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Biodiversity of micro-eukaryotes in Antarctic Dry Valley soils with <5% soil moisture ☆

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Abstract

Soils in the Dry Valleys of Antarctica are considered to be among the world's most extreme environments. These soils are old, cold and dry with low contents of organic carbon and nitrogen. Habitats adjacent to water (lakes and ice melts) have significant biological activity as demonstrated by the presence of algal mats, lichens and small invertebrates, particularly nematodes, tardigrades and rotifers. In contrast, there are extensive areas in the Dry Valleys that are extremely dry with less than 5% moisture content. These soils are often salty and appear to be barren of life as they have a coarse texture due to their lack of plant organic material. In contrast, molecular techniques (DNA extraction from soils, cloning and rDNA sequence analysis) demonstrated the presence of a complex micro-eukaryotic food web whose structure and composition varied with moisture content and location. Micro-eukaryotic communities in soils with 0.2–1.3% moisture were represented by species of the yeast genus *Trichosporon* and an unidentified clade of micro-eukaryotes, whereas levels from 3.1% to 4.9% contained complex food webs including primary producers (chlorophytes and stramenopiles), symbionts (lichen associated fungi), saprophytes (fungi), predators (alveolates and cercozoans) and fungal nematode parasite/pathogens. The soils had a diversity of species (80 species from 15 sites) with a restricted number (3–21 species) at each site. The sensitive and measurable community structure of the low moisture Dry Valley soils provides an unparalleled opportunity to examine local and global environmental effects on micro-eukaryotic community dynamics with multiple trophic levels.

Keywords: Less than 5% soil moisture; Chlorophytes; Ascomycetes; Basidiomycetes; Cercozoans; Alveolates; Stramenopiles

1. Introduction

Soils in the Dry Valley, Antarctica offer a unique opportunity to investigate the intrinsic to global environmental effects on community structures of microbial food webs. Dry Valley soils are considered to be the oldest (thousands to millions of years), coldest (−20 °C average temperature) and driest (<10 cm precipitation yr^{-1}) with the lowest organic carbon content (0.03 wt%) and biological activity of any soils on earth (Burkins et al., 2001) to the extent that 35% of the soils are reported to have no animals present (Freckman and Virginia, 1998). Some of the soils, which are in direct contact with water, virtually teem with life including cyanobacterial mats, protozoa, chlorophytes, diatoms, nematodes, tardigrades, rotifers, and springtails. These soils may lay dormant for considerable periods of time and then spring to life with the introduction of water. Consequently, they reflect the ecological axiom "where there is water, there is life" (McKnight et al., 1999). In contrast to habitats adjacent to streams and glacial melts, there are soils in the Dry Valleys that do not have the luxury of significant quantities of water, their moisture levels are below 5%, which is considered to be the separation point between wet and dry soils (Freckman and Virginia, 1998). The extent of micro-eukaryotic food webs in these dry soils is relatively unknown.

Most of the ecological studies of Dry Valley soils are based on traditional capture (or culture) methods and

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phenotypic examination of the organisms. In contrast, molecular studies are changing our view of ecosystems, particularly our concepts regarding the presence and activities of micro-scale organisms. The pioneering study of microbial communities in the Dry Valleys was presented by Torre et al. (2003), who examined prokaryotes in sandstone rocks. The first molecular study of Antarctic soil eukaryotes was undertaken by Lawley et al. (2004) from the Antarctic Peninsula region and in the La Gorce Mountains.

Based on the simplicity of the food web in the Dry Valley soil ecosystem, we addressed a basic mycological question: what extent of the soil mycoflora consists of uncultured (or unculturable) species? The question is posed due to a potential analogy with bacterial ecology. In most environments, the majority of the bacterial metabolic activity is derived from uncultured organisms, rather than from species represented on growth media. Our study, which focused on fungi, included other micro-eukaryotes in $<5\%$ moisture soils at 14 sites in the Taylor Valley and one site in the Wright Valley. The techniques were based on extraction of DNA from soils, followed by cloning and sequence analysis of the D1D2 region of the large sub-unit of the ribosomal DNA. Phylogenetic diversity was further examined by small sub-unit rDNA analysis with samples from six of the sites.

2. Methods

2.1. Sites

The Dry Valley landscape includes three large valleys (Taylor, Victoria, and Wright), several smaller valleys as well as glaciers and upland areas in the McMurdo Dry Valley region. This landscape can be divided into three basic zones: coastal, intermediate, and interior (Marchant and Denton, 1996). Steep gradients exist for carbon sources, salts, moisture availability and temperature from the coastal through the interior regions as well as with increased elevations up the valley sides (Cameron, 1972; Campbell et al., 1998). These soils are low in organic content and weakly developed. A paucity of clay and organic material may lead to a low soil buffering capacity, therefore the salt concentration directly effects the soil pH (Campbell et al., 1998) and the distribution of soil biota has been shown to be non-uniform (Powers et al., 1995, 1998). The climate within the McMurdo Dry Valleys is more extreme than the maritime or sub-Antarctic. This large ice-free area ($\sim$4800 km^2) is dominated by low temperatures and strong winds (for reviews see Cameron, 1972; Campbell et al., 1998). Soil surface temperatures during the summer can vary from $-15\,^{\circ}$C to $+27\,^{\circ}$C in 3 h (Cameron and Morelli, 1974) and many freeze-thaw cycles can be experienced in a matter of minutes when clouds pass over (Friedmann et al., 1987).

The soil was collected and carried out in backpacks (the trips typically lasted hours) to the field station at Lake Fryxell for temporary storage to await transport to the McMurdo laboratory (a helicopter trip across the Ross Sea) for processing. During the transportation process the samples were stabilized at $0\,^{\circ}$C to avoid ambient and transportation temperature variations. The temperature of $0\,^{\circ}$C was not different from temperatures that the organisms experienced in their original habitat. Stabilization at $0\,^{\circ}$C helped to avoid changes in the community structure, cell lysis and death of organisms from high temperatures if the soil was allowed to remain warmer than normal for extended periods of time.

Samples were collected during the austral summers of 2002–2003 and 2003–2004 (November–February) at 14 locations in Taylor Valley and one location in Wright Valley (Fig. 1). The sites were chosen to posses a diversity of geomorphic features that ranged from near marine influence to dry and salty (Lake Bonney Basin), including sites near lakes or ponds as well as ephemeral streams. The samples represent a range in soil moisture from 0.2% to 1.3% and from 3.1% to 4.9% (soils with intermediate values were not analyzed). The sites spanned a horizontal distribution from the Taylor Valley floor to above the level of Lake Washburn (>350 m), which is an ecological legacy that consists of ancient pools of organic nutrients from communities in past climate regimes (Doran et al., 1994). The organics are potential relics, which functionally link ancient and modern communities (Moorhead et al., 1999).

