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Review

Pelagic and benthic communities of the Antarctic ecosystem of Potter Cove: Genomics and ecological implications

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ABSTRACT

Molecular technologies are more frequently applied in Antarctic ecosystem research and the growing amount of sequence-based information available in databases adds a new dimension to understanding the response of Antarctic organisms and communities to environmental change. We apply molecular techniques, including fingerprinting, and amplicon and metagenome sequencing, to understand biodiversity and phylogeography to resolve adaptive processes in an Antarctic coastal ecosystem from microbial to macrobenthic organisms and communities. Interpretation of the molecular data is not only achieved by their combination with classical methods (pigment analyses or microscopy), but furthermore by combining molecular with environmental data (e.g., sediment characteristics, biogeochemistry or oceanography) in space and over time. The studies form part of a long-term ecosystem investigation in Potter Cove on King-George Island, Antarctica, in which we follow the effects of rapid retreat of the local glacier on the cove ecosystem. We formulate and encourage new approaches to integrate molecular tools into Antarctic ecosystem research, environmental conservation actions, and polar ocean observatories.

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1. Introduction: Environmental change in the northern maritime peninsula region

One of the most dramatic consequences of anthropogenically induced climate warming is the vast retreat of coastal ice sheets and melting of the glacial ice caps currently observed in the Northern sector of the West Antarctic Peninsula (WAP) (Vaughan, 2006; Osmanoğlu et al., 2013; Osmanoglu et al., 2015). Rapid deglaciation is opening shallow water coastal environments (defined here as down to ~150m) of highly fragmented bathymetry and substrate type (from rocks and bolder fields to sandy and muddy areas) to new colonization (Wölfl et al., 2014; Quartino et al., 2013). This includes growth of new coastal biomass from macroalgal beds with associated epiphytes to pioneering assemblages of migratory and sessile fauna with capacity for larval dispersal.

Physical and environmental change in WAP shallow marine areas is fast. As an example, sea surface temperature (SST) at our study site, the shallow Potter Cove on King George Island (South Shetlands), has increased by approximately 0.36 °C per decade [6, based on 20-year time series of SST data], accelerating microbial and heterotrophic turnover rates. As a consequence, benthic and pelagic species and communities are facing highly dynamic environmental conditions as they move inland and colonize newly uncovered areas. Summer meltwater waves released from climate sensitive coastal ice sheets (Osmanoğlu et al., 2013) cause freshening of the shallow water bodies, which impact pelagic biodiversity. Scouring by drifting icebergs and ice growlers, and calving through the glacial fronting lines can interfere with rapid and sustained benthic colonization in glacial proximity. Melting ice masses and thawing of coastal permafrost areas further mobilize lithogenic particle transport with surface meltwater plumes effecting shading and constraining primary and secondary production in melt water impacted areas (Schloss et al., 2012; Khim et al., 2007; Yoo et al., 2015; Fuentes et al., 2016).

Species colonizing or transiently surviving (pelagic) in present day glacial cove and fjord environments have managed through Pliocene and Pleistocene glaciation cycles by shifting between habitable areas of polynyas or ice fracture zones under former or extant Antarctic ice shelves (Weihe & Abele, 2008; Azam et al., 1991). Hence, they are fit to deal with low light intensities, low temperatures, and extreme scarcity of food. Only few sessile (sponges, ascidians and bryozoans) or slowly moving species (e.g. limpets, echinoderms, polychaetes, isopods, amphipods and pygnogonids), frugal enough to survive such conditions, colonize the areas in front of melting glaciers. As a consequence, macrobenthic species richness in present day melt impacted bays and coves in the West Antarctic is lower compared to ice free shelf areas or coastal sectors less influenced by glacial disturbance (Quartino et al., 2013; Thatje et al., 2008; Siciński et al., 2012; Dayton & Oliver, 1977).

Antarctic intertidal environments and rock pools show considerable daily variations of abiotic parameters such as temperature, salinity, and light climate, especially when low tides are around noon. Aerial exposure at freezing temperature is a natural stressor, that is tolerated by only few Antarctic macrofauna and algae species (Waller et al., 2006; Weihe & Abele, 2008), but it remains an open question whether this enhanced stress tolerance is generic to the majority of species in these coastal community.

We summarize emerging knowledge of shallow water community composition and species specific functional responses to Antarctic coastal change obtained through genomic analyses of the major organismal groups. The work was done in Potter Cove on King-George Island (Isla 25 de Mayo, South Shetland Archipelago), a 10 km^2 shallow (maximum depth 80 m) fjord, strongly impacted by glacial melting. This review is structured according to the major organism groups for which we have obtained genomic data, starting with the prokaryotes (Archaea and Bacteria in water column and the marine sediments). In this section we include results from whole genome sequencing projects of three Antarctic bacterial strains with potential implications for biotechnological application, coastal ecosystem management, and the mitigation of Antarctic coastal contaminations. We then present the results from molecular surveys of the most important groups of coastal microeukaryote plankton, and finally discuss two typical macrofauna keyspecies for which we analyzed response to environmental change at the level of gene expression.

Work was conducted in the frames of a long-term ecosystem research program that was started 25 years ago as initiative between the Instituto Antártico Argentino (IAA) and the Alfred Wegener Institute Helmholtz Center for Polar and Marine Science, Germany (AWI). The project builds on various cooperative programs and EU supported networking projects. Supported by these affirmative actions, we established an Argentine-European research group collaborating at Dallmann Laboratory, an annex to the Argentine Carlini Station on King-George Island. Abiotic change and ecological shifts in this coastal ecosystem are continuously investigated by gathering multi-layered knowledge in different system compartments (glaciology, hydrography, bathymetry, geology, biogeochemistry and biology) in space and over time. A major aim within the biological studies in our current EU networking project IMCONet (EU FP7 IRSES, action no. 318718, www.imconet.eu) was, to understand how glacial melting affects the Antarctic coastal environment and its communities.

Our approach in Potter Cove was therefore dual and included

(A) **The characterization of community composition as a function of environmental variability**. We combine spatial and time series (eco)system observations (e.g. climatology, glacial retreat and fast ice dynamics, hydrography, biogeochemistry, a.o.) with seasonal samplings of pelagic and benthic microbiota communities, using molecular techniques such as fingerprinting, clone libraries and recently also amplicon and metagenome sequencing (work in progress and not included in this review). This produces new knowledge on local biodiversity and connectivity, and also on the functional response of the communities to abiotic and biotic changes.

(B) **Functional genomics/phenomics of polar species**. We combine analysis of genetic connectivity and geographical provenance with comparisons of population specific traits *in situ* and with controlled stress exposure experiments to understand evolutionary limits of species/population specific stress response. In these experiments we use ecologically important key species (e.g. macroalgae and molluscs) of known geographic habitat expansion. Individuals are exposed to defined stress levels and their physiological and genetic stress response investigated, either based on transcriptomic analysis or through a combined analysis of stress genes and physiological stress markers.

