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GENOMIC AND TRANSCRIPTOMIC DIVERSITY OF ANTARCTIC ORGANISM



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Understanding *Pseudogymnoascus* sp. Response towards Temperature Stress : A Proteomic Approach

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Mass spectrometry (MS)-based proteomics is a powerful tool that has helped researchers to identify and quantify complex protein mixtures in various cells. Under temperature stress, fungi will undergo numerous physiological changes and metabolic modifications for survival. Changes in protein abundances can give an overview of the complex protein response under high and low temperature stress. In this study, *Pseudogymnoascus* sp. (isolate HND16 R1-1 sp2), a fungus isolated from Arctic soil, was selected as a model organism, in an attempt to provide an overview of how polar fungi respond towards temperature stress via a proteomic approach. Firstly, an optimisation of protein extraction protocols was carried out. Three different chemical extraction methods; 1) TCA-acetone, 2) TCA-acetone-phenol, and 3) phenol-guanidine hydrochloride were used. The quantity and quality of proteins extracted were found to be best when using TCA-acetone extraction. This was demonstrated through total protein content and 1D SDS-PAGE resolution. Subsequently, *Pseudogymnoascus* sp. was exposed to three different temperatures that represented optimal (15°C), low (5°C) and high (25°C) temperature stress for 5 days and TCA-acetone was used to extract proteins for comparative analysis by 2-dimensional (2D) gel electrophoresis. There were significant differences in the proteome profiles between samples exposed to low and high temperature stress, thus showing the physiological response of *Pseudogymnoascus* sp. via its proteome. This will be followed by the identification of proteins that change in abundance using tandem mass spectrophotometry.

Transcriptional Analysis of the Thermal Stress Response In Polar Pseudogymnoascus Spp. Soil Fungi

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Global temperatures are predicted to rise due to climate change. In polar systems, studies to link shifting environmental parameters to physiological response and thus functional activity are lacking in soil fungi. Pseudogymnoascus fungi, commonly isolated from polar soils, have been shown to secrete high levels of extracellular hydrolase enzymes, suggesting a role as important decomposers. In this study, we sought to measure transcriptional changes in these fungi in response to a transient heat. Arctic, Antarctic and temperate strains were cultured at 15°C (ideal growth temperature) for 5 days then transferred to 25°C (heat challenge temperature) for 2 hours. Possible orthologs of *Saccharomyces cerevisiae* HSP70 and HSP90 genes were identified among available Pseudogymnoascus spp. protein predictions, and their expression following heat treatment was measured by quantitative real-time PCR. Consistently across all strains, two HSP70 homologs and the HSP90 homolog were upregulated, of which two were statistically significant in polar strains. Conversely, one HSP70 homolog was downregulated in all strains, demonstrating functional differences of homologous genes in the HSP70 family. RNAseq analysis of one of the Arctic strains revealed 2,992 differentially expressed genes (DEGs), many of which are involved in pathways of protein synthesis and metabolism. Based on BLASTP search against ESR genes described in the yeasts *Saccharomyces cerevisiae* and *Lachancea kluyveri*, 169 out of these DEGs are genes involved in Environmental Stress Response (ESR) (FDR < 0.001). Taken together, our findings suggest that short-term exposure to thermal stress elicits an extensive transcriptional response in Pseudogymnoascus.

Pseudogymnoascus spp as a potential fungal model for climate change studies

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Fungal species from the genus *Pseudogymnoascus* are commonly found in soils from temperate and polar regions. *Pseudogymnoascus* spp are psychrophilic, tolerant to a wide range of stresses, including desiccation, hyper-salinity, solar radiation, and low temperatures by developing functional strategies. Despite the general lack of knowledge on their ecological role, adaptation mechanisms at low temperatures and extreme environments, results from our preliminary studies of environmental stressors on these polar strains have provided some insights. The activation energy (E_a) based on relative growth rates (RGRs) of the fungal isolates vary with nutrient conditions and declined with increasing temperature (E_a was negative). Only certain extracellular hydrolytic enzymes (EHs) showed activities across the experimental temperature range, indicating trade-off between growth and enzyme activity. As for UVB radiation experiments, exposure to unweight UVB significantly reduced the RGRs and conidia production. Pigments were not simulated under UVB radiation. UVB-induced DNA damage and repair experiments may suggest the nucleotide excision repair (NER) is the primary repair pathway. With these findings, supported with other recent studies, we will justify why *Pseudogymnoascus* spp is a good fungal model candidate for climate change studies. We will also highlight the importance of filling the knowledge gaps concerning long term data, large scale data, data from multiple simultaneous drivers of change, and the application of genomic, transcriptomic and proteomic approaches.

Fine scale genetic diversity associated with contrasting thermal tolerances between populations of the Antarctic collembolan, *Gomphiocephalus hodgsoni*

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Assessing the resilience of Antarctic biota to climate change is integral to predicting the likely impacts of rising temperatures on the survival, diversity and distribution of Antarctic communities. Collembola are key components of terrestrial Antarctic soil systems and are also sensitive to environmental disturbances, making them ideal biological indicators of climate change. The limited dispersal capabilities of Collembola and consequent low levels of gene flow have created highly genetically structured communities which present opportunities to test whether that genetic diversity confers differential survival capabilities. Previous studies have identified two distinct COI lineages of the springtail *Gomphiocephalus hodgsoni* within Taylor Valley and postulated that the currently dominant upper valley lineage is more cold adapted while the coastal lineage more warm adapted. This project aimed to test this idea by measuring critical thermal maxima and supercooling points of an upper Taylor Valley population and a coastal population from Botany Bay. Our data demonstrate that indeed the coastal population has on average higher upper thermal limits (overall mean: 31.3 °C, range of means: 28.0-32.9 °C) compared to the upper valley population (overall mean: 27.2 °C, range of means: 22.2-31.5 °C). The coastal population also had on average higher supercooling points, further reinforcing this pattern (coastal mean: -14.3 °C; upper valley mean: -22.6 °C). These findings highlight the potential that as temperatures rise warm adapted populations may start to proliferate at the expense of more cold adapted groups leading to overall changes in community structure and a decline in genetic diversity.

Phylogeny of the gastropod mollusk *Nacella concinna* and mollusk-associated bacteria from the water area of the Argentine Islands, Graham Land, West Antarctica

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Three morphotypes of the Antarctic limpet *Nacella concinna* were found in the water area of the Vernadsky Station (Ukraine). The morphotypes were very diverse in their shell surface and coloration. A phylogenetic analysis of the limpets was conducted using 12S, 16S and CO1 mitochondrial genes. Both p-distances and phylogenetic trees suggest that all three morphotypes belong to the same species. *Nacella concinna* is phylogenetically close to South American species and probably originates from that region, and has spread along subantarctic islands and the Antarctic Peninsula due to currents. The high ecological plasticity of *N. concinna* to various factors such as wave loads, currents, nutrition, microflora and underwater landscapes may be manifested in the structure of its shell.

