**Spatial pattern in Antarctica: what can we learn from Antarctic bacterial isolates?**

**Running Title: Spatial pattern in Antarctica**

**ABSTRACT**

A range of small- to moderate-scale studies of patterns in bacterial biodiversity have been conducted in Antarctica over the last two decades, most suggesting strong correlations between the described bacterial communities and elements of local environmental heterogeneity. However, very few of these studies have advanced interpretations in terms of spatially-associated patterns, despite increasing evidence of patterns in bacterial biogeography globally. This is likely to be a consequence of restricted sampling coverage, with most studies to date focusing only on a few localities within a specific Antarctic region. Clearly, there is now a need for synthesis over a much larger spatial to consolidate the available data. In this study, we collated Antarctic bacterial culture identities based on the 16S rRNA gene information available in the literature and the GenBank database (n > 2000 sequences). In contrast to some recent evidence for a distinct Antarctic microbiome, our phylogenetic comparisons show that a majority (~75%) of Antarctic bacterial isolates were highly similar (≥ 99% sequence similarity) to those retrieved from tropical and temperate regions, suggesting widespread distribution of eurythermal mesophiles in Antarctic environments. However, across different Antarctic regions, the dominant bacterial genera exhibit some spatially distinct diversity patterns analogous to those recently proposed for Antarctic terrestrial macroorganisms. Taken together, our results highlight the threat of cross regional homogenization in Antarctic biodiversity, and the imperative to include microbiota within the framework of biosecurity measures for Antarctica.

**INTRODUCTION**

Based on thermal, climatic and floristic characteristics, Antarctica can be broadly simplified and divided into three biogeographic zones, namely the sub-Antarctic, maritime Antarctic and continental Antarctic (Convey, 2013). The sub-Antarctic conventionally includes a ring of oceanic islands located between 45˚S and Antarctic Polar Frontal Zone (Convey, 2007; Selkirk, 2007). The islands of the sub-Antarctic experience relatively higher precipitation and milder and less variable temperatures in comparison with the maritime and continental zones. At higher southern latitudes, the maritime Antarctic includes the various Scotia arc archipelagos and the bulk of the coastal Antarctic Peninsula. Crytogramic fellfield is perhaps the most typical vegetated habitat of the maritime Antarctic, with vegetation being particularly well-developed on the nitrogen-rich ornithogenic gelisols formed near areas of vertebrate influence (Smith, 1984; Michel et al., 2006). Finally, continental Antarctica comprises the eastern and southern parts of the Antarctic Peninsula, along with the bulk of the Antarctic continent. Terrestrial ecosystems within this region are restricted to small isolated “islands” of ice-free ground located mainly either in the low-lying coastal zones, or in the form of isolated nunataks and the higher altitudes of inland mountain ranges (Convey et al., 2012a), with the striking exception of the McMurdo Dry Valleys in Victoria Land which cover an area of c. 40,000 km2, or just over 90% of the total area of ice-free ground in Antarctica.

Understanding how regional and local variability influence diversity is an important step towards clarifying the functioning of Antarctic ecosystems (Chown & Convey, 2007; 2012). Further, improving knowledge on the spatial scales at which these ecosystems operate provides the basis of the framework for conservation planning required in Antarctica (Hughes & Convey, 2010; Terauds et al., 2012). Such questions have attracted considerable research interest over the last two decades. For instance, in comparisons of the distribution patterns of the major groups of terrestrial invertebrates and vegetation (moss and lichen), the majority showed patterns specific to the Antarctic “sector” of origin (Peat et al., 2007; Pugh & Convey, 2008). In addition, the existence of an ancient biogeographical boundary separating the Antarctic Peninsula and the remainder of Antarctic continent was proposed by Chown and Convey (2007), and is also consistent with the presence of region-specific cyanobacterial and green algal diversity in Antarctica (De Wever et al., 2009; Vyverman et al., 2010). The biogeographical complexity of Antarctica is increasingly being appreciated, and has most recently been re-enforced by Terauds et al. (2012), who identified 15 biologically distinct ice-free zones, termed ‘Antarctic Conservation Biogeographic Regions (ACBRs)’, across Antarctica.

