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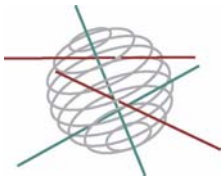

Part 2:
Global change, Ecosystems and Biodiversity

FINAL REPORT

LATE QUATERNARY CLIMATE HISTORY OF COASTAL ANTARCTIC ENVIRONMENTS: A MULTI-PROXY APPROACH (LAQUAN)

EV/01

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Januari 2007

BELGIAN SCIENCE POLICY

D/2007/1191/9
Published in 2007 by the Belgian Science Policy
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ABSTRACT

Novel biological proxies and inference models were developed to reconstruct past environmental changes in Antarctic ice-free regions. Reference datasets of cyanobacterial sequences, diatoms and pigments were constructed in order to study the present diversity and distribution of biota in benthic microbial mats from Antarctic lakes. These datasets were subsequently used for comparison between living and fossil floras or to develop inference models to quantitatively reconstruct past environmental changes in East Antarctica. Paleolimnological analyses and application of the models revealed the history of late Quaternary variation in climate, ultraviolet (UV) radiation, and relative sea-level in the Larsemann Hills.

The study of modern cyanobacterial diversity showed that each lake is quite unique in terms of diversity. Every single lake studied resulted in the discovery of new Operational Taxonomic Units (OTUs), which suggests that there is a lot more diversity to discover. The majority of the genotypes are restricted to Antarctica and sometimes, even present only in one sample, which hints to the existence of endemic cyanobacteria. A taxonomic inventory of the diatom flora from the Larsemann Hills similarly revealed that Antarctic endemics account for about 40 % of all freshwater and brackish taxa.

Fossil cyanobacterial sequences were found in layers of up to 9000 years old. The validation of fossil sequences of Progress and Heart Lake cores by both laboratories allowed us to assess that a majority of cyanobacterial sequences found in sedimentary core layers were really from fossil organisms. Comparison between the modern and fossil diversity revealed that most fossil sequences were also present in modern samples. The main problems encountered were related to the presence of good-quality bacterial DNA that act as competitor of fossil DNA during PCR, downcore degradation of fossil DNA, and the selective, group-specific resistance of cyanobacterial DNA to degradation.

The main paleoenvironment-related results can be summarized as follows. During the Last Glacial Maximum one of the main peninsulas in the Larsemann Hills was only partly glaciated, as evidenced by uniquely long lake sedimentary records extending into the previous interglacial period (Eemian). Diatom-based inference models revealed that this interglacial was probably warmer and wetter than the Holocene, which was further supported by the presence of currently sub-Antarctic endemics in the Eemian diatom flora. The last glacial period was characterized by dry and cold conditions prevailing over the Larsemann Hills. The levels of the cyanobacterial UV-screening compound scytonemin in fossil microbial communities from this period were three times higher than the present-day values. Shortly after 13,500 yr BP, deglaciation of the Larsemann Hills and the continental shelf in Prydz Bay intensified. The collapse of this part of the East Antarctic Ice Sheet (EAIS) thus

coincided and may have contributed to melting water pulse 1A, which was one of the most rapid sea-level rises since the end of the last glacial period. During the Holocene, several warm periods were detected, coinciding with more productive coastal waters. Marine sediments in isolation basins from these periods are characterized by open water taxa and high chlorophyll a concentrations. Based on a relative sea level curve, we inferred that during the last warm period (the Hypsithermal) increased moisture supply to this part of the EAIS might have contributed to the global sea level fall between 4000 and 2500 yr BP. The high sediment accumulation rate in the isolation lakes further enabled us to identify several dry episodes and periods of higher UV radiation penetration during the past 2000 years.

Together, our results highlight the potential of coastal Antarctic lakes for the reconstruction of past environmental changes and underscore the need for continued studies of lacustrine sediment sequences from this climate sensitive region.

KEY-WORDS: *Paleolimnology, Antarctica, fossil DNA, diatoms, cyanobacteria, fossil pigments, climate change, ice sheet volume, ultraviolet radiation, sea-ice extent, moisture balance, deglaciation, Eemian, Last Glacial Maximum, Holocene*

INTRODUCTION

The study of past environments and climates is essential to obtain a comprehensive understanding of the influence of climate changes on ecosystems and how the various components of the climate system interact to produce these anomalies. As the instrumental record only spans a short period of time, proxy records in natural stratigraphic archives (e.g., ice sheets, marine and lake sediments, speleotherms, loess) are being used to reconstruct spatio-temporal patterns of naturally occurring past climate changes. These reconstructions are extremely important, as they are needed to test and calibrate the climate models that are used to forecast future conditions, and to inform policy-making (e.g., Weaver et al. 2003; Mix 2003). As local climates are diverse, it is essential to establish a global network of accurate, trustworthy climate reconstructions (Mix 2003). In particular, there is an urgent need for high-resolution records from various latitudes especially in the Southern Hemisphere. These are currently lacking, in contrast to the intensively studied Northern Hemisphere (Stocker 2003). The Southern Hemisphere, and Antarctica in particular, are however key sites for present and past global climate (e.g., Weaver et al. 2003; Knorr & Lohmann 2003). Antarctica derives its importance to the climate system from its high reflectivity (albedo) for solar radiation, its low thermal conductivity, its large thermal inertia and, especially, its critical role in driving deep ocean water circulation. Furthermore, because the ice sheets store a large amount of water, variations in their volume are a potential source of sea level variations (IPCC 2001). In addition, some of the recently observed climate changes are amplified at the Poles (e.g., temperature rise, Vaughan et al. 2003) or even restricted to these regions, like ice shelf collapse (e.g., Doake et al. 1998; De Angelis and Skvarca 2003) and the "ozone holes" (Farman et al. 1985). As a result, ecosystems of these remote regions are the "canaries in the coalmine" and provide a "natural experiment" with simple community structures (Robinson et al. 2003), with which we can reveal biological responses (at scales between cell biochemistry and whole ecosystem) to recent climate change, including temperature rise, change in precipitation-evaporation and radiation (Convey et al. 2002).

Paleo-climate and -environmental studies in Antarctica have to date been largely focused on geomorphological evidence and the stratigraphic records in ice cores (e.g., Petit et al. 1999; Masson et al. 2000; EPICA community members 2004), marine sediment cores (e.g., Taylor & McMinn 2002; Taylor et al. 2001; Cremer et al. 2003, Hodgson et al. 2003) and lacustrine sediment cores from currently ice-free regions (e.g., Roberts & McMinn 1998; Roberts et al. 2001). The latter are also critical for evaluating the impact of past climate changes (as detected in ice cores) on terrestrial and lacustrine environments, (e.g., Bomblies et al. 2001; Quayle et al. 2002). Although paleo-environmental and -climatic research has experienced an

explosive growth over the past decennia in Antarctica and continues to enjoy rapid progress, some major questions still need to be answered.

First, little is known on how long-term climate changes detected in ice cores (e.g., Petit et al. 1999; EPICA members 2004) have affected the terrestrial ecosystems, as most Antarctic oases have only become ice-free since the beginning of the present interglacial. However, the recent discovery that currently ice-free oases escaped the last glaciation (Gore et al. 2001) gives us the opportunity to study the past environments of the preceding glacial period and the previous interglacial. Second, although the political interest in the so-called "ozone hole" is high (Solomon 2004) and a vast number of studies are dealing with how organisms are affected by the higher UVR fluxes as a result of the human induced ozone depletion, little is known about the historical evolution of UVR (Rozema et al. 2002). What is known comes mainly from historical records (based on observations of sunspot activity as there is a direct link between solar activity and ozone column depth, Reid 1999), and since the 1950's, from direct measurements. There is thus an urgent need to establish a context of natural variability against which the biological significance of elevated UVR irradiances, resulting from the recent ozone holes, can be determined. A detailed knowledge of the extent, thickness and dynamics of the continental ice sheet covering the glaciated areas is also lacking (Bentley 1999). The Antarctic Ice Sheet is currently the biggest wildcard in the calculation of the post-glacial sea level rise of c. 120m (Peltier 2002).

The project LAQUAN addressed several of these issues. We used diatoms, pigments, molecular markers, and non-biological proxies in lake-sediment cores to reconstruct the paleolimnological history of lakes in the Larsemann Hills (east Antarctica). The specific aims of the project are (1) the development of (new) proxies enabling the reconstruction of past environmental changes, (2) the evaluation of these proxies (3) a proxy reconstruction of local climate change, (4) the reconstruction of past UVR fluxes in the region, and (5) the reconstruction of the relative sea history for the region.

GENERAL DESCRIPTION OF THE STUDY AREAS

Different lakes in different ice-free areas were investigated within the framework of the LAQUAN project, namely the Larsemann Hills, the Vestfold Hills, the Rauer Islands, the Bølingen Islands, the Windmill Islands, the McMurdo Dry Valleys, and Byers Peninsula.

The Larsemann Hills (LH)

The majority of the paleolimnological research was focused on the Larsemann Hills (LH, 69°23' S, 76°53' E) in Prydz Bay, a 50 km^2 large ice-free area on the Ingrid Christensen Coast, Princess Elizabeth Land, located approximately midway between the eastern extremity of the Amery Ice Shelf and the southern boundary of the Vestfold Hills. The region consists of two main peninsulas (Stornes and Broknes), together with a number of scattered offshore islands. More than 150 freshwater lakes are found in the hills (Gillieson et al. 1991) ranging from small ephemeral ponds to large water bodies. Five lakes were the subject of a detailed paleolimnological analysis using our different proxies, namely Heart L., Pup Lagoon, Kirisjes Pond, L. Reid and Progress L.

The Vestfold Hills (VH)

The Vestfold Hills (VH, 68°30' S, 78°00' E) form a 400 km^2 ice-free area on the Prydz Bay coast, consisting of three main peninsulas (Mule, Broad and Long Peninsula) and a number of offshore islands. Water chemistry, morphometry and diatom composition from 33 lakes out of the approximately 300 lakes in the region are described in Roberts and McMinn (1996). Typically, the majority of the lakes in this dataset are deep, saline, meromictic and large. Detailed descriptions of the physical and biological characteristics of many of the lakes (e.g., Ace L.) in the Vestfold Hills have been published (e.g., Laybourn-Parry 2003). The modern cyanobacterial diversity was studied for one sample of Ace L.

The Rauer Islands (RI) and Bølingen Islands (BI)

The Rauer Islands (RI, 68°45' S - 68°55' S and 77°30' E - 78°00' E) and Bølingen Islands (BI, 69°23' S – 75°50' E) are ice-free coastal archipelagos in Prydz Bay. The Rauer group includes 10 major islands and promontories together with numerous minor islands covering a total area of some 300 km^2 and is situated approximately 30 km away from the Vestfold Hills. A detailed description of the region and of the microbial communities inhabiting 10 out of more than 50 shallow lakes and ponds are given in Hodgson et al. (2001a). The Bølingen Islands (BI, 69°30'S – 75°50'E) are situated approximately 10 km away from the Larsemann Hills and north of the Publications Ice Shelf. Three lakes were the subject of a detailed inventory of the

cyanobacterial diversity using molecular tools, namely Firelight L. (BI), L. Rauer 2 and L. Rauer 8 (RI, see Hodgson et al. 2001a, for a detailed description of the physical environment of the latter two).

The Windmill Islands (WI)

The Windmill Islands (WI, 66°20' S- 110° 30 E) are a group of ice-free islands and peninsulas on the East Antarctic coast. An analysis of the limnology and diatom composition of 14 saline to freshwater lakes and ponds from Beall, Holl, Peterson and Warrington Islands and Browning Peninsula is given in Roberts et al. (2001b). Geology and glaciology of the region is described by Goodwin (1993) and Paul et al. (1995).

The McMurdo Dry Valleys (DV)

The McMurdo Dry Valleys are located on the western coast of McMurdo Sound (77°00'S 162°52'E) and form the largest relatively ice-free area (approximately 4800 km²) on the Antarctic continent. The perennially ice-covered lakes, ephemeral streams and extensive areas of exposed soil within the McMurdo Dry Valleys are subject to low temperatures, limited precipitation and salt accumulation. The cyanobacterial diversity in a microbial mat sample of L. Fryxell was studied by morphological and molecular approaches. L. Fryxell (77°37' S, 163°07' E) is located at the Eastern end of the Taylor Valley in Southern Victoria Land, Antarctica. It is a 7 km², 18.5 m deep brackish and meromictic lake, perennially ice-covered by 3-4.5 m thick ice. However each summer, a peripheral moat area opens up around the ice cover (Spigel & Priscu 1998).

Byers Peninsula – Antarctic Peninsula (AP)

Byers Peninsula is an extensive, largely ice-free area at the Western end of Livingston Island (South Shetland) and centred on 62°37'S, 61°03'W. The approximate area is 65.7 km^2. The cyanobacterial diversity was studied in one microbial mat sample from a meltwater (sample 41 in Triangular) and one sample from a water-flooded shore of a small stream (sample C1). In both cases, only a few millimeters of water were flowing over the mats that were exposed to high light and UV intensities in summer. This work was carried out during the Antarctic campaign of the LIMNOPOLAR project (collaboration with Prof. A. Quesada, UAM, Spain)

PART 1: THE DEVELOPMENT AND EVALUATION OF (NEW) BIOLOGICAL PROXIES FOR ENVIRONMENTAL RECONSTRUCTION

A. DIATOMS AND FOSSIL PIGMENTS

A.1. INTRODUCTION

Diatoms and fossil pigments are currently widely used as proxies to reconstruct past environmental changes in regions with different climatic settings. In Antarctica, the use of diatoms in paleolimnological studies is particularly well established, as they form an important fossilizable component of the benthic lacustrine microbial communities (Spaulding & McKnight 1999). The use of fossil pigments in Antarctic paleolimnology is only recently becoming established and was to a considerable extent developed within the EU MICROMAT and the current BelSPO LAQUAN projects. The following chapter is divided into three parts, which are dedicated to the use of (i) diatoms and (ii) pigments in Antarctic paleolimnology, and (iii) the critical assessment of the preservation of both proxies in lake-sediment cores.

Despite the (long-lasting) scientific interest for Antarctic diatoms, we came across numerous inconsistencies and misidentifications in the Antarctic diatom literature, apparently caused by the application of diatom literature from the Northern Hemisphere to Antarctic environments (see also Jones 1996; Spaulding & McKnight 1999). It is however imperative that identifications, which are the key to ecological and physiological information in the literature, are unambiguous and verifiable (i.e. clear illustrations and descriptions, and preferably references to voucher materials, should be provided). These inconsistencies and misidentifications not only jeopardise the use of paleoecological inference models, but have also led to the notion that cosmopolitanism prevails in the Antarctic region (Jones 1996). In the first part of this chapter we therefore provide the results of a detailed analysis of the taxonomic composition and the geographic distribution of the diatom communities in the study regions. Subsequently, we extend existing diatom calibration datasets from the Vestfold Hills (Roberts & McMinn 1996, 1998) and the Windmill Islands (Roberts et al. 2001b) with our results, in order to understand the factors controlling extant species composition and structure of the microbial communities. To this end we link the diatom taxa to ambient environmental conditions using multivariate statistics and use the extended dataset to develop diatom-based inference models (or transfer functions) to quantitatively reconstruct changes in the moisture balance of east Antarctic lakes. The moisture balance in dry regions in general, and more specifically in east Antarctic oases, is known to be climate dependent (e.g., Roberts and McMinn 1998; Spaulding and McKnight 1999). In the Larsemann Hills, higher temperatures

lead to enhanced melting of snow banks in the catchment area and to a positive moisture balance (i.e., rising lake level and decreasing salinity), conversely, lower temperatures lead to reduced lake levels and increasing salinity. A similar response has been reported in lakes in the nearby Vestfold Hills (Gibson et al. 1995).

In contrast to diatoms (and some other protists), most algae do not leave recognizable fossils in the sedimentary record. Biogeochemical markers such as fossil pigments might be good alternatives and are increasingly used as proxies of past and present production and algal community composition (e.g., Bianchi et al. 2002a). Fossil pigments have shown their potential in a diversity of applications as indicators of algal and bacterial composition, food-web interactions, lake acidification, mass flux within lakes, past UV radiation and a wide range of anthropogenic impacts (Leavitt and Hodgson 2001 and references therein). In the framework of the LAQUAN project we test the use of pigments and pigment ratios for paleolimnogical reconstructions in Antarctica and relate the presence of group-specific biomarkers to environmental conditions.

Although both proxies are often reliable indicators in paleolimnological studies, differential preservation in sediments can prevent quantitative reconstruction of algal/diatom community production and composition. Taphonomical processes related to diatom dissolution can be high in several environments, such as saline lakes or lakes where the pore waters in the sediments are under-saturated in SiO_2 (Ryves et al. 2001). In the case of pigments, the most rapid degradation occurs during sinking in environments where grazing, microbial processing and the exposure to irradiance, heat and oxygen is high (Louda et al. 1998; Cuddington and Leavitt 1999). While fossil pigments offer potentially valuable information about past changes in gross biomass partitioning and ecosystem function, there has been no direct validation of pigments as biomarkers over longer timescales (>centuries). Until now, many investigators feel that the best way to assess the accuracy of pigments as proxies of algal production is to compare changes in pigment composition of sediments with long-term ecological datasets that span the same time period (e.g., Leavitt and Findlay 1994). Here we evaluate the long-term preservation of fossil pigments and diatoms by comparing changes in biomarker concentrations with estimates of absolute diatom abundance in two sediment cores from two East Antarctic lakes. Combining both proxies additionally further enables us to differentiate between past benthic and planktonic diatom production.

A.2. METHODS

A.2.1. Diatom and pigment analysis of modern samples

Sediment samples and limnological data were collected for the Larsemann Hills, the Rauer Islands and the Bølingen Islands during November and December 1997;

detailed sampling and analysis procedures are given in Hodgson et al. (2001a, 2004) and Sabbe et al. (2004). For the Windmill Islands and the Vestfold Hills full methodological description can be found in Roberts and McMinn (1996) and Roberts et al. (2001b).

A combined East Antarctic (EA) dataset of 113 lakes was constructed on the basis of the calibration datasets described above, hereby ensuring taxonomic consistency after detailed light and scanning electron microscopy (see Sabbe et al. 2003). Diatom slides were made according to a slightly modified protocol by Renberg et al. (1990). For each diatom calibration dataset the analysis was done following the methodology described in Verleyen et al. (2003). Taxonomic details are given in Sabbe et al. (2003).

Pigments were analyzed following standard protocols described in detail in Leavitt and Hodgson (2001) and Squier et al. (2002). The HPLC system was calibrated using authentic pigment standards from the US Environmental Protection Agency and compounds isolated from reference cultures following SCOR protocols (Jeffrey et al. 1997). Chlorophylls and carotenoids were expressed as organic matter-specific concentrations (ng g^{-1}TOC). The taxonomic affinities of the pigments were derived from Jeffrey et al. (1997).

A.2.2. Sediment cores

Sediments were recovered from the deepest part of the lakes using a combination of a Glew gravity corer (Glew 1991) for surface sediments and a Livingstone corer (Wright 1967) for intermediate to basal sediments. The cores were sectioned in the field into 1 cm sections, sealed in sterile 'Whirl-pack' bags and stored at -40^{o}C for transport. A detailed description of the materials and methods can be found in Verleyen et al. (2004a) and Hodgson et al. (2005a).

A.2.3. Diatom and pigment analysis of fossil samples

Diatoms and pigments were analysed according to the methods described above. Quantitative fossil diatom analyses (cf. Battarbee & McKneen 1982) were additionally performed in order to assess the total diatom biomass (TDB), which was calculated by multiplying the absolute valve concentration (g^{-1} dry weight) of each taxon by the surface area of its frustule, and then adding together this data from all taxa present in the sample. In order to evaluate the amount of diatom dissolution, a dissolution index (%dissolution) was calculated following the morphological index of Ryves et al. (2001). This index, assessed microscopically, expresses the ratio between the number of diatoms with visible signs of dissolution and the total number of counted valves, which exceeded 50 in each sample.

Different pigment groups were clustered and ratios were calculated to assess the amount of diatom production and the influence of light on pigment preservation

(see Verleyen et al. 2004b for a detailed description of the ratios used). We estimated the total production of photosynthetic organisms as total chlorophyll (TChla) by calculating the sum of bacteriochlorophylls, chlorophyll *a* and their derivatives. Total diatom carotenoid concentration (TDC) was estimated as the sum of the diatom biomarkers fucoxanthin (and its derivatives), diatoxanthin and diadinoxanthin. The ratios TDC/TChla and TDB/TChla were used to compare the pigment based and microfossil based estimates of diatom production. To assess the influence of light on the preservation conditions for pigments, two indicators of mean irradiance were calculated. These were based on ratios of the sum of the xanthophylls [diadinoxanthin (DD)+ diatoxanthin (DT)]/TChla and the carotenoid ß carotene (Bcar)/TChla which have previously been shown to indicate irradiance conditions experienced by diatoms and all algal groups respectively (cf. Sigleo et al. 2000).

A.2.4. Multivariate analysis

In all statistical analyses, species and pigment data were log (x+1) transformed to reduce the influence of dominant taxa/pigment classes and all environmental variables (except pH) were log (x+1) transformed to reduce or remove skewness.

Standard multivariate analyses were performed to explore the distribution of diatom taxa and specific pigment classes (indirect ordinations), and their relationship to the environmental variables (direct ordinations). All ordinations were performed using CANOCO 4.5 for Windows (ter Braak and Smilauer 2002). For a more complete description of the different techniques used the reader is referred to Sabbe et al. (2004).

A.2.5. Inference model development

Only taxa occurring with a relative abundance of at least 2% in at least one sample were incorporated in the diatom-based inference models. The weighted averaging (WA) and weighted averaging partial least squares (WA-PLS) algorithms were selected (see Birks 1998) in the CALIBRATE 1.01 software program (Juggins and ter Braak 1998). Outliers were detected according to Jones & Juggins (1995). The optimal number of components in the WA-PLS model was determined following the criteria in Verleyen et al. (2003). An inference model for salinity was constructed based on the entire dataset (111 lakes; two outliers excluded) and a transfer function for lake-water depth in oligo-saline lakes (salinity ≤ 2‰) was based on a subset containing 55 freshwater lakes.

A.2.6. Time series analysis

Statistical relationships among time series (sediment-core samples) were explored using basic time-series procedures in SYSTAT v. 10 software (SPSS Chicago,

Illinois, USA). Preliminary analyses indicated that all predictor and response variables at both sites were not normally distributed and exhibited substantial temporal autocorrelations. Consequently, all variables were transformed sequentially using $\log_{10}(X+1)$ and first difference transformations to normalize variance and remove autocorrelations. Subsequent cross correlation analyses indicated that only lag=0 correlations were significant and substantial. Therefore we report these correlations as Pearson correlation coefficients for transformed variables.

A.3. RESULTS

A.3.1. Environmental settings of the lakes in the Larsemann Hills

In order to explore the relationship between the diatom taxa and the environmental conditions, and to extend the existing reference dataset, we assessed the basic limnology of the lakes in the Larsemann Hills and Bølingen Islands following standardized techniques (see Table 1 in Sabbe et al. 2004).

Principal Component Analysis (PCA) of the physical, chemical and morphometric data showed that the limnological diversity of the lakes was primarily determined by variation in conductivity and lake morphometry. Dissolved oxygen was strongly negatively correlated with conductivity, while dissolved and total organic carbon (DOC, and TOC respectively), dissolved silicate and pH were positively correlated with this variable. In most lakes, TOC levels were below detection limits (0.01 $mg.L^{-1}$). However, in the oligosaline lakes BAL, L70, L71 and BFI, and the freshwater lake Sunset (BSU), TOC concentrations ranged between 8.8-21.5 $mg.L^{-1}$. Particulate organic matter concentration (calculated as the difference between TOC and DOC) was very low in most lakes; dissolved organic matter contributed to more than 97 % of TOC (except in BFI: 88 %).

During sampling, a number of macroscopically recognizable mat types (growth forms), which occurred over different parts of the lake depth gradient, were distinguished and described (Fig.1). Finely laminated (FILM) prostrate mats were restricted to deeper lakes (>7m).

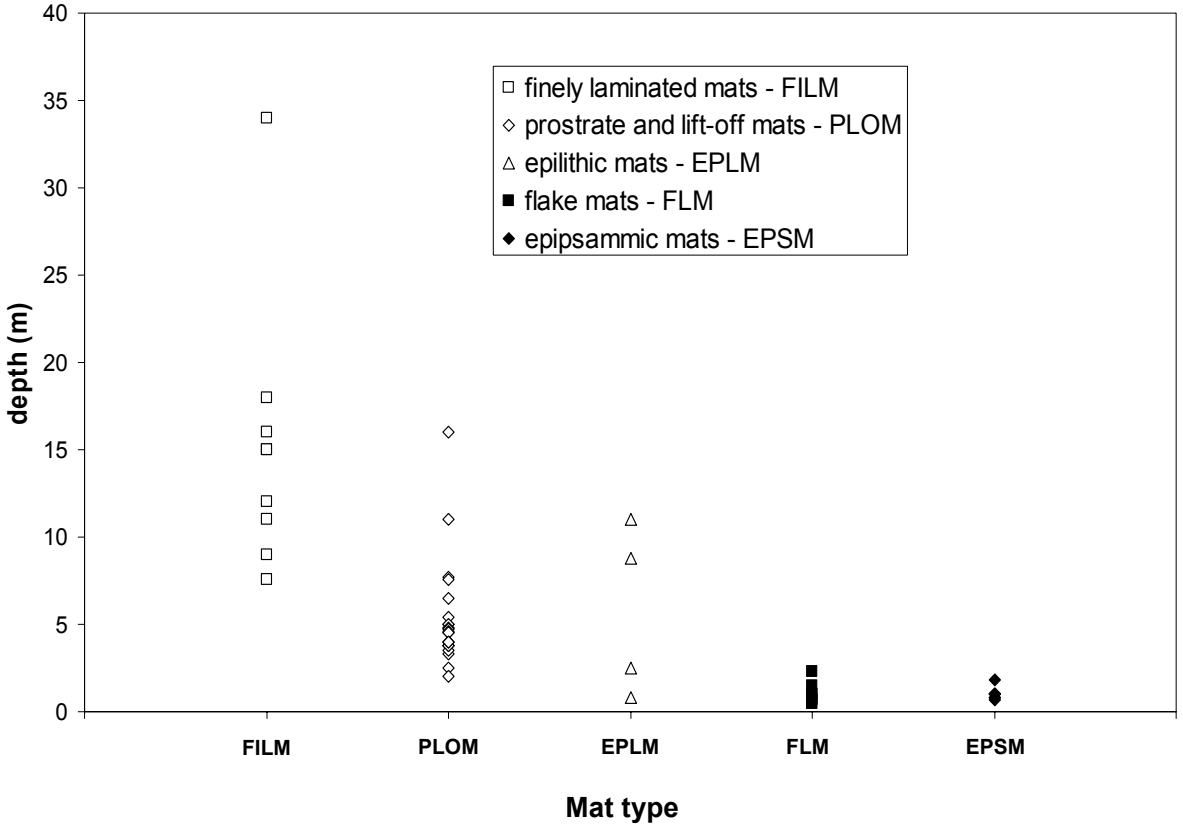

Fig. 1: *Depth zonation of microbial mat types in lakes of the Larsemann Hills and Bølingen Islands.*

Less structured prostrate mats (PLOM) occurred between 2 m and 16m, and parts of these mats sometimes lifted off, due to bubble formation and physical disturbance by wind and ice (cf. Simmons et al. 1993). Flake mats (FLM), consisting of small (1-2 cm) plate-like growths of cyanobacteria, were confined to shallow lakes up to 2 - 3 m deep. They consisted of 17-73 % inorganic sediment (mean 35.2 %). Epipsammic (interstitial; EPSM) communities consisted of an organic matrix with a high amount of embedded inorganic sediment (32-99%, mean 78.6%); they were observed in shallow waterbodies only (<1.8m). Epilithic mats (EPLM) were found on rocks on both shallow and deeper lakes in the region.

A.3.2. Diatoms

A.3.2.1. Floristic and taxonomic study of the diatom floras

The newly collected diatom samples from 66 freshwater and saline lakes and pools in the Larsemann Hills, Rauer Islands and Bølingen Islands were the subject to a detailed floristic and taxonomic survey (see Sabbe et al. 2003 for a full description of the results). A total of 31 taxa were distinguished. Marine species such as

Fragilariopsis and *Cocconeis* spp., listed in the species list of the Rauer Island group (Hodgson et al. 2001a) have not been included here, as it is uncertain whether these taxa actually form part of the autochthonous diatom flora of the lakes or whether it concerns deposition of dead, airborne marine material.

Ten taxa could not be identified to species (9) or even generic (1) level, either because they have most probably not yet been described or because they belong to species complexes that are in need of revision. Four new combinations are proposed, namely *Craspedostauros laevissimus* (West & West) Sabbe comb. nov., *Planothidium quadripunctatum* (Oppenheim) Sabbe comb. nov., *Psammothidium metakryophilum* (Lange-Bertalot & Schmidt) Sabbe comb. nov. and *Psammothidium germainii* (Manguin) Sabbe comb. nov. *Nanofrustulum shiloi*, *P. stauroneioides* and *Planothidium quadripunctatum* are reported for the first time from continental Antarctica. Three species are reported for the first time from continental Antarctica, while another three are for the first time confirmed for eastern Antarctica.

A.3.2.2. Factors regulating diatom community structure in East Antarctic lakes

The salinity gradient in the combined dataset (113 lakes) ranges between 0 ‰ and 165 ‰, comprising all major types of freshwater to saline lakes. A total of 68 species was identified (see Appendix 1). The number of species in the saline meromictic lakes in the VH is generally higher compared with the shallower holomictic lakes (see Table 1 in Verleyen et al. 2003 for additional information).

