The limnology and biology of the Dufek Massif, Transantarctic Mountains 82° South.

D. A. Hodgson**,** P. Convey, E. Verleyen, W. Vyverman, S. J. McInnes, C. J. Sands, R. Fernández-Carazo, A. Wilmotte, A. De Wever, K. Peeters, I. Tavernier, A. Willems.

In: Polar Science Journal, Volume 4, 103-113, 2010.

http://dx.doi.org/10.1016/j.polar.2010.04.003

The limnology and biology of the Dufek Massif, Transantarctic Mountains 82° South

Dominic A. Hodgson[1*†], Peter Convey[1†], Elie Verleyen[2], Wim Vyverman[2,], Sandra J. McInnes[1], Chester J. Sands[1], Rafael Fernández-Carazo[3], Annick Wilmotte[3], Aaike De Wever[1], Karolien Peeters[4], Ines Tavernier[1], Anne Willems[4]

[1]British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge, CB3 0ET, UK

[2]Laboratory of Protistology and Aquatic Ecology, Ghent University, B-9000 Gent, Belgium

[3]Centre for Protein Engineering, Institute of Chemistry B6, University of Liège, B-4000 Liège, Belgium

[4]Laboratory of Microbiology, Fac. Science, Ghent University, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium

[†]These authors contributed equally to this work

*Corresponding author: Dominic Hodgson

Running title: Limnology and biology of the Dufek Massif

Abstract

Very little is known about the higher latitude inland biology of continental Antarctica. In this paper we describe the limnology and biology of the Dufek Massif, using a range of observational, microscopic and molecular methods. Here two dry valleys are home to some of the southernmost biota on Earth. Cyanobacteria are the dominant life forms, being found in lakes and ponds, in hypersaline brines, summer meltwater, relict pond beds and in exposed terrestrial habitats. Their species diversity is the lowest yet observed in Antarctic lakes. Green algae, cercozoa and bacteria are present, but diatoms were absent except for a single valve; likely windblown. Mosses were absent and only one lichen specimen was found. The Metazoa include three microbivorous tardigrades (*Acutuncus antarcticus*, *Diphascon sanae* and *Echiniscus* (cf) *pseudowendti*) and bdelloid rotifer species, but no arthropods or nematodes. These simple faunal and floral communities are missing most of the elements normally present at lower latitudes in the Antarctic which is probably a result of the very harsh environmental conditions in the area.

Key words: Antarctic, cyanobacteria, biogeography, endemism, refugia.

Introduction

The evolutionary history and geographical isolation of the Antarctic continent have produced a unique environment, inhabited by species adapted to its extreme conditions. However, very little is known about the higher latitude inland limnology and biology of continental Antarctica and the few data that do exist are largely based on opportunistic non-specialist collections made during geological field studies.

The southernmost aquatic systems studied to date are in the Brown Hills and Darwin Glacier region (~80°S), about 300 km south of McMurdo Sound (Vincent and Howard-Williams, 1994). Proglacial lakes are also known to be present as far south as 85°S in the Patuxent Range, and in the Mt. Heekin area of the Transantarctic Mountains.

In the International Geophysical Year (1957) geologists discovered the Davis Valley and 'Forlidas Valley' (the name is unofficial) - unique, over-deepened dry valleys occupying an area of 53 km^2 in the northern Dufek Massif (Behrendt et al., 1974) (Figs. 1 and 2). Although less than 1% of the area of the McMurdo Dry Valleys, Davis Valley and Forlidas Valley are nevertheless the largest ice-free valley system found south of 80°S in the sector between 90°W and 90°E. They contain a near-unprecedented geomorphological record of glacial history of the ice sheet (Boyer, 1979), including evidence of up to seven distinct phases of glaciation (Hodgson et al. in prep.). The area also contains a number of proglacial lakes and ponds and one land-locked pond; the remnant of a once much larger proglacial lake (Fig. 2). The latter, to our knowledge, is the southernmost example of this type of water body. Despite the

cold and dry climate of this region of continental Antarctica the presence of these lakes and ponds showed that liquid water is available which could potentially support a biota. More remarkable were reports from the International Geophysical Year of a 'primitive leafy-type water plant' (Neuburg et al., 1959) and a 'strange pinkish plant that is somewhat leafy' (Behrendt, 1998). This is a reference to the abundant cyanobacterial mats found there, which grow as foliose clumps and would resemble plants to a non biologist.

After nearly 50 years since these first observations we revisited the Davis Valley to describe and measure the limnology of the lakes and ponds and carry out the first biological inventory. The area is particularly interesting for biological studies as a result of the remarkable lack of human impacts, being only briefly visited as part of a traverse in the International Geophysical Year (1957), by the US Geological Survey in 1978-79 and for 9 days by the authors in December 2003. The primary research objective was to determine whether, compared with lower latitude and coastal Antarctic studies (eg. Convey and Stevens, 2007; Convey et al., 2008), the terrestrial and freshwater biota present at these high continental latitudes are post-glacial colonists or long-term survivors.

Site description

The Dufek Massif (Fig. 1) is a range of peaks in the Pensacola Mountains (part of the Transantarctic Mountain range), centred at 82°24' S 52°12' W. It is situated approximately mid-way between the Support Force Glacier and the Foundation Ice Stream, two of the major glaciers draining northwards from the Polar Plateau into the

Ronne-Filchner Ice Shelf. Approximately 60 km to the southeast is the Forrestal Range (also part of the Pensacola Mountains), which is separated from the Dufek Massif by the Sallee Snowfield. The Ford Ice Piedmont separates the Dufek Massif from the Ronne and Filchner Ice Shelves, about 50 km to the northwest and 70 km to the northeast respectively. The nearest significant mountain chains are the Ellsworth Mountains 800 km to the west-north-west and the Shackleton Range 400 km to the north-east, both of which do not form part of the Transantarctic Mountains.

The total area of the Dufek Massif is 11,668 km^2 and its highest point is England Peak (2150 m). Its geology consists of a middle Jurassic differentiated stratiform mafic igneous complex overlain in the lower parts of the valleys by a glacial drape sorted into polygons by freeze thaw processes. The geology has been described by Behrendt et al. (1974), Ford (1976; 1978; 1990) and remotely by Ferris et al. (1998). Cosmogenic isotope surface exposure age dating and geomorphological data point to a complex glaciological history with the repeated exposure of ice free surfaces for at least the last 1.6 million years (Hodgson et al., in prep). Meteorological studies are limited, but mean annual temperatures inferred from nearby ice boreholes lie between -24.96°C, 32 km due north of Forlidas Pond on the Ford Ice Piedmont measured in December 1957 (Aughenbaugh et al., 1958), and -9°C measured in December 1978 in the Enchanted Valley, 26 km to the south (Boyer, pers. comm.). Near surface winds in winter are predominantly from the west-north-west with modeled mean winter velocities of c. 10 m s^{-1} (van Lipzig et al., 2004). Many geomorphological features related to wind erosion such as ventifacts and tafoni are present. Regionally, it has been identified as an ablation area comprising two 'ablation types' (van den Broeke et al., 2006). Type 1 includes erosion-driven ablation areas, caused by 1-D and/or 2-D

120 divergence in the katabatic wind field where solid precipitation and sublimation are
121 small but where divergence in the snowdrift transport can be considerable. Type 2
122 dominates in the Davis and Forlidas Valleys and includes sublimation-driven ablation
123 areas occurring at the foot of steep topographic barriers, where temperature and wind
124 speed are high and relative humidity low, with individual glacier valleys serving as
125 gates for air drainage from the plateau to the Ronne-Filchner Ice Shelf. Strongest
126 sublimation rates occur on these localized glaciers in the Transantarctic Mountains,
127 where widespread blue ice areas are present (van den Broeke et al., 2006).
128
129 Combined, the Davis and Forlidas Valleys are approximately 7 km north to south and
130 7 km west to east (Figs. 2 and 3). Their northern extent in the Davis Valley is defined
131 by the blue ice lobes that form part of the southern margin of the Ford Ice Piedmont
132 (Fig. 4), and southern limit rises to escarpments breached by outlet glaciers, the
133 largest of which is the Edge Glacier which extends approximately 4 km into the Davis
134 Valley from the Sallee Snowfield (Fig. 2). The western and eastern margins are
135 enclosed by Forlidas Ridge and Wujek Ridge (Figs. 2 and 3).
136
137 Both valleys contain frozen and liquid water bodies. In Forlidas Valley there is
138 Forlidas Pond (51°16'48"W, 82°27'28"S), a 90.3 m diameter, shallow pond (1.83 m
139 deep) with a perennially frozen water column and evidence of an occasional
140 freshwater moat. It is an isolated remnant of a formerly much more extensive
141 proglacial lake, which had mid-late Holocene water levels up to 17.7 m above present
142 delineated by an upper limit of salt efflorescence, an absence of well-developed frost-
143 sorted polygons, and a series of lake terraces at 11.6 m, 8.61 m, 4.16 m and 1.25 m
144 above the present water level (Fig. 4 and Hodgson, in prep). An ephemeral frozen

145 melt water pond also occurs where the valley meets the Ford Ice Piedmont. A series
146 of meltwater ponds also occurs along the blue-ice margin of the northern Davis
147 Valley at 51º 05.5'W, 82º 27.5' S and 51º 07' W, 82º 27.55' S (Fig. 6), whilst inland of
148 this a number of relict pond beds mark the position of former proglacial ponds likely
149 formed during periods of ice advance into the valley. Edge Lake (Fig. 2), a
150 perennially frozen proglacial lake at the terminus of the Edge Glacier is surrounded
151 by a series of 4-5 depositional proglacial lake ice-push shorelines cut into the valley
152 side, particularly near the eastern side of the terminus of the Edge Glacier, indicating
153 higher lake ice levels in the past. The surface of the lake has an uneven, slightly
154 domed, topography suggesting that it has accumulated from successive surface
155 meltwater refreezing events, but experiences enhanced ablation at the lake margins.
156 Seasonal melt water streams were observed on the eastern margin of the glacier
157 during the field sampling campaign.
158
159 Incised dry stream channels and water erosion features are evident within the ice-free
160 area. Some are fed by seasonal supraglacial melt water, but others appear to be relict
161 features. The presence of liquid water at or near the surface of all the water bodies,
162 and even the small glacial melt streams at the margin of the Edge Glacier, illustrates
163 the ability of the relatively large areas of bare rock and soil to absorb solar radiation
164 and emit heat causing local ice and snow melt.
165
166 Soils are not well-developed in the area and generally lack a significant organic
167 component. Parker *et al*. (1982) collected a soil sample (S7) that was light brown in
168 color, resulting from gravel weathering predominantly to muscovite. The soil
169 comprised sand (81%) with silt (14%) and clay (5%); a composition different from

other sites in the Pensacola Mountains where the clay proportions of six samples ranged from 0.4% to 1.6%. The soil sample from the Davis Valley had a pH of 6.4 (Parker et al., 1982). Nitrate was the primary nitrogen ion and orthophosphate-P concentration was below the detection limit of 0.01 μg g^{-1}. Microbial analyses of soil cultures showed that one pseudomycelium-forming yeast and measurable numbers of viable, aerobic heterotrophic microorganisms were present including Gram-negative rods, but these were not identified further (Parker et al., 1977; Parker et al., 1982).

