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IDENTIFICATION OF A PSYCHROPHILIC GREEN ALGA FROM LAKE BONNEY ANTARCTICA: *CHLAMYDOMONAS RAUDENSIS* ETTL. (UWO 241) *CHLOROPHYCEAE*[1]

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An unusual psychrophilic green alga was isolated from the deepest portion of the photic zone (<0.1% of incident PAR) at a depth of 17 m in the permanently ice-covered lake, Lake Bonney, Antarctica. Here we identify and report the first detailed morphological and molecular examination of this Antarctic green alga, which we refer to as strain UWO 241. To determine the taxonomic identity, UWO 241 was examined using LM and TEM and partial sequences of the small subunit (SSU), internal transcribed spacer (ITS) 1 and ITS2 regions (including the 5.8S) of the ribosomal operon. These data were compared with those of previously described taxa. We identified UWO 241 as a strain of *Chlamydomonas raudensis* Ettl (SAG 49.72). *Chlamydomonas raudensis* is closely related to *C. noctigama* Korshikov (UTEX 2289) as well as foraminifer symbionts such as *C. hedleyi* Lee, Crockett, Hagen et Stone (ATCC 50216). In addition, its morphology, pigment complement, and phototactic response to temperature are reported. *Chlamydomonas raudensis* (UWO 241) contains relatively high levels of lutein and low chl *a*/*b* ratios (1.6 ± 0.15), and the phototactic response was temperature dependent. The Antarctic isolate (UWO 241) included the typical photosynthetic pigments found in all chl *a*/*b* containing green algae. It possesses a small eyespot and, interestingly, was positively phototactic only at higher nonpermissive growth temperatures. Comparison of SSU and ITS rDNA sequences confirms the identification of the strain UWO 241 as *C. raudensis* Ettl and contradicts the previous designation as *C. subcaudata* Wille.

Key index words: **Antarctica; *Chlamydomonas; Chlamydomonas hedleyi; Chlamydomonas noctigama;* Chlamydomonas raudensis*; Chlamydomonas subcaudata;* Lake Bonney; phototaxis; phylogeny; psychrophile; systematics; UWO 241**

Abbreviations: ITS, internal transcribed spacer; SSU, ribosomal small subunit

Antarctica consists of many harsh and extreme environments, and despite the general increase in air temperature at the Earth's surface, the air temperatures of the McMurdo Dry Valleys have cooled at the rate of 0.7° C per decade from 1986 until 2000 (Doran et al. 2002). This region is one of the driest and coldest deserts on Earth, and it houses the only permanently ice-covered lakes on our planet (Priscu 1998). Average annual precipitation is less than 10 cm, and the average annual air temperature is approximately −20° C (Priscu et al. 1999). Lake Bonney is situated in the Taylor Valley, which is located within the McMurdo Dry Valley system, southern Victoria Land, Antarctica. The lake is 7 km long and 1 km wide at the widest point (Fritsen and Priscu 1999). To the north and south of the lake lie peaks that are over 3000 m above sea level, and the Taylor Glacier is positioned to the west of the lake. The lake is unique because of the strong vertical stratification of its physical, chemical, and biological attributes. It consists of two lobes, each approximately 40 m deep and permanently covered by 3–4.5 m of ice (Fritsen and Priscu 1999). The ice cover prevents wind mixing of the water column, resulting in vertical motion only at the molecular scale; vertical mixing time for Lake Bonney is approximately 50,000 years (Moorhead et al. 1999). Temperature differences are typically the cause for vertical stratification in aqueous

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systems, although in this lake stratification occurs as a result of strong salinity gradients that produce distinct density differences (Spigel and Priscu 1996). The salinity of the lake ranges from fresh water at the surface to a hypersaline brine (more than five times seawater) at the bottom (Spigel and Priscu 1998). The temperature profile is unusual, with a range from 0° C at the ice–water interface to a maximum of 6.1° C at a depth of 14 m. The minimum temperature is −1.0° C at the bottom of the lake (Priscu et al. 1999). Temperature and thermoclines remain constant year round due to the ice cover and lack of vertical mixing.

The light environment in and around the lake is also distinct. There are four months of darkness during the austral winter, and in Lake Fryxell, another permanently ice-covered lake in the McMurdo Dry Valleys, algal cells remain in the vegetative stage during the winter months, generating the required energy through heterotrophy (McKnight et al. 2000). There are no data as yet on the overwintering strategies of Lake Bonney's phytoplankton. During the austral summer the ice cover on Lake Bonney attenuates between 97% and 99% incident PAR and absorbs all wavelengths greater than 600 nm (Howard-Williams et al. 1998). Therefore, photosynthetic inhabitants of the water column below the ice are never exposed to saturating light levels and have adapted to a shade environment with a spectral distribution in the blue-green range (480–520 nm) (Lizotte and Priscu 1994).

Biological stratification in Lake Bonney results in discrete phytoplankton assemblages (Koob and Leister 1972, Priscu et al. 1999). The first detailed biological limnologic characterization of the east lobe of Lake Bonney was performed during the austral summer of 1965–1966 (Koob and Leister 1972). Carbon fixation was found to occur in the lake, and chl *a* concentration maxima were obtained at depths of 4 and 12 m (Koob and Leister 1972). Two important planktonic populations were found to dominate the lake. The first was a unicellular flagellated green alga that Koob and Leister (1972) identified as *Chlamydomonas subcaudata* because of its morphology and to previous reports of this species in Antarctic regions. The first reference to *C. subcaudata* (SAG 12.87) was made in 1908 by J. Murray, who reported its occurrence in Pony Lake, Victoria Land, Antarctica (Hirano 1965). The second most abundant planktonic population consisted of an unidentified unicellular coccoid alga (Koob and Leister 1972). In addition, a large population of cyanobacteria was found at the ice–water interface, and five spatially distinct bacterial populations were found down to 15 m below the ice (Koob and Leister 1972). Parker et al. (1977) continued the limnological studies of the east lobe of Lake Bonney during the austral summers of 1972–1973 and 1973–1974. Two major communities were uncovered, a planktonic one containing cryptophyte, chrysophyte, and chlorophyte algae; heterotrophic bacteria and yeasts; viruses; microfauna such as ciliates and rotifers and a benthic algal mat consisting of oscillatorious blue-green algae, protozoa, bacteria, fungi, and microfauna (Parker et al. 1977, Priscu et al. 1999).

In the 1990s, an enigmatic green alga (UWO 241) was isolated from the east lobe of Lake Bonney and based on its Antarctic location and the existing literature it was believed to be *C. subcaudata* (Neale and Priscu 1995). We designate this Antarctic isolate hereafter as UWO 241. The isolate UWO 241 was the dominant species in a stratified layer 17 m below the ice where the average temperature is between 4 and 6° C and irradiance is below 15 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (Lizotte and Priscu 1994). This green alga is an obligate psychrophile because it does not grow at temperatures above 16° C (Morgan et al. 1998). Furthermore, the photosynthetic apparatus in this green alga is unusual in its composition and function (Neale and Priscu 1995, Morgan et al. 1998, Morgan-Kiss et al. 2002). Compared with *C. reinhardtii* (UTEX 89), UWO 241 has an unusually low chl *a/b* ratio ($\leq$2.0) and low levels of PSI and PSI-associated proteins relative to PSII (Morgan et al. 1998). Interestingly, this shade-adapted psychrophilic alga has the capacity to acclimate physiologically to high light, although it is not able to undergo short-term photoacclimatory state transitions that balance energy distribution between PSII and PSI; the psychrophile is locked in state one (Morgan et al. 1998, Morgan-Kiss et al. 2002).

