JT SCIENCE

A newsletter to promote a better understanding of the natural, physical, & cultural environment in Joshua Tree National Park

JT SCIENCE

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FALL 2022

OUR MISSION
Joshua Tree National Park Association works in partnership with Joshua Tree National Park to help in its achievement of programming goals in education and interpretation, along with scientific and historical research and activities.

BEING A PART OF THE ADVENTURE
We operate four visitor centers and one park store that are often the first stop for visitors from around the world. We also offer a field institute, with classes taught by experts in natural sciences, cultural history, and the arts; and we raise funds via donations and our membership program. Visit a park store to learn about wildflower identification, birding, geology, stargazing, native plants and local history; pick up climbing and hiking guides; or sign up for a Desert Institute field class and make the park your classroom.

YOUR MEMBERSHIP MATTERS
As a member you support scientific research, the park's historical collections, youth programs, and assist in the preservation of our fragile desert. Email **membership@joshuatree.org** to find out more!

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Editor: Tasha La Doux
Design: Andrea Good
Oversight Committee:
Jane Rodgers, Jacqueline Guevara, Michael Vamstad, Jay Goodwin, and Anna Tegarden

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Questions?
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Welcome to JT Science

The mission of JT Science is to promote a better understanding of the natural, physical, and cultural environment in Joshua Tree National Park (JTNP). Each issue highlights scientific endeavors being conducted in JTNP and provides an opportunity for scientists, land managers, and the general public to know and appreciate this desert region.

In support of this mission, the Joshua Tree National Park Association not only provides funding to publish this science-based newsletter for the public, but it also provides annual funding for the **Graduate Student Research Grant Program**. The goals of the grant program are to support graduate student researchers conducting independent field studies in JTNP and more specifically to support high priority research needs that inform park management of socio-cultural, natural, and wilderness resources identified by park management.

Grant awardees will provide lectures and workshops for the Desert Institute, manuscripts for JT Science, public outreach materials for JTNP Interpretation staff, as well as final reports summarizing their findings. By offering up to $5,000 to each student, this program provides opportunities for the student to gain experience with grant and report writing skills, budget management, and most importantly, to demonstrate how their research can apply to land management issues.

To date, we have supported over 30 students, through which we have gained insight on such things as visitor awareness of tortoises, conservation implications for fringe-toed lizards and Joshua Trees, impacts to soil crust, inventories for bees and wasps, and discoveries of new-to-science species of poppies and green algae. The impact of this program and the contributions made by these scientists has been richly rewarding.

Enjoy the Newsletter!

Exploring the Microbial Diversity in Biological Soil Crusts at Joshua Tree National Park

Nuttapon Pombubpa[1], Tania Kurbessoian[1], Jason E. Stajich[1], Nicole Pietrasiak[2]

INTRODUCTION

Up to 40% of the global land surface consists of desert environments (also known as dryland regions, semi-arid and arid lands) (Belnap et al. 2016). Due to the sparse water availability, deserts are typically devoid of dense vegetation and the spaces between plants may appear barren at first glance. These plant interspaces, however, are often occupied by a microscopic world forming soil surface structures recognized as "biological soil crusts (or biocrusts)" (Belnap et al. 2001). These biocrusts form at the uppermost millimeters to centimeters of soil and are an aggregation of minerals and microorganisms. In dryland regions, biocrusts can cover up to 70% of the land surface area (Belnap et al. 2016), including the desert floor of Joshua Tree National Park (JTNP). Within biocrust, a huge variety of microorganisms can coexist and cooperate as a community. For example, biocrusts can be made up of bryophytes, lichens, eukaryotic algae, cyanobacteria, bacteria, and fungi, which all interact cooperatively to create a protective and productive community on the soil surface (*Figure 1*) (Belnap et al. 2001, Belnap et al. 2016). Forming a hot zone of biodiversity at the soil surface, biocrust can be thought of as a "living skin" on top of the soil, where each microbial member is an essential contributor to the ecology of desert environments (Belnap et al. 2016).

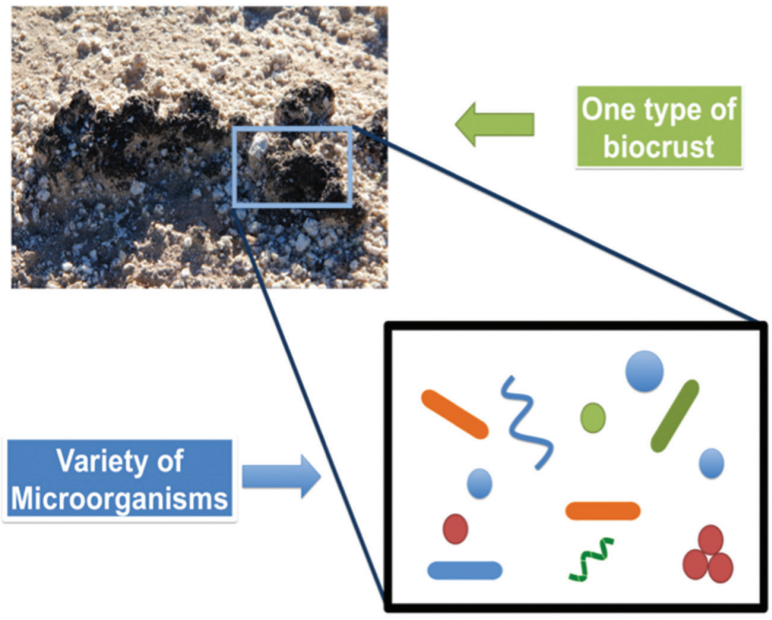

Figure 1. Biocrusts are like a "living skin" on top of arid lands soil. They consist of a living community of microorganisms such as bryophytes, lichens, eukaryotic algae, cyanobacteria, bacteria, and fungi. These complex communities contribute to healthy desert ecosystems by promoting nutrient cycling and reducing soil erosion.

Biocrusts play important roles in desert ecosystems.

For instance, they facilitate carbon and nitrogen cycling through biochemical processing performed by the microorganisms that inhabit the crusts. They prevent soil erosion, improve regeneration, and stabilization of vegetation, therefore creating a healthy and stable ecosystem (Belnap and Gardner 1993, Belnap et al. 2001, Belnap et al. 2016, Weber et al. 2016). To better understand these complex microbial communities, studies of microorganisms that inhabit and enable the functions of biocrusts are needed.

The goals of this article are threefold: 1) to provide a basic understanding of biocrusts and the microbial diversity found within them; 2) to summarize the findings from previous research conducted in JTNP by a team of scientists over the last two decades; and 3) to build upon that knowledge base by presenting additional data collected by the authors of this paper. More specifically, we developed protocols that further investigate the microbial components found in the biocrusts at JTNP such as algae, bacteria, and especially fungi, as this was one of the major knowledge gaps regarding microbial diversity in the park.

BIOCRUSTS AT JTNP

Within the hot Mojave and Sonoran desert biomes, including the area of JTNP, biocrusts can be challenging for the untrained eye to notice. These cryptic communities may appear to be bare unconsolidated soil, as they are generally difficult to identify from a distance. However, if we take a minute and look closer at the ground, biocrusts are frequently encountered, especially in the park. A hand lens or magnifying glass can reveal the telltale microscopic structures that are distinctive for many types of biocrusts (*Figure 2*). First, one will notice the consolidated nature of the crust aggregate that will hold the soil together cohesively as shown in Figure 2A. Biocrusts are

[1]Department of Microbiology & Plant Pathology and Institute of Integrative Genome Biology, University of California, Riverside, Riverside, California 92521
[2]Plant and Environmental Sciences Department, New Mexico State University, Las Cruces, New Mexico 88003

also characterized by dangling filamentous components that can be seen among the subsurface of biocrust (*Figure 2A*). Those "danglies" represent biological filaments of cyanobacteria or fungi that can appear like micro-roots; they are often seen with a few soil particles adhering to them.

Biocrusts can be classified based on the dominant photosynthetic microorganisms present in the crust, which are the components that can perform photosynthesis just like plants fixing carbon dioxide from the atmosphere and producing oxygen. Photosynthetic microorganisms include cyanobacteria, other algae, lichens, liverworts or mosses (Pietrasiak et al. 2013). Light algal crusts (LAC) are the most common biocrust types in hot desert ecosystems, including the Mojave and Colorado Deserts at JTNP, and are dominated by cyanobacteria and other algae that quickly turn green in the presence of water (*Figure 3*; Pietrasiak et al. 2011a,b). Another type of biocrust is referred to as lichen crusts (*Figure 2B*), these consist of fungal and algal/cyanobacterial components. A lichen crust may have multiple species of lichen-forming or lichen associated fungi co-occurring within one crust structure (*Figure 2B and 2C*). Lichen crusts can also be found throughout the desert Southwest, but have a more patchy distribution, particularly in JTNP (Pietrasiak et al. 2013). Two primary types of lichen biocrusts are found in JTNP: 1) a fungus with a cyanobacterial partner (Cyano-Lichen Crusts; CLC) or 2) a fungus with a green algal partner (Green Algal Lichen Crusts; GLC). Finally, moss dominated crusts are much more limited on the landscape, especially in JTNP. They occur preferentially in moist microhabitats, such as under the protective rock ledge of a boulder or along the shady banks of a riparian corridor; anywhere that moisture drips or runs off hard surfaces and can be trapped for longer periods (Pietrasiak et al. 2011a,b).

ALGAL AND CYANOBACTERIAL DIVERSITY IN BIOCRUSTS

Most biocrust communities have a basic architecture that includes two main structural components: 1) primary producers that perform photosynthesis, and therefore produce carbohydrates and oxygen; and 2) associated heterotrophic consumers such as fungi and microscopic animals, which then live off the carbon-rich products that the primary producers generate. In JTNP, the most common primary producers in biocrust are cyanobacteria and other algae (*Figure 3*). These algae are making up the majority of the photosynthetic component in the LAC found throughout the park (Flechtner et al. 2013, Pietrasiak et al. 2011a,b, 2013). Under certain conditions microalgae may partner with fungi or mosses to create more complex biocrust communities such as lichen or moss crusts.

Algae are very interesting organisms. The term "algae" refers to a very diverse group of organisms spanning across the entire tree of life, including members from the Prokaryotes (Kingdom: Monera) and Eukaryotes (Kingdom: Protista). While "algae" does not represent a technical taxonomic term and there is no agreed upon definition of what most people think of as "algae," it generally refers to organisms that can photosynthesize, but aren't plants. Examples of "algae" include groups of organisms like diatoms, green algae, yellow algae, and blue green algae also known as cyanobacteria (*Figure 4*). The latter are the only Prokaryotes (Bacteria) that photosynthesize like plants. Each of these groups of algae represents their own lineage in the tree of life and they differ in cell organization and structure, pigmentation, and ecosystem roles. What unites the groups is the presence of the green pigment chlorophyll in their cells which enables the ability to perform photosynthesis while producing oxygen, and the lack of the reproductive organs and vegetative tissues found in multicellular plants (Graham et al. 2009).

Cyanobacteria are one group of algae that are extremely abundant in desert soils. They represent the majority of photosynthetically active biomass in biocrusts. These bacteria play essential roles in the desert environment. For example, cyanobacteria are adept at excreting sugary polymeric compounds around their cells that are very sticky and function as fibrous glue holding soil grains in place (Belnap and Gardner 1993). This sticky glue is very important in desert interspaces, where plants are lacking and only a few roots are available to stabilize the seemingly bare soil.

The aggregation of soil enabled by the cyanobacteria helps reduce erosion.

The stickiness of these cyanobacteria also traps dust, which often contains essential micronutrients that can be incorporated into to the soil (Belnap and Gardner 1993, Hu et al. 2002). Another important function performed by cyanobacteria is called nitrogen fixation, which brings substantial amounts of nitrogen into the nutrient poor desert soil (Belnap 2002,

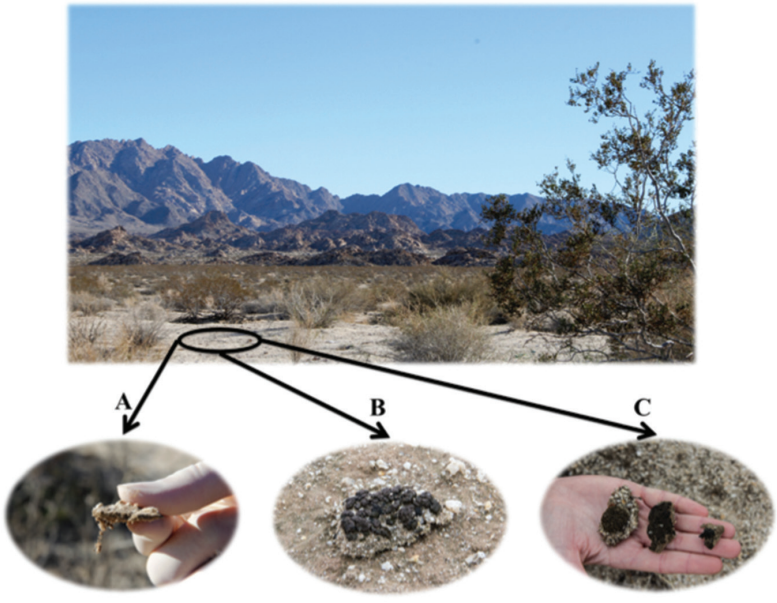

Figure 2. Plant interspaces in JTNP contain a variety of biocrusts but are dominated by light algal crusts and lichen crusts, both of which will have characteristic filaments in the subsurface. The filaments look like tiny roots dangling from under the crust (A). Light algal crusts (A) and lichen crusts (B, C) are commonly found in JTNP. The majority of lichen crusts in JTNP are composed of two different fungal species: *Collema coccophorum* (B), which is a cyano-lichen crust and *Clavascidium lacinulatum* (C), which is a green algal lichen crust.