For the first time, pure bacteria strains were isolated from the mantle and the digestive system of three *N. concinna* morphotypes for 16S rRNA barcoding. It was found that the microbiota of *N. concinna* belongs to marine Proteobacteria (*Pseudoalteromonas*, *Psychrobacter*, *Shewanella*, *Halomonas*, *Cobetia*, *Psychromonas*), Bacteroidetes (*Bizionia*, *Formosa*) and Firmicutes (*Oceanobacillus*). A Maximum Likelihood tree demonstrated that some *Pseudoalteromonas* strains phylogenetically close to species occurring in the Arctic and Pacific Oceans, which suggests their bipolar distribution.

Shedding light on the molecular clock of the Antarctic krill *Euphausia superba*

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Euphausia superba is a high-latitude pelagic organism which plays a central role in the Southern Ocean ecosystem. Krill show daily and seasonal rhythms in physiology and behaviour, which are synchronized with the extremely variable environmental cycles of their habitat. Over the past few years we have focus our efforts to shed light on the molecular architecture and functioning of krill's circadian clock machinery. Our findings describe an ancestral circadian clock, with both mammalian and insect features and a free-running period shorter than 24 hours, involved in the temporal orchestration of gene expression, physiology (energy-storage pathways, oxygen consumption) and behaviour (diel vertical migrations). Moreover, we generated the most comprehensive transcriptome database of krill that allowed us to deeply study gene expression (RNA-Seq and microarray) in adult and larval krill under different natural or simulated photoperiodic conditions. Our results suggest that the photoperiod plays a major role in the entrainment of krill circadian functions when a robust day/night cycle is present. But, when the day/night cycle is strongly biased towards full light or constant darkness, photoperiodic cues might not be sufficient and alternative Zeitgebers might be required. Interestingly, a short free-running period could allow the entrainment of the clock to alternative Zeitgebers characterized by 12 hours periods, such as the morning/evening light transitions or the tidal rhythms. A deeper understanding of the functioning of clock machinery in other polar organisms could reveal whether high-latitude clocks have developed similar strategies to cope with polar environment.

Benthic Sediment Based Community Compositional Profiling from Underneath the Ross Ice Shelf, Antarctica, as part of the New Zealand Ross Ice Shelf Project (NZRISP)

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Microorganisms in benthic sediments play an important role in regulating the marine ecosystem through complex biogeochemical pathways. The Ross Ice Shelf (RIS) is the largest ice shelf in Antarctica, spanning over 500,000 square kilometers and operating as a prominent physical barrier against sunlight penetration to the underlying water column and benthic sediments below. Current knowledge surrounding how microbial communities within the benthos survive and drive these vital nutrient processes in a carbon limited and sunlight deprived system remain unknown. As part of the NZRISP, this project sought to undertake one of the first studies to examine the composition and structure of benthic microbial communities underneath the RIS. This was achieved using a complex hot-water drilling system to sample sediment cores from the seafloor from two disparate regions of the RIS. Our hypothesis maintains that the different sites will be compositionally distinct from one another as a result of variation in under-ice shelf hydrological processes and nutrient cycling dynamics. Current molecular genetic techniques were used to assess both community composition (DNA-barcoding) and function (Metagenomics). Standard taxonomy database searches revealed that over 40% of the bacterial community were comprised of unknown phyla. Thaumarchaeota were shown to be the top-dominating taxa, an organism typically reflective of an open-ocean system. Species abundance and diversity were strongly correlated with sediment depth, whereby the greatest species richness occurred within the top ~0.5cm. This study aims to be the first of its kind to compare the bacterial communities between two previously unexplored and isolated regions of Antarctica.

Diversity and Ecology of Uncultured Chlorophyta (Viridiplantae) Assemblages in Protected and Non-Protected Sites in Deception Island (Antarctica, South Shetland Islands) Investigated Using an NGS Approach

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Assessment of the diversity of algal assemblages in Antarctica has until now largely relied on traditional culturing approaches. Here we used DNA metabarcoding through high throughput sequencing (HTS) to assess the uncultured algal diversity at two sites on Deception Island, Antarctica. The first was a relatively undisturbed site within an Antarctic Specially Protected Area (ASP 140) and the second was a site heavily impacted by human visitation, the Whalers Bay historic site. We detected 57 distinct algal taxa, 29 from within ASP 140 and 50 from Whalers Bay. Of these taxa, 22 were common to both sites, and 35 only occurred at one site. Algal richness was about six times greater than reported in previous studies using culture methods. A high proportion of DNA reads obtained was assigned to the highly invasive species *Caulerpa webbiana* at Whalers Bay, and the potentially pathogenic genus *Desmodesmus* was found at both sites. Our data demonstrate that important differences exist between protected and human impacted sites on Deception in terms of algal diversity, richness, and abundance. The western Antarctic Peninsula, including the South Shetland Islands, has experienced considerable effects of climate change in recent decades, while warming through geothermal activity on Deception itself makes this island one of the most vulnerable to colonization of non-native species. The detection of DNA of taxa native to different parts of the world highlights concerns about how the human impacts, both tourism and national operations, and it may influence future biological colonization processes in Antarctica.

Fungal community present in rocks of Ellsworth Mountain, Continental Antarctica

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In the present study, we focused the characterization of the fungal community present in saline crust and the interior of seven different rocks collected in the Heritage Range in the Southern part of the Ellsworth Mountain system, continental Antarctica. Fragments of the saline crust and inner rocks were obtained using mini drill. One (1 g) of each sample was diluted in 0.85% NaCl and 100 μ L of a 10⁻¹ inoculated into different culture media (MEA, MEA+17% NaCl, DG18, and DRBC) and incubated at 10 °C for 60 days. A total of 34 fungal isolates were obtained, 23 from the saline crust and 11 from the inner rocks. Fungi were identified using molecular biology methods in taxa of the genera *Penicillium*, *Cladosporium*, *Naganishia* and *Filobasidium*. Our results indicate that rocks of continental Antarctica shelter a restricted fungal community composed by cold adapted cosmopolitan taxa, which may be adapted to high extreme conditions of continental Antarctica.

The whole-genome sequence of the endemic Antarctic fungus *Antarctomyces pellizariae* reveals an ice-binding protein and provides insights on Leotiomyces phylogeny.

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Extreme environments, such as the snow-covered surfaces of Antarctica offer the conditions that select the development of life forms with adaptations to extreme conditions. The ability to survive, adapt, and grow at low temperatures may be due to the capabilities to produce antifreeze proteins and ice-binding proteins, which have properties that attenuate the effects of intense cold. In this study, we sequenced and reconstruct the nuclear and mitochondrial genomes of the endemic Antarctic fungus *Antarctomyces pellizariae* UFMGCB 12416. We identified a putative ice-binding protein-coding gene, determined the protein three-dimensional structure by homology modeling, compared its structure with other publicly available ice-binding proteins that contain the DUF3494 domain, and reconstructed the phylogenetic relationships with others Leotiomyces from the alignment of thousands of orthologous proteins. Our results will be useful for a better understanding of microbial ice-binding proteins and genomic aspects of psychrophilic fungi.

