

JOSHUA TREE SCIENCE

February 2020

In this Issue:

“Long-term Butterfly Monitoring at Joshua Tree National Park.”

J. Zarki and M. Lutz; Page 1.



“A preliminary report on the bees of Joshua Tree National Park, with special focus on Anthophora, Digger Bees.”

M.C. Orr; Page 13.



“Exploring the Microbial Diversity in Biological Soil Crusts at JTNP.”

N. Pombubpa, T. Kurbessoian, J.E. Stajich, N. Pietrasiak; Page 25.



“The advantages of having green stems in arid environments.”

E. Avila-Lovera and L.S. Santiago; Page 37.

Long-term Butterfly Monitoring at Joshua Tree National Park

Joe Zarki¹ and Marilyn Lutz¹

The national parks comprise the very centerpiece of butterfly conservation. As the rest of the American landscape changes around us, the parks and monuments at least remain intact— a reliable core of the continent and its habitats where butterflies persist and survive.

Robert Pyle, author, *Handbook for Butterfly Watchers*, founder of the Xerces Society, former park ranger, Sequoia National Park

Despite butterflies' importance, few personnel at national parks pay much attention to these invertebrates, and even the largest and oldest parks seldom possess a species list. Much of what is known about butterflies within parks depends on the individual interest of a staff member or volunteer.

Roland Wauer, former Chief of Resource Management, National Park Service

Relatively few studies of butterflies have been carried out in Joshua Tree National Park (JTNP or park), and most of those were short-term in duration. Consequently, many questions about butterfly distribution and the status of park butterfly populations remain unanswered. For the past 24 years, the authors have engaged in a volunteer effort to collect data on butterflies in JTNP. This long-term monitoring is described here along with some of the project's more interesting findings.

Butterflies and moths belong to the Order Lepidoptera and occur on all continents except Antarctica. Worldwide there are between 18,000-20,000 species of

butterflies with an estimated 250,000 species including all known moths; approximately 725 species are found in North America (NABA, 2018).

Butterflies are insects that exhibit a four-part life cycle progressing from egg to larva to pupa to the adult form called imago. Butterflies are generally warm weather creatures with the greatest diversity occurring in tropical regions. Most butterflies have fairly short lives in their adult form, a few weeks to a month for many smaller species whereas a few larger species such as the monarch and mourning cloak may live nine months or more. Adult butterflies largely feed on flower nectar that they

obtain through a flexible anatomical tube called a proboscis (Figure 1). They have taste receptors on their feet that enable them to identify specific plants on which to lay their eggs.

With a lifestyle based on visiting flowers for nectar, butterflies are known as important pollinators. They are also highly sensitive to environmental changes and thus are important indicators of environmental quality. Most butterfly species have populations that are localized to a specific region or habitat and thus respond quickly to local conditions such as fires, drought, and fluctuating seasonal precipitation levels. Their rapid response to environmental



Figure 1. Three common generalist species: the painted lady (left), orange sulphur (middle), and gray hairstreak (right); all obtain food and lay their eggs on a wide variety of plants. Butterflies largely feed on flower nectar using their proboscis, as all three species demonstrate here. Photos by Joe Zarki.

stresses makes them good candidates for climate-change monitoring efforts.

Desert ecosystems, though hot and arid, offer butterflies a wide range of plants and nectar sources. The Mojave and Sonoran Desert floristic components at JTNP support a diverse butterfly fauna representing all the major North American butterfly families. While generalist species such as the painted lady (*Vanessa cardui*), orange sulphur (*Colias eurytheme*), and gray hairstreak (*Strymon melinus*) are able to use many different kinds of plants (Figure 1), some species are more specialized, often using just one or a few larval host plants and/or adult nectar sources. For example, pallid dotted-blue (*Euphilotes pallescens elvirae*) feeds only on *Eriogonum* as an adult and larvae, whereas tiny checkerspot (*Dymasia dymas*) and ‘Loki’ juniper hairstreak (*Callophrys gryneus loki*) are specialized at the larval stage only (Figure 2). The tiny checkerspot (*Dymasia dymas*) uses only a few species in the Acanthaceae family (Monroe, 2004; Figure 2). One of these plants, chuparosa (*Justicia*

californica), occurs in JTNP, but only along low elevation bajadas and desert washes within the Sonoran Desert sections of the park. Nevertheless, when chuparosa is blooming in March and April, tiny checkerspots become one of the most locally common butterflies at JTNP. Larvae of the ‘Loki’ juniper hairstreak feed only on California juniper (*Juniperus californica*) while adults take nectar from many annual and perennial flowers.

National parks protect relatively large, intact ecosystems that serve as important refuges for butterfly populations that represent a wide spectrum of North America’s lepidopteron fauna. Data collection efforts through citizen science programs have expanded the ability of parks to gather data on a wide range of park resources and science-related questions regarding the impacts of climate change or population trends for individual species or groups of organisms. Although, these volunteer-driven data collection efforts have recognized strengths and weaknesses they can be valuable additions to a

park inventory and monitoring program (Wilson, et.al. 2017).

Since 1985, the authors have been involved with creating volunteer-led data collection efforts on butterfly populations at three national parks: Yellowstone, Badlands, and Joshua Tree. By obtaining National Park Service (NPS) research permits and establishing monitoring protocols using the Seasonal Count Program of the North American Butterfly Association, long-term data collection has improved each park’s understanding of its invertebrate pollinators (NABA, 2018b).

The North American Butterfly Association (NABA) Count Program uses a methodology first developed (www.naba.org/ftp/naba18us.pdf) by the National Audubon Society for its popular Christmas Bird Count, which is one of the oldest ongoing citizen science programs in existence. Under the NABA protocol, a 15-mile diameter circle is established as the area to be surveyed. Each count has one or more volunteer compilers who organize and share the count data with the national database.



Figure 2. Specialist species are limited to specific plant taxa within a genus or family. For example, larvae of the ‘Loki juniper hairstreak (left) feed only on California juniper (*Juniperus californica*), whereas tiny checkerspot larvae (center) feed only a few species in the Acanthaceae family; this female (center) is shown ovipositing on chuparosa, JTNP’s only member of the Acanthaceae. All stages of pallid dotted-blue (right), shown here mating, are restricted to a few *Eriogonum* species. Photos by Joe Zarki.

Compilers also determine the date each year when participants will fan out and, during a single 24-hour period, find and identify any butterflies they encounter. While most individual butterflies will be noted in their adult phase, participants also record butterflies found as eggs, larvae, and pupae if they can be correctly identified. In addition, the number of individual butterflies (in all stages) is counted or estimated. Count dates are selected to assess butterfly populations at their times of peak activity and diversity. For most North American sites, this date will be in summer, but in the Southwest, peak seasons of biodiversity occur in spring and fall in response to seasonal rainfall.

The first nationwide survey, the 4th of July Butterfly Count, was started by the Xerces Society in 1975. Since 1993, the program has been administered by NABA. The emphasis for the NABA Count Program is to conduct non-consumptive studies of butterflies. Identification, data collection, and appreciation of butterflies are the main drivers for the program. Butterfly count compilers are responsible for recording their count data in NABA's on-line database, and an annual report is issued that contains each count's results. An average of 450 counts are conducted each year. At present, 19 NPS units are either the primary site or are partially covered by 26 active NABA

counts (NABA, 2018). Eight counts are in the southeastern U.S. and 16 take place in the western U.S., all but three of those are located in California (Table 1).

In JTNP, three butterfly counts are currently run each year. The oldest count, simply called 'Joshua Tree, CA,' was first run in 1995 and has been conducted for 25 consecutive years between mid-April and early May. The Joshua Tree count circle is centered at Hidden Valley Campground (Figure 3). The second count, referred to as the "Joshua Tree Fall count," was started in 2014. It uses the same count circle but takes place between late September and mid-October. A third count called 'Cottonwood Spring, CA' was added in 2006 and takes place earlier in the spring, most often in late March. The Cottonwood Spring count is centered near the Cottonwood Visitor Center (Figure 3).

An initial problem with starting a NABA butterfly count at JTNP was one of establishing trust. In 1995, a major federal investigation on the commercial sale of butterflies illegally collected from national parks broke into the news (Laufer, 2009). Suspicion was high surrounding any new activity involving the study of butterflies in national parks. Eventually, after assurances that park butterfly counts would rigorously adhere to all restrictions and conditions, a research permit was issued. Illegal collecting is still a worldwide conservation issue, and it remains a concern at many national park units.

Challenging field conditions sometimes lead to dramatic up and down swings in our count results. The timing of seasonal

| Park | Count Name | Season | Years Held (through 2018) |
|------------------------------------|---|--------|---------------------------|
| Apostle Islands National Lakeshore | Cornucopia, WI | summer | 33 |
| Big Bend National Park | Big Bend National Park, TX | fall | 1 |
| Big Cypress National Preserve | Pincrest (Tri-Co.), FL | summer | 16 |
| Blue Ridge Parkway | Blue Ridge Parkway, VA | summer | 27 |
| | Peaks of Otter, VA | summer | 26 |
| Bryce Canyon | Bryce Canyon, UT | summer | 13 |
| Cape Cod National Seashore | Truro, MA | summer | 14 |
| Everglades | Homestead, FL | spring | 18 |
| | Shark Valley, FL | spring | 12 |
| | Shark Valley, FL | summer | 13 |
| Golden Gate | Marin Co., CA (includes Muir Woods) | summer | 30 |
| | San Francisco, CA | summer | 24 |
| John Muir National Historic Site | Benecia, CA | summer | 18 |
| Joshua Tree (includes 2019) | Cottonwood Spring, CA | spring | 14 |
| | Joshua Tree, CA | spring | 25 |
| | Joshua Tree, CA | fall | 6 |
| Lassen Volcanic | Mount Lassen | summer | 12 |
| Lava Beds | Lava Beds National Monument, CA | summer | 10 |
| Marin County, CA | Marin County, CA | summer | 30 |
| Pinnacles | Pinnacles National Park, CA | summer | 19 |
| Point Reyes | Point Reyes, CA | summer | 14 |
| Shenandoah | Island Ford, VA (partially within park) | summer | 20 |
| | Shenandoah National Park, VA | summer | 21 |
| Tuzigoot National Monument | Cottonwood, AZ (includes entire park) | summer | 3 |
| Yellowstone | Yellowstone National Park, MT | summer | 14 |
| Yosemite | Yosemite National Park, CA | summer | 6 |

Table 1. NPS Units with Active NABA Butterfly Counts. A number of counts have been started and discontinued at other NPS units.

wildflower blooms and local weather conditions heavily influences butterflies. Dry years can lead to greatly reduced flights of adult butterflies; in addition, cold temperatures, cloudy skies, and high winds all depress butterfly activity. It is possible on some spring days to find no butterflies at all. Over the years, some counts have been cancelled and rescheduled on fairly short notice when late season storms produced poor conditions for butterfly activity. The variability of desert weather makes setting count dates an annual challenge.

Count participants are divided into parties of 2-4 people and assigned specific areas to survey. It is essential that every party have at least one person able to identify nearly all of the butterflies that might be encountered. Since the count

program relies on identification through recognition of field marks, most identification must occur in the moment of observation. Occasionally, netting and releasing butterflies will be done in the field to help identify an individual. Photographs can be used to identify or confirm an identification later, but obtaining usable photos is not always possible, as some species are skittish and highly elusive. Over time, dedicated volunteers can become proficient at field identification. In developing a group of skilled volunteers for butterfly counts, we often recruited from local birdwatchers and wildflower enthusiasts; these amateur naturalists often make good butterfly observers since they already have developed skills of pattern recognition and looking for very specific field

marks to enable correct identifications. Many experienced birders are also familiar with the NABA data collection protocol through their participation in Christmas Bird Counts.