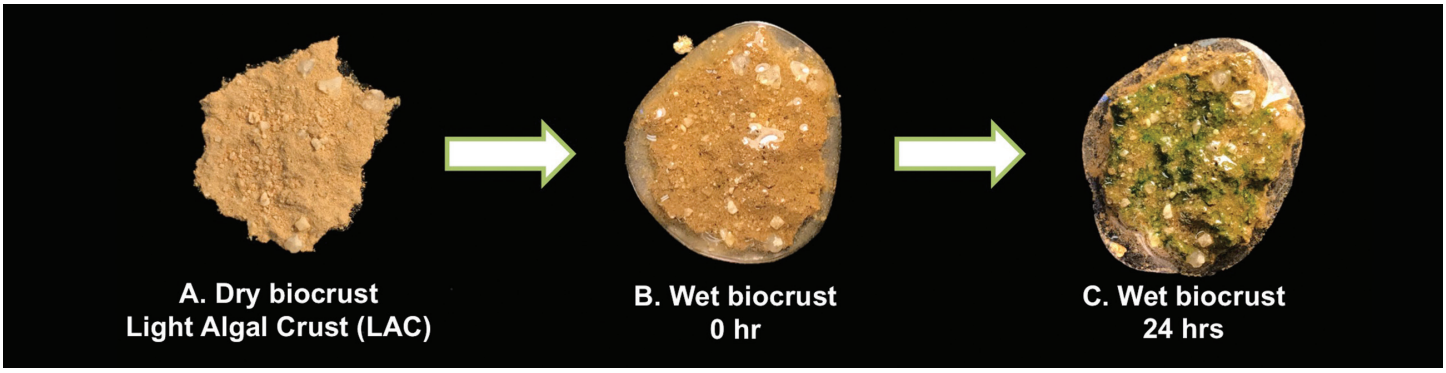

Figure 3. Light algal crusts (LAC) are the most common biocrust types in JTNP. They are dominated by cyanobacteria and other algae that quickly turn green once water is added; within 24 hours the algae and cyanobacteria become active (*far right*), but without the water are invisible to the naked eye (*far left*).

Pietrasiak et al. 2013). Through biochemical reactions performed by cyanobacteria, inert atmospheric nitrogen is incorporated into specialized cell compartments or cell types, where it is assimilated into organic molecules through a process called biological nitrogen fixation. Through these actions cyanobacteria enrich the desert topsoil with essential nutrients and help create a thin, fertile, and biologically active skin of the desert that we call biocrust.

> Previous research has laid the foundation for the importance of cyanobacteria in desert soils, but we only have scratched the surface in understanding the breadth of species diversity.

Prior studies on the diversity of cyanobacteria in JTNP have discovered many new species (Řeháková et al. 2007, Mühlsteinová et al. 2014, Osorio-Santos et al. 2014, Pietrasiak et al. 2014, Bohunická et al. 2015). Within the last 11 years a team of phycologists under the supervision of Jeffrey Johansen (John Carroll University, OH) and Nicole Pietrasiak (New Mexico State University, NM) described and published two new cyanobacterial genera (*Mojavia*, *Roholtiella*) and 5 new species (*Mojavia pulchra*, *Roholtiella mohaviensis*, *Oculatella coburnii*, *Trichocoleus desertorum*, *Symplocastrum flechtnerae*) from JTNP soils (*Figure 4*) using culture dependent methods. Additional assessment of the biocrusts of JTNP, among other desert systems, will certainly be needed to describe all the cyanobacterial members present in desert systems. The application of culture independent assessment using DNA-based description of biodiversity is likely to uncover additional genera and certainly new species of cyanobacteria in JTNP biocrusts. This discovery of taxa then opens the door to unraveling the various functions contributed to the ecosystem by these microorganisms.

Very little is known about the biodiversity of eukaryotic algae, including diatoms, green algae, and yellow algae in biocrusts, not to mention the ecosystem roles of these organisms. The few studies that have been done on eukaryotic algae in biocrust, indicate that the contribution of these groups of algae to biomass is rather low but their diversity is much greater than in cyanobacteria (Büdel et al. 2016). To date, the only published study investigating the eukaryotic algal diversity of JTNP used a culture dependent approach (Flechtner et al. 2013). In this study, the authors isolated living algal strains by placing soil on culture medium and observing and isolating the organisms which grew. The authors intensively studied 95 algal isolates from 18 locations within JTNP using morphological observations and DNA sequence information from these isolates. This study revealed 28 unique lineages in the families of Chlorophyceae (17), Trebouxiophyceae (7), Xanthophyceae (3) and Eustigmatophyceae (1). Most did not match any described algal species in published taxonomic keys, highlighting the poor knowledge we currently have about these organisms, but more importantly, their great potential for discovery of new species (unknown to science). In the same year, Fučíková et al. (2013) included selected JTNP algal isolates in an extensive revision of the green algal genus *Bracteacoccus* and newly described 5 *Bracteacoccus* species from dryland environments. This monograph reports several records of *Bracteacoccus* species from JTNP including: four records of the established *Bracteacoccus pseudominor* found at three locations within the Colorado Desert portion of the park, one record of the newly described *Bracteacoccus deserticola* from the Pinto Basin, one record of the newly described *Bracteacoccus glacialis* found in the Wonderland of Rocks, and nine records of the newly described *Bracteacoccus occidentalis* from five locations within JTNP. In 2014, a new genus and species of green algae, *Rotundella rotunda*, was discovered on the alluvial fans near Eagle Mountain and named by Fučíková et al. (2014). Many more discoveries of enigmatic algae from biocrusts can be anticipated in the future.

FUNGAL DIVERSITY IN BIOCRUSTS

Studies of biocrusts have primarily focused on algal and bacterial communities as part of understanding the microbiological composition of crusts classified by morphology. Exploration of the fungi in the biocrust systems is still an emerging research area and is needed to better understand the functional roles they play in the morphological properties and nutrient cycling activities of soil surfaces (Maier et al. 2016, Maier et al. 2018). Currently there are limited published reports on fungal diversity in biocrusts and no previous fungal study in biocrust has been done at JTNP. Most studies were conducted with culture dependent approaches, which underestimate microbial diversity. One study examined fungi in crusts using denaturing gradient gel electrophoresis (DGGE) (Bates and Garcia-Pichel 2009), which is used as a fingerprinting method to estimate environmental microbial diversity. DGGE has only limited utility as it does not allow

direct identification of species, only a pattern of the sizes of DNA bands can be matched between sample sites to look for similarities. To overcome this, DNA sequence based approaches are applied to estimate diversity and identify fungal species directly from environmental samples of biocrusts (Steven et al. 2014, Steven et al. 2015, Maier et al. 2018).

MECHANISMS FOR DISCOVERING MICROORGANISMS

Technology advances have enabled and simplified sampling methods for DNA sequencing that allow us to explore microbial diversity found in nature, very little research has focused on conducting inventory type studies of biocrust. There are two ways to assess the biological diversity found within a biocrust. The first approach, called "culture dependent," results from using cultured strains of organisms that were present in the soil sample. This is achieved by plating biocrust soils onto semi-solid microbiological media in order to further isolate life strains and eventually obtain individual isolates of each taxonomic entity. The media consists of nutrients for the organisms to grow and agar to provide a surface to visualize and observe morphologies of the isolates. The nutrient content of the media can be adjusted to favor the growth of one microorganism over another. Specifically, a serial dilution is used to isolate the microbes from the soil (*Figure 5*). This method involves making a soil slurry by suspending the soil sample with increasingly higher proportions of water or media in order to get a low starting concentration of cells and reduce the number of species growing on the Petri dishes to a manageable count. In order to isolate single organisms, 100 uL of biocrust-water suspensions are spread on a variety of media and the growth of microbial colonies is scored over the course of a few days. An alternative method used with the serial dilutions is called pour plating, where the crust-water solution can be poured to differentiate among microorganisms based on their oxygen dependence. The top layer obviously favors oxygen-loving microbes, whereas the bottom layer, which is submerged in water, selects for oxygen sensitive strains (Clark 1967). Once axenic cultures have been obtained, DNA sequence information can be generated directly from isolated microorganisms from this culture dependent method.

Although microbial diversity in biocrust can be explored to some extent using a culture dependent method, basic growth media may

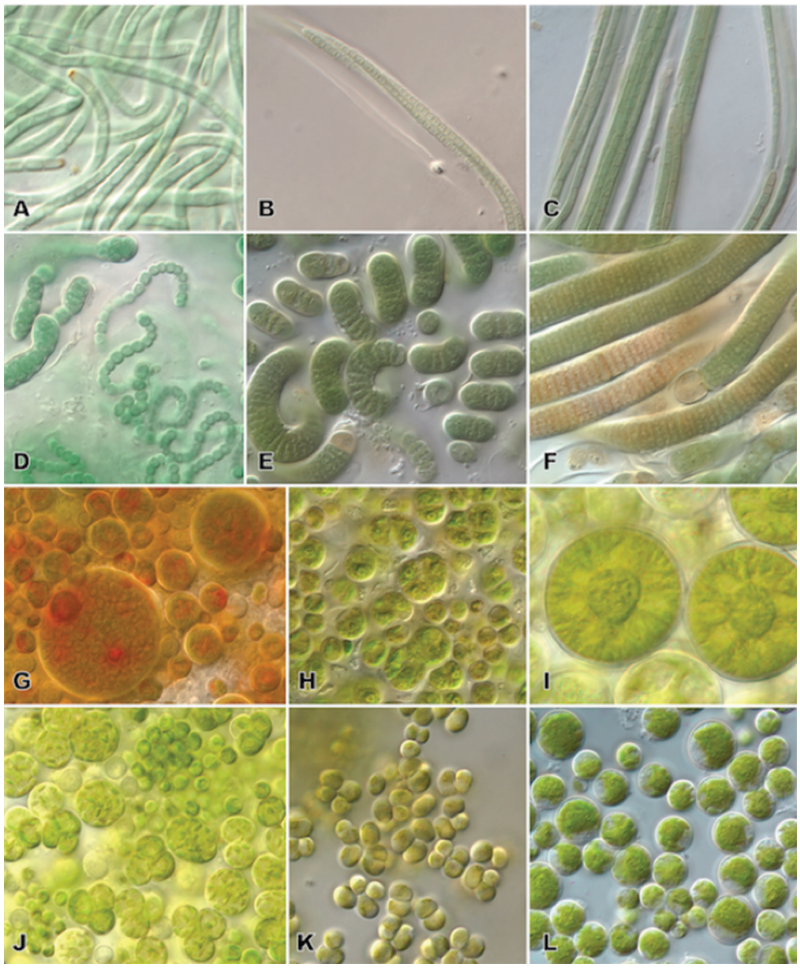

Figure 4. Cyanobacterial (A-F) and eukaryotic algal (G-L) diversity of cultures isolated from JTNP biocrusts. Photographs show: A) *Oculatella coburnii* (Synechococcales clade), B) *Trichocoleus desertorum* (Synechococcales clade), C) *Symplocastrum flechtnerae* (Oscillatoriales clade), D) *Nostoc* sp. (Nostocales clade), E) *Spirirestis rafaelensis* (Nostocales clade), F) *Hassallia* sp. (Nostocales clade), G) *Bracteacoccus* sp., H) *Chlorosarcinopsis* sp., I) cf *Actinochloris* sp., J) *Myrmecia* sp., K) *Stichococcus* sp., and L) *Parietochloris* sp.