Reports of regionalisation are also becoming evident in studies of Antarctic bacterial communities (Yergeau et al., 2007b; Vyverman et al., 2010; Chong et al., 2012b), suggesting that the evolutionary timescales underlying the development of biogeographical patterns in Antarctica may be similar for both prokaryotic and eukaryotic biota. Furthermore, it is increasingly being recognised that, even with the scales of isolation of Antarctic terrestrial ecosystems, the dangers of irreversible human-mediated contamination of Antarctic microbial ecosystems, and consequential compromising of future microbial studies given the rapidly developing technologies in this field, are very real and serious (Cowan et al., 2011a; Hughes et al., 2011, 2013). Nevertheless, detailed regional scale syntheses similar to those conducted for the terrestrial eukaryotes (micro- and macro- invertebrate fauna) of Antarctica have not been attempted for the continent’s bacterial assemblages (see Chong et al., 2012b). This discrepancy is perhaps primarily underlain by the scarcity of bacterial studies in Antarctica. For instance, of the 14,078 species recorded in Scientific Committee for Antarctic Research (SCAR) Antarctic Biodiversity Database (ABD) (<http://data.aad.gov.au/aadc/biodiversity/taxon_drilldown.cfm>) (accessed 28 May 2013), bacterial species account for only approximately 5% of the total records. Additionally, compilation and comparison of the available bacterial spatial data is difficult due to inconsistencies in the methodologies used to describe this diversity (e.g. culture-dependent vs. culture-independent approaches; diversity profiling vs gene sequencing).

Despite the well accepted limitations of the culture dependent strategy and the recent development of high-throughput sequencing techniques, cultures remain as an important resource in assessing the biodiversity, functional role and interactions of the bacterial community in Antarctic natural ecosystems. Owing to the arid and cold setting typical of the Antarctic terrestrial environment, DNA degrades at a much slower rate than is typical of tropical or temperate regions. This longevity increases the chance of the “legacy” DNA (from dead or non-viable cells) from being co-extracted and amplified, masking the signal of the living or active populations (Cary et al., 2010; Stomeo et al., 2012). Although it is possible to address this problem by targeting less stable RNA (Kowalchuk et al., 2006), there is currently no information available on how much faster RNA degrades in comparison to DNA under Antarctic field conditions, while the utility of these approaches is constrained in comparison with soil DNA analyses by the rapidity and practicality of protocols required at the point of collection.

Although not as comprehensive as the data available for Antarctic macroogranisms, the number of culture-dependent Antarctic bacterial studies has increased considerably over the last two decades (Fig. 1). Encouraged by the success of previous workers in deriving spatial patterns based on Antarctic eukaryotic data, we hypothesize that the available Antarctic bacterial 16S rRNA gene sequences are also capable of revealing significant patterns in the biogeographic distribution of bacteria in Antarctica, as well as identifying similarities in distribution patterns between bacteria, plants and animals, as has been proposed elsewhere (Green & Bohannan, 2006).

In this study, we collated Antarctic bacterial culture identities based on the 16S rRNA gene information available in the literature and the GenBank database. Using this, we compare the sequence variation based on the “Antarctic provinces” used in previous studies of Antarctic macroorganism biogeography (Peat et al., 2007; Pugh & Convey, 2008). In addition, we also compare the sequence novelty of the 16S rRNA gene sequences of Antarctic bacterial isolates with isolates obtained from elsewhere. Finally, based on the available data, we identify Antarctic provinces that remain understudied, and that now require focused study in order to address major ecological questions.

