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NEWLY REVEALED DIVERSITY OF GREEN MICROALGAE FROM WILDERNESS AREAS OF JOSHUA TREE NATIONAL PARK (JTNP)

Valerie R. Flechtner[1], Nicole Pietrasiak[2], and Louise A. Lewis[3]

ABSTRACT.—Documentation of the biodiversity of eukaryotic algae from desert systems is sparse. Our objective was to characterize microalgae from soil samples collected throughout Joshua Tree National Park, California, USA. Morphological, life-cycle, and DNA sequence data were collected for 100 microalgal isolates distributed over 18 sites in Joshua Tree National Park. Phylogenetic analysis of the 18S rDNA data separated the green algae into 15 major clades—10 in the class Chlorophyceae and 5 in the class Trebouxiophyceae—containing 2 or more lineages plus 9 lineages represented by a single isolate. Five isolates belonging to the class Xanthophyceae and 2 isolates belonging to Eustigmatophyceae were also identified. Some green algal isolates could be placed with confidence in known genera including *Bracteacoccus*, *Chlorosarcinopsis*, *Myrmecia*, *Neochlorosarcina*, *Scenedesmus*, and *Stichococcus*, whereas several green isolates could not be assigned to known genera based on morphological or molecular data. Both morphological and molecular data were important to identifying this biodiversity. Due to the paucity of informative morphological characters, morphology alone does not capture the species diversity found at sites. Molecular data are a richer source of characters with which to identify the algae, but more representative sequences of soil algae are needed in public databases to make identification of any new taxa straightforward. Overall, our data suggest that the biodiversity of these hot deserts still is largely unknown and unexplored.

RESUMEN.—La documentación sobre la biodiversidad de las algas eucariotas de los sistemas de desierto es escasa. Nuestro objetivo fue caracterizar las microalgas de muestras de suelo colectadas a lo largo del Parque Nacional Joshua Tree en California (Estados Unidos). Se recopilaron datos sobre la morfología, el ciclo de vida y las secuencias de ADN de 100 microalgas aisladas de 18 puntos del Parque Nacional Joshua Tree. El análisis filogenético del gen rADN 18S separó a las algas verdes en 15 clados principales—10 en la clase Chlorophyceae y cinco en la clase Trebouxiophyceae—que contienen dos o más linajes más nueve linajes representados por un solo aislado. También se identificaron cinco aislados que pertenecen a la clase Xanthophyceae y dos aislados que pertenecen a la clase Eustigmatophyceae. Algunos aislados de algas verdes pudieron ubicarse con certeza en géneros conocidos, incluyendo a *Bracteacoccus*, *Chlorosarcinopsis*, *Myrmecia*, *Neochlorosarcina*, *Scenedesmus* y *Stichococcus*, mientras que varios aislados de algas verdes no pudieron asignarse a géneros conocidos con base en su información morfológica o molecular. Tanto la información morfológica como la molecular fueron importantes para identificar esta biodiversidad. La morfología por sí sola no captura la diversidad de las especies que se encontró en los sitios debido a la escasez de características morfológicas informativas. Los datos moleculares son una fuente más abundante de rasgos con las cuales se pueden identificar a las algas, pero se necesitan secuencias más representativas de algas terrestres en bases de datos públicas para identificar de manera inequívoca cualquier nuevo taxón. En general, nuestros datos sugieren que la biodiversidad de estos desiertos de elevadas temperaturas aún permanece mayormente desconocida e inexplorada.

Few comprehensive studies exist focusing on the characterization of nondiatom eukaryotic algal flora in arid and semiarid deserts of North America. Arid and semiarid deserts are separated on the basis of annual precipitation; arid deserts experience from 60–100 mm to 150–250 mm annual precipitation, while semiarid deserts experience from 150–250 mm to 250–500 mm precipitation (Meigs 1953). In most of the previous studies, algal identification was made on the basis of morphological and life-cycle data. Using these techniques, Johansen et al. (1993) identified 90 algal taxa, including 47 chlorophyte and 9 xanthophyte taxa, in a semiarid sagebrush steppe community in the Lower Columbia Basin (WA). Flechtner et al. (2008) identified 56 algal taxa, including 16 nondiatom eukaryotic algal taxa, in samples from San Nicolas Island, the largest of the Channel Islands off the coast of California; they described 2 new chlorophyte species.

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Particularly in hot deserts, such as the Mojave and the Colorado Deserts, floristic studies are sparse. Only a few soil studies, concentrated during the 1960s, were conducted in this region (Cameron 1960, 1964, Durrell 1962, Shields and Drouet 1962, Hunt and Durrell 1966). Floristic emphasis was placed on cyanobacteria or diatoms; only a small number of green algae were identified. More recently, Flechtner and Lewis have demonstrated that considerable eukaryotic algal diversity can exist in soils from hot, dry deserts in the southwestern United States and in Mexico (Flechtner et al. 1998, Lewis and Flechtner 2002, Lewis and Lewis 2005) and have described 3 new species of *Scenedesmus* based on a combination of morphological and DNA sequence data (Lewis and Flechtner 2004).

Morphological approaches have traditionally been accepted as important tools in defining the algal flora of a given locale, and some investigators still rely solely on these techniques (Flechtner et al. 1998, 2008, Škaloud 2009). But light microscopy has limitations. Many algae need to be examined from cultured materials in order to observe motile stages. Some algal species (e.g., *Chlorella*) are small, have very simple morphology, and do not produce alternative life stages such as gametes or zoospores. Morphological plasticity influenced by nutritional components or the physical form of the substrate medium has been documented for members of the genera *Scenedesmus* (Trainor and Egan 1990) and *Pleurastrum* (Sluiman and Gärtner 1990), respectively; environmental factors can also affect algal morphology (Luo et al. 2006). Where plasticity exists, the placement of an isolate in the correct taxonomic position using morphological traits is difficult. It is often necessary, therefore, to supplement morphological data obtained using light microscopy with ultrastructural characteristics or DNA sequence analysis.

During a study investigating the distribution and abundance of various types of microbiotic soil crusts, Pietrasiak et al. (2011) collected surface soil samples from 75 sites in undisturbed wilderness areas within Joshua Tree National Park (JTNP). The availability of a large sample set made possible a detailed study of the algal flora present in the soils. This manuscript focuses primarily on the green algae identified in a subset of these samples. We used a combination of DNA sequence analysis and light microscopy examination of vegetative and motile phases to investigate the diversity of the green algal flora in 18 sites in JTNP. Our research goals were (1) to characterize the new eukaryotic algal isolates using morphological traits; (2) to incorporate molecular phylogenetic analysis to further identify the species and place the green algal isolates into a larger known green algal tree of life; and (3) to enrich our knowledge of the diversity of eukaryotic soil algae of Joshua Tree National Park.

METHODS

Study Area

Joshua Tree National Park (JTNP) is located in southern California about 140 miles east of Los Angeles. The park encompasses almost 800,000 acres (3238 km^2). Its southern boundary lies in the Colorado Desert, and its northern boundary lies in the Mojave Desert. It was declared a national monument in 1936, and presently, significant portions of the park are wilderness areas that are protected from anthropomorphic disturbance. This protection makes the park an ideal site for the study of the distribution of microbiotic soil crusts and their algal components.

Sampling

In 2006, Pietrasiak et al. (2011) conducted an extensive ecological survey of microbiotic soil crusts by characterizing 75 sites representing all wilderness segments of JTNP. Composite surface soil samples (0–1 cm) were collected along transects. From this pool of 75 surveyed sites, we chose 18 sites for an extensive floristic study on free-living (nonlichenized) green algae that represent the spatial extent of the park (Fig. 1). Universal Transverse Mercator coordinates for the 18 study sites appear in Appendix 1.

Characterization of Eukaryotic Algae

Composite surface soils were plated onto agar-solidified Z8 and Bold's Basal Medium (BBM), and colony-forming units of algae were isolated (Flechtner et al. 1998). Representative colonies selected for further study were subcultured in either liquid BBM or agar-solidified BBM plates. Unialgal stock cultures of selected isolates were maintained on agar-solidified slants. Each isolate was identified by indicating the site from which it was

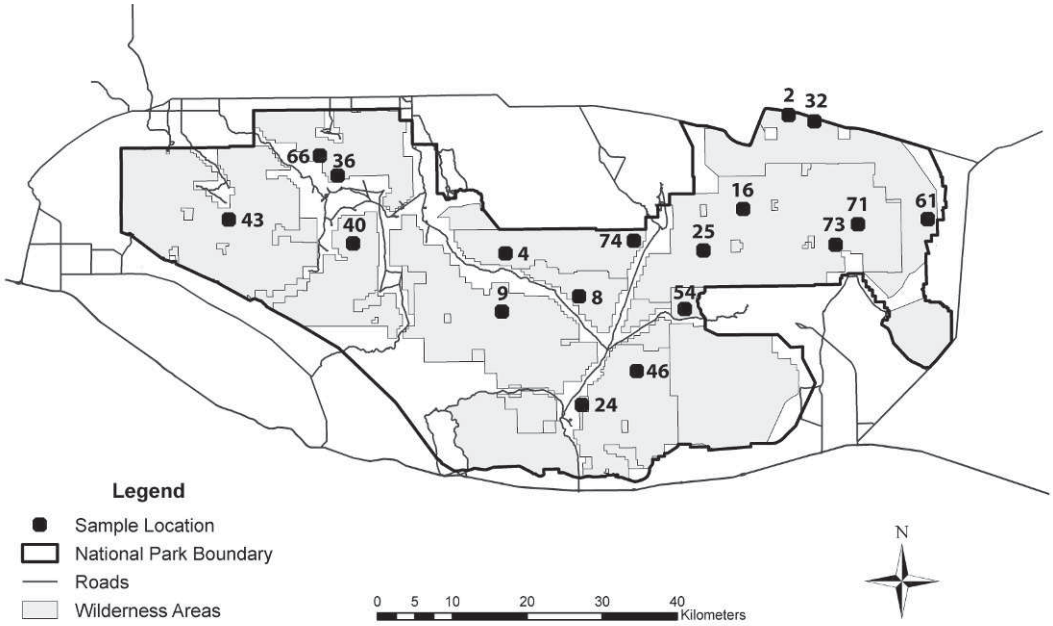


Fig. 1. Map of Joshua Tree National Park showing the locations of study sites within the park.

isolated followed by the strain number; thus, the designation WJT36VFNP5 indicates the fifth isolate obtained from site 36.

The presence of an extracellular matrix was determined by suspending plate-grown cultures in India ink. Starch production was determined by staining with Gram's iodine. Zoospore production was often difficult to achieve. We found the best procedure was to (1) heavily streak small plates of agar-solidified BBM and incubate them until confluent growth had been achieved (2–5 days); (2) make a heavy suspension of cells in a small volume (0.5–1.0 mL) of sterile water in a small sterile glass tube in the late afternoon; (3) wrap the tubes in aluminum foil and place them in a beaker that was subsequently wrapped in foil; (4) incubate the cultures overnight in the dark at 25 °C; and (5) unwrap and examine the cultures individually after 15–19 h incubation. Alternatively, for some cultures, zoospore production was best in cultures freshly inoculated onto agar-solidified BBM and incubated on a 15-h light : 9-h dark cycle for 1–3 days. Once the presence of motile zoospores had been detected, zoospores were fixed in 3% formalin by adding 4 μL of a 10% formalin solution to 9 μL of the zoospore suspension for morphological characterization. Specimens were examined using an Olympus BH-2 photomicroscope with Nomarski DIC optics and photographed using an Olympus DP25 camera. Taxonomic identifications were made using a standard key (Ettl and Gärtner 1995) or primary literature.

DNA Sequence Analysis

DNA was extracted using DNEASY or MOBIO PowerPlant extraction kits following the manufacturer's protocol. For most isolates, the 3′ half of the 18S rDNA gene region (approximately 800 nucleotides) was targeted. In some cases, nearly complete gene sequences (over 1700 nucleotides) were obtained. Primers for PCR amplification followed Lewis (1997). Double-stranded amplified product was sequenced in 20-μL volumes using 18S sequencing primer with an ABI PRISM system by reading electrophoresed labeled base calls. Verification of base call reads was done through forward- and reverse-strand sequencing (2 copies for each direction of DNA strands), and a consensus sequence was created using Sequencher Software (Gene Codes Corp.). The consensus was compared to the GenBank public database of sequences using BLAST (Altschul et al. 1990) to check against contaminant sequences and to pinpoint close published

sequences for inclusion in phylogenetic analysis. GenBank accession numbers appear in Appendix 2.