Fungi present on the surface of archaeological materials from different Antarctic sealers sites

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In the current study, we identified fungi present on the surface of different archaeological materials (tissue fragment, skin tissue, wood fragments and whalebone) of the sealers sites from the beginning of the century XIX. Using sterile disposable loops, smears were made on the material in different parts of the pieces, striated on Sabouraud agar and incubated at 10 and 25 °C, according to the storage temperature of the artifacts. We isolated 30 fungi, 19 from the wood fragment, three from the tissue, four from the skin tissue, and four from the whalebone. *Penicillium* sp. 1 was the most abundant taxa. The genus *Talaromyces* and *Penicilium* were recovered from the surface of the wood fragment. In the tissue fragment and skin tissue, species of *Trichoderma* were predominant, followed by endemic genera *Antarctomyces*. *Penicillium* and *Mortierella* species were isolated from whalebone. The identification of fungi in archaeological artifacts may contribute for the control the biological degradation, one of the most common problems in organics materials, improving consequently the preservation strategies of archaeological collections that constitute the polar heritage.

IcemiRs - Evolution of gene regulation by microRNAs in temperate, Antarctic red-blooded, and Antarctic white-blooded notothenioids

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As Antarctic waters chilled, red-blooded notothenioid ancestors adapted to the cold and subsequently the unique white-blooded icefishes evolved. Gene losses, duplications, and changes in expression patterns are likely sources of adaptation, including to a cold environment and to the transition to the hemoglobin-null phenotype of icefishes. microRNAs (miRNAs), yet underappreciated in Antarctic fish, are endogenous small molecules modulating translation of targeted messenger RNAs. We enquired whether miRNA repertoires and expression patterns evolved during 1) the cold adaptation of Antarctic fish, and 2) the evolutionary remodeling of icefish organs.

To address these questions, we performed high-throughput Illumina smallRNA-sequencing on a panel of organs in three temperate red-blooded notothenioids, two Antarctic red-blooded notothenioids, and two white-blooded Antarctic icefishes. We analyzed results using our miRNA data analyzing tool, Prost!, and recently published and as yet unpublished genome assemblies for each species.

Our analysis revealed that the notothenioid miRNA repertoire remained relatively stable over evolution of the group with only a few losses and duplications and the gain of a few novel miRNA genes throughout the phylogeny with no strong association with cold adaptation or icefish evolution. A comparison of miRNA expression patterns between temperate notothenioids, Antarctic notothenioids and Antarctic icefish organs, however, revealed organ-specificity for many miRNAs and expression level changes between species consistent with a potential role of miRNA regulation in cold adaptation, response to temperature change, and icefish evolution.

The metagenomic and metatranscriptomic responses of coastal phytoplankton assemblages to decreased relative irradiance: an in situ incubation experiment in the Western Antarctic Peninsula

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Antarctic phytoplankton provide vital contributions to carbon cycling and ecosystem trophic interactions in the Southern Ocean via their highly productive seasonal periods. As a result, phytoplankton serve as the basal layer that dictates rates of energy flow and carbon sequestration in Antarctic waters based primarily on their community dynamics. This pivotal role in biochemical processes make the factors influencing key phytoplankton community like composition, production, and nutrient usage crucial to understanding how future phytoplankton assemblages may shift under a fluctuating polar climate. Given already observable shifts in coastal phytoplankton community compositions, abundances, and carbon cycling over the past 20 years, this understanding is even more pertinent in modern times. Explorations into probable abiotic factors have created recent questions on how the effects of light may influence community parameters. This study aims to use meta-omic methods to approach these questions and examine how natural phytoplankton assemblages of the Western Antarctic Peninsula respond to a regime of decreasing ambient irradiance under in situ conditions. To do so, we are utilizing metagenomic, metatranscriptomic, and metalipidomic analyses to gain an in-depth understanding of how the specific diversity, function, and energy content of the communities will change under the experimental conditions. By utilizing a gradient of decreased light to simulate a deepening mixed layer, and using meta-omic tools to quantify dynamic shifts on a high-resolution scale, the results of this study will be significant to predicting changes in coastal phytoplankton community dynamics in the future Antarctic environment.

Disentangling the role of microbiomes in subantarctic carbon export South of Australia.

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The Southern Ocean (SO) is the most effective oceanic CO₂ sink globally driven in part by the biological carbon pump. The role played by phytoplankton in carbon export is increasingly well known. However, we lack a mechanistic understanding regarding the microbial community dynamics, ecology and evolutionary patterns especially across understudied biogeographic regions such as the SO. This is crucial for understanding the potential impact of global change on biogeochemical cycling in marine environments and potential feedbacks associated with these processes. We elucidate the role played by microbial communities by analysing samples collected from the subantarctic zone, using a Marine Snow Catcher (MSC) deployed 10 m below the MLD at the Southern Ocean Time Series (SOTS) south of Tasmania. A combination of metagenomics and nutrient analysis was used to clarify microbe mediated carbon export. Preliminary data show increased levels of particulate organic carbon (POC) from suspended to sinking fraction, implying that carbon export occurs within the first 2-hour under gravitational sedimentation. Metagenomic data from these samples are currently being used to generate metagenome assembled genomes (MAGS) following established pipelines. Functional annotation of these MAGS will reveal the repertoire of genes linked to carbon export. Taken together, these findings will provide a clearer understanding of the role of microbial communities on carbon export.

Colobanthus quitensis under CO₂ limitation: The response of oxalate oxidase and calcium oxalate crystals.

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The leaves of the Antarctic plant *Colobanthus quitensis* exhibit a reduced CO₂ diffusion, resulting in photosynthetic limitations. Nonetheless, this plant optimizes its carbon assimilation to survive the harsh Antarctic environment; but the mechanism used for *C. quitensis* to counteract the CO₂ diffusion is unclear. We tested whether *C. quitensis* possess oxalate oxidase enzymes (OxO), whose activity could be associated with the decomposition of calcium oxalate crystals to obtain CO₂, maintaining a basal level of photosynthesis, as it has been recently evidenced in other plants and proposed as an “Alarm photosynthesis”. Putative OxO enzymes were identified from the transcriptome by *in silico* analysis using phylogeny and docking tools. In addition, *C. quitensis* plants were placed in airtight chambers injected either with ambient air (~400ppm CO₂) or soda lime filtered air (~10ppm CO₂) for 10 hours. Crystal’s areas in leaves were monitored using polarized-light microscopy and digital image analysis; measurements of electron transport rate (ETR) and OxO activity were also performed for both treatments. A significant reduction in the leaf crystals area was observed in the CO₂-limited condition at the end of the experiment. Crystal decomposition was accompanied by increased OxO activity and a slight decrease in the ETR. Our results suggested that a CO₂ limiting condition is directly related with CaOx crystals decomposition. Consequently, the CaOx crystal decomposition might play a role as a complementary endogenous mechanism facilitating the CO₂ supply in the Antarctic plant *C. quitensis* to compensate the reduced CO₂ diffusion of leaves developed under its natural habitat.

Do Antarctic giant isopod juveniles have the molecular repertoire to cope with Southern Ocean warming?

