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ORIGINAL PAPER

Late Holocene changes in cyanobacterial community structure in maritime Antarctic lakes

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Abstract Despite the dominance of cyanobacteria in polar freshwater aquatic ecosystems, little is known about their past biodiversity and response to climate and environmental changes. We explored the use of light microscopy of microfossils, high performance liquid chromatography of the fossil pigment composition and denaturing gradient gel electrophoresis of fossil 16S rRNA genes to study past and present-day differences in cyanobacterial community structure in response to climate changes in two adjacent maritime Antarctic lakes with contrasting depths (4 and 26 m) and light climates. Light microscopy was of limited use because of degradation of cell structures. Fossil cyanobacterial pigment concentrations were below the detection limits of our method in several sediment samples in the deep lake, but abundant and diverse in the sediment core from the shallow pond, probably as a consequence of increased light availability and/or a more diverse and abundant benthic cyanobacterial flora. Total carotenoid and chlorophyll concentrations were highest in both lakes between ca. 2,950 and 1,800 cal yr BP, which coincides with the late Holocene climate optimum recognised elsewhere in maritime Antarctica. Cyanobacterial molecular diversity was higher in the top few centimeters of the sediments in both lakes. In deeper sediments, the taxonomic turnover of cyanobacteria appeared to be relatively small in response to past climate anomalies in both lakes, underscoring the broad tolerance of cyanobacteria to environmental variability. This, however, may in part be explained by the low taxonomic resolution obtained with the relatively conserved 16S rRNA gene and/or the preferential preservation of particular taxa. Our results highlight the potential of fossil DNA in lake sediments to study colonization and succession dynamics of lacustrine cyanobacteria and warrant further investigation of the factors that affect preservation of cyanobacterial DNA.

Rafael Fernandez-Carazo and Elie Verleyen contributed equally to this work.

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Introduction

Fossil or ancient DNA (aDNA) is increasingly becoming part of the paleoecological tool-box (Savichtcheva et al. 2011) and has enabled, for example, refined reconstructions of past climate changes, inferred past changes in microbial and multicellular community structure in lakes, terrestrial habitats and the marine environment (Coolen et al. 2004; Anderson-Carpenter et al. 2011; Boere et al. 2011), and study of the colonization-extinction dynamics in particular taxonomic groups (Mergeay et al. 2007). The use of aDNA in paleoecology is, however, impeded by its stability and preservation potential in sediments, which depend on interacting factors such as microbial activity, adsorption of DNA to substrates, salinity, oxygen concentration, temperature and pressure (Boere et al. 2011). Nevertheless, aDNA holds particularly great potential for organisms that do not leave recognizable fossils, such as cyanobacteria. For example, ancient cyanobacterial DNA has been successfully detected in 5.9-Ma-old evaporites from the Mediterranean Sea (Panieri et al. 2010) and changes in cyanobacterial community structure have been studied in a sediment core from Ace Lake (Vestfold Hills, East Antarctica), spanning the past 10,450 cal yr (Coolen et al. 2008). In the latter study, two *Synechococcus*-like sequences were recovered and their presence appeared to be dependent upon lake-water salinity, which varied in response to relative sea level changes and the subsequent establishment of meromictic conditions.

Cyanobacteria are the most abundant photosynthetic organisms in Antarctic lacustrine habitats and play a critical role in ecosystem function (Vincent 2000). Filamentous cyanobacteria typically form benthic microbial mats and dominate primary production in shallow lakes and the littoral zone of deep lakes where light conditions are relatively high and sometimes reach levels that induce photo-inhibition and photoprotective strategies (Hodgson et al. 2004). Small unicellular taxa, such as *Synechococcus*, can constitute an important component of the picoplankton in deeper lakes (Whitton and Potts 2000; Laybourn-Parry and Pearce 2007; Coolen et al. 2008). By contrast, diatoms and sometimes mosses dominate primary production in those parts of deeper lakes where low light conditions prevail (Hodgson et al. 2004; Wagner and Seppelt 2006). For example, Wharton et al. (1983) described a shift from cyanobacteria-dominated towards diatom-dominated benthic communities with increasing depth in the lakes of the McMurdo Dry Valleys.

Recent studies have shown that polar and alpine regions harbor a flora that is in part composed of typical cold-adapted microbes (Jungblut et al. 2009; Souffreau et al. 2013), including taxa that are (potentially) endemic to Antarctica (Sabbe et al. 2003; Taton et al. 2006a). Many of these regions have recently entered a period of rapid warming, which has already had an impact on the physical and chemical structure, biodiversity and functioning of their aquatic ecosystems (Quayle et al. 2002; Smol et al. 2005; Smol and Douglas 2007). Polar lakes have therefore been recognised as 'early warning systems' because they respond very quickly to climate-induced environmental changes (Hodgson and Smol 2008). The consequences of the rising temperatures include increased lake water-column stability and chemical properties (Hodgson et al. 2006; Parker et al. 2008; Verleyen et al. 2012), ecological reorganizations of algal and invertebrate communities, including the dominance of planktonic over benthic diatoms (Smol et al. 2005), invasions by low-altitude and low-latitude species (Rautio et al. 2008), increased nutrient concentrations and primary production (Quayle et al. 2002) and even complete desiccation (Smol and Douglas 2007). In contrast to these ecosystem-level changes, which were mainly studied in Arctic lakes, comparatively little is known about changes in the diversity and community structure of the dominant photoautotrophs in Antarctic water bodies, i.e. cyanobacteria, brought about by climate and environmental changes, particularly on longer time scales.

Here, we aimed to evaluate the use of: (1) light microscopy, (2) high performance liquid chromatography (HPLC) of fossil pigments and (3) a novel protocol using cyanobacterial-specific PCR primers and denaturing gradient gel electrophoresis (DGGE) based on the 16S rRNA genes, to study Holocene changes in the cyanobacterial community structure during the past ca. 6,000 years in two adjacent isolation lakes with contrasting water depths and light climates in the Antarctic Peninsula (Fig. 1). DGGE allows relatively rapid and inexpensive inventory of the most abundant

taxa in microbial communities and was successfully used to study present-day cyanobacterial communities in Antarctic lakes (Taton et al. 2003; Verleyen et al. 2010). It separates PCR products of equal length, but different sequence (and thus melting temperature), on an acrylamide gel containing a gradient of chemical denaturants (Muyzer 1999). In particular, we aimed to study the response of cyanobacteria to past climate changes, including the late Holocene climate optimum, which has been recorded in lake sediments from regions north of the Antarctic Peninsula (Bentley et al. 2009) and in Prince Gustav Channel, including Beak Island (Sterken et al. 2012).

Materials and methods

Sampling sites

Beak Island (63°36′S, 57°20′W) is part of a remnant volcano in Prince Gustav Channel (northeastern Antarctic Peninsula) and is currently free of permanent snow fields, ice caps or glaciers (Fig. 1). Lake Beak-1 (BK1, unofficial name) (63°36′39.2349″S, 57°20′39.5940″W) is the largest (400 m diameter) and deepest (26 m) lake on the island, with an elevation of 10.95 m a.s.l. The lake was formed in a depression created by a secondary eruption vent, after isostatic uplift and isolation from the sea. Lake Beak-1 has a circum-neutral pH (6.87–7.37) and a summer water temperature (January 2006) between 4.29 and 4.82 °C (bottom and surface of the lake, respectively). The specific conductance ranges between 280 and 293 μS cm^{-1} throughout the water column. The extinction coefficient for PAR (W m^{-2}) was determined using a Spectrosense meter and equaled 1.1 (Fig. 2). The total dissolved nitrogen concentration was 140 μg L^{-1} and the PO_4-P concentration was 17 μg L^{-1}. The lake is ice-covered for 8–9 months per year. Moss banks occur on the northwestern shores of the lake. The bottom of the lake is covered by an aquatic moss bed, which is dominated by *Cratoneuropsis chilensis*. A shallow outlet stream with abundant benthic microbial mats connects Lake Beak-1 with Lake Beak-2 (BK2, unofficial name) (63°36′51.5235″S, 57°21′03.9619″W). Beak-2 is an irregularly shaped and shallow isolation pond with an approximate surface area of 0.06 km^2. Located ~6–7 m a.s.l., its mean depth is approximately 2 m and the maximum depth is 4 m. The summer temperature (January 2006) of the water column was 12.2 °C. The pH and specific conductance were 8.61 and 250 μS cm^{-1}, respectively. The total dissolved nitrogen concentration was 490 μg L^{-1} and the PO_4-P concentration was 6 μg L^{-1}. The bottom of Lake Beak-2 is covered by cyanobacterial mats. A more complete description of the limnological conditions and methods used can be found in Sterken et al. (2012).