Canonical correspondence analysis (CCA) with forward selection and Monte Carlo permutation tests revealed that the variance in diatom composition could be significantly ($P \leq 0.05$) explained by salinity, lake-water depth, reactive silicate and dissolved inorganic phosphate (Fig. 2).

The diatom flora can be divided into two distinct, salinity-determined groups, namely (a) oligo-and hypo-saline lakes situated on the negative side of the first ordination axis and (b) meso- and hyper-saline lakes situated on the positive side of the first ordination axis (Fig. 2). Within both groups a distinction can be made between deep lakes, situated on the negative side of the second ordination axis and shallow ponds and lakes, situated on the positive side of the second ordination axis.

The deep, meso- and hyper-saline lakes from the VH are dominated by planktonic *Chaetoceros* species, and marine and sea-ice associated species (e.g., *Navicula directa* and *Fragilariopsis cylindrus*). The shallow saline- and hyper-saline ponds in the RI are characterised by the Antarctic endemic *Navicula* cf. *shackletoni*, with *Luticola muticopsis, Navicula phyllepta* and *P. microstauron* as co-dominants*. L. muticopsis* and *Pinnularia cymatopleura* dominate in the meso- and hyper-saline ponds in the WI.

Aerophilic diatoms, which grow in wet terrestrial habitats (e.g., *Pinnularia borealis*, *D.* cf. *perpusilla* and *L. muticopsis*), dominate the diatom composition in the

shallow oligo-saline ponds; *Psammothidium abundans* dominates in the deeper lakes. *Stauroforma inermis* is abundant in the freshwater lakes with a water depth between 3 and 5 m. The overriding effect of lake water depth on the freshwater diatom communities is confirmed by a separate CCA a dataset containing only lakes with salinity values below 2 ‰ (mainly lakes from the Larsemann Hills). Lake water depth, salinity, reactive silicate and elevation significantly ($P \leq 0.05$) explain 21.4 % of the total variance in diatom assemblages in the oligo-saline lakes. Depth is the most important variable and explains 8.4 % of the total variance in the diatom.

A.3.2.3. Transfer functions for salinity and lake-water depth

As salinity and lake water depth significantly explain changes in the diatom communities, both variables can be used to develop diatom based inference models to reconstruct changes in the moisture balance of east Antarctic lakes.

Two lakes were left out of the salinity model because their WA residuals exceeded the standard deviation of log-transformed salinity (0.74). None of the WA-PLS components shows a decrease in RMSEP, implying simple WA should be used for modelling. Jack-knifed r^2 and apparent r^2 are high in the East Antarctic transfer-function, 0.83 and 0.86 respectively and RMSEP is relatively low (0.31), implying the transfer function is relatively robust. The optima and tolerances of the different taxa are given in Appendix 1.

A transfer function for lake water depth is based on the calibration dataset of oligo-saline lakes (salinity ≤ 2 ‰) from East Antarctica in order to infer changes in the moisture balance in sediment cores extracted from these lakes. At these low salinities, species turnover is low, compared with the rapid change in species composition between hypo-, meso- to hyper-saline lakes (see Table 3 in Verleyen et al. 2003). Five lakes were identified as outliers, because the jack-knifed residuals exceeded the standard deviation of log transformed z-max (0.36). Leave-one-out cross-validation (RMSEP and jack-knifed r^2) was used in order to select the number of components in the model and not the apparent statistics (RMSE and r^2). WA-PLS outperforms simple WA as the jack-knifed r^2 is 6.8 % higher (0.76) and RMSEP is 8.2 % lower (0.22) in WA-PLS with two components. The lake-water depth inference model is similarly robust and the optima and tolerances of the different taxa are given in Appendix 2.

A.3.3. Pigments

To compile a reference data for paleolimnological studies using fossil pigments, we examined the extent to which environmental variables, gross mat morphology (Fig.3) and species composition influence the modern pigment content of *in situ* microbial communities in 62 east Antarctic lakes.

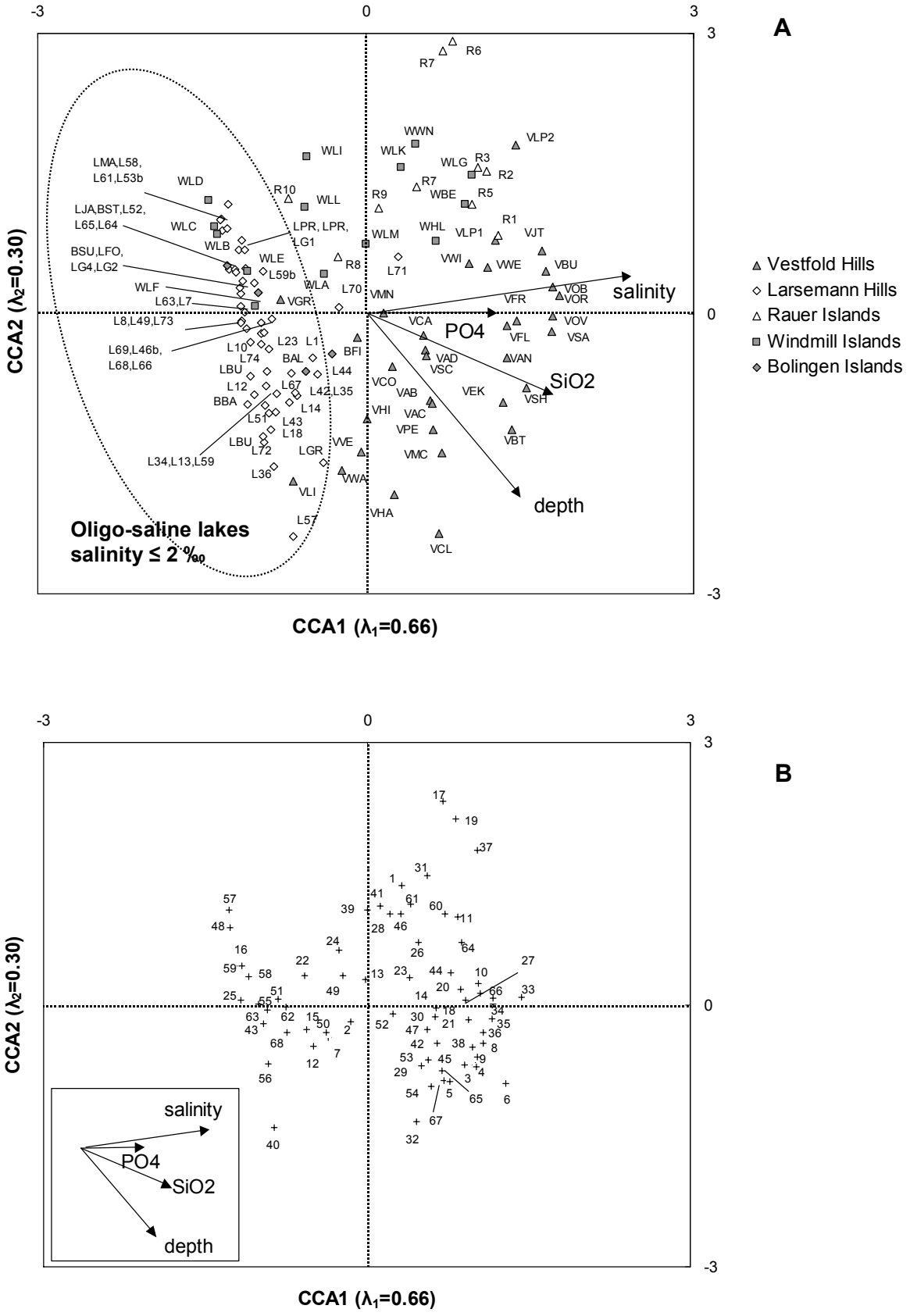

Fig.2: *CCA ordination biplot for the first two axes showing (A) the lakes and (B) species of the inter-calibrated East Antarctic calibration dataset (see Appendix 1 for species numbers).*

Most benthic microbial mat samples contained a mixture of chlorophylls and carotenoids and scytonemins (see Hodgson et al. 2004 for detailed information). Scytonemin, in both oxidized (yellow-green), and reduced (red) forms together with some derivatives, was most abundant in lakes between 0-2 m depth and absent or rare in lakes over 4 m depth (Fig.3). Of the 36 samples containing scytonemin only 4 occurred at depths of greater than 5 m. This indicates that the depth zone between 2-4 m is an important ecological boundary in terms of UV-stress to which the microbial community is exposed. Cyanobacterial carotenoids were, on average, in slightly higher concentrations in intermediate depth lakes (2-10 m depth), while chlorophyll *a* concentrations were high relative to all other pigments in the deeper lakes (>4m depth). Chlorophyll *b* (from the green algae) was less abundant with the exception of two lakes (R12, L69) in the 2-4 m category. Ratios of all major pigment classes thus strongly suggest that pigment content and composition are related to lake depth (as a proxy for light climate; Fig.3). This is further confirmed in the CCA where it was revealed that lake water depth, turbidity, latitudinal and longitudinal position, dissolved oxygen, conductivity, and sulphate significantly (P≤0.05) explain the variance in pigment data. RDA revealed that lake water depth explained more than 20% of the variance in scytonemin content.

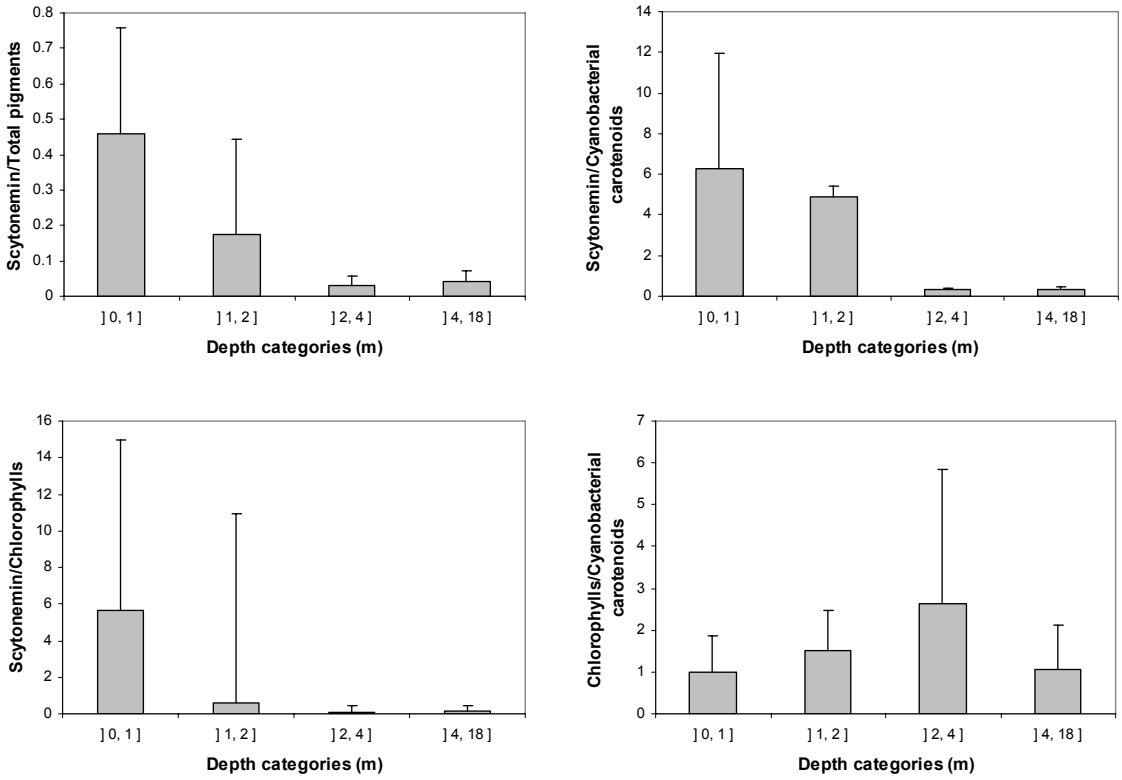

Fig.3: *Pigment ratios in aggregated lake depth categories, non-zero means for scytonemins and standard deviation as error bars.*

A.3.4. Pigment and diatom preservation conditions

Diatom and pigment data in sediment cores from two lakes, Heart L. (HL) and Pup Lagoon (PL), were compared to critically assess changes in long-term proxy preservation.

A.3.4.1. Diatom assemblages in Pup Lagoon

Based on fossil diatom assemblages, the PL sediment core can be divided into three zones spanning the past *c*. 5800 years (Fig. 4; Verleyen et al. 2004a). First, a marine zone between 302 and 150 cm is characterised by marine and sea-ice associated diatoms. Second, a well-marked transition zone from marine to freshwater conditions between 150 and 140 cm is characterised by stomatocysts from Chrysophyceae and lacustrine brackish water diatoms. Third, a freshwater zone between 140 cm and the top of the core is characterised by lacustrine freshwater and brackish water diatoms.

A.3.4.2. Preservation conditions in Pup Lagoon

Both morphological and biogeochemical proxies for past diatom production (TDB and TDC; see methods section) showed higher values in the marine core section than during the lacustrine period (Fig. 4). Following correction of time series for temporal autocorrelation, statistical analyses revealed that TDB and TDC were more strongly correlated in the marine interval (r^2=0.358, $p < 0.0001$) compared with the lacustrine period (r^2=0.223, $p = 0.001$). Ratios of TDB/TChla declined less than TDC/TChla during the transition from the marine to the lacustrine interval (Fig.4). Overall, the average ratio of TDB/TDC was thus 4.4 times lower in the marine core section compared with the lacustrine period.

The first irradiance index [(DD+DT)/TChla] was higher in the marine interval with lower and occasionally zero values in the lacustrine core section (Fig.4). In contrast, the second irradiance index, Bcar/TChla was highly variable in both sections (Fig.4). In the marine interval, diatom dissolution was minimal, whereas it was relatively high during the lacustrine period.

A.3.4.3. Diatom assemblages in Heart Lake

Based on fossil diatom assemblages, the HL sediment core can be divided into five zones spanning the past *c.* 10,000 years (Fig.5). 1) A marine zone between 361 and 355 cm is characterised by marine diatoms; 2) a glacial till/diamicton zone between 355 and 275 cm contained only rare and fragmented diatoms (note: as a consequence, pigment data from this part of the core were not included in statistical analyses); 3) A freshwater zone between 275 and 245 cm characterised mainly by lacustrine diatoms, except at 262 cm where marine diatoms are abundant, and at 270 cm, where lacustrine and marine diatoms co-occurred; 4) Another marine zone between 245 and 25 cm containing marine diatoms; and 5) A freshwater zone

between 25 and 0 cm containing lacustrine diatoms.

A.3.4.4. Preservation conditions in Heart Lake

TDB concentrations remained relatively constant throughout the core (Fig.5). In contrast, TDC values were extremely low in the lacustrine intervals, contributing to a greater variation in TDC/TChla ratios between marine and lacustrine sections than that recorded for TDB/TChla ratios (Fig.5). Both morphological and biogeochemical proxies for past diatom production (TDB and TDC) were positively correlated throughout the entire core (r^2=0.187, $p < 0.0001$); however, correlations were highly significant in the marine intervals (r^2=0.557, $p < 0.0001$) but not in the lacustrine core sections (r^2=0.102, $p = 0.111$). The average ratio of TDB/TDC was thus 32.2 times lower during the marine period compared with the lacustrine interval.

The first irradiance index [(DD+DT)/TChla] was low in the lacustrine zones and higher in the marine intervals. In contrast, the second irradiance index (Bcar/TChla) showed no clear differences between marine and lacustrine sections. As in the PL core, diatom dissolution was relatively high in the lacustrine zones and low or even zero (i.e., no visible signs of dissolution) in the marine sections (Fig.5).

A.4. DISCUSSION

A.4.1. Floristic and taxonomic study of the diatom floras

The total number of taxa from the Larsemann Hills and Bølingen Islands is comparable to the species numbers reported from inland freshwater and saline lakes from other continental Antarctic locations (e.g. Jones 1996 and references therein). However, it is much lower than the numbers reported from maritime Antarctica and Subantarctica, which is in accordance with the previously observed trend of decreasing species numbers as one moves southwards in the Antarctic (Van de Vijver & Beyens 1999). This phenomenon of low species diversity has been attributed to the harshness of the environment and geographic isolation, as well as to factors related to latitude such as period of ice cover and light intensity (Jones 1996).

Analysis of literature data on Antarctic lacustrine diatoms shows that taxonomic practice has a profound influence on the assessment of distribution patterns. Force-fitting of European and North American names to Antarctic taxa and erroneous identifications have contributed to an underestimation of endemism in the diatom flora of Antarctic inland waters. In addition, changing concepts on species boundaries during the last decade influence the interpretation of biogeographic patterns. The application of a more fine-grained taxonomy will almost certainly reveal a higher degree of endemism in Antarctica, and especially continental Antarctica.

The present case-study shows that in the Larsemann Hills Antarctic endemics account for about 40 % of all freshwater and brackish taxa, while the biogeographic

distribution of about 26 % is unknown, mainly due to their uncertain taxonomic identity. This contradicts the view that cosmopolitanism prevails in Antarctic diatoms. A detailed description and discussion of the biogeographic distribution of the diatoms in the Larsemann Hills can be found in Sabbe et al. (2003).

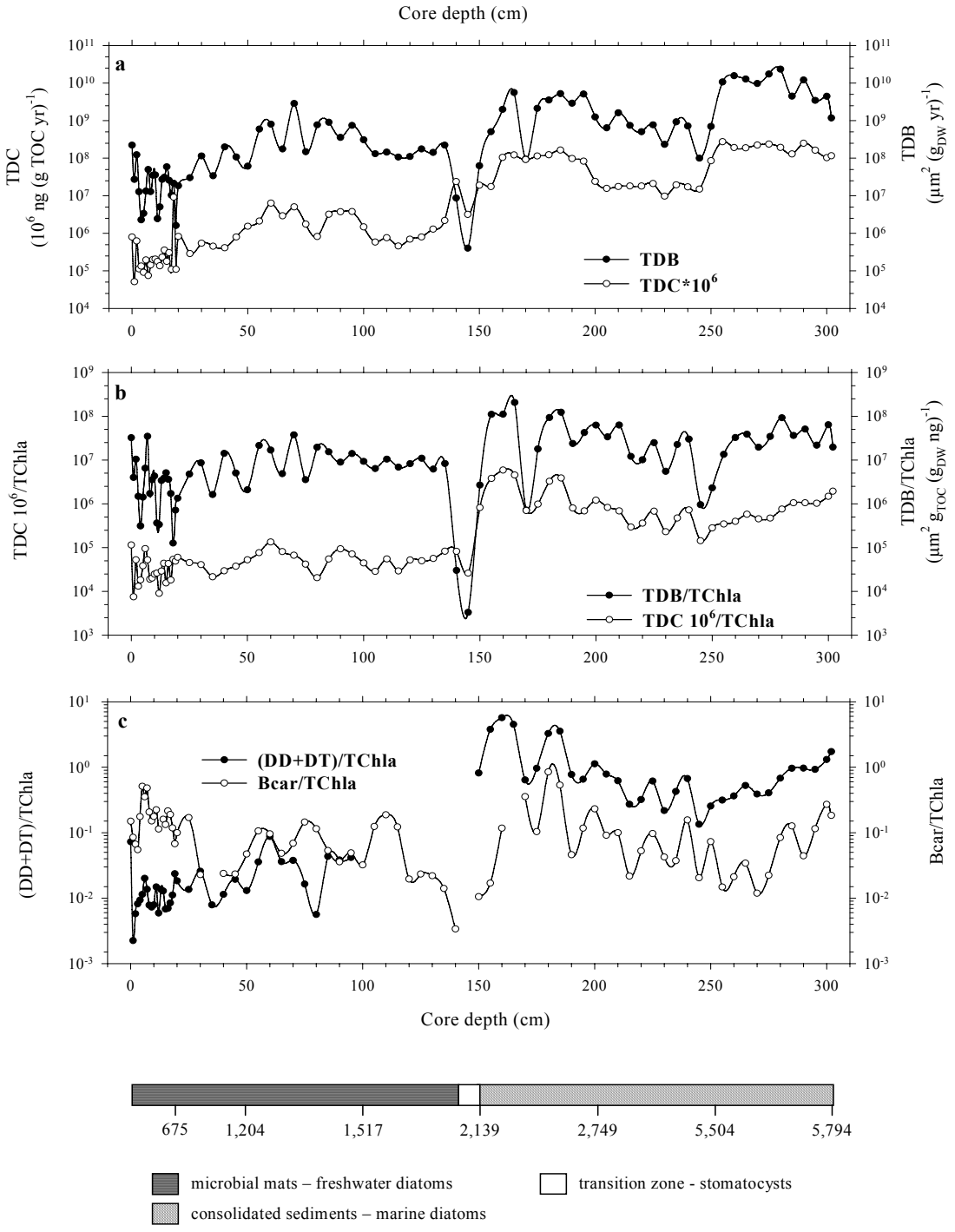

Fig.4: *Proxy reconstructions of environmental changes in PL. a) Total diatom biovolume (TDB) and total screening pigments for diatoms (TDC). b) Total diatom biovolume and total diatom carotenoids divided by total chlorophyll a (TDB/TChla; TDC/TChla). c) Sum of diadinoxanthin and diatoxanthin and ß-carotene divided by total chlorophyll a (Bcar/TChla).*

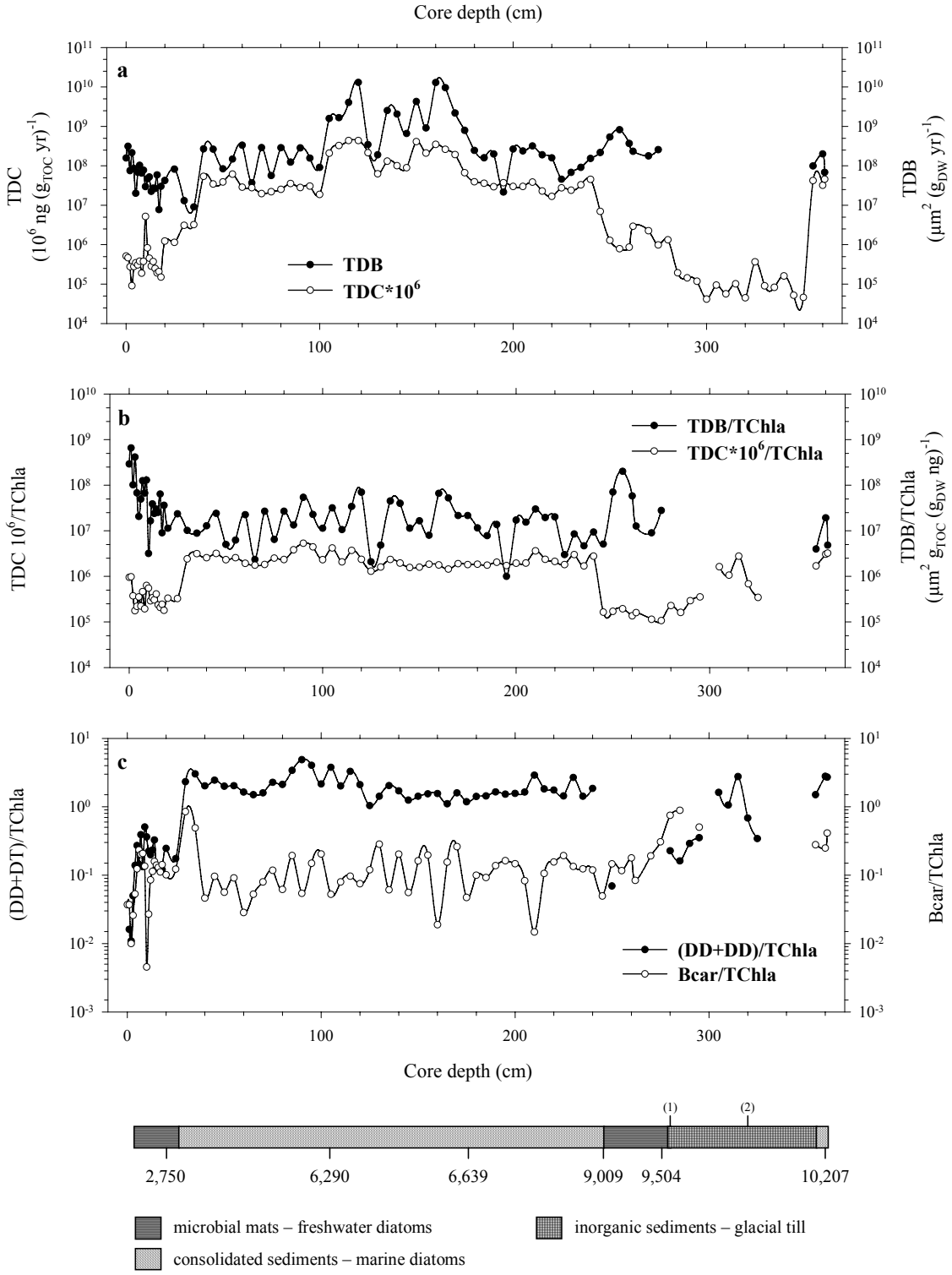

Fig.5: *Proxy reconstructions of environmental changes in HL. a) Total diatom biovolume (TDB) and total screening pigments for diatoms (TDC). b) Total diatom biovolume and total diatom carotenoids divided by total chlorophyll a (TDB/TChla; TDC/TChla). c) Sum of diadinoxanthin and diatoxanthin and ß-carotene divided by total chlorophyll a (Bcar/TChla).*

A.4.2. Factors influencing the diatom communities in east Antarctic lakes

Multi-variate analyses revealed that diatom communities show a high degree of similarity in analogous types of lakes in east Antarctica and that salinity and lake depth are the main variables structuring these communities (Fig.2). In the hypo-saline lakes and ponds the same brackish water diatoms occur (e.g., *Amphora veneta* and *Craspedostauros laevissimus*). Although dominated by different taxa, the flora of the shallow hyper-saline ponds in the Rauer Islands has taxa in common with the ponds in the Windmill Islands, whereas both floral assemblages are significantly different from the diatom community in the deep, in general meromictic, meso- and hyper-saline lakes of the Vestfold Hills. The occurrence of the planktonic marine species in lakes in the latter region is remarkable. Possibly, some marine and sea-ice diatoms (e.g., *Fragilariopsis cylindrus* and *Fragilariopsis curta*) with a broad salinity tolerance are able to colonise the deep and saline water bodies in the VH leading to higher species diversity and the presence of planktonic taxa.

Even at genus level, differences existed between deep and shallow freshwater assemblages. Genera with many aerial representatives like *Hantzschia*, *Luticola* and *Diadesmis*, which are also commonly reported from terrestrial and stream environments in Antarctica (Broady 1996), occurred in the shallow ponds. The presence of these taxa may be due to the shallow nature of the ponds or they may be flushed into the lakes through the inflow of melt water from the catchment area. In the shallow ponds, abrasion by ice and freezing probably act to prevent the development of prostrate mats, which are characteristic of deeper lakes. The higher likelihood of anoxia and freeze-out of salts in shallow lakes may also constitute a stress factor for the benthic communities. The dominant species in the deep freshwater lakes were *Stauroforma inermis* or *Psammothidium abundans*. *Stauroforma inermis* forms short chain-like colonies and appears to prefer somewhat shallower lakes.

Distinct zonation of microbial mat types and of benthic diatom communities with depth has been documented for many Antarctic lakes [e.g., Wharton et al. (1983)]. Although many, although not mutually exclusive, different factors may contribute to this zonation [e.g., differential responses of species to light regime, degree of physical disturbance by lake ice, intracellular ice formation and occurrence of seasonal anoxia], the resulting depth intervals over which particular diatom species occur are highly predictable. In Antarctic lakes, where planktonic diatoms are virtually absent (Jones 1996) changes in species composition of benthic diatoms may therefore provide information on lake level variations.

A.4.3. Diatom-based transfer functions for salinity and lake-water depth

Previously, a diatom based transfer function for salinity was constructed on the basis of a calibration dataset comprising saline and hyper-saline lakes in the VH (Roberts

and McMinn 1996, 1998). Although a broad salinity gradient was sampled, only two oligo-saline lakes were included in the dataset (VLI and VGR). Unlike the deeper saline lakes in the VH, most Antarctic lakes are freshwater and relatively shallow (Ellis-Evans 1998). In order to construct a salinity transfer function enabling the reconstruction of climate-related changes in sediment cores from oligo- to hyper-saline lakes in East Antarctica, we extended the salinity gradient by incorporating the lakes in the Larsemann Hills.

The model to reconstruct lake-water depth in oligo-saline lakes is particularly relevant, because these widespread freshwater bodies have not yet been exploited for quantitatively inferring changes in the moisture balance in Antarctica. Both transfer functions therefore provide useful tools for inferring changes in the climate-dependent moisture balance in all types of lakes in East Antarctic oases between 102 and 75°E (see Verleyen et al. 2003 for a full discussion).

A.4.4. Pigments to reconstruct past environmental change

In order to establish a reference dataset for paleolimnological studies using fossil pigments, we examined the extent to which environmental variables, gross mat morphology (Fig.1) and species composition influence the modern pigment content of *in situ* microbial communities in 62 east Antarctic lakes. Our study differs from previous studies by examining the combined influences of environmental variables, gross morphology and species composition on the pigment contents of *in situ* microbial mats and avoiding artificially imposed experimental conditions. The advantage of this approach is that it identifies the variables that have an over-arching influence on pigment contents and that are environmentally relevant rather than induced, or enhanced, by artificial reduction in a laboratory.

