RECLAMATION
Managing Water in the West

Genetic Investigation of Listed Vernal Pool Plants and their Communities, Merced County

Environmental Assessment

U.S. Department of the Interior
Bureau of Reclamation

May 2017

Mission Statements

The Department of the Interior protects and manages the Nation's natural resources and cultural heritage; provides scientific and other information about those resources; and honors its trust responsibilities or special commitments to American Indians, Alaska Natives, and affiliated island communities.

The mission of the Bureau of Reclamation is to manage, develop, and protect water and related resources in an environmentally and economically sound manner in the interest of the American public.

List of Abbreviations and Acronyms

CDFW	California Department of Fish and Wildlife
CESA	California Endangered Species Act
CFR	Code of Federal Regulations
CNDDB	California Natural Diversity Database
CVP	Central Valley Project
CVPCP	Central Valley Project Conservation Program
eDNA	environmental DNA
ESA	Endangered Species Act
NHPA	National Historic Properties Act
RADseq	restriction site-associated DNA sequencing
SNP	single nucleotide polymorphism
UC Merced	University of California, Merced
UC Merced Preserve	UC Merced Vernal Pool and Grassland Reserve
USFWS	U.S. Fish and Wildlife Service

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Section 1 Introduction

1.1 Background

In conformance with the National Environmental Policy Act of 1969, Council on Environmental Quality regulations (40 CFR 1500-1508), and Department of the Interior Regulations (43 CFR Part 46), the Bureau of Reclamation (Reclamation) prepared this Environmental Assessment to evaluate and disclose potential environmental impacts associated with providing $389,831 to University of California, Merced (UC Merced) to conduct field surveys and to develop methods for identifying vernal pool plants from environmental DNA (eDNA) in soil samples. The data collected would be used to improve vernal pool plant species richness estimates, develop genetic markers for the observed vernal pool plant species, and understand the population genetics of those species.

Staff from UC Merced would design methods for detecting vernal pool plant species from the soils of vernal pools located in Eastern Merced County. More broadly, the staff will identify vernal pool habitat parameters important to species diversity, and which parameters are associated with high biodiversity and listed vernal pool plant species. This information can then be used to prioritize vernal pools for conservation. In addition, they will examine genetic variability in three federally listed vernal pool plant species, Colusa grass (*Neostapfia colusana*), San Joaquin Orcutt grass (*Orcuttia inaequalis)*, and hairy Orcutt grass (*O. pilosa*), and perform a detailed population genetic investigation of Colusa grass to describe its genomic diversity.

1.2 Need for the Proposal

The Central Valley Project Conservation Program (CVPCP) program helps Reclamation mitigate past impacts of Reclamation's Central Valley Project (CVP) on threatened and endangered species, and help minimize future impacts. The UC Merced project contributes to the CVPCP objectives and helps Reclamation meet its commitments to the conservation and recovery of listed species impacted by the CVP. The project was identified by the Sacramento Field Office of the U.S. Fish and Wildlife Service (FWS) as a priority action for the CVPCP in that the topic reflects the most current evaluation of research needs for federally listed vernal pool plant species that is complimentary to other on-going conservation actions within the Central Valley. The research will provide information to be used during surveys and for other purposes to identify critical habitat conservation and restoration activities leading to the improvement of vernal pool plant populations, particularly those listed by the FWS as endangered or threatened under the Endangered Species Act.

The FWS identified the research need as a priority because the identification of listed vernal pool plants based on field surveys has limitations. Field surveys are time-consuming and are limited to time of year (flowering periods), and by the surveyors'

identification skills. Whereas, sampling eDNA can be a quick method for collecting information about the presence or absence of species of interest. More efficient sampling methods allow for more vernal pools to be surveyed. In turn, this can allow resources to be focused on vernal pools with positive results for listed species and contribute to habitat management by prioritizing vernal pools to be studied.

Section 2 Alternatives Including the Proposed Action

2.1 No Action Alternative

Under the no action alternative, Reclamation would not provide $389,831 to UC Merced to conduct field surveys and develop eDNA sampling methods for vernal pool plant species. UC Merced would be required to obtain the funds from other public or private sources to conduct this research. If alternative funding cannot be secured, the Proposed Action would not move forward.

2.2 Proposed Action

Reclamation would provide $389,831 to UC Merced to conduct field surveys and develop eDNA sampling methods for vernal pool plant species. UC Merced would complete the following tasks:

Task 1 - Conduct vernal pool plant surveys and soil sampling

Plant surveys would be conducted within the UC Merced Vernal Pool and Grassland Reserve (UC Merced Preserve) which includes the Campus Natural Reserve (CNR) and the Virginia Smith Trust Preserve (VST) (Figure 1). Plant surveys would collect presence/absence data and be conducted for two seasons after surface water in the vernal pools has dried, and plant species are flowering (April—May). A total of nine samples will be collected per vernal pool at a total of 36 vernal pools. All commonly identified species and percent cover will be recorded.