**MATERIALS AND METHODS**

**Antarctic culturable bacteria 16S rRNA gene database**

An initial search for Antarctic culturable bacterial 16S rRNA gene sequences was carried out using a GenBank nucleotide search. A total of 1766 entries were detected using the boolean search string “(((((Antarctic) AND Bacteria) NOT sea) NOT marine) AND 16S) NOT uncultured” (accessed on 15 Feb 2013). In order to account for Antarctic bacterial 16S rRNA gene sequences that were not selected using the search string, we also carried out an exhaustive search of the Antarctic literature. The two lists were combined, and the geographic origin of the isolates was recorded based on the latitude and longitude data reported either in the GenBank record or the original literature. For records that did not provide precise GPS data, we approximated the sample origin based on the formal GPS location of the reported sampling site, accepting that this gave an approximately 0.5 degree latitude/longitude margin of error. If both GPS data and sampling location name were absent, the sequences were not included in our database. Our final database consisted of 2089 distinct entries (Table S1) obtained from various Antarctic terrestrial habitats including soil, rock surface, snow, lake, glacier surface and cryoconite. The sequences were classified into the closest genus at 80% cutoff confidence using classifier tools from the ribosomal database project website (http://rdp.cme.msu.edu/) (Cole et al., 2009), and were grouped according to the genus and sampling sites into an EXCEL spreadsheet (Tables S1 and S2).

**16S rRNA gene sequence homology**

To assess the novelty of the Antarctic isolates’ 16S rRNA gene signatures, all 2089 entries in our database were subjected to separate nucleotide-BLAST search against the GenBank nucleotide collection (nr/nt) excluding uncultured or environmental sample sequences. We segregated the sequences into two groups: sequences which did not show any significant similarity, along with those that achieved ≥99.0% match with deposited sequences obtained from the Antarctic alone, were regarded as ‘novel’, while those that showed at least one ≥99.0% match with sequences from other regions were regarded as ‘cosmopolitan’. The 99% homology threshold was chosen based on its utility in species delineation (Peeters et al., 2011a). The sequences were assigned into different genera using an 80% bootstrap confidence level according to the RDP Naïve Bayesian classifier (Wang et al., 2007).

**Spatial pattern determination**

We selected 6 core genera, known to be common in Antarctic terrestrial habitats (*Arthrobacter, Brevundimonas,* *Flavobacterium*, *Pseudomona*s, *Psychrobacter* and *Sphingomona*s) (Yergeau et al., 2007b; Cary et al., 2010; Pearce et al., 2012) and which showed the highest geographic spread in our database, for spatial analyses.

Sequences from each genus were aligned separately using the ‘align.seqs’ command available in the Mothur program (Schloss et al., 2009). The alignment was carried out by comparing the sequences to the SILVA reference alignment provided by the Mothur website (http://www.mothur.org/wiki/Silva\_reference\_files). In brief, the latter included 50,000 columns and consisted of 14,956 sequences.

To compensate for differences in sequence length and lack of overlap between the sequenced 16S rRNA gene region across the studies, the phylogenetic tree was constructed with parsimony insertion tools using the ARB software (Ludwig et al., 2004). This option “fits” the various sequences into a guide tree by comparing the overlapping region between the target sequences and the sequences in the guide tree. For this study, the guide tree was built using the SILVA non-redundant (NR) SSU reference dataset (Pruesse et al., 2007) that contained 286,858 sequences (SSU Ref NR111). The phylogenetic tree was visually inspected and exported into newick format for subsequent analyses.

The newick format tree was uploaded into FastUniFrac (Hamady et al., 2010) (http://bmf2.colorado.edu/fastunifrac/) to calculate the pairwise UniFrac distances between taxa from each original sampling site. This approach compares the fraction of branch length between sites that leads to a set of taxa that occur uniquely in one site but not another (Lozupone & Knight, 2005). In short, if two sites contain mutually similar taxa, the UniFrac distance between them will be minimum while if the two sites encompass exclusively different taxa, the UniFrac distance will be maximum.

