on questionable taxonomy, and three locations were moved based on descriptions from original field notes.

We acquired a suite of environmental predictors believed to influence *Dipodomys venustus* distribution: four climatic variables, three topographic variables, and one vegetation type (Figure 1). We acquired climatic water deficit, mean annual precipitation, mean daily maximum temperature and mean daily minimum temperature from the California Basin Characterization Model (v. 8, Flint et al. 2018; Thorne, pers. comm.) (resolution = 270m). We acquired elevation from the Shuttle Radar Topography Mission (resolution = 90m). Elevation was coarsened to match the resolution of the climatic data. We then calculated slope and topographic position index using the “terrain” function in package “raster” (Hijmans 2023). Topographic position index was calculated at scale three with a rectangular window. Vegetation types were acquired from CalFire FRAP (California Department of Forestry and Fire Protection 2015) (resolution = 30m), which is based on the “best available” land cover data, most often derived from the California Wildlife Habitat Relationships database. We calculated the proportion of shrub cover within each 270 m raster cell (i.e. the number of 30m cells categorized as shrub divided by the number of cells within each 270m cell). Shrub cover was defined as any vegetation in the following categories: montane chaparral, mixed chaparral, chamise-redshank chaparral, juniper, sagebrush, low sage, and undetermined shrub. Annual precipitation and maximum temperature were removed as too highly correlated with climatic water deficit.

We generated 10,000 random background points within a 100 km buffer of *D. venustus* occurrences (Figure 2). We then ran MaxEnt using the ENMEval package in R (Kass et al. 2021). We used all feature types and tested regularization multipliers between 0.5 and 3.0 at intervals of 0.5. The regularization multiplier is a parameter that determines how finely tuned the model outcome is. Higher regularization multipliers lead to more general models, lower values lead to better fit models. We used AICc and model selection to identify the model that best predicted the occurrence data while controlling for over-fitting.

We then used the best model to identify trapping sites, stratified by suitability. With the best model we identified quantiles of habitat suitability at known *D. venustus* locations. “Unsuitable” was defined as anywhere with values lower than 5% of all sutiability values at *D. venustus* locations. “Low suitability” was defined as areas with suitability between 5% and 50%; high suitability areas were places that had suitability higher than 50% of known *D. venustus* locations. We generated 1km hex grids throughout the study area, which were then subset to grids that were at least partially within public lands (based on the California Protected Areas Database). Hex grids were assigned “high” “low” or “no” suitability categories based on the mean suitability score within the polygon based on the thresholds described above. We then used the “grts” function in package “spsurvey” (Dumelle et al. 2023) to stratify these grids, with 80% grids selected as high and 20% in low. We generated 150 total hex grids, with the expectation that not all would be accessible.

The data collected at the camera and kangaroo rat live-trapping locations from this study will be used to refine the final habitat suitability model. Table 1 shows the locations that have been trapped during the study so far, organized by reporting period (2022-23, 2023-24, 2024-25).

Results

The best model included all predictors and all feature types, with a regularization multiplier of 2. Suitability was highest when climatic water deficit was approximately 500 mm, with steep declines bast 1,000 mm; similarly, suitability was maximized at median daily minimum temperatures (Figure 3). Suitability was positively correlated with elevation, strongly negatively correlated with slope, and mostly unrelated to shrub cover or topographic position index (Figure 3). Highest suitability areas were found in the most mountainous areas of the study area (Figure 4).

<u>Objective 2 Develop habitat suitability models:</u>

Field work and data collection for Habitat Suitability Models has been completed. We sampled 46 total hex grids. This fell short of the estimated 80-100 proposed for this study, due to a combination of unanticipated trap-shyness and limited site access. However, we sampled every major population of *D. venustus* and have sufficient locations to build robust Habitat Suitability Models. An ensemble modeling method was used via the TidySDM package in R. This allowed us to include machine learning methods such as Maxent and Boosted Regression Trees which are robust to spatial bias, while also including regression-based methods such as GLM and Multivariate Adaptive Regression Splines (MARS) that would help keep the models appropriately generalized.

All Habitat Suitability Models have been developed and completed. We created 5 Habitat Suitability Models for the narrow-faced kangaroo rat and its three confirmed subspecies. These models are based on the available genetic data that has been completed so far. While the Henry Coe State Park population does appear to be its own subspecies based on the preliminary genetic data, we did not have enough independent locations to create its own model. Narrow-faced kangaroo rat occurrence data collected between 2023-2024 (hereafter referred to as modern-only occurrences) were identified to species from tissue samples sent to the UC Davis genetics Lab. The modern occurrences were then thinned to one location per $270m^2$ raster cell to reduce spatial autocorrelation (n = 50), and then buffered (r = 80,000m) to delineate the biologically relevant study extent. I then generated 10,000 random pseudo-absence points within the buffer. All subspecies datasets were constructed from the modern-only occurrences (Table 1). Each dataset was then thinned by raster cell and buffered before 10,000 pseudo-absence points were created in the respective study areas. The Santa Lucia kangaroo rat data was buffered by 80,000m, the Santa Cruz kangaroo rat data was buffered by 50,000m, and the Elephant-eared kangaroo rat data was buffered by 60,000m.

Due to the low sample of the modern occurrences, I created a second range-wide dataset that incorporated both modern occurrences and historical occurrences (hereafter modern-plus-historical occurrences). Historical occurrences were obtained from the Berkely MVZ, Cal Academy, Michigan, and Cal Poly Museum repositories with collection dates between 1892-1991 and identified to species by UC Davis (Statham 2025, Unpublished). These locations were then collated with the modern occurrences, thinned by raster cell (n = 90), and buffered before 10,000 pseudo-absence points were created.

The modern-only range-wide model included all statistical algorithms except GLM and a highly restricted projection of ecological niche (Figure 1). Distance to Manzanita and Shrub Cover were the most important predictor variables, though all other variables still had some level of impact on the model. Distance to Manzanita had a drastic effect on model prediction, with distances greater than 8,000 meters having no predictive performance; average distance was approximately 1500 meters. Additionally, the narrow-faced kangaroo rat was associated with lower Climatic Water Deficits (< 750), higher Percent Sand Content of Soils (> 50%), higher Percent Shrub Cover (> 50%), and higher Topographic Positions (> 25). Lower Percent Slope (0-20%) and Minimum Annual Temperature (5-8 degrees Celsius) were only slightly associated with model prediction.

The modern-plus-historic range-wide model included all four statistical algorithms and a more general ecological niche projection (Figure 2). Distance to Manzanita was the most important predictor variable, but all other variables retained a moderate level of impact on the model. Distance to Manzanita model prediction was nearly identical to the modern-only range-wide, with distances greater than 10,000 meters having no predictive performance; average distance was approximately 1000 meters. Additionally, the narrow-faced kangaroo rat was still associated with lower Climatic Water Deficits (< 750), higher Percent Sand Content of Soils (> 50%), and higher Topographic Positions (> 25). Lower Percent Slope (0-20%), Minimum Annual Temperature (5-8 degrees Celsius), and higher Percent Shrub Cover (> 50%) had a small associated with model prediction.

The Santa Cruz kangaroo rat model included only Boosted Regression Trees statistical algorithm and a heavily restricted projection of ecological niche (Figure 3). Distance to Manzanita and Percent Shrub Cover were the most important predictor variables, with all other variables having almost no impact on the model. Low Distance to Manzanita (< 1,000m) and higher Percent Shrub Cover (>65%) had strong model prediction. Lower Climatic Water Deficit (<300) and higher Topographic Positions (>50) had a small signal in model prediction. All other predictors had almost no signal in model prediction.

The Santa Lucia kangaroo rat model included all statistical algorithms and predicts considerable high niche suitability (Figure 4). Distance to Manzanita and Percent Shrub Cover were important predictor variables. Percent Slope had a small amount of relative feature importance. Minimum Annual Temperature, Climatic Water Deficit, Percent Sandy Content of Soils, and Topographic Position Index had almost no feature importance. Lower Distance to manzanita (< 2,000m) retained its strong predictive performance compared to the other models (Figure 15). In addition, low Climatic Water Deficit (< 500) and high Percent Shrub Cover (> 75%) also showed moderate average model prediction. Lower Percent Slope (10-25%), higher Topographic positions (>25), higher Percent Sand Content of Soils (>75%), and lower Minimum annual Temperature (<6 Degrees) had only a slight effect on model prediction.

The Elephant-eared kangaroo rat model included all statistical algorithms and a considerably site-restricted projection of ecological niche (Figure 5). Distance to Manzanita was the most important predictor variable, with Minimum Annual Temperature a close second. All other variables had no feature importance in the model. Low Annual Minimum Temperature (< 6C) and low Distance to Manzanita (< 2,000m) had large predictive performances in the model. Higher Topographic Positions (>50) had small model predictive performance. All other variables had almost no relationship with average model prediction.

