Supplemental Information

"Protist diversity in a permanently ice-covered Antarctic lake during the polar night transition"

Methods

Site Description

The McMurdo Dry Valleys (77º00′S, 162º52′E) are the largest ice-free area in Antarctica and represent a polar desert with an average yearly temperature of -15°C to -20°C. Lake Bonney is one of several ice-covered lakes which are the only perennial source of liquid water in dry the valleys, supporting year-round biological activity. Lake Bonney has two 40 m deep lobes (east lobe, ELB; west lobe WLB), which are separated by a 13 m deep bedrock sill that allows exchange of the fresh surface waters, but prevents mixing of the deep saline waters between the two basins. The water temperature reaches a maximum in the middle of the water column and never exceeds 4.9 ºC in ELB and 2.2 ºC in the WLB. The lowest temperatures recorded during this study in ELB and WLB were -2.1 and -4.3 ºC, respectively, and occurred just above the bottom (~38 m) (Figure SI 1A, B). ELB and WLB both have steep mid-depth conductivity gradients with values ranging from 0.36 to 109.13 mS cm^{-1} and 0.74 to 79.60 mS cm^{-1}, respectively. The water column of Lake Bonney does not mix on an annual scale (mixing time is about 50,000 years; Spigel and Priscu, 1996) and probably has not done so for more than 1000 years (Lyons *et al.*, 2006; Spigel and Priscu, 1998). Both lobes of Lake Bonney showed distinct chlorophyll-a maxima at 13.5 m, just above the chemocline (Figure SI 1 C, D). Chlorophyll levels in WLB reached 15.5 μg l^{-1}, which was almost twice those in the deep maxima of ELB reflecting higher levels of phytoplankton primary production in the former (Priscu and

Neale, 1995). Relatively little chlorophyll-a was present below 20m, a depth where insufficient light exists to support photosynthesis (i.e., the bottom of the trophogenic zone). The dissolved oxygen in the trophogenic zone of both lobes of Lake Bonney was highly supersaturated with respect to the mixing ratio in air above the lake and show maxima that coincides with the chlorophyll-a maxima (Figure SI 1 C, D). Oxygen levels decreased precipitously beneath the chemoclines. Phytoplankton populations in the trophogenic zone above the chemocline have been shown to be P-deficient (Figure SI 1 E, F) and supported to a large extent by upward diffusing nutrients across the chemocline (Dore and Priscu, 2001; Priscu, 1995).

Sampling

Temperature and conductivity were measured with a Seabird model 25 profiler as described by Spigel and Priscu (Spigel and Priscu, 1998). Chlorophyll-a was determined with a bbe Moldaenke profiling spectrofluorometer. This instrument was lowered at a rate that produced ~10 measurements m^{-1}, which was adequate to define the highly layered phytoplankton species. Discrete water samples for nutrient analysis were collected at 1-3 m intervals throughout the water column, immediately filtered through Whatman GF/F filters, and stored at -20 ^{o}C for no longer than 2 months before processing. All inorganic nitrogen species were determined with a Lachat autoanalyzer using methods described by Priscu (Priscu, 1997). Owing to the low levels of soluble reactive phosphorus (SRP) in these systems, SRP was analyzed manually using the antimony-molybdate method (Stickland and Parsons, 1972) with a 10 cm pathlength cuvette. Dissolved oxygen was measured on an unfiltered aliquot of the discrete samples immediately upon collection using Winkler titrations.

Samples for phylogenetic analysis were collected at 6, 13, 18, and 20m in the east lobe (ELB) and 10, 13, 15, and 20m in the west lobe (WLB) of Lake Bonney from February to April 2008. All collection depths are relative to the piezometric water level in the ice borehole and represent microbial populations throughout the trophogenic zone (Priscu, 1995). Samples (1 liter) were vacuum concentrated (5 kPa) onto 47 mm 0.45 µm Durapore polyvinylidene fluoride membrane filters (Millipore). The filters were frozen immediately in liquid nitrogen and transported frozen to McMurdo Station, where they were stored at -80ºC until DNA extraction. DNA was isolated from a whole filter using the MP DNA kit (MP Biomedicals, CA) following the kit's instruction.