The UniFrac method also overcomes the problem that varying definitions of operational taxonomic units (OTUs) were adopted in each individual study. For instance, RFLP was used by Saul et al. (2005), 97% nucleotide cutoff by Aislabie et al. (2006a), and a 99% cutoff by Peeters et al. (2011a). In theory, the inclusion of identical or closely similar sequences will contribute very little to the branch length in the phylogenetic tree (Lozupone & Knight, 2005). For subsequent analyses, the UniFrac distance matrix was exported into the PERMANOVA+ add-on of the PRIMER6 multivariate data analysis package (Plymouth Marine Laboratory, UK) and the R program ([www.r-project.org](http://www.r-project.org)).

The presence of spatial patterns was analysed using two complementary methods. First, PERMANOVA was carried out to infer the occurrence of pattern at large spatial scale. In brief, each location was grouped into one of the 13 conventional Antarctic geographic sectors as used by Pugh and Convey (2008) (Fig. 2). Significance was calculated by a 999 permutation test, and corrected using the Monte Carlo correction. We also visualized the relationship between the biogeographical provinces using principle component analysis (PCO) of the distance matrix obtained from the pairwise PERMANOVA analysis. Second, the presence of spatial pattern at moderate scale was tested using a mantel correlogram. This was carried out by using the ‘mantel.correlog()’ option available in VEGAN package, R program. We specifically chose to evaluate distance classes at a 50-100 km interval as this approximates to a 1 degree latitude or longitude difference at 50-70°S. A smaller distance interval could not be selected due to inadequate sample sizes being available to enable statistically significant inference. The two analyses (PERMANOVA and mantel correlogram) were repeated for all six selected genera.

**RESULTS**

**Overall spatial coverage and sequence homology**

At least one record of culturable bacterial 16S rRNA gene sequence was retrieved from each of the 13 predefined geographical sectors (Table 1). However, the sampling intensity across the different sectors was very inconsistent. This was especially the case for the Palmer and South Sandwich Islands sectors, each of which contained only a single lat/long cluster, including a total of only 8 sequences. Relatively higher sampling intensity was available for the Scott, Graham, Enderby and South Shetland Islands sectors (Fig. 2), which together accounted for more than 60% of the total sequences in our database. However, even for the best-studied area (Scott Sector), the lat/long boxes with at least one record covered less than 30% of the ice-free area (estimated based on Peat et al., 2007, Table 1).

Approximately 26% of the sequences included in the database were “novel” to Antarctica based on our definition of novelty (see Materials and Methods). The highest percentage of sequence novelty was observed in the sub-Antarctic (33%), followed by the maritime Antarctic (28%) and continental Antarctica (27%) (Table 1).

**Genera-specific spatial pattern and sequence novelty**

A total of 157 different genera were resolved from our database, and 12% of the genera contributed more than 1% of the sequences in the database (Table S2). Among them, the most widespread genera were identified as *Arthrobacter*, *Brevundimonas*, *Flavobacterium*, *Psychrobacter*, *Pseudomonas* and *Sphingomonas*, together contributing c. 37% of the total records in database.

*Flavobacterium*

In Antarctica, *Flavobacterium* is generally isolated from aquatic sources such as benthic microbial mats and freshwater lakes (Pearce et al., 2003; Van Trappen et al., 2004), and habitats influenced by marine vertebrates such as penguin rookeries (Yi et al., 2005). They are capable of degrading high molecular mass organic matter in cold environments and are prevalent in penguin guano mineralization processes (Zdanowski et al., 2005; Van Trappen, 2009). Further, several strains of Antarctic *Flavobacterium* are able to express antibacterial activities and may play a role in maintaining community stability (Rojas et al., 2009).