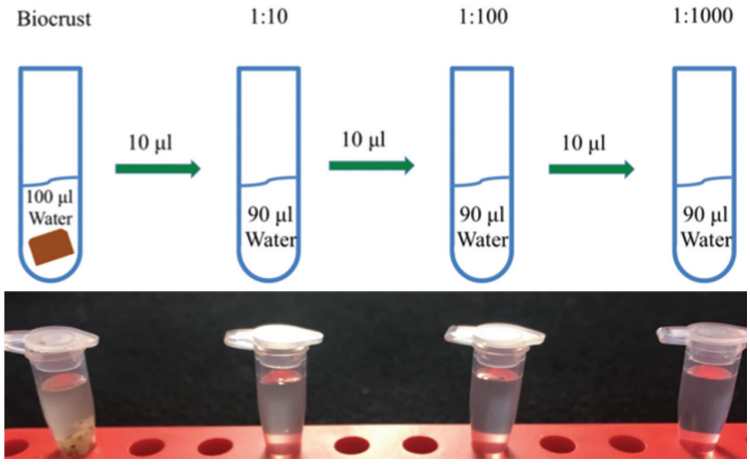

Figure 5. Serial dilution method involves a dilution series of biocrust water suspensions are prepared by adding biocrust to 100 ul of sterile water. To dilute the biocrust water suspension, 10 ul of original solution is transferred and combined with 90 ul of sterile water (1:10 dilution). Additional dilutions are created by repeating this step: 1:100 and 1:1000, accordingly.

not provide suitable conditions for the growth of the majority of microorganisms. Direct DNA sequencing from soil samples can be used to capture greater microbial diversity and represent a "culture independent" method for assessing biocrust microbial communities. These molecular and genomic technologies are contributing tremendously to achieve a better understanding of the microbial diversity and composition of many environments from the human body to the open ocean. Polymerase Chain Reaction (PCR) amplification and Next Generation Sequencing (NGS) are used to assay regions of the genome which are found in all organisms. One of these, the 16S ribosomal RNA (rRNA) gene is used to survey Bacteria and Archaea while the Internal Transcribed Spacer (ITS) regions can be used to identify fungi present in a sample. These molecular markers have proven successful in recovering a broad range of bacterial and fungal diversity in soil microorganisms (Caporaso et al. 2012, Smith and Peay 2014). Broad sampling and fine scale analysis with NGS can be used to effectively compare biodiversity among biocrusts from different locations or classified as different morphological types. These studies can help identify the core taxonomic composition of biocrusts and indicate key organisms that may play important roles in the formation and ecological functions of biocrust.

CURRENT RESEARCH RESULTS

We have used NGS sequencing (amplicon sequencing) of the 16S gene on collections we made of LAC and CLC biocrusts in JTNP to test whether there are differences in species that comprise these crust types. DNA samples from both crust samples were extracted, amplified with PCR targeting the 16S gene marker and sequenced using Illumina MiSeq. This sequencing captured a broad range of bacterial diversity and using bioinformatics analyses we focused first on the observed diversity of Cyanobacteria. Comparing these sequences with a collection of previously generated sequences from Cyanobacteria cultures compiled by Drs. Johansen and Pietrasiak, we constructed a phylogenetic tree representing the diversity of the JTNP cyanobacteria (*Figure 6*). Our analysis found that Cyanobacteria from nearly all known described groups (major clades) are present in the biocrust samples. Our work also demonstrates that both culture dependent and culture independent methods can equally recover the broad phylogenetic diversity found in biocrusts for Cyanobacteria

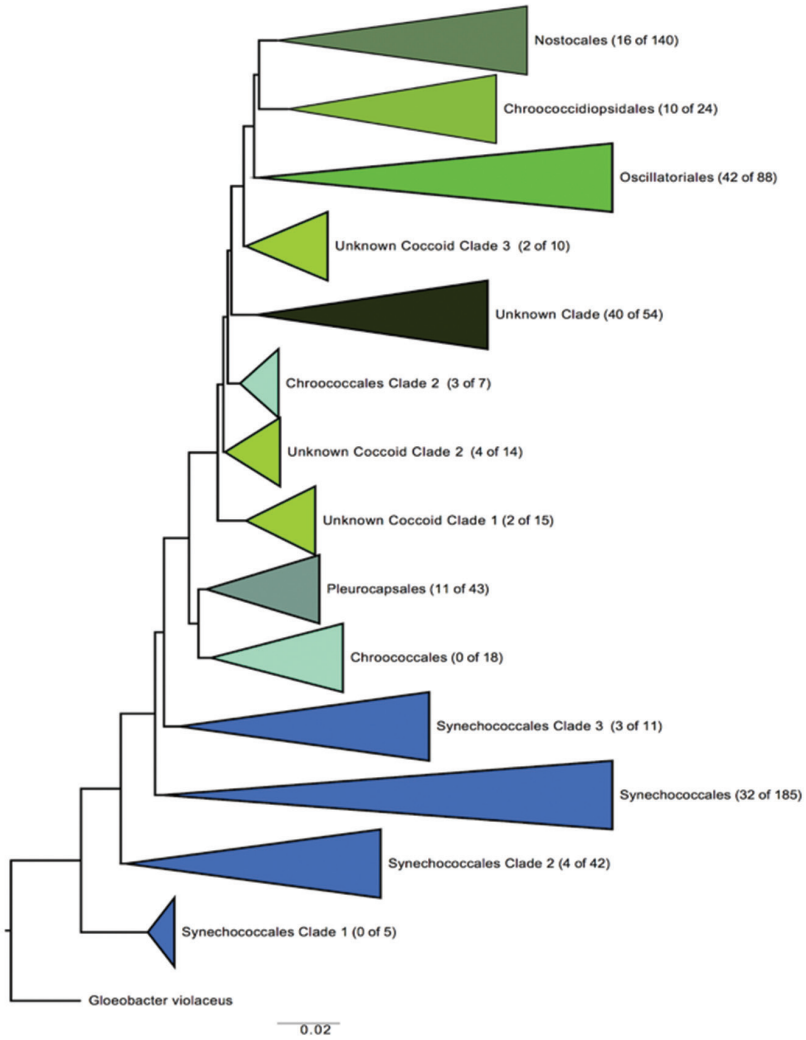

Figure 6. Phylogenetic tree of Cyanobacteria with all major clades shown. The number of lineages found in JTNP biocrusts out of the total number of known lineages is shown in parentheses to the right of the clade. These data are based on amplicon sequences and they illustrate that members of nearly all (12 of 14) of the major terrestrial cyanobacteria clades have been recovered from biocrusts in JTNP.

(*Figure 6*). Further work on additional groups of Bacteria will help determine the extent of novel taxa which can be observed from the culture independent methods.

We have also assessed the composition of fungal communities using amplicon sequencing of biocrust DNA. The ITS ribosomal gene region was amplified with PCR to inventory the fungi present in the biocrust samples using primers (Smith and Peay 2014) that are also being used to identify fungi in the Earth Microbiome Project (Walters et al. 2016). The PCR products are sequenced on an Illumina MiSeq to produce sequence fragments ~300 bp long. The DNA sequence fragments are compared to each other to collect them into groups which all represent sequences that are mutually similar based on the percentage of DNA bases that match. These clusters of sequences represent a guess of a fungal species or strain that is present in the biocrust sample. To determine what might be the name of this fungus, the sequence is matched against a database of known sequences. A curated database called UNITE is one of the best references for fungi and contains an enormous library of fungal ITS sequences and corresponding species names (Nilsson et al. 2015).

The ITS rDNA marker is sometimes referred to as a "barcode" as the sequence is often different between closely related species so that each species can have its own nearly unique signature. However, there can still be

challenges with the marker as it still may be invariant among some groups of species. It is also difficult to use ITS sequences when they do not match any known Fungi, as it can be difficult to guess if it is a new species or species group not previously seen before. As we have seen in this and many other studies of fungi from the environment, there is vast, unsampled biodiversity that is only now being revealed through amplicon sequencing which leads to many sequenced ITS sequences assigned as an Unknown Fungus.

Analysis of our samples identified that biocrusts of different morphological classifications (e.g. LAC and CLC) are comprised of varied fungal taxa that differ at the genera and family level but are fairly consistent when comparing the presence of major phyla. The observed groups that dominate the crusts include Dothideomycetes, Eurotiomycetes, and Sordariomycetes within the Ascomycota and Agaricomycetes and Tremellomycetes from the Basidiomycota (*Figure 7*). These results are similar to previously reported types of fungi found in biocrusts using other methods (Bates and Garcia-Pichel 2009 and Steven et al. 2015). Within the light algal crust (LAC), the three most abundant Ascomycota genera were *Alternaria*, *Phoma*, and *Elasticomyces*; whereas, the top three fungal genera from Basidiomycota were *Coprinellus*, *Cryptococcus*, and *Clitopilus*. While mushrooms do inhabit some arid regions, we did not observe any fruiting in the regions where we sampled and were surprised to see the high abundance of some of these basidiomycetes. It may be that taxa are from spores that have blown in and are dormant awaiting a rain event.

Our efforts have provided a high-resolution look at the fungal taxa that can be present in biocrusts and one arid region.

The species reported from our study are only examples of some of the most abundant types of fungi living in biocrusts. We expect that many more fungi contribute to the biocrust community (*Figure 8*). Identifying the less abundant species will be undertaken with additional sequencing and robust analyses to confirm the presence of these organisms and compare their abundances across biocrust environments.

Traditional culture dependent methods to isolate fungi generally use a nutrient rich media and are kept at room temperature. Because JTNP represents an extremely dry and cyclically hot environment, we experimented to find optimal growth conditions that might favor the more extremophilic species. We attempted growing the fungi in a range of temperatures, salt concentrations, and pH; we were successful in isolating fungi in the phyla Ascomycota and Basidiomycota with a range of growth rates (*Figure 9*). Examples of the fungal species we have brought into culture from biocrusts are *Phoma* sp., *Didymella* sp., *Ustilaginales* sp., *Didymella* sp., *Aspergillus* sp., *Alternaria* sp., and *Knufia* sp. Several of these fungal species match high abundance fungal gene markers that we identified from our culture independent amplicon sequencing of DNA from biocrust collected in the field. Using these starting cultures, we can test physiology, enzyme and biochemical properties, and interactions with algae and bacteria to better understand the roles these fungi play in the ecosystems.

CONCLUSION

We are only in the beginning stages of exploring the microbial diversity of biocrusts at JTNP. Based on the limited research that has been done in the park to date, we know that these microbial communities are very diverse and likely harbor many new species to science across all microbial lineages. In addition to knowing very little about the biological diversity found in these communities, we know even less about the specific adaptations of these microbes or the synergistic roles they play to contribute to ecosystem functions. Culture dependent and independent methods can both yield valuable information in the quest for more information regarding microbial diversity and the basic biology/ecology of these microorganisms. Culture dependent methods enable detailed studies of the biology of cyanobacteria, eukaryotic algae, and fungi found in these arid lands. However, culture dependent approaches are limited to the subset of organisms that can grow on culture media, therefore research that only focuses on these may miss a sizeable fraction of the actual microorganisms living in the soil. New sequencing technologies allow culture independent evaluation of microbial community diversity. Using NGS, we can produce a broader sampling of the microbial community diversity than is possible in the culture dependent approach. For the first

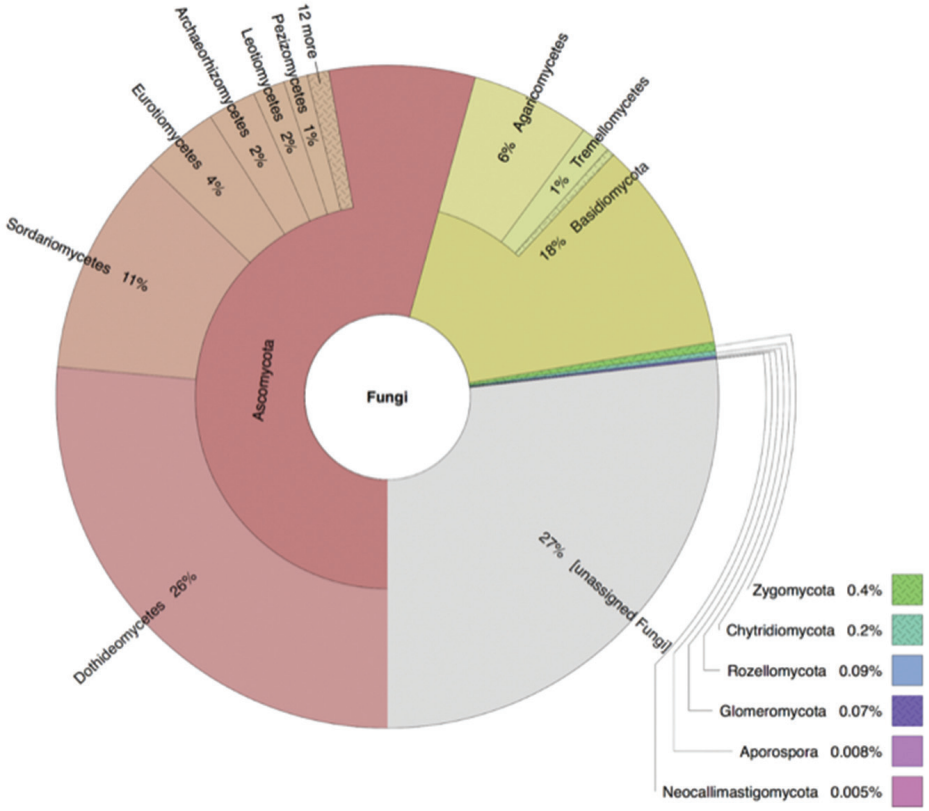

Figure 7. *Fungal diversity in light algal crust from Joshua Tree National Park. Using Krona for visualization (Ondov et al. 2011), main fungal phyla are Ascomycota and Basidiomycota.*

time, additional abundant, but unculturable species, can be documented as part of the biocrust microbial community. Before we can hope to understand the function and processes that dominate these biocrusts, we must first document and identify the diversity present. Only then we can begin to quantify or explore how these microorganisms might contribute to ecosystem functions and/or how they respond to different environmental conditions. Finally, a better understanding of the microbial community can be used to develop management and monitoring strategies to assess the health of desert ecosystems and therefore to develop bioremediation strategies, such as the addition of missing community members. Overall, understanding the interactions and diversity of microbes that support biocrust formation and persistence are key aspects of desert land conservation.