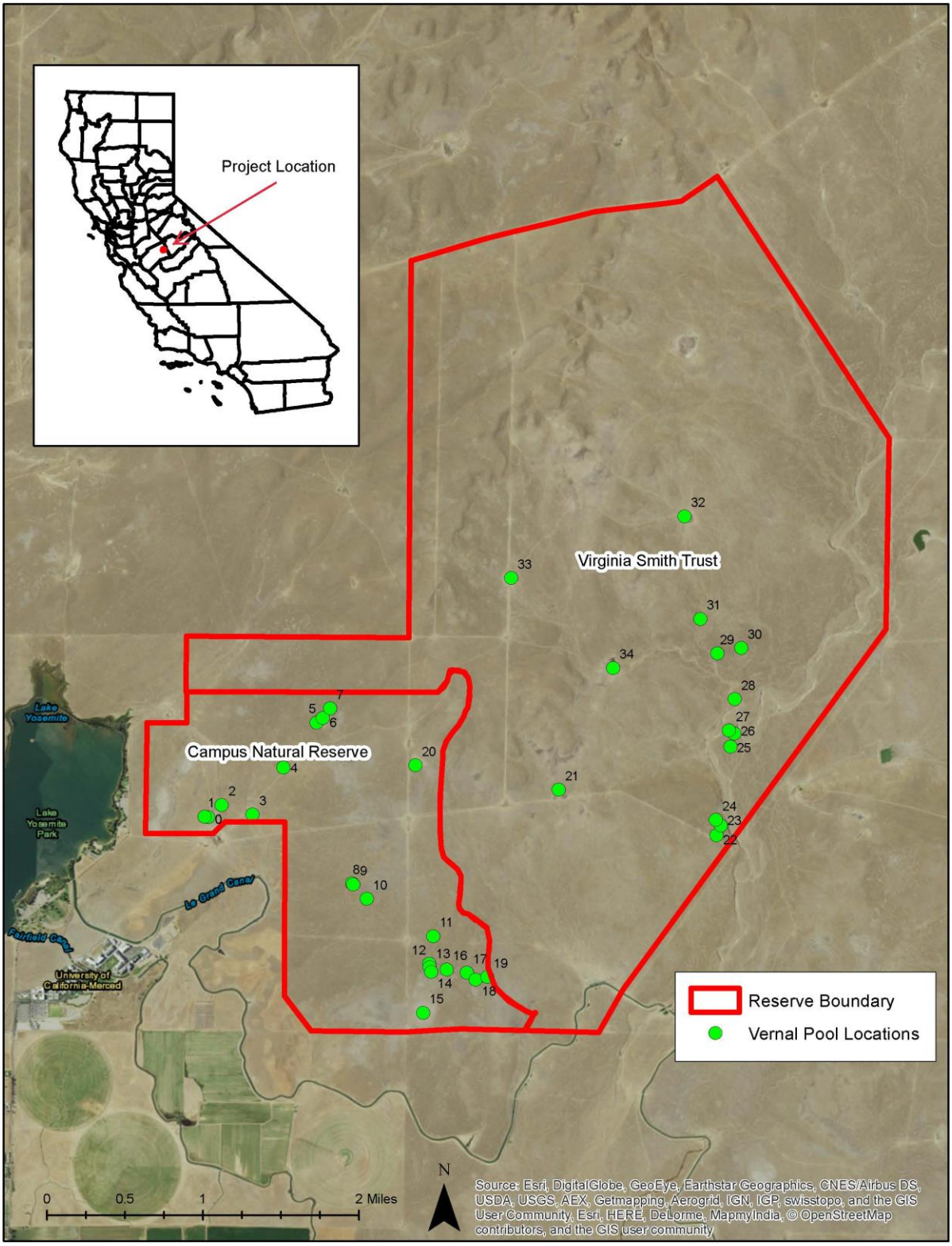

Figure 1. Project Location

Voucher specimens (specimens retained for future reference) would be collected for all species and sampled for DNA to create reference barcode sequence libraries for global access. The voucher specimens would be placed on herbarium sheets and housed at UC Merced. Following peak flowering time, soil samples will be collected. An auger up to a five inch diameter will be used to sample the top 2 inches of soil. Samples will be collected from each vernal pool, for a total of 36 soil samples.