Individual Trebouxiophyceae and Chlorophyceae alignments were prepared manually, in Text Wrangler, using the rDNA data from the JTNP isolates and a selection of nearest NCBI Blast matches. The chlorophycean alignment included 155 taxa in total and 1788 characters, and the trebouxiophycean alignment had 62 taxa and 1777 characters. Homologies in the alignment were informed through an estimated secondary structure (using MARNA—Siebert and Backofen 2005), and when homology could not be unambiguously assessed, sites were excluded. A total of 85 and 96 characters were excluded from the chlorophycean and trebouxiophycean analyses, respectively. Phylogenetic analyses were performed using maximum likelihood in PAUP (Swofford 2002) with the GTR + gamma model of substitution. In addition, Bayesian phylogenetic analyses were performed to obtain branch posterior probabilities (MrBayes—Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) under a GTR + I + gamma model. For each data set, 2 independent runs were done, each using 1 cold and 3 heated chains. The analysis was run for 10^7 generations, with trees sampled every 1000 generations. The output was examined in Tracer v1.4.1 (Rambaut and Drummond 2003) in order to examine convergence of the runs. The first 200 trees of each run were discarded as burnin, and the remaining trees were used to produce the 50% majority-rule consensus tree with branch support values.

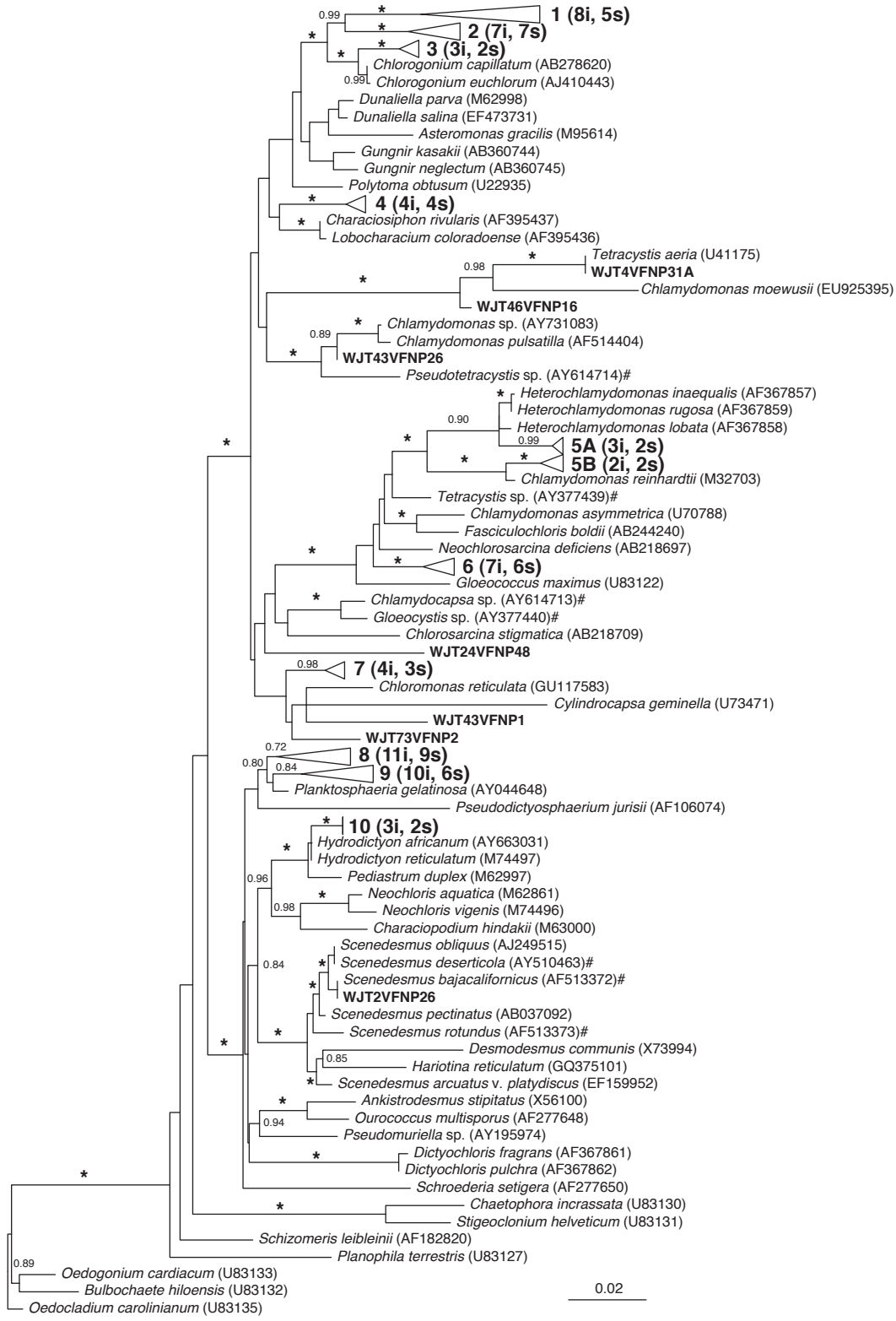
Results and Discussion

Morphological data was collected for approximately 300 isolates. From these 300, ninety-five isolates from the classes Chlorophyceae and Trebouxiophyceae and 5 isolates from the classes Xanthophyceae and Eustigmatophyceae were selected for DNA sequence analysis. Separate phylogenetic analyses of the chlorophycean taxa and trebouxiophycean taxa were performed, with each of the resulting phylogenetic trees showing relationships of the JTNP taxa to one another and to published 18S rDNA sequences. The phylogenetic analyses produced trees containing 15 major JTNP lineages (those having multiple isolates represented), including 10 major chlorophyte (Fig. 2) and 5 major trebouxiophyte (Fig. 3) algae.

Chlorophyceae and Trebouxiophyceae

TAXONOMIC ASSIGNMENT OF CHLOROPHYCEAN ISOLATES.—Approximately 75% of the green algal isolates ($n = 69$) and 10 of the 15 clades identified on the basis of 18S rDNA sequence analysis fall into the class Chlorophyceae; 3 of the chlorophycean clades (clades 2, 6, and 8) could be reliably assigned to known genera on the basis of morphological and molecular data (Figs. 2, 4). JTNP isolates belonging to clade 2 are aligned with certain species of the genus *Chlorosarcinopsis* (Fig. 4) and were commonly seen (Fig. 2). Members of this clade have a parietal chloroplast with a pyrenoid surrounded by an entire starch hull, produce packets of 2–8 cells by dividing vegetatively, produce naked zoospores, develop carotenoid pigment in stationary phase cultures, and are embedded in an extracellular matrix, at least in young cultures (Fig. 5A–5C). Phylogenetically, these isolates are most closely related to the species *C. arenicola*, *C. eremi*, and *C. variabilis* and are part of the A(c) clade of Watanabe et al. (2006). Several morphotypes exist within clade 2, a level of variation that suggests the presence of several putative new species. Members of clade 6 belong to the genus *Neochlorosarcina* clade D(b) of Watanabe et al. (2006). This genus is similar to the genus *Chlorosarcinopsis* in having spherical cells with a parietal chloroplast and pyrenoid with an entire starch hull, forming packets through vegetative cell division, and producing carotenoid in stationary phase cultures (Fig. 5D). The diacritical feature separating the genus *Neochlorosarcina* from the genus *Chlorosarcinopsis* is the nature of the zoospore (Watanabe 1983). Zoospores of *Neochlorosarcina* possess a thin cell wall and round up very slowly once they stop moving, whereas the zoospores of *Chlorosarcinopsis* are naked and round up

Fig. 2. Phylogenetic relationships among the Joshua Tree National Park (JTNP) green algae and selected related published sequences from algae in class Chlorophyceae. One of 2 maximum likelihood trees is shown (tree score, –lnL = 13621.42). Ten clades of JTNP algae are represented as collapsed triangles, with details of each presented in Fig. 4. Taxon labels indicate previously obtained sequences from desert isolates by a pound sign (#). Bayesian posterior probabilities are shown for groupings, with an asterisk (*) indicating values of 1.0. The scale bar indicates the expected number of substitutions per site.



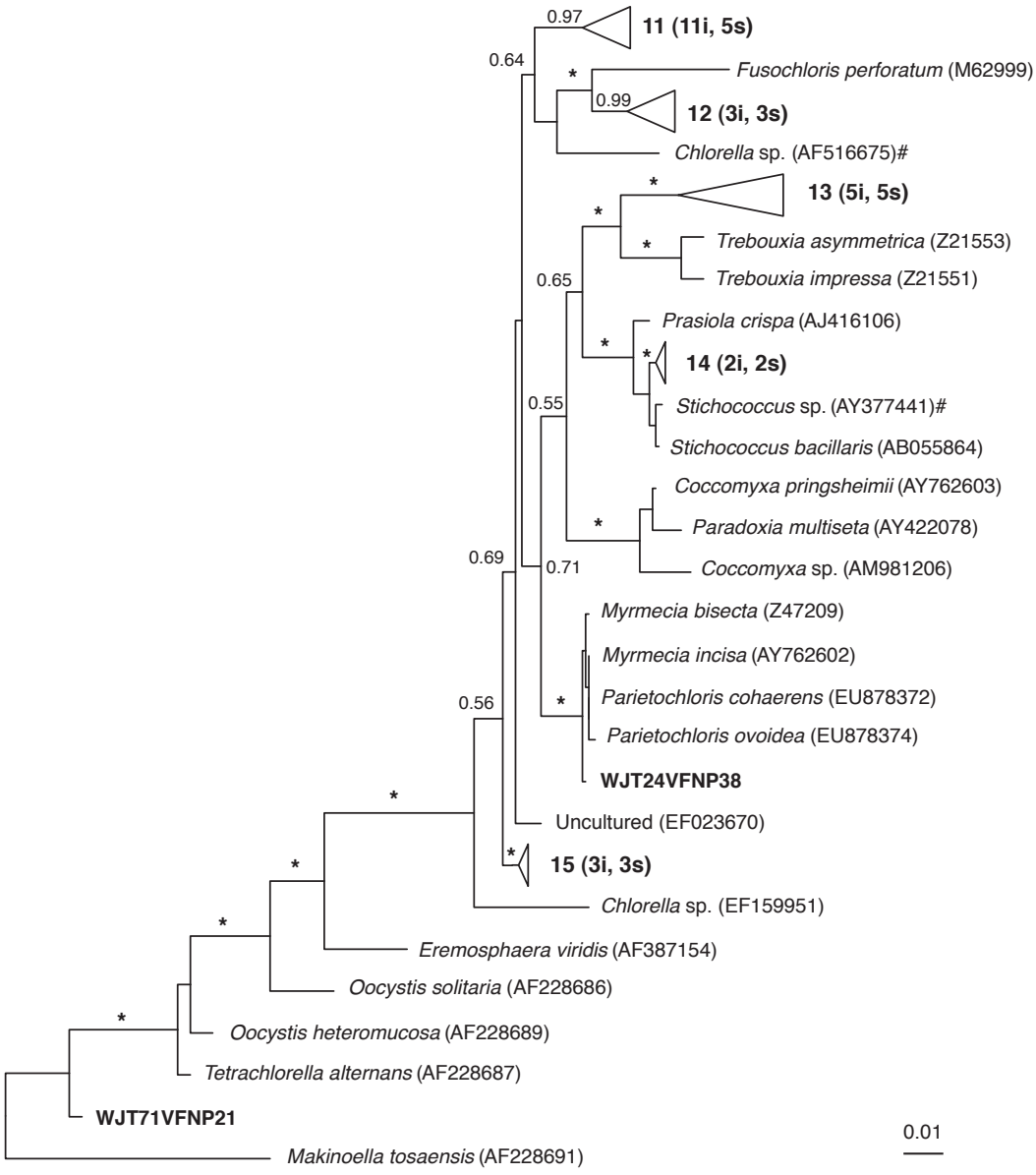


Fig. 3. Phylogenetic relationships among the Joshua Tree National Park (JNTP) green algae and selected related published sequences from algae in class Trebouxiophyceae. One of 44 maximum likelihood trees is shown (tree score, –lnL = 6872.001). Five clades of JTNP algae in this class are represented as collapsed triangles, with details of each presented in Fig. 4. Taxon labels indicate previously obtained sequences from desert isolates by a pound sign (#). Bayesian posterior probabilities are shown for groupings, with an asterisk (*) indicating values of 1.0. The scale bar indicates the expected number of substitutions per site.

very quickly. Our isolates meet all of the morphological criteria of the genus *Neochlorosarcina*. DNA sequence analysis shows that 4 isolates group with *N. negevensis* (Fig. 4), and the morphological characteristics of these isolates are consistent with the known species.