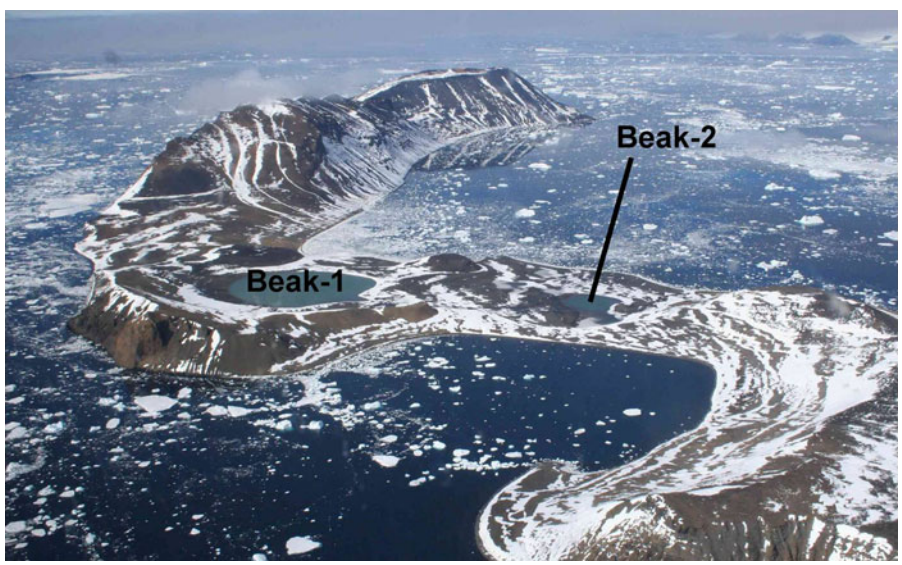

Fig. 1 Lakes Beak-1 and Beak-2 in Beak Island, seen from Duse Bay, looking towards the southeast

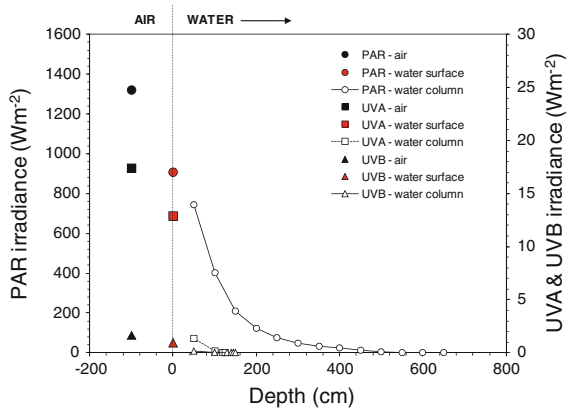

Fig. 2 Light profiling of the water column in Lake Beak-1. Photosynthetic active radiation reaches almost zero at ~5 m depth. UV radiation is undetectable below ~1 m depth

Sediment core extraction

Sediment cores were taken from near the deepest part of the lakes using both a UWITEC gravity corer and Livingstone piston corer. The sediment cores were described, photographed and sliced in the field at 0.5-cm intervals. The subsamples were stored in sterile Whirlpak® bags, frozen at −20 °C and kept in the dark until analysis. The sediment cores extracted from Lakes Beak-1 and Beak-2 were 165 and 245 cm long, respectively. The sediment in Lakes Beak-1 and Beak-2 consisted of a bottom zone (respectively, 95 and 89 cm) comprising marine muds and brackish lacustrine sediments, overlain by respectively, 70 and 156 cm of freshwater sediments composed of muds and/or decayed microbial mats and moss beds. The freshwater sediments in Lake Beak-1 and Lake Beak-2 were deposited after the lakes became isolated from the sea, 6,990 and 2,920 cal yr BP, respectively (Roberts et al. 2011; Sterken et al. 2012). This transition from marine to lacustrine sediments was determined using fossil diatoms and pigments (Roberts et al. 2011). In Beak-1, the sediment core section between 70 and 45 cm depth consisted of light greenish-grey to olive green, clay-rich laminated muds, whilst an olive green, fine organic mud was present between 45 and 30 cm (Fig. 3a). A unit of olive green, laminated fine organic mud is present between 28 and 5 cm and there were abundant moss shoots in the uppermost 5 cm of the core. Moss macrofossils were also present at 17.5–15 and 33–32 cm depth. In Beak-2, organic muds were present between 156 and 144 cm (Fig. 3b). The upper 144 cm consisted of an orange, decomposed microbial mat. No visible moss remains were present in the sediment core from Lake Beak-2.

In this study, we focused on the lacustrine sediments, because cyanobacteria are abundant in freshwater environments in Antarctica (Hodgson et al. 2004), whereas they are rare in the Southern Ocean (Wilmotte et al. 2002). The samples were selected to assess the effect of the main climate anomalies in this part of Antarctica (i.e. late Holocene climate optimum and the recent temperature rise) on the cyanobacterial community structure. We therefore focused on sediments that were deposited when the effect of lake isolation and related salinity changes were negligible, as reflected by the absence of saline and brackish water diatoms (Roberts et al. 2011).

Geochronology

Ages were obtained using ^{14}C AMS at the NERC Radiocarbon Laboratory and Beta Analytic Inc. (USA). Dates were calibrated using: (1) CALIBomb SH1, a compilation of Southern Hemisphere datasets (Hua and Barbetti 2004), from the absolute percentage of modern carbon (pMC) data corrected using $^{13}C/^{12}C$ isotopic ratios from measured pMC, or (2) Intcal04. 14C atmosphere (http://calib.qub.ac.uk/calib/calib.html). Age/depth curves were developed using CLAM software (Blaauw 2010). A full description of the methods used and chronology can be found in Roberts et al. (2011) and Sterken et al. (2012).

Microscopy

Seven layers from Lake Beak-1 (0, 1, 2.5, 6, 23.5, 24 and 46 cm depth) and eight layers from Lake Beak-2 (1, 5, 10, 60, 64, 132, 146 and 156 cm depth) were analyzed using a Leica DM LB2 microscope (Leica Microsystems GmbH, Wetzlar, Germany) coupled to a Deltapix Invenio Camera (Deltapix, Maalov, Denmark). Samples were screened for the presence of cyanobacterial remains.