One way to increase the expertise factor within park-based citizen-science programs is to rely on knowledgeable staff, volunteers, and partners who work in non-science disciplines, but who have the interest and dedication to employ their experience in field-based data collection activities. Where individuals already possess detailed intimate knowledge of an NPS unit, its resource base, and its approaches to resource management, they bring advantages many other volunteers may lack. Networking with NABA members can also generate skilled volunteers, as

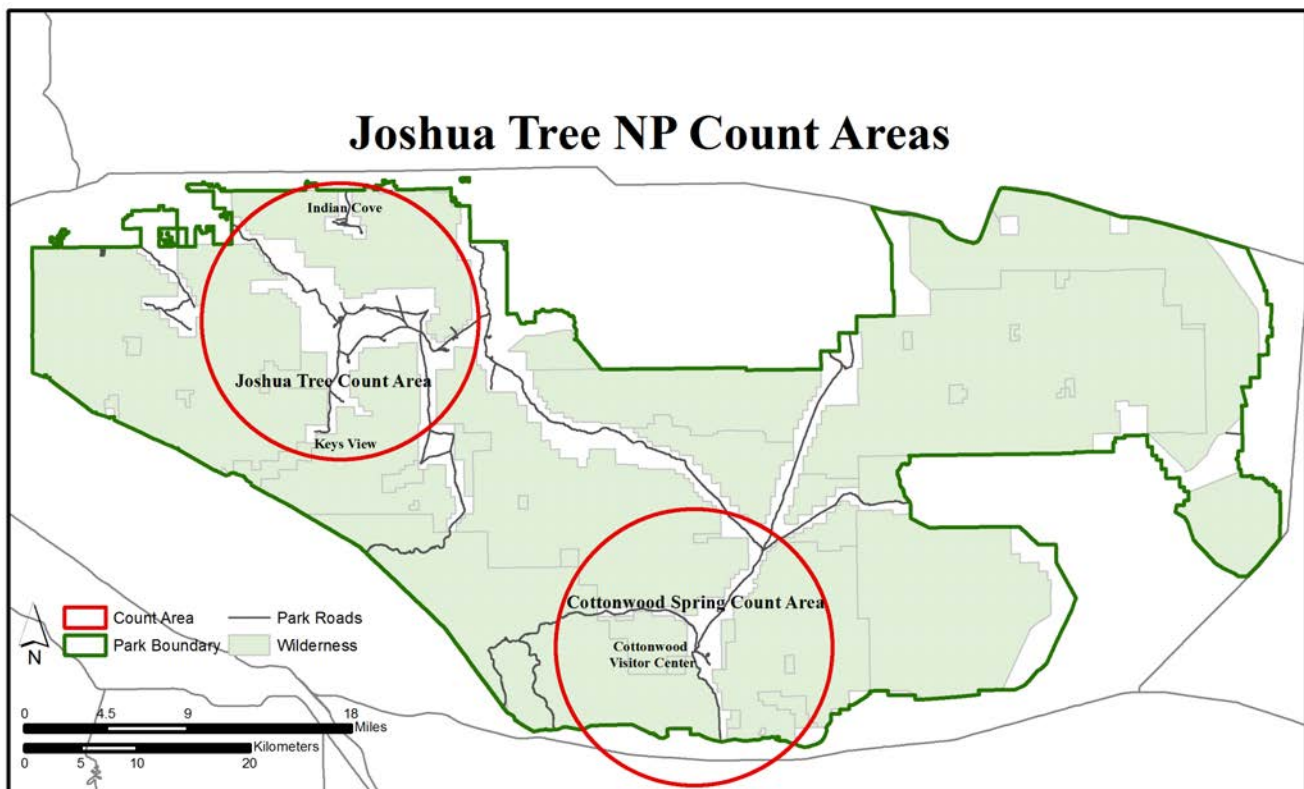


Figure 3. Map of JTNP showing the two areas used for the annual NABA butterfly counts. The “Joshua Tree” count is conducted in spring and fall, since 1995 and 2014, respectively. The Cottonwood Spring count was started in 2006 and is conducted in early spring.

was the case at JTNP. For several years, Fred Heath, a one-time President of NABA's Board of Directors and co-author of the field guide *Introduction to Southern California Butterflies*, took part in the Joshua Tree spring count. Also, becoming active in NABA regionally can benefit a local effort. For example, Marilyn Lutz became a regional editor for NABA's Southern California (Region 1) counts. This led to improved awareness of JTNP's butterfly counts within the California butterfly community and among NABA's national leadership.

To increase the effectiveness of our early counts, a literature search was conducted to create a checklist of species known, or likely to occur, at JTNP. We reviewed past butterfly studies conducted in and around JTNP in order to gather records on park butterflies, for example:

1. Elbert Sleeper (1959) published the results of early invertebrate surveys that included lepidoptera at Joshua Tree National Monument.
2. Thomas and John Emmel (1973) included considerable information about park

butterflies in their seminal publication, *Butterflies of Southern California*.

3. David Eiler (1985) documented his butterfly sightings from Joshua Tree National Monument and the nearby Big Morongo Canyon Preserve.
4. Drs. Gordon Pratt and John Emmel conducted field studies at the park from 1969 through 1998, and their field notes have been invaluable resources (Emmel 2018 and Pratt 1995).
5. Wanda Dameron (1997) summarized butterfly records for Joshua Tree National Monument and the Coachella Valley.
6. Walter Sakai (1999) submitted reports on annual inventories conducted by his Santa Monica College field ecology classes that included butterfly specimens from JTNP. Sakai also included published data from the early NABA counts at JTNP.
7. The butterfly specimens from the park museum collection, comprising 48 species collected within the park, were also an important source.

Based on these sources and the

early years of observational data from our counts, a rough checklist of park butterflies numbering about 80 species gradually emerged around 2007. One important outcome of this first checklist was that it highlighted a number of "rare" species. Drs. Pratt and Emmel had recorded a number of species that, even after 25 years of count data, have still not been relocated in the park. This rarity can be due to a number of things from inherent scarcity due to biological or ecological reasons to a lack of appropriate field efforts at the right time or in the proper habitat.

Today, the current park butterfly checklist numbers 85 species. The taxonomy used for the checklist generally follows the North American Butterfly Association's *Checklist of North American Butterflies* (NABA 2001), but for some species and groups, we have chosen to use Warren, et. al. (2013). An additional 14 species occur close to JTNP, but they have not yet been found within the park.

During the twenty-five years of conducting NABA counts, participants have observed and recorded 68 species of butterflies.

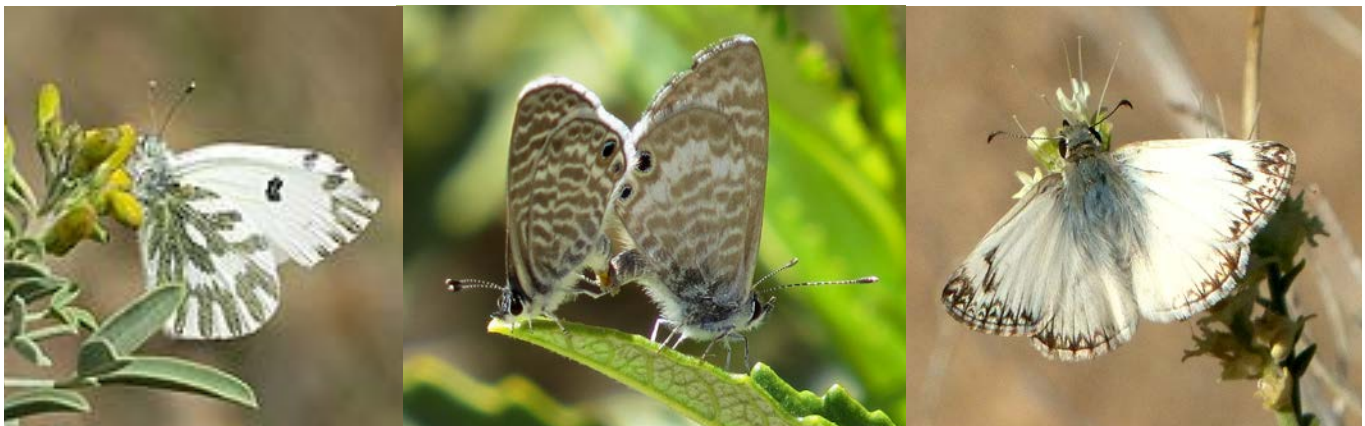


Figure 4. During the 25 years of conducting NABA butterfly counts at JTNP, seven species stand out as being the most frequently observed (21 of 25 years): checkered white, Becker's white (left), marine blue (center), variable checkerspot (not shown), northern white skipper (right), sleepy orange (Figure 2), and western pygmy-blue. Photos by Joe Zarki.

While no single species has been recorded every year, seven have been found in 21 of 25 years (Figure 4): checkered white (*Pontia protodice*), Becker's white (*Pontia beckerii*), sleepy orange (*Abaeis nicippe*), western pygmy-blue (*Brephidium exilis*), marine blue (*Leptotes marina*), variable checkerspot (*Euphydryas chalcedona*), and northern white skipper (*Heliopetes ericetorum*). Nineteen other species have been recorded in at least 15 of the 25 years that counts have been run. Conversely, 27 species have been recorded five or fewer times, and eight species have been found on only a single count.

The annual NABA Count Report includes a list of high yearly totals and record high totals for each North American species. Because the park

conducts its counts in the spring and fall seasons, park butterfly counts are sometimes able to produce high totals for the nation for certain species that have their primary flight period outside the summer season. In 2017, the park had high annual totals for five species: 412 individuals of Sara orangetip (*Anthocharis sara*); 2 individuals of Wright's metalmark (*Calephelis wrightii*); 96 individuals of sagebrush checkerspot (*Chlosyne acastus*; Figure 5); 427 individuals of tiny checkerspot (*Dymasia dymas*); and 53 individuals of northern white skipper (*Heliopetes ericetorum*). The 2017 total of 412 Sara orangetips (Figure 5) set NABA's all-time record high for this species. The two Wright's metalmark sighted would appear to be a small number, but it

represented the high for all NABA counts in 2017. This is partly a reflection of the limited range of this species minimizing the number of counts where it can be found. The park also set a record high in 2016 of 147 Mormon metalmarks (*Apodemia mormo*; Figure 5). It is of interest to note that many authorities have split *Apodemia mormo* into a number of separate taxa, at both the subtaxon and species levels (Pratt, et. al. 2011; Warren, et. al. 2013). If we follow this more recent taxonomy, JTNP has three distinct taxa from this complex of *Apodemia mormo* butterflies: the desert Mexican metalmark (*Apodemia mejicanus deserti*), Behr's metalmark (*Apodemia virgulti mojavelimbus*), and the Mormon metalmark (*Apodemia mormo mormo*). However, NABA does not follow this taxonomy, therefore we count all *Apodemia mormo* taxa as one species for the NABA counts.

One of the most exciting discoveries during a JTNP butterfly count was finding a tagged monarch butterfly in the Smithwater Canyon area (Figure 5). This exceptional find occurred on October 9, 2016 during the fall Joshua Tree count. Studies have shown that recovery rates for tagged monarch butterflies average only about 1% of all individuals that are tagged (Taylor, et. al. 2016). Careful photographs of the hind wing enabled the tag number to be read, and it was determined through a program called Southwest Monarch Study (swtag.org) that this individual was tagged in southeast Arizona on September 20, 2016, and travelled approximately 350 miles WNW to JTNP in 20 days.

Including other fieldwork done outside the NABA Count



Figure 5. Significant numbers of five butterfly species were observed in 2017, two are shown here: Sagebrush checkerspot, *Chlosyne acastus* (top left), Sara orangetip, *Anthocharis sara* (top right). In 2017, the park observed a record high of Mormon metalmark, *Apodemia mormo mormo* (bottom right). Also in 2016, a tagged monarch was observed (bottom left). Photos by Joe Zarki.

| Common Name | Scientific Name | Date Observed | Observer(s) |
|----------------------------|--------------------------------|---------------|---------------------------------------|
| Pipevine swallowtail | <i>Battus philenor</i> | 10/13/2014 | Bill Truesdell |
| Giant swallowtail | <i>Papilio cresphontes</i> | 7/10/2007 | Marilyn Lutz |
| Large orange sulphur | <i>Phoebis agarithe</i> | 3/27/2015 | Marilyn Lutz |
| Mexican yellow | <i>Eurema mexicana</i> | 10/24/2014 | Joe Zarki |
| Silver-banded hairstreak * | <i>Chlorostrymon simaethis</i> | 4/30/2017 | Bob Cullen, Tom Haworth, Donna Thomas |
| Mallow scrub-hairstreak | <i>Strymon istapa</i> | 3/29/2014 | Robb Hannawacker |
| Variiegated fritillary | <i>Euptoieta claudia</i> | 4/26/1997 | Marilyn Lutz |
| Arizona powdered-skipper | <i>Systasea zampa</i> | 5/5/2001 | Joe Zarki |
| Mojave sootywing | <i>Hesperopsis libya</i> | 5/6/2000 | Marilyn Lutz |
| Fiery skipper ** | <i>Hylephila phyleus</i> | 9/29/2015 | Tom Haworth |
| Sandhill skipper | <i>Polites sabuleti</i> | 5/5/2001 | Marilyn Lutz |

Table 2. Eleven species have been added to the inventory through citizen science observations posted on iNaturalist. *There is a record for *C. simaethis* from April 16, 1992 by Kelly Richers on Utah Trail near the park’s North Entrance, however it cannot be determined if the location was within the park’s 1992 boundaries. **An exotic species.

program, 79 butterfly species have been documented within the park by volunteer observers. Many of these were photographed and posted to the park’s “Arthropods of Joshua Tree National Park Project” on iNaturalist.org. As of October 2019, there were 519 observations in the Arthropods Project, 433 are butterfly records representing 71 different species.

Citizen science fieldwork at JTNP has documented the first park records for 11 butterfly species (Table 2, Figures 6 and 7). For example, in 2017, park volunteers Bob Cullen, Tom Haworth, and Donna Thomas documented the first confirmed park record for silver-banded hairstreak (*Chlorostrymon simaethis*; Figure 6). Thanks to years of butterfly fieldwork, they had the presence

of mind to recognize that this Lycaenid was not like any others they had seen before. Former JTNP park ranger Robb Hannawacker also made many significant observations including the discovery of the first mallow scrub-hairstreak (*Strymon istapa*; Figure 6) ever found in the park on March 29, 2014, near Cottonwood.