The station numbers (Fig. 1) refer to the year of sampling and specific location, for example 02T11D1. Specifically, 02 refers to the field season of November 2002–February 2003; 03 is the Nov 03–Feb 04 field season. T is the Taylor Valley, 1–30 are cross valley stations, A1 and D1 are quadrant designations with the pit number. CW is the Commonwealth Glacier, Lab2 is the Labyrinth Pond Number 2 (Dauphin Pond) in the Wright Valley and YB refers to Stream YB in the Taylor Valley.

Station locations (Table 1, Fig. 1) *Summer 2002/3*: stations 02T11D1 (north side of valley), 02T16A1, 02T17A1 (south side of valley) are in the Lake Bonney basin at elevations of 335–430 m. Station 02T14A1 is near the bottom of the valley at 100 m near a relatively high traffic area. Sites 02T21D1, 02T22D1, 02T23A1 are along a cross valley transect near Lake Fryxell. Station 02T21D1 is on the north side of the valley at a high elevation (410 m), 02T22D1 is at a medium elevation (144 m) and 02T23A1 is close to the lake at a low elevation (25 m). Sites 02T34D1 and 02T35D1 are in the New Harbor area, which is a marine influenced basin. Sites 02T34D1 (70 m) and 02T35D1 (100 m) are in the north and south valleys (respectively).

Summer 2003/4: Site 03CW1A1 is off the tip of the Commonwealth Glacier near a small pond that contains actively growing algal mats. There were no visible algal mats within the sampling area. 03LAB2 is in the Labyrinths at the side of the Dauphin Pond. 03T10A1 is a high elevation site on the north side of the Taylor Valley within 300 m of site 02T11D1. Site 03T27A1 is in the

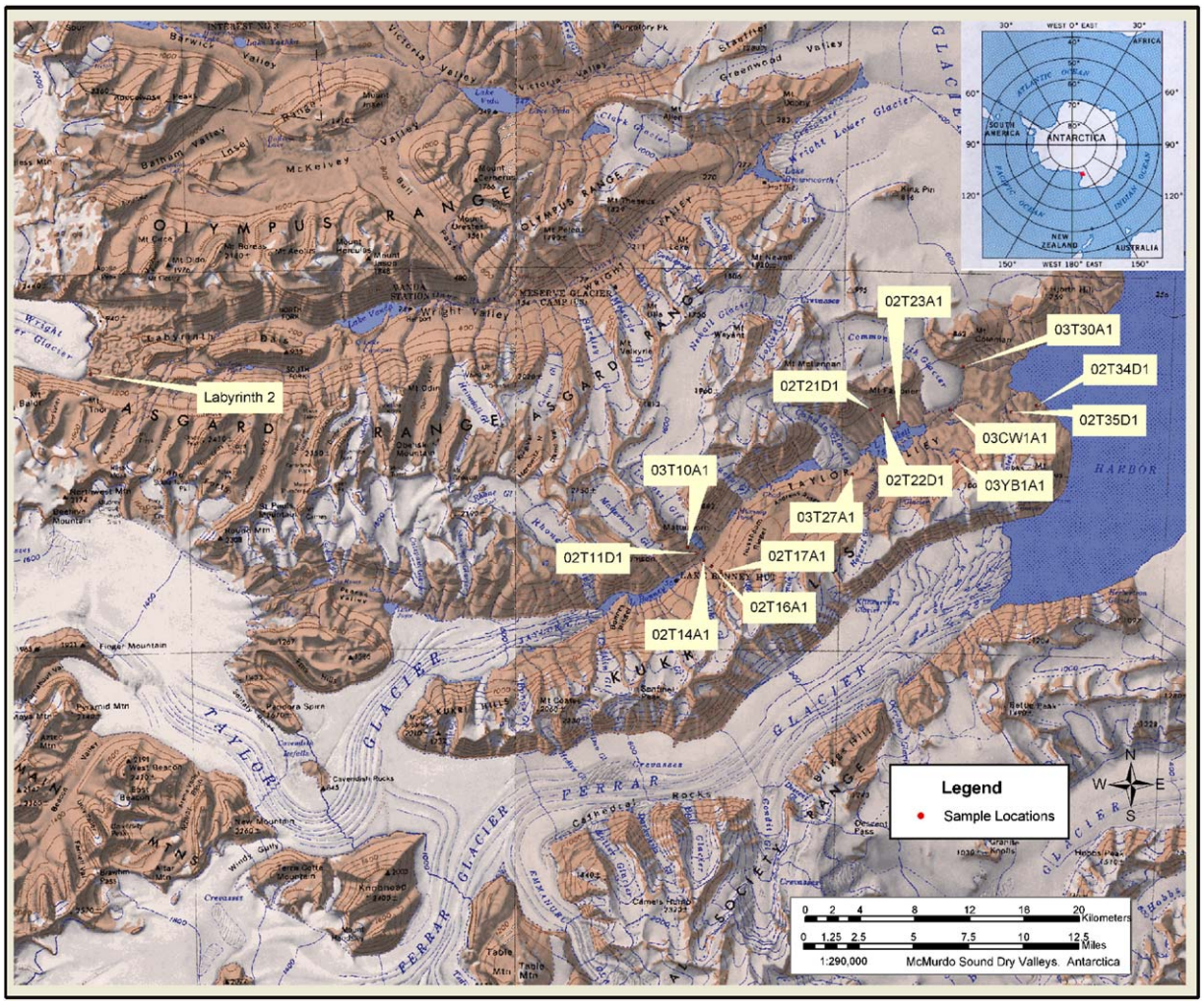

Fig. 1. Station locations in the Dry Valley, Antarctica. Station legend: O2 = field collections Nov 2002–Feb 2003; 03 = Nov 03–Feb 04 field collections; T = Taylor Valley; CW = Common Wealth Glacier; LAB2 = Labyrinth Pond No. 2; YB = Stream YB; 1–35 = Transect location; A1 & D1 = quadrant and pit numbers.