Fossil pigments

Fossil pigments were extracted from bulk sediments following standard protocols (Wright et al. 1991). All samples were freeze-dried, followed by immediate pigment extraction by sonication (30 s at 40 W) in

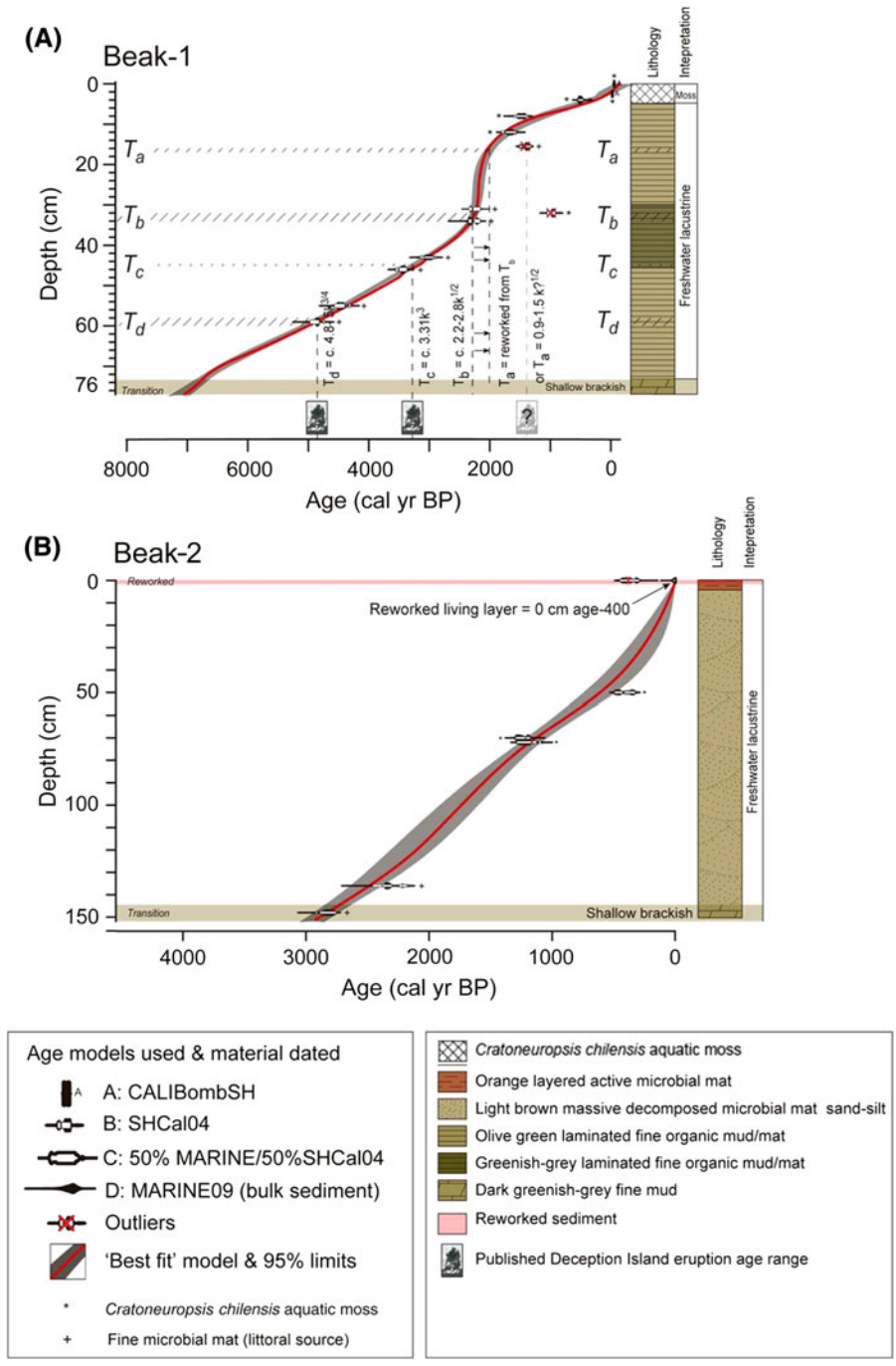

Fig. 3 Stratigraphic age-depth plot undertaken in CLAM (Blaauw 2010) and lithology of the Lake Beak-1 (**a**) and Lake Beak-2 (**b**) sediment cores. CLAM settings: smooth spline (smoothing 0.3); 1,000 iterations weighted by calibrated probabilities at 95 % confidence ranges and resolution 1-year steps

2–5 mL of high-performance liquid chromatography (HPLC)-grade acetone (90 %), and filtration of the extracts through a nylon filter (mesh size 0.20 µm) to remove fine particles. Pigments were separated and quantified using an Agilent Technologies 1100 series HPLC with an auto-sampler cooled to 4 °C, a diode array spectrophotometer (400–700 nm) and absorbance and fluorescence detectors. A reversed-phase Spherisorb ODS2 column was used (internal diameter: 4.6 mm, particle size of 5 µm) with a gradient of three solvents (Wright et al. 1991; Method A). The HPLC system was calibrated using authentic pigment

standards and compounds isolated from reference cultures following Scientific Committee on Oceanic Research (SCOR) protocols (Jeffrey et al. 1997). Pigments were identified based upon the shape of the absorption spectrum and the retention time. Compounds were considered as derivatives in cases for which (1) the absorption spectrum matched, but the retention time differed from that of the standards, or (2) the maxima in the absorption spectra were slightly offset, but the retention time was comparable with that of the pigment standard (Table S1). Retention times and absorption maxima of unknown pigments that were grouped as 'unknown carotenoids,' are given in Table S1. Retention times of unknown carotenoids and chlorophylls were sometimes similar, which caused problems with identifying both fractions. In those cases, the pigment concentration was calculated for the mixture (Table S1) and the compounds were grouped as 'chlorophyll/carotenoid mixture.' For pigment derivatives, the response factor of the native pigment was used. For unidentified carotenoids and chlorophylls, we applied a mean carotenoid and mean chlorophyll response factor, respectively. For the chlorophyll/carotenoid mixture, we used the averaged response factor of all chlorophylls and carotenoids. The total carotenoid concentration equalled the sum of all carotenoids, carotenoid-like pigments and all other compounds that had no affiliation with chlorophylls. The relative abundance of the individual carotenoids and unknown compounds was calculated as a percentage by dividing their concentration by the total carotenoid concentration. The total sum of chlorophylls equalled the sum of the concentrations of chlorophylls and their derivatives as well as the chlorophyll/carotenoid mixture. The relative abundance of the different chlorophylls was calculated by dividing the concentration of the individual compound by the total sum of chlorophylls. Total chlorophyll and carotenoid concentrations were expressed on a dry mass basis to enable direct comparison with other independent measures of lake primary production, such as loss-on-ignition and the total diatom concentration (Sterken et al. 2012). This approach can be used because the wet density is more or less constant in the lacustrine part of both sediment cores (Roberts et al. 2011). Hence, dilution effects from the input of allochthonous inorganic matter or inorganic precipitates, which potentially bias these ratios (Leavitt and Hodgson 2001), are negligible. The taxonomic affinities of the pigments are summarized in Table S2.

DNA extraction and amplification

Strict precautions were taken to prevent contamination. DNA extractions and pre- and post-PCR steps were carried out in separate rooms, with dedicated material. All the manipulations involving aDNA were completed in a laminar flow hood. Two layers of disposable gloves were used. The laminar flow hood and all instruments were disinfected with sodium hypochlorite solution, alcohol and RNase Away (MβP, San Diego, USA). All disposable material was sterilized by UV (20′) before use, as was the entire room (weekly). DNA extraction was done in sterile and DNA-free tubes (Eppendorf "Biopur"; Hamburg, Germany) and PCR reactions in sterilized "PCR clean" Eppendorf tubes (free of PCR inhibitors, human DNA and DNases). Moreover, a negative control consisted of water extracted in parallel. DNA was extracted using the FastDNA Spin Kit for Soil (QBiogene, USA), following the manufacturer's instructions.

Amplification of ~410 bp partial sequences of 16S rRNA genes for subsequent DGGE was performed using the two primer pairs 16S369F-16S781RGC(a) and 16S369F-16S781RGC(b). The PCR program was similar to that reported in Fernandez-Carazo et al. (2011) with the following modifications: 1.2 units of Super *Taq* Plus (HT Biotechnology, Cambridge, UK) and 3–4 μL of DNA were used per reaction. All the primers are listed in Taton et al. (2003) except 16S369F (GGCAGCAGTGGGGAATTTTC), which was designed and tested to improve the specificity of the cyanobacterial amplification. The T and C at positions 366 and 369 (*Escherichia coli* positions), respectively, are specific for cyanobacteria and different in many other bacteria. In primer 16S369F they are closer to the 3′ end of the primer, which enables better discrimination against bacteria (Kwok et al. 1990) than in primer CYA359F of Nübel et al. (1997).