In a broader context, we now



Figure 6. Citizen science fieldwork at JTNP has documented the first park records for 11 butterfly species, three of which are shown here: the silver-banded hairstreak (*Chlorostrymon simaethis*) was observed in 2017 (left); the mallow scrub-hairstreak (*Strymon istapa*) was observed in 2014 (middle); and the variegated fritillary (*Euptoieta claudia*) was observed in 2011 (right). Photos by Bob Cullen, Robb Hannawacker, and Joe Zarki.

have over 25 years of count data for 68 butterfly species (Table 3). This kind of long-term data allows the park to document interesting ecological observations, as well as develop monitoring programs to assess distribution and population trends.

they have coevolved such a partnership. This can affect the distribution of the butterflies within the park since their caterpillars may not be able to survive without protection from the ants. Even in areas with otherwise suitable habitat, a butterfly species may be absent

blue larvae in the park. Once the ant species is identified, it may be possible to align the distribution of the *pallidescens*-coevolved ants with yucca buckwheat stands to better predict locations for pallid dotted-blues throughout JTNP.

Certain butterflies are known to exhibit irruptive life cycles; the painted lady (*Vanessa cardui*) is one well-known example. For the most part, we do not understand the cause for these long-term population cycles where a species will be completely absent from the park for many years at a time, but then be present by the millions in other years. The California tortoiseshell (*Nymphalis californica*) is a species that exhibits irruption cycles. Rarely seen at JTNP, these large and boldly marked Nymphalids were reported from many locations across southern California in 2017. This led to their discovery in the Lower Covington area of the park where they were present in modest numbers through the summer and fall of that year.

Joshua Tree National Park's geographic overlap with the Sonoran Desert along its southeastern boundary means that the park is home to a subset of species generally associated with the Sonoran Desert. Following monsoonal rains, typically between July and September, various semi-tropical butterflies will exhibit migrational movements that result in incursions into southern California. Successful breeding causes some individuals to push out beyond the edges of their normal range and bring them into the park. One reason a fall count was started was to capture the presence of some of these species such as the Mexican yellow (*Eurema mexicana*; Figure 7). For



Figure 7. Several species in the park are best observed during the fall season, for example: Mexican yellow (top left), Dammer's dotted-blue (top right), red admiral (bottom right), and American snout (bottom left). Photos by Robb Hannawacker (bottom left) and Joe Zarki.

Butterflies lead much more complex lives than might be suspected given their small size. For example, many hairstreaks and blues have symbiotic relationships with ants, in some cases with a single ant species (Ballmer and Pratt, 1991). These butterflies have evolved anatomical structures enabling them to generate semiochemicals that mimic ant pheromones and secretions containing sugars and amino acids. Some ant species respond positively to these secretions and will actively protect the caterpillars with which

without the presence of its caterpillar protector.

The pallid dotted-blue (*Euphilotes pallescens elvirae*) is an example of a species that exhibits this relationship. Based on the data we have collected, it is currently known from only a few locations within the park even though it's host plant, yucca buckwheat (*Eriogonum plumatella*), occurs widely across the park (however, only a small part of the suitable habitat within the park has been examined). At present, it is not known which ant species attends to pallid dotted-



Figure 8. Four vulnerable species of hairstreaks have been identified in the park: Behr's hairstreak (left), hedgerow hairstreak (middle); mountain mahogany hairstreak (right), and thicket hairstreak (not pictured). All occur within narrow ecological zones and are adapted to one, or only a few, caterpillar host plants. Photos by Joe Zarki and Tom Haworth (right).

other resident species of both the Mojave and Sonoran Deserts, fall is their normal flight time, and you will miss them completely unless you are looking from late summer into early November. Dammer's dotted-blue (*Euphilotes enoptes dammersi*; Figure 7) and the Mormon metalmark (*Apodemia mormo*; Figure 5) are among these fall flyers at JTNP. Some species may fly in spring, but are nonetheless, more likely to be encountered during the fall season. Mallow scrub-hairstreak (*Strymon istapa*, Figure 6), American snout (*Libytheana carinenta*; Figure 7), and red admiral (*Vanessa atalanta*; Figure 7) are among the butterflies that are more frequently found in fall.

Joshua Tree National Park is subject to a variety of long-term environmental stresses that may have long-term impacts on butterfly populations. Among these stresses are climate change, exotic plant species, wildfires, air pollution, changes in native plant communities, and urban development along park boundaries. A number of

butterfly species found in the park's higher elevations, along its western reaches, have populations that may be at risk if the park becomes warmer and drier over time. Hopkins (2018) in the Inland Deserts Region Report of California's Fourth Climate Change Assessment noted, "A warming and likely drier climate, with more intense droughts, would clearly stress populations anywhere. For those species already at or near their presumed physiological limits living in deserts, there is reason for concern..."

Currently, we have identified four species of hairstreaks (Table 4) that depend on host plants that already have restricted ranges across the park's higher mountains. These species: Behr's hairstreak (*Satyrium behrii*), mountain mahogany hairstreak (*Satyrium tetra*), hedgerow hairstreak (*Satyrium saepium*), and thicket hairstreak (*Callophrys spinetorum*), all occur within narrow ecological zones and are adapted to one, or only a few, caterpillar host plants. If

populations of their host plants decline, it is possible that local populations of these butterflies may also decline and could one day be extirpated within the park. During fieldwork at the park in the 1980s and 1990s, Drs. John Emmel and Gordon Pratt made numerous observations of these species (Emmel, 2018; Pratt, 1995). However, no observations of these rare hairstreaks have been made during the authors 25 years of field work, and little is known about the present park populations of these four butterflies. However, more focused and systematic searches of suitable habitat could produce future observations of these species and shed further light on their status with the park.

Overall, the data collected over the years through the NABA counts and other citizen science efforts has established a much-needed inventory of the butterfly species known to occur in JTNP. In addition, the data has provided baseline information regarding fluctuations in abundance and frequency of these species. Importantly, however, now that

we have an inventory and basic population data for the park, the hope is to encourage additional research on the many species that occur here.

There remains a critical need for a better understanding of the

population status and trends for most park species. In 2001 when Roland Wauer made the statement that leads this article, only four national parks were participating in NABA count surveys and just a handful more

had other efforts in place to document and assess their lepidopteron fauna (Wauer, 2001). Inventory and monitoring programs within the NPS are now more robust and all-taxa surveys have been conducted at a growing

| | Joshua Tree (Spring Counts) | Cottonwood (Spring Counts) | Joshua Tree (Fall Counts) | Total (All counts combined) |
|----------------------------|--|---------------------------------------|--------------------------------------|--|
| Total # Counts: | 25 | 14 | 6 | 45 |
| # SPECIES RECORDED: | | | | |
| Total | 59 | 43 | 46 | 68 |
| High | 36 | 29 | 28 | 36 |
| Low | 3 | 8 | 18 | 3 |
| Median | 24 | 22 | 25 | 23 |
| Average | 22 | 19 | 23 | 21 |
| INDIVIDUALS: | | | | |
| High | 2,626 | 4,476 | 957 | 4,476 |
| Low | 3 | 39 | 79 | 3 |
| Median | 303 | 499 | 299 | 308 |
| Average | 576 | 884 | 372 | 645 |

Table 3. Joshua Tree National Park NABA Seasonal Count Summary, 1995-2019. Three counts are conducted in the park each year, two in the spring and one in the fall. Over the last 25 years, we have conducted 45 counts and recorded a total of 68 species. The highest number of individuals ever recorded in one count was 4,476. On average, we observe 645 individual butterflies representing 21 species during our counts.

| SPECIES | HOST PLANT(S) | HABITAT |
|--|---|--|
| Behr's hairstreak <i>Satyrium behrii</i> | <i>Purshia tridentata, Purshia glandulosa</i> | Chaparral, pinyon-juniper-oak woodlands |
| Mountain mahogany hairstreak <i>Satyrium tetra</i> | <i>Cercocarpus betuloides</i> | Chaparral, mountain hillsides |
| Hedgerow hairstreak <i>Satyrium saepium</i> | <i>Ceanothus sp.</i> | Chaparral, hillsides, canyons |
| Thicket hairstreak <i>Callophrys spinetorum</i> | <i>Arceuthobium sp.,</i> dwarf mistletoe on conifers | Pinyon pine woodlands |

Table 4. Vulnerable butterfly species in Joshua Tree National Park. Four species of hairstreaks have been identified in the park as vulnerable due to the restricted distribution of their host plant(s) and habitat in the park. All occur within narrow ecological zones and are adapted to one, or only a few, caterpillar host plants.

number of parks.

Yet invertebrates remain among the poorest known and least studied faunal groups within the National Park System. However, Rep. Raul Grijalva (AZ) recently sponsored the Extinction Prevention Act of 2019, a component of which is the North American Butterfly Conservation Act (H.R. 2918). This legislation would establish a butterfly conservation fund to promote the research, conservation, and education of Lepidoptera and dedicate up to \$5,000,000 annually toward those objectives. While the legislation's fate is uncertain, if enacted, it would provide a much-needed boost to severely underfunded efforts by scientists and volunteers on behalf of butterflies at Joshua Tree, and elsewhere.

References

- Ballmer, G.R. and G.F. Pratt. 1991. Quantification of ant attendance (Myrmecophily) of Lycaenid larvae. *Journal of Research on the Lepidoptera* 30(1-2):95-112.
- Dameron, W. 1997. Searching for butterflies in southern California. Flutterby Press: Los Angeles, California USA.
- Eiler, D.L. 1985. David L. Eiler butterfly records, Joshua Tree National Monument and nearby locations, 1984-85. Unpublished Report. David L. Eiler, Personal correspondence. North Manchester, Indiana, USA.
- Emmel, T.C. and John F. Emmel. 1973. The butterflies of southern California. Natural History Museum of Los Angeles County Contributions in Science Series 26: 1-148.
- Emmel, J.F. 2018. Field notes, Joshua Tree National Monument/National Park, March, 1969-June, 1998. Unpublished Report. Dr. Gordon Pratt, E-mail Correspondence, Jan. 30, 2018. Anza, California, USA.
- Frakes, Neil. 2018. Invasive plant management at Joshua Tree National Park. National Park Service: Joshua Tree National Park, Division of Science and Resource Stewardship. Downloaded October 17, 2018: <https://www.cal-ipc.org/wp-content/uploads/2018/02/Cal-IPC-Symposium-2017-Managing-Invasives-Joshua-Tree-Neil-Frakes.pdf>.
- Hopkins, Francesca. (University of California, Riverside). 2018. *Inland Deserts Summary Report*. California's Fourth Climate Change Assessment. Publication number: SUM-CCCA4-2018-008.
- Laufer, P. 2009. The dangerous world of butterflies, the startling subculture of criminals, collectors, and conservationists. The Lyons Press. Guilford, Connecticut, USA.
- Lepidopterist Society, 2018. Frequently asked questions, "how many butterflies and moth are there in the world? In specific parts of the world?" <https://www.lepsoc.org/content/frequently-asked-questions#9>. The Lepidopterist Society, San Francisco, California, USA.
- Mast, Katie. 2013. Volunteers track migrations of declining monarch populations. *High Country News*, September 2, 2013. <https://www.hcn.org/blogs/goat/volunteers-help-track-western-monarch-migration>.
- Monroe, Lynn and Gene. 2004. Butterflies and their favorite flowering plants. Anza-Borrego Desert State Park and environs. Merryleaf Press. Lyons, Colorado, USA.
- NABA. Butterfly counts. 2018a. http://www.naba.org/butter_counts.html. North American Butterfly Association, Morristown, New Jersey, USA.
- NABA. 2018b. About NABA. <http://www.naba.org/aboutNABA.html>. North American Butterfly Association, Morristown, New Jersey, USA.
- NABA. 2018c. Butterfly questions and answers. <https://www.naba.org/qanda.html>. North American Butterfly Association, Morristown, New Jersey, USA.
- NABA. 2018. NABA Butterfly counts, 2017 Report. Sharon Wander, Ed., North American Butterfly Association, Morristown, New Jersey, USA.
- NABA. 2001. North American Butterfly Association (NABA) Checklist & English names of North American butterflies, 2nd Ed. <http://www.naba.org/pubs/checklst.html>. North American Butterfly Association, Morristown, New Jersey, USA.
- Pratt, G. F. 1995. Field notes, Joshua Tree National Monument/National Park & Big Morongo Canyon Preserve, 1994-1995. Unpublished Report. National Park Service: Joshua Tree National Park Library, Twentynine Palms, California, USA.
- Pratt, G.F. J.F. Emmel, and G. Bernard. 2011. The buckwheat metalmarks. *American Butterflies* 19:2/3/4, pp. 4-31. North American Butterfly Association, Morristown, New Jersey, USA.
- Pyle, Robert Michael. 1984. Audubon Society handbook for butterfly watchers. Charles Scribners & Sons: New York, New York, USA.
- Sakai, W.H. and N.D. Hogg. 2000. Animal inventories in Joshua Tree National Park with special emphasis on sensitive species, sensitive areas and lands newly added to the park under the Desert Protection Act. Santa Monica College: Santa Monica, California, USA.
- Sleeper, E.L. 1959. The distribution of the insects and some insect allies of Joshua Tree National Monument. California State College, Long Beach, California, USA.
- Taylor, et. al. 2016. Is the monarch decline due to an increase in mortality during the fall migration? Monarch Watch, Kansas Biological Survey: University of Kansas, Lawrence, KS.
- Walker, Dennis, 2007. Southern California butterflies. (<http://www.socalbutterflies.com>)

Warren, A.D., K.J. Davis, E.M. Strangeland, J.P. Pelham, and N.V. Grishin. 2013. Illustrated lists of American butterflies [21-XI-2017]. <http://butterfliesofamerica.com/index.html>.

