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# Biodiversity of Microscopic Green Algae from Desert Soil Crusts

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# BIODIVERSITY OF MICROSCOPIC GREEN ALGAE FROM DESERT SOIL CRUSTS

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**Abstract**

In the desert ecosystem, the ground is covered with soil crusts. Several organisms exist here, such as cyanobacteria, lichens, mosses, fungi, bacteria, and green algae. This most superficial layer of the soil contains several primary producers of the food web in this ecosystem, which stabilize the soil, facilitate plant growth, protect from water and wind erosion, and provide water filtration and nitrogen fixation. Researching the biodiversity of green algae in the soil crusts can provide more context about the importance of the soil crusts. Little is known about the species of green algae that live there, and through DNA-based phylogeny and microscopy, more can be understood. In this study, DNA was extracted from algal cultures newly isolated from desert soil crusts in New Mexico and California. Through PCR reactions, the 18S and *rbcL* genes were amplified and sequenced for analysis. A phylogenetic tree was constructed to observe biodiversity in the collection site and genetic relatedness to previously collected soil algae from New Hampshire. The analyzed strains belong to the classes Chlorophyceae and Trebouxiophyceae, representing several genera and potentially novel species. These results show that biodiversity of soil crusts, both desert and temperate, is still in need of further study and may yield new discoveries in the future.

**Introduction**

Microscopic green algae are simple plants, so they are characteristically green in color due to chlorophyll, have a cell wall, mitochondria, chloroplasts which perform photosynthesis, and store energy in the form of a starch. Green algae occur in a variety of habitats as solitary unicells, colonies of individual cells which are enclosed in mucilage, or as filaments (Lewis and McCourt, 2004). However, the biodiversity of the species, the role they play in the ecosystem, and their geographic distributions are still poorly understood.

Most people commonly associate algae with green, lush moist areas, like on the surface of a pond. However, algae are found also in non-aquatic habitats, such as tree bark, soil, man-made surfaces, snow and ice, and deserts (Evans and Johansen, 1999). Algae are very resilient and can grow on a variety of substrates (Rindi et al., 2008). Deserts, which are of particular interest to biologists due to their extreme environmental conditions, can be separated into four categories: hot and dry, semiarid, coastal, and cold (UCMP, 2000). The Chihuahuan Desert (New Mexico/ Mexico), the Atacama Desert (Chile), and the Mojave Desert (southwestern United States) are categorized within the hot and dry deserts, and are the location of where algal samples were previously collected. The hot and dry desert type is very hot during the summers and has minimal rainfall during the winters. When it does rain, the evaporation rate sometimes exceeds the rainfall rates, causing rain to evaporate before it even touches the ground (UCMP, 2000). Since there is minimal rainfall, there is also minimal plant life, and herbivorous animals. There is little humidity in the atmosphere to deflect the sun's rays and temperatures can range from 43.5-49 degrees Celsius during the day (UCMP, 2000). However, organisms have adapted to live there, and likewise, plants and algae have adapted to these harsh environmental conditions.

The most superficial layer of the desert soil is called the soil crust and can have varying thickness, integrity, and structure (Figure 1). Even though desert algae are not thought about often, they perform a crucial role in the desert habitat, especially in the microbial soil crusts. Microbial soil crusts help shape ecosystems through nitrogen fixation, soil stabilization, and water filtration (Cable and Huxman, 2003). These functions are unique to the microorganisms that live there. This foundation helps promote biodiversity, which builds a more stable ecosystem. More specifically, they protect against water and wind erosion and help facilitate plant growth. The soil crusts are fragile and after damage would require years to be reestablished (Cardon, 2008). The crusts can be damaged by bike or vehicle traffic or footsteps, which break the crusts apart. The wind can scatter the pieces, making recovery difficult. It usually takes about 5-7 years to recover and 50 years to strengthen a damaged crust (NPS, 2018).

A)  B) 

C)  D) 

**Figure 1.** A) A very thin and brittle soil crust with little moisture from one collection site at White Sands National Monument in New Mexico. B) A thicker soil crust with more moisture and green algae that is visible to the naked eye at another collection site at White Sands National Monument. C) A more compacted and dry soil crust with visible lichen growth at Jornada Experimental Range in New Mexico. D) A crust with larger grains of soil and loosely packed with visible growths of mosses at Aguirre Springs National Recreation Area in New Mexico.