DGGE and phylogenetic analysis

DGGE was run in 6.5 % acrylamide gels with a (40–65 %) denaturing gradient using a Dcode gene system (Bio-Rad), as described in Taton et al. (2003). DGGE gels were performed two times for most of the samples to assess reproducibility and detect potential contamination. When several bands from the same sample showed identical sequences, we selected the

longest one with the best quality to sequence in both directions using primers 16S378F and 16S781R. When several bands from different samples migrated at the same position, at least two bands were sequenced. The sequences from this study were deposited in Genbank, with the following accession numbers (JF748835 to JF748872).

Partial sequences (~ 410 bp) were used for BLAST analyses (18-05-11). A distance tree was constructed with the software package TREECON for Windows 1.3b (Van de Peer and De Wachter 1997), with an alignment of 441 positions (*E. coli* 359–800). The dissimilarity values were corrected for multiple substitutions by the model of Jukes and Cantor (1969) and were used to calculate a distance matrix. A distance tree was constructed by the Neighbor-joining method (Saitou and Nei 1987). Indels were not taken into account. A bootstrap analysis was performed that involved construction of 1,000 resampled trees. The tree combines the sequences obtained in this study, as well as their two most similar strain sequences and two uncultured sequences obtained using the option Seqmatch from RDPII (18-05-11) (http://rdp.cme.msu.edu) (Cole et al. 2009). To determine the similarity of our sequences with those of Coolen et al. (2008), a second distance tree, based on *Synechococcus* 16S rRNA sequences, was also constructed following the same methodology, but with the following exceptions: (1) *E. coli* positions (359–805) were used and (2) it included the five closest uncultured sequences, the five closest cultured sequences from RDPII (07-09-11) and the *Synechococcus* sequences from Coolen et al. (2008). The cyanobacterial sequences were grouped into operational taxonomic units (OTUs) with the software DOTUR, using the average-neighbor method (Schloss and Handelsman 2005). We define the OTUs as groups of sequences that exhibit more than 97.5 % similarity with each other, using *E. coli* positions 405–780, except in some cases where the sequences were slightly shorter (Taton et al. 2003).

Results

Geochronology

Calibrated radiocarbon dates in the Lake Beak-1 sediment cores were generally in stratigraphic order or overlapped at two-sigma error (Table 1; Fig. 3a). The lacustrine sediment unit analysed in this study dates from ca. 6,400 cal yr BP, with the top 2.5 cm most likely deposited since ca. AD 1980 (Sterken et al. 2012).

Top sediments of the Beak-2 core date to ca. 300–400 cal yr BP, which suggests that some mixing of the surface sediments has occurred in this shallow lake (Table 1; Fig. 3b). A reservoir effect is very unlikely because the lake water is derived from Lake Beak-1 (no reservoir effect), through an inflow stream. This, together with the fact the shallow lake is ice-free for several months per year, ensures that the isotopic composition of the lacustrine carbon pool is in equilibrium with atmospheric CO_2. Dates are in stratigraphic order (Table 1). More details regarding the geochronology can be found in Roberts et al. (2011) and Sterken et al. (2012).

Microscopy

Cell structures related to filamentous cyanobacteria were sparse, usually damaged and therefore difficult to identify. Thin filamentous structures from the order Oscillatoriales were observed in the surface layer and at 6 cm depth of the Beak-1 core (Fig. S1), but cells were compressed and deformed, impeding accurate identification. A third structure related to a filamentous cyanobacterial sheath from the order Oscillatoriales appears at 8 cm depth. At 46 cm depth, an empty sheath that could belong to cyanobacteria or green algae was observed.

Similarly, samples from the Beak-2 sediment core did not possess cyanobacterial structures conserved well enough to allow unambiguous identification. Filaments related to Cyanobacteria were observed at 1 cm depth (Fig. S1), but the cells were deformed and their size likely modified from their original morphologies. Different morphotypes, possibly related to the Oscillatoriales, were present at 5 and 64 cm depth, but they also lacked diacritical characters.

Fossil pigments

The number of cyanobacterial marker pigments is low and generally under the detection limit of our method in the Beak-1 core, with only zeaxanthin and a pigment with a retention time similar to that of the UV-screening compound scytonemin (listed as 'unknown compound') being present at specific depths (Fig. 4; Table S1). The majority of the carotenoids are

