New research proposal to Western National Parks Association (WNPA)

The information supplied should be limited to the space provided and submitted on these forms.
A proposal received in any other format will be discarded. Additional attachments are not permitted.

Title of project: Unveiling a potential new species of California Jewelflower endemic to Pinnacles National Park.	Park(s) in which research is to be conducted: Pinnacles National Park (PINN)
Name, address, and phone number of principal investigator (PI)(s): Natalia Ivalu Cacho, PhD. Assistant Professor. Twin Lakes Rd. Boulder, CO 80301; phone: 530- 304-5391. Alejandra Vasco, PhD. Research Botanist. 1700 University Dr., Fort WorthTexas 76107; phone: 332-4441 ext. 262	Payee information (individual name and address or institution's name and address required): Botanical Research Institute of Texas (BRIT) 1700 University Drive, Fort Worth 76107
Is this a multiyear project? ◯ YES | NO ◉ Total amount requested: This year $ 10,000 If multiyear project, estimated amount: Second year $ _____ Third year $ _____	Desired start date: April 01, 2020 Note: no earlier than October 15, 2019
Project duration: Project final completion date: April 30, 2020 (see research guidelines)	
Name(s) of research participant(s) who will acquire advanced degree(s) as a result of working on this project, if any:	Product(s) of research (articles, theses, maps, checklists, etc.) in addition to final report to WNPA (see research guidelines): one research article on the systematics and status of Streptanthus in the Pinnacles National Park.

Abstract to be provided by PI(s). Do not exceed the half-page space provided below.

The objective of this project is to elucidate the status of what appears to be a new species of jewelflower (Streptanthus) recently discovered at Pinnacles National Park (PINN). We aim to provide a solid framework for a taxonomic assessment and description (including a formal name) for this potentially new species of jewelflower by integrating morphology, evolutionary history, genetic diversity and systematics.

The occurrence of individuals of Streptanthus has been recorded in PINN for some time, but their species assignation is unclear, and populations from the park have not been formally studied from either a morphological or a molecular standpoint. Morphologically, individuals of Streptanthus from PINN seem to be intermediate between S. glandulosus and S. insignis, with some resemblance to C. coulteri. Current phylogenetic analyses of DNA sequence data indicate that S. insignis is a close relative of S. glandulosus and rather distant of C. coulteri. However, the relationships of S. glandulosus to S. insignis and close relatives remain currently unresolved to the point that this clade is referred to as the 'Glandulosus Complex'. Thus, to identify the closest relatives of Streptanthus occurring at PINN, and infer where Streptanthus at PINN came from, it is necessary to resolve the evolutionary relationships of the Glandulosus Complex.

We will integrate morphological analyses with population genetic and phylogenetic approaches to evaluate whether the PINN jewelflower belongs to one of the Streptanthus species already described, or if represents a genetic and morphological cohesive entity deserving the species rank and as such a new species should be described.

(1) JUSTIFICATION (to be provided by submitting park): This section should specify the following: 1) Are NPS-appropriated funds available for the project (yes/no)? 2) Where does this project rank in the submitting park's research priorities for all funding sources? 3) Was this proposal solicited by the park? If not, why is this project important to the park? 4) How will this research enrich visitors' understanding of the park? 5) What are the implications for resource management?

1. NPS funding is not available for the project. However, if the researcher needs temporary accommodations PINN will provide housing if it is available to complete this project. The park will facilitate the required NPS research permit, conduct field surveys, and collect samples to be used for genetic analysis.

2. The park considers confirming that the undescribed jewelflower is indeed a new species or subspecies and describing it as such as one of our highest research priorities. If, as believed, this is a new species, it has significant management implications (see 5). Other NPS Natural Resource funding prioritizes management-focused projects rather than research, therefore, WNPA funding is the best fit for this project.

3. This proposal was solicited by the park's Vegetation Ecologist in consultation with the park Superintendent and the researcher was solicited by the park directly as an expert in this genus of plants.

4. This research to determine the species status of the Pinnacles jewelflower has the potential to engage visitors in an exciting sense of discovery. Describing a newly found plant species in the park is a compelling story that needs to be shared with park visitors, stakeholders, and partners. Highlighting its discovery illustrates how we are still learning about our natural world, even though PINN has been a protected area for more than 100 years. It also showcases the importance of conducting scientific research in parks because new discoveries can be–and are–being made throughout the national park system. The wilderness character and diligent stewardship of these lands to protect the park for future generations make these sorts of discoveries possible.

