J. Phycol. **43,** 78–89 (2007)

DOI: 10.1111/j.1529-8817.2006.00310.x

DISTRIBUTION, PHYLOGENY, AND GROWTH OF COLD-ADAPTED PICOPRASINOPHYTES IN ARCTIC SEAS[1]

Connie Lovejoy[2], *Warwick F. Vincent*

Département de Biologie, Université Laval, Québec City, Québec, Canada, G1K 7P4

Sylvia Bonilla

Sección Limnología, Facultad de Ciencias, Universidad de la República, 11400 Montevideo, Uruguay

Suzanne Roy

Institut des Sciences de la Mer, Université du Québec à Rimouski, 310 Allée des Ursulines, Rimouski, Québec, Canada, G5L 3A1

Marie-Josée Martineau, Ramon Terrado, Marianne Potvin

Département de Biologie, Université Laval, Québec City, Québec, Canada, G1K 7P4

Ramon Massana, and Carlos Pedrós-Alió

CSIC CIMIMA, Departament de Biologia Marina i Oceanografia, Institut de Ciències del Mar, CMIMA, CSIC, Passeig Marítim de la Barceloneta, 37-49 Barcelona, Spain

Our pigment analyses from a year-long study in the coastal Beaufort Sea in the western Canadian Arctic showed the continuous prevalence of eukaryotic picoplankton in the green algal class Prasinophyceae. Microscopic analyses revealed that the most abundant photosynthetic cell types were *Micromonas*-like picoprasinophytes that persisted throughout winter darkness and then maintained steady exponential growth from late winter to early summer. A *Micromonas* (CCMP2099) isolated from an Arctic polynya (North Water Polynya between Ellesmere Island and Greenland), an ice-free section, grew optimally at 6°C–8°C, with light saturation at or below 10 μmol photons $\cdot m^{-2} \cdot s^{-1}$ at 0°C. The 18S rDNA analyses of this isolate and environmental DNA clone libraries from diverse sites across the Arctic Basin indicate that this single psychrophilic *Micromonas* ecotype has a pan-Arctic distribution. The 18S rDNA from two other picoprasinophyte genera was also found in our pan-Arctic clone libraries: *Bathycoccus* and *Mantoniella*. The Arctic *Micromonas* differed from genotypes elsewhere in the World Ocean, implying that the Arctic Basin is a marine microbial province containing endemic species, consistent with the biogeography of its macroorganisms. The prevalence of obligate low-temperature, shade-adapted species in the phytoplankton indicates that the lower food web of the Arctic Ocean is vulnerable to ongoing climate change in the region.

Key index words: **Arctic Ocean; biogeography; climate change; phytoplankton; picoeukaryotes; picoplankton; pigments; polar; prasinophytes; psychrophiles**

Abbreviations: **FISH, fluorescent *in situ* hybridization**

A widely held oceanographic paradigm is that photosynthetic picoplanktonic cyanobacteria, such as *Synechococcus* or *Prochlorococcus*, are continuously abundant in the sea (Scanlan and West 2002). Larger-celled eukaryotes including diatoms, prymnesiophytes, and dinoflagellates rise above this phototrophic background and produce seasonal blooms under specific hydrographic conditions (Smetacek et al. 1990, Li 2002). An unusual feature of Arctic and Antarctic marine ecosystems is that the background population of picocyanobacteria is conspicuously absent or sparse (Li 1998, Vincent 2000), suggesting the existence of ecological filters or geographic barriers that give rise to biogeographic patterns in the global distribution of marine microbes. In the Arctic Ocean, high concentrations of minute, chl-containing eukaryotes (picoeukaryotes; nucleus-containing cells less than 2–3 μm in diameter) have been reported in the picoplankton (Booth and Horner 1997, Booth and Smith 1997, Sherr et al. 2003). Earlier studies (Throndsen and Kristiansen 1991) using dilution culture assays noted that the small prasinophyte identified as the monotypic species *Micromonas pusilla* (Butcher) Manton et Parke was numerically abundant in European Arctic waters. More recently, the application of genera-specific fluorescence *in situ* hybridization (FISH) nucleotide probes confirmed that *Micromonas* was dominant in late summer in these waters along with another monotypic picoprasinophyte, *Bathycoccus prasinos* Eikrem et Throndsen (Not et al. 2005).

[1]Received 17 April 2006. Accepted 2 November 2006.
[2]Author for correspondence: e-mail connie.lovejoy@bio.ulaval.ca.

Earlier results from the North Water Polynya (NOW) between Ellesmere Island and Greenland, using HPLC analysis, showed that prasinophytes were present in the phytoplankton from April to July (Vidussi et al. 2004). During that same field study, we isolated a picoprasinophyte identified at the time by light microscopy and SEM as *M. pusilla*. This isolate (CCMP2099) was subsequently maintained in culture and deposited in the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP). A phylogenetic survey of marine prasinophytes that did not include CCMP2099 found that cultured isolates identified as *M. pusilla* fell into three distinct clades (Guillou et al. 2004). More recently, a molecular comparison of multiple genes (18S, ITS1, 5.8S, and ITS2 ribosomal genes; β-tubulin; RUBISCO *rbc*1; and cytochrome c1 oxidase subunit 1 *cox*1) of morphological *M. pusilla* isolates from throughout the World Ocean including CCMP2099 confirmed that this taxon consists of multiple lineages. The authors suggested that the morphological species *M. pusilla* consists of at least five distinct clades (Šlapeta et al. 2006). In that study, the NOW isolate CCMP2099 was placed in clade E along with one isolate from the Gulf of Naples (CCMP1646). However, these two isolates were genetically distinct from each other, and Šlapeta et al. (2006) suggested that clade E may represent an ancient, isolated lineage that diverged from other micromonads ~60 million years ago. The existence of a relict *Micromonas* lineage in the Arctic would be consistent with the persistence of the Arctic Ocean as a semienclosed basin for 60–100 million years (Golonka et al. 2003, Backman et al. 2004) and slow exchange of surface waters with other oceanic regions throughout this time (Jakobsson et al. 2003). The Arctic has been in its current stable cold state for the last 4 million years (Piepenburg 2005).

The overall aim of this study was to examine the biogeography, diversity, and growth characteristics of small prasinophytes in the Arctic. Our first objective was to determine their distribution and genetic diversity in Arctic seas by way of 18S rRNA clone libraries. Our second objective was to assess the seasonal importance of picophytoplankton, including the *Micromonas* morphotype that has been widely reported in Arctic surface waters (Booth and Horner 1997, Sherr et al. 2003, Not et al. 2004). The third objective was to define the psychrophilic growth characteristics of Arctic *Micromonas* CCMP2099, which differs from all other isolates of this genus in that it cannot be maintained in culture at 20°C. The sensitivity of Arctic plankton to warmer temperatures is of major interest in view of current observations and model results that the Arctic is continuing to warm at much faster rates than elsewhere (Stroeve et al. 2005).

MATERIALS AND METHODS

Sample collection. Sampling was conducted within five separate oceanographic missions using four different ships (Fig. 1). In the Greenland Norwegian Barents Sea (GNB) (stations M09 and Z59), sampling was from the Norwegian survey ship F/F *Johan Hjort* using a rosette CTD system fitted with 5 L Niskin bottles (OceanTest Equipment Inc., Fort Lauderdale, FL, USA). Sampling in Canadian Arctic waters in 2002 was from the icebreaker platforms CCGS *Louis St. Laurent* (stations AO-NW01 and AO-NW08) and CCGS *Pierre Radisson* (stations MD65, CA66, and CA72) using rosette CTD systems fitted with 12 L Niskin bottles. An additional inshore sample from the western Canadian Arctic for clone library construction was collected in Dease Straight, Northwest Passage (DS07), from the chl maximum in September 2005 as part of the Marine Microbial Biodiversity of the Arctic Ocean and Seas program aboard the icebreaker CCGS *Amundsen*, with sample collection using a rosette CTD system equipped with 12 L Niskins. The overwintering (2003–2004) sampling was part of the Canadian Arctic Shelf Exchange Study (CASES) in which CCGS *Amundsen* was frozen into Franklin Bay in the coastal Beaufort Sea (Station FB). Upper mixed-layer microbial plankton communities were sampled 5 m below the water surface using the ship CTD rosette system as above during openwater conditions. During the time that the ship was frozen into Franklin Bay from December 2003 to early June 2004, samples were taken using a 5 L Niskin bottle from 3 m below the bottom ice through a 300 mm hole that had been drilled 500 m upcurrent of the ship.