Table 1
Stations location, chemical and physical data

Year	Sample ID	Longitude	Latitude	Soil moisture (%)	Chlorophyll a (μg/kg)	Organic carbon (mg/g)	Soil cond (ms/cm)	Soil salinity (ppt)	Soil pH	Elevation (m)
2003	03CW1A1	163.3328	−77.5885	4.8	51.69	0.54	0.2	0.5	9.8	25
2003	03Labyrinth 2	160.7135	−77.5650	4.9	231.34	0.23	0	0.1	8.1	810
2003	03T10A1	162.5352	−77.6811	3.5	0	0.37	0.5	1.1	8.7	430
2002	02T11D1	162.5615	−77.6850	0.3	0	0.44	2.39	1.2	8.3	240
2002	02T14A1	162.5855	−77.6894	0.2	12.19	0.23	0.6	0.3	9.3	100
2002	02T16A1	162.6102	−77.6933	0.2	0	0.21	2.19	1.1	8.3	255
2002	02T17A1	162.6388	−77.6967	0.7	0	0.26	5.51	3.3	8.7	425
2002	02T21D1	163.0893	−77.5891	1.1	3.10	0.44	0.29	0.1	9.2	410
2002	02T22D1	163.1270	−77.5927	0.3	7.70	0.59	0.0932	0.1	9.7	145
2002	02T23A1	163.1744	−77.5974	1.3	39.17	1.69	0.665	0.3	9.5	25
2003	03T27A1	163.0366	−77.6317	3.3	4.73	0.33	0	0.1	9.9	60
2003	03T30A1	163.3677	−77.5600	3.7	4.59	0.45	0.1	0.2	10.2	130
2002	02T34D1	163.5941	−77.5841	3.5	4.63	0.59	0.604	0.3	9.3	70
2002	02T35D1	163.5193	−77.5888	3.1	1.19	0.60	1.108	0.6	9.2	100
2003	03YB1A1	163.3574	−77.6218	3.5	19.88	0.49	0	0.1	9.9	25

lowlands at a dry, sandy soil location at the tip of the Canada Glacier. 03T30A1 is on the marine side of the Columbia Glacier near the valley floor. Site 03YB1A1 is on the south side of the Taylor Valley between two streams fed by small hanging glacier runoffs. The elevation is partway up the side of the slope in the Lake Fryxell drainage with some marine influence.

At each site a sampling area (2 m circle) was marked and divided into quadrants with a north/south and an east/west axis. Within each quadrant, soil samples of 1 kg were aseptically collected from circular pits ~20 cm in diameter to the depth of permafrost and placed in plastic bags. A separate 50 g sample was collected for chlorophyll-*a* analysis in a sterile 50 ml screw top tube and covered immediately with foil to eliminate photo bleaching of chlorophyll. All samples were stored at 0 °C in a freezer at the Lake Fryxell hut for up to 5 days until transported to the McMurdo Crary laboratory where they were processed within 1 day of arrival. Each sample was mixed thoroughly in the plastic sample bag and divided into sub-samples.

2.2. Chemical and physical tests

Soil moisture: A sub-sample of approximately 50 g was removed, placed in a pre-weighed aluminum dish and weighed on a balance accurate to 0.01 g. This sample was dried at 105 °C for 24 h, placed in a desiccator to cool and re-weighed. These data were used to calculate water content of the soil.

Soil pH, conductivity and salinity: Soil salinity was estimated from soil conductivity measurements (Rhodes, 1982). Soil pH was determined from a 1:2 solution (soil:distilled deionized water), which was allowed to rehydrate for 2 h at 20 °C. Values were measured directly at 20 °C with a bench top Orion 720A pH meter (Forester, 1998). Electrical conductivity was measured with a Corning 311 conductivity meter.

Organic carbon and total nitrogen analysis: A sub-sample of each soil was analyzed for organic C and total N using a Carlo Erba 1500 element analyzer (Carlo Erba, Milan Italy) (Nelson and Sommers, 1982).

Chlorophyll-a analysis: The sub-sample for chlorophyll-*a* was frozen until ready for analysis by the acetone/dimethyl sulfoxide incubation method adapted by Burkins et al. (2000).

2.3. Molecular methods

DNA extraction: there are two basic methods of DNA isolation and identification from soil viz., (1) the cell extraction method where concentration and extraction of microbial cells from soils precedes the DNA extraction and (2), the direct lysis method. All methods introduce their own bias. Typically the direct lysis method has been used in temperate soils with higher biomass because it yields more DNA and presumably a less biased sample of the community. A major draw back is that more PCR-inhibitory material is co-extracted with DNA in the direct lysis method. Because the Dry Valley soils are low in biomass, we used a modified method to concentrate organisms prior to isolation of DNA directly from concentrated soil. All steps for cell concentration were carried out at 4 °C (shaking in water and centrifugation). The tubes were kept on ice between steps. Culturable fungi and bacteria were used as monitors of cell concentration during protocol development. Most bacteria and all culturable fungi were in the cell pellet (not the supernatant) after centrifugation. This pellet was then used for DNA extraction. DNA concentrations from the supernatant were either very low or not detectable. Extracted DNA levels from the pellet were suitable for PCR-ased analysis. The following procedure was employed: total DNA was extracted from 200 g of soil. Each sample was placed in a 250 ml flask with 200 ml distilled water on a New Brunswick G 24 gyrotory horizontal shaker at 120 rpm for 1 h. The liquid slurry was decanted into a 50 ml centrifuge tube and centrifuged for 30 min at 5125*g* in a table-top IEC Clinical centrifuge or until the supernatant became clear, at which time the supernatant was removed and more slurry added to the tube. This was continued until a 10 g pellet was recovered from the centrifuged slurry. Soil DNA extraction from the pellet used the hot detergent and bead beating method of the MO BIO UltraClean Soil DNA kit Mega Prep (MO BIO Laboratories) following manufacturer's instructions.

Large sub-unit rDNA sequence analysis: DNA samples from the 15 sites were amplified in the D1D2 domains of the large sub-unit (LSU) rDNA between primers FG1 (5′TGTTTGGGAATGCAGCTC3′) and R635 (5′GGTCCGTGTTTCAAGACGG3′) (Fell et al., 2000). Eppendorf HotMaster Mix (Eppendorf North America) was used according to the manufacturer's instructions. The optimized amplification program (Fell et al., 2000) consisted of one denaturation cycle at 94 °C for 5 min followed by thirty 1 min denaturation at 94 °C, 1 min annealing at 55 °C and 1 min extension at 72 °C cycles. The final extension at 70 °C was 8 min. Samples of the amplicons produced ~348–579 bp bands after electrophoresis on 2% agarose minigels with 1KB ladders. The amplicons were purified (QIAquick PCR Purification Kit, Qiagene Inc.), then ligated and transformed in competent cells (TA Cloning Kit with INV$\alpha F'$ chemically competent *Escherichia coli*, Invitrogen Corp.) using the manufacturers' protocols. Sixteen plasmids from each sample were purified (Wizard SV 96 Plasmid DNA Purification System, Promega Corp.) and cyclo-sequenced with primers M13F (M13/pUC universal primer code 1, Sambrook and Russell 2001) (5′GTAAAACGACGGCCAGT3′) and M13R (5′GGAAACAGCTATGACCATG3′), which is a 3 nucleotide 5′ extension of M13/pUC universal primer code 4 (Sambrook and Russell 2001). Sequencing employed an AB13730 DNA Analyzer (Applied Biosystems). The sequences were analyzed with Seqman 5.51 for MacOSX

(DNASTAR Inc), visually corrected and compared with GenBank database (http://www.ncbi.nlm.nih.gov/) with nucleotide–nucleotide BLAST (blastn) searches. Known and unknown sequences were aligned with Clustal W (DNAStar Megalign) followed by parsimony analysis with a heuristic search (PAUP4.01b, Sinauer Associates). LSU sequences were submitted to GenBank (Table 2).