In this paper we summarize first results obtained and published with combinations of molecular and genomic techniques and discuss their implications for the investigation of environmental processes typical for the complex Potter Cove ecosystem. More extensive analyses are currently ongoing so that the limited amount of published knowledge will grow significantly in the near future. Hence, it appears timely to propose here and now prioritization of approaches that integrate extended use of molecular genomics in ecological research to fill existing knowledge gaps and to develop the best practice of ecosystem monitoring in the future. We are increasingly able to include targeted use of cost intensive molecular studies in the development of event driven ecosystem observation strategies.

2. Microbes: Archaea, bacteria and viruses in Potter Cove

It was only in 1991 that the importance of organic matter and nutrient cycling through the Antarctic microbial web has received full appreciation when Azam and coworkers published their seminal paper, describing the role of the microbial loop for Antarctic pelagic environments (Azam et al., 1991). In the absence of primary production during the Antarctic winter period, bacterial productivity, supported by the dissolved organic carbon (DOC) pool from the previous summer, becomes the pivotal food source for the heterotrophic grazer communities (Azam

et al., 1991; Hodson et al., 1981). The concept of the Antarctic microbial loop was primarily conceived for open ocean waters characterized by low primary production and high nutrient levels (HNCL regions). It essentially excluded coastal zones in Antarctica where intensive spring and early summer blooms are recorded, except in areas that receive a major input from glacial melt waters and eroded sediments (Dierssen et al., 2002; Schloss et al., 2014). Potter Cove, where chlorophyll-a values over 3 mg m^{-3} are still an exception (Schloss et al., 2014) is a model for these low productivity, melt impacted systems. As climate change and especially the rising mean water temperatures in Potter Cove are bound to affect local microbial growth and community composition, our present analysis addresses current community structure and seasonal dynamics. We employ different culture independent molecular techniques to analyze the dominant microbial groups of the pelagic Potter Cove ecosystem. A seasonal survey was carried out to understand the influence of glacier melting on the microbial community composition, of which we here summarize recently published knowledge, especially on the Achaean communities. To our knowledge this is the first project attempting a year round analysis of microbial communities, including archaeal and bacterial populations in coastal Western Antarctica.

2.1. Prokaryotic community structure and seasonal dynamic in surface waters

For the analysis of the prokaryote communities of Potter Cove (Lat: −62°14′S, Lon: −58°40′W), surface water samples were taken at three different stations in the interior part of the cove behind the major glacial moraine (E1), in the outer cove (E2), as well as in front of one of the major melt water rivers (E3) within a 15 months period from December 2007 to February 2009. Sampling intervals were biweekly in spring and summer and once per month during the rest of the year except for station E3, where meltwater discharge freezes during winter (see Fig. 1).

2.1.1. Archaea

Environmental genomic DNA (gDNA) was extracted from the water samples and five archaeal 16S rRNA gene clone libraries were constructed, using the universal primer set Arc21F and Arc958R [for further details of sampling and DNA isolation see 19]. The libraries revealed high dominance (449 out of 467 sequences) of members of the phylum *Thaumarchaeota* (Hernández et al., 2015) within the archaeal communities of Potter Cove. Ninety-six percent of the archaeal sequences fell into the thaumarchaeotal clade together with the well-known *Nitrosopumilus maritimus* and other ammonium-oxidizing archaea (Fig. 2). It is noteworthy that a first massive sequencing of 16S rRNA genes from surface sediment samples in the inner Potter Cove at station E1, and hence close to the melting glacier, also displayed dominance of *Thaumarchaeota* within the *Archaea* domain (Matos et al., 2016).

Dominance of *Thaumarchaeota* is frequently observed among archaeal populations and has been reported for Southern Ocean and Arctic surface waters (Amano-Sato et al., 2013). The same dominance pattern is seen in Antarctic sediments and soils (Signori et al., 2014; Cameron et al., 2012; Richter et al., 2014), sea ice (Cowie et al., 2011) and even in symbiont populations colonizing Antarctic sponges (Rodríguez-Marconi et al., 2015) and underpins the ecological importance of *Thaumarchaeota* in Antarctic ecosystems. Members of this lineage are chemolithoautotrophic ammonia-oxidizers (Venter et al., 2004; Könneke et al., 2005), suggesting a role of *Thaumarchaeota* in the biogeochemical cycling of carbon and nitrogen in the water column and surface sediments. The phylogenetic tree of the *Thaumarchaeota* obtained in our study comprises several clusters which are composed of sequences so far exclusively found in Potter Cove (Hernández et al., 2015) whereas other sequences were readily available in public

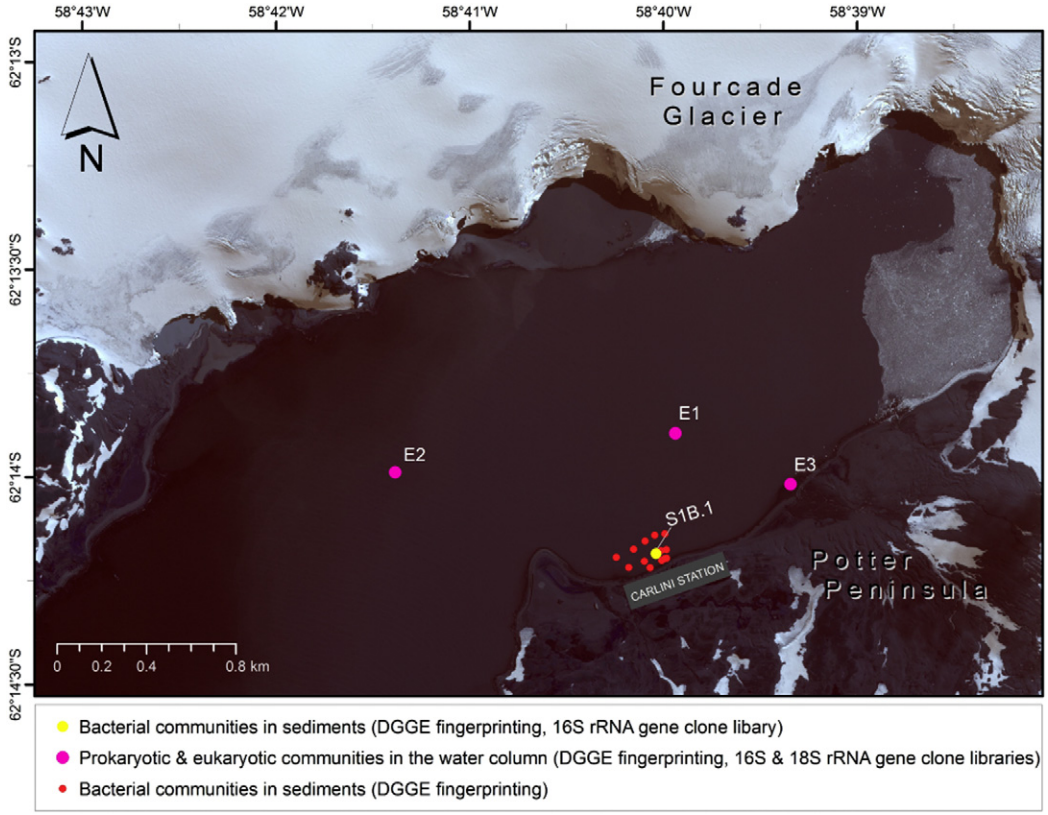

Fig. 1. Satellite image of Potter Cove (DIGITALGLOBE 2014. WorldView-2 scene 103001001F612100, Image Courtesy of/Copyright© DigitalGlobe - Longmont, Colorado. All rights reserved., Catalog ID: 103001001F612100, Acq Date: 2013/03/07, Sensor: WV02, Band Info: Pan_MS1_MS2, Resolution 0.5 × 0.5 m) with different color dots indicating sampling stations for the genetic analyses the environmental microbiomes (see image legend).