5. Because this jewelflower species appears to be a very narrow endemic with a very small range, it is extremely rare and of great conservation concern. NPS Management Policy 4.4.2.3 directs NPS to "inventory other native species that are of special management concern to parks (such as rare, declining, sensitive, or unique species and their habitats) and will manage them to maintain their natural distribution and abundance." However, the Pinnacles jewelflower is not yet recognized as a species. In order to inventory this species and confer this species the protections or planning considerations as directed by our management policies, it is imperative that the species be formally recognized.

(2) CONCISE STATEMENT OF RESEARCH OBJECTIVES, DESIGN, AND METHODOLOGY: This section should include the facilities and sites to be used. Note: limit this section to the two pages provided.

RESEARCH OBJECTIVES

The objective of this project is to elucidate the status of what appears to be a new species of jewelflower (Streptanthus) discovered at Pinnacles National Park (PINN). We aim to provide a solid framework for a taxonomic assessment and description (including a formal name) for this potentially new species of jewelflower by integrating morphology, evolutionary history, genetic diversity and systematics of Streptanthus from PINN. Specifically, this research will:

1. Clarify the evolutionary relationships of the Streptanthus at PINN using phylogenetic analyses of genomic data.
2. Infer population structure and genetic diversity for Streptanthus occurring at PINN.
3. Use detailed morphological analyses to place Streptanthus from PINN in the context of the Glandulosus Complex.

California Jewelflowers are mustards (Brassicaceae) in the genus Streptanthus and allied genera Caulanthus and Guillenia with their center of diversity in California. An urn-shaped calyx, strong bilateral symmetry and wide variation in color sets apart Streptanthus flowers from the yellow cruciform flowers typical of Brassicaceae. Streptanthus is also remarkable in the high ecological diversity it exhibits, especially associated with bare habitats of peculiar geologies. As many as 30% of the species in CA are endemic to serpentine soils; others are associated with gabbro, basaltic or granitic substrates. The occurrence of individuals of Streptanthus has been recorded in PINN for some time, but their species assignation is unclear, and populations from the park have not been formally studied from either a morphological or a molecular standpoint.

Morphologically, individuals of Streptanthus that have been collected in PINN seem to be intermediate between S. glandulosus and S. insignis; this last species was originally described in the Jepson Manual as "intermediate between S. glandulosus an C. coulteri" (Hickman, 1993). Our current understanding of evolutionary relationships within Streptanthus, 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 close relatives distributed loosely around the San Francisco Bay area and the South Coast Ranges such as S. hispidus, S. niger, S. callistus and S. albidus remain currently unresolved to the point that this clade 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 PINN colonized from), it is necessary to resolve the evolutionary relationships among the six species that conform Glandulosus Complex.

Due to the recent origin of the Glandulosus complex, and the fact that eight molecular regions do not suffice to confidently resolve relationships within it, we advocate the use of genomic data to tackle this issue. In particular, we propose to perform a RadSeq approach, which has been employed successfully for phylogeographical (Emerson et al, 2010), phylogenetic (Eaton & Ree, 2013; Hipp et al, 2014) and population structure (Zellmer et al, 2012) inference, and laboratory and bioinformatic methods are well developed (Etter & Johnson, 2012; Eaton 2014).

RESEARCH METHODOLOGY

Taxonomic sampling– We will include multiple accessions of Streptanthus from PINN (from different provenances within the Park if possible) and representatives of S. hispidus, S. niger, S. callistus and S. albidus, all species within the Glandulosus Complex. As outgroups we will include accessions from the various clades of Streptanthus and relatives as outgroup. We want to highlight that throughout the years, we have assembled a collection of plant tissue in silica gel with representatives of S. hispidus, S. niger, S. callistus and S. albidus; this collection is a valuable contribution in time, effort and funds that would be otherwise destined to collecting.

We propose to include 20-30 samples from Streptanthus occurring at PINN from as many populations as possible, in addition to at least two individuals of narrow endemics S. hispidus, S. albidus and S. callistus, a wide sampling of S. glandulosus throughout its range (e.g., ~50 accessions, from ~7-8 populations, 6-7 individuals / population), and key outrgroups, such as one representative of each of the major clades of Streptanthus, for a total of 95 samples.