On the grounds that the area contains some of the most southerly freshwater ponds known in Antarctica that contain plant life which would be threatened by possible contamination by human activity (Behrendt, 1998), Forlidas Pond and Davis Valley Ponds were designated as an Antarctic Specially Protected Area (ASPA). ASPA No.23 lies within Specially Reserved Area No.1, proposed by the USA and adopted at the Antarctic Treaty Consultative Meeting XVI (Bonn, 1991; http://cep.ats.aq/cep/apa/aspa/sites/aspa119/summary.html).

Methods

Surveying and environmental measurements

Topographic maps were compiled by the Mapping and Geographical Information Centre (BAS) at 1:50,000 scale using aerial photographs from the United States Geological Survey (Lassiter Station 1-16 and 1-17, 01.02.1958), GPS-surveyed ground control and differential geodetic GPS survey transects using a Trimble 5700 base station and a Magellan ProMark 10CM rover unit. Altitudes were referenced to

195 the WGS84 reference ellipsoid, and included accurate photogrammetric height
196 measurements of key landforms. Gemini Tinytag Plus data loggers were deployed to
197 measure temperature and relative humidity at the sampling sites from 3-15 December
198 2003. Loggers were placed over snow and rock to measure the influence of advected
199 radiation, with the sensors oriented to shield them from direct sunlight and data
200 recorded at 30 minute intervals. Lake water conductivity, temperature and oxygen
201 saturation were measured using a SOLOMAT WP4007 water quality meter and
202 803PS Sonde. Water chemistry analyses followed the protocols described in Hodgson
203 et al. (2009). Briefly, sodium, potassium, calcium, magnesium, iron, aluminium,
204 manganese and silicon were determined by ICP-OES. Anions nitrate, chloride and
205 sulphate were determined from direct analysis of aqueous solutions by Ion
206 Chromatography. Total dissolved nitrogen was determined on a Shimadzu TNM-1
207 analyser equipped with a thermal conductivity detector DOC and TOC were
208 determined by Shimadzu TOC-Vcph analyser with a detection limit of 0.5mg/l.
209 Nutrients phosphate and ammonium were determined by colorimetry.
210
211 *Biological sampling*
212 The biological sampling programme is summarised in Table 1. To minimise
213 contamination we followed the protocols outlined in the Management Plan for
214 Antarctic Specially Protected Area No. 119. All sampling equipment was scrubbed
215 with Virkon multi-purpose disinfectant before use, and subsamples were collected in
216 sterile WhirlPak bags or acid-washed bottles. Water samples were collected directly
217 into acid-washed bottles (surface waters) or via a UWITEC water sampler (Brine
218 layer). Biological material was collected manually from lake benthic, littoral and
219 catchment areas at each of the study sites. Samples for microscopic investigation were

preserved in Lugol's iodine solution or ethanol and those for molecular analysis were frozen.

The presence of mosses and lichens was assessed. Due to the extreme rarity of lichens in the area (and its protected status) we did not remove samples, but instead photographed them for later taxonomic study.

A combination of microscopic and molecular methods was used to identify the microbiota. Microscopic methods included analyses of water samples in UWITEC plankton counting chambers, and analyses of natural samples and cultures. Representative samples obtained for microbial analyses included the hypersaline brine at the bottom of the Forlidas Pond (sample TM1), a cyanobacterial mat that was actively growing in the littoral zone (air bubbles on the mat surface and trapped under ice were taken as evidence of recent photosynthetic activity) situated under 15 cm of ice and 15 cm of water (TM2), and a sample of a red-orange foliose clump of terrestrial cyanobacterial mat located 20 metres from the shoreline and probably corresponding to "plant-like" organisms reported previously (Neuburg et al., 1959; Behrendt, 1998) (TM3).

Cyanobacteria were analysed by a combination of morphological and molecular methods, as described in detail by Fernández-Carazo et al. (in prep.). Briefly, they involved the observation of cultures by microscopy with reference to the taxonomic works of Komárek and Anagnostidis (2005) and the diacritical morphological traits described by Taton et al. (2006a). DNA extraction methods were slightly modified from Taton et al. (2003). Following this, DGGE, construction of clone libraries and

245 isolation and characterisation of the strains were carried out (Taton et al., 2003; Taton
246 et al., 2006b). Sequencing was performed by GIGA (http://www.giga.ulg.ac.be/)
247 (Liège, Belgium) using an ABI 3730 xls DNA analyser (Applied Biosystems, Foster
248 City, USA). A distance tree was constructed with the software package TREECON
249 for Windows 1.3b (Van de Peer and De Wachter, 1997) by the Neighbor-joining
250 method (Saitou and Nei, 1987) using 379 positions covered by most sequences. The
251 formula of Jukes and Cantor was used to correct for multiple mutations. The tree
252 comprised DGGE bands, clones and strains' sequences from Forlidas Pond samples as
253 well as their 3 most similar strain sequences, and 5 uncultured sequences selected
254 using Seqmatch from RDP (http://rdp.cme.msu.edu). A bootstrap analysis was
255 performed involving the construction of 500 resampled trees. The OTUs were
256 calculated using DOTUR (Schloss and Handelsman, 2004), with a threshold at 97.5%
257 .16S rRNA similarity to define OTUs. In the case of OTU 16ST80, the sequence of
258 sdG4 is exactly at the limit of similarity, and it was included in this OTU.
259
260 In order to study the cultivable bacterial diversity, isolates of a littoral sample (TM2)
261 were grown on a selection of heterotrophic media (Peeters et al., in preparation). The
262 isolates were screened for duplicates and grouped by rep-PCR using primer (GTG)5
263 as described in Gevers et al. (2001). Representative isolates were identified by partial
264 16S rRNA gene sequencing using primer BKL 1 (Coenye et al., 1999). A fragment of
265 approx. 400 to 450 bp from the 5' end was obtained and compared with the EMBL
266 database using FASTA (http://www.ebi.ac.uk/Tools/fasta33) for preliminary
267 identification.
268

To study the uncultivable diversity of bacteria, green algae and cercozoa, we first optimized the protocols to extract DNA from environmental samples by removing extracellular DNA (Corinaldesi et al., 2005) prior to bead-beating extraction. For the Denaturing Gradient Gel Electrophoresis (DGGE) analysis of the bacteria, we followed the protocols as described in Van der Gucht et al. (2001). For the green algae, we used a nested PCR approach using the primer combinations Euk1A-CHLO02r and Euk1A-Euk516r-GC (Díez et al., 2004). These primers are known to also detect cercozoa (Zhu et al., 2005), hence these taxa were also included in the DGGE analysis. Excised bands were sequenced and identified after re-extraction and amplification. A nucleotide BLAST search (Altschul et al., 1997) was performed in order to obtain sequences that were most similar.

Samples for diatom analysis were prepared following a slightly modified protocol from Renberg (1990) and embedded in Naphrax®. The slides were screened for the presence of frustules at 1000x magnification using a Zeiss Axioplan II microscope.

Fauna were extracted using Baermann and Tullgren extractions on return of the collected substrata to the BAS Rothera Research Station, having been kept under field conditions in the intervening ~ two week period. Tardigrades from the Baermann funnel extractions were permanently mounted on microscopes slides (using de Faures medium) for identification. Individual tardigrades were grouped into morphotypes under 400x magnification. A representative of each morphotype was mounted and examined under high power (1000x) magnification for detailed taxonomic identification. It was not possible to extract tardigrade DNA from ethanol preserved samples from the Dufek Massif, so our specimens were compared with DNA from

morphologically congruent dried meiofaunal samples collected from Lake Lundström, in the adjacent Shackleton Range (400 km distant). Meiofauna were separated from the substrate by homogenising and centrifugation of samples using an Optiprep™ gradient solution (see Sands et al. 2008 for detailed methods). Specimens were lifted into individual tubes with 5 µL double distilled H_2O and kept stored frozen (-80 °C). DNA was released from individual tardigrades by disrupting the tissue by a series of freeze thaws followed by a 15 minute incubation at 95 °C in a 5% chelex solution. Three genes were amplified, a fragment of mitochondrial cytochrome c oxidase (CO1), the near complete small ribosomal sub unit (18S), and a fragment of the *Wingless* gene (Wnt). Details of the above protocol, including primers and amplification strategy, and results are given in Sands et al. (2008).

Results and Discussion

Environmental measurements and observations

Microclimate temperatures over snow from 3-15 December 2003 ranged from a maximum of +12.8ºC to a minimum of –14.5ºC, with an average over the period of –0.56ºC. Microclimate temperatures over rock ranged from a maximum of +16.0ºC to a minimum of –8.6ºC, with an average over the period of +0.93ºC (Fig. 7). Relative humidity recorded over snow ranged from a maximum of 80.4% to a minimum of 10.8%, with an average over the period of 42.6%. Over rock surfaces, relative humidity ranged from a maximum of 80.9% to a minimum of 5.6%, with an average over the period of 38.7% (Fig. 7).

Being in an ablation area (van den Broeke et al., 2006) evaporation and sublimation dominate over precipitation. As a result Forlidas Pond has evaporated down to a small remnant of a once much larger proglacial lake (Fig. 5). The pond was frozen almost completely to its base, with a thin layer of hypersaline slush at the lake bottom. The depth of the pond was 1.83 and the thickness of the ice between 1.63 and 1.83 m. The conductivity of the hypersaline slush was 142.02 mS cm^{-1}, approximately four times greater than seawater. The ionic order of the brine layer was Cl-Na-Mg-SO_4-Ca-K and its temperature was -7.67 ºC (Table 2). At the margins of the pond, liquid water was present in a moat area under 10-15 cm of ice. At Forlidas Pond this moat water had a freshwater ion sum of 178 mg L^{-1}compared with the 111942 mg L^{-1} of the brine layer (Table 2). The Davis Valley Ponds also had shallow freshwater littoral moats that, at the time of sampling, were either locally ice free, frozen with liquid water present under ice, or frozen to the bed. The surface morphology of the pond ice suggested that that these moats could have been 1-2 m more extensive during warmer years.