As anticipated, the pigment composition of the microbial mats is strongly influenced by the lake water depth gradient (cf. the microbial mat type and the diatom composition in the lakes), presumably on account of its impact on the light climate (Fig.4). The cyanobacteria in the shallow lakes thus receive so much PAR and UVR that a major cellular effort is invested in synthesising a range of PAR and UVR screening compounds, such as xanthophylls and scytonemins. Xanthophylls disperse excess energy (heat) from the cells using the xanthophyll cycle and the scytonemins are responsible for UVR protection and stress response preventing the UV dependent bleaching of chlorophyll and damage to DNA (Garcia-Pichel & Castenholz 1991).

As the low attenuation values measured for UVR in the lakes allow UVR penetration beyond maximum lake depth in the shallower lakes (Ellis-Evans et al. 1998), cyanobacteria with UVR screening capabilities appear to have an advantage over those phototrophs without UVR screening capabilities. During periods of elevated UVR fluxes a marked shift in species composition is expected to occur,

towards cyanobacteria that produce UVR screening compounds. These changes are preserved in the sedimentary archive, which will allow the reconstruction of past variations in UVR receipt (see below).

A.4.5. Pigment and diatom preservation conditions

Although pigments are widely used in paleolimnology (see Leavitt and Hodgson 2001 and references therein), few attempts have been made to compare fossil pigments with alternative fossil proxies for algal production, particularly in marine environments (e.g., Bianchi et al. 2002), yet, similar correlations are found in freshwater environments (e.g., Leavitt and Findlay 1994). Here, we compared absolute diatom counts with pigment-derived estimates of diatom production. In doing so we were able to evaluate long-term changes in pigment and/or diatom preservation in both marine and freshwater sediments and evaluate the relative influence of the different preservation environments.

In both lakes, ratios of TDC/TDB were higher in the marine zones compared with the lacustrine zones (Figs.4,5). The difference in the pigment-based (TDB/TChla) and biovolume-based (TDC/TChla) ratios between these contrasting environments may arise from variations in diatom dissolution, cellular pigment quotas, physiological response to significantly altered light regimes, and/or pigment preservation. Our results indicate that better pigment preservation in the marine sections is likely the overriding factor beyond this difference between both environments (see Verleyen et al. 2004b for a full discussion). In general, pigment preservation is reduced by prolonged exposure to elevated oxygen concentration, high irradiance, temperature, grazing or microbial processing (Louda et al. 1998, 2002; Cuddington and Leavitt 1999). We therefore speculate that improved preservation in marine sediments may have arisen as a result of anoxia under sea-ice (cf. McMinn 1995). In support of this hypothesis, we note that bacteriochlorophylls were abundant in the marine intervals of the Pup Lagoon core, but not during freshwater episodes (Verleyen et al. 2004a). Such bacteriochlorophylls are produced by obligate anaerobic sulphur bacteria. In contrast, oxygen concentrations have been shown to reach 120-170% of air-equilibrium values in the upper 5 mm of microbial mats in freshwater systems near McMurdo Sound (Vincent et al. 1993). Together, these patterns suggest that pigment preservation (and also diatom dissolution), may vary substantially between marine and lacustrine sedimentary environments in Antarctica, and that photo-protective compounds such as diadinoxanthin and diatoxanthin need to be used with caution if they are to serve as a quantitative measure of the contribution of diatoms to primary production or as measures of irradiance. Clearly, more research concerning pigment stability and preservation in different sedimentary environments is needed.

We suggest that the combination of pigments and diatom biovolume estimates

is a useful tool for distinguishing historical trends arising from production and preservation artefacts during ecosystem changes which is a key goal of many paleo-ecological studies.

B. CYANOBACTERIA, MOLECULAR MARKERS AND FOSSIL DNA

B.1. INTRODUCTION

Cyanobacteria are the dominant photoautotrophic organisms in freshwater ecosystems in Antarctica, where they accumulate conspicuous biomasses (Vincent 2000). They are present in both planktonic and benthic microbial communities in lakes, where they are important primary producers, and have been recorded from as far south as the Darwin Glacier area (80ºS) (Vincent and Howard-Williams 1994). About 500 species have been identified in Antarctica on morphological grounds.

Early studies on the diversity and biogeographical distribution of cyanobacteria were based on the identification of the organisms using morphological criteria. Unfortunately, cyanobacteria have often quite simple morphologies and some of these characters exhibit plasticity, so that their taxonomic usefulness can be limited. The unsatisfactory state of the cyanobacterial taxonomy based on morphology is reflected in current revisions (e.g., Komárek & Anagnostidis 1989). In addition, a number of identifications of Antarctic cyanobacteria were made with floral guides written for temperate species without taking into account their ecology (Komárek 1999), which could give the impression that mostly cosmopolitan taxa were found on the continent. A similar problem was experienced for diatom identification (see A.1). Out of 68 species found in various microbiotopes of ice-free areas of King George Island (AP), Komárek (1999) determined that about 60% were probably endemic to Antarctica. The problems encountered in identifying cyanobacteria on the basis of morphological criteria have prompted the use of molecular tools for diversity studies. Looking at genotypes instead of morphotypes is more informative and allows to map the geographical and ecological distributions of cyanobacteria with more accuracy. The first publication on a joint study of morphological and genotypic diversity of cyanobacteria in one microbial mat sample from L. Fryxell (DV) was made in the laboratory of one of the partners (Taton et al. 2003). Later, Jungblut et al. (2005) carried out a similar study on microbial mats in 3 ponds of the McMurdo Ice Shelf (MMIS). Taton et al. (2006a) have extended their precedent study with 5 samples from 4 lakes of the Larsemann Hills, Vestfold Hills and Rauer Islands.

At the bottom of lakes and ponds, sediments have accumulated, which are composed of inorganic material and fossilized benthic microorganisms (dominated by cyanobacteria). Some organisms kept their morphological structure and can be studied by microscopic analyses, such as diatoms. In the case of cyanobacteria, no

morphologically recognizable structures are left, implying that they must be studied by molecular methods. The use of fossil DNA to reconstruct paleoenvironments from Holocene and Pleistocene sediments has been shown to be possible and useful, even in the absence of obvious macrofossils (Willerslev et al. 2003). A study of DNA durability and degradation in permafrost samples indicated a limit of 400 thousand years for successful PCR amplifications (Willerslev et al. 2004a). Few studies have been done on fossil DNA in lake sediments (Coolen et al. 2004a,b) and, to our knowledge, this is the first investigation on cyanobacterial fossil DNA. One of the most important criteria to allow for reliable studies is that the quality and the quantity of fossil DNA is sufficient, which was shown to be one of the difficulties to use fossil cyanobacterial DNA in sedimentary cores of Antarctic lakes. In order to ascertain the lack of contamination by modern cyanobacterial DNA, precautions and controls were carried out, and a cross-validation between two laboratories was carried out for a few samples (Willerslev et al. 2004b).

Here, we aimed to (i) assess the microbial diversity and community structure of cyanobacteria in living Antarctic microbial mats to serve as reference data set and calibration for paleoecological studies, and (ii) to extract and analyse fossil DNA preserved in lake-sediment cores from three lakes, and compare the paleodiversity with the modern communities.

B.2. METHODS

B.2.1. Origin of modern cyanobacterial samples

Surface sediment samples from lakes were collected for the Larsemann Hills (L. Reid, Progress L., Heart L.), the Rauer Islands (L. Rauer2, Rauer8,) and the Bølingen Islands (Firelight L.) using a glew corer (Glew 1991) during November and December 1997. A second sample for L. Reid was taken in 1998 (Taton et al. 2006b). Detailed sampling and limnological analysis procedures are given in Hodgson et al. (2001a, 2004) and Sabbe et al. (2004). The Ace L. sample (VH) was collected using a scraping device through a hole drilled in the ice at a depth of 2 m in 1998/1999. Microbial mat samples from L. Fryxell (McMurdo Dry Valleys) were collected in January 1999 from a shallow, moated area of the lake close to the inflow of Huey Creek and Canada Stream. A sample from an artificial mat grown in a Benthic Gradient Chamber using a subsample as inoculum was analysed (Taton et al. 2003; Buffan-Dubau et al. 2001).

Microbial mat samples from Byers Peninsula (Antarctic Peninsula) were collected in very shallow waters. One sample was obtained from a laminated microbial mat in a meltwater (Triangular) (62 38'47''S, 61 06'43''W) on a plateau situated at 64 m altitude above sea level in February 2004. It was divided in two subsamples, namely 41T representing the orange-red pigmented upper layer and

41B representing the greenish bottom lower layer. The Campamento mat sample (C1) was collected from the shore of a small stream on South Beaches (62º39'47''S, 61º05'53''W) in January 2003.

B.2.2. Origin of fossil cyanobacterial samples

Sedimentary cores were sampled using a glew and a modified livington corer in three lakes from the Larsemann Hills (L. Reid, Heart L., Progress L.). The cores are described by Verleyen et al. (2004c) and Hodgson et al. (2005a; 2006) and a simplified lithologies can be found in Fig. 6. In the L. Reid core a sample at 3 cm depth, in the Progress L. core at 2, 7 and 8 cm depth and in the Heart L. core at 2, 4, 14, 231; 254, and 346 cm depth were the subject to DNA extraction and analyses. For each core, the surface layers with living mats were integrated in the study of fossil layers, using the same protocols, even if they had been already studied by other methods.

B.2.3. Molecular methods

For modern samples, DNA was extracted as described by Taton et al. (2003, 2006a). In summary, a mechanical lysis was carried out with glass beads and followed by a deproteinisation with phenol and a purification step with the Wizard DNA Clean-up system (Promega, USA). The UltraClean^{TM} Soil DNA Isolation Kit (MoBio Laboratories, USA) was used for Rauer8. For Rauer2, the Wizard DNA purification system for food (Promega, USA) was used to extract DNA. For the modern and fossil layers of Heart L., DNA was extracted with the Nucleospin Plant kit (Macherey Nagel, Germany) or the Wizard DNA purification system for food (Promega, USA). The latter kit was also used for the fossil layers of Progress L. and Reid L.

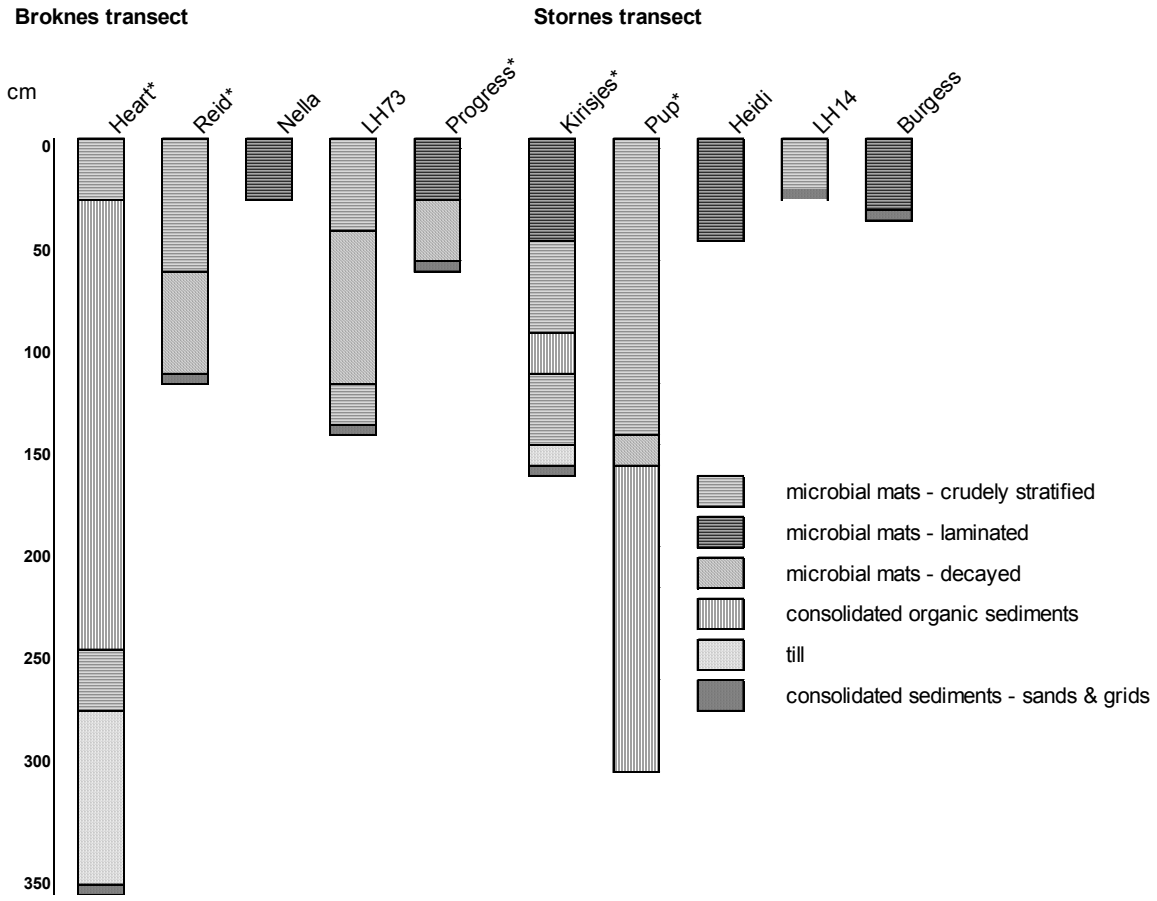

Fig.6: *Core depth and simplified lithologies of the lake-sediment cores used in the LAQUAN project. Cores indicated with an *asterisk were the subject to a detailed paleolimnological analysis (see below).*

For modern and fossil samples, after PCR with primers specific for cyanobacteria (Taton et al. 2003), clone libraries were constructed using the TOPO TA cloning kit (Invitrogen BV, NL). Denaturating Gradient Gel Electrophoresis (DGGE) was also performed as described by Boutte et al. (2006), but with a linear gradient of 40 to 65% denaturant. Partial 16S rRNA sequences were obtained, and analysed as described by Taton et al. (2003). A cross-validation was performed for the cores of Progress L. and Heart L., as described later.

B.2.4. Phylogenetic analyses

B.2.4.1. Diversity analysis

For the study of modern diversity, sequences from clone libraries and strains were used. In the study of past diversity, we mixed sequences obtained with the clone libraries and by DGGE. Modern diversity was analysed as follows. The corrected sequences were grouped in OTU (Operational Taxonomic Units) using the computer program DOTUR (Schloss and Handelsman 2005). An OTU is defined as a group of

sequences sharing more than 97.5% of similarity with each other, similar to the bacterial species definition (Stackebrandt & Göbel 1994). Each OTU might contain one or more species, but is surely distinct from other OTUs at the species level.

Fossil diversity was analysed by BLAST. Each fossil sequence was compared with the modern OTUs and when the similarity was higher than 97.5%, they were included in these OTUs. If the similarity was lower, we dealt with new OTUs.

B.2.4.2. Distance trees

Distance trees were built separately for modern and fossil diversity. For modern diversity, the distance tree was built with the software package ARB for Linux (Ludwig et al. 2004), using a Neighbour joining method with the Jukes and Cantor correction for multiple mutations. Aligned partial 16S rRNA sequences corresponding to positions 405-780 of *E. coli* were used. The tree includes one representative per OTU and per sample for sequences determined in this study, one representative per OTU for Antarctic sequences available in GenBank, the most similar sequences found by BLAST analysis, (when it was a non cultivated sequence, the first strain sequence was added). Finally, one sequence per cluster as determined in the Bergey's Manual by Wilmotte and Herdman (2001) was included as reference.

For the fossil diversity, two distance trees were constructed: one with cyanobacterial and plastid sequences and one with bacterial sequences. The trees include the corrected sequences obtained in this study by cloning and by DGGE plus their most similar sequence found by BLAST and the reference sequences. The trees were constructed by Neighbour-joining based on partial 16S rRNA data, corresponding to *E. coli* sequence positions 485-725 and using the software package TREECON (Van De Peer and De Wachter, 1997). Bootstraps were performed, involving the construction of 100 resampled trees.

B.2.5. Multivariate analysis

In statistical analyses, OTUs were log (x+1) transformed to reduce the influence of dominant groups. Standard multivariate analyses were performed to explore the distribution of cyanobacterial OTUs. Our modern clone and strain sequences were analysed with DCA (Detrended Correspondence Analysis) using CANOCO 4.5 for Windows (ter Braak and Smilauer, 2002) and with a cluster analysis (Sörensen – group average) in PC-ORD 4.0.

B.2.6. Validation study

Twelve layers from the sedimentary cores of Progress and Heart L. were studied in both laboratories by DGGE analyses. Subsamples of the same layers were obtained independently in the laboratories of the two partners and analysed in an identical fashion to enable the comparison. Due to the impossibility to reproduce exactly

identical migration conditions in both laboratories, the PCR products of both laboratories were exchanged and run side by side on the DGGE gels. All the sequences obtained were compared layer per layer. The criterium for validation was the belonging to the same OTU, as defined above. The sequences that were found in both laboratories cannot be the result of contaminations during the experiments (see distance tree in appendix 4).

B.3. Results

B.3.1. Modern microbial diversity

A total of 78 OTUs were obtained when all our 583 clone and strain sequences are combined with all available Antarctic sequences in ULg lab and in Genbank. A plot showing the relative abundance of each OTU per sample is given in appendix 3. Forty-six OTUs are found only in one sample and are presented in white. The number of OTUs belonging to the categories 'Antarctic endemic' and 'found in non Antarctic regions' was calculated for the clone libraries of each of the three regions (AP, PB and SVL) as shown in Figure 7.

The distance tree in appendix 5 shows the genotypic relationships and the composition of the 78 OTUs. Sixteen OTUs were qualified as 'Antarctic' OTUs because they contain only sequences already reported from Antarctica but never found somewhere else (indicated by the ending 'Ant' after the name). 36 OTUs were designated as 'novel' OTUs because their sequences were obtained for the first time during our studies and they are, of course, Antarctic (indicated by the ending 'New' after the name). Finally, the distance tree also comprises 21 'cosmopolitan' OTUs with sequences that were also found in non-Antarctic regions. Five OTUs contain only Antarctic sequences deposited in GenBank by other authors and are not described here.

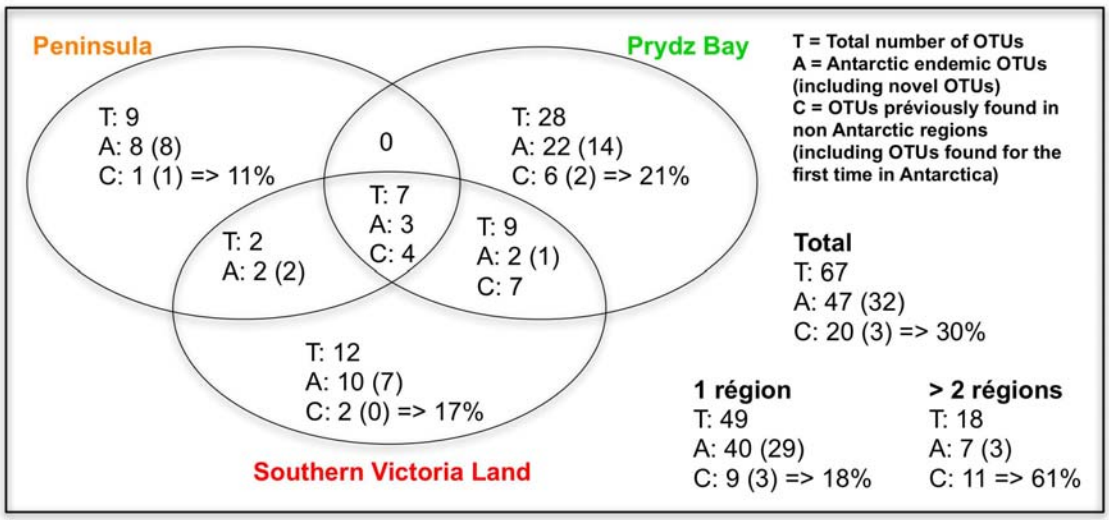

Fig.7: *Geographical distribution of OTUs on the basis of the clone libraries. T: Total number of OTUs - A: Antarctic endemic OTUs (including novel OTUs) - C: OTUs found in non Antarctic regions (including OTUs found for the first time in Antarctica). South Victoria Land (SVL) includes Dry Valleys (DV) and the Mc Murdo Ice Shelf (MMIS), Prydz Bay (PB) contains LH, VH, RI, BI and WI.*

B.3.1.1. OTUs already reported from Antarctica but nowhere else

Eleven Antarctic OTUs are found in only one sample. Five others include sequences from different lakes and regions. OTU21Ant contains our clone sequences from C1 meltwater (AP), Heart L. and L. Reid (LH), L. Fryxell (DV), and the clone CSC1 isolated from cryoconite holes (DV; Christner et al. 2003). OTU19Ant contains our clone sequences from meltwaters C1 and 41 (AP), from L. Reid and Progress L. (LH), and L. Fryxell (DV). In addition, there is one strain sequence from a pond in the LH (Taton et al. 2006b) and 2 clones (LB3-46 from DV (Priscu et al. 1998) and OraP10 from MMIS (Jungblut et al. 2005)). OTU06Ant contains our clone sequences from meltwaters C1 and 41 (AP), Heart L. (LH) and L. Fryxell (DV) and two clone sequences from the Dry Valleys (LB3-76 (Priscu et al. 1998) and CSC14 (Christner et al. 2003)). OTU11Ant contains two strain sequences (LH) and one clone LB3-53 (DV) (Priscu et al. 1998). OTU02Ant contains our clone sequences from Heart L., Progress L., L. Reid (LH), and one clone FreP07 from Fresh Pond (MMIS) (Jungblut et al. 2005).

B.3.1.2. Novel OTUs

Twenty-nine new OTUs are unique to the sample in which they were found. Seven OTUs contain sequences present in several samples from one or several geographic regions. OTU14New contains sequences from meltwaters C1 and 41 (AP). OTU16New contains uniquely sequences from the Larsemann Hills (Heart L., L. Reid and Progress L.). This is also the case for OTU01New, but only with sequences from Progress L. and L. Reid. OTU07New contains sequences from L. Reid (LH) and one sequence from L. Fryxell (DV). OTU04New is composed of sequences from the

meltwaters C1 and 41 (AP) and from L. Fryxell (DV). OTU27New contains one clone sequence from meltwater 41 (AP) and one strain sequence (LH). OTU10New contains one clone sequence from Heart L. (LH) and one strain sequence of L. Gentner (LH). OTU15New includes two strain sequences from different lakes in LH.

B.3.1.3. OTUs also found in non-Antarctic regions

Twenty-two cosmopolitan OTUs and one bipolar OTU are observed. The non-polar sequences includes strains and clones sequences from a variety of habitats (e.g. soil crusts in North-American deserts, rivers in Chile, England, Finland and France, lichens' symbionts, cryptoendoliths in Yellowstone Park travertine, Canada, lakes in France, Ireland and USA, marine coastal sediment, European harbours, stromatolites in Shark Bay, alkaline soil). OTU55 contains both Antarctic and Arctic sequences.

B.3.1.4. Diversity in upper and lower layers of a laminated mat

The separate study of the two layers of sample 41 from the Triangular meltwater (AP) has shown that in the upper layer 30% of the clones were chimeric (instead of a few percents as usual). This phenomenon is generally observed when the DNA is very damaged (Hebsgaard et al. 2005), which is probably due to the high UV levels to which surface layers are exposed. The bottom layer, on the contrary, seems to contain intact DNA, probably because it is efficiently shielded from high UV radiations by the pink-orange coloured surface layer. A total of 15 OTUs was recovered from sample 41, 13 OTUs in the surface layer and 9 in the bottom zone. About half of the OTUs (7) are common to both layers. Unfortunately, we cannot infer the metabolic state of the organisms to which the DNA corresponds as it is impossible to distinguish by PCR between DNA from living organisms, free DNA and DNA from lysing cells.

B.3.1.5. Differences in cyanobacterial sequences between lakes and regions

The cyanobacterial sequences from clone libraries of our 13 Antarctic mat samples were analysed by DCA (Fig. 8). Cluster analysis (not shown) revealed the presence of three distinct groups. Group I includes 3 samples from oligo- to hyposaline lakes from the LH taken at water depth of more than 5 m. Group II contains samples from sites which experience high light intensities and high UV irradiances during the Antarctic summer. Group II consists of the 2 samples taken in the littoral zone (depth less than 1 m) from oligo- to hyposaline lakes in the LH (L. ReidD) and the DV (Fryxell L.), the artificial mat inoculated with a sample from L. Fryxell, as well as the 3 samples from meltwaters and rivers of the AP. Group III includes 4 lakes with relatively high salinities from the BI, VH and RI that are clearly separated from all other samples (Fig. 8). These results imply that regional difference in cyanobacterial communities do exist and suggest that salinity and depth (correlated to light

exposure) could be important parameters for the distribution of cyanobacterial OTUs.

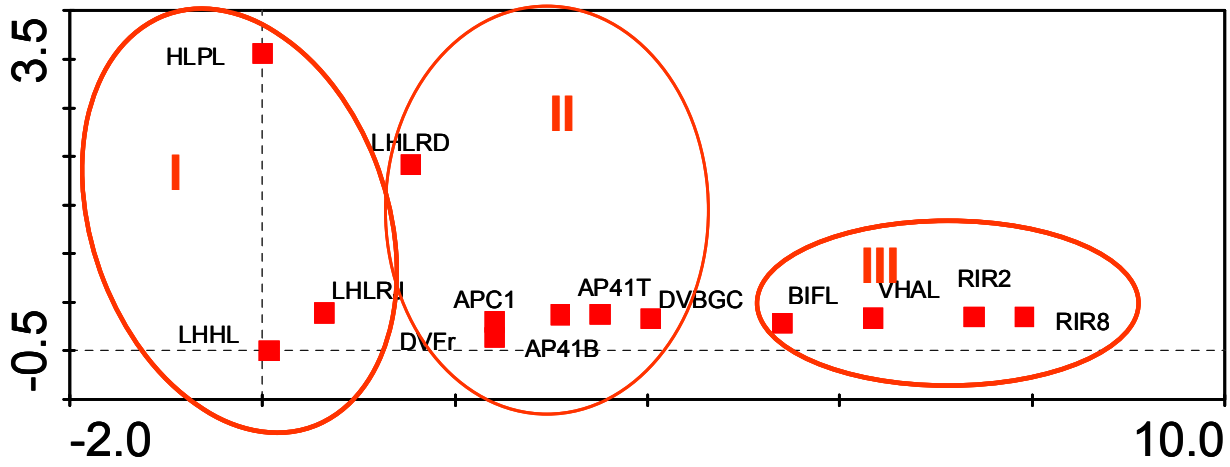

Fig. 8: *Modern cyanobacterial sequences obtained in this study analysed by DCA and plotted by sample. LHHL (Heart L.), LHPL (Progress L.), LHRJL (L. Reid2), LHRDL (L. Reid1), DVFL (L. Fryxell), DVBGC (L. Fryxell artificial mat), APC1 (meltwater C1), AP41T (meltwater 41 upper layer), AP41B (meltwater 41 lower layer), BIFIL (Firelight L.), VHAL (Ace L.), RIR2L (L. Rauer2), RIR8L (L. Rauer8)*

B.3.2. Fossil DNA – testing of molecular tools in Antarctic paleolimnology

The main problem encountered with the analysis of fossil DNA was that bacterial sequences were amplified, even though the primers used were specific for cyanobacteria. The distance tree constructed for the fossil sequences is shown in Appendix 4. A distance tree for bacterial fossil sequences has also been constructed but is not shown in this report.

The numbering of OTUs is identical to the one for modern cyanobacterial diversity. Four additional novel OTUs have been found in the fossil layers (OTU79New to OTU82New) of Heart and Progress L., whereas the novel OTU83New was observed for the first time in LHPSLb (a surface layer of the Progress L. core). No new OTU has been found for L. Reid but only one fossil layer was studied.

In the Progress L. core, the majority of the surface sequences (layers LHPSLa,b,c) are related to cyanobacteria and diatoms, while the sequences from layers at 2, 7, 8, 18, 20 and 27 cm depth are mainly of bacterial origin. Most of the sequences of the mineral and sandy layer LHPSLa are clustered with a plastid from the diatom *Gyrosigma fasciola*. The layer below the sandy layer is blue-green and contains many filamentous cyanobacteria, green algae and a few diatoms (LHPSLb,c). The few cyanobacterial sequences that can still be obtained from the deeper layers (up to 8 cm depth) are affiliated to filamentous (OTU19, 80) or unicellular cyanobacteria (OTU82). The bacterial sequences in the layers up to 8 cm

depth are related to aerobic bacteria, while sequences from layers at 18 till 27 cm depth appear to be related to anaerobic bacteria. We thus observe an aerobic-anaerobic transition from the surface to bottom of the core.

Cyanobacterial sequences in the Heart L. core were found in layers of up to 9500 years old, perhaps due to the good preservation conditions as a result of seawater incursion in the basin and subsequent rapid burial of the layers between c. 9500 and 2500 years ago. Cyanobacterial sequences obtained at 14 cm depth were affiliated to filamentous cyanobacterial sequences (OTU10, the halotolerant OTU63, OTU76 (*Pseudanabaena sp.),* the new OTU79), to one *Nostoc* group (OTU33), and to unicellular sequences related to *Synechococcus* spp. (OTU59 and 58). In addition, DGGE sequences of a new plastid group were found and seem to be distantly related to *Chlorella mirabilis*. In the deeper freshwater layers at 254 cm depth, only OTU58 was found. Notably, this unicellular OTU is also found in the marine layer at 231 cm depth and in the glacial till zone at 346 cm depth. In addition, this OTU includes sequence AY303360, an unpublished DGGE sequence from freshwater Holocene sediments in Ace L. in the nearby Vestfold Hills. In the marine layer at 231 cm depth, in addition to OTU58, sequences related to a typical marine diatom (*Chaetoceros socialis)* plastid were found. The glacial till in the oldest layer (LHHL346) consists of glacially derived rocks of various origins, including potentially terrestrial material. Interestingly, a *Calothrix* sequence is present in this layer, which was also retrieved at 2 cm depth from the same core whereas it has not been observed before in microbial mats from Antarctic lakes.

