A *Phaeocystis globosa* bloom associated with upwelling in the subtropical South Atlantic Bight

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We observed an unusual, subtropical bloom of Phaeocystis globosa Scherffel during a strong upwelling event and confirmed its identity using morphological, physiological and genetic traits. This low-latitude bloom of P. globosa colonies occurred on the South Atlantic Bight continental shelf during the summer of 2003. Maximum chlorophyll a concentration measured during this subsurface bloom was 11.37 $\mu g\,L^{-1}$ at 31 m depth. Divers reported abundant flocculent material below the steep thermocline, and this material was identified as Phaeocystis sp. using microscopy. Using morphological and physiological traits as well as the sequence of the 18S small subunit ribosomal RNA gene (GenBank Accession # EF100712), this phytoplankton was identified as P. globosa. Interactions of physical and biological conditions may restrict low-latitude P. globosa blooms to strong upwelling events.

INTRODUCTION

Phytoplankton within the genus *Phaeocystis* form massive blooms in many parts of the world's oceans (reviewed in Baumann *et al.*, 1994; Lange *et al.*, 2002). *Phaeocystis* blooms are important to pelagic food web structure as well as to high latitude carbon cycling (Smith *et al.*, 1991, Arrigo *et al.*, 1999). Although *Phaeocystis* species are widely distributed, *Phaeocystis* blooms are common only in high-latitude environments: they rarely occur in low-latitude environments such as the subtropics (except Chen *et al.*, 2002). Subtropical blooms appear "enigmatic" (Verity and Medlin, 2003) since physical conditions, such as temperature and nutrients, at low latitudes should support *Phaeocystis* blooms. The infrequent occurrence of low-latitude *Phaeocystis* blooms complicates their study and prevents a thorough understanding of their ecology.

Due to the rarity of *Phaeocystis* blooms at low latitudes, the species identity of *Phaeocystis* in these environments is largely unknown (but see Chen *et al.*, 2002). Although

a variety of techniques, including microscopy, culturing and genetic analyses, are now used to identify *Phaeocystis* spp., earlier studies distinguished between these species using only morphological features of colonies as determined with light microscopy. However, many *Phaeocystis* spp. display a remarkable degree of morphological plasticity, alternating between multiple forms of solitary cells and colonies, and overlapping distributions (Medlin *et al.*, 1994; Vaulot *et al.*, 1994; Chen *et al.*, 2002). This plasticity generates uncertainty in species-level identifications (Baumann *et al.*, 1994; Vaulot *et al.*, 1994). For example, Baumann *et al.* (Baumann *et al.*, 1994) noted ambiguous species identifications for all seven of the studies to report *Phaeocystis* occurrence in tropical marine areas. More recently, modern molecular techniques have been used to provide unambiguous identifications of *Phaeocystis* species (Baumann *et al.*, 1994; Medlin *et al.*, 1994; Chen *et al.*, 2002). Such identifications could be critical to the ecology of *Phaeocystis* spp. blooms.

During the summer of 2003, we observed a *P. globosa* bloom at a subtropical latitude (~32°N) off the coast of Savannah, GA, USA. From this bloom, we isolated a *P. globosa* colony, grew it in culture and confirmed its identity by sequencing its 18S small subunit ribosomal RNA gene. Our goal was to identify this species using morphological, physiological and genetic traits.

METHOD

During the bloom, between July and August 2003, we observed and collected *P. globosa* using scientific divers, benthic cores and Niskin bottle water samples. CTD data including temperature (°C) and chlorophyll *a* (Chl *a*) were collected during several across-shelf, transect cruises from the coast of Savannah to the continental shelf. Chl *a* was determined by *in vivo* fluorometry with a WET Labs WETStar chlorophyll fluorometer integrated into a Sea-Bird Electronics SBE 25 Sealogger CTD/SBE 32 Carousel Water Sampler. The fluorometer response was determined from extracted chlorophyll samples collected during the same cruise. Using bilinear interpolation on 1 m bin averaged data, we report Chl *a* and temperature along a single transect gathered during six CTD casts on a cruise during 29 July 2003 (Fig. 1). The transect started at the sea buoy at 31°51.55′N, 80°53.01′W and proceeded southeasterly. A single *P. globosa* colony was isolated from combined samples from 24.9 and 38.1 m on 7 August 2003 by repeatedly transferring the colony to autoclaved, GF/F filtered seawater. This isolate was cultured in L-Si medium (Guillard and Hargraves, 1993) at 20°C at $100–150\ \mu$mole m^{-2} sec^{-1} at a light:dark cycle of 14:10. Maximum light levels measured 1 week earlier from a Licor datalogger mounted to a benthic chamber at 27 m were 25 μmole m^{-2} sec^{-1} (J. Nelson; Skidaway Institute of Oceanography; unpublished data). This strain was deposited in the Provasoli-Guillard National Center for Culture of Marine Phytoplankton culture collection in Maine (CCMP2754).

