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**GENOMIC INSIGHTS INTO PAST AND
PRESENT ANTARCTIC BIODIVERSITY**



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ABSTRACTS SUBMITTED TO THE (CANCELLED) SCAR 2020 OSC IN HOBART

Biodiversity in Antarctic shallow benthic hard-bottom communities: metabarcoding as a tool for biodiversity assessment

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Marine benthic communities living in three-dimensional structures built mostly by sessile suspension feeders, the so-called “animal forests”, are among the most diverse ecosystems in the Biosphere and play a crucial role for planetary ecosystem services. Documenting the diversity of marine life is challenging because many species are cryptic, small, and/or rare, and belong to poorly known groups. Exhaustive and consistent qualitative or quantitative biodiversity assessments are particular challenging in complex hard substrates of Antarctica. Biodiversity assessment methods have recently been revolutionized by the application of metabarcoding techniques. In the present study, we used metabarcoding to characterize the complex communities inhabiting marine hard substrates in Antarctica for obtaining baseline inventories for future monitoring and management. Samples were taken by scuba diving along the West Antarctic Peninsula and the South Shetland Islands. All rocky-bottom communities (three replicates each) were sampled by carefully scraping a 25 × 25 cm quadrat and the samples were separated into three size fractions (mega, macro, and meiobenthos). A total of 99 samples were metabarcoded with the COI marker. The number of total MOTUs detected from all samples by Bayesian clustering was about 3000 from which around 2000 (66%) could be taxonomically assigned to the level of phylum or lower. The undetectability of some minor groups in this study is possibly related to the incompleteness of reference databases for Antarctic organisms. The application of metabarcoding techniques to characterize marine hard bottom communities will contribute to a reproducible eukaryotic biodiversity assessment of structurally complex communities.

Reconstructing past Antarctic ecosystems using ancient DNA

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The study of ancient DNA from sediments (sedaDNA) is a new tool to characterise past marine ecosystems from deep ocean sediments, and has great potential for paleoclimate research. Recent improvements in ship-board sediment coring procedures have allowed far greater levels of contamination control and, along with refinements in sedaDNA sample processing, sequencing and bioinformatic techniques, now make the application of ancient DNA techniques to marine sediments eminently realistic. Our previous research has shown that sedaDNA from marine eukaryotes (including phytoplankton and higher organisms) is invariably low (~1.5% of metagenomic shotgun data when using the taxonomically informative marker gene 'small subunit ribosomal RNA' as reference). Therefore, we developed an optimised protocol for the extraction of ancient eukaryote DNA from marine sediments, achieving a broad eukaryotic biodiversity signal while retaining highly-damaged small DNA fragments, which are characteristic of ancient DNA. Using our new technique and further optimised bioinformatic pipelines, we were able to extract and analyse sedaDNA from deep ocean sediment cores collected off East and West Antarctica (Totten Glacier/IN2017_V01, Iceberg Alley/IODP Exp. 382). Our preliminary genetic community data revealed the presence of major groups of phytoplankton (e.g., diatoms, dinoflagellates) and microzooplankton (e.g., ciliates, foraminifera), as well as higher organisms such as crustaceans, molluscs and cnidarians, which are not normally part of the fossil record. These results demonstrate the breadth of biodiversity estimates possible through the application of novel sedaDNA techniques to Antarctic marine sediments, enabling the reconstruction of marine ecosystems across the food-web and through time in this climatically important region.

Out of Africa and across the sub-Antarctic: an enigmatic beetle radiation unveiled

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The origin and evolution of Southern Ocean Island faunas have vexed naturalists for centuries. In an unprecedented genomic effort for the region's fauna, we have unravelled the evolutionary history of one of the most enigmatic species radiations across these islands: that of the endemic, flightless Ectemnorhinini weevils. Analysis of 515 genes from more than 80 weevil species globally reveals that the closest relatives of the Ectemnorhinini are African, providing support for early ideas about Southern Indian Ocean island affinities. Molecular dating provides a crown age for the group in the Eocene (approx. 40 Ma), in keeping with some of the oldest known subaerial portions of the Kerguelen islands. Evolution has thus been sustained in situ throughout island uplift, submergence, volcanism and glaciation, although a high rate of extinction is also evident from the phylogeny. Molecular biogeographic analysis reveals the Crozet islands to be a central source of diversity for the group, again in line with previous theories and suggestive of significant within-archipelago speciation. Despite their flightlessness, the weevils have dispersed extensively throughout their evolutionary history; not only from Africa but also repeatedly between islands and often against the prevailing West Wind Drift. Thus, we consider birds a likely mode of transport for these species. Whereas most weevils globally have radiated in parallel with flowering plants, this unique tribe diversified as the climate cooled and bryophyte-dominated fellfield habitat came to dominate the sub-Antarctic.

Antarctic Biocrusts: Unravelling their composition and functionality in the cold drylands

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Antarctica is characterized by having some of the most severe climatic conditions for life. Despite this, it is possible to find microorganisms and plants growing under these adverse conditions. In this environment, one of the most conspicuous biological formations are the biocrust, which are microbial communities keystone in the ecosystem functioning where they are developing, however in the Antarctica the studies of this formations are limited. We aimed to address these following questions: Who organisms to participate in the formation of the biocrusts? What is the role of biocrusts in the nutritional aspects of soils? What is the effect of biocrusts on the growth of the vascular plants? To address these questions, samples of biocrust and individuals of *Colobanthus quitensis* were obtained from King George Island. To identify composition of biocrust, DNA was extracted and characterized using metagenomics sequencing of 16S and ITS. Additionally, manipulative soil experiments with and without biocrusts were conducted in order to assess the role in the nutritional quality of soils as well as on the growth of *C. quitensis*. Our results showed high taxonomic variability in the composition of the biocrust, with dominance of proteobacteries, and cyanobacterial. In relation to nutrient content levels were higher in soils with biocrusts compared to bare soil; additionally, this higher nutrient content was associated with a greater final biomass of *C. quitensis*. Thus, our results suggest that biocrusts have a positive impact in Antarctic plants and soils by improving nutrient content, with significant impacts in the plant Antarctic communities.