FUTURE RESEARCH DIRECTIONS

Our research shows that biocrusts are made up of very complex and unique microbial communities and we have just started to reveal the secret of these communities. Although eukaryotic algae, cyanobacteria, bacteria, and fungi have been found inhabiting biocrusts, their interactions and ecosystem functions are still under investigations.

> As we learn more about these microorganisms, important species will be revealed along with their functions.

However, matching cooperating microbes and their relationships will be very challenging at this early stage since a fraction of microorganisms cannot be isolated using current culture dependent methods. By exploring microbial diversity while developing both culture dependent and independent methods, microbial interactions experiments can be conducted in the near future and will show how these microorganisms help each other to build the biocrust community. Lastly, focusing more on filamentous and mycorrhizal fungi may help us evaluate the connections between biocrust microbial communities and vascular plants in drylands. This has already been recognized as the "fungal loop hypothesis" (Collins et al. 2008).

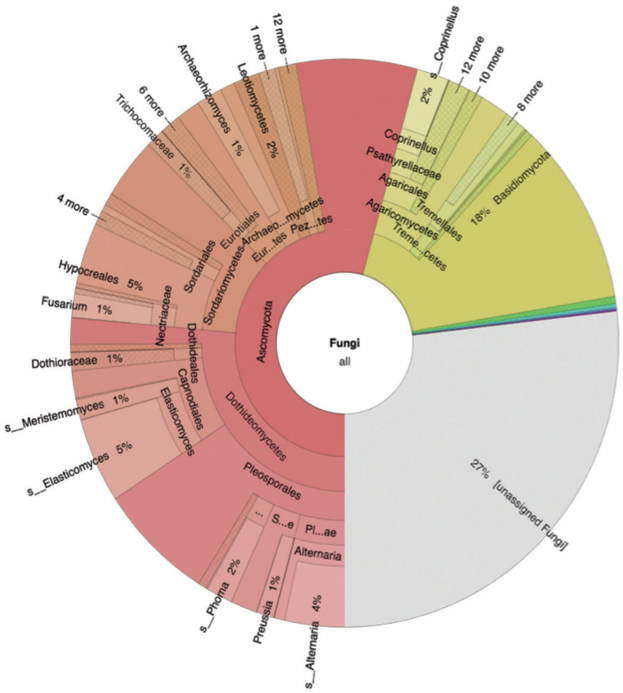

Figure 8. Light algal crust (LAC) from Joshua Tree National Park contains a complex fungal composition. A variety of fungal species are represented with several dominant species from Basidiomycota (yellow) and Ascomycota (red).

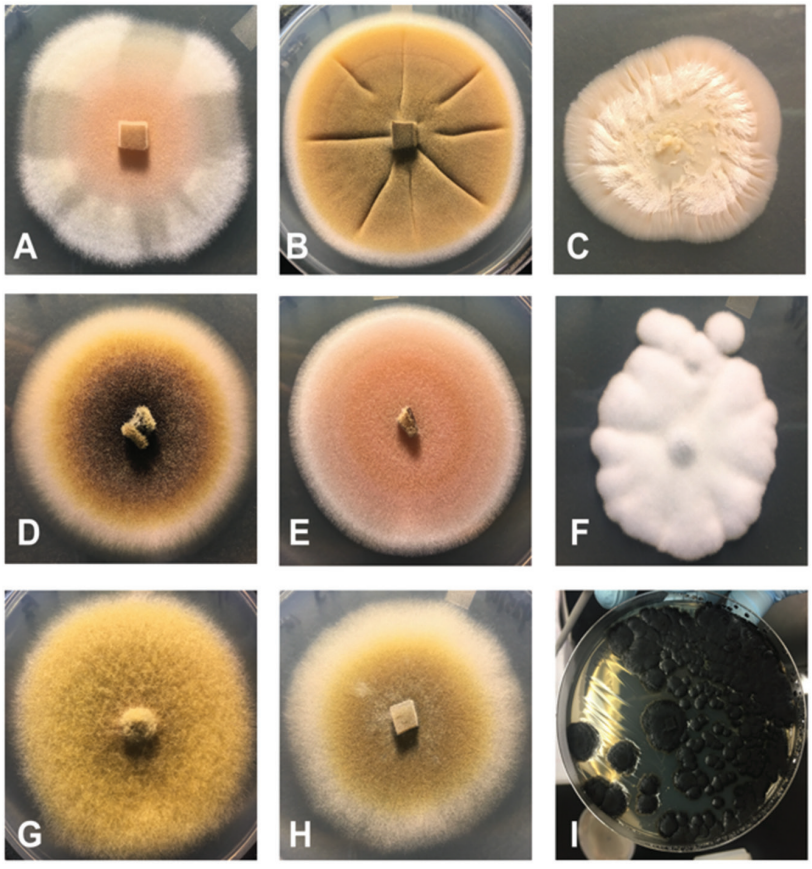

Figure 9. Depiction of a small sample of the variety of fungi isolated from the JTNP biocrusts by using a culture dependent method. The genera depicted include: A) Phoma sp., B) Didymella sp., C) Acremonium sp., D) Didymella sp., E) Aspergillus sp., F) Aspergillus sp., G) Alternaria sp., H) Phoma sp., I) Knufia sp..

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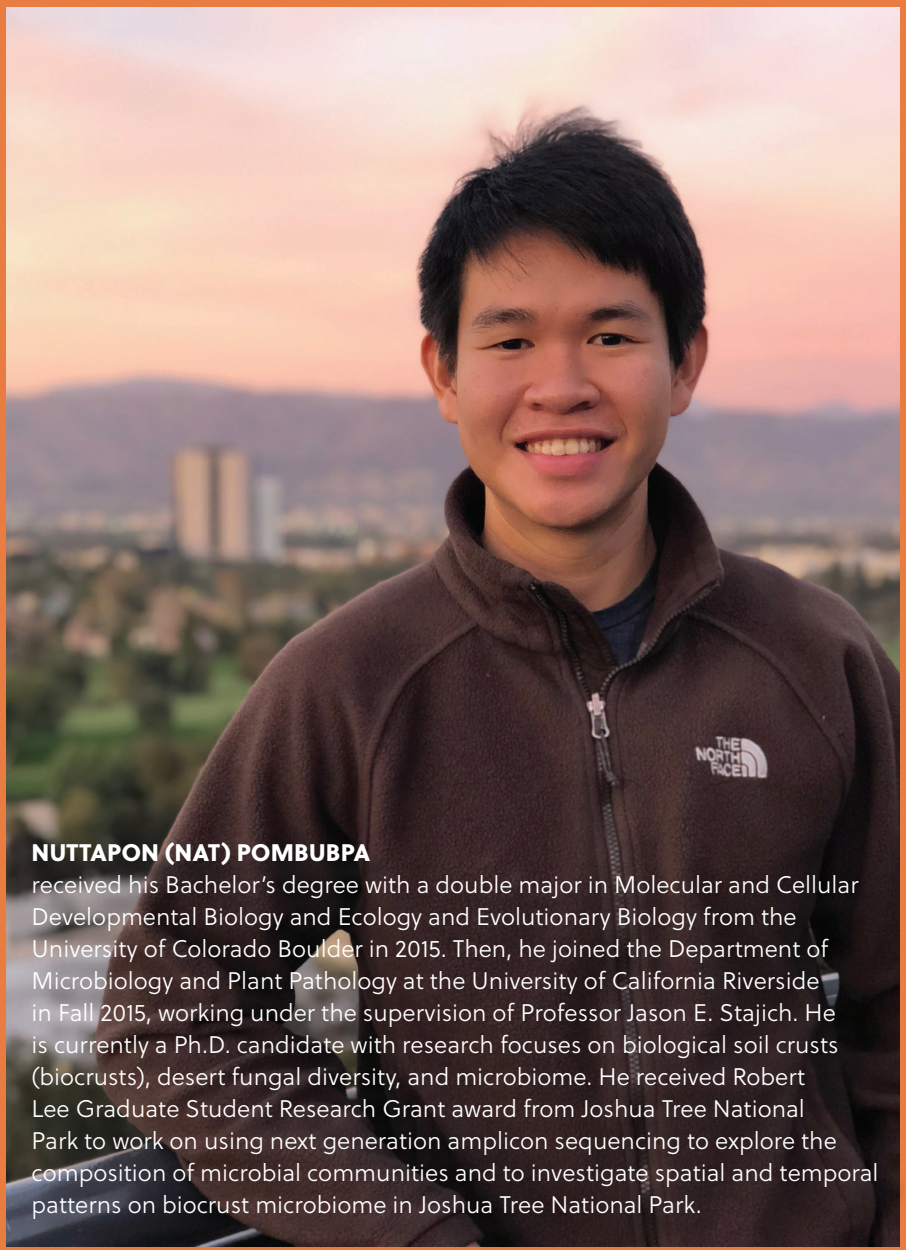

NUTTAPON (NAT) POMBUBPA
received his Bachelor's degree with a double major in Molecular and Cellular Developmental Biology and Ecology and Evolutionary Biology from the University of Colorado Boulder in 2015. Then, he joined the Department of Microbiology and Plant Pathology at the University of California Riverside in Fall 2015, working under the supervision of Professor Jason E. Stajich. He is currently a Ph.D. candidate with research focuses on biological soil crusts (biocrusts), desert fungal diversity, and microbiome. He received Robert Lee Graduate Student Research Grant award from Joshua Tree National Park to work on using next generation amplicon sequencing to explore the composition of microbial communities and to investigate spatial and temporal patterns on biocrust microbiome in Joshua Tree National Park.

The Advantages of Having Green Stems in Arid Environments

Eleinis Ávila-Lovera[1] and Louis S. Santiago[1]

Figure 1. Plants with green stems are categorized by one of three types of stem photosynthesis syndromes. *1*) Cactoids, like *Opuntia basillaris* (above) take up carbon dioxide at night; *2*) Sarcocaulescent plants, e.g. *Dudelya saxosa* (above) recycle carbon; *3*) Retamoids, like *Ephedra californica* (above), have stomata in the stem epidermis allowing for gas exchange with the atmosphere.

Photos by Tasha La Doux

INTRODUCTION

All plants need carbon to grow, and most plants get carbon when they take up carbon dioxide through photosynthesis with their leaves. However, in arid ecosystems, green-stemmed plants, which are able to take up carbon dioxide through photosynthesis with their stems, are common. The main limitation to photosynthesis in arid conditions is that when plants open their stomata, the tiny pores through which they absorb carbon dioxide, they also lose water (Lambers et al. 2008). Water is extremely valuable in arid ecosystems; it is the main resource that limits plant productivity on land (Chaves and Pereira 1992; Chaves et al. 2002). Reducing water loss during photosynthesis is a major advantage that green-stemmed plants have over species with normal bark (Ehleringer et al. 1987; Osmond et al. 1987; Nilsen and Sharifi 1997). Another advantage is that they can still absorb carbon dioxide for growth even when they are leafless or during dry seasons (Smith and Osmond 1987; Nilsen and Bao 1990; Tinoco-Ojanguren 2008). Such advantages might also be important during extreme drought because photosynthesis is often limited during water deficit, but can be critical for plant survival. Therefore, evaluating the costs and benefits of having green stems is important to determine how stem photosynthesis alters the balance of carbon gain and water loss during drought. Understanding these cost-benefits can also aid in predicting which species may survive future extreme droughts.

Plants with green stems are categorized by one of three types of stem photosynthesis syndromes (*Figure 1*). Retamoids include leafless or almost leafless woody plants that have stomata in the stem epidermis allowing for gas exchange with the atmosphere (Schaedle 1975). The other two groups of plants that photosynthesize with stems are sarcocaulescent and cactoid, with fleshy and succulent stems, respectively. These differ from the retamoids in that sarcocaulescent plants usually recycle carbon whereas cactoids take up carbon dioxide at night. Our focus in this study is on retamoid plants that have green photosynthetic stems (*see species list in Table 1*), and we compared them with non-green-stemmed plants. It has been noted that plants bearing green stems belong to at least 26 unrelated plant families (Nilsen 1995; Gibson 1996), which suggest that the syndrome evolved independently in different taxa, likely due as a response to life in arid environments.

Climate change can have strong effects on plant distribution and vegetation processes by changing composition and structure of the plant community. How plants die during drought can be a consequence of carbon starvation or hydraulic failure or both (McDowell et al. 2008; McDowell 2011). Recent studies have shown an interdependency of these mechanisms (McDowell 2011; McDowell et al. 2011, 2013; Sevanto et al. 2014), making it even more difficult to try to identify the cause. However, because each species responds differently to drought based on its

[1]Department of Botany and Plant Sciences, University of California, Riverside, CA 92521

physiological strategies, we are limited in our ability to predict how contrasting species will respond. California's recent severe drought highlights the need to understand the physiology of desert species, specifically related to traits involved in water use and conservation.

Green stems are considered part of a key suite of drought-survival traits (Pivovaroff et al. 2016; Santiago et al. 2016).

> The ability of stems to photosynthesize after leaf loss may promote plant carbon balance and prolong survival during drought.