Members of clade 8 belong to the genus *Bracteacoccus*. Cells are usually spherical, contain multiple chloroplasts without pyrenoids, and produce naked zoospores with 2 flagella of uneven length (Fig. 5E–5F). Molecular data shows them aligning with *Bracteacoccus* (Fig. 4).

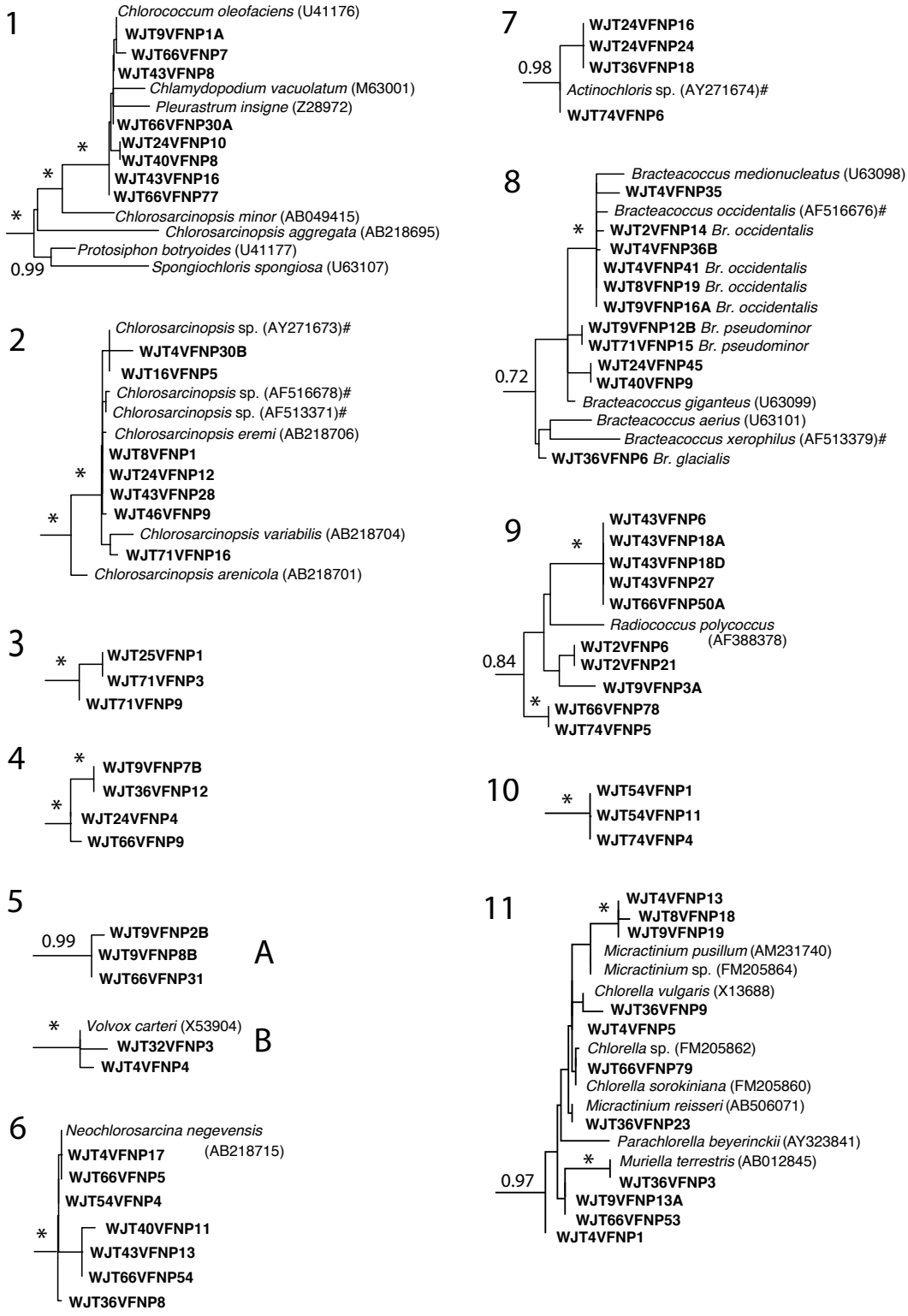


Fig. 4. Caption and panels 12–15 on page 50.

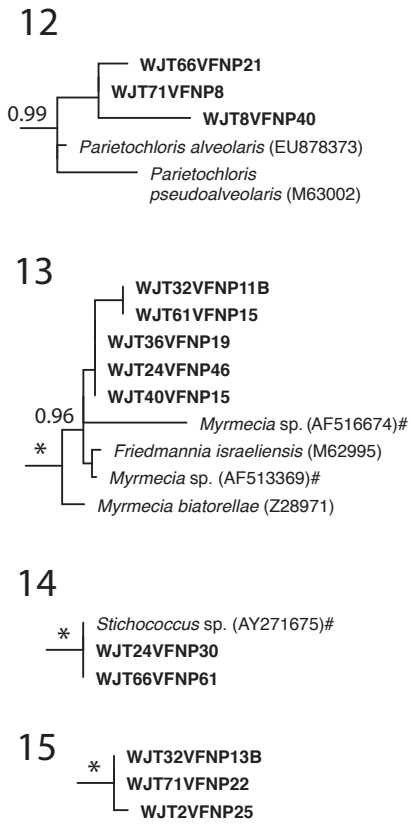


Fig. 4. Details of the phylogenetic relationship within fifteen "collapsed" clades in Figures 1 and 2, each representing 2 or more isolates of Joshua Tree National Park (JTNP) green algae and any related published algal sequences. Taxon labels indicate previously obtained sequences from desert isolates by a pound sign (#). Bayesian posterior probabilities are shown for groupings, with an asterisk (*) indicating values of 1.0.

These isolates, along with other taxa isolated from a variety of locales in North America and Europe, are part of a manuscript reevaluating the genus *Bracteacoccus*. WJT isolates fall into 1 known species (*B. pseudominor*) and 3 new *Bracteacoccus* species (Fučíková et al. in press).

Unlike algae in clades 2, 6, and 8, clade 7 contains isolates identified on morphological basis only as belonging to the genus *Actinochloris.* Vegetative cells of these isolates (Fig. 5G) are spherical to more rarely ovoid, large (30–80 μm in diameter), and multinucleate; they possess an asteroid chloroplast containing a pyrenoid with multiple starch grains, have a cell wall which thickens with age, and produce autospores and walled zoospores. Isolates WJT24VFNP24 and WJT74VFNP6 contain one or more very large inclusions in the cytoplasm (Fig. 5H). Molecular analysis shows sequence similarities among the isolates. It is not possible to verify the generic placement of these isolates identified as *Actinochloris* because there are no 18S rDNA sequences for known species of this genus in the GenBank public database.

Several JTNP isolates are phylogenetically allied and share morphological and molecular similarities to one or more known genera. Whereas we cannot at this time confidently place these isolates into a specific genus, the morphological features of our isolates are consistent with the general characteristics of some of the known taxa with which they are phylogenetically allied.

Clade 1 contains 8 isolates and known species of the genera *Pleurastrum*, *Chlorococcum*, *Chlamydopodium*, *Chlorosarcinopsis*, *Protosiphon*, and *Spongiochloris* (Fig. 4). WJT24VFNP10 died before a thorough morphological study could be performed. The remaining 7 have similar morphological characteristics. Mature vegetative cells are spherical or, less often, ovoid, typically 8–20 μm in diameter, and are uninucleate, at least in young cultures (Fig. 5I). The chloroplast is parietal with typically one pyrenoid; red or orange pigment is produced as the culture ages (Fig. 5J). Asexual reproduction occurs through autospores and, where observed, walled zoospores that are 3–5 μm wide and 5.5–8 μm long (Fig. 5K–5L). These characteristics are most consistent with members of the genus *Chlorococcum*, but additional molecular and morphological data are necessary to confirm this placement.

Five isolates that we identified as chlamydomonads on the basis of a motile vegetative phase and production of walled zoospores form a bipartite phylogenetic lineage (clades 5A and 5B). Genus assignments were problematic. Within clade 5A, isolates WJT9VFNP2B and WJT9VFNP8B (Fig. 5O) are morphologically similar. Cells are ovoid when young and remain ovoid or, more rarely, become spherical in older cultures. They are embedded in a gelatinous extracellular matrix and have a parietal chloroplast with one pyrenoid covered with multiple starch grains. Young cultures are composed primarily of sporangia or flagellated walled zoospores that are rarely motile; sporangia produce 2 (more rarely 4 or 8) zoospores. The morphology of WJT66VFNP31 (Fig. 5M)