Warren, A.D., K.J. Davis, E.M. Strangeland, J.P. Pelham, and N.V. Grishin. 2013. Riodinidae. <http://butterfliesofamerica.com/list.htm#RIODINIDAE>.

Wauer, R. 2001. Wings of change. *National Parks*, May/June 2001. National Parks Conservation Association. Washington, District of Columbia, USA.

Wilson, A., K. Bacher, I. Breckheimer, J. Lundquist, R. Rochefort, E Theobald, L. Whiteaker, and J. HilleRisLambers. Monitoring wildflower phenology using traditional science, citizen science, and crowdsourcing approaches. *Park Science* 33(1):17-26.

Author Biographies

Originally from Maryland, Joe Zarki thoroughly enjoyed a 38-year career as a National Park Service ranger. He had stints at Death Valley, Denali, Tuzigoot National Monument, Yellowstone, and he served as Chief of Interpretation at Badlands National Park and at Joshua Tree (1995-2013). Since his retirement in 2013, Joe has authored a number of publications including the popular history, *Images of America, Joshua Tree National Park*. He has also co-authored publications on birds of Joshua Tree and on the reptiles and amphibians of the park. In 2019, he was named to the Board of Directors of the Joshua Tree National Park Association. Long active in citizen activities, Joe coordinates Christmas Bird Counts at Joshua Tree National Park and Morongo Valley. Together with his wife Marilyn Lutz, he helps organize butterfly counts at Joshua Tree National

Park and at Big Bear Lake. Joe volunteers at the Big Morongo Canyon Preserve and also plays guitar in several local music projects.

Marilyn has been interested in butterflies since she first assisted Dr. Gillian Bowser with fieldwork for a butterfly phenology project in Yellowstone NP in 1984. She compiled the first North American Butterfly Association (NABA) count in Yellowstone in 1990, followed by counts in Badlands NP 1991-94, and Joshua Tree 1995 to present. While at the Badlands, she began assisting NABA as a count editor for the Northern Plains Region. After moving to Joshua Tree, she switched to editing the Southern California Region. Marilyn and Joe met in Yellowstone in 1985 and have been birding and butterflying together ever since. Marilyn is a Facility Management Specialist and GSA Fleet Manager for the National Park Service at Joshua Tree National

Park. When not butterfly watching, she enjoys cooking, reading, running, and yoga.



A preliminary report on the bees of Joshua Tree National Park, with special focus on *Anthophora*, Digger Bees

Michael C. Orr¹

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.

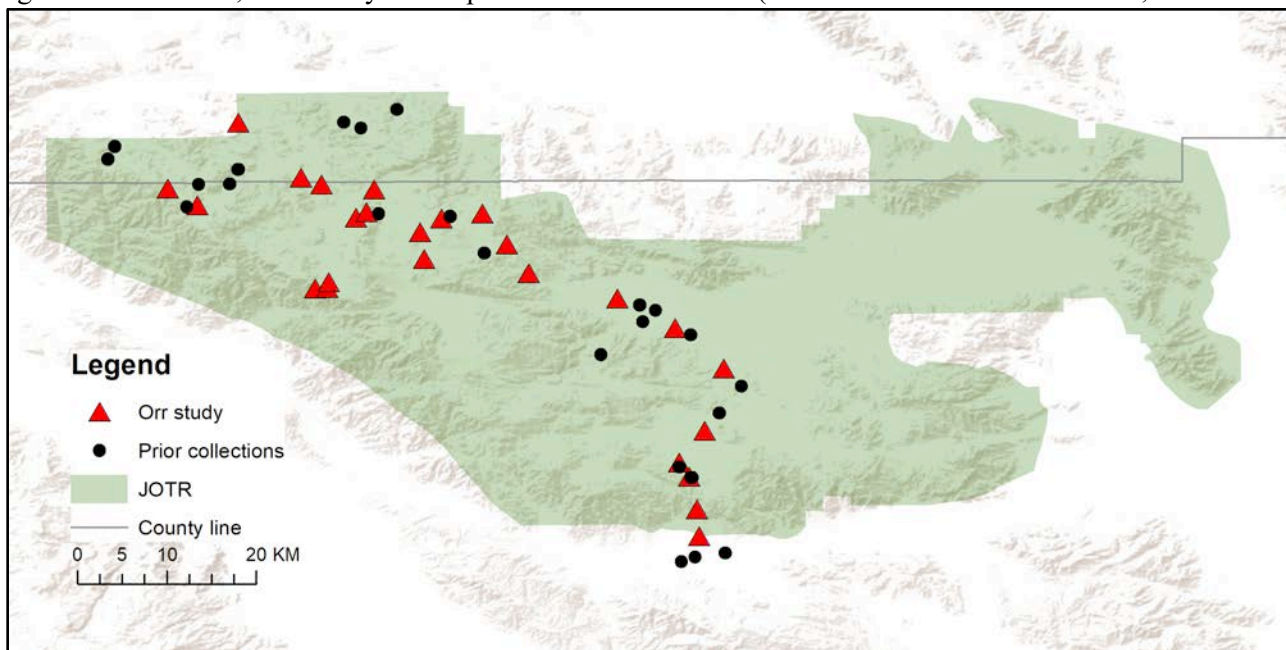


Figure 1. Collection sites within and around JTNP for specimens used to create the inventory for this study. The present-day area of JTNP is indicated as a green polygon. Localities from the present study are given as red triangles and prior collections are given as black circles. County lines are given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

¹Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing, P.R. China.

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 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). 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



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. Left: *Psorothamnus* specialist *Anthophora hololeuca* was newly detected within JTNP in this study; Center: *Anthophora pueblo* peeking out of its nest in a sandstone bank; Right: *Anthophora urbana*, a supergeneralist species, collecting pollen from *Ericameria* (Asteraceae). Photos by M. Orr.

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 or 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.

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

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 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

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

country continue to database their specimens, it seems likely that these gaps will be filled.

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 is one of only two *Anthophora* 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



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).

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.

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



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.

study. This is a testament to the incredible invertebrate diversity yet to be discovered in the Desert Southwest: nearly 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

| <i>Anthophora</i> species: | | | |
|-----------------------------------|----------------------|------------------------|------------------------------|
| <u>Subgenus</u> | <u>species</u> | <u>Documentation</u> | <u>Notes</u> |
| <i>Anthophoroides</i> | <i>californica</i> | Expected, not Observed | |
| <i>Lophanthophora</i> | <i>neglecta</i> | Expected, not Observed | |
| <i>Micranthophora</i> | <i>mortuaria</i> | Expected, not Observed | |
| <i>Micranthophora</i> | <i>columbariae</i> | Both | |
| <i>Micranthophora</i> | <i>curta</i> | Both | |
| <i>Micranthophora</i> | <i>estebana</i> | Both | |
| <i>Micranthophora</i> | <i>petrophila</i> | Both | |
| <i>Mystacanthophora</i> | <i>urbana</i> | Both | |
| <i>Paramegilla</i> | <i>centrifformis</i> | Both | |
| <i>Paramegilla</i> | <i>fulvicauda</i> | Both | |
| <i>Anthophoroides</i> | <i>signata</i> | Orr | |
| <i>Lophanthophora</i> | <i>coptognatha</i> | Orr | |
| <i>Lophanthophora</i> | <i>dammersi</i> | Orr | |
| <i>Micranthophora</i> | <i>abroniae</i> | Orr | |
| <i>Micranthophora</i> | <i>hololeuca</i> | Orr | |
| <i>Pyganthophora</i> | <i>vannigera</i> | Orr | |
| <i>Micranthophora</i> | <i>pachyodonta</i> | Prior | |
| <i>Anthophoroides</i> | <i>cinerula</i> | Expected, not Observed | Undescribed at time of study |
| <i>Anthophoroides</i> | <i>pueblo</i> | Expected, not Observed | Undescribed at time of study |
| <i>Micranthophora</i> | <i>striata</i> | Expected, not Observed | Undescribed at time of study |
| <i>Micranthophora</i> | <i>parkeri</i> | Orr | Undescribed at time of study |
| <i>Micranthophora</i> | <i>timberlakei</i> | Orr | Undescribed at time of study |

Table 3. Species of *Anthophora* found or expected to occur in JTNP. Documentation refers 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.

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 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



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 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

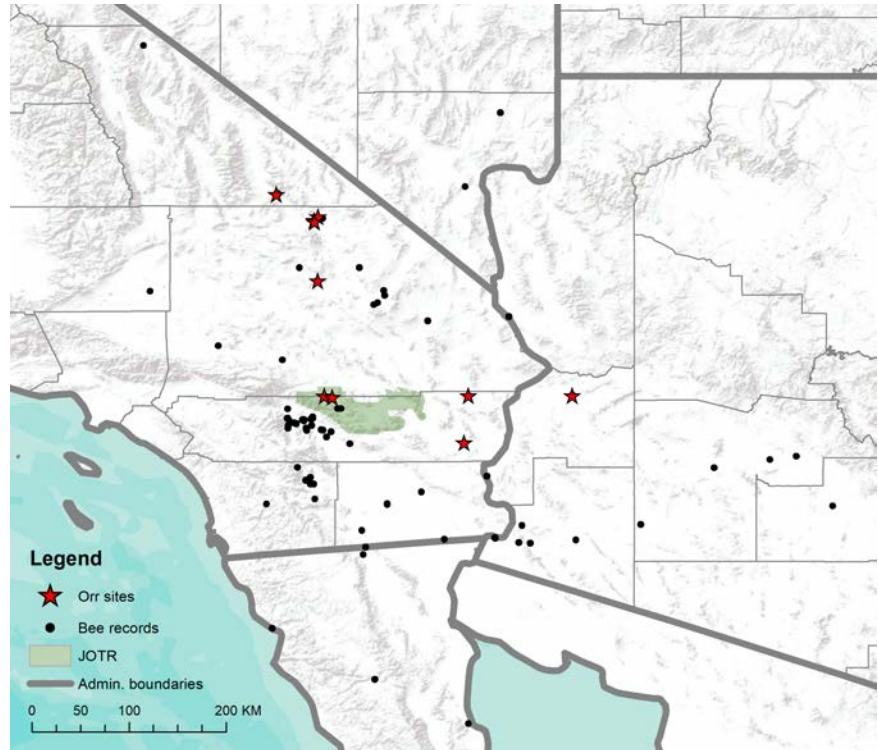


Figure 6. All known collection sites of *Anthophora abroniae* (black circles). *Anthophora abroniae* was collected by the author at 10 sites, all of which had *Abronia villosa* in bloom (red stars). Joshua Tree National Park is shown as a green polygon; state and county boundaries are shown as gray lines.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

good places to document bee diversity (Figure 7). In extreme desert environments, where precipitation can be quite limited, areas with more consistent bloom 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*, *Psoralea 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.