PCR amplification and RFLP

18S rRNA genes were amplified from DNA with universal eukaryote primers: EK-82F (5′-GAAACTGCGAATGGCTC) and EK-1520R (5′-CYGCAGGTTCACCTAC) (Lopez-Garcia *et al.*, 2001) to generate products for cloning. PCR was performed in triplicate using 25 cycles of 95°C for 1 min, 52°C for 1 min, and 72°C for 2 min. Gel purified PCR products were ligated into pGEM-T Easy Vector (Promega, WI) and transformed into TOP 10 cells. Colonies were selected for PCR amplification to screen the presence of inserts using standard M13 primers and the products were subjected to restriction fragment length polymorphism (RFLP) analysis. Positive PCR products were digested with the restriction enzyme *Hae*III (Fermentas, MD) for 3 h at 37ºC. RFLP patterns were visualized in 2.5% agarose gels. Each unique RFLP pattern was sequenced at least once for phylogenetic identification.

Phylogenetic analysis

Sequencing reactions were performed using the BigDye Terminator v3.1 cycle sequencing kit (ABI, CA) with M13R primer and the fragments were sequenced on an Applied Biosystems 3730×l DNA Analyzer (ABI, CA). Nucleotide-nucleotide BLAST (www.ncbi.nlm.nih.gov/BLAST/) was used to search GenBank for nearest relative sequences. BLAST results and representatives for each archive were aligned by using CLUSTAW from the MEGA 4.1. Phylogenetic trees were constructed by Neighbor-joining method with a Kimura two-parameter distance model using MEGA 4.1 software. Bootstrapping was used to estimate reliability of phylogenetic trees with 1,000 replicate trees. The 18S rRNA gene sequences reported in the current study have been deposited in GenBank under accession numbers GU969060 to GU969102.

Operational taxonomic unit (OTU) richness was compared by rarefaction analysis according to Kenneth et al. (Kenneth *et al.*, 1975) within each 18S rRNA clone library as well as within libraries pooled by lake depth. An OTU was defined as a clone exhibiting a unique RFLP pattern. All unique OTUs were confirmed by sequencing. Species diversity was calculated using the Shannon-Wiener index (Krebs, 1989).

Real Time PCR

Quantification of 18S rRNA was performed using real-time quantitative PCR (qPCR) a Bio-Rad iCycler iQ detection system using the primers EUK345f (AAGGAAGGCAGCAGGCG) and EUK499r (CACCAGACTTGCCCTCYAAT) (Zhu *et al.*, 2005). QPCR was performed in duplicate in a 25-µL reaction mixture containing 1 µL of cDNA, 1.5 µL of each primer (10 pmol μL^{-1}) and 12.5 µL iQ SYBR Green

Supermix (Bio-Rad, CA). Amplification conditions were 5 min at 95°C followed by 35 cycles of 1 min at 94°C, 20 s at 60°C, and 30 s at 72°C. To determine the melting temperature and PCR product specificity, a melting curve was acquired by heating from 50°C to 95°C. The threshold cycle (Ct) was defined as the cycle number at which a statistically significant increase in fluorescence was detected. Standard curves for real-time PCR were developed from plasmids containing the target insert.

Figure SI 1. Environmental data for the water columns of the east and west lobes of Lake Bonney collected on 12 and 16 March 2008, respectively. All depths are from the piezometric water level within the sampling hole. SRP = Soluble Reactive Phosphorus; chlorophyll-a levels are based on high resolution (~10 cm depth intervals) *in situ* spectrofluorometry. The ice cover at this time was ~ 3.5 m thick.