Objective 3 Design robust monitoring framework for detecting occupancy trends:

We had proposed to design an occupancy monitoring and modeling framework for tracking trends in *D. venustus* through time. This was based on assumptions about the status of the species; our ability to identify the species on camera and in hand; and high capture probabilities as with other species of kangaroo rat. Based on lessons learned from this project, we recommend a more complex monitoring framework consisting of both occupancy and mark-recapture estimates:

1. Conduct regular (annual if possible, or semi-annual) monitoring of populations associated with the Santa Cruz and *elephantinus* groups (sub-clades A-C, and E-F, respectively below). We found that both clades appear to be restricted to a small number of discrete locations, such that changes in occupancy would be too coarse a metric for population monitoring, and therefore changes in population size, growth, or other demographics are needed for these range-restricted and likely very small, unique clades.
2. Prioritize occupancy trapping at locations already known to be occupied by *D. venustus*. Trap success with this species was considerably lower compared to others within the genus (e.g., the giant kangaroo rat (*D. ingens*), Heermann's kangaroo rat (*D. heermanni*), and San Joaquin kangaroo rat (*D. nitratoides*). We also tested the use of fecal pellets for species identification, both collecting pellets from the surface and using specialized bait boxes (following protocols described in Aylward et al. 2023). However, based on camera work (K. Hickman, pers. comm.) and our tests, *D. venustus* appears not to defecate aboveground, and they instead likely use latrines within their burrow systems. Live capture and subsequent analysis of fecal pellets or tissue to confirm species ID is therefore the only reliable method to determine *D. venustus* presence. Given the large effort needed to detect *D. venustus*, we recommend balancing two considerations: maximizing the number of sites where the species is found while continuing to sample areas of lower suitability to further refine the habitat model. Previous work with giant kangaroo rats (Bean et al. 2019) suggested that a stratified sampling design, with a higher proportion of sites targeted towards high suitability areas, would be capable of detecting unbiased trends in range-wide occupancy with approximately 50 independent locations. When the final range-wide distribution model is completed, we will identify 50 unique hex grids for long-term monitoring, with 80% in high suitability areas and 20% in low suitability areas. Due to access limitations, these grids will primarily be taken from areas sampled during this project. We will simulate changes in occupancy using the distribution model to ensure that the trapping design can detect unbiased trends in occupancy. This work is expected to be completed by September, 2025.

References

Bean, W.T., H.S. Butterfield, R. Stafford, and M. Westphal. 2019. Range-wide giant kangaroo rat surveys and monitoring optimization. Technical report in fulfillment of a California Department of Fish & Wildlife Species Conservation and Recovery Grant.

Aylward, C., L. Barthman-Thompson, W.T. Bean, D.A. Kelt, B.N. Sacks, and M.J. Statham. 2023. Patch size and connectivity predict remnant habitat occupancy by an endangered wetland specialist, the salt marsh harvest mouse. Landscape Ecology 38:2053-2067.

Objective 4 Resolve the taxonomic status of named subspecies:

Subobjective (1): To resolve the modern taxonomy and population structure within D. venustus.

We have extracted DNA from 178 modern kangaroo rat tissue samples from Henry Cowell State Park, Sierra Azul, Santa Lucia North (Hastings Natural History Reservation), Pozo/La Panza, Santa Lucia Central, Santa Lucia South, and the Gabilan and Diablo Ranges. This comprises sites from each of the *D. venustus* subspecies, as well as other species within the kangaroo rat genus (*Dipodomys*) and kangaroo mouse genus (*Microdipodops*). We also obtained samples and extracted DNA from 55 frozen muscle samples from museums. This brings us to 233 of 200 modern tissue samples proposed for this study. The muscle samples from the museums are relatively recent and contain high quality DNA and yield more comprehensive genetic analyses. Therefore, the muscle samples are considered part of this subobjective looking at modern taxonomy.

We have completed the DNA sequencing of this project. Initially we conducted PCR amplification and DNA sequencing on a small section of mitochondrial DNA (the D-loop and later a small fragment of the cytochrome b gene). We used the resulting DNA sequences to facilitate species identification. This enabled us to weed out misidentified *D. heermanni* from both the live trapping and museum specimens.

We have recovered whole mitochondrial genomes (>16,435 bp [base pairs]) from both modern and historical specimens. As far as we are aware we are the first to recover whole mitochondrial genomes for *D. venustus*, *D. agilis*, *D. californicus*, *D. simulans*, *D. ingens*, *D. heermanni*, *M. pallidus*, and *M. megacephalus*. This data has allowed us to produce a high-resolution phylogenetic tree and to assess divergence times among kangaroo rat species as well as among *D. venustus* populations (Figures 7 and 9).

We have identified two clades (1 and 2) within *D. venustus*. Specimens of *D. agilis* and *D. simulans* formed distinct subclades within Clade 2 indicating a close relationship among all three species (Figure 8). The subclades K, L, and M were primarily restricted to single species; however, we identified two *D. venustus* from Pozo within the northern *D. agilis* subclade (M), and one *D. agilis* within the *D. simulans* subclade M. This data indicates historical hybridization among species at zones of contact.

Within *D. venustus*, we identified geographically discrete subclades. This is indicative of long-term distinct populations of the species. Indeed, our divergence time estimation indicated that the majority of geographically discrete subclades were formed during the last glacial period of the Pleistocene (Wisconsin Glacial Period) c. 115,000-11,700 years ago when the climate would have been colder and less suitable for the species. Many subclades last shared a common ancestor with neighboring subclades during the Sangamonian interglacial period (c. 130-115 thousand years ago) when the climate was more suitable and the species would have had wider geographic range, which would have facilitated gene flow across a greater geographic area. We identified multiple geographically discrete subclades within each named subspecies, thus indicating phylogeographic partitioning beyond the currently recognized subspecies.

Nuclear DNA

Thus far we have only discussed analyses of mitochondrial DNA, which represents a single maternally inherited portion of DNA. We have also generated a nuclear DNA dataset of thousands of loci, which represents the input from both maternal and paternal lines, as well as their ancestors. This data will give us a more complete and highly resolving picture than with the mitochondrial DNA alone. Specifically, we generated a highly resolving genotyping by sequencing dataset from 203 individuals, with a sequencing depth of >15x, and comprising 63,995 independent SNP (single nucleotide polymorphism) loci across the genome.

At the species level, we identified a close relationship among *D. venustus, D. agilis,* and *D. simulans* (Figure 11). While *D. heermanni* and *D. ingens* were well differentiated from one another, and from *D. venustus* etc. These findings are consistent with the mtDNA. We used the discrete clusters of individuals to determine the range extent of *D. venustus, D. agilis,* and *D. simulans*. Admixture analyses (not shown here) indicated a small proportion of shared ancestry among the three species where their geographic ranges were closest to one another.

Within *D. venustus* we identified strong population substructure evident in both Admixture analysis (data not shown) and a multi-dimensional scaling (MDS) plot (Figure 12). We identified genetically differentiated populations in the Gabilan Range, the San Benito Mountains of the Diablo Range, further north in the Diablo Range at Henry Coe State Park, and in Sierra Azul and Henry Cowell State Park in the Santa Cruz Mountains.

Within *D. v. sanctiluciae*, while we continue to identify population differentiation, there appears to be a cline, where the northern most and southern most populations are most differentiated, while those in the middle are genetically intermediate. The genetically discrete populations we have identified is broadly consistent with the clustering of suitable habitat identified with the wide ecological niche modelling (Figure 1).

Subspecific Taxonomy within *D. venustus*

Both the nuclear DNA and mtDNA indicate subdivision within *D. venustus* beyond the currently recognized subspecies. Both marker types indicate deep genetic differentiation within the currently recognized subspecies *D. v. elephantinus*. The genetic units we have identified are on adjacent mountain ranges that are separated by a valley associated with the San Andreas Fault. Each of these populations have endemic mitochondrial clades that last shared a common ancestor 1.56 million years ago (Figure 9). Thus, these population should be considered distinct Evolutionary Significant Units (ESUs) and potentially subspecies. Within *D. v. venustus* the populations at Sierra Azul and Henry Cowell were distinct, but relatively closely related, which is consistent with them being isolated populations within the same mountain range. In contrast the newly identified population at Henry Coe State park is more genetically differentiated. This is consistent with the location of these populations on different mountain ranges (the Cruz Mountains and the Diablo Range respectively) either side of a wide valley. The populations on each of these mountain ranges have endemic subclades that last shared a common ancestor ~130,000 years ago. Based on the nuclear DNA, the population at Henry Coe State Park is one of the most differentiated within the species. It is a similar genetic distance from populations in Sierra Azul and the San Benito Mountains, both of which belong to different subspecies.

Thus, the taxonomic affinity of the Henry Coe population is uncertain. It is consistent with being a distinct ESU and it may comprise a distinct subspecies. Populations within the Range of *D. v. sanctiluciae* appear to form a cline of genetic differentiation. Populations to the North and South are most differentiated from one another, while geographically intermediate populations appear to be genetically intermediate. The two main subclades within the range of *D. v. sanctiluciae* are deeply divergent (~1.5 million years ago), and are geographically partitioned North and South. Thus, the populations considered to belong to the subspecies *D. v. sanctiluciae* may reflect secondary contact between two ancient divergent entities.

Hybridization between species.
We identified evidence of hybridization between *D. venustus*, *D. agilis*, and *D. simulans* with both the mtDNA and nuclear DNA datasets. With nuclear DNA we identified evidence of gene flow between *D. venustus* and *D. agilis* within the geographically closest populations (to the east of the city of Santa Maria). Notability we identified *D. agilis* mtDNA subclade K in two southern *D. venustus*. Similarly, we identified one *D. agilis* individual with a mtDNA haplotype belonging to the *D. simulans* subclade M (Figure 9). The nuclear DNA also identified shared ancestry

The data interpretation above is preliminary. Interpretation may change as new data is analyzed.