Table 1 Radiocarbon dates for the sediment cores from Lakes Beak-1 and Beak-2

Lab/Publication Code	Core ID & depth	Stratigraphic depth (cm)	Summary description	Carbon source	Marine / Lacustrine	Carbon content (wt %)	$\delta^{13}C_{VPDB}$ (‰)	pMC / ^{14}C enrichment (% modern ± 1σ)	Conventional radiocarbon age (years BP ± 1σ)	2σ calibrated age data (A=yr AD; B-D=cal yr BP) Min. - Max.	Rel. Prob.	Median prob.	Age Model
BEAK 1													
SUERC-12385	BK1E:0-1	0 - 1	Fine strands aquatic moss *Cratoneuropsis chilensis*	Macrofossil	Lacustrine	44.2	-32.4	108.6 ± 0.5	modern	>2004 AD or	1.00	>2004 AD	A
								107.9 ± 0.5*		1957 - 1958	0.00	1958 AD	A x
BETA-288864	BK1E:2.5	2.5 - 3	Fine strands aquatic moss *Cratoneuropsis chilensis*	Macrofossil	Lacustrine	26.8	-26.5	122.2 ± 0.5	modern	1982 - 1986	0.86	1984 AD	A
								122.5 ± 0.5*		1961 - 1962	0.11	1961 AD	
BETA-288865	BK1E:4	4 - 4.5	Fine strands aquatic moss *Cratoneuropsis chilensis*	Macrofossil	Lacustrine	43.0	-27.1*	94.1 ± 0.5	490 ± 40	451 - 545	0.98	505	B
BETA-288866	BK1E:8.5	8 - 8.5	Fine strands aquatic moss *Cratoneuropsis chilensis*	Macrofossil	Lacustrine	4.2	-25.4	81.4 ± 0.5	1650 ± 50	1368 - 1607	1.00	1477	B
BETA-288867	BK1E:12	12 - 12.5	Olive green fine organic mud/mat	Mat (TOC)	Lacustrine	2.8	-28.4*	79.8 ± 0.4	1810 ± 40	1557 - 1742	0.90	1663	B
										1753 - 1811	0.10		
SUERC-12394	BK1E:15.5	15.5 - 16	Greenish-grey laminated fine organic mud	Mat (TOC)	Lacustrine	1.4	-26	82.3 ± 0.4	1563 ± 35	1311 - 1424	0.78	1390	B #
										1457 - 1516	0.19		
Not analysed	BK1E:15.5	15.5 - 16	Very fine strands aquatic moss *Cratoneuropsis chilensis*	Macrofossil	Lacustrine	*Insufficient material for AMS dating; 2 attempts*							- x
SUERC-12395	BK1E:31	31 - 32	Olive green fine mud / light grey clay laminations	Mat (TOC)	Lacustrine	9.3	-29.8	75.1 ± 0.3	2305 ± 35	2153 - 2277	0.69	2235	B
										2289 - 2344	0.31		
SUERC-12386	BK1E:32-33	32 - 33	Fine strands aquatic moss *Cratoneuropsis chilensis*	Macrofossil	Lacustrine	30.0	-28.9	87.2 ± 0.4	1101 ± 35	914 - 1015	0.87	957	B #
										1023 - 1055	0.13		
SUERC-12396	BK1E:34	34 - 35	Consolidated medium-dark olive green mud/mat	Mat (TOC)	Lacustrine	11.4	-31.4	74.7 ± 0.3	2345 ± 35	2297 - 2355	0.54	2307	B
										2157 - 2265	0.46		
SUERC-12397	BK1E:43.5	43 - 44	Light grey-olive green fine organic mud/mat	Mat (TOC)	Lacustrine	6.7	-30.4	69.4 ± 0.3	2940 ± 35	2918 - 3160	0.95	3018	B
SUERC-12398	BK1E:46	46 - 47	Light grey-olive green fine organic mud/mat	Mat(TOC)	Lacustrine	4.0	-29.3	66.5 ± 0.3	3280 ± 35	3370 - 3511	0.89	3441	B
										3518 - 3557	0.11		
SUERC-12401	BK1E:55	55 - 56	Light grey-olive green fine organic mud/mat	Mat (TOC)	Lacustrine	4.1	-29.9	60.4 ± 0.3	4048 ± 35	4384 - 4571	0.96	4473	B
SUERC-12402	BK1E:59	59 - 60	Light grey-olive green fine organic mud/mat	Mat (TOC)	Lacustrine	3.0	-30.2	58.2 ± 0.3	4346 ± 35	4816 - 4972	0.97	4857	B
SUERC-12403	BK1E:73.5	73 - 74	Medium-light olive green fine organic mud/mat	Mat (TOC)	Transition end	9.6	-26.7	47.3 ± 0.2	6010 ± 36	6674 - 6888	1.00	6783	B
SUERC-12564	BK1D:8.5	73 - 74	Medium-light olive green fine organic mud/mat	Mat (TOC)	Transition end	8.7	-27.8	46.8 ± 0.2	6098 ± 35	6781 - 7001	0.99	6890	B
SUERC-12404	BK1E:78.5	78 - 79	Dark greenish-grey/black fine organic mud	Bulk (TOC)	Transition start	3.1	-22.4	43.2 ± 0.2	6735 ± 35	6797 - 7088	0.97	6944	C
SUERC-12565	BK1D:14.5	79 - 80	Dark greenish grey laminated organic mud	Bulk (TOC)	Transition start	1.2	-22.5	39.8 ± 0.2	7393 ± 36	7508 - 7672	1.00	7596	C
BEAK LAKE 2													
SUERC-12568	BK2A:0-1	0 - 1	Orange microbial mat (living) layer	Mat (TOC)	Lacustrine	6.8	-14.7	95.9 ± 0.4	339 ± 35	300 - 461	1.00	389	B
Not analysed	BK2A: 16-17	16 - 17	*Cratoneuropsis chilensis* 'leaves' & microbial mat	Macrofossil	Lacustrine	*Insufficient material for AMS dating; 2 attempts*							- x
SUERC-12387	BK2A:50-51	50 - 51	*Cratoneuropsis chilensis* 'leaves' & microbial mat	Macrofossil	Lacustrine	49.4	-21.6	95.1 ± 0.4	400 ± 35	324 - 415	0.52	410	B
										426 - 496	0.46		
SUERC-12388	BK2A:70-71	70 - 71	*Cratoneuropsis chilensis* moss in microbial mat	Macrofossil	Lacustrine	22.3	-19.5	84.2 ± 0.4	1377 ± 35	1178 - 1300	1.00	1256	B
SUERC-12391	BK2A:72-73	72 - 73	*Cratoneuropsis chilensis* moss in microbial mat	Macrofossil	Lacustrine	46.4	-25 (est)	84.8 ± 0.4	1329 ± 35	1166 - 1289	0.89	1217	B
										1124 - 1163	0.08		
SUERC-12569	BK2B:43-44	136 - 137	Laminated organic mat/mud	Mat (TOC)	Lacustrine	6.1	-16.9	74.4 ± 0.3	2374 ± 35	2301 - 2460	0.80	2338	B
										2178 - 2244	0.19		
SUERC-12946	BK2B:55-56	148 - 149	Dark-light olive green mat	Mat (TOC)	Transition end	3.8	-16.1	70.8 ± 0.3	2780 ± 35	2753 - 2888	0.97	2821	B
SUERC-12570	BK2B:66-67	159 - 160	Black fine organic mud	Bulk (TOC)	Transition start	1.7	-17.2	63.6 ± 0.3	3632 ± 35	3055 - 3335	1.00	3191	C

Data correspond to Roberts et al. (2011); * absolute pMC (percent modern carbon); highest probability age data shown for clarity. All calibrated age ranges are available on request. Calibration models: A = Samples were calibrated using CALIBomb SH1, a compilation of Southern Hemisphere datasets (Hua and Barbetti 2004), from absolute percentage of modern carbon (pMC) data, corrected according to $^{13}C/^{12}C$ isotopic ratios from measured pMC, and where 'modern' = 1950 and 100 pMC and present day (2010) pMC value = 107.5; B = Intcal04.14C atmosphere (http://calib.qub.ac.uk/calib/calib.html); $B^{\#}$ = calibrated using INTCAL09 (Reimer et al. 2009), which produces the same calibration ages as Intcal04.14C; C = Intcal04.14C 50 % atmosphere/50 % marine mixed model; x indicates age not used in age-depth model

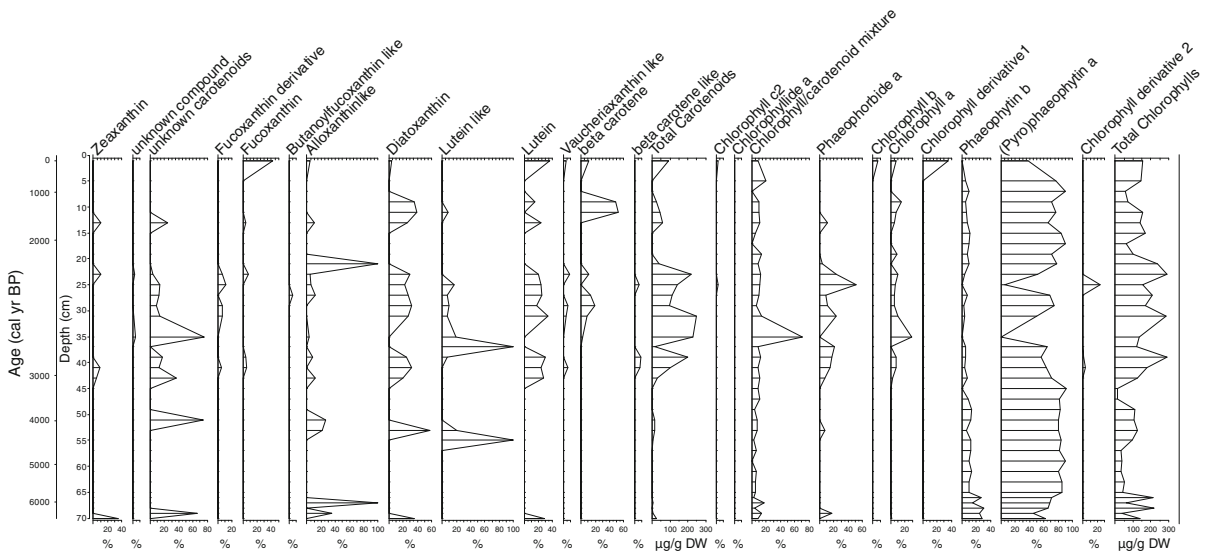

Fig. 4 Pigment stratigraphy from the Beak-1 sediment core showing the general absence of cyanobacterial marker pigments, except zeaxanthin. The retention times and absorption maxima of the pigment derivatives and the mixtures of unidentified pigments can be found in Table S1

derived from diatoms, cryptophytes and green algae (Table S2). The total carotenoid concentration is relatively high between 41 and 23 cm (ca. 2,845–2,160 cal yr BP) and the total chlorophyll concentration similarly peaks between 44 and 20 cm (ca. 3,170 and 2,120 cal yr BP).

In the Beak-2 core, the cyanobacterial pigment composition is relatively diverse and composed of myxoxanthophyll, echinenone, a carotenoid with an absorbance spectrum comparable to that of canthaxanthin, and a mixed peak that contains zeaxanthin and lutein (Fig. 5). Myxoxanthophyll appears from 100 cm depth onwards, whereas zeaxanthin was detected in all but one sample. A compound with a similar retention time to that of scytonemin (listed as 'unknown compound') was also present. The remaining carotenoids are derived from diatoms and green algae. The total carotenoid concentration and chlorophyll concentration is relatively high between 151 and 101 cm, which corresponds to the period between ca. 2,920 and 1,750 cal yr BP.