Micromonas (CCMP2099) was isolated in 1998 from 75 m in the North Water Polynya (NOW98; Fig. 1) between Ellesmere Island and Greenland (latitude 76.283° N; longitude 74.75° W) with a dilution method using six- and 24-well Corning plates (Corning Inc., Corning, NY, USA). Initial seawater samples were collected using sterile 50 mL syringes directly from the Niskin bottles. The syringes were adjusted to allow 1 mL of head space and capped, and then the seawater community was maintained under 24 h low irradiance at 0°C while onboard the ship for the subsequent 1–3 weeks. On return to land, 1–2 mL of the syringe contents was transferred into well plates containing different culture media. Successive transfers after 2–6 weeks over 1 year led to the isolation of numerous unialgal cultures, which were deposited with CCMP. The *Micromonas* CCMP2099 culture was maintained at 2°C under low light (12:12 light:dark) in K media (Keller et al. 1987). Culture experiments were undertaken in K-media in Sanyo growth incubators (Sanyo Canada Ltd., Concord, ON, Canada) under cool-white fluorescent light at 0, 2, 4, 6, 8, 10, 12.5, and 15°C and under three irradiance levels (10, 50, and 100 μmol photons $\cdot m^{-2} \cdot s^{-1}$). The cultures were shaken by hand every day, and cell concentrations were measured by optical density (OD) at 750 nm in subsamples removed at 1- to 3-day intervals. These OD values were converted to cell concentrations with a calibration curve based on OD measurements and hemocytometer cell counts of a dilution series. The cultures were preincubated for several days, and growth rates were determined for the subsequent exponential phase by log-linear regression. Samples were taken during the late exponential phase for pigment analysis. The cells were filtered onto 25 mm GF/F glass fiber filters (Whatman Inc., Florham Park, NJ, USA), and the pigments were then extracted and measured by HPLC, as for the marine samples in 2003–2004. Chl *a* for field samples in 2002 and 2005 was estimated using standard techniques; details for the 2002 samples are given in Lovejoy et al. (2006), and the 2005 chl *a* concentrations were estimated as in Nusch (1980).

Microscopy. Samples for fluorescence microscopy were preserved immediately after collection with EM-grade glutaraldehyde (Canemco & Marivac, Quebec; 1% final concentration) in the dark at 4°C for <24 h. Samples were then filtered onto 0.6 μm pore-size 25 mm black polycarbonate filters (Nuclepore) and stained with 4′6-diamino-2-phenylindol (DAPI, 5 μg $\cdot mL^{-1}$ final concentration) and subsequently examined by fluorescence microscopy. Counts were

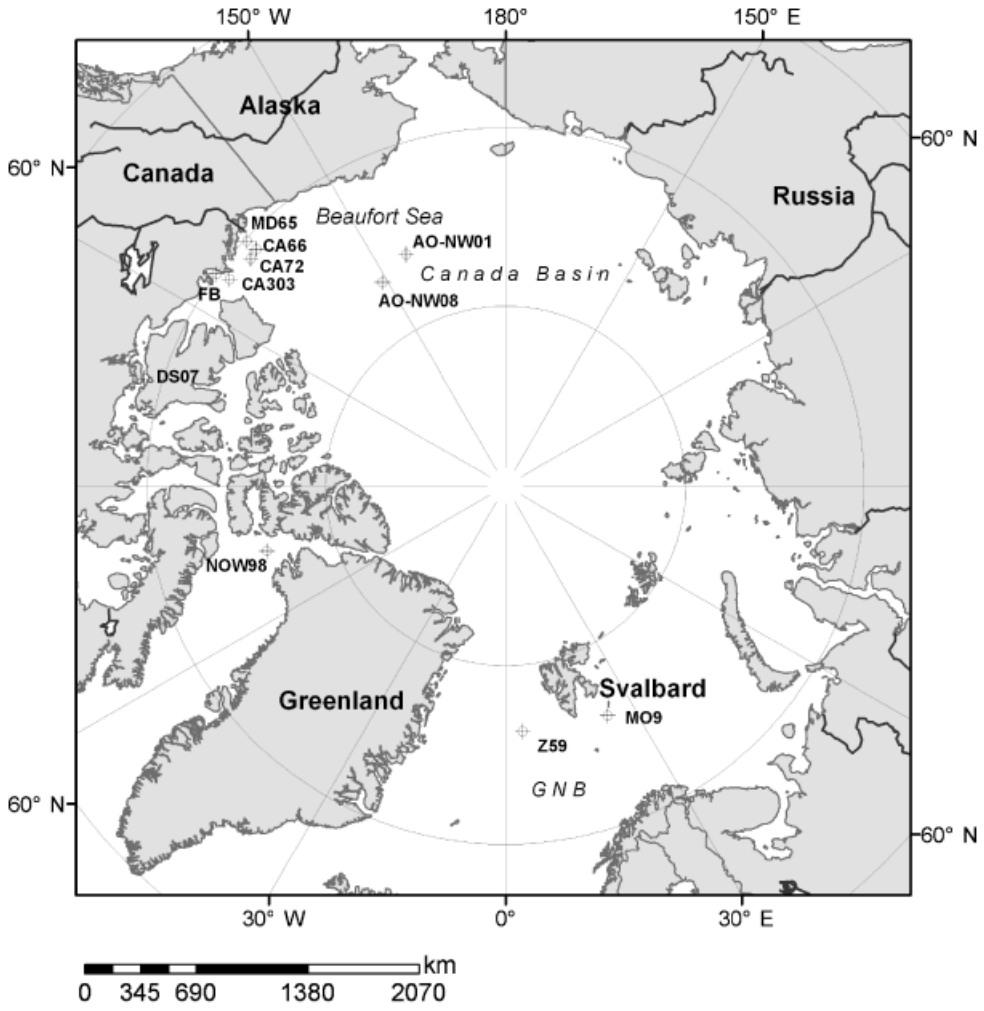

FIG. 1. Sampling stations in the present study: Amundsen Gulf, Franklin Bay, (FB); Arctic Ocean, North Wind Ridge stations (NW01 and NW08); Beaufort Sea, Canadian Arctic Shelf Exchange Study stations (CA303, CA72, CA66); Greenland, Norwegian, Barents Seas (GNB M09, Z59); Mackenzie Delta Offshore Station (MD65); North Water Polynya (NOW98); North West Passage, Dease Straight, (DS07).

performed with a Zeiss Axiovert 100 (Carl Zeiss Canada Ltd., Toronto, ON, Canada) or an Olympus BX51 microscope (Olympus America Inc., Melville, NY, USA) equipped with UV, blue, and green filter blocks. All reniform or oval cells of 1.5–2.5 μm diameter with a single parietal chloroplast (seen as red fluorescence under blue excitation) were counted as *Micromonas*-like cells. While specific FISH probes may have decreased the possibility of misidentification, FISH protocols are more time consuming than direct microscope counts, with potential problems related to cell loss, cellular deformation, chloroplast bleaching, and nontarget labeling of unknown organisms.