Subobjective (2) - To resolve the historical range of species/subspecies and historical diversity within D. venustus

We have sampled and processed 94 kangaroo rat historical specimens (claw, toe, skin, bone, and tissue in formalin) from five museums (California Academy of Sciences, Museum of Vertebrate Zoology (UC Berkeley), the Museum of Wildlife and Fish Biology (UC Davis), the University of Michigan, and California Polytechnic State University, San Luis Obispo). This completes our sampling of historical specimens for this study.

The analyses of these data are presented in the phylogenetic trees (Figure 7 and 8). Analyses of historical specimens has allowed us to substantially expand the geographic scope of our sampling, especially in areas where the species has been extirpated. Analyses of the combined modern and historical specimens has allowed us to resolve the geographic scope of multiple distinct lineages within the species. For example, our data indicate that the historical range of the Santa Cruz kangaroo rat (*D. v. venustus*) extended north throughout much of the Santa Cruz Mountains. At the outset of this project, there was uncertainty regarding the subspecies present at Fremont Peak. Based on the data thus far, specimens in this area are distinct from those in the Santa Cruz subspecies. Similarly, at the outset of the project, three historical specimens from near Mt Hamilton in the Diablo range were assigned to the Santa Cruz subspecies. Based on the disjunct distribution of these samples, there was uncertainty of their species, let alone subspecies. Our genetic analyses of the historical specimens confirmed that they were indeed *D. venustus*. This surprise result led to some last-minute trapping and sampling in Henry Coe State Park, also in the Diablo Range. Whole mitochondrial DNA sequencing of both the historical and modern specimens indicate that animals in this area belong to an endemic subclade and were last connected to the animals in the Santa Cruz Mountains approximately 130,000 years ago.

<u>Objective 5 (Engage Midpeninsula Regional Open Space District and California State Parks)</u>

Kangaroo rat trapping has been conducted on both Midpen and State Parks lands. These organizations facilitated access to trapping locations as well as support. In addition, Midpen funded personnel at UC Santa Cruz in 2023 to conduct additional trapping and sampling, which has contributed to this study.

7. TRACS Supplemental Questionnaire (briefly summarize questions 2-6 below)

Q2 Please describe and justify any changes in the implementation of your objective(s) or approach(es).

We will develop a more robust monitoring framework which includes not just monitoring of occupancy but also mark-recapture estimates to provide estimates of density and survival for two clades (Santa Cruz and *elephantinus*).

Q3 If applicable, please share if the project resulted in any unexpected benefits, promising practices, new understandings, cost efficiencies, management recommendations, or lessons learned.

Based on our mitochondrial DNA results thus far, the geographic ranges for the subspecies *D. v. sanctiluciae* and *D. v. elephantinus* may be considerably smaller than previously thought.

The narrow-faced kangaroo rat is much less "trap happy" than other species of kangaroo rats, requiring greater effort for monitoring, both using occupancy or mark-recapture estimates. We suggest that, given the limited range of *D. v. elephantinus and D. v. venustus*, these two clades be monitored using more robust mark-recapture estimates rather than simply occupancy. Range-wide, we recommend implementing a consistent occupancy monitoring protocol, with survey sites biased towards high suitability areas, to detect population trends.

Q4 For Survey projects only: If applicable, does this project continue work from a previous grant? If so, how do the current results compare to prior results? (Grantees may elect to add attachments such as tables, figures, or graphs to provide further detail when answering this question.)

N/A

Q5 If applicable, identify and attach selected publications, photographs, screenshots of websites, or other documentation (including articles in popular literature, scientific literature, or other public information products) that have resulted from this project that highlight the accomplishments of the project.

N/A

Q6 Is this a project you wish to highlight for communication purposes?
No

8. **Discuss differences:** None

9. **List any publications or in-house reports resulting from this work.** None so far

10. **Name, title, phone number, and e-mail address of person compiling this report**

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Annual Performance Report 2024/2025
Figures and Tables

Table 1: All locations sampled for the project. Blue = 2022-23, Peach = 2023-24, and Seafoam = 2024-25.

2023-2024 DIVE Sampling Sites

REGION	SITE	DATE	DIVE	CREW	LATITUDE	LONGITUDE
Santa Cruz Coastal	Henry Cowell 1	**2023-02-17**	Present	UC Santa Cruz	37.037655	-122.050432
Santa Cruz Coastal	Henry Cowell 2	**2023-02-19**	Present	UC Santa Cruz	Not Collected	Not Collected
Santa Cruz Coastal	Henry Cowell South	**2023-03-17**	Present	UC Santa Cruz	37.026128	-122.04859
Santa Lucia North	Hastings Natural Reserve Firebreak	**2023-03-25**	Present	UC Santa Cruz	36.382862	-121.541864
Sierra Azul	Cathermola Road	**2023-04-05**	Present	UC Santa Cruz	37.133962	-121.902263
Sierra Azul	Mt. Umunhum-Loma Prieta Road	**2023-04-13**	Present	UC Santa Cruz	37.123243	-121.867542
Sierra Azul	William's Property	**2023-04-29**	Present	UC Santa Cruz	37.144982	-121.925681
Santa Lucia Corridor	Santa Rita Ranch	**2023-05-05**	Absent	Cal Poly	35.51485	-120.8332
Santa Lucia North	Hastings Natural Reserve Grassland South	**2023-05-26**	Absent	UC Santa Cruz	36.362133	-121.564377
Sierra Azul	Mt. Thayer	**2023-06-15**	Present	UC Santa Cruz	37.162731	-121.917166
Pozo/La Panza	Pozo/La Panza 2	**2023-07-03**	Present	Cal Poly	35.362907	-120.340341
Pozo/La Panza	Pozo/La Panza 3	**2023-07-04**	Present	Cal Poly	35.379966	-120.349167
Pozo/La Panza	Pozo/La Panza 4	**2023-07-11**	Present	Cal Poly	35.351224	-120.246092
Sierra Azul	Loma Prieta Highpoint	**2023-07-19**	Absent	UC Santa Cruz	37.103797	-121.847924
Sierra Azul	Loma Prieta Way	**2023-07-19**	Present	UC Santa Cruz	37.104559	-121.849013
Pozo/La Panza	Pozo/La Panza 1	**2023-07-20**	Present	Cal Poly	35.40957	-120.32367
Santa Lucia Corridor	West Cuesta Ridge 1	**2023-07-24**	Absent	Cal Poly	35.392948	-120.707328
Santa Lucia Central	Fort Hunter Liggett 1	**2023-07-31**	Absent	Cal Poly	35.896688	-121.057414
Santa Lucia Central	Fort Hunter Liggett 2	**2023-07-31**	Absent	Cal Poly	35.991675	-121.176818
Santa Lucia Central	Fort Hunter Liggett 3	**2023-08-02**	Present	Cal Poly	35.888497	-121.283048
Santa Lucia Central	Big Sur Prewitt Ridge	**2023-08-14**	Absent	Cal Poly	35.972486	-121.454549
Santa Lucia Central	Big Sur South Coast Ridge Road	**2023-08-14**	Absent	Cal Poly	36.007234	-121.450649
Santa Lucia Central	Big Sur Nacimiento-fergusson Road	**2023-08-15**	Present	Cal Poly	36.002452	-121.385395
Santa Cruz Coastal	Woodcutter's Trail	**2023-08-15**	Absent	UC Santa Cruz	37.018243	-122.104534
Santa Cruz Coastal	BLM Coastal	**2023-08-24**	Absent	UC Santa Cruz	37.003082	-122.166048
Diablo Range	Clear Creek BLM 1	**2023-09-04**	Absent	Cal Poly	36.296963	-120.658613
Diablo Range	Clear Creek BLM 2	**2023-09-04**	Absent	Cal Poly	36.306428	-120.644208

Table 1 Continued

Santa Cruz Coastal	Wilder Ranch	**2023-09-05**	Absent	UC Santa Cruz	36.975311	-122.081822
Santa Lucia South	Pine Mountain 2	**2023-09-10**	Absent	Cal Poly	35.025933	-120.030958
Santa Lucia South	Pine Mountain 3	**2023-09-11**	Absent	Cal Poly	35.05404	-120.047855
Santa Lucia South	Pine Mountain 1	**2023-09-12**	Absent	Cal Poly	35.03473	-120.039944
Gabilan Range	Pinnacles National Park	**2024-05-06**	Absent	Cal Poly	36.484491	-121.18631
Gabilan Range	Pinnacles National Park	**2024-05-14**	Absent	Cal Poly	36.486321	-121.159577
Gabilan Range	Pinnacles National Park	**2024-05-14**	Present	Cal Poly	36.495141	-121.162421
Gabilan Range	Pinnacles National Park	**2024-05-20**	Present	Cal Poly	36.493602	-121.144454
Gabilan Range	Pinnacles National Park	**2024-05-27**	Present	Cal Poly	36.524723	-121.155036
Diablo Range	Clear Creek Management Area	**2024-06-24**	Present	Cal Poly	36.384436	-120.691193
Carrizo	Chimineas Ranch	**2024-07-01**	Absent	CDFW	35.154701	119.978792
Carrizo	Chimineas Ranch	**2024-07-01**	Absent	CDFW	35.160559	119.989134
Gabilan Range	Fremont Peak State Park	**2024-07-02**	Absent	Cal Poly	36.760323	-121.50195
Gabilan Range	Hollister Hills State Park	**2024-07-08**	Present	Cal Poly	36.777562	-121.469933
Santa Lucia North	Big Sur Chews Ridge 1	**2024-07-15**	Present	Cal Poly	36.31472	-121.557876
Santa Lucia North	Big Sur Chews Ridge 2	**2024-07-15**	Absent	Cal Poly	36.314941	-121.575503
Santa Lucia North	Big Sur Tassajara Road	**2024-07-22**	Present	Cal Poly	36.277421	-121.55604
Santa Lucia Corridor	West Cuesta Ridge 2	**2024-08-13**	Present	Cal Poly	35.396042	-120.715875
North Diablo Range	Henry Coe State Park	**2024-10-26**	Present	Cal Poly	37.17148	-121.451666

