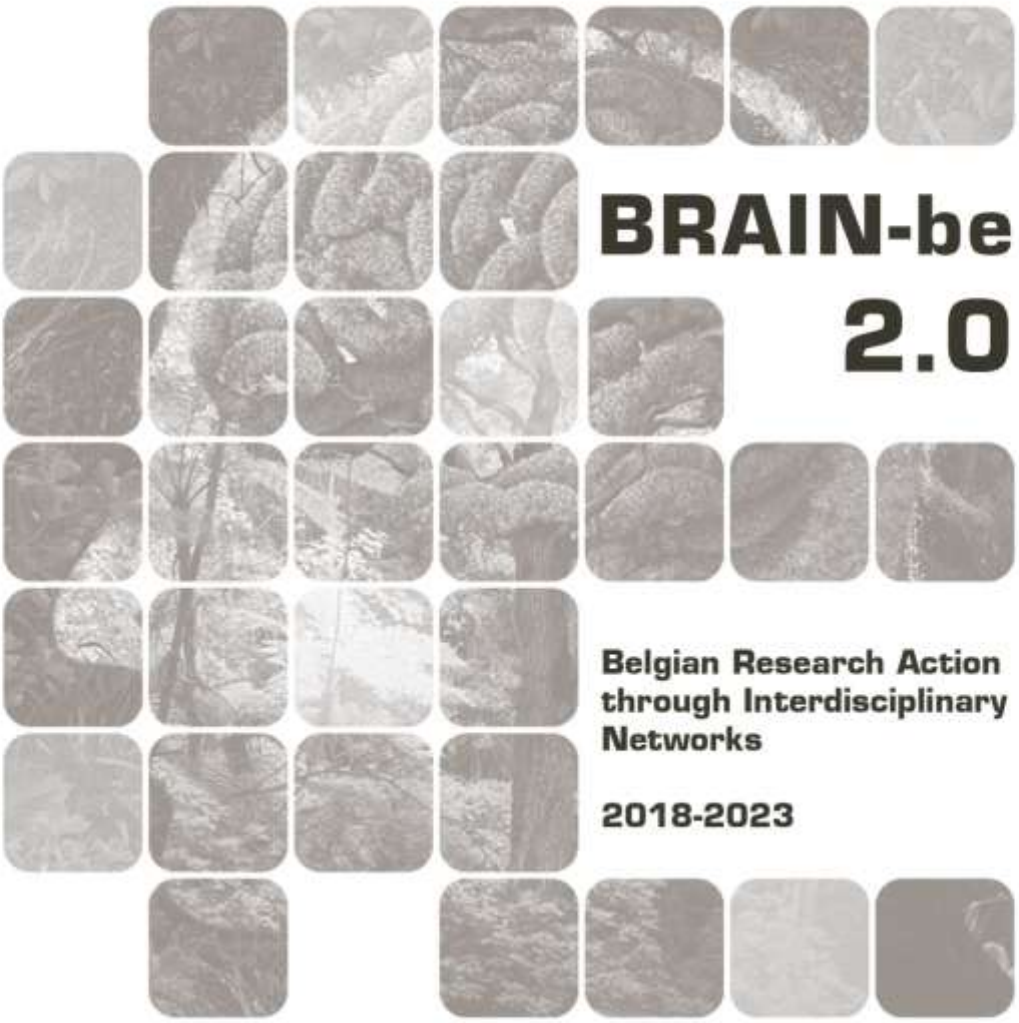

COPE

Conservation management of polar ecosystems: using genomic approaches to study connectivity across spatial and functional scales

SCHÖN, ISA (RBINS) - AERTS, DORIEN AUTHOR 2 (RBINS) - VERHEYE, MARIE (KU Leuven) - VAN DE PUTTE, ANTON (RBINS; ULB) – VOLCKAERT, FILIP (KU Leuven)

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NETWORK PROJECT

COPE

Conservation management of polar ecosystems: using genomic approaches to study connectivity across spatial and functional scales

Contract - B2/191/P1/COPE

FINAL REPORT

PROMOTORS: SCHÖN, ISA (RBINS)
VOLCKAERT FILIP (KU Leuven)

AUTHORS: SCHÖN, ISA (RBINS)
AERTS, DORIEN (RBINS)
VERHEYE, MARIE (KU Leuven)
VAN DE PUTTE, ANTON (RBINS; ULB)
VOLCKAERT FILIP (KU Leuven)

natural sciences
KU LEUVEN
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.be

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WTCIII
Simon Bolivarlaan 30 bus 7
Boulevard Simon Bolivar 30 bte 7
B-1000 Brussels
Belgium
Tel: +32 (0)2 238 34 11
http://www.belspo.be
http://www.belspo.be/brain-be

Contact person: David Cox
Phone: +32 (0)2 238 34 03

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ABSTRACT

Context

The high degree of endemism and special cold adaptations make Antarctic organisms highly vulnerable to global change. Given the current fast warming and loss of ice sheets, protection of the unique Antarctic biodiversity becomes a most urgent issue. The delimitation of Marine Protected Areas (MPAs) is one of the most important tools to protect marine habitats. Nine MPA domains have been suggested by the Convention on the Conservation of Antarctic Marine Living Resources (CCAMLR) in the Southern Ocean (SO) but only two have been realized so far and four more are in the planning stage. The COPE project provides data on genetic connectivity of endemic *Charcotia* amphipods and *Trematomus* fishes to optimize the delimitation of MPAs in the Southern Ocean and identify regions of particular concern.

Objectives

The COPE project has five major objectives:

1. Delimitate genetic species of selected amphipod and fish morphospecies for subsequent population genetic analyses and the identification of cryptic diversity.

2. Obtain genomic data on neutral genetic variability, connectivity and population structure of two key taxa of the SO, scavenging *Charcotia* amphipods and *Trematomus* fish at a circum-Antarctic scale.

3. Use outlier analyses from genomic data to test for local adaptation to abiotic factors that characterize the seascape.

4. Compare patterns of genetic connectivity in both taxa groups to their life histories including dispersal capacities and seascapes.

5. Model the distribution of *Charcotia* and *Trematomus* species in the SO from existing occurrence data and environmental data layers.

Conclusions

The COPE project successfully achieved its objectives. Most analyses were based on existing collections in the two partner institutions because of reduced ship time linked to the COVID-19 pandemic. We recommend the extension of databases and datasets with abiotic and biodiversity information to further develop the scarce biological knowledge, and, for example, to feed into Species Distribution Models and dispersal models linked to hydrodynamic models. We made several important contributions to the field: we detected additional cryptic diversity in both target taxa, contributing to realistic estimates of endemic diversity in Antarctic marine ecosystems. Reconstructions of the realized ecological niche in amphipod sister species revealed pronounced inter- and intraspecific differences, illustrating trophic plasticity of scavenging Antarctic amphipods. The project provided the first estimates of genome sizes for 16 Antarctic amphipod species, confirming large differences and the presence of giant genomes in amphipods. This database forms an important base for genomic research. The most relevant and novel results are estimates of genetic population differentiation and structuring in two amphipod and six *Trematomus* species at various geographic scales in the Southern Ocean. The genome-wide data revealed various patterns, even among closely related species. We found evidence for the absence of population structure within the Eastern Weddell Sea in three *Trematomus* fish species. In contrast, we observed patterns of geographic

isolation in two other *Trematomus* species and the amphipod *Charcotia obesa*. There is also evidence for population differentiation at small scales, for example along the Western Antarctic Peninsula or within the Eastern Weddell Sea. Observed patterns of putatively adaptive genetic variation suggest that chromosomal rearrangements might play a key role in adaptation of *Trematomus* fishes. Putatively adaptive patterns observed in *Charcotia obesa* resulted from two geographically close locations in the Western Antarctic Peninsula. These locations fall within the same bioregion, which suggests that the adaptation is driven by local conditions. Some patterns of genetic connectivity are new and surprising, as for example the connection between one side of the Filchner Trough and the western Weddell Sea, which might be linked to oceanic currents. The connectivity results are highly relevant for the delimitation of MPAs in the Southern Ocean; they identify the Filchner Trough as potential stepping stone region, requiring special protection. Our results also emphasize the need of networks of connected MPAs to accommodate large scale differences but also the incorporation of adjacent bioregions in MPAs to protect local genetic differentiation. We plan to draft a science communication paper for the next meeting of the Working Group on Ecosystem Monitoring and Management (WG-EMM) in preparation of the 2025 CCAMLR meeting to be sure that our results are valorised for science policy and the conservation of the Southern Ocean.

Keywords

Amphipoda, connectivity, cryptic diversity, fish, genomics, hydrodynamics, Notothenioidei, population structure, population differentiation, RAD sequencing, seascape, Southern Ocean, species distribution models.