Acknowledgements

I first thank Harold W. Ikerd and Tasha La Doux for their identifications and general support. Zachary M. Portman is also thanked for his

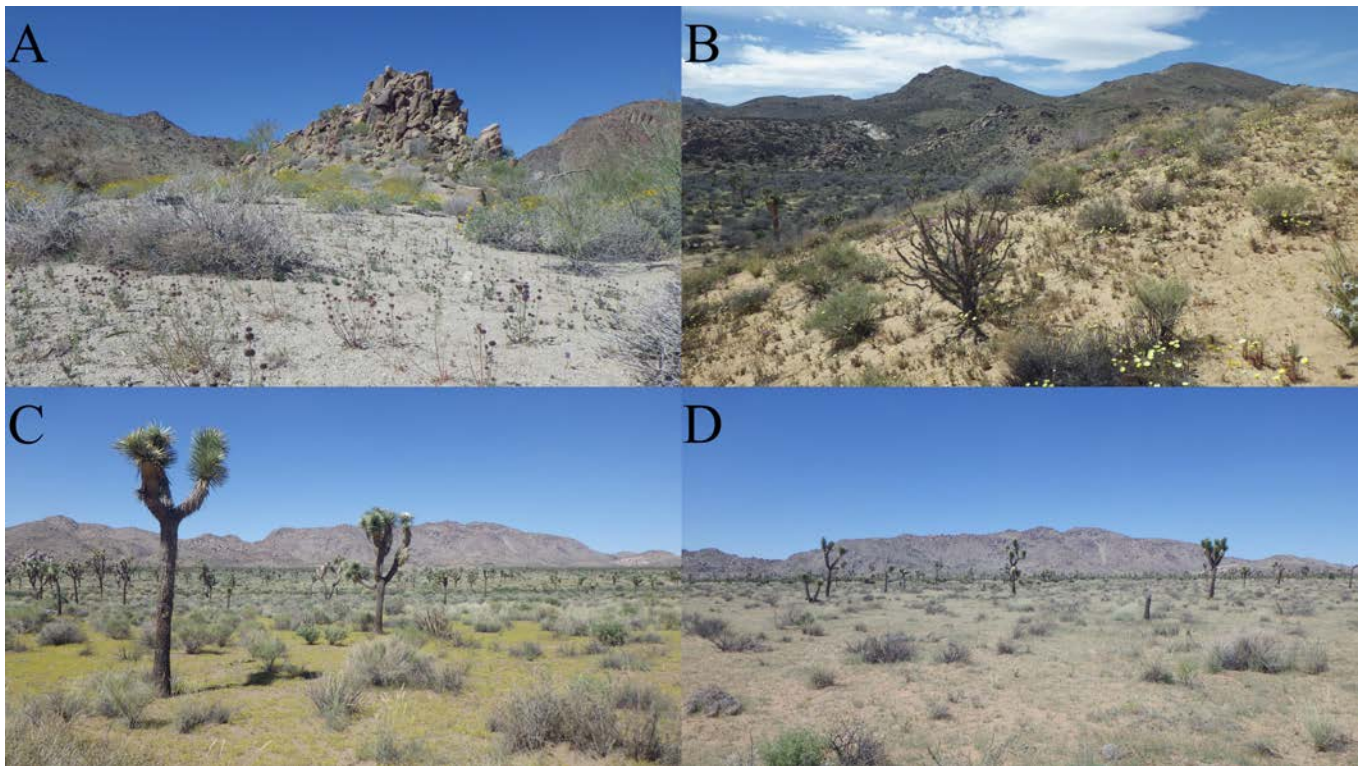


Figure 7. The best sampling localities from this study. **A)** Cottonwood Wash, where *Chaenactis* sp., *Encelia farinosa*, *Larrea tridentata*, *Lupinus* sp., *Malacothrix* sp., *Parkinsonia florida*, *Phacelia campanularia*, *Salvia columbariae* were in bloom during spring 2016. **B)** Sandy bench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

identifications, as well as his input on *Perdita* biology. Terry Griswold, Vincent J. Tepedino, and Amber D. Tripodi are thanked for valuable discussions. Four anonymous reviewers are also thanked for their many improvements. This project was carried out with funding awarded to MCO by Joshua Tree National Park Association's Robert Lee Graduate Student Research Program and The Community Foundation's Desert Legacy Fund under permission from National Park Service study JOTR-00237, permit JOTR-2015-SCI-0006.

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.

References

- Adondakis, S., and D.L. Venable. 2004. Dormancy and germination in a guild of Sonoran Desert annuals. *Ecology* 85(9):2582–2590.
- Bartomeus, I., J.S. Ascher, D. Wagner, B.N. Danforth, S. Colla, S. Kornbluth, and R. Winfree. 2011. Climate-associated phenological advances in bee pollinators and bee-pollinated plants. *Proceedings of the National Academy of Sciences* 108(51):20645–20649.
- Brooks, R.W. 1988. Systematics and Phylogeny of the Anthophorine Bees (Hymenoptera; Anthophoridae; Anthophorini). *The University of Kansas Science Bulletin* 53:436–575.
- Cane, J.H., and S. Sipes. 2006. Characterizing floral specialization by bees: analytical methods and a revised lexicon for oligolecty. Pages 99–122 in N.M. Waser and J. Ollerton, editors. *Plant-pollinator interactions: from specialization to generalization*. University of Chicago Press, Chicago, USA.
- Danforth, B.N. 1999. Emergence dynamics and bet hedging in a desert bee, *Perdita portalis*. *Proceedings of the Royal Society of London B: Biological Sciences* 266(1432):1985–1994.
- DL. 2017. DiscoverLife.org. <http://www.discoverlife.org/> (Accessed 22–24 November 2017.)
- Forrest, J.R.K. 2015. Plant-pollinator interactions and phenological change: what can we learn about climate impacts from experiments and observations? *Oikos* 124:4–13.
- GBIF. 2017. Global Biodiversity Information Facility. <https://www.gbif.org/> (Accessed 22–24 November 2017.)
- Gerst, K.L., and D.L. Venable. 2017. Phenology mediates reproductive success in the desert annual *Chylismia brevipes*. *Mojave National Preserve Science Newsletter*, April:8–12.
- Hegland, S.J., A. Nielsen, A. Lázaro, A.L. Bjerknes, and Ø. Totland. 2009. How does climate warming affect plant-pollinator interactions? *Ecology letters* 12(2):184–195.
- Hurd, P.D. 1957. Notes on the autumnal emergence of the vernal desert bee, *Hesperapis fulvipes* Crawford (Hymenoptera, Apoidea). *Journal of the Kansas Entomological Society* 30:10–10.
- Jurado, E., and M. Westoby. 1992. Germination biology of selected central Australian plants. *Austral Ecology* 17(3):341–348.
- Kemp, P.R. 1983. Phenological Patterns of Chihuahuan Desert Plants in Relation to the Timing of Water Availability. *Journal of Ecology* 71(2):427–436.
- Kopec, K., and L.A. Burd. 2017. Pollinators in Peril: A systematic status review of North American and Hawaiian native bees. Center for Biological Diversity.
- Litman, J.R., B.N. Danforth, C.D. Eardley, and C.J. Praz. 2011. Why do leafcutter bees cut leaves? New insights into the early evolution of bees. *Proceedings of the Royal Society of London B: Biological Sciences* 278:3593–3600.
- Michener, C.D. 1979. Biogeography of the bees. *Annals of the Missouri botanical Garden* 66(3):277–347.
- Michener, C.D. 2007. *The Bees of the World*. Second Edition. Johns Hopkins University Press, Baltimore, Maryland, USA.
- Minckley, R.L., J.H. Cane, and L. Kervin. 2000. Origins and ecological consequences of pollen

specialization among desert bees. *Proceedings of the Royal Society of London B: Biological Sciences* 267:265–271.

Nelson, R.A., and T.L. Griswold. 2015. The floral hosts and distribution of a supposed creosote bush specialist, *Colletes stepheni* Timberlake (Hymenoptera: Colletidae). *Journal of Melittology* 49:1–12.

NPIC. 2017. USDA-ARS National Pollinating Insects Collection, Logan, UT. (Accessed 22 November 2017.)

Olson, D.M., E. Dinerstein, E.D. Wikramanayake, N.D. Burgess, G.V.N. Powell, E.C. Underwood, J.A. D'Amico, I. Itoua, H.E. Strand, J.C. Morrison, C.J. Loucks, T.F. Allnutt, T.H. Ricketts, Y. Kura, J.F. Lamoreux, W.W. Wettengel, P. Hedao, and K.R. Kassem. (2001) Terrestrial ecoregions of the world: a new map of life on Earth. *Bioscience* 51:933–938.

Orr, M.C., J.B. Koch, T.L. Griswold, and J.P. Pitts. (2014). Taxonomic utility of niche models in validating species concepts: A case study in *Anthophora* (Heliophila) (Hymenoptera: Apidae). *Zootaxa* 3846(3):411–429.

Orr, M.C., T. Griswold, J.P. Pitts, and F.D. Parker. 2016. A new bee species that excavates sandstone nests. *Current Biology* 26(17):R792–R793.

Orr, M.C., J.P. Pitts, and T. Griswold. (2018). Revision of the bee group *Anthophora* (Micranthophora) (Hymenoptera: Apidae), with notes on potential conservation concerns and a molecular phylogeny of the genus. *Zootaxa* 4511(1):1–193.

Portman, Z.M., M.C. Orr, and T. Griswold. (2019). A review and updated classification of pollen gathering behavior in bees (Hymenoptera,

Apoidea). *Journal of Hymenoptera Research* 71:171–208.

Portman, Z.M., and V.J. Tepedino. 2017. Convergent evolution of pollen transport mode in two distantly related bee genera (Hymenoptera: Andrenidae and Melittidae). *Apidologie* 48(4):1–12.

Potts, S.G., V. Imperatriz-Fonseca, H.T. Ngo, M.A. Aizen, J.C. Biesmeijer, T.D. Breeze, L.V. Dicks, L.A. Garibaldi, R. Hill, J. Settele, and A.J. Vanbergen. 2016. Safeguarding pollinators and their values to human well-being. *Nature* 540(7632):220–229.

Rafferty, N.E., C.D. Bertelsen, and J.L. Bronstein. 2016. Later flowering is associated with a compressed flowering season and reduced reproductive output in an early season floral resource. *Oikos* 125(6):821–828.

Ritchie, A.D., R. Ruppel, and S. Jha. 2016. Generalist Behavior Describes Pollen Foraging for Perceived Oligolectic and Polylectic Bees. *Environmental Entomology* 45(4):909–919.

Settele, J., J. Bishop, and S.G. Potts. 2016. Climate change impacts on pollination. *Nature Plants* 2:16092.

Tevis, L. 1958. Germination and Growth of Ephemerals Induced by Sprinkling a Sandy Desert. *Ecology* 39(4):681–688.

Timberlake, P.H. 1956. A revisional study of the bees of the genus *Perdita* F. Smith, with special reference to the fauna of the Pacific Coast (Hymenoptera, Apoidea) Part II. University of California Press 11(5):247–350.

Tropicos. 2017. <http://www.tropicos.org/> (Accessed 23 November 2017.)

Weislo, W.T., and J.H. Cane. 1996. Floral resource utilization by solitary bees (Hymenoptera: Apoidea) and exploitation of their stored foods by natural enemies. *Annual Review of Entomology* 41:257–286.

Wilson, J.S., J.P. Pitts, and C.D. von Dohlen. 2009. Lack of variation in nuclear genes among isolated populations of the sand dune restricted bee *Colletes stepheni* (Hymenoptera: Colletidae). *Journal of the Kansas Entomological Society* 82(4):316–320.

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

Nuttapon Pombubpa¹, Tania Kurbessoian¹, Jason E. Stajich¹, Nicole Pietrasiak²

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).

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 contribute to effective soil nutrient cycles, 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

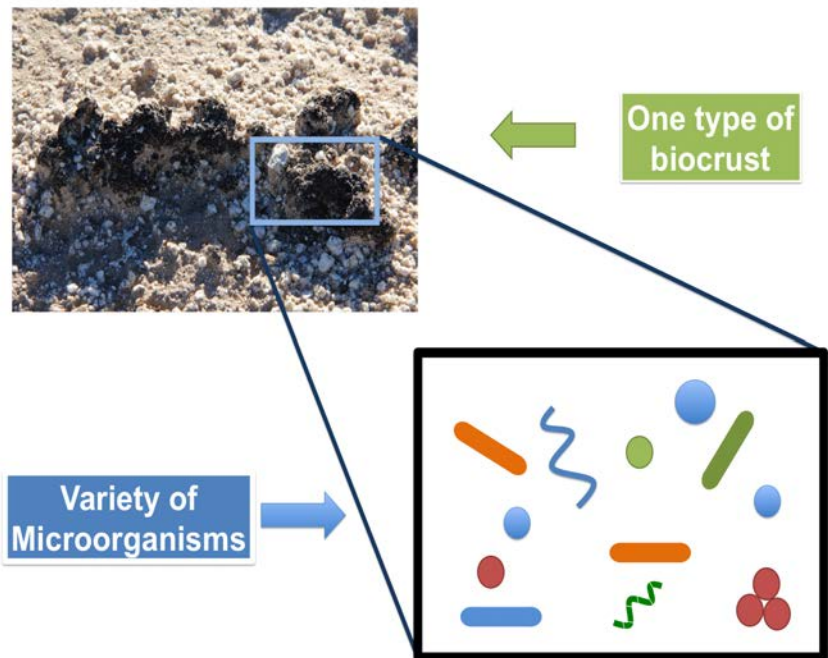


Figure 1. Biocrusts are like a “living skin” on top of arid lands soil. They consist of a living community of microorganisms such as bryophytes, lichens, eukaryotic algae, cyanobacteria, bacteria, and fungi. These complex communities contribute to healthy desert ecosystems by promoting nutrient cycling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

¹Department of Microbiology & Plant Pathology and Institute of Integrative Genome Biology, University of California, Riverside, Riverside, California 92521

²Plant and Environmental Sciences Department, New Mexico State University, Las Cruces, New Mexico 88003

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 hard 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 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 (Flehtner et al. 2013,

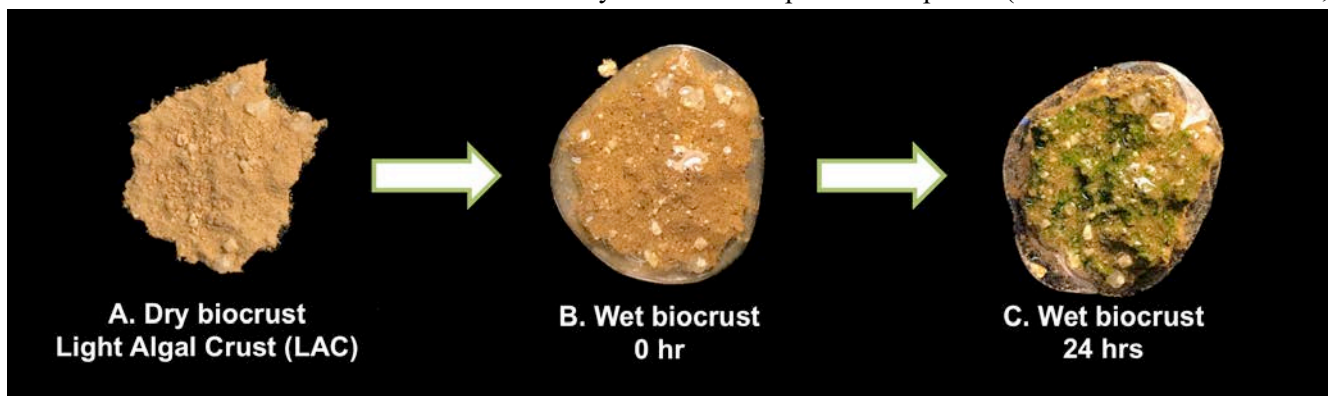


Figure 2. Light algal crusts (LAC) are the most common biocrust types in JTNP. They are dominated by cyanobacteria and other algae that quickly turn green once water is added; within 24 hours the algae and cyanobacteria become active (far right), but without the water are invisible to the naked eye (far left).