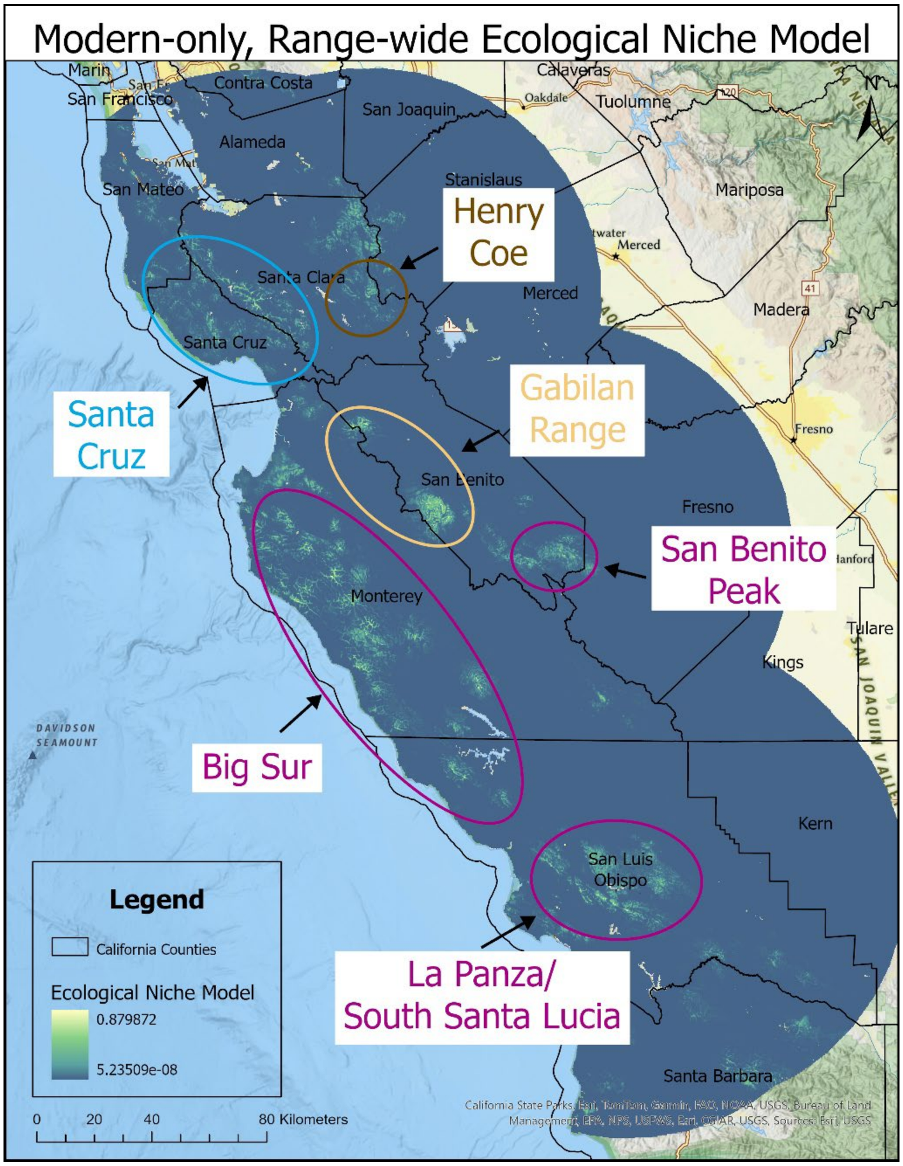

Figure 1: Narrow-faced kangaroo rat ensemble, range-wide ecological niche model using modern occurrence data only. Dark colors represent low suitability, and light colors represent high suitability. Contemporary Narrow-faced kangaroo rat range marked and annotated with color corresponding to subspecies. Purple = Santa Lucia kangaroo rat, blue = Santa Cruz kangaroo rat, yellow = Elephant-eared kangaroo rat, and brown = unverified Henry Coe population.

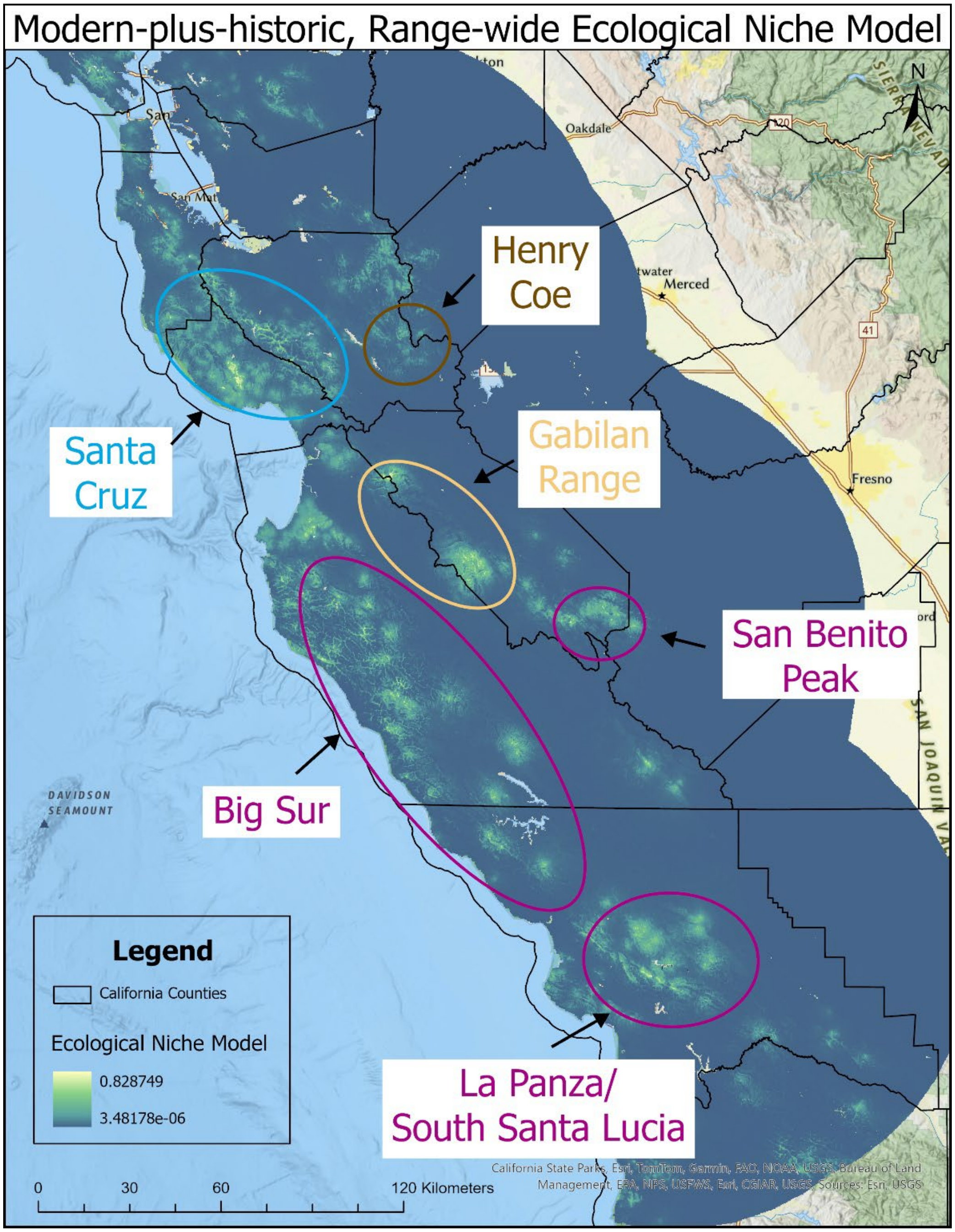

Figure 2: Narrow-faced kangaroo rat ensemble, range-wide ecological niche model using modern-plus-historic occurrence data. Dark colors represent low suitability, and light colors represent high suitability. Narrow-faced kangaroo rat range marked and annotated with color corresponding to subspecies. Purple = Santa Lucia kangaroo rat, blue = Santa Cruz kangaroo rat, yellow = Elephant-eared kangaroo rat, and brown = unverified Henry Coe population.