Besides this possible advantage of green stems, little is known about their water cost compared to non-green stems. Most green-stemmed species keep their young epidermis, which is less resistant to water loss than non-green bark tissue. In other words, during drought a green-stemmed plant might continue to lose water through their outer layer even if all stomata are closed, whereas, non-green stemmed species develop a waterproof layer in their stem that limits their water loss. Our main objective was to compare carbon dioxide uptake and loss of water vapor between leaves and stems of green- and non-green-stemmed species. Normally, non-green stems only lose carbon dioxide to the atmosphere through respiration, whereas green stems can either take up carbon dioxide from the atmosphere in a process known as stem net photosynthesis (SNP) or re-assimilate internally respired carbon dioxide in a process known as stem recycling photosynthesis (SRP) (Nilsen 1995; Ávila et al. 2014). We hypothesized that plants with green stems lose more water than non-green stemmed plants. However, the advantage of having an extra carbon income could offset this cost.

MATERIALS AND METHODS

Our study was performed in a desert wash in the Mojave Desert at Joshua Tree National Park (34° 03' 50.5" N, 116° 03' 16.3" W), near the California Riding and Hiking Trail by the north entrance of the park. The study site has a mean annual air temperature (MAT) of 18.6 °C and mean annual precipitation (MAP) of 119.1 mm. However, 2016 MAT was 19.4°C whereas precipitation was 82.3 mm. The community is dominated by creosote bush (*Larrea tridentata*) (*Figure 1*) (Ávila-Lovera et al. 2019). We selected species with and without visually green stems (*Table 1, Figure 2*). Plant water status was measured as water potential with a pressure chamber, leaf and stem gas exchange of carbon dioxide was measured using an infrared gas analyzer, and water vapor loss through the epidermis was measured using bench dehydration. These traits were measured every six weeks from spring 2016 (February) to spring 2017 (March), spanning two wet seasons and one dry season.. Traits recorded for leaves and green stems included photosynthetic rate, stomatal conductance, and water-use efficiency; for the non-green stems we measured respiration rate and non-stomatal conductance. We also measured carbon and oxygen isotopic composition in photosynthetic tissues of leaves and stems at the end of both wet and dry seasons. The carbon isotopic composition of photosynthetic tissues is related to long-term water-use efficiency, with high values indicating higher long-term water-use efficiency, whereas oxygen isotopic composition is related to how dry the air was during the growing season, where higher values indicate drier air.

RESULTS AND DISCUSSION

Plants with green stems relied on their stem as the sole organ for carbon assimilation for most of the study period (Ávila-Lovera et al. 2019). All woody species with green stems had small

Figure 2. Left: View of the field site, a desert wash at Joshua Tree National Park near the North Entrance. Michael Torres, Eleinis Ávila-Lovera and Antonio Zerpa collecting data on one of the green-stemmed species, *Senna armata* (Fabaceae). In the background, many individuals of *Larrea tridentata* (Zygophyllaceae), a non-green stemmed species. Right: *Menodora spinescens* var. *mohavensis* (Oleaceae) showing its green stems and some inflated fruits in October 2015. **Photos by Mark E De Guzman**

leaves during the spring of 2016 (*Figures 2 and 3*) and did not have any leaves all summer and fall until the following year's winter.

Plants with green stems had slightly higher water potential than plants without green stems, indicating that they maintained a better water status.

However, both groups of plants experienced lower water potentials during the dry season. Green stems had higher photosynthetic rate, stomatal conductance and water loss through the epidermis than leaves of non-green-stemmed plants when normalized per area of exposed tissue, which yielded similar intrinsic water-use efficiency in both types of organs (*Figure 3*). When looking at whole-plant integrated annual carbon gain, calculated from photosynthetic rate measures integrated across the year, we found no differences between green stems and leaves of non-green stemmed species.

We found partial support for higher water-use efficiency in stems than leaves based on the carbon isotopic composition data (Ávila-Lovera et al. 2019). Furthermore, carbon isotopic composition of green stems was statistically higher than that of leaves of the same species in only one of eight green-stemmed species studied that had both leaves and green stems during the wet season of 2016. Nitrogen content in leaves and stems of green-stemmed species was also higher than in leaves and stems of non-green-stemmed species, which partially explains the higher photosynthetic performance in green stems than leaves of non-green stemmed species.

Green stems had higher water loss through the epidermis than leaves and stems of non-green-stemmed plants (Ávila-Lovera et al. 2019). This result raises questions about the possible trade-off between carbon gain and water loss through the epidermis in green stems and how this may affect plant responses to current and future droughts (Ávila-Lovera et al. 2019). However, considering that the plants in this study inhabit a wash, they may be tapping deep, relatively stable water sources throughout the year.

Models that predict drought-induced plant mortality are needed to predict the relative

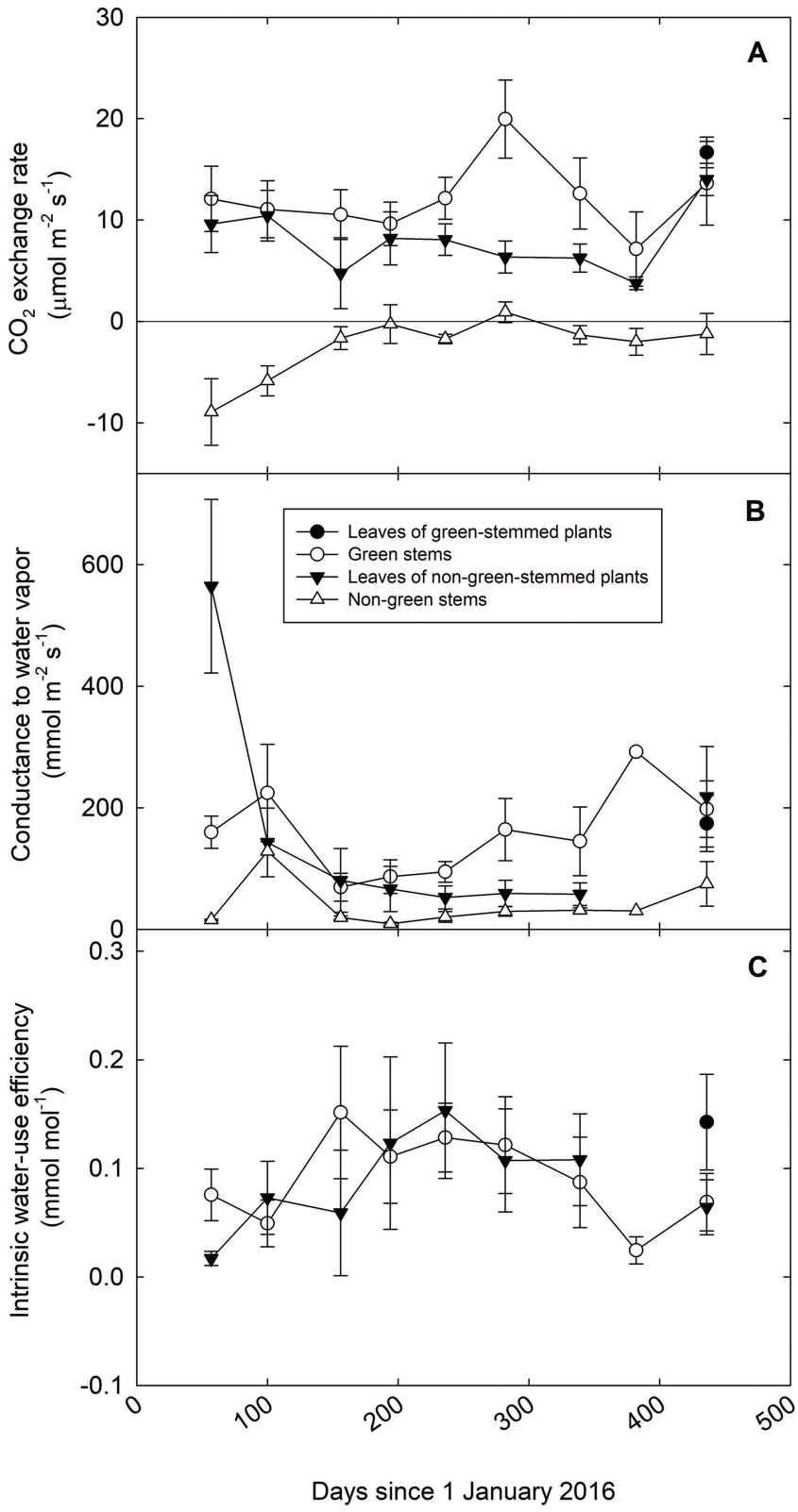

Figure 3. Gas-exchange data for leaves (when present) and stems of green-stemmed species, and leaves and stems of non-green-stemmed species during nine sampling campaigns from February 2016 to March 2017. (*a*) CO_2 exchange rate, where positive values indicate net carbon uptake and negative values indicate respiration. (*b*) Stomatal conductance to water vapor of leaves and green stems, and non-stomatal conductance to water vapor of non-green stems. (*c*) Intrinsic water-use efficiency for leaves and green stems. Values shown are means of all species within each stem-type group ± standard error. Taken and modified from (Ávila-Lovera et al. 2019)

vulnerability of these plant types and to better understand the mechanisms behind physiological responses to changes in climate. Multiple models have been used for simulating physiology of plants in order to predict mortality (McDowell et al. 2013). However, none of these models have accounted for the extra carbon income derived from stem photosynthesis, which could prolong survival during drought. Our work increased our knowledge of the physiology of desert green-stemmed species and their possible responses to climate change by showing the greater water loss of green stems. In addition, studying the costs and benefits of green stems in other ecosystems will increase our understanding of the way plants cope with drought, and help to predict which plants might die first. If species with green stems can maintain photosynthetic carbon dioxide uptake during drought, even at a low rate, they would not be as likely to die of carbon starvation. However, if species with green stems also lose substantially more water through their epidermis during drought, they might be at an increased risk of hydraulic failure.

Finally, models used so far can be re-parameterized to account for stem carbon dioxide assimilation and to test if carbon starvation, hydraulic failure, or both mechanisms are responsible for mortality of green-stemmed plants.

FUTURE DIRECTIONS

The results of this study can be used in screening programs to select species that are better adapted to face more intense, longer and more frequent droughts, which are the predictions of climate change in our desert and Mediterranean climate ecosystems. These plants can be successfully used in restoration practices of degraded arid lands, as it has been done in a tropical semi-arid ecosystem (Fajardo et al. 2013). Also, many of the plant species in JTNP are native to California, and their conservation and the management of the land they occupy is essential if we want to preserve its great biodiversity.

Species	Family	Common name[a]	Stem type
Ambrosia dumosa	Asteraceae	Burro weed	Non-green
Ambrosia salsola	Asteraceae	Burrobrush, cheesebush	Green
Bebbia juncea	Asteraceae	Sweetbush	Green
Stillingia linearifolia	Euphorbiaceae	Linear leaved stillingia	Green
Psorothamnus arborescens	Fabaceae	Mojave indigo bush	Non-green
Senna armata	Fabaceae	Desert senna	Green
Senegalia greggii	Fabaceae	Catclaw	Non-green
Krameria bicolor	Krameriaceae	White rhatany	Green
Condea emoryi	Lamiaceae	Desert lavender	Non-green
Scutellaria mexicana	Lamiaceae	Mexican bladdersage	Green
Menodora spinescens	Oleaceae	Spiny desert olive	Green
Eriogonum inflatum	Polygonaceae	Desert trumpet	Green
Thamnosma montana	Rutaceae	Turpentine broom	Green
Simmondsia chinensis	Simmondsiaceae	Jojoba	Non-green
Larrea tridentata	Zygophyllaceae	Creosote bush	Non-green

Table 1. List of fifteen plant species studied in a desert wash (34°03'50.5" N, 116°03'16.3" W) at Joshua Tree National Park, CA, USA. Family, common name, and stem type is indicated.
[a] Common name information taken from Calflora (www.calflora.org)

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DR. ELEINIS ÁVILA-LOVERA is a plant ecophysiologist interested in understanding the process and advantages of stem photosynthesis. Her research focuses on studying plants from arid and semi-arid ecosystems in southern California and the Tropics, where green stems are advantageous. She has found that stem photosynthesis is very similar to leaf photosynthesis and that plants bearing green stems can continue assimilating carbon during the dry season, when most plants in deserts are leafless.