Flora

Visible biota was limited within the study area, and macroscopic vegetation appeared to be restricted to cyanobacterial mats, found both in lakes and terrestrial habitats, and a very sparse occurrence of small (~ mm scale) yellow and black crustose lichens deep within crevices on larger boulders (Fig. 8), as previously observed by Neuburg *et al.* (1959). Often, only the black apothecia were visible. Through analyses of the photographs the species has been identified as *Lecidea cancriformis* Dodge & Baker; one of a few lichens which occurs in the severest environments of continental Antarctica, especially on far inland nunataks as far as 86°S, to high altitudes

(Ovstedal and Lewis-Smith, 2001, page 220, Plate 48). The British Antarctic Survey Plant Database also reports *Blastenia succinea* Dodge & Baker and *Xanthoria elegans* (Link.) Th. Fr. in samples from elsewhere in the Dufek Massif; although these have not been independently verified by us. Previous anecdotal reports of the possible occurrence of mosses within the area could not be substantiated, and it is probable that the rich cyanobacterial mat growth was previously mistaken for bryophytes by non-specialists. The cyanobacterial community is the most abundant biota and is present in at least three distinct environments:

(1) In the permanent water bodies; particularly in the moat of Forlidas Pond, at the bottom and littoral zones of the Davis Valley Ponds, and in the seasonally wetted perimeter of Edge Lake. These habitats were extensively covered by red-brown cyanobacterial mats (Fig 9a). These were actively photosynthesizing, as evidenced by gas bubbles trapped against the lower ice surfaces, and bubbles incorporated into the ice. Because perennially ice covered lakes have elevated concentrations of dissolved O_2 gas, the microbial mats growing on the bottom can become buoyant and start to float off the bottom as 'lift-off' mats, (cf. Doran et al., 2004, p. 480), or become incorporated into the base of the lake ice when it makes contact with the bed. In Forlidas Pond and the Davis Valley Ponds lift off mats frozen into the base of the lake ice eventually migrate up through the ice profile (cf. Adams et al., 1998). In the Davis Valley, this appeared to take place over several years with each summer marked by the development of a 2-3 cm melt-cavity formed by the upward progression of the clump thorough the lake ice due to preferential heating of its upper surface (Fig. 9b). These clumps eventually break out at the surface and are dispersed by wind onto the shoreline, or further afield. In the littoral zone of Forlidas Pond, melting and

369 refreezing of the moat has resulted in cyanobacterial mats being incorporated under
370 shoreline boulders. Fossil examples of this type of mat were also found buried under
371 boulders between the present and previous (higher) shorelines. Cyanobacteria were
372 also present in the hypersaline brine of Forlidas Pond as single cells and as small
373 flakes. A strain corresponding to the morphology of *Leptolyngbya antarctica* was
374 isolated from the saline slush of TM1 (Fernandez-Carazo et al. in prep.).
375
376 (2) In exposed terrestrial locations, particularly at the edge of larger rocks and within
377 the boundary crevices of frost sorted polygons. These were generally very foliose in
378 form, mid brown in colour, and best developed at the edge of larger rocks with depths
379 of at least 10-15 cm (Fig. 9c & 9d). Nearly all clumps were completely dry on
380 discovery, although those near to melting snow were damp and some had lower thalli
381 that were often deep green in colour. Particularly good examples of this growth form
382 were found in the mid valley floor of Forlidas Valley and in Davis Valley (near a
383 large snow gully where it meets the second major terrace above Edge Lake);
384
385 (3) In a series of dry pond beds, two of up to 50 m diameter, in the Davis Valley (Fig.
386 2), which have extensive areas of almost continuous cyanobacterial mat on the former
387 pond floors (Fig 8d). These pond beds and gullies occupy depressions and therefore
388 may accumulate snow in winter, permitting the cyanobacteria to take advantage of the
389 wet and protected environment within the snow patches (Cockell et al., 2002).
390
391 Analyses of the cyanobacterial molecular diversity in and around Forlidas Pond
392 showed that the richness obtained in the current study was lower (2 to 5 OTUs per
393 sample) than in Antarctic coastal lakes (4 to 12 OTUs per sample (Fernandez-Carazo

et al. in prep.). The spatial distribution of the cyanobacterial OTU's showed that TM1 (hypersaline brine at the bottom of the pond) and TM2 (mats in the littoral zone) shared two OTUs, 16ST63 and 16ST14 (Table 1, Fig. 10). In addition, TM2 shared OTUs 16ST44, 16ST49 and 16ST80 with the terrestrial mat (TM3). These data support the idea that the cyanobacterial diversity is not limited to specific aquatic or terrestrial habitats, but that aquatic species are able to colonise the surrounding terrestrial niches, and *vice versa* (cf. Gordon et al., 2000). On the basis of the geomorphological evidence of the former presence of a larger proglacial lake that evaporated, it is possible that the terrestrial cyanobacteria were once aquatic, but still survive in the dried lake bed. Another hypothesis could be that the foliose clumps were made by submersed aquatic cyanobacteria that died subsequently, but whose undegraded DNA has contributed to the 16S rRNA survey for TM3.

Microscopic analyses revealed that no diatom communities were present. One solitary valve of the diatom *Pinnularia microstauron* (Ehr.) Cl. was detected but, as this is a common windblown subaerial diatom, we do not consider it as evidence of an extant community. A study of ponds in the Darwin Glacier region (79.7°S) reported a similar absence of diatoms (Vincent and Howard-Williams, 1994). Analysis of the DGGE bands in the environmental samples revealed only a single green algal sequence (*Urospora* sp.; E30) and two cercozoans (a Heteromitidae; E12_5 and a *Paulinella* sp.; E23). Bacteria identified by DGGE bands included *Cyanobacteria* (*Nostocales, Oscillatoriales, Chroococcales, Gloeobacteriales* , Bacteroidetes (*Sphingobacteriales* and *Flavobacteriales*), *Firmicutes* (*Clostridiales*) and *Gammaproteobacteria* (*Pseudomonadales*; *Psychrobacter*) (Tables 1 and 3). Preliminary data based on partial 16S rRNA gene sequence analysis of the isolated bacteria in cultures revealed

419 a large diversity. Of more than 330 isolates sequenced, 33% belonged to the
420 *Firmicutes* (low %GC Gram-positive bacteria), 23% were *Bacteroidetes,* 25% were
421 *Alphaproteobacteria*, 9% were *Actinobacteria* (high %GC Gram-positive bacteria)
422 and 8% were *Betaproteobacteria*. *Gammaproteobacteria* (1.5%) and Deinococci
423 (0.3%) were present in smaller numbers.
424
425 Viable yeast species have previously been recorded in the soil, along with the
426 cyanobacterium *Oscillatoria* sp., and the algae *Trebouxia* sp. and *Heterococcus* sp.
427 (Parker et al., 1982). Chasmoendolithic cyanobacteria have been recorded in rocks on
428 the west spur of Walker Peak at about 1070 m in the Dufek Massif (Friedman, 1977),
429 although we found no evidence of their presence within the dry valley areas included
430 in our survey, suggesting that endolithic organisms are not widespread here.
431
432 *Fauna*
433 The invertebrate fauna within the area was equally impoverished, with both the
434 diversity and abundance of organisms being extremely limited compared with lower
435 latitude and coastal Antarctic sites. A total of 50 Tullgren extractions of terrestrial
436 cyanobacterial mat and soil substrates generated no arthropods. Although a negative
437 result, this strongly suggests that these groups are absent from the area as there are no
438 other obvious habitats which might be expected to harbour them. A total of 130
439 Baermann extractions revealed three species of the tardigrade from two Classes:
440 *Echiniscus* (cf) *pseudowendti* Dastych, 1984 (Heterotardigrada), *Acutuncus*
441 *antarcticus* (Richters, 1904) and *Diphascon sanae* Dastych, Ryan and Watkins, 1990
442 (Eutardigrada) and a few unidentified bdelloid rotifers (Table 1). Tardigrades were
443 commonly found (c. 40-60 per cc) while rotifers were rarer (c. 5-15 per cc); although

444 not a quantitative extraction technique, these values are low in comparison with those
445 obtained from similar extractions from other Antarctic locations. Surprisingly, the
446 most productive sites for these organisms were not the aquatic environments of the
447 permanent lakes, but the former pond beds in the Davis Valley. *Acutuncus antarcticus*
448 is an Antarctic species that occurs in semi-permanent damp/wet habitats throughout
449 the Antarctic continent and sub-Antarctic islands, but has not been reported from any
450 of the close neighbour continents. *Echiniscus* (cf) *pseudowendti* and *Diphascon sanae*
451 found in samples from Forlidas Pond are also endemic to the Antarctic, with restricted
452 distributions. For example, *Echiniscus* (cf) *pseudowendti* has been found in the
453 maritime regions of the Antarctic Peninsula, Droning Maud Land (Heimefrontfjella)
454 and Enderby Land (Thala Hills), and *Diphascon sanae* from Droning Maud Land
455 (Robertskollen), Enderby Land (Prince Charles Mountains and Mawson Station) and
456 Ellsworth Land (for a discussion of the molecular data see Sands et al., 2008).
457
458 Avifauna was sparse. A single snow petrel (*Pagadroma nivea*) was noted flying
459 around one of the peaks above Davis Valley.
460
461 *Biological interactions with the physical environment*
462 As "dry valley" ecosystems, the Davis and Forlidas Valleys share environmental
463 features with the Dry Valleys of Victoria Land. However, they are 2100 km distant
464 from them and 570 km further south (82°S vs. 77°S). The climate of the Dufek Massif
465 is that of an Antarctic cold desert, experiencing limited precipitation and rapid
466 ablation. This restricts the potential for metabolic activity and prevents growth for
467 much of the year, possibly even requiring dormancy on multi-year timescales.
468 However, summer microclimate temperatures measured during the field campaign

were relatively high, ranging from –14.5 ºC to +12.8 ºC over snow and –8.6 ºC to +16.0 ºC over rock during our visit. The valleys also have many features related to wind erosion but some of these may be relatively ancient, as they occur mostly on rock surfaces above the glacial drift limits, whilst the foliose terrestrial cyanobacterial growth forms remain intact on the valley floor.

With these relatively harsh conditions and physical isolation, it is perhaps not surprising that the valleys appear to lack many of the components typical of the Victoria Land ecosystems (see Adams et al. 2006), including nematodes, arthropods and mosses, and that there is an extremely sparse development of lichens. Cyanobacteria are the dominant phototrophs, in common with other aquatic and terrestrial polar ecosystems (cf. Vincent, 2000), and benefit from a range of biochemical adaptations for survival in shallow water and terrestrial habitats (Hodgson et al., 2004). In the Dufek Massif their greatest biomass was found in the microbial mats that form in the benthic and littoral zones of lakes and ponds and in terrestrial habitats.

The abundance and macroscopic growth-form of the terrestrial cyanobacteria despite apparent limited water availability is something of a paradox. At the time of our visit there was very little snow within either valley bottom. However, water is required for the mats to be metabolically active and is likely derived from within snow patches or from snow melt focussed into depressions and in the lee of boulders, with metabolically active periods being short and unpredictable. Alternatively, later in the season there may be periods of increased supraglacial meltwater flowing off the local ice sheet and outlet glaciers, which could potentially provide a source. Although there

494 was no implication of this process occurring during our visit, we located deep
495 footprints from a previous visit (i.e. 20-45 years old), which indicated that some
496 ground was waterlogged at that time.
497
498 The most productive terrestrial habitats appeared to be the dried areas of mat on the
499 beds of the relict proglacial ponds (Fig. 2) and in cracks, crevices, the lee side of
500 boulders (Fig. 9c), and in the shallower parts of the ponds (Fig 9a). This is a likely
501 function of these environments accumulating snow cover in winter which persists into
502 spring. Previous studies suggest that such under snow habitats can be biologically
503 very active in Antarctica (Cockell et al., 2002), and they also have the advantage of
504 protecting the biota from exposure to wind abrasion. Extractions from samples taken
505 from within these areas were found to yield the greatest numbers of rotifers and
506 tardigrades.
507
508 Mats collected within freshwater ponds did not generate larger numbers or diversity
509 of invertebrates. Rotifers, at least, were present in clumps of mat that had travelled
510 upwards through the pond ice – it is plausible that they have been present throughout
511 the upwards journey, although colonisation while on the ice surface is also possible.
512
513 *Species diversity and endemism*
514 The very limited species diversity in the Forlidas and Davis Valleys supports the
515 hypothesis that, in the more extreme and remote regions of continental Antarctica,
516 species assemblages present would be characterised by a low biodiversity, taxa
517 tolerant of extreme cold and dry conditions, and a certain degree of endemism. For
518 Antarctic microbial communities, molecular studies have shown that their