Pietrasiak et al. 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. 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 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, 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.

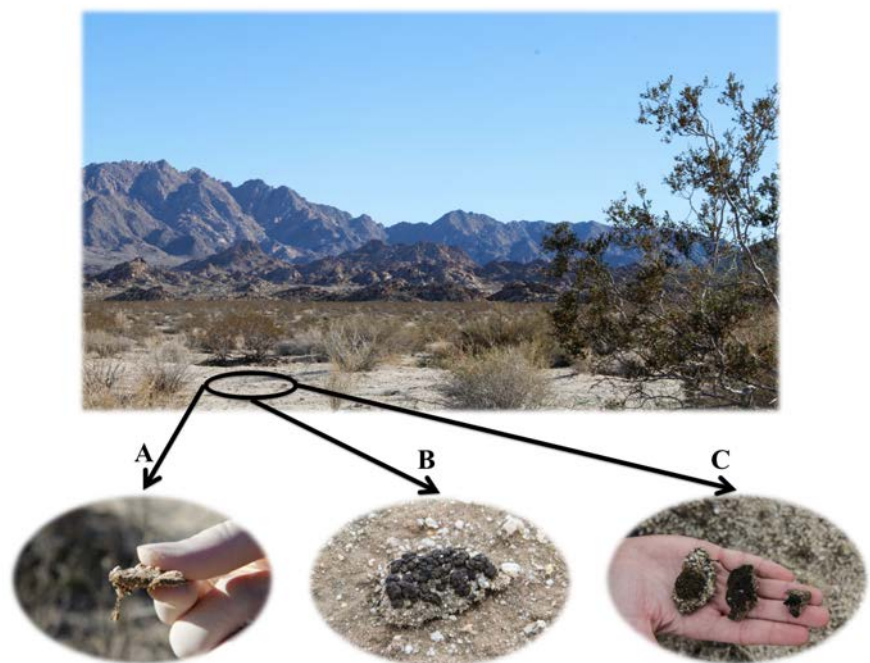


Figure 3. Plant interspaces in JTNP contain a variety of biocrusts but are dominated by light algal crusts and lichen crusts, both of which will have characteristic filaments in the subsurface. The filaments look like tiny roots dangling from under the crust (A). Light algal crusts (A) and lichen crusts (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.

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, and 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

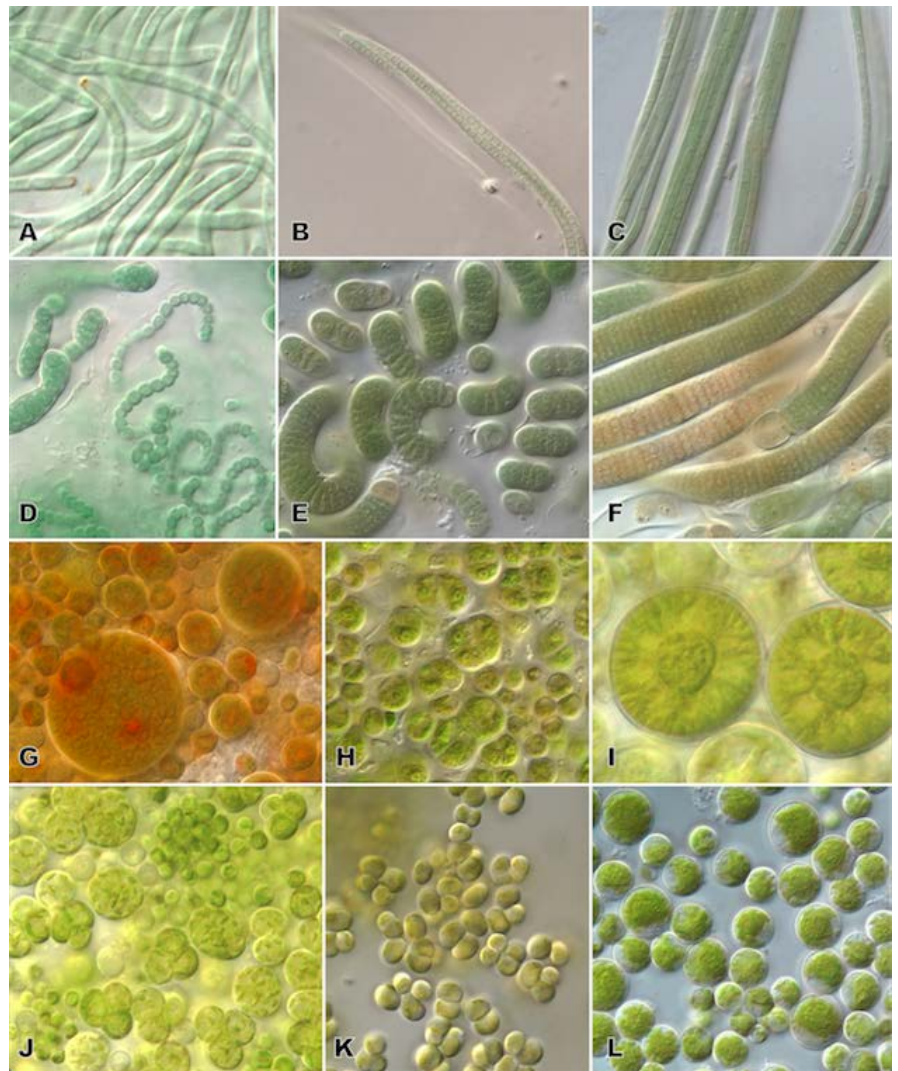


Figure 4. Cyanobacterial (A-F) and eukaryotic algal (G-L) diversity of cultures isolated from JTNP biocrusts. Photographs show: A) *Oculatella coburnii* (Synechococcales clade), B) *Trichocoleus desertorum* (Synechococcales clade), C) *Symplocastrum flechtnerae* (Oscillatoriales clade), D) *Nostoc* sp. (Nostocales clade), E) *Spirirestis rafaelsensis* (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.

Bracteacoccus and newly described 5 *Bracteacoccus* species from dryland environments. This monographs reports several records of *Bracteacoccus* species from JTNP including: 4 records of the established *Bracteacoccus pseudominor* found at 3 locations within the Colorado desert portion of the park, 1 record of the newly described *Bracteacoccus deserticola* from the Pinto Basin, 1 record of the newly described *Bracteacoccus glacialis* found in the Wonderland of Rocks, and 9 records of the newly described *Bracteacoccus occidentalis* from 5 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.

Fungi 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 sizes of DNA bands run on a gel that 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 spores and reduce to manageable counts the number of species growing on the Petri dishes. In

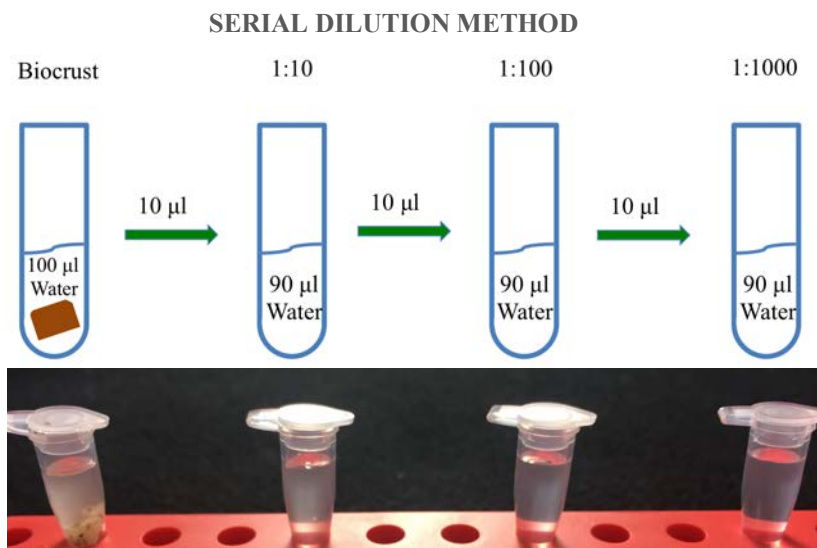


Figure 5. Serial dilution method involves a dilution series of biocrust water suspensions are prepared by adding biocrust to 100 ul of sterile water. To dilute the biocrust water suspension, 10 ul of original solution is transferred and combined with 90 ul of sterile water (1:10 dilution). Additional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

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 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. 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 Joshua Tree National Park to test whether there are differences in species

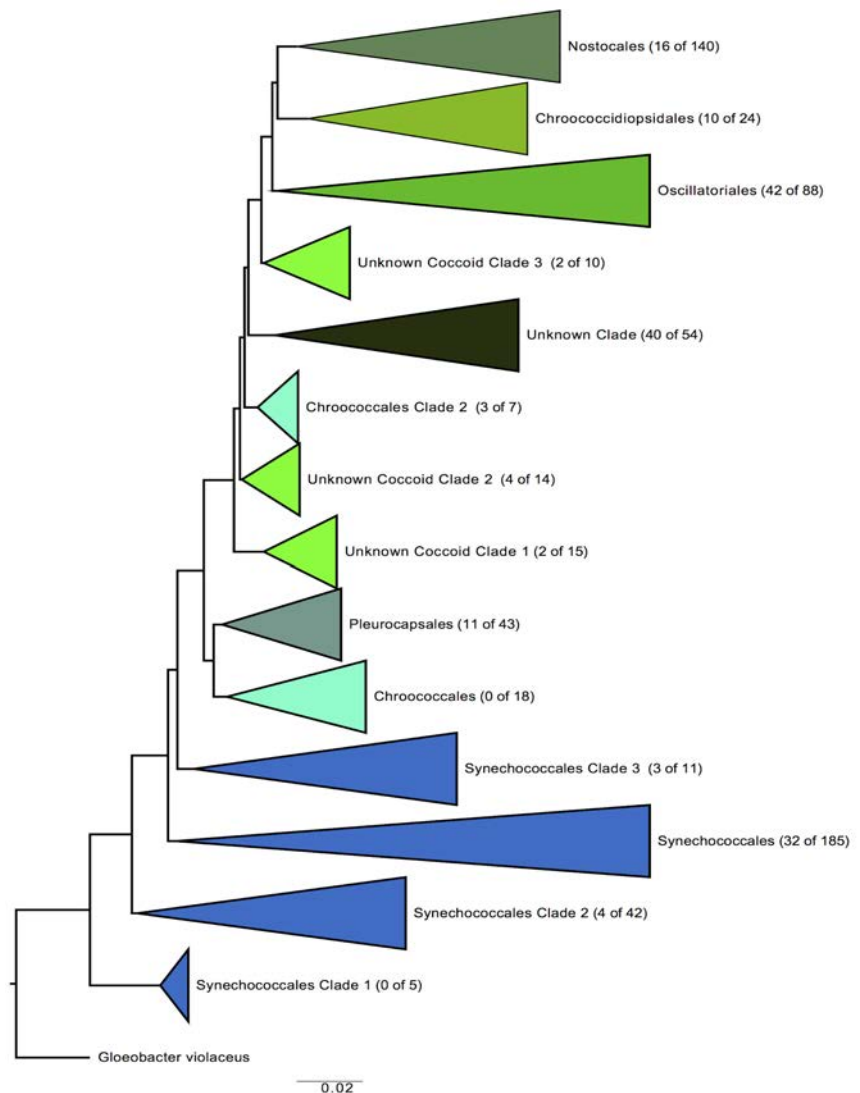


Figure 6. Phylogenetic tree of Cyanobacteria with all major clades shown. The number of lineages found in JTNP biocrusts out of the total number of known lineages is shown in parentheses to the right of the clade. These data are based on amplicon sequences and they illustrate that members of nearly all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

that comprise these crust types. DNA samples from both crust samples were extracted, amplified with Polymerase Chain Reaction (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

dependent and culture independent methods can equally recover the broad phylogenetic diversity found in biocrusts for Cyanobacteria (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

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 name (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