Eleinis Ávila-Lovera collecting stem samples at the chaparral – desert ecotone near Morongo Valley. (Photo by Mark E. De Guzman)

A Preliminary Report on the Bees of Joshua Tree National Park, with Special Focus on *Anthophora* (Digger Bees)

Michael C. Orr[1]

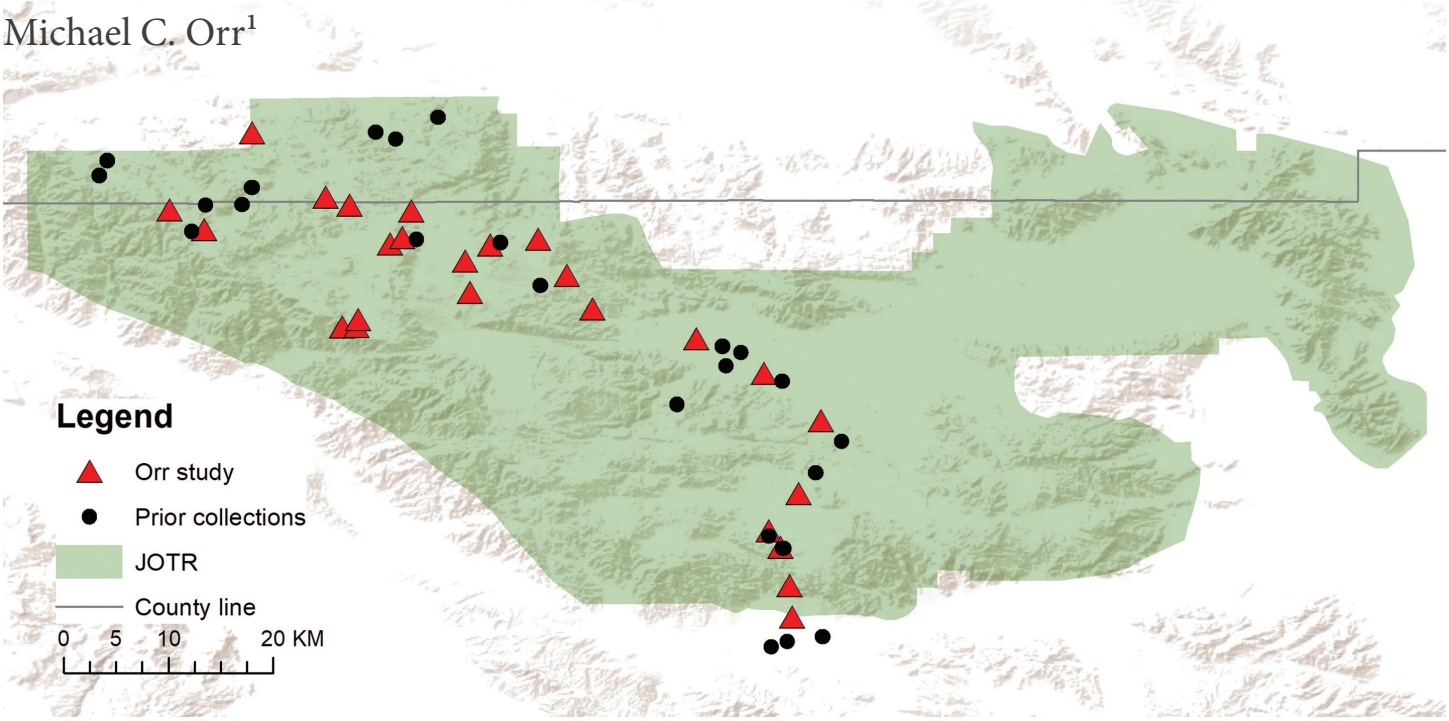

Figure 1. Collection sites within and around JTNP for specimens used to create the inventory for this study. The present-day area of JTNP is indicated as a green polygon. Localities from the present study are given as red triangles and prior collections are given as black circles. County lines are given in gray (San Bernardino County north, Riverside County south). Scale 1:600,000

BACKGROUND

Deserts are extreme environments that force organisms to adapt and compete for resources that are limited in both time and space. By studying organisms that thrive in deserts, we can better our knowledge of how environments drive evolution.

Bees are an exceptionally rich study system because of their close relationship with flowers, which are, in deserts, tied to limited and stochastic water resources (Michener 1979, Michener 2007, Minckley et al. 2000). Despite the challenges that deserts pose, bees attain their greatest species richness in xeric areas, possibly a result of their hypothesized origin in an arid region of Gondwana, where they would have accumulated many strategies for surviving such harsh conditions (Litman et al. 2011, Michener 1979). Regardless of the cause, xerophilic bees exhibit a number of apparent adaptations for desert life, including highly opportunistic emergence times and the ability to wait multiple years to emerge, both of which enable better tracking of local floral resources (Danforth 1999, Hurd 1957, Orr et al. 2016). It has also been suggested that deserts cause a higher degree of floral specialization than is seen in other environments (Minckley et al. 2000).

Floral specialists, or oligoleges, are those bees that consistently use a certain subset of the total flowering plant species available to them (Cane and Sipes 2006, Wcislo and Cane 1996). In deserts, it has been suggested that floral specialists are better able to track their specific floral hosts than generalists can track floral resources overall (Minckley et al. 2000). This seems intuitive when one considers that different plant species use disparate cues for seed germination and flowering times, therefore bees need to adapt to these shifting cues in the same way the plant does (Adondakis and Venable 2004, Jurado and Westoby 1992, Kemp 1983, Tevis 1958). Unfortunately, the floral preferences of many bees remain unknown, and recent reports call into question some past assignments of specialization (Cane and Sipes 2006, Nelson and Griswold 2015, Ritchie et al. 2016, Wilson et al. 2009). Clearly, more work is needed to understand the basic biology of these vital pollinators.

Situated at the interface of the Mojave and Sonoran Deserts, Joshua Tree National Park (JTNP) is an ideal area for studying desert bee ecology and evolution (*Figure 1*). Further, studies in protected areas such as this are vital for an informed balance between conservation and recreation. Detailed in this article are data collected during preliminary bee surveys in

[1]Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing, P.R. China, and Entomologie, Staatliches Museum für Naturkunde Stuttgart, Stuttgart, Germany.

JTNP, including the documentation of several rare species and significant range extensions. Because the author's primary research focus is on the xerophilic genus Anthophora (Brooks 1988, Orr et al. 2014), greater focus is given to this group of bees in this article, especially the floral preferences of *Anthophora*, the Digger Bees. *Anthophora* (*Figure 2*) is a large genus of solitary bees, commonly referred to as Digger Bees because nearly all of these bees are strong nest excavators, typically of soil (Brooks 1988).

These bees are found worldwide, however, they are clearly more prevalent in arid areas, making JTNP an ideal habitat.

OBJECTIVES

1. Compile an initial inventory of bees known to occur in Joshua Tree National Park.

2. Document exceptionally rare bee species or unusual range extensions.

3. Learn more about the floral specialists present in the park, with focus on the genus *Anthophora*.

METHODS

Given the spatial and temporal stochasticity of both precipitation and flowering time, opportunistic sampling was used to target unique or promising locations within JTNP (*Figure 1*), with special emphasis on documenting the species richness of *Anthophora* within the park. Sweep net sampling from plants most likely to be visited by bees was conducted. A total of 295 specimens were collected and subsequently deposited in the USDA-ARS National Pollinating Insects Collection (NPIC). During 2015-2016, a total of eight days were spent sampling within the park boundaries, not including those days during which sampling was impossible due to inclement weather or insufficient bloom (>20 days were spent in the park overall). Sampling dates include the following days during spring: 24–25 March 2016 and 19–21 April 2016. Fall collections took place on the following days: 23–25 August 2015. To augment the data collected through field surveys, specimen records with adequate georeferenced localities (*Figure 1*) from the NPIC were included in the data set for compiling the inventory list (NPIC 2017).

Figure 2. The *Anthophora* genus is a diverse group of solitary bees that are known for ground nesting, though many also use banks, hence their common name, Digger Bees.

Top: *Psorothamnus* specialist *Anthophora hololeuca* was newly detected within JTNP in this study;

Center: *Anthophora pueblo* peeking out of its nest in a sandstone bank;

Bottom: *Anthophora urbana*, a supergeneralist species, collecting pollen from Ericameria *(Asteraceae)*.

Photos by M. Orr

All specimens that were located within any 25 km grid within or entering JTNP's boundaries were included on the inventory (*Table 1*). Identification of field-sampled specimens was done to the genus or species level then deposited in the NPIC. Among bees, it is not unusual for specimens to be unidentifiable to species because many of them remain undescribed or because identification resources for many groups are entirely lacking.

Additional information regarding rarity, distribution, and/or interesting biology and ecology for each taxon was gleaned from public databases, such as DiscoverLife (DL 2017) and Global Biodiversity Information Facility (GBIF 2017); however, the bulk of this information was compiled based on the author's, and other experts', knowledge of these groups (Michener 2007). For example, in analyzing the inventory produced by this effort, it is important to look at which taxa were expected to be observed, but were not. The absent taxa from our inventory could be a result of many things, but most likely it falls into one of three categories: 1) the taxon is naturally rare on the landscape spatially or temporally, or simply low in numbers (abundance); 2) as a result of insufficient sampling (due to lack of flowering, poor weather conditions, etc); or 3) the biology or ecology of the bee makes them difficult to capture (e.g. kleptoparasites). In an effort to facilitate future collecting efforts, these taxa were identified (*Table 2*).

Photographs were taken with a Pentax Optio WG-2 and a Nikon D3300 (not all pictures were taken within the park). Any microscope images were taken with a Keyence VHF-500x Digital Microscope. Maps were generated with ArcMap 10.3 as well as ESRI basemaps and shapefiles.

RESULTS

Twenty-five distinct locations were successfully sampled throughout the park in this study, though over 40 were visited (*Figure 1*). To maximize the time spent collecting, areas adjacent to roads were prioritized. Sites that were visited multiple times include Cottonwood Wash, Key's View, Quail Springs Valley, Queen Valley, and various stops within the Pinto Basin. In the future, special focus should be given to less accessible southern localities that fall within the Sonoran Desert ecoregion. For the current study, only the two southernmost localities (*Figure 1*) in the park fell within this ecoregion (as described by Olson et al. (2001)). In addition, future work should focus on areas with unique habitats that were not sampled in this study.

Species richness is a fundamental ecological metric; it refers to the number of taxa found in an area.

> Within JTNP, it is expected that bee species richness will be high due to its geographic size and habitat diversity.

As a comparison, Michener (1979) predicted that about 500 species are expected in the adjacent Palm Springs area; because JTNP is superior in both size and habitat diversity, it seems likely that bee richness in the park is nearer to 600 species in approximately 65 genera. During this study, 295 specimens were collected, representing six families and 41 genera. The complete dataset is based on 459 total specimens, which includes 164 prior collections; it documents six families and 43 genera (*Table 1*). As discussed earlier, insect inventory studies often have many taxa that cannot be identified to species level, thus discussions about diversity often focus on a broader level, such as the genus or family, as is done in this case.

The field sampling efforts conducted during this project recovered 41 bee genera, the majority of these (27/43, 63%) were new for the park inventory. This means that with only eight days of field collecting, this study nearly tripled the known genera for the park (*NPIC 2017; Table 1*). Prior efforts had only documented 16 genera, two of which were not observed during this study. For a number of reasons, as mentioned above, some genera are harder

Family	Genus	Source	Family	Genus	Source
Andrenidae	*Andrena*	Orr	Colletidae	*Colletes*	Both
	Calliopsis	Orr		*Hylaeus*	Orr
	Macrotera	Orr	Halictidae	*Augochlorella*	Orr
	Megandrena	Both		*Conanthalictus*	Both
	Perdita	Both		*Dieunomia*	Orr
	Pseudopanurgus	Orr		*Dufourea*	Both
Apidae	*Anthophora*	Both		*Lasioglossum*	Orr
	Anthophorula	Orr		*Nomia*	Orr
	Apis	Both		*Xeralictus*	Orr
	Centris	Orr	Megachilidae	*Anthidiellum*	Prior
	Ceratina	Orr		*Anthidium*	Both
	Diadasia	Orr		*Ashmeadiella*	Both
	Ericrocis	Orr		*Atoposmia*	Both
	Eucera	Orr		*Coelioxys*	Orr
	Exomalopsis	Orr		*Dianthidium*	Both
	Habropoda	Orr		*Hoplitis*	Both
	Melissodes	Orr		*Megachile*	Both
	Peponapis	Orr		*Osmia*	Both
	Svastra	Orr		*Stelis*	Prior
	Tetraloniella	Orr		*Trachusa*	Orr
	Triepeolus	Orr	Melittidae	*Hesperapis*	Both
	Xeromelecta	Orr			

Table 1. List of bee genera known to occur in JTNP. This inventory documented 6 families and 43 genera; importantly, 63% of the genera documented were new for the inventory. "Source" identifies whether the taxon was observed during this study only (Orr), prior studies only (Prior), or both.

to observe in nature. In addition, there can be sampling bias in the field or represented in museum collections as a result of research interests of particular entomologists. For example, the highest percent of shared genera between this collection effort and past efforts was seen in the family Megachilidae (7/11, 64%), which is the group of bees that the NPIC collection manager, Dr. T. Griswold, is an expert on. Interestingly, the two genera that were not documented during this field survey also belonged to this family. It seems likely that further data mining efforts in other institutions will raise the number of taxa found in JTNP, but, more importantly, promoting field collections and insect surveys will certainly add significantly to the inventory of invertebrates for the park.

Despite the current and past inventory efforts, there are a number of expected genera that remain unknown from JTNP (*Table 2*). There are 24 genera that have been identified as being "likely present" in the park (*Michener 2007, NPIC 2017; Table 2*) based on a variety of things, such as expert knowledge, known occurrences just outside the park boundary, the presence of good habitat and host plants, and/or low detection rates for the type of bee. The fact that exceedingly common groups are absent from the collection record, such as *Agapostemon* and *Halictus*, demonstrates that insufficient sampling effort could account for many of these missing taxa. Conversely, for seven of these genera, the relative rarity of these groups may explain their absences, in other words, these rare bees are hard to find even under ideal conditions. There are also 11 kleptoparasitic genera among the "likely present" taxa that have yet to be collected, compared to only five in the known genera (*Figure 3*). In this case, the bees are detected infrequently because they are most easily found patrolling for the nest sites that they invade (rather than needing to collect pollen for themselves); in other words, kleptoparasites are not as often found around flowers, where most collections took place in this study (Michener 2007). Additional fieldwork that focuses on capturing these types of bees would certainly yield more taxa for the inventory. In addition, as insect collections across the country continue to database their specimens, it seems likely that these gaps will be filled.