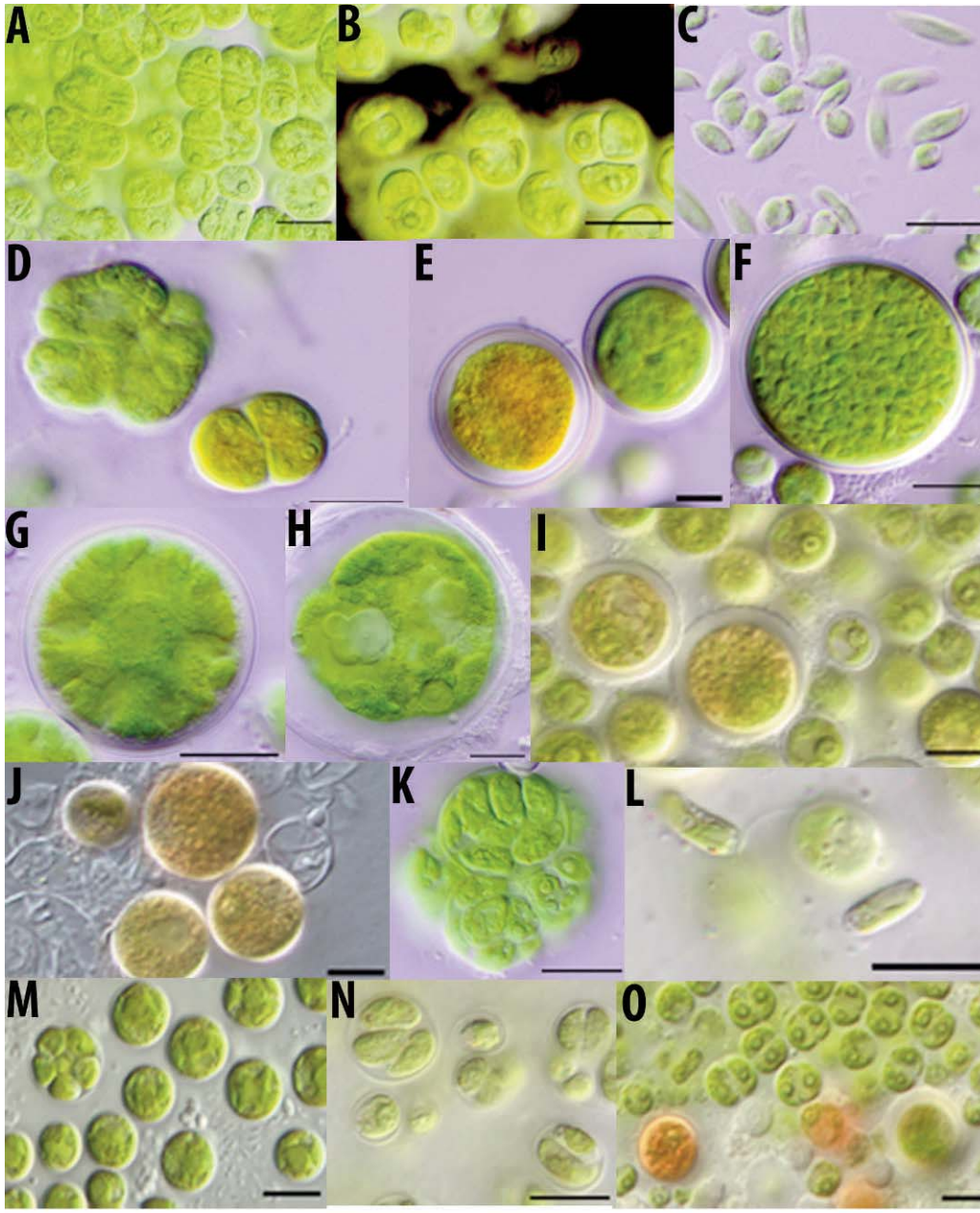


Fig. 5. Representative chlorophyte taxa from clades 1, 2, 5, 6, 7, and 8 (strain numbers are indicated in parentheses). Panels A–C, clade 2, *Chlorosarcinopsis:* **A,** packets formed by vegetative cell division (WJT71VFNP5); **B,** extracellular matrix revealed by India ink staining (WJT16VFNP5); **C,** naked zoospores (WJT71VFNP5). Panel **D,** clade 6, *Neochlorosarcina* (WJT66VFNP5). Panels E–F, clade 8, *Bracteacoccus:* **E,** stationary phase cells with thickened walls (WJT4VFNP41); **F,** vegetative cell with multiple chloroplasts (WJT2VFNP14). Panels G–H, clade 7, *Actinochloris:* **G,** vegetative cells with asteroid chloroplast and pyrenoid covered with starch grains (WJT36VFNP18); **H,** thick-walled cell with inclusion (WJT24VFNP24). Panels I–L, clade 1: **I,** vegetative culture with young cells and mature cells with thickened cell walls (WJT43VFNP16); **J,** pigmented cells and persistent cell walls (WJT66VFNP30A); **K,** several generations of sporangia (WJT9VFNP1A); **L,** walled zoospores (WJT43VFNP16). Panels M–N, clade 5a, *Heterochlamydomonas:* **M,** vegetative cells and sporangia (WJT66VFNP31); **N,** sporangia and young vegetative cells in extracellular matrix (WJT9VFNP2B). Panel **O,** clade 5b, *Chlamydomonas,* dividing vegetative cells and older pigmented cells (WJT32VFNP3). Panels A–M and O: scale bar = 10 μm; panel N: scale bar = 5 μm.

is different from the other 2 isolates in this clade. While young cells are ovoid, mature vegetative cells are primarily spherical. The pyrenoid is large and central with a thick hull; the chloroplast is often divided into thick strands or lobes. Walled flagellated zoospores are also produced, as well as broadly ovoid or spherical nonflagellated cells that may be autospores. Molecular analysis also shows that this isolate has 18S rDNA sequence similarities to *Heterochlamydomonas,* and the general morphology of these isolates is consistent with those of this genus. A diacritical feature that defines the genus *Heterochlamydomonas* is the presence of walled zoospores with flagella of uneven length. This feature is difficult to ascertain. To determine whether flagella are equal or unequal in length, one needs to observe a flagellated cell with the flagella lying parallel. While we did see flagellated cells, they were not plentiful, and in no case were we able to observe parallel flagella. Our isolates are embedded in an obvious gelatin layer. The presence of gelatin is mentioned for only one known species of *Heterochlamydomonas* (*H. inaequalis*) and is designated as being associated with the wall of individual cells (Cox and Deason 1969). Our isolates form a clade associated with but separate from the 3 known species and *Heterochlamydomonas,* and it is possible that they are new species in the genus. The correct generic placement of these isolates requires further study. The 2 isolates in clade 5B are spherical, are of similar size (5–16 μm in diameter), contain a chloroplast with a pyrenoid, and are embedded in an extracellular matrix. Some cells have either a thickened cell wall or an individual gelatin hull (Fig. 5N). Older cells accumulate carotenoid pigment. Asexual reproduction is via walled zoospores that are most often produced in pairs (more rarely 4 or 8); mating and zygote production were also observed. The 18S rDNA sequence data groups these isolates with *C. reinhardtii* and *Volvox carteri.* The isolates are similar to members of the genus *Chlamydomonas* in having a motile vegetative phase, walled zoospores, and sexual reproduction; they may represent palmelloid stages of *Chlamydomonas* species.

The 10 isolates in clade 9 share the morphological traits of being spherical, large (25–75 μm in diameter), and multinucleate and having a textured chloroplast with one to multiple pyrenoids. Isolates differ in cell diameter and chloroplast morphology, including the number of pyrenoids present (Fig. 6A–6E). Production of zoospores is rare, and in those isolates where zoospores have been detected, they are naked (Fig. 6F). DNA analysis groups the isolates in clade 9 with *Radiococcus polycoccus* (Fig. 4), but the morphology of these isolates is not consistent with the morphological characteristics of this genus. Our isolates resemble members of the genera *Spongiochloris* and *Neochloris* that are separated morphologically on the basis of chloroplast structure and molecularly on the basis of 18S rDNA sequences. Within the genus *Spongiochloris,* a variety of chloroplast morphologies are described. Molecular data are available for single species of *Spongiochloris* (*S. spongiosa*) and for 2 species of *Neochloris* (*N. aquatica* and *N. vigenis*), and shows that *S. spongiosa* is well separated phylogenetically from *Neochloris.* Additional sequence data, including sequences from other known *Spongiochloris* species, is necessary to clarify the generic assignment of the isolates in this clade.

The 3 WJT isolates forming clade 3 are morphologically similar. Cells are 5–16 μm in diameter, ovoid to spherical, have a parietal chloroplast with a textured surface and one pyrenoid, are uninucleate, and produce morphologically similar walled zoospores (Fig. 6G). Molecular analysis shows these 3 taxa grouping with members of the genus *Chlorogonium* (Fig. 2). The morphology of our isolates is inconsistent with that of *Chlorogonium,* which are spindle-shaped and 5–15 times as long as they are wide. The morphology of our isolates does not fit any known chlorophycean genus, and clade 3 may therefore represent a genus new to science. Additional research is required to resolve this issue.

Isolates in clade 4 do not fall into any known genus based on morphological or molecular data. Cells are spherical to ovoid, 7–30 μm in diameter, and uninucleate (Fig. 6H). The chloroplast fills the cell; the surface has folds or shallow striations and multiple pyrenoids; the hull of the pyrenoid is thin and irregular (Fig. 6I). Reproduction occurs via walled zoospores, and autospores produced 16–64 per sporangium (Fig. 6J). Although these strains are related on the basis of 18S rDNA sequence data, morphological differences among the isolates suggest they may represent several different species.

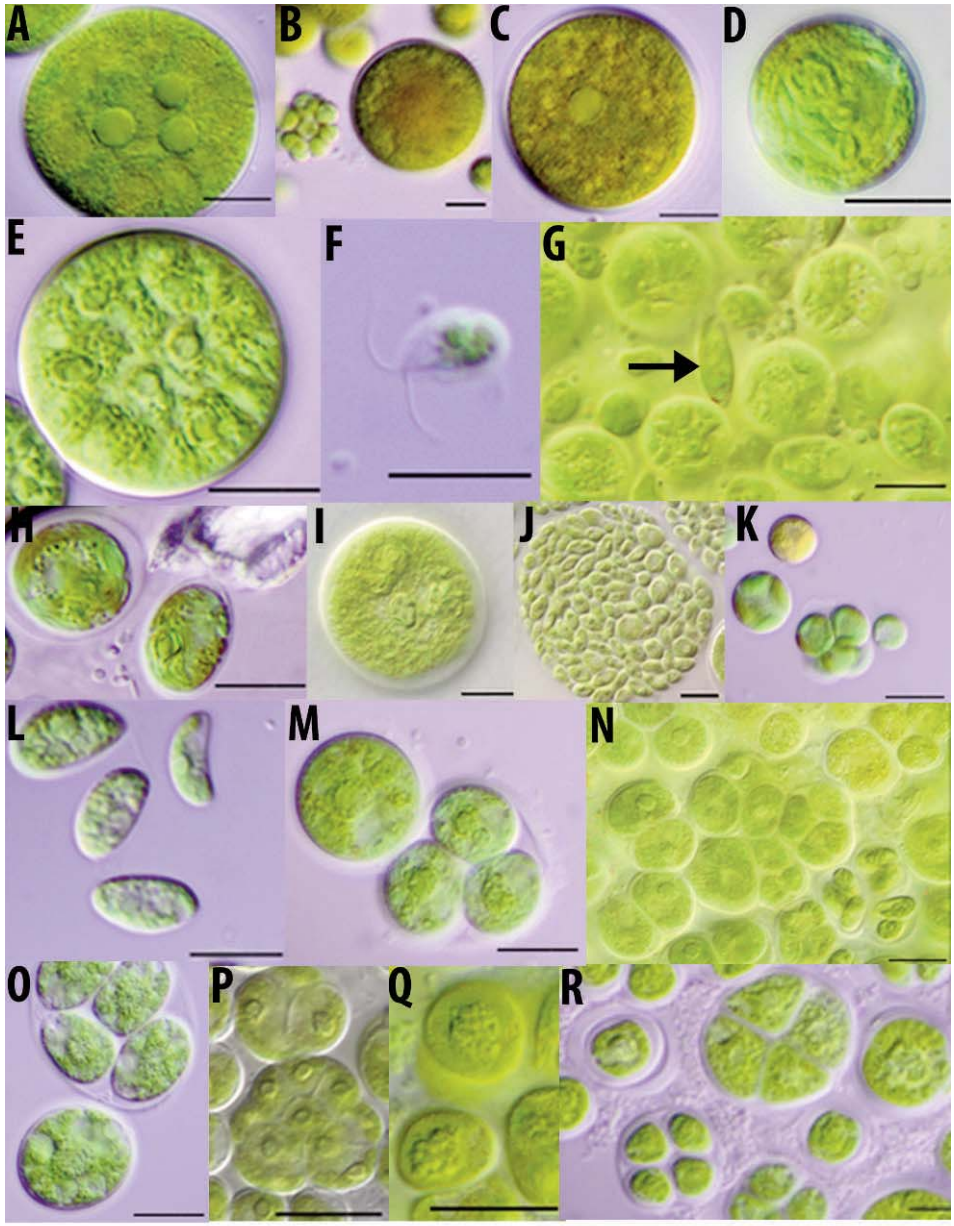


Fig. 6. Representative chlorophyte taxa from clades 3, 4, 9, and 10, as well as chlorophyte taxa that are not closely matched to existing published 18S rDNA sequences (strain numbers are indicated in parentheses). Panels A–F, various chloroplast structures seen in members of clade 9: **A,** multiple pyrenoids (WJT2VFNP6); **B,** pigmented vegetative cells and autospores (WJT66VFNP78); **C,** spongy chloroplast surface (WJT2VFNP21); **D,** striated chloroplast surface (WJT4VFNP18D); **E,** striated chloroplast surface with multiple pyrenoids (WJT74VFNP5); **F,** naked spore (WJT2VFNP6). Panel **G,** clade 3, vegetative cells and walled zoospore indicated by arrow (WJT25VFNP1). Panels H–J, clade 4: **H,** vegetative cells showing thickened outer hull (WJT9VFNP7B); **I,** vegetative cells with multiple pyrenoids (WJ24VFNP4); **J,** sporangium with zoospores (WJT36VFNP12). Panel **K,** clade 12, vegetative cells and autospores (WJT54VFNP11). Panels L–R, taxa which do not fall into clades: **L,** *Scenedesmus* sp. (WJT2VFNP26); **M,** *Tetracystis* sp. showing mature vegetative cells and autospores (WJT46VFNP16); **N,** *Tetracystis aeria* showing vegetative cells and sporangia (WJT43VFNP31A); **O,** unknown chlorophyte showing mature vegetative cells and sporangium with 4 daughter cells (WJY43VFNP1); **P,** unknown chlorophyte showing vegetative cell division (WJT43VFNP26); **Q,** unknown chlorophyte showing pyrenoid with multiple large starch grains (WJT24VFNP48); **R,** unknown chlorophyte showing cells with thick hulls embedded in gelatin (WJT73VFNP2). Panels A–F, H, and J–R: scale bar = 10 μm; panels G and I: scale bar = 5 μm.