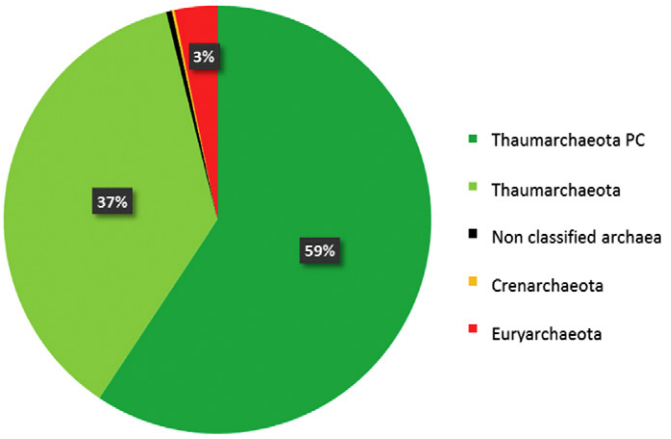

Fig. 2. Percentage of pelagic archaean phyla in Potter Cove (stations E1, E2 and E3) based on 467 ribosomal (16S rRNA) sequences. Thaumarchaeota PC (dark green): lineages exclusively detected in Potter Cove. Thaumarchaeota (light green): lineages that have been reported from other shelf and open ocean areas.

databases. One of the mixed clusters contained the sequence of the well-studied and completely sequenced *Nitrosopumilus maritimus* [NCBI accession number SAMN00000032, see 29]. Interestingly, four of the clusters were exclusively composed of PC thaumarchaeotal sequences. It is noteworthy that one of these PC clusters represented 62% of the overall thaumarchaeotal sequences, with most sequences sharing 99% similarity, indicating dominance of a single phylotype.

The 18 archaeal 16S rRNA gene sequences not affiliated with the thaumarchaeotal clade were distributed across 5 different branches. Fourteen sequences appear to represent a novel lineage within the *Archaea* domain that grouped into a not well supported (Neighbor-joining method, bootstrap value <50) clade related to the order *Thermoplasmatales*. This clade was named "Putative Cold Marine Water Euryarchaeota" (PCMWE) (Hernández et al., 2015). Using the Maximum Likelihood method, the PCMWE cluster was assigned as sister group (bootstrap value of 80) to the order *Thermoplasmatales*. This group encompasses iron oxidizing Archaea, including *Ferroplasma cupricumulans* and *Ferroplasma acidiphilum* (a nonthermophilic acidophile genus), as well as *Picrophilus oshimae*, *Thermoplasma acidophilum* and *Thermogymnomonas acidicola* (Emerson et al., 2010). Members of this *archaean* group are therefore likely involved in the oxidation of ferrous iron Fe(II) originating from subglacial erosion or oozing from the suboxic, ferruginous sediments in Potter Cove (Monien et al., 2014). This is all the more interesting, since the South Shetland archipelago is one of the sites of most intensive geothermal activity south of 60°S (Smith, 2005).

2.1.2. Bacteria

Proteobacteria and *Bacteroidetes* are the two dominant phyla in surface waters of the Southern and Antarctic Ocean (Luria et al., 2014; Straza et al., 2010; Grzymski et al., 2012) and this also applies to Potter Cove coastal waters. The initial study of pelagic bacteria in Potter Cove was based on only one clone library that showed a dominance of *Proteobacteria* with 50% of the clones affiliated to the class *Alphaproteobacteria*, 31% to the *Gammaproteobacteria*, and 3% to the *Betaproteobacteria* (Landone Vescovo et al., 2014). Furthermore the *Bacteroidetes* were represented by 5% of clone sequences. Another 10% of clones remained unclassified. The absence of *Cyanobacteria* in this sample appeared conspicuous. This study of the Potter Cove pelagic communities is currently continued based on five clone libraries constructed from the material sampled at the same spatial stations E1, E2 and E3 and using the same gDNA-extracts as for the analysis of the Archaean groups (Hernandez E. pers. communication).

Half of the *Alphaproteobacteria* in Potter Cove belong to the family *Rhodobacteraceae*, with one cluster (Rhodo-B) that is likely to be specific to Antarctic waters (Landone Vescovo et al., 2014). Rhodobacteria are an interesting group, capable of switching between photosynthetic energy production under anoxic conditions and chemoheterotrophy when oxygen is present. In other coastal areas of sub-Antarctic islands and the WAP *Rhodobacterales* have a marked seasonal variation (Ghiglione & Murray, 2012), with greater dominance in summer compared to the low light winter season, suggesting a special adaptation of this group to the extreme light regime in Antarctic coastal waters. As *Cyanobacteria* are rather uncommon in Antarctic marine waters (Koh et al., 2012) including Potter Cove (Landone Vescovo et al., 2014), although they are often the main primary producers of Antarctic freshwater ecosystems (lagoons, meltwater streams (Kumari et al., 2009)), Rhodobacteria may play an important role as primary producers in these coastal low light areas. Indeed a few *Rhodobacterales* from the marine genera *Roseobacter* and *Erythrobacter* are capable of aerobic anoxygenic photosynthesis at low light levels and at wavelengths in the near infrared spectrum (Sato-Takabe et al., 2012). Future studies in Potter Cove will address the role of *Rhodobacteria* under climate change conditions.

2.2. Bacterial communities from Potter Cove sediments

In the frames of our Potter Cove ecosystem studies, part of the efforts were dedicated to the understanding of the spatial structure of bacterial communities in sediments and the biogeochemical cycles of organic matter degradation. Analysis of total bacterial communities was carried out mainly to study the response of sedimentary microbiota to small quantities of diesel spilled on soils and into the coastal areas of Carlini station (Vazquez et al., 2017, in revision), but some aspects from these analyses are of general ecological interest and hence reported here. Samplings close to the shore in front of Carlini station were carried out in 2010 and 2011 (Fig. 1), and the structure of sediment bacterial communities was assessed using the fingerprinting technique DGGE. Moreover, 16S rRNA gene clone libraries were constructed for surface samples from one site (site S1B.1) using the universal primers 27F and 1492R (GenBank accession numbers KY190789-KY190–953). The fingerprinting showed that the 10 km^2 cove harbors a rich and diverse sedimentary microbial community with differences between the sandy shallow coastal and the central muddy areas (Wölfl et al., 2014). Environmental parameter analysis included TOC and TN for all sites, with molar TOC/TN ratios representative of marine organic matter (Vazquez et al., 2017, in revision). Partial 16S ribosomal gene sequencing revealed a remarkable dominance of a few high rank phylogenetic lineages, represented by several low-rank taxonomic groups that occurred in low abundance, revealing rather diverse and evenly distributed bacterial communities. At site S1B.1, *Proteobacteria* (represented by α-, γ- and to a minor extent δ-*Proteobacteria*) and *Bacteroidetes* (dominated by members of the family *Flavobacteriaceae* and the genus *Haliscomenobacter*) were the dominant phyla, followed by *Planctomycetes* (mainly members of the family *Planctomycetaceae*), and *Verrucomicrobia*. The predominance of *Proteobacteria* and *Bacteroidetes* in sediments resembles the situation observed in the water column (see 2.1.2 Bacteria), although the representative lineages in both environments are different. About half of the sequences obtained in the clone libraries could not be taxonomically assigned at the genus level and were hence identified only at the phylum level. Moreover, of the total OTUs identified in the clone libraries, 67% were represented by only one or two sequences, reinforcing the presence of rare microbiota. The resulting high metabolic diversity of the bacterial communities in Potter Cove sediments speaks for their ability to respond to sudden environmental change.