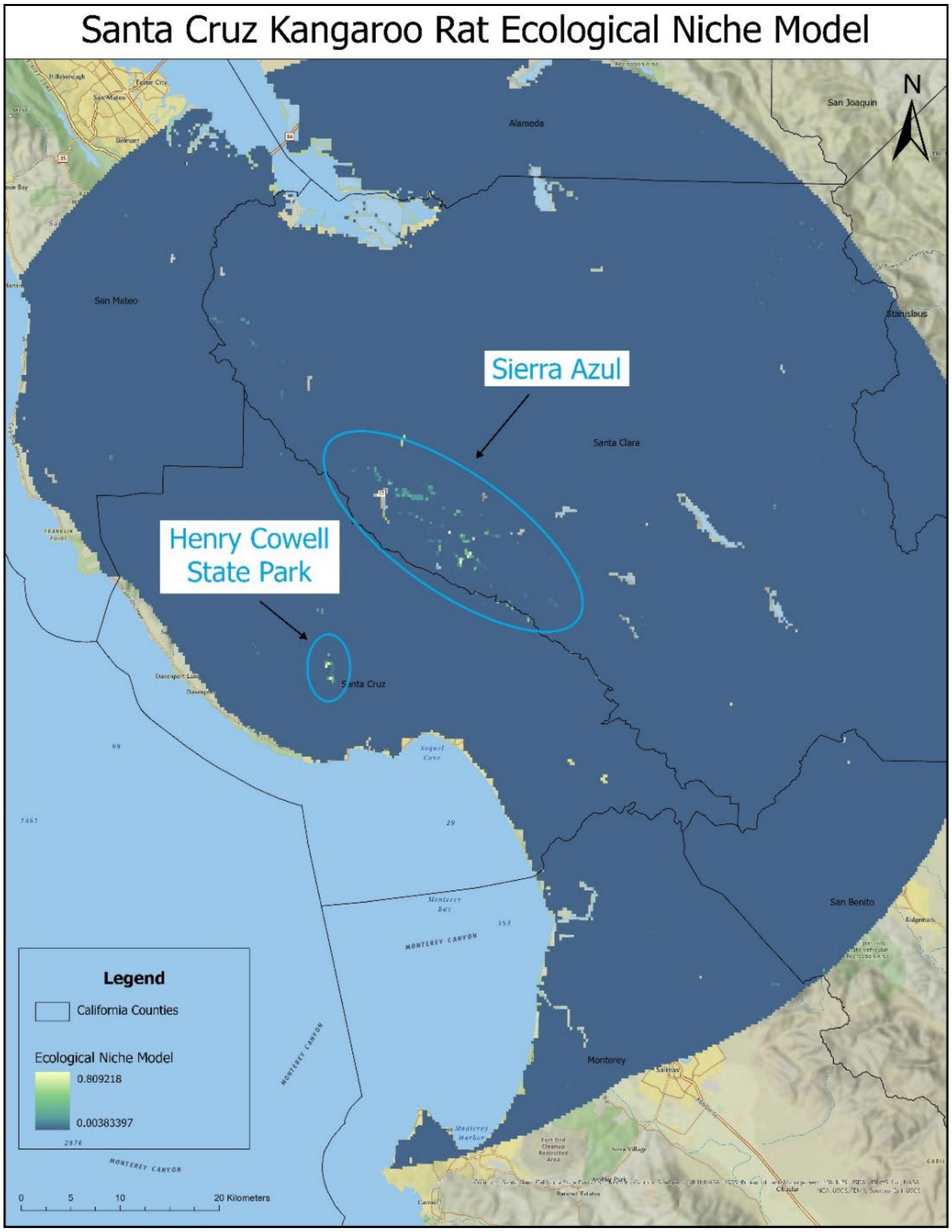

Figure 3: Santa Cruz kangaroo rat ensemble, ecological niche model using modern occurrences only. Dark colors represent low suitability, and light colors represent high suitability. Key Santa Cruz kangaroo rat locations marked in light blue and annotated

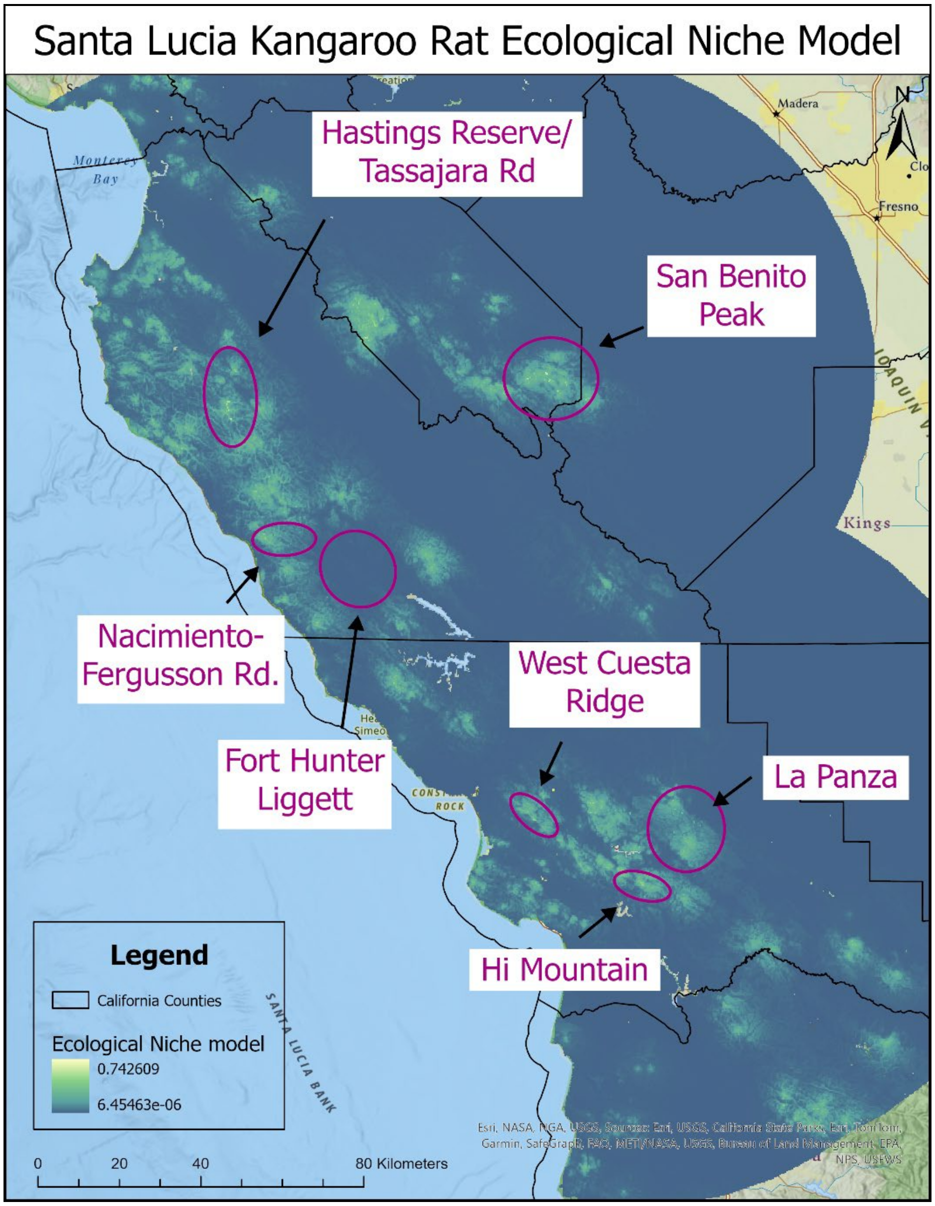

Figure 4: Santa Lucia kangaroo rat ensemble, ecological niche model. Dark colors represent low suitability, and light colors represent high suitability. Key Santa Lucia kangaroo rat locations marked in purple and annotated.