Species living in the soil crusts include lichens, mosses, micro-fungi, bacteria, cyanobacteria, and green algae (NPS, 2018). Green algae communities often form a thin layer above the soil (Cardon, 2008).

The morphologies of green microalgae are often simple, round, green, unicellular and cannot be reliably used to distinguish one species from another (Fucikova et. al., 2014). However, microscopy along with DNA data can be used to identify and characterize microalgal species. The unique DNA sequences can be compared to one another for a more accurate estimate of diversity and phylogenetic relationships, which can potentially lead to the discovery of new microalgal species.

Through microscopy and DNA-based phylogeny, the biodiversity of the isolates from the Southwestern United States deserts were examined and compared to previously collected strains from temperate soil crusts from New Hampshire and Massachusetts. By extracting the DNA from the species and sequencing the 18S and *rbcL* genes, biodiversity from different localities can be compared to one another in a reliable, quantitative way. This research adds to existing research done on desert species of algae and suggests the possibility of new species to be discovered. This information will also lead to more research on how to protect the desert environments.

**Methods**

In early July 2019, samples were collected from New Mexico in White Sands National Monument (32.7872° N, 106.3257° W), Dripping Springs Natural Area (32.32256° N, 106.57388° W), and Aguirre Springs National Recreation Area (32.370378° N, -106.560876° W). At each site, roughly 10 grams of soil was accumulated and approximately 12 samples were collected (Table 1).

These samples were rehydrated with distilled water and after a two-week incubation in rehydrated state they were spread onto a Bold's Basal Medium (BBM) media plate. The BBM media was made with 5 milliliters of $NaNO_3$, $MGSO_4.7H_2O$, NaCl, $K_2HPO_4$, $KH_2PO_4$, $CaCl_2.2H_2O$ stock solutions, 470 milliliters of distilled $H_2O$ (or 420 milliliters of distilled $H_2O$ and 50 milliliters of goat manure extract), and 7.5 grams of agar, following Bischoff and Bold (1963). The cultures were left on a sunny windowsill to allow for growth. Once some biomass accumulated, 3-5 colonies of green algae were isolated, and each put on a new plate. These isolates were then given a unique strain code (Table 1). The colonies that were selected were green and distanced from other colonies on the plate, so it could be ensured that only pure strains of green algae were present on the new plates. These plates of isolates were left on a windowsill to allow for growth in preparation for DNA extraction.

Once a sufficient amount of biomass accumulated (about 50 micrograms), it was scraped off the BBM media plate to prepare for extraction. DNA extraction was performed following the steps outlined in the Qiagen DNeasy® PowerPlant® Pro Kit (50). These extraction products were tested with NanoDrop to find the concentrations of DNA extracted.

After a successful DNA extraction, a PCR reaction was set up. For the reaction of the 18S gene and corresponding primers, (SSU1, SSU2, and N18G [Shoup and Lewis, 2003]), 52 °C was the annealing temperature, and held at 95 °C for 3 minutes. For 35 cycles, it was held at 95 °C for 30 seconds, the 52 °C for 30 seconds, and 72 °C for 3 minutes. Then the reactions were held at 72 °C for 10 minutes and finally held at 10 °C for 10 minutes. For the PCR of the *rbcL* gene and the corresponding primers (M28, M1390, M650, M1161, M379, and M636), the procedure described in McManus and Lewis (2011) was used.

Once the PCR reaction was done, an agarose gel was made for electrophoresis. The gel was made with 0.36 grams of agarose and 40 milliliters of TBE or TAE and microwaved at 30 second intervals until all visible particles were dissolved. The solution was poured into the electrophoresis rig to harden. For the 1Kb ladder, 10 microliters of the ladder solution were mixed with 1 microliter of loading dye/ Gel Red dye mix. For each sample, 4 milliliters of the PCR sample were mixed with 1 milliliter of loading dye/ Gel Red. Five microliters of each mixture was added to each well of the gel. The gel was run for 30-45 minutes. A photo was taken of the gel using UV imaging (BIO-RAD Molecular Imager ChemiDoc™ XRS+).