There were 92 16S rRNA gene sequences from Antarctic terrestrial *Flavobacterium* isolatesin our database. A large fraction of these sequences (53.3%) was novel and distantly related (< 99% similar) to other non-Antarctic *Flavobacterium* sequences. Significant effect was detected (PERMANOVA pseudo-F5,26 = 1.740, PMC = 0.0280) when comparing the 16S rRNA gene heterogeneity between the *Flavobacterium* isolated from different Antarctic regions. A closer inspection of the data under the PCO of the PERMANOVA distance (Fig. 3A) suggests that the separation was mainly due to the uniqueness of *Flavobacterium* representatives obtained from Graham Sector. In addition, we were also able to detect a significant spatial autocorrelation under the spatial class of 50 km (*P* = 0.040).

*Arthrobacter*

*Arthrobacter* is a common soil bacterial genus globally which accounts for approximately 0.3-0.5% of the clones retrieved in soil-based clone library studies and 5-40% of the total clones isolated on culture media (Janssen, 2006). This abundance is perhaps attributable to the genus’ typically high resistance to desiccation stress (Potts, 1994). In Antarctica, other than soil, representatives of *Arthrobacter* have also been isolated from habitats such as pond, lake, cryoconite and rocks (Reddy et al., 2002; de la Torre et al., 2003; Peeters et al., 2011b). In general, the psychrophilic *Arthrobacter* differ from their mesophilic counterparts by containing glucose as the cell wall sugar and in their inability to hydrolyse starch (Reddy et al., 2002). Psychropilic *Arthrobacter* are producers of cold-active enzymes such as β-galactocidase and dehydrogenase (de Pascale et al., 2012).

Among the 192 retrieved *Arthrobacter* 16S rRNA gene sequences, around 22% were not affiliated to *Arthrobacter* from other geographical regions, suggesting a pool of novel *Arthrobacter* in Antarctica. Further, a marginally significant regional effect (PERMANOVA pseudo-F7,53 = 1.335, PMC = 0.065) was detected between *Arthrobacter* isolated from SOI, SSI, Graham and Palmer Sectors, and those retrieved from Ronne, Maud, Enderby, Wilkes, Scott and Byrd Sectors (Fig 3B). Further, marginally significant (*P* = 0.072) and significant (*P* = 0.036) spatial autocorrelations were detected using spatial distance classes of 50 and 100 km.

*Pseudomonas*

*Pseudomonas* showed the highest abundance (268 sequences) and widest geographical spread across Antarctica (26 lat/long boxes), and was obtained from various habitats including soil, lake, glacier, ice and sandstone rock. The high prevalence of *Pseudomonas* in Antarctica is generally associated with its ability to counter cold-shock by accumulation of intracellular polymers such as polyhydroxyalkanoates (PHA) and polyhydroxbutyrate (PHB) (Ayub et al., 2009; Goh & Tan, 2012). Additionally, *Pseudomonas* has been reported to be particularly abundant in hydrocarbon-contaminated soil (Saul et al., 2005), and some representatives are known to degrade polycyclic aromatic hydrocarbons at low temperature (Aislabie et al., 2000; Ma et al., 2006).

Overall, Antarctic *Pseudomonas* 16S rRNA genes showed very high sequence similarity to those of isolates obtained elsewhere. Almost all sequences (~96%) isolated from Antarctica had at least one close representative (≥99% homology) from other geographical regions. Homogeneous distribution was also apparent when comparing *Pseudomonas* sequences from the different Antarctic regions (PERMANOVA pseudo-F7,53 = 1.172, PMC = 0.2583). Further, no spatial autocorrelation was detected at distance classes of 50 and 100 km.

*Brevundimonas*

*Brevundimonas* are aerobic gram-negative bacilli which share close phylogenetic relationship with the genus *Pseudomonas*. As an oligotroph, the genus is commonly found in freshwater and marine habitats with low levels of nutrients (Tayeb et al., 2008). The *Brevundimonas* sequences in our database were dominated (~60%) by isolates sourced from freshwater, snow and lake sediments. These sequences covered 18 lat/long boxes spread over eight sectors (SSI, SOI, Graham, Ronne, Bryd, Maud, Scott and Enderby).