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The effects of warming in early life stages of Antarctic marine invertebrates have rarely studied. Some studies evaluating the upper thermal limits of early juveniles compared to adults, suggest that juveniles are more resistant than adults; however, the molecular basis of this resistance has not yet been elucidated. Juveniles of the isopod *Glyptonotus antarcticus* were extracted from the marsupium to conduct differential gene expression analysis. Using a RNA-Seq approach, the response to thermal stress was evaluated by using pools of five isopods that were exposed to 5 ° C for 24 hours evaluating the response at 1, 6, 12 and 24 hours. Two control groups (1 and 24 h) were maintained at 1 ° C. In total, 18 separate RNAseq libraries from each temperature treatment were generated using the Illumina HiSeq2000 platform. Differentially expressed genes (between control and heat stress animals) were functionally enriched with processes relating to cellular stress, antioxidants and ubiquitination pathways. The classical chaperone response was not induced and some small Hsps were up-regulated. The cathepsin L and cuticle proteins related to molting and development were down-regulated genes. These results show that the transcriptomic response of *G. antarcticus* juveniles changes over time during thermal stress, however, they may not have the physiological plasticity necessary to deal with a further warming scenarios expected for future decades.

Genomics and Genome Mining of Extremophile Lichens in Antarctica

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Lichens represent some of the oldest and most diverse symbioses on Earth. Lichens consist of a photobiont (cyanobacterium/alga), a mycobiont (fungus), which together form a unique structure called the thallus in a symbiosis with multiple microorganisms. Lichens play a vital role in ecosystems as they are essential in soil formation, naked soil colonization, and nutrient uptake and release for plants. Lichens can colonize a wide range of substrates, from natural surfaces to man-made materials. They can also tolerate extreme environmental conditions and even resist outer space conditions.

Lichens in Antarctica are able to adapt to the cold and dry conditions through robust resistance to frost and photosynthetic capabilities. However, no study has attended to identify potential genes that might confer the capacity to survive such harsh conditions, and the functional roles of these genes in the symbiosis.

Here we analyzed varied species of lichens collected across locations in the South Shetland Islands and Graham Land in February 2020. We analyzed the lichens microbiome, identified extremophile microbes, and annotated genes that might be related to extreme conditions. Additionally, we found multiple unknown biosynthetic gene clusters (BGCs) that are related to the production of antimicrobials, indicating that lichens from Antarctica could be a source of new antimicrobials. Altogether, these results not only expand our knowledge of Antarctic's biodiversity and serve as a baseline for further multi-omics studies in lichens, but also evidence the potential of lichens in bioprospection and exobiology.

A comparative metagenomic analyses of the microflora present in both wild and captive colonies of Gentoo and Chinstrap penguins

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Penguin colonies in Antarctica leave their stamp on the land in the form of large guano deposits. The microbiota found within this largesse can tell us quite a bit about the nutritional and physiological well being of these penguin populations--both those found on around the Antarctic peninsula as well as their relatives in captivity--as this microbial flora is plays a significant role in metabolism, synthesis, and secretion of vital nutrients that sustain their host.

In February 2020, multiple samples of guano were collected from colonies of Gentoo (*Pygoscelis papua ellsworthi*) and Chinstrap penguins (*Pygoscelis antarcticus*) along Graham Land and the South Shetland Islands. Then, guano samples were likewise collected from colonies cared for in captivity at Central Park Zoo in New York City.

Our metagenomic analysis comparing the gut microbial flora present in those colonies that inhabit the Antarctic peninsula to those in captivity offers insight into the influence of containment and diet upon gut microbial population. This study is intended as a pilot toward an expanded, longitudinal investigation of additional colonies and species in the wild and in captivity.

Transcriptome analysis of hormone related genes induced by temperature changes in the Antarctic marbled rockcod *Notothenia rossii*

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Antarctic marbled rockcod *Notothenia rossii* belong to Antarctic notothenioid which is the most dominant fish fauna in the Southern Ocean. Antarctic notothenioid teleost have evolved to adapt to thermal stable and cold Antarctic Ocean. In this study, we examined differentially expressed hormone related genes regulated by temperature changes using RNA sequencing method. We investigated transcriptional differences of three different organs (liver, blood and brain) at heat shock stress (from 0°C to 4°C). We found that 19 steroid related genes and 31 hormone related genes were differentially expressed in brain and nine steroid related genes and 12 hormone related genes were differentially expressed in blood. Further in-depth study are need to correlate genetic information between hormonal metabolites and receptors according to temperature conditions and determine hormone types and receptor based metabolic functions by hormone profiling.

Multiple application potential of Antarctic *Micractinium* KSF0031

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Antarctic microalgae have several advantages. Their evolutionary adaptation to a wide range of habitats and extreme environments has allowed them to have an abundance of biological and genetic diversity, as well as to produce a variety of bioactive molecule. We have been conducting research to apply Antarctic microalgae from basic to applied research for multiple purposes.

We here introduce Antarctic freshwater microalga KSF0031, one of new species of the genus *Micractinium* collected on the snow surface on the South Shetland Islands, Antarctica. Based on the morphological and molecular characteristics, it was named *Micractinium variabile* sp. Nov. KSF0031 (hereafter referred to as KSF0031). We analyzed the transcriptome of KSF0031 and used nanopore sequencing to produce 4,617,230,585 bp and an average read length 4,957bp. The contig number was 103 and N50 contig size is 1.9Mbase. We are especially analyzing unsaturated fatty acid-related genes and gene coding low-temperature active enzyme proteins. Also, we are investigating the chemical composition and the biological function to reveal the antioxidant, anti-inflammatory, and anticancer potentials of Antarctic microalga KSF0031.

Comparative analysis of key genomic features identified in Antarctic blackfin icefish to the genome of the Antarctic bullhead notothen

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Antarctic fish (Notothenioidei) have adapted and evolved with developing cold-adapted genomic signatures, phenotypes, and physiology to survive in the earth's coldest marine environment. Notothenioideis are recognized in two types based on different physiological features, including the presence or absence of hemoglobin in their bloods. Antarctic bullhead notothen, *Notothenia coriiceps* comprises red-blooded fish (having hemoglobin, myoglobin and red blood cells in the blood) and has been used as a good model to understand the adaptation to the Southern Ocean due to high biomass and a wide range of distribution in Antarctica. Icefish such as Antarctic blackfin icefish, *Chaenocephalus aceratus* have colorless blood due to lack of hemoglobin and myoglobin gene clusters in the genome with the absence of red blood cells in the blood. To compensate for the lack of oxygen carrier protein, icefish have evolved to possess a large volume of blood, scaleless skin, enlarged head, and enhanced vascular system. Since the genome information of *N. coriiceps* and *C. aceratus* is available, comparative genomics and bioinformatics enable us to figure out how the key molecular pathways are different between Antarctic fish as well as in comparison with temperate fish. In this study, we compared two Antarctic fish genomes (*N. coriiceps* and *C. aceratus*) to discover commonly developed genomic features and unique characteristics. This result can be helpful to understand unique evolutionary trajectory of each Antarctic fish.