Task 2 – Identify species and determine species diversity in the vernal pools

Genomic DNA extraction methods would be used to remove eDNA from the soil samples. After the eDNA has been extracted, metabarcoding[1] analysis would be used to determine the species composition from the soil samples. With this data, species-level identification can be made. Once the number of species in each vernal pool and their abundance is known, species diversity can be established.

Developing a reference barcode sequence may be necessary if there are species found in the soils samples that do not already have DNA barcode sequences in a reference database. A reference barcode sequence would be developed by extracting genomic DNA from vouchered specimens collected in the field or from herbarium specimens. If a reference barcode sequence is developed, the information would be added to reference databases. Metabarcoding sequencing would be conducted at UC Merced or the University of California, Davis Genome Center.

Task 3 – Comparison of results

Species identified from metabarcoding would be compared to the plant species observed during the field surveys. Habitat characteristics (i.e. inundation timing, duration, depth) would be evaluated and compared with plant species diversity (i.e. mean number of species per vernal pool, variation of species between vernal pools, and species diversity among the vernal pools). Statistical analysis would be used to determine if any common plant species may be used as surrogates for predicting biodiversity. Analysis of results would be conducted at UC Merced.

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[1] Metabarcoding is an extension of the more common DNA barcoding technique. It identifies species by sequencing a standardized region of DNA and comparing it against an established reference database. Barcode regions are sections of DNA that vary little among individuals from the same species but vary extensively among individuals of different (even closely related) species

Task 4 - Develop single nucleotide polymorphism (SNP) markers for target species

A single nucleotide polymorphism (SNP) is a single base pair mutation and acts as a genetic fingerprint which can be used in identification. Since SNP markers are like a fingerprint, they are used to identify a particular sequence of DNA in a pool of unknown DNA. SNP marker identification for the three target species (Colusa grass, San Joaquin Orcutt grass, and hairy Orcutt grass) would be developed using restriction site-associated DNA sequencing (RADseq). Plant tissue from five individual plants, for each of the three target species (total of 15 individual plants) would be sequenced to find SNP markers. In addition, a population genetic study of Colusa grass would be conducted to examine its diversity and genetic structure. Plant tissue samples would be collected from a minimum of 300 plants.

Permits

Dr. Jason Sexton and Dr. Molly Stephens have obtained valid State permits for the proposed activities funded through this action under the Fish and Game Code section 2081, subdivision (a) and California Code of Regulations, Title 14, section 786.9, subdivision (c) from the California Department of Fish and Wildlife. Unless renewed by CDFW, this Permit's authorization shall expire on December 31, 2019.

2.3 Environmental Commitments and Best Management Practices

As part of the Proposed Action, the following environmental commitments and best management practices would be implemented to avoid and minimize potential effects to the environment:

- Project activities will occur only during daylight hours (one half hour following sunrise and one half hour prior to sunset).
- Access routes will be along established roads and driveways.
- All trash items will be disposed of in securely closed containers and removed daily from the project site.
- No pets will be permitted at the project site.
- Work will be limited to dry conditions (i.e. work will not occur during rain-events).

Section 3 Affected Environment and Environmental Consequences

3.1 Resources Not Analyzed in Detail

Department of the Interior Regulations, Executive Orders, and Reclamation guidelines require a discussion of the following items when preparing environmental documentation:

3.1.1 Cultural Resources

The Proposed Action is an undertaking as defined at 36 CFR § 800.16(y). Reclamation determined the undertaking has no potential to cause effects on historic properties, pursuant to 36 CFR § 800.3(a)(1), and, therefore, would result in no significant impacts to cultural resources. As such, Reclamation has no further obligations under 54 U.S.C. § 306108, commonly known as Section 106 of the National Historic Preservation Act. (Appendix A).

3.1.2 Indian Trust Assets

Indian Trust Assets are legal interests in assets that are held in trust by the United States for federally recognized Indian tribes or individuals. There are no Indian reservations, rancherias or allotments in the project area. The nearest Indian Trust Asset is a public land allotment (a parcel of land or real estate holding that may or may not be affiliated with a particular tribe or is in the process of being recorded) located approximately 28 miles to the east of the project site. Therefore, the Proposed Action does not have a potential to affect Indian Trust Assets (Appendix B).

3.1.3 Indian Sacred Sites

Executive Order 13007 (May 24, 1996) requires that federal agencies accommodate access to and ceremonial use of Indian sacred sites by Indian religious practitioners, and avoids adversely affecting the physical integrity of such sacred sites. The proposed action would not be located on Federal lands and therefore would not affect access to or use of Indian sacred sites.