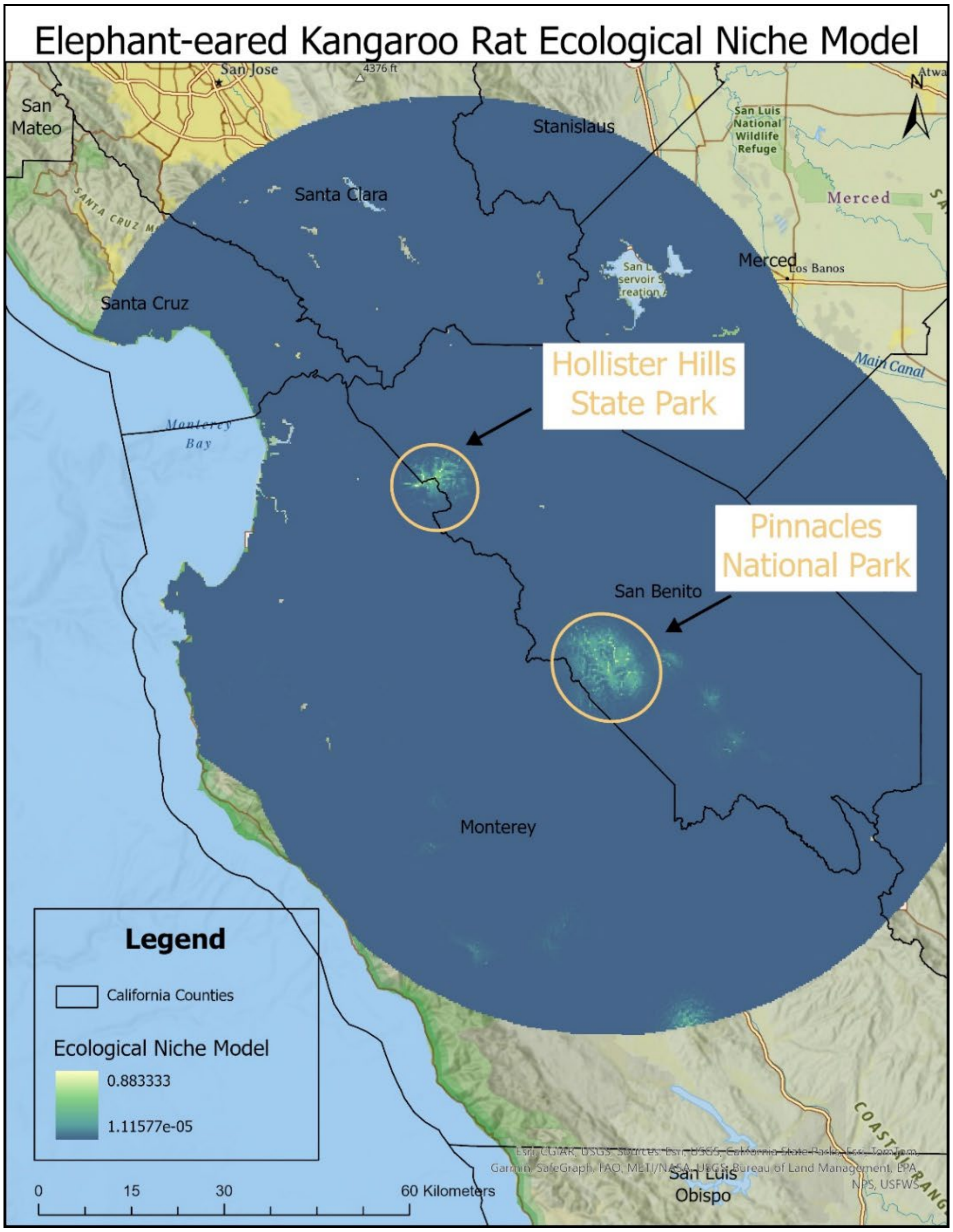

Figure 5: Elephant-eared kangaroo rat ensemble, ecological niche model. Dark colors represent low suitability, and light colors represent high suitability. Key Elephant-eared kangaroo rat locations marked in yellow and annotated.

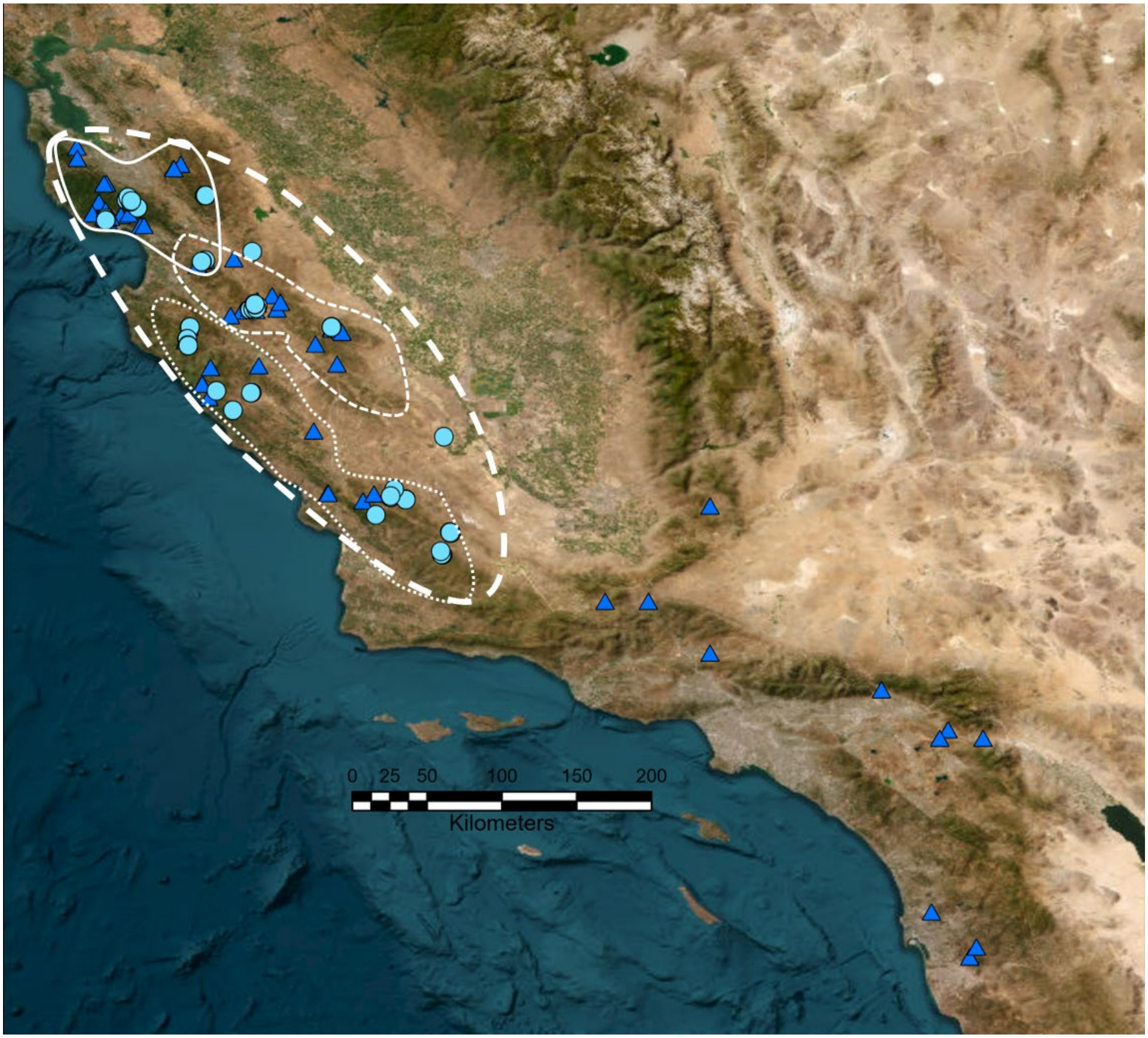

Figure 6. Genetic samples of *D. venustus* (and related species) collected and analyzed in this study. Circles are modern specimens, triangles are historical (museum) specimens. The approximate geographic scope of *D. venustus* is indicated by the larger polygon. The smaller internal polygons are *D. venustus venustus* (northern), *D. v. elephantinus* (middle), and *D. v. sanctiluciae* (southern).

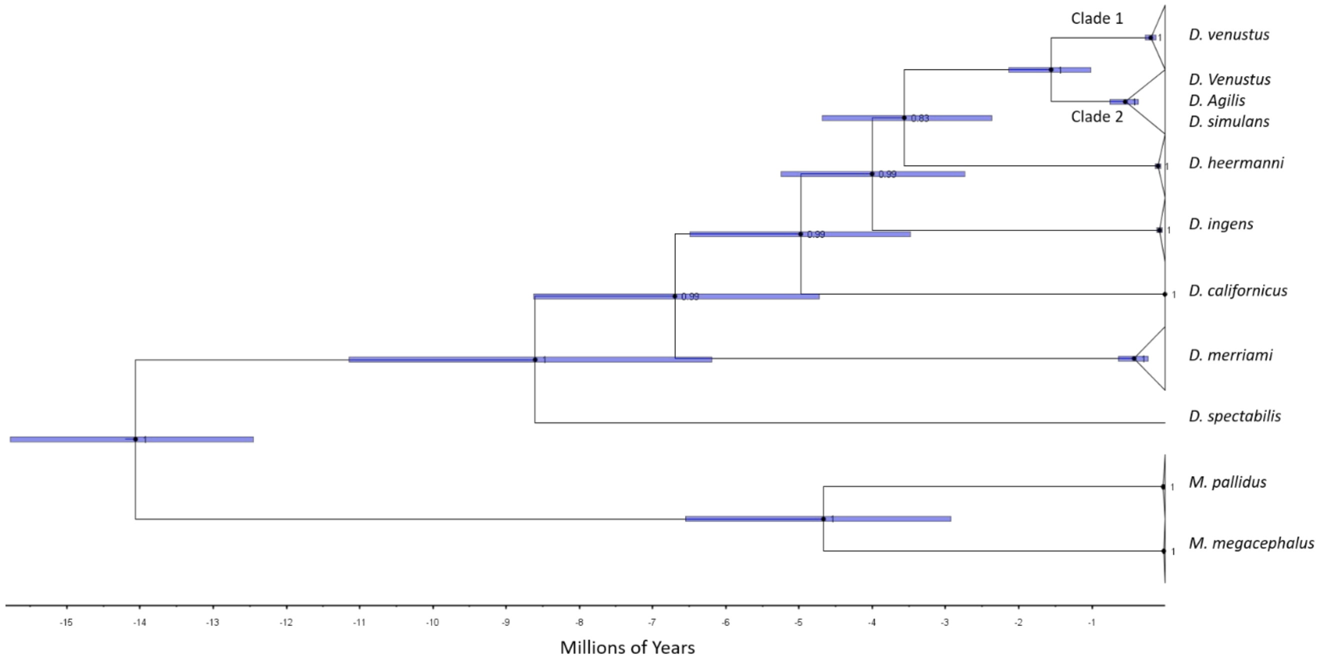

Figure 7. Phylogenetic tree of kangaroo rat species based on whole mitochondrial DNA genomes. Bayesian support values are provided at the nodes (from 0-1). Estimated divergence times are indicated at the nodes (with blue bars indicating 95% confidence intervals), and the scale bar in millions of years at the bottom.