1. INTRODUCTION

The Southern Ocean (SO) south of 60° S and surrounding the Antarctic continent has existed for more than 60 million years (myr; (Clarke & Crame 2010; Lawver et al. 2014). Its temperature changed from warm conditions in the Paleocene to the current low temperatures some 15 myr ago. The SO is isolated from the other oceans by the Antarctic Circumpolar Current, which originated about 12 myr ago. Because of its long evolutionary history and its isolation, there is a high degree of endemism among the marine taxa of the SO (De Broyer et al. 2014; De Broyer & Danis 2011, 2013). The speciation rate of fish, for example, is higher in the polar regions including Antarctica than in the tropics (Rabosky et al. 2018). The SO and its ecosystems are currently exposed to various anthropogenic stressors such as increasing temperature, UV exposure and acidification of seawater, overfishing, tourism, pollution and invasive species threatening its ecosystem functioning (summarized by Constable et al. 2023). The Western Antarctic Peninsula (WAP) is heavily affected by atmospheric warming, which is happening 50 times faster than elsewhere on the globe (Steig et al. 2009). While this is causing fast ice loss in the Western part of the continent, land ice masses are increasing in other regions on the Antarctic continent https://www.theclimateadaptationcenter.org/2024/05/21/whats-going-on-the-antarctic-2024-update. Purich and Dorddridge reported a record of low sea ice coverage from Antarctica in 2023 with indications for an altered sea ice state. Unique features of Antarctic taxa including high levels of endemism (Griffiths et al. 2009; Kaiser et al. 2013; Saucede et al. 2014), special thermal adaptations and sensitivity to increasing temperatures (Cheng & William 2007; Pörtner et al. 2007; Peck 2016) are making them especially vulnerable to global change.

2. STATE OF THE ART AND OBJECTIVES

"Effective regional and local protection is critical to safeguard ecosystems [of the SO] against the effects of climate change that are already underway" (Constable et al. 2023). One of the most important tools for the conservation of marine habitats is the establishment of Marine Protected Areas (MPAs). In 2005, members of the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) committed to establishing a network of MPAs to ensure the long-term conservation and sustainable use of the marine environments and resources of the SO (see Liu 2024 for a recent overview of CCAMLR's process to establish MPAs). Developing and managing such a network within CCAMLR requires the setup of dynamic, ecosystem-based approaches that take advantage of state-of-the-art methods in systematic conservation planning and spatial ecology. CCAMLR has suggested nine MPA domains in the SO, of which two have been delimited so far, the South Orkney Islands Southern Shelf MPA and the Ross Sea region MPA. Together, they cover about 3% of the CCAMLR-managed area, while global Aichi targets set the minimal coverage of these areas at 10%. Proposals for three additionally MPAs are currently considered in East Antarctica, in the Weddell Sea and around the Western Antarctic Peninsula. MPAs play a critical role in protecting marine biodiversity, including ecosystem structure and function (O'Leary et al. 2018). Potential benefits of MPAs are diverse and include the maintenance of genetic diversity and moderation of the impacts of fishing on biodiversity (Pinsky & Palumbi 2014), increasing the ability of marine ecosystems to withstand environmental perturbations, and protecting emblematic biodiversity such as marine mammal populations and seabirds. Size, spacing, location and configuration are key factors for designing networks that simultaneously enhance biological conservation and increase fishery yield and profit (Gaines et al. 2010). Highly connected networks generally improve resilience in complex systems such as MPAs (Gao et al. 2016). Connectivity, the extent to which individual elements are linked into a network, consists in the natural environment of an ecological-demographic and an evolutionary-genetic component. Ecological connectivity focuses on the exchange of individuals in a contemporary context while evolutionary connectivity does so in a historical context (Waples & Gaggiotti 2006). Ecological connectivity is best understood at the level of the seascape (Douglass et al. 2014). The seascape of the Antarctic, based on ecoregions (Spaulding et al. 2007), species habitats, or ecosystem functions, is commonly known and used by managers, but evolutionary and population connectivity remains understudied in Antarctic taxa.

COPE's focal taxa are endemic species of benthic amphipods and bentho-pelagic fishes, typical fauna of the SO. Brooding amphipods have a constrained mobile dispersal phase, which makes them more sensitive to environmental shifts (Ingels et al. 2012); they form an important link between lower and higher trophic levels (Havermans & Smetacek 2018). Notothenioid fish have a larger dispersal potential and are higher level predators (Duhamel et al. 2014). The choice of model organisms followed a long research tradition among the two partners guaranteeing the availability of existing samples.

COPE had **five major objectives**:

1. Delimitate genetic species of selected amphipod and fish morphospecies for subsequent population genetic analyses and the identification of possible cryptic diversity.

2. Obtain genomic data on neutral genetic variability, connectivity and population structure of two key taxa of the SO, scavenging *Charcotia* amphipods and *Trematomus* fish at a circum-Antarctic scale.

3. Use outlier analyses from genomic data to test for local adaptation to abiotic factors that characterize the seascape.

4. Compare patterns of genetic connectivity in both taxa groups to their life histories including dispersal capacities and oceanic seascapes.

5. Model distribution of *Charcotia* and *Trematomus* species in the SO from existing occurrence data and layers of environmental data.

The COPE project focused on the sustainable management of polar ecosystems through the integration of evolutionary connectivity in the design of marine protected areas (MPAs). This was achieved through connectivity assessment and distribution modelling of key taxa of the polar seas, accounting for the influence of the seascape. A conceptual scheme of the project, with its interactions between global change and conservation of the polar seas is presented below (Figure 1).

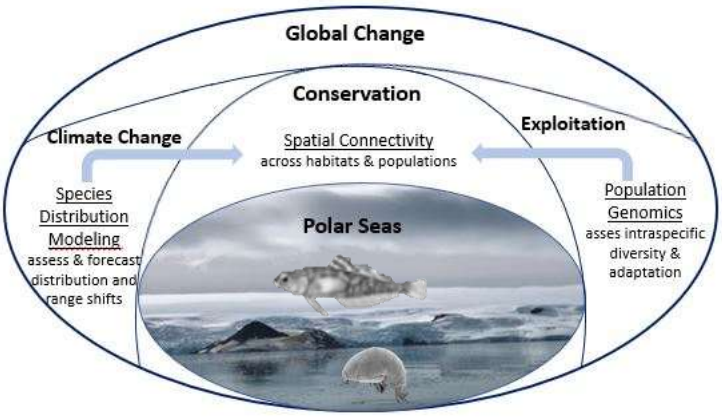


Figure 1. Polar seas are sentinels and gatekeepers of the planet's oceans. In a context of global change, including climate change and exploitation, knowledge of the ecological niche and genetic structure and adaptation is required in a spatial context for effective conservation measurements.

3. METHODOLOGY

The COPE project took a multidisciplinary approach. This included molecular techniques to extract DNA from *Charcotia* amphipods and *Trematomus* fish; DNA sequencing targeted single markers like the mitochondrial DNA barcoding marker Cytochrome I Oxidase (COI; Hebert et al. 2003) to identify cryptic biodiversity, and also reduced-representation sequencing (Christiansen et al. 2021) of parts of the genome of amphipods and fish with RAD (Restriction site Associated DNA)-based methods. The latter technique provides a much finer resolution than DNA barcoding and unravels population structure, variation and connectivity without the need for a reference genome. For non-model organisms of genetic research, RAD sequencing needs to be carefully optimized based on multiple parameters, including genome size (Christiansen et al. 2021). Depending on the position of restriction sites in the genome, RAD sequencing can provide data for neutral or adaptative genetic diversity. Seascape genomic analyses links genetic diversity to environmental factors and provide insights into local adaptation (Selkoe et al. 2016; Liggins et al. 2020). The evolutionary history of both target taxa was reconstructed with various phylogenetic methods. The phylogenetic trees were used, together with estimates of genetic diversity, to statistically delimitate genetic species.

For the first time, the COPE project provided genome size estimates of 16 Antarctic amphipod species with Feulgen staining and/or Flow Cytometry. Both methods have been widely validated in other taxa (Rees et al. 2007; Mulligan et al. 2014).

The trophic ecology of two amphipod morphospecies was investigated with stable isotope analyses. Ratios of the stable isotopes of carbon and nitrogen are commonly used to investigate the trophic ecology of organisms based on the close relationship between assimilated stable isotope ratios in an organism and its diet. Carbon stable isotope ratios (13C:12C) can determine the source of primary carbon while the stable isotope ratio of nitrogen is used to assess the trophic position in the food web.

Based on occurrence data and abiotic parameters, we also modelled species occurrences at the scale of the entire Southern Ocean and more regionally. We will explain details of the methodology more extensively below when presenting and discussing our scientific results and making recommendations.

4. SCIENTIFIC RESULTS AND RECOMMENDATIONS

4.1 Sample acquisition and participation in expeditions

Because of COVID-19 restrictions, the scientific COPE team could not participate in any Antarctic expedition in 2020. In 2021, postdoc Anton Van de Putte managed to take part in the R/V *Polarstern* expedition PS 219. Dorien Aerts had been assigned as back-up for the same cruise but could not participate in the end.

Additional amphipod samples were obtained in the course of 2022. Most important to continue preparation for the molecular work of WP3 was the collection of snap-frozen amphipod specimens during *Polarstern* expedition PS119 for genome size estimates with flow cytometry.

Dorien Aerts and Anton Van de Putte submitted a project proposal for the Peruvian research vessel R/V *B.A.P. Carasco* to join the 2023 Antarctic expedition (ANTARXXIV) in February and March 2023. Both projects were accepted, and contacts were made between the Peruvian representative in Belgium and the chief scientist. All preparations for the material, permits and the selection of Peruvian Master students were finalized. However, due to political uncertainties in Peru, participation of non-Peruvian researchers in this expedition was cancelled on short notice in late December 2022 (two months before departure).

Thus, the project had to rely mainly on existing samples of amphipods and fish from the collections of the RBINS and the Laboratory of Biodiversity and Evolutionary Genomics at the KU Leuven.