3.1.4 Environmental Justice

Executive Order 12898 requires each Federal agency to identify and address disproportionately high and adverse human health or environmental effects, including social and economic effects of its program, policies, and activities on minority populations and low-income populations. Reclamation has not identified adverse human health or environmental effects on any population as a result of implementing the Proposed Action. Therefore, implementing the Proposed Action could not have a significant or disproportionately negative impact on low-income or minority individuals within the Proposed Action area.

3.2 Biological Resources

3.2.1 Affected Environment

The UC Merced Preserve supports annual grassland habitat. Annual grasslands in the project area are dominated by naturalized non-native Mediterranean grasses and forbs, but they also include a component of native species. Wetlands in the project area includes vernal pools, swales, pool/swale complexes, mound areas, clay slope wetlands, and clay playas. These seasonal wetland types occur on soils with low permeability and support wetland species. Artificial wetlands include stock ponds, irrigation canals, and tailing areas (generally formed by water ponding against or leaking from adjacent irrigation canals). Artificial wetlands generally do not support typical vernal pool plants, although some stock ponds are primary breeding areas used by California tiger salamanders (Airola 2008).

A listing of federally listed endangered, threatened, proposed, and candidate species (listed species) and critical habitat was obtained for Merced County via the FWS website. In addition, a search of the California Natural Diversity Database (CNDDB) conducted. The following listed species were identified in the web-search as having the potential to occur within the project area:

Scientific Name	Common Name	Federal Status	Habitat
Plants			
Castilleja campestris var. succulenta	succulent owl's-clover	Threatened	Occurs in vernal pools.
Neostapfia colusana	Colusa grass	Threatened	Occurs in vernal pools.
Orcuttia inaequalis	San Joaquin Valley Orcutt grass	Threatened	Occurs in vernal pools.
Amphibians			
Ambystoma californiense	California tiger salamander	Threatened	Breeds in vernal pools, stock ponds, and other seasonal wetlands that are inundated for an average of 3–4 months annually. Salamanders use aestivation sites, primarily in soil crevices and burrows of ground squirrels and other fossorial mammals, during the nonbreeding season.
Crustaceans			
Branchinecta conservatio	Conservancy fairy shrimp	Endangered	Conservancy fairy shrimp occurs mainly in large, turbid alkaline pools; vernal lakes; and vernal pools. The sole occurrence of Conservancy fairy shrimp on UCM Conservation Lands is within the CNR, where it occupies a large vernal pool.
Branchinecta lynchi	vernal pool fairy shrimp	Threatened	Occupies vernal pools and a variety of other seasonal wetland types, including artificial depressions and drainages with

			suitable hydrology on the Preserves.
Lepidurus packardi	vernal pool tadpole shrimp	Endangered	Occurs in seasonal wetland habitats of widely varying sizes and conditions. A small amount of occupied habitat occurs on the VST Preserve.
Mammals			
Vulpes macrotis mutica	San Joaquin kit fox	Endangered	Inhabit grasslands and scrublands. Oak woodland, alkali sink scrubland, and vernal pool and alkali meadow communities also provide habitat for kit foxes. Dens are scarce in areas with shallow soils because of the proximity to bedrock, high water tables, or impenetrable hardpan layers.

Other listed plant species including Greene's Tuctoria (*Orcuttia greenei* aka *Tuctoria greenei*, Federally Endangered), Hairy Orcutt Grass (*Orcuttia pilosa*, Federally Endangered), Hartweg's golden Sunburst *(Pseudobahia bahiifolia*, Federally Endangered), and Hoover's spurge *(Chamaesyce hooveri*, Federally Threatened) have been identified in surveys in Eastern Merced County or outlying areas or were historically present in the county but lack recent verified records (Airola 2008).

Habitat suitability in eastern Merced County for the San Joaquin kit fox *(Vulpes macrotis mutica*, Federally Endangered*)* was characterized in the UC Merced Preserve's Conservation Strategy Plan (2008) on the basis of land cover type, slope, and adjacent land uses. The known distribution of San Joaquin kit fox is limited to a few areas in eastern Merced County (approximately 2 miles south of UC Merced Preserve). However, UC Merced Preserve considered its property suitable for kit fox residency and dispersal.

3.2.2 Environmental Consequences

Voucher specimens of Colusa grass, San Joaquin Orcutt grass, and hairy Orcutt grass would be collected and used for DNA sequencing. The voucher specimens would be placed on herbarium sheets and housed at UC Merced. Plant tissue from five individual plants, for each of the three species (total of 15 individual plants) would be collected and sequenced to find genetic markers. In addition, plant tissue samples of Colusa grass would be collected from a minimum of 30 individual plants at each sampling location. A total of 10 locations would be sampled (10 sampled population locations with 30 individuals per population = 300 individual plant tissue samples). Genetic material would be used to complete a fine-scale genetic study of the population.