If one bright, clear band was present, the samples were sent to the Macrogen for purification and sequencing. The raw sequencing reads were trimmed, paired and assembled into contigs, which were then analyzed using DNA Subway. To generate a phylogenetic tree, the MUSCLE tool was used to align the sequences and the PHYLIP NJ and PHYLIP ML tools to construct the tree and compute 100 bootstrap pseudoreplicates to assess branch support. Separate alignments and phylogenies were generated for the two represented green algae classes – Chlorophyceae and Trebouxiophyceae.

Using a light compound microscope Olympus BH2 equipped with an Amscope digital camera MU1000, photographs were taken of the samples with successful DNA extractions and PCR reactions. The micrographs were taken at 400x magnification in bright field.

Additional isolates from previous field collections were also sequenced. These samples were provided by Dr. N. Pietrasiak (NMSU) and represent other Southwestern US deserts as well as one sample from Atacama (Chile). For comparison to temperate soil biota, sequences obtained by former members of the Fucikova lab were added to the phylogenetic analyses. These sequences represent soil crust collections from southern New Hampshire and western Massachusetts.

**Table 1. shows the strains sequenced (either the 18S or *rbcL* gene), where the strain was collected, and if it was collected or from a previous collection.**

| Strain | Locality | Collected | 18s | *rbcL* |
|---|---|---|---|---|
| WTN9MI20 | Wapack Trail Northbound | N | - | M28-M1390 |
| WTN3AI1 | Wapack Trail Northbound | N | - | M28-M1390 |
| ATA34QKH8 | South American Atacama Desert | N | - | M28-M1390 |
| Gse-v-8ea | Grand Staircase-Escalante National Monument | N | - | M28-M1390 |
| MOHKF1A | Mohawk State Forest | N | - | M28-M1390 |

| | | | | |
|---|---|---|---|---|
| WJT43-27B | Joshua Tree National Park | N | - | M28-M1390 |
| WJT66VFNP78 | Joshua Tree National Park | N | N18G-SSU2 | M28-M650 |
| WS2 | White Sands National Monument | Y | - | M28-M1390 |
| WS1KF6 | White Sands National Monument | Y | - | M28-M650 |
| WS3VW3 | White Sands National Monument | Y | - | M28-M650 |
| WS3K6 | White Sands National Monument | Y | N18G-SSU2 | M28-M1390 |
| WTN10-MI17 | Wapack Trail Northbound | N | - | M28-M1390 |
| Gse-07F4 | Grand Staircase-Escalante National Monument | N | - | M28-M1390 |
| Gse-07F5 | Grand Staircase-Escalante National Monument | N | N18G-SSU2 | M28-M1390 |
| WTN10-MI18 | Wapack Trail Northbound | N | - | M379-M1161 |

| | | | | |
|---|---|---|---|---|
| WJT66VFNP78 | Joshua Tree National Park | N | - | M379-M1161 |
| WJT43VFNP18D | Joshua Tree National Park | N | - | M379-M1161 |
| CMT1BRIN43 | Clark Mountains | N | - | M379-M1161 |
| WJT66VFNP50A | Joshua Tree National Park | N | - | M379-M1161 |
| WTN8-MI7 | Wapack Trail Northbound | N | - | M28-M1390 |
| MMD1DS1 | Mt. Monadnock Dublin Trail | N | - | M28-M1390 |
| WTS1KF1 | Wapack Trail Southbound | N | N18G-SSU2 | - |
| WTN4AI12 | Wapack Trail Northbound | N | N18G-SSU2 | - |