A genome reduced-representation approach (3RAD) to understanding the population structure of the Antarctic toothfish in the Weddell Sea and beyond

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The Antarctic toothfish (*Dissostichus mawsoni*) has a critical role in Southern ecosystems as a top fish predator. There is a near total lack of knowledge of the life history and population structure of Antarctica toothfish in the Weddell Sea. Based on the hypotheses that have arisen from the Ross Sea, a possible life cycle of Antarctic toothfish comprises juvenile development on nutrient rich continental shelf areas, followed by passive transport via gyre systems to offshore sea mounts, where spawning occurs, prior to completion of the cycle as fish are passively transported back towards the coast.

Genetics approaches offer the possibility to test between population hypotheses using the metric of relatedness between individuals. RADseq methods reduce the complexity of a genome in order to highlight interindividual variation without the need for whole genome sequencing. While many variants of this approach have been developed, our group has optimized the implementation of the 3RAD approach, which addresses many of the limitations of existing RADseq approaches including cost, adapter dimer and chimera formation, and DNA quantity required.

This presentation will share the results of our development of 3RAD in Antarctic toothfish, with the goal that our approach be available for use in the community in order to address gaps in our knowledge of Antarctic toothfish genetics structure in the Weddell Sea and on a circumpolar scale.

A deep look into the Photosynthetic Pico- and Nano-eukaryotic diversity across Antarctic (Ross Sea) and subantarctic waters (Campbell Plateau) of the SW Pacific sector

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Photosynthetic pico-eukaryotes (PPE) and photosynthetic nano-eukaryotes (PNE) are key components of the Southern Ocean. Traditional genomic techniques used to describe these communities tend to overlook the small phytoplanktonic fraction and hence we still know little about the composition and functionality of this component. In this work, we investigated the taxonomic composition of PPE and PNE communities in two contrasting oceanic regions: the subantarctic region of Campbell Plateau (autumn) with distinct HNLC conditions (ON vs. OFF of C. Plateau) and the oceanic and slope region of the Ross Sea, surveyed during two oceanographic voyages conducted in March 2017 and February-March 2018, respectively. We compared the molecular diversity obtained from PPE and PNE populations sorted by flow cytometry (FCM) as well as from the whole community collected in 0.8 µm filters (Filtered) using high throughput sequencing of the V4 region of 18S rRNA. We obtained a total of 5825159 sequences and 1515 Amplicon Sequence Variant (ASV) for the sorted samples. Chao1 and Shannon diversity index were overall greater for the sub-Antarctic region. Samples from the Antarctic region were dominated by 8 ASV corresponding to Ochrophyta, Haptophyta and Dinoflagellata, with the main abundance of *Phaeocystis antarctica* and *Fragilariopsis* sp. While the Subantarctic region were mainly dominated by 2ASV, corresponding to Chlorophyta (mamiellales) and Haptophyta (phaeocystales). FCM-sorted samples denote a higher sequencing coverage among the PPE and PNE community compared to Filtered samples. Overall this approach allows a better understanding of the diversity of the small photosynthetic eukaryotes on the Antarctic and Subantarctic ecosystem

Unraveling the core-genome of the Antarctic picocyanobacterial lineage

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Antarctic cyanobacteria are widespread in aquatic biotopes and often dominate the phototrophic biomass. Evaluation of the influence of environmental selection and historical processes on their current diversity and distribution is challenging, especially in relation to the question of endemism. Recent studies have shown that depending on taxonomic resolutions and molecular methodologies, the answer could be different.

Here, we focus on molecular traits involved in mechanisms underlying the ecological success of the picocyanobacterial lineage in Antarctica. Based on an extensive sampling of *Cyanobium*/*Synechococcus* genomes, we perform a comparative analysis to determine genes that are specific to Antarctic genomes and find the ones linked to functional adaptations.

We reconstructed the genomes of 8 strains from the BCCM-ULC Cyanobacteria Culture Collection (Liège, Belgium). 19 genomes assemblies were then retrieved from NCBI database to conduct a pan-genome analysis (5 Antarctic, 1 Arctic, and 21 non-polar genomes). Phylogenomic tree based on an alignment of sequences of 36 concatenated ribosomal proteins confirmed that the Antarctic genomes represent a distinct lineage.

From the 7286 groups of orthologous genes (OGs) defined for these 27 genomes, 1840 (25,3 %) constituted the Antarctic-specific core-genome and are mainly shared with non-Antarctic genomes. Further analyses are being carried out to analyze in more detail the core-genome, genes only found in Antarctica, gene duplication events and to determine potential selective pressures occurring on these genes. From an environmental protection perspective, in which the question of endemism is decisive, highlighting the specificity of Antarctic genomes could be determining.

Characterization of the microbial community of the Dalton polynya

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The Southern Ocean plays a pivotal role in the global carbon cycle. Antarctic coastal polynyas especially represent hotspots for primary production, harbouring the highest biomass of primary producers on the continental shelf. Climatic changes in this region have potential to impact on the magnitude of the Southern Ocean to act as a carbon sink with consequences that will reverberate across global ecosystems. The Dalton polynya in the Sabrina Coast is within the most active polynyas for East Antarctica. However, the continuous input of melting freshwater from the Totten glacier has created a highly stratified environment. The effects of these changes on the composition of the microbial community and primary producers has not been examined. As such identifying the community composition and the factors influencing their distribution is of extreme importance in order to understand the role of the Southern Ocean in past, present and especially future marine biogeochemical cycle. Here we provide the first detailed characterization of the microbial communities across environmental gradients in the Dalton polynya using both molecular and quantitative approaches. High-throughput flow cytometry was applied for a quantitative integrated study of the viral, bacteria and photosynthetic communities, coupled with the sequencing of the 16s rRNA and 18s rRNA for qualitative characterization of the bacterial and eukaryotic community diversity. Preliminary results revealed how specific phytoplankton species were differentially distributed along the continental shelf. These measurements provide essential context to understand and begin to model the effect that climate change will have in this important region for East Antarctica.