To identify this species of *Phaeocystis*, we examined the morphology of colonies using light microscopy and compared this to the morphology of other *Phaeocystis* spp. (Baumann *et al.*, 1994; Zingone *et al.*, 1999). We also determined a conservative temperature range at which *P. globosa* grew. However, colony morphology and temperature range can be poor traits for distinguishing species of *Phaeocystis* (Kornmann, 1955; Sournia, 1988; Baumann *et al.*, 1994; Medlin *et al.*, 1994). To determine the identity of our *Phaeocystis* isolate more precisely, we cloned and sequenced a 1761 bp fragment of the small subunit ribosomal RNA (SSU rRNA) gene. To
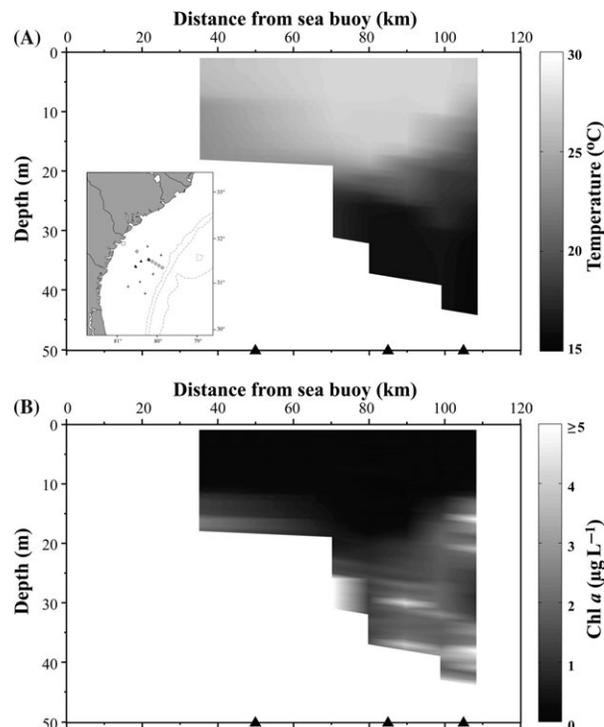
Fig. 1. Cross-continental shelf contour plots of (**A**) temperature and (**B**) Chl *a* concentration during the *Phaeocystis globosa* bloom. Plots were generated from six CTD casts along a southeasterly transect starting from the sea buoy near Savannah, GA and moving across the continental shelf on 29 July 2003. Inlay shows CTD casts (diamonds), dive sites (triangles), sea buoy (square) and military towers (plus signs). Distances along the *x*-axes refer to distance from the sea buoy. Arrows denote locations where divers observed *P. globosa* in the water column. Subsurface Chl *a* maxima were associated with colder, upwelled regions.

accomplish this, 5 mL of an exponential phase culture was collected onto a 0.8 μm Supor filter (PALL Life Sciences, East Hills, NY) and total DNA was extracted from the filter as previously described (Allen *et al.*, 2005) using the MoBio UltraClean soil DNA isolation kit (MoBio Laboratories, Inc). The 1761 bp fragment of the SSU rRNA gene was PCR amplified, cloned and sequenced essentially as previously described (Frischer *et al.*, 2002). PCR amplification was accomplished using the universal 18S rDNA targeted PCR primers UnivF-15 (5′ ctg cca gta gtc ata tgc) and UnivR-1765 (5′ acc tgg tta cga ctt tac) as previously described (Frischer *et al.*, 2002). To facilitate sequencing, amplified 18S rRNA gene PCR products were cloned into the pCR 2-TOPO cloning vector using a TOPO Cloning Kit, Version J (Invitrogen) following the manufacturer's instructions. Automated sequencing was completed using a Beckman CEQ8000XL DNA Analysis System. Sequencing reactions were facilitated using a CEQ

DTCS dye terminator cycle sequencing quick start kit. A total of six sequencing primers were utilized such that the complete fragment sequence was determined in the forward (primers M13-20F[18]; 18S-570F; 18S-1138F) and reverse (primers M13-48R[24]; 18S-570R; 18S-1138R) directions as previously described (Gruebl *et al.*, 2002).