In addition to photoacclimation or photoprotection, motile green algae can adjust the amount of light energy absorbed through phototaxis. Phototaxis involves the sensing of light and the directed movement toward (positive) or away from (negative) a light source. *Chlamydomonas* species possess eyespots that contain an optical system composed of carotenoid-rich lipid globules and opsin-related retinal-binding proteins, including chlamyopsin (Fuhrmann et al. 2001). Traditionally, the number and position of pyrenoid, the cell and chloroplast shape, and the presence or absence and the size of eyespots had been used as characters defining *Chlamydomonas* species (Ettl 1976, Hoham et al. 2002). However, caution must be taken because variation in chloroplast morphology occurs between individual species within the same population complex as do the presence or absence and the size of eyespots (Hoham et al. 2002). Through an interesting set of experiments, Priscu and Neale (1995) examined whether phototaxis was responsible for maintaining the stratification of the discrete *Chlamydomonas* populations in Lake Bonney. The 4-m population maintains its place in the water column through phototaxis; the 12-m population does not, but phototaxis can be induced by exposure to higher irradiance. Finally, there was no phototactic response in the 18-m population *in situ* or when incubated at a higher irradiance (Priscu and Neale 1995). This led the authors to conclude that the maintenance of biologically stratified layers in Lake Bonney is the result of a set of complex physiological responses to an equally complex set of environmental variables.

The UWO 241 strain from Lake Bonney plays an important ecological role by providing the ecosystem with its energy requirements during the austral summers. The unique habitat of this organism coupled with its unusual physiology and reduced eyespot spurred us to question and establish its phylogeny. In this report, we compare DNA sequences of the small subunit (SSU) rDNA from the Antarctic alga UWO 241 with those of other chlorophytes (including the new sequences of *C. hedleyi*, *C. subcaudata*, and *C. noctigama*). In addition, we sequenced the internal transcribed spacer (ITS) 1 and ITS2 rDNA (including the 5.8S rDNA) of UWO 241 and the authentic strain ("type strain") of *C. raudensis* (SAG 49.72). These data indicate that UWO 241 is identical with *C. raudensis*, and this species has never before been identified in Antarctica. Furthermore, we describe morphological and physiological characteristics and phototactic responses to temperature for UWO 241. Finally, comparisons of these characters were made with those of its closest phylogenetic relatives, *C. noctigama* (UTEX 2289) and *C. raudensis* (SAG 49.72) as well as with *C. subcaudata* (SAG 12.87) and the model organism, *C. reinhardtii* Dangeard (UTEX 89, CC-400).

MATERIALS AND METHODS

Algal cultures. Strains were obtained from the Sammlung von Algenkulturen, Universität Göttingen, Germany (SAG, Schlösser 1994); the Culture Collection of Algae, University of Texas at Austin, USA (UTEX); the Provasoli-Guillard National Center for Culture of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine, USA (CCMP, Anderson et al. 1997); and the Environmental Stress Biology Group at the University of Western Ontario (NPA Huner). The strains examined are as follows:

- UWO 241: Unidentified *Chlamydomonas species* from Lake Bonney, Antarctica (here identified as *C. raudensis* UWO 241), deposited at the Culture Collection of Algae and Protozoa, Scotland, UK (CCAP 11/131).
- CCMP 1619: *Chlamydomonas* spec. (here identified as *C. raudensis*) coll. by Lizotte and Lesser, isol. by Jacobsen, from Lake Bonney, Antarctica.
- UTEX 89: *Chlamydomonas reinhardtii* Dangeard isol. by Smith, from potato field, Amherst, MA, USA.
- SAG 33.72: *Chlamydomonas noctigama* Korshikov isol. by Strehlow, from garden basin Bot. Gard. Univ. Berlin, Germany.
- UTEX 2289: *Chlamydomonas noctigama* Korshikov isol. by H. Ettl, from pond, Nordmähren, Czechoslovakia.
- SAG 12.87: *Chlamydomonas subcaudata* Wille isol. by Ichimura, Muroran/Hokkaido, Japan.
- SAG 49.72: *Chlamydomonas raudensis* Ettl (authentic strain) isol. by Ettl, from meadow pool near Rudná, Nordmähren, Czech Republic.
- ATCC 50216: *Chlamydomonas hedleyi* (authentic strain) isol. by Lee, from foraminiferan *Archais angulus*, Key Largo Sound, FL, USA.

Growth conditions. Cultures were grown in 250-mL pyrex culture tubes suspended in thermoregulated aquaria at a temperature and light regime of 8° C/20 μmol photons · $m^{-2} \cdot s^{-1}$ for *C. raudensis* (UWO 241) and 29° C/20 μmol photons · $m^{-2} \cdot s^{-1}$ for *C. reinhardtii* (UTEX 89) and *C. noctigama* (UTEX 2289). All cultures were continuously aerated under ambient CO_2 conditions. Light was supplied by fluorescent tubes (Sylvannia CW-40, GE Lighting, Mississuaga, Canada) and was measured with a quantum sensor (model LI-189, Licor Inc., Lincoln, NE, USA). *Chlamydomonas reinhardtii* (UTEX 89) and *C. noctigama* (UTEX 2289) were grown in a modified Bold's basal medium, and UWO 241 was grown in a modified Bold's basal medium supplemented with 0.7 M NaCl to simulate the high salt content in Lake Bonney (Nichols and Bold 1965). *Chlamydomonas subcaudata* (SAG 12.87) was grown at 16° C on nutrient agar plates.

DNA extraction, amplification and sequencing. The amplified sequences included the SSU, ITS1, and ITS2 (including the 5.8S) rDNA. The primers used in the reactions are listed in Table 1. The DNA from *C. raudensis* UWO 241 was amplified from either whole cells according to Lachance et al. (1999) or purified nuclear DNA according to Piskur (1989). DNA preparations were dried and stored at –70° C. All DNA sequences have been deposited in the EMBL Nucleotide Sequence Data Base.

Phylogenetic analyses. The SSU phylogenetic tree was inferred using distance, parsimony, and maximum likelihood criteria using PAUP, version 4.0b10 (Swofford 1998). The data set used the alignment (1726 unambiguously aligned positions) of 68 taxa of Chlorophyceae, including six sequences of *Chaetophora*-clade *sensu* Pröschold et al. (2001) as outgroup. To decide on the evolutionary model that best fit our data, we used the program Modeltest 3.06 (Posada and Crandall 1998), which uses two statistics, the likelihood ratio test and the Akaike information criterion (Akaike 1974). Based on the results of these tests, the model selected by the hierarchical likelihood ratio test was the Tamura-Nei

TABLE 1. List of oligonucleotides primers used for PCR amplification and sequencing of the SSU rDNA, the D1/D2 domain of the large subunit (LSU) rDNA, and the ITS1 region of the rDNA.