Genomic insight into the extent and drivers of genetic differentiation in *Eudyptes* penguin species and populations

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Around 8 species of *Eudyptes* penguins are described. There is no consensus in the exact number possibly due a recent diversification process that challenges species delimitation when there is low genetic or morphological differentiation. *Eudyptes* penguin species breed on several islands separated by vast open ocean extensions along Antarctic, sub-Antarctic and sub-Tropical waters. This water masses are delimited by oceanic fronts in which drastic biotic and abiotic changes occur, leading to their conception of oceanic barriers to dispersal for several taxa. Geographical distance between breeding colonies and the presence of oceanic fronts may lead to a reduction in gene flow in *Eudyptes* penguin species however their contribution to population differentiation can vary depending on the species. Using SNPs we studied contemporary patterns of connectivity in five *Eudyptes* penguin species; Macaroni (*E. chrysolophus*), royal (*E. schelegeli*), northern (*E. moseleyi*), southern (*E. chrysocome*) and eastern (*E. filholi*) rockhopper penguins. We also evaluated genetic differentiation in two groups of taxa under taxonomic debate (macaroni/royal and northern/southern/eastern rockhoppers). We found that even across vast distances, genetic differentiation among *Eudyptes* penguin populations within each species was low however, at least at some degree, it may be imposing a reduction in gene flow. The same is true for the presence of the Antarctic polar front separating macaroni penguin populations, in which we found two genetic clusters. Genetic differentiation was scarce between macaroni/royal penguins suggesting they represent a single evolutionary unit. Conversely, genetic differentiation between northern/southern/eastern rockhoppers supports the designation of three separated species.

Introducing POLA₃R, an online portal to discover microbial polar 'omics data

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High throughput DNA sequencing techniques have completely revolutionized microbial ecological research at the poles. Like museum collections, the vast wealth of microbial 'omics data that these studies generated needs to be archived in a way that makes the data Findable, Accessible, Interoperable and Reusable (FAIR) for future research. However, the complexity of 'omics data, which typically includes geographical and environmental components besides the sequencing data, as well as laboratory protocol metadata, poses serious challenges for data archiving on international nucleotide and biodiversity databases such as GenBank and Global Biodiversity Information Facility (GBIF). The Microbial Antarctic Resource System (mARS) represented a first step to achieve this in an Antarctic context. But now we are expanding that scope to all Polar regions. Therefore, we introduce the Polar 'Omics Links in Arctic-Antarctic-Alpine (A₃) Research, or POLA₃R for short. On this online platform datasets that are enriched with metadata and environmental information are made publicly accessible, and are linked to the associated publications and the sequences on nucleotide databases such as GenBank. To allow interoperability with other systems, the portal is designed to operate between different data archiving standards, such as the Minimum Information on any (x) Sequence (MIxS) as well as DarwinCore. Datasets that are listed on POLA₃R are also registered on GBIF to increase their discoverability. As such, POLA₃R aims to provide a hub for the polar scientific community, where you can discover high quality and complete molecular biodiversity data.

Biogeographical patterns in Southern Ocean near-shore marine benthic mollusks

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The biogeography of the Southern Ocean (SO) biota and its current bioregionalization are the consequence of major tectonic, oceanographic and climate changes since the Mesozoic. Here, we present biogeographical comparisons in different near-shore benthic mollusks with contrasting developmental modes across the SO. Through phylogenetic and phylogeographic approaches we aim to further understand the role of historical and contemporary processes in the recorded evolutionary patterns. We include direct developers (*Laevitorina*, *Margarella* and *Siphonaria*) and broadcast-spawners (*Nacella*, *Mytilius* and *Aequiyoldia*). We estimated uncorrected p-distances, divergence time analyses and maximum parsimony genealogies. Very levels of genetic diversity characterize the analyzed mollusks as a consequence of the Quaternary glacial processes that deeply affected their population sizes. Similarly, most of the analyzed groups (*Nacella*, *Laevitorina*, *Aequiyoldia*, *Margarella*) showed high degree of genetic divergence between Antarctic and sub-Antarctic provinces supporting the role of the Polar Front as an effective biogeographic barrier. The effective separation of Antarctic and sub-Antarctica lineages occurred less than 10 Ma, long after the physical separation of the continental landmasses or to the initiation of the Antarctic Circumpolar Current. Surprisingly, direct developers (*M. vioclea*, *S. lateralis*, *S. fuegiensis*) exhibited high levels of genetic identity between geographically distant sub-Antarctic provinces supporting the role rafting as an important biogeographic mechanism. In contrast, broadcast-spawners (*Nacella* and *Aequiyoldia*) showed marked genetic differences across the sub-Antarctic. Finally, current biogeographical patterns in SO mollusks are not related to particular groups or developmental modes but to historical oceanographic/climatic processes, as well as contemporary ones including the likelihood of long-distance dispersal.

Genomes and transcriptomes help elucidate the evolutionary drivers and biodiversity of Antarctic marine invertebrate fauna

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Our understanding of marine invertebrate fauna found on Antarctic continental-shelf regions has changed dramatically due to the application of molecular tools. There is far more biodiversity than previously recognized and genetic admixture around the continent and to other regions is better characterized. We extended these earlier studies with RAD-tag based SNP (single nucleotide polymorphism) efforts to study evolutionary patterns of marine invertebrate adaptation and dispersal in the Southern Ocean. Currently the primary hypothesis explaining such diversity invokes glaciation and genetic drift as forces of speciation. To test this hypothesis, we are using transcriptomes and genomes to look for signatures of selection versus genetic drift. We have sequenced more than a dozen transcriptomes from Antarctic organisms (with an emphasis on echinoderms). These transcriptomes are not unusual compared to transcriptomes from more temperate animals, but genes of interest have been identified. For example, putative antifreeze proteins (large glycoproteins) occur in taxa such as *Odontaster validus*. Moreover, genomes of commonly studied Antarctic marine taxa (including the echinoderms *Sterechinus neumayeri* and *Astrotoma agassizii*) are being sequenced. Although an initial 10X Genomics sequencing approach was not successful at producing high quality genomes (presumably due to heterozygosity issues with the assembler), we are scaffolding these runs with PacBio data. The resulting genomes will be paired with RAD-based Single Nucleotide Polymorphism (SNP) data and sliding window analyses to identify genomic regions that have been subject to positive selection or pronounced admixture. Additionally, we will report on the taxa sequenced and the availability of these resources.