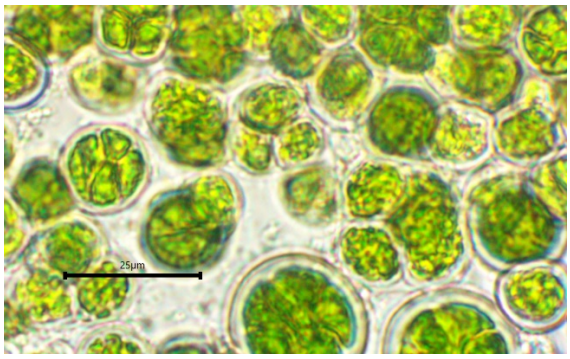
**Results**

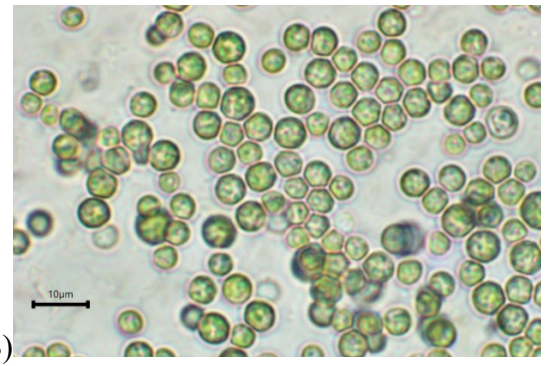
Morphologically, the isolates appear to be very similar to one another, even though they are from different localities with varying soil crusts (Figure 2). The cells of most isolates are roughly 10-25 μm in length, round, green, and enclosed in mucilage (Figure 3). It is difficult to categorize and name algae based on physical appearance alone.

A)  B) 

C) 

**Figure 2.** A) Dripping Springs collection site in New Mexico. This site had soil crusts with moisture from the spring water and had coarse textured soil. B) White Sands National Monument collection site in New Mexico. This locality had very thin and brittle soil crusts with fine textured sand. C) Aguirre Springs National Recreation Area collection site in New Mexico. This site had soil crusts with larger grains of soil and loosely packed with visible growths of mosses.

A) 


B) 

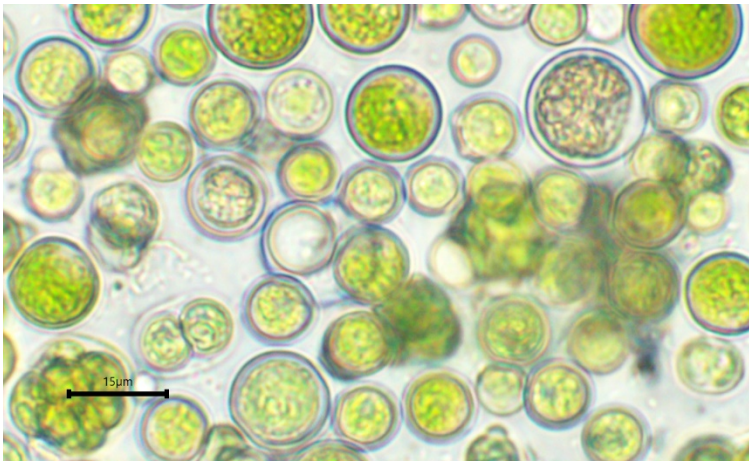

C) 


**Figure 3.** A) light micrograph of a green algae (*Acutodesmus*) sample from White Sands National Monument (WS3 KF6), with a scalebar of 25 µm. B) light micrograph of a green algae sample (*Desmodesmus*) from the Grand Staircase-Escalante National Monument (gse-v-8ea), with a scalebar of 10 µm. C) light micrograph of a green algae (unknown species, potentially related to *Pharao* or *Follicularia*) sample from Joshua Tree National Park (WJT43) with a scalebar of 15 µm.

Of the samples analyzed, there were 12 successfully sequenced. In the Chlorophyceae tree, there are about 9 different genera represented in the samples analyzed (Figure 4). Three of these sequences were from the samples newly collected in New Mexico. WS3VW3 is most closely related to the genus *Chloromonas* and WTN10MI17 to *Chlamydomonas*. WTN8MI7 is most closely related to *Heterochlamydomonas*. CMT1BRIN43 and WJT66VFNP50A are closely related to each other and both are most related to *Pharao desertorum*. WS3KF6 is most closely related to *Acutodesmus*. WTN10MI18 is most closely related to *Coelastrella*. GSE07F4 and GSEV8ea are most closely related to *Desmodesmus*. ATA34OKH8, WJT43_27B, and

WJT66VFNP78 are closely related to each other and on their own branch of the tree, not particularly closely related to any other chlorophycean genus. NIWKF3 is most closely related to *Chlorococcum*.