Primer	5′ Sequence 3′
SSU rDNA	
SSU 1 forward	GGTTGATCCTGCCAGTAGTCATA
SSU 2 reverse	GTGAACCTGCGA AGG ATCAT
SSU 3 forward	ATTGGAGGGCAAGTCTGGTG
SSU 4 reverse	GGTGCATGGCCGTTCTTAGT
D1/D2 domain LSU rDNA	
NL1 (D1 domain forward)	GCATATCAATAAGCGGAGGAAAAG
NL4 (D2 domain reverse)	GGTCCGTGTT TCAAGACGG
ITS	
a	GGGATCCGTTTCCGTAGGTGAACCTGC
b	GGGATCCATATGCTTAAGTTCAGCGGGT

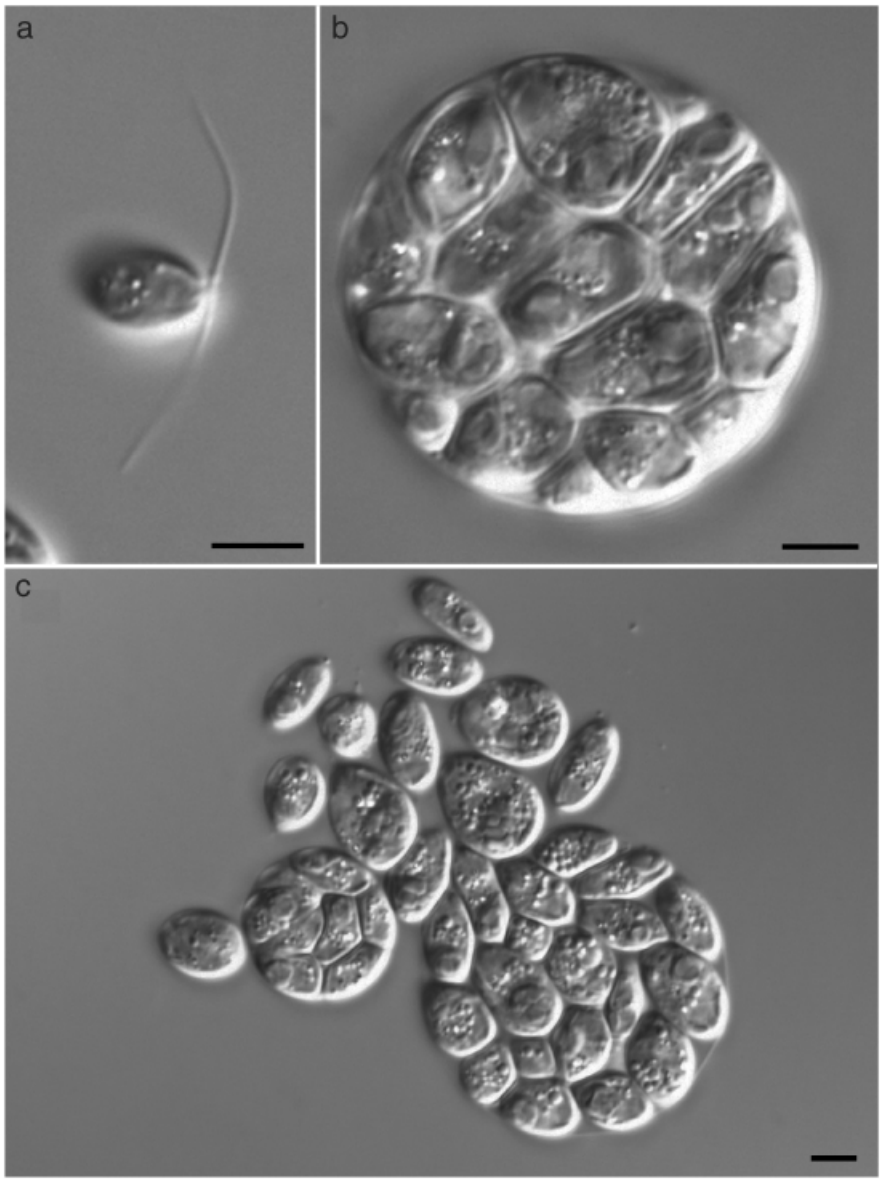


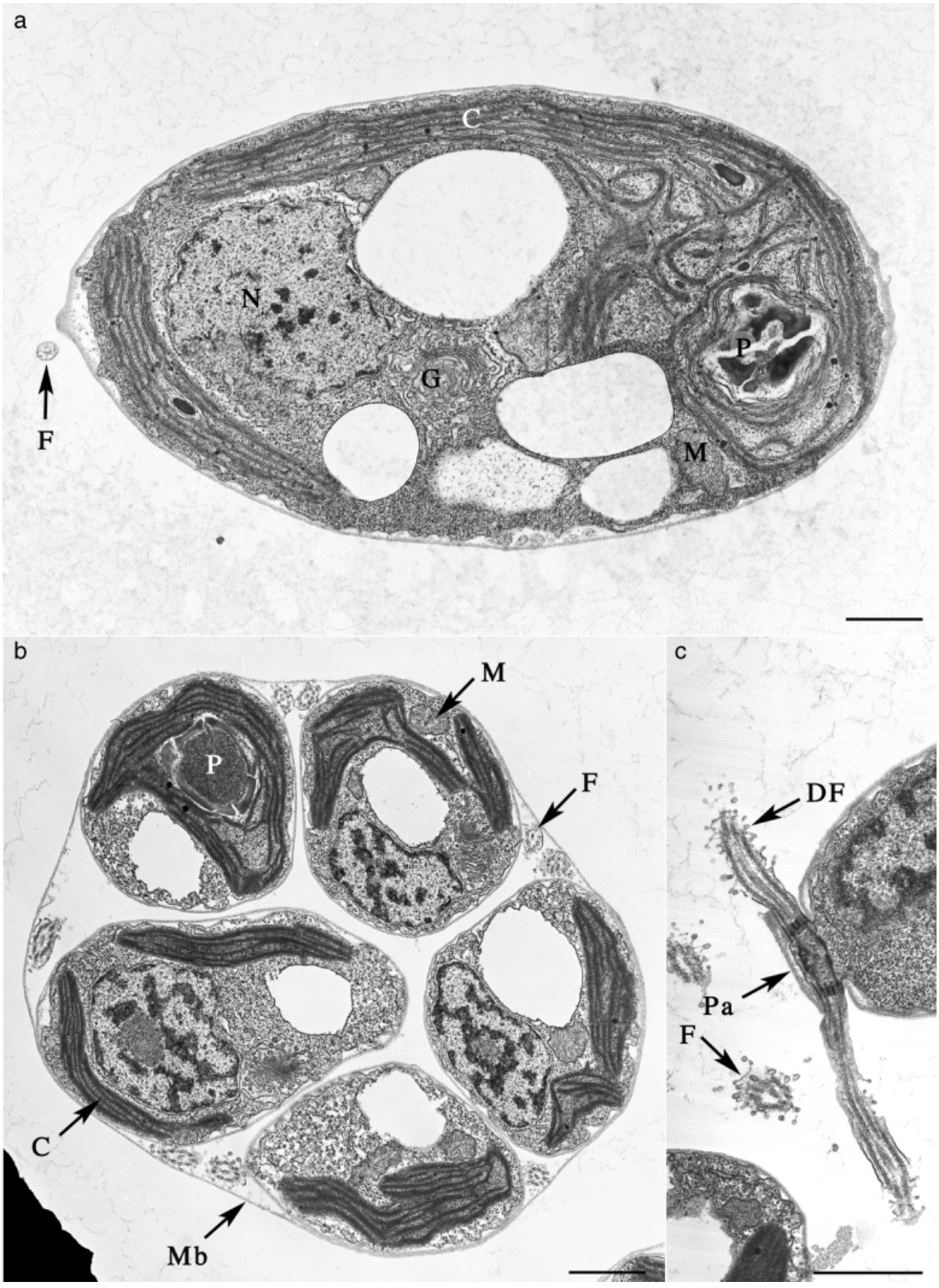
FIG. 1. Light microscope images of *Chlamydomonas raudensis* (UWO 241). (a) Single cell, (b) colony. Note the two long flagella in a. (c) The colony in b ruptured while under the microscope. Magnification 100 × . Scale bars, 5 μm.