The observed dominance of chemoheterotrophic aerobic bacteria at site S1B.1 is indicative of sediments rich in organic matter, but with enough oxygen present at the sediment surface to support its aerobic decomposition. In conclusion, the bacterial communities in Potter

Cove sediments are rich and diverse and, based on the observed groups, functionally versatile and capable to respond to environmental change.

2.3. Whole genome sequencing of culturable bacteria from Potter Cove

De novo genome sequencing of environmental microorganisms opens up new frontiers in microbial genomics and provides insight into both, functional capabilities and biodiversity. As part of a deeper understanding of the biodiversity, functional potential and genetic resources of West Antarctic coastal areas, three bacterial strains were isolated from Potter Cove and fully sequenced. Below these strains are described and some characteristics derived from their genomes are highlighted within the ecological context.

2.3.1. Bizionia argentinensis JUB59, a new species from Potter Cove

Members of the phylum *Bacteroidetes* with the dominant class *Flavobacteria* are one of the most abundant bacterial types in Potter Cove. The genus *Bizionia* belongs to the marine clade of the family *Flavobacteriaceae* and currently comprises eleven species isolated from seawater and from marine invertebrate homogenates. A yellow pigmented bacterial strain (JUB59) isolated from Potter Cove surface waters represents a novel member of the genus *Bizionia* and was named *Bizionia argentinensis* (Bercovich et al., 2008).

B. argentinensis JUB59^T cells are Gram-negative, non-motile aerobic rods, 0.2–0.4 mm in diameter and 2–3 mm in length. As *Bizionia* and other members of the *Flavobacteriaceae* family play an important role in the turnover of organic matter in marine environments, whole genome analysis was undertaken for a better understanding of the ecological role of *Flavobacteria* at the Antarctic study site. Whole-genome sequencing of strain JUB59^T (Roche 454 GS FLX system) resulted in a genome size of 3.29 MB. The unclosed draft genome has a coding density of 85.09% and a GC content of 33.77% [for a detailed genome description see 42 and DDBJ/EMBL/ GenBank: accession number AFXZ01000000]. Although a great number of genes can still not be assigned to known functions, genome analysis revealed that JUB59^T belongs to the denitrifying facultative anaerobes, capable of assimilatory-dissimilatory nitrite reduction and the use of NO_2^- as electron acceptor, a capacity that is rarely reported for members of the *Flavobacteriaceae* family (Jones et al., 2008). A set of genes coding for the biosynthesis of unsaturated fatty acids indicates cold adaptation. Furthermore, *B. argentinensis* is able to secrete peptidases to the environment, potentially to use proteins from the DOC pool as carbon source, evidenced by 24 genes belonging to peptidase families in Pfam or Tigrfam and carrying signal peptides. Hence, the ecological role of *B. argentinensis* appears to consist of carbon and nitrogen cycling in the marine environment of Potter Cove, as proposed for other marine *Bacteroidetes*.

2.3.2. Shewanella frigidimarina Ag06–30

The second complete genome was obtained from *Shewanella frigidimarina* Ag06–30, a bacterial strain isolated from intertidal seawater near a southern elephant seal resting area (Di Noto et al., 2016). The full genome sequence of *S. frigidimarina* Ag06–30 is available in the GenBank database under the accession number LRDC00000000. It consists of 4,799,218 base pairs with a GC content of 41.24%. Comparative genome analysis revealed that this strain is closely related to *S. frigidimarina* NCIMB400 (Bozal et al., 2002). Members of the genus *Shewanella* are gram-negative bacteria that thrive in aquatic niches and have highly versatile respiratory chain systems. For some mesophilic (temperate) *Shewanella* species a function as opportunistic pathogens is discussed (Fredrickson et al., 2008; Tsai et al., 2008). *Shewanella* spp. has a very plastic genome with abundant mobile genetic elements (MGE), key to bacterial genome evolution and adaptation. Furthermore, some *Shewanella* species are reservoirs of antimicrobial resistance genes, such as carbapenemases, that support their survival in the native niche while competing with other microorganisms. We therefore looked for similar resistance patterns in the *S. frigidimarina* Ag06–30 genome and found the carbapenemase gene SFB-1 along with other antimicrobial resistance genes, as well as several MGEs, such as group II introns, insertion sequences and an integron. Together, these elements contribute to the continuous adaptation and evolution of the species within its ecological niche.

The role and participation of *Shewanella* spp. in shaping Antarctic microbial ecology has yet to be clarified. However, this bacterium has two major metabolic abilities that can have direct implication for the ecosystem: a complex extracellular electron transfer (EET) network and multiple carbon metabolic pathways. While the EET network is reducing metals such as Fe(III), the capacity to use different carbon sources indicates that *Shewanella* spp. can metabolize complex organic compounds yielding smaller organic products, rendering them available to other bacteria. Our analyses showed that *S. frigidimarina* Ag06–30 has all metabolic pathways previously described, as well as several unique regions (Reid & Gordon, 1999; Darling et al., 2004). We thus propose that strain Ag06–30 is capable of extracellular electron transfer for the respiratory reduction of organic compounds and the reduction of different metals. Based on these features, *Shewanella* spp. is of potential interest for the biotechnological development of microbial fuel cells, but also for bioremediation purposes of contaminated environments.