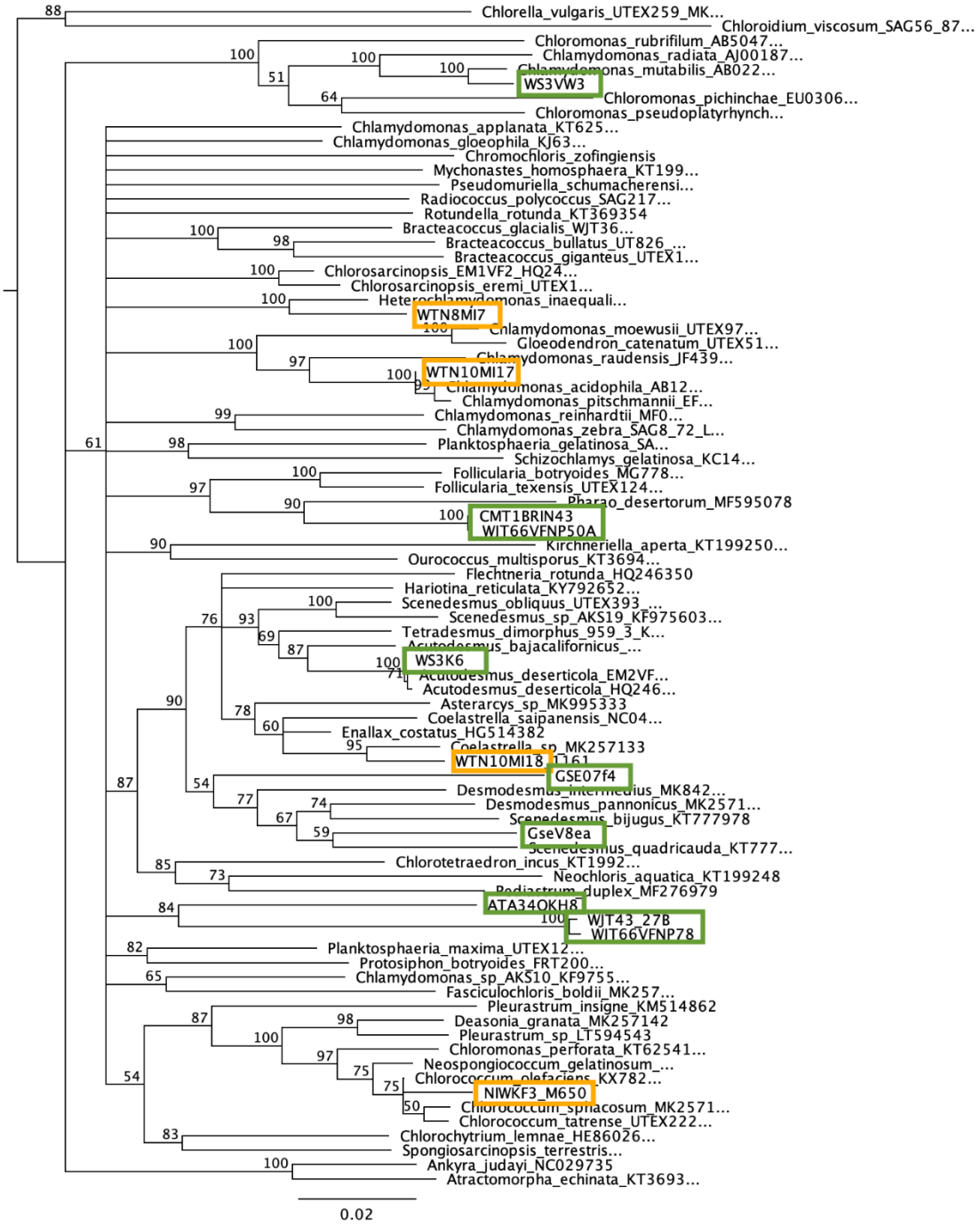


**Figure 4.** Phylogenetic relationship in the class Chlorophyceae with the collected samples and previous known sequences of species, sequenced for the *rbcL* gene. The tree was inferred using the Neighbor Joining method with the bootstrap pseudoreplicates to assess statistical support for branches. The green boxes are samples from the Southwest and South American deserts. The yellow boxes are samples that are from New England.

In the Trebouxiophyceae tree, there are about 11 different genera represented in the samples analyzed (Figure 5). Therefore, in total for the *rbcL* gene sequences there are 20 different genera represented.