HPLC pigment analysis. One- to two-liter samples of water were filtered either directly onto Whatman GF/F filters or through 3 μm pore-size polycarbonate filters and then onto the GF/Fs. The filters were stored frozen at −80°C until analysis. Phytoplankton pigments on the GF/Fs were extracted following sonication (twice for 30 s at 10 W) in 3 mL of 95% MeOH. The extracts were cleared by centrifugation (10 min, 3400*g*), filtered through 0.2 μm Acrodisc filters® (Pall Corp., Mississauga, ON, Canada), and stored under argon gas at 4°C in darkness until HPLC analysis within 24 h of extraction. Following this, 50–100 μL of the extracts was injected into a Varian ProStar HPLC (Mulgrave, Australia) equipped with a Symmetry C8 column (3.5 μm pore-size, 4.6 mm × 150 mm; Waters Corporation, Milford, MA, USA).

The HPLC peaks were detected by diode-array spectroscopy (350–750 nm) with the instrument set to a slit width of 1 nm. Absorbance chromatograms were obtained at 440 (for chls) and 450 nm (for carotenoids). Chlorophylls were also detected by fluorescence (excitation: 440 nm; emission: 650 nm). Standards for identification and quantification of pigments (chl *a*, *b*, c_2, and c_3; β,β-carotene; diadinoxanthin; fucoxanthin; lutein; prasinoxanthin; antheraxanthin; and zeaxanthin) were obtained from commercial suppliers (Sigma Inc., St. Louis, MO, USA; and DHI Water & Environment, Hørsholm, Denmark) to calibrate our HPLC. Standards of many pigments found in Prasinophyceae are not available for purchase. These pigments [uriolide, micromonal, *cis*-neoxanthin, and Mg-2,4-divinyl pheoporphyrin a5 monomethyl ester (MgDVP)] were identified by their chromatographic and spectral characteristics, and quantification was based on the response factor of prasinoxanthin. Owing to the low signal obtained in the chromatograms, it was not possible to confirm diadinoxanthin in the samples, and the most likely peak is indicated as diadinoxanthin-like in Fig. 2. The HPLC solvent protocol was based on gradient dilution with two solvent mixtures (Zapata et al. 2000): a methanol, acetonitrile, and aqueous pyridine (50:25:25 v:v:v) solution; and a methanol, acetonitrile, and acetone (20:60:20 v:v:v) solution. The flow rate was 1 mL · min^{-1}, and the equilibration time was 7 min. CHEMTAX analysis (as in Not et al. 2005) was performed on samples from three stations sampled in CASES (2002) for which there were complete suites of pigment data for the total phytoplankton and the <3 μm fraction. Pigment concentrations during the seasonal study in Franklin Bay were often too low for the photodiode array to provide a full set of

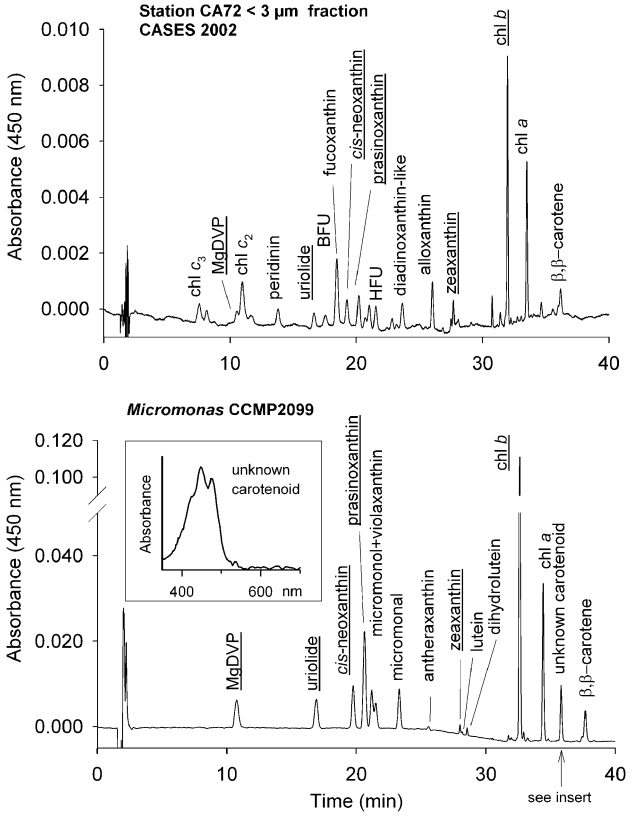

FIG. 2. HPLC chromatograms for picoplankton in the Beaufort Sea (upper panel; surface water, station CA72, sampled October 5, 2002) and for *Micromonas* CCMP2099 from the North Water Polynya (lower panel). The underlined pigments are the characteristic signature for the order Mamiellales of the green algal class Prasinophyceae. Inset: absorption spectrum for the unknown carotenoid (shoulder at 423 nm, peaks at 449 and 476 nm) at 36 min.

carotenoid data for CHEMTAX; however, the chl pigments could be detected and quantified throughout the year using the more sensitive HPLC fluorescence detector.

Clone libraries and DNA analysis. To investigate the genetic identities of picoeukaryotes and to extend our knowledge of their geographical distribution in Arctic seas, we constructed 11 clone libraries from the following sites (Fig. 1): GNB Station Z59 (latitude 76.326° N, longitude 3.985° E), sampled at 5 and 60 m on August 28, 2002; Arctic Ocean Northwind Ridge Station NW01 (75.986° N, 156.873° W) at 5 and 50 m, and Canada Basin NW08 (76.777° N, 148.959° W), sampled at 5 and 50 m August, 26–30, 2002; Mackenzie Shelf Station, MD65 in the Beaufort Sea (70.140° N, 133.520° W), sampled at 5 m on October 2, 2002; coastal Beaufort Sea Station, FB in Franklin Bay (70.037° N, 126.301° W), sampled at 3 m on January 17, 2004, an additional CASES Beaufort Sea Station, CA303 (70.789° N, 126.934° W), sampled at 5 m on June 20, 2004, at the start of the seasonal diatom bloom; and the Northwest Passage Dease Straight Station DS07 (68.580° N, 106.570° W), sampled from the deep chl maximum at 30 m on August 30, 2005. Our 2005 shipboard fluorescence microscope observations showed that the deep chl maximum layer throughout the Beaufort Sea and the Northwest Passage was dominated by small *Micromonas*-like cells. The Dease Straight Station, where water from the Beaufort Sea flows eastward through the Northwest Passage, was selected as a site that was rich in micromonads to investigate their genetic variation at the 18S rDNA level in detail. We did not recover prasinophyte clones from three libraries: GNB Station M09 (76.318° N, 23.740° E) at 5 m, Z59 at 60 m, and NW08 at 5 m.

At all stations of the four regions—Beaufort Sea, Northwest Passage (Dease Straight), Canada Basin, and GNB—water samples for DNA analysis were transferred directly from the Niskin bottles to polycarbonate bottles. Before filling with sample water, the bottles were rinsed with 10% hydrochloric acid (HCl), then three times with Milli-Q H_20, and three times with sample water. For the 2002 Canadian samples, microbial biomass was collected by filtering 1–2 L of seawater under <5 mm Hg pressure. For Canadian samples in 2004 and 2005 and the Norwegian samples, 4–6 L of water was filtered through 0.22 µm Sterivex filter (Millipore Canada Ltd., Mississauga, ON, Canada) units using a peristaltic pumping system. All samples were prefiltered through 3 µm pore-size polycarbonate filters. All environmental DNA sample filters were stored in buffer (40 mM EDTA, 50 mM Tris-HCl, 0.75 M sucrose) and frozen at −70°C until nucleic acid was extracted in phenol chloroform as in Diez et al. (2001).