Below we summarize all samples and their geographic origin.

4.1.2 Overview of analysed samples

4.1.2.1 Amphipods

Altogether, 320 specimens of *Charcotia* amphipods were morphologically identified. Wherever possible, these specimens were also used for DNA extractions, DNA sequencing, analyses of stable isotopes and estimates of genome sizes. Most of the analysed amphipods (n = 234) belonged to the morphospecies *C. obesa*. Most of these samples originated around the Western Antarctic Peninsula and the Adélie Coast (south-east), although there were also samples from offshore Eastern Antarctica (Figure 1). For *C. amundseni*, we obtained 81 samples coming from three locations off the Northern part of the Antarctic continent (Figure 2).

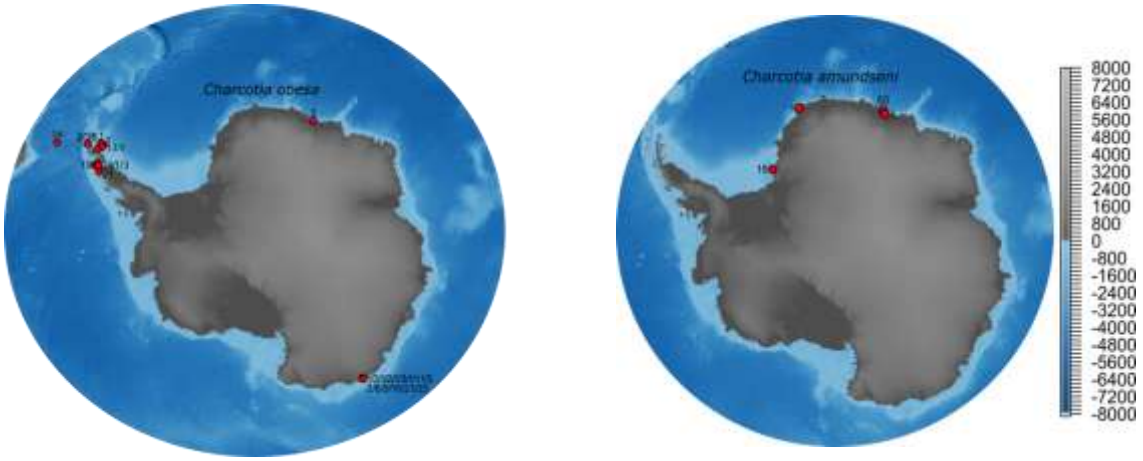


Figure 2. Overview of samples of *Charcotia obesa* (left) and *Charcotia amundseni* (right). Shades of blue indicate bathymetry of the Southern Ocean. Sample locations are indicated by red dots; the numbers next to the dots show the number of samples from each location.

4.1.2.2 *Trematomus* Fish

Fish specimens analysed in the COPE project (n=547) originated from different areas of the SO with a focus on the Western Antarctic Peninsula and the North-East coast of Antarctica (Figure 3). Most samples were obtained from five morphospecies (Table 1), although we had additional fish from another six *Trematomus* species with lower sample numbers. The latter were not further analysed.

Table 1. Overview of all samples of *Trematomus* fish. Only the first five morphospecies with sample numbers above 70 were further analysed. N = number of samples. * See below why these two morphospecies were merged here.

***Trematomus* species**	**N**
T. eulepidotus	146
*T. loennbergii/lepidorhinus**	100
T. scotti	78
T. newnesi	96
T. borchgrevinki	2
T. bernacchii	40
T. hansoni	60
T. pennellii	20
T. tokarevi	5

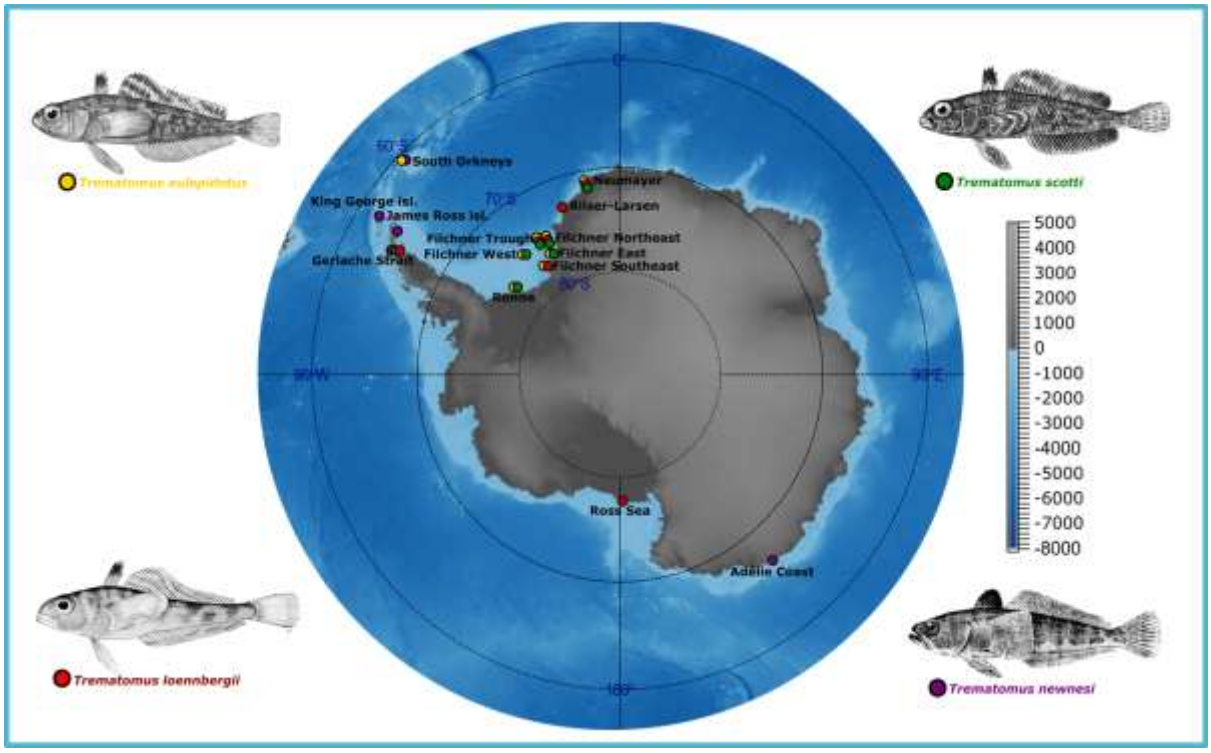


Figure 3. Overview of samples of four *Trematomus* morphospecies. Species identity is indicated with circles of different colours. Shades of blue indicate bathymetry of the Southern Ocean.

4.2 Species Distribution Models

Species distribution models (SDMs) are powerful tools to predict the likely distribution of species and investigate the relationships between species and their environment (Elith & Leathwick 2009). Key for a reliable model are high-quality, unbiased data at appropriate spatial and temporal resolution. Although SDMs have been successfully constructed for various taxa in the Southern Ocean (Guillaumot et al. 2019, 2020a, b; El-Gabbas et al. 2021; Gonzales et al. 2024), its remoteness represents a challenge. As part of WP4, we used existing occurrence and environmental data to model current and future distributions of fish and amphipod species and populations. For this objective, we followed the workflow of Guillaumot et al. (2018, 2019, 2020a). Under supervision of Dorien Aerts and in close collaboration with Charlène Guillaumot, specialist in niche modelling of the SO, Vera Claes, bachelor student from UHasselt, reconstructed SDMs with the currently available amphipod data. We generated four models, one model each of the entire SO for both *Charcotia* species (Figure 4) and one regional SDM each for the West Antarctic Peninsula for each species (Figure 5). Occurrence data were collected from online databases (OBIS, GBIF and Biodiversity.aq) for *C. obesa*, *C. amundseni* and *Waldeckia obesa* (since not all databases have been updated with the latest taxonomic nomenclature). We filtered the data based on the depth distribution of the species (<150 m *C. obesa* and >150 m *C. amundseni*), which was suboptimal because of overlap in depth distribution, but the only approach currently possible with online data. The dataset was supplemented with local datasets of *Charcotia* in the master thesis of Tim Plevoets (VUB; 2018-2019), a previous local dataset of Anton Van de Putte (checked by Cedric D'Udekem D'Acoz for species delimitation) and specimens collected by Dorien Aerts. This resulted in a final dataset of 658 occurrence records for *C. obesa* (Figure 4A) and 134 occurrence records for *C. amundseni* (Figure 4B).

The environmental descriptors were collected using the 'blueant' package in R (https://australianantarcticdivision.github.io/blueant/articles/SO_SDM_data.html), which is a collection of data source definitions defining a range of environmental and other data sources for SO studies. We selected environmental descriptors most suitable for benthic species (for example, depth, seafloor-_current, sediment, seafloor_temp, …). After checking for collinearity using the Spearman correlation and the VIF method, a dataset of 17 descriptors was selected. Further model calibration was done using the Kernel Density Estimate layer, clock4_crossvalidation and generation of Boosted Regression Trees to select the parameter set for the final SDM. The final model performance was checked with AUC, COR, TSS, maxSSS extrapolation, with the necessary correction of environmental descriptors.