GSE07F5 is most closely related to *Chlorella*. WTN3AI1 is most closely related to *Parietochloris*. MMD1DS1 is most closely related to *Lobosphaera*. NIP2KF2 is most closely related to *Heveochlorella*. WTN9MI20, MOHKF1A, WTN0DS7_1390, WTN9MI19_1390, and WTN2MI5_1390 are most closely related to *Coccomyxa*. WS1KF6 is most closely related to *Lagerheimia*, *Oocystella*, *Oocystidium*, *Quadricoccopsis*, and *Tetrachlorella*. WS2 is most closely related to *Ecballocystopsis*.

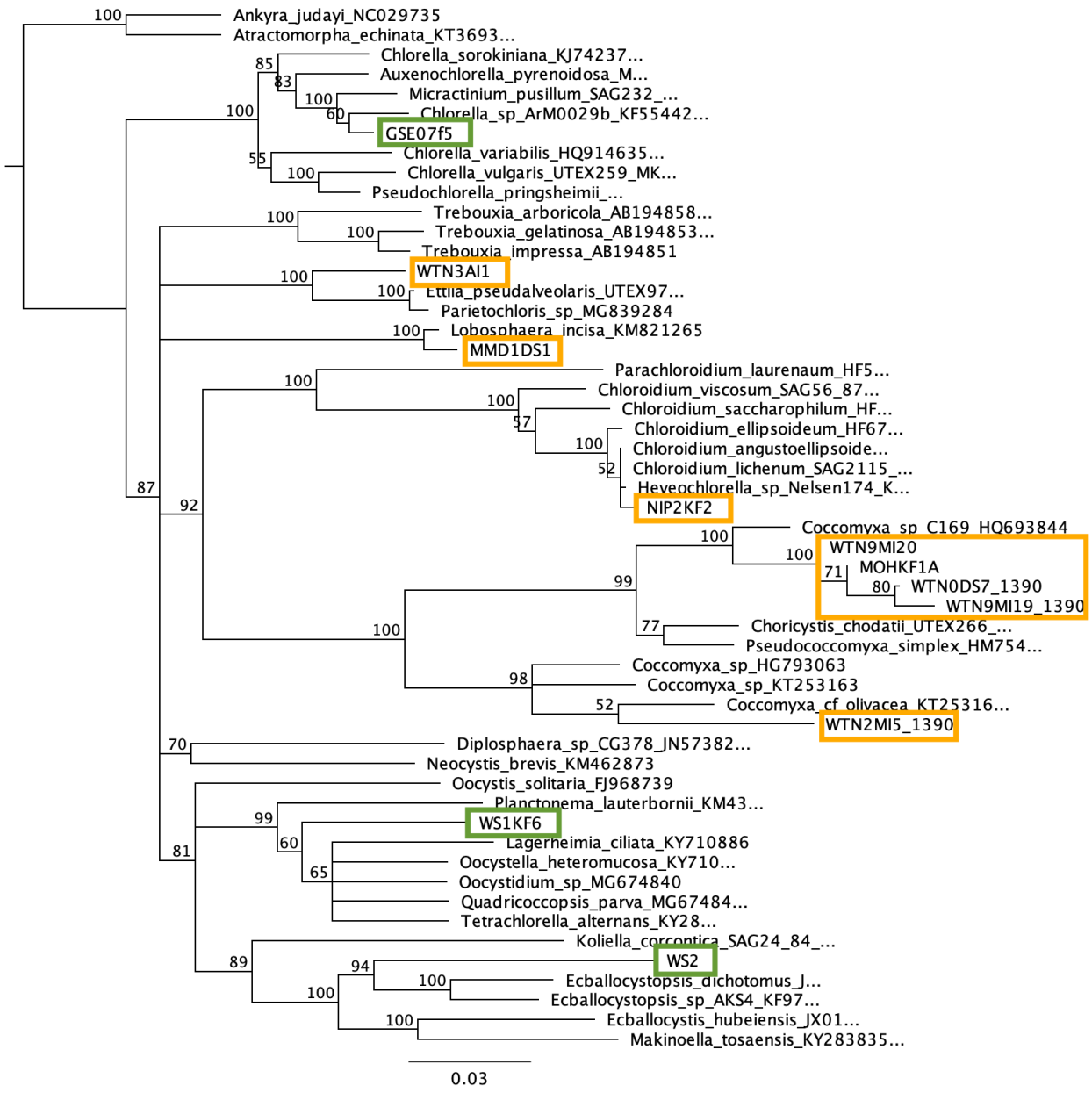



**Figure 5.** shows the phylogenetic relatedness in the class Trebouxiophyceae with the collected samples and previous known sequences of species, sequenced for the *rbcL* gene. The tree was inferred using the Neighbor Joining method, with bootstrap pseudoreplicates to assess statistical support for branches. The green boxes are samples from the Southwest deserts. The yellow boxes are samples that are from New England.