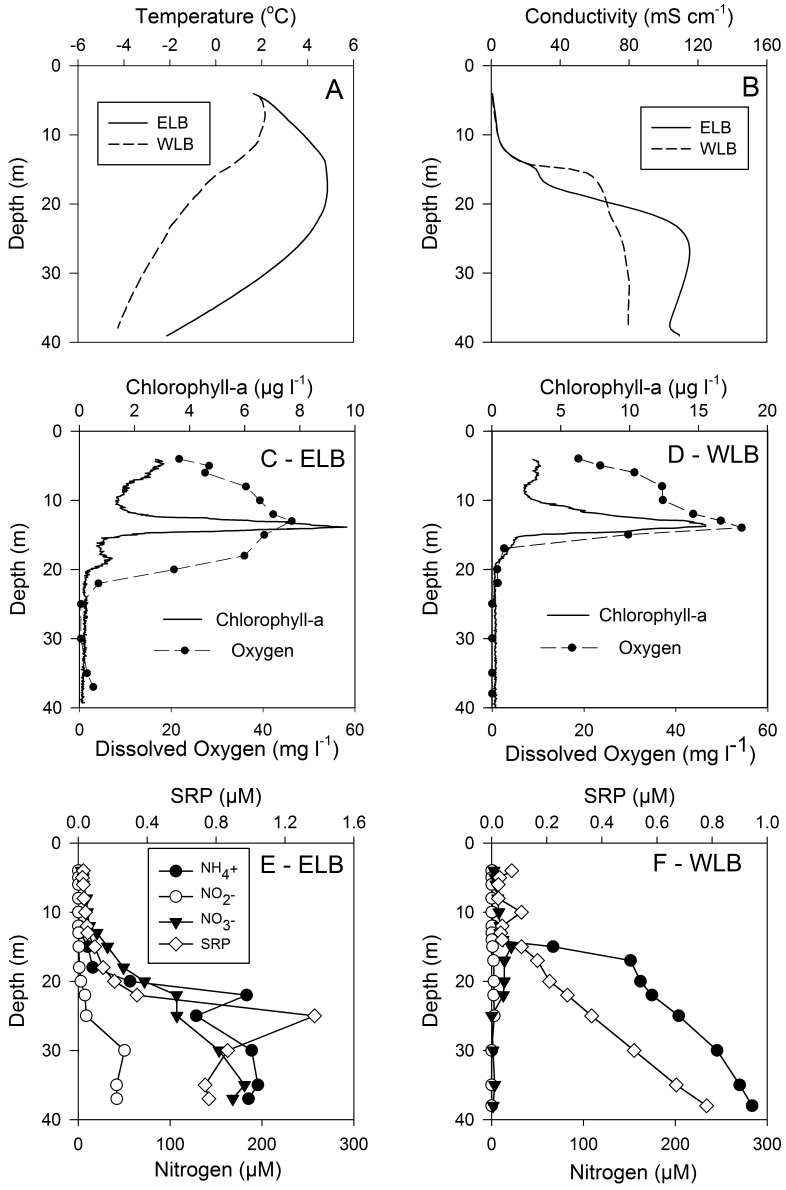

Figure SI 2A. Phylogenetic tree showing the position of Lake Bonney phylotypes from based on small subunit ribosomal RNA sequences. The tree was constructed by neighbor-joining of partial (~1000 bp) 18S rRNA sequences using MEGA 4 software. The numbers at the nodes show bootstrap support at percentages based on 1,000 replicates. Only bootstrap values greater than 50% are given. Bars to the right represent number of clones detected as a percentage of the total across all clone libraries. B. Sub-tree of chlorophytes. C. Sub-tree of stramenopiles.