519 composition includes both cosmopolitan OTUs, and also a greater number of
520 Antarctic endemic species than has been estimated by traditional morphological
521 methods (Taton et al., 2006a; DeWever et al., 2009). Our molecular data for
522 cyanobacteria in Forlidas Valley show a depleted diversity, with only 2 - 5 OTUs per
523 sample compared with the greater number of OTUs found in other regions of
524 Antarctica (Taton et al., 2006a). This is likely a product of geographical isolation (cf.
525 Vyverman et al., 2007; Verleyen et al., in press) combined with multiple
526 environmental stressors such as salinity and seasonal desiccation, and UV radiation
527 (Bowman et al., 2000; Taton et al., 2006a). However some of the cyanobacteria, such
528 as OTU 16ST63 from the brine of Forlidas Pond, are related to sequences from other
529 hypersaline Antarctic lakes such as Rauer 8 in the Rauer Islands (for location see
530 Hodgson et al., 2001) and Ace Lake in the Vestfold Hills (16ST23 in Taton et al.,
531 2006a), but are also present in desert crusts on sand dunes in Israel (AM398947) and
532 in saline lakes in Chile (EF633019). OTU 16ST07 only includes sequences from
533 different sampling locations on the Antarctic continent and sequences from Tibetan
534 glaciers, which could indicate that this OTU has particular adaptations to glacial
535 conditions (Fig. 10). The six cyanobacterial OTUs are found outside Antarctica, but in
536 addition, they are distributed in more than one location within the continent (Fig. 10).
537 This is in agreement with previous studies where the cosmopolitan OTUs have been
538 found to be more widespread on the continent than endemics. For example, Taton et
539 al. (2003; 2006a; 2006b) studied the molecular diversity in benthic cyanobactera in
540 lakes from different and geographically separated Antarctic biotopes, including Lake
541 Fryxell (McMurdo Dry Valleys) and coastal lakes in the Prydz Bay region (East
542 Antarctica). In addition, two meltwater samples from Livingston Island (Antarctic
543 Peninsula) have recently been studied (unpubl. data). Using clone libraries based on

544 16S rRNA sequences, 70% of OTUs were only found in Antarctica. This suggests a
545 rather high degree of endemism, though the influence of geographic gaps in the
546 database might be biasing the data. However, within this dataset, a higher proportion
547 of the cosmopolitan genotypes were found in multiple Antarctic regions (47%,
548 compared to 16% for the apparently 'endemic' sequences). These cosmopolitan
549 genotypes are likely to possess resistance capacities (Taton et al., 2006b) that will also
550 be beneficial during dispersal to and between different Antarctic regions (Zakhia et
551 al., 2007).
552
553 At Forlidas Pond the two OTUs found in the hypersaline brine (TM1) were also
554 present in the littoral zone (TM2), and three of the OTUs from the littoral zone were
555 also found in the terrestrial sample (TM3), suggesting that although the diversity is
556 low some OTU's have a wide environmental tolerance (Fernandez-Carazo et al. in
557 prep.). Contrary to some of the observations in Wright and Burton (1981), who
558 reviewed the biology of Antarctic saline lakes, the cold brine in Forlidas Pond does
559 not appear to be incompatible with cyanobacterial growth, although salinity and
560 freezing conditions are likely to limit metabolic processes as they do in some of the
561 more saline ponds on the McMurdo Ice Shelf (cf. Vincent, 2000). The dominance of
562 the cyanobacteria over the green algae agrees with other studies which have suggested
563 that continental cyanobacteria are more resistant to freezing and desiccation regimes
564 than sub-Antarctic taxa, and are more abundant than green algae whose membranes
565 are poorly adapted to freeze-thaw processes (Šabacká and Elster, 1996).
566
567 Of the cultivated bacterial isolates characterized from sample TM2, 13.5% had less
568 than 97% similarity to known sequences in the EMBL database, indicating that these

569 taxa represent organisms that have not been reported previously and are potentially
570 new to science. This observation is in line with previous reports (Brambilla et al.,
571 2001; Van Trappen et al., 2002) and is not unexpected in view of the limited amount
572 of studies using cultivation to study Antarctic bacterial diversity, and the estimate that
573 only a small fraction of bacterial species have so far been described (Schloss and
574 Handelsman, 2004).
575
576 The degree of endemism of all microbial groups in Antarctica is still debated. A
577 consistent problem faced by researchers interested in the possibility of microbial
578 endemism is the paucity of Antarctic data relating to microbial diversity and
579 distribution (Wynn-Williams, 1996). As a broad generalisation, microbiota are
580 thought not to face the same dispersal limitations as do many larger organisms or their
581 propagules. This has led to the development of the 'global ubiquity hypothesis'
582 (Finlay, 2002), whereby their small size means that they can easily enter and remain
583 in the air column and thereby reach all parts of the planet. This has also been
584 rationalised in terms of 'everything is everywhere, and the environment selects', in
585 other words that dispersal is not a limiting factor on species distribution, which is
586 rather controlled by possession of appropriate adaptations to allow survival,
587 development and reproduction under the conditions imposed by the 'recipient'
588 environment. This is consistent with previous descriptions of a largely cosmopolitan
589 microbial flora, for example as applied to classical morphological studies of the
590 eukaryotic algae (Broady, 1996) and many of the diatom studies cited in Jones et al.
591 (1996).
592

593 However, some authors suggest that microbial endemism is still possible because of
594 the long isolation of Antarctica from other parts of the world, the fact that dispersal
595 processes which favour local species are more efficient than long distance dispersal
596 processes and that there has probably been strong environmental selection for
597 adaptive strategies (Vincent, 1988; Franzmann, 1996). The application of molecular
598 biological techniques of identification has led recently to an increase in records of
599 microbial diversity through sequence data (e.g. Adams et al., 2006), although an
600 inherent weakness of these approaches, as with more classical culture techniques, is
601 that sequence presence and detection does not automatically prove biological activity
602 or functional significance within the ecosystem. In the absence of baseline microbial
603 diversity data against which to compare, assessment of endemism, or indeed of post
604 colonisation adaptation, remains difficult. However, Lawley et al. (2004) argued that
605 there was circumstantial support for Antarctic endemism in diverse microbial groups
606 based on very limited overlap in OTU composition between different locations in a
607 comparative study based on the same soil habitat, and Boenigk et al. (2006) argued
608 that there is evidence for considerable ecophysiological specialization within
609 Antarctic strains of certain microbial taxa, only possible if these have been isolated
610 for long periods of evolutionary time.
611
612 Few studies have attempted to assess directly the mechanism of dispersal employed
613 by cyanobacteria in Antarctica, although representatives of the group have been
614 recorded in simple aerobiological trapping studies at locations on the Antarctic
615 Peninsula (Hughes et al., 2004; Pearce and Galand, 2008). However, the mechanisms
616 of dispersal in Antarctica are unlikely to be different to those commonly recorded
617 elsewhere, with the major difference for terrestrial biota relating to the paucity and

618 isolation of suitable establishment sites. Thus, the major routes of dispersal are likely
619 to be through transport in the air column, incidental attachment to other biota,
620 transport in fresh water flows (on a local scale within Antarctica) and, more recently,
621 human transportation (Frenot et al., 2005; Hughes et al., 2006; Convey, 2008)
622
623 In the Dufek Massif, we found no evidence of local endemic organisms isolated there
624 for long periods of evolutionary time. This is consistent with the glaciological history
625 which suggests the repeated exposure of ice free surfaces for about 1.6 million years
626 (Hodgson et al., in prep) which is insufficient time for in-situ speciation, which for
627 bacterial 16S rRNA genes, is c. 50 million years for 1% divergence. Instead, there are
628 some groups of taxa present that are endemic to the Antarctic continent (tardigrades,
629 lichen and possibly some bacteria), and others which are cosmopolitan (for example
630 none of the green algal, cercozoan or bacterial DGGE-bands are unique to
631 Antarctica). For the cyanobacteria, only cosmopolitan cyanobacterial OTUs were
632 found, and among the bacterial strains obtained in cultivation about 13.5% represent
633 potentially new species some of which may be endemic. These results imply that the
634 Dufek Massif has not functioned as a biological refuge over long timescales (cf.
635 Convey et al., 2008; Convey et al., 2009). Instead it has been colonised in the
636 Quaternary by a combination of Antarctic endemic and cosmopolitan taxa whose
637 distribution, dispersal and establishment has been dependent upon life cycle
638 characteristics (e.g., formation of resting spores and resistance to the extreme
639 environmental conditions).
640
641
642 **Conclusions**

There has been recent recognition that levels of endemism and/or molecular evolutionary differentiation are considerably greater than previously appreciated across most of the groups of terrestrial biota (with the exception of bryophytes) that currently dominate Antarctic terrestrial communities (Convey and Stevens, 2007; Peat et al., 2007; Convey et al., 2008; Pugh and Convey, 2008). These are interpreted as supporting long-term terrestrial biological presence in Antarctica through glacial cycles and even in some cases back to the breakup of Gondwana. Furthermore, this includes considerable regionalisation within Antarctica itself (Chown and Convey, 2007; Pugh and Convey, 2008). Although we found no evidence in this study of regionalisation within the Dufek Massif, we did find a mix of Antarctic endemic and cosmopolitan species that have colonised ice free land there during the Quaternary period. We also found that the Dufek Massif contains some of the most reduced metazoan terrestrial and freshwater ecosystems known from Antarctica with autotrophs limited to cyanobacteria, plus a few green algae and lichens. This pristine and low diversity flora and fauna, only 800 km from the South Pole reinforces the importance of the area's designation as an ASPA, and highlights a particular vulnerability to human impacts.

Acknowledgements

This study contributes to SCAR EBA and IPY MERGE and was supported by the Natural Environment Research Council, British Antarctic Survey and the BELSPO projects AMBIO (SD/BA/01A and SD/BA/01B) and HOLANT (SD/CA/01A), and the FNRS Credit 1.5.104.04. Elie Verleyen is a post doctoral research fellow with the Fund for Scientific Research, Flanders. Ines Tavernier is funded by the Institute for the Promotion of Science and Technology in Flanders. Thanks are due to the

668 Fundación Alfonso Martin Escudero for funding Rafael Fernández. Annick Wilmotte
669 is Research Associate of the FRS-FNRS (National Funds for Scientific Research of
670 Belgium). The British Antarctic Survey provided logistic support and Richard Burt
671 provided field support. Ron Lewis-Smith is thanked for assistance in lichen
672 identification (based on our photographs). Some of the information in this paper has
673 been included in the Management Plan for Antarctic Specially Protected Area No.
674 119 – Davis Valley and Forlidas Pond, Dufek Massif which was produced by Colin
675 Harris of Environment Research and Assessment in collaboration with DH and PC.
676 Maps were produced by Peter Fretwell and Andreas Cziferszky at the Mapping and
677 Geographic Information Centre (MAGIC), British Antarctic Survey. We are
678 particularly grateful for the quality and depth of the reviews.
679
680

References

Adams, B., Bardgett, R.D., Ayres, E., Wall, D.H., Aislabie, J., Bamforth, S., Bargagli, R., Cary, C., Cavacini, P., Connell, L., Convey, P., Fell, J., Frati, F., Hogg, I.D., Newsham, N., O'Donnell, A., Russell, N., Seppelt, R. and Stevens, M.I., 2006. Diversity and distribution of Victoria Land biota. Soil Biol and Biochem 38, 3003-3018.

Adams, E.E., Priscu, J.C., Fritsen, C.H., Smith, S.R. and Brackman, S.L., 1998. Permanent ice covers of the McMurdo Dry Valley lakes, Antarctica: bubble formation and metamorphism. In: J.C. Priscu (Editor), Ecosystem dynamics in a polar desert, the McMurdo Dry Valleys, Antarctica. American Geophysical Union, Washington, D.C., pp. 281-295.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J.H., Zhang, Z., Miller, W. and Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25, 3389-3402.