Table 2
Genbank numbers for D1D2 large sub-unit rDNA sequences detected in Dry Valley soils

Unknown	
GT 16A1 4 II	DQ111711
UE T10A1 16 III	DQ111719
E T10A1 1 III	DQ111709
E T35D1 11 II	DQ111705
T T23A15II	DQ111716
T YB1A1 8 III	DQ111717
G YB1A1 9 III	DQ111713
T LAB2 12 III	DQ111715
T LAB2 4 III	DQ111714
T YB1A1 16 III	DQ111718
FC CW1A1 11 III	DQ111708
FC T27A1 3 III	DQ111710
E T30A1 11 III	DQ111704
G T27A1 2 III	DQ111712
E T35D1 16 II	DQ111706
E YB1A1 11 III	DQ111707
UE T27A1 8 III	DQ111724
FC YB1A1 14 III	DQ111722
Chlorophytes	
Chlamydomonas sp.	DQ111726
Chlorococcum sp.	DQ111727
Acrosiphonia sp.	DQ111728
CS T35D113 II	DQ111723
Fungi	
T30A114 III	DQ111735
T30A1 4 III	DQ111736
SP T30A1 2 III	DQ111737
Caloplaca saxicola	DQ111738
Coniochaeta lignaria	DQ111739
Cochliobolus heliconiae	DQ111740
Phaeosphaeria sp.	DQ111741
LM T30A1 1 III	DQ111725
Trichosporon domesticum	DQ111746
Trichosporon loubieri	DQ111745
Trichosporon sp.	DQ111744
Trichosporon ovoides	DQ111747
Cryptococcus curvatus	DQ111748
Cryptococcus arrabidensis	DQ111749
Acanthobasidium sp.	DQ111742
Hohenbuehelia sp.	DQ111743
Malassezia globosa	DQ111750
Stramenopiles	
Pauliella sp. 1	DQ111729
Pauliella sp. 2	DQ111730
Hantzschia sp.	DQ111731
NL YB1A1 3 III	DQ111732
Chrysolepidomonas sp.	DQ111733
VV CW1A1 6 III	DQ111720
VV T34D1 11 I	DQ111721
Heterococcus sp.	DQ111734

Bootstrap analyses were based on 500 replicates using a full heuristic search with TBR branch swapping (PAUP4.01b)

Ninety-six sequences, representing 22 different genotypes, aligned with GenBank data in the initial ~150 bp adjacent to the F primer, which corresponds to ~50% of the relatively conserved D1 region. However, the remaining ~200–400 bp, which includes the remainder of the D1 region and the less conserved D2 region, did not align with GenBank sequences. Three different strategies were explored to eliminate the possibility of PCR or random cloning artifacts: (1) Cloning was repeated for samples T23A1 and T34D1 using the same procedures and products. (2) Amplicons from samples CW1A1, LAB2, T23A1, T30A1, T34D1,and T35D1 were gel electrophoresed, individual bands were cut from the gels, purified with Qiagene Qiaex II Gel Extraction Kit and sequenced without cloning. (3) Sequence-specific reverse primers, which were used in association with FG1, were created for three representative unidentified sequences in Unknown lineage 1: primer 529R (5′CGATTGACTCAGCGTA TCACTGG3′) to represent sequence GT16A1 4 II was tested with genomic sample T30A1; primer EE1 (5′AGCGATTGACTCAACGTATCACTG3′) which represented sequence ET35D111II was tested with samples 02T21D1, 02T34D1, 03T30A, and 03CW1A1, and primer EE2 (5′GCGATTGACCCAGCGTATCAC3′) for sequence ET10A1 1III was tested with samples 02T23A1, 03T10A1, and 03T30A1.

SSU sequence analysis: to confirm the phylogenetic diversity in the LSU results, the SSU region was examined from stations 03CW1A1, 03LAB2, 03T10A1, 03T27A1, 03T30A1, and 03YB1A1. Procedures were identical to LSU with the exception that SSU primers were employed for amplification and sequencing: SSU 598 m (5′CGGTAATTCCAGCTCCAATAGC3′), which is a shortened version of SSU 598 (Willerslev et al., 1999) and EUK Bm (5′TCCTTCTGCAGGTTCACC3′), a shortened version EUK B (Medlin et al., 1988). Amplicons obtained and sequenced were ~1200 bp in length. The sequence segment initiated at a position ~598 bp from the 5′ end of the SSU.

3. Results

The LSU and SSU data (Table 3) demonstrated a phylogenetic diversity of micro-eukaryotes, which is illustrated in the trees (Figs. 2 and 3) derived from parsimony analysis. The trees were constructed to illustrate (a) the relationships of individual sequences to published GenBank data and (b) the relationships of these sequences/species within lineages. Sequences derived from our cloning and sequencing experiments are represented by bold letters in the trees. GenBank sequences are distinguished by their GenBank numbers.

Table 3
Biodiversity of microeucaryotes at Dry Valley stations (SSU = small subunit, LSU = large subunit rDNA)