Ancient DNA PCR amplification, DGGE and distance tree analysis

PCRs with primers 16S369F-781RGC (a&b) enabled aDNA amplification at all depths. Contamination controls were always negative.

In total, 17 cyanobacterial representative sequences were selected from the DGGE gels and subsequently used to construct a distance tree (Fig. 6). Representative sequences were sequenced in both directions to obtain approximately 370-bp-long sequences, with the exception of four DGGE bands that were sequenced in only one direction (Table 2). All the sequences were submitted to a BLAST analysis to find their most related sequences and they were 98.3–100 % similar to sequences from modern microorganisms (indels and ambiguous bases were not taken into account) (Table 2). The cyanobacterial sequences were distributed into six OTUs (Fig. 6) that were related to sequences already found in other locations outside Antarctica and therefore considered to have a ubiquitous distribution (Table S3). The cyanobacterial OTUs belonged to the orders Oscillatoriales (16ST44), Nostocales (16ST33, 16ST34, 16ST101 and 16ST102) and Chroococcales (16ST58). The analysis of the band classes is summarized in Table 3.

In Lake Beak-1, the uppermost 2.5 cm (BK1–0 to BK1–2.5) (past 2–3 decades) are characterized by the presence of sequences from Nostocales (OTU 16ST101 and 102, *Anabaena/Nostoc*), Oscillatoriales (OTU 16ST44, *Phormidium autumnale*) and unicellular cyanobacteria related to *Synechococcus* (16ST58). Interestingly, only OTU 16ST58 is present in sediments exceeding 2.5 cm depth. This OTU (16ST58) is not related to any previously recorded Antarctic

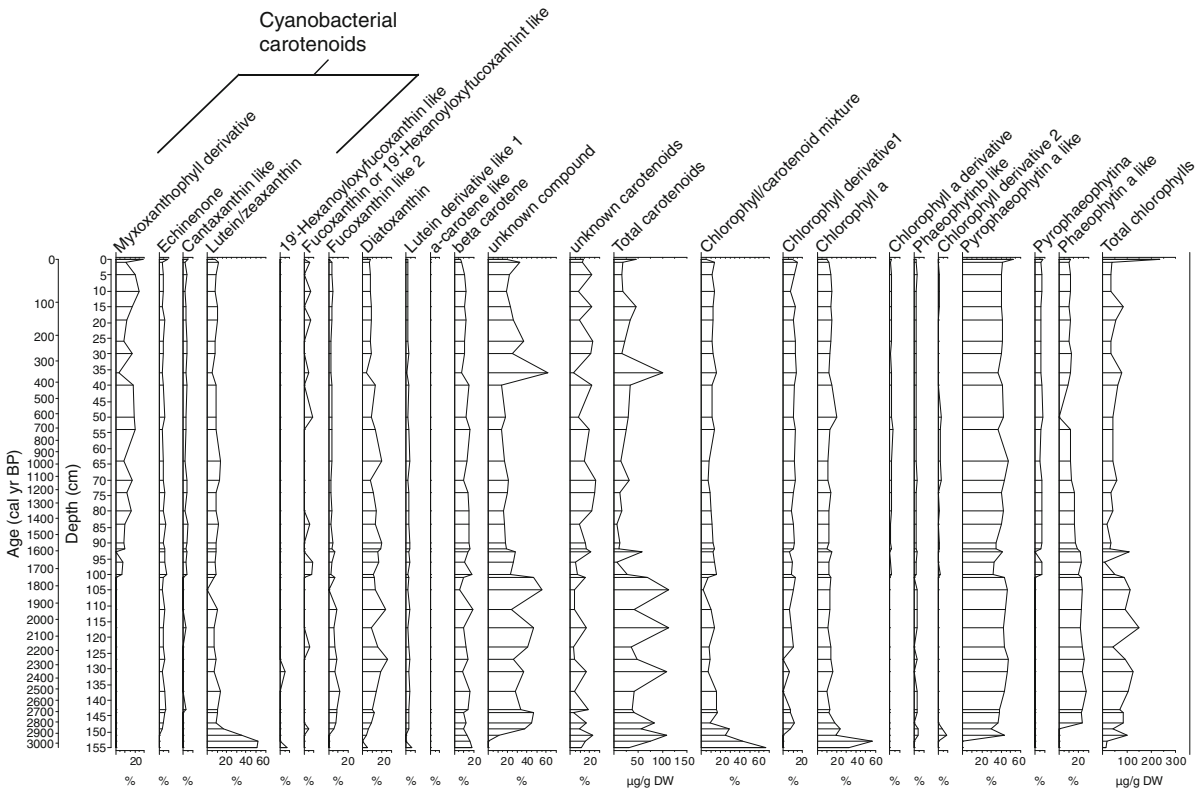

Fig. 5 Pigment stratigraphy from the Beak-2 sediment core. The retention times and absorption maxima of the pigment derivatives and the mixtures of unidentified pigments can be found in Table S1

Synechococcus sequences (Fig. S2), but rather to *Synechococcus* sp. LS0535, isolated from Lake Superior (USA) or to *Synechococcus* sp. PCC 7009, isolated from a low-salinity brine pond in California (USA).

The cyanobacterial diversity was higher in Lake Beak-2. Two OTUs belonging to Nostocales (16ST33, *Nostoc* sp. and 16ST102, *Nostoc* sp.) were recorded in all the samples analyzed, whereas one Nostocales OTU (16ST34, *Anabaena* sp.) and one Oscillatoriales OTU (16ST44, *P. autumnale*) were detected only at 5-cm (16ST34, 16ST44) and 10-cm (16ST44) depth (Table 3). The OTU (16ST58) related to *Synechococcus* sp. was detected throughout the entire core, similar to Lake Beak-1.

Discussion

Microscopic observations of the sediment core layers did not yield recognizable cyanobacterial structures. The lack of a protective layer around the cell wall, as in, for example, pollen and some green algae (Kodner et al. 2009; Bennett and Willis 2001), likely makes cyanobacterial cells and structures highly vulnerable to bacteria-mediated degradation in lake sediments (Räsänen et al. 2006). In general, the preservation of cyanobacterial cells is highest under fast crystallization, rapid exclusion of water, and stable anoxic conditions, which slow down enzymatic and bacterial degradation of the ultra-structures, or by carbonaceous compression and cellular replacement by precipitated minerals (Hebsgaard et al. 2005; Tomescu et al. 2009). If these conditions are met, cyanobacterial cells can be preserved in a range of environments, including evaporites (Panieri et al. 2010), siltstone (Tomescu et al. 2009) or peritidal areas (Golubic et al. 2006).