Morphological innovations in brittle stars driven by sub-Antarctic glacial refugia

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The Pleistocene glacial period has been widely hypothesised to be challenging for benthic fauna to persist in the Southern Ocean. One of the key ecological disturbances at the time was that most continental shelf habitats were repeatedly eroded by ice sheet expansions. While limited molecular data has hinted where benthic fauna might have persisted in ice-free areas (i.e. refugia) in the Southern Ocean, how these past extreme environmental changes influenced evolutionary processes remains unclear. Since their recent speciation in the late Pleistocene, new genetic (partial cytochrome c oxidase subunit I) and genomic (double digest restriction-site associated DNA) data suggest the Antarctic brittle stars *Ophionotus victoriae* persisted in deep sea refugia, while *O. hexactis* persisted in sub-Antarctic refugia. Incomplete lineage sorting between *O. victoriae* and *O. hexactis* was also detected, highlighting that the time in isolation between deep sea and sub-Antarctic refugia appears to be insufficient for monophyly to be established under coalescence. Ecological shifts related to environmental fluctuations within sub-Antarctic refugia might have promoted morphological innovations in *O. hexactis* (an increase in arm number and a switch to brooding from broadcast spawning). Our results contribute to a better understanding of how environmental extremes could influence how species evolve and diversify and also highlight the resilience of Southern Ocean fauna through ecological adaptation.

Hind-wing morphology in the sub-Antarctic diving beetle *Lancetes angusticollis* (Curtis, 1839) (Coleoptera: Dytiscidae)

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The diving beetle *Lancetes angusticollis* is found in lakes in southern South America and in South Georgia. The geographical isolation of these populations suggests they have been separated on sufficiently long timescales to permit the evolution of adaptive changes in morphology. One of the more commonly reported morphological variations associated with island isolation is the reduction of wings, generally linked to different requirements in terms of energy reserves and lower predation pressure. We tested the hypothesis that the South Georgian populations of *L. angusticollis* would have reduced hind wings in comparison with their mainland counterparts. To test this, we documented geometric morphometrics, analyzing the data with Principal Component Analyses, to assess body and wing size and shape. Beetles from South Georgia had significantly longer heads, elytra and hind leg lengths, and shorter pronotum length, although they did not differ in overall body length. The centroid size did not vary, meaning that the overall size of the wings was not different, but the calculated wing loads showed that hind wings were of different shapes, with the main differences being in the costal, jugal and posterior margins of the wings along with the cubital cells. However, all of these observed differences in wing shape were subtle and do not clearly link with the hypothesis being tested. Based on this study, we suggest the most likely reasons for the slight differences in morphology found to be founder effect and genetic drift.

Phylogeography of the winged Antarctic midge *Parochlus steinenii* (Gercke, 1889) (Diptera: Chironomidae: Podonominae)

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The chironomid midge *Parochlus steinenii*, one of only two holometabolous insects occurring in Antarctica (South Shetland Islands), is also found around lakes in southern South America and in sub-Antarctic South Georgia. Previously published evidence, based on a small number of sequences of the 28S rDNA gene, inferred divergence between South American populations and those in South Georgia and Antarctica around seven million years ago. To further test the divergence hypothesis, we extracted DNA from over 150 specimens of *P. steinenii* from 13 different lakes across most of the species' known range. In addition to obtaining further 28S sequences, we expanded the analysis to include the mitochondrial COX1 gene. No variation was present among the new 28S sequences, in contrast to the previous study. However, sufficient variation was present amongst the COX1 sequences to permit phylogeographic analysis and the generation of a haplotype network and molecular phylogeny. These analyses confirm the presence of distinct clades from each region and corroborate the deep genetic separation of South American from South Georgian and Antarctic clades.

A global phylogeny of the shrimp family Benthesicymidae: from Equator to Subpolar areas and from benthic to mesopelagic.

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Benthesicymidae is a family of pelagic shrimps distributed across all principal oceanic biotopes, within wide geographical (from Equator to subpolar areas of both Hemispheres) and depth (5 km) ranges. Benthesicymidae provide a unique opportunity to examine how evolutionary trends mirror so different environmental factors and how morphological traits favor colonization of these principal oceanic biotopes. We present the first phylogenetic study to include all species of the family (all 37 valid species), which is based on six molecular markers and 105 morphological characters. Molecular methods provide better resolution of deeper nodes and generally higher support of the clades, while morphological methods allow analyses of all valid species of the global fauna. We suggest a new phylogenetic systematics of the family including two new subfamilies and five new genera. We analyzed morphological traits within benthic and pelagic clades and showed supremacy of copulatory structures. We show that the petasma greatly evolved both in the benthic and pelagic clades, while thelycum significantly evolved only in the pelagic clades. We propose a model explaining this difference. Along with previous studies, our results confirm the idea that the elaboration of the copulatory structures is a key to successful colonization of the pelagic realm. We also discuss possible pathways of various clades of Benthesicymidae into medium and great depths, into low and high latitudes.

A phylogeny of krill (Crustacea: Euphausiacea): pathways into high latitudes, near-shore habitats, and deep sea

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The order Euphausiacea, widely known as krill, is a commercially and ecologically important group of marine crustaceans, dominating in the World Ocean and in the Antarctic. Krill serves an important role in the sequestration of organic carbon from the upper ocean layers and it has been shown that the sinking of krill fecal pellets and moult casts are the primary agents of the downward flux of carbon. We present a phylogenetic study of the global krill fauna based on molecular markers (4) and morphological characters (168) and scrutinize the taxonomy of the whole order. We revealed groups of morphological characters, which are likely coupled with same biological role and thus interlinked evolutionarily. We analyze the evolutionary pathways of the clades into main oceanic biotopes and discuss morphological adaptations most likely coupled to this process. We pay a special attention to pathways into extreme biotopes: high latitudes of the Arctic and the Southern Ocean, near-shore habitats, and the deep sea.

Applying genomic tools to answer phylogeographic questions for Antarctic benthic marine invertebrates.