The isolates in clade 10 are mostly solitary, spherical with a thin cell wall, and 4–16 μm in diameter (Fig. 6K). Young cells are uninucleate; older cells appear to be multinucleate. The chloroplast is parietal, often lobed and with finger-like projections or thick strands, and lacks a pyrenoid. Sporangia producing 2–8 autospores that can be retained in the mother cell wall are seen in all 3 strains; naked zoospores were observed in WJT74VFNP4. DNA sequence data shows these 3 isolates to be most closely related to the genera *Pediastrum* and *Hydrodictyon* (Fig. 2). These genera, which are composed of closed coenobia forming a sac-like network of very large (up to 15 mm in length) cells or a plate of cells, occur in freshwater habitats. The JTNP isolates bear no morphological similarity to these genera.

Several isolates do not show 18S rDNA sequence similarity to any of the other WJT isolates examined in this study. Of these, 2 can be assigned to known genera; for others, there is not clear generic affiliation. WJT2VFNP26 (Fig. 6L) aligns with the genus *Scenedesmus* based on both morphological and molecular data. Several taxa in this genus (MX7VF7, YPGChar, LG2VF16, and SEV3VF49) are newly described taxa isolated from desert soils in North America. WJT2VFNP26 is similar to *S. bajacalifornicus* and *S. deserticola* in morphology but is separated well enough from known *Scenedesmus* species in the tree that it is probably a new species within the genus.

WJT4VFNP31A (Fig. 6N) fits in the genus *Tetracystis* based on 18S rDNA gene data (Fig. 2) and morphology. The isolate produces autospores that are retained in a stretched mother cell wall and walled zoospores with 2 flagella of equal length. Molecular analysis shows isolate WJT4VFNP31A to be closely related to *T. aeria,* and the morphological characteristics of the isolate are consistent with those reported for this species. The morphology of isolate WJT46VFNP16 is also consistent with its placement in the genus *Tetracystis.* The vegetative cells of WJT46VFNP16 are uninucleate, ovoid to spherical, and are 7.5–32 μm in diameter (Fig. 6M). The parietal chloroplast is asteroid in young cells with a single pyrenoid that is initially covered with small starch grains and then develops a thick hull. Granules accumulate in the cytoplasm and enlarge with age, obscuring the details of chloroplast structure in older cells. Vegetative reproduction is via autospores that are produced 4–8 per sporangium and retained in the stretched mother cell wall and in walled zoospores. WJT46VFNP16 does not cluster closely with WJT4VFNP31A based on 18S rDNA sequence data and is not linked to any known *Tetracystis* species; it may be a new species in this genus.

DNA sequence analysis identified several isolates that did not group with any other JTNP taxa and were not aligned with known genera. Isolate WJT43VFNP26 forms vegetative cells that are spherical and uninucleate. Cells divide to form diads, tetrads, or octets and are found in groups or retained in a mother cell wall (Fig. 6P); solitary cells are seldom seen. The chloroplast is parietal and robust, filling the cell. The chloroplast surface is without structure. There is one pyrenoid with a thick hull, entire or composed of several large starch grains. The cell wall forms a structured matrix that remains after vegetative cells die; this matrix is somewhat thickened. Production of walled zoospores, 3.2–5.5 μm wide and 8–10 μm long, is copious. Zoospores are torpedo shaped with an anterior nucleus, 2 anterior contractile vacuoles, and median stigma. Sequence data shows isolate WTJ43VFNP26 grouping with 2 species of *Chlamydomonas* (Fig. 2), but the morphology of this isolate is not consistent with placement in this genus.

Isolate WJT24VFNP48 forms spherical cells with a parietal chloroplast containing one pyrenoid; this isolate divides vegetatively to form packets of 2 or 4 cells, and forms zoospores that round up when they stop moving. This isolate has several noteworthy characteristics. The pyrenoid is covered with several large, robust starch grains (Fig. 6Q). The zoospores are metabolically active; they move with a snake-like motion and bend and round up slowly when they stop moving. Red pigment is produced as the culture ages. The cells appear to be embedded in a matrix, and in some cases, gelatin hulls can be seen around individual cells. DNA sequence data shows this isolate to be well separated from other *Chlorosarcinopsis* and *Neochlorosarcina* species (Fig. 2); it may therefore represent a new algal genus.

Two isolates, WJT43VFNP1 (Fig. 6O) and WJT73VFNP2 (Fig. 6R), have morphological characteristics similar to those described for members of the genus *Chloromonas,* but neither shares 18S rDNA sequence similarities with any known member of this genus (Fig. 2).

Vegetative cells are uninucleate, ovoid to spherical, and contain an asteroid with 1–2 pyrenoids covered with large starch grains; the cell wall can thicken somewhat with age. Vegetative reproduction is via autospores or walled zoospores that are produced 4–8 per mother cell.

TAXONOMIC ASSIGNMENT OF TREBOUXIOPHYCEAN ISOLATES.—Approximately 25% of the isolates described in this study ($n = 26$) fall into the class Trebouxiophyceae. Clade 12 is composed of isolates of several morphological types. Two of the WJT isolates, WJT8VFNP40 and WJT71VFNP8 (Fig. 7B), have lobed chloroplasts, but the chloroplast morphology of WJT71VFNP8 is more irregular than that of WJT8VFNP40; in both cases, a pyrenoid covered with multiple starch grains is present. Both produce autospores and naked zoospores (Fig. 7C). WJT66VFNP21 (Fig. 7A) has a lobed chloroplast but lacks a pyrenoid; this isolate also produces motile zoospores. Molecular analysis of the 3 WJT isolates in clade 12 places them in a cluster with 2 known members of the genus *Parietochloris* (Figs. 3, 4). The morphologies of isolates WJT8VFNP40 and WJT71VFNP8 are consistent with the characteristics of this genus.

Members of clade 13 have a lobed chloroplast with no pyrenoid (Fig. 7D). Zoospore production was detected in only WJT32VFNP11B; zoospores were naked and no stigma was observed. Molecular data places them in the genus *Myrmecia* (Fig. 3), which now includes *M. astigmatica*, *M. biatorella*, and *M. israeliensis* (Friedl 1995); the morphology of the isolates is consistent with this placement.

Clade 14 contains 2 isolates which group with members of the genus *Stichococcus* based on 18S rDNA sequence data (Figs. 3, 4). The morphology of WJT66VFNP61 (Fig. 7E) is consistent with this placement. WJT24VFNP30 forms small packets (Fig. 7F), and this morphology is more consistent with the genus *Diplosphaera* than with the genus *Stichococcus.*

The isolates that compose clade 11 are small spherical or ovoid algae with a parietal chloroplast and a thin cell wall, and they do not produce zoospores; a pyrenoid is present in all taxa except WJT66VFNP53. These characteristics, coupled with their placement in Trebouxiophyceae on the basis of 18S rDNA data, suggests that they are members of the family Chlorellaceae. This family has been the focus of extensive study by several groups during the last 12 years (Huss et al. 1999, Krienitz et al. 2004, Luo et al. 2006, 2010). Based on molecular data (18S and ITS), the family is divided into 7 clades: *Actinastrum*, *Chlorella*, *Didymogenes*, *Hegewaldia*, *Meyerella*, and *Micractinium* (Krienitz et al. 2004, Luo et al. 2010). Most of the taxa examined in this study are planktonic; edaphic soil isolates were found only in the genera *Chlorella* and *Micractinium.*

Molecular analysis (Fig. 4) shows the isolates in clade 11 (Fig. 6G–6K) to be most closely related to members of the genera *Chlorella* and *Micractinium*, genera shown by several investigators to be closely related phylogenetically (Krienitz et al. 2004, Luo et al. 2006, 2010). Initially, the relationship to *Micractinium* seemed unlikely since 2 diacritical features of this genus are growth in colonies and the formation of spikes on the cell wall. But Luo et al. (2006) have shown that strains of *Micractinium pusillam* possess these characteristics only when a specific grazer, the rotifer *Brachionus calciflorus*, is present in the medium. Since this grazer was not present in our cultures, it is possible that some of our isolates, which are identified as *Micractinium*, are indeed members of that genus, whose members are capable of forming spikes but do not do so under the culture conditions employed in this study. Further study is needed to resolve the generic placement of these isolates.

Clade 15 contains 3 isolates that are related to each other based on 18S rDNA sequence data but do not show affiliation with any known genera (Figs. 3, 4). WJT2VFNP25 and WJT32VFNP13B (Fig. 7L) are morphologically similar to *Chlorosarcina brevispinosa* in that they divide vegetatively to form packets, produce thick-walled cells with surface extensions, and have a parietal chloroplast lacking a pyrenoid; they produce naked zoospores that lack a stigma (Fig. 7M). The morphology of WJT71VFNP22 is different. Most cells of this isolate have one or more inclusions that resemble naked pyrenoids that do not stain with iodine and lack surface extensions (Fig. 7N). No zoospore production was observed.

Originally, the genus *Chlorosarcina* was described as containing 3 species (*C. brevispinosa*, *C. longispinosa*, and *C. stigmatica*), and it was placed in the class Chlorophyceae with *C. stigmatica* as the type species (Deason 1959). Subsequently, Deason and Floyd (1987) separated *C. stigmatica* from *C. longispinosa* and *C. brevispinosa* based on ultrastructural