model (TrN, Tamura and Nei 1993), with the proportion of invariable sites (I) and the gamma shape parameter (G) for among site variation was calculated from the data set (TrN + I + G; base frequencies and substitution parameters were estimated by Modeltest). The phylogenetic tree based on ITS1 and ITS2 was calculated by PAUP using the TrN + I + G model. The confidence of branching in distance (using the same evolutionary model; TrN + I + G) and parsimony methods was assessed using 1000 bootstrap resamplings of the data set (Felsenstein 1985). The ITS rDNA sequences were aligned with the program DNAMAN, version 4.1 (Lynnon BioSoft, Vaudreuil, Québec, Canada) and compared with published sequences in GenBank. The sequences were aligned, and the neighbor-joining tree was constructed (1000 bootstrap iterations) using the DNAMAN algorithm, CLUSTAL W (Thompson et al. 1994).

EM. Cells of C. *subcaudata* (SAG 12.87) harvested off of agar plates and 5 mL each of *C. raudensis* UWO 241, *C. noctigama* (UTEX 2289), and *C. reinhardtii* (UTEX 89) cultures were centrifuged, fixed with equal parts of fresh 4% (v/v) glutaraldehyde and 2% (v/v) osmium tetroxide buffered with 0.1 M sodium cacodylate, pH 6.8, and 0.7 M NaCl, and stained with 2% (v/v) OsO_4. Pelleted cells were resuspended in agar, stained with 3% (w/v) uranyl acetate, and dehydrated with acetone. The agar blocks were infiltrated and embedded in Epon-Araldite (EM Sciences, Hatfield, PA, USA).

Pigment analysis. Five milliliters of cell culture were harvested during mid-exponential phase and centrifuged. Pigments were extracted from the pellet in 100% cold acetone at 4° C according to Morgan et al. (1998). Chl was extracted in 90% (v/v) cold acetone and the concentration determined spectrophotometrically (Jeffrey and Humphrey 1975).

Phototaxis. A phototactic dish test was performed on UWO 241 and *C. reinhardtii* (UTEX 89). Algal cultures harvested during stationary phase were exposed to 3000μmol photons $\cdot m^{-2} \cdot s^{-1}$ of white light for 30 min under controlled measuring temperatures of 7, 18, and 25° C. All samples were dark adapted for 2 h before the experiment. One half of the Petri dish was kept in the dark throughout


a
C
N
G
P
M
F
b
M
P
F
C
Mb
c
DF
Pa
F

the experiment. Dark controls were performed at all experimental temperatures for both species.

RESULTS

LM and EM of UWO 241 compared with species diagnoses of described Chlamydomonas. The morphology of UWO 241 has been investigated under standard culture conditions (see Methods) by LM and EM. Light microscope analysis showed that UWO 241 exists as biflagellate single cells approximately 10–15 μm long and 5–12 μm wide and having an ellipsoid to ovoid cell shape (Fig. 1a). The flagella are 1.5 times as long as the cell (Fig. 1a). During asexual reproduction the cells form sporangia with 16 to 32 zoospores that are approximately 30 μm in diameter (Fig. 1, b and c). Sexual reproduction of UWO 241 is unknown.

The observation that our UWO 241 exists both as single cells and within colonies was corroborated by transmission electron micrographs (Fig. 2). The UWO 241 strain exhibits one cup-shaped parietal chloroplast with the nucleus centrally or anteriorly located between the chloroplast lobes (Fig. 2a). Starch is accumulated as plates within and surrounding the basally located pyrenoid (Fig. 2a). The sporangia contain single motile cells as observed from the cross-sections of the flagella (Fig. 2b). The flagella are decorated with vesicular outpocketings of the flagellar membrane, and flagellar cross-sections reveal the typical $9+2$ tubule construction (Fig. 2b). The apical papilla in this psychrophile is flat and broad (Fig. 2c).

Despite belonging to the same clade (Fig. 3), UWO 241 is morphologically distinct from strains of *C. noctigama*. The latter strain is surrounded by a wall-bound extracellular matrix, has a rounded apical papilla maintained within this matrix, its plasma membrane is irregular and globose, and starch accumulation is not restricted to the pyrenoid but found throughout the chloroplast (Fig. 4).

The eyespots in UWO 241 were small and elliptic and occurred in an anterior position within the chloroplast. They were difficult to locate due to their small size; one of the largest observed was 1.1 μm long (Fig. 5a). They were different from the spatially separated rows of globules observed in *C. noctigama* (UTEX 2289), the very long ones in *C. subcaudata* (SAG 12.87) and the multirowed eyespot in *C. reinhardtii* (UTEX 89) (Fig. 5, b–d).

Comparisons with species diagnoses of described *Chlamydomonas* species (Ettl 1976) showed that UWO 241 has similar cell morphology to that given in the original description of *C. raudensis* (Ettl 1976). However, the cells of *C. raudensis* (SAG 49.72) are smaller (6–12 × 3–8 μm; Ettl 1976) than UWO 241.