**Discussion**

Twelve samples were successfully sequenced. There were 9 different genera represented on the Chlorophyceae tree and 11 on the Trebouxiophyceae tree, for a total of at least 20 taxa/ species represented using the *rbcL* data. The species that were found in the Chlorophyceae tree were *Chlamydomonas* sp., *Chloromonas* sp., *Heterochlyamydomonas* sp., *Pharao desertorum*, *Acutodesmus* sp., *Coelastrella* sp., *Desmodesmus* sp., *Planktosphaeria* sp., *Chlorococcum* sp. and an uncertain species represented by three strains. The species that were found in the Trebouxiophyceae tree were *Chlorella* sp., *Parietochloris* sp., *Lobosphaera* sp., *Heveochlorella* sp., *Coccomyxa* sp., *Lagerheimia* sp., *Oocystella* sp., *Oocystidium* sp., *Quadricoccopsis* sp., *Tetrachlorella* sp., and *Ecballocystopsis* sp.

*Chlamydomonas* and *Heterochlyamydomonas* are green algae species in the order Volvocales that have been previously found in the Mojave Desert of North America (Lewis and Lewis, 2005). Volvocales are normally colony-forming, flagellated organisms, but in soils they are also known to live as non-motile unicells. WS3VW3 was collected at the White Sands National Monument, but WTN10MI17 and WTN8MI7 were collected at the Wapack Trail Northbound. *Chlorococcum* is another volvocalean species of green algae with a cosmopolitan distribution, which includes lakes in North America (Prescott, 1962), but is also known from soils. NIWKF3, collected in a New Hampshire soil crust, most likely belongs to the genus.

The order Sphaeropleales is represented in Figure 4 by several soil crust taxa. *Desmodesmus* and *Acutodesmus* are commonly found in the soil crusts of western American deserts (Lewis and Flechtner, 2004). GSE07F4 and GSEV8EA were collected at the Grand Staircase-Escalante National Monument, which is a desert ecosystem. *Pharao desertorum* is a

species of desert algae was discovered in the Western Desert of Egypt, but there is little research on the green algae in this taxon. CMT1BRIN43 (Clark Mountains) and WJT66VFNP50A (Joshua Tree National Park) were both collected in desert ecosystems, and while they are related to *Pharao desertorum*, they are genetically distinct and likely represent a new species. *Protosiphon* and *Planktosphaeria* are freshwater algae species found in North American moist soils (John, 2003; John and Tsarenko, 2002), and were not represented in any of the samples studied here. However, ATA34OKH8 (Atacama Desert), WJT43_27B (Joshua Tree National Park), and WJT66VFNP78 (Joshua Tree National Park) were collected from desert soils and while on the tree they appear in the proximity of *Protosiphon* and *Planktosphaeria*, they are not related to them phylogenetically. Instead, other analyses (not shown) place these strains closer to *Follicularia* and *Pharao* – two desert soil genera. It is likely that ATA34OKH8, WJT43 27B, and WJT66VFNP78 represent two new species.

*Parietochloris* is a species of trebouxiophycean green algae that is typically found in forest soils of temperate area (Maltsev et al., 2018) and WTN3AI1 was collected from the Wapack Trail Northbound, which is in New England and therefore represents a temperate region as well. *Lobosphaera*, a species typically found in alpine soil (Watanabe, 1996) and MMD1DS1 (Mt. Monadnock Dublin Trail) was collected in New England, even though Mt. Monadnock is not that high in elevation, the ecology is otherwise consistent with that known for *Lobosphaera*. *Coccomyxa* is an extremely common, widespread genus that colonizes various aquatic and terrestrial habitats, such as the soil crusts of New England (Darienko et al., 2015). WTN9MI20 (Wapack Trail Northbound), MOHKF1A (Mohawk State Forest), WTN0DS7 (Wapack Trail Northbound), WTN9MI19 (Wapack Trail Northbound), and WTN2MI5 (Wapack Trail Northbound) were all collected from soil crusts in New England and represent at least two