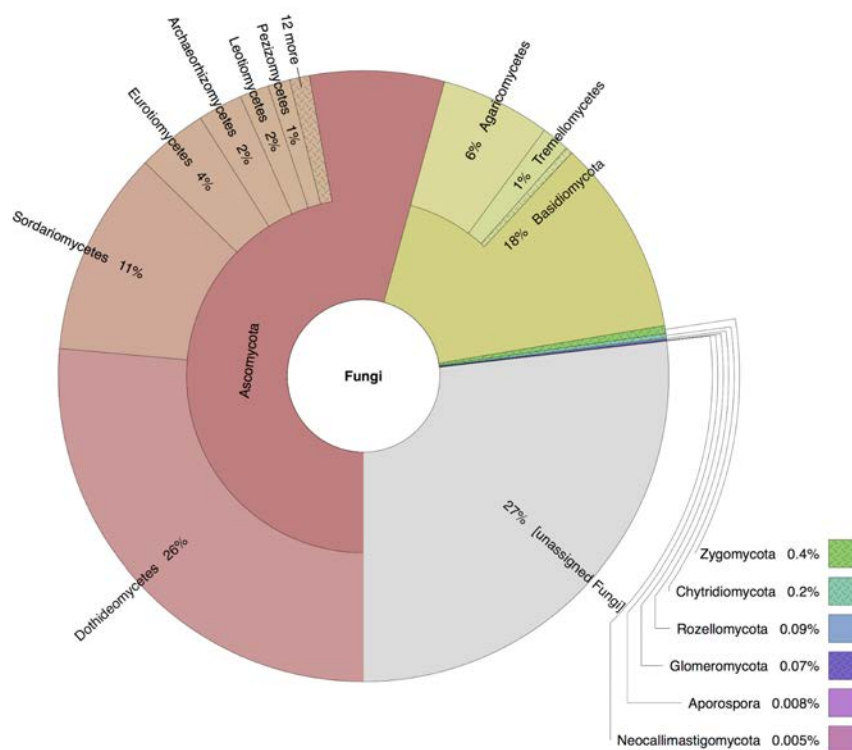


Figure 7. Fungal diversity in light algal crust from Joshua Tree National Park. Using Krona for visualization (Ondov et al. 2011), main fungal phyla are Ascomycota and Basidiomycota.

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

Internal Transcribed Spacer (ITS) 1 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

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), but to identify 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

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

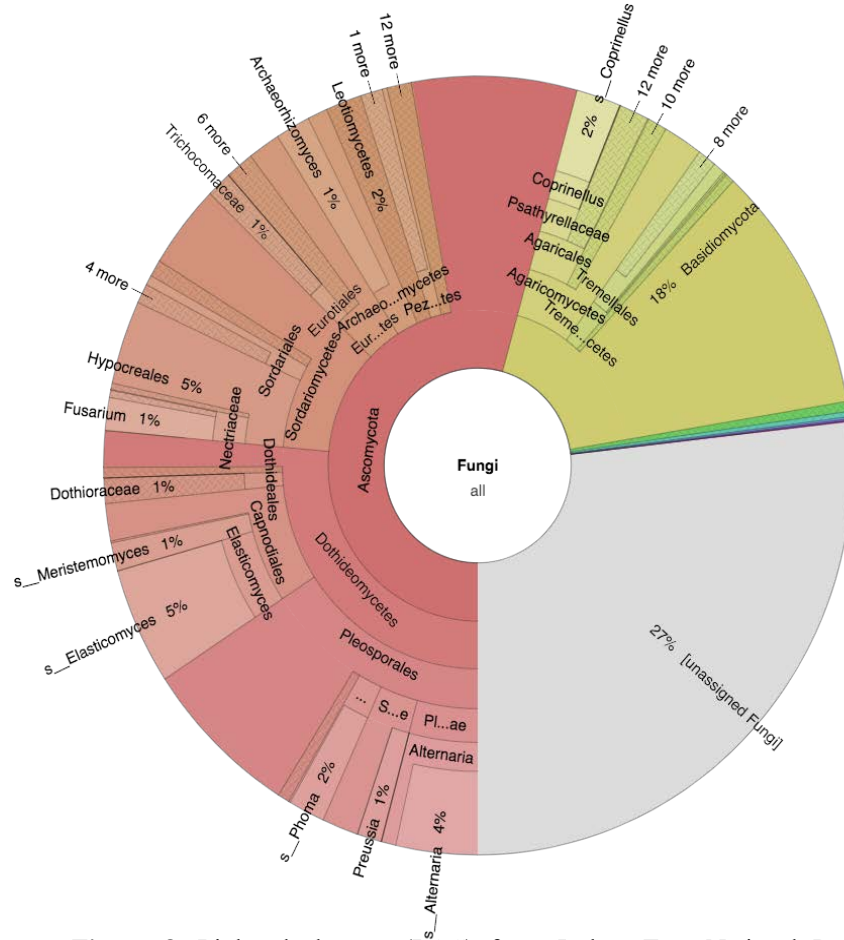


Figure 8. Light algal crust (LAC) from Joshua Tree National Park contains a complex fungal composition. A variety of fungal species are represented with several dominant species from Basidiomycota (yellow) and Ascomycota (red).

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

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 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 their cooperating microbes and relationships are very challenging at this early stage, since a fraction of microorganisms cannot be isolated using current culture dependent method. By exploring microbial diversity while developing both culture dependent and independent method, microbial interactions experiment can be conducted in the near future and will show how these microorganisms help each

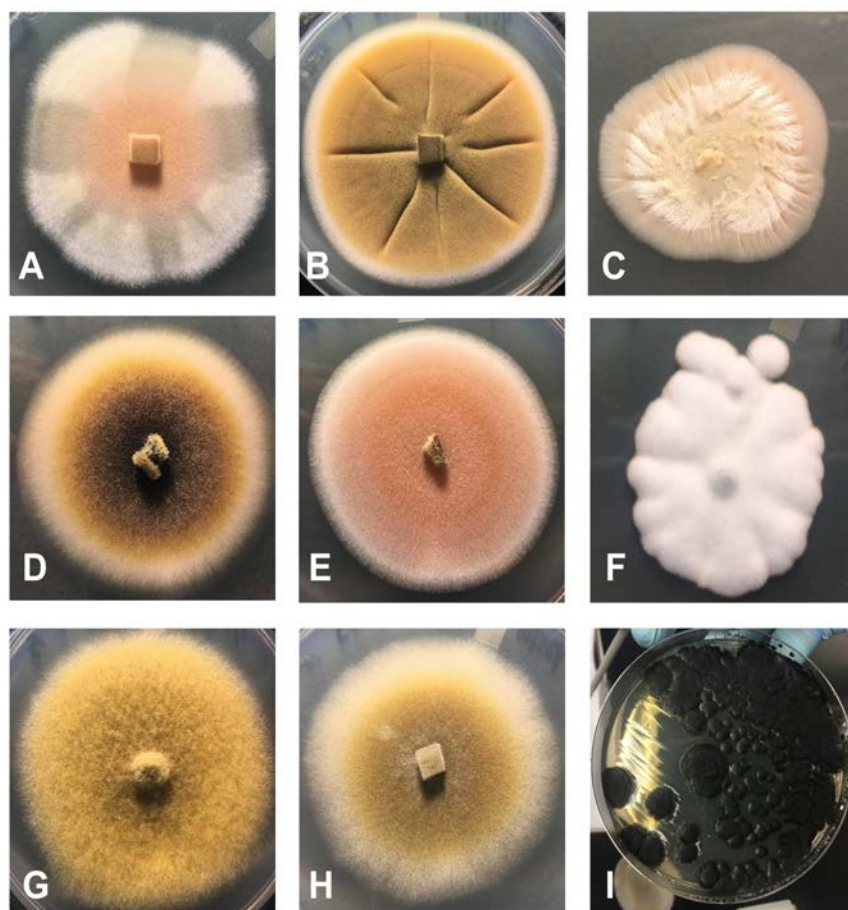


Figure 9. Depiction of a small sample of the variety of fungi isolated from the JTNP biocrusts by using a culture dependent method. The genera depicted include: A) *Phoma* sp., B) *Didymella* sp., C) *Acremonium* sp., D) *Didymella* sp., E) *Aspergillus* sp., F) *Aspergillus* sp., G) *Alternaria* sp.,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

other to build 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 which has already been recognized as the fungal loop hypothesis (Collins et al. 2008).

Acknowledgement

This research was supported by funds from: the USDA Agriculture Experimental Station at University of California, Riverside and NIFA Hatch project CA-R-PPA-5062-H to J.E.S.; the California Desert Research Fund at The Community Foundation awarded to N. Pietrasiak; and Robert Lee Graduate Student Research Grants awarded to Nuttapon Pombubpa and Nicole Pietrasiak. Primer sequences and arrayed barcodes were provided by Alfred P. Sloan Foundation Indoor Microbiome Project. We thank Joshua Tree National Park for permits to allow us to conduct research. We thank Aurapat Ngamnithiporn, Derreck Carter-House, and Sangsan Warakkagun for assistance with biocrust sampling and transportation. We would also like to thank all 3 reviewers for their substantial suggestions and comments. Nuttapon Pombubpa was supported by Royal Thai Government Scholarship.

References

Bates, S.T. and Garcia-Pichel, F., 2009. A culture-independent study of free-living fungi in biological soil crusts of the Colorado Plateau: their diversity and relative contribution to microbial biomass. *Environmental Microbiology*, 11(1), pp.56-67.

Belnap, J. and Gardner, J.S., 1993. Soil microstructure in soils of the Colorado Plateau: the role of the cyanobacterium *Microcoleus vaginatus*. *The Great Basin Naturalist*, pp.40-47.

Belnap, J., Eldridge, D.J., Kaltenecker, J.H., Rosentreter, R., Williams, J. and Leonard, S., 2001. Biological soil crusts: ecology and management. US Department of the Interior, Bureau of Land Management. US Geological Survey, Technical Reference, 2, pp.1-110.

Belnap, J., 2002. Nitrogen fixation in biological soil crusts from southeast Utah, USA. *Biology and fertility of soils*, 35(2), pp.128-135.

Belnap, J., Weber, B. and Büdel, B., 2016. Biological soil crusts as an organizing principle in drylands. In *Biological Soil Crusts: An Organizing Principle in Drylands*. Springer, Cham, pp.3-13.

Bohunická, M., Pietrasiak, N., Johansen, J.R., Gómez, E.B., Hauer, T., Gaysina, L.A. and Lukešová, A., 2015. *Roholtiella*, gen. nov. (Nostocales, Cyanobacteria)—a tapering and branching cyanobacteria of the family Nostocaceae. *Phytotaxa*, 197(2), pp.84-103.

Büdel, B., Dulić, T., Darienko, T., Rybalka, N. and Friedl, T., 2016. Cyanobacteria and algae of biological soil crusts. In *Biological Soil Crusts: An Organizing Principle in Drylands*. Springer, Cham, pp.55-80.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M. and Gormley, N., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME journal*, 6(8), p.1621.

Clark, D.S., 1967. Comparison of pour and surface plate methods for determination of bacterial counts. *Canadian journal of microbiology*, 13(11), pp.1409-1412.

Collins, S.L., Sinsabaugh, R.L., Crenshaw, C., Green, L., Porras-Alfaro, A., Stursova, M. and Zeglin, L.H., 2008. Pulse dynamics and microbial processes in aridland ecosystems. *Journal of Ecology*, 96(3), pp.413-420.

Flehtner, V.R., Pietrasiak, N. and Lewis, L.A., 2013. Newly revealed diversity of green microalgae from wilderness areas of Joshua Tree National Park (JTNP). *Monographs of the Western North American Naturalist*, 6(1), pp.43-63.

Fučíková, K., Flehtner, V.R. and Lewis, L.A., 2013. Revision of the genus *Bracteacoccus* Tereg (Chlorophyceae, Chlorophyta) based on a phylogenetic approach. *Nova Hedwigia*, 96(1-2), pp.15-59.

Fučíková, K., Lewis, P.O. and Lewis, L.A., 2014. Putting incertae sedis taxa in their place: a proposal for ten new families and three new genera in Sphaeropleales (Chlorophyceae, Chlorophyta). *Journal of phycology*, 50(1), pp.14-25.

Graham, L.E. Wilcox, L.W., Graham, J. 2009. *Algae*. 2nd ed. San Francisco: Benjamin Cummings.

Hu, C., Liu, Y., Song, L. and Zhang, D., 2002. Effect of desert soil algae on the stabilization of fine sands. *Journal of Applied Phycology*, 14(4), pp.281-292.

Maier, S., Muggia, L., Kuske, C.R. and Grube, M., 2016. Bacteria and Non-lichenized Fungi Within Biological Soil Crusts. In *Biological Soil Crusts: An Organizing Principle in*

Drylands. Springer, Cham, pp.81-100.

Maier, S., Tamm, A., Wu, D., Caesar, J., Grube, M. and Weber, B., 2018. Photoautotrophic organisms control microbial abundance, diversity, and physiology in different types of biological soil crusts. *The ISME journal*, p.1.

Mühlsteinová, R., Johansen, J.R., Pietrasiak, N., Martin, M.P., Osorio-Santos, K. and Warren, S.D., 2014. Polyphasic characterization of *Trichocoleus desertorum* sp. nov. (Pseudanabaenales, Cyanobacteria) from desert soils and phylogenetic placement of the genus *Trichocoleus*. *Phytotaxa*, 163(5), pp.241-261.