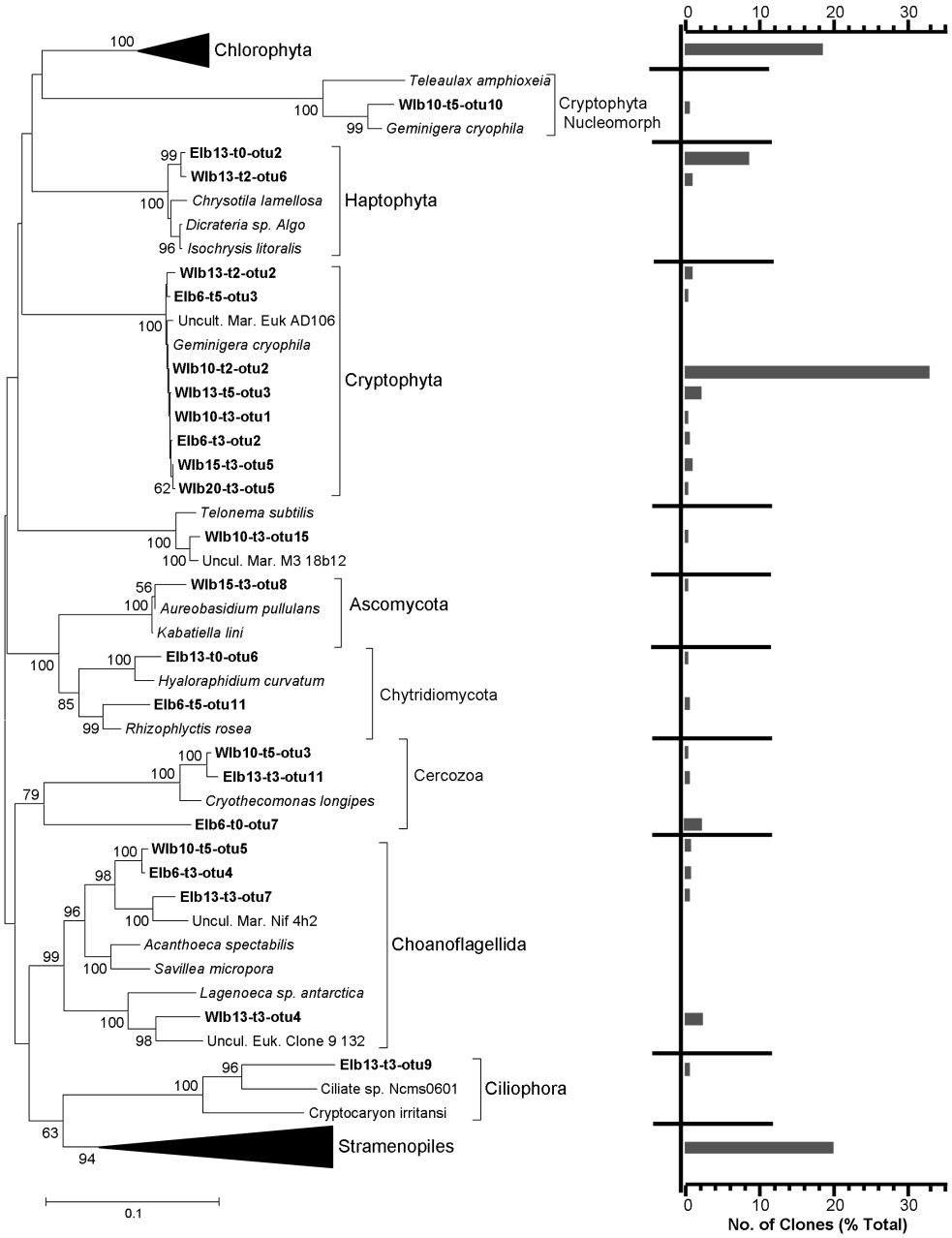

Figure SI 2B.