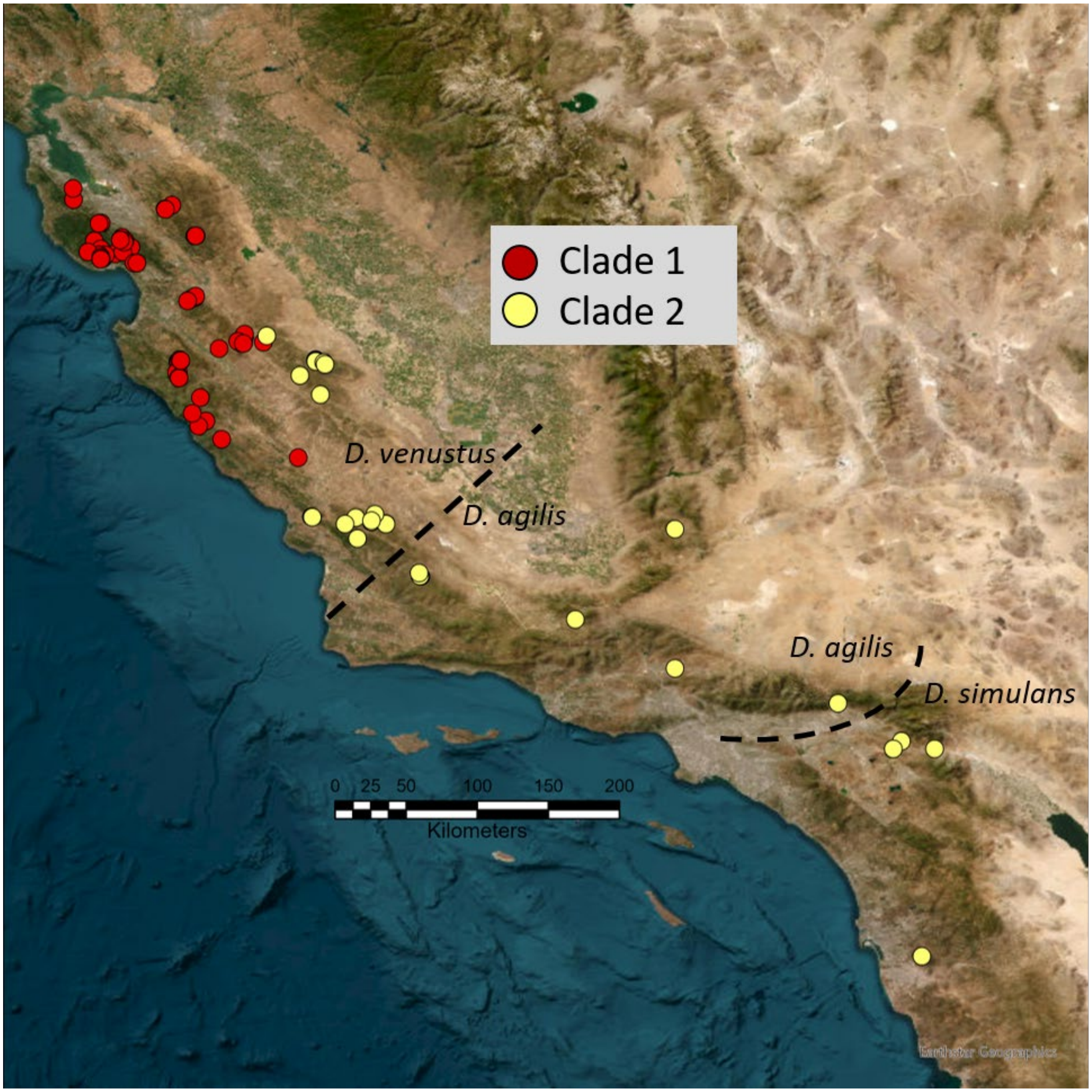

Figure 8. Map of *Dipodmys* samples indicating their assigment to either mitochondrial Clade 1 or 2. The boundary of the species is based on previous natural history records as well as our nuclear DNA analyses.

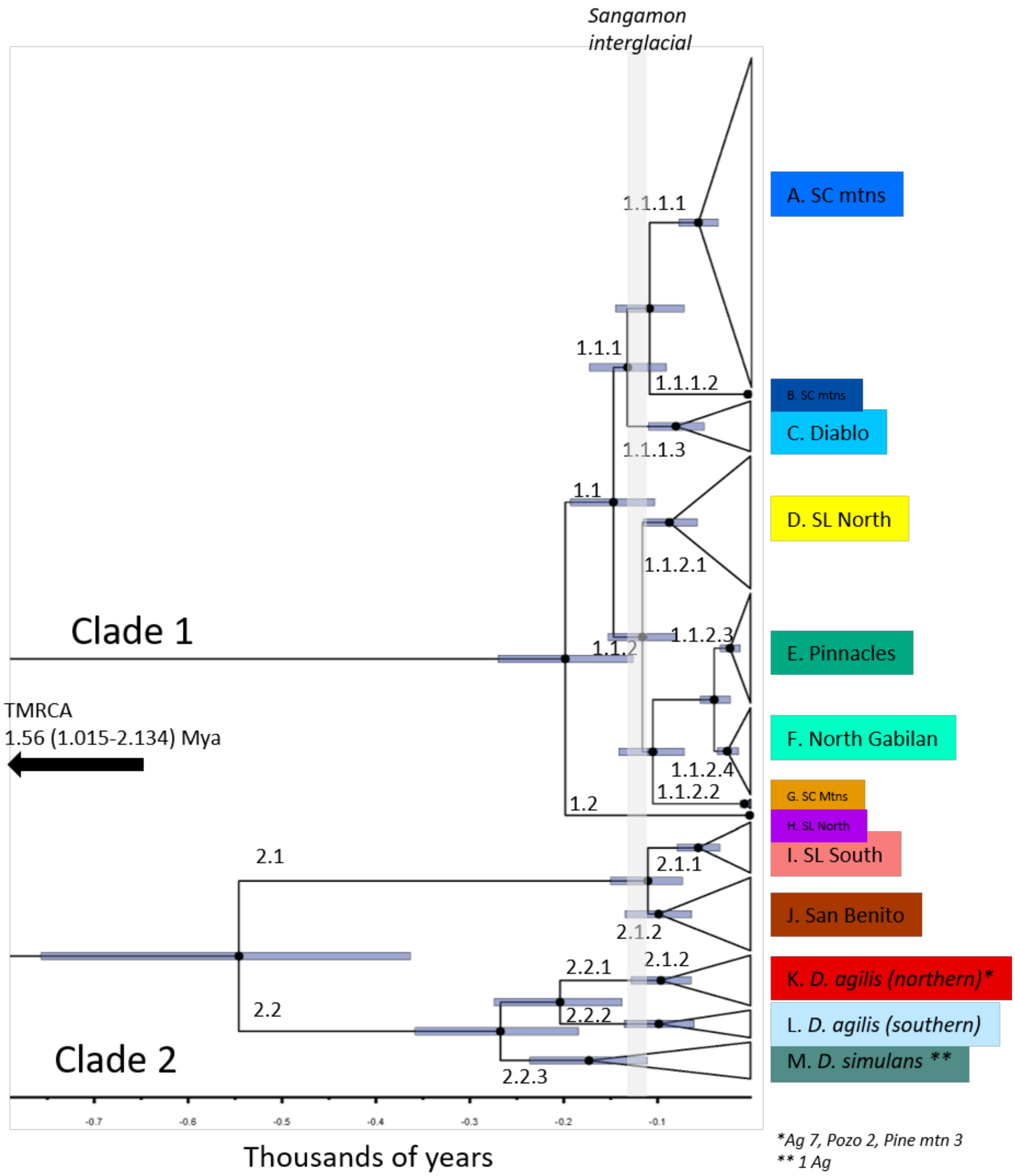

Figure 9. Phylogenetic tree of *D. venustus* and the close relatives *D. agilis* and *D. simulans* species based on whole mitochondrial DNA genomes. Estimated divergence times are indicated at the nodes (with blue bars indicating 95% confidence intervals), and the scale bar in thousands of years at the bottom. Subclades are color coded and named, and their geographic occurrence is provided in the map in Figure 10. The approximate

time period of the last interglacial period (the Sangamonian Interglacial) is indicated by a grey vertical bar.