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Benthic invertebrates living in the marine continental shelf environment in Antarctica show remarkable diversity. Historically, studies employing mitochondrial genes or gene fragments (e.g., amplicons from cytochrome c oxidase subunits I and II, 16S, cytochrome b) described numerous unknown, yet genetically distinct, lineages in several invertebrate taxa including isopods, sea spiders, echinoderms, and nemerteans. Despite this increased appreciation of diversity from the region and the early application of molecular tools to study them, we are still trying to explain bigger picture phylogeographic patterns around the Antarctic and the factors that produce such patterns. Efforts to improve our knowledge of Antarctic phylogeography include the application of new and novel genomic tools, along with increased sampling efforts around the continent. In this, we are improving our understanding of patterns of biodiversity around the continent and throughout the Southern Ocean. The development and application of new molecular methods, such as whole genome scanning techniques (e.g., RADSeq) and mitogenomics on organisms from the region, is allowing us to address questions relating to scenarios that may have impacted the current distributions of Antarctic shelf marine fauna. In this presentation, we will discuss our ongoing work that continues to investigate major these patterns around the continent for a number of organisms, including mitogenomics in sea spiders and population genomics of the pycnogonid *Nymphon australe* and the nemertean *Parborlasia corrugatus*. We expect that knowledge of organismal diversity through the implementation of additional 'omic studies that include increased sampling efforts will have tangential impacts throughout Antarctic biological research efforts.

Grasping the diversity of the Doris 'kerguelenensis' species complex within the Southern Ocean

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The Southern Ocean benthic sea slug, Doris 'kerguelenensis', was long-thought to be a single, widespread species that showed reduced dispersal potential. Belonging to the Dorididae family, this slug is a direct-developing, simultaneous hermaphrodite that can only crawl along the seafloor as an adult. More recently however, a combination of mitochondrial and nuclear DNA sequencing combined with metabolomics, has revealed a multitude of highly divergent lineages within that 'species', signalling an explosive marine adaptive radiation comprising of over 32 putative species. This radiation was thought to be caused by a unique combination of selection and allopatry facilitated through millions of years of episodic glacial cycles. This resulted in the segregation of the animals into smaller, reduced populations, which in turn, increased vulnerability to predation pressure. Through Sanger sequencing of the single mitochondrial gene, Cytochrome Oxidase I (COI), this project has generated a single gene phylogeny consisting of over 1,000 samples, resulting in up to 70 putative mitochondrial lineages. Using available transcriptomic data, we will next characterise and confirm the placement of these mitochondrial lineages within the D. 'kerguelenensis' species complex as well as incorporate secondary metabolite profiles into this phylogenetic reconstruction in order to further delimit species. By resolving this phylogeny and mapping the distribution of these metabolites across the topology of the phylogeny, we will be able to produce important genetic resources that can be used in future work.

Evidence of strong small-scale population structure in the Antarctic freshwater copepod *Boeckella poppei* in lakes on Signy Island, South Orkney Islands

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Environmental conditions were particularly severe during the Last Glacial Maximum, altering the distribution of the Southern Hemisphere biota, particularly at higher latitudes. The copepod *Boeckella poppei* is the only macroscopic continental invertebrate species known to be distributed today across the three main biogeographic regions in Antarctica as well as in southern South America. Signy Island (South Orkney Islands) is a unique location for the study of Antarctic freshwater ecosystems due to its location and geographic isolation; it contains 17 lakes in several low altitude catchments. We conducted phylogeographic and demographic analyses using the *cox1* gene on 84 individuals of *B. poppei* from seven lakes across Signy Island. We recorded low levels of genetic diversity and a strong genetic differentiation signal between the eastern and western valleys within the island. Phylogeographic structure and demographic inference analyses suggested at least one asymmetrical dispersal event from west to east. Demographic inference detected a strong signal of population growth during the deglaciation process, which may have followed either (1) a strong genetic bottleneck due to a reduction in population size during the last glacial period, or (2) a founder effect associated with postglacial recolonization of Signy Island from elsewhere. The genetic architecture of this island's populations of *B. poppei* shows that historical events, rather than continuous dispersal events, likely played a major role in the species' current distribution. Finally, our study considers possible mechanisms for dispersal and colonization success of the most dominant species in the Antarctic freshwater community.

Towards an understanding of genetic structure among *Lepidonotothen squamifrons* (Teleostei: Nototheniidae) populations distributed around the Southern Ocean

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Lepidonotothen squamifrons is among the notothenioid fish that are widely distributed around the Southern Ocean. This distribution have created number of taxonomic issues, which still exist regardless of the number of research that attempted to solve them. The present study aimed to address this problem by investigating genetic structure among *L. squamifrons* populations that are found in the three ocean sectors of the Southern Ocean. The genetic structure was assessed by analysing the mitochondrial COI gene and six microsatellite markers. The COI data set produced 13 haplotypes and four of these are shared while nine are private. Two of the shared haplotypes consisted of specimens from the Atlantic Ocean only; one was shared between the Atlantic and Pacific Oceans specimens; and the last haplotype consisted of the Indian Ocean specimens only. Analyses of genetic differentiation using AMOVA, for both COI and microsatellite data sets, showed higher genetic variations within specimens of the same locality. A haplotype network tree revealed that Atlantic Ocean specimens contributed more towards these variations as specimens in this ocean were clustered in different haplotypes. However, the observed genetic variations do not represent the presence of two species within this population. The present study further showed that there is gene flow between Atlantic and Pacific Oceans populations, while Marion Island population (representative of Indian Ocean) differ slightly from these two oceans.

Environmental drivers of diversity in Antarctic terrestrial plants and animals: using genomic approaches to discover broad-scale patterns

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Understanding the drivers of Antarctic biodiversity patterns is crucial if we are to mitigate the effects of rapid environmental change. Pressing questions remain largely unanswered including: where are biodiversity hotspots in Antarctica, and are Antarctic terrestrial populations biologically isolated or well connected? The rapid development of genomic techniques provides exciting new opportunities to address these and related questions. In a broadscale new research project, we are using environmental DNA (eDNA) metabarcoding analyses of soil, combined with spatial environmental analyses, to investigate the drivers of diversity in terrestrial communities over both local (10s to 100s km) and continental scales. In this talk, we will present an overview of the progress of the project to date, including results of preliminary eDNA metabarcoding analyses of soils from East Antarctica (including Larsemann and Vestfold Hills), testing whether geothermal areas support higher biodiversity than non-geothermal areas, on local scales. Geothermal areas have been proposed to have helped life to survive Pleistocene glacial maxima. If they have, we expect to see higher biological diversity close to geothermal areas, and lower diversity further away. Furthermore, we present a summary of methodological trials aimed at optimising Antarctic soil eDNA analyses. Our findings will shed light on the processes that have shaped – and continue to shape – the evolution and diversity of Antarctic terrestrial communities.