In the L. Reid core only one fossil layer of c. 6000 yrs old was studied. The fossil sequences were related to Antarctic cyanobacterial clones from Fryxell (FR121 (OTU07) and FR397 (OTU19)). OTU19 corresponds to a *Leptolyngbya* sp. present in all three Antarctic regions (Appendix 5).

The sequences of L. Reid core appear in the same OTUs as sequences of the Progress L. core, whereas samples from the Heart L. core have a different diversity. OTU58 appears to be characteristic for the latter core. It includes *Synechococcus*-like strains from temperate freshwater and marine coastal habitats that probably present a certain halotolerance.

B.4.Discussion

B.4.1. Modern cyanobacterial diversity

The study of modern cyanobacterial communities revealed that each lake is quite unique in terms of diversity and taxonomic composition; every single lake studied resulted in the discovery of new Operational Taxonomic Units (OTUs). There is thus a lot more cyanobacterial diversity to discover, which definitely merits further taxonomic inventories of Antarctic lakes using molecular techniques.

Due to different methodologies and protocols, it is difficult to compare our data with results from other polar and temperate biotopes and regions. However, we observed a relatively larger number of OTUs in our samples than could be expected under the extreme environmental conditions knowing to occur in Antarctica. The diversity was particularly high in the freshwater lakes; the number of OTUs varied between 8 and 13 in dilute lakes, whereas it was lower in the samples from the more saline lakes (4 OTUs in L. Rauer 8 and 5 in Ace L). This indicates that salinity has a profound effect on cyanobacterial diversity in Antarctica. The potential structuring role of salinity is also evident when the community structure is compared between the different samples. The cyanobacterial flora varies between saline and freshwater lakes implying that both lake types cluster in different groups in the ordination and cluster analyses.

Our results furthermore indicate that shallow littoral zones or meltwaters from different geographic regions are relatively similar in terms of taxonomic composition, probably as a result of the occurrence of taxa that are well adapted to high light and UV intensities in these environments. Salinity and water depth could thus be important factors in structuring the Antarctic cyanobacterial communities. It is however not clear if environmental conditions alone can explain the differences in community composition. Samples from lakes in the same geographic region, yet, with different ecological characteristics (L. Reid and Heart L.; Fig.8) are relatively similar in terms of cyanobacterial taxonomic composition. The provinciality might thus indicate that geographical, dispersal related factors are important in structuring cyanobacterial communities.

The importance of dispersal limitation can also be inferred from a comparison of Antarctic OTUs with non-polar sequences in Genbank (about 4300 available). In total, 55 out of 78 OTUs were restricted to Antarctica. Some genotypes were even present only in one sample. This hints to the existence of endemics, which implies that some cyanobacteria are probably not cosmopolitan and are thus limited in their dispersal capacity. It is however uncertain if the apparent endemism is real because the cyanobacterial sequences available in Genbank do not reflect the entire diversity of this phylum, nor the whole range of biotopes in which they occur. More in particular, it is unfortunate that there are no sequences from sub-Antarctic regions that could be used to better assess the geographical range and distribution of our OTUs (Gibson et al. 2006). The same remark holds true for the lack of sequences from cold alpine lakes with quite similar environmental conditions, and the small number of Arctic sequences that hinder the detection of potential bipolar OTUs.

On the basis of clone libraries, 55 % of the 'cosmopolitan' OTUs are distributed in at least two Antarctic regions whereas only 15% of the Antarctic OTUs occur in more than two sites. Our hypothesis is that non-polar OTUs had to be well adapted to deal with harsh conditions during dispersion and colonization of the

Antarctic biotopes. These adaptations could similarly have been quite useful in spreading to various habitats in different geographic regions across Antarctica.

OTUs distributed in several of our lakes are often already found by other authors in other biotopes than benthic microbial mats, namely terrestrial mats, dust inclusions in ice cover, cryoconites, and cryptoendolithic habitats in quartz stones. This is in agreement with the observation of the existence of a continuum between terrestrial and aquatic biotopes by Gordon et al. (2000).

B.4.2 Past cyanobacterial diversity

It is likely that changes in the distribution of OTUs with sediment core depth record changes in the cyanobacterial community composition and ecology of the lake. As the sediments in these lakes are cold but not frozen, it is probable that the spontaneous chemical decay of DNA continues at a slow pace and the effect of fossilisation processes should thus be considered as well. The taphonomy of organisms might be group- and even species-specific. Differential fossilization processes might therefore lead to a selection of sequences which remain available for amplification by PCR. For example, DNA from gram-negative bacteria is degraded earlier than DNA from gram-positive Actinobacteria (Willerslev et al. 2004a). In addition, many groups of bacteria seem to be living in the cores, and their DNA (of better quality) is isolated at the same time as fossil cyanobacterial DNA and can act as competitors during PCR, which might introduce additional bias.

In general, sequences from older layers appear to be a subset of those from younger layers. Only 5 new OTUs were obtained (OTU79New to OTU83New), that have no close relatives in Genbank, except for OTU82 that is closely related to PCC7502 isolated from a *Sphagnum* bog in Switzerland. OTU79 to 82 were exclusively fossil, but OTU83 was also found in a surface layer of Progress L. with living cyanobacteria. Noteworthy, OTU81 contains heterocystous cyanobacteria while only two heterocystous OTUs were observed among the fossil sequences. The other 8 OTUs in the fossil layers were already known from Antarctic modern mat sequences.

A validation of the results was performed for 5 layers of the Progress L. core (from the surface till 8 cm depth) and for 7 layers of the Heart L. core (from the surface till 346 cm depth) by comparing the data obtained in the laboratories from the two partners. To this end, we aimed to ensure that the sequences obtained were really from fossils layers and not the result of a contamination with modern samples. The DGGE method is very difficult to standardise as many parameters can affect the separation of the PCR products on a denaturating gradient, and the possibility to obtain band sequences. Therefore, we were not able to get good quality, comparable fingerprints in all cases, nor to extract all bands. However, when sequences obtained in both labs belonged to the same OTU, they could be assumed to be free from

experimental contaminations. This was the case for 75% of the sequences in the modern layers and 55 % in the fossil layers.

The OTUs that were found in the oldest layers in which cyanobacterial sequences could still be retrieved were OTUs19 for L. Reid (6000 years old), OTU58 and 81 for Heart L. (over 9500 years old), OTU19 and 82 for Progress L. (c. 2000 years old). The first two are widespread in Antarctica (OTU19) or cosmopolitan and halotolerant (OTU58). The latter two appear as novel fossil sequences. The organisms from which DNA was still amplifiable after thousands of years were thus either 'generalists' with a wide ecology or 'specialists' which were for the first time observed in Antarctica. In addition, the oldest layer with a plastid sequence was the marine core level LHHL231 (c. 7500 years old). As plastid sequences can be amplified with the cyanobacterial-specific primers, they yield additional information on the past algal diversity.

The present study was a first attempt to use fossil cyanobacterial DNA to reconstruct the paleodiversity of the populations. As such, it has identified the problems associated with the use of fossil DNA, namely (1) the presence of good-quality bacterial DNA that act as competitor of fossil cyanobacterial DNA during PCR, (2) the continuing degradation of fossil DNA, and (3) the resistence against degradation of cyanobacterial DNA might be group specific. However, only two cores could be extensively studied, implying that the data set is too restricted to make conclusions on the representativity of the obtained sequences and the possibility to compare modern and fossil diversity.

PART 2: THE INTEGRATED PALEOECOLOGICAL ANALYSIS OF THE LARSEMANN HILLS

A. CLIMATE HISTORY

A.1. INTRODUCTION

The evolution of past climate changes spanning over 740,000 years in Antarctica are becoming well documented from stable isotopes in ice cores (e.g. EPICA members 2004). However, little is known about how long-term climate changes were expressed in the terrestrial environment. This gap in knowledge exists mainly because most Antarctic coastal oases were covered by continental ice sheets at the Last Glacial Maximum (LGM), eliminating the terrestrial paleorecord. However, recent evidence that some areas (e.g., the Bunger Hills; Gore et al. 2001) were ice free at the LGM and during Termination I (last glacial–interglacial transition) has provided an opportunity to examine environmental change over longer time scales. In these ice-free oases, lake sediments have accumulated evidence of physical processes and biological activity from which it is possible to piece together a paleoenvironmental record.

In this study we present a continuous reconstruction of climate-driven changes in the second largest eastern Antarctic oasis, the Larsemann Hills, through the last glacial cycle (Eemian to Holocene), employing multiple proxies (sedimentology, geochronology, fossil pigments, diatoms) in lake-sediment cores from a combination of isolation lakes (Pup Lagoon, Heart L., Kirisjes Pond) and proglacial lakes (L. Reid, Progress L.). Paleoclimate is inferred from both the lacustrine and marine sediments in the isolation lakes. In the lacustrine sediments, the moisture balance is quantitatively inferred using the diatom based transfer functions described above (see also Verleyen et al. 2003). In the marine sections of the cores, coastal sea surface conditions are reconstructed using the presence and abundance of diatoms characteristic of open water conditions (e.g., *Chaetoceros* resting spores) or of sea-ice cover (e.g., *Fragilariopsis* species) in combination with productivity data based upon fossil pigments.

A.2. MATERIALS AND METHODS

Lithology and geochronology

The cores were taken as described above, photographed, macroscopically described, and analysed for wet density, dry weight, organic content and carbonate content using standard methods. A chronology was established using a combination of radiometric (^{137}Cs, ^{210}Pb), radiocarbon (^{14}C) and thermoluminescence dating.

Where possible, samples for radiocarbon were derived from discrete biological remains of cyanobacterial mats at distinct stratigraphic boundaries (Hodgson et al. 2001b). Dates up to 20,265 ^{14}C yr BP (limit of calibration curve) are reported as conventional radiocarbon years BP and as calibrated years BP (cal. yr BP relative to AD 1950) using the atmospheric decadal dataset in CALIB 4.3 (option A, Stuiver and Reimer 1993). A reservoir correction is applied to radiocarbon dates of marine samples, by subtracting 1300 years, following recent conventions for the Southern Ocean (Ingólfsson et al. 1998). No reservoir correction is applied to dates from lacustrine sediments, because the surface sediment dates indicate that ^{14}C in modern freshwater surface sediments are in near equilibrium with modern atmospheric CO_2 (Hodgson et al. 2001b; Appendix 2). Age depth curves were constructed to extra- and interpolate major changes in the core.

In order to confirm the age of sediments older than the AMS radiocarbon dating limit (i.e., c. 45,000 yr BP) further analyses were carried out using thermoluminescence dating. The dates are presented as wide age ranges as the sediments have some unusual TL properties, which may influence the accuracy of the chronological information. More in particular, an unusual glow curve shape, with a greater than normal ratio of high temperature to low temperature TL was observed, together with a supra-linear shape in the early stage of the growth curve and an unusually high TL sensitivity to alpha radiation. We therefore use these dates with caution. The results are reported as TL yr BP.

Siliceous microfossils and fossil pigments

Diatoms, stomatocysts and fossil pigments were analysed using the methods described above. At least 400 valves (>2/3 intact) and/or stomatocysts were counted in each sample. The diatom-based transfer functions were used to reconstruct historical lake salinity and water depth (see part 1). Further high resolution pigment separations were carried out on selected sediment layers using HPLC and APCI LC-MS (Squier et al. 2005).

Ecological modeling and statistical analyses

Fossil diatom assemblages from the core were compared with the reference data set of modern assemblages (see above), using standard ordination techniques in CANOCO 4.5 for Windows (ter Braak and Smilauer 2002). Stratigraphic data were zoned using CONISS, a stratigraphically constrained cluster analysis following squared root transformation and plotted using TILIA 2.0b4, TILIA*GRAPH 2.20 and TGView version 1.1.1.1 (Grimm 2001). Only taxa which are found at more than 4 % relative abundance in the isolation lakes and 2 % in the proglacial lakes were incorp

A.3. RESULTS

A.3.2. Lithology and chronology of the sediment cores

A lithological diagram can be found in Fig.6. A detailed description of the sediment stratigraphy can be found in Verleyen et al. (2004c) for Heart L., Pup Lagoon and Kirsjes Pond, in Hodgson et al. (2005a) for L. Reid, and in Hodgson et al. (2006) for Progress Lake. All ^{14}C dates are summarized in Appendix 4 and published and discussed in Hodgson et al. (2001b); additional TL dates are given in Verleyen et al. (2004c) and Hodgson et al. (2005a).

A.3.1. Deglaciation history of the Larsemann Hills

Measurements of the present and former ice flow indicators, coupled with the dating program, permit us to present the likely scenario for the deglaciation history of the region (Fig.9). The finding that the Larsemann Hills were ice-free during the LGM, implies that lakes in this region may contain unique and some of the oldest continuous sediment sequences from Antarctica (see below).

The deglaciation history of the Larsemann Hills was reconstructed based on geomorphological evidence (glacial striae directions) and radiometric (^{210}Pb and ^{137}Cs), radiocarbon (AMS ^{14}C) and uranium series (^{238}U) dating techniques in sediment cores from 11 lakes on two coast to sea transects. More specifically we aimed to test the hypotheses that parts of the Larsemann Hills were ice-free during the Last Glacial Maximum (LGM) as previously suggested in Burgess et al. (1994), but in contrast to earlier findings by Gillieson (1991).

A.3.3. Climate change on a glacial-interglacial timescale in the Larsemann Hills

Climate change during the past glacial-interglacial cycle were reconstructed using sediment cores from lakes situated on Broknes, namely L. Reid and Progress L., as this peninsula was shown to be ice-free during the LGM (see Fig.9).

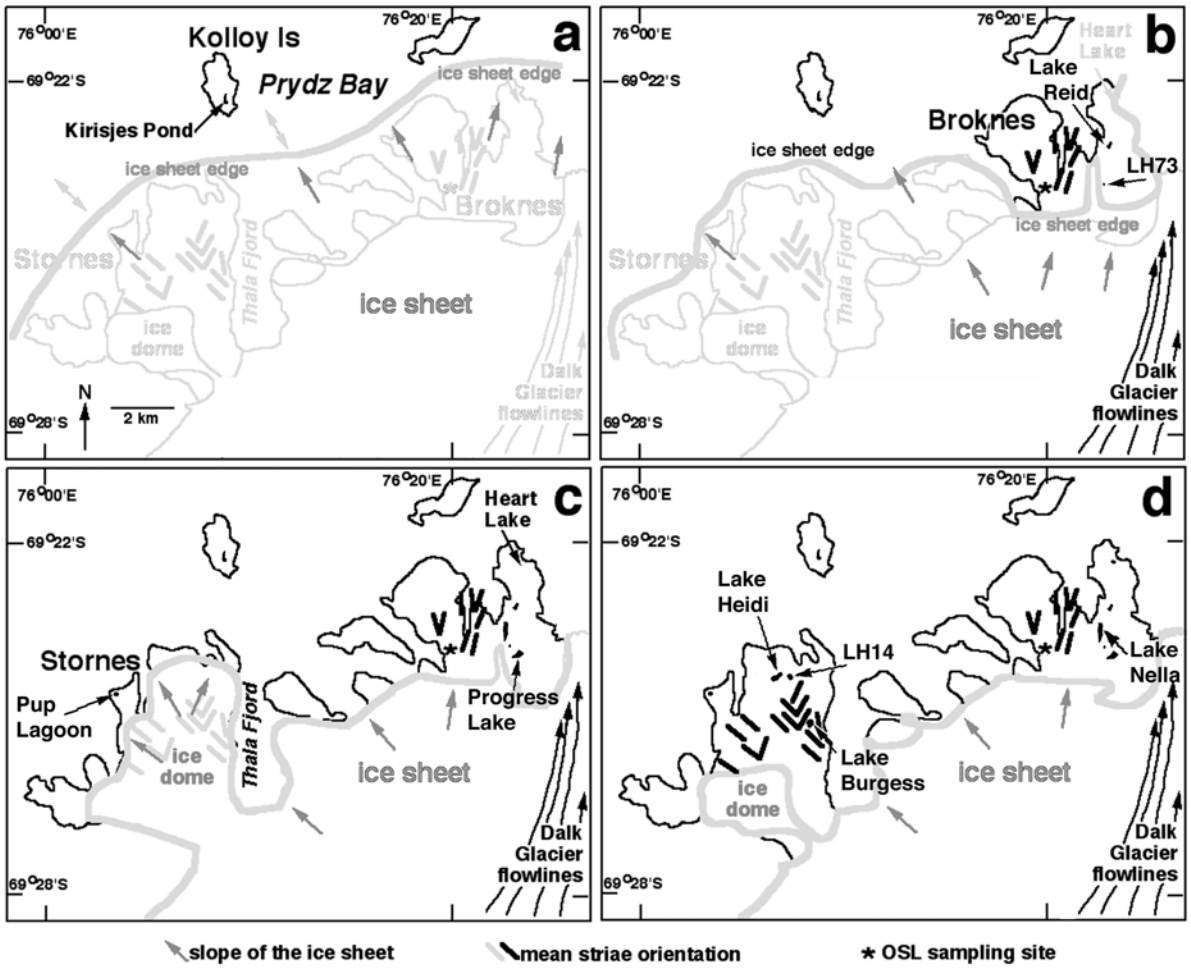

Fig.9: *Interpretive deglaciation history of the Larsemann Hills. (a) Sometime preceding 40 ka BP the ice sheet covered both peninsulas, we are uncertain of the location of the ice edge and the timing of the advance is unknown. Ice advanced across Thala Fjord, deriving fossiliferous Pliocene marine muds and smearing them across Stornes. (b) At some time preceding 40 ka BP and lasting until at least 20 ka BP, an expanded ice sheet covered most of Stornes. Islands to the north were exposed. In contrast, Broknes remained largely free of ice with a minor encroachment of the Dålk Glacier to the northeast and a minor readvance of the ice sheet margin in the vicinity of Progress Lake until sometime after 20.9 ka BP. A lobe of stagnant ice lay in the Lake Nella basin. (c) By ~4 ka BP the ice sheet had receded from Stornes, leaving the periphery of the peninsula exposed, but with most of the area covered by an ice dome which exhibited radial flow. On Broknes, the Lake Nella ice lobe had receded (after 6.6 ka BP, or 1.6 ka BP) and the ice sheet margin was similar to that of today. (d) 0 ka BP. The remnants of the Stornes ice dome now occupy a minority of the area.*

The Lake Reid core

The diatom stratigraphy in the L. Reid core is divided into four zones based on CONISS cluster analysis (Fig.10,11). Zone 1 (104–114 cm) is dominated by *P. abundans*, a diatom currently found in deep freshwater lakes. The ratio of stomatocysts/diatoms is high, reconstructed lake water depth is deeper than present

and reconstructed salinity fluctuates around 2 ppt (Fig.11). In Zone 2 (102–76 cm) the percentage of *P. abundans* declines and is replaced by *D.* cf. *perpusilla*, a species characteristic of shallow ponds, and *S. inermis*.

Pinnularia microstauron appears for the first time and there is an increased percentage of the aerophilic diatom *L. muticopsis*. A number of other species are present, characteristic of freshwater to brackish tolerant taxa together with a few in-blown marine taxa. The stomatocyst/diatom ratio is highest in this zone. Reconstructed lake water depth is variable and lower than in Zone 1, with a minimum recorded at 96 cm (Fig.10). In Zone 3 (74–40 cm) most species decline rapidly and are replaced by *S. inermis*. The stomatocyst/diatom ratio is lowest in this zone of the core. Reconstructed lake water depth is extremely constant, reflecting the near monospecific diatom assemblage. Finally, at the beginning of Zone 4 (38–0 cm) there is a rapid increase in species diversity with a decline in *S. inermis* being offset by an increase in the brackish water tolerant taxa including *A. veneta*, *P. microstauron*, *C.* cf. *molesta* and *L. muticopsis*. There is an underlying trend of increasing salinity from 20 cm towards the top of the core this zone (Fig.10).

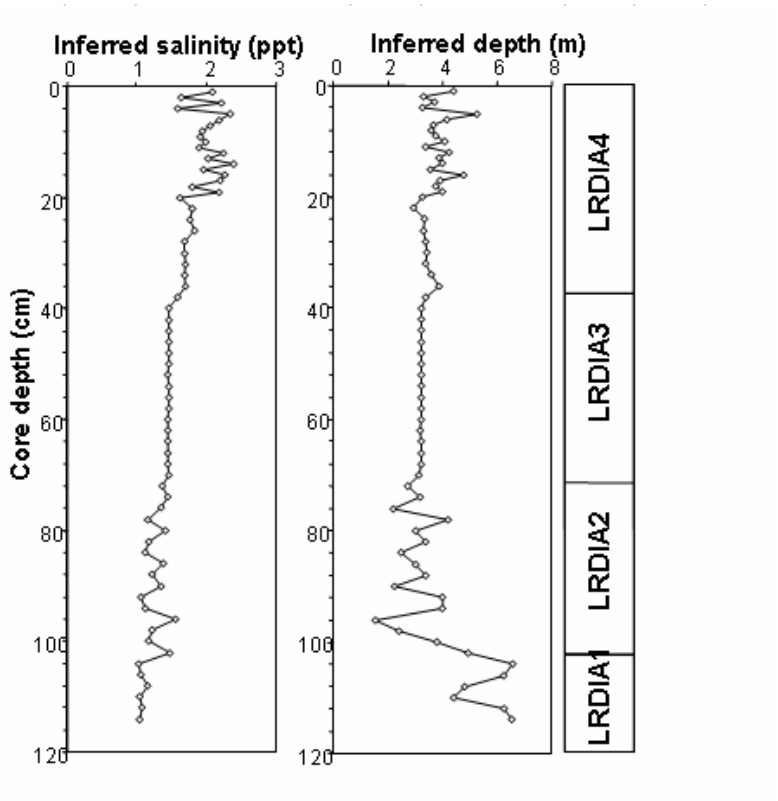

Fig.10: *Reconstructed salinity and lake water depth in the L. Reid core using the diatom-based inference models.*

The core contains a mixture of chlorophylls, carotenoids, bacteriochlorophylls and scytonemins derived from a primary producer community comprising both oxygenic and anoxygenic photoautotrophs. There are a number of common taxon-specific

pigments, including diatoxanthin (diatoms), myxoxanthophyll (filamentous cyanobacteria), lutein-zeaxanthin (green algae and cyanobacteria), scytonemin, echinenone, canthaxanthin and myxoxanthophyll (cyanobacteria) and chlorophyll b (green algae and mosses). Plots of total chlorophyll and carotenoid concentrations (Fig.12) show a similar trend to the diatom data, suggesting that the whole oxygenic photoautotrophic community responded in a similar manner to changes in the environment. The relative abundance of the chlorophyll a-derivatives changes throughout the core, with some being restricted to the lower Zones 1–3 (pyrophaeophorbide). The carotenoid composition is mainly derived from cyanobacteria, diatoms and green algae. Diatoxanthin is relatively concentrated in Zones 1, 2 and 3. Ratios of all carotenoids are relatively stable in Zones 1–3 with more variability evident in Zone 1. The concentration of scytonemin, an extra-cellular sunscreen pigment produced by shallow-water cyanobacteria to protect against harmful UV-radiation, is markedly higher in Zones 2 and 3 (Fig.12). Low pigment concentrations were detected in samples at 72 and 74 cm.

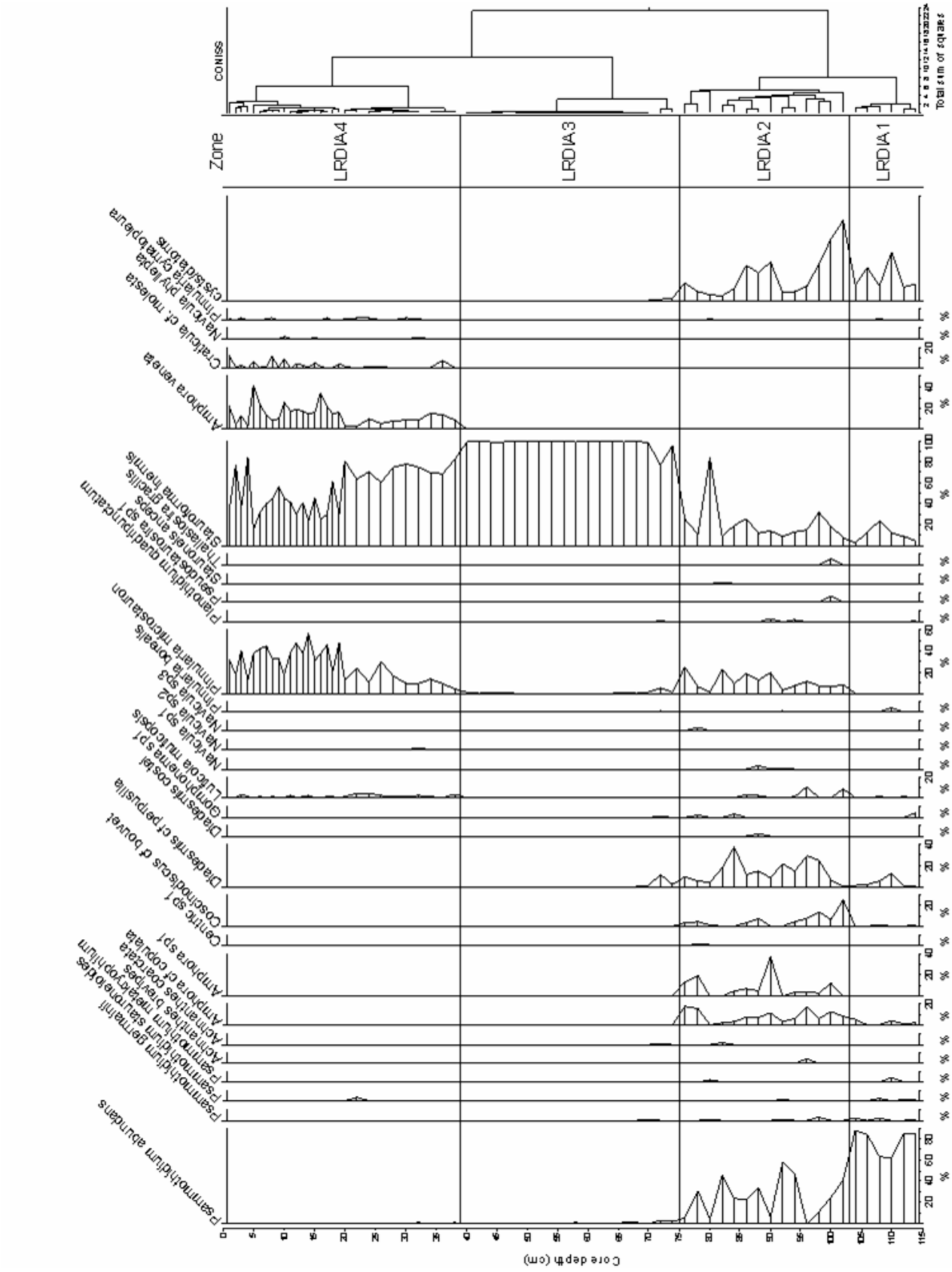

Fig.11: *Diatom stratigraphy (%) and stomatocyst/diatom ratio in the Lake Reid core. Zoning is based on CONISS constrained cluster analysis.*

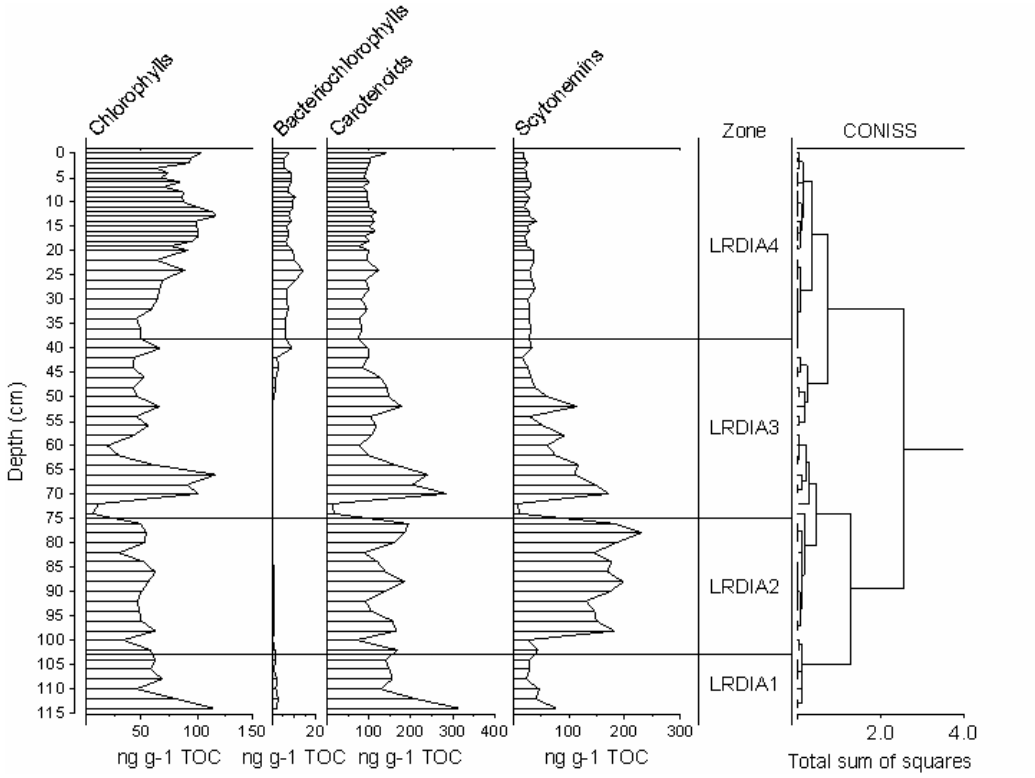

Fig.12: *Pigment stratigraphy. (a) Total chlorophyll, carotenoid, scytonemin and bacteriochlorophyll stratigraphy down the core expressed as organic matter specific concentration per gram TOC (ng g^{-1} TOC).*

The Progress Lake core

Based on stratigraphically constrained cluster and ordination analyses, the diatom stratigraphy in Progress Lake was divided into two zones (Fig.13), which correspond with the lithological units (Fig.6). In accordance with other continental Antarctic datasets, there was low diatom species diversity (Shannon-Weaver diversity index) despite relatively high concentrations of diatoms. Diatom concentration and species diversity were significantly negatively correlated (r^2=0.924, $p < 0001$). The lower unit of the core (Zone 1) had the highest species diversity. Interestingly, some of the taxa from Zone 1 are not currently found in the Larsemann Hills or the modern East Antarctic reference dataset (see above and Verleyen et al. 2003). Of these, at least three (*Diadesmis costei, Diatomella balfouriana and Psammothidium manguinii*), and possibly as many as nine taxa are believed to be absent from the entire landmass of East Antarctica (cf. Kellogg and Kellogg, 2002) and include diatoms currently found in the sub and maritime Antarctica, including a species considered as a sub-Antarctic endemic (e.g. *D. costei*, Van de Vijver et al. 2002). Of these taxa, some are associated with soil and moss habitats (*D. costei, D. balfouriana*) and others with aquatic mosses (e.g. *P. manguinii*). With the exception of a few isolated colonies, mosses are rare in the Larsemann Hills today. A peak in *Gomphonema* cf. *parvulum* occurred at 41 cm and resulted in a sharp decline in the diatom species diversity. In Zone 2 there was a substantial decrease in species diversity and a change in species

composition. The diatom assemblage included 10 of the 31 lacustrine species described from the present day flora of 66 regional lakes and ponds and is characteristic of deeper lakes in the region (Sabbe et al. 2003). The assemblage was dominated by *P. abundans,* with *S. inermis* and *P. microstauron* being sub-dominant. All diatom taxa in Zone 2 are found in the present day East Antarctic reference dataset (Verleyen et al. 2003).

