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Antarctic eukaryotic soil diversity of the Prince Charles Mountains revealed by high-throughput sequencing

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ABSTRACT

Studies of Antarctic eukaryotes have been hampered by their morphological conservatism, small size and the logistical constraints of remote field work, resulting in a deficiency of baseline biodiversity information about Antarctic terrestrial environments. The application of high throughput sequencing (HTS) in metataxonomic approaches is a promising alternative. Here, we apply such HTS approaches to the hitherto largely unsurveyed micro-eukaryote fauna of the Prince Charles Mountains, East Antarctica. We sequenced 18S rDNA amplicons of twelve Antarctic bulk-soil DNA extracts, retrieved from three sampling regions (four bulk-soil extracts per sampling region). After isolating eukaryotic phylotypes with a stringent filtering approach and initial network visualization, we firstly used rarefied data to compare four α diversity metrics between the three regions. Weighted and unweighted inter-sample UniFrac distances were then used for β diversity comparisons among rarefied data. Furthermore, we analysed the distribution of the most abundant phylotypes and phylotype groups. Lastly, we checked the validity of species-level taxonomic assignments using different sets of reference data in conjunction with two different taxonomy assignment approaches. Phylotype numbers in un-rarefied data compared across regions were lowest for Mount Menzies (73°S; 3330 m), intermediate at Mawson Escarpment (73°S; 807 m) and highest at Lake Terrasovoje (70°S; 173 m), likely due to low biological load at the higher latitude and elevation inland sites. Analysis of rarefied data was difficult due to low sequence coverage particularly from Mount Menzies, but indicated differences in Shannon diversity between Mawson Escarpment and Lake Terrasovoje. PCoA of weighted UniFrac distances between samples from Mawson Escarpment and Lake Terrasovoje indicated changes in community composition in relation to elevation of the sampling locations. The most widespread phylotypes were fungal, followed by non-algal protists. Species-level assignments included known Antarctic taxa in all sampling regions. We show that HTS can provide a rapid survey of the micro-eukaryote fauna to provide baseline biodiversity information for remote, harsh, and hitherto largely unsurveyed environments in the Prince Charles Mountains.

1. Introduction

The biodiversity of remote Antarctic habitats is a key issue in understanding the history of the Antarctic continent, the biological effects of climate change, as well as for conservation efforts, but many regions of Antarctica remain unsurveyed (Kennicutt et al., 2015; McGeoch et al., 2015). Antarctic soils are home to organisms including bacteria, unicellular eukaryotes, fungi, lichen, cryptogamic plants and invertebrates (Convey et al., 2014). These soil communities are distinct from other soil biota as a consequence of long-term persistence under harsh environmental conditions; furthermore their long history of isolation is responsible for a high degree of endemism (Convey et al., 2008). Simplicity and endemism make Antarctic soil communities interesting for a variety of ecological questions. Changes in biodiversity patterns in simple

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communities such as increasing population densities of mites (Kennedy, 1994) and nematodes (Convey, 2003) can be important indicators of human impact and environmental change in terrestrial Antarctica (Nielsen and Wall, 2013). Identifying such indicators in terrestrial Antarctica could also help to understand human-mediated biodiversity changes in more complex temperate and tropical ecosystems and their effect on ecosystem processes, such as decomposition and energy flow (Wall and Virginia, 1999). Studies on the endemicity of Antarctic biota revealed that many terrestrial habitats might have become available for re-colonisation since the beginning of the current inter-glacial period (17,000 years ago) (Stevens and Hogg, 2003; Magalhães et al., 2012). However, there is also evidence that some regions remained ice-free and inhabited for much longer (Convey and Stevens, 2007). Today, human influence increasingly threatens the unique Antarctic soil communities through human-mediated climate change, increasing risk of pollution, and the introduction of non-indigenous organisms which may outcompete endemics in an increasingly accommodating environment. Successful conservation of Antarctic environments in the face of these threats requires biodiversity information (Chown et al., 2012; Terauds et al., 2012; Turner et al., 2013), Unfortunately, this information is missing for many remote ice-free areas of continental Antarctica, such as Dronning Maud Land, large regions of the Transantarctic Mountains (McGaughran et al., 2011), and the Prince Charles Mountains (Terauds et al., 2012).

Biodiversity research in Antarctica is complicated for two main reasons: Firstly, logistic difficulties exacerbated by the harsh environmental conditions typically limits biological research in Antarctica to the proximity of stations when extensive field work is required (Convey, 2010). Secondly, traditional soil biodiversity assessments including manual sorting and morphological identification of organisms are time consuming and require taxonomic expertise, especially for the cryptic soil fauna of Antarctica (Velasco-Castrillón and Stevens, 2014). Molecular methods are better suited for the study of Antarctic biota (Rogers, 2007), but may lack resolution when sequence information is not considered (e.g. in analysis of Terminal Restriction Fragment Length Polymorphisms – TRFLPs; Makhalanyane et al., 2013, Dreesens et al., 2014) or may be work intensive (e.g. Sanger-sequencing) (Lawley et al., 2004; Fell et al., 2006; Velasco-Castrillón and Stevens, 2014).