3. *Rhodococcus* sp. ADH

Human presence in the Antarctic, including commercial and scientific shipping and land based operations at coastal research stations increase the risks for local contamination of soils and the coastal marine environment. When a pristine area suffers sudden contamination, the low in-situ abundance of "contaminant competent" microbiota creates a need to think of new biotechnological processes such as bioaugmentation (i.e., addition of cultivated autochthonous contaminant-competent bacteria) to accelerate the biodegrading activity of soils and sediments and, if applied soon after the spill, to reduce the time for remediation (Stallwood et al., 2005). To develop new strategies to mitigate ecological damage caused by oil spills, we isolated several hydrocarbon-degrading bacteria from the Potter Cove environment. *Rhodococcus* sp. ADH (Fig. 3), a bacterium with high degradative activity for aliphatic hydrocarbons, was isolated from surface soil contaminated with diesel at Carlini Research Station, and its genome sequenced using the Illumina HiSeq 1500 platform. The estimated genome size was 7.1 Mbp with an average GC content of 62.3% and a total of 6383 protein-coding sequences (GeneBank WGS master accession LJIS00000000,

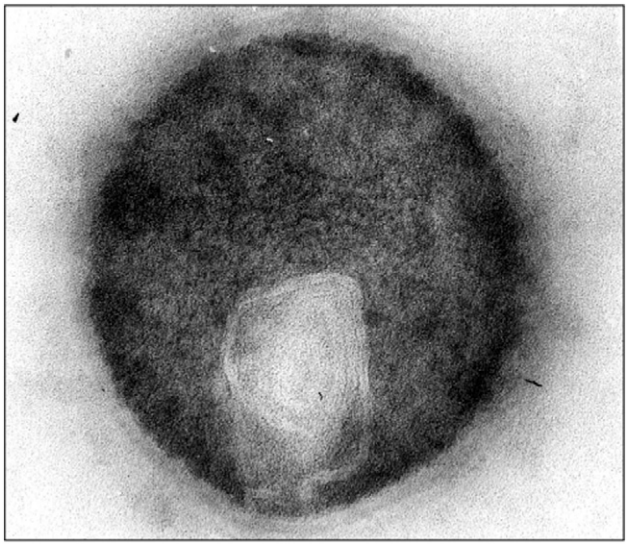

Fig. 3. *Rhodococcus* sp. ADH. TEM image of a cell growing in n-hexadecane as sole carbon and energy source. 120.000× magnification. Internal membrane structures were observed only in cells growing on hydrocarbons.

PRJNA294916; IMG accession 2,639,762,848). A comparison of the 16S rRNA gene sequence to sequences present in the prokaryote database EzTaxon and GenBank indicated 100% identity with *R. qingshengii* djl-6^T (DQ090961 and NR_043535) and *R. jialingiae* djl-6-2^T (DQ185597). Genes encoding for physiological properties that are discriminating between the two closely related species within the genus (Wang et al., 2010) are currently not sufficiently clear to allow taxonomic assignment of the *Rhodococcus* ADH strain to species level.

The high alkane degradation capacity of the ADH strain in liquid cultures with hydrocarbons as the sole carbon and energy source (Ruberto et al., 2005) is consistent with the presence of multiple genes in its genome encoding putative cytochrome P450 hydroxylases, ferredoxin and ferredoxin reductases, and with at least 6 alkane monooxygenase gene homologs, all involved in the aerobic degradation of alkanes. Other genes that were identified encode for urea decomposition, nitrate and nitrite ammonification and sulphate assimilation. On the other hand, genes involved in the dissimilatory use of nitrate or sulphate were not detected, supporting a strictly aerobic metabolism of this strain. All of these features are relevant for the use of the ADH strain in alkane bioremediation actions where nitrates, urea and sulphates are usually added as nutrients or terminal electron acceptors. Further into depth analysis of the *Rhodococcus* sp. ADH genome is currently under way to substantiate the metabolic pathways that enable growth in soils containing different aliphatic hydrocarbons and at low temperatures with the goal to facilitate bioremediation processes in polar areas.

4. Microeukaryotic planktonic communities

Roughly at the turn of this century, molecular tools were introduced to study planktonic protist assemblages from marine Antarctic habitats. Since then, the number of studies has rapidly increased (Diez et al., 2001; Díez et al., 2004; López-García et al., 2001; Gast et al., 2004; Gast et al., 2006; Piquet et al., 2008; Piquet et al., 2010; Piquet et al., 2011; Wolf et al., 2013; Wolf et al., 2014), yet they remain rather underrepresented when compared with marine polar prokaryotes (De La Iglesia & Trefault, 2012).

For the coastal areas in the WAP region, the number of published studies so far also remains limited. Piquet et al. (Piquet et al., 2011) and Rozema et al. (Rozema et al., 2016) used molecular tools such as DGGE, clone libraries and Illumina MiSeQ sequencing to unravel the coupling between protist and bacterial dynamics at Rothera Research station (Adelaide Island). More to the north (in the Palmer LTER region), Luria and coauthors (Luria et al., 2014) compared microbial communities, including Archaea, Bacteria and Eukaryota. They found differences among microbial eukaryote assemblages between northern and southern stations within the Palmer-LTER sampling grid during summer, based on 454-Genome sequencing. In the coastal regions of King George Island (northern WAP), Luo et al. (Luo et al., 2016) and Moreno-Pino et al. (Moreno-Pino et al., 2016) described molecular diversity of microbial eukaryotes. Luo et al. (Luo et al., 2016) performed Illumina Seq2000 sequencing of the 18rRNA gene on environmental samples <20 μm from two coves on King George Island (Great Wall Cove and Ardley Cove, Fildes Peninsula). Apart from the dominant phyla (from high to low fraction of total reads: dinoflagellates; cryptomonads, Prymnesiophyceae, diatoms, Incertae sedis and Chlorophyta) a large numbers of reads belonged to Stramenopile lineages from MAST-1, MAST-2, MAST-7 and MAST-8 (Luo et al., 2016). Alveolates consisted mainly of dinoflagellates belonging to the genera *Gyrodinium*, *Karlodinium* and *Protoperidinium*, besides the categories uncultured and unclassified *Dinophyceae*. Other abundant reads from other phyla were represented mainly by the genera *Geminigera* (Cryptophyceae), *Thalassiosira*, *Fragilariopsis*, *Chaetoceros* and other diatoms (Stramenopiles), *Pyramimonas* and *Micromonas* (Chlorophyta), as well as *Phaeocystis* and *Chrysochromulina* (Haptophyta). Both coves are characterized by high microbial eukaryote diversity but differ in community composition (Luo et al., 2016). When taking together the few published molecular studies in Antarctic marine waters, a remarkable diversity is revealed with a variety of novel and previously unobserved groups/lineages, and this also applies to the WAP region.

Long term climatic trends as observed in Potter Cove (Schloss et al., 2012) may have far reaching consequences for both structure and functioning of the microbial eukaryotes, specifically for the photosynthetic phytoplankton. Early studies were exclusively based on microscopic observations and showed that phytoplankton communities in Potter Cove are dominated by diatoms (Schloss & Ferreyra, 2002; Schloss et al., 2002). Pigment fingerprinting (HPLC-CHEMTAX) revealed only a small fraction of coastal phytoplankton to belong to (phyto) flagellate groups such as haptophytes, cryptophytes, dinoflagellates and prasinophytes (van de Poll et al., 2011). It remained unknown how the high glacial meltwater input during summer would influence phytoplankton production, diversity and species composition in Potter Cove. Hence, research carried out during the CLIPOPEN (IPY), IMCOAST and IMCONET EU programs was largely based on molecular approaches and generated a plethora of novel insights into microbial eukaryotic diversity (M.Krikke and M. van de Loosdrecht, University of Groningen, unpubl. res.). During an initial field campaign in summer 2010/2011 samples were collected at 0 m and 10 m water depth along a three station transect perpendicular to the meltwater plume of the Fourcade Glacier in Potter Cove. Microbial eukaryote diversity and composition as a function of environmental parameters were analyzed using molecular fingerprinting (DGGE), cloning and sequencing of the 18SrRNA gene supplemented with microscopy (G. Almandoz, Universidad Nacional de La Plata, Argentina). In addition to universal eukaryote specific primers (EUK1A and 516R), the dinoflagellate specific primer set 18ScomF1 and Dino18SR1 and the diatom specific primer set 1209F and Diatom18SR1 were used. Results gave a first clear evidence of the impact of glacial meltwater in the inner cove which is characterized by low surface water salinities and high turbidity near the glacier front. Yet, for all groups alike, “time” (i.e., seasonal differences) was the main shaping factor.