Eukaryotic 18S rRNA genes were amplified by PCR with eukaryote-specific forward primer A and reverse primer B (Medlin et al. 1988), which amplifies the 18S rRNA gene. We carried out four PCR reactions for each sample using increasing amounts of template (2, 4, 6, and 8 µL). Other than using Promega Taq (Madison, WI, USA) in 2002, and iTaq DNA polymerase (BIO-RAD Laboratories, Hercules, CA, USA) in 2004 and 2005, all reactions were carried out as in Diez et al. (2001). The resulting amplified gene products from the four individual PCRs per sample were pooled and then cleaned using a Qiaquick PCR Purification kit (Qiagen Inc., Mississauga, ONT, Canada), and the PCR products were cloned using TA cloning kits (Invitrogen, Carlsbad, CA, USA) following the manufacturer's recommendations. For all of the 2002 libraries, positive clones were amplified with the original Euk A and B primers and then screened by RFLP using HAEIII (New England Biolabs, Ipswich, MA, USA). Unique RFLP patterns were sequenced with the Euk 528f primer (Diez et al. 2001) and Big Dye (3.1) terminator ready reaction mix (Applied Biosystems, Foster City, CA, USA) to obtain a 700–800 base pair (bp) segment covering conserved and rapidly evolving regions of the 18S rRNA gene (Wuyts et al. 2000). Additional methodological details and results for all 2002 clone libraries are given in Lovejoy et al. (2006). For the 2004 CASES and 2005 Dease Straight libraries, the inserted 18S rDNA sequences were amplified with the vectors' M13 primers. The PCR products were screened on an agarose gel, and 50–60 clones among those that were the correct size were randomly chosen to be sequenced using the 528f eukaryotic primer. The 2002 clone libraries were sequenced at the Serveis Científico—Tècnics, Universitat Pompeu Fabra (Barcelona), with an ABI3100 automated sequencer (Applied Biosystems). Sequences from the environmental CASES (CA040111 and Ce303 s) and Dease Straight (ds070) libraries were sequenced at the Centre Génomique de Québec. The two complete sequences from the ds070 library were obtained using the vectors' T7p universal primer and the eukaryotic 528f and 1055f primers. The NOW *Micromonas* isolate, which had been extracted using the Qiagen DNAeasy Plant Mini Kit, was sequenced by the Université Laval (Québec, Canada) Sequencing Service with universal 18S rDNA eukaryotic primers (Euk 516r, 336f, 528f, and 1055f) to obtain a nearly complete 18S rDNA sequence.

Phylogenetic analysis. Poor-quality sequences and suspected chimeras were checked by using the Chimera Check program at Ribosomal Data Project II (Michigan State University, East Lansing, http://35.8.164.52/cgis/chimera.cgi?su=SSU) and by separately submitting two or three sequence segments to NCBI BLAST (Altschul et al. 1990). All sequences that were >97% similar to our shortest environmental se-

quence (NW617.07 with 686 bp 100% match to CCMP2099) were retrieved from NCBI GenBank and used for the phylogenetic comparison. Many of these were unpublished environmental sequences, which were also screened for chimeras as above. Only sequences from cultures and unambiguous environmental sequences were subsequently used in our phylogenetic analysis. Sequences were aligned using Clustal X (Thompson et al. 1997) and then manually checked using Bioedit v.5.0.9 (Hall 1999). An initial dendrogram was constructed (details are given below) using all of our partial prasinophyte sequences >720 bp. An additional dendrogram using 1700 bp 18S rDNA sequences was constructed using all nonchimeric, nearly full-length environmental sequences that were >97% similar to our full-length sequences; all cultured *Micromonas* sequences that were included in the study by Šlapeta et al. (2006); and two full-length environmental sequences from our Dease Straight library. We next searched for similarities between our Arctic sequences and the ~500 bp environmental sequences that clustered near *Micromonas* group E, retrieving ME1-2, RA010613.139, RA101613.139, RA000412.153, RA010516.35, RA000907.63, RA000907.9, RA000907.39, and BL010320.1. Finally, we searched the NCBI environmental_nt data base for any matches to CCMP2099.

All dendrograms were constructed after initial alignment using Clustal X. These alignments were manually checked using Bioedit. Tree construction was performed with PAUP v 4.0b10 (Sinauer Associates Inc., Sunderland, MA, USA), using the neighbor-joining and maximum-likelihood methods as in Guillou et al. (2004). Clade credibility was checked with a heuristic search using MrBayes v3_0b4 (Ronquist and Huelsenbeck 2003).

RESULTS

Culture studies. Strain CCMP2099 was isolated from shipboard enrichments at Station NOW98 (Fig. 1) during the North Water Polynya (NOW) study in April 1998. It is a 1.5–2 µm diameter prasinophyte that is morphologically identical to *M. pusilla*. HPLC analysis of its accessory pigments confirmed the typical composition for prasinophytes in clade II of the order Mamiellales (Latasa et al. 2004): chl *b*, MgDVP (a chl *c*–like pigment), uriolide, *cis*-neoxanthin, prasinoxanthin, micromonal, antheraxanthin, lutein, zeaxanthin, dihydrolutein, and an unknown carotenoid eluting after chl *a* (Fig. 2).

Strain CCMP2099 showed exponential growth in batch culture over a wide range of temperatures and irradiance levels (Fig. 3). Optimal growth rates of 0.55 day^{-1} were achieved at 6°C–8°C at moderate irradiances, with no difference in growth between 50 and 100 µmol photons $\cdot m^{-2} \cdot s^{-1}$. Growth persisted at 40% of these optimal rates at 0°C, with no difference between 10, 50, and 100 µmol photons $\cdot m^{-2} \cdot s^{-1}$. Growth rates at the higher irradiances sharply decreased at 12.5°C, and at 15°C there was disintegration and loss of cells in all cultures.

Population growth in the Beaufort Sea. Our initial sampling in the Beaufort Sea in September to October 2002 showed that the picophytoplankton fraction accounted for a large component of the total pigment stocks and contained the prasinophyte clade II suite of accessory pigments that were also observed in our CCMP2099 cultures (Fig. 2). Analysis of carotenoids and chls from the surface waters of three stations [Fig. 1; CASES stations MD65, CA66 (70.852°N, 133.652°W), and CA72 (70.982°N, 131.810°W)], sampled at 5 m in October 2002) by CHEMTAX indicated that prasinophytes accounted for 38 ± 5% (mean ± SD) of the total phytoplankton chl *a* and 55 ± 13% of the <3 µm fraction. Many of the carotenoids were close to our limits of detection by the photodiode array, and these values should be interpreted with caution.

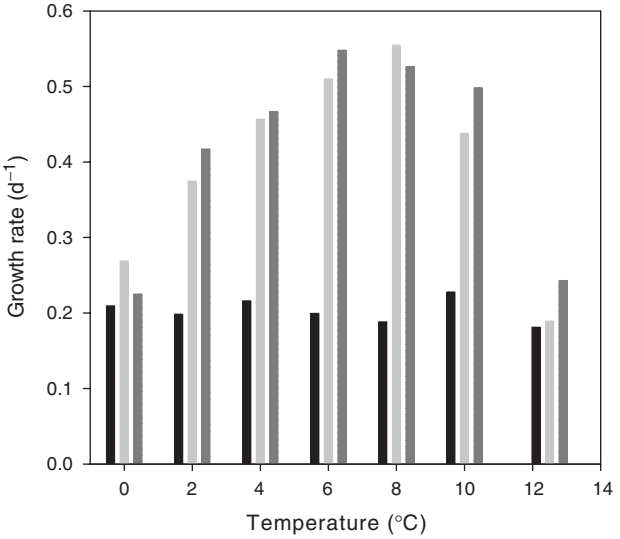

FIG. 3. Growth of *Micromonas* CCMP2099 from the North Water Polynya as a function of temperature. The cultures were incubated under irradiances of 10 µmol photons $\cdot m^{-2} \cdot s^{-1}$ (black bars), 50 µmol photons $\cdot m^{-2} \cdot s^{-1}$ (light gray bars), and 100 (dark gray bars) µmol photons $\cdot m^{-2} \cdot s^{-1}$. Note that at 15°C, all cultures decreased in cell concentration after inoculation.