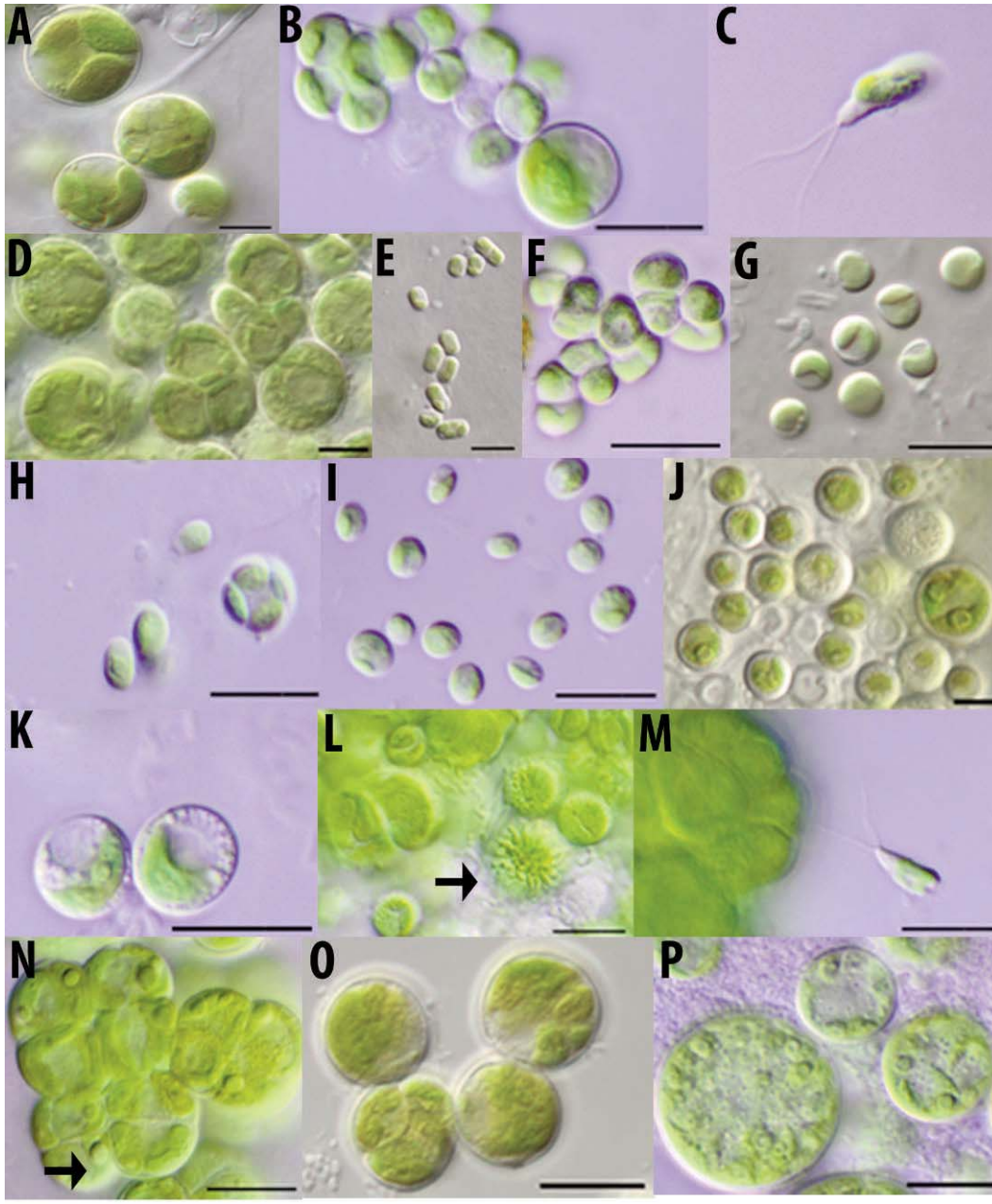


Fig. 7. Representative trebouxiophyte taxa (strain numbers indicated in parentheses). Panels A–C, clade 12, *Parietochloris:* **A,** lobed chloroplast (WJT66VFNP21); **B,** autospores at upper left, pyrenoid covered with starch grains at lower right (WJT71VFNP8); **C,** naked zoospore (WJT71VFNP8). Panel **D,** clade 13, *Myrmecia* showing lobed chloroplast and autospores (WJT32VFNP11B). Panels E–F, clade 14, *Stichococcus:* **E,** single cells (WJT66VFNP61); **F,** packets (WJT24VFNP30). Panels G–K, clade 11: **G,** single cells with parietal chloroplast (WJT36VFNP23); **H,** single cells with pyrenoid and sporangium with 4 daughter cells (WJT4VFNP5); **I,** spherical and ovoid cells (WJT36VFNP9); **J,** spherical cells with thickened cell walls (WJT9VFNP19); **K,** cells with large vacuole (WJT8VFNP18). Panels L–N, clade 15: **L,** single and dividing vegetative cells, some with extensions indicated by arrow (WJT32VFNP13B); **M,** naked zoospore (WJT32VFNP13B); **N,** packet of dividing cells, some with an inclusion indicated by arrow (WJT71VFNP22). Panel **O,** unknown trebouxiophyte showing single cells and sporangia with 4 autospores (WJT24VFNP38). Panel **P,** unknown trebouxiophyte showing multiple chloroplasts, each with its own pyrenoid (WJT71VFNP21). Panels B–C and E–P: scale bar = 10 μm; panels A and D: scale bar = 5 μm.

characteristics and placed *C. longispinosa* and *C. brevispinosa* in the Pleurastrophyceae (now Trebouxiophyceae) based on several characteristics, including counterclockwise orientation of the flagellar apparatus components. In their study evaluating the phylogenetic relationships and taxonomy of sarcinoid green algae, Watanabe and coworkers did not deal specifically with 3 *Chlorosarcina* species (*C. brevispinosa*, *C. longispinosa*, and *C. halophila*) because "they are excluded from the Chlorophyceae" (Watanabe et al. 2006). If the placement of *C. brevispinosa* and *C. longispinosa* in the Trebouxiophyceae is valid, then isolates WJT2VFNP25 and WJT71VFNP22 may be members of the genus *Chlorosarcina.* Since there are no available 18S rDNA sequences from *C. brevispinosa* or *C. longispinosa,* it is not possible to use sequence homology to verify the placement of these isolates in *Chlorosarcina* at this time.

Isolate WJT24VFNP38 groups with *Myrmecia bisecta, M. incisa, Parietochloris cohaerens,* and *P. ovoidea* based on 18S rDNA sequence similarity (Fig. 3); no other WJT isolates fall into this group. Morphologically, the isolate resembles *Myrmecia* in that it has spherical cells with a lobed chloroplast lacking a pyrenoid and forming 4 autospores which are retained in the sporangium (Fig. 7O). Friedl (1995) excluded *M. bisecta* from the genus *Myrmecia* based on zoospore morphology and 18S rDNA sequence data. Zoospore formation was not observed for this isolate, and so it is not possible at this time to determine whether WJT24VFNP38 forms the same type of zoospores as *M. bisecta.* Additional morphological and molecular data are necessary to establish the correct phylogenetic placement of this isolate.

Isolate WJT71VFNP21 is not closely related to any other JTNP isolate. The18S rDNA sequence data places it in the class Trebouxiophyceae but fails to define genus placement (Fig. 3). The cells of this isolate are spherical, large (more than 40 μm in diameter), and multinucleate. The cell wall is thin in young cells, thickening slightly with age. In young cells, the chloroplast is comprised of strands that traverse the cytoplasm, with multiple pyrenoids encased in a robust entire hull. Later, individual chloroplasts, each with its own pyrenoid, are seen (Fig. 7P). These characteristics are consistent with the genus *Follicularia.* Further study is required to establish the correct phylogenetic placement of this isolate.

Xanthophyceae and Eustigmatophyceae

Xanthophytes and Eustigmatophytes were rarely encountered in our sites. Five isolates, 3 Xanthophytes and 2 Eustigmatophytes, were selected for DNA sequence analysis. No tree has been constructed for these taxa because so few were isolated from our sites.

TAXONOMIC ASSIGNMENT OF XANTHOPHYTE ISOLATES.—WJT43VFNP18B and WJT43VFNP32 were assigned to the genus *Capitularia* based on cell morphology. They form short, unbranched filaments that extend along the agar rather than forked, upright filaments that are characteristic of the related genus *Heterococcus* (Fig. 8A–8B). Single cells 10–24 μm in diameter are also observed frequently (Fig. 8D). Cells contain multiple pyrenoids (Fig. 8D). Autospores and zoospores were produced in large spherical sporangia (Fig. 8C); free zoospores were rarely observed (Fig. 8D). A search of DNA sequences in public databases reveals isolate WJT43VFNP18B as being most similar to *H. caespitosus* and WJT43VFNP32 as being most similar to *H. chodatii.* No 18S rDNA sequence data exists for the single *Capitularia* species, *C. radians.* Ettl and Gärtner (1995) consider the differentiation of the 6 genera in the family Heterococcaceae to be artificial. The morphological characteristics of our isolates are most similar to *H. chodatii* (*H. viridis*), and it may be that our isolates are members of the genus *Heterococcus.*

Cells of both WJT43VFNP24 (Fig. 8E) and WJT40VFNP19 (Fig. 8F) are spherical, 5–10 μm (occasionally to 18 μm) in diameter, and uninucleate, with multiple small chloroplasts lacking a pyrenoid. Autospore production was observed; no zoospore production was detected. DNA sequence analysis shows WJT43VFNP24 being related to *P. meiringensis.* The cell morphology of our isolate is consistent with this species, but *P. meiringensis* forms zoospores, and we observed no zoospore formation with our isolate. We did not obtain sequence data for WJT40VFNP19.

A single isolate from the genus *Xanthonema* was recovered; this isolate is morphologically similar to *X. hormidioides* (Fig. 8G). We did not obtain sequence data for this isolate.

TAXONOMIC ASSIGNMENT OF EUSTIGMATOPHYTE ISOLATES.—The 2 Eustigmatophyte

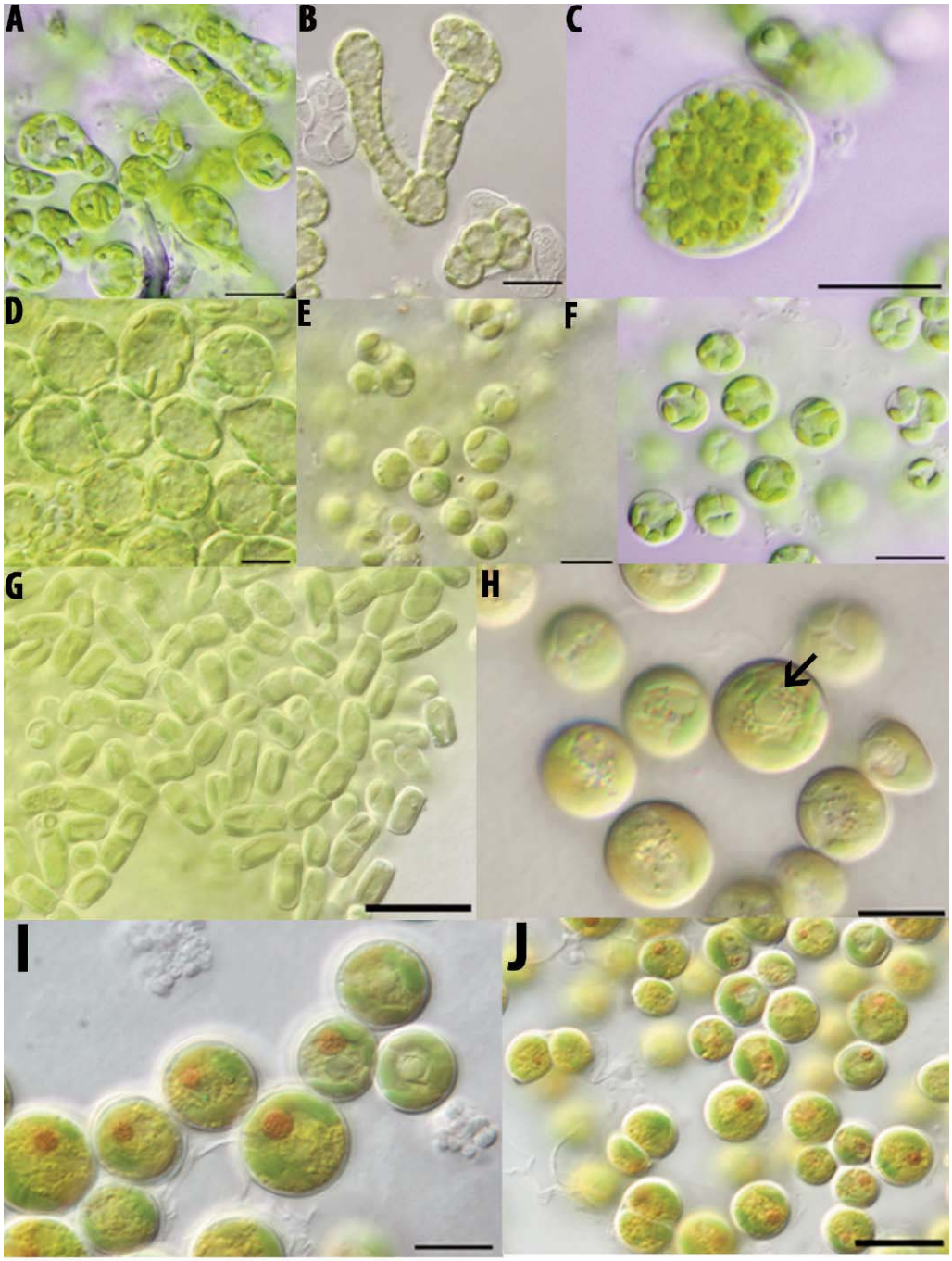


Fig. 8. Representative xanthophyte and eustigmatophyte taxa (strain numbers are indicated in parentheses). Panels A–D, *Capitularia:* **A,** short filaments with bulbous end (WJT43VFNP18B); **B,** short filament and individual cells (WJT43VFNP32); **C,** sporangium with zoospores (WJT43VFNP32); **D,** vegetative cells with multiple pyrenoids and free zoospores at lower left (WJT43VFNP32). Panels E–F, *Pleurococcus:* **E,** vegetative cells with lobed chloroplast and sporangia producing 4 daughter cells (WJT43VFNP24); **F,** vegetative cells and sporangia (WJT40VFNP19). Panel **G,** *Xanthonema* (WJT36VFNP5). Panels H–J, *Eustigmatos:* **H,** vegetative cells with pyrenoid indicated by arrow (WJT66VFNP74); **I,** vegetative cells showing pigment and lobed chloroplast (WJT24VFNP32); **J,** vegetative cells and dividing cells (WJT24VFNP32). Panels A–D, F–G, and I–J: scale bar = 10 μm; panels E and H: scale bar = 5 μm.

isolates for which we have molecular data (WJT24VFNP32 and WJT66VFNP74) show close affinity to the species *Eustigmatos vischeria.* The isolates have chloroplasts with multiple deep lobes (Fig. 8I) and a polyhedral pyrenoid (Fig. 8H). WJT24VFNP32 accumulates droplets of red pigment (Fig. 8I). Both isolates produce autospores as diads or tetrads (Fig. 8J); zoospore formation was not observed.