Identification of AP2 transcription factors responsible for the cold tolerance of the dominant Antarctic moss *Sanionia uncinata*, based on de novo genome assembly and genetic transformation approach

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Mosses in Antarctica grow mostly in coastal areas and are expected to have developed various unique physiological/molecular mechanisms to survive in extreme environments. *Sanionia uncinata* (Hedw.) Loeske is a dominant moss species in the maritime Antarctic and considered as a good target to investigate genes associated with abiotic stress tolerance of mosses. Here, we aimed to select and characterize function of a key transcription factor which induces the cold tolerance process in *S. uncinata* at the molecular level. At first, we report the draft genome sequence of an Antarctic *S. uncinata*, obtained using third-generation PacBio sequencing technology. About 1 million reads were attained from four Sequel sequencing runs and merged together into a single dataset of 21 Gb. The de novo assembly produced 673 contigs comprised of 621 Mb with an N50 contig length of 2.2 Mb and the longest contig length of 10.3 Mb, and a total of 28,651 coding genes were inferred. Based on the transcriptome analysis, we could find twenty genes containing AP2 DNA binding domain, showing transcriptional induction in response to cold stress treatment (2°C). Then to characterize their functions related to cold tolerance, we generated transgenic plants which constitutively express SuAPL genes using the model moss *Physcomitrella patens*, exhibiting significantly lower cold tolerance than the Antarctic moss. Some of transgenic mosses displayed enhanced freezing tolerance in terms of electrolyte leakage under freezing conditions, compared to wild-type. These results suggest that SuAPL have important functions in cold adaption of *S. uncinata* to extreme Antarctic environments.

The seasonal transcriptome dynamics of Antarctic moss, *Sanionia uncinata* (Hedw.) Loeske, throughout the year in natural environments, reveals the molecular adaptation of extremophile plants

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Maritime Antarctic is covered with snow and ice for most of the year, but during the austral summer season, snow melts and a variety of vegetation is revealed. During this short growing season, terrestrial plants actively perform cellular activities under the prolonged daytimes. But even in this short summer, the sub-zero temperatures, dryness, continuous strong light, and UV radiation are very extreme conditions for plant growth. And after these summers are over, the snow starts to pile up and the plants stop growing and enter the dormancy state in a long and dark winter. Here, in this study, we investigated the annual transcriptome response of extremophile plants in a natural environment. During the 2015-2016 winter/summer station study, we had sampled *Sanionia uncinata*, one of the dominant bryophyte species of the maritime Antarctic, naturally habituated in the King George Island (62°14' S; 58°44' W), on a monthly basis, and conducted transcriptome analysis. As a result, we could identify groups of genes that fluctuate with rhythmicity according to the seasonal changes. The weighted co-expressed gene network analysis enabled to construct a correlation network on climate-gene expression and to identify the groups of genes responding to changing climatic factors in a natural environment.

Mitochondrial diversity and physico-chemical environment modulate gene expression in the Southern Ocean bivalve *Aequiyoldia eightsi* species complex

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Regulation of gene expression is a pivotal adaptive mechanism of organism facing environmental variation. This regulatory capacity is constrained, however, by attributes inherent to organisms (genotypes). In this study, we analysed gene expression patterns and Single Nucleotide Polymorphisms (SNPs) across South American and Antarctic populations of *Aequiyoldia* shallow marine bivalves. We explored gene expression patterns in response to 1) natural environmental conditions on continental and local scales (1000 km vs 1 km) as well as 2) nuclear and mitochondrial genotypes. We validated our results by contrasting the observed magnitudes of differential expressed genes (DEGs) with magnitudes expected by chance in randomized group comparisons. Trans-Drake comparison resulted in highly distinct nuclear and mitochondrial SNP compositions coupled with striking differences in DEGs. Mitochondrial SNPs divided Antarctic animals into two groups, each composed by organisms featuring mitochondrial homoplasmy and heteroplasmy (in total: four mitotypes). Mitotype pattern was not reflected in nuclear SNPs. Habitat comparison (animals from rocky, sandy, muddy grounds) revealed clear differences in DEGs at local scale in Potter Cove, without significant nuclear or mitochondrial genetic structure between stations. Interestingly, differential expression analysis between mitotypes resulted in a number of DEGs in the same order of magnitude as driven by the environment, distinguishing heteroplasmic from homoplasmic organisms at a level well above the number of DEGs expected by chance. Field-based transcriptomics provide an unprecedented understanding of the mechanics of local adaptation and will prove especially valuable in times of rapid climate change.

Functional resilience of nitrogen fixation over a salinity gradient on the McMurdo Ice shelf

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The ability of ecosystems to accommodate to environmental change is dependent on the ability of keystone taxa and communities to respond to perturbations through resistance or resilience mechanisms. Functional resilience describes sustained critical ecological processes through shifts in composition or physiological flexibility in the face of environmental change, critical to ecosystem integrity. In Antarctic terrestrial aquatic environments, cyanobacteria dominated microbial mats are fundamental components providing biomass, productivity and critical biogenic habitat. In many locations this includes driving nitrogen dynamics through nitrogen fixation. In this study we analyse the ability of microbial mats to sustain nitrogen fixation capacity across a range of environmental conditions. The work was carried out on the McMurdo Ice Shelf in ponds with diverse chemical compositions, the 5 selected ponds spanned a range of conductivities from 400 to 28,400 $\mu\text{S}/\text{cm}$, all with biomass dominated by microbial mats. In these ponds there is evidence to show ponds can rapidly shift to more saline or fresh with changing hydrologic balance. In each pond mat nitrogen fixation was measured using the acetylene reduction assay, and mat community composition characterised using 16s rRNA gene sequencing and microscopy. Nitrogen fixation was sustained at comparable rates over the entire salinity range, while the dominant nitrogen fixing cyanobacteria transitioned from Nostocales to Nodulariales as salinity increased. We conclude that, in respect of nitrogen fixation, microbial mats in Antarctic ponds show functional resilience to changes in pond salinity through turnover of dominant diazotrophs.

Molecular insights into phytoplankton assemblages along natural iron gradients at the West Antarctic Peninsula

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The West Antarctic Peninsula (WAP) is a highly productive coastal environment where phytoplankton, mainly diatoms, form the base of a rich, polar marine food web. However, the WAP has experienced significant warming over the last 50 years and may serve as a sentinel for climate change in Antarctica. Long-term studies of phytoplankton community diversity, using high-performance liquid chromatography or the 18S rDNA gene marker, have suggested transient limitation by the micronutrient iron (Fe); however, the impact of climate change on phytoplankton molecular ecophysiology, using gene expression, remains underexplored. To better understand whether diatoms, and other phytoplankton functional groups differ in their gene expression responses to changes in Fe availability, a comparative analysis of phytoplankton assemblages from inshore and offshore regions of the WAP were performed using metatranscriptomic approaches. By using next-generation sequencing technology, we obtained thousands of differentially expressed genes representing the full complement of iron metabolism and transport, as well as photosynthesis, iron homeostasis, and N assimilation. Additionally, specific gene expression patterns (e.g. iron starvation induced proteins and genes encoding iron-free proteins) corresponded to diatom iron physiological status, thus representing potential molecular indicators for iron limitation. This research provides new insights on the mechanisms that may underpin physiological responses of ecologically relevant WAP phytoplankton species to variations in iron availability and their potential effects on ecosystem processes and carbon export.