In contrast to the microfossil evidence, fossil pigments allowed detection of cyanobacteria during the late Holocene in both lakes. As with microfossils, however, the absence of marker pigments does not rule out the presence of cyanobacteria because pigments are susceptible to degradation (Leavitt and Hodgson

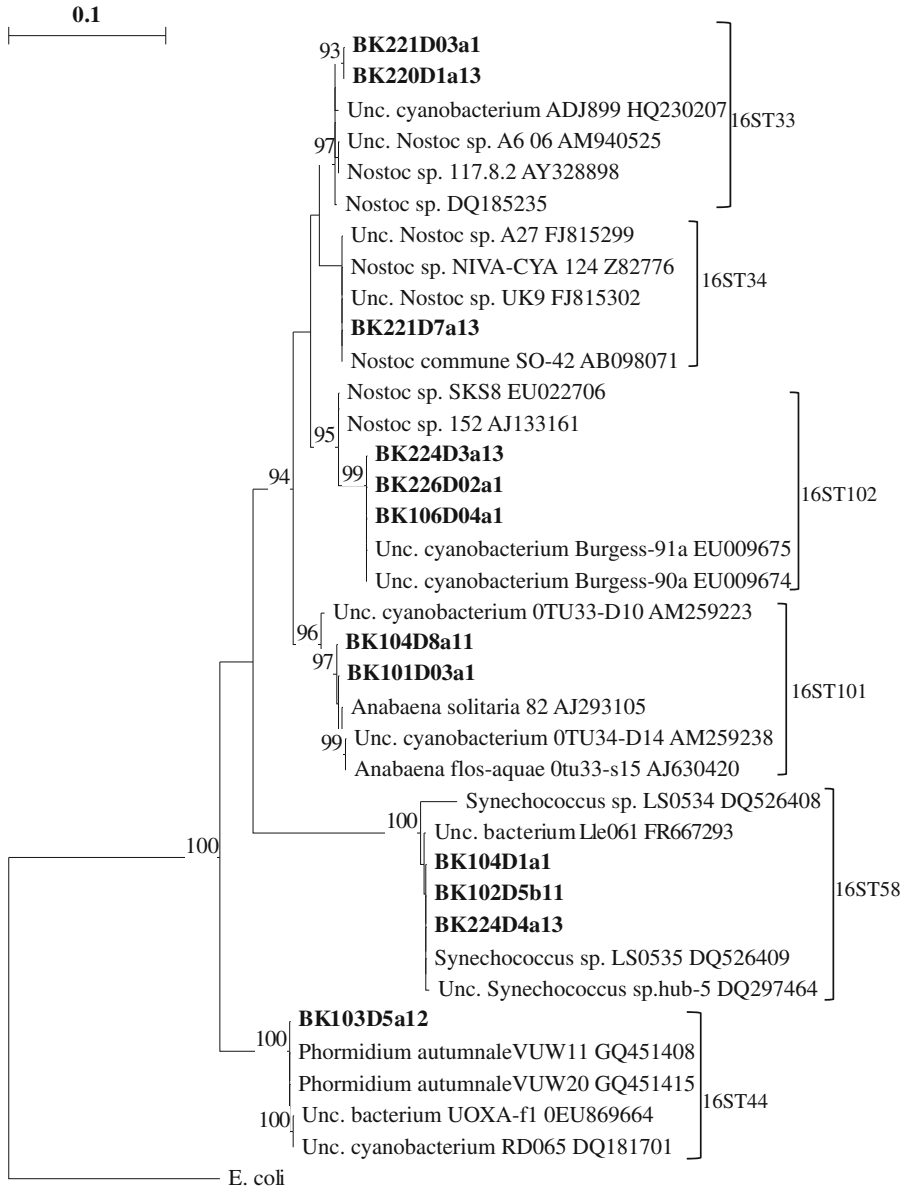

Fig. 6 Distance tree based on cyanobacterial 16S rRNA sequences (*E. coli* positions 359–800) constructed by the Neighbor-joining method (Saitou and Nei 1987) using TREECON (Van de Peer and De Wachter 1997). A bootstrap analysis was performed that involved construction of 1,000 resampled trees (values indicated at the *node*). The branches with less than 85 % bootstrap are shown as unresolved. Beak Island sequences are in *bold* type

2001). Similarly, a pigment-based estimation of the relative abundance of different algal groups might be biased as a result of high variability in the degree of pigment preservation between different compounds (Verleyen et al. 2004). Hence, we cautiously link the Beak-1 pigment stratigraphy to the presence of diatoms, green algae/mosses and cryptophytes during the past 6,400 cal yr. This is in agreement with the present-day benthic flora, which is dominated by mosses and diatoms (Sterken et al. 2012). In contrast, the pigment composition in the Beak-2 core contained a relatively large amount of cyanobacterial marker pigments, which indicate a well established, benthic cyanobacterial flora during the past ca. 3,200 years. This also agrees well with the present-day situation, as surface sediments of Lake Beak-2 were covered by

Table 2 Analysis of the cyanobacterial sequences

Representative sequences	Most closely related GenBank uncultured sequence (% similarity)	Most closely related GenBank strain (% similarity)	OTU
BK220D1a13, **BK221D03a1**, BK226D1a1	Uncultured *Nostoc* sp. (AM940525) 99.15 %	*Nostoc commune* (AB251860) 99.15 %	16ST33[f]
BK221D7a13	Uncultured *Nostoc* sp. clone UK9 (FJ815302) 99.6 %	*Nostoc commune* CYN52 (EU586722) 99.6 %	16ST34[f]
BK103D5a12, BK103D6a12	Clone UOXA-f10 (EU869664) 100 %	*P. autumnale* VUW8 (GQ451424) 100 %	16ST44[f]
BK102D5b11, BK115D6b11, **BK104D1a1**, BK104D5b11, BK117D2a12, **BK224D4a13**	Uncultured *Synechococcus* sp. hub-5 (DQ297464) 100 %	*Synechococcus* sp. LS0535 (DQ526409) 100 %	16ST58[u]
BK101D03a1, BK104D8a11	Clone 0TU27CN45 (AM259248) 99.6 %	*Anabaena solitaria* (AJ293105) 99.4 %	16ST101[f]
BK106D04a1, BK224D3a13, BK226D02a1	Clone H04 (FJ59286) 98.9 %	*Nostoc* sp. SK8 (EU022706) 98.9 %	16ST102[f]

Sequences are grouped in OTUs. Names in bold correspond to DGGE bands sequenced in both directions. [f] indicates the OTU is related to filamentous cyanobacteria. [u] indicates the OTU is related to unicellular cyanobacteria

living microbial mats dominated by cyanobacteria. Based on the molecular evidence, the cyanobacterial community structure also differed between the two lakes. Three OTUs (16ST44, 16ST58 and 16ST102) were found in both lakes, whereas OTU 16ST101 was detected only in Lake Beak-1 and OTUs 16ST33 and 16ST34 were detected only in Lake Beak-2. In general, heterocystous cyanobacteria of the order Nostocales were more diverse in shallower Lake Beak-2 (four OTUs instead of two in Beak-1). These differences in community structure of cyanobacteria between the two lakes were likely related to differences in lake water depth, which has a profound influence on the light climate, which in turn is one of the major factors influencing the structure, diversity and formation of microbial mat communities in Antarctic lakes (Hodgson et al. 2004; Verleyen et al. 2005, 2010). Many benthic cyanobacteria are adapted to high light levels, compared to, for instance, diatoms and mosses. Wharton et al. (1983) showed a shift from cyanobacterial to diatom communities with increasing depth in the lakes of the McMurdo Dry Valleys. Moreover, Antarctic cyanobacteria are known to produce high amounts of screening pigments that protect them against high and sometimes damaging light levels (Hodgson et al. 2004), which makes them well-adapted to thrive in shallow-water conditions. Mosses can grow at water depths exceeding 80 m in East Antarctic lakes (Wagner and Seppelt 2006). This could explain the dominance of pigments related to mosses and diatoms, and the low abundance of cyanobacteria and cyanobacterial marker pigments in Lake Beak-1, which experiences low amounts of light at its bottom (Fig. 2). By contrast, Lake Beak-2 is dominated by cyanobacterial marker pigments and a relatively large diversity of sequences related to heterocystous cyanobacteria, which is likely related to the shallow nature of the lake.