Family	Genus	Ecological Notes	Reason
Andrenidae	*Ancylandrena*		2
Apidae	*Bombus*		2
	Epeolus	Kleptoparasite	3
	Holcopasites	Kleptoparasite	3
	Martinapis	Rare	1
	Melecta	Kleptoparasite	3
	Neolarra	Kleptoparasite	3
	Neopasites	Rare, Kleptoparasite	1, 3
	Nomada	Kleptoparasite	3
	Oreopasites	Kleptoparasite	3
	Townsendiella	Rare, Kleptoparasite	1, 3
	Xylocopa		2
	Zacosmia	Kleptoparasite	3
Halictidae	*Agapostemon*		2
	Halictus		2
	Protodufourea	Rare	1
	Sphecodes	Kleptoparasite	3
	Sphecodosoma	Rare	1
Megachilidae	*Chelostoma*		2
	Dioxys	Rare, Kleptoparasite	1, 3
	Heriades		2
	Lithurgus		2
	Protosmia		2
Melittidae	*Melitta*	Rare	1

Table 2. List of genera expected to occur in JTNP but missing from the inventory data. Reasons for missing taxa from the current database include, but are not limited to: 1) low abundance; 2) insufficient sampling; or 3) ecological restrictions.

RARE BEES, RANGE EXTENSIONS, AND SPECIES OF SPECIAL INTEREST

Any attempt to compile an inventory will lead to interesting findings; for example, field collections can lead to the discovery of a new species to science. Or, more commonly, a species is discovered in a place it was unknown prior - this is called a range extension. Rarity is a term that is generally reserved for species that naturally occur in low numbers (low abundance) or have a narrow geographic range.

The tricky part about defining rarity for a species is that often there is a paucity of data to pull from to fully explain why there are very few collections of a certain taxon in an area.

In other words, rarity might be due to their biology (e.g., restriction to a specific host plant or nesting substrate) or an artifact of insufficient sampling. Below is a list of species pulled from the inventory that are considered rare or of special interest based on the data thus far. They are listed alphabetically by family then species.

Andrenidae (Mining Bees)

Perdita bebbiae: Prior to this study, this extremely rare species was only known from one location: south of Ocotillo, CA, in San Diego County (Timberlake 1956). During this effort, a second specimen was documented from JTNP east of Squaw Tank. In both instances it was collected on *Bebbia juncea* (sweetbush); this may be its primary or sole

host, however, with so few collection records, it would be premature to declare this. If it is limited to *Bebbia juncea*, floral limitation could not explain its rarity, as *Bebbia juncea* is both widespread and common throughout the southwestern U.S. and northwestern Mexico (Tropicos 2017). A better explanation for its rarity may be that its nesting habits are unusually restrictive, which would mean the species becomes less abundant away from its nesting habitat.

Perdita mucronata: The biology of this rare species is exceptionally poorly known. There is only one known location for this species in the world: near Cottonwood Springs. Unfortunately, the only collection is from 1963 and it lacks any kind of information about the habitat or associated plants it was found near (Timberlake 1956). It was not observed or collected during the current study.

Apidae (Apid Bees)

Anthophora columbariae: Recently, Kopec and Burd (2017) listed this relatively uncommon species as "declining," although the exact methods used in this study were not published and a mechanism of decline is not evident. Interestingly, it was found plentifully throughout JTNP (33 specimens from seven sites). Its conservation status remains uncertain (Orr et al. 2018). This species is primarily known from more mild environments in California's coastal ranges. Two other primarily "coastal" species found in JTNP are *Conanthalictus nigricans* (Halictidae) and *Habropoda tristissima* (Apidae). A number of additional species are known from the coastal ranges and higher elevations of the Mojave Desert (especially the Mojave National Preserve), which suggests that the park's upper elevations might be an important transitional zone between these environments.

Anthophora signata: This uncommon species represents one of only two *Anthophora* lineages known to nest in wood, whereas all but two of the other 400+ taxa in this genus are ground-nesting (Brooks 1988). Fitting for this current inventory study, it was previously recorded nesting in Joshua Trees (*Yucca brevifolia*). However, according to Brooks (1988), it may also nest in the ground; it may be that this atypical wood-nesting behavior is opportunistic in nature, as related species do not use wood.

Halictidae (Sweat Bees)

Dufourea snellingi: This taxon was originally described in 1980 based on specimens collected from Upper Covington Flat in JTNP. Since then, it has only been reported from a few disjunct locations in the California desert. With so few recorded observations of this species, very little is known about it. This genus contains many floral specialists that use a wide variety of plants, and it seems likely that this species is also a specialist (Michener 2007). Unfortunately, this bee was not observed during this project and the host plant for this species remains unknown.

Figure 3. *Ericrocis lata*, one of the more photogenic kleptoparasitic bees collected in JTNP, resting upon a stem of *Bebbia juncea*. Several additional individuals were also present. This bee invades nests of *Centris*, of which three species were collected (more are expected).

Megachilidae (Leafcutter and Mason Bees)

Trachusa autumnalis: The distribution of this species reaches over a large area geographically, but is only known from three isolated patches: from Vidal Junction (near the AZ-CA border), Baja California Sur, and now JTNP. This species may specialize on Asteraceae, as it was collected on this family in 2/3 cases (on *Bebbia juncea* in JTNP), but both *Bebbia juncea* and Asteraceae in general are common in the desert, meaning this alone could not explain its rarity in collections. One interesting fact about these collections is that they all occurred during late summer and fall (Aug-Oct), when temperatures are high and any flowering that is happening then will be due to monsoonal rain events. Asteraceae includes many summer-blooming plants, so this bee may have evolved to emerge in response to hot summer rains necessary for plant germination. This would easily account for a lack of specimens because summer blooms are hard to track and most people do not think of collecting during the hottest time of the year. As bee collectors generally time their trips with periods of high bloom, avoidance of late summer could bias sampling against species that are specifically active during these times.

THE *ANTHOPHORA* OF JTNP

As with many bees, *Anthophora* (Digger Bees) attains its highest number of species in deserts (Michener 1979). Almost 60 of the 400 described species of *Anthophora* occur north of Mexico in the Western Hemisphere, most of which (>75%) reside in the xeric Southwest. Prior to this study only eight species of *Anthophora* had been documented in JTNP; now, there are a total of 16 unique taxa documented in the park based on a total of 113 *Anthophora* specimens (*Table 3*).

> Six additional species are likely present in JTNP, based on nearby observations, but have yet to be documented.

Consequently, total sampling efforts have recovered 73% of the expected *Anthophora* in JTNP. Of more significance is that two of the 16 documented *Anthophora* species are undescribed taxa and three of the six "expected" taxa were also undescribed at the time of this study. This is a testament to the incredible invertebrate diversity yet to be discovered in the Desert Southwest: nearly

Figure 4. Left: A female *Anthophora abroniae* foraging on *Abronia villosa*. Right: The unusually elongate and hairy galea of *Anthophora abroniae*, which is used to remove pollen (yellow dots attached to hairs) from the narrow flowers of *Abronia villosa*. For reference, the mandibles are on the right.

one-quarter of all documented *Anthophora* taxa known to occur in JTNP were undescribed when discovered.

FLORAL SPECIALISTS, A CASE EXAMPLE IN THE SUBGENUS *MICRANTHOPHORA*

The subgenus *Micranthophora* specializes on a remarkable diversity of plants, especially considering how few species it contains. Some of these species, such as the *Psorothamnus* specialist *Anthophora hololeuca* (*Figure 2*), newly detected within JTNP in this study, will forage on just a few similar species. As a comparison, the genus *Perdita* consists mostly of oligoleges that will only collect pollen from a few closely related species or genera of plants. The genus *Perdita* includes 636 species but they only use 21 plant families, this works out to be roughly 0.03 plant families per species (Portman and Tepedino 2017).

In contrast, *Micranthophora* contains 26 species that use 9 different plant families, i.e. 0.35 plant families per species (Orr et al. 2018) – a much higher ratio than seen in other groups of oligoleges. In part, this ratio is high because while the majority of *Micranthophora* are likely or confirmed Asteraceae specialists (17/26: 65%), the remaining nine species utilize plants from eight different families: Boraginaceae, Capparaceae, Cleomaceae, Fabaceae, Lamiaceae, Nyctaginaceae, Zygophyllaceae, and possibly Solanaceae. Efforts to determine host breadth are ongoing within this group of floral specialists, as the specific host plant(s) for many species remains uncertain, but one notable exception is discussed below.

ANTHOPHORA ABRONIAE AND ITS FLORAL HOST, *ABRONIA VILLOSA*

The association of these two species appears to be the tightest of any *Micranthophora*, and it also ranks highly among bees in general, given the rarity of true monolecty (bees that visit only one host plant; Cane and Sipes 2006). The

Figure 5. A male *Anthophora petrophila* grooms itself while perched on a stem by its mandibles, similar to how female *Anthophora abroniae* behave when moving pollen to their scopae (the place where pollen is stored on the legs).

Anthophora species			
Subgenus	**Species**	**Documentation**	**Notes**
Anthophoroides	*californica*	Expected, not Observed	
Lophanthophora	*neglecta*	Expected, not Observed	
Micranthophora	*mortuaria*	Expected, not Observed	
Micranthophora	*columbariae*	Both	
Micranthophora	*curta*	Both	
Micranthophora	*estebana*	Both	
Micranthophora	*petrophila*	Both	
Mystacanthophora	*urbana*	Both	
Paramegilla	*centriformis*	Both	
Paramegilla	*fulvicauda*	Both	
Anthophoroides	*signata*	Orr	
Lophanthophora	*coptognatha*	Orr	
Lophanthophora	*dammersi*	Orr	
Micranthophora	*abroniae*	Orr	
Micranthophora	*hololeuca*	Orr	
Pyganthophora	*vannigera*	Orr	
Micranthophora	*pachyodonta*	Prior	
Anthophoroides	*cinerula*	Expected, not Observed	Undescribed at time of study
Anthophoroides	*pueblo*	Expected, not Observed	Undescribed at time of study
Micranthophora	*striata*	Expected, not Observed	Undescribed at time of study
Micranthophora	*parkeri*	Orr	Undescribed at time of study
Micranthophora	*timberlakei*	Orr	Undescribed at time of study

Table 3. Species of *Anthophora* found or expected to occur in JTNP. Documentation refers to voucher specimens collected during this study (Orr), prior studies (Prior), or both. "Expected, not Observed" refers to species that are likely present in JTNP, based on nearby observations, but have yet to be documented. Five taxa are new to science and have yet to be described, two of which were first documented during this study.

vast majority of female *Anthophora abroniae* specimens have been collected on *Abronia villosa*, Sand Verbena (Orr et al. 2018; *Figure 4*). Interestingly, females of this species have an unusually elongate mouthpart, called a galea, which is covered with mop-like hairs (*Figure 4*). This structure is used for manipulating flower parts and in this case is highly specialized to remove pollen from the narrow flowers of *Abronia villosa*.

These highly modified mouthparts enable *Anthophora abroniae* to very quickly and efficiently forage for pollen on this plant, though they would prove unwieldy and poorly-suited for most other plants. Consistently observed in the California deserts, including JTNP, females land on a cluster of *Abronia villosa* and move between flowers, quickly dipping their galea into each flower before moving to the next. As is common among many bees, the females will then groom pollen off of their hairy galea with their legs, either midflight or while holding onto the stem of a plant by their mandibles (*Figure 5*; Portman et al. 2019).

During this study, *Anthophora abroniae* was found at both Quail Springs Valley and Queen Valley, and it was collected prior to this study in the Pinto Basin. It was only collected or seen during spring, consistent with all but one record of this species (Orr et al. 2018), and consistent with the primary blooming period for *Abronia villosa*. Only three specimens were taken from JTNP because of how easily this species can be identified, thereby enabling better observation of its behavior. Over the course of approximately two hours of total observations at Quail Springs Valley, no *Anthophora abroniae* females were ever witnessed visiting plants other than *Abronia villosa*. In fact, the author has only ever witnessed a female *Anthophora abroniae* visiting an alternative plant (*Palafoxia arida*) once during >10 total hours of observation throughout the Desert Southwest. During the observation period in JTNP, males were repeatedly seen perching beside host plants and chasing each other away (along with other insects of adequate size).