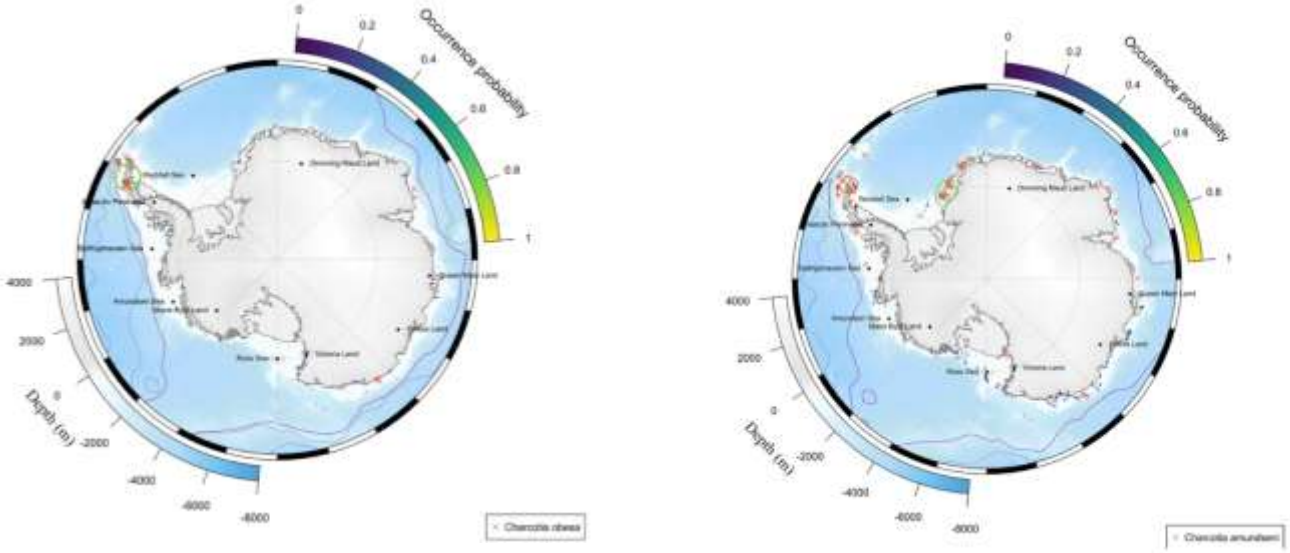


Figure 4. A) SDM of *Charcotia obesa* over the entire SO showing a medium to high (>0.3) predictive occurrence probability on the west side of the WAP and near Brabants Island and Anvers Island (Green circle). B) SDM of *C. amundseni* over the entire SO showing a high predictive to medium (>0.3) occurrence probability at the northern tip of the WAP (orange circle), at the brunt ice shelf in the Weddell Sea (green circle) and nearby Kemp land (North-East Antarctica).

Conclusions from these SDMs constructed at species level are that model performance on a local scale (Figure 5) was better than on the scale of the entire SO (Figure 4). Model performance (based on AUC, COR, TSS and maxSSS) of *C. obesa* was generally poor with lower statistical support (Figure 4A & Figure 5A), regardless of the scale. This is probably due to the narrow depth range (<150m) of this amphipod species and the lack of environmental descriptors for this shallow depth layer of the Southern Ocean. Future improvements for SDMs of amphipods should extend the occurrence data. To predict future occurrences under climate change, more recent data for the environmental descriptors should be used as soon as these will become available, since a lot of the layers date from 2005-2012. Alternatively, abiotic information from recent cruises could also be used in future SDMs together with the occurrence data of *Charcotia* from these cruises, including for example in shallow coastal areas as during the Belgica121 expeditions with the Australis (Danis et al. 2021).

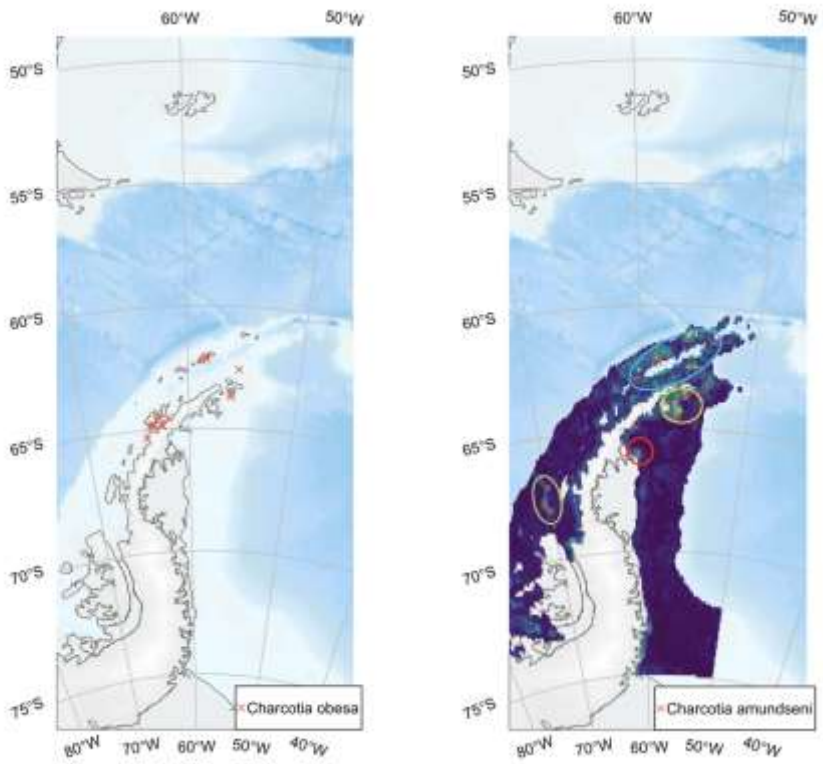


Figure 5. SDMs around the Western Antarctic Peninsula of A) *Charcotia obesa* showing no predictive occurrence probabilities and B) *Charcotia amundseni* with high occurrence probabilities (> 0.6): Yellow = James Ross and Seymour Island; Blue = South Shetland Islands and Elephant Island; Green = Adelaide and Alexander Island; Red = Larsen Ice Shelf.

For fish, SDMs have been run on *Trematomus eulepidotus* and *T. loennbergii*, the most promising candidates with the most occurrence data across the SO. Modelling was conducted in principle as described in Guillaumot et al. (2018) and Christiansen et al. (2021). In brief, assembled occurrence data and a subset of environmental data were used for predictive SDMs using boosted regression trees (BRT). Environmental variables were thinned using variation inflation factor, biological knowledge, and correlation plots. BRT parameters were optimized following Elith et al. (2008) and a four-fold 'CLOCK' method was used to train and test the model following Guillaumot et al. (2019). Similarly, we used kernel density estimation layer (Philipps et al. 2009) and a multivariate environmental similarity surface index (Elith et al. 2010) as described in Guillaumot et al. (2019). All these corrections were used to make sure conservative estimates are retrieved and distributions are not predicted in areas with little to no available background data. Model evaluation was conducted using Area Under the receiver operating Curve (AUC; Fielding & Bell 1997) and by assessing the number of presence test data correctly classified as suitable habitat by the model predictions.

Predicted species distributions for *Trematomus eulepidotus* and *T. loennbergii* generally fitted well with known, documented occurrences (Figures 6 & 7). *Trematomus eulepidotus* seemed overall more widely distributed in the investigated part of the SO (Figure 6) than *T. loennbergii* (Figure 7). However, the latter species lives at greater depths (Eastman 2017), so this observation may in fact relate to less available habitat. Occurrence probability for both species was surprisingly low at the Filchner Trough region, although we know that both species occur there. As this also corresponds to a putative genetic break (see below), the results may indicate that population-level SDMs could more accurately predict

current and future occurrence for *Trematomus* species as hypothesized at the onset of the COPE project.

From our currently available data, sample sizes of the different population genetic entities of both amphipods and fish are too limited to successfully generate statistically supported, population-genetic-based SDMs as originally planned and for example applied elsewhere (Lovrenčić et al. 2022). This kind of models could be constructed in the future when the population genetic structures of more species of the SO are available.

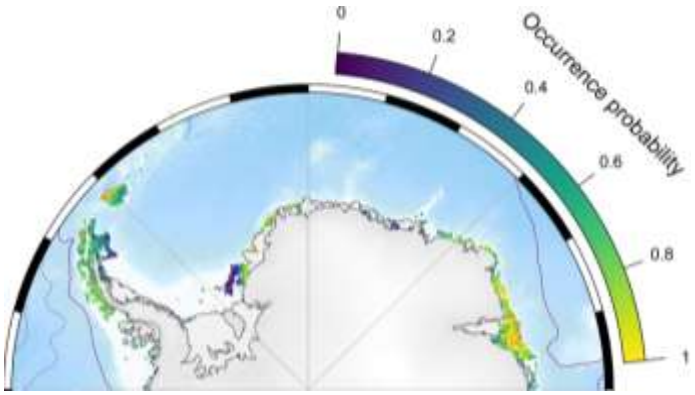


Figure 6. Species distribution probability of *Trematomus eulepidotus* at the West Antarctic Peninsula, the Weddell Sea, and the Atlantic and Indian Ocean sectors of the Southern Ocean. Observed occurrences shown as orange dots.