Amplification of short, ~410-bp fragments was successful for both lakes, but larger fragments (~1,700 bp) could not be amplified (data not shown). This is in agreement with previous studies, which showed that the fragment size of aDNA is relatively short (Willerslev and Cooper 2005). The conditions enabling preservation of aDNA were, apparently, relatively good in both lakes. Even in Lake Beak-1, where cyanobacteria constitute only a minor component of the photoautotrophic communities, as suggested by fossil pigment analysis, cyanobacterial DNA was detected in sediments as old as ~3,000 years. DNA degradation in fossil cells generally starts immediately after cell death, and is the result of several reactions, such as spontaneous alkylation, hydrolysis or oxidation (Willerslev and Cooper 2005). Enzymes released by dead cells can completely degrade the DNA and this decay increases with time. It is very likely that the relatively low temperatures, darkness, and low oxygen concentrations in the sediments resulted in relatively good preservation conditions. These factors, together with low liquid water availability and high salt concentrations have been shown to

Table 3 Analysis of the DGGE band classes from Lakes Beak-1 and Beak-2

		Depth	OTU						Age*
			16ST33	16ST34	16ST44	16ST58	16ST101	16ST102	
Sample	BK1	BK1-0				■	■		modern
		BK1-0.5				■	■		
		BK1-1			■	■	■		
		BK1-1.5				■	■		ca. 1980 AD - present day
		BK1-2				■	■		
		BK1-2.5				■		■	
		BK1-5.5				■			540-960
		BK1-6				■			
		BK1-6.5				■			
		BK1-7.5				■			
		BK1-8				■			1150
		BK1-27				■			2200
		BK1-38				■			2600
		BK1-42				■			3000
	BK2	BK2-1	■			■		■	2
		BK2-5	■	■	■	■		■	30
		BK2-10	■		■	■		■	70
		BK2-60	■			■		■	870
		BK2-64	■			■		■	970
		BK2-132	■			■		■	2400
		BK2-146	■			■		■	2800
		BK2-156	■			■		■	3050

Shading indicates the presence of an OTU in the sample. * Estimated ages (cal yr BP) based on the stratigraphic age-depth plot (Fig. 3)

slow the degradation of DNA (Willerslev and Cooper 2005). Our results, however, also indicate that the preservation potential of aDNA from cyanobacteria might be genus- or species-specific. Nostocales-related OTUs have been mainly found in the upper layers of the Beak-2 sediment core (16ST34), but in particular,

also in the Beak-2 deeper layers (16ST33 and 16ST102) (Table 3). Their better preservation might be related to the formation of resistant cells (akinetes), although we did not detect akinetes by microscopy. Akinetes have thicker cell walls, contain storage products for long-term survival, and are viable for at least 120 years (Wood et al. 2008). Other filamentous cyanobacteria related to the Oscillatoriales, which do not produce akinetes and which are usually the major mat-forming organisms in these environments, were only found in the upper layers of both lakes, suggesting rapid degradation over time. The only OTU present in all the studied core depths was related to unicellular *Synechococcus* spp. (16ST58). This is in agreement with other studies (Coolen et al. 2008; Savichtcheva et al. 2011) in which *Synechococcus* sequences were detected along the entire sediment cores. The members of this OTU thrive in various habitats, including marine environments (Baltic Sea, AF330254) (97.8 % similarity) and freshwater lakes (100 % similarity) (Lake Superior, USA; DQ526409) (Fig. S2). Other sequences assigned to this genus were detected in Antarctic lakes and sediment cores (Vincent et al. 2000; Powell et al. 2005; Coolen et al. 2008; Verleyen et al. 2010), but the sequences from Beak Island were different from those observed previously (Fig. S2). Coolen et al. (2008) found different *Synechoccocus*-related sequences in all sediment core depths in Ace Lake (Vestfold Hills). They represented the only cyanobacterial aDNA detected, though earlier studies showed that other filamentous cyanobacteria, such as *Phormidium murrayi*, *P. priestleyi*, *P. pseudopriestleyi*, *Nodularia* cf. *harveyana*, *Geitlerinema deflexum* and *Leptolyngbya antarctica* are present in the contemporary benthic microbial mats of this lake (Taton et al. 2006a, b). Together with the fact that the limnological conditions remained relatively stable during the past 2,760 year in Ace Lake (Coolen et al. 2008), this might point to the preferential preservation of the DNA of *Synechococcus* spp. The small cell size of this taxon possibly results in higher DNA concentration in a reduced physical space, or better protection from degradation.

The taxonomic composition of the cyanobacterial communities remained more or less constant, with no apparent big changes in species composition, throughout the period of increased total carotenoid and chlorophyll concentrations in both lakes (between 3,170 and 2,120 cal yr BP). This period likely corresponds with the late Holocene climate optimum recorded at the north of the Antarctic Peninsula (Hodgson et al. 2005; Bentley et al. 2009; Sterken et al. 2012). This climate optimum is reflected in multiple indicators of primary production in the Beak-1 sediment core between ca. 3,170 and 2,120 cal yr BP (Sterken et al. 2012). These indicators include increased concentrations of diatoms, organic carbon content and chlorophyll *a*, proxies traditionally used in paleolimnological studies in Antarctica (Verleyen et al. 2012). The lack of turnover in cyanobacterial communities contrasts with the diatoms in the Beak-1 core, which showed changes in species relative abundance and diversity (Sterken et al. 2012). The relative insensitivity of cyanobacterial species might be accounted for by three mutually non-exclusive factors. First, it might be related to the broad ecological niches of cyanobacteria (Whitton and Potts 2000), which would result in changes in productivity, but not in community composition. Second, the relatively small changes in cyanobacterial community structure might be related to the taxonomic marker used in our analysis; 16S rRNA is a relatively conserved gene and might not be ideally suited to delineate cyanobacteria at a finer taxonomic scale and hence preclude observation of more subtle changes in community structure (Cho and Tiedje 2000). Third, taxon-dependent differences in the preservation potential of aDNA might result in the selective degradation of particular sequences, which in turn affects the taxonomic turnover in the core.

In the uppermost sediments of Beak-1, distinct changes in cyanobacterial community structure were present and coincided with a high turnover in the diatom communities, associated with a rise in tychoplanktonic species (Sterken et al. 2012). The increasing OTU richness of heterocystous, nitrogen-fixing cyanobacteria might point to an increased light penetration in the lake, possibly as a result of a decrease in internal nutrient loading as a consequence of the colonization of the lake bottom by mosses. In addition, the ratio between total dissolved nitrogen and PO_4-P concentration equals 8.2, which is below the Redfield ratio (16), suggesting the possibility of nitrogen limitation, which could favor the growth of nitrogen-fixing cyanobacteria. Alternatively, the appearance of heterocystous cyanobacteria in the uppermost sediments could be related to the better preservation of more recent aDNA. Whereas these changes might reflect the first ecological responses of the lakes to the recent period of rapid warming, they might also be related to natural lake succession processes. For example, aquatic moss communities

become abundant during a period that precedes anthropogenic-induced climate warming and slightly precedes the shifts in the diatom communities. Hence, it remains uncertain whether these changes are part of the natural lake succession or are related to climate warming.

Conclusions

Light microscopy was of limited use in identifying past cyanobacterial species assemblages in freshwater Antarctic lakes, similar to most freshwater systems elsewhere. By contrast, HPLC of sedimentary pigments enabled us to reconstruct tentatively the abundance of cyanobacteria relative to other autotrophs. Differential pigment preservation might, however, bias this estimate. DGGE of aDNA allowed the identification of cyanobacterial OTUs and an assessment of their presence and absence through time. Our data showed that cyanobacterial species assemblages remained relatively stable during and after the late Holocene climate optimum. By contrast, cyanobacterial diversity increased in the most recent sections of both cores, possibly related to recent climate warming and subsequent reorganization of lake communities and/or the better preservation of more recent aDNA. We conclude that fossil DNA can be a powerful tool to reconstruct past changes in cyanobacterial community structure in Antarctic lakes. In order to utilize the full potential of aDNA as a paleolimnological proxy, however, we need to better understand the preservation potential of DNA in lake sediments and the taxon-dependent differences in degradation of DNA.

Supplementary material

ESM Fig. 1 (1) Retention time of the pigment derivatives; (2) taxonomic affinity of the different pigments; (3) geographical location of the sequences from the OTUs detected in this study; (4) cyanobacteria-related structures observed by light microscopy; (5) *Synechococcus* sp. distance tree, indicating the position of the *Synechococcus* sequences from this study related to other Antarctic and non-Antarctic *Synechococcus* sequences.

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