Diversity, structure and functions of Antarctic vascular plant rhizosphere microbiomes from the central maritime Antarctic

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Deschampsia antarctica and *Colobanthus quitensis* are two native Antarctic vascular plants resisting severe conditions of maritime Antarctic, while the mechanisms of its adaptation are still debated. Microorganisms in rhizosphere soil have a significant role in plants' adaptation and distribution.

The rhizosphere microbiomes of *Deschampsia antarctica* and *Colobanthus quitensis*, from the central maritime Antarctic were studied. Rhizosphere microbiomes of *D. antarctica* had high taxon richness (Shannon 5.2–9.3), while of *C. quitensis* had much lower diversity (Shannon 0.9). The bacteria in the rhizosphere communities of the hairgrass mainly affiliated to Proteobacteria, Bacteroidetes and Actinobacteria. Rhizosphere of pearlwort was inhabited by Actinobacteria. The microbiomes included high proportions of unique OTUs (19.6% to 63.3%) and there was high heterogeneity between the samples at the OTUs' level. Soil parameters were not the reason for the heterogeneity, and it can be caused by microscale effects of environmental conditions such as edaphic or microclimate factors.

Metabolic predictions in silico using PICRUSt 2.0 indicated that the microbiomes of *D. antarctica* were similar by the predicted functional repertoire, despite the overall differences in diversity. About 4.3–10.7% of MetaCyc ontology pathways of the microbiomes were involved in degradation of polymeric and aromatic substrates. KEGG orthologs involved in siderophores (0.08–0.15%), indole acetic acid (0.2–0.5%) and ACC-deaminase synthesis (0.03–0.05%) were among the predicted functional groups. The data obtained indicate that these communities are involved in the primary processes of soil development in the central maritime Antarctic and may be beneficial for the growth of Antarctic vascular plants.

A first assessment of genetic variability of *Lyallia kerguelensis* an endemic species of Kerguelen islands: should this species be considered as threatened?

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Genetic diversity is well-recognized as a major determinant to species adaptation and resilience. Although sub-Antarctic plant species are facing changes in environmental conditions (e.g. an increase in variability of rainfall), few studies have assessed the amount and population patterns of their genetic variability. Genetic studies were carried out on *L. kerguelensis*, a strict endemic species from Kerguelen Islands, which distribution is scarce, extremely fragmented and restricted to fellfield or moraines. Most populations do not contain more than a hundred or even dozens of individuals. This species has possibly suffered from herbivory pressure caused by the recent introduction of rabbit. The following questions were addressed: i) what is the ploidy level of this species? This question matters since polyploidy can drive rapid adaptation. ii) what is the level of genetic diversity within populations and what is the population genetic structure across the whole Kerguelen archipelago? Microsatellite markers were de novo developed. Fifteen of them were used to assess the level of genetic polymorphism in 20 populations across Kerguelen Islands. Four whole chloroplast genomes from four populations sampled at remote locations were also sequenced. Original data showed this species is octoploid and displayed an uncommonly low genetic diversity at microsatellite markers. Only three SNP were found among the ~156 Kbp of the chloroplast genome. Results strongly suggested *L. kerguelensis* has undergone a very strong and recent (possibly at LGM) and/or protracted demographic bottleneck. Together with other demographic and ecological observations, this result questions whether this species should be considered as threatened

Phylogeography of Antarctic Soil Invertebrates

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Terrestrial Antarctic ecosystems are populated by diverse yet understudied invertebrate communities, essential for healthy ecosystem functioning. As healthy ecosystems are built from the ground up, it is essential to understand how environmental and biogeographic conditions can influence contemporary distribution patterns. Soil mite, springtail and nematode specimens collected from islands off the Antarctic Peninsula between 2014-2016 will be analysed using comparative phylogeographic techniques that combine genetic and geographical datasets to capture their distribution and evolutionary histories. Soil communities from temperate Australian islands along a comparable latitudinal transect with different biogeographic histories will help disentangle the biogeographic, climatic and environmental drivers of soil faunal communities. Comparing gene flow within species between remnant (e.g. isolated by rising sea-levels) and pioneering populations (dispersers to off-shore islands) will highlight the factors that enable soil fauna to overcome isolation and ecosystem fragmentation. In summary, detailed analysis will also reveal the drivers of distribution at the a) local scale; environmental and biotic variables, b) regional scale; climatic influences and gene flow; and, c) temporal scale; evolution and dispersal in Antarctic and temperate invertebrate communities. These will improve our capabilities of conserving these essential ecosystem operators under mounting pressures from local and global environmental change.

Hidden diversity in Antarctica

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The Southern Ocean is one of the most isolated marine ecosystems, characterized by high levels of endemism, diversity, and biomass. Ascidians are among the dominant groups in Antarctic benthic assemblages, thus recording the evolutionary patterns of this group is crucial to improve our current understanding of the assembly of this polar ocean. We studied the genetic variation within *Cnemidocarpa verrucosa sensu lato*, one of the most widely distributed abundant and studied ascidian species in Antarctica. Using a mitochondrial and a nuclear gene (COI and 18S), the phylogeography of fifteen populations distributed along the Antarctic Peninsula and South America (Burdwood Bank/MPA Namuncurá) was characterized, where the bimodal distribution of the genetic distance suggested the existence of two species within the nominal *C. verrucosa*. When re-evaluating morphological traits to distinguish between genetically defined species, the presence of a basal disc in one of the genotypes could be a morphological trait to differentiate the species. These results are surprising due to the large research that has been carried out with the conspicuous *C. verrucosa* with no differentiation between species. Furthermore, it provides important tools to distinguish species in the field and laboratory. But also, these results give new insights to patterns of differentiation between closely related species that are distributed in sympatry, where the permeability of species boundaries still needs to be well understood.