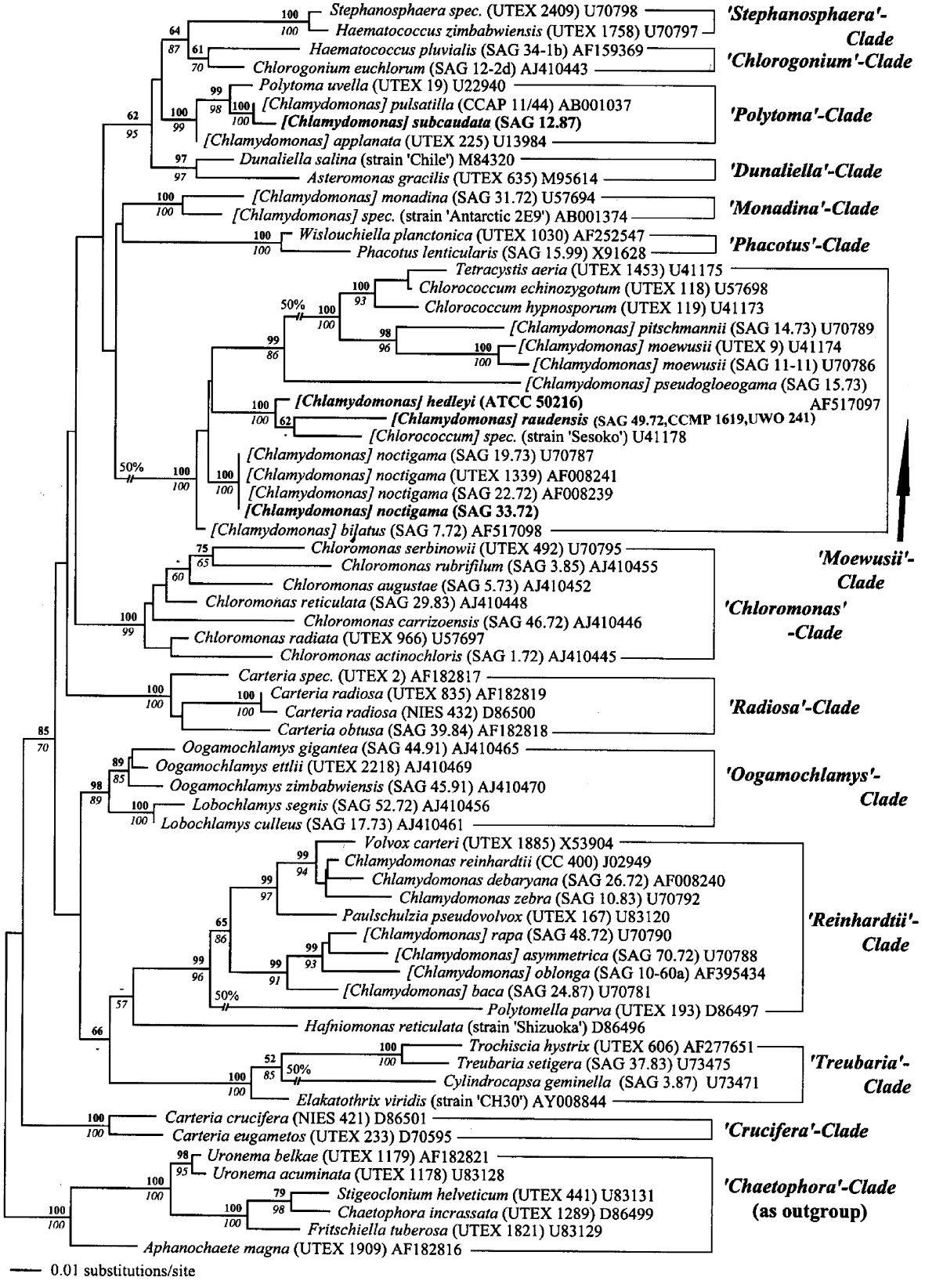
Phototactic properties. Before exposure to light, a homogeneous distribution of cells was apparent (Fig. 6a). Although UWO 241 did not display a phototactic response at 7° C, a weak response was observed at 18° C where cells just moved from the edge of the dish, and a strong positive phototactic response was observed at 25° C (Fig. 6, b–d). Control cells kept in the dark at 18° C and 25° C moved away from the edge of the dish, suggesting that the weak phototactic response observed at 18° C was a temperature rather than a light response (data not shown). In contrast to *C. reinhardtii* (UTEX 89) (data not shown), positive phototaxis in UWO 241 increased with increasing temperature.

Photosynthetic pigments. UWO 241 possessed the typical photosynthetic pigments found in the green algae. Lutein was the most abundant carotenoid (282 ± 5.1 mmol/mol chl *a*) followed by β-carotene (95 ± 15.2 mmol/mol chl *a*), neoxanthin (89 ± 11.5 mmol/mol chl *a*), violaxanthin (70 ± 8.4 mmol/mol chl *a*), antheraxanthin (12 ± 5.5 mmol/mol chl *a*), and, finally, zeaxanthin (5 ± 2.4 mmol/mol chl *a*). The chl *a*/*b* ratio for UWO 241 was unusually low at 1.6 ± 0.2 relative to the typical values for *C. reinhardtii* of approximately 3.0. The chl *a*/*b* ratio for UWO 241 is lower than *Chlamydomonas* green cells collected from Hermit Island, Antarctica (2.2) (Bridigare et al. 1993), and this is most likely due to growth of *C. raudensis* at low irradiances.

Taxon sampling, alignment, and phylogenetic analysis. The goal of this study was to identify UWO 241, and this allowed us to establish the phylogenetic position of UWO 241 based on SSU and ITS1 and ITS2 rDNA sequences. For comparison purposes, we also sequenced the SSU of *C. noctigama* (SAG 33.72), *C. subcaudata* (SAG 12.87), and *C. hedleyi* (ATCC 50216).

We included these four sequences in a representative data set (62 taxa) of the CW (basal bodies clockwise displaced) group of the Chlorophyceae *sensu* Pröschold et al. (2001), aligned them according to their secondary structure, and used the six taxa of the *Chaetophora* clade *sensu* Pröschold et al. (2001) as an outgroup (Fig. 3). The phylogenetic analysis showed that UWO 241, the Antarctic strain CCMP 1619, *C. raudensis* (SAG 49.72)m and *C. noctigama* (SAG 33.72), together with *C. bilatus*, an unidentified strain of *Chlorococcum* (*Cl*), are the sister group to *C. moewusii* subclade within the "*Moewusii*" clade (Fig. 3). Sequence analysis of the ITS1 and D1/D2 domain of the large subunit rDNA confirmed the placement of UWO 241 in the "*Moewusii*" clade (data not shown). *Chlamydomonas subcaudata* (SAG 12.87) is a member of the "*Polytoma*" clade and is not closely related to UWO 241 (Fig. 3). The clades are provisionally named after a representative taxon and follow the designations according to Pröschold et al. (2001). In this broad phylogenetic tree, UWO 241 is sister to the unidentified strain of *Chlorococcum*

FIG. 2. Electron micrographs of *Chlamydomonas raudensis* (UWO 241). (a and c) Single cell, (b) colony. Note the starch accumulation within the pyrenoids (P), the membrane (Mb) surrounding the flagellated daughter cells in the colony, the flat apical papilla (Pa), and the vesicular outpocketings of the flagellar membrane (DF). C, chloroplast; N, nucleus; G, golgi apparatus; M, mitochondrion; F, flagellum. Scale bars, 1.0 μm.