Under federal law, activities that may impact federally-listed plant species are subject to regulation under the Endangered Species Act (ESA). However, destruction, damage or relocation of protected plants is not prohibited unless these activities take place on federal lands or are otherwise in violation of state law on other lands. Collecting would be conducted at vernal pools located in UC Merced Preserve.

State listed plant species are regulated by the California Department of Fish and Wildlife (CDFW). The California Endangered Species Act prohibits the transport, take, possession, purchase, or sale of any part or product of a listed species. The Native Plant Protection Act prohibits the take and possession of any native plant that is designated as endangered or rare. CDFW, however, may authorize the take or possession of such species by permit for scientific, educational, or management purposes. The applicants have obtained valid State permits for the proposed activities funded through this action under the Fish and Game Code section 2081, subdivision (a) and California Code of Regulations, Title 14, section 786.9, subdivision (c) from the California Department of Fish and Wildlife.

Soil samples collected from the vernal pools could contain cysts of the vernal pool crustaceans. Collection of cysts is considered take under the Endangered Species Act. Plant and soil samples will be collected by UC Merced staff who are being added as authorized individuals to the U.S. Fish and Wildlife Service, Region 8 recovery permit for Colusa grass, hairy Orcutt grass, San Joaquin Orcutt grass, and vernal pool branchiopods under Section 10(a)(1)(a) of the Endangered Species Act (ESA), 16 U.S.C. §1531 *et seq*. The collection of soil samples would be conducted in accordance with the permits. Therefore, the proposed action would have no additional adverse effects or exceed take from what was previously authorized by the Federal Recovery Permits (Appendix C).

Researchers would access the project area in vehicles using existing roads, and access to vernal pool collection sites would be on foot. Environmental commitments and best management practices listed in the Proposed Action (Section 2.3) would be implemented to avoid effects to the San Joaquin kit fox and California tiger salamanders.

Section 4 Consultation and Coordination

4.1 Agencies and Persons consulted during preparation of the EA

UC Merced staff were consulted in the development of this EA.

4.2 Central Valley Project Conservation Program Technical Team

CVPCP managers are guided by a Technical Team of biologists and natural resource specialists from Reclamation, the USFWS, and the California Department of Fish and

Wildlife. During the period of October 12, 2015 through January 21, 2016, members of the Technical Team reviewed and scored proposals submitted to Reclamation for consideration for funding. The UC Merced genetic investigation project proposal ranked in the top tier of proposals and was recommended for funding following evaluation by the Team. Reclamation and USFWS management subsequently approved the Technical Team's recommendation for funding of the proposed action.

Section 5 References

Airola, D. A. 2008. Management Plan for Conservation Lands and Adjacent Campus Buildout Lands for the University of California, Merced. September. Sacramento, CA. Prepared for University of California, Merced, Physical Planning, Design and Construction, Merced, CA.

California Natural Diversity Database (CNDDB). January 2017 data.

U.S. Fish and Wildlfie Service (USFWS). 1998. Recovery plan for upland species of the San Joaquin Valley, California, Region 1, Portland, OR. 319 pp.

Sexton, Jason and Dr. Molly Stephens. 2015. Genetic investigation of listed vernal pool plants and their communities in Merced County Project Proposal.

Appendix A Cultural Resources Compliance

CULTURAL RESOURCES COMPLIANCE
Division of Environmental Affairs
Cultural Resources Branch (MP-153)

MP-153 Tracking Number: 17-SCAO-114

Project Name: Grant for Genetic Investigation of Listed Vernal Pool Plants and Their Communities in Merced County

NEPA Document: EA

NEPA Contact: Jamie LeFevre, Natural Resources Specialist; Dan Strait, Central Valley Project Program Manager

MP-153 Cultural Resources Reviewer: BranDee Bruce, Architectural Historian

Date: March 28, 2017

Reclamation proposes to award a grant to conduct species surveys of above-ground vernal pool species/community composition and to characterize vernal pool habitat using the survey data. Activities to be covered in the grant include identifying and sampling species, analyzing them in a laboratory to identify and compare species present, and developing a single nucleotide polymorphism marker for a number of grass types. No construction or modification of facilities are need to accomplish this project.

Reclamation has determined that the proposed action is the type of activity that does not have the potential to cause effects on historic properties pursuant to 36 CFR § 800.3(a)(1). As such, Reclamation has no further obligations under Section 106 of the National Historic Preservation Act (54 U.S.C. § 306108). Based on analysis of the project activities, the proposed action would have no significant impacts on properties listed, or eligible for listing on the National Register of Historic Places.