How the Genus *Eusirus* amphipods evolved and speciated in the Antarctic

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Antarctica has been subjected to different climatic changes in the past requiring the marine fauna to either adapt to novel environmental conditions, migrate to better conditions or become extinct. Currently, warming of Antarctica is happening at an unprecedented rate. Understanding how these organisms have managed to survive in the past will thus allow us to predict their possible responses to future climate changes. Here, the amphipod Genus *Eusirus* will be used as model organisms since these amphipods are very diverse and knowledge on their ecology and biogeography is still very limited. In this study, molecular, morphological and ecological data of *Eusirus* will be collected. Because no reference genome exists for this group, we will use a Next generation sequencing approach to obtain the complete mitochondrial genome (mitogenome) of these amphipods. Draft mitogenomes have been assembled and annotated from skim sequencing for two *Eusirus* species. From these mitogenomes, we designed primers for long-range PCRs to amplify the entire mitogenome in several pieces. Complete mitogenomes will allow us to estimate genetic divergence amongst different species and check for specific temperature adaptations of mitochondrial genes. The obtained mitogenome data will also provide better supported phylogenies for reconstructing evolutionary history of *Eusirus*. In order to get a better understanding of adaptive and/or non-adaptive processes which led to the current diversity of *Eusirus* amphipods, we will explore how morphological and ecological diversity are partitioned along the resulting phylogeny, concurrently with potential changes in lineage diversification through time.

Are genetic and species diversities turnover correlated in lichen-forming fungi and their photobionts along a latitudinal gradient in the Transantarctic Mountains?

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Lichens are the most diverse group of macroscopic organisms in Continental Antarctica. Their occurrence is known up to 86°S and reduced communities are known from most ice-free rocky areas visited in the Transantarctic Mountains. However, little is known about the processes behind the assembly of those communities, although random dispersal has been proposed as the likely main driver. The study of the species-genetic diversity correlation and the turnover at both diversity levels across space may shed light on the main processes shaping communities when a neutral marker is used to measure genetic diversity. We have sequenced the putative neutral internal transcriber spacer (ITS) of both myco- and photobionts (Trebouxioid taxa) more than 700 lichen samples from 5 regions in continental Antarctica along a latitudinal transect from 76° S to 84° S. Further, we delimited operational taxon units in both taxa using an algorithm based on genetic distances (ABGD). Results pointed to a decoupled turnover between mycobionts and photobionts, with a high turnover of species and ITS haplotypes in the former, but not so pronounced in the latter.

Phylogenetic diversity of lichen symbiotic fungi inhabiting the Continental Antarctica

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Lichen symbioses have been very successful in colonizing a broad range of extreme environments; one of these is the Antarctic continent. Indeed, lichens – lichen forming fungi – are the most diverse and visually common eukaryotic organism followed by mosses in all Antarctic regions. Since the phenotypic features are highly plastic in lichens including non-lichenized fungi, we used DNA sequence based sample identification to resolve their diversity and evaluate endemism in terrestrial mycobiota of continental Antarctica (Victoria Land). We used the internal transcribed spacer – ITS – sequences as this is the universally accepted DNA barcodes of fungi including lichens. We sequenced and analyzed a total of 54 samples from 9 localities along the Victoria Land coast line representing different genera of broad phylogenetic range (Order to Family) . While some widespread morphospecies e.g. *Rhizoplaca melanophthalma* were monophyletic, the others, for example, *Umbilicaria aprina*, *U. decussata*, *Physcia caesia* and *P. dubia* appear polyphyletic. Moreover, our results underline significantly higher endemism in the continental Antarctica than previously assumed. In addition, our study provides a baseline for further investigations on tracing the migratory route of Antarctic populations of widespread lichen species especially during past periods of excessive warming. The sample identification based on morphological and chemical features was confirmed with the ITS sequences.

The brooding brittle star *Astrotoma agassizii* does not always brood as it is a species complex: genomic and life history characters provide clues to a turbulent evolutionary history

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The Southern Ocean holds a wealth of diversity that is particularly rich along the shallower continental shelf regions of Antarctica, Kerguelen Plateau and the Scotia Arc including the Patagonian Shelf. Cryptic diversity is not uncommon among species from these regions, hugely increasing the actual diversity and richness compared to the recognised diversity. The cryptic diversity can in part be explained by vicariance, or allopatric speciation due to large distances between suitable habitat, strong oceanographic features such as the Polar Front that at least partially inhibit gene flow, and historical events fragmenting populations. A possible example of population fragmentation is the bulldozing of benthic life off the continental shelf by grounded glaciers during the last glacial maximum leaving few isolated refugia in which pockets of the previous assemblage survived. The large and charismatic brittle star *Astrotoma agassizii* is one such example of a species distributed across the Southern Ocean and in which there has been evidence of cryptic diversity. New evidence is suggesting the diversity is substantially greater than recognised in previous work, with complexity identified in life history traits and morphology that add a new dimension to understanding the evolutionary history of this enigmatic species group.

Antarctic coastal plankton metabarcoding: a long term and low-cost approach to monitor intra and inter-annual dynamics by taking advantage of research base desalination plant filters

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One of the main requirements for a sound biological monitoring is the availability of long term and possibly temporal fine-grained data. This is often difficult to be achieved, especially in Antarctica, due to the existing logistic and economic constraints. In the framework of the Italian PNRA project "TNB-CODE" (Terra Nova Bay barCODding and mEtabarcoding of Antarctic organisms from marine, terrestrial and limnetic environments), we have focused on the use of filters of the Mario Zucchelli base desalination plant to monitor coastal plankton communities. Filters of different mesh sizes are used to decrease the amount of organisms and debris before desalination processes and are in use continuously to be changed just before clogging. Thus, they naturally collect the spectrum of species present in the surrounding water masses. Analysis of data from the desalination plant diary from 2001 to 2019 showed a consistent higher rate of filter replacement in coincidence with the phytoplanktonic blooms. Molecular data obtained from 5 µm mesh filters sampled during January in 2012 and 2013 demonstrated the ability of metabarcoding in characterising nanoplankton communities, highlighting both inter- and intra-annual dynamics, even when changes occur in a few days. These fine-grained data would not have been possible by using standard sampling approaches as they would have required a continuous sampling at sea. This method, by combining a cost-effective sampling and molecular techniques, represents a viable solution that guarantees the acquisition of long term data for coastal plankton monitoring.