The changing distribution of pigment biomarkers, derived from both oxygenic and anoxygenic primary producers, records changes in the community composition and ecology of the lake. The highest concentrations of chlorophylls, bacteriochlorophylls, carotenoids, and xanthophylls occurred in Zone 1. There was a diverse assemblage of chlorophyll pigments dominated by chlorophyll a derivatives with further contributions from chlorophyll b and bacteriochlorophyll a-derived components. The carotenoids were derived from green algae and/or mosses cyanobacteria, diatoms, and possibly chrysophytes. Lutein (green algae and mosses) and nostoxanthin (cyanobacteria) were dominant in this zone and showed several clear periods of alternating dominance. Scytonemin (cyanobacteria) was present in low abundance in Zone 1. The uppermost (living) layers consisted of two discrete sub-millimeter thick laminae consisting of a thin layer of diatoms (fucoxanthin, chlorophyll a) together with green algae (chlorophyll b), which appeared to represent growth through the late summer and winter months. About a millimeter below this was a layer representing the previous early spring and early summer growth period where concentrations of chlorophylls and carotenoids reach a maximum of 41 and 55 ng g^{-1}TOC respectively. The composition in this spring and early summer growth period consisted of green algae (chlorophyll b), green algae and cyanobacteria (lutein, zeaxanthin), cyanobacteria (echinenone, nostoxanthin) and diatoms (fucoxanthin).

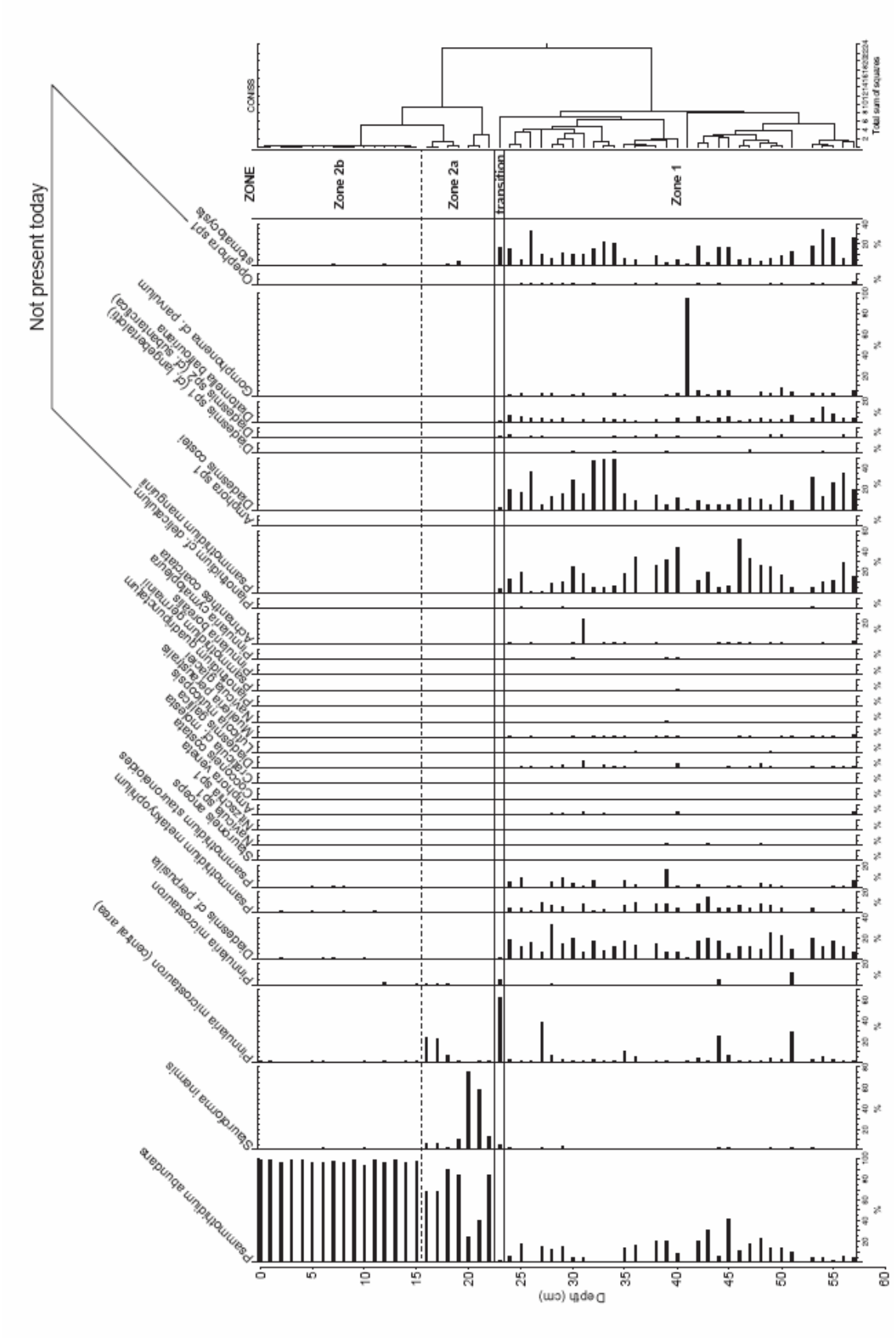

Fig.13: *Diatom stratigraphy of Progress Lake. Zoning is based on CONISS analysis.*

A.3.2. Holocene climate history of the Larsemann Hills

The Holocene climate history is mainly based on the analysis of the sediment record in the isolation lakes. ^{14}C dating revealed that the accumulation rate was high in some of the water bodies, allowing a relatively high resolution study to be made. Four sedimentological zones can be recognized in the cores (Fig. 14), namely a marine zone, a lacustrine zone, a transition zone between marine and lacustrine and a zone of unstructured diamicton, which we interpret as a glacial till. In the latter, diatoms are rare and highly fragmented, the material is well-mixed and organic content is low. The siliceous flora in the transition zone is dominated by stomatocysts (presumably resting stages of chrysophytes).

Pup Lagoon core

In the Pup L. (PL) core three main diatom zones were identified, namely a marine PLDA1, a freshwater PLDA2 and a transition zone (PLDA3; Fig.14, see also Verleyen *et al*. 2004c). PLDA1 (302 - 150 cm, *c*. 5800 - 2140 cal. yr BP) is dominated by marine and sea ice associated diatoms (e.g., *F. cylindrus*, *Fragilariopsis curta* and *Navicula glaciei*). The number of frustules per gram sediment dry weight is significantly higher in this zone compared with the rest the core. PLDA1 is subdivided in three zones, PLDA1A, PLDA1B and PLDA1C. These closely correspond to the zones identified in the pigment analysis. *Chaetoceros* resting spores are relatively abundant in PLDA1A, at 225 cm and in the basal levels of PLDA1C. The upper layers of the latter zone are dominated by the brackish water diatoms *Navicula phyllepta* and *C. laevissimus*. High productivity conditions were thus inferred from the presence of *Chaetoceros* resting spores between 5800 and 5500 cal. yr BP and between 2750 and 2200 cal. yr BP. PLDA2 (150 - 140 cm, *c*. 2140 - 2000 cal. yr BP) is dominated by stomatocysts, presumably siliceous resting stages of Chrysophyceae and by lacustrine brackish water diatoms. The diatom assemblage in PLDA3 (140 - 0 cm, *c*. 2000 cal. yr BP - present) is dominated by the freshwater diatom *S. inermis* and by *P. microstauron*. The euryhaline diatom *A. veneta* is more abundant in the lower levels of this zone (PLDA3A, 140 – 95 cm, c. 2000 – 1500 cal. yr BP). In a single sample at 100 cm depth *Planothidium quadripunctatum* occurs, together with freshwater (*P. abundans*), and brackish water (*C. laevissimus*) diatoms and some marine and sea ice associated diatoms (*F. curta*). Reconstruction of the moisture balance using the diatom based transfer function (Verleyen et al. 2003) revealed that lake water depth remained nearly constant from until present. The lake has since 1500 cal. yr BP thus probably been an open with an outflow stream and annually flushed during spring and summer by melt water from snow banks in the catchment, probably comparable with the present day situation.

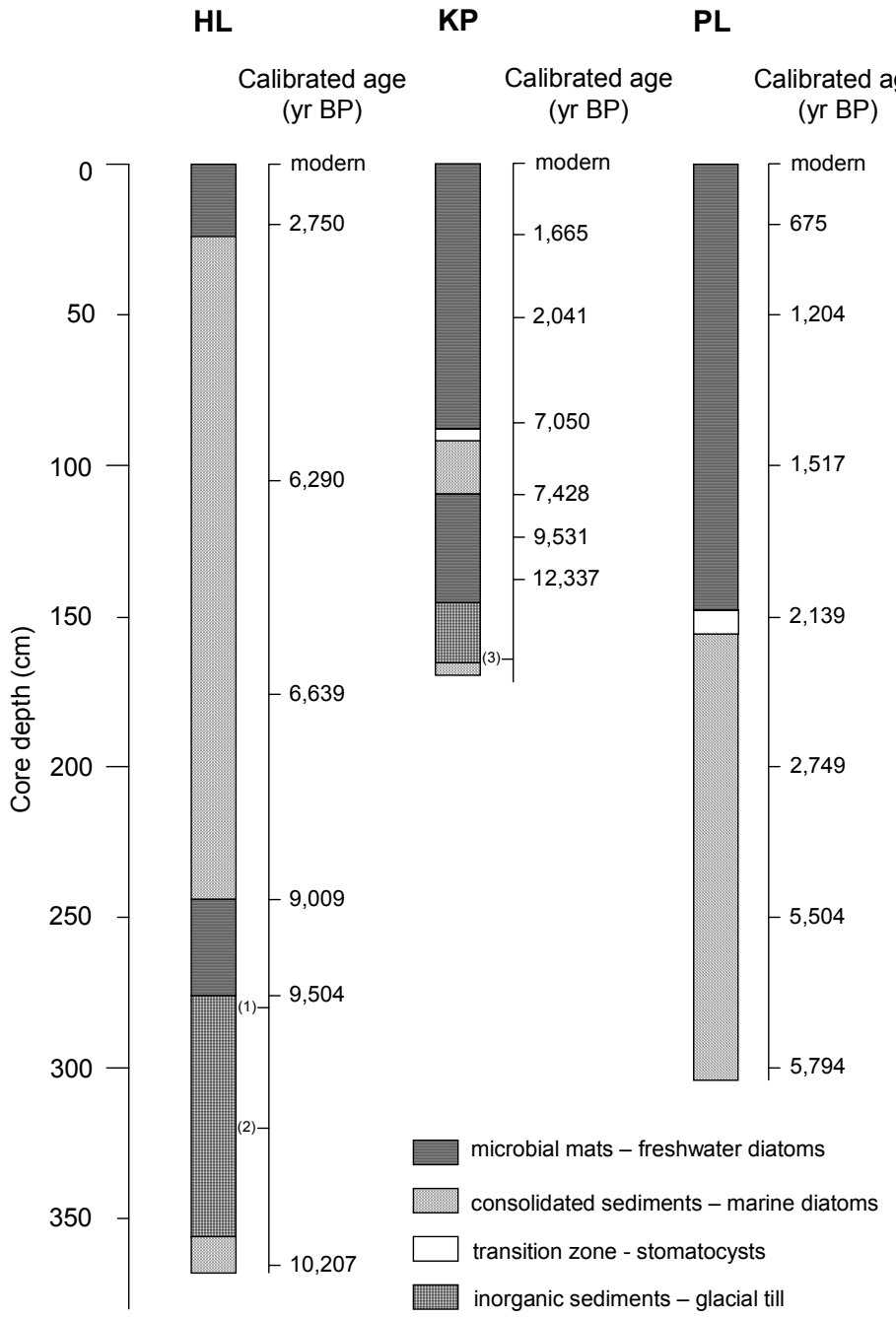

***Fig.14**: Stratigraphy of the Heart Lake (HL) and Kirisjes Pond (KP) core and the previously published (Verleyen et al. 2004a) Pup Lagoon core (PL). Zoning is based on diatom data. Radiocarbon dates in HL at 280 cm [(1)] and 320 cm [(2)] and in KP at 156 cm [(3)] are respectively 21,780, 25,460 yr BP and <43,200 yr BP. TL dates in the HL core at 275-280 cm and 330-335 cm range respectively between 4,100 – 8,900 TL yr BP and 65,000 – 98,000 TL yr BP and in the KP core, the age of the glacial till zone falls within the interval of 24,000-35,000 TL yr BP (at 148-150 cm) and 30,000-43,000 TL yr BP (at 152-154 cm).*

Kirisjes pond core

Kirisjes Pond (KP) is, in contrast to PL, currently a closed lake (with an inactive outflow channel situated 1.5-2 m above the present lake level). This gives it the potential to register changes in the climate driven moisture balance by applying the transfer function. Salinity and water depth are significantly and negatively correlated (R^2=-0.63, P=0.000) throughout the lacustrine sediments (Fig.15), which is in agreement with the general pattern of rising lake level leading to decreasing salinity. The majority of the sediments are deposited under lacustrine conditions, with some minor marine transgressions and glacial deposits interrupting them (Fig.14,15), giving the potential to quantitatively reconstruct almost the entire Holocene moisture balance.

The core can be divided into six main diatom zones (Fig.14). The diatom assemblage in the first zone (KP I, 158-156 cm, > 43,200 yr BP) consists of a mixture of marine and brackish water diatoms (*Tryblionella marginulata, F. curta* and *Thallasiora oestroepii*) together with some freshwater taxa as co-dominants (*P. microstauron*). The second zone (KP II, 156-144 cm, *c.* 43,200 yr BP-13,500 cal. yr BP) is an inorganic diamicton with low diatom abundance and fragmented valves (subsequently referred to as a glacial till zone). A freshwater zone (KP III) is situated between 144 and 112 cm (*c.* 13,500-7700 cal. yr BP), with *S. inermis* being the dominant diatom. Modelled salinity and depth are highly constant between *c.* 13,540 and 9,530 cal. yr BP (Fig.15). The relative abundance of *S. inermis* is near 100% between *c.* 13,500 and 11,500 cal. yr BP, whereas *P. microstauron* becomes sub-dominant from *c.* 11,500 until 9,530 cal. yr BP. Drier conditions (low lake level and high salinities) are present between *c*. 9530 and 8030 cal. yr BP. Marine taxa (*Chaetoceros* resting spores, *Fragilariopsis* spp.) dominate between 112 and 94 cm (KP IV, *c.* 7700-7080 cal. yr BP). A well-marked transition zone (KP V, 94-88 cm) between marine (KP IV) and lacustrine conditions (KP VI) is recognized with stomatocysts being the dominant siliceous fossils together with brackish water diatoms (e.g., *T. marginulata*), similar to the transition zone in the Pup Lagoon core (Verleyen *et al*. 2004a). From 88 until 0 cm (KP VI, 7080 cal. yr BP-present) the siliceous assemblage is composed of lacustrine diatoms.

In the early stages of the lake's development (KP VI), water level and salinity are positively correlated (in contrast with the rest of the core) possibly due to seawater incursion leading to higher salinity and rising lake level whilst the lake sill was still within the maximum tidal range. After complete isolation, salinity and lake water level are mainly controlled by the evaporation-precipitation balance and meltwater input from the catchment area, leading to negative correlations between both variables (Fig.15). Between *c.* 4100 and 3810 and between *c.* 2925 and 2015 cal. yr BP a more positive water balance can be recognized during the, in general, relatively dry interval from *c.* 6,000 until 1,700 cal. yr BP. From 50cm (*c.* 2,040 cal. yr

BP) until present, the sedimentation rate is relatively high, allowing high-resolution reconstructions to be made. From *c.* 1665 cal. yr BP until present averaged lake level is higher and averaged salinity lower than in the rest of the core, suggesting more melt water input. In this zone, three periods with a negative water balance can be recognized, namely around *c*. 1180, between *c*. 760 and 690 and between c. 280 and 140 cal. yr BP.

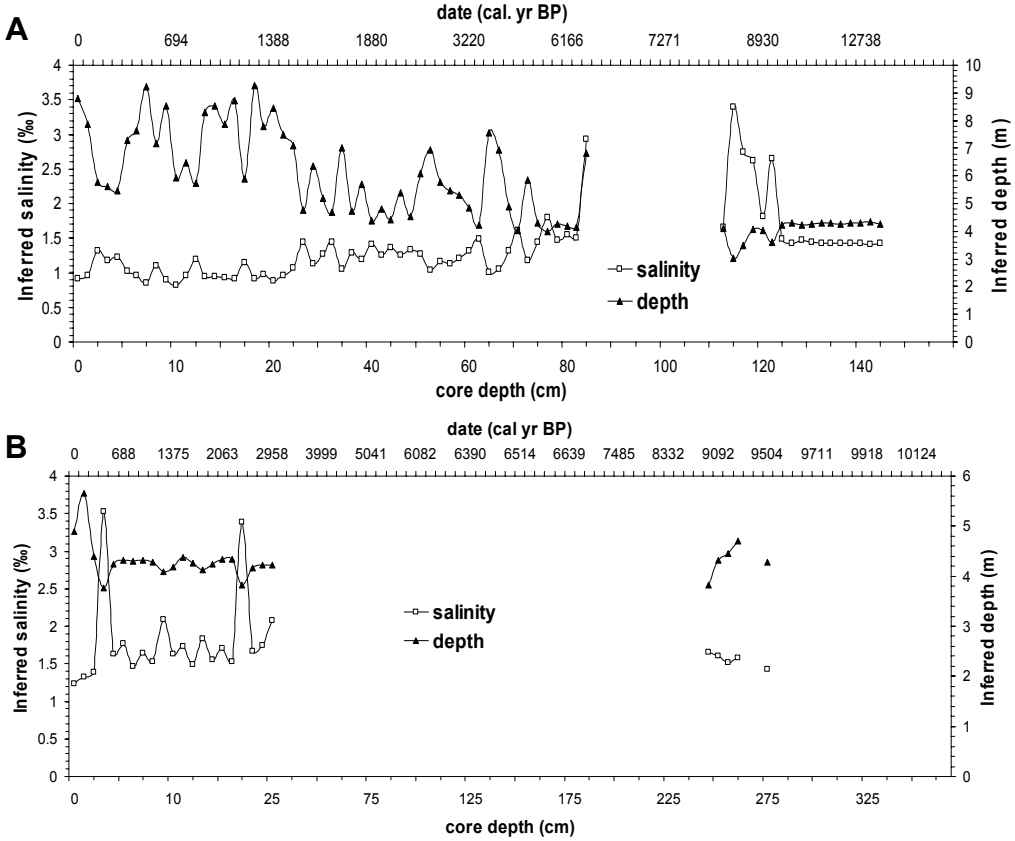

Fig.15*: Reconstructed lake-water depth and salinity in the Kirisjes Pond (A) and Heart Lake (B) sediment cores using the diatom-based inference models.*

Heart Lake core

The Heart Lake core can be divided into five distinct zones based on lithology and diatom analyses (Fig. 14). The basal core levels (HL I, 361-355 cm, *c*. 10,200 cal. yr BP) are dominated by marine and sea ice associated diatoms (e.g., *F. cylindrus*, *F. curta*, *N. glaciei* and *Synedropsis laevis*). The second zone (HL II, 350-280 cm) is a diamicton which we interpret as a glacial till, with extremely low carbon content. The sediments in this zone are radiocarbon dated (on bulk material) between 21,780 and 25,460 ^{14}C yr BP and overlay the marine sediments dated 10,314 ^{14}C yr BP (*c*. 10,200 cal. yr BP after correction for the reservoir effect). These old radiocarbon dates are probably related to the presence of old carbon, which is transported from the continent into the basin by means of iceberg discharge. However, thermoluminescence dating however indicated that the age of this glacial till zone

falls within the range of 4,100 – 8,900 TL yr BP (275-280 cm) and 65,000 – 98,000 TL yr BP (330-335 cm) so we cautiously interpret this as comprising a mixture of washed-in material of Holocene and pre-Holocene age. The third zone (275-245 cm, *c*. 9500-9000 cal. yr BP) is characterized by lacustrine diatoms. A brief marine transgression, marked by the presence of *Fragilariopsis* species, occurs in this zone between 262 and 270 cm. Between 245 and 25 cm (HL IV, *c*. 9000-2950 cal. yr BP) marine and sea-ice associated diatoms are present, with an assemblage comparable with the first zone (HL1). From 25cm to the top of the core (HL V, *c*. 2950 cal. yr BP-present) lacustrine diatoms dominate the assemblage. Unlike the Pup Lagoon and Kirisjes Pond cores, no transition zone between marine and freshwater conditions can be recognized.

Although there is an outflow above the present water level, below this threshold, Heart Lake functions as a closed lake with salinity and lake water depth dependent on the precipitation-evaporation balance and melt water input from the catchment area (Fig.15). Both variables are significantly negatively correlated (R^2=-0.57, P=0.002) throughout the entire lacustrine zone, similar to Kirisjes Pond. Dry (and cold) conditions (indicated by an increase in salinity and a decrease in lake water depth) are present around *c.* 2340, 1,240 and around *c.* 413 cal. yr BP. From *c.* 413 cal. yr BP until present salinity gradually declines with a peak in lake water depth at 1 cm (Fig.15).

A.4. DISCUSSION

A.4.1. Climate change on a glacial-interglacial timescale

Four different periods were recognised during the pre-Holocene period spanning the past 120,000 years. These periods mainly correspond to the diatom zones identified in the Lake Reid core. The information gathered for the Eemian in this core is further extended with data from the Progress Lake core.

The Eemian interglacial (c. 125,000-115,000 yr BP)

Based on radiocarbon and thermoluminescence dates we cautiously speculate that Zone 1 in LR (104–114 cm) and PR (57-24 cm) are likely to correspond to the previous interglacial (MIS 5e *c.* 125,000 to 115,000 yr ago) a period with similar climate boundaries as the present conditions (see Hodgson et al. 2005a, 2006).

Diatom-inferred lake water depth is higher than present in the bottom zone in LR core (Fig.11). The unusual combination of deep lake water and a high stomatocyst/diatom ratio in this zone are likely linked with the presence of an extensive littoral area and wet seepages in the catchment. Together these data suggest wetter summer conditions. This is supported by the diatom composition in PR, where a greater diatom diversity was observed than in the most recent core

levels. Furthermore, a number of these diatom taxa are not presently found in east Antarctica, and some are endemics nowadays associated with the warmer sub- or maritime Antarctic biome (e.g., *D. balfouriana, P. manguinii*). Auto-ecological studies (Van de Vijver et al. 2002) show that some of these currently non-indigenous taxa are epiphytic and associated with areas where terrestrial and aquatic moss banks accumulate. The significant negative correlation between the total number of diatom valves and the diversity index may thus suggest that the increases in diversity results from the in-wash of diatoms from the littoral zone and from aerophilic catchment habitats (such as moss banks). With the exception of a few peripheral colonies, moss accumulations are sparse in the Larsemann Hills today. Together all this evidence is in favour of a warmer and wetter Eemian interglacial compared with the present warm period.

The last glacial period (c. >42,000–33,000 yr ago)

Despite a thickening of the coastal ice sheet during the last glacial period and its extension onto the continental shelf (Domack et al. 1998), Lake Reid was apparently not scoured by ice. The start of the last glacial period is indicated in the LR core by a trend towards shallower, and thus more arid, conditions, as evidenced by an increase in diatom-inferred salinity and a decrease in diatom-inferred lake water depth, which reaches a minimum at 96 cm. Many stomatocysts are also found in this zone. These are characteristic of shallow ponds in the Larsemann Hills, particularly those where the water column freezes down to the sediments in winter (Sabbe et al. 2004).

While the chlorophyll and carotenoid concentrations remain generally low, sustained high relative abundances of scytonemin occur in Zone 2 (and Zone 3) and have been linked to a high ultraviolet radiation environment in the lake (see below). During the lake water low stand (96 cm), extremely dry and cold conditions can be inferred and one could tentatively link this with MIS 5d, the cold stage after the MIS5e interglacial. At this time, the deuterium excess in the Vostok ice core (Vimeux et al. 2001) reveals that evaporation in lower latitudes was a relatively more important source of precipitation over Antarctica than evaporation at higher latitudes, in contrast to the other cold stages during the last glacial. Whether these changing conditions affected the amount of precipitation (leading to the lake water minimum in Lake Reid), is not yet clear. However, ice-core evidence from the interior does show an overall decline in the accumulation rate during the last glacial period.

The Last Glacial Maximum and Termination1 (33,000 ^{14}C yr BP–10,800 cal yr BP)

The striking feature of Zone 3 is not the monospecific assemblage of *S. inermis* but the near absence of all other diatom taxa (including littoral species). Pigment analyses using HPLC-MS have detected the presence of purple photosynthetic

bacteria in this zone (Squier et al. 2004) that may indicate a degree of oxygen depletion consistent with perennial ice cover. With, at best, limited periods of open water (possibly only at the margins of the lake) the growing season and thus species succession were probably extremely short, leading to the low species richness. This arid period peaks between 56 and 64 cm in the LR core and it may correspond with the LGM.

The stable diatom-inferred lake water level shortly before, during and after the LGM (and during Termination I) is remarkable. A similar evolution can be inferred from the Kirisjes Pond core where lake water depth and salinity are highly stable during transition from the last glacial to the Holocene (KP, Fig.15). Despite the constant lake water depth, this transition period is characterized by relatively rapid climate changes in both hemispheres (Stocker 2003). The gradual increase in temperature recorded in Antarctic ice cores is interrupted by a short cooling period, the Antarctic Cold Reversal (ACR; Jouzel et al. 2001), that coincides with a rapid rise in global sea level (MWP1A; Clark et al. 2002). In Antarctica, temperature increased immediately after the ACR, leading to deglaciation of the currently ice-free coastal oases. In the LR core however, lake water level remains quasi constant, implying little variation in the moisture balance (and thus precipitation-evaporation balance and meltwater regime) during Termination I. Together these data imply that the terrestrial environment in this part of East Antarctica responded to slowly changing temperatures after Termination I, possibly due to the buffering effect of ice sheets that remained grounded on some parts of the continental shelf of Prydz Bay before 14,000 cal yr B.P. (Domack et al. 1998; Verleyen et al. 2005a) and the presence of firnified snow and ice in the Broknes valleys. The slow response of both lakes is in contrast to present-day lakes in the maritime Antarctic, which have been shown to amplify climate changes during deglaciation through increases in their effective catchment areas leading to higher inputs of nutrients (Quayle et al. 2002).

A.4.2. Holocene climate evolution

From *c*. 11,500 until *c*. 9,500 cal. yr BP fragmented frustules of *P. microstauron* are present in the Kirisjes Pond core, suggesting inflow from the catchment area (Fulford-Smith and Sikes 1996), lake-ice melting during spring and summer (comparable to the present day situation) and thus warmer conditions. This period of melt water input and open water conditions coincide with the widespread early Holocene climate optimum (11,500 – 9,000 yr BP) detected in East Antarctic ice cores (Masson et al. 2000). Around *c.* 9,500 cal. yr BP a glacial till zone was deposited in the Heart Lake basin, which is likely derived from iceberg discharge from the Dålk Glacier (east of Heart Lake) and the in-wash of sub-glacial tills from the margins of this glacier. This iceberg calving or rapid re-advance and retreat of

glaciers is possibly linked to higher temperatures (and/or higher precipitation) during the early Holocene climate optimum.

Between *c*. 9,500 and 7,800 cal. yr BP diatoms in both lacustrine (Kirisjes Pond and part of Heart lake) and marine sediments (part of the Heart Lake core) are characteristic of cooler and/or drier climate conditions. This is evidenced by the low lake level and higher salinities in the lakes and the dominance of sea ice diatoms in the marine sediments of Heart Lake at this time.