Woods et al. (2023) could successfully use the database Myctobase to model distributions of the eight most common myctophid fish species. In contrast to our study, they relied on occurrence and environmental data from 1905 fish trawls, a much larger database than what was available for the model organisms of COPE. We recommend extending data collection of the occurrences of Antarctic taxa significantly to provide a basis for more meaningful SDMs. Especially sampling outside the known hotspots like the conclusions of Guillaumot et al. (2021). Likewise, filling knowledge gaps on taxonomic identities including cryptic diversity (see below) are required. Until such extended databases become available for more taxa of the SO, evaluating and communicating model uncertainties remain vital (Guillaumot et al. 2021).

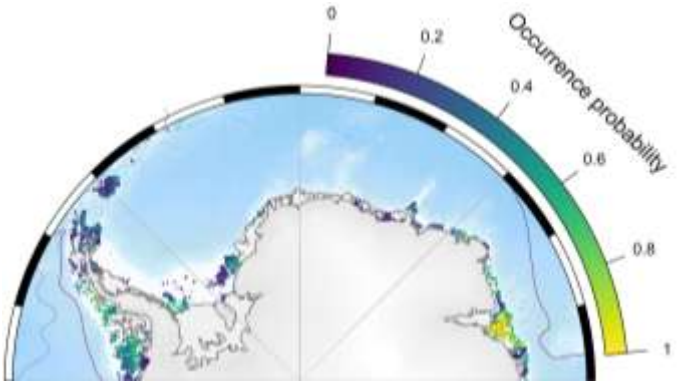


Figure 7. Species distribution probability of *Trematomus loennbergii* off the West Antarctic Peninsula, in the Weddell Sea, and the Atlantic and Indian Ocean sectors of the Southern Ocean. Observed occurrences shown as orange dots.

4.3 Trophic ecology of *Charcotia* amphipods

Analyses of stable isotopes are commonly used to investigate trophic ecology of organisms also from the SO (e.g. Guerreiro et al. 2015; Michel et al. 2019), based on the close relationship between assimilated stable isotope ratios in an organism and its diet (DeNiro & Epstein 1978, 1981). Food sources at the primary trophic level can vary in their stable isotope composition (Wing et al. 2018; Zenteno et al. 2019), making it possible to use the ratios of stable isotopes of carbon (13C:12C) to determine the source of primary carbon. To assess the trophic position of an organism in a food web, the stable isotope ratio of nitrogen (15N:14N; δ15N) can be measured as indicator. Consumers are usually enriched in 15N through their diet, resulting in a sharp increase of δ15N values with each trophic level (Nienstedt & Poehling 2004). To compare isotopic niches as proxies of the trophic niche between species, ratios of C and N isotope are combined (Newsome et al. 2007).

Initially, the trophic ecology of *Charcotia* amphipods was not planned. However, given its complementary to the tasks on cryptic diversity and genomic investigations, trophodynamics provides a more complete picture of variation and connectivity among amphipod species and populations of the SO. Lyssianoid amphipods are known to differ in trophic ecology, even among closely related species (Havermans et al. 2010; Havermans et al. 2018). There have been few investigations on the trophic ecology of *Waldeckia obesa* (Chapelle et al. 1994; Dauby et al. 2001; Janeck & Rakusa-Suszczewski 2005), the taxonomic name under which *Charcotia* amphipod species were known until 2018 when d'Udekem d'Acoz formally described *C. amundseni* as a new species to distinguish it from *C. obesa*. Given the overlap in depth distribution of both *Charcotia* species, the taxonomic identity of amphipods from previous studies is not clear. Therefore, we identified *Charcotia* specimens morphologically and genetically to the species level (see above, sample acquisition) first, followed by estimates of stable isotope compositions to unravel the realized ecological niche of both species.

We found little overlap in the standard ellipses of N and C isotope ratios between the two species (Figure 8). Also, the inferred trophic positions differed by one level with *C. amundseni* having a higher estimated trophic level (TP) of 5 (Figure 9). Within each species, we found geographic differences in stable isotope ratios (not shown). For *C. obesa*, populations from Dumont D'Urville Sea differed significantly from those around the Southern Shetland Islands or the Western Antarctic Peninsula. For *C. amundseni*, we found a significant difference in stable isotope ratios between the populations in the Filchner area as compared to Breid Bay and Crown Bay off Queen/Monning Maud Land.

Our results illustrate that the two *Charcotia* amphipod species occupy rather high trophic positions, much higher than the TP of 2.4 that Michel et al. (2019) estimated for *C. obesa* or Zenteno et al. (2019) found for other, smaller scavenging amphipods. Such a difference could suggest a higher trophic plasticity of *Charcotia* amphipods than initially assumed. It furthermore indicates that scavenging amphipods show regional differences in the prey they consume and that this prey is sourced from different trophic positions in the Antarctic food web (Smale et al. 2007).

The results on trophic ecology of *Charcotia* amphipods have been compiled into a scientific publication which is currently undergoing its second round of reviews (Aerts et al. under review).

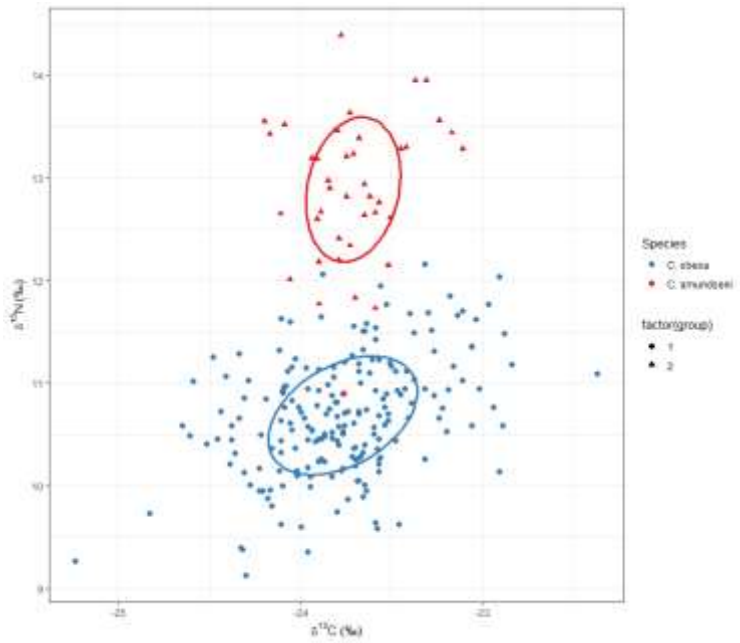


Figure 8. Standard Ellipse Area of stable isotope ratios of 15N and 13C and their respective isotopic niche of *Charcotia obesa* (blue) and *Charcotia amundseni* (red).

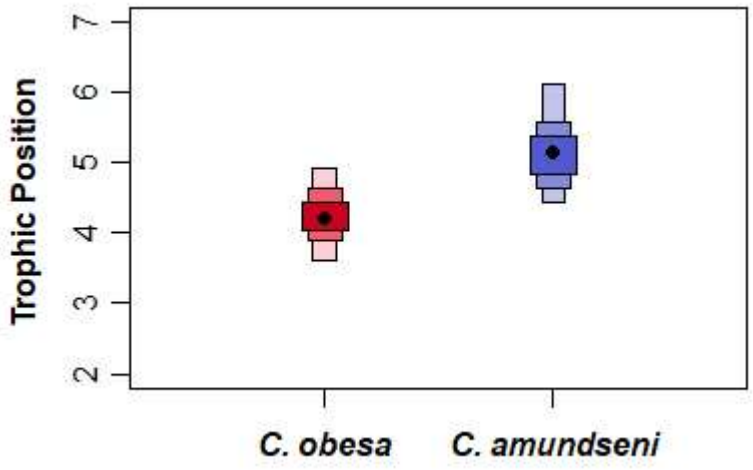


Figure 9. Trophic positions of *Charcotia obesa* (red) and *Charcotia amundseni* (blue). Boxplots show credibility intervals of 50, 75 and 95 % of posterior estimations. Black dots denote the statistical mode of each species' trophic position.

4.4 Genome sizes of Antarctic amphipods

Amphipods are among the arthropod groups with an exceptionally large range of genome sizes varying from 0.68 to 64.62 pg (Table 2). Genome sizes of amphipods seem to be larger in extreme environments such as the Arctic or the Deep Sea (Bachmann & Rheinsmith 1973; Libertini et al. 2000; Rees et al. 2007; Ritchie et al. 2017). With one recent exception for krill (Shao et al. 2023), no estimates of genome size from Antarctic amphipods are available. Knowledge on genome size is important to further test the hypothesis on the effects of extreme environments and to optimize genomic tools like Reduced Representing Sequencing (Christiansen et al. 2021).

Here, we applied two methods to estimate genome size, flow cytometry, which requires fresh tissue or well-conserved flash-frozen tissues, and Feulgen Imaging Densitometry (staining of nuclei) which is also possible on alcohol and formol preserved samples from museum collections. Because we obtained data from more species with the first method, only these results are described and discussed here. Our study provided for the first time estimates of genome sizes from Antarctic amphipods from 16 species belonging to 9 families. We could confirm the huge variation in genome size from other studies. Antarctic amphipods had genomes with sizes ranging from 0.42 to 58.2pg. *Ampelisca richardsoni* had the maximum estimate of 58.24pg (see Table 2 below), a truly giant genome. The only other available estimate for genome sizes of Antarctic amphipods comes from Antarctic krill (Shao et al. 2023) based on genomic sequence data, they estimated a genome size of 48 Gb or 49 pg, which falls inside our range. On average, Antarctic amphipods had a genome size of 11.38 pg. We did not observe an overall larger genome size in Antarctic amphipods than in other marine amphipods, also not from greater depths (Figure 10). Our results do thus not support the hypothesis on extreme environments although this needs to be further confirmed with additional data from non-extreme habitats. Still, we found differences according to Antarctic habitats between benthic and pelagic amphipods (not shown). Large genome sizes are usually attributed to abundant repetitive genomic features (Rutz et al. 2023) such as satellite DNAs and transposable elements (Shao et al. 2023). This could be further investigated in the future once high-quality genomes of Antarctic amphipods become available. We are currently preparing a scientific publication of these results.