Subsequent analysis of samples from our overwintering platform CCGS *Amundsen* stationed in the coastal Beaufort Sea in 2003–2004 provided a unique multiseasonal record of size-fractionated phytoplankton dynamics (Fig. 4). The picoplankton fraction accounted for 40%–80% of total chl *a* in all months sampled, with the exception of July during peak phytoplankton biomass (Fig. 4A). Chl *b*–containing picoplankton were well represented throughout the year, even during continuous winter darkness (Fig. 4B), and MgDVP was also commonly found in the samples. This pigment appears in many algal groups, although it is in higher amounts in the order Mamiellales. On average, 82% (SD = 12.6) of the chl *b* was in the <3 µm fraction, and chl *b* + MgDVP contributed 69% (SD = 9.7) of the total accessory chls. Chl c_2 (characteristic of chromophytes including dinoflagellates and diatoms) was the third most abundant chl (after chl *a* and *b*). From first light onward (February), there was a steady decline in the picoplankton contribution to total pigment concentrations, and during peak phytoplankton biomass in summer, most of the chl c_2 was in the >3 µm fraction (Fig. 4C). Small quantities of chl c_3 (characteristic of prymnesiophytes

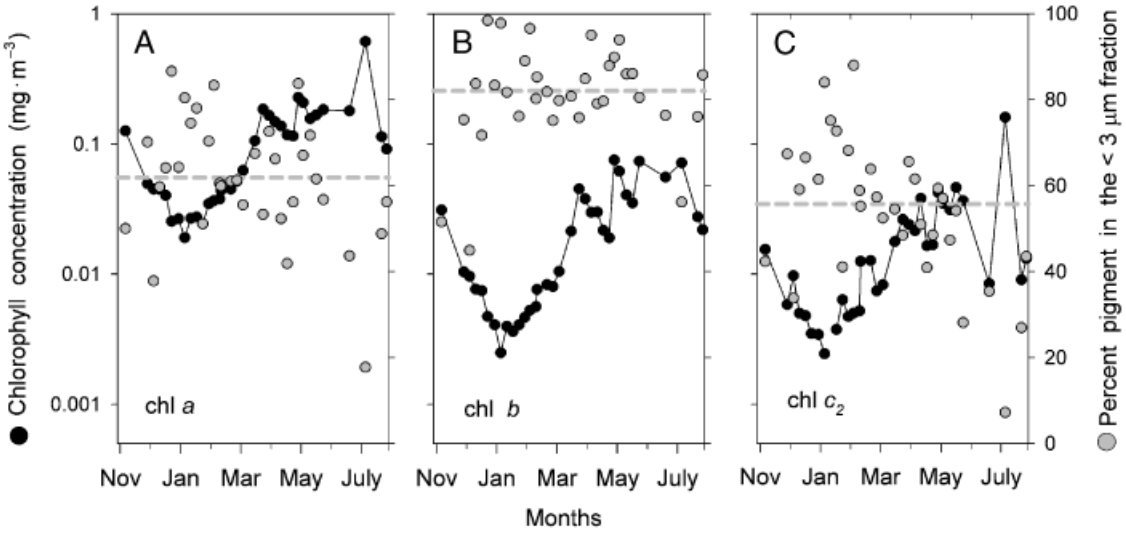

FIG. 4. Seasonal dynamics of the main photosynthetic pigments in the near-surface waters of Franklin Bay, Beaufort Shelf, Arctic Canada. Sampling was from November 19, 2003, to August 6, 2004, at Station FB, except for three dates in the vicinity of station CA303. (A) Total chl *a* concentrations (closed circles) and percent contribution of cells <3 µm (gray circles; dashed gray line = average). (B) Total chl *b* (closed circles) and % contribution of cells <3 µm to total chl *b* (gray circles; dashed gray line = average). (C) Chlorophyll c_2 concentrations (closed circles) and percent contribution of cells <3 µm (gray circles; dashed gray line = average).

and pelagophytes) were also recorded throughout the year, including during winter.

Analysis of the CASES Beaufort Sea samples by epifluorescence microscopy confirmed the low cell concentrations of picocyanobacteria (<600 cells · mL^{-1}) relative to other oceanic regions, and the consistent presence of 1–2 µm eukaryotic cells with a single acentric chloroplast (Fig. 5, inset). Some of these cells had the distinctive emergent flagellum of *Micromonas*; however, this feature was not always observed. Following our protocol, using DAPI to stain the nucleus and viewing under epifluorescence microscopy did not allow us to separate *Bathycoccus* and *Micromonas* reliably. Counts were therefore pooled as total picoprasinophytes. Other single-chloroplast prasinophytes are larger or have more evident flagella (e.g., *Mamiella, Mantoniella, Resultor, Nephroselmis*, and *Pseudoscourfieldia*).

Concordant with our pigment data, we collected these highly fluorescent, chl-containing small cells throughout winter, with an exponential increase of this population over spring (Fig. 5). The steadiness of this net population growth is especially striking, given the large variations in irradiance during this period. It is consistent, however, with the low saturation irradiance that

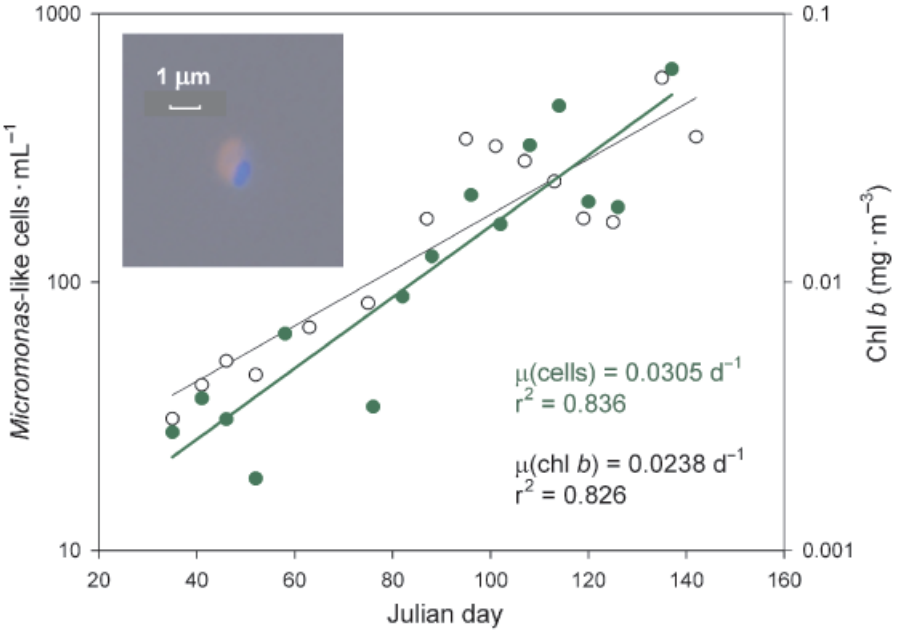

FIG. 5. Exponential growth of picoprasinophytes over 100 days from late winter to early summer in the surface (sub-ice) waters of the coastal Arctic Ocean (station FB) as measured by chl *b* in the <3 µm fraction, and by epifluorescence cell counts. Inset: photomicrograph of a typical *Micromonas*-like cell, stained with 4′6-diamino-2-phenylindol and viewed with a UV filter block, in one of the chl maximum samples from Station DS07.

TABLE 1. Details of sampling sites that were the source of the clone libraries.