(2) CONCISE STATEMENT OF RESEARCH OBJECTIVES, DESIGN, AND METHODOLOGY (continued):

Molecular Methods– We will extract genomic DNA with the Plant DNeasy Kit (Qiagen, Valencia, CA), and perform DNA quality check and quantification with a combination of visualization in agarose gels and fluorimetric methods using a Qbit. DNA will be normalized to a final concentration of ~50ng/µL, prior to shipping to the sequencing facility. RadSeq, as other reduced representation methods, relies on adequate coverage for reliable SNP identification, typically 20-30X. Coverage is a function of the genome size, restriction enzyme used, size selection performed, and the number of sequencing reads obtained (Etter & Johnson, 2012; Monson-Miller et al., 2012). The genome size of Streptanthus ranges from 0.75-1.9 pg (Cacho et al, in prep.), which are on the smaller size for plants and therefore amenable for being addressed with this kind of methods. Barcoding, library construction, and Illumina sequencing will be performed at Floragenex (www.floragenex.com) following standard protocols.

Sequence processing and analyses– After quality check, sequences (reads) will be processed with iPyRad (Eaton, 2014), a pipeline specifically developed to process RadSeq data in the absence of a reference genome to infer Rad Loci, which will be the basis for phylogenetic and population genetic analyses.

Phylogenetic analyses will be based on SNPs that are fixed within but variable among populations, and inference will be done under both, likelihood and Bayesian frameworks, using RAXML (Stamatakis, 2014) and MrBayes(Ronquist et al, 2012). We will assess genetic diversity of Streptanthus populations from the PINN using STRUCTURE v.2.3 (Pritchard et al, 2000), and compare it to the genetic diversity of its close relatives. We will also explore newer methods that relax a fully bifurcating tree approach by incorporating drift and migration (e.g., TreeMix; Pickrell & Pritchard, 2012), or even incorporate a network framework (Solís-Lemus & Ané, 2016). These more complex models, in combination with models that explicitly incorporate information on the geographic location of populations (e.g.GENELAND, Guillot et al, 2005; TESS, Durand et al, 2009) allow us to evaluate hypothesis of geographic range expansion of streptanthoids based on population genetic and phylogenetic patterns.

Morphological Data and Analyses– We will perform analyses with morphological measurements that include basal and cauline leaf length and width, petal and sepal dimensions and color, anther morphology, style shape, seed and fruit morphology (dimensions, shape of joint). We will use multivariate analyses (e.g., Principal Components Analyses) to assess and detect morphological discontinuities among streptanthoids that would support recognizing the PINN jewelflower as a new distinct species.

The integration of morphology with population genetic and phylogenetic approaches will allow us to evaluate whether Streptanthus from PINN belongs to one of the Streptanthus species already described, or if represents a genetic and morphological cohesive entity deserving the species rank and as such a new species should be described.

SELECTED REFERENCES (rest available upon request):

Cacho et al 2014. Novel nuclear markers inform the systematics and the evolution of serpentine use in Streptanthus and allies (Thelypodieae, Brassicaceae). doi:10.1016/j.ympev.2013.11.018. • Cacho & Strauss. 2013. Single-Copy nuclear gene primers for Streptanthus and other Brassicaceae from .... doi:10.3732/apps.1200002. • Eaton 2014. PyRAD: Assembly of de novo RADseq loci for phylogenetic analyses. doi:10.1093/bioinformatics/btu121. • Eaton & Ree. 2013. Inferring phylogeny and introgression using RADseq: an example from flowering plants.... doi: 10.1093/sysbio/syt032. • Hickman 1993. The Jepson manual: Higher plants of California. University of California Press, Berkeley, CA, USA. • Ronquist et al 2012. Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. doi:10.1093/sysbio/sys029. • Solís-Lemus & Ané 2016. Inferring phylogenetic networks with Maximum Pseudolikelihood under ILS. doi: 10.1371/journal.pgen.1005896. • Stamatakis 2014. RAxML v8: A tool for phylogenetic analysis and postanalysis of large phylogenies. doi: 10.1093/bioinformatics/btu03 • Pritchard et al 2000. Inference of population structure using multilocus genotype data. webID: PMC1461096.