	CW1A1	LAB2	T10A1	T11D1	T14A1	T16A1	T17A1	T21D1	T22D1	T23A1	T27A1	T30A1	T34D1	T35D1	YB1A1
Clorophyta															
Acrosiphonia sp.		LSU													
Chlamydomonas sp.											LSU				
Chlorococcum sp. 1	LSU	LSU													
CST35D1 13 II														LSU	
CW1A1 16	SSU														
CW1A1 2b	SSU														
CW1A1 9b	SSU														
CW1A1 15b	SSU														
Dunaliella sp.												SSU			
LAB2 9b		SSU													
LAB2 15b		SSU													
T27A1 7b											SSU				
T30A110											SSU				
T30A1 12												SSU			
Trebouxia asymmetrica															SSU
Trebouxia sp.												SSU			
YB1A1 11															SSU
YB1A1 15															SSU
Stramenopiles															
Chrysolepidomonas sp.									LSU						
Hantzschia sp.	LSU												LSU		
Heterococcus sp.	LSU														
Nitzschia sp.															LSU
Pauliella sp. 1	LSU														
Pauliella sp. 2	LSU														
Spumella-like Flagellate			SSU												
T10A1 6b			SSU												
WCW1A1 6 III	LSU														
WT34D1 11 I													LSU		
Alveolates															
Colpodella sp.												SSU			
Cyrtohymena citrina	SSU														
Orthamphis breviseries	SSU														
Spathidium sp.	SSU														
T10A1 6			SSU												SSU
YB1A1 1															SSU
YB1A1 13b															SSU
Asco pezizomycota															
Caloplaca saxicola												LSU			
Cochliobolus heliconiae													LSU		
Coniochaeta lignaria														LSU	
LMT30A1 1 III												LSU			
Phaeosphaeria sp.															LSU
SPT30A1 2 III												LSU			
T30A1 4 III												LSU			
T30A1 14 III												LSU			
Basidio															
Acanthobasidium sp.										LSU					
Cryptoc. arrabidensis													LSU	LSU	LSU
Cryptoc. curvatus													LSU		
Hohenbuehelia sp.												LSU			
Malassezia globosa			LSU								LSU				
Trichosp. domesticum			LSU	LSU	LSU	LSU	LSU	LSU	LSU	LSU	LSU		LSU		
Trichosporon sp.							LSU								
Trichosp. louberii				LSU	LSU	LSU	LSU		LSU	LSU			LSU		
Trichosp. ovoides				LSU	LSU	LSU	LSU	LSU	LSU	LSU					
Nematodes															
Pungentus sp. 1															SSU
Pungentus sp. 2															SSU
YB1A1 7b															SSU
Cercozoans															
T10A114			SSU												

Table 3 (*continued*)

	CW1A1	LAB2	T10A1	T11D1	T14A1	T16A1	T17A1	T21D1	T22D1	T23A1	T27A1	T30A1	T34D1	T35D1	YB1A1
T10A1 15			SSU												
T10A1 3			SSU												
T10A1 3b			SSU												
T27A1 8b											SSU				
YB1A16															SSU
YB1A1 8b															SSU
Unidentified lineage															
ET10A1 1 III			LSU							LSU		LSU	LSU		LSU
ET30A1 11 III												LSU			
ET35D1 11 I	LSU							LSU				LSU	LSU	LSU	
ET35D1 16 II														LSU	
EYB1A1 11 III															LSU
FCCW1A1 11 III	LSU														
FCT27A1 3 III											LSU				
FCYB1A1 14 III															LSU
GT16A1 4 II						LSU					LSU	LSU	LSU	LSU	LSU
GT27A1 2 III											LSU		LSU		
GYB1A1 9 III															LSU
TLAB2 12 III		LSU													
TLAB2 4 III		LSU													
TT23A1 5 II						LSU				LSU					
TYB1A1 16 III															LSU
TYB1A1 8 III															LSU
UET10A1 16 III			LSU							LSU					
UET27A1 8 III											LSU				

3.1. Lineages and clades of micro-eukaryotes: LSUrDNA

The LSU data (Fig. 2) include an unknown clade, chlorophytes, ciliates, fungi (ascomycetes and basidiomycetes) and stramenopiles. The unknown clade is comprised of 96 sequences, which represent 22 genotypes (Table 3). Members of the unidentified clade were found at all but two of the 15 stations and they were the prevalent eukaryotes, in concert with basidiomycetes, at sites with moisture levels of 0.2–1.3% (Fig. 4). The unifying characteristic of this clade is the similarity of the initial 5′ ~150 nucleotide fragment, which aligned with basidiomycetes in GenBank analyses. The 5′ sequence regions in the 99% bootstrap cluster (Fig. 2) aligned (93–94% identity) with *Termitomyces*-related basidiomycetes that inhabit the nests of termites in Thailand (Taprab et al., 2002). The GenBank list included (94% identity) the smut fungal genus *Entorrhiza* (Begerow et al., 1997) and other basidiomycetes. The remaining members of the unknown clade aligned (93–96% identity) with the genus *Entorrhiza*, with the exception of UE T27A1 8III, which aligned with the genus *Clavulinopsis* (96%), a macroscopic basidiomycetous coral fungus (Larsson et al., 2004).

The majority of the remaining ~200–400 nucleotide fragment did not align with fungi or any other micro-eukaryotes except for the terminal 3′ 30–32 nucleotides (with the universal reverse primer), which aligned with a wide range of micro-eukaryotes. These results indicate that the sequences do not represent introns inserted into the primary structure. Also, our data do not suggest that the sequences represent chimeras, as indicated by the presence of identical sequences from samples at several environmental collection sites. For example, sequence ET10A1 1 III was found at 5 stations (Table 3). In addition, the authenticity of the sequences was confirmed by three methods: repeated cloning and sequencing, direct sequences of excised amplicons from gels and design/testing of species-specific primers with the genomic DNA. Repeated cloning and excised gel bands resulted in sequences identical to those obtained from the first round of cloning and the sequence-specific primers produced the appropriate sized amplicons (data not shown).

As a consequence of a lack of alignment of the major part of the D1D2 sequence with known organisms, the sequences appear in a weakly supported clade. There are four additional unidentified LSU sequences, which share the alignment characteristics and appear on branches attached to or within specific phylogenetic lineages. The affinity is due to close similarities in the 5′ ~150 nucleotides, which is in the D1 region. The sequences are: CST35D1 13 II attached to the Chlorophytes, LMT30A1 1 II adjacent to the Ascomycetes, and VVCW1A 16 III and VVT36A1 6 I within the Stramenopiles.