Microscopic results showed relatively high abundances of cryptophytes and diatoms during the summer melt season at all stations alike. The dinoflagellate community was dominated by small gymnodinoids. Sequence analysis evidenced high abundances of *Haptophyceae*, *Bacillariophyceae* and *Peridinea*. The most abundant sequences were associated (≥97% similarity) with *Thalassiosira nordenskioldii*, *Thalassiosira oestrupii*, *Thalassiosira antarctica* and *Fragilariopsis cylindrus*. The *Haptophyceae* were represented by *Phaeocystis antarctica*, *Phaeocystis jahnii* and *Chrysochromulina simplex* sequences. The most abundant dinoflagellate sequences (≥97% similarity) were associated with *Gyrodinium spirale*, *Pentapharsodinium* sp., *Karlodinium micrum* and *Takayama acrotrocha*. Overall the molecular data indicate that Potter Cove hosts a dynamic protist community with high temporal variability and with additional glacial influence. These data closely match the sequences recorded in the nearby Fildes Bay (Luo et al., 2016; Moreno-Pino et al., 2016). In view of the high contribution of dinoflagellates to total sequences in our and other studies, and their inherent taxonomic and functional diversity, research efforts directed to this important group in Antarctic coastal waters have recently been intensified. In the Ross Sea, a highly abundant dinoflagellate was suggested to represent a new genus, closely related to toxic genera *Karenia* and *Karlodinium* (Gast et al., 2006). These latter genera were also found in Potter Cove in our recent studies (M. van de Loosdrecht, University of Groningen, unpubl. results) using dinoflagellate specific primers, DGGE and clone libraries. In this particular project, samples were analyzed over a 14 months period, between December 2007 and February 2009, along the same three stations transect described above. Eukaryote specific primers Euk1A and Euk 516r and dinoflagellate specific primers 18Scom F1 and Dino18SR1 were used to partially amplify the 18S rRNA genes of the total eukaryote and dinoflagellate communities. Clone libraries and subsequent sequencing were based on a selection of samples, representative of seasons and locations.

Sequencing results revealed that the highest proportion of sequences belonged to the *Gymnodiniales*. This group comprised genera closely related to *Karenia*, *Karlodinium* and *Takayama* (all belonging to *Kareniaceae*), besides Gymnodiniaceae (*Gyrodinium spirale*, *G. fusiforme*, *Lepidodinium* spp.). Peridiniales encompassed *Peridinium* spp., *Protoperidinium bipes* and *Lessardia elongata*. In general, the earlier findings of (Gast et al., 2006) were supported by these data, which moreover demonstrated the high dinoflagellate diversity at Potter Cove.

In conclusion, fundamental components of Antarctic protist assemblages that were identified with the use of molecular tools have remained undetected in earlier work that exclusively relied on traditional methods (Gast et al., 2006). The unidentified components include the highly diverse dinoflagellates, but also picoeukaryotes belonging to alveolates and stramenopiles (López-García et al., 2001; Massana et al., 2004). At the same time, most molecular analyses have not yet linked DNA sequence information to the corresponding morphological features. Hence the identities of many sequence entries remain undescribed. In future work, molecular information should urgently be coupled to culture information, to better link morphological and genetic identification (Gast et al., 2006). We furthermore recommend an integration of methodologies for Antarctic microbial eukaryote studies. These are preferably HPLC-Chemtax or flow cytometry for quick and rough group level analyses, in combination with more detailed species information obtained by high throughput sequencing and whole genome studies, which need to be supported by classical microscopic observations.

5. Benthic key species: molecular analysis of their adaptive capacities

The benthic environment of the Antarctic continental shelf is the coldest and most stable marine habitat on earth, where many endemic macroinvertebrates have evolved strictly cold adapted genotypes since the mid Cenozoic (initiation of the Polar Front, see (Clarke et al., 2009; Clarke et al., 2005) Mackensen 2004 see Poulin 2014). The benthic communities in our study area in the northern sector of the WAP typically combine a large number of Antarctic endemics (e.g. the Antarctic bivalves *Laternula elliptica* and *Yoldia eightsi* (Glover et al., 2008)), mixed with a set of species endemic to the Antarctic Peninsula and neighboring regions. These "peninsula-endemics" typically occur in high abundances in the coastal shallows of the WAP and the (sub)antarctic islands, including the South Shetlands and KGI. While the pattern of coexistence of species with continental and more regional distribution areas is stable in Potter Cove, the decision which species fall into either of the two categories is less stable. The application of molecular methods, in particular molecular barcoding, has identified numerous cryptic species even in well-studied species, effectively transferring species once believed to have circum-Antarctic distributions into the category of locally distributed species (Held, 2014). Among them we find the isopods *Glyptonotus antarcticus* sensu strictu (Held & Wägele, 2005) and *Ceratoserolis trilobitoides* sensu strictu (Held, 2003), both of which have their closest relatives in the Southern Ocean. Also a typical colonizer of the Antarctic intertidal zone, the Antarctic limpet *Nacella concinna* has its closest relatives across the Drake Passage in Southern South America (*N. deaurata/magellanica*). Other species have been confirmed to occur on a continental scale (e.g. some mysids and lysianassoid amphipods (Baird et al., 2012; Havermans et al., 2013)), with many more awaiting a detailed analysis of their taxonomic status.

In our approach we are relating the evolutionary background, e.g. knowledge on phylogeny or phylogeography in sibling species, to their phenotypic capacities to respond to the constraints typical for shallow cove and bay environments surrounded by tidewater glaciers. Phenotypic traits can be morphological and behavioral, as well as physiological response patterns, including molecular markers such as gene expression and cellular stress and damage indicators.