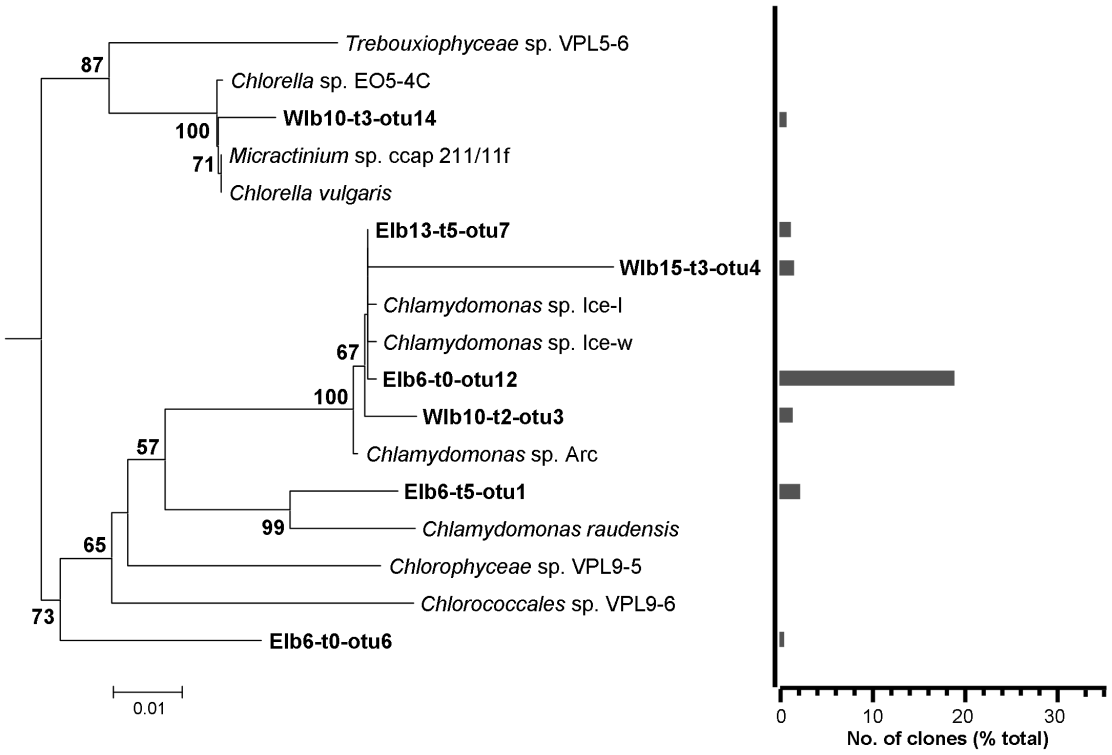
Trebouxiophyceae sp. VPL5-6
87
Chlorella sp. EO5-4C
Wlb10-t3-otu14
100
71
Micractinium sp. ccap 211/11f
Chlorella vulgaris
Elb13-t5-otu7
Wlb15-t3-otu4
Chlamydomonas sp. Ice-I
Chlamydomonas sp. Ice-w
67
Elb6-t0-otu12
100
Wlb10-t2-otu3
57
Chlamydomonas sp. Arc
Elb6-t5-otu1
99
Chlamydomonas raudensis
65
Chlorophyceae sp. VPL9-5
Chlorococcales sp. VPL9-6
73
Elb6-t0-otu6
0.01
0
10
20
30
No. of clones (% total)

Figure SI 2C.

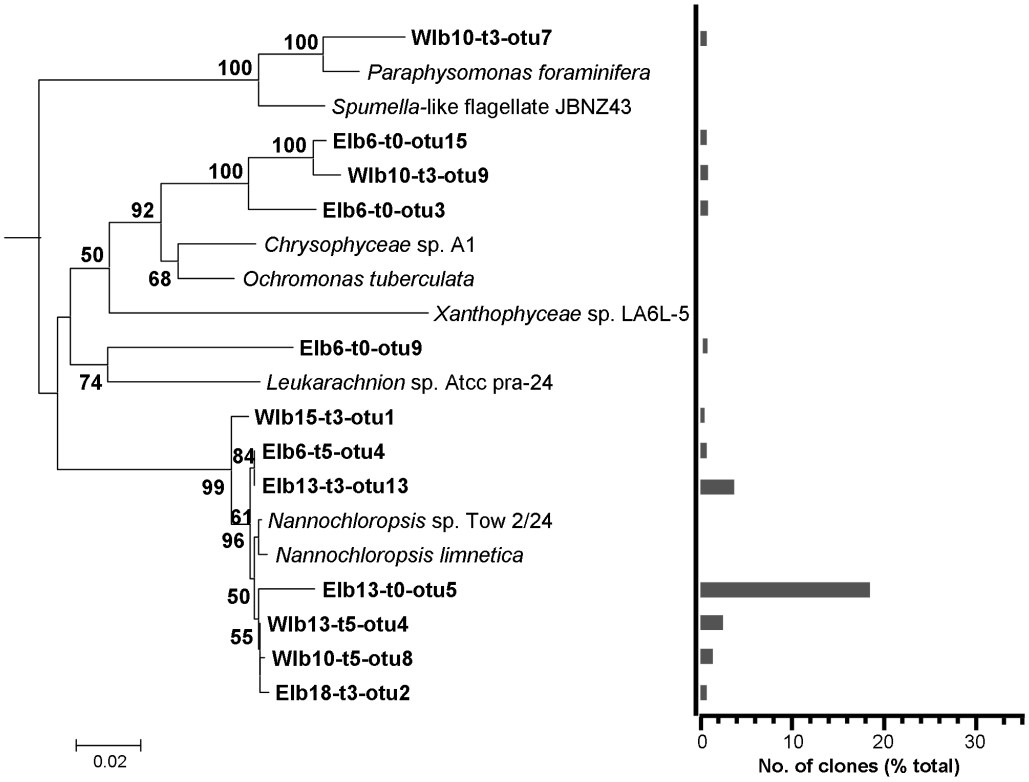
100
Wlb10-t3-otu7
100
Paraphysomonas foraminifera
Spumella-like flagellate JBNZ43
100
Elb6-t0-otu15
100
Wlb10-t3-otu9
92
Elb6-t0-otu3
Chrysophyceae sp. A1
50
68
Ochromonas tuberculata
Xanthophyceae sp. LA6L-5
Elb6-t0-otu9
74
Leukarachnion sp. Atcc pra-24
Wlb15-t3-otu1
84
Elb6-t5-otu4
99
Elb13-t3-otu13
61
Nannochloropsis sp. Tow 2/24
96
Nannochloropsis limnetica
50
Elb13-t0-otu5
Wlb13-t5-otu4
55
Wlb10-t5-otu8
Elb18-t3-otu2
0.02
0
10
20
30
No. of clones (% total)