CONCLUSIONS

We have demonstrated that Joshua Tree National Park (JTNP) supports a diverse green algal flora. Based on phylogenetic analyses of 18S rDNA sequence data, 24 distinct lineages of green algae were determined, 17 in Chlorophyceae and 7 in Trebouxiophyceae. Nine lineages are represented by a single isolate, whereas most are represented by more than one isolate (the 15 collapsed clades in Figs. 2 and 3, and shown in detail in Fig. 4). Members of Xanthophyceae and Eustigmatophyceae were encountered much less frequently, yet these represented 4 different genera. When a given lineage is represented by more than one isolate, morphological variation is evident among the isolates, and often molecular variation is present as well. Together, morphological and molecular variation suggest untapped taxonomic diversity. Other investigators have recorded extensive diversity of green microalgae in aquatic environments (Fawley et al. 2004, Škaloud 2009) and soils (Johansen et al. 1993, Flechtner et al. 1998, 2008). Indeed, Fawley et al. (2004) reported isolation of 273 strains from 4 different sites in North Dakota and Minnesota. Sequence analysis revealed 93 different 18S rDNA sequences among these isolates; of these 93, only 4 sequences corresponded to sequences in GenBank. Collectively, these studies demonstrate that microalgae, including those in JTNP soils, are poorly understood and need further investigation.

Sequence data from the 3′ end of the 18S rDNA gene was used as a phylogenetic marker in this study because ribosomal sequence data are a common starting point in phylogenetic studies (e.g., Nakada et al. 2008), and a number of 18S sequences are available in public databases. But 18S data are a coarse-grained estimator of diversity, particularly for comparison of species in a single genus. Recently, Hall et al. (2010) assessed the efficacy of 7 molecular markers (cytochrome oxidase I, the ITS region of the nuclear rRNA operon, a portion of the chloroplast 23S rRNA gene, the plastid encoded rbcL, and *tuf*A) that are more variable than the 18S rDNA gene to serve as bar codes in freshwater green algae (Charophyceae, Chlorophyceae, and Zygnematophyceae). They were unable to identify any marker that was useful in all 3 groups, but they concluded that the ITS, rbcL, and *tuf*A loci were able to differentiate closely related chlorophyte species. A case in point is our study of *Scenedesmus* species. When we used 18S sequences to compare newly isolated *Scenedesmus* strains recovered from arid sites in the western United States and Baja California, Mexico, with known *Scenedesmus* species, only a few well-supported clades were recovered, reflecting a lack of diversity in 18S sequences in this genus. But when we expanded our sequence analysis to include internal transcribed spacer (ITS1, 5.8S, and ITS2) sequence data, bootstrap analysis resolved 5 distinct clades, demonstrated that *Scenedesmus* lineages from arid soils align separately from their aquatic relatives, and allowed us to describe 3 new species in this genus (Lewis and Flechtner 2004). Molecular phylogenetic analysis of the isolates reported in this study employing more variable makers (e.g. ITS or rbcL) may reveal a higher level of diversity than is suggested by our initial findings.

The problems associated with identification of microalgae at the genus and species levels have been thoroughly discussed in a recent review by Pröschold and Leliaert (2007). These authors emphasize the importance of a multiphasic approach involving morphological characterization using a light microscope, ultrastructural characterization using an electron microscope, and DNA sequence analysis (e.g., Fučíková et al. in press). Both morphological and molecular data were necessary to uncover the diversity observed in this study. Use of morphology alone would have masked the true diversity of genera such as *Bracteacoccus* and *Chlorosarcinopsis*, both of which contain species that can be differentiated only on the basis of molecular data. On the other hand, obtaining DNA sequence data from new microalgal isolates does not assure the identification of that isolate to the genus and species. In some instances (e.g., clades 7 and 15), isolates fit the morphological description of a

known genus (*Actinochloris* and *Chlorosarcina*, respectively), but DNA sequence data for known members of the genus is not available from GenBank. For some genera where 18S rDNA sequence data are available, the data are often limited to a single species (e.g., *Spongiochloris spongiosa*); this limitation is particularly problematic where the known genus may be polyphylectic.

Many of the JTNP isolates examined in this study do not align with known species of green algae. These isolates may represent species or even genera new to science. In order to fully clarify the diagnosis of new isolates placed provisionally in a known genus on the basis of morphology, it is necessary to obtain type strains and get comparable data, as was done for *Scenedesmus* (Lewis and Flechtner 2004), *Chlorosarcinopsis* (Watanabe et al. 2006), *Chlorella* (Luo et al. 2010), and *Bracteacoccus* (Fučíková et al. in press).

Researchers attempting to identify newly isolated microalgal taxa from North American soils face additional challenges. Traditionally, European researchers have been more active in this field than have American researchers, and as a consequence, most of the authoritative texts (e.g., Ettl and Gärtner 1995) describe European taxa and are not available in English. Aquatic habitats have received more attention than terrestrial habitats; as a consequence, many of the algal genera and species for which good morphological descriptions and molecular data exist are aquatic. In addition, many earlier papers describing taxa from arid and semiarid North American locales that would be most useful for those researchers are out of print and difficult to obtain.

There is currently no centralized reference source describing terrestrial algae from arid and semiarid regions of North America. Many of the taxonomic studies providing detailed morphological and physiological descriptions of algal species are old and out of print. Algaebase (www.algaebase.com) is a useful public access database containing over 127,000 algal species and over 10,800 images, but the number of taxa listed for North America is small. We have developed our own web site (http://hydrodictyon.eeb.uconn.edu/bcp/) as a research and teaching resource, and we continue to update this site as new data become available. The site includes micrographs, descriptions and links to sequence data for numerous isolates from California, Utah, New Mexico, San Nicolas Island, and Baja, California, Mexico. It is our hope that further analysis of the taxa isolated from JTNP will not only provide a good understanding of the algal flora of this park, but will be of use to researchers and teachers working to identify the microalgae present in desert soils and will also contribute to our understanding of the phylogenetic relationships among green algae.

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LITERATURE CITED

ALTSCHUL, S.F., W. GISH, W. MILLER, E.W. MYERS, AND D.J. LIPMAN. 1990. Basic local alignment search tool. Journal of Molecular Biology 215:403–410.

CAMERON, R.E. 1960. Communities of soil algae occurring in the Sonoran Desert in Arizona. Journal of the Arizona Academy of Science 1:85–88.

_____. 1964. Terrestrial algae of southern Arizona. Transactions of the American Microscopical Society 83: 212–218.

COX, E.R., AND T.R. DEASON. 1969. *Heterochlamydomonas*, a new alga from Tennessee. Journal of the Tennessee Academy of Science 44:105–107.

DEASON, T.R. 1959. Three Chlorophyceae from Alabama soil. American Journal of Botany 46:572–578.

DEASON, T.R., AND G.L. FLOYD. 1987. Comparative ultrastructure of three species of *Chlorosarcina* (Chlorosarcinaceae, Chlorophyta). Journal of Phycology 23: 187–195.

DURRELL, L.W. 1962. Algae of Death Valley. Transactions of the American Microscopical Society 81:267–278.

ETTL, H., AND G. GÄRTNER. 1995. Syllabus der Boden-, Luft-, und Flechtenalgae. Gustav Fisher Verlag, Stuttgart. 721 pp.

FAWLEY, M.W., K.P. FAWLEY, AND M.A. BUCHHEIM. 2004. Molecular diversity among communities of freshwater microchlorophytes. Microbial Ecology 48:489–499.

FLECHTNER, V.R., J.R. JOHANSEN, AND J. BELNAP. 2008. The biological soil crusts of the San Nicolas Island:

enigmatic algae from a ecologically isolated ecosystem. Western North American Naturalist 68:405–436.

FLECHTNER, V.R., J.R. JOHANSEN, AND W.C. CLARK. 1998. Algal composition of microbiotic crusts from the Central Desert of Baja California, Mexico. Great Basin Naturalist 58:295–311.

FRIEDL, T. 1995. Inferring taxonomic positions and testing genus level assignments in coccoid green lichen algae: a phylogenetic analysis of 18S ribosomal RNA sequences from *Dictyochloris reticulta* and from members of the genus *Myrmecia* (Chlorophyta, Trebouxiophyceae cl. nov.). Journal of Phycology 31: 632–639.

FUČÍKOVÁ, K., V. FLECHTNER, AND L.A. LEWIS. In press. Revision of the genus *Bracteacoccus* Tereg (Chlorophyceae, Chlorophyta) based on a phylogenetic approach. Nova Hedwigia.

HALL, J.D., K. FUČÍKOVÁ, C. LO, L.A. LEWIS, AND K.G. KAROL. 2010. An assessment of proposed DNA barcodes in freshwater green algae. Cryptogamie, Algologie 31:529–555.

HUELSENBECK, J.P., AND F. RONQUIST. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17: 754–755.

HUNT, C.D., AND I.W. DURRELL. 1966. Distribution of fungi and algae. U.S. Geological Survey Professional Paper 509:55–66.

HUSS, V.A.R., C. FRANK, E.C. HARTMAN, M. HIRMER, A. KLOBPICEL, B.M. SEIDEL, P. WENSELER, AND E. KESSLER. 1999. Biochemical taxonomy and molecular phylogeny or the genus *Chlorella* sensu lato (Chlorophyta). Journal of Phycology 35:587–598.

JOHANSEN, J.R., J. ASHLEY, AND W.R. RAYBURN. 1993. Effects of range fire on soil algal crusts in semiarid shrub-steppe of the lower Columbia Basin and their subsequent recovery. Great Basin Naturalist 53:73–88.

KRIENITZ, L., E.H. HEGEWALD, D. HEPPERLE, V.A.R. HUSS, R. ROHR, AND M. WOLF. 2004. Phylogenetic relationship of *Chlorella* and *Parachlorella* gen. nov. (Chlorophyta, Trebouxiophyceae). Phycologia 43: 529–542.

LEWIS, L.A. 1997. Diversity and phylogenetic placement of *Bracteacoccus* Tereg (Chlorophyceae, Chlorophyta). Journal of Phycology 33:279–285.

LEWIS, L.A., AND V.R. FLECHTNER. 2002. Green algae (Chlorophyta) of desert microbiotic crusts: diversity of North American taxa. Taxon 51:443–451.

_____. 2004. Cryptic species of *Scenedesmus* (Chlorophyta) from desert soil communities of western North America. Journal of Phycology 40:1127–1137.

LEWIS, L.A., AND P.O. LEWIS. 2005. Unearthing the molecular phylodiversity of desert soil green algae (Chlorophyta). Systematic Biology 54:936–947.

LUO, W., S. PFLUGMACHER, T. PRÖSCHOLD, N. WALZ, AND L. KRIENTIZ. 2006. Genotype versus phenotype variability in *Chlorella* and *Micractinium* (Chlorophyta, Trebouxiophyceae). Protist 157:315–333. Available from: http://www.elsevier.de/protis

LUO, W., T. PRÖSCHOLD, C. BOCK, AND L. KRIENITZ. 2010. Generic concept of *Chlorella*-related coccoid green algae (Chlorophyta, Trebouxiophyceae). Plant Biology 12:245–253.

MEIGS, P. 1953. World distribution of arid and semi-arid homoclimates. Pages 203–209 *in* Reviews of Research on Arid Zone Hydrology: Paris, United Nations Educational, Scientific, and Cultural Organization, Arid Zone Programme-1.

NAKADA, T., K. MISAWA, AND H. NOZAKI. 2008. Molecular systematics of Volvocales (Chlorophyceae, Chlorophyta) based on exhaustive 18S rDNA phylogenetic analysis. Molecular Phylogenetics and Evolution 48: 281–291.