Further supporting the close association of this bee and plant, the distribution of the

Anthophora abroniae fits well within that of the host plant (*Figure 6; Tropicos 2017*). Over the course of several years, the author has reviewed thousands of specimens from 30 institutions and visited numerous locations throughout the Desert Southwest where *Abronia villosa* occurs; *Anthophora abroniae* was never documented at a site that didn't also have *Abronia villosa* in bloom (*Figure 6*). Most remarkable is a site near Bouse, AZ, where a single *Abronia villosa* plant was found (it was less than 8 cm in height with only one cluster of flowers) and even there, an *Anthophora abroniae* male was seen waiting beside the plant. With the many hours of observation and strong collection records now in place for *Anthophora abroniae*, this highly specialized relationship between these two species has only been reinforced and the groundwork has been laid for many interesting research opportunities. In the future, this system may prove useful for examining the ramifications of narrow host specialization in bees.

MANAGEMENT IMPLICATIONS

It is still too early to formally describe the structure of bee communities across the park, nor is it possible to accurately ascertain the species richness of bees found in JTNP. Without a doubt, further sampling throughout the various habitats in the park will prove valuable, as much of the park remains unexplored by entomologists (*Figure 1*). For example, in the higher elevations of the Little San Bernardino Mountains there are likely to be several genera commonly found in mild environments (e.g., *Bombus*, *Dioxys*, *Protosmia*), as well as other bee species that are generally found in the cismontane regions of southern California. In addition, many plant communities associated with the Sonoran Desert barely reach into the park along the southern and eastern boundaries, therefore providing an opportunity to document bees associated with these plant assemblages will likely be productive.

Mesic habitats, such as palm oases, or ephemeral springs and washes are generally good sites for bee collecting, as these are also places where plants are more likely to bloom even in relatively dry years. As an example, Cottonwood Wash, Quail Springs Valley, and Queen Valley were all awash with bloom during at least one visit during this project and therefore were good places to document bee diversity (*Figure 7*). In extreme desert environments, where precipitation can be quite limited, areas with more consistent bloom

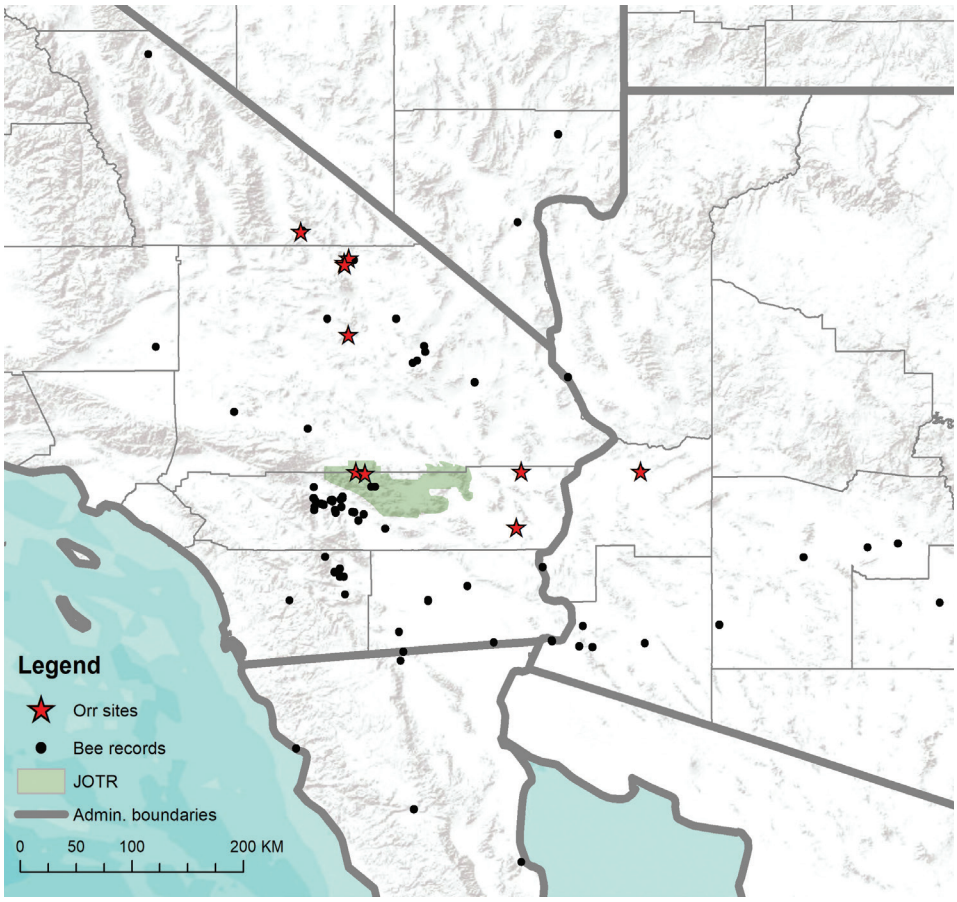

Figure 6. All known collection sites of *Anthophora abroniae* (black circles). *Anthophora abroniae* was collected by the author at 10 sites, all of which had *Abronia villosa* in bloom (red stars). Joshua Tree National Park is shown as a green polygon; state and county boundaries are shown as gray lines. Scale 1:4,000,000.

are likely important reservoirs for bee species richness during dry years. However, some desert bees can wait multiple years to emerge, and, if widespread across many species, this trait could make consistent bloom less important to bee species richness in deserts (Danforth 1999, Orr et al. 2016). Any future sampling efforts should also focus on sampling during the summer and fall blooming periods, as this will undoubtedly add a number of taxa to the inventory, and perhaps even lead to additional species being discovered.

> Counterintuitively, a plethora of flowers often makes for poor bee collecting.

This is because a given year's bee abundance is a consequence of the prior year's floral resources, save for those species with multiple generations in a year (their second generations may be much larger than the first due to more resources within a year). This phenomenon was most evident on 26 March 2016 at the southern edge of JTNP, south of Cottonwood Wash. There, a wide array of flowers were in plentiful, peak bloom: *Bebbia juncea, Chaenactis spp., Chylismia spp., Cryptantha spp., Encelia farinosa, Eriogonum spp., Eschscholzia spp., Larrea tridentata, Lupinus spp., Malacothrix glabrata, Mentzelia involucrata, Mentzelia nitens* (likely), *Nama demissa*, *Parkinsonia florida, Penstemon sp., Phacelia campanularia, Psorothamnus arborescens*, and *Salvia columbariae*. Over the span of two hours at this site, however, a mere five bee specimens were collected, with only a few (<5) more observed. The combination of a poor bloom in spring 2015 (T. La Doux, pers. comm.) and many flowers in 2016 could dilute what few bees were present, while also decreasing their fidelity to any given plant; this would explain why there were so few bees despite the presence of many types of flowers.

An alternative explanation for this phenomenon is more harrowing. In recent years, climate change has been a central focus for studies of bee decline (Bartomeus et al. 2011, Forrest 2015, Potts et al. 2016, Settele et al. 2016). Phenological mismatches, where plants and pollinators no longer sync up in their seasonal activity, have been intensely explored in other habitats, but very few of these studies have occurred in deserts (Gerst and Venable 2017). Although results have been mixed, with many plants and pollinators responding to similar cues, some work has shown that plants may be impacted by mismatches (Bartomeus et al. 2011, Forrest 2015, Hegland et al. 2009, Rafferty et al. 2016). As many of these studies have focused on montane or relatively mild environments, it's unclear what to expect in deserts, though one might expect pollinators that evolved in environments with high resource stochasticity to be better able to track their floral hosts under changed conditions. In the event that phenological mismatches are occurring, it is unclear how this issue might be alleviated.

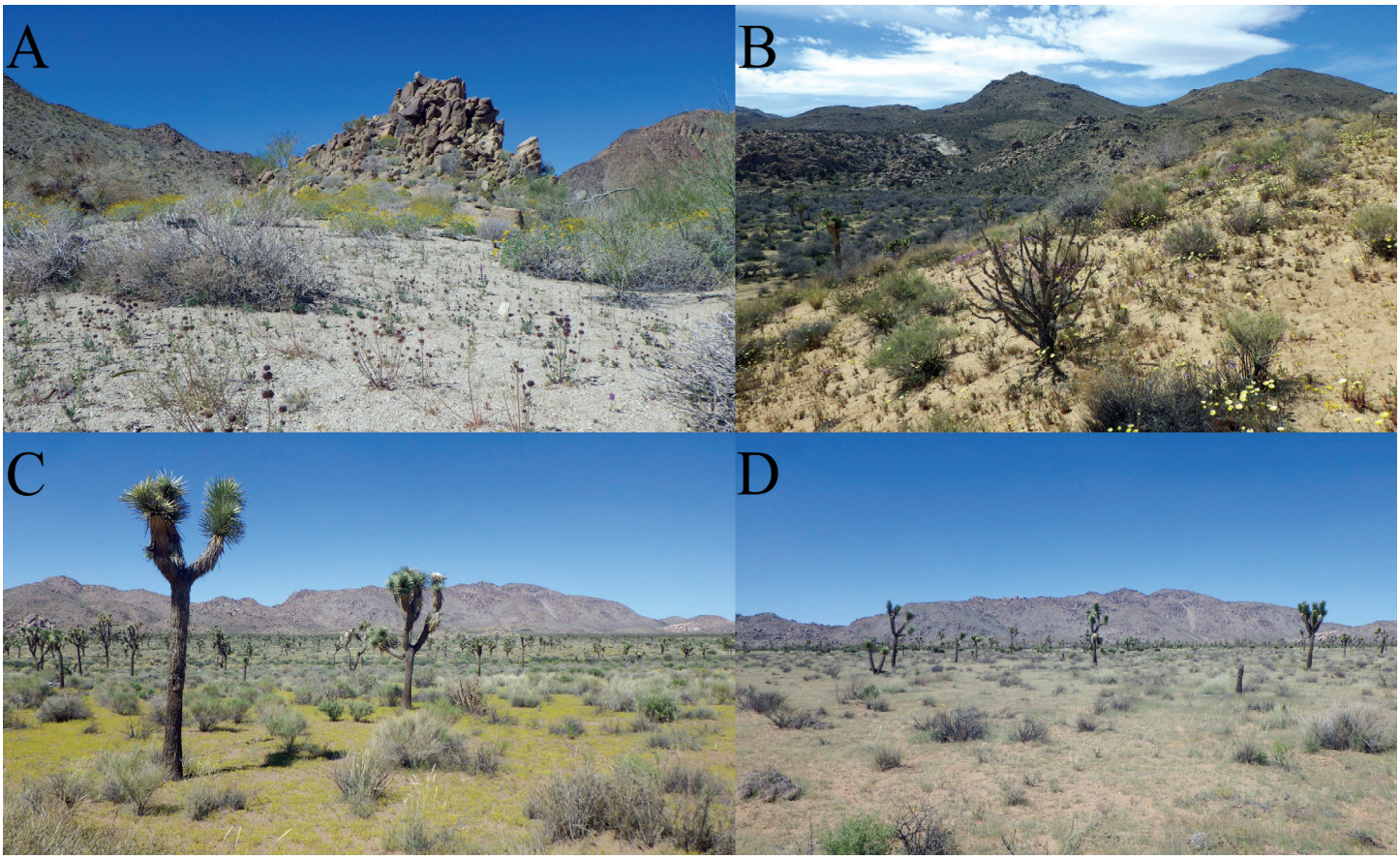

Figure 7. The best sampling localities from this study. A) Cottonwood Wash, where *Chaenactis sp., Encelia farinosa, Larrea tridentata, Lupinus sp., Malacothrix sp., Parkinsonia florida, Phacelia campanularia,* and *Salvia columbariae* were in bloom during spring 2016.

B) Sandy bench along Quail Wash upon which *Abronia villosa, Amsonia tomentosa, Baileya pleniradiata, Camissonia spp., Chaenactis spp., Cryptantha spp., Krameria bicolor, Malacothrix glabrata, Phacelia distans,* and *Salazaria mexicana* were blooming in spring 2016.

C) Queen Valley during fall (8/23/2015), with many flowering *Pectis papposa, Abronia villosa, Chilopsis linearis, Encelia sp., Larrea tridentata, Nicolletia occidentalis,* and *Sphaeralcea ambigua* were also in bloom.

D) Different location in Queen Valley during fall (8/23/2015); this demonstrates the stochasticity of floral resource availability in the desert: the bloom is completely absent in this photo, which is less than one km away from the abundant bloom shown in (C).

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MICHAEL C. ORR, PhD
As a postdoctoral researcher at the Chinese Academy of Sciences, Michael studies the systematics and evolutionary relationships of the bee group Anthophorini, with special focus on their biogeography and the evolution of various species traits. He focused on the desert bee group *Anthophora* for his PhD at Utah State, and during this time Michael's sampling efforts contributed to a bee inventory for Joshua Tree National Park.

Front cover: *Anthophora urbana* (Urbane Digger Bee), a common ground-nesting bee. Image by **Michael Orr.**
Back cover: *Astragalus coccineus* (scarlet milkvetch), an uncommon herbaceous perennial in Joshua Tree NP. Image by **Tasha La Doux.**

JOSHUA TREE NATIONAL PARK ASSOCIATION

74485 National Park Drive
Twentynine Palms, CA 92277

www.joshuatree.org

Email mail@joshuatree.org
Phone (760) 367-5525

LINKS AND CONTACTS

JT Science www.nps.gov/jotr/learn/nature/jtnp-science
jtresearchgrant@joshuatree.org

Grant Program www.nps.gov/jotr/learn/nature/grantprogram
jtresearchgrant@joshuatree.org

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