Aughenbaugh, N., Neuburg, H. and P., W., 1958. Report 825-1-Part I, October 1958, USNC-IGY Antarctic Glaciological Data Field Work 1957 and 1958. Ohio State University Research Foundation, World Data Center for Glaciology at Boulder, Colorado.

Behrendt, J.C., Henderson, J.R., Meister, L. and Rambo, W.K., 1974. Geophysical investigations of the Pensacola Mountains and adjacent glacierized areas of Antarctica. U.S. Geological Survey Professional 844.

Behrendt, J.C., 1998. Innocents on the ice; a memoir of Antarctic exploration, 1957. University Press of Colorado, Boulder.

Boenigk, J., Pfandl, K., Garstecki, T., Harms, H., Novarino, G. and Chatzinotas, A., 2006. Evidence for geographic isolation and signs of endemism within a protistan morphospecies. Appl. Environ. Microbiol. 72(8), 5159-5164.

Bowman, J.P., McCammon, S.A., Rea, S.M. and McMeekin, T.A., 2000. The microbial composition of three limnologically disparate hypersaline Antarctic lakes. FEMS Microbiological Letters 183(1), 81-88.

Boyer, S.J., 1979. Glacial geological observations in the Dufek Massif and Forrestal Range, 1978-79. Antarctic Journal of the United States 14(5), 46-48.

Brambilla, E., Hippe, H., Hagelstein, A., Tindall, B.J. and Stackebrandt, E., 2001. 16S rDNA diversity of cultured and uncultured prokaryotes of a mat sample from Lake Fryxell, McMurdo Dry Valleys, Antarctica. Extremophiles 5, 23-33.

Broady, P.A., 1996. Diversity, distribution and dispersal of Antarctic terrestrial algae. Biodiversity and Conservation 5, 307-335.

Chown, S.L. and Convey, P., 2007. Spatial and temporal variability across life's hierarchies in the terrestrial Antarctic. Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences 362, 2307-2331.

Cockell, C.S., Rettberg, P., Horneck, G., Wynn-Williams, D.D., Scherer, K. and Gugg-Helminger, A., 2002. Influence of ice and snow covers on the UV exposure of terrestrial microbial communities: dosimetric studies. Journal of Photochemistry and Photobiology B: Biology 68(1), 23-32.

Coenye, T., Falsen, E., Vancanneyt, M., Hoste, B., Govan, J.R.W., Kersters, K. and Vandamme, P., 1999. Classification of Alcaligenes faecalis-like isolates from the environment and human clinical samples as Ralstonia gilardii sp. nov. . International Journal of Systematic Bacteriology 49, 405-413.

Convey, P. and Stevens, M.I., 2007. Antarctic Biodiversity. Science 317, 1877-1878.

729 Convey, P., 2008. Non-native species in Antarctic terrestrial and freshwater
730 environments: Presence, sources, impacts and predictions, Non-native species
731 in the Antarctic. Gateway Antarctica Special Publication 0801, pp. 97-130.
732 Convey, P., Gibson, J.A.E., Hillenbrand, C.-D., Hodgson, D.A., Pugh, P.J.A.,
733 Smellie, J.L. and Stevens, M.I., 2008. Antarctic terrestrial life - challenging
734 the history of the frozen continent? Biological Reviews 83, 103-117.
735 Convey, P., Stevens, M.I., Hodgson, D.A., Smellie, J.L., Hillenbrand, C.-D., Barnes,
736 D.K.A., Clarke, A., Pugh, P.J.A., Linse, K. and Cary, S.C., 2009. Exploring
737 biological constraints on the glacial history of Antarctica. Quaternary Science
738 Reviews 00, 00-00.
739 Corinaldesi, C., Danovaro, R. and Dell'Anno, A., 2005. Simultaneous recovery of
740 extracellular and intracellular DNA suitable for molecular studies from marine
741 sediments. Appl. Environ. Microbiol. 71, 46-50.
742 DeWever, A., Leliaert, F., Verleyen, E., Vanormelingen, P., Van der Gucht, K.,
743 Hodgson, D.A., Sabbe, K. and Vyverman, W., 2009. Hidden levels of
744 phylodiversty in Antarctic Green algae: further evidence for the existence of
745 glacial refugia. Proceedings of the Royal Society B: Biological Sciences 276,
746 3591-3599.
747 Díez, B., Massana, R., Estrada, M. and Pedrós-Alió, C., 2004. Distribution of
748 eukaryotic picoplankton assemblages across hydrographic fronts in the
749 Southern Ocean, studied by denaturing gradient gel electrophoresis.
750 Limnology and Ocenanography 49, 1022-1034.
751 Doran, P.T., Priscu, J.C., Berry Lyons, W., Powell, R.D., Andersen, D.T. and Poreda,
752 R.J., 2004. Paleolimnology of extreme cold terrestrial and extraterrestrial
753 environments. In: R. Pienitz, M.S.V. Douglas and J.P. Smol (Editors),
754 Developments in Palaeoenvironmental Research. Volume 8. Long-term
755 Environmental Change in Arctic and Antarctic Lakes. Springer, Dordrecht, pp.
756 475-507.
757 Ferris, J., Johnson, A. and Storey, B., 1998. Form and extent of the Dufek intrusion,
758 Antarctica, from newly compiled aeromagnetic data. Earth and Planetary
759 Science Letters 154(1-4), 185-202.
760 Finlay, B., 2002. Global dispersal of free-living microbial eukaryote species. Science
761 296, 1061-1063.
762 Ford, A.B., 1976. Stratigraphy of the layered gabbroic Dufek intrusion, Antarctica.
763 Contributions to stratigraphy. Geological Survey Bulletin 1405-D.
764 Ford, A.B., Schmidt, D.L. and Boyd, W.W., 1978. Geologic map of the Davis Valley
765 quadrangle and part of the Cordiner Peaks quadrangle, Pensacola Mountains,
766 Antarctica. U.S Geologic Survey Antarctic Geological Map A-10.
767 Ford, A.B., 1990. The Dufek intrusion of Antarctica. Antarctic Research Series 51.
768 American Geophysical Union, Washington, DC, 15-32.
769 Franzmann, P.D., 1996. Examination of Antarctic prokaryotic diversity through
770 molecular comparisons. Biodiversity and Conservation 5, 1295-1305.
771 Frenot, Y., Chown, S.L., Whinam, J., Selkirk, P.M., Convey, P., Skotnicki, M. and
772 Bergstrom, D.M., 2005. Biological invasions in the Antarctic: extent, impacts
773 and implications. Biological Reviews 80, 45-72.
774 Friedman, E.I., 1977. Microorganisms in antarctic desert rocks from dry valleys and
775 Dufek Massif. Antarctic Journal of the United States 12, 26-30.
776 Gevers, D., Huys, G. and Swings, J., 2001. Applicability of rep-PCR fingerprinting
777 for identification of Lactobacillus species. FEMS Microbiology Letters 205,
778 31-36.

779 Hodgson, D.A., Vyverman, W. and Sabbe, K., 2001. Limnology and biology of saline
780 lakes in the Rauer Islands, eastern Antarctica. Antarctic Science 13(3), 255-
781 270.
782 Hodgson, D.A., Vyverman , W., Verleyen, E., Sabbe, K., Leavitt, P.R., Taton, A.,
783 Squier, A.H. and Keely, B.J., 2004. Environmental factors influencing the
784 pigment composition of *in situ* benthic microbial communities in east
785 Antarctic lakes. Aquatic Microbial Ecology 37, 247-263.
786 Hodgson, D.A., Roberts, S.J., Bentley, M.J., Smith, J.A., Johnson, J.S., Verleyen, E.,
787 Vyverman, W., Hodson, A.J., Leng, M.J., Cziferszky, A., Fox, A.J. and
788 Sanderson, D.C.W., 2009. Exploring former subglacial Hodgson Lake. Paper
789 I: Site description, geomorphology and limnology. Quaternary Science
790 Reviews(28), 2295-2309.
791 Hodgson, D.A., in prep. Lake high stands in the Pensacola and Shackleton Mountains
792 Antarctica, 4500-2300 yr BP.
793 Hodgson, D.A., Bentley, M.J., Schnabel, C. and Cziferszky, A., in prep. Glacial
794 geomorphology and cosmogenic 10Be and 26Al nuclide exposure ages in the
795 northern Dufek Massif, Transantarctic Mountains.
796 Hughes, K., Ott, S., Bölter, M. and Convey, P., 2006. Colonisation processes. In:
797 D.M. Bergstrom, P. Convey and A.H.L. Huiskes (Editors), Trends in Antarctic
798 Terrestrial and Limnetic Ecosystems: Antarctica as a Global Indicator
799 Springer, Dordrecht, pp. 35-54.
800 Hughes, K.A., McCartney, H.A., Lachlan-Cope, T.A. and Pearce, D.A., 2004. A
801 preliminary study of airborne microbial biodiversity over peninsular
802 Antarctica. Cell Mol Biol 50, 537-542.
803 Jones, V.J., 1996. The diversity, distribution and ecology of diatoms from Antarctic
804 inland waters. Biodiversity and Conservation 5, 1433-1449.
805 Komárek, J. and Anagnostidis, K. (Editors), 2005. Cyanoprokaryota. Teil/ 2nd Part:
806 Oscillatoriales. Süsswasserflora von Mitteleuropa 19/2, Vol. 2.
807 Elsevier/Spektrum, Heidelberg.
808 Lawley, B., Ripley, S., Bridge, P. and Convey, P., 2004. Molecular analysis of
809 geographic patterns of eukaryotic diversity in Antarctic soils. Applied and
810 Environmental Microbiology 70, 5963-5972.
811 Neuburg, H.A.C., Thiel, E., Walker, P.T., Behrendt, J.C. and Aughenbaugh, N.B.,
812 1959. The Filchner Ice Shelf. Annals of the Association of American
813 Geographers 49(2), 110-119.
814 Ovstedal, D.O. and Lewis-Smith, R.I., 2001. Lichens of Antarctica and South
815 Georgia: A Guide to their Identification and Ecology. Studies in Polar
816 Research xii. Cambridge University Press, 411 pp.
817 Parker, B.C., Ford, A.B., Allnutt, T., Bishop, B. and Wendt, S., 1977. Baseline
818 microbiological data for soils of the Dufek Massif. Antarctic Journal of the
819 United States 12(5), 24-26.
820 Parker, B.C., Boyer, S., Allnutt, F.C.T., Seaburg, K.G., Wharton Jr., R.A. and
821 Simmons Jr., R.A., 1982. Soils from the Pensacola Mountains, Antarctica:
822 physical chemical and biological characteristics. Soil Biology and
823 Biochemistry 14, 265-271.
824 Pearce, D.A. and Galand, P.E., 2008. Microbial biodiversity and biogeography. In:
825 W.F. Vincent and J. Laybourn-Parry (Editors), Polar Lakes and Rivers -
826 Limnology of Arctic and Antarctic Aquatic Ecosystems. Oxford University
827 Press, Oxford, UK, pp. 213-231.