Library	Station	Z_{lib}	Z_{max}	Temperature	Salinity	Chl *a*
NW415	AO-NW08	5	3473	−1.4	27.02	0.07
NW414	AO-NW08	50		−1.0	31.77	0.53
NW617	AO-NW01	5	801	−1.4	27.05	0.19
NW614	AO-NW01	50		−0.8	31.87	0.25
NOR26	M09	5	67	4.5	33.17	2.81
NOR46	Z59	5	3231	6.1	33.49	0.61
NOR50	Z59	60		2.8	34.46	0.09
MD65	MD65	5	33	−0.44	26.49	0.21
CA040116	FB	3	220	−1.62	30.01	0.04
Ce303s	303	5	270	1.09	30.86	0.65
ds07	DS07	30	117	−0.68	27.96	0.28

Station locations are shown in Fig. 1. Z_{lib} is the depth (m) of the sample used for library construction, and Z_{max} (m) is the maximum depth of the water column at that station. Temperature (°C) and salinity (PSU) are from CTD profiles, and chl *a* concentrations (in $\mu g \cdot L^{-1}$) were measured in extracts of filtered samples.

we measured in the CCMP2099 culture experiments at 0°C (Fig. 3). The net *in situ* growth rate estimated from log-linear regression of the cell count data versus Julian day for the period February to May 2004 (Fig. 5) gives a population doubling time (calculated as ln 2/μ) of 23 days. A calculation based on log-linear regression of the <3 μm chl *b* pigment stocks for the same period (Fig. 5) gives a slightly longer estimate of 29 days.

Pan-Arctic molecular analyses. We constructed 18S rDNA clone libraries from four Arctic regions that included offshore as well as nearshore sites (Table 1). Results for the sampling at these sites in 2002 for taxa other than picoprasinophytes are given in Lovejoy et al. (2006). The <3 μm cell fraction showed the frequent presence of prasinophyte 18S rDNA sequences. These fell into three genera in the order Mamiellales: *Micromonas, Bathycoccus*, and a single occurrence of *Mantoniella* (Fig. 6). *Micromonas* was the most frequently retrieved phylotype among these prasinophytes, and it clustered apart from known temperate latitude strains of this genus (Figs. 6 and 7) with a distinct 10 bp nucleotide arrangement (indel: GTTAACGCTC) 716 bp from the 5′ end of *Saccharomyces cerevisiae* RDN18-1, within the V4 region of the eukaryotic SSU rRNA. *Micromonas* CCMP2099 from the NOW, ~2000 km to the east of our Beaufort Sea study and ~2000 km west of the GNB sites, clustered with the Arctic environmental phylotypes and had the same distinct 10 bp indel. The indel was not found in any other environmental sequences other than the environmental clone ANT37-4 sequence from Antarctica. However, this sequence was different in other regions, with only 97% similarity (726/741 bp, bit score 1282) to our Arctic clones. ANT37-4 was not included in our dendrograms because there was only an ~20 bp overlap with this sequence and the other partial sequences used in the analysis. The closest match of CCMP2099 found in the GenBank environmental_nt data base was to environmental sequence IBEA_CTG_2087931 (AACY01075624.1) from a Sargasso Sea shotgun library, with a similarity of <94% (1331/1409, bit score 1732), indicating the presence of *Micromonas* in that library but not the Arctic clade.

The 2005 ds070 library from Dease Straight was dominated by micromonads all belonging to the CCMP2099 cluster (Figs. 6 and 7, cluster Ea). Direct microscope counts of samples from this site gave concentrations of *Micromonas*-like cells of 6730 (SD 1397) $cells \cdot mL^{-1}$ (average of four separate sample bottles taken from the chl maximum layer). The micromonads accounted for 90% of all cells (both photosynthetic and non-chl-containing) in these samples. For this library, we used the 528f primer to screen 50 random sequences from the library before RFLP screening. From this, we obtained 33 good-quality sequences, of which 16 had their closest BLAST matches to CCMP2099 (bit scores 1742–1935). No other prasinophytes were retrieved. The second most abundant ribotype was a marine stramenopile (four clones) and a dinoflagellate (four clones); the remaining were single ribotypes with BLAST matches to diatoms, other dinoflagellates, and ciliates. After sequencing, the clones were screened using HAIII-RFLP as for the earlier clone libraries, and we found that all 16 micromonads had the same unique pattern.

B. prasinos was also widely distributed and was retrieved from our midwinter (CA040111) clone library for the Beaufort Sea. This phylotype, at least at the level of 18S rDNA, did not appear to be restricted to Arctic waters.

DISCUSSION

The observations reported in this study, in combination with records from earlier studies of the Arctic Ocean phytoplankton, provide broad evidence that picoprasinophytes are spatially and temporally prevalent throughout the Arctic region (Table 2). Small green flagellates have been reported in the Barents Sea, where dilution culture assays showed that *M. pusilla* was prominent in spring and early summer (Throndsen and Kristiansen 1991). Epifluorescence microscopy has also been used in several studies to