Table 2. Estimates of haploid genome size (pg) for 17 species of Antarctic amphipods generated by flow cytometry (FCM), including number of individuals tested (n), body size and habitat. NA: not available.

	FCM (pg)			
Species	Mean ± SD	n	Body size (mm)	Habitat
Family Cyllopodidae				
Cyllopus lucasii	0.48	1	23	Pelagic
Family Phrosinidae				
Primno macropa	1.28	1	15.25	Pelagic
Family Hyperiidae				
Hyperoche capucinus	1.21	1	18	Pelagic
Hyperoche medusarum	0.42	1	NA	Pelagic
Hyperiella dilatate	0.57	1	NA	Pelagic
Hyperiella antarctica	1.19	1	NA	Pelagic
Hyperia macrocephala	4.31	1	28.3	Pelagic
Themisto gaudichaudii	0.61	1	16.3	Pelagic
Family Eusiridae				
Eusirus sp.	5.68	1	NA	Pelagic
Eusirus laticarpus	3.38	1	15	Pelagic
Eusirus microps	1.09	1	32.67	Pelagic
Family Epimeriidae				
Epimeria georgiana	5.38	1	37	Benthic
Family Lysianassidae				
Charcotia amundseni	9.27	1	27	Benthic
Family Uristidae				
Uristes murrayi	24.2 ± 5.69	6	30.57	Benthic
Uristes adarei	18.48	1	30	Benthic

Family Iphimediidae				
Gnathiphimedia sexdentata	6.92	1	23	Benthic
Family Ampeliscidae				
Ampelisca richardsoni	58.2	1	26.67	Benthic
Unknown amphipod	21.08	1	NA	NA
Unknown amphipod	29.32	1	NA	NA
Unknown amphipod	5.68	1	NA	NA
Unknown amphipod	1.35	1	NA	NA

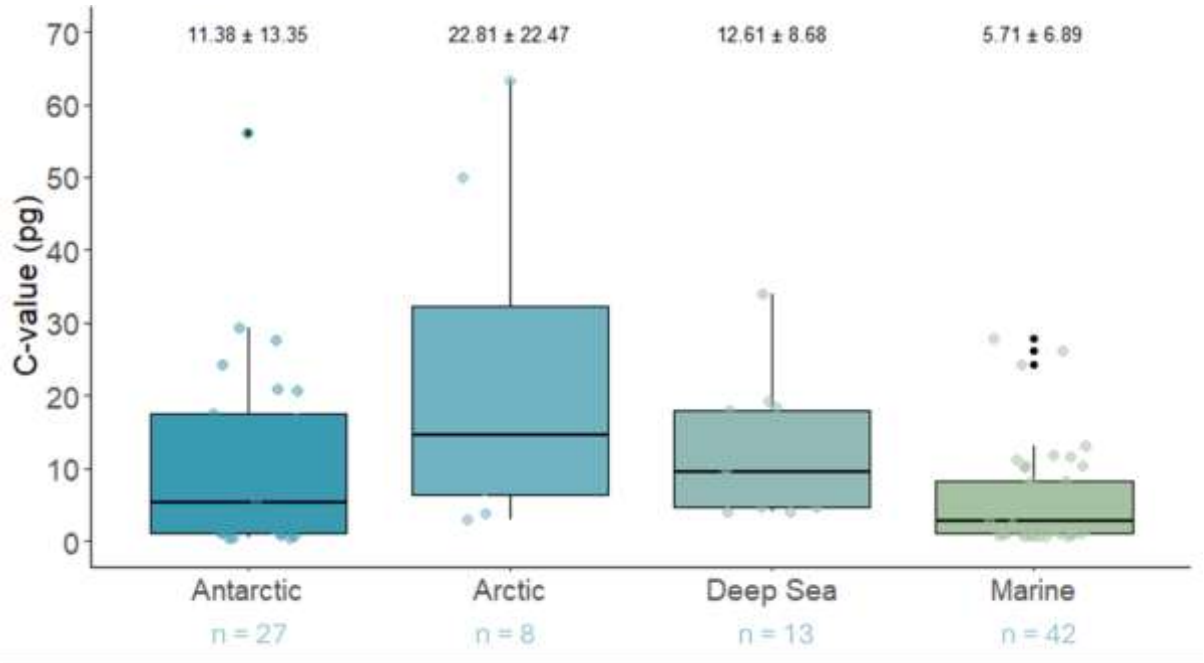


Figure 10. Estimates of average haploid genome sizes for amphipods from various habitats. n= number of analysed species. The figure includes original Antarctic data, and published data for marine taxa (Bachmann & Rheinsmith 1973; Rheinsmith et al. 1974; Libertini et al. 2000, 2003, 2008; Krapp et al. 2006; Jeffery & Gregory 2014; Hultgren et al. 2018); arctic taxa (Rees et al. 2007), and the deep-sea (Ritchie et al. 2017).

4.5 Cryptic diversity in *Charcotia* amphipods

Cryptic diversity, genetic species that lack morphological differences, is widespread, also in the SO, and has been reported from taxa such as Polychaeta (Brasier et al. 2016), Echinodermata (Moreau et al. 2018; Jossart et al. 2020) and fish (Christiansen et al. 2018). Likewise, multiple examples of cryptic diversity are also known in marine (Beerman et al. 2018) and Antarctic amphipods (Baird et al. 2011; Havermans et al. 2018), which often consist of species complexes (D'Udekem d'Acoz & Verheye 2017).

We successfully obtained DNA sequences of the COI locus from 216 specimens of *C. obesa*. After initial data cleaning, aligning and trimming we obtained COI fragments of 681 bp. Based on the structure of the obtained phylogenetic tree, the haplotype network (Figure 11) and the results of three different statistical methods for the delimitation of genetic species, we found that the number of identified

genetic species varied between two (ASAP, bPTP) and nine (4theta). When using a conservative approach, we accepted that the morphospecies *C. obesa* consists at least of two genetic species. No obvious morphological differences have been observed among specimens of *C. obesa* (d'Udekem d'Acoz et al. 2018) indicating that these genetic species could indeed be two cryptic species; however, to finally confirm that this diversity is indeed cryptic and not owing to micromorphological differences, we recommend that additional morphological analyses are conducted on multiple specimens of the two different genetic species. The haplotype network showed that there was no obvious geographic pattern in the occurrence of the two genetic species (Figure 11).

When estimating the genome size of three individuals each from the two genetic species with Feulgen staining, we found no clear differences in genome size among the two genetic species (Figure 12). One of the two genetic species showed in fact a rather wide variation in genome size, ranging from 7.8 to 13.3 pg. Given the small sample size for which we could obtain these estimates because of lack of time, we recommend expanding this analysis with a larger number of individuals in future research.