This document conveys the completion of the cultural resources review and Section 106 process for this undertaking. Please retain a copy with the administrative record for this action. Should the proposed action change, additional review under Section 106, possibly including consultation with the State Historic Preservation Officer, may be required.

Appendix B Indian Trust Assets Compliance

Indian Trust Assets
Request Form

**Please send your request to: Kevin Clancy

Date:

Requested by	Jamie LeFevre, x 5035
Fund	15XR0680A3
WBS	RX086349991000000
Cost Center	2015200
Region # **(if other than MP)**	(NA)
Project Name	Genetic Investigation of Listed Vernal Pool Plants and their Communities, Merced County
CEC or EA Number	
Project Description	University of California, Merced (UC Merced) staff will to conduct field surveys and develop methods for identifying vernal pool plants from environmental DNA (eDNA) in soil samples. The data collected would be used to improve vernal pool plant species richness estimates, develop genetic markers for the observed vernal pool plant species, and understand the population genetics of those species.
***Project Location (Township, Range, Section, e.g., T12 R5E S10, or XY cords)**	Vernal pools are located in Eastern Merced County in the Virginia Smith Trust and the UC Merced Campus Natural Reserve. (Figure 1) 37°23'07.8"N 120°21'43.5"W

*Please include map with request, if available.

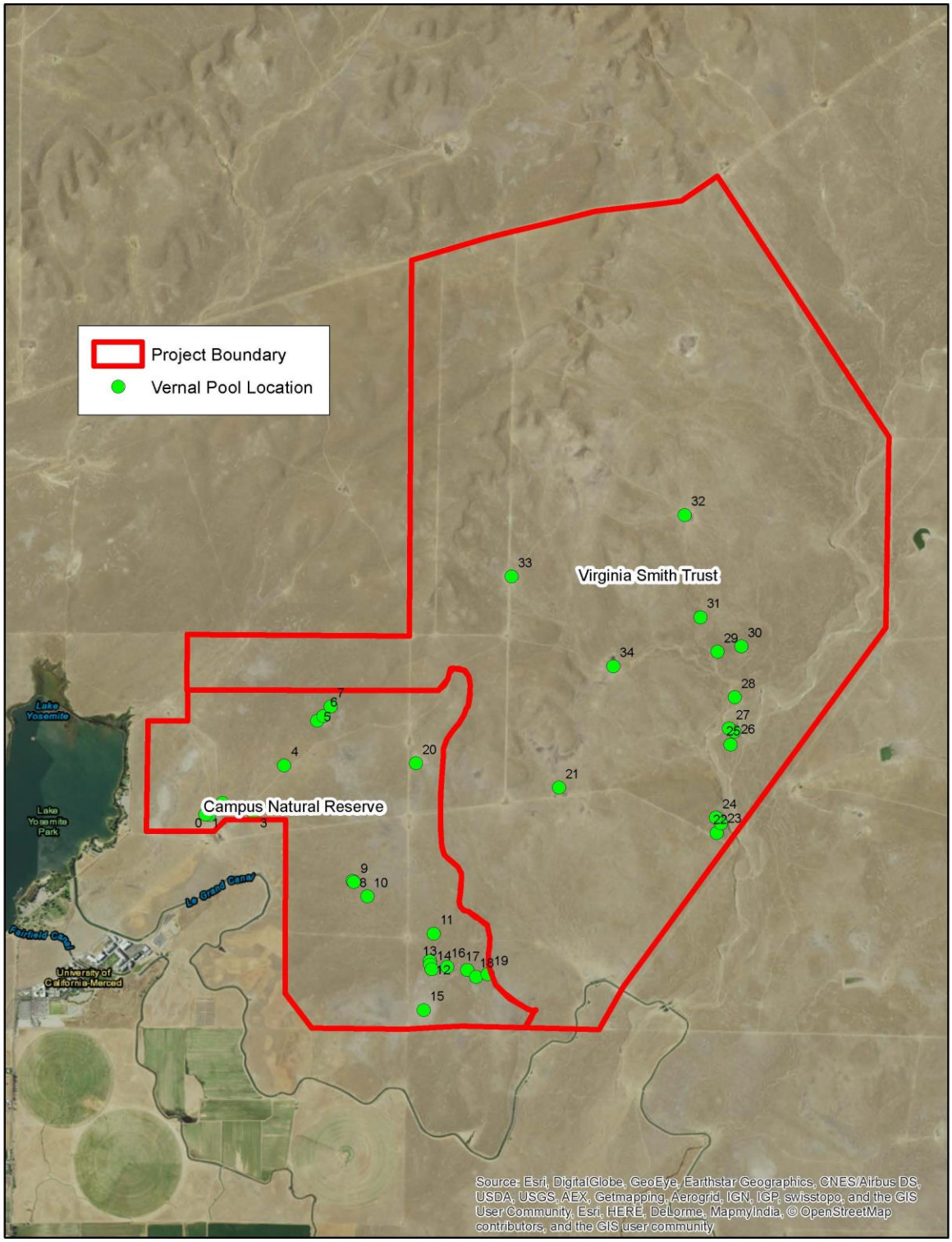
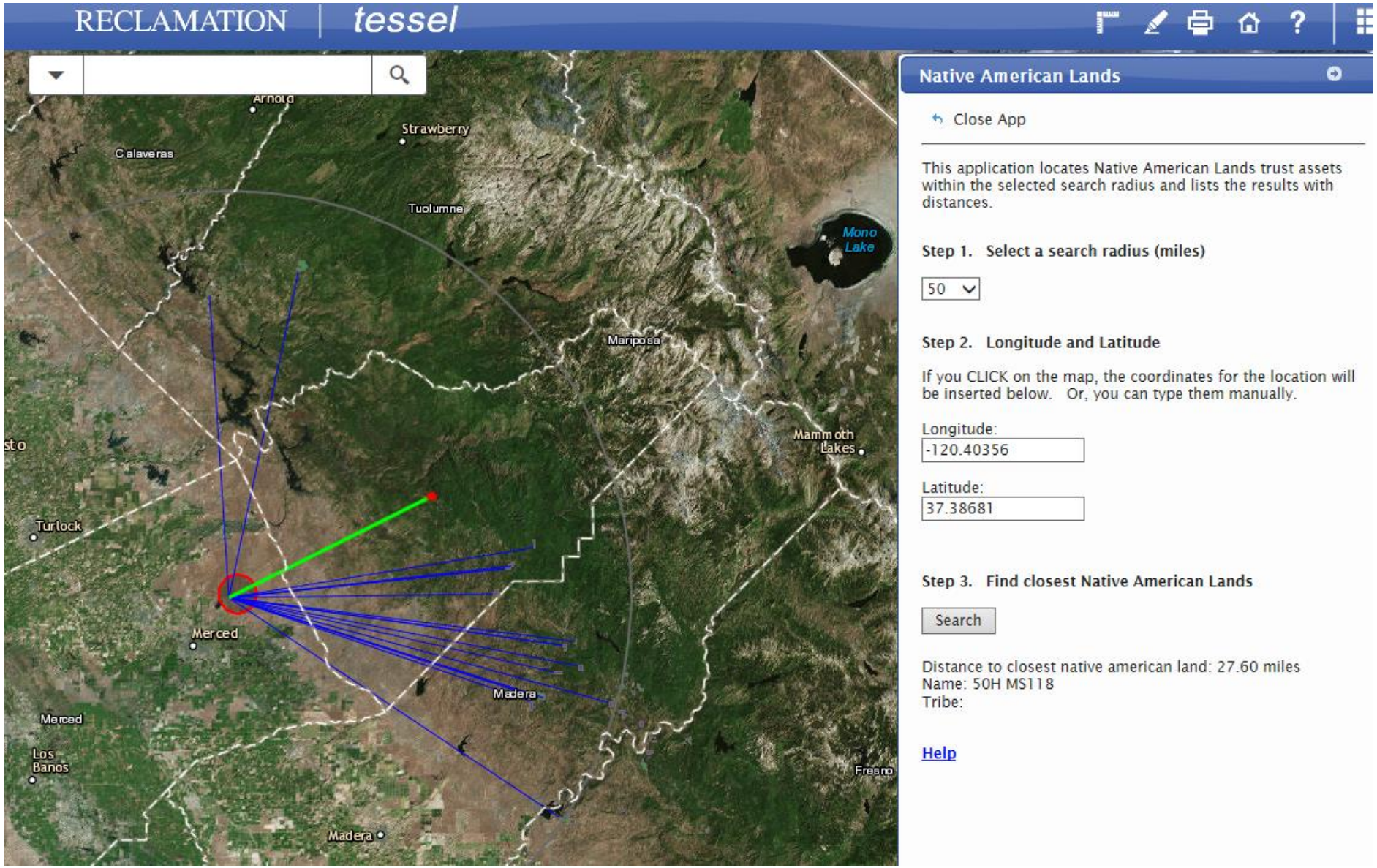


Figure 1. Location of Selected Vernal Pools



ITA Determination:

The closest ITA to the proposed Genetic Investigation of Listed Vernal Pool Plants and their Communities Project is public land allotment (a parcel of land or real estate holding, that may or may not be affiliated with a particular tribe or is in the process of being recorded) which is 28 miles east of the project site. (See attached image).