The identity of the SAB *Phaeocystis* sp. isolate was determined by phylogenetic comparison with 17 other *Phaeocystis* sp.18S rRNA genes available in the public databases GenBank (www.ncbi.nlm.nih.gov) and the Ribosomal Database Project (RDP; Maidak *et al.*, 2000). For phylogenetic comparisons, we restricted our analysis to *Phaeocystis* spp. known to form colonies or aggregates. After trimming all sequences to the shortest sequence used in the phylogenetic comparisons (*P. globosa* Santou97 AJ279499, 1004 bp), sequences were aligned using the Clustal W version 1.8 multiple sequence alignment algorithm (Thompson *et al.*, 1994). Alignments were viewed and refined based on secondary structure considerations (Relman *et al.*, 1996) using BioEdit v7.0.5.3 (Hall, 1999). Phylogenetic trees were inferred and drawn using the Treecon for Windows software package version 1.3b (Van de Peer and de Wachter, 1994, 1997) using the Kimura two parameter model for inferring evolutionary distance (Jin and Nei, 1990). Bootstrap estimates (1000 replicates) of confidence intervals were also made using the algorithms in Treecon.

RESULTS

Upwelling conditions resulted in temperatures over $8°C$ colder than average on the South Atlantic Bight continental shelf during 2003 (Aretxabaleta *et al.* 2006; Fig. 1A). These conditions appeared to facilitate a subsurface phytoplankton bloom (Fig. 1B). Maximum Chl *a* concentration was $11.37\ \mu g\ L^{-1}$ at 31 m at 70.531 km from the sea buoy. Niskin bottle samples from this location that were enriched with media (L-Si) produced *P. globosa* colonies. Diving and laboratory observations suggest that colonial *P. globosa* dominated this bloom. On 28–30 July 2003, scientific divers noticed flocculent material in the water column 58 km ($31°30.06'$N, $80°26.14'$W) and 94 km ($31°37.90'$N, $79°55.47'$W) from the sea buoy for Wassaw Sound (Savannah, GA, USA). A sample of this material was collected by a diver and identified later as a *P. globosa* colony using light microscopy. Then, on 7 August 2003, benthic cores from 38.1 m ($31°22.73'$N, $80°18.59'$W) and 24.9 m depth ($31°33.72'$N, $80°31.84'$W) contained flocculent material on their surfaces. Some of this material was collected from four cores and identified later as *P. globosa* colonies using light microscopy. Finally, on 27 August–29 August 2003, no colonies were observed in several water samples collected with Niskin bottles during a transect from coastal Savannah waters to the continental shelf. However, *P. globosa* was present at this time since whole water samples collected 4 m off the seafloor in 20 m of water ($31°37.20'$N, $80°35.95'$W) and enriched with media developed abundant *P. globosa*-like colonies (described in Baumann *et al.*, 1994) after 26 days. Colonies were not originally observed in these samples suggesting that the bloom had dissipated or simply that the dominant morphology of *P. globosa* shifted from colonies to solitary cells.

The spherical colonies collected *in situ* consisted of cells ($\sim$5 μm in diameter) evenly spaced around each colony. Thus, these colonies were morphologically similar to a previously identified *P. globosa* strain (CCMP 627), but their large size ($\sim$15 mm in diameter) was more similar to colonies of the Chinese isolate of *P. globosa* ($\sim$30 mm in diameter, Chen *et al.*, 2002) than to colonies of the common, temperate varieties of *P. globosa* (8–9 mm in diameter, Baumann *et al.*, 1994). Although this strain (CCMP 2754) initially formed colonies, it no longer forms spherical colonies characteristic of *P. globosa* (J. Sexton; Provasoli-Guillard National Center for Culture of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences; Maine; personal communication). Similarly, two strains of Norwegian *P. pouchetii* stopped forming colonies in culture (Jacobsen, 2002). The temperature range for growth of this *Phaeocystis* sp. was at least 16–20°C, since *Phaeocystis* sp. were collected by divers at 16°C and were cultured in the laboratory at 20°C. This temperature range is within the growth range of temperatures reported for *P. globosa* (4–30°C, Jahnke and Baumann, 1987; Chen *et al.*, 2002). However, this range is conservative since we did not test the ability of *P. globosa* to grow at extreme temperatures.