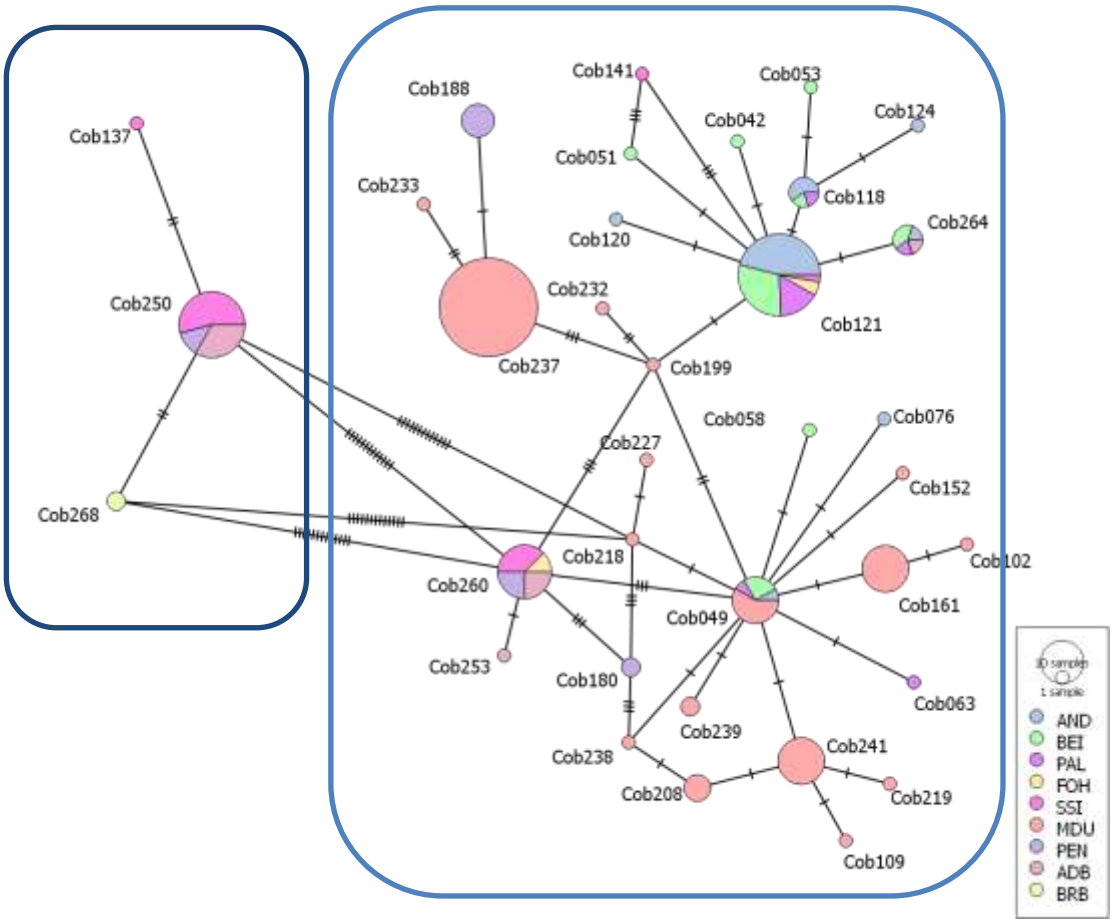


Figure 11. Haplotype network of *Charcotia obesa*, based on COI DNA sequence data. The size of the circles is proportional to the number of individuals with this haplotype; colours indicate the geographic origin of the specimens (AND: Andvord, BEI: Berthelot Islands, PAL: Palmer Station, FOH: Foyn Harbor, SSI: South Shetland Islands, MDU: Dumont D'Urville Sea, PEN: Tip WAP, ADB: Admiralty Bay, BRB: Breid Bay) while horizontal lines represent mutational steps. The two different genetic species are indicated by two different coloured quadrangles.

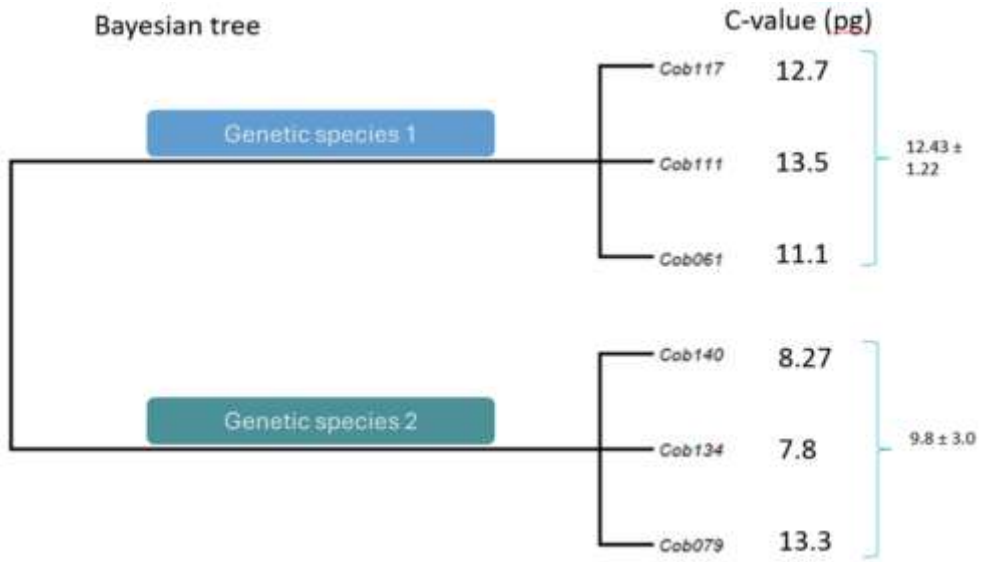


Figure 12. Bayesian phylogenetic tree of a subset of the two genetic species of *Charcotia obesa* with estimates of their genome sizes. Estimates were obtained using the Feulgen Imaging Densitometry technique and based on 15 nuclei each.

For *C. amundseni*, we successfully extracted DNA from 73 samples. Following the same species delimitation methods as for *C. obesa*, we found that in contrast to its sister species, *C. amundseni* shows a much higher number of genetic species of up to 9, depending on the method that we used to delimitate genetic species. When following the same conservative approach as for *C. obesa*, we still identified six genetic species in *C. amundseni*; these are also obvious from the structure of the phylogenetic tree and the haplotype network (Figure 13). For *C. amundseni*, there is also no obvious phylogeographic pattern of the six genetic species although this should be confirmed with additional samples, also from more locations in the SO.

Again, at least three individuals from each genetic species were selected to estimate their genome sizes. Here, it seems that genetic species of *C. amundseni* might show small differences in their genome size from each other (Figure 14). Several genetic species are represented by single individuals though, which makes it impossible to obtain reliable estimates of genome sizes for these genetic species. Similarly to *C. obesa*, we would recommend estimating the genome sizes of more samples of the various genetic species of *C. amundseni*, which was not possible in the framework of the COPE project, also because the research part on genome sizes was now much more extensive than originally foreseen in the project.

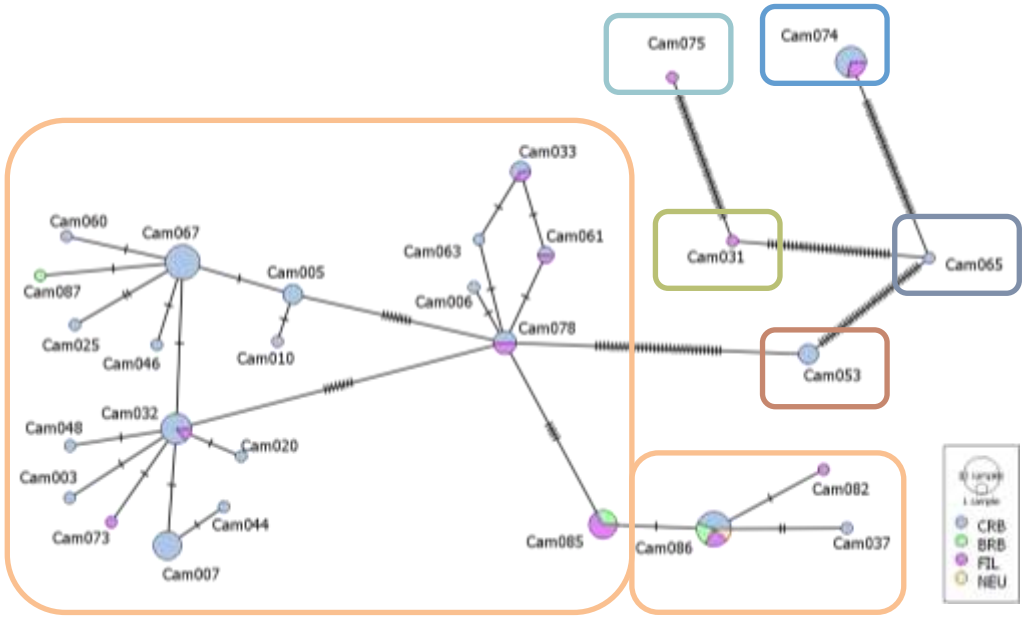


Figure 13. Haplotype network of *Charcotia amundseni*, based on COI DNA sequence data. The size of the circles is proportional to the number of individuals with this haplotype; colours indicate the geographic origin of the specimens (CRB: Crown Bay, BRB: Breid Bay, FIL: Filchner Area, NEU: Neumayer Station) while horizontal lines represent mutational steps. The six different genetic species are indicated by different coloured quadrangles.

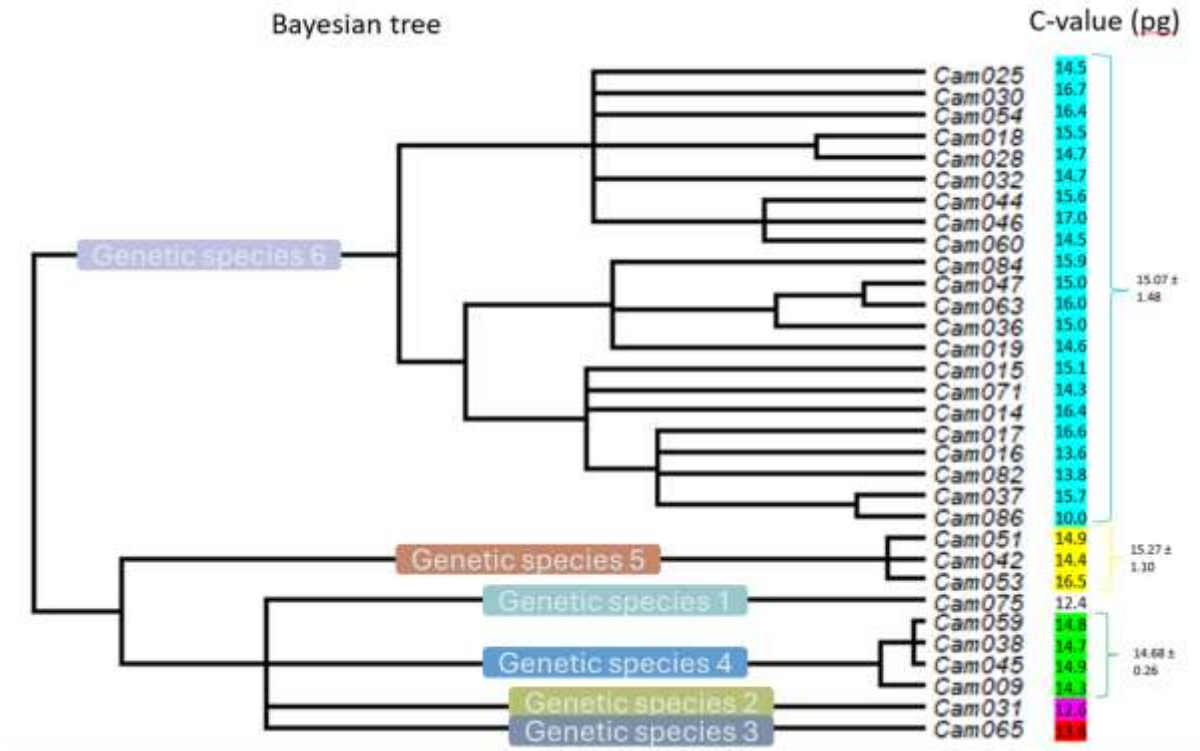


Figure 14. Bayesian phylogenetic tree of a subset of the six different genetic species of *Charcotia amundseni* with estimates of their genome sizes. Estimates were obtained using the Feulgen Imaging Densitometry technique and based on 15 nuclei each.