Among the 62 reported isolates, a large majority are cosmopolitan species with only 2 showing potential endemism. We did not detect significant regionalization using the UniFrac Distance-based PERMANOVA (PERMANOVA pseudo-F7,25 = 0.6813, PMC = 0.8942), but a significant autocorrelation (*P* = 0.036) was observed at the distance class of 100 km.

*Sphingomonas*

Members of *Sphingomonas* have been isolated from various Antarctic habitats including snow, soils, rock surfaces, glaciers and lakes (Brambilla et al., 2001; Busse et al., 2003; Hughes & Lawley, 2003; Aislabie et al., 2008). Representatives are able to degrade a wide range of aromatic hydrocarbons at low temperature (Baraniecki et al., 2002), and are capable of maintaining membrane fluidity through the production of carotenoids (Jagannadham et al., 2000).

We found 53 records of Antarctic *Sphingomonas* isolates which originated from 20 separate lat/long boxes. Among them, seven showed a high level of novelty and were not closely related to *Sphingomonas* sequences from other geographical regions. However, no spatially explicit pattern was detected using the region-based PERMANOVA (PERMANOVA pseudo-F8,21 = 0.7850, PMC = 0.7721) and mantel correlograms.

*Psychrobacter*

*Psychrobacter* are gram-negative, oxidase-positive, non-motile, coccoid bacteria that are generally psychrotolerant. They have been reported to be prevalent in diverse cold environments, including deep sea trenches (Maruyama et al., 2000), Siberian permafrost (Bakermans et al., 2006), Himalayan mountain ranges (Shivaji et al., 2011), and Antarctic coastal and ornithogenic habitats (Bowman et al., 1996, 1997; Bozal et al., 2003). Although *Psychrobacter* are also observed in temperate and tropical soils, they occur at significantly lower abundance and species diversity in comparison with the Arctic and Antarctica (Rodrigues et al., 2009).

Records of Antarctic terrestrial *Psychrobacter* strains currently available in GenBank were restricted to only five sectors (SSI, Scott, Graham, Enderby and Ronne), despite the fact that the genus has frequently been detected in clone libraries obtained from Antarctic soil and aquatic samples (Mikucki & Priscu, 2007; Shravage et al., 2007; Aislabie et al., 2008). Among the 98 sequences in our database, 9 (9.2%) showed low relationship to lineages detected in non-Antarctic regions. Further, neither regionalisation nor spatial correlation were detected using PERMANOVA (pseudo-F4,22 = 1.1163, PMC = 0.3254) and mantel correlogram.

**DISCUSSION**

Despite the presence of clear regionalisation in the Antarctic terrestrial micro- and macro-invertebrate fauna (Chown & Convey, 2007; Convey et al., 2008; Pugh & Convey, 2008), and the frequent reports of localized bacterial diversity in Antarctica (e.g. Pointing et al., 2009; Chong et al., 2012a; Lee et al., 2012; Stomeo et al., 2012), we were only able to detect a significant regional effect in *Flavobacterium* amongst the top six most studied genera in Antarctica*.* This may be partly due to the flat distribution of the relative diversity of the Antarctic genera, with even the most ‘common’ group accounting for only a small percentage of the overall diversity. Additionally, the general lack of pattern is also influenced by the low sampling intensity within each Antarctic province, even accepting that some are particularly poorly sampled (Fig. 2).

**Potential for bacterial regionalisation in Antarctica**

Notwithstanding the overall lack of sampling coverage, our data analyses indicate that the distributions of *Flavobacterium* and, potentially, *Arthrobacter* are analogous with those of various multicellular organisms across the recognisedAntarctic geographical provinces identified by Pugh and Convey (2008). The regional effect was more pronounced when comparing the isolates retrieved from Scotia arc and Antarctic Peninsula (Graham and Palmer) against representatives from the remaining sectors of continental Antarctica (Fig 3A and 3B). Such separation is also consistent with the Gressitt Line biogeographical boundary, which is proposed to be a reflection of Antarctic historical contingency (Convey & Stevens, 2007).