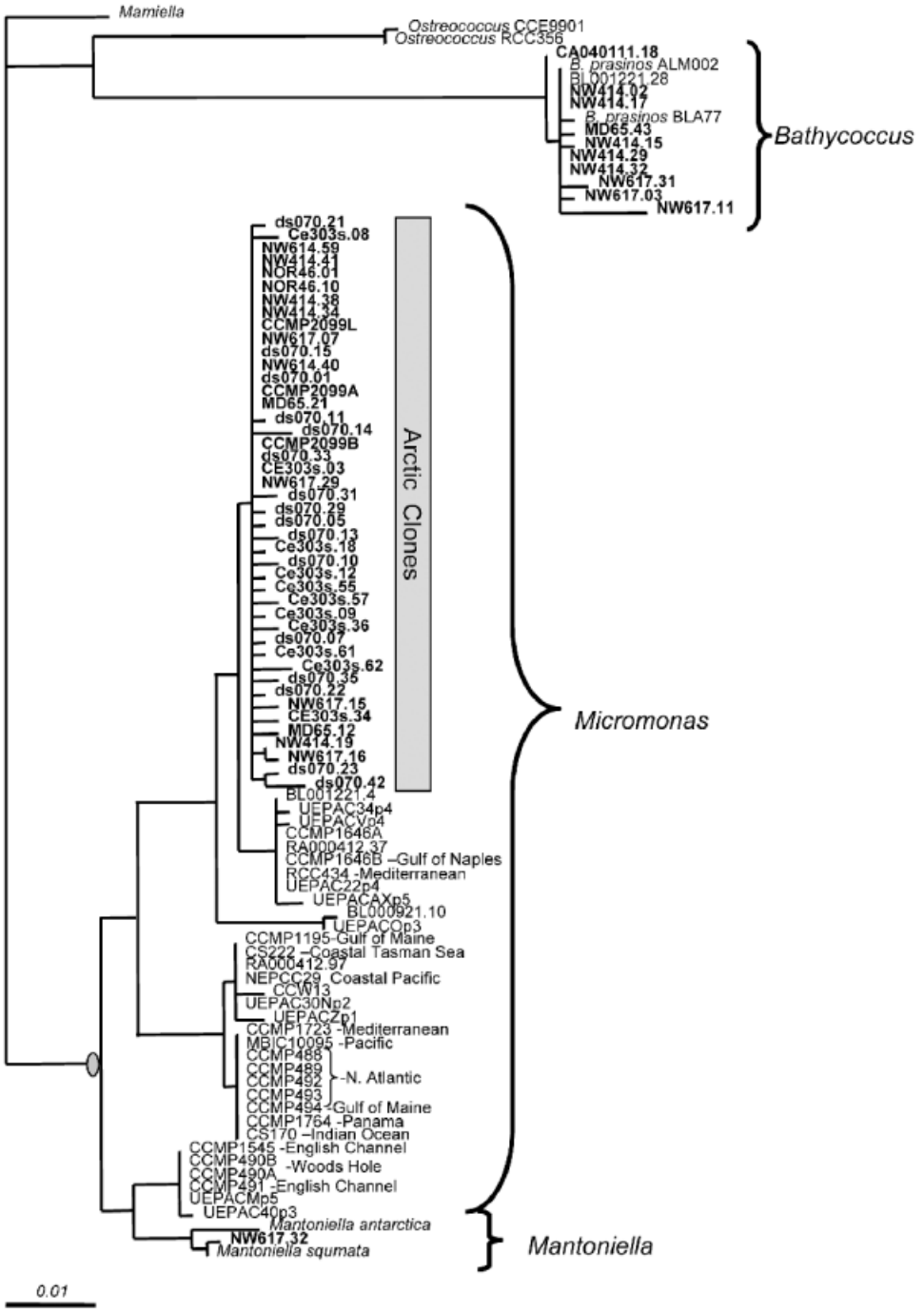

FIG. 6. Maximum-likelihood tree for partial (720 bp beginning at the conserved motif TTAAAAAGCTCGTAG) of the 18S rRNA gene in the prasinophyte order Mamiellales, rooted with *Mamiella*. Sequences in boldface are from this study for environmental clones (GenBank accession numbers DQ055149–DQ055172 and DQ835538–DQ835561) from North Wind Ridge and Canada Basin (prefix NW); Mackenzie delta in the Beaufort Sea (MD); CASES station Franklin Bay in winter (CA040111), and Station 303 in summer (Ce303s); nonredundant ribotypes from Dease Straight (ds070) and the Norwegian-Greenland-Barents Seas (NOR); and three sequences for *Micromonas* isolate CCMP2099 from the North Water Polynya (L from this study DQ025753) A and B from Šlapeta et al. (2006). Arctic locations are shown in Fig. 1, and further site details are given in Table 1. Isolate origins for other cultured strains are given. Origins of environmental strains listed are as follows: BL, Blanes Bay Mediterranean; RA and RC, English Channel, France; NA, North Atlantic; CCW, Sippewisset Salt Marsh, Maine, USA; and UEPAC, Pacific Ocean. The small oval indicates the position of Fig. 7.

quantify small eukaryotes in Arctic seas. In the "Arctic Ocean Section" from the Chukchi Sea to Makarov Basin, an ~2 μm flagellate, unidentified but resembling *M. pusilla*, was the most abundant phototroph in epifluorescence analyses at all stations and on average accounted for 93% of total autotrophic cells and 36% of autotrophic biomass (Booth and Horner 1997). This species has also been reported as the phytoplankton dominant in Norwegian Arctic seas in late summer (Not et al. 2005). The SHEBA/JOIS drift experiment, from November 1997 to August 1998 over latitudes 75°–80° N and longitudes 143°–166° W, showed that a 2 μm diameter micromonad (among other phytoplankton) was present in epifluorescence counts throughout winter and represented a major constituent of the spring bloom beneath the pack ice (Sherr et al. 2003). The study by Not et al. (2005) used genus-level probes and showed that *Bathycoccus* and *Micromonas* were dominant picoeukaryotes in Arctic-influenced waters of the GNB. In the present study, we confirmed that *Micromonas* is abundant and widely distributed, but we also determined that this microbial phototroph is a unique pan-Arctic ecotype that differs genetically and in terms of growth characteristics from *M. pusilla*

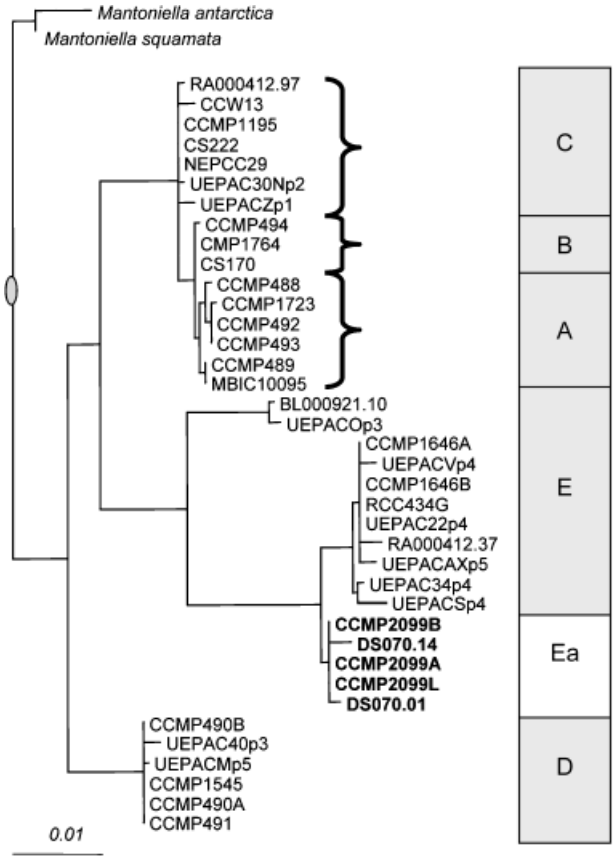

FIG. 7. Maximum-likelihood dendrogram for the 18S rRNA gene incorporating all nearly full-length sequences (>1700 bp) available in GenBank July 3, 2006, with 97% or greater matches to CCMP 2099. Sequences in boldface are from the Arctic. Origins of clones and cultures are as in Fig. 6. Neighbor-joining (NJ) and Bayesian tree topology was substantially the same; bootstrap NJ and Bayesian support was 100% and >60, respectively, for all branches. Clade designations A–E are from Šlapeta et al. (2006). Clade E branches into additional clades including clade Ea, containing Arctic micromonads. The small oval indicates the position of this tree in relation to Fig. 6.

clades collected elsewhere in the world. The genetic variability of *B. prasinos* was much less than for *Micromonas* at the level of the 18S rRNA gene, consistent with reports elsewhere (Guillou et al. 2004).

In the Arctic Ocean, the North Polar *Micromonas* ecotype and *B. prasinos* replace picocyanobacteria as the baseline community that persists throughout all seasons. Biomass peaks of larger cells, as well as the sea-ice algal community, are superimposed on this background, but the continuous, widespread distribution of these taxa means that picoplankton-size prasinophytes are among the most abundant photosynthetic cell types in northern seas. Polar cyanobacteria tend to be cold tolerant rather than psychrophilic, with slow growth under cold ambient temperatures (Tang et al. 1997). The replacement of picocyanobacteria by picoprasinophytes in the Arctic Ocean and surrounding seas likely reflects the superior growth of these cold-adapted ecotypes under the stable low temperatures that characterize the North Polar ocean. Our continuous seasonal observations also show their ability to retain their pigments and survive throughout winter darkness at reduced population densities, and to resume net population exponential growth at the earliest return of light in late winter–early spring.

Guillou et al. (2004) observed that although *Micromonas* seems morphologically uniform (small cell with one distinct flagellum), it comprises a genetically diverse species complex. Subsequent genomic analysis has confirmed clades of micromonads among cultured strains (Šlapeta et al. 2006). In that study, five distinct clades (A–E) were identified using five genes. Clade E was made up of two isolates: one from the Mediterranean and CCMP2099 from the Arctic. Our phylogenies recovered the E clade, and we were able to distinguish clades within E, one of which was composed of strictly Arctic clones (Fig. 7, clade Ea). The remainder of clade E was composed of representatives from the Mediterranean, the English Channel, and the Pacific. Our extensive molecular survey, covering much of the Arctic at both inshore and offshore sites and during different seasons, indicates the absence of temperate latitude strains of *Micromonas* in Arctic seas. Our targeted database search of micromonad-related cultures and environmental sequences found no sequences from other regions of the World Ocean that clustered with our Arctic ribotypes. In contrast, other *Micromonas* clades distinguished originally by Šlapeta et al. (2006) had members from widely dispersed regions (Figs. 6 and 7). A subsequent survey using denaturing gradient gel electrophoresis in late summer 2005 across the Canadian Archipelago, from the North Water Polynya to the Beaufort Sea, confirmed the widespread occurrence of the Ea clade represented by the CCMP2099 phylotype and the lack of other *Micromonas* clades (A. Hamilton and C. Lovejoy, Université Laval, Canada, unpublished data). These results, in combination with our laboratory growth experiments, support the notion of a cold-adapted, geographically restricted ecotype or geotype (Papke and Ward 2004). Ecotypes of the eukaryote *Ostreococcus* have recently been described (Rodriguez et al. 2005), and cyanobacterial *Synechococcus* and *Prochlorococcus* ecotypes are widely reported (Rocap et al. 2002, Scanlan 2003). However, these are mostly associated with defined depth and light regimes that change over oceanic provinces but not specifically by geography (Bouman et al. 2006, Johnson et al. 2006).