Two major phylogenetic groups of fungi were detected: ascomycetes and basidiomycetes. Ascomycetes were found at stations 03T30A1, 02T34D1, 02T35D1, and 03YB1A1. Species included general saprophytes *Phaeosphaeria* sp., *Coniochaeta lignaria* and *Cochiobolus heliconiae* and species in the lichen-forming genus *Caloplaca*. There were three unidentified ascomycetes, T30A1T 14 III, T30A1 4 III, and

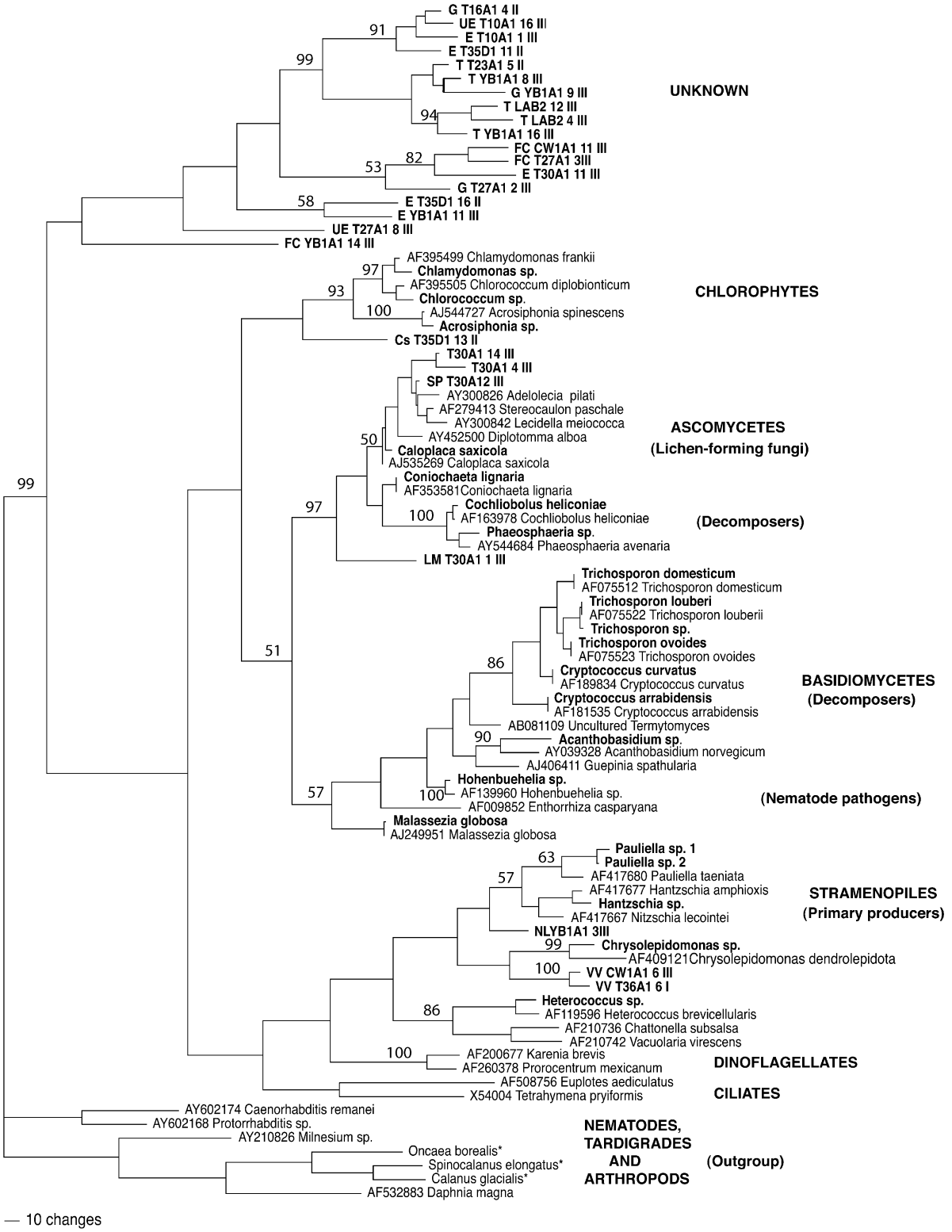

Fig. 2. Sequence analysis of the D1D2 region of the large sub-unit rDNA to illustrate the relationship of sequences obtained from cloned DNA to closest relatives from GenBank BLAST alignments. The tree represents one of 100 equally parsimonious trees. Numbers on branches are the bootstrap percentages (>50%) from 500 full heuristic replications (PAUP 4.0b10). Bold letters indicate sequences obtained in this study, other sequences represent reference data obtained from GenBank. Outgroup = nematodes, tardigrades, and arthropods.

SPT30A1 12 III. The clade relationship to *Stereocaulon* and associated genera suggests that these sequences represent lichen-forming fungi. The unidentified sequence, LM T30A11 III, appears as a separate branch attached to the ascomycetes. All of these ascomycetes are filamentous fungi; ascomycetous yeasts were not recorded.