We speculate that the similarities in distribution patterns may also reflect trophic interactions between different elements of the Antarctic biota (Hogg et al., 2006). For instance, soil bacteria are involved in the mineralization of marine vertebrate excreta, and play an important role in releasing vital nutrients in the rhizosphere supporting microbial and plant growth (Zdanowski et al., 2005; Berg & Smalla, 2009). Previous studies have suggested a strong correlation between the presence of vegetation and animals and the underlying soil bacterial community composition (Yergeau et al., 2007a; Chong et al., 2010). As the current study was not designed to test for the presence of trophic interactions, this is an area requiring further work.

Our finding of evidence for at least some regionalization and spatial autocorrelation suggests that biogegraphic patterns of bacterial distribution exist in the Antarctic microbial community (Vyverman et al., 2010; Bahl et al., 2011; Chong et al., 2012b). Although it remains unclear whether the pattern detected is driven primarily by spatial separation or environmental heterogeneity, we suggest that a pure environmental effect (“the environment selects”, as advocated by the global ubiquity hypothesis; Finlay, 2002) is unlikely to cause the patterns seen. This is supported by the absence of clustering in relation to the isolation sources in the PCO ordination based on UniFrac distance (Fig S1). Further, if the distribution of Antarctic bacteria was driven solely by environmental parameters, then no spatial pattern would be discernible from the bacterial culture approach. This is because most of the culture media used in the original studies contained very similar nutrient composition (i.e. yeast extract and peptone), and would therefore be likely to select for similar species, hence yielding very low UniFrac distances.

**Endemism and the existence of a distinct Antarctic microbiome**

Natural dissemination rates of bacterial and other microscopic propagules from neighbouring ecosystems (i.e. South America, Africa, Australia, sub-Antarctic islands) into Antarctica are expected to be very low compared to those typical of other regions of the globe (Marshall, 1996; Marshall & Convey, 1997; Cowan et al., 2011a). In order to transfer and establish successfully, bacterial propagules, as with colonising propagules generally, are dependent on the availability of suitable means of translocation (wind, ocean current or biological vector), their ability to withstand harsh conditions during long distance travel, their capacity to survive Antarctic conditions on arrival at a suitable location, and the availability of suitable resources for reproduction and population maintenance (Pearce et al., 2009; Hughes & Convey, 2010). Due to the lack of effective transfer mechanisms and long history of geographical isolation (Convey et al., 2008), both of which facilitate evolutionary divergence processes and speciation, and in contrast with the global ubiquity hypothesis, it is therefore reasonable to hypothesise that Antarctica might harbour a novel microbiome distinct from other major continental regions (Staley & Gosink, 1999; Vincent, 2000; Tindall, 2004; Cowan et al., 2011a).

Indeed, the presence of distinct cyanobacterial and algal diversity in Antarctica has been reported previously (Bahl et al., 2011; De Wever et al., 2009; Namsaraev et al., 2010). For instance, Taton et al. (2006) suggested that approximately 70% of the Antarctic Cyanobacteria are endemic to the continent while De Wever et al. (2009) estimated that the majority (~60%) of Antarctic green algae lineages have ages of 17 to 84 Ma, consistent with the timing of ancient tectonic processes such as the opening of the Drake Passage (30-45 Ma).

Both direct characterization of 16S rRNA gene sequences from environmental DNA samples (e.g. Aislabie et al., 2008; Niederberger et al., 2008; Pearce et al., 2010) and bacterial isolates (this study) have generally shown that a majority (>50-75%) of the retrieved sequences were highly similar (~98-99%) to those from tropical and temperate regions, suggesting a widespread distribution of eurythermal mesophiles (sensu Barton 2005) in Antarctica (Cowan et al., 2011a). Also, the optimum growth temperature of many Antarctic bacterial isolates has been found to be higher (>15 ˚C) than that of true psychrophiles (4-8˚C) (Aislabie et al., 2008; Peeters et al., 2011a), despite the fact that most isolation was carried out under low temperature conditions (≤10 ˚C) in the original studies (Table S3). Taken together, these observations suggest that the cross-continental transfer of bacterial propagules is also an important contributor to bacterial diversity in Antarctica.