The prevalence of the CCMP2099 phylotype (clade Ea) over distance scales of several thousand kilometers implies that the Arctic Basin comprises a single microbial province (as defined by Martiny et al. 2006) with endemic and cosmopolitan species, consistent with the macroorganism biogeography of the Arctic. This may reflect the limited dispersal abilities of *Micromonas*, for example, the apparent absence of resting cysts in this taxon, in combination with the environmental pressures imposed by the polar environment. The isolation of the Arctic Basin has provided a long-term oppor-

TABLE 2. Records of picoprasinophytes in the Arctic Ocean.

Location	Nature of the record	Concentration (cells · mL^{-1})	Reference
Greenland, Norwegian, and Barents Seas	Clone library analysis; FISH cell counts	4–9073	This study, Not et al. (2005)
North Water Polynya	Isolate CCMP2099, HPLC analysis (NOW)	–	This study, Vidussi et al. (2004)
Beaufort Sea, NW Passage	Clone library, HPLC analysis, epifluorescence microscopy (CASES, MMBOAS)	7–9475	This study
Canada Basin, Northwind Ridge	Clone library analysis (JWACS)	–	This study
Canada Basin, Chukchi Plateau, and Mendeleyev Basin	Epifluorescence microscopy (SHEBA/JOIS)	1000–28,000	Sherr et al. (2003)
Chukchi Sea to Makarov Basin	Epifluorescence microscopy (Arctic Ocean Section)	1300–10,020	Sherr et al. (1997)
Chukchi and Eastern Beaufort	HPLC analysis	–	Hill et al. (2005)
Barents Sea	Dilution culture assays	–	Throndsen and Kristiansen (1991)
Northeast Water Polynya	HPLC and epifluorescence microscopy	3–380,200	Booth and Smith (1997)

CASES, Canadian Arctic Shelf Exchange Study; FISH, fluorescent *in situ* hybridization; JWACS, Joint Western Arctic Climate Study; MMBOAS, Marine Microbial Biodiversity of the Arctic Ocean and Seas; NOW, North Water Polyna.

tunity for genetic differentiation, selecting for a *Micromonas* ecotype with optimal growth in thermally stable, cold ice-covered seas and loss of ability to survive in warmer temperatures. A recent review of 16S rDNA sequences in GenBank has revealed that several bacterial ribotypes (conservatively estimated at 97% similarity) may be restricted to the North Polar region (Pommier et al. 2005), in keeping with our conclusion that the Arctic is a marine microbial province. Much more detailed phylogenetic surveys are required, however, to extend the polar data bases and to confirm this conclusion.

Our culture experiment with *Micromonas* CCMP2099 showed that this isolate has a number of distinctive phenotypic attributes, including a growth optimum at 6°C–8°C. It was unable to grow at 15°C, and even at 12.5°C, it showed impaired growth rates at the higher irradiances. At temperatures of 2°C and above, much faster growth was observed at 50 µmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}, which appeared to be at or above the onset of light saturation (E_k). At 0°C, a temperature closer to that typically experienced by this species in Arctic seas, growth rates were as high as those under much warmer temperatures, and light saturation was at or below 10 µmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}. This interaction between temperature and irradiance optima is well known in other phototrophs, and an increasing E_k value with increasing temperature is attributable to the much greater effect of warming on maximum photosynthetic rates (P_{max}) relative to light-limited photosynthesis (e.g., Henley 1992, Rae and Vincent 1998). The relatively fast growth of *Micromonas* CCMP2099 under low light and low temperatures likely confers a strong selective advantage on this genotype in the perennially cold, ice-covered Arctic Ocean.

The occurrence of a psychrophilic, shade-adapted prasinophyte in the Arctic Basin is consistent with the widespread success of green algae in several types of low-temperature habitats. For example, *Chlamydomonas nivalis* (F. A. Bauer) Wille, *Chloromonas* spp., and *Raphidonema nivale* Lagerh. commonly occur in snow, pack ice, and glaciers (Mueller et al. 2001), while species of the prasinophyte genus *Pyramimonas* occur in sea ice (Daugbjerg and Moestrup 1993, Ikavalko and Gradinger 1997) and perennially ice-covered Antarctic lakes (Vincent 1988). An ecotype of *C. raudensis* H. Ettl. isolated from Antarctic Lake Bonney shows an upper growth limit of 15°C, optimal growth at 8°C, and an ability to grow under extreme shade conditions (Morgan et al. 1998). The persistence of low irradiances beneath the ice and prolonged winter darkness may be factors, in addition to extreme cold, selecting for specific genotypes in polar lakes and seas. Mixotrophy, the use of both phototrophic and heterotrophic strategies for energy acquisition, could be one mechanism allowing this and other phytoplankton to survive under this low-energy regime.

Small chl *b*–containing prasinophytes often co-occur with picocyanobacteria in a variety of coastal marine environments (Ypma and Throndsen 1996, Zingone

et al. 1999, Not et al. 2004). In this study, we determined that picoprasinophytes were much more common than cyanobacteria and were abundant not only in Arctic coastal waters but also beyond the shelf up to several hundred kilometers offshore, including in the remote Canada Basin, an oligotrophic gyre with a maximum depth of over 5000 m. The Arctic region is currently experiencing major climatic change and is susceptible to much greater impacts in the future (Meehl et al. 2005, Stroeve et al. 2005). Our results from throughout the Arctic show that the most abundant photosynthetic cells include a single high-latitude *Micromonas* ecotype that has a narrow thermal niche (Hochachka and Somero 2002) in keeping with the stable cold-temperature regime of high-latitude seas. Characterization of this ecologically important taxon and the other picoprasinophytes will require more extensive genomic analysis and may reveal finer differentiation, as has been reported in the picoeukaryote *Ostreococcus*, with depth (Rodriguez et al. 2005). Our finding that a cold-adapted lineage is temporally and spatially prevalent in Arctic seas implies that the lower food web community of these pelagic ecosystems will be vulnerable to future warming and loss of ice cover.

This research was supported by the Natural Sciences and Engineering Research Council of Canada; the Strategic Science Fund from Fisheries and Oceans, Canada; the Japan Marine Science and Technology Center, Tokyo, Japan; the Canada Climate Action Fund; ARTIC (REN2001-4909-E/ANT, MCyT) and ESTRAMAR (CTM2004-12631/MAR, MEC) Spain; GEN-μMar (CTM2004-02586/MAR), European Union; and Fonds québécois de recherche sur la nature et la technologie, Québec, Canada. The authors thank E. Carmack and W. Eikrem for facilitating the sampling. We also thank the chief scientists, officers, and crew of the CCGS *Pierre-Radisson*, CCGS *Louis St. Laurent*, CCGS *Amundsen*, and F/F *Johan Hjort*. We are also grateful to J. Pouliot, P. Galand, V. Balague, V. Farjalla, C. Nemecz-Wieltschnig, C. Martineau, L. Miller, M. Rautio, and M.-È. Garneau for their roles in this research, and to J. Corbeil, L. Johnson, and P. Galand for critically reading the manuscript. This is a contribution to the Canada Arctic Shelf Exchange Study (CASES), the Joint Western Arctic Climate Study (JWACS), and the Canada Research Chair program in aquatic ecosystem studies.

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