828 Peat, H.J., Clarke, A. and Convey, P., 2007. Diversity and biogeography of the
829 Antarctic flora. Journal of Biogeography 34, 132-146.
830 Pugh, P.J.A. and Convey, P., 2008. Surviving out in the cold: Antarctic endemic
831 invertebrates and their refugia. Journal of Biogeography 35(12), 2176-2186.
832 Renberg, I., 1990. A procedure for preparing large sets of diatom slides from
833 sediment cores. Journal of Paleolimnology 4, 87-90.
834 Šabacká, M. and Elster, J., 1996. Response of Cyanobacteria and Algae from
835 Antarctic wetland habitats to freezing and desiccation stress Polar Biology
836 30(1), 31-37.
837 Saitou, N. and Nei, M., 1987. The neighbor-joining method: A new method for
838 reconstructing phylogenetic trees. Molecular Biology and Evolution 4, 406-
839 425.
840 Sands, C.J., Convey, P., Linse, K. and McInnes, S.J., 2008. Assessing meiofaunal
841 variation among individuals: an example using Tardigrada. BMC Ecology
842 8(1), 7.
843 Schloss, P.D. and Handelsman, J., 2004. Status of the Microbial Census.
844 Microbiology and Molecular Biology Reviews 68, 686-691.
845 Taton, A., Grubisic, S., Brambilla, E., De Wit, R. and Wilmotte, A., 2003.
846 Cyanobacterial diversity in natural and artificial microbial mats of Lake
847 Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular
848 approach. Applied & Environmental Microbiology 69, 5157-5169.
849 Taton, A., Grubisic, S., Balthasart, P., Hodgson, D.A., Laybourn-Parry, J. and
850 Wilmotte, A., 2006a. Biogeographical distribution and ecological ranges of
851 benthic cyanobacteria in East Antarctic lakes. FEMS Microbial Ecology 57,
852 272-289.
853 Taton, A., Grubisic, S., Ertz, D., Hodgson, D.A., Piccardi, R., Biondi, N., Tredici, M.,
854 Mainini, M., Losi, D., Marinelli, F. and Wilmotte, A., 2006b. Polyphasic study
855 of Antarctic cyanobacterial strains. Journal of Phycology 42, 1257-1270.
856 Van de Peer, Y. and De Wachter, R., 1997. Construction of evolutionary distance
857 trees with TREECON for Windows: accounting for variation in nucleotide
858 substitution rate among sites. Computer Applications in the Biosciences 13,
859 227-230.
860 van den Broeke, M., van de Berg, W.J., van Meijgaard, E. and Reijmer, C., 2006.
861 Identification of Antarctic ablation areas using a regional atmospheric climate
862 model. Journal of Geophysical Research 111D18110,
863 doi:10.1029/2006JD007127.
864 Van der Gucht, K., Sabbe, K., De Meester, L., Vloemans, N., Zwart, G., Gillis, M.
865 and Vyverman, W., 2001. Contrasting Bacterioplankton Community
866 Composition and Seasonal Dynamics in Two Neighbouring Hypertrophic
867 Freshwater Lakes. Environmental Microbiology 3, 680-690.
868 van Lipzig, N.P.M., Turner, J., Colwell, S.R. and van Den Broeke, M.R., 2004. The
869 near-surface wind field over the Antarctic continent. International Journal of
870 Climatology 24(15), 1973-1982.
871 Van Trappen, S., Mergaert, J., Van Eygen, S., Dawyndt, P., Cnockaert, M. and
872 Swings, J., 2002. Diversity of 746 heterotrophic bacteria isolated from
873 microbial mats from ten Antarctic lakes. Systematic and Applied
874 Microbiology 25, 603-610.
875 Verleyen, E., Sabbe, K., Hodgson, D.A., Grubisic, S., Taton, A., Cousin, S.,
876 Wilmotte, A., De Wever, A., Van der gucht, K. and Vyverman, W., in press.

The structuring role of climate-related environmental factors on Antarctic microbial mat communities. Aquatic Microbial Ecology.

Vincent, W.F., 1988. Microbial Ecosystems of Antarctica. Cambridge University Press, 320 pp.

Vincent, W.F. and Howard-Williams, C., 1994. Nitrate-rich inland waters of the Ross Ice Shelf region, Antarctica. Antarctic Science 6(3), 339-346.

Vincent, W.F., 2000. Cyanobacterial dominance in the polar regions. In: B.A. Whitton and M. Potts (Editors), The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp. 321-340.

Vyverman, W., Verleyen, E., Sabbe, K., Vanhoutte, K., Sterken, M., Hodgson, D.A., Mann, D.G., Juggins, S., Van de Vijver, B., Jones, V.J., Flower, R., Roberts, D., Chepurnov, V.A., Kilroy, C., Vanormelingen, P. and De Wever, A., 2007. Historical processes constrain patterns in global diatom diversity. Ecology 88(8), 1924-1931.

Wright, S.W. and Burton, H.R., 1981. The biology of antarctic saline lakes. Hydrobiologia 82, 319-338.

Wynn-Williams, D.D., 1996. Antarctic microbial diversity : the basis of polar ecosystem processes. Biodiversity and Conservation 5, 1271-1293.

Zakhia, F., Jungblut, A.-D., Taton, A., Vincent, W.F. and Wilmotte, A., 2007. Cyanobacteria in cold systems (Chapter 8). In: R. Margesin, F. Schinner, J.-C. Marx and C. Gerday (Editors), Psychrophiles: From Biodiversity to Biotechnology. Springer, Heidelberg, pp. 121-135.

Zhu, F., Massana, R., Not, F., Marie, D. and Vaulot, D., 2005. Mapping of picoeucaryotes in marine ecosystems with quantitative PCR of the 18S rRNA gene. FEMS Microbial Ecology 52, 79-92.

906 **Tables**
907
908 Table 1. Biological sampling program in the Davis and Forlidas Valleys: groups of taxa identified and the methods used.
909

Description	Method	Number of samples	Number of taxa	Taxa
Bryophyta	Observational survey	0	0	n/a
Lichens	Observational survey	1	1	*Lecidea cancriformis* Dodge & Baker
Bacillariophyceae / Diatoms	Survey under light microscope	2	1	*Pinnularia microstauron* (Ehr.) Cl.††
Cyanobacteria	Clone library, DGGE + band sequencing, isolation of strains+ sequencing (microscopy)	3	6	Sample TM1: 16ST63, 16ST14 Sample TM2: 16ST63, 16ST14, 16ST44, 16ST49, 16ST80 Sample TM3: 16ST44, 16ST49, 16ST80, 16ST07
Chlorophyta /Green algae	DGGE + band sequencing	2	1	*Urospora* sp.
Rhizaria/ Cercozoa	DGGE + band sequencing	2	2	Heteromitidae, *Paulinella* sp.
Bacteria	DGGE + band sequencing	2	32	Cyanobacteria: Nostocales, Oscillatoriales, Chroococcales, Gloeobacteriales** Bacteroidetes: Sphingobacteriales, Flavobacteriales Firmicutes: Clostridiales Gammaproteobacteria: Pseudomonadales, Psychrobacter
Bacteria	Isolation of strains + sequencing	1	330 isolates	*Firmicutes* 33%, *Bacteroidetes* 23%, *Alphaproteobacteria* 25%, *Actinobacteria* 9%, *Betaproteobacteria*. 8%, *Gammaproteobacteria* 1.5%, Deinococci 0.3%
Arthropods	Tullenberg	50	0	n/a
Invertebrates	Baermann extractions	130	3	See Tardigrades (below)

Tardigrades	Light microscope (Molecular†)	14 20	3 1	*Echiniscus* (cf) *pseudowendti* Dastych, 1984 (Heterotardigrada), *Acutuncus antarcticus* (Richters, 1904) *Diphascon sanae* Dastych, Ryan and Watkins, 1990 (Eutardigrada)
Rotifers	Tullenberg and light microscope	130	present	Bdelloid rotifers
Soil bacteria and algae	Cultured (Parker et al., 1982)*	1	3	Cyanobacteria: *Oscillatoria* sp. Algae: *Trebouxia* sp., *Heterocous* sp. (viable yeasts present)
Avifauna	Observation	n/a	1	Snow petrel (*Pagadroma nivea*)

910 *previously published, ** tentative identification based on about 100 bases, †analyses carried out on morphologically congruent samples from
911 the Shackleton Range, †† not considered as evidence of an extant community
912
913

914 Table 2. Water chemistry of Forlidas Pond
915

Parameter	Brine layer	Freshwater moat
Sample depth (m)	1.63	0.2
Conductivity (mS cm^{-1})	142.37	2.22
Temperature (°C)	-7.7	0.7
pH	7.3	8.15
Al (mg L^{-1})	0.834	<0.002
Fe (mg L^{-1})	0.363	0.004
Mg (mg L^{-1})	10500	13.9
Ca (mg L^{-1})	1240	11.4
K (mg L^{-1})	908	1.36
Na (mg L^{-1})	31000	45
Si (mg L^{-1})	3.1	0.222
Cl (mg L^{-1})	65700	88.6
SO_4-S (mg L^{-1})	2590	17.5
Ratio SO_4-S/Mg	0.25	1.26
Ratio Mg/Ca	8.48	1.22
Ion sum (mg L^{-1})	111942	178
TN (mg L^{-1})	3800	4.3
TOC (mg L^{-1})	53	0.97
DOC (mg L^{-1})	55.3	1.04
NO_3-N (mg L^{-1})	3600	4.42
NH_4-N (mg L^{-1})	2.71	0.043
PO_4-P (mg L^{-1})	0.078	<0.005

916
917
918

Table 3. Bacteria (uncultured diversity)

	TM1	TM2	Total
Unsequenced or unidentified DGGE bands	16	11	21
Bacteroidetes	3	2	3
Cyanobacteria	2	3	5
Firmicutes	0	1	1
Gammaproteobacteria	1	1	2
Total	22	18	32

925 **Figure captions**

926

927 Fig. 1. Location map

928

929 Fig. 2. Topographic map of the study area

930

931 Fig. 3. Aerial panorama of the Davis and Forlidas Valleys looking south.

932

933 Fig. 4. Davis Valley looking east from Forlidas Ridge showing the blue ice lobes of
934 the Ford Ice Piedmont.

935

936 Fig. 5. Forlidas Valley and Forlidas Pond looking west northwest from the air. The
937 area of the valley floor without frost-sorted polygons marks the upper Holocene water
938 level, 17.7 m above the present day pond.

939

940 Fig. 6. Davis Valley Ponds from the blue ice lobes of the Ford Ice Piedmont.

941

942 Fig 7. Microclimate temperature (°C) and relative humidity (%) data for the Dufek
943 Massif. Logging was at 30 minute intervals from 12.52 pm on the 3rd Dec 2003 to
944 17.22pm on 11th Dec 2003. Sensors were oriented to partially shield them from direct
945 sunlight but were not located within a Stevenson screen.

946

947 Fig. 8. Lichen *Lecidea cancriformis* in crevice (1cm diameter lip balm container for
948 scale).

949

950 Fig. 9. Main cyanobacterial habitats (a) cyanobacterial mat under ice, Forlidas Pond
951 moat (b) cyanobacterial mat melting up through the ice of the Davis Valley in annual
952 increments of 2-3 cm. The ice is more than 2 m deep. Lens cap for scale (c)
953 cyanobacteria around a boulder in the Davis Valley. Ice axe for scale (d) detail of
954 terrestrial cyanobacteria (e) cyanobacteria in relict proglacial pond beds, Davis
955 Valley.