Molecular phylogenetic reconstruction of the evolutionary relationship between our isolate and available *Phaeocystis* species based on the comparison of a 1004 bp region of the 18S rRNA gene sequence confirmed the identity of our isolate as a *P. globosa* strain (Fig. 2). Average nucleotide similarity of this region between *P. globosa* str *robertsonii* and other *Phaeocystis* spp. 18S rDNA sequences including *P. globosa* *P. pouchetii*, *P. Antarctica*, *P. cordata* and *P. jahnii* was 99.4% (99.3–99.7%), 97.9%, 98.4%, 96.7% and 95.3%, respectively. The similarity between *P. globosa* str *robertsonii* and *E. huxleyi* is 92.9% for this gene fragment. The 1761 bp 18S rDNA sequence determined in this study was submitted to GenBank (Accession # EF100712).
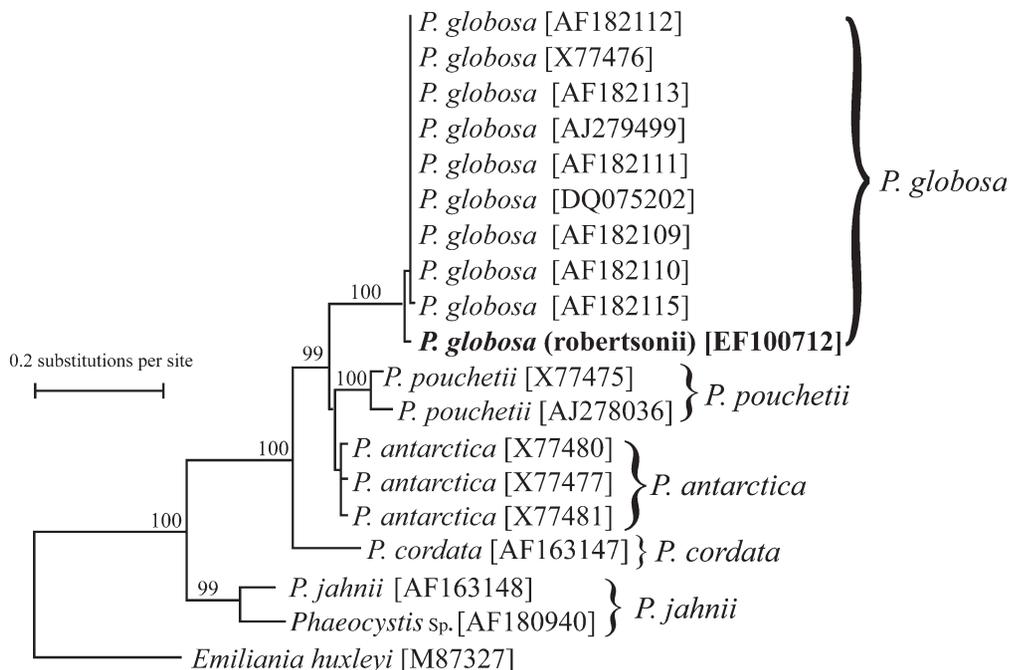
Fig. 2. Inferred taxonomic relationship between colony- or aggregate-forming *Phaeocystis* species *P. globosa*, *P. pouchetii*, *P. antarctica*, *P. cordata* and *P. jahnii* based on 18S ribosomal RNA gene sequences. The *P. globosa* strain isolated in this study is in bold. The tree was rooted with the 18S rRNA gene from *Emiliania huxleyi*. GenBank accession numbers are provided in brackets. The scale bar indicates 0.02 nucleotide substitutions per site. Numbers refer to bootstrap values (from 1000) for each node.

DISCUSSION

Several observations support the conclusion that a bloom of *P. globosa* colonies occurred off the coast of Savannah, GA, USA, during summer 2003. First, divers encountered abundant *P. globosa*-like colonies in the subsurface Chl *a* maximum during July 2003. Second, the colony morphology (spherical colonies with regularly spaced cells on the colony periphery) of this *Phaeocystis* sp. matches the morphology of other *P. globosa* colonies. Third, the temperature range of this species was within the range previously reported for other *P. globosa* strains. Fourth, *P. globosa* is probably the most common *Phaeocystis* species in temperate and subtropical waters (Vaulot *et al.*, 1994). Finally, phylogenetic reconstruction based on 18S rRNA gene sequences identified our isolate as a *P. globosa* strain.