High-Throughput Sequencing (HTS) of environmental samples poses an interesting opportunity to generate biodiversity information from remote Antarctic habitats (Chown et al., 2015b), as it is faster than clonal Sanger sequencing, and field work is simple in comparison to traditional morphological surveys (Bohmann et al., 2014). Hence, such HTS based metataxonomic studies (*sensu* Marchesi and Ravel, 2015) have been used to monitor invasive species, survey biodiversity over large spatial scales, and provide valuable snapshots of biodiversity for future conservation efforts (Gutt et al., 2012; Bohmann et al., 2014; Drummond et al., 2015; Chown et al., 2015b). Metataxonomics in Antarctica have been used to examine viruses (López-Bueno et al., 2009), bacteria in hypolithic communities (Makhalanyane et al., 2013), soil (Teixeira et al., 2010), air (Bottos et al., 2014), as well as fungi and other eukaryotes (Pointing et al., 2009; Rao et al., 2012; Dreesens et al., 2014; Niederberger et al., 2015).

Here we apply a metataxonomic HTS approach to explore the micro-eukaryotic soil biodiversity in three ice-free regions of the Prince Charles Mountains (PCMs) in Eastern Antarctica (Fig. 1). With few exceptions (Cremer et al., 2004; Wagner et al., 2004; Skotnicki et al., 2012), the PCMs remain biologically unsurveyed, hindering conservation planning (Terauds et al., 2012). Amplicons of nuclear 18S ribosomal DNA (18S) were generated from bulk soil extracts and sequenced using the Illumina MiSeq platform. Using these data we aimed to: (i) determine any differences in eukaryotic diversity among the sampling regions (ii) determine whether highly abundant phylotypes in individual samples are widespread or restricted to particular regions, and (iii) examine the validity of species-level taxonomic assignments of Antarctic phylotypes using two different sources of reference data (well-curated, but potentially less comprehensive, and potentially less well curated, but more comprehensive) in conjunction with appropriate taxonomy assignment approaches.

2. Methods and materials

2.1. Fieldwork, soil storage and DNA extraction

Fieldwork was conducted during the austral summer of November 2011/January 2012 at Mount Menzies, Mawson Escarpment and Lake Terrasovoje (Fig. 1). Satellite imagery was used to determine several soil-sampling locations within each of the three regions based on broader glaciological and geological properties (bedrock, moraine lines and altitude). Within each region, four sites were then opportunistically chosen for sampling, twelve samples in total. At each sampling site a maximum of 500 g of soil was collected from the top 10 cm of the substratum by combining five subsamples from the corners and centre of a 1 m square quadrat into a sterile WhirlPak bag (Nasco, Fort Atkinson, US-WI; protocol after Magalhães et al., 2012). Sample contamination was minimised by wearing nitrile gloves and cleaning equipment with 70% ethanol. In the field, samples were stored at −30 to +4 °C in insulated containers (Coleman, Wichita, US-KS). Samples were transported and stored at −20 °C.

DNA extraction was performed at the South Australian Research and Development Institute (SARDI) using a method optimised for the retrieval of DNA from different soil types and the retrieval of invertebrates in agricultural ecosystems for plant pathogen detection (Pankhurst et al., 1996; Ophel-Keller et al., 2008; Haling et al., 2011; Huang et al., 2013), and processes 400 g of starting material. Cross contamination during extraction was detected by measuring the concentration of blank extractions. DNA was stored at −20 °C (SARDI) and at −60 °C (University of Adelaide).

2.2. Amplification and library generation

PCR and sequencing primer sequences were sourced from the 18S rRNA amplification protocol 4.13 of the Earth Microbiome Project, as well as groups specializing in developing HTS methods (Gilbert et al., 2010; Parfrey et al., 2014). Fusion primers were designed for use with the Illumina platform (project specific design detailed in Supplemental information). Twofold PCR replication was chosen to evaluate the feasibility of amplifying large numbers of samples in subsequent projects. Amplifications were carried out in a volume of 20 μl, with 2 μl of template, 1.5 mM $MgCl_2$, 1× AmpliTaq Gold buffer (Thermo Fisher Scientific, Waltham, US-MA), 0.25 mM of each dNTP, 0.5 μM of forward and reverse primer and 1.25 units AmpliTaq Gold (Thermo Fisher Scientific, Waltham, US-MA). After initial denaturation at 94 °C for 3 min, PCR was performed with 35 cycles of 94 °C for 45 s, 57 °C for 1 min, and 72 °C for 1:30 min, with final elongation of 10 min at 72 °C. To monitor and remove contamination, no-template controls were included in the amplification, sequencing and analysis procedure. Amplicons were visualised on 2% agarose gels, then duplicates were pooled and purified using Agencourt AMPure XP beads (Beckman Coulter, Brea, US-CA). Amplicon DNA concentrations were quantified using a Qubit 2.0 fluorimeter (dsDNA HS Assay -Thermo Fisher Scientific, Waltham, US-MA) and a 2200 TapeStation with High Sensitivity D1K ScreenTapes (Agilent Technologies, Santa Clara, US-CA). Amplicons were then combined in equimolar ratios (4.5 pmol,