Diversity, function and biogeography of the gut microbiota of the antarctic heart urchin (Spatangoida) *Abatus agassizii*

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Abatus agassizii is an irregular sea urchin species that inhabits shallow waters of South Georgia and South Shetlands Islands, and feeds by ingestion of the surrounding sediment. Despite the low complexity of its feeding habit, it harbors a long and twice-looped digestive tract suggesting that it may host a complex bacterial community. We focused two *A. agassizii* populations at the south of the King George Island in the West Antarctic Peninsula. Through a metabarcoding approach targeting the 16S rRNA gene, we characterized the *Abatus* microbiota composition and putative functional capacity, evaluating its differentiation among the gut content and the gut tissue in comparison with the external sediment. Additionally, we aimed to define a core gut microbiota between *A. agassizii* populations to identify potential keystone bacterial taxa. Our results show that *A. agassizii* acts as a selective filter of the diversity and the composition of the surrounding sediment bacterial community, at both genetic and predicted functional levels. Specific bacterial taxa, belonging mostly to Planctomycetacia and Spirochaetia, were differently enriched in the gut content and the gut tissue, respectively. Predictive functional profiles revealed higher abundance of specific pathways, as the sulfur cycle in the gut content and the amino acid metabolism, in the gut tissue. Further, the definition of a core microbiota allowed the identification of potential keystone taxa assigned to the *Desulfobacula* and *Spirochaeta* genera as potentially host selected. Finally, by exploring the genetic structure of these keystone taxa in other *Abatus* species we revealed evidence of biogeographic patterns.

Out from Antarctica: Population genetics suggest recent diversification in the monogeneric family Harpagiferidae (Nototheniidae) along the Southern Ocean.

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Notothenioid fishes dominate Antarctica in diversity, abundance and biomass since the local extinction of most of the Eocene ichthyofauna. From these, the monogeneric family Harpagiferidae represents an interesting biogeographical model. According to the current taxonomy, this genus includes a single Antarctic species, *Harpagifer antarcticus*, restricted to the western Antarctic Peninsula and several shallow-water sub-Antarctic species, geographically assigned to southern South America, Falkland/Malvinas, South Georgia, Marion, Crozet, Kerguelen and Macquarie islands. Moreover, *Harpagifer* species exhibits a Pleistocene divergence (1.7, 0.8Ma) between South America and Antarctica, being more recent than the ones recorded in marine benthic invertebrates, macroalgae and even other notothenioids fishes. Such results suggest that the separation of the Antarctic and sub-Antarctic lineages could be related to recent dispersal events. Here, we analyzed >5,000 GBS-SNPs and COI sequences obtained from 260 individuals from seven species of *Harpagifer* in the Southern Ocean. Our main results suggest the presence of three genetic groups: Antarctica (Antarctica, South Georgia, Signy Island), South America (Patagonia, Falkland/Malvinas Islands) and sub-Antarctic Islands (Kerguelen, Marion). Levels of genetic distance between the recorded groups were surprisingly low, and it was not possible to identify significant evolutionary units. Patterns of genetic structure here recorded seem to be associated to microevolutionary processes (i.e. local adaptation) than historical divergence. Assuming the Antarctic origin of *Harpagifer*, our results suggest that long-distance dispersal played an important role in the recent Quaternary biogeography of *Harpagifer* from Antarctica to sub-Antarctica, a process that was followed by local adaptation processes.

An integrated study of three limno-terrestrial Tardigrade species groups, Eutardigrada: Mesobiotus, Acutuncus and Heterotardigrada: Pseudechiniscus, in Antarctica show ancient origins, strict spatial scales and morphological plasticity to environmental factors.

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Antarctica's long history of isolation and glaciation has led to the fragmentation and adaptation to extreme environments of its surviving biota, creating the high endemism seen in Antarctica today. Antarctica is divided into East and West continental Antarctica (separated by the Transantarctic Mountains), and the Maritime Antarctic (Antarctic Peninsula and Scotia Arc) separated from Continental Antarctica by the Gressitt Line, with the geological history differing significantly between these areas. There is a striking difference in species composition between Continental and Maritime Antarctica in multiple invertebrate groups, however this has yet to be shown in tardigrades. Tardigrades are an ancient lineage with a cosmopolitan distribution. They have developed physiological adaptations enabling them to survive in extreme environments, and they are a particularly important component of the depauperate Antarctic terrestrial fauna. This study performed an integrated analysis of the 18S and COX1 gene regions with morphological characteristics from multiple areas and habitat types of the Antarctic continent including the Maritime, East and Transantarctic regions. To test for population differences between biogeographic areas three species groups were used, Eutardigrada (Mesobiotus and Acutuncus) and Heterotardigrade (Pseudechiniscus). Phylogeographic analysis using both Maximum Likelihood and Bayesian methods has shown genetically distinct populations between biogeographic zones, with haplotype networks showing little interaction across populations. Molecular dating has shown these separation events to have occurred approximately 40Ma, pre-dating geographic isolation and the last glacial maximum. Morphological analysis between differing habitat types of genetically identical populations has shown significant differences indicating a plasticity to environment independent of speciation.

Mapping the microbial diversity of the South Pacific

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Marine microbes are ubiquitous, both phylogenetically and functionally diverse and are key regulators of marine biogeochemical pathways. High resolution baseline census datasets are an important resource towards understanding the identity and function of marine microbes, but these datasets are scarce in the Southern Hemisphere. This is especially true for microbial surveys that include both vertical and latitudinal distributions and are accompanied by corresponding contextual information vital towards microbial ecology and biogeography studies. We present a methodologically standardised, phylogenetic amplicon sequencing dataset describing Bacteria, Archaea and microbial Eukarya assemblages from a latitudinal transect along the South Pacific. Samples are collected from eight depth points from surface to bottom waters every 0.5° latitude from 0° to 66°S along the GO-SHIP P15S sustained hydrographic section. The sequence data covered a wide range of key oceanographic fronts and water masses and are linked to an extensive set of globally standardised, publicly available physical and chemical oceanographic contextual information. The 1032 microbial samples have allowed us to visualise the microbial ecosystem structure of the southern Pacific basin, provide a benchmark against which we can assess assemblage changes especially towards the effects of climate change, as well as validate and constrain oceanic models. In addition to enriching existing global oceanic observations, this dataset harbours an immense array of potential. We envision that this dataset, when complemented with other global efforts such as GEOTRACES, will be a powerful tool aiding in filling many knowledge gaps of the unseen microbial biodiversity in the Southern Hemisphere.

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