Nilsson, R.H., Tedersoo, L., Ryberg, M., Kristiansson, E., Hartmann, M., Unterseher, M., Porter, T.M., Bengtsson-Palme, J., Walker, D.M., De Sousa, F. and Gamper, H.A., 2015. A comprehensive, automatically updated fungal ITS sequence dataset for reference-based chimera control in environmental sequencing efforts. *Microbes and Environments*, 30(2), pp.145-150.

Ondov, B.D., Bergman, N.H. and Phillippy, A.M., 2011. Interactive metagenomic visualization in a Web browser. *BMC bioinformatics*, 12(1), p.385.

Osorio-Santos, K., Pietrasiak, N., Bohunická, M., Miscoe, L.H., Kováčik, L., Martin, M.P. and Johansen, J.R., 2014. Seven new species of *Oculatella* (Pseudanabaenales, Cyanobacteria): taxonomically recognizing cryptic diversification. *European Journal of Phycology*, 49(4), pp.450-470.

Pietrasiak, N., Johansen, J.R., LaDoux, T. and Graham, R.C., 2011a. Comparison of disturbance impacts to and spatial

distribution of biological soil crusts in the Little San Bernardino Mountains of Joshua Tree National Park, California. *Western North American Naturalist*, 71(4), pp.539-552.

Pietrasiak, N., Johansen, J.R. and Drenovsky, R.E., 2011b. Geologic composition influences distribution of microbiotic crusts in the Mojave and Colorado Deserts at the regional scale. *Soil Biology and Biochemistry*, 43(5), pp.967-974.

Pietrasiak, N., Regus, J.U., Johansen, J.R., Lam, D., Sachs, J.L. and Santiago, L.S., 2013. Biological soil crust community types differ in key ecological functions. *Soil Biology and Biochemistry*, 65, pp.168-171.

Pietrasiak, N., Mühlsteinová, R., Siegesmund, M.A. and Johansen, J.R., 2014. Phylogenetic placement of *Symplocastrum* (Phormidiaceae, Cyanophyceae) with a new combination *S. californicum* and two new species: *S. flechtnerae* and *S. torsivum*. *Phycologia*, 53(6), pp.529-541.

Řeháková, K., Johansen, J.R., Casamatta, D.A., Xuesong, L. and Vincent, J., 2007. Morphological and molecular characterization of selected desert soil cyanobacteria: three species new to science including *Mojavia pulchra* gen. et sp. nov. *Phycologia*, 46(5), pp.481-502.

Smith, D.P. and Peay, K.G., 2014. Sequence depth, not PCR replication, improves ecological inference from next generation DNA sequencing. *PloS one*, 9(2), p.e90234.

Steven, B., Yeager, C., Belnap, J. and Kuske, C.R., 2014. Common and distinguishing features of the bacterial and fungal communities in biological soil crusts and shrub root zone

soils. *Soil Biology and Biochemistry*, 69, pp.302-312.

Steven, B., Hesse, C., Gallegos-Graves, L.V., Belnap, J. and Kuske, C.R., 2015. Fungal diversity in biological soil crusts of the Colorado plateau. In *Proc 12th Biennial Conf Science Management Colorado Plateau*.

Walters, W., Hyde, E.R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A., Gilbert, J.A., Jansson, J.K., Caporaso, J.G., Fuhrman, J.A. and Apprill, A., 2016. Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. *Msystems*, 1(1), pp.e00009-15.

Weber, B., Belnap, J. and Büdel, B., 2016. *Synthesis on Biological Soil Crust Research*. In *Biological Soil Crusts: An Organizing Principle in Drylands*. Springer, Cham, pp.527-534.



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 Avila-Lovera¹ and Louis S. Santiago¹

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 sarcocaulous and cactoid, with fleshy and succulent stems, respectively. These differ from the retamoids in that sarcocaulous 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



Figure 1. Plants with green stems are categorized by one of three types of stem photosynthesis syndromes. 1) Cactoids, like *Opuntia basilaris* (left) take up carbon dioxide at night; 2) Sarcocaulous plants, e.g. *Dudelya saxosa* (center) recycle carbon; and 3) Retamoids, like *Ephedra viridis* (right), have stomata in the stem epidermis allowing for gas exchange with the atmosphere. Photos by Tasha La Doux.

¹Department of Botany & Plant Sciences, University of California, Riverside, Riverside, California 92521

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 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 (Pivovarov 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



Figure 2. Left: View of the field site, a desert wash at Joshua Tree National Park near the North Entrance. Field technicians 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.

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*) (Fig. 1) (Ávila-Lovera et al. 2019). We selected species with and without visually green stems (Table 1, Fig. 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 leaves during the spring of 2016 (Fig. 2-3) 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 (Fig. 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.

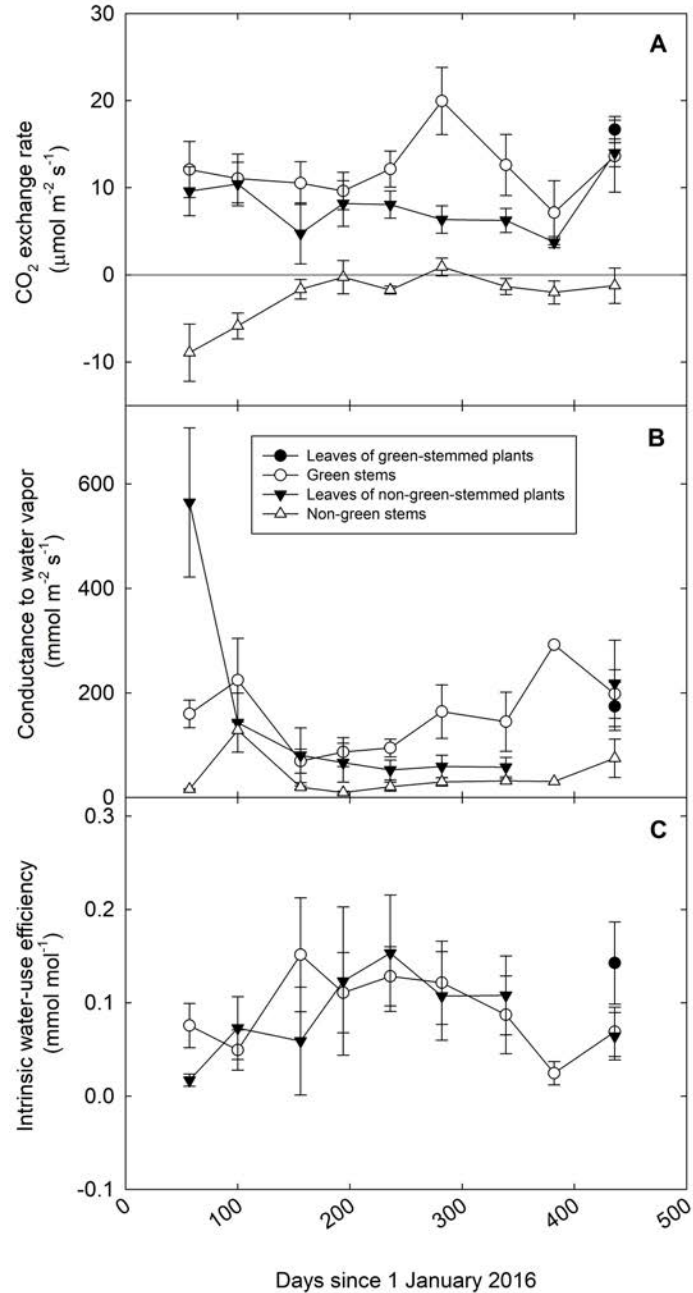


Figure 3. Gas-exchange data for leaves (when present) and stems of green-stemmed species, and leaves and stems of non-green-stemmed species during nine sampling campaigns from February 2016 to March 2017. (a) CO₂ exchange rate, where positive values indicate net carbon uptake and negative values indicate 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 \pm standard error. Taken and modified from (Ávila-Lovera et al.)

Models that predict drought-induced plant mortality are needed to predict the relative 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 Joshua Tree National Park 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.

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).

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

References

- Ávila E, Herrera A, Tezara W (2014) Contribution of stem CO₂ fixation to whole-plant carbon balance in nonsucculent species. *Oecologia* 152:3–15
- Ávila-Lovera E, Haro R, Ezcurra E, Santiago LS (2019) Costs and benefits of photosynthetic stems in desert species from southern California. *Functional Plant Biology* 12.
- Chaves MM, Pereira JS (1992) Water stress, CO₂ and climate change. *Journal of Experimental Botany* 43:1131–1139.
- Chaves MM, Pereira JS, Maroco J, et al (2002) How plants cope with water stress in the field? Photosynthesis and growth. *Annals of Botany* 89:907–916.
- Ehleringer JR, Comstock JP, Cooper TA (1987) Leaf-twig carbon isotope ratio differences in photosynthetic-twig desert shrubs. *Oecologia* 71:318–320
- Fajardo L, Rodríguez JP, González V, Briceño-Linares JM (2013) Restoration of a degraded tropical dry forest in Macanao, Venezuela. *Journal of Arid Environments* 88:236–243.
- Gibson AC (1996) Structure-function relations of warm desert plants. Springer Berlin Heidelberg, Berlin, Heidelberg
- Lambers H, Chapin FS, Pons TL (2008) *Plant Physiological Ecology*. Springer New York, New York, NY
- McDowell N, Pockman WT, Allen CD, et al (2008) Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to drought? *New Phytologist* 178:719–739.
- McDowell NG (2011) Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. *Plant Physiology* 155:1051–1059. <https://doi.org/10.1104/pp.110.170704>
- McDowell NG, Beerling DJ, Breshears DD, et al (2011) The interdependence of mechanisms underlying climate-driven vegetation mortality. *Trends in Ecology & Evolution* 26:523–532.
- McDowell NG, Fisher RA, Xu C, et al (2013) Evaluating theories of drought-induced vegetation mortality using a multimodel-experiment framework. *New Phytologist* 200:304–321.
- Nilsen E, Sharifi M (1997) Carbon isotopic composition of legumes with photosynthetic stems from Mediterranean and desert habitats. *American Journal of Botany* 84:1707–1713
- Nilsen ET (1995) Stem photosynthesis: extent, patterns, and role in plant carbon economy. In: Gartner BL (ed) *Plant stems: physiology and functional morphology*. Academic Press, San Diego, pp 223–240
- Nilsen ET, Bao Y (1990) The influence of water stress on stem and leaf photosynthesis in *Glycine max* and *Sparteum junceum* (Leguminosae). *American Journal of Botany* 77:1007–1015.
- Osmond CB, Smith SD, Gui-Ying B, Sharkey TD (1987) Stem photosynthesis in a desert ephemeral, *Eriogonum inflatum*. Characterization of leaf and stem CO₂ fixation and H₂O vapor exchange under controlled conditions. *Oecologia* 72:542–549
- Pivovarovoff AL, Pasquini SC, De Guzman ME, et al (2016) Multiple strategies for drought survival among woody plant species. *Functional Ecology* 30:517–526.
- Santiago LS, Bonal D, De Guzman ME, Ávila-Lovera E (2016) Drought survival strategies of tropical trees. In: Goldstein G, Santiago LS (eds) *Tropical Tree Physiology*. Springer International Publishing, Cham, pp 243–258
- Schaedle M (1975) Tree Photosynthesis. *Annual Review of Plant Physiology* 26:101–115.
- Sevanto S, McDowell NG, Dickman LT, et al (2014) How do trees die? A test of the hydraulic failure and carbon starvation hypotheses. *Plant, Cell & Environment* 37:153–161.
- Smith SD, Osmond CB (1987) Stem photosynthesis in a desert ephemeral, *Eriogonum inflatum*. Morphology, stomatal conductance and water-use efficiency in field populations. *Oecologia* 72:533–541
- Tinoco-Ojanguren C (2008) Diurnal and seasonal patterns of gas exchange and carbon gain contribution of leaves and stems of *Justicia californica* in the Sonoran Desert. *Journal of Arid Environments* 72:127–140.

Dr. Eleinis Ávila-Lovera is a plant ecophysiologicalist 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)

JOSHUA TREE SCIENCE

Joshua Tree Science is a publication devoted to promoting a better understanding of the natural, physical, and cultural environment in Joshua Tree National Park. Each issue highlights scientific endeavors being conducted in the park and provides an opportunity for scientists, land managers, and the general public to know and appreciate this desert region.

EDITORIAL COMMITTEE:

Jane Rodgers
Neil Frakes
Michael Vamstad
Jay Theuer
Luke Sabala

LEAD EDITOR:

Tasha La Doux

PUBLICATION TEAM:

Johanna Lombardo
??

Questions regarding this publication
should be directed to: ???

LEARN MORE ABOUT JTNP HERE:

TO DO RESEARCH IN JTNP, REQUEST A SCIENTIFIC PERMIT HERE:

IF YOU ARE INTERESTED IN WRITING AN ARTICLE FOR JOSHUA TREE SCIENCE PLEASE CONTACT: ???