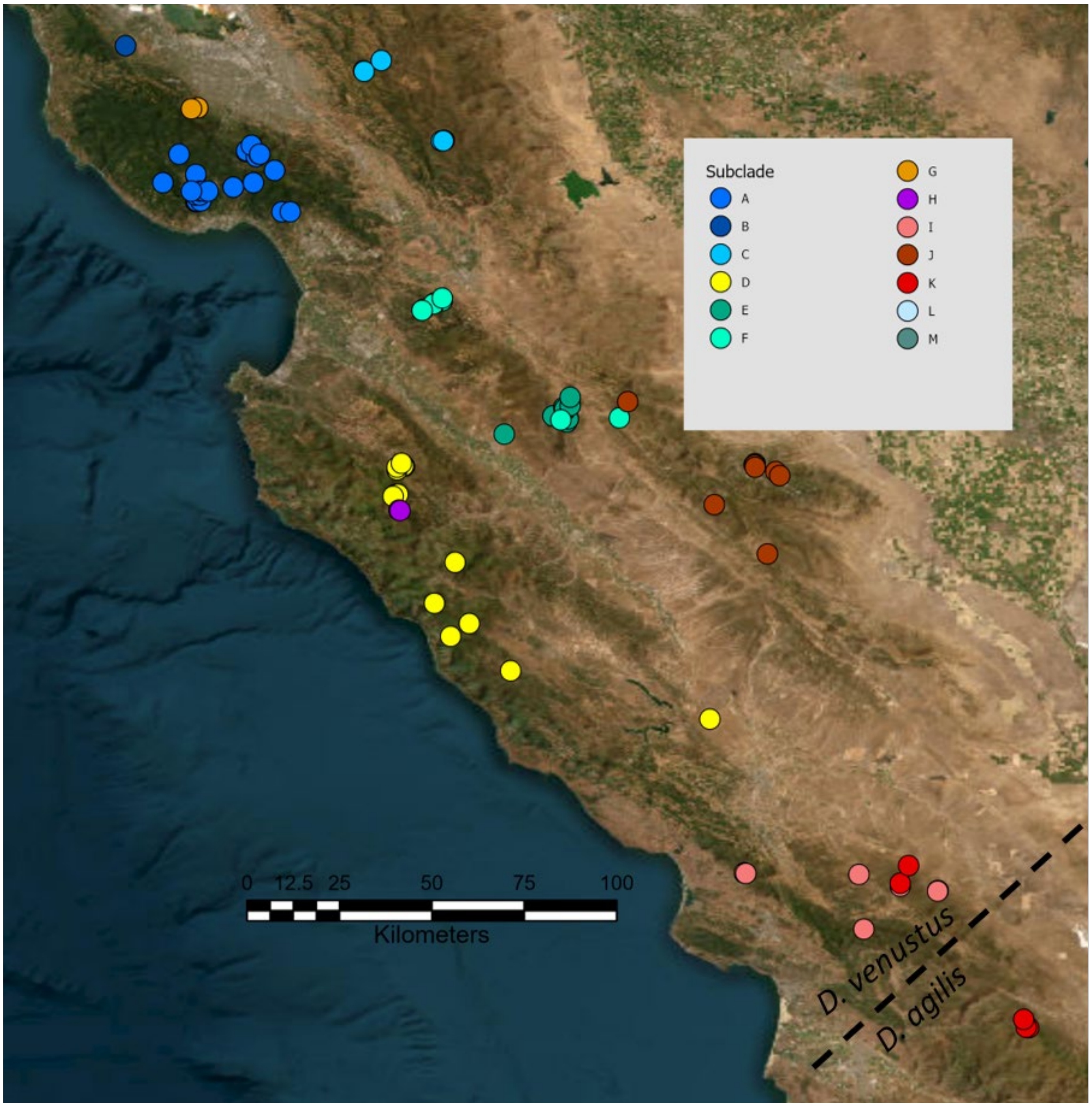

Figure 10. Map of subclades within D. venustus. The relationship among the subclades is indicated in Figure 9.

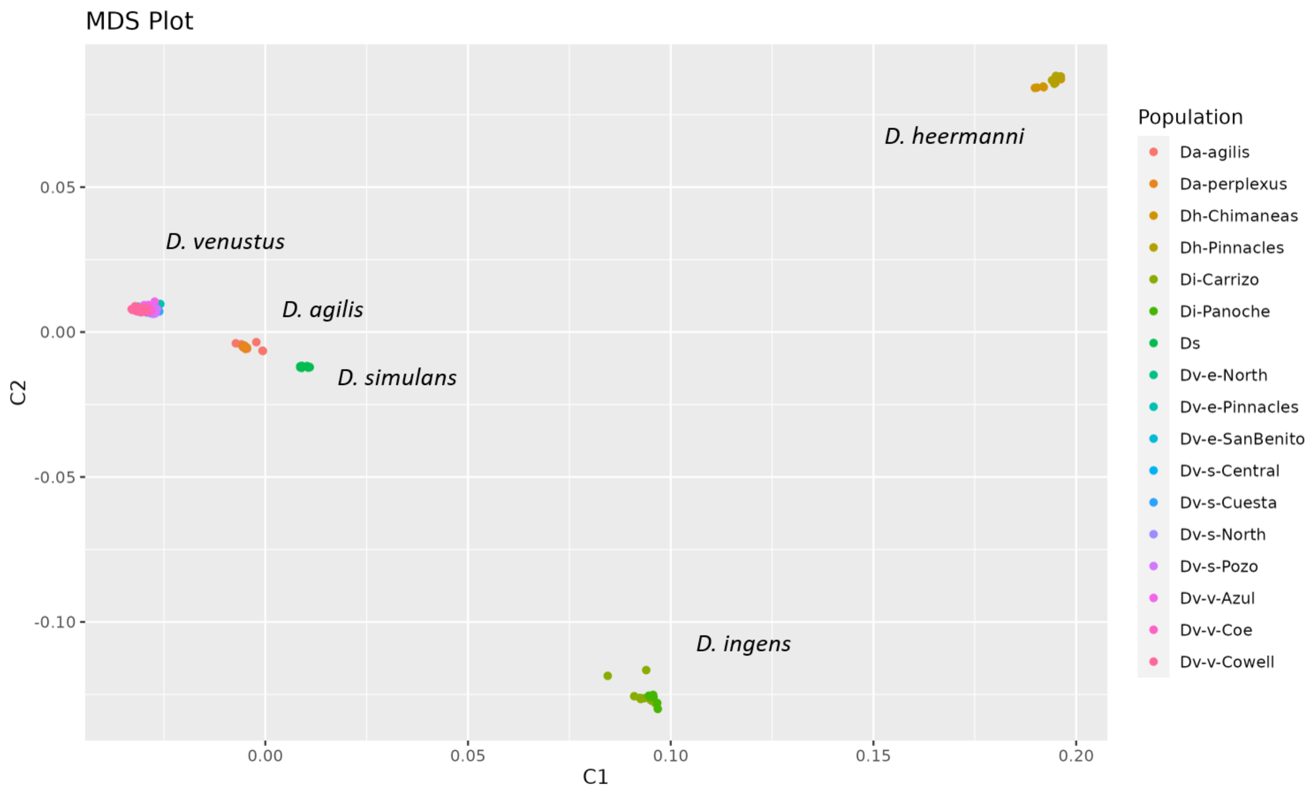

Figure 11. Ordination plot of five *Dipodomys* species showing the relatively close relationship among *D. venustus, D. agilis*, and *D. simulans*. Each point represents an individual animal, which is color-coded by subspecies and geographic location.

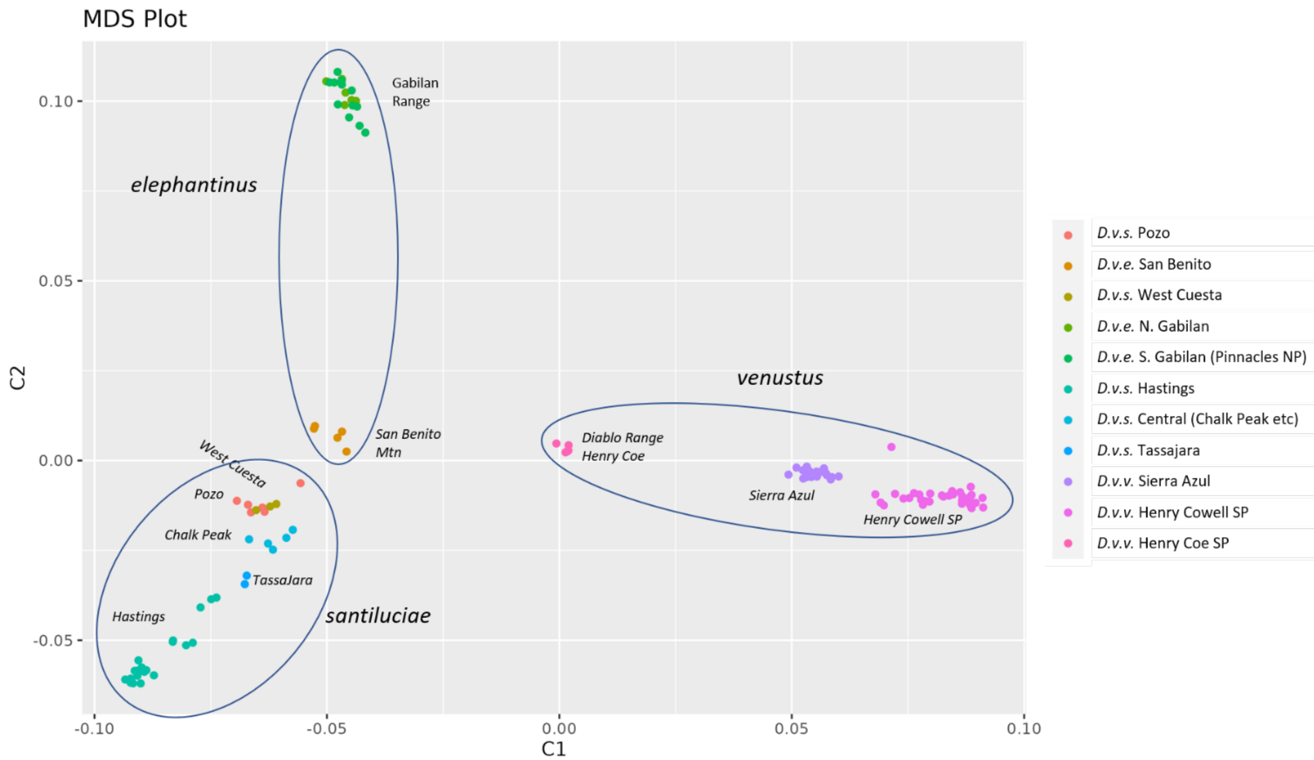

Figure 12. Ordination plot of *Dipodomys* venustus. Each point represents an individual animal, which color-coded by subspecies and geographic location.