Based on the nature of the planned work it does not appear to be in an area that will impact Indian hunting or fishing resources or water rights nor is the proposed activity on actual Indian lands. It is reasonable to assume that the proposed action will not have any impacts on ITAs.

K. Clancy	Kevin Clancy	03/31/2017
Signature	Printed name of approver	Date

Appendix C Endangered Species Act Compliance

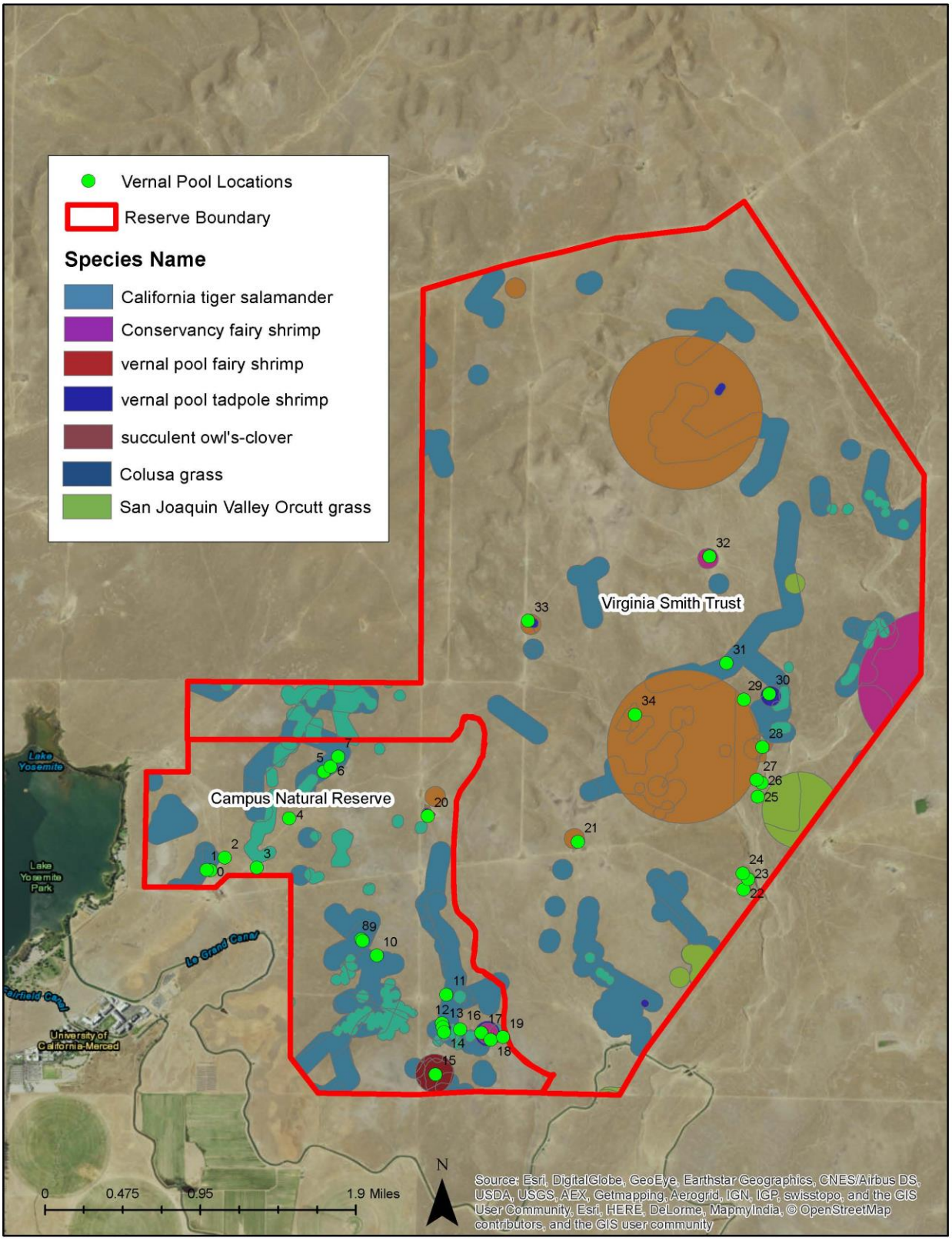


Figure 1. Location of listed species in the Project Area based on CNDDB data.