Stephanosphaera spec. (UTEX 2409) U70798
Haematococcus zimbabwiensis (UTEX 1758) U70797
'Stephanosphaera'-Clade
Haematococcus pluvialis (SAG 34-1b) AF159369
Chlorogonium euchlorum (SAG 12-2d) AJ410443
'Chlorogonium'-Clade
Polytoma uvella (UTEX 19) U22940
[Chlamydomonas] pulsatilla (CCAP 11/44) AB001037
[Chlamydomonas] subcaudata (SAG 12.87)
[Chlamydomonas] applanata (UTEX 225) U13984
'Polytoma'-Clade
Dunaliella salina (strain 'Chile') M84320
Asteromonas gracilis (UTEX 635) M95614
'Dunaliella'-Clade
[Chlamydomonas] monadina (SAG 31.72) U57694
[Chlamydomonas] spec. (strain 'Antarctic 2E9') AB001374
'Monadina'-Clade
Wislouchiella planctonica (UTEX 1030) AF252547
Phacotus lenticularis (SAG 15.99) X91628
'Phacotus'-Clade
Tetracystis aeria (UTEX 1453) U41175
Chlorococcum echinozygotum (UTEX 118) U57698
Chlorococcum hypnosporum (UTEX 119) U41173
[Chlamydomonas] pitschmannii (SAG 14.73) U70789
[Chlamydomonas] moewusii (UTEX 9) U41174
[Chlamydomonas] moewusii (SAG 11-11) U70786
[Chlamydomonas] pseudogloeogama (SAG 15.73) AF517097
[Chlamydomonas] hedleyi (ATCC 50216)
[Chlamydomonas] raudensis (SAG 49.72,CCMP 1619,UWO 241)
[Chlorococcum] spec. (strain 'Sesoko') U41178
[Chlamydomonas] noctigama (SAG 19.73) U70787
[Chlamydomonas] noctigama (UTEX 1339) AF008241
[Chlamydomonas] noctigama (SAG 22.72) AF008239
[Chlamydomonas] noctigama (SAG 33.72)
[Chlamydomonas] biljatus (SAG 7.72) AF517098
'Moewusii'-Clade
Chloromonas serbinowii (UTEX 492) U70795
Chloromonas rubrifilum (SAG 3.85) AJ410455
Chloromonas augustae (SAG 5.73) AJ410452
Chloromonas reticulata (SAG 29.83) AJ410448
Chloromonas carrizoensis (SAG 46.72) AJ410446
Chloromonas radiata (UTEX 966) U57697
Chloromonas actinochloris (SAG 1.72) AJ410445
'Chloromonas'-Clade
Carteria spec. (UTEX 2) AF182817
Carteria radiosa (UTEX 835) AF182819
Carteria radiosa (NIES 432) D86500
Carteria obtusa (SAG 39.84) AF182818
'Radiosa'-Clade
Oogamochlamys gigantea (SAG 44.91) AJ410465
Oogamochlamys ettlii (UTEX 2218) AJ410469
Oogamochlamys zimbabwiensis (SAG 45.91) AJ410470
Lobochlamys segnis (SAG 52.72) AJ410456
Lobochlamys culleus (SAG 17.73) AJ410461
'Oogamochlamys'-Clade
Volvox carteri (UTEX 1885) X53904
Chlamydomonas reinhardtii (CC 400) J02949
Chlamydomonas debaryana (SAG 26.72) AF008240
Chlamydomonas zebra (SAG 10.83) U70792
Paulschulzia pseudovolvox (UTEX 167) U83120
[Chlamydomonas] rapa (SAG 48.72) U70790
[Chlamydomonas] asymmetrica (SAG 70.72) U70788
[Chlamydomonas] oblonga (SAG 10-60a) AF395434
[Chlamydomonas] baca (SAG 24.87) U70781
Polytomella parva (UTEX 193) D86497
Hafniomonas reticulata (strain 'Shizuoka') D86496
'Reinhardtii'-Clade
Trochiscia hystrix (UTEX 606) AF277651
Treubaria setigera (SAG 37.83) U73475
Cylindrocapsa geminella (SAG 3.87) U73471
Elakatothrix viridis (strain 'CH30') AY008844
'Treubaria'-Clade
Carteria crucifera (NIES 421) D86501
Carteria eugametos (UTEX 233) D70595
'Crucifera'-Clade
Uronema belkae (UTEX 1179) AF182821
Uronema acuminata (UTEX 1178) U83128
Stigeoclonium helveticum (UTEX 441) U83131
Chaetophora incrassata (UTEX 1289) D86499
Fritschiella tuberosa (UTEX 1821) U83129
Aphanochaete magna (UTEX 1909) AF182816
'Chaetophora'-Clade (as outgroup)
0.01 substitutions/site

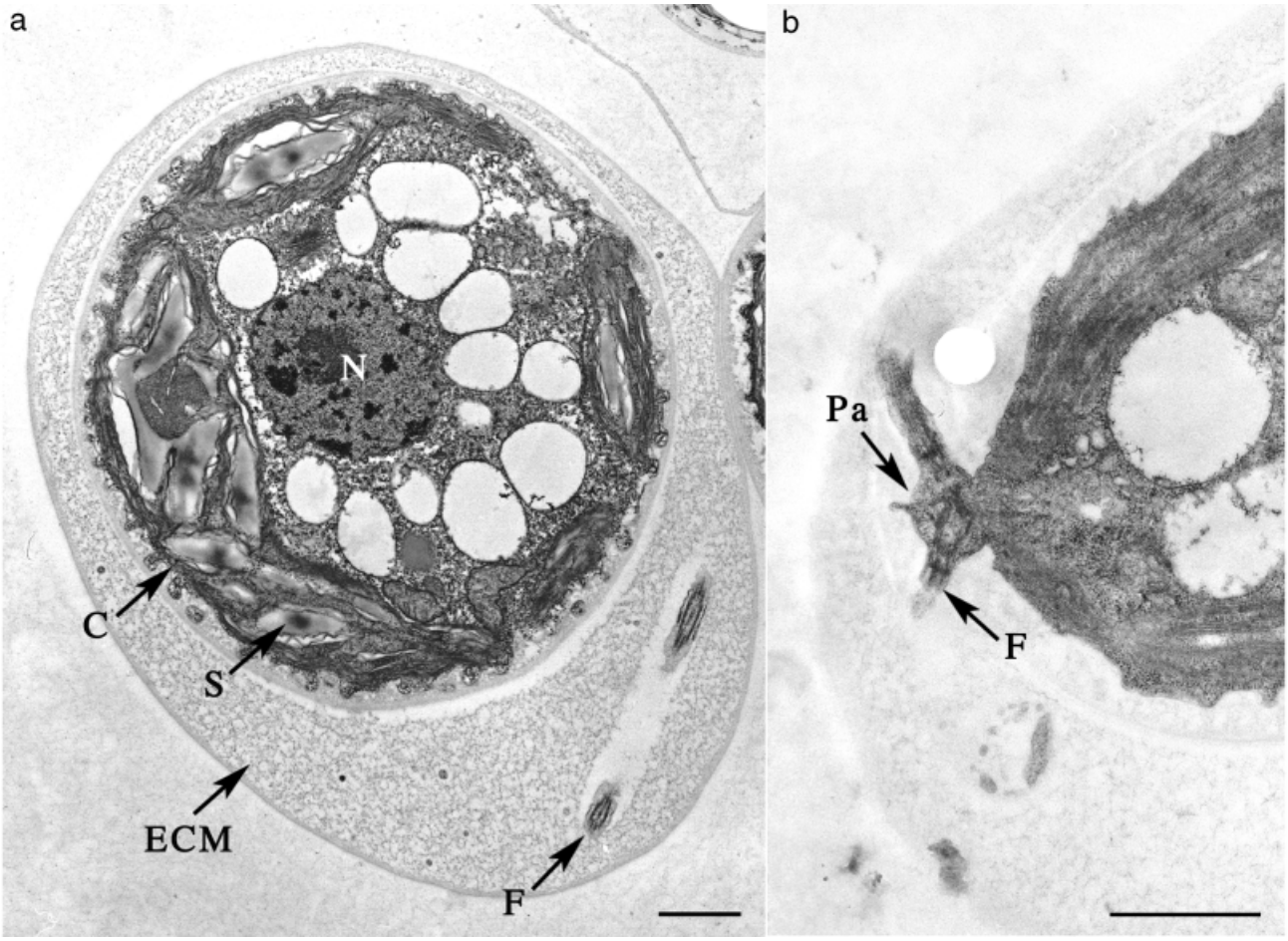


FIG. 4. Electron micrographs of *Chlamydomonas noctigama* (UTEX LB 2289). (a) Single cell, (b) close up of apical region. Note the extracellular matrix (ECM) surrounding the irregular plasma membrane, the rounded apical papilla (Pa), and the distribution of starch (S) throughout the chloroplast (C). N, nucleus; F, flagellum. Scale bars, 1.0 μm.