Fungi present in lacustrine sediments of Antarctic Peninsula

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In lacustrine ecosystems of Antarctica fungi play a fundamental role in decomposition of organic matter suspended in water. Considering that little is known about the diversity and ecological roles of fungi in these habitats, we evaluated 8 different lakes of Peninsula Antarctica. A total of 195 fungal isolates were recovered, and among them 42 taxa were classified in phyla Ascomycota, Basidiomycota, and Mortierellomycota. The fungal assemblages were rich and diverse, composed principally of cold cosmopolitan and psychrophilic endemic taxa recognized as decomposers, symbiotics and pathogens. Some of the identified species such as *Thelebolus globosus*, *Antarctomyces psychrotrophicus*, *Pseudogymnoascus verrucosus*, *Vishniacozyma victoriae*, and *Phenoliferia* sp. were frequently in our sediment samples. Other potential new species were also detected in this work. In addition, we detected fungal taxa and isolates able to produce bioactive compounds that may represent the source of prototype molecules for applications in medicine and agriculture. The dynamics and balance of this fungal community represents an interesting aquatic web model for further ecological, evolutionary and biotechnological studies under extreme conditions and potential climate changes in these regions.

Assessment of uncultured fungal diversity in lake sediments of Vega Island, Antarctica

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Vega Island has many lakes and ponds which are a potential archive for studies of climate and environmental changes in Antarctica. Historically, Vega also hosts many species of lichens, plants, animals and associated microbiota. In addition, the island is considered an important place for paleontology studies. In this pioneering study we evaluate fungal diversity in lake sediments, using DNA metabarcoding through high throughput sequencing (HTS), never mentioned in the literature for Vega Island. A sample of Copepodo and Pan Negro lakes were collected using pvc pipes, and 500 mg of sediment, from the central portion of the core, were used for DNA extraction. The previous taxonomic annotation showed OTUs associated with the phyla Ascomycota, Basidiomycota, Mortierellomycota and the zoosporic fungi Rozellomycota. A total of 111 taxa were found and clustered in 11 classes, 26 orders, 44 families, 69 genera, and 67 species, and among them 32 species were shared between the two lakes, and some of them were not yet described for lake environments from Antarctica. The most abundant taxa were *Pseudogymnoascus appendiculatus*, *Leotiomyces* sp. and *Pseudogymnoascus roseus*, and 44 taxa did not correspond with species deposited on UNITE database and can represent species that still unknown to the Antarctic environment.

Identification of fungi present in a lake environment on King George Island, Antarctica

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In order to know the microbial portion present in the Antarctic lakes, the present study aimed to assess the diversity of the fungal community present in string baits suspended in a lake in Punta Hennequin, King George Island, Antarctica. Polystyrene bags containing three fragments of sterilized cotton string were launched and fixed at two points on the lake, where they remained for two years. The string fragments were processed in duplicates using Dichloran agar, minimal medium, Marine agar, and malt extract agar. Forty-eight filamentous fungi and 49 yeasts were obtained. Among these, 83 were identified as belonging to the genera *Thelebolus*, *Dactylaria*, *Goffeauzyma*, *Phenoliferia*, *Vishniacozyma*, *Chalara*, *Pseudogymnoascus*, *Mortierella*, *Mrakia*, and *Leucosporidium*. Our results indicate that the Antarctic lakes represent habitats for endemic and cosmopolitan fungi, which can present several physiological adaptations under extreme conditions, which may be useful to use in biotechnological processes.

Uncultured fungal diversity in Antarctic marine sediments

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In the present study, we evaluate the uncultivable fungal diversity of marine sediments of Maxwell Bay, South Shetlands, Antarctica using DNA metabarcoding through high throughput sequencing (HTS). Marine sediments of 150 m and 250 m depth were sampled using a gravity corer. Aliquots of 15 g of sediment were suspended in 0.9% saline and the supernatant filtered in 0.44 µm membrane, which was used for the DNA extraction. The taxonomic annotation showed OTUs associated with the phyla Ascomycota, Basidiomycota, Mortierellomycota, and Mucoromycota. OTUs of 107 different taxa were detected and clustered in 14 classes, 29 orders, 44 families, 56 genera, and 54 species. Some of these species such as *Malassezia globosa*, *Malassezia restricta*, *Mortierella alpine*, *Mortierella fimbricystis*, *Mortierella gamsii*, *Phaeosphaeria dennisiana*, *Pseudogymnoascus roseus* and *Rhodotorula mucilaginosa* occurred in the sediments of 150 and 250 m depth. *Leotiomyces* sp., *Pseudogymnoascus roseus* and *Diaporthales* sp. were the most abundant taxa (15734, 11330, 3721 reads respectively). In addition, 53 OTUs did not correspond with any species deposited on database and may represent fungi propagules that still unknown to the Antarctic environment.

Uncultured fungal diversity in permafrost of the Antarctic Peninsula

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The aim of the present work was to access the fungal permafrost community of the Deception and King George islands, Antarctica using the DNA metabarcoding through high throughput sequencing (HTS). Twenty-three operational taxonomic units (OTUs) were detected. In the permafrost of Deception Island, *Aspergillus restrictus*, *Scheffersomyces amazonenses*, *Rhodotorula mucilaginosa*, *Mortierella* sp. 1, and *Mortierella* sp. 2 were detected. In permafrost of King George Island, *Aspergillus westerdijkiae*, *Penicillium brasilianum*, *Penicillium chrysogenum*, *Pseudogymnoascus* sp., *Candida* sp., *Leucosporidium creatinivorum*, *Rhodotorula mucilaginosa*, *Mortierella* sp. 1, and *Mortierella* sp. 2 were found. Some taxa detected represent the first records in Antarctic permafrost. Fifteen OTUs represented unclassified taxa might represent new species. The diversity, richness, and abundance between the permafrost of the two islands were significant and further studies are necessary increase the knowledge of the real fungal community in Antarctic permafrost.

Identification of fungi present in biofilm of Kroner Lake, Deception Island, Antarctica

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In the Antarctic environment, lakes represent untapped habitats that can be used to study microbial diversity and ecology in extreme conditions. The present study evaluated the diversity of the fungal community present in biofilm sampled at Kroner Lake, Deception Island, Antarctica. The biofilm was collected in 15 points of the lake and the samples processed in the culture media Sabouraud dextrose agar, Marine agar, and incubated at 10° C. Forty-four filamentous fungi and 22 yeasts were obtained. Of these, 50 were identified as belonging to the genera *Metschnikowia*, *Antarctomyces*, *Pseudogymnoascus*, *Mortierella*, *Tricellula*, *Leucosporidium*, *Holtermanniella*, *Vishniacozyma*, *Arthroderma*, *Rhodotorula*, and *Debaryomyces*. The species found in the biofilm include endemic or cosmopolitan taxa and may be responsible for the primary decomposition process of organic materials.