(3) CONCISE STATEMENT OF HOW YOUR RESEARCH CAN ENHANCE THE INTERPRETIVE MISSION OF THE PARK: Also include one paragraph describing the plan for an interpretation-related product of the research. Use this page only.

This research will engage the park's visitors through a sense of excitement at the discovery of a new species.

Many people assume that most species have already been found. The discovery and sharing of a new species is a tangible expression of a very important aspect of the scientific process: that new information is always being learned that changes or enhances our understanding. The thrill of discovery has the potential to capture the imagination of the public, especially children, and awaken them to the fact that they, too, might one day discover a new species.

The fact that this is appears to be an endemic species presents the opportunity to elucidate several other scientific concepts such as speciation, specialization, and biodiversity. We propose to create a short video or park bulletin about this species and use it to expand upon the above-described ecological concepts and put this discovery in the framework of other species that occur only at Pinnacles (species endemic to Pinnacles area).

A key "take away" will be that by protecting national parks like Pinnacles, we are not only protecting beautiful places, but we are protecting scientific discoveries that have yet to be made in the future. This short video or bulletin would help visitors connect the discovery of the Pinnacles jewelflower with the larger role of National Parks as scientific laboratories and sources of discovery.

(4) QUALIFICATIONS OF THE PI(S) CONDUCTING THE RESEARCH: Use this page only. List only those qualifications directly related to this grant request. Include a list of other WNPA-funded research conducted by this PI.

1. PI Natalia Ivalu Cacho, PhD.
Assistant Professor, Botany Department, Instituto de Biología, UNAM.
3er Circuito de Ciudad Universitaria, Deleg. Coyoacán, CDMX 04510, México.
-and-
4955 Twin Lakes Rd. Boulder, Colorado 80301, USA.
Phone: +1 (530) 304-5391 (US number); ivalu.cacho@ib.unam.mx, ivalu.cacho@gmail.com

PI Cacho is a leading expert on Streptanthus phylogenetic systematics and evolutionary ecology. She is responsible for developing markers appropriate to increase phylogenetic resolution in Streptanthus and close relatives (Cacho & Strauss, 2013), and for the most comprehensive phylogenetic analyses of this group today (Cacho et al., 2014). Currently she has collaborations to develop a genomic perspective on the phylogeny of Streptanthus and relatives. Also, PI Cacho has used the phylogenetic framework developed to address a series of questions on the evolutionary ecology of these diverse group of mustards, including associations to peculiar soils (Cacho & Strauss, 2015), evolution of floral scents (Weber, Cacho et al., 2018) and evolution of plant defense (Cacho et al., 2015).

2, Co-PI Alejandra Vasco, PhD.
Research Botanist, Botanical Research Institute of Texas.
1700 University Drive, Fort Worth, Texas 76107-3400, USA.
Phone: +1 (817) 332-4441 ext. 262; email: avasco@brit.org

PI Alejandra Vasco is an expert on plant systematics and evolution. PI Vasco has extensive experience on plant taxonomy and leaf evolution, which is of high importance for this grant because one of the axes where Streptanthus found on PINN seems to be distinctive is on its leaf morphology.

Selected References:

Cacho, NI & SY Strauss. 2013. Single copy nuclear gene primers for Streptanthus and other Brassicaceae from genomic scans, published resources, and ESTs. Applications in Plant Sciences 1(7): 1200002. DOI: 10.3732/apps.1200002. • Cacho, NI, AM Burrell, A Pepper, and SY Strauss. 2014. Novel markers inform the systematics and the evolution of serpentine use in Streptanthus and allies (Thelypodieae, Brassicaceae). Molecular Phylogenetics and Evolution 72: 72-81. DOI: 10.1016/j.ympev.2013.11.018. • Cacho, NI & S.Y. Strauss. 2014. Adaptation to bare habitats: an evolutionary precursor to soil specialization in plants. Proceedings of the National Academy of Sciences 11(42): 15132–15137. DOI: 10.1073/pnas.1409242111. • Cacho, NI, DJ Kliebenstein, & SY Strauss. 2015. Macroevolutionary patterns of glucosinolate defense and tests of defense-escalation and resource availability hypotheses. 2015. New Phytologist. 208 (3): 915-927. DOI: 10.1111/nph.13561. • Weber, MJ, NI Cacho, MJQ Phan, C Disbrow, SR Ramírez & SY Strauss. 2018. The evolution of floral signals in relation to range overlap in a clade of California Jewelflowers (Streptanthus s.l.). Evolution DOI: doi:10.1111/evo.13456.