956

957 Fig.10. Distance tree based on cyanobacterial partial 16S rRNA sequences (*E. coli*
958 positions 380 to 757) (constructed by the Neighbor-joining method (Saitou and Nei,

1987). A bootstrap analysis was performed that involved construction of 500 resampled trees (values indicated at the node), and the branches with less than 70% bootstrap support are drawn as unresolved. The tree comprised the sequences of 7 DGGE bands, 8 clones and 4 strains from the Forlidas Pond samples (in bold italics) and their 3 most similar strain sequences and 5 uncultured sequences from rdpII (http://rdp.cme.msu.edu). The sequences found in Antarctic samples are in bold. *E. coli* sequence is the out group. The OTU numbers are indicated on the right. The evolutionary distance between two sequences is obtained by adding the lengths of the horizontal branches connecting them and using the scale bar (0.1 mutation per position).

Figure 1

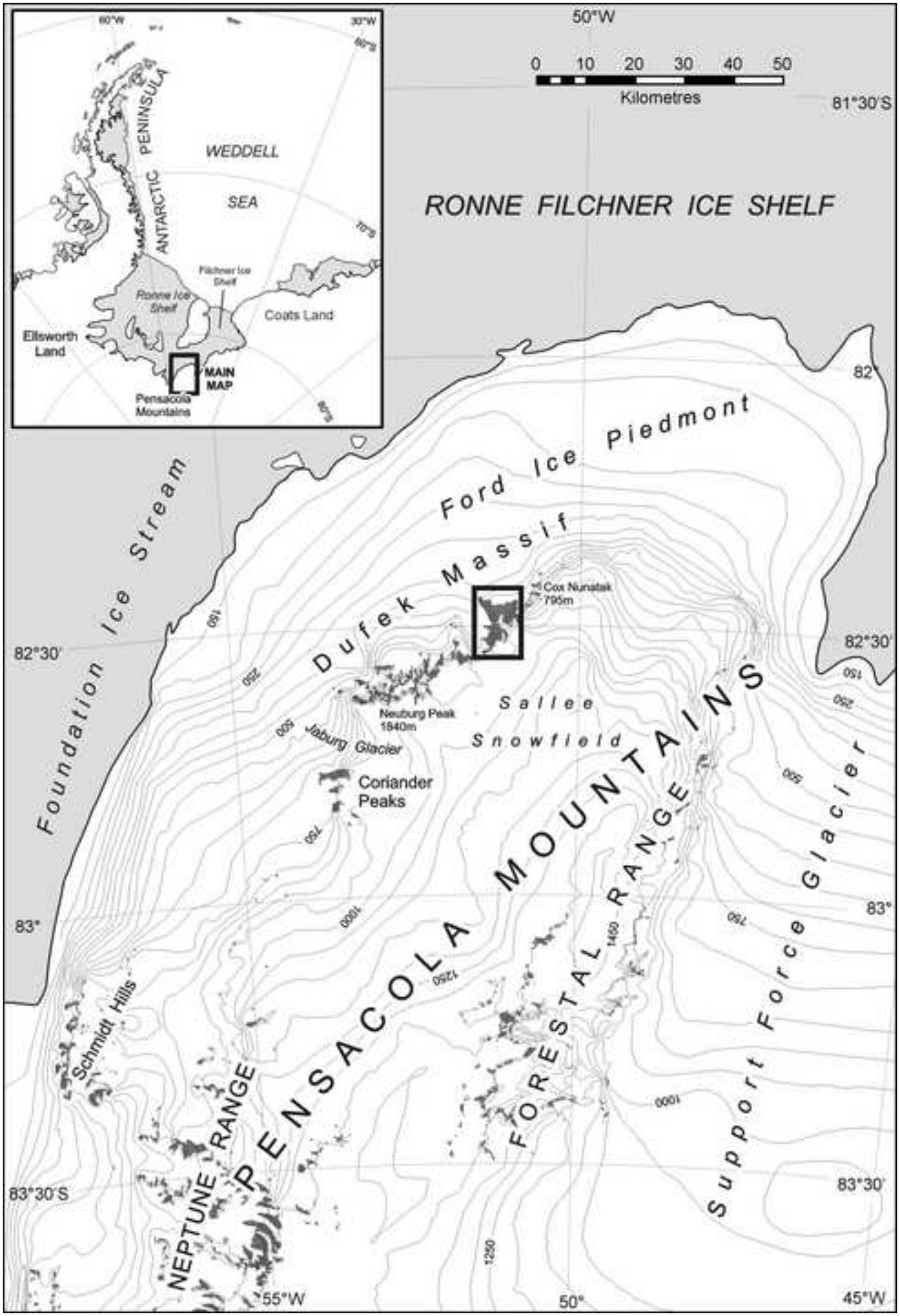

50°W
0 10 20 30 40 50
Kilometres
81°30'S
RONNE FILCHNER ICE SHELF
WEDDELL SEA
ANTARCTIC PENINSULA
Ronne Ice Shelf
Filchner Ice Shelf
Coats Land
Ellsworth Land
MAIN MAP
Pensacola Mountains
82°
82°30'
Foundation Ice Stream
Ford Ice Piedmont
Dufek Massif
Cox Nunatak 795m
Neuburg Peak 1840m
Jaburg Glacier
Coriander Peaks
Sallee Snowfield
PENSACOLA MOUNTAINS
FORESTAL RANGE
Support Force Glacier
83°
Schmidt Hills
NEPTUNE RANGE
83°30'S
83°30'
55°W
50°
45°W

Figure 2

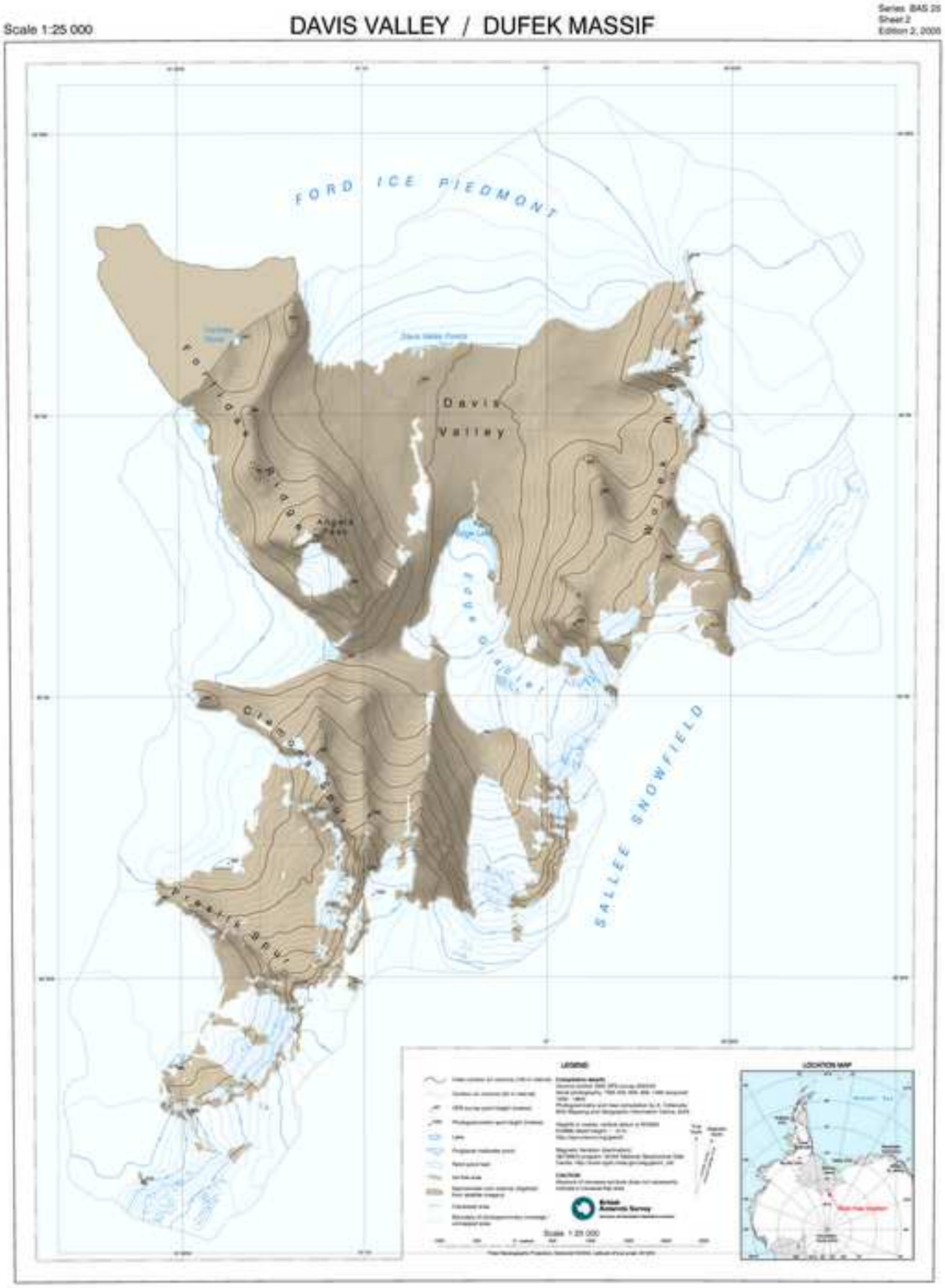

Scale 1:25 000
DAVIS VALLEY / DUFEK MASSIF
Series BAS 25
Sheet 2
Edition 2, 2000
FORD ICE PIEDMONT
Davis Valley
SALLEE SNOWFIELD
LEGEND
LOCATION MAP
British Antarctic Survey
Scale 1:25 000

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

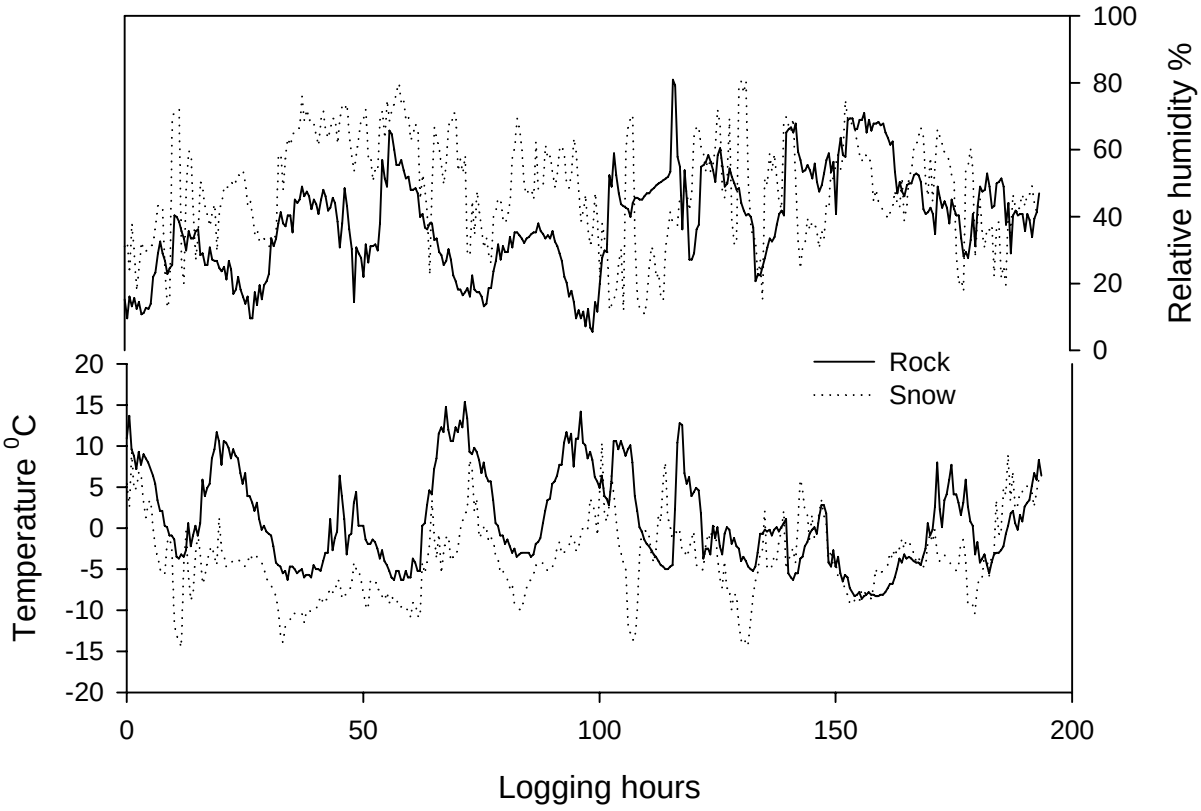

Relative humidity %
100
80
60
40
20
0
Rock
Snow
Temperature ^{0}C
20
15
10
5
0
-5
-10
-15
-20
0
50
100
150
200
Logging hours