PIETRASIAK, N., J.R. JOHANSEN, AND R.E. DRENOVSKY. 2011. Geologic composition influences distribution of microbiotic crusts in the Mojave and Colorado deserts at the regional scale. Soil Biology and Biochemistry 43:967–974.

PRÖSCHOLD, T., AND F. LELIAERT. 2007. Systematics of the green algae: conflict of classic and modern approaches. Pages 123–154 *in* J. Brodie and J. Lewis, editors, Unravelling the algae: the past, present, and future of algal systematics. CRC Press. 402 pp.

RAMBAUT, A., AND A. DRUMMOND. 2003. Tracer: MCMC trace analysis tool. University of Oxford, Oxford, United Kingdom.

RONQUIST, F., AND J.P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

SHIELDS, L.M., AND F. DROUET. 1962. Distribution of terrestrial algae within the Nevada Test Site. USDA SSIR1. U.S. Government Printing Office, Washington, DC. 63 pp.

SIEBERT, S., AND R. BACKOFEN. 2005. MARNA: multiple alignment and consensus structure prediction of RNAs based on sequence structure comparisons. Bioinformatics 21:3352–3359.

ŠKALOUD, P. 2009. Species composition and diversity of aero-terrestrial algae and cyanobacteria of the Boreč Hill ventaroles. Fottea 9:65–80.

SLUIMAN, H.J., AND G. GÄRTNER. 1990. Taxonomic studies on the genus *Pleurastrum* (Pleurastrales, Chlorophyta). I. The type species, *P. insigne*, rediscovered and isolated from soils. Phycologia 29:133–138.

SWOFFORD, D.L. 2002. PAUP* 4.0: Phylogenetic Analysis Using Parsimony [software]. Version 4.0b10. Distributed by Sinauer Associates, Fitchburg, MA.

TRAINOR, F.R., AND P.F. EGAN. 1990. Phenotypic plasticity in *Scenedesmus* (Chlorophyta) with special reference to *S. armatus* unicells. Phycologia 29:461–469.

WATANABE, S. 1983. New and interesting green algae from soils of some Asian and Oceanian regions. Arch. Protistenkdr. 127:223–270.

WATANABE, S., K. MITUSUI, T. NAKAYAMA, AND I. INOUYE. 2006. Phylogenetic relationships and taxonomy of sarcinoid green algae: *Chlorosarcinopsis, Desmotetra, Sarcinochlamys* gen. nov., *Neochlorosarcina,* and *Chlorosphaeropsis* (Chlorophyceae, Chlorophyta). Journal of Phycology 42:679–695.

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APPENDIX 1. Universal Transverse Mercator GPS coordinates for the site sampled within Joshua Tree National Park. Coordinates listed are within zone 11S, NAD83 datum.

Site ID	Easting	Northing	Site ID	Easting	Northing
WJT2	639079	3775129	WJT40	580981	3758029
WJT4	601307	3756724	WJT43	564402	3761209
WJT8	611132	3750951	WJT46	618806	3741017
WJT9	600837	3748948	WJT54	625202	3749282
WJT16	633011	3762629	WJT61	657603	3761283
WJT24	611479	3736523	WJT66	576564	3769728
WJT25	627695	3757108	WJT71	648298	3760591
WJT32	642508	3774289	WJT73	645317	3757879
WJT36	578916	3767071	WJT74	618410	3758393

APPENDIX 2. Algal strains used in this study, organized into major taxonomic groups, with their corresponding localities (JTNP Area, as detailed in Appendix 1). For the isolates that were included in phylogenetic analyses, the GenBank accession numbers and corresponding numbered clades illustrated in Figures 2–4 are reported. Previously published sequences are indicated with an asterisk.

	JTNP Area	Strain number	Taxon	18S rDNA GenBank accession number
CHLOROPHYCEAE CLADE				
1	9	WJT9VFNP1A	*Chlorococcum* sp.	JX446402
1	24	WJT24VFNP10	*Chlorococcum* sp.	JX446403
1	40	WJT40VFNP8	*Chlorococcum* sp.	JX446404
1	43	WJT43VFNP8	*Chlorococcum* sp.	JX446405
1	43	WJT43VFNP16	*Chlorococcum* sp.	JX446406
1	66	WJT66VFNP7	*Chlorococcum* sp.	JX446407
1	66	WJT66VFNP30A	*Chlorococcum* sp.	JX446408
1	66	WJT66VFNP77	*Chlorococcum* sp.	JX446409
2	4	WJT4VFNP30B	*Chlorosarcinopsis* sp.	JX446410
2	8	WJT8VFNP1	*Chlorosarcinopsis* sp.	JX446411
2	16	WJT16VFNP5	*Chlorosarcinopsis* sp.	JX446412
2	24	WJT24VFNP12	*Chlorosarcinopsis* sp.	JX446413
2	43	WJT43VFNP28	*Chlorosarcinopsis* sp.	JX446414
2	46	WJT46VFNP9	*Chlorosarcinopsis* sp.	JX446415
2	71	WJT71VFNP16	*Chlorosarcinopsis* sp.	JX446416
3	25	WJT25VFNP1	Chlorophycean green alga	JX446417
3	71	WJT71VFNP3	Chlorophycean green alga	JX446418
3	71	WJT71VFNP9	Chlorophycean green alga	JX446419
4	9	WJT9VFNP7B	Chlorophycean green alga	JX446420
4	24	WJT24VFNP4	Chlorophycean green alga	JX446421
4	36	WJT36VFNP12	Chlorophycean green alga	JX446422
4	66	WJT66VFNP9	Chlorophycean green alga	JX446423
5A	9	WJT9VFNP2B	*Heterochlamydomonas* sp.	JX446424
5A	9	WJT9VFNP8B	*Heterochlamydomonas* sp.	JX446425
5A	66	WJT66VFNP31	*Heterochlamydomonas* sp.	JX446426
5B	4	WJT4VFNP4	*Chlamydomonas*-like green alga	JX446427
5B	32	WJT32VFNP3	*Chlamydomonas*-like green alga	JX446428
6	4	WJT4VFNP17	*Neochlorosarcina* sp.	JX446429
6	36	WJT36VFNP8	*Neochlorosarcina* sp.	JX446430
6	40	WJT40VFNP11	*Neochlorosarcina* sp.	JX446431
6	43	WJT43VFNP13	*Neochlorosarcina* sp.	JX446432
6	54	WJT54VFNP4	*Neochlorosarcina* sp.	JX446433
6	66	WJT66VFNP5	*Neochlorosarcina* sp.	JX446434
6	66	WJT66VFNP54	*Neochlorosarcina* sp.	JX446435
7	24	WJT24VFNP16	*Actinochloris* sp.	JX446436
7	24	WJT24VFNP24	*Actinochloris* sp.	JX446437
7	36	WJT36VFNP18	*Actinochloris* sp.	JX446438
7	74	WJT74VFNP6	*Actinochloris* sp.	JX446439
8	2	WJT2VFNP14	*Bracteacoccus occidentalis*	GQ985407*
8	4	WJT4VFNP35	*Bracteacoccus* sp.	JX446440
8	4	WJT4VFNP36B	*Bracteacoccus* sp.	JX446441
8	4	WJT4VFNP41	*Bracteacoccus occidentalis*	JX455835
8	8	WJT8VFNP19	*Bracteacoccus occidentalis*	JQ259951*

APPENDIX 2. Continued.

	JTNP Area	Strain number	Taxon	18S rDNA GenBank accession number
8	9	WJT9VFNP16A	*Bracteacoccus occidentalis*	JQ259948*
8	9	WJT9VFNP12B	*Bracteacoccus pseudominor*	JQ259953*
8	24	WJT24VFNP45	*Bracteacoccus* sp.	JX446442
8	36	WJT36VFNP6	*Bracteacoccus glacialis*	JQ259940*
8	40	WJT40VFNP9	*Bracteacoccus* sp.	JX446443
8	71	WJT71VFNP15	*Bracteacoccus pseudominor*	HQ246324*
9	2	WJT2VFNP6	*Radiococcus* sp.	JX446444
9	2	WJT2VFNP21	*Radiococcus* sp.	JX446445
9	9	WJT9VFNP3A	*Radiococcus* sp.	JX446446
9	43	WJT43VFNP6	*Radiococcus* sp.	JX446447
9	43	WJT43VFNP18A	*Radiococcus* sp.	JX446448
9	43	WJT43VFNP18D	*Radiococcus* sp.	JX446449
9	43	WJT43VFNP27	*Radiococcus* sp.	JX446450
9	66	WJT66VFNP50A	*Radiococcus* sp.	JX446451
9	66	WJT66VFNP78	*Radiococcus* sp.	JX446452
9	74	WJT74VFNP5	*Radiococcus* sp.	JX446453
10	54	WJT54VFNP1	Hydrodictyaceae green alga	JX446454
10	54	WJT54VFNP11	Hydrodictyaceae green alga	JX446455
10	74	WJT74VFNP4	Hydrodictyaceae green alga	JX446456
NA	2	WJT2VFNP26	*Scenedesmus* sp.	JX446457
NA	4	WJT4VFNP31A	*Tetracystis* sp.	JX446458
NA	24	WJT24VFNP48	Chlorophycean green alga	JX446459
NA	43	WJT43VFNP1	Chlorophycean green alga	JX446460
NA	43	WJT43VFNP26	Chlorophycean green alga	JX446461
NA	46	WJT46VFNP16	Chlorophycean green alga	JX446462
NA	73	WJT73VFNP2	Chlorophycean green alga	JX446463
TREBOUXIOPHYCEAE CLADE				
11	4	WJT4VFNP1	Chlorellaceae green alga	JX446464
11	4	WJT4VFNP5	Chlorellaceae green alga	JX446465
11	4	WJT4VFNP13	*Micractinium* sp.	JX446466
11	8	WJT8VFNP18	*Micractinium* sp.	JX446467
11	9	WJT9VFNP13A	Chlorellaceae green alga	JX446468
11	9	WJT9VFNP19	*Micractinium* sp.	JX446469
11	36	WJT36VFNP3	Chlorellaceae green alga	JX446470
11	36	WJT36VFNP9	Chlorellaceae green alga	JX446471
11	36	WJT36VFNP23	Chlorellaceae green alga	JX446472
11	66	WJT66VFNP53	Chlorellaceae green alga	JX446473
11	66	WJT66VFNP79	Chlorellaceae green alga	JX446474
12	8	WJT8VFNP40	*Parietochloris* sp.	JX446475
12	66	WJT66VFNP21	*Parietochloris* sp.	JX446476
12	71	WJT71VFNP8	*Parietochloris* sp.	JX446477
13	24	WJT24VFNP46	*Myrmecia* sp.	JX446478
13	32	WJT32VFNP11B	*Myrmecia* sp.	JX446479
13	36	WJT36VFNP19	*Myrmecia* sp.	JX446480
13	40	WJT40VFNP15	*Myrmecia* sp.	JX446481
13	61	WJT61VFNP15	*Myrmecia* sp.	JX446482
14	24	WJT24VFNP30	*Stichococcus* sp.	JX446483
14	66	WJT66VFNP61	*Stichococcus* sp.	JX446484
15	2	WJT2VFNP25	Trebouixiophyceae green alga	JX446485
15	32	WJT32VFNP13B	Trebouixiophyceae green alga	JX446486
15	71	WJT71VFNP22	Trebouixiophyceae green alga	JX446487
NA	24	WJT24VFNP38	Trebouixiophyceae green alga	JX446488
NA	71	WJT71VFNP21	Trebouixiophyceae green alga	JX446489
XANTHOPHYCEAE				
	43	WJT43VFNP18B	*Heterococcus* sp.	JX446490
	43	WJT43VFNP32	*Heterococcus* sp.	JX446491
	43	WJT43VFNP24	*Pleurococcus* sp.	JX446492
	40	WJT40VFNP19	*Pleurococcus* sp.	isolate only
	36	WJT36VFNP5	*Xanthonema* sp.	isolate only
EUSTIGMATOPHYCEAE				
	24	WJT24VFNP32	*Eustigmatos* sp.	JX446493
	66	WJT66VFNP74	*Eustigmatos* sp.	JX446494