(U41178) (Fig. 3). A more specific neighbor-joining tree constructed from the ITS1 rDNA sequences placed UWO 241 as a sister species to *C. hedleyi* (ATCC 50216) in subclade B (Fig. 7). Interestingly, all the other species in this arbitrarily named subclade B, including *C. hedleyi* (ATCC 50216), are symbionts found in foraminifers (Pawlowski et al. 2001).

According to the morphological investigations shown here, UWO 241 was identical with *C. raudensis* (SAG 49.72). For confirmation, we sequenced the ITS1 and ITS2 rDNA (including the 5.8S rDNA) of UWO 241, *C. raudensis* (SAG 49.72), and *Chlamydomonas* sp. CCMP 1619 that, similarly to UWO 241, was isolated from Antarctica. These three rDNA sequences were 100% identical, leading us to conclude that our Antarctic psychrophile (UWO 241) and CCMP 1619 are strains of *C. raudensis* Ettl (data not shown).

FIG. 3. Phylogram based on nuclear-encoded SSU rDNA sequence comparisons. The strains whose SSU rDNA were determined in this study are shown in bold. Bootstrap percentage values above the branches are given for neighbor-joining (>50%) using the HKY85 model (bold) and the values determined using the Tamura and Nei model (not bold). The estimated gamma shape parameters and the proportion of invariable sites were determined as the best model for the data set by Modeltest 3.04. and unweighted maximum parsimony (italics, under branch). Brackets refer to the GenBank accession numbers. Seven sequences of the *Chaetophora* clade were used as the outgroup.

DISCUSSION

One of the most striking physiological characteristics of the Antarctic strain UWO 241 is that it is an obligate psychrophile (Morgan et al 1998). However, morphologically and phylogenetically, UWO 241 is identical to CCMP 1619 and the authentic strain of *C. raudensis* (Ettl 1976, Schlösser 1994). Henceforth, we call our Antarctic psychrophile *C. raudensis Ettl* (strain UWO 241). *Chlamydomonas raudensis* (UWO 241) lives in a discrete layer in the lake at depths of between 10 and 17 m, which is a transition zone between an oxygen-rich layer where O2 production exceeds respiratory O2 uptake and the deeper oxygen-deficient region (Koob and Leister 1972).

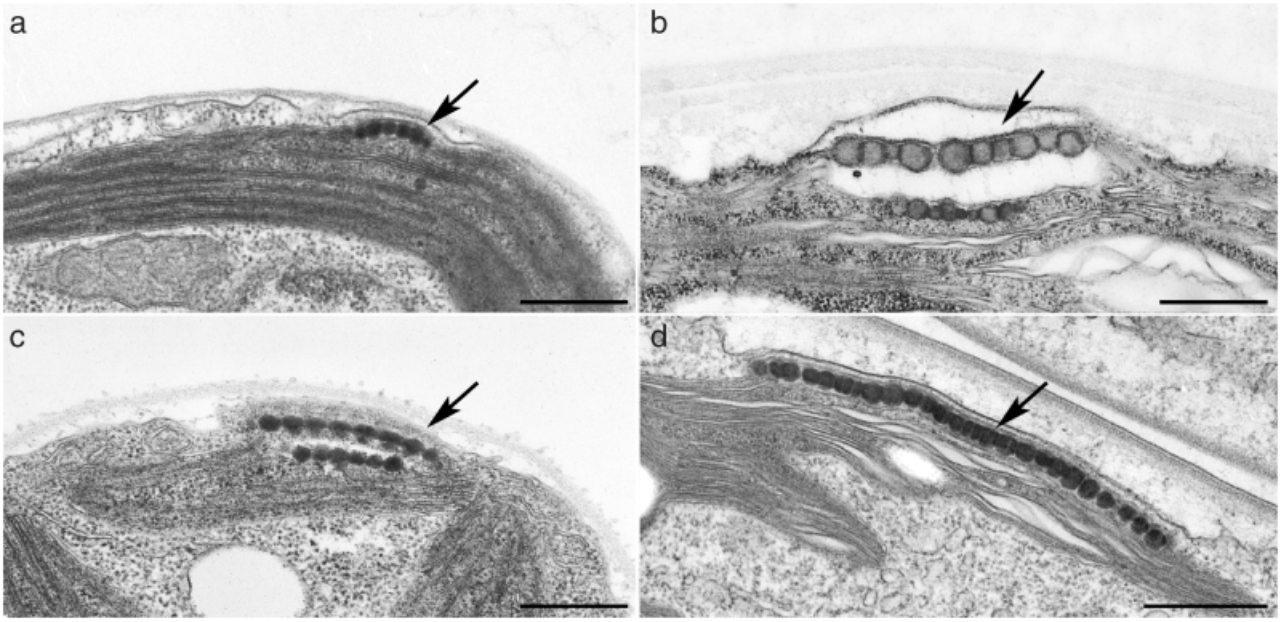


FIG. 5. Electron micrographs showing the eyespots (arrows) of (a) *Chlamydomonas raudensis* (UWO 241), (b) *C. noctigama* (UTEX 2289), (c) *C. reinhardtii* (UTEX 89), and (d) *C. subcaudata* (SAG 12.87). The eyespots were located at the interface between the thylakoid membranes within the chloroplast and the plasma membranes. Scale bars, 0.5 μm.

A question that arose during the analysis was how *C. raudensis* (UWO 241) fits in phylogenetically with other psychrophilic or psychrotolerant *Chlamydomonas* species. Three regions of rDNA were sequenced, and all agreed in placing *C. raudensis* (UWO 241) in the *"Moewusii"* clade as proposed by Pröschold et al. (2001) or the *"C. eugametos"* lineage described by Buchheim et al. (1997). Hoham et al. (2002) used SSU rDNA and *rbc*L sequence data to examine the phylogeny of cold-tolerant *Chloromonas* and *Chlamydomonas* species from cold habitats and found that the 21 taxa examined occurred in four different clades. This led to the conclusion that cold habitats have been invaded at least five times, and cold-tolerant species are not necessarily closely related. According to the phylogenetic tree based on 18S sequences presented in Hoham et al. (2002), *C. raudensis* (UWO 241) is closely related to *C. bilatus*, a cold tolerant *Chlamydomonas* species isolated from an alpine pool in the High Tatra mountains, Czech Republic.