Figure 8

Figure 9a

Figure 9b

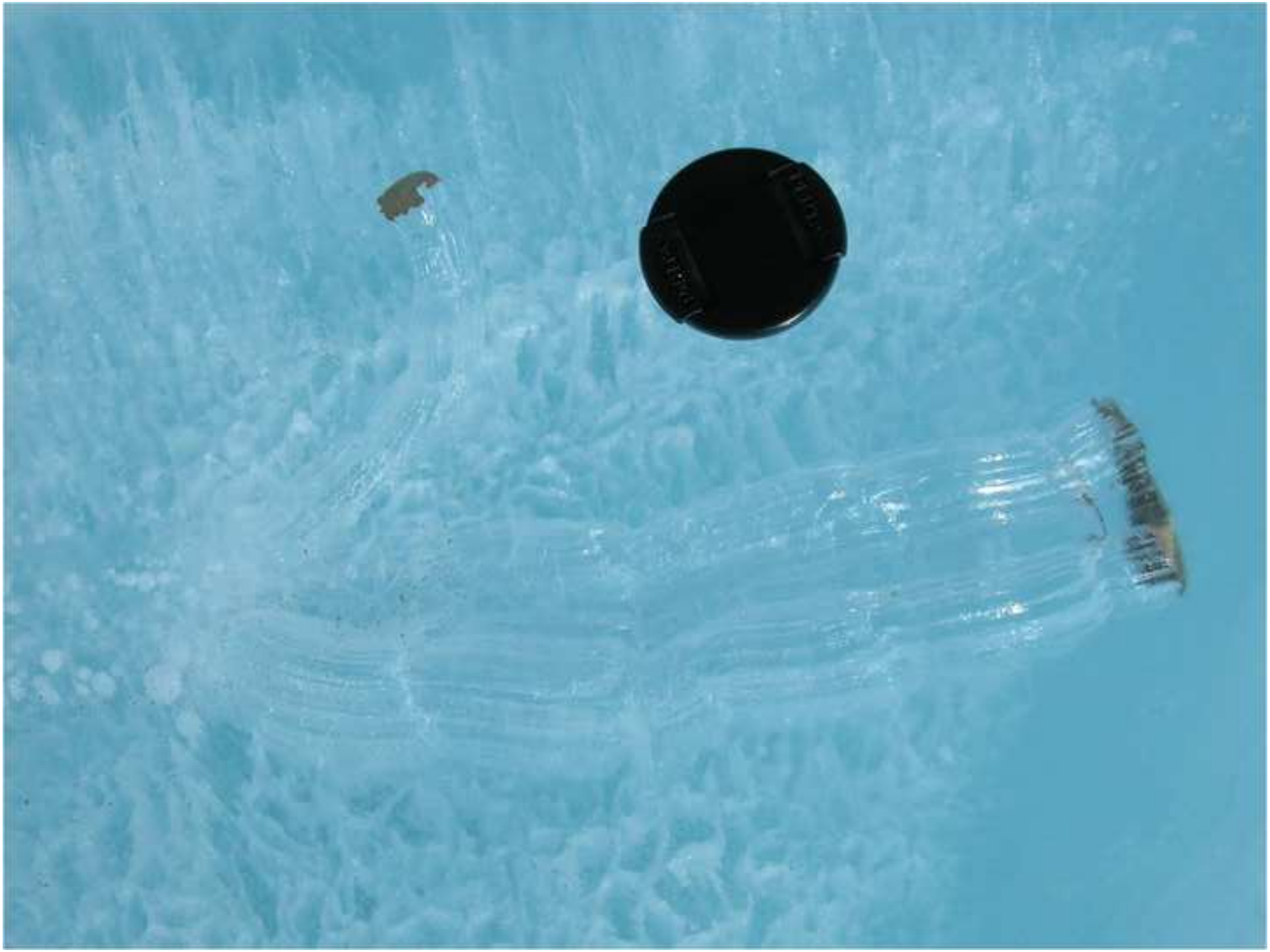
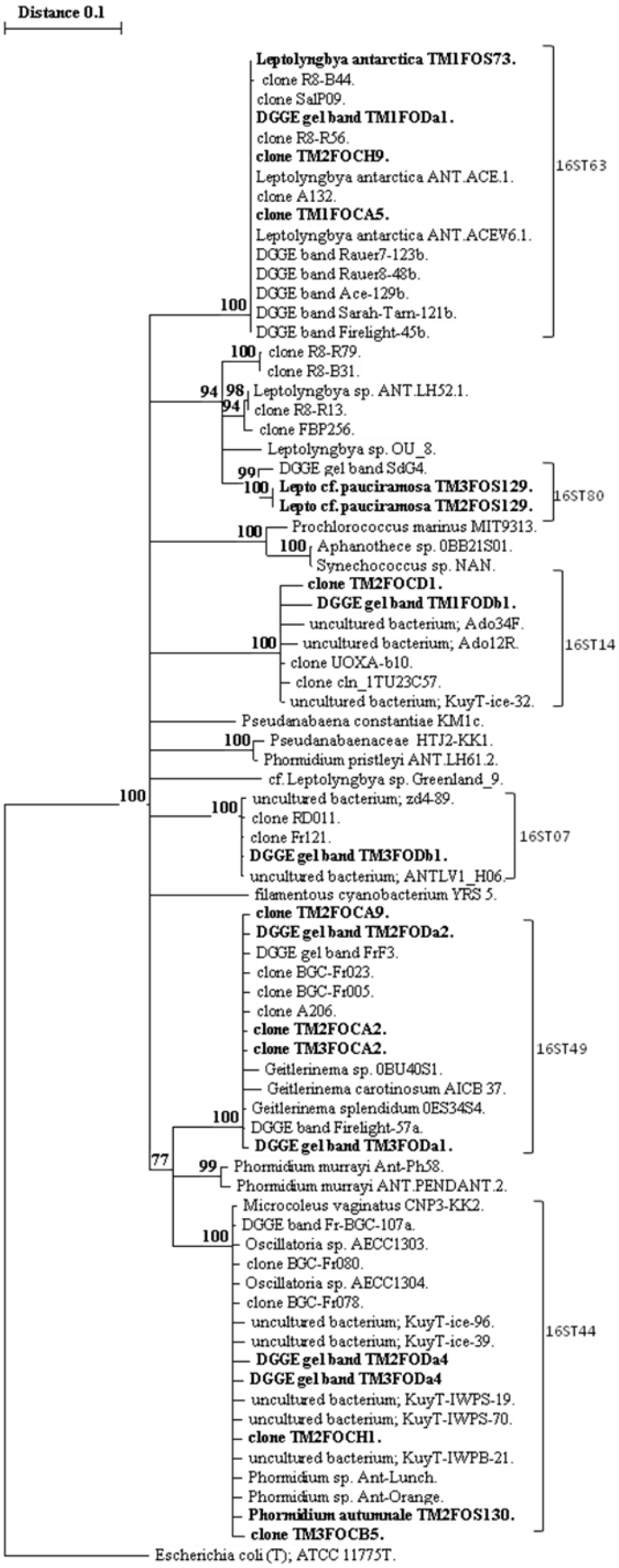


Figure 9c

Figure 9d

Figure 10


Distance 0.1
Leptolyngbya antarctica TM1FOS73.
clone R8-B44.
clone SalP09.
DGGE gel band TM1FODa1.
clone R8-R56.
clone TM2FOCH9.
Leptolyngbya antarctica ANT.ACE.1.
clone A132.
clone TM1FOCA5.
Leptolyngbya antarctica ANT.ACEV6.1.
DGGE band Rauer7-123b.
DGGE band Rauer8-48b.
DGGE band Ace-129b.
DGGE band Sarah-Tarn-121b.
DGGE band Firelight-45b.
16ST63
clone R8-R79.
clone R8-B31.
Leptolyngbya sp. ANT.LH52.1.
clone R8-R13.
clone FBP256.
Leptolyngbya sp. OU_8.
DGGE gel band SdG4.
Lepto cf. pauciramosa TM3FOS129.
Lepto cf. pauciramosa TM2FOS129.
16ST80
Prochlorococcus marinus MIT9313.
Aphanothece sp. 0BB21S01.
Synechococcus sp. NAN.
clone TM2FOCD1.
DGGE gel band TM1FODb1.
uncultured bacterium; Ado34F.
uncultured bacterium; Ado12R.
clone UOXA-b10.
clone cln_1TU23C57.
uncultured bacterium; KuyT-ice-32.
16ST14
Pseudanabaena constantiae KM1c.
Pseudanabaenaceae HTJ2-KK1.
Phormidium pristleyi ANT.LH61.2.
cf. Leptolyngbya sp. Greenland_9.
uncultured bacterium; zd4-89.
clone RD011.
clone Fr121.
DGGE gel band TM3FODb1.
uncultured bacterium; ANTLV1_H06.
16ST07
filamentous cyanobacterium YRS 5.
clone TM2FOCA9.
DGGE gel band TM2FODa2.
DGGE gel band FrF3.
clone BGC-Fr023.
clone BGC-Fr005.
clone A206.
clone TM2FOCA2.
clone TM3FOCA2.
Geitlerinema sp. 0BU40S1.
Geitlerinema carotinosum AICB 37.
Geitlerinema splendidum 0ES34S4.
DGGE band Firelight-57a.
DGGE gel band TM3FODa1.
16ST49
Phormidium murrayi Ant-Ph58.
Phormidium murrayi ANT.PENDANT.2.
Microcoleus vaginatus CNP3-KK2.
DGGE band Fr-BGC-107a.
Oscillatoria sp. AECC1303.
clone BGC-Fr080.
Oscillatoria sp. AECC1304.
clone BGC-Fr078.
uncultured bacterium; KuyT-ice-96.
uncultured bacterium; KuyT-ice-39.
DGGE gel band TM2FODa4
DGGE gel band TM3FODa4
uncultured bacterium; KuyT-IWPS-19.
uncultured bacterium; KuyT-IWPS-70.
clone TM2FOCH1.
uncultured bacterium; KuyT-IWPB-21.
Phormidium sp. Ant-Lunch.
Phormidium sp. Ant-Orange.
Phormidium autumnale TM2FOS130.
clone TM3FOCB5.
16ST44
Escherichia coli (T); ATCC 11775T.