Budget for New Research Proposal

Project title and submitting park: _____

PERSONNEL EXPENSES

PRINCIPAL INVESTIGATOR(S)	Funds requested from WNPA	Cash or in-kind contribution (Please specify which type and source.)
1 PI Cacho (cash, salary, UNAM)		2,000
2 PI Vasco (cash, salary, BRIT)		2,000
3		

OTHER PERSONNEL (Specify number in brackets. Specify duties to be performed to earn funds on next page.)	Funds requested from WNPA	Cash or in-kind contribution (Please specify which type and source.)
1 PINN ecologist: in-kind		
2 Intern	1,200	
3		
4		
5		
TOTAL PERSONNEL COSTS	1,200	4,000

OTHER EXPENSES

OTHER COSTS

1 Supplies and material	1,500	1,500 (in-kind and cash)
2 Consulting services		
3 Computer services		
4 Subcontracts (itemize on next page.)	6,800	
5 Equipment (itemize equipment costing more than $100 each on next page)		
6 Travel and subsistence (itemize on next page)	500	1,000 (cash)
TOTAL OTHER COSTS	8,800	2, 500
TOTAL PROJECT COSTS If multiyear project, summarize estimated subsequent year(s') budget(s) on next page.	10,000	6,500

COSTS (continued): Be sure to explain here the duties that will be performed by any funded individual.

We request funds to help defray molecular work, sequencing and salary for an intern to develop interpretative materials.

Plant collection will be performed by PINN's ecologist Amelia Ryan, and samples will be shipped to the Botanical Institute of Texas (BRIT) where DNA will be extracted, quantified and normalized by PIs Cacho and Vasco. Data analyses and writeup will be lead by PI Cacho; PI Vasco will take lead on taxonomical and leaf morphology sections. Interpretative materials will be developed by collaboration between PIs, park personnel, and an intern.

Supplies and materials (TOTAL: $1,500.00):
$522.00 ..... DNA Extraction Kit (Qiagen Plant Mini Kit 50, cat. no. 69104): 2 units @ $236 each + shipping.
$348.00 ..... Qubit dsDNA HS Assay Kit 500 (cat. no. Q32854): 1 unit @ $298 + shipping.
$230.00 ..... Pippette tips, tubes, agarose, buffers, and other laboratory supplies.
$400.00 ..... Materials for interpretative product.

Subcontracts (TOTAL : $6,800.00)
$6650.00 ..... RADseq sequencing, including library preparation and sequencing for one plate (95 samples)*.
$150.00 ..... Shipping of samples (tissue and DNAs) through Fedex overnight service.
* from (www.floragenex.com/pricing).

Travel: We request $500.00 to help defray expenses for PI Cacho to travel to Texas to perform molecular work at BRIT.

We are leveraging our funding requests with: (a) existing collections of various populations of key taxa for this project, including S. glandulosus, S. insignis, and S. coulteri that represent various thousands of dollars and several months of collecting efforts; (b) specialized equipment and supplies (fully equiped and working lab at BRIT); and, (c) taxonomic and molecular expertise of PIs.

A special note for researchers and the park superintendent:

WNPA is the funder of this grant on behalf of the NPS, and WNPA monitors progress, administers the payment schedule, and determines successful completion or default.

All other decisions regarding the conduct of this research grant (park access, laws, safety, protocols, etc.) and uses of the research, data, and its products (release of information, publication, intellectual property, etc.) rest in the hands of the NPS and are the responsibility of the NPS. Researchers and the NPS should clarify any questions or assumptions before accepting the grant.

Due to several factors, all WNPA grants are for ONE (1) YEAR ONLY; however we welcome and will carefully consider applications for second or third years following a successful first year.

Best wishes and hopes for a successful project. Thank you from WNPA.

I have read and agree to abide by the research guidelines in effect at the time of this application.

Alejandra Vasco G. — August 27, 2019
Signature of Principal Investigator(s) — Date

Blanca Alvarez Stanley — 8/28/19
Signature of Park Superintendent — Date

RA RL — 8/28/19
Signature of Chief of Interpretation — Date

For WNPA use only

WNPA Research Committee Review: Action and Date:

Amount Granted: