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Two genetic and ecological groups of *Nostoc commune* in Victoria Land, Antarctica, revealed by AFLP analysis

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Abstract: Microscopy, DNA sequencing, and amplified fragment length polymorphism (AFLP) were used to examine variation within *Nostoc commune* from collections between 72 and 78°S in Victoria Land, Antarctica. Although there is considerable bias of collected material towards southern latitudes, and this material varies greatly in age (collected between 1984 and 2004), an important new phylogeographic pattern was found. DNA sequencing of the tRNA^{leu}(UAA) region, used recently to define form species *N. commune*, revealed little variation between collections. AFLP analysis, however, split the collected material according to habitat (irrigated soil communities versus ponds), rather than latitude. These results suggest that environmental factors linked to latitude are not the greatest drivers of genetic variation in Victoria Land. These may operate at a lower level but would require intensive sampling within narrowly defined habitat types at a range of latitudes to uncover. We advocate extensive sampling across local environmental gradients based on water availability, comparative culturing, and development of sequence characterised amplified regions (SCARs) across a range of latitudes in future seasons of the Latitudinal Gradient Project.

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Key words: DNA, Latitudinal Gradient Project, phylogenetic analysis, phylogeography, taxonomy, ultrastructure

Introduction

A key question of the Latitudinal Gradient Project (LGP) concerns changes in the diversity and complexity of ecosystems with latitude in the Ross Sea region (Howard-Williams *et al.* 2006). This region of Antarctica can be separated into three climatic and latitudinal zones. Green algae and cyanobacteria dominate non-marine habitats in all three zones (Broady 1996). One of the most widespread and abundant taxa is the colony-forming cyanobacterium *Nostoc commune* Vaucher. The distribution, dispersal, and genetics of this species are of prime significance in investigating any relationship between biodiversity and latitude in the sites selected for the LGP. Furthermore, the importance of *N. commune* in both polar regions (Vincent 2000a) and *Nostoc* species generally world-wide (Potts 2000) make phylogeographic patterns in this genus of general interest.

Although more complex than many cyanobacteria, *N. commune* is still characterized by relatively simple morphology. The trichomes contain intercalary heterocytes and are embedded in a dense polysaccharide matrix; these two properties - the ability to fix atmospheric nitrogen and to resist desiccation - may explain its dominance in terrestrial habitats of continental Antarctica.

The simple morphology of *N. commune* has led to two conjectures: first, that the species *N. commune* as traditionally described may not represent a monophyletic taxon, and secondly, that organisms corresponding to this description from polar and temperate regions may be genetically distinct (J. Komárek, personal communication 1997, Vincent 2000b, B. Büdel, personal communication 2005). Phylogenetic reconstruction using the region of the *N. commune* genome encoding tRNA^{leu}(UAA) now indicates that historical collections of organisms identified as *N. commune* are interspersed with organisms identified as other species of *Nostoc* and the genus *Anabaena*, and furthermore, that *N. commune* collected from polar regions can share sequence identity in tRNA^{leu}(UAA) with *N. commune* collected from other areas of the world (Wright *et al.* 2001). Three collections of the species from Antarctica (Mars Oasis on the Antarctic Peninsula, Ross Ice Shelf, and Lake Bonney) were included in the study of Wright *et al.* (2001), but only two of these grouped in their "cluster IV", which they ascribed to form species *N. commune*, and none shared complete sequence identity. It is evident, therefore, that genetic diversity exists within Antarctic *N. commune* populations, but the extent and drivers of this diversity have not previously been investigated.

The technique of amplified fragment length polymorphism (AFLP; Vos *et al.* 1995) has previously been used to examine genetic diversity of *N. linckia* (Roth) Bornet & Flah. under a short (< 1 km) environmental gradient in Israel (Satish *et al.* 2001). Here, we adapt this technique to examine genetic diversity of *N. commune* along the latitudinal gradient between 72 and 78°S in Victoria Land, Antarctica (> 600 km). This covers all three zones identified for study in the LGP. Although the official project has utilized only the Cape Hallett site to date,

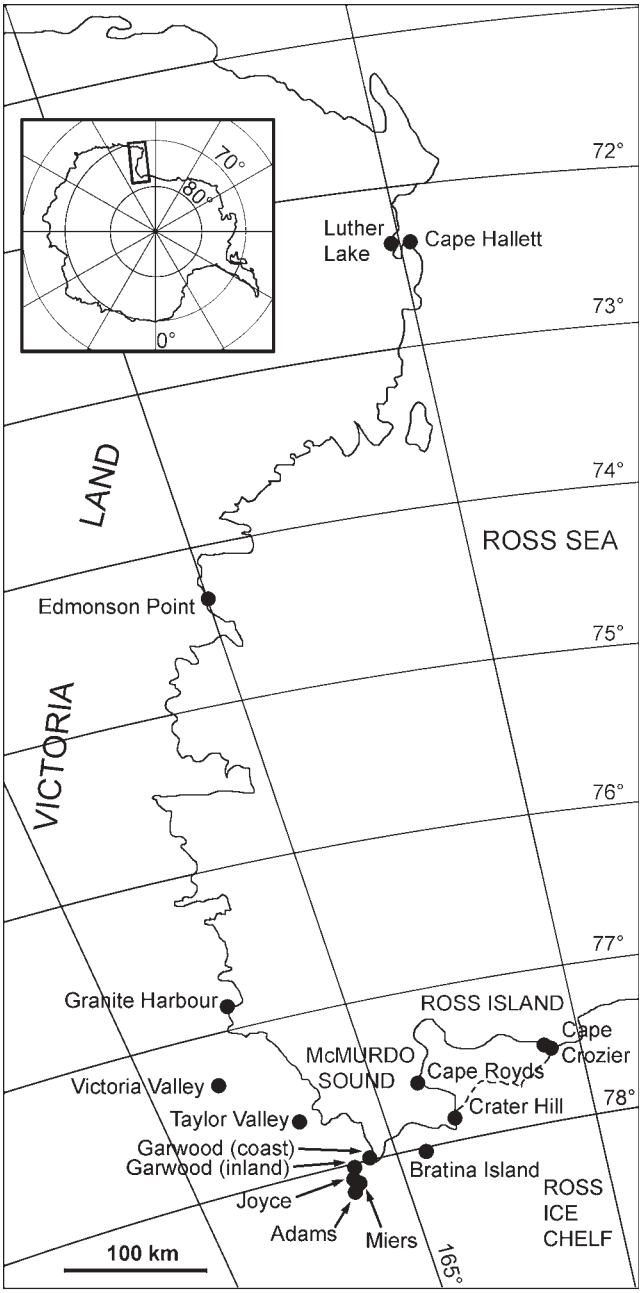

Fig. 1. Map of locations in Victoria Land, Antarctica, from which *Nostoc commune* was collected and used in this study. Inset: Antarctica, indicating the area shown in the main figure.

additional material has been obtained through support provided to other science events in different areas and from additional collectors. We examine the relationship between genetic diversity of *N. commune*, latitude, and other factors, and comment on variation in morphology in the organism found across 16 collections from a range of latitudes and habitats.

Method

Sample collection and examination

Small quantities (< 50 g wet weight) of macroscopic colonies of *N. commune* were collected from the sites shown in Fig. 1 and listed in Table I. These were kept cold and in the dark, frozen on return to Scott Base, and stored at -20°C in the laboratory (except the Taylor Valley sample, which was stored in desiccated condition). Electrical conductivity, pH, and water temperature were recorded at the time of sample collection in many cases, the former two parameters measured using a Hanna HI9812 combination meter and standard procedures.

Detailed morphology of *N. commune* colonies was examined at 7.1 times magnification using a Leica MZ16 stereomicroscope. Trichomes and cells were examined at 400 and 1000 times magnification using a Leica DMLB compound microscope with Nomarksi Differential Interference Contrast optics. Images were recorded using a Leica DC500 digital camera.

DNA extraction, PCR, and sequencing

DNA was extracted from field material using the Qiagen DNeasy Plant Mini Kit in accordance with the manufacturer's instructions, except that the final centrifugation step was extended to 5 min. Samples giving DNA that subsequently proved recalcitrant to complete digestion with restriction enzymes were re-extracted from the original material as follows. An extraction buffer was made from 3.6 ml PCR-grade water, 0.4 ml 0.5 M EDTA, 1 ml 1 M Tris buffer at pH 8.0, 2 ml 10% CTAB, and 3 ml 5 M NaCl, and heated to 65°C in a heat block. Colonies of *N. commune* were ground using a pre-heated mortar and pestle in 1 ml of extraction buffer. The resulting mixture was incubated for 30 min at 65°C, then centrifuged at 13 000 rpm for 5 min. Supernatant was transferred to 1.5 ml Eppendorf tubes containing 750 µl chloroform/isoamyl alcohol (Sambrook *et al.* 1989), mixed thoroughly, and centrifuged for 5 min at 13 000 rpm. The top layer was transferred to a new tube containing 375 ml buffered phenol and 375 ml chloroform/isoamyl alcohol, mixed, and centrifuged for 5 min at 13 000 rpm. Chloroform was removed by repeating the chloroform/isoamyl alcohol step and the top layer removed to a new tube, at which point the Qiagen DNeasy Mini Kit protocol was followed from the addition of buffer AP3/E.

PCR of the $tRNA^{leu}$(UAA) region was carried out using the primers LEU1 and LEU3 of Wright *et al.* (2001), and their PCR program and conditions. Sequences of forward and reverse strands were determined for all taxa. Capillary separation of Big Dye Terminator 3.1 reactions and sequencing were carried out by the Allan Wilson Centre, Massey University, Palmerston North, New Zealand. Electropherograms were checked using Sequencher 4.5

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Table I. Samples used in latitudinal analysis of *Nostoc commune*.

Site	Latitude	Altitude (m)	Notes[1]	Season collected	Collector	Accession no. for tRNA^{leu} sequences[2]
Cape Hallett	72°19'16"S	~5	Cape Hallett SPA, cyanobacterial-dominated area of irrigated soil, EC = 160, pH = 6.1, T = 9.0	2003–04	P. Novis	DQ320637
Luther Lake	72°22'17"S	~200	Little Luther Lake, EC = 260, pH = 9.1, T = 3.0	2003–04	P. Novis	DQ320639
Edmonson Point	74°20'00"S	ND	Frozen pond in moraine depression, fresh, no other chemistry data	1984–85	P. Broady	DQ320634
Granite Harbour	76°53'00"S	ND	Pond in moraines, no chemistry data	1993–94	P. Broady	DQ320636
Victoria Valley	77°16'00"S	ND	Small pond near river in valley bottom, no chemistry data	1990–91	P. Broady	DQ320643
Cape Crozier A	77°31'00"S	~300	Large frozen pond, no chemistry data	1984–85	P. Broady	DQ320632
Cape Crozier B	77°31'00"S	ND	Small pond bottom edge snow field, no chemistry data	1984–85	P. Broady	DQ320633
Cape Royds	77°31'00"S	ND	Small pond, EC = 730, no other chemistry data	1984–85	P. Broady	DQ320641
Taylor Valley	77°37'00"S	~100	Soil polygons 100-500 m west of Canada Glacier near Lake Fryxell, thalli in furrows at edge of soil polygons, no chemistry data	2003–04	B. Büdel	DQ320642
Crater Hill	77°50'00"S	300	Pond Crater Hill summit, no chemistry data	1990–91	P. Broady	DQ320631
Bratina Island	78°00'00"S	ND	Pool east of Bratina Island, Ross Ice Shelf	1987–88	P. Broady	DQ320630
Garwood Valley (inland)	78°01'24"S	368	Irrigated soil fan east of Lake Colleen, EC = 300, pH = 7.5, T = 1.0	2004–05	P. Novis	DQ320635
Garwood Valley (coastal)	78°01'59"S	~10	Small pool, lower valley near coast, EC = 1210, pH = 8.9, T = 9.0	2002–03	P. Novis	ND
Joyce Valley	78°04'06"S	545	Lake on Joyce side of Miers Pass, EC = 520, pH = 7.0, T = 5.0	2002–03	P. Novis	DQ320638
Lake Miers	78°06'01"S	165	Inlet of Lake Miers, EC = 70, pH = 8.2, T = 6.0	2002–03	P. Novis	DQ320640
Adams Valley	78°07'09"S	~400	Pond near Adams Glacier, EC = 700, pH = 10.1, T = 7.0	2002–03	P. Novis	DQ320629

[1] EC = Electrical conductivity (µS cm^{-1}), T = water temperature (°C).
[2] Sequences from the study of Wright *et al.* (2001) that were included in the phylogenetic analysis (Fig. 4) correspond to the following GenBank accession numbers: LBP (Lake Bonney Pond), AF204070; RISA (Ross Ice Shelf), AF204071; RISB (Ross Ice Shelf), AF20472; MOA (Mars Oasis), AF20473.
ND = No data.

(Gene Codes Corporation, Michigan, USA). Multiple sequence alignments were constructed using ClustalX 1.8 (Thompson *et al.* 1997) and were based on the alignments of Wright *et al.* (2001) obtained from GenBank. Sequence differences between the Antarctic taxa and those previously published were evaluated in ClustalX. GenBank accession numbers for these sequences are provided in Table I.

AFLP

AFLP was carried out using the method of Vos *et al.* (1995) with some modifications. DNA was digested with the restriction enzymes MseI and EcoRI for 3 h at 37°C. Each 20 µl reaction contained T4 DNA ligase buffer, 0.05 µmol NaCl, 5 nmol MseI adaptor, 0.5 nmol EcoRI adaptor, 1 µl

Table II. Characteristics of *Nostoc commune* specimens evaluated with light microscopy.

Collection site	Colony surface at 7x magnification	Trichome density[1]	Frequency of heterocytes (per 200 cells)	Most common vegetative cell shape	Vegetative cell size (length x width, µm)
Cape Hallett	Smooth	High	5	Near-spherical	3.6–4.7 x 3.1–4.6
Luther Lake	Moderately convoluted	Low	6	Near-spherical	4.1–4.9 x 3.1–4.3
Edmonson Point	Smooth	Moderate[2]	5	Ellipsoidal	[3]
Granite Harbour	Smooth	Low	2	Ellipsoidal	4.4–4.9 x 3.9–6.4
Victoria Valley	Moderately convoluted	Moderate	12	Near-spherical	4.0–4.9 x 3.3–5.0
Cape Crozier (A)	Smooth	Low	4	Near-spherical	4.2–4.7 x 3.3–4.6
Cape Crozier (B)	Smooth	Low[2]	5	Near-spherical	4.7–5.2 x 3.7–4.9
Cape Royds	Smooth	Moderate	6	Near-spherical	4.1–5.2 x 3.4–4.6
Taylor Valley	Smooth	Low	6	Near-spherical	3.8–4.7 x 2.2–3.2
Crater Hill	Heavily convoluted	Low	6	Near-spherical	4.3–5.2 x 3.3–5.0
Bratina Island	Smooth	Low	5	Near-spherical	3.8–4.1 x 3.5–4.5
Garwood (inland)	Smooth	Moderate	11	Near-spherical	3.9–4.8 x 3.4–4.1
Garwood (coastal)	Smooth	Moderate	4	Near-spherical	4.5–5.6 x 2.3–5.3
Joyce	Smooth	Low	7	Ellipsoidal	3.7–4.2 x 3.9–5.5
Lake Miers	Smooth	Moderate	6	Ellipsoidal	3.5–4.6 x 3.5–5.2
Adams Valley	Moderately convoluted	Low[2]	4	Near-spherical	4.3–4.9 x 3.5–5.4

[1] Assessed approximately by eye. For examples see Fig. 2d & e.
[2] Mucilage degradation made this category difficult to evaluate for these specimens.
[3] This category was not evaluated owing to obvious cell shrinkage in these specimens.

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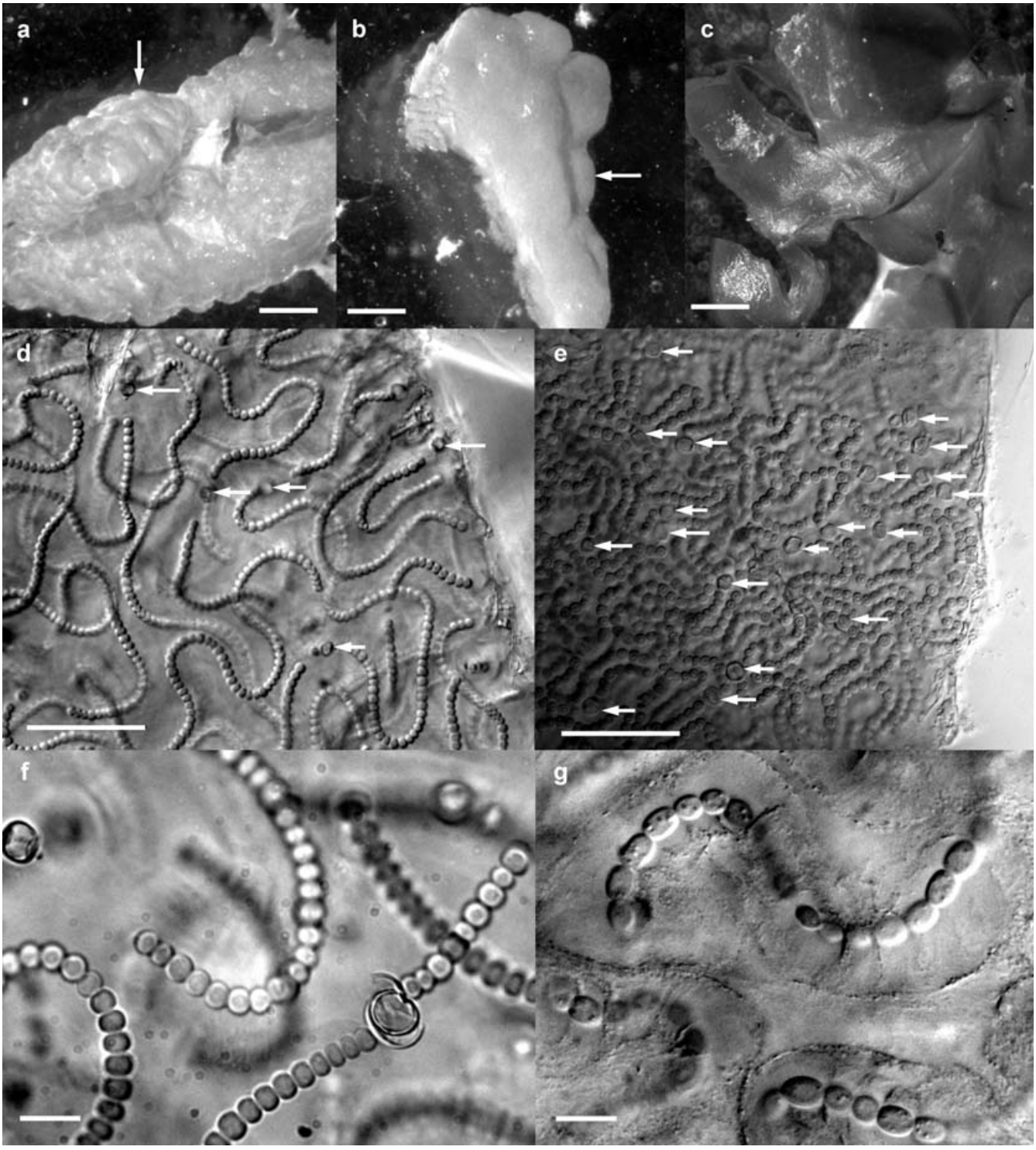

Fig. 2. Examples of morphological variation observed in *Nostoc commune* from sites throughout Victoria Land: **a.** surface of colony from Crater Hill pond, showing extensive convolutions (arrow; scale = 1 mm), **b.** surface of colony from Luther Lake, showing fewer larger convolutions (arrow; scale = 1 mm), **c.** surface of colony from Cape Hallett, showing more typical smooth surface with frequent dissections (scale = 1 mm), **d.** loosely packed trichomes in material from Taylor Valley soil polygons, with heterocytes indicated (arrows; scale = 50 mm), e. tightly packed trichomes in material from Cape Hallett, also with heterocytes indicated (arrows; scale = 50 mm), **f.** near-spherical cells in material from Taylor Valley soil polygons (scale = 10 mm), **g.** ellipsoidal cells from Joyce Valley pond (scale = 10 mm).

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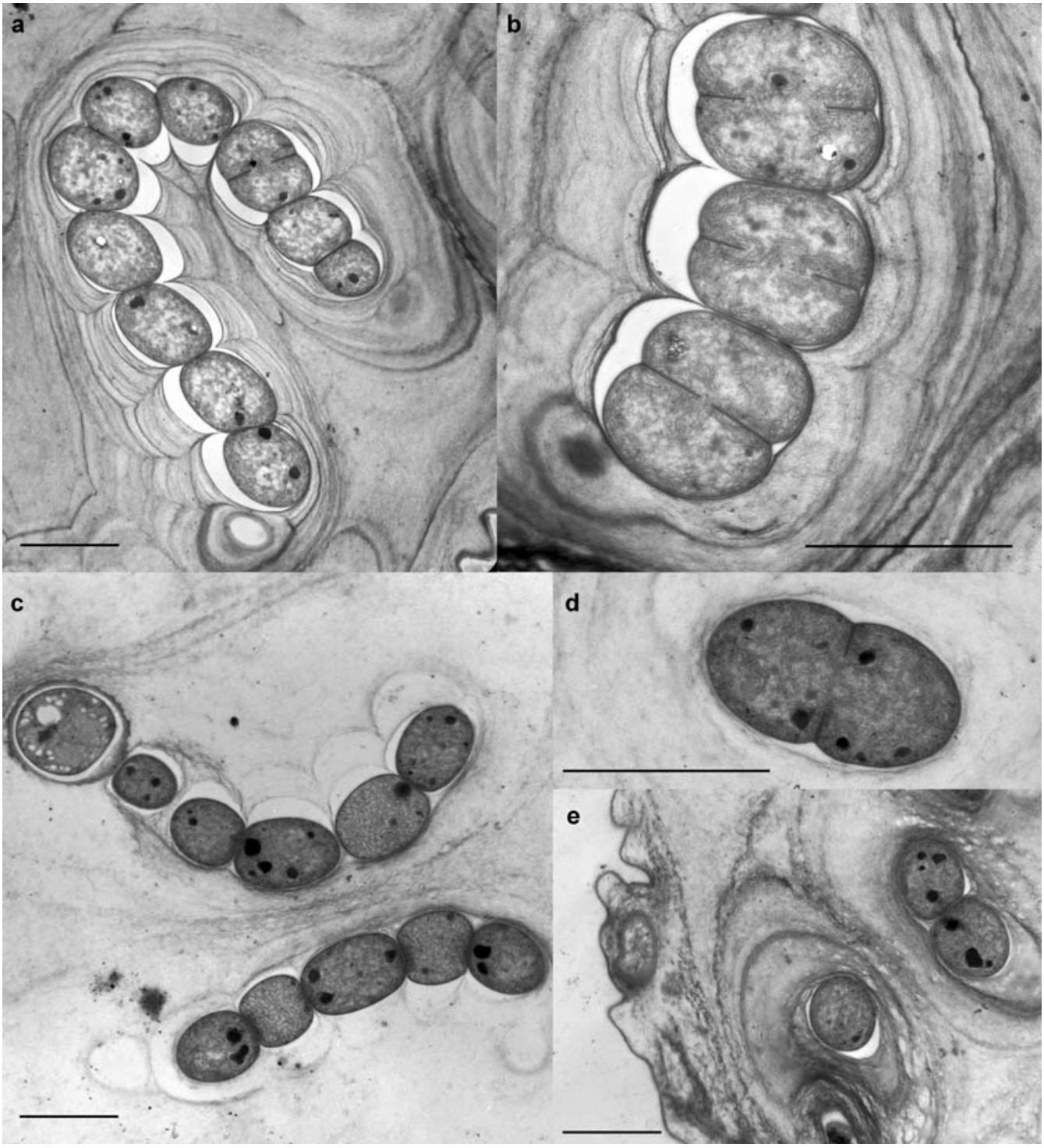

Fig. 3. Transmission electron micrographs of *Nostoc commune* from Victoria Land: **a.** trichome from Garwood inland collection (irrigated soil) showing near-spherical cells and conspicuously layered mucilage, **b.** dividing cells from Garwood inland collection, **c.** trichomes from Joyce pond collection showing ellipsoidal cells and mucilage with less conspicuous layering, **d.** dividing cell from Joyce pond collection, **e.** edge of colony from Joyce pond collection showing surface folds in section. All scale bars = 5 μm.

BSA (1 mg ml^{-1}), 10 units EcoRI, 2 units MseI, 0.2 units T4 DNA ligase, and approximately 3 ng of DNA (quantified by agarose gel electrophoresis against standards). Preselective amplification was carried out using EcoRI primers with an addition of a single base (A) and MseI primers with the addition of a single base (C) at the 3' end. PCR conditions

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for amplification were 72°C for 120 s followed by 20 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 120 s. For selective amplification, 12 combinations of 1–3 nucleotides added to EcoRI and MseI primers were used. These were screened for suitability using four randomly selected taxa. The best primer combination was Eco+ACT/Mse+C; only these data were analysed. AFLP results were visualized on a 6% polyacrylamide gel which was silver stained. Bands were scored for each sample directly from the gel. Scorable bands were defined as those unambiguously present in both or neither of the replicates for all samples; this resulted in 21 reliable characters.

Phylogenetic reconstruction

To illustrate the sequence diversity in the tRNA^{leu}(UAA) dataset, and relate the collections obtained to the broad-scale study of Wright *et al.* (2001), two unrooted neighbour-joining trees were constructed using the number of nucleotide differences between taxa as the distance model in MEGA2.1 (Kumar *et al.* 2001). The first dataset contained all the taxa of Wright *et al.* (2001) and all the taxa reported here, and the second dataset omitted the taxa of Wright *et al.* (2001) that were not collected from Antarctica.

AFLP fragments were coded as binary data and analysed by split decomposition based on Hamming distances in Splitstree 3.2 (Huson 1998). Split decomposition was chosen because splits graphs visualise conflicting signals in the data, rather than force them into a bifurcating structure which is not necessarily appropriate for the data (Lockhart *et al.* 2001).

Transmission electron microscopy (TEM)

Three samples (Joyce Valley, Garwood Valley (inland), Taylor Valley; see Table II) were selected for TEM based on morphological observations and AFLP results. Cells were prepared for TEM by fixing in 2% OsO_4, 0.1 M cacodylate buffer for 30 min. The specimens were dehydrated in ethanol series and embedded in Spurr's resin. Sections were cut, stained in 2% uranyl acetate and lead citrate for 30 min each, and examined with a JEOL 1200 EX microscope.

Results

Morphological diversity

Considerable variation was observed using light microscopy in samples of *N. commune* collected from different sites. This is presented in Table II, with examples shown as follows. Colony surface varied from heavily convoluted (Crater Hill, Fig. 2a) to moderately convoluted (Luther Lake, Fig. 2b) or relatively smooth (Cape Hallett, Fig. 2c). Trichomes ranged from very loosely packed (Taylor Valley, Fig. 2d) to very tightly packed (Cape Hallett, Fig. 2e). Intercalary heterocytes ('heterocysts') were

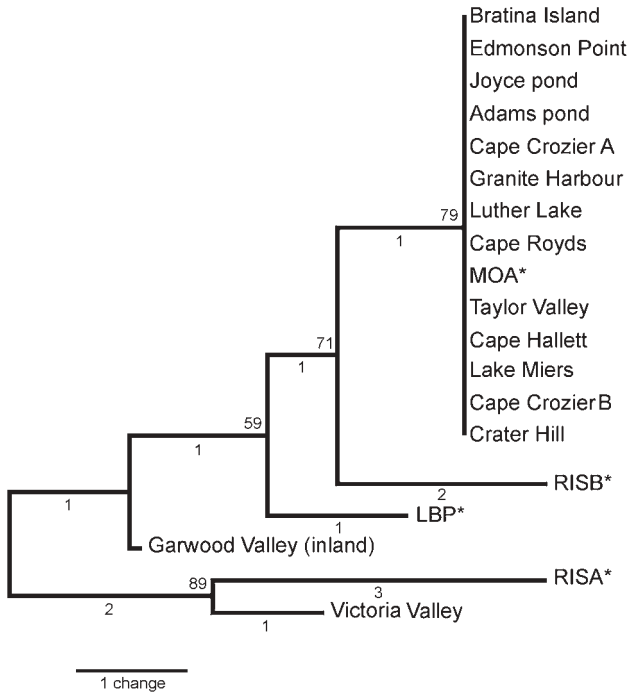

Fig. 4. Neighbour-joining tree from tRNA^{leu}(UAA) sequences in *Nostoc commune*, based on the number of nucleotide differences between samples. Abbreviated taxa marked with an asterisk are from the dataset of Wright *et al.* (2001). Labels above nodes are bootstrap percentages (100 replicates). Edge labels and scale bar represent number of nucleotide differences.

observed in all material, but varied from rare (2 per 200 cells, Granite Harbour, not shown) to common (12 per 200 cells, Victoria Valley, not shown). Heterocytes are indicated in Fig. 2d & e. Cell shape in most samples was mostly near-spherical (e.g. Taylor Valley, Fig. 2f) but some specimens were composed mainly of ellipsoidal cells (e.g. Joyce Valley pond, Fig. 2g).

Variation in mucilage structure was apparent between samples from Garwood (inland) and Joyce under TEM (Fig. 3). In specimens from the former site, extensive mucilage layering occurred around short, near-spherical cells (Fig. 3a & b), whereas the latter trichomes contained near-ellipsoidal cells (Fig. 3c & d), with mucilage lacking extensive layering, but possessing a conspicuously folded external surface (Fig. 3e). Cell shape differences were consistent in both mature and dividing cells (compare Fig. 3a with c, and Fig. 3b with d). Thylakoid distribution did not appear to differ consistently between Joyce and Garwood (inland) specimens. The mucilage and cell shape features in the Garwood (inland) specimens were also observed in the Taylor Valley specimens (not shown).

Sequencing of tRNA^{leu}(UAA) region

Little sequence variation was found between most specimens, including a group of 14 collections with no

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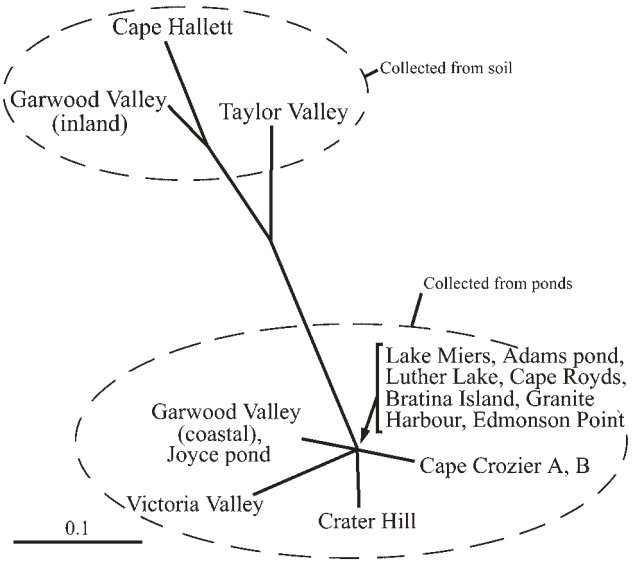

Fig. 5. Graph obtained from split decomposition of AFLP data based on Hamming distances in Antarctic *Nostoc commune*. Scale bar represents Hamming distance.

nucleotide differences, identical to the sequence reported by Wright *et al.* (2001) for their taxon MOA, from the Mars Oasis (Antarctic Peninsula). This is shown in Fig. 4; non-Antarctic taxa of Wright *et al.* are omitted from this tree, which is presented to illustrate diversity in the dataset since the branch lengths in this analysis correspond to number of nucleotide differences between taxa. The remaining two specimens lacked sequence identity with any taxa discussed by Wright *et al.* (2001); in addition, the Victoria Valley sample contained a 14 base pair intron that was not shared by any other known taxa, and this is not accounted for in the tree-building analysis.

AFLP analysis

A graph derived from split decomposition of the 21 AFLP characters shows a major split separating the Taylor, Garwood (inland) and Cape Hallett collections from the remaining samples (Fig. 5). Specimens from these three sites account for all those collected from soil (as opposed to ponds and lakes; Table I). No structure in the molecular data available was found to correlate with latitude, nor could the two groups (derived from soil and ponds) be distinguished on the basis of morphology (Table II).

Discussion

Potential utility of morphological characters

Much of the morphological diversity in *N. commune* found in different sites (Fig. 2) is probably due to environmental variation. In particular, the abundance of heterocytes (Table II; Fig. 2d & e) is well known to vary according to nitrogen supply, although their development may differ between taxa (Schlangstedt *et al.* 1987). Density of trichomes is perhaps related to the amount of mucilage secreted; if mucilage is produced partly to conserve water in desiccating environments (Potts 1999), then trichome density should be related to water stress. However, there is no obvious correlation between the most water-stressed habitats (Garwood Valley inland, Taylor Valley, and Cape Hallett, where specimens were collected from irrigated mineral soil rather than from ponds) and trichome density (Table II), although it is possible that the relationship is more complicated through the interaction of other factors including pH, nitrogen source, and CO_2 availability influencing mucilage production (Potts 2004).

The surface features of the colonies collected from Crater Hill Pond (Fig. 2a) seemed quite different from all other samples and are harder to explain by environmental effects, given that many other pond samples were included in the study from Ross Island and elsewhere (Table I). Supporting this conclusion, Komárek & Anagnostidis (1989, p. 256) stated "thallus morphology [in the Nostocales] can be influenced slightly by the environment, but the principal structure is always the same in natural conditions". Similarly, any environmental stimuli leading to ellipsoidal cells (Fig. 2g) rather than spherical cells (Fig. 2f) are unknown. The stability of these characters could be evaluated using a dedicated culture study, notwithstanding the difficulties outlined by Komárek & Anagnostidis (1989, pp. 248, 259). It could be that some easily recognized, stable morphotypes exist within the traditional concept of *N. commune* in Victoria Land.

Genetic structure of N. commune *populations in Victoria Land*

Some expert cyanobacterial taxonomists doubt whether polar and temperate cyanobacteria with identical morphology are likely to be closely related to the exclusion of other forms (e.g. Komárek & Anagnostidis 1989, pp. 248, 274, J. Komárek, personal communication 1997, B. Büdel, personal communication 2005). Implicit in this point of view is the unknown ability of cyanobacteria to disperse in a viable state across large distances, and if this occurs, how frequently (Broady 1996). There has been considerable historical debate over species concepts in the cyanobacteria (Castenholz 1992), including advocates for ecological data to be included in species definitions (Komárek & Anagnostidis 1989 pp. 274–277). The latter authors would perhaps argue that morphological differences discernible between our specimens are good evidence for ecological adaptation to specialized niches, and without improved water availability and chemistry data we are unable to identify these niches at present. Water chemistry can vary enormously between Antarctic ponds, and this may be reflected in cyanobacterial diversity (e.g. Jungblut *et al.*

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2005). The simple environmental data available for our collections (Table I) show considerable variation, but this does not correspond to the two genetic groups identified (see below). Rather than extensive specialization of genotypes to niches, recent phylogenetic work on *N. commune* suggests the opposite: that genotypes from different geographic localities (even hemispheres) are largely intermixed, implying extensive dispersal and no specialization of genotypes to any particular niche (Wright *et al.* 2001).

We advocate considerable caution in adopting the extreme of either of these views, at least regarding specimens from Victoria Land. Firstly, the morphology in culture of different Antarctic *Nostoc* field morphotypes has received scant attention, and interpreting all field variation as representing distinct taxa seems risky, despite a general lack of evidence for ecophenes within this species (Garrick 1981, p. 72, Komárek & Anagnostidis 1989, p. 256). Secondly, the apparent lack of sensitivity in the $tRNA^{leu}$(UAA) region (Fig. 4) compared with AFLP (Fig. 5) suggests that drawing extensive phylogeographic conclusions from the phylogeny of Wright *et al.* (2001) is unwarranted. Although there is reason to doubt the finer detail of our AFLP results, owing to difficulties analysing the older material (see below), the main split separating specimens collected from soil and those collected from ponds (Fig. 5) was very obvious and seems robust, as well as ecologically meaningful. Omitting the older specimens with DNA more recalcitrant to digestion (notably Edmonson Point and Bratina Island) did not affect this split. However, there is no suggestion of such a split from the sequence data (Fig. 4), a conflict that in our view implies that the utility of $tRNA^{leu}$(UAA) for phylogeographic investigations of *N. commune* within Antarctica is limited. We also suggest that the separation of Antarctic collections by temperate ones in the phylogeny presented by Wright *et al.* (2001) may be artifactual, but would require extensive temperate collections for AFLP analysis to test this. Genetic segregation of Antarctic and Arctic picocyanobacteria has been documented previously (Vincent *et al.* 2000), suggesting at least some polar restriction in groups more readily dispersed than *N. commune*. Discrepancies between phylogenies of heterocystous cyanobacteria constructed using $tRNA^{leu}$(UAA) versus other sequences have been noted elsewhere (e.g., 16S rRNA, Oksanen *et al.* 2004).

The finding that soil and pond populations of *N. commune* may be genetically distinct (Fig. 5) is a new and important result. The three soil populations span almost the entire latitudinal gradient, with nearby pond sites in each case placed in a different genetic group. Insufficient resolution exists to determine if latitude-related structure exists within each of these groups, but the main split between habitat types rather than latitude seems to confirm relatively frequent dispersal of airborne *N. commune* between 72 and 78°S. The evidence for such dispersal has been reviewed by Vincent (2000b), and includes spore trap data (Marshall & Chalmers 1997) and observation of propagules in the cloud layer over the Ross Ice Shelf (Saxena 1982). Survival of Antarctic cyanobacterial mats under freezing and desiccating conditions is well known (Hawes *et al.* 1992), and their successful dispersal under these conditions seems equally likely. Other studies in which environmental gradients have been shown to correlate with genetic segregation of bacterial populations include Rohwer *et al.* (2002), Hewson & Fuhrman (2004), Horner-Devine *et al.* (2004), and Yannarell & Triplett (2005).

To uncover any patterns correlated with the latitudinal gradient, it is now clear that local gradients of water availability (and possibly others, such as enrichment or salinity) must be better accounted for. The present results suggest that genetic differences within local gradients of water availability in Victoria Land should be detectable using AFLP; in fact, this has already been demonstrated in Israel for *N. linckia* by Satish *et al.* (2001). If this can be done, and replicated in several sites at different latitudes in future seasons of the LGP, a phylogeographic model for *N. commune*, one of the most frequently observed non-marine organisms in Victoria Land, will be imminent.

In analysing these data we have attempted a very conservative approach, dictated by the somewhat deteriorated nature of some of the material available (although without this material, addressing latitudinal relationships would have been severely compromised). This analysis could be improved. Higher quality DNA, which could be obtained by fresh collections of the Ross Island, Victoria Valley, Edmonson Point, and Bratina Island samples, or possibly by initiating and purifying cultures from the old samples, would most probably result in many more scorable characters and considerably greater phylogeographic resolution. Morphological variation has been found previously among isolates cultured from identical field material corresponding to *N. commune* (Garrick 1981). This may reveal greater correlation of genotypes with ecological conditions and morphology, and identify latitudinal gradients driving the distribution of genotypes within pond and soil habitats. Sequencing the variable fragments isolated from the AFLP gels to develop SCAR (Sequence Characterised Amplified Region) markers (e.g. Lockhart & McLenachan 1997) would likely provide a resource for future sequencing studies, thereby overcoming many of the DNA quality issues experienced to date. These approaches exceed the scope of this small project. However, the present results show that the environmental gradients linked to latitude are not the strongest drivers of genotype distribution in *N. commune* from Victoria Land.

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