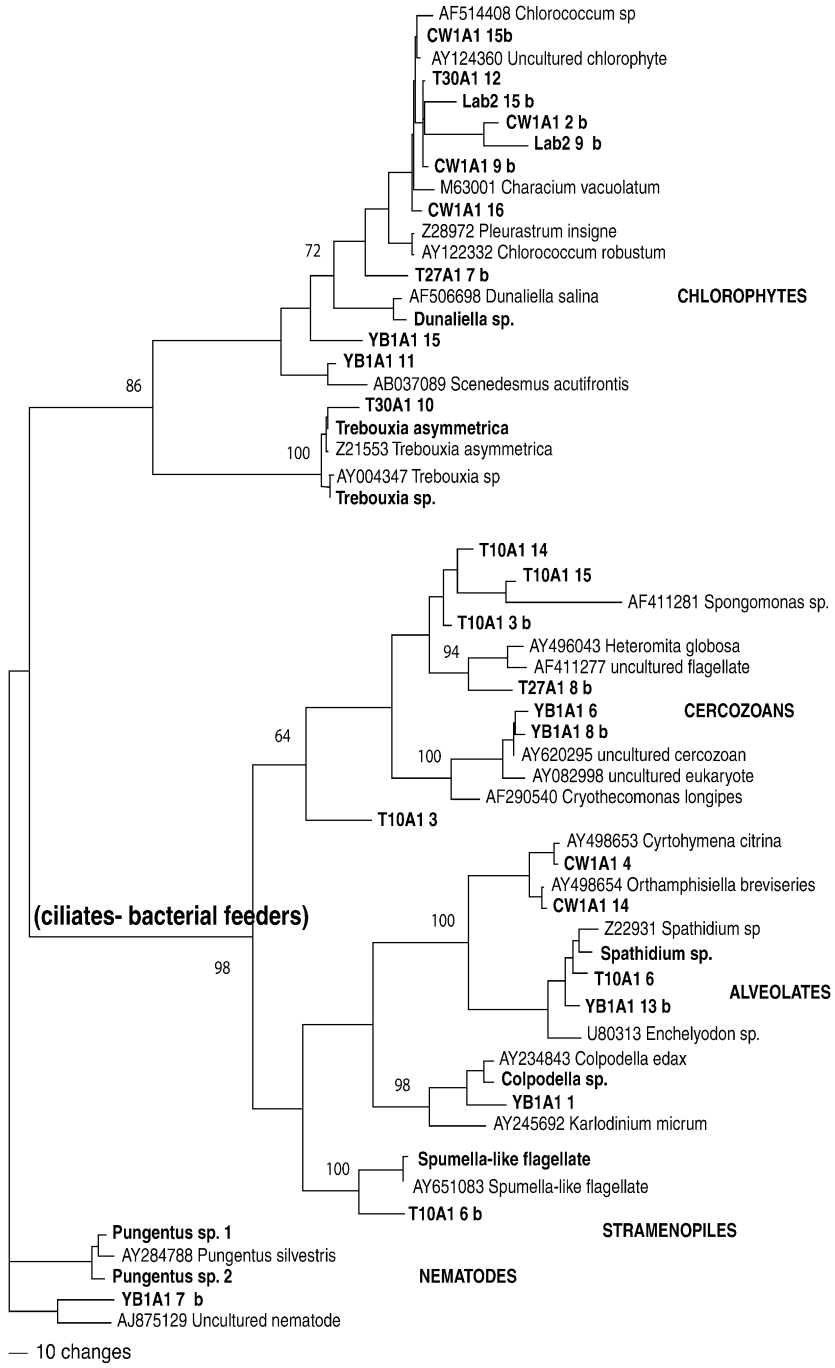

Fig. 3. Sequence analysis of ~1200 bp region the small sub-unit rDNA to illustrate the relationship of sequences obtained from cloned DNA to closest relatives from GenBank BLAST alignments. Bold letters indicate sequences obtained in this study, other sequences represent reference data obtained from GenBank. The tree represents one of 100 equally parsimonious trees. Numbers on branches are the bootstrap percentages (>50%) from 500 full heuristic replications (PAUP 4.0b10). Outgroup = nematodes.

Basidiomycetes occurred at the majority of the study sites. The prevalent species belong to the yeast genus *Trichosporon*, which is found worldwide in soils. Some members of the genus, including *Trichosporon ovoides* and *Trichosporon louberi*, are opportunistic human pathogens. *Malassezia* (stations T10A1 and T27A1) causes skin diseases in humans and other animals, particularly dogs. The yeast has been reported from nematodes in soils (Renker et al., 2003) and, in association with nematodes, as the causative agent of bovine parasitic otitis (Duarte et al., 2001). Cultivation of strains of *Malassezia* requires the addition of oils (e.g. olive oil), which are not usually included in standard growth media (Yarrow, 1998). Members of the genus *Hohenbuehelia* are wood decomposers and nematophagus fungi. These fungi have "sticky knobs" on their hyphae, which attach to nematodes and form hyphae that penetrate and digest the nematode. The other basidiomycetes, *Acanthobasidium* and *Cryptococcus*

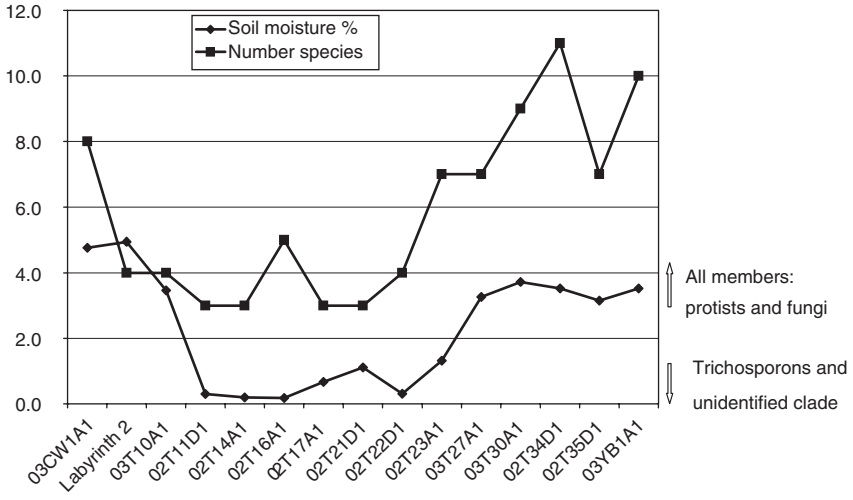

Fig. 4. Comparison of soil moisture levels (Table 1) and number of LSU species at collection sites (Table 3).

spp., which were found in this study, are considered to be general saprophytes.

In addition to the fungi, primary producers were also identified from the LSU sequences. Stramenopiles, in the microalgal and diatom genera *Hantzschia*, *Paulellia*, *Heterococcus* and *Chrysolepidomonas* were present at stations CW1A1 at T10A1, T22D1, and T34D1. One unidentified stramenopile (NL YB1A1 3 III) was found at site YB1A1. Similarly, chlorophytes with the closest affinities to the genera *Chlamydomonas*, *Chlorococcum*, and *Acrosiphonia* were observed at the same stations.

3.2. Lineages of microeukaryotes: SSUrDNA

Comparative cloning and sequence analysis were undertaken with samples from stations 03CW1A1, 03LAB2, 03T10A1, 03T27A1, 03T30A1, and 03YB1A1. The SSU (Fig. 3) and LSU results provide different views of the micro-eukaryotic communities. Notably, fungi were not found in the SSU analyses. In contrast, the numbers of species of chlorophytes, ciliates and other alveolates, were expanded by the SSU investigation, and two additional lineages were found: nematodes and cercozoans. The majority of the SSU sequences aligned with major clades, some sequences appear to be related to known species, whereas other sequences may represent unknown species. We did not undertake a successful continuum sequence analysis of the SSU, ITS, and LSU regions, which might have provided some identity to the large unknown LSU clade.

Tardigrades and rotifers were not present in our study, due perhaps to the locations sampled or the DNA methods. These invertebrates are rare in Dry Valley soils (Freckman and Virginia, 1998). Based on their life styles, these animals are probably limited to regions that are moist and rich in vegetation, such as mosses.

3.3. Relationship of biodiversity to soil moisture content (Fig. 4)

The two parameters were plotted and organisms were found at the tested moisture levels: 0.2–1.3% and 3.1–4.9%. The prevalent species at 0.2–1.3% moisture levels were *Trichosporon* spp. and members of the unidentified clade. These same species were also found at nearly all stations with higher amounts of moisture. The moisture levels of 3.1–4.9% contained the greatest trophic level—and species—diversity. Biodiversity did not appear to be related to any other parameter that was measured (Table 1).