Between *c.* 7,730 and 5,230 cal. yr BP warm conditions can be inferred based on higher water levels in the Kirisjes Pond core and the relatively high abundance of *Chaetoceros* resting spores in marine sediments of Heart Lake and Pup Lagoon cores. *Chaetoceros* spp. are related to high productivity (e.g., Leventer 1992, 1998) and stratified, open water conditions during summer and spring in Antarctica (Whitehead et al. 2001). These conditions are probably linked to a second climate optimum during the Holocene detected in various other archives in Antarctica (e.g. Masson et al. 2000, Cunningham et al. 1999).

The presence of sea-ice associated taxa in the Pup Lagoon and Heart L. sediment cores between 5,230 and 2700 cal. yr BP suggests that coastal oceanographic conditions are similar to the present situation. In the Kirisjes Pond core, lake water depth is relatively low during this period except around 3,800 cal. yr BP, which coincides with a lake level rise in the Vestfold Hills (Roberts and McMinn 1998) and a warm period around 3,800 yr BP in East Antarctica and 3,000 yr BP in West Antarctica (Masson et al. 2000). The short optimum in the Larsemann Hills is interrupted by low lake levels stands, but between 3,000 and 2,000 cal. yr BP lake level is high in Kirisjes Pond and between 2,700 and 2,200 cal. yr BP more open water taxa are found in the Pup Lagoon core, suggesting warmer conditions. This period of a general warmer climate in Antarctica is detected in several proxy-climate records of Antarctica and is being considered as a potential analogue to study some aspects of the recent temperature rise in the Antarctic Peninsula (Vaughan et al. 2003).

In contrast, Late Holocene dry conditions are present around *c.* 2,000 cal. yr BP in Kirisjes Pond, concomitant with a rapid cooling in Antarctic ice cores (Masson et al. 2000) and sediment cores in the open marine environment (Taylor and McMinn 2002) and marine embayments (McMinn et al. 2001) in Prydz Bay, possibly linked with Neoglacial cooling. In addition, short dry periods in Kirisjes Pond are recognized between c. 760 and 690 cal. yr BP and between c. 280 and 140 cal. yr BP. The latter may be related to a cold period seen in the Byrd and Taylor Dome ice cores, affiliated to an Antarctic Little Ice Age (LIA) as suggested by Masson et al. (2000).

B. PAST FLUXES IN ULTRAVIOLET RADIATION (UVR)

B.1. INTRODUCTION

Since the discovery of the Antarctic spring-time ozone hole (Farman et al. 1985), there have been many studies dealing with the depletion of the ozone layer and the impact of increased ultraviolet radiation (UVR) transmission on the biota of high-latitude terrestrial and aquatic ecosystems (e.g., Robinson et al. 2003). However, it is still unclear if the biota are experiencing higher UVR during recent times (i.e., since the discovery of the ozone hole) than they have in the period before instrumental measurements of ozone started (in 1957). Reconstructions of longer-term changes in UVR flux are only recently becoming available and are based on a combination of historical, indirect measurements (sunspots; Rozema et al. 2002) and direct proxy data, including flavonoids in pollen and p-coumaric acid content in sporopollenin (Rozema et al. 2001). Paleolimnological approaches can also be used to reconstruct past UVR receipt (e.g., Leavitt et al. 1997, 2003; Pienitz and Vincent 2000).

In East Antarctic lacustrine ecosystems, low turbidity and low concentrations of DOM in the lakes due to the absence of vegetation in the catchments, give rise to very transparent waters and very low vertical attenuation coefficients (Kd_{PAR} 0.18-0.47m^{-1}, Kd_{UVRa} 0.21-0.28 m^{-1}, Kd_{UVRb} 0.27-0.35 m^{-1} ; Ellis-Evans et al. 1998). The effect of cDOM on the UVR flux can thus be expected to be relatively low in these lakes in contrast to lakes with extensive vegetation in the catchment area. Collectively, this implies that these water bodies are particularly well-suited to the reconstruction of past atmospheric UVR flux, provided that past variations in lake-specific properties affecting the light climate, such as lake depth, snow cover, lake-ice properties and DOM concentrations, can be accounted for. The Antarctic region is particularly relevant to reconstructions of past UVR fluxes, as it is currently subject to the most severe ozone depletion on Earth during the spring-time ozone hole, which has resulted in an increase in UVR-B fluxes of 6-14 % since 1980 (WMO 2002).

The aim of this study is to infer changes in past UVR penetration during the Late Holocene on a relative high resolution timescale and during a glacial-interglacial cycle on a lower resolution timescale. Therefore, we analysed fossil pigments and siliceous microfossils in a radiocarbon-dated sediment core from two shallow lakes in the Larsemann Hills, namely Pup Lagoon and L. Reid.

B.2. MATERIALS AND METHODS

The procedures for core retrieval, radiocarbon, pigment and diatom analyses are described above. In order to evaluate pigment preservation in the core, total diatom biomass (TDB) was compared with total diatom carotenoid concentration (TDC) in each sample (cf. Verleyen et al. 2004b, see above).

A proxy for photosynthetic active radiation (PAR) and UVR intensity was

developed by dividing the total concentration of the photoprotective pigment zeaxanthin (Zea; Holt et al. 2005), present in cyanobacteria and green algae in the lakes in the Larsemann Hills, by total chlorophyll a concentrations (TChla) to exclude the influence of the organic-matter-specific concentration.

A proxy for light intensity in the UVR wavelengths was based upon the scytonemin concentration and its derivatives (TScyt). Scytonemin is a sheath pigment of certain cyanobacteria which absorbs in the UV spectrum of light and is formed as a protection against harmful UVR (Proteau et al. 1993). The UVR proxy was obtained by dividing TScyt by the total cyanobacterial carotenoid concentration (TCC; including, where present, nostoxanthin, echinenone, canthaxanthin and anthaxanthin).

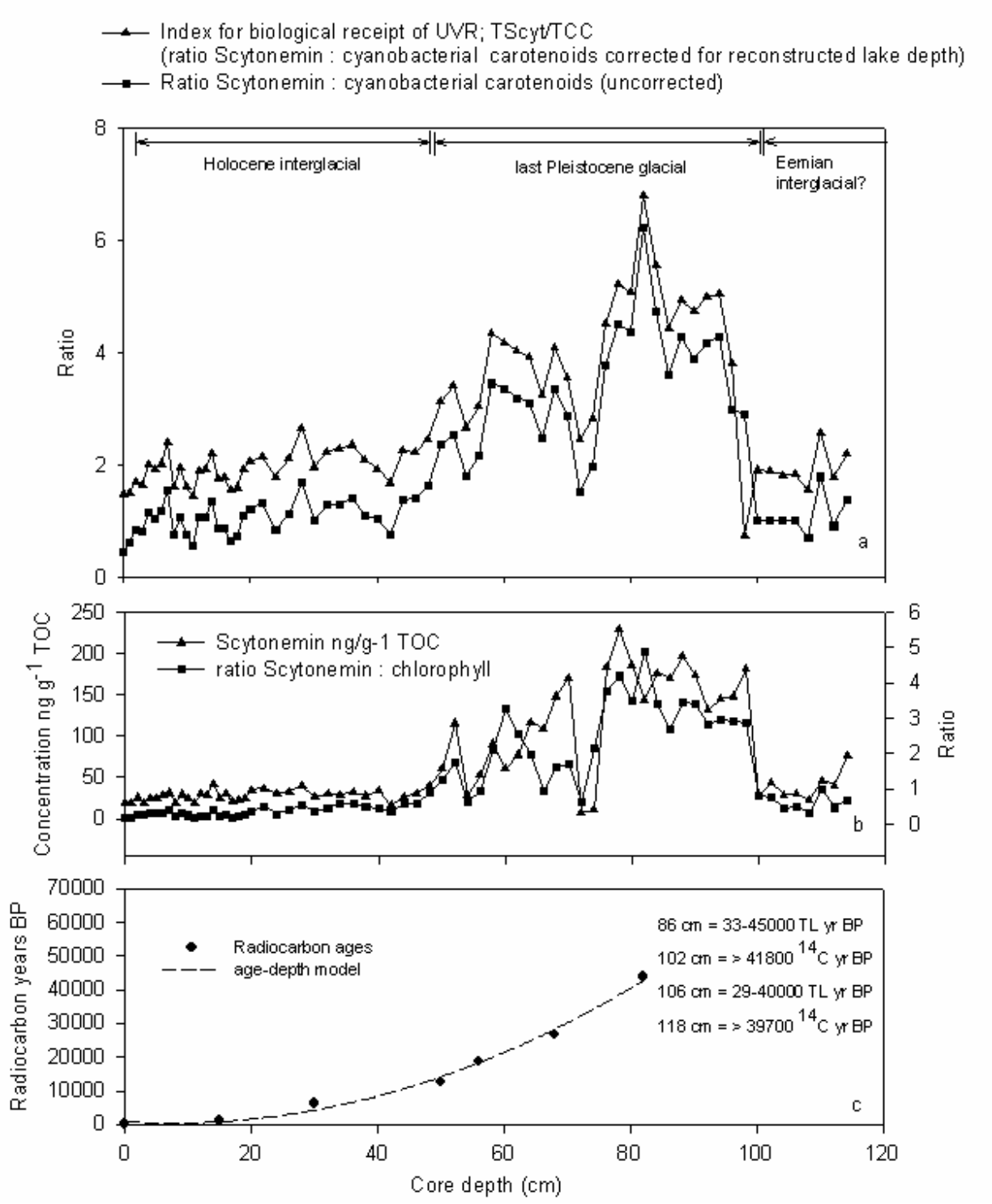

Fig.16: *(a) Ratio of scytonemin to cyanobacterial carotenoids (TScyt/TCC) in a sediment core from Lake Reid, (b) Scytomenin concentration, and the ratio of scytonemin to chlorophyll. (c) Chronological data for the sediment core.*

Comparison between the UVR and UVR+PAR proxies throughout the sediment core was used to estimate the relative changes in UVR in relation to total incoming light intensities. Coincident changes in both proxies are likely linked with changes in factors affecting both PAR and UVR penetration, such as changes in lake-ice

properties, snow cover, DOM concentrations and cloud cover, rather than changes exclusively affecting the UVR penetration (e.g., ozone column depth).

Historical changes in salinity and lake depth in Pup Lagoon and L. Reid were inferred with the diatom-based transfer functions (see above). The influence of lake depth on the UVR proxy was in addition excluded by a simple correction based on a non-linear regression linking modern scytonemin:cyanobacterial carotenoids ratios ($TScyt/TCC_{mod}$) with lake depth in 28 lakes in the Larsemann Hills (Hodgson et al. 2004). All data were logarithmically transformed before correlation analyses in Statistica 6.0. For a detailed description of the methods used the reader is referred to Verleyen et al. (2005a).

B.3. RESULTS

B.3.1. Glacial-interglacial changes in UVR

Analyses of sedimentary pigments revealed that UVR receipt (as TScyt:TCC) was c. 340% higher during the last glacial (mean ratio 2.4 $\pm$ 1.11 SD) compared with the Holocene and the previous (MIS5e) interglacials (0.7 $\pm$ 0.25 SD, and 0.7 $\pm$ 0.3 SD respectively, Fig. 16a). Similarly, mean ratios of scytonemin to chlorophyll were 490% greater during the last glacial than in the Holocene, suggesting that the metabolic effort of benthic cyanobacteria during growth periods was geared towards photo-protection rather than photosynthesis (Fig 16b). Increases in both ratios arose because of elevated concentrations of UVR-screening pigments rather than declines in other pigments. In contrast, the PAR + UVR increase was less marked with a TCC:TChl ratio of c. 0.25 that of the UVR index (Fig 16a). These ratios suggest that the elevated irradiance was a combination of UVR and PAR but with the ratio of UVR:PAR ratio being relatively higher during the glacial period.

B.3.2. Changes in UVR during the late Holocene

Overall, four well-defined maxima of higher $TScyt/TCC_{corr}$ (and TScyt/TCC) are present at 6, 14, 65 and 85-90 cm depth in the PL core spanning the last c. 1600-1800 years (Fig.17). Three moderately high values are present at 16-17, 30-35 and 100 cm; the latter is coincident with the rise in salinity associated with the brief marine incursion and a salinity rise. The maxima correspond, after application of the age-depth model, with higher values of UVR penetration around 1820-1780, 1580-1490, 790-580 and 680-440 AD respectively. Periods of elevated light intensity can be inferred based on the Zea/TChla ratio between 80-100 cm. The proxy for UVR and the proxy for UVR+PAR follow a similar pattern between 0 and 4 cm and between 85 and 105 cm. Both proxies are weakly correlated (r^2=0.4315, p<0.005) but the correlation breaks down when the samples between 0 and 4 cm (past 110 – 135 years) and between 85 and 105 cm (310 – 680 AD) are excluded from the correlation

analysis (r^2=0.0177, p<0.925).

B.4. DISCUSSION

Several lines of evidence suggest that changes in inferred UVR receipt did not arise from changes in within-lake controls of UVR exposure such as changes in the preservation environment, lake depth, species turnover linked to salinity and temperature, or optical properties of the water column as discussed in detail in Hodgson et al. (2005b) and Verleyen et al. (2005b).

The only remaining within-lake controls that could have influenced the increase in the concentration of UVR-absorbing pigments (e.g., during the last glacial period) are the timing of the periods of photosynthetic activity, changing snow cover on the lake ice, and behavioural adaptations to low light intensity. Ice core evidence also suggests a decline in snow precipitation (as accumulation rate) on the interior Antarctic plateau during the last glacial (Petit et al. 1999). There are no data for the coast, but a reduction in snow cover on the lake ice, together with a decline in cloud cover (both consistent with increased glacial sea ice coverage of the ocean and cooler conditions) could have increased UVR and PAR transmission as long as it could offset the greater duration and thickness of lake ice cover that we assume during the glacial. Some cyanobacteria may also have increased their exposure to UVR by colonizing the underside of lake ice, a behavioural adaptation to low light intensity.

A final set of factors that must be considered is that increased biological receipt of UVR arose because of changes in UVR transmission to the Earth's surface through changes in cloud cover and atmospheric turbidity, or stratospheric ozone column depth. Climate observations show that extensive sea ice results in reduced cloud cover and precipitation over Antarctica (King & Turner 1997) and enhanced transmission of UVR through the atmosphere (Nichol et al. 2003): a mechanism consistent with the more extensive ice shelves and sea-ice of the last glacial. This extensive glacial ice cover may also have increased surface albedo on the Antarctic continent and back scattering of UVR in the lower atmosphere which can increase diffuse down-welling irradiance by as much a 10% (Nichol et al. 2003).

Alternatively, ozone depletion during the last glacial might have resulted from the steeper thermal gradients between low and high latitudes, which may have intensified the circumpolar vortex (a key physical element required for polar ozone depletion in spring). More persistent vortices, combined with enhanced stratospheric cooling, might also reduce stratospheric temperatures below the –80°C threshold at which polar stratospheric clouds form. These clouds are essential for rapid depletion of ozone during spring (Roscoe & Lee 2001).

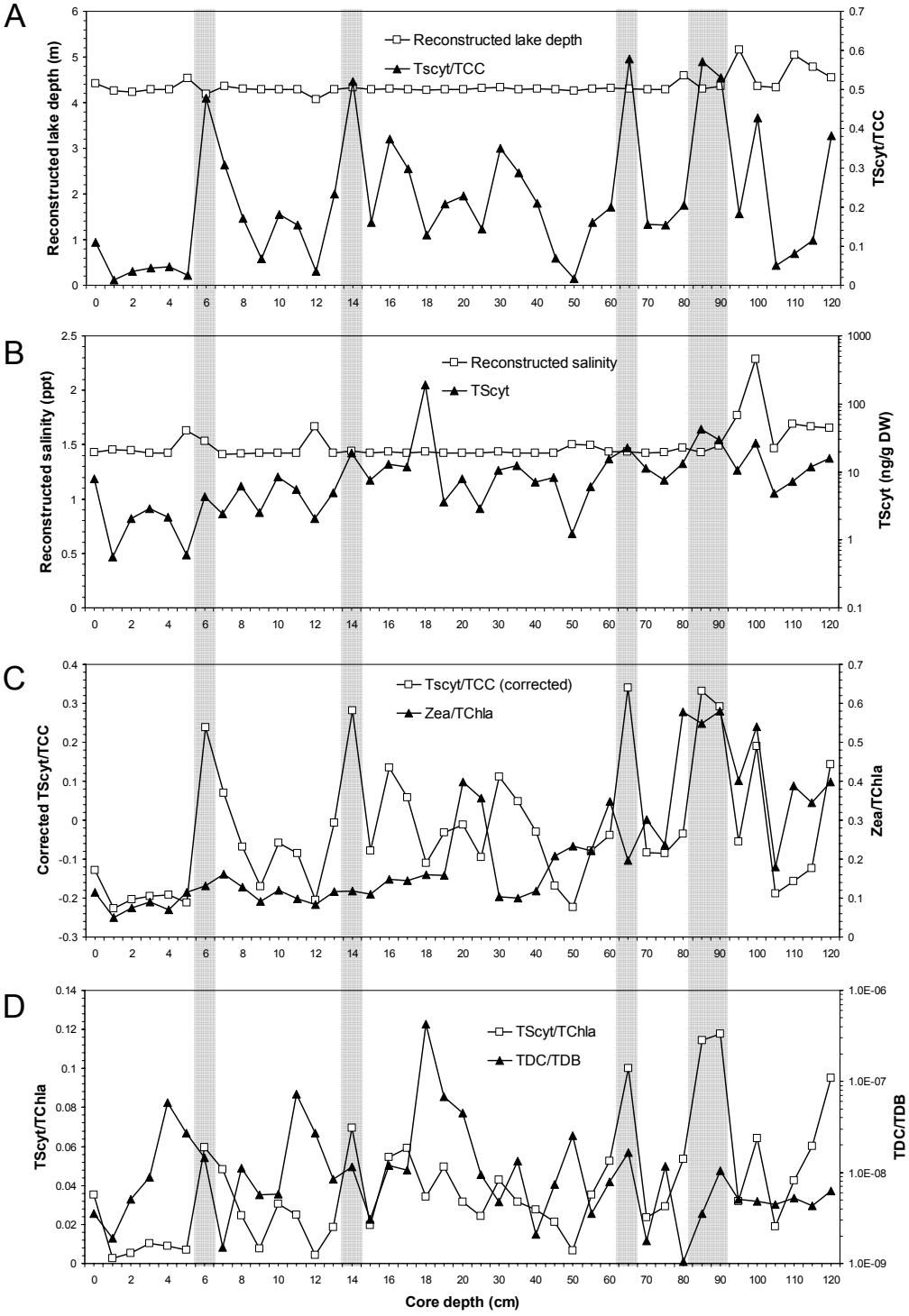

Fig. 17: *(A) Variations in inferred lake depth (m) using the diatom based WA-PLS transfer function (Verleyen et al. 2003) and TScyt/TCC, (B) Variations in inferred salinity (ppt) using the diatom based WA transfer function (Verleyen et al. op. cit.) and TScyt (ng/g TOC), y-axis of the latter on logarithmic scale, (C) variations in the UVR proxy (TScyt/TCC$_{corr}$) and the proxy for PAR + UVR (Zea/TChla), (D) variations in TDC/TDB (y-axis on logarithmic scale) and TScyt/TChla in order to evaluate preferential pigment preservation in the core.*

Long-term changes in solar output cannot be tested or predicted by global climate models because UVR production is assumed to vary by less than 1% during a typical solar cycle (Haigh 1996). However, several of the periods of high UVR during the late Holocene (within the error of the chronological model) coincide with solar minima as reconstructed from historical records and proxy data (Fig. 17). Minima of solar activity are found at the Dalton Minimum (1795-1820 AD), Maunder Minimum (1645-1715 AD), Sporer Minimum (1416-1534 AD), Wolf Minimum (1282-1342 AD) and Oort Minimum (1010-1090 AD; Camuffo et al. 2000) and as recently compiled by Pang and Yau (2003) based upon Asian historical records, around *c*. 580-800, 400-500 and 200-300 AD. This may possibly imply that changes in UVR are to some extent related to changes in solar activity, yet this link is far from certain. A higher resolution study with sufficient radiocarbon dates could potentially be compared with records of past temperature change and solar activity (e.g., the cosmogenic nuclides ^{14}C and ^{10}Be in ice cores).

C. CHANGES IN THE RELATIVE SEA HISTORY

C.1. INTRODUCTION

The Lambert Glacier-Amery Ice Shelf system occupies the largest glacier catchment on Earth and comprises one-eighth of the total area of the East Antarctic (part of the Antarctic) Ice Sheet (EAIS; Wiliams et al. 2001). It is known to play an important role in the production of Antarctic Bottom Water (Meredith et al. 2000) and has been proposed as one of four key areas of Last Glacial Maximum (LGM) ice sheet expansion in Antarctica (Huybrechts, 2002). During the past ~5 million years it has accumulated and discharged vast quantities of ice and its terminus has fluctuated > 500 km several times during its history (Hambrey and McKelvey, 2000). However, less is known about the thickness and extent of ice in the Lambert-Glacier catchment during the LGM and the subsequent deglaciation.

An important source of information on former ice thickness and extent is changes in relative sea level (RSL; Zwartz et al. 1998). In Antarctica, however, there are few RSL data, due largely to the lack of coastal ice-free areas, where organic material for radiocarbon dating can accumulate.

Two principal methods are used to obtain the elevation and age data necessary to construct RSL curves (see Bentley et al. 2005 for an overview). The first method is based on direct indicators of former sea level, such as raised beaches that can be dated using incorporated fossiliferous material. The second method uses sediment cores from isolation basins with a known sill height and dates the transitions from lacustrine to marine and marine to lacustrine sediments.

In the LAQUAN project we developed a new RSL history for the Larsemann Hills, based on the analysis of marine and freshwater transitions recorded in the

sediments of three isolation basins. Previously published geological evidence of RSL and ice sheet history from the continental shelf zone and the nearby Vestfold Hills is incorporated into the discussion to infer the timing and extent of regional deglaciation.

C.2. MATERIALS AND METHODS

The three previously described isolation lakes (Heart L., Pup Lagoon, and Kirisjes Pond) in the Larsemann Hills were selected at different elevations to study the history of marine transgression and regression in the region. Procedures for sediment coring, description and chronology are described above. Marine and freshwater transitions were identified using a combination of sedimentology, diatoms and pigments. Sill elevations of the lakes were surveyed by the Australian Antarctic Data Centre with a spot-elevation accuracy of < 0.5 m.

C.3. RESULTS AND DISCUSSION

C.3.1. Timing of the last deglaciation

The minimum age of deglaciation is *c.* 12,800 - 11,940 cal yr BP (10,400 +/- 65 ^{14}C yr BP), based on the presence of biogenic sediments at 138 cm in the Kirisjes Pond core (see above). However, the precise onset of biogenic sedimentation occurs above a diamicton at 144 cm. Thus, if the calibrated date in the sediment core at 138 cm is extrapolated to 144 cm, assuming a linear sedimentation rate between 124, 138 and 144 cm, one can infer that deglaciation at this site started between *c.* 14,490 and 12,660 cal yr BP (averaged 13,580 cal yr BP; fig.3) depending on whether the maximum or minimum sedimentation rate is used for the extrapolation. Lacustrine sediments are not only present in Kirisjes Pond at this time, but have also been reported in Ace Lake (Vestfold Hills) from c. 13,800 - 13,000 cal yr BP (11,380 corrected ^{14}C yr BP, Roberts and McMinn, 1999). Therefore, regional recession of the ice sheet from these sites and the onset of biogenic lake sedimentation commenced sometime before *c.* 12,660 cal yr BP.

Offshore, the exact timing of retreat of the ice margin from the Prydz Bay Channel is not well known, but evidence from marine cores suggests that the ice sheet had already withdrawn from the middle to outer continental shelf at *c.* 68.8°S before *c.* 13,800 - 13,020 cal yr BP (12,680 +/- 110 ^{14}C yr BP, Domack et al. 1998). This date however, is 4-7 cm above the transition to siliceous mud and diatomaceous ooze (open marine conditions) in the marine sediment core. An extrapolation based on a mean-sedimentation rate in this section of the core yields a retreat age between *c.* 15,370 - 13,690 and 15,160 - 13,440 cal yr BP (*c.* 13,440 - 13,120 uncorrected ^{14}C yr BP). In combination with the data from the Larsemann Hills (Kirisjes Pond, this study) and the Vestfold Hills (Ace Lake, Roberts and McMinn, 1999), this indicates

that regional retreat of the ice-margin retreat took place between *c.* 15,370 – 13,440 cal yr BP (retreat from the continental shelf) and 14,490-12,660 cal yr BP (retreat from Kirisjes Pond in the Larsemann Hills and Ace Lake in the Vestfold Hills).

C.3.2. Relative sea level changes during the Holocene

The multiple proxies analysed in the three sediment cores record distinct transitions between marine and freshwater phases (Fig.18). By dating these transitions, correcting for the marine reservoir effect, and calibration, we have been able to constrain the RSL curve back to *c*. 10,360 - 9,910 cal yr BP (10,314 +/- 65 ^{14}C yr BP, fig. 3), extending the previous curve constructed in the nearby Vestfold Hills (Zwartz et al. 1998).

Our interpretation is that the sea-level data between *c.* 10,360 - 9,910 cal yr BP and 9,260 – 8,650cal yr BP indicate that local effects on RSL (dominated by crustal uplift) are in equilibrium with the eustatic sea-level rise associated with ongoing global deglaciation. From *c.* 9,260 - 8650cal yr BP until *c.* 7,570 - 7,270 cal yr BP, the increase in RSL is quite rapid and is probably related to a decline in the rate of isostatic uplift. Our Holocene RSL highstand (between *c.* 7,570 - 7,270 cal yr BP and 7,250 - 6,950 cal yr BP) is coincident with the high stand in the Vestfold Hills (Zwartz et al. 1998), as well as with data from other East Antarctic regions (e.g., Miura et al. 1998).

From the time of the highstand to the present, local isostatic uplift is likely the dominant contributor to the observed RSL fall, as eustatic sea level has remained essentially constant in this period. Thus, if local crustal uplift reflected only the isostatic response to the last deglaciation of the East Antarctic ice sheets (EAIS), we would expect an exponential decay in uplift rate since 7,250 - 6,950 cal yr BP. Instead, our data suggest an increase in the rate of RSL fall starting around *c.* 2,847 – 2,509 cal yr BP, thus indicating a more complex EAIS history during the Holocene. If proven to be robust, this feature may be linked to local glacier readvance during the mid-Holocene, which would temporarily have reduced crustal uplift rates. Based on geomorphological evidence, Adamson and Pickard (1986) speculated that a readvance of the Sørsdal Glacier in the Vestfold Hills (the Chelnok Glaciation) occurred around 3,000 yr BP (not corrected and not calibrated). Similarly, Domack et al. (1991) reported glacier thickening and grounding line advance of the Lambert Glacier Amery Ice Shelf between 7,000 and 3,800 yr BP (not calibrated). These data are consistent with previously reported glacier readvance and thickening of the EAIS near the Windmill Islands and consequent global sea-level lowering (*c.* 0.7 +/- 0.1m between 4,000 and 2,500 cal yr BP; Goodwin, 1998), linked with increased precipitation during the late Holocene climate optimum (Hypsithermal). The global sea level fall of *c.* 0.7 m (Goodwin, 1998), thus implies that the RSL lowering in the Larsemann Hills between *c.* 4,000 and 2,500 cal yr BP (fig. 3) is likely to be

dominated by the eustatic sea-level fall rather than isostatic uplift. The latter was thus at a virtual standstill during this period, as RSL fall in the Larsemann Hills was equal to global sea level fall.

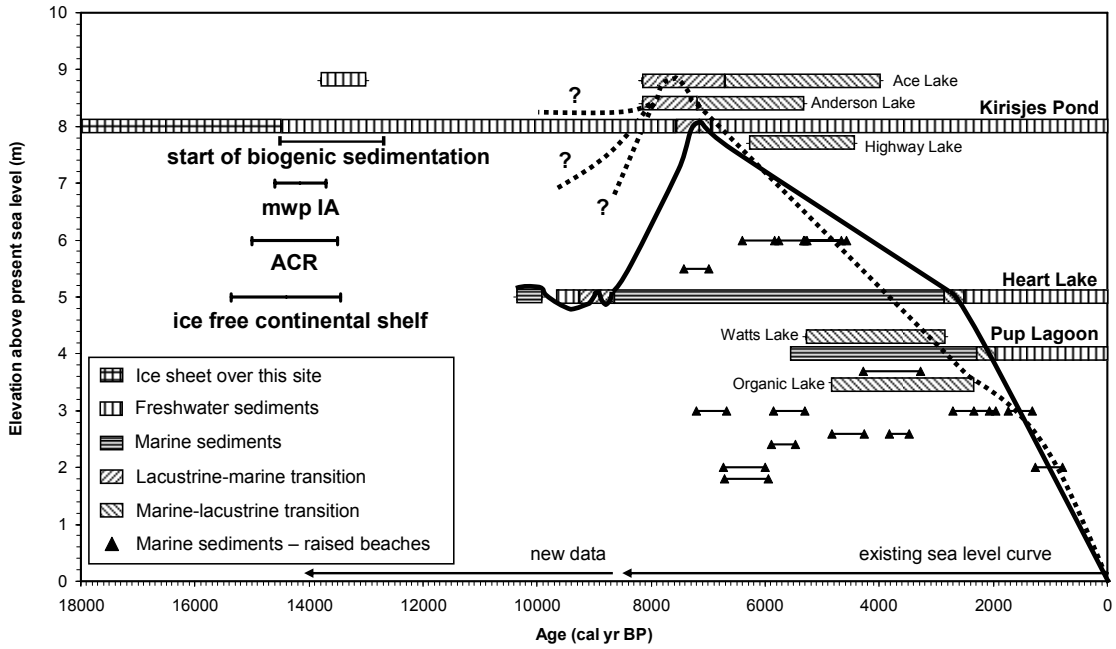

Fig.18: *RSL curves for the Larsemann Hills (LH, black line) and the Vestfold Hills (VH, dashed line). The LH curve is derived from sediment cores represented by horizontal bars with different shading representing marine and freshwater sediments. The transition zones take error ranges of calibrated dates and the sedimentation rate models (VH) into account. Horizontal bars under the curve represent sediment deposits containing marine assemblages. Horizontal bars above the curve represent lake-sediment deposits containing freshwater assemblages. Triangles under the curve represent dated raised beaches. The dashed lines with question marks show the range of RSL constraints predicted by the Vestfold Hills ice-sheet model (Zwartz et al. 1998). The timing and duration (maximum ranges) of mwp-IA, the ACR, the ranges of calibrated dates of ice retreat from the continental shelf and the start of biogenic sedimentation in Kirisjes Pond are shown as horizontal bars.*

C.3.3. The possibility of an East Antarctic contribution to meltwater pulse IA

Field evidence indicates that retreat of the East Antarctic (part of the Antarctic) ice sheet in the Prydz Bay region occurred between *c.* 15,370 - 13,440 (age of the deglaciation of the continental shelf) and *c*. 13,580 +/- 920 cal yr BP (the start of biogenic sedimentation in Kirisjes Pond). At its maximum extent, the ice margin in the region is thought to have advanced more than ~200 km seaward from the Larsemann Hills to the continental shelf break (Domack et al. 1998). If the subsequent ice retreat was rapid, as suggested by the field evidence, then it is possible that this event might

have made a contribution to meltwater pulse IA (mwp-IA), a controversial episode of rapid sea-level rise identified in far-field sea-level records (e.g. Hanebuth et al. 2000). This significant meltwater event was of a magnitude of ~25 m (sea level equivalent) and occurred within the period between *c.* 14,600 - 14,200 and 14,100 - 13,700 cal yr BP (Clark et al. 2002).