species of *Coccomyxa*. *Ecballocystopsis* is typically found growing on wet surfaces (Xia et al., 2013), but WS2 was collected from the White Sands National Park in the desert ecosystem. This may indicate that WS2 is a new, ecologically distinct species of *Ecballocystopsis*, and that the genus has a broader ecological tolerance than previously thought. The unique gypsum substrate in White Sands National Park likely harbors numerous new species yet to be discovered. *Chlorella*, *Heveochlorella*, and *Tetrachlorella*, despite their similar names represent distinct phylogenetic lineages. *Chlorella*-like algae are typically able to withstand high and varying temperatures, similar to that found in the desert ecosystem, but can also be found in ponds (Masojídek and Torzillo, 2008). GSE07F5 (Grand Staircase-Escalante National Monument), relative of *Chlorella*, and WS1KF6 (White Sands National Park), relative of *Tetrachlorella* and *Oocystella* were collected from the desert ecosystem. *Lagerheimia*, *Oocystella*, and *Oocystidium* are typically known as freshwater species (Guiry, 2012). *Quadricoccopsis* is a type of green algae common to inland water (Liu et al., 2018), but their relative WS1KF6 was collected from the White Sands National Park, which is a desert ecosystem. Thus, this group of algae also likely has a broader ecological tolerance than previously thought, as it includes a desert representative. NIP2KF2 is related to *Heyeochlorella* and *Chloroidium* and was collected from soil crusts in New England. The *Heyeochlorella* clade is known to colonize various terrestrial substrates in a variety of climates (Darienko et. al., 2010).

Desert green algae have a unique biodiversity, and at least in our study, the temperate samples had no species overlap with the desert samples. Many of our strains potentially represent new species. This conclusion parallels that of Lewis and Lewis (2005), where this research found that, when compared to aquatic species of green algae, desert green algae represented numerous new taxa, distinct from their aquatic relatives. Likely, many green algal genera contain species of

which some are aquatic and some have colonized and adapted to terrestrial environments, including desert soil crusts.

The gypsum soil substrate has a unique chemistry. Unlike the soil crusts in New England, which are derived from an acidic granite-based bedrock, the gypsum soil is calcium based and moderately soluble in water. Thousands of years ago, the area of the White Sands National Park was a sea. However, due to tectonic plate movement and environmental temperature increase, the water evaporated, leaving behind a chalky substrate, which is gypsum. Over the years, wind eroded the large particles into the sandy consistency that exists today (Knapp, 2016). This unique, calcium-based substrate is a habitat that could produce unique organisms and microorganisms that are specifically adapted to that soil chemistry. Little is known about microalgal dispersal but once they settle in a new location, they likely become reproductively isolated from other populations and evolve independently, and thus new species arise. Many green algal species mostly reproduce asexually, so reproductive isolation can happen rapidly.

The biodiversity of green algae is previously poorly studied, and many species new to science are still likely to be discovered. To continue this research, more samples could be added to this data. Other samples that were collected in July 2019 will continue to be isolated and sequenced. These sequences could be added to the phylogenetic tree, through the same methods previously stated to evaluate the biodiversity of the desert soil crusts further.

Biodiversity is important because it promotes more stable ecosystems (Cardinale et al., 2012). A diversity of species means that the systems are more resilient to disturbance, and they have a better chance of recovery. Biodiversity also helps remove pollution from the ecosystems and habitats (Cardinale et al., 2011). Plants keep the soil and air clean, produce oxygen and absorb carbon dioxide, which brings about the next point of a food source. In the food web, the

animals are codependent on each other for food. Plants play a huge role as the primary producers because they are able to perform photosynthesis and are well understood as the base of the food web. They also provide a stable food option to herbivorous animals above them in the web. However, plants themselves are supported by soil and the organisms within it, and this biota is often overlooked in the ecosystem analyses. Without this sturdy foundation, then the entire food web will collapse.

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