Sex determination mechanisms in Antarctic Notothenioid fish

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Antarctic notothenioid fishes provide an iconic example of a marine species flock. What evolutionary mechanisms propelled this spectacular burst of biodiversity? Reproductive isolating mechanisms can include the origin of new sex determination genes and new sex chromosomes. To uncover sex-linked loci in notothenioids, we used RAD-seq and pool-seq to identify sex-specific genetic polymorphisms in populations of males vs. females in several notothenioid species and mapped reads along genome sequences. For blackfin icefish (*Chaenocephalus aceratus*), results identified a polymorphism on ancestral notothenioid chromosome-9 (chrAN9) that was fully linked to phenotypic sex. This sex-linked locus resides between tandem duplicates of *bmpr1ba* (called *bmpr1ba.1* and *bmpr1ba.2*), which, along with *bmpr1bb*, represents a teleost duplicate of human *BMPR1B*. The mammalian *Bmpr1b* protein is part of the *Amh* receptor dimer, the other part of which (*Amhr2*) is the major sex determination gene in several fish species. A male-specific single nucleotide polymorphism in *bmpr1ba.2* is predicted to strongly alter protein function, likely acting as a dominant, Y-chromosome-linked, constitutive activator of testis development. For South Georgia icefish (*Pseudochaenichthys georgianus*), we found a sex-linked locus on chrAN12 that contains a tandem duplication of three genes, including *gsdf* (gonadal soma derived factor), variants of which are the major Y-chromosome sex determination gene in several fish species. All icefishes examined have tandem duplicates of both *bmpr1ba* and *gsdf*, suggesting that different lineages appropriated different pre-existing tandem duplications to become sex determination genes. This finding is predicted if new sex determination genes helped promote lineage diversification in the Antarctic notothenioid radiation.

Comparative genomics in the search for adaptations to life on low temperatures in the *Metschnikowia* genus

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The *Metschnikowia* genus comprises ascomycetous yeasts from diverse environments. Besides the flower associated species there are also aquatic species, macroalgae associated and micro crustaceans' parasites. *M. bicuspidata*, the most widespread, is found on almost every ocean and many temperate lakes. Despite being freeze tolerant, *M. bicuspidata* is not present in Antarctic Ocean. *M. australis*, a closely related species, occupies its niche on these southern waters. Also freeze tolerant, *M. australis* performs better at low temperatures, as we show in this study. We have sequenced *M. australis* genome and investigated it together with *M. bicuspidata* and other 33 publicly available *Metschnikowia* genomes searching for freeze tolerance associated elements. All genomes were predicted and annotated using the same pipeline, and 1317 common single copy orthologous genes were used to reconstruct these yeasts phylogeny. We observed that *M. australis* has a smaller genome, with less predicted coding sequences and repetitive content in comparison to *M. bicuspidata*. We have also developed a homology-based network approach to visualize and identify orthologous genes shared among genomes, which shows paralogous expansions shared by *M. australis* and *M. bicuspidata* genomes and also exclusive to each organism, which may relate to adaptations to cold environments. 249 *M. australis* exclusive CDSs were analyzed by 3 Antifreeze protein classifiers to select the 17 most prominent candidates for in vivo detection. We found that 14 of those are expressed at 4°C. Most have no similarity to any known gene, and future analyses will be done to identify their influence in freeze tolerance.

Uncultured cryptic fungal diversity in soils of Deception Island, Antarctica: taxonomy and distribution in protected and non-protected areas using an NGS approach

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We assessed the soil micro-fungal diversity in soils from two sites on Deception Island, Antarctica using DNA metabarcoding through high throughput sequencing (HTS). The first site was within an Antarctic Specially Protected Area (ASPA 140), representing an undisturbed area, and the second was at Whalers Bay, a historic site that is popular with visitors. We detected 346 fungal OTUs dominated by the phyla Ascomycota Basidiomycota, Mortierellomycota, and Mucoromycota. We also detected taxa of the rare phyla Chytridiomycota and Rozellomycota, which occurred with moderate frequency in both areas and are often difficult to detect using traditional isolation methods. A total of 127 OTUs were detected in the ASPA, 142 from Whalers Bay, and 113 were common to both areas. The fungal assemblages at both sites displayed high diversity, richness, and dominance indices. Sixty-five OTUs were considered dominant (those with >1,000 reads), with *Cladosporium* sp., *Pseudogymnoascus roseus*, *Leotiomyces* sp. 2, *Penicillium* sp., *Mortierella* sp. 1, and *Mortierella* sp. 2 achieving >45,000 reads). Additionally, 23 OTUs were identified only at higher taxonomic levels (phylum, class, order, and family) and may represent new taxa and/or new records to Antarctica. Our data indicated that the soil micro-fungal diversity in the protected area was similar to that in the highly visited area. The overall diversity detected included a combination of apparently endemic and cosmopolitan taxa.

Cultured fungal diversity in permafrost of Antarctic Peninsula

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We accessed the diversity and distribution of the cultivable fungi present in permafrost and active layer obtained from Robert, Livingston, and Deception islands, Antarctica. Using different culture media, 58 fungal taxa were identified, which 27 from permafrost and 31 from the active layer; however, only 5 occurred in both microhabitat. *Mortierella* and *Pseudogymnoascus* were the most and abundant taxa. The fungal diversity found in the different sites was moderate to high. Forty taxa could not be identified in species level and may represent new species. Our results demonstrate that permafrost shelter viable fungal species with moderate to rich diversity assemblages across the Antarctic Peninsula. The Antarctica Peninsula represents the major area on earth under effects of the global warm climate changes, and permafrost represents a concern with the release of trapped gases and microorganisms. Along with the physico-chemical analysis, the results obtained in this study suggest that the geological and biological history of these soils is the factor responsible for shaping the fungal community in these environments.

Southern Ocean Acidification impacts: calcifying vs non-calcifying species

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Southern Ocean organisms are thought to be particularly vulnerable to ocean acidification (OA), as they inhabit cold waters where calcite-aragonite saturation states are naturally low. It is also generally assumed that calcifying animals would be more affected by the OA. In this context, we proposed to study the impacts of low-pH on two dominant species from an Antarctic fjord, one non calcifying, the ascidia *Cnemidocarpa verrucosa* and the bivalve *Yoldia eightsii*. We report results of ~2 months of experiment, comparing the molecular mechanisms underlying responses under two pCO₂ treatments (ambient and low-pH). Enzymatic activity (spectrophotometry) and RNAseq using mass sequencing techniques were performed. Significant effects were found in caspase (Mann-Whitney; W=26.00; p=0.02) in *C. verrucosa* at 2 months in low-pH condition. There was an increase in the regulation of 224 genes and a decrease in the expression of 111 (FC \geq 2; p-value \leq 0.05). Particularly, low-pH caused an up-regulation of genes involved in the immune system and antioxidant response. While in the infaunal bivalve *Y. eightsii* less differentially expressed genes were observed, an up-regulation of 34 genes and a decrease in the expression of 69 genes were found (F \geq 2; p-value \leq 0.05). This work addresses the effect of OA in two abundant species from Potter Cove and surprisingly showed a more pronounced response in the non-calcifying species. The present approach is being carried out in other species, such as corals, limpets and snails, which in turn will allow us to increase the understanding of the response capacity of Antarctic coastal ecosystems to climate change.