Resolving the magnitude of the contribution of the Antarctic ice sheet to mwp-IA is a task of first order importance, because rapid increases in the freshwater flux to the Southern Ocean, if targeted at areas of deep-water formation, may influence the thermohaline circulation and cause regional [Antarctic Cold Reversal, Clark et al. (2002)] or inter-hemispheric [Bølling-Allerød warm period, Weaver et al. (2003)] climate change.

GENERAL CONCLUSIONS

Novel biological proxies and inference models were developed to reconstruct past environmental changes in Antarctic ice-free regions. Reference datasets of cyanobacterial sequences, diatoms and pigments were constructed in order to study the present diversity and distribution of biota in benthic microbial mats from Antarctic lakes. Our results showed that each lake is quite unique in terms of cyanobacterial diversity and that every single lake studied resulted in the discovery of new Operational Taxonomic Units (OTUs). This suggests that there is a lot more diversity to discover. The majority of the genotypes are restricted to Antarctica and sometimes, even present only in one sample, which hints to the existence of endemic cyanobacteria. A taxonomic inventory of the diatom flora from the Larsemann Hills similarly revealed that Antarctic endemics account for about 40 % of all freshwater and brackish taxa.

These datasets were subsequently used for comparison between living and fossil floras or to develop inference models to quantitatively reconstruct past environmental changes in the Larsemann Hills (East Antarctica). We also made a first attempt to use fossil cyanobacterial DNA to reconstruct the paleodiversity of the populations. The main problems encountered were related to the presence of good-quality bacterial DNA that act as competitor for fossil DNA during PCR, continuing degradation of fossil DNA, and probably selective decay of cyanobacterial DNA from certain taxa. Paleolimnological analyses and application of the models revealed the history of late Quaternary variation in climate, ultraviolet (UV) radiation, and relative sea-level. Together, our results highlight the potential of coastal Antarctic lakes for the reconstruction of past environmental changes and underscore the need for continued studies of lacustrine sediment sequences from this climate sensitive region.

ACKNOWLEDGEMENTS

This research is part of the Vth Phase of the Belgian Science Policy Office funded Antarctic program and the British Antarctic Survey Signals in Antarctica of Past Global Changes Program (SAGES). Logistical support was provided by the Australian Antarctic Division (ASAC projects 2112, 562 and 1071). We also thank Antonio Quesada for the possibility to sample in Livingston Island, in the frame of the project LIMNOPOLAR. We take the opportunity to thank Dominic Hodgson, Andrew McMinn, Donna Roberts, Peter Leavitt, Glenn Milne, James Smith and Mike Bentley for valuable discussions.

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Appendix 1: species list and reference number with salinity and lake depth optima and tolerances

Reference number and taxon		salinity opt. (‰)	salinity tol. (‰)	depth opt. (m)	depth tol. (m)
1	*Achnanthes brevipes* Agardh	20.21	2.19	-	-
2	*Achnanthes taylorensis* Kellogg, Stuiver, Kellogg & Denton	4.65	4.49	0.7	2.05
3	*Amphora* sp. a	33.15	4.09	-	-
5	*Amphora* sp. c	18.42	1.25	-	-
6	*Amphora* sp. d	55.45	0.98	-	-
7	*Amphora veneta* Kützing	2.55	2.49	4.87	2.26
8	*Chaetoceros spp.*	41.75	2.08	-	-
9	*Chaetoceros* resting spore	35.94	2.79	-	-
10	*Cocconeis costata* Gregory	46.39	2.3	-	-
11	*Cocconeis fasciolata* (Ehrenberg) Brown	44.56	1.99	-	-
12	*Cocconeis pinnata* Gregory	1.85	1.88	3	2.05
13	*Cocconeis* sp. a	7.37	5.88	2.58	2.21
14	*Craspedostauros laevissimus* (West & West 1911) Sabbe	20.6	2.44	-	-
15	*Craticula* cf. *molesta* (Krasske) Lange-Bertalot & Willmann	1.68	1.28	4.1	2.57
16	*Diadesmis* cf. *perpusilla* (Grunow) D. G. Mann	0.17	0.13	1.2	2.16
17	*Diploneis splendida* (Gregory) Cleve	57.55	1.07	-	-
18	*Fragilaria* sp. a	34.14	2.81	-	-
19	*Fragilariopsis angulata* Hasle	66.42	0.8	-	-
20	*Fragilariopsis curta* (Van Heurck) Hasle	34.96	2.16	-	-
21	*Fragilariopsis cylindrus* (Grunow ex Cleve) Hasle	35.94	2.41	-	-
22	*Hantzschia cf. amphioxys* (Ehrenberg) Grunow	2.02	4.86	0.9	1.29
23	*Hantzschia virgata* (Roper) Grunow	15.64	5.27	1	2.05
24	*Luticola muticopsis* Van Heurck	4.95	4.02	0.91	1.52
25	*Muelleria peraustralis* (West & West) Spaulding & Stoermer	0.1	1.62	3.24	1.27
26	*Nanofrustulum shiloi* (Lee, Reimer & McEnery) Round, Hallsteinsen & Paasche	18.34	2.86	-	-
27	*Navicula adminii* Roberts & McMinn	47.66	1.91	-	-
28	*Navicula* cf. *cancellata* Donkin	15.28	2.07	-	-
29	*Navicula* cf. *dentata* Hustedt	13.26	0.86	-	-
30	*Navicula* cf. *salinarum* Grunow	19.23	2.55	2.02	2.93
31	*Navicula* cf. *shackletoni* West & West	31.93	3.17	1	2.05
32	*Navicula collersonii* Roberts & McMinn	9.95	0.47	-	-
33	*Navicula cryptotenella* Lange-Bertalot	88.95	1.76	-	-
34	*Navicula cryptotenelloides* Lange-Bertalot	55.16	2.36	-	-
35	*Navicula directa* (W. Smith) Ralfs	51.66	2.05	-	-
36	*Navicula glaciei* Van Heurck	41.06	2.44	-	-
37	*Navicula incertata* Lange-Bertalot	81.62	0.07	-	-
38	*Navicula perminuta* Grunow	33.18	1.77	-	-
39	*Navicula phyllepta* Kützing	9.76	3.31	1	2.05
40	*Navicula (?)* sp. 1	0.17	0.06	-	-
41	*Navicula* sp. 2	12.4	1.62	-	-
42	*Navicula* sp. 3	15.2	0.04	-	-
43	*Nitzschia commutata* Grunow	0.4	1.62	3.8	2.05
44	*Nitzschia lecointei* Van Heurck	32.27	1.74	-	-
45	*Nitzschia perminuta* Grunow	28.07	2.54	-	-
46	*Nitzschia stellata* Manguin	18.66	1.98	-	-
47	*Pinnularia lundii* Hustedt	16.98	2.15	-	-
48	*Pinnularia borealis* Ehrenberg	0.15	0.09	0.62	1.22
49	*Pinnularia cymatopleura* West & West	4.88	2.67	0.82	1.2
50	*Pinnularia microstauron* (Ehrenberg) Cleve	2.44	1.93	2.86	2.71
51	*Pinnularia microstauron* var. *microstauron* (Ehrenberg) Cleve	0.87	1.34	1.2	1.91
52	*Pinnularia quadratarea* (Østrup) Heiden	9.73	2.43	-	-
53	*Pinnularia viridis* (Nitzsch) Ehrenberg	14.26	1.16	-	-
54	*Pinnularia viridis* var. "*constricta*" (Østrup) Heiden	15.8	1.62	-	-
55	*Planothidium quadripunctatum* (Oppenheim) Sabbe	0.39	0.26	2.85	2.68
56	*Psammothidium abundans* (Manguin) Bukhtiyarova & Round	0.32	0.35	5.21	2.25

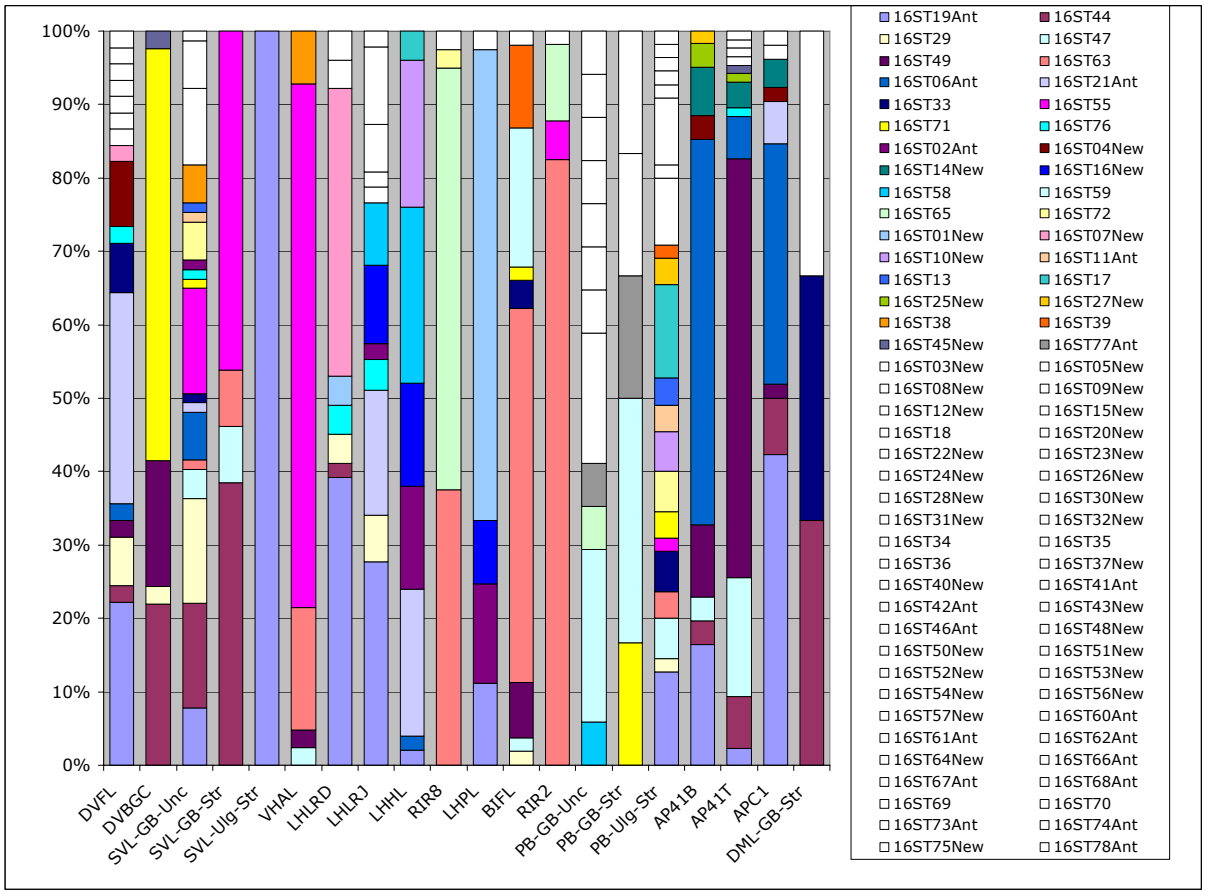
57	*Psammothidium germainii* (Manguin) Sabbe	0.24	0.13	0.58	1.39
58	*Psammothidium metakryophilum*(Lange-Bertalot & Schmidt) Sabbe	0.34	1.48	1.69	3.06
59	*Psammothidium stauroneioides* (Manguin) Bukhtiyarova	0.2	0.18	1.04	2.06
60	*Pseudostaurosira sp. 1*	71.51	1.14	-	-
61	*Sellaphora seminulum* (Grunow) Mann	21.28	1.53	-	-
62	*Stauroforma inermis* Flower, Jones & Round	1	1.68	3.24	2.19
63	*Stauroneis anceps* Ehrenberg	0.45	0.85	1.27	2.39
64	*Stauroneis salina* W. Smith	39.39	1.53	-	-
65	*Thallasiosira antarctica* Comber	19.69	2.82	-	-
66	*Trachyneis aspera* (Ehrenberg) Cleve	59.58	2.65	-	-
67	*Tryblionella marginulata* Grunow) Mann	20.66	1.78	-	-
68	Centric diatom sp. 1	0.89	0.41	1.29	3.23

Appendix 2: AMS ^{14}C dating of lake sediments from the Larsemann Hills

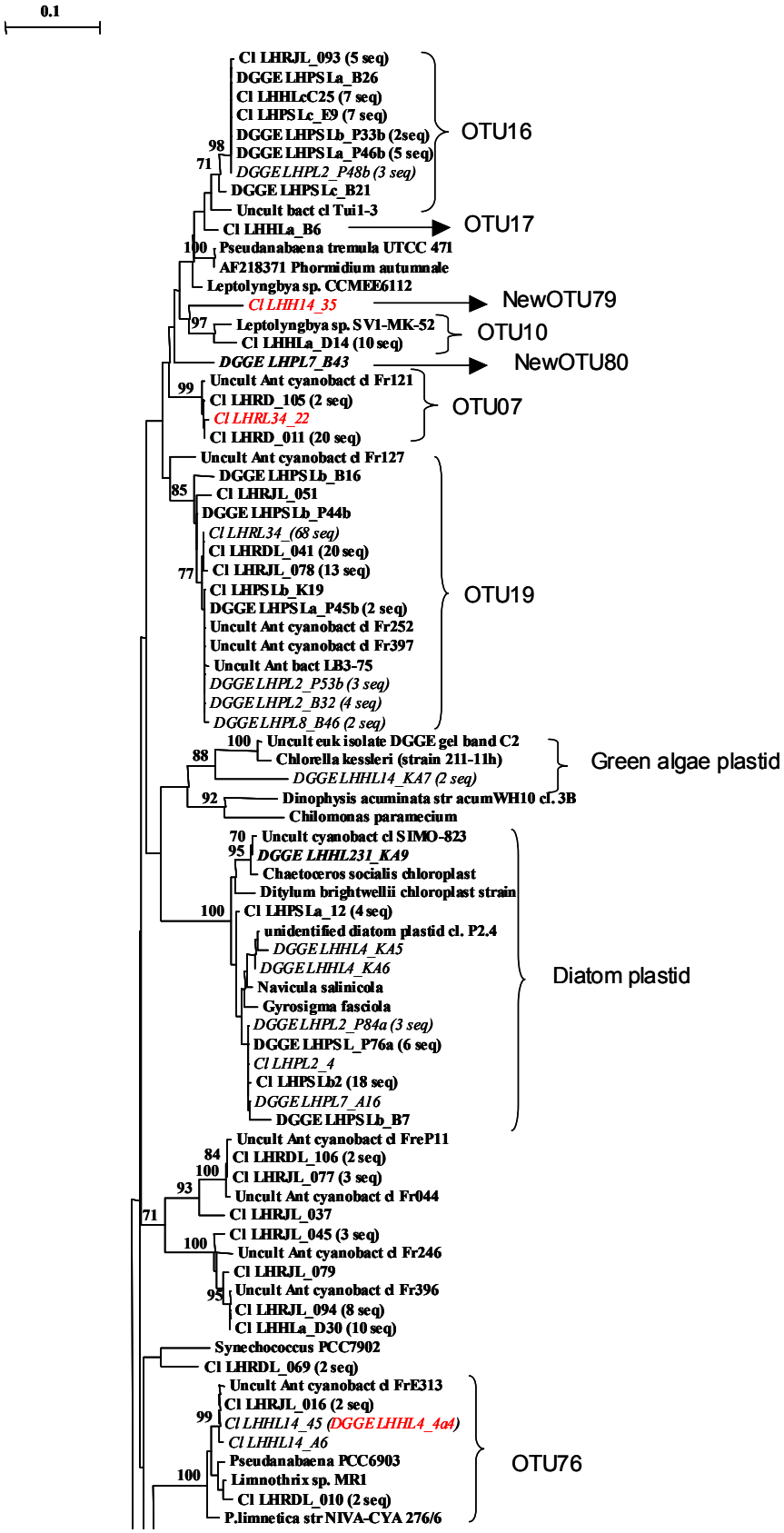
Sediment depth (cm)	Publication Code	Sample Material	^{14}C Enrichment (%Modern +/- 1σ)	^{14}C Enrichment (%Modabsolute +/- 1σ)	Radiocarbon age (years BP +/- 1σ)	Carbon content# (% by wt.)	$d^{13}C_{PDB}$ ‰ +/- 0.1
Broknes transect: Heart Lake							
0	AA-35716	C	109.80 +/- 0.55	109.15 +/- 0.55	modern	21.8	-16.9
20	AA-35736	C DE	72.16 +/- 0.39		2620 +/- 45	1.7	-13.2
105	AA-35737	O DE	42.91 +/- 0.29		6795 +/- 55	0.6	-20.0
175	AA-35738	O DE	41.26 +/- 0.28		7110 +/- 55	1.7	-20.5*
245	AA-35739	O DE	36.63 +/- 0.33		8070 +/- 75	3.0	-20.9
275	AA-41164	O DE	34.68 +/- 0.26		8508 +/- 59	9.3	-10.0
280	AA-35740	D	6.64 +/- 0.14		21780 +/- 160	0.04	-20.5*
320	AA-35741	D	4.20 +/- 0.12		25460 +/- 230	0.05	-23.3
356-360	AA-41633	O DE S	27.7 +/- 0.23		10314 +/- 65	0.3	-18.8
Lake Reid							
0	AA-35720	C	101.97 +/- 0.49	101.37 +/- 0.49	modern	25.2	-12.1
15	AA-35722	C DE	87.19 +/- 0.44		1100 +/- 40	18.2	-11.0
30	AA-35723	C DE	46.39 +/- 0.31		6170 +/- 55	8.1	-10.1
50	AA-35724	C DE	20.95 +/- 0.2		12555 +/- 75	2.6	-12.4
56	AA-35725	O DE	9.64 +/- 0.15		18790 +/- 120	0.8	-15.1
68	AA-35726	O DE	3.68 +/- 0.12		26520 +/- 260	1.5	-14.0*
82	AA-35727	O DE	0.43 +/- 0.11		43800 +/-	2.8	-13.8
102	AA-35728	O DE	0.55 +/- 0.1		41800 +/-	1.8	-20.2
116-118	CAMS-50381	O DE S	**		>39700	1.8	-20.5
Progress Lake							
0	AA-35721	C	113.33 +/- 0.53	112.66 +/- 0.53	modern	21.3	-9.6
6	AA-35754	C	80.54+/- 0.43		1740 +/- 40	1.1	-17.7
10	CAMS-64374	C	68.82 +/- 0.25		3000 +/- 30	0.5	-16.20
17	AA-35755	C	65.93 +/- 0.38		3345 +/- 45	1.9	-18.8
22-24	AA-41165	C	34.68 +/- 0.14		20,920 +/- 150	1.2	-26.0
26	AA-35756	C S	0.46 +/- 0.11		43200 +/- 1900	0.3	-25.8
34	AA-35757	C S	<0.55		>41800	0.5	-26.0
44	AA-35758	C S M	0.26 +/- 0.11		47800 +/- 3300	0.8	-26.0
52	AA-35758	C S M	<0.45		>43400	2.3	-27.2
56-58	CAMS-50376	C S M	**		>43200	0.7	-25
Stornes transect: Kirisjes Pond							
0	AA-35717	C	107.35 +/- 0.51	106.72 +/- 0.51	modern	7.4	-15.8
24	AA-35742	C	80.39 +/- 0.42		1755 +/- 40	2.8	-19.9
52	AA-35743	C DE	77.12 +/- 0.43		2085 +/- 45	1.7	-20.0*
86	AA-35744	C DE	46.19 +/- 0.3		6205 +/- 50	34.0	-14.6
110	AA-35745	O DE	37.76 +/- 0.32		7825 +/- 70	11.0	-23.0
124	AA-35746	C DE	34.51 +/- 0.26		8545 +/- 60	7.6	-17.0
138	AA-35747	C DE O S	27.4 +/- 0.23		10400 +/- 65	11	-16.5
156-158	CAMS-50376	O S	**		>43200	0.7	-25
Pup Lagoon							
0	AA-35718	C	104.45 +/- 0.51	103.83 +/- 0.51	modern	21.3	-13.1
20	AA-35748	C DE	91.93 +/- 0.46		675 +/- 40	36.0	-13.9
50	AA-35749	C DE	85.39 +/- 0.44		1270 +/- 40	26.0	-13.8
100	AA-35750	C DE	82.02 +/- 0.49		1590 +/- 45	8.2	-14.9
150	AA-35751	O DE	76.53 +/- 0.41		2150 +/- 45	13.0	-19.3
200	AA-35752	O DE	61.42 +/- 0.36		3915 +/- 45	6.4	-21.6
250	AA-35753	O DE S	46.88 +/- 0.3		6085 +/- 50	0.06	-19.5
300-302	CAMS-50377	O DE S	45.17 +/- 0.25		6380 +/- 50	0.7	-20.7

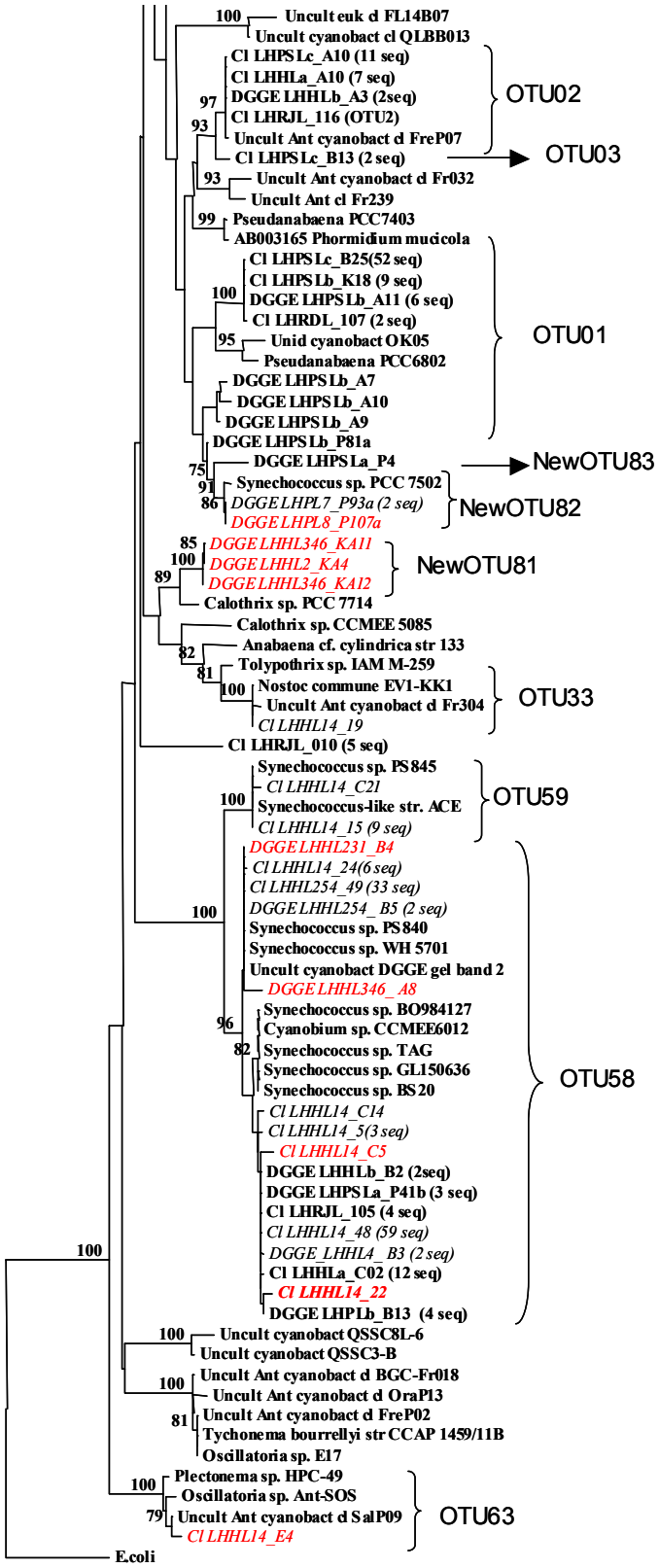
Sample material: C=filamentous cyanobacteria, O=unknown organic fraction, S=silts, sands, M=moss layer, D=diamicton, DE=degraded. **indistinguishable from background at 2 s.d. results reported as > conventional radiocarbon age and/or < ^{14}C enrichment were indistinguishable from background.