Previous reports of *Phaeocystis* spp. blooming in the colony form are rare for lower latitudes but are common for higher-latitude temperate and polar regions (Baumann *et al.*, 1994). The colony-forming *Phaeocystis* species, *P. globosa*, *P. antarctica*, *P. pouchetii*, and *P. jahnii*, are reported to occur exclusively in colder waters or form blooms of colonies primarily at the higher latitudes within their range (Baumann *et al.*, 1994; Zingone

et al., 1999; Verity and Medlin, 2003). Reigman and van Boekel (Reigman and van Boekel, 1996) suggest that colonial blooms of *P. globosa* form in NO_3-rich waters typical of upwelling conditions. However, pigment analysis and sequencing of natural plankton samples suggest that *Phaeocystis* spp. may be common in tropical waters (Moon-van der Staay *et al.*, 2000). Two studies identified *Phaeocystis* spp. from samples collected from the South Atlantic Bight near our study site. A bloom of *P. pouchetii* was reported in the Gulf Stream waters off the coast of Florida (Atkinson *et al.*, 1978), but this was later identified as a colony forming diatom (G. Paffenhöfer; Skidaway Institute of Oceanography; GA, USA; personal communication). Whole water samples collected from 31°11′N, 79°37′W contained colonies of *Phaeocystis* sp. after multiple-day incubations (Verity and Medlin, 2003). Although *Phaeocystis* may occur in tropical and subtropical waters (reviewed in Baumann *et al.*, 1994), it is unclear which species bloomed in these areas and whether the bloom contained colonies. As an exception, Chen *et al.* (Chen *et al.*, 2002) provided convincing evidence that a bloom of colonial *P. globosa* occurred in Chinese waters. Similarly, we observed a *Phaeocystis* bloom in the subtropics and identified the species as *P. globosa*.

Although blooms of *Phaeocystis* colonies occur less frequently in lower latitude environments, there is growing evidence that *Phaeocystis* spp., especially *P. globosa*, can bloom in these environments under certain conditions. Blooms of *Phaeocystis* sp. colonies at lower latitudes may be associated with periods of wind-driven upwelling events along the coast (Garrison *et al.*, 1998; Verity and Medlin, 2003; this study). For example, *Phaeocystis* colonies were reported from the Arabian Sea especially during periods of upwelling (Garrison *et al.*, 1998). Similarly, we observed a bloom of *P. globosa* colonies during a strong upwelling event in 2003 that brought cold water from the deep waters of the Gulf Stream onto the continental shelf of the South Atlantic Bight. The physical characteristics of the upwelling event are described fully in Aretxabaleta *et al.* (Aretxabaleta *et al.*, 2006). This upwelling resulted in temperatures up to $8°C$ colder than average (Aretxabaleta *et al.* 2006; Fig. 1A). These conditions may have facilitated a subsurface phytoplankton bloom that appeared as a subsurface Chl *a* maximum (Fig. 1B). Colonial *Phaeocystis* sp. dominated this bloom (personal observation). The study of lower latitude blooms of *Phaeocystis* spp. will be facilitated if we can predict their occurrence by predicting bloom-favorable conditions (e.g. upwelling events).

Several factors may be responsible for low-latitude, *P. globosa* colony blooms during upwelling events. Upwelled waters may supply a stock of *P. globosa* from which blooms develop. Alternatively, *P. globosa* may always be present in these areas in solitary cell forms (but is overlooked because of its small size) and the upwelling of cold waters provides favorable conditions for colony formation and bloom development. For example, upwelled waters may stimulate colony formation by enhancing nitrate levels (Riegman *et al.*, 1992). However, a range of nutrient levels are present in subtropical waters where *P. globosa* blooms rarely occur (Verity and Medlin, 2003) and the role of nutrients in colony formation is controversial (Verity *et al.*, 1991; Hegarty and Villareal, 1998). Several laboratory studies describe colony formation at higher temperatures (Chen *et al.*, 2002; Verity and Medlin, 2003; Qi *et al.*, 2004; this study) and low nutrients (Verity *et al.*, 1991) suggesting that the cue for bloom development is not simply due to the physical conditions associated with upwelling events (i.e. low temperatures and high nutrients).

Although the physical conditions alone do not explain the occurrence of *P. globosa* blooms at lower latitudes during strong upwelling events, these factors may interact with biotic factors to enhance *P. globosa* blooms. During typical conditions at lower latitudes, the greater density and metabolism of zooplankton may limit *P. globosa* production (Verity and Medlin, 2003) and

prevent the transformation from solitary cells to colonies (Long *et al.*, 2007), a critical feature of bloom initiation (Peperzak *et al.*, 2000). Compared with the mesozooplankton populations at temperate and polar latitudes, the higher abundances and grazing rates of mesozooplankton at lower latitudes may, therefore, directly and indirectly suppress bloom development. The intrusion of waters over $8°C$ colder than ambient temperatures during strong upwelling events could significantly affect the behavior and physiology of zooplankton grazers of *P. globosa* thereby supporting favorable bloom conditions. For example, zooplankton may avoid these waters perhaps because colder temperatures decrease metabolism, as suggested for important consumers at our study sites, including copepods and doliolids (Haskell *et al.*, 1999).

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