Diversity of total and cultivable siderophore producing marine bacteria: influence on phytoplankton growth in the Indian sector of Southern Ocean

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Indian sector of Southern Ocean (ISO) is a climate-sensitive region for global biogeochemical cycles and ocean warming in response to climate change. Microbes are key drivers of important biogeochemical processes. Yet, diversity and abundance of microorganisms and their interactions with phytoplankton population remain poorly studied in ISO, particularly with regards to diversity of total bacterial forms and cultivable forms of siderophore producing bacterial population. Here, we present first set of amplicon sequencing of 16S rDNA to identify total bacterial population diversity. We also present the cultivable siderophore producing bacterial diversity from ISO. Gammaproteobacteria was found to be dominant group in various fronts of ISO, while light harvesting alphaproteobacteria dominated in polar stations. Bacteroidetes, Cyanobacteria and Deferribacteres were dominant bacterial phyla other than Proteobacteria. The cultivable bacterial population was predominantly derived from Gamaproteobacteria, Actinobacter and Bacilli and tested positive for siderophore production. Selected siderophore producing bacterial isolates were grown along with two most abundant phytoplankton *Gephyrocapsa oceanica*, a coccolith and diatom (*Skeletonema costatum*) from different oceanic environments to test the effect of siderophores and iron on plankton growth and plankton bacterial interaction. Results clearly indicate that growth of *Gephyrocapsa oceanica* was always stimulated in presence of bacteria with or without iron and siderophores. In case of the diatom, presence of bacteria suppressed the growth of the diatom. Our experiment shows that bacterial-phytoplankton interactions are species specific and same bacterial species elicit variable response to different phytoplankton species. Such species-specific responses can have wider implications in Southern Ocean biogeochemistry.

Integration of comparative and functional genomics as a tool for linking phylogeny to function in cold temperature environments

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Traditionally microbial surveys have been based off from 16S rRNA genes which have been used to infer the phylogenetic identity and functional potential of organisms. While these surveys are easily completed it is not resolved whether organisms with similar 16S rRNA sequences behave similarly in the environment. In contrast to 16S rRNA surveys genomics facilitates the study of the genetic information of an individual organism. To understand how the selective pressures of the Antarctic influence genetic adaptations we investigated the genomic traits of a *Janthinobacterium* spp. in comparison to genomes of 35 published *Janthinobacterium* species. We hypothesized that genome shrinkage and specialization to narrow ecological niches would be energetically favorable for dwelling in an ephemeral Antarctic stream, however, the genome of *Janthinobacterium* sp. CG23_2 was on average 1.7 ± 0.6 Mb larger and predicted 1411 ± 499 more coding sequences compared to the other *Janthinobacterium* spp. Putatively identified horizontal gene transfer events contributed 0.92 Mb to the genome size expansion of *Janthinobacterium* sp. CG23_2. We aimed to determine whether genome size was correlated to increased functional capacity. Our data suggest that genome plasticity and the abundant complementary genes for sensing and responding to the extracellular environment supported the adaptation of *Janthinobacterium* sp. CG23_2 to this extreme environment. Additionally, to understand the functional role of individual organisms in Antarctic environments we have recently been focused on developing strategies to analyze active fractions of microbial assemblages in order to determine the ecological function of individual organisms.

Evolution of the immune system in two Antarctic Notothenioids

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Fish rely significantly on innate immune response, extremely important for survival in aquatic environments, rich in bacteria, parasites, fungi and viruses. Antarctic notothenioid fish evolved from 25 Myears ago in a very cold and stable environment through adaptive radiation, likely developing specific immune responses and host-parasite interactions. Little is known about the functioning and adaptability of their immune system, an issue as climate change can lead to occurrence of novel pathogens or disruption of microbial balance in Antarctic Ocean.

Experiments with *Notothenia coriiceps* and *N. rossii* were performed in Great Wall Station, King George Island, in Antarctic summers of 2017 and 2019. The experimental design included groups kept at 2°C and 6 °C (n=6/8), treated with LPS or Poly:IC in different exposure time/routes. Plasma and tissues involved in the immune process were collected, and functional assays performed. Lysozyme and antitrypsin show differential responses to LPS and Poly:IC at both temperatures. Transcriptomic analysis in headkidney showed that gene networks related to WBC production are active in LPS-challenged fish and genes related to innate response are differentially expressed in all interface tissues. Skin and gut microbiomes revealed tissue specific diversity and different community responses to these challenges, with skin showing higher variability. Comparative in silico analysis of TLRs gene family showed that several genes evolved under positive pressure of the extreme conditions and novel TLR genes were characterised.

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Sex identification from distinctive gene expression patterns in Antarctic krill (*Euphausia superba*)

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Antarctic krill (*Euphausia superba*) is a highly abundant keystone species of the Southern Ocean ecosystem, directly connecting primary producers to high-trophic level predators. Sex ratios of krill vary remarkably between swarms and this phenomenon is poorly understood, as identification of krill sex relies on external morphological differences that appear late during development. Sex determination mechanisms in krill are unknown, but could include genetic, environmental or parasitic mechanisms. Similarly, virtually nothing is known about molecular sex differentiation. The krill genome has to date not been sequenced, and due to its enormous size and large amount of repetitive elements, it is currently not feasible to develop sex-specific DNA markers. To produce a reliable molecular marker for sex in krill and to investigate molecular sex differentiation we therefore focused on identifying sex-specific transcriptomic differences. Through transcriptomic analysis, we found large gene expression differences between testes and ovaries and identified three genes exclusively expressed in female whole krill from early juvenile stages onwards. The sex-specific expression of these three genes persisted through sexual regression, although our regressed samples originated from a krill aquarium and may differ from wild-regressed krill. Two slightly male-biased genes did not display sufficient expression differences to clearly differentiate sexes. Based on the expression of the three female-specific genes we developed a molecular test that for the first time allows the unambiguous sex determination of krill samples lacking external sex-specific features from juvenile stages onwards, including the sexually regressed krill we examined.

Gene expression pattern analysis provides insights into the cold-adaptation mechanisms of an Antarctic yeast, *Glaciozyma antarctica* PI12

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Psychrophilic yeast, *Glaciozyma antarctica* PI12 from the sea ice in the Southern Ocean has adapted well to grow and survive at low temperatures. However, the information on its adaptation strategies and mechanisms at the molecular level is limited. Hence this project was carried out to sequence and analyze the whole transcriptome to understand the cold-adaptation strategies of *G. antarctica* PI12. *G. antarctica* PI12 cells, grown in YPD medium at its optimal growth temperature of 12°C were exposed to freeze stress at 0 and -12°C for 6 h and 24 h. A hundred and sixty-eight genes were differentially expressed and the gene expression patterns were dependent on the severity of the cold. More genes differentially expressed at -12°C when compared to 0°C. *G. antarctica* PI12 was found to share some common adaptation strategies with other yeasts, namely, *Saccharomyces cerevisiae*, and *Mrakia* spp. At the same time, it has some unique strategies and mechanisms too. Among the strategies were the production of anti-freeze protein to prevent ice-crystallization inside and outside the cell. Additionally, several molecular chaperones, detoxifier of reactive oxygen species (ROS), and transcription and translation genes were constitutively expressed in *G. antarctica* PI12 to ensure the cells were always prepared to endure the fluctuating freezing temperatures. Unexpectedly, *G. antarctica* PI12 was found to use nitrite as an alternative terminal acceptor of electrons when the oxygen level was low. These mechanisms coupled with several other common mechanisms ensured *G. antarctica* PI12 adapted well to the cold temperatures.

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