4. Discussion

Early studies in Dry Valley soils noted an absence of in situ primary productivity and concluded that the energy that drives soil food webs was dependent on reserved organic matter (Moorehead and Priscu, 1998). In addition to the reserved organics, Freckman and Virginia (1998) theorized the presence of a self-contained food web, including algal derived carbon. This theory was supported by Burkins et al. (2001), who demonstrated significant summer rates of photosynthesis, which could replenish the organic carbon pool. The hypothesis of autotrophically derived carbon helps to explain the source of energy to support the invertebrate biomass in these soils: nematodes 5–42 mg dry wt m^{-2}, flagellates and amoeba at 7 mg dry wt m^{-2} (Freckman and Virginia, 1998). Although these numbers are low compared to other habitats, long term (decades to centuries) turnover rates require significant C inputs, which could include materials transported from exogenous sources. Our exploration with DNA-based analyses of $<5\%$ moisture soils, demonstrated potential contributions to carbon input and turnover through the presence of soil micro-eukaryote communities composed of primary producers and consumers. Torre et al. (2003)

stated that C fixation in the Dry Valleys was due to a combination of lichens and cyanobacteria, consequently, based on our results, photosynthetic protists can be added to this duo.

The initial focus of the study was to ascertain if uncultured fungi represent a significant percentage of the mycoflora. The question is not easy to pursue in temperate and tropical environments, due to the presence of high phylogenetic diversity and large biomass of fungi in soils and waters. The Dry Valley system, with low biotic diversity, provided an ideal study site. The results, which are significant for Dry Valley research, were not conclusive regarding the presence or absence of uncultured fungi. The members of the unidentified clade showed sequences whose initial 5′ 150 bp region demonstrated the presence of basidiomycetous fungi, however, the lack of alignment by the remaining ~200–400 bp sequence drew that observation into question. In contrast, the unidentified fragment did not align with any known organisms, which would suggest that the sequences could represent unknown fungi or a known group of fungi whose sequences have not been submitted to GenBank. The unidentified clade does not appear to represent a single phylogenetic lineage, although there is one cluster (Fig. 2, bootstrap value 99%), which is comprised of closely related sequences. The appearance of phylogenetic diversity is based on the absence of bootstrap support for the entire clade (values of 50% or less were not included) and the presence of sequences (CST35D1 13 II, LMT30A1 1 III, VVCW1A 16 III and VVT36A1 6 III) with similar characteristics dispersed among the Chlorophytes, Ascomycetes, and Stamenopiles. Clearly, this is a phylogenetically diverse group of organisms that is widely distributed in the soils of the Dry Valleys. Their biology and role in food webs deserves additional investigation through sequence analysis of other genes, microscopy and growth studies with a variety of culture media.

The study also generated information on the presence of a protist/fungal food web in Dry Valley soils. Due to biases in DNA extraction methods and PCR primers, neither the LSU nor the SSU studies fully represented the complexity of the food web. The PCR bias was exemplified by the species and lineage diversities represented in Figs. 2 and 3. Consequently, the study cannot be viewed as a complete analysis of the eukaryotic soil inhabitants in totality or at a specific site. The study does indicate that all, or nearly all, soils contain micro-eukaryotes. The most widely distributed microbes were members of the unidentified clade and several species of basidiomycetous yeasts.

The basidiomycetous yeasts found in this study (*Malassezia, Cryptococcus,* and *Trichosporon*) have either opportunistic or obligate animal and human associations. *Malassezia* has always been isolated in association with man or animals. In contrast, *Trichosporon* is considered to be a soil saprophyte, which can produce serious opportunistic trichosporonosis in man (Kwon-Chung and Bennett, 1992; Middelhoven et al., 2004). Another species of interest, *Cryptococcus curvatus*, which is a member of the Trichosporonales, is also associated with human diseases (Fell and Tallman, 1998). The phenotypic generic distinction between *Trichosporon* and *Cryptococcus* is the presence, in *Trichosporon*, of arthroconidia, which are distinctive disarticulating hyphal elements. Based on our available distribution data, *C. curvatus* appears to differ from *Trichosporon* species by a limitation to habitats with moisture contents of 3.1–3.5%. The occurrence of opportunistic pathogens in Dry Valley soils raises the question as to their role in soils. The known association of *Malassezia* with nematodes suggests that *Trichosporon* spp. and *C. curvatus* may have similar roles with members of the food web.

Soil samples with a moisture content of 3.1–4.9% had a diversity of protist and fungal lineages. Lawley et al. (2004) observed a similar diversity in soils from the Antarctic Peninsula and LaGorce Mts. A direct comparison of our study with the Lawley study is not feasible. The Lawley study used SSU sequence analysis, but did not appear to have submitted the sequences to an electronic database. Their use of the extensive SSU database provided the opportunity to delineate a more detailed phylogenetic structure than is available with the limited LSU data. Lawley et al. did not demonstrate any unknown lineages, which suggests that the members of the unknown clade in our study may belong to known groups of micro-eukaryotes, whose LSU sequences are not present in GenBank.

Lawley et al. (2004) and our study can be compared based on the relationships of moisture contents and fungal populations. The higher moisture content (9.7–27.7%) of the Lawley et al. study is reflected by the presence of zoosporic fungi at the majority of the stations. Zoosporic fungi, which require presence of moisture for dispersal, were not found in our study of Dry Valley soils. Ascomycetes were found, in our study, at 4 sites with 3.1–4.9% moisture. In contrast, Lawley et al. reported ascomycetes at all sites in frequencies of 13–38% of the community structure. Basidiomycetes were rare in the Lawley et al. (2004) study, whereas they were prevalent members of the biota in the Dry Valley soils.

An important aspect of the two studies is the presence of complex food webs that differ in structure with environmental and climatic conditions. Soils in the Dry Valleys are not uniform; depending on location, the soils have different levels of moisture content. Consequently, the microbial community structure will vary as demonstrated with the 0.2–1.3% and 3.1–4.9% moisture communities. Similarly, Treonis et al. (1999) reported a relationship between the invertebrate community structure and moisture, but not a relationship between total abundance of invertebrates and moisture.

The presence of organisms in low moisture samples does not infer either activity or origin. Potentially, some, or all, of these organisms could be wind blown transients from present or past (even ancient) environmental events. Future research will be required to address this question, however,

the available data (Fig. 4) would suggest that distributions are not the result of recent wind activity. If wind is the source for distribution, the anticipation would be that all species would be everywhere, in contrast to the moisture-related distribution depicted in Fig. 4. Some of these species may be endemic to the Antarctic. In contrast, the cosmopolitan nature of genera such as *Trichosporon* and *Malassezia* suggests one or more external origins such as man, animals or wind. If the yeasts are temporary transients, they might have considerable difficulty surviving the daily and yearly environmental extremes, for example freeze-thaw cycles from −15 °C to 27 °C within a few minutes. The counter hypothesis is that the organisms, regardless of origin and time, have adapted to this environment and they are capable of metabolic and reproductive activities. The Dry Valley soils provide a unique environmental laboratory to study the origin and activities of microbial communities, in contrast to highly complex tropical and temperate soils where studies must be limited to a few select species.

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