Figure SI 3. Depth profiles of photosynthetically available radiation (PAR) in the east (A) and west (B) lobes of Lake Bonney during the polar night transition. Note that the 125axes for PAR are different between the two panels.

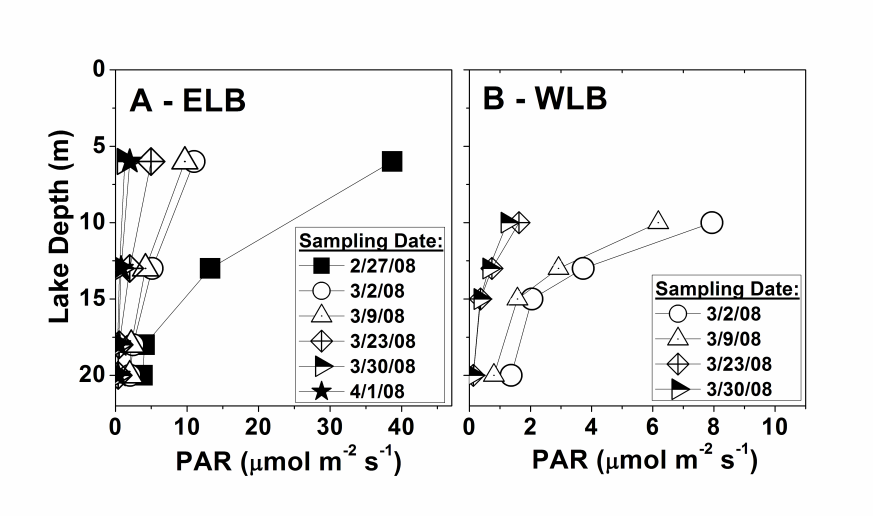

Table SI 1. List of 18S rRNA sequences used in this study.