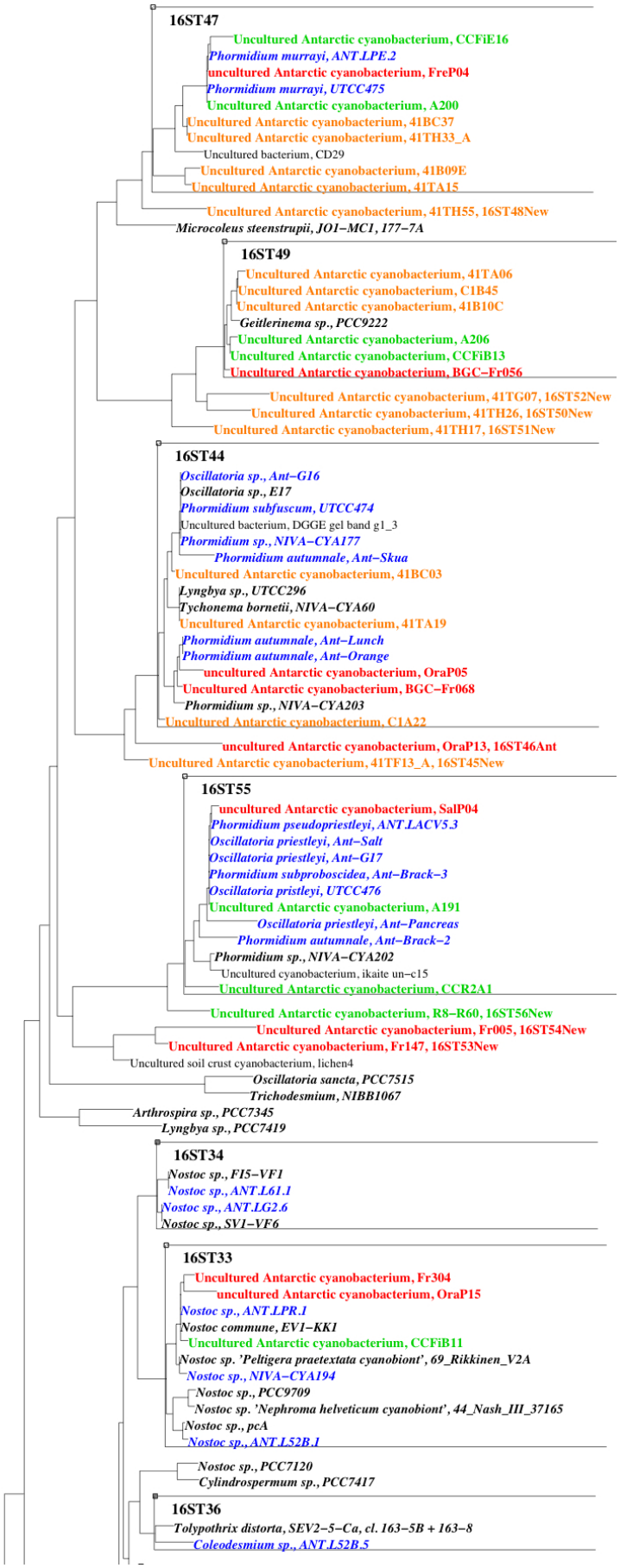
Appendix 3. Relative abundance of each cyanobacterial OTU per sample. List of abbreviations: DVFL ➔ L. Fryxell – DVBGC ➔ Benthic gradient artificial mat from L. Fryxell - SVL ➔ Southern Victoria Land - GB ➔ sequences in GenBank - Unc ➔ Uncultured - Str ➔ Strain - Ulg ➔ sequences obtained at ULg – VHAL ➔ Ace L. – LHLRD ➔ L. Reid1 – LHRJL ➔ L. Reid2 – LHHL ➔ Heart L. – RIR8 ➔ L. Rauer8 – LHPL ➔ Progress L. – BIFIL ➔ Firelight L. in Bolingen Islands – RIR2L ➔ L. Rauer2 - PB ➔ Prydz Bay – AP41B ➔ meltwater 41 lower layer – AP41T ➔ meltwater 41 upper layer – APC1 ➔ meltwater C1 - DML ➔ Dronning Maud Land



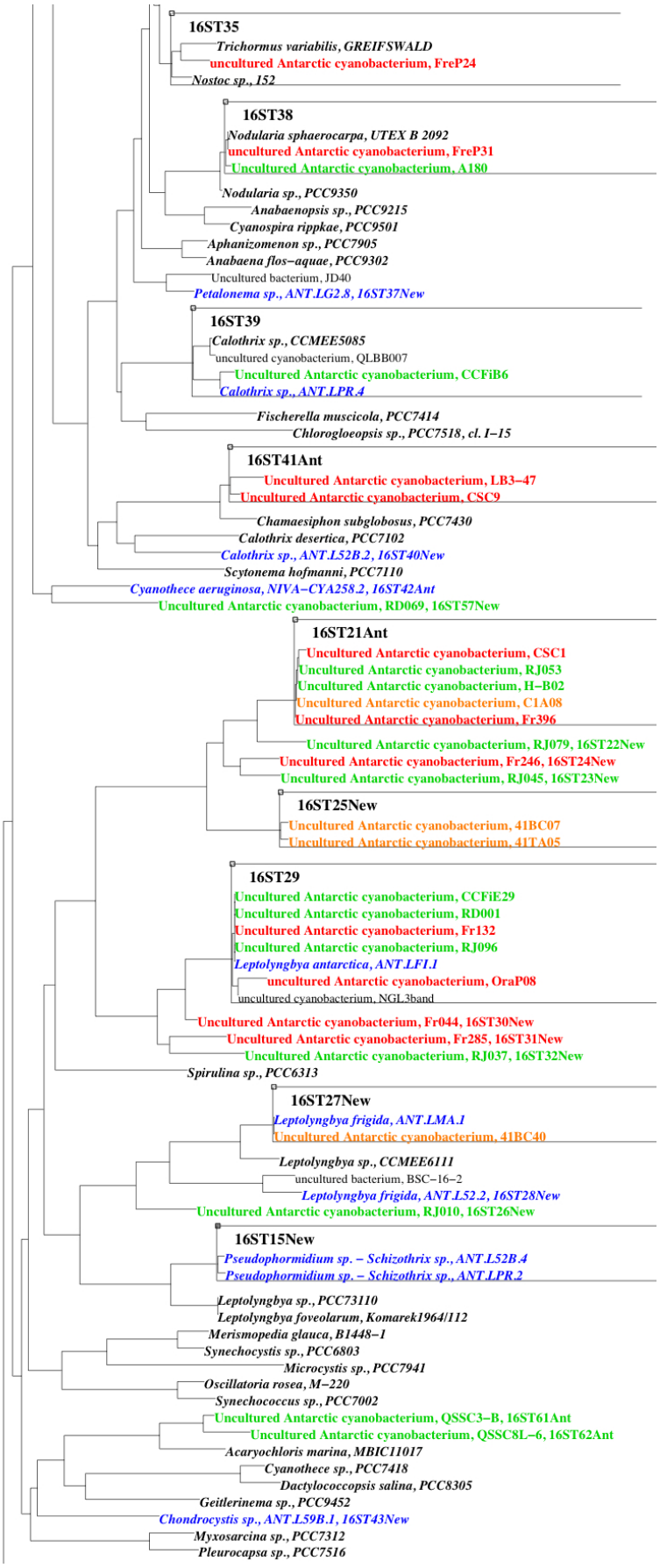
Appendix 4: p. 74-76: Neighbor-joining tree constructed for the study of fossil cyanobacterial diversity on partial 16S rRNA sequences (245 positions). It includes the corrected sequences from this study (clones and DGGE bands) plus their most similar sequences in Genbank and references. Bootstrap analysis was performed, involving the construction of 100 resampled trees, and values higher than 70% are shown besides the concerned nodes. All fossil sequences are in italics and the ones not validated by both laboratories are in red.

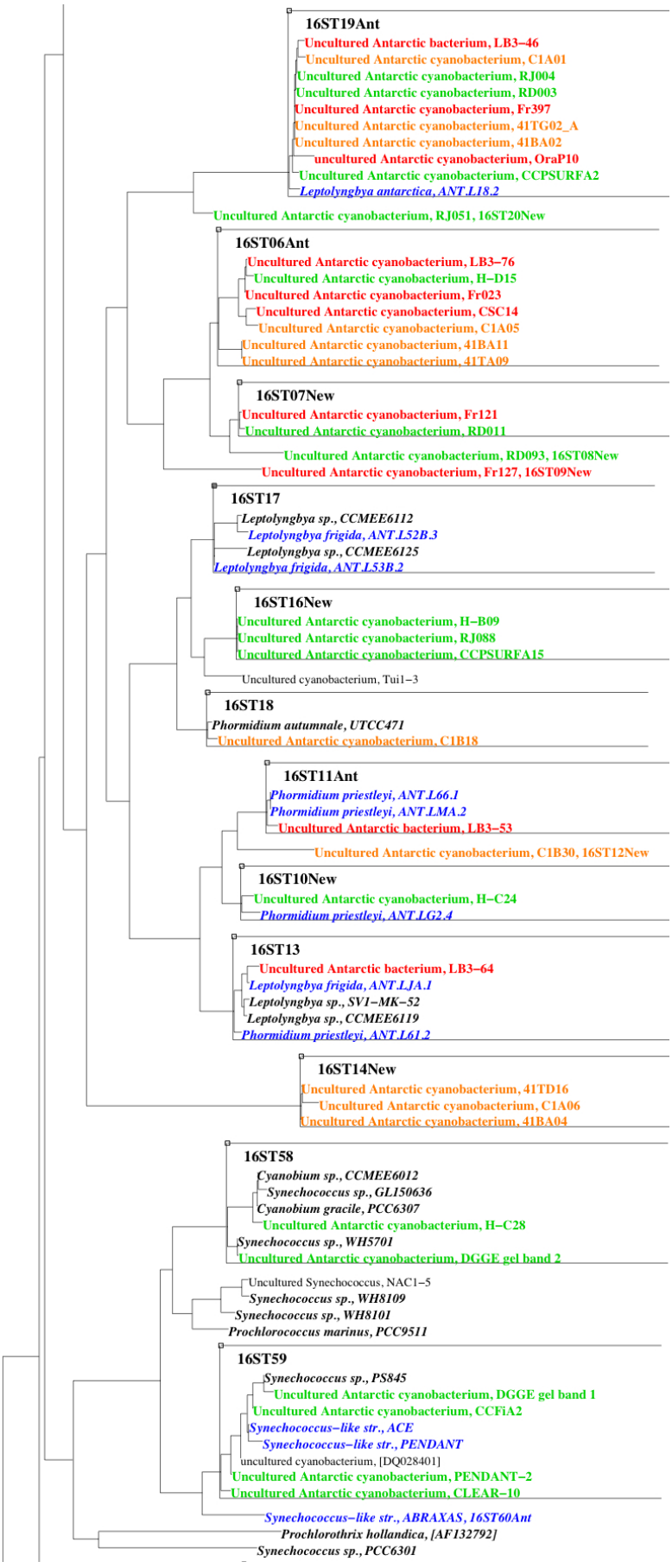

0.1
Cl LHRJL_093 (5 seq)
DGGE LHPS La_B26
Cl LHHLcC25 (7 seq)
Cl LHPS Lc_E9 (7 seq)
DGGE LHPS Lb_P33b (2seq)
DGGE LHPS La_P46b (5 seq)
DGGE LHPL2_P48b (3 seq)
DGGE LHPS Lc_B21
Uncult bact cl Tui1-3
OTU16
Cl LHHLa_B6
OTU17
Pseudanabaena tremula UTCC 471
AF218371 Phormidium autumnale
Leptolyngbya sp. CCMEE6112
Cl LHH14_35
NewOTU79
Leptolyngbya sp. SV1-MK-52
Cl LHHLa_D14 (10 seq)
OTU10
DGGE LHPL7_B43
NewOTU80
Uncult Ant cyanobact cl Fr121
Cl LHRD_105 (2 seq)
Cl LHRL34_22
Cl LHRD_011 (20 seq)
OTU07
Uncult Ant cyanobact cl Fr127
DGGE LHPS Lb_B16
Cl LHRJL_051
DGGE LHPS Lb_P44b
Cl LHRL34_(68 seq)
Cl LHRDL_041 (20 seq)
Cl LHRJL_078 (13 seq)
Cl LHPS Lb_K19
DGGE LHPS La_P45b (2 seq)
Uncult Ant cyanobact cl Fr252
Uncult Ant cyanobact cl Fr397
Uncult Ant bact LB3-75
DGGE LHPL2_P53b (3 seq)
DGGE LHPL2_B32 (4 seq)
DGGE LHPL8_B46 (2 seq)
OTU19
Uncult euk isolate DGGE gel band C2
Chlorella kessleri (strain 211-11h)
DGGE LHHL14_KA7 (2 seq)
Green algae plastid
Dinophysis acuminata str acumWH10 cl. 3B
Chilomonas paramecium
Uncult cyanobact cl SIMO-823
DGGE LHHL231_KA9
Chaetoceros socialis chloroplast
Ditylum brightwellii chloroplast strain
Cl LHPS La_12 (4 seq)
unidentified diatom plastid cl. P2.4
DGGE LHHL4_KA5
DGGE LHHL4_KA6
Navicula salinicola
Gyrosigma fasciola
DGGE LHPL2_P84a (3 seq)
DGGE LHPS L_P76a (6 seq)
Cl LHPL2_4
Cl LHPS Lb2 (18 seq)
DGGE LHPL7_A16
DGGE LHPS Lb_B7
Diatom plastid
Uncult Ant cyanobact cl FreP11
Cl LHRDL_106 (2 seq)
Cl LHRJL_077 (3 seq)
Uncult Ant cyanobact cl Fr044
Cl LHRJL_037
Cl LHRJL_045 (3 seq)
Uncult Ant cyanobact cl Fr246
Cl LHRJL_079
Uncult Ant cyanobact cl Fr396
Cl LHRJL_094 (8 seq)
Cl LHHLa_D30 (10 seq)
Synechococcus PCC7902
Cl LHRDL_069 (2 seq)
Uncult Ant cyanobact cl FrE313
Cl LHRJL_016 (2 seq)
Cl LHHL14_45 (DGGE LHHL4_4a4)
Cl LHHL14_A6
Pseudanabaena PCC6903
Limnothrix sp. MR1
Cl LHRDL_010 (2 seq)
P.limnetica str NIVA-CYA 276/6
OTU76

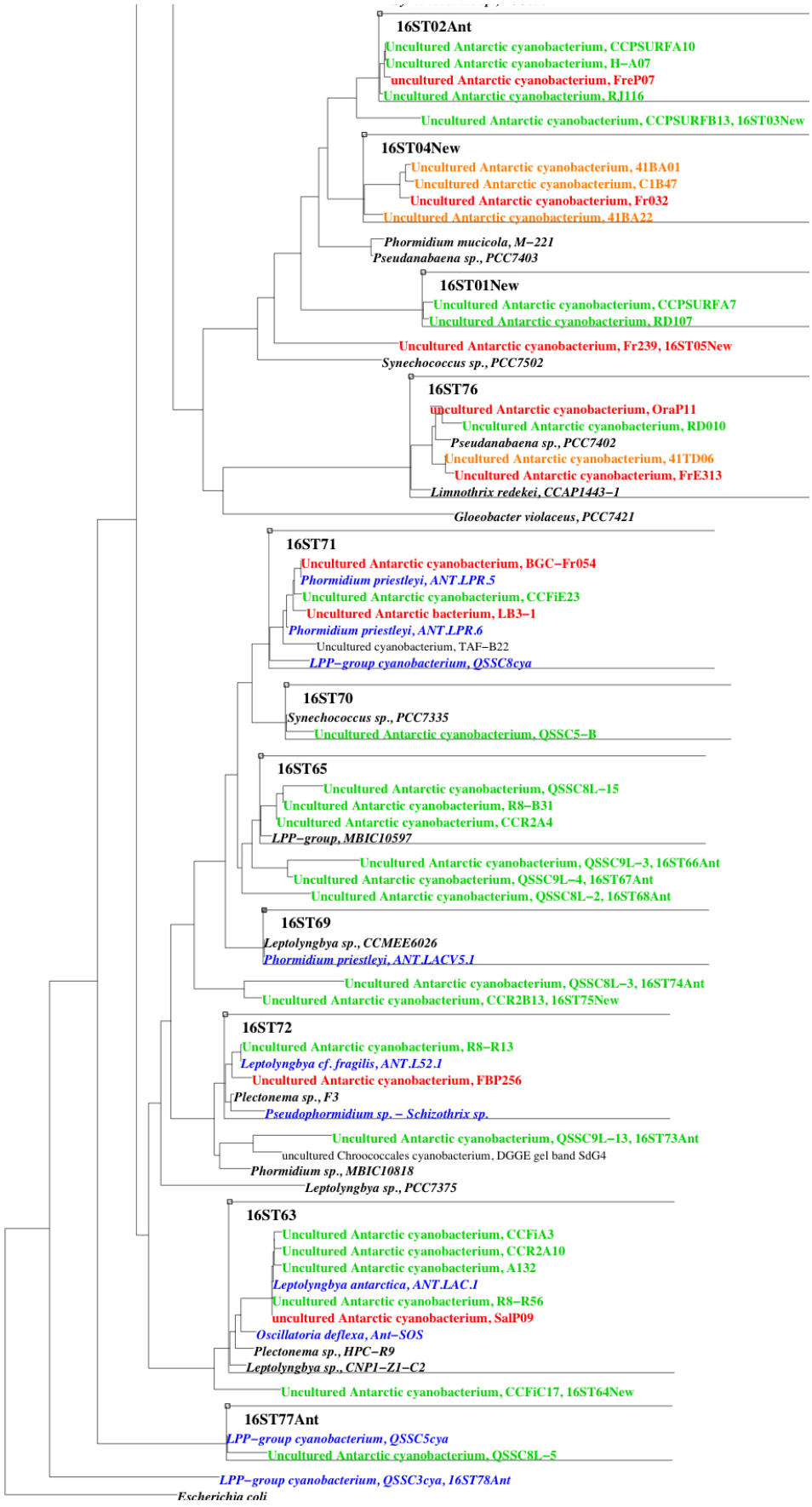

Uncult euk cl FL14B07
Uncult cyanobact cl QLBB013
Cl LHPS Lc_A10 (11 seq)
Cl LHHLa_A10 (7 seq)
DGGE LHHLb_A3 (2seq)
Cl LHRJL_116 (OTU2)
Uncult Ant cyanobact cl FreP07
OTU02
Cl LHPS Lc_B13 (2 seq)
OTU03
Uncult Ant cyanobact cl Fr032
Uncult Ant cl Fr239
Pseudanabaena PCC7403
AB003165 Phormidium mucicola
Cl LHPS Lc_B25(52 seq)
Cl LHPS Lb_K18 (9 seq)
DGGE LHPS Lb_A11 (6 seq)
Cl LHRDL_107 (2 seq)
Unid cyanobact OK05
Pseudanabaena PCC6802
OTU01
DGGE LHPS Lb_A7
DGGE LHPS Lb_A10
DGGE LHPS Lb_A9
DGGE LHPS Lb_P81a
DGGE LHPS La_P4
NewOTU83
Synechococcus sp. PCC 7502
DGGE LHPL7_P93a (2 seq)
DGGE LHPL8_P107a
NewOTU82
DGGE LHHL346_KA11
DGGE LHHL2_KA4
DGGE LHHL346_KA12
NewOTU81
Calothrix sp. PCC 7714
Calothrix sp. CCMEE 5085
Anabaena cf. cylindrica str 133
Tolypothrix sp. IAM M-259
Nostoc commune EV1-KK1
Uncult Ant cyanobact cl Fr304
Cl LHHL14_19
OTU33
Cl LHRJL_010 (5 seq)
Synechococcus sp. PS845
Cl LHHL14_C21
Synechococcus-like str. ACE
Cl LHHL14_15 (9 seq)
OTU59
DGGE LHHL231_B4
Cl LHHL14_24(6 seq)
Cl LHHL254_49 (33 seq)
DGGE LHHL254_ B5 (2 seq)
Synechococcus sp. PS840
Synechococcus sp. WH 5701
Uncult cyanobact DGGE gel band 2
DGGE LHHL346_ A8
Synechococcus sp. BO984127
Cyanobium sp. CCMEE6012
Synechococcus sp. TAG
Synechococcus sp. GL150636
Synechococcus sp. BS20
Cl LHHL14_C14
Cl LHHL14_5(3 seq)
Cl LHHL14_C5
DGGE LHHLb_B2 (2seq)
DGGE LHPS La_P41b (3 seq)
Cl LHRJL_105 (4 seq)
Cl LHHL14_48 (59 seq)
DGGE_LHHL4_ B3 (2 seq)
Cl LHHLa_C02 (12 seq)
Cl LHHL14_22
DGGE LHPLb_B13 (4 seq)
OTU58
Uncult cyanobact QSSC8L-6
Uncult cyanobact QSSC3-B
Uncult Ant cyanobact cl BGC-Fr018
Uncult Ant cyanobact cl OraP13
Uncult Ant cyanobact cl FreP02
Tychonema bourrellyi str CCAP 1459/11B
Oscillatoria sp. E17
Plectonema sp. HPC-49
Oscillatoria sp. Ant-SOS
Uncult Ant cyanobact cl SalP09
Cl LHHL14_E4
OTU63
E.coli



Appendix 5: Neigbor-joining tree constructed for the study of modern cyanobacterial diversity on partial 16S rRNA sequences (375 positions). It includes one representative per OTU and per sample for sequences of strains and clones, one representative per OTU for the Antarctic sequences available in Genbank, the most similar sequences in Genbank and reference sequences. The OTUs are indicated on the tree. Antarctic strain sequences are in blue, AP sequences are in orange, DV sequences are in red, PB sequences are in green and the non-Antarctic sequences are in black.


16ST35
Trichormus variabilis, GREIFSWALD
uncultured Antarctic cyanobacterium, FreP24
Nostoc sp., 152
16ST38
Nodularia sphaerocarpa, UTEX B 2092
uncultured Antarctic cyanobacterium, FreP31
Uncultured Antarctic cyanobacterium, A180
Nodularia sp., PCC9350
Anabaenopsis sp., PCC9215
Cyanospira rippkae, PCC9501
Aphanizomenon sp., PCC7905
Anabaena flos−aquae, PCC9302
Uncultured bacterium, JD40
Petalonema sp., ANT.LG2.8, 16ST37New
16ST39
Calothrix sp., CCMEE5085
uncultured cyanobacterium, QLBB007
Uncultured Antarctic cyanobacterium, CCFiB6
Calothrix sp., ANT.LPR.4
Fischerella muscicola, PCC7414
Chlorogloeopsis sp., PCC7518, cl. I−15
16ST41Ant
Uncultured Antarctic cyanobacterium, LB3−47
Uncultured Antarctic cyanobacterium, CSC9
Chamaesiphon subglobosus, PCC7430
Calothrix desertica, PCC7102
Calothrix sp., ANT.L52B.2, 16ST40New
Scytonema hofmanni, PCC7110
Cyanothece aeruginosa, NIVA−CYA258.2, 16ST42Ant
Uncultured Antarctic cyanobacterium, RD069, 16ST57New
16ST21Ant
Uncultured Antarctic cyanobacterium, CSC1
Uncultured Antarctic cyanobacterium, RJ053
Uncultured Antarctic cyanobacterium, H−B02
Uncultured Antarctic cyanobacterium, C1A08
Uncultured Antarctic cyanobacterium, Fr396
Uncultured Antarctic cyanobacterium, RJ079, 16ST22New
Uncultured Antarctic cyanobacterium, Fr246, 16ST24New
Uncultured Antarctic cyanobacterium, RJ045, 16ST23New
16ST25New
Uncultured Antarctic cyanobacterium, 41BC07
Uncultured Antarctic cyanobacterium, 41TA05
16ST29
Uncultured Antarctic cyanobacterium, CCFiE29
Uncultured Antarctic cyanobacterium, RD001
Uncultured Antarctic cyanobacterium, Fr132
Uncultured Antarctic cyanobacterium, RJ096
Leptolyngbya antarctica, ANT.LFI.1
uncultured Antarctic cyanobacterium, OraP08
uncultured cyanobacterium, NGL3band
Uncultured Antarctic cyanobacterium, Fr044, 16ST30New
Uncultured Antarctic cyanobacterium, Fr285, 16ST31New
Uncultured Antarctic cyanobacterium, RJ037, 16ST32New
Spirulina sp., PCC6313
16ST27New
Leptolyngbya frigida, ANT.LMA.1
Uncultured Antarctic cyanobacterium, 41BC40
Leptolyngbya sp., CCMEE6111
uncultured bacterium, BSC−16−2
Leptolyngbya frigida, ANT.L52.2, 16ST28New
Uncultured Antarctic cyanobacterium, RJ010, 16ST26New
16ST15New
Pseudophormidium sp. − Schizothrix sp., ANT.L52B.4
Pseudophormidium sp. − Schizothrix sp., ANT.LPR.2
Leptolyngbya sp., PCC73110
Leptolyngbya foveolarum, Komarek1964/112
Merismopedia glauca, B1448−1
Synechocystis sp., PCC6803
Microcystis sp., PCC7941
Oscillatoria rosea, M−220
Synechococcus sp., PCC7002
Uncultured Antarctic cyanobacterium, QSSC3−B, 16ST61Ant
Uncultured Antarctic cyanobacterium, QSSC8L−6, 16ST62Ant
Acaryochloris marina, MBIC11017
Cyanothece sp., PCC7418
Dactylococcopsis salina, PCC8305
Geitlerinema sp., PCC9452
Chondrocystis sp., ANT.L59B.1, 16ST43New
Myxosarcina sp., PCC7312
Pleurocapsa sp., PCC7516


16ST19Ant
Uncultured Antarctic bacterium, LB3–46
Uncultured Antarctic cyanobacterium, C1A01
Uncultured Antarctic cyanobacterium, RJ004
Uncultured Antarctic cyanobacterium, RD003
Uncultured Antarctic cyanobacterium, Fr397
Uncultured Antarctic cyanobacterium, 41TG02_A
Uncultured Antarctic cyanobacterium, 41BA02
uncultured Antarctic cyanobacterium, OraP10
Uncultured Antarctic cyanobacterium, CCPSURFA2
Leptolyngbya antarctica, ANT.L18.2
Uncultured Antarctic cyanobacterium, RJ051, 16ST20New
16ST06Ant
Uncultured Antarctic cyanobacterium, LB3–76
Uncultured Antarctic cyanobacterium, H–D15
Uncultured Antarctic cyanobacterium, Fr023
Uncultured Antarctic cyanobacterium, CSC14
Uncultured Antarctic cyanobacterium, C1A05
Uncultured Antarctic cyanobacterium, 41BA11
Uncultured Antarctic cyanobacterium, 41TA09
16ST07New
Uncultured Antarctic cyanobacterium, Fr121
Uncultured Antarctic cyanobacterium, RD011
Uncultured Antarctic cyanobacterium, RD093, 16ST08New
Uncultured Antarctic cyanobacterium, Fr127, 16ST09New
16ST17
Leptolyngbya sp., CCMEE6112
Leptolyngbya frigida, ANT.L52B.3
Leptolyngbya sp., CCMEE6125
Leptolyngbya frigida, ANT.L53B.2
16ST16New
Uncultured Antarctic cyanobacterium, H–B09
Uncultured Antarctic cyanobacterium, RJ088
Uncultured Antarctic cyanobacterium, CCPSURFA15
Uncultured cyanobacterium, Tui1–3
16ST18
Phormidium autumnale, UTCC471
Uncultured Antarctic cyanobacterium, C1B18
16ST11Ant
Phormidium priestleyi, ANT.L66.1
Phormidium priestleyi, ANT.LMA.2
Uncultured Antarctic bacterium, LB3–53
Uncultured Antarctic cyanobacterium, C1B30, 16ST12New
16ST10New
Uncultured Antarctic cyanobacterium, H–C24
Phormidium priestleyi, ANT.LG2.4
16ST13
Uncultured Antarctic bacterium, LB3–64
Leptolyngbya frigida, ANT.LJA.1
Leptolyngbya sp., SV1–MK–52
Leptolyngbya sp., CCMEE6119
Phormidium priestleyi, ANT.L61.2
16ST14New
Uncultured Antarctic cyanobacterium, 41TD16
Uncultured Antarctic cyanobacterium, C1A06
Uncultured Antarctic cyanobacterium, 41BA04
16ST58
Cyanobium sp., CCMEE6012
Synechococcus sp., GL150636
Cyanobium gracile, PCC6307
Uncultured Antarctic cyanobacterium, H–C28
Synechococcus sp., WH5701
Uncultured Antarctic cyanobacterium, DGGE gel band 2
Uncultured Synechococcus, NAC1–5
Synechococcus sp., WH8109
Synechococcus sp., WH8101
Prochlorococcus marinus, PCC9511
16ST59
Synechococcus sp., PS845
Uncultured Antarctic cyanobacterium, DGGE gel band 1
Uncultured Antarctic cyanobacterium, CCFiA2
Synechococcus–like str., ACE
Synechococcus–like str., PENDANT
uncultured cyanobacterium, [DQ028401]
Uncultured Antarctic cyanobacterium, PENDANT–2
Uncultured Antarctic cyanobacterium, CLEAR–10
Synechococcus–like str., ABRAXAS, 16ST60Ant
Prochlorothrix hollandica, [AF132792]
Synechococcus sp., PCC6301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uncultured Antarctic cyanobacterium, FreP07
Uncultured Antarctic cyanobacterium, RJ116
Uncultured Antarctic cyanobacterium, CCPSURFB13, 16ST03New
16ST04New
Uncultured Antarctic cyanobacterium, 41BA01
Uncultured Antarctic cyanobacterium, C1B47
Uncultured Antarctic cyanobacterium, Fr032
Uncultured Antarctic cyanobacterium, 41BA22
Phormidium mucicola, M–221
Pseudanabaena sp., PCC7403
16ST01New
Uncultured Antarctic cyanobacterium, CCPSURFA7
Uncultured Antarctic cyanobacterium, RD107
Uncultured Antarctic cyanobacterium, Fr239, 16ST05New
Synechococcus sp., PCC7502
16ST76
Uncultured Antarctic cyanobacterium, OraP11
Uncultured Antarctic cyanobacterium, RD010
Pseudanabaena sp., PCC7402
Uncultured Antarctic cyanobacterium, 41TD06
Uncultured Antarctic cyanobacterium, FrE313
Limnothrix redekei, CCAP1443–1
Gloeobacter violaceus, PCC7421
16ST71
Uncultured Antarctic cyanobacterium, BGC–Fr054
Phormidium priestleyi, ANT.LPR.5
Uncultured Antarctic cyanobacterium, CCFiE23
Uncultured Antarctic bacterium, LB3–1
Phormidium priestleyi, ANT.LPR.6
Uncultured cyanobacterium, TAF–B22
LPP–group cyanobacterium, QSSC8cya
16ST70
Synechococcus sp., PCC7335
Uncultured Antarctic cyanobacterium, QSSC5–B
16ST65
Uncultured Antarctic cyanobacterium, QSSC8L–15
Uncultured Antarctic cyanobacterium, R8–B31
Uncultured Antarctic cyanobacterium, CCR2A4
LPP–group, MBIC10597
Uncultured Antarctic cyanobacterium, QSSC9L–3, 16ST66Ant
Uncultured Antarctic cyanobacterium, QSSC9L–4, 16ST67Ant
Uncultured Antarctic cyanobacterium, QSSC8L–2, 16ST68Ant
16ST69
Leptolyngbya sp., CCMEE6026
Phormidium priestleyi, ANT.LACV5.1
Uncultured Antarctic cyanobacterium, QSSC8L–3, 16ST74Ant
Uncultured Antarctic cyanobacterium, CCR2B13, 16ST75New
16ST72
Uncultured Antarctic cyanobacterium, R8–R13
Leptolyngbya cf. fragilis, ANT.L52.1
Uncultured Antarctic cyanobacterium, FBP256
Plectonema sp., F3
Pseudophormidium sp. – Schizothrix sp.
Uncultured Antarctic cyanobacterium, QSSC9L–13, 16ST73Ant
uncultured Chroococcales cyanobacterium, DGGE gel band SdG4
Phormidium sp., MBIC10818
Leptolyngbya sp., PCC7375
16ST63
Uncultured Antarctic cyanobacterium, CCFiA3
Uncultured Antarctic cyanobacterium, CCR2A10
Uncultured Antarctic cyanobacterium, A132
Leptolyngbya antarctica, ANT.LAC.1
Uncultured Antarctic cyanobacterium, R8–R56
uncultured Antarctic cyanobacterium, SalP09
Oscillatoria deflexa, Ant–SOS
Plectonema sp., HPC–R9
Leptolyngbya sp., CNP1–Z1–C2
Uncultured Antarctic cyanobacterium, CCFiC17, 16ST64New
16ST77Ant
LPP–group cyanobacterium, QSSC5cya
Uncultured Antarctic cyanobacterium, QSSC8L–5
LPP–group cyanobacterium, QSSC3cya, 16ST78Ant
Escherichia coli