A BLAST search with ITS1 rDNA sequences of *C. raudensis* (UWO 241) showed a close relationship to *C. hedleyi* (ATCC 50216) and other *Chlamydomonas* isolates. All these latter species are symbionts in large miliolid foraminifera (Lee et al. 1974, Pawlowski et al. 2001). *Chlamydomonas raudensis* (UWO 241) is closely related to *C. noctigama*, another member in the "*Moewusii*" clade. From their data, Pawlowski et al. (2001) suggested that *C. noctigama* is the ancestor to foraminiferal symbionts, which raises interesting questions regarding the evolutionary history of *C. raudensis* (UWO 241) because the latter occurred between the foraminifer symbiont branch and the *C. noctigama* branch of the ITS1 phylogram. At present, the classification of

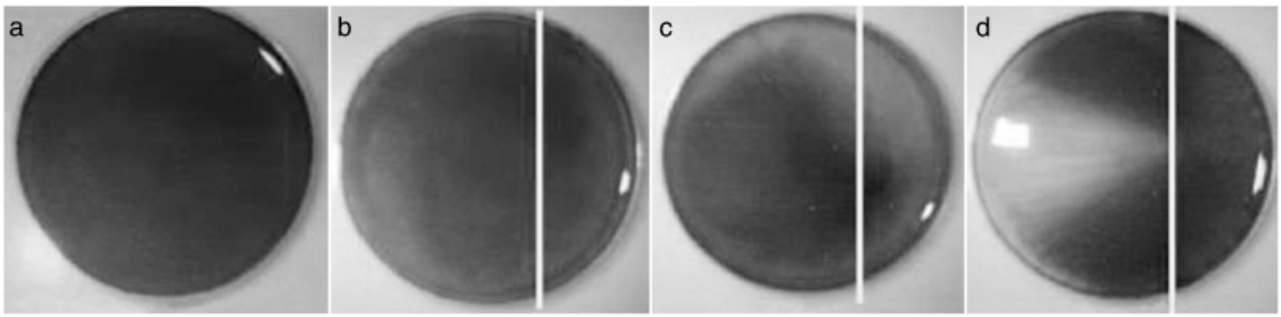


FIG. 6. Phototactic response of *Chlamydomonas raudensis* (UWO 241) after a 30-min exposure to 3000 μmol photons $\cdot m^{-2} \cdot s^{-1}$ of white light under the controlled temperatures of (b) 7° C, (c) 18° C, and (d) 25° C. Cells of *C. raudensis* (UWO 241) were grown at 8° C as described in Materials and Methods. (a) A representative control plate before the treatment. The light source was at the right-hand side of the plates. The lines represent the portion of the plate kept in the light (right side) and the area of the plate kept in darkness (left side). All cells were dark adapted for 30 min before exposure to light.

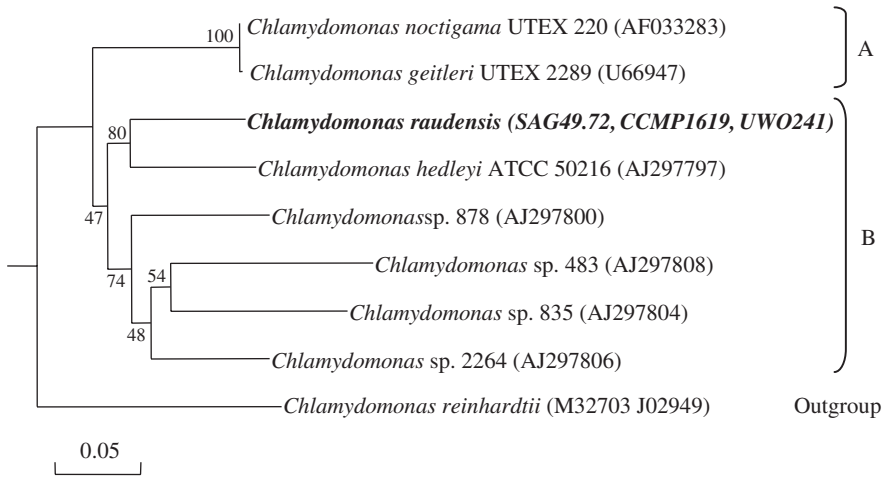


FIG. 7. Phylogram based on ITS1 and ITS2 (including 5.8S) rDNA sequences showing the nearest relatives of *Chlamydomonas raudensis* (UWO 241) inferred using the neighbor-joining method. The numbers above and below the branch points refer to bootstrap values from 1000 resamplings. Sequences were aligned with the program DNAMAN, version 4.1 (Lynnon BioSoft, Vaudreuil, Québec, Canada) and compared with published sequences in GenBank. Brackets refer to the GenBank accession num-

C. noctigama is in a state of flux. Buchheim et al. (1997) reevaluated this species using SSU rDNA sequences. Their data support the previous morphological, biochemical, and phylogenetic placement of this taxon into the vegetative lytic enzyme group 14 (VLE-14; Schlösser 1976). However, because of the lethality and infertility of zygotic meiotic products, they suggest that *C. noctigama* as well as other members in VLE-14 do not fit the biological species concept and are in the process or early stages of reproductive isolation.

The comparison with original descriptions of *Chlamydomonas* species has shown that our psychrophilic Antarctic strain (UWO 241) is identical to *C. raudensis* Ettl (SAG 49.72). In addition, the Antarctic strain CCMP 1619 is also identical to *C. raudensis* (SAG 49.72). The previous designation of an Antarctic strain as *C. subcaudata* in Seaberg et al. (1979) and the designation in the recent literature of our Antarctic psychrophile as *C. subcaudata* contradict the original description of this species. For *C. subcaudata* (SAG 12.87), the cells have an ellipsoid to fusiform shape and a size of 15–43 × 8–23 μm. The cell walls are very thick, and the papilla is broad and two humped. The flagella are as long as the cell, and the chloroplast is cup shaped, has ridges on the outsite, and a single pyrenoid at the basal position of the chloroplast (Ettl 1976). The eyespot is large, elliptic, and is situated within the chloroplast at the equator of the cell. The phylogenetic position of *C. subcaudata* (SAG 12.87) within the *"Polytoma"* clade confirmed that UWO 241 indeed is not *C. subcaudata*.

Despite the small eyespot in *C. raudensis* (UWO 241), it is phototactic, although this positive response appears to be temperature regulated. The strong positive phototactic response is only observed at high nonpermissive growth temperatures. This could explain why Priscu and Neale (1995) were unable to elicit a phototactic response in their experiments and supports the conclusion that some other factor, perhaps chemotaxis, is responsible for keeping *C. raudensis* (UWO 241) cells in their vertical position within the stratified water column (Koob and Leister 1972, Priscu and Neale 1995).

Chlamydomonas raudensis (UWO 241) possesses various morphological features that make it resemble other *Chlamydomonas* species, and this could cause misidentification. However, our examination reveals that it is morphologically distinct from its closest relatives, *C. hedleyi* (ATCC 50216) and *C. noctigama* (UTEX 2289, SAG 33.72). For example, in contrast to *C. raudensis* (UWO 241), *C. hedleyi* can contain up to two pyrenoids and *C. noctigama* (UTEX 2289) is surrounded by an extracellular wall-bound matrix (Lee et al. 1974). *Chlamydomonas raudensis* (UWO 241) is also distinct from its former namesake, *C. subcaudata*, on morphology and rDNA sequences. In addition, *C. raudensis* (UWO 241) exists as free motile single cells and nonmotile colonies that carry flagellated daughter cells. The occurrence of these two developmental stages suggests that the unidentified coccoid algae observed by Koob and Leister (1972) could be the colonial stage of the free-living green algae found in Lake Bonney.

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