Name	Accession Number	Reference
Acanthoeca spectabilis	EU011922	(Carr *et al.*, 2008)
Aureobasidium pullulans	AY225165	(Prasongsuk *et al.*, 2005)
Blastocladiales sp. TJ-2007a	EF565163	Unpublished
Chlamydomonas raudensis	AJ781313	(Pocock *et al.*, 2004)
Chlamydomonas sp. Arc	EF537906	Unpublished
Chlamydomonas sp. Ice-L	AY731082	(Liu *et al.*, 2006)
Chlamydomonas sp. Ice-w	AY731083	(Liu *et al.*, 2006)
Chlorella sp. EO5-4C	FJ946889	(De Wever *et al.*, 2009)
Chlorella vulgaris	GQ122370	Unpublished
Chlorococcales sp. VPL9-6	FJ946907	(De Wever *et al.*, 2009)
Chlorophyceae sp. VPL9-5	FJ946901	(De Wever *et al.*, 2009)
Chrysophyceae sp. A1	EF432525	Unpublished
Chrysotila lamellosa	AM490998	(Medlin *et al.*, 2008)
Ciliate sp. NCMS0601	AM412525	(Kim *et al.*, 2007)
Cryothecomonas longipes	AF290540	(Kühn *et al.*, 2000)
Cryptocaryon irritans	FJ167510	Unpublished
Dicrateria sp. ALGO	AM490997	(Medlin *et al.*, 2008)
Geminigera cryophila	DQ452092	Unpublished
Geminigera cryophila nucleomorph	DQ452091	Unpublished
Hyaloraphidium curvatum	Y17504	(Ustinova *et al.*, 2000)
Isochrysis galbana	GQ118682	Unpublished
Isochrysis litoralis	AM490996	(Medlin *et al.*, 2008)
Kabatiella lini	EU707925	(Loncaric *et al.*, 2009)
Lagenoeca sp. antarctica	DQ995807	(Nitsche *et al.*, 2007)
Leukarachnion sp. ATCC PRA-24	FJ356265	(Grant *et al.*, 2009)
Micractinium sp. ccap 211/11f	FM205877	(Luo *et al.*, 2006)
Nannochloropsis limnetica	AF251496	(Krienitz *et al.*, 2000)
Nannochloropsis sp. JL2/4-1	DQ977727	(Fawley and Fawley, 2007)
Nannochloropsis sp. Tow 2/24	DQ977728	(Fawley and Fawley, 2007)
Ochromonas marina	EF165138	Unpublished
Ochromonas tuberculata	AF123293	(Andersen *et al.*, 1999)
Paraphysomonas foraminifera	AF174376	(Atkins *et al.*, 2000)
Powellomyces variabilis	AF164243	(James *et al.*, 2006)
Protaspis grandis	DQ303924	(Hoppenrath and Leander, 2006)
Rhizophlyctis rosea	AY635829	(James *et al.*, 2006)
Savillea micropora	EU011928	(Carr *et al.*, 2008)
Selenophoma mahoniae	EU754114	(de Gruyter *et al.*, 2009)
Spumella-like flagellate JBNZ43	AY651095	(Boenigk *et al.*, 2005)
Teleaulax amphioxeia	AB364287	(Nishitani *et al.*, 2008)
Telonema subtilis	AJ564772	Unpublished
Trebouxiophyceae sp. VPL5-6	FJ946891	(De Wever *et al.*, 2009)
Uncul FrWater Crypto LG07-11	AY919703	(Richards *et al.*, 2005)
Uncul. Cercozoan MLB84.164	EU143890	(Chen *et al.*, 2008)
Uncul. Euk. Clone D7	FN263278	(Piwosz and Pernthaler)
Uncul. Euk.RE5in36	AY082999	(Amaral Zettler *et al.*, 2002)
Uncul. Mar. M3 18B12	AY149178	Unpublished
Uncult. Mar. Euk. AD106	DQ781323	(Dalby *et al.*, 2008)

Table SI 2. Richness and diversity estimates from 18S rRNA clone libraries from lake 130samples collected during the transition to polar night. No. of clones, number of 18S rRNA clones from each library screened by RFLP; SW, the Shannon-Wiener diversity index, calculated as described in (Krebs, 1989) ; MaxSW, maximum SW diversity, calculated as ln(number of OTUs); Evenness, the product of SW/MaxSW.

Lake	Depth (m)	No. of clones	No. of libraries	OTUs	SW	Max-SW	Evenness
ELB	6	210	4	20	1.48	3	0.49
ELB	13	129	4	20	2.18	3	0.73
ELB	18	93	2	6	0.69	1.79	0.39
ELB	20	49	1	4	0.99	1.39	0.71
WLB	10	109	3	21	1.66	3.04	0.55
WLB	13	144	4	12	1.84	2.48	0.74
WLB	15	65	2	8	1.2	2.08	0.58
WLB	20	61	1	6	1.45	1.79	0.81

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