5.1. Laternula elliptica

the Antarctic soft shell clam, is one of the most widely distributed (circum-antarctic) and abundant shallow water Antarctic macroinvertebrates and, therefore, one of the best-studied organisms. Large clams can burrow down to 50 cm sediment depth and, as bioturbate filter feeders, are of central importance for benthic carbon deposition and turnover in the upper sediment layers. Ventilating and working the sediment, *Laternula elliptica* is an important habitat builder for macro- and meiofauna in Potter Cove sediments. Age in bivalves can be read from shell ring counts so that maximum lifespan (36 y at King George Island), lifetime growth rates, and age at maturity can be inferred across populations. This enabled us to describe lifetime energy allocation to growth, reproduction and cellular metabolism under different stress conditions and to investigate the age-dependent response of *Laternula* to stressors typical for glacial melt and coastal disturbance scenarios. The stressors we tested included enhanced sediment deposition, transient food shortage, removal from sediment, injury by ice scour, as well as hypoxia as a consequence of increased detritus deposition in surface sediments. We combined physiological analysis with gene expression to understand what supports the better fitness in younger specimens (<5 cm shell length) that can easily rebury into the sediments and better survive experimentally inflicted shell and tissue damage (Philipp et al., 2011; Husmann et al., 2014; Husmann et al., 2016). We used the transcriptome-based approach and pyrosequencing (Genome Sequencer FLX System, 454 Life Sciences Branford, CT, USA, at the Institute of Clinical Molecular Biology in Kiel, Germany) to generate a broad enough cDNA library for L. *elliptica* based on RNA extractions across different tissues (digestive gland [DG]; gills [G]; syphon [SY], hemocytes [HC]) from young/small and old/large animals exposed to the following experimental conditions: food deprivation (food (−)), feeding (food (+)) and injury, as well as their combination (see Husmann et al. 2014 (Husmann et al., 2014)). The RNA sequence database was deposited as sequence read archive study ERP001323 and Project number PRJEB33 of the European Nucleotide Archive (ENA). Accession numbers please see in the original papers (Husmann et al., 2014; Husmann et al., 2016). Gene expression profiles in different treatments and age groups were analyzed by qPCR.

Only young bivalves were able to induce stress genes after injury as long as food was made available to them, but not under food deprivation (Husmann et al., 2014). Hemocyte cells of young animals mounted a stronger immune response (measured as oxidative burst reaction) upon exposure to bacterial infection mimetics and, consequently, young specimens survived injury infliction better than older bivalves (Husmann et al., 2011). Upon experimental food deprivation, energy saving strategies manifest only in younger, fast growing specimens (downregulation of cell cycling genes and enhanced transcription of autophagic factors), potentially stabilizing the general physiological condition and the hepatosomatic index (HSI), which rapidly dwindled in food deprived large animals, indicating starvation. In another age dependent study of hypoxic stress we used a custom made microarray for L. *elliptica* together with colleagues from British Antarctic Survey and qPCR for the analysis of Hsp70 expression (Clark et al., 2013). Exposure to acute hypoxia caused upregulation of stress response genes in both age groups (antioxidants, HSP70 members). Differential gene expression patterns evidenced induction of muscle function and energetics in young and immune defense genes in older animals (Clark et al., 2013). Both data sets combined emphasize better energetic and tissue maintenance to support stress tolerance in actively burying young bivalves. Already now the Potter Cove L. *elliptica* populations under the glacial melting plume show signs of disturbance with 30% lower animal density compared to undisturbed locations outside the plume extension. Philipp et al. (Philipp et al., 2011) reported older animals (>5 cm shell length) to reduce respiration when exposed to current summer sediment concentrations, potentially causing these animals to become transiently hypoxic. Analysis of growth rates read as "shell ring width" of L. *elliptica* shell

collections from Potter Cove between 1989 and 2010 indicates a massive reduction of shell and somatic growth and of gonad productions between the 1960s and present (Brey et al., 2011). In fact the derived growth model suggests respiration and gonad production to have declined most pronouncedly in animals older than 25 years of age, the reproductively most active individuals in the population (Guy et al., 2014), which brings up the question whether Potter Cove will be a suitable habitat for L. *elliptica* under continuing glacier melting and permafrost thawing.

The **Antarctic patellogastropod limpet *Nacella concinna*** is a typical and abundant colonizer of shallow water environments along the Antarctic Peninsula, including tide pools and moist, rocky crevasses where temperature can be well above 0 °C in summer. During low tides the limpets prevent desiccation by clamping shells down to the rocky surface which causes the concentration of oxygen in shell water to fluctuate between full saturation and hypoxia (Weihe & Abele, 2008). This entails a relatively broad tolerance to intertidal conditions including thermal and salinity extremes as well as hypoxia and freezing during periods of aerial exposure (Waller et al., 2006). The majority of our experimental work with *Nacella concinna* was accordingly aimed at specifying its plasticity and ecophysiological and morphological adaptive capacities to deal with the vagaries of life in the Antarctic intertidal (Weihe & Abele, 2008). Additionally, we analyzed the heat shock response (HSR) of Hsp70 genes: Hsp70A, Hsp70B, HSC70, using qPCR and comparing three allopatric *Nacella* species, the two South American limpets *N. magellanica* (intertidal) and *N. deaurata* (subtidal) from two locations in Patagonia (Puerto Montt and Punta Arenas) and *N. concinna* (intertidal and subtidal population) from Potter Cove, Antarctica (Koenigstein et al., 2013). The comparison revealed i) an inducible HSR in both Patagonian limpets with a more pronounced induction of Hsp70A than Hsp70B upon air exposure in both species, and ii) quasi absence of inducible HSR in the Antarctic limpet *N. concinna* which had however significantly higher expression of the constitutive protection through higher expression of Hsc70 especially in intertidal animals. The unexpected variability in the expression patterns of genes typically regarded as stable in combination with the lack of an inducible response in genes regarded as variable requires special precaution for the calibration and interpretation of qPCR data. Careful testing of the utility of a set of candidate reference genes using multiple methods proved vitally important in establishing a trustworthy baseline (Koenigstein et al., 2013). Initiation of the inducible HSR happened faster in subtidal limpets (*N. deaurata*: induction within 2 h) than in the pre-accustomed intertidal *N. magellanica* (induction after 4 h) and moreover changed along a biogeographical gradient of thermal adaptation: stronger response in Puerto Montt at 16–24 °C than in Pta Arenas at 9–17 °C. No significant HSR was found Antarctic limpets upon air exposure at 0 °C. However, all three tested Hsps are present (in the genome) and expressed in the Antarctic limpets from King George Island, and other samples have been collected to investigate the response to conditions more stressful than 6 h of air exposure at 0 °C. Interruption of genetic connectivity across the Drake Passage through long distance larval transport has been dated to 3.7 Ma BP based on haplotype networks constructed from COI sequences by Poulin and colleagues (Poulin et al., 2014). Our own genetic clock model based on COI, 16S and 18S rRNA gene fragments of all three limpets and using the Australian genus *Cellana* as an outgroup for calibration (separation confirmed at 35.14 Ma BP during the early Oligocene and the opening of the Tasman and Drake Antarctic gateways) dated the split at 6.5 Ma BP between the South American limpets and the Antarctic *N. concinna* (K. Pöhlmann and C. Held unpublished results). This is in close agreement with (Poulin et al., 2014) and indicates *Nacella concinna* to be a good example for studies of relatively recent adaptation to Antarctic conditions and the limitations posed by long lasting connectivity to its South American counterpart/s (e.g. higher energy requirement, dependency on macroalgae and epiphytic microalgae for feeding and reproduction, adaptation to temperate and variable climate).