It is also important to note that the techniques currently applied in the estimation of biodiversity in Antarctica might themselves inherently bias towards the selection of cosmopolitan species. For instance, the universal PCR primers generally used in molecular profiling and sequencing may have lower affinity towards novel bacterial species, as they are usually designed based on the described sequences available in the public domain (e.g. Winsley et al., 2012). In addition, novel Antarctic bacteria may have different specific nutrient requirements to those available in commonly-used culture media (e.g. R2a, TSA and NA), and require a specific isolation strategy instead of the common methodology (Stingl et al., 2008; Niederberger et al., 2010). It remains a challenge to capture the complete diversity of an ecosystem, and it is estimated that Antarctic isolation studies generally reveal only 5% of the total diversity (number of unique isolated phylotypes as a proportion of estimated phylotype diversity) (Aislabie et al., 2006b) while the majority of molecular assessments of Antarctic soil bacteria only achieve a coverage of 40-70% (Chong et al., 2012b).

**Future directions**

It has been suggested that spatially distinct and strongly regionalised biodiversity in Antarctica may be vulnerable to contamination through intra- and inter regional anthropogenic transfer of propagules (Tin et al., 2009; Cowan et al., 2011a; Hughes et al., 2011, 2013). The increasing indication of regionalization and evolutionary divergence in bacterial diversity in Antarctica, through studies such as described here and those of de Wever et al. (2009) and Vyverman et al. (2010), supports the imperative for inclusion of microbiota in the framework for biosecurity measures applying to Antarctica (see Convey et al. (2012b) and Hughes et al. (2013) for discussion of Antarctic conservation, environmental protection and governance). Currently, with the exception of risks associated with microbial pathogens of vertebrates, codes of conduct relating to invasive species control cover only the macroscopic flora and fauna (Hughes & Convey, 2010; 2012). It is likely that many more non-indigenous bacterial species may be capable of adapting to Antarctic conditions than previously thought, as it is now appreciated that at least some Antarctic communities are dominated by cosmopolitan mesophilic species.

Even now, our ability to answer the key ecological questions raised for the Antarctic bacterial community is hindered by several important limitations (Table 2). A major constraint remains the lack of spatially explicit data at sufficient sampling intensity across the continent. For instance, a large fraction of bacterial community studies carried out in Antarctica remain at the opportunistic exploratory stage, and are highly dependent on the logistic support of the nearest scientific station. As a result, most sampling has been carried out in the vicinity of the most active Antarctic stations (Hughes et al., 2011), inevitably leading to low sampling in sectors such as Ronne and Byrd where no research stations are established. There is also a lack of sampling in the high altitude areas around the Transantarctic Mountains, and in the continental interior beyond 80˚S. These limitations apply not only for microbial studies, and the biodiversity of these areas is poorly researched in general (Peat et al., 2007; Pugh & Convey, 2008; Vyverman et al., 2010; Hughes & Convey, 2012).

There is clearly a need for systematic and targeted large-scale bacterial sampling across Antarctica, similar to that carried out on British soil (Griffiths et al., 2011), the hypolithic microbial community of the McMurdo Dry Valleys (Cowan et al., 2011b) and Darwin Mountains, Transantarctic Mountain (Magalhães et al., 2012), notwithstanding the logistic and financial challenge this presents. One practicable approach may be to acquire and compare the biodiversity of several signature ecotypes within the continent, with selection guided by the ‘Antarctic Conservation Regions’ recently defined by Terauds et al. (2012).The future availability of such data is crucial to facilitate understanding of the bacterial biodiversity and biogeography in Antarctica, and in refining current biosecurity and conversation management strategies.

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