All in all, the dispersability of species across surprisingly large distances in the West Antarctic seems to be less limiting than previously thought. The role of major currents such as the ACC or the Antarctic Coastal Current as an oceanographic feature connecting populations is illustrated by a multi-locus study on three populations of *Septemserolis septemcarinata*, which unlike the *Nacella* limpets, is a brooding species without free-living larval dispersal stages or the ability to swim as an adult. Populations separated by >2000 km of uninhabitable deep sea between them and no interspersed suitable habitats proved to be conspecific without signs of cryptic speciation among them. Despite a shallow genetic differentiation characterizing the *S. septemcarinata* populations from South Georgia, Bouvetoya and Marion Island, testing models of dispersal in a Bayesian statistical framework suggests that asymmetrical and recurrent gene flow in the direction of (and likely mediated by) the Antarctic Circumpolar Current is by far the most likely scenario to explain the observed patterns of neutral genetic variation in the loci examined (Leese et al., 2010). This in turn means that (1) dispersal across major barriers even by seemingly ill-adapted species is possible on an evolutionary timescale and (2) any differences we observe in functional traits affording adaptation to local environmental conditions are less likely aided by random genetic drift, but emphasize the role of strong natural selection on the basis of the adaptive value of genetic variation. To understand the adaptability of more complex macroinvertebrates (and vertebrates) to Antarctic coastal conditions, and to predict their potential to migrate and colonize new habitats as the glaciers retreat and to survive under warmer and more hypoxic conditions, we need to combine population genomic markers with functional studies and population specific gene expression profiles.

6. Integrating marine genomics across scales – resolving seasonality and patchiness and monitoring events

Implementation of environmental genomics into the long-term monitoring and ecological programs running in Potter Cove has leveraged our understanding of Antarctic coastal biodiversity and system function under conditions of glacier melt. Using these - by now widely accepted - molecular tools has enhanced our knowledge of taxonomic composition and of the seasonal community dynamics at different ecosystem levels from microbes to phytoplankton. Sanger sequencing-based molecular tools capture the overall biodiversity of dinoflagellate blooms that build up during the summer months in glacier proximal zones of the cove, as a complement of traditional HPLC and microscopic methods. In the next step, meta-genomic studies will allow to draw conclusions towards the genetic potential of important species and groups, without applying cultivation techniques that would ignore the non-cultivable components of the ecosystem. This will further enhance the applicability of the results for the understanding of ecosystem functions. A first step in this direction was recently taken when the presence of sequences encoding two monooxygenase biocatalysts (Baeyer–Villiger and bacterial cytochrome P450 monooxygenases) were identified and described based on marine sediments metagenomes from Potter Cove and other cold regions, using an “in silico” approach (Musumeci et al., 2017).

Evolutionary adaptations of Antarctic microbes to the conditions prevailing at Fourcade glacier are potentially reflected in newly identified lineages, not previously described elsewhere (e.g. Potter Cove “specific” clusters of Thaumarchaeota PC in Fig. 2). This is a good reason to start comparative studies in neighboring and remote areas prone to deglaciation. More generally, non-classified results within the clone libraries (e.g., of new microbial ribotypes) will expand database knowledge available to comparative ecological studies in other areas.

Molecular analyses are insightful tools in analyzing system response to change, such as the responses of the Potter Cove microbial communities to seasonal ecosystem dynamics with winter freezing and ever more warming summer melt conditions. Metagenomics was performed to understand seasonal microbiome biodiversity in its response to

biogeochemical fluxes and biological cycles in space and time. Specific functions, such as the putative iron-oxidizing capacity of the newly identified clade of PCMWE archaea, potentially bear significance beyond the concrete local conditions of first description. Potter Cove, with its surface and subglacial erosion of Fe containing suspended particles and see page of reduced iron from organic matter enriched suboxic sediments, serves as a showcase for conditions that will - as a consequence of increased glacial melting - become more prominent in other coastal areas of the Antarctic. It also encourages comparative work in other WAP regions with high (e.g., Deception Island) and lower (e.g., Marguerite Bay) Fe concentrations.

Another major finding in the seasonal analysis of the pelagic microbiomes in the cove is the striking difference between the meltwater outlet area (E3) and the two more central sampling stations (E1 and E2). The future will be to combine metagenomics with meta-transcriptomics in Potter Cove, i.e., information on community structure and functional diversity with analysis of gene expression, to clearly resolve functional activities and interaction networks (Toseland et al., 2013). The first step in this direction was motivated by the need to leverage ecosystem conservation and management in an Antarctic coastal ecosystem impacted by >60 years of human presence around the Jubany/Carlini research station. Combinations of fingerprinting, metagenomics and functional analysis (Vazquez et al., 2017 in revision) in high resolution (S sites in front of Carlini station) and in potentially more polluted and eutrophicated areas are required to investigate anthropogenic impact, and to understand the role of functional biodiversity in the ecosystem response to human impact and to climate change.

Contrary, sequence-based assessments of natural communities are currently still limited by the lack of sequencing technology for the large genome size of most multi-cellular organisms, such as zooplankton or macrobenthos. These can, at the moment, best be studied by transcriptomics as a framework to effectively access functional sequence information. Arguably, this approach ignores the larger, non-coding part of the whole genome, which is currently still difficult to incorporate into the functional interpretation. Future sequencing technologies may overcome these limitations by cheaper approaches offering higher throughput, longer reads, and, accordingly, less algorithmic demands for genome and transcriptome assembly (nanopore sequencing). However, the lack of genomic sequence information for non-model organisms is no longer inhibiting molecular and functional studies, since transcriptomic tools already offer insights into processes and mechanisms under experimentally controlled treatments (gene expression studies, e.g (Clark et al., 2013).). Additionally, comparative transcriptomics in field studies, i.e., with non-cultivated organisms, may offer new clues to adaptive traits at the sequence level when applied to different populations within their specific environments (Heinrich et al., 2016).

A different outlook can be developed for community analyses, e.g., extending the amplicon sequencing to a multi-locus approach (Bybee et al., 2011), however, this approach is still hampered by the lack of reference sequences that would be needed for the analyses of complex environmental samples. Hence, it appears more promising to apply phylogenetic methods directly on meta-transcriptomic sequence data (Hanekamp et al., 2007; Moustafa et al., 2010), connecting community analyses and functional analyses within a single framework. Last but not least, the description of new functional traits and the link to the coding features in the respective genomes are urgently required, as the sequencing of whole genomes from environmental isolates produces a high number of open reading frames without reliable assignment to putative function. This applies especially to the lack of annotated sequences in the databases corresponding to the huge number of yet undescribed (micro-) organisms in polar environments.

In summary, we propose three main method fields to be further explored for Potter Cove molecular studies:

Firstly, regular sequencing approaches like genomics observatories (Davies et al., 2014) are needed to provide basic information on temporal community dynamics in time series.

Secondly, event-driven sequencing approaches (i.e., following the first day of fast ice break up, following the development of major events etc.) should be performed to resolve exceptional states and processes in more detail, since those are most likely not observed on the time-frequencies of genomic observatory samplings. Therefore, data analysis techniques for abiotic sensors may serve as a tool to trigger a “smart sampling”, possibly involving automatic sampling devices (— > Ferryboxes, automated filtration devices (AutoFIMs) which collect particles close to the surface) to record species inventory, biomass and community structure.

Lastly, multi-station sampling will resolve patchiness of habitat/niche specific processes and community structures. Overall, this would constitute a massive increase of sequencing data volumes, for which knowledge extraction tools would be the ultimate challenge to generate new, ground-breaking ecological models of Potter Cove, applicable to further locations under global change influence.

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