When comparing the levels of cryptic diversity of the two investigated *Charcotia* species to other Antarctic amphipods, they are for *C. amundseni* in the same order of magnitude as in *Eusirus* where three cryptic species were reported for *E. perdentatus* and four to five for *E. giganteus* (Verheye & d'Udekem d'Acoz 2021), respectively. In contrast, the cryptic diversity of *C. obesa* is lower than in *Eusirus* and also lower than in its sister morphospecies *C. amundseni.*

4.6 Cryptic diversity in *Trematomus* fish

Cryptic diversity has been suspected to occur within the *Trematomus genus* (Eastman & DeVries 1997; Bernardi & Goswami 1997). Phylogenetic analysis based on the ddRAD dataset (21,270 SNPs, Figure 15) of COPE revealed two distinct clades within *T. eulepidotus*. We furthermore observed separated clades within the *T. loennbergii/lepidorhinus*, group, one including only the *T. loennbergii* morphotype (*T. loennbergii* 1) and a second one comprising morphotypes of both *T. loennbergii and T. lepidorhinus* (*T. loennbergii* 2/*lepidorhinus*) (Figure 15).

Trematomus lepidorhinus and *T. loennbergii* have often been confused as they have very similar morphologies; they are mainly distinguished by scales on the snout, lower jaw, and preorbital (scaled vs. naked), and the snout length ratio (Lautredou et al. 2010; deWitt et al. 1990). They could not be discriminated in previous studies by any molecular markers, including mitochondrial or nuclear markers (Lautredou et al. 2010; Kuhn et al. 2009), and can thus not be discriminated based on the novel ddRAD dataset either. Unfortunately, the most recent *Trematomus* genomic phylogenies (Bista et al. 2023; Rayamajhi et al. 2024) only include one specimen of *T. loennbergii* and could thus not further test the monophyly of these two morphospecies. From the present data, we conclude for now that the morphological species *T. loennbergii* and *T. lepidorhinus* are not distinct species and should be synonymized.

The two separate ddRAD genetic lineages observed in *T. eulepidotus* and *T. loennbergii/lepidorhinus*, which are in both cases found in sympatry in some sampling stations of the Eastern Weddell Sea (see maps Figure 3), suggest the presence of distinct cryptic species or ecotypes within these two groups. Genomic rearrangements could maintain nuclear divergence among the lineages by suppressing recombination in some genomic regions. A high chromosomal number diversity (2n = 24–58) characterizes trematomids, and especially the group *T. loennbergii/lepidorhinus*. *Trematomus lepidorhinus* has a diploid chromosome number of 47 in males and 48 in females (Gigliotti et al. 2015; Ozouf-Costaz et al. 1991), while *T. loennbergii* has diploid chromosome numbers ranging between 26 and 33 without any sex-specific differences (Ghigliotti et al. 2015; Ozouf-Costaz et al. 1997; Morescalchi et al. 1992), although both have 52 chromosomal arms. The latter suggests that chromosomal rearrangements, notably chromosomal fusions, fissions, translocations and inversions frequently occur within the group (Auvinet et al. 2020; Rayamajhi et al. 2024). Transposable elements were hypothesized to have facilitated nonhomologous recombination, thereby increasing genomic rearrangements (Auvinet et al. 2020). Such rearrangements could putatively be at the origin of the distinct nuclear lineages that we observe if specific nuclear regions experience reduced gene flow, due to chromosomal incompatibilities. This could be investigated soon, notably thanks to the first published high-quality genomes of 24 Antarctic notothenioid fishes (Bista et al. 2023). As the divergence between the *T. loennbergii/lepidorhinus* and *T. lepidorhinus* lineages is not observed with mitochondrial COI data, the two lineages might still be hybridizing; or alternatively, the genetic resolution of this small gene fragment is insufficient to differentiate between the different lineages.

Hybridization has also been suggested in other Antarctic fish species such as *Chionodraco* (Marino et al. 2013; Schiavon et al. 2021).

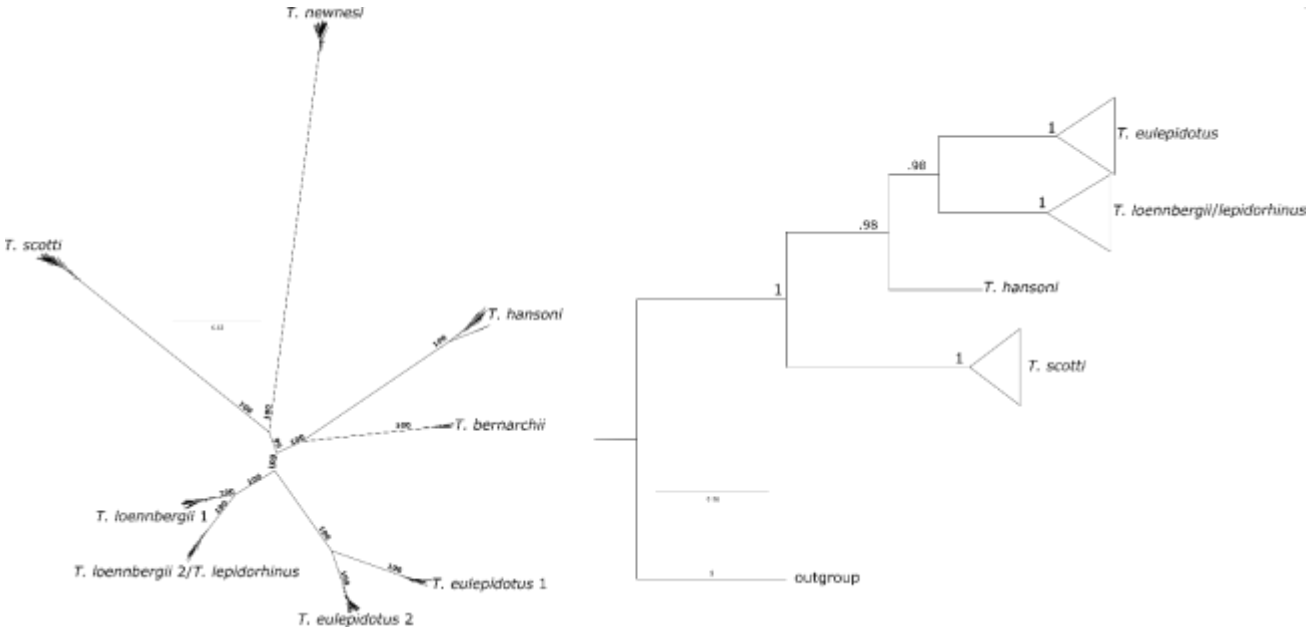


Figure 15. Phylogenies of the analysed *Trematomus*. A. Phylogeny constructed with Maximum Likelihood approaches and based on the ddRAD sequencing dataset (21,270 SNPs), with bootstrap support values above the corresponding nodes (left). B. Bayesian phylogeny based on COI (626 bp), with posterior probabilities shown above the corresponding nodes (right).

4.7 Population genetic structure and connectivity of *Charcotia*

4.7.1 *In silico* analyses for *Charcotia* amphipods

In silico computations were performed by Dorien Aerts using the SimRAD package, based on scripts of Christiansen et al. (2021). Digestions were performed on a simulated genome (500 Mb) scaled to the genome size of the target species (6.65 pg for *Charcotia obesa* (Feulgen Imaging estimate) and 8.66 pg for *C. amundseni (*flow cytometry estimate)) and on a reference genome of the closest related amphipod (*G. roeselli*, 3.5 pg). Simulations were used for three different protocols, Genotyping by Sequencing (GBS) with single digestions of the restriction enzymes *PstI* or *ApeKI*, or double digest (dd)RAD with the two restriction enzymes *EcoRI-MspI.* Numerous size windows were selected to calculate the number of fragments within these windows. Further analyses included estimates of marker density of the selected size windows. In these *in silico* digestions, we aimed for a coverage of 40x to provide a buffer for the actual digestions (where we aimed for a coverage of 20x). Based on these results, there were a few size windows for which sufficient coverage could be obtained with double digestion using *EcoRI-MspI*. Based on the simulation results, the final decision was made to conduct ddRAD with *EcoRI-MspI* in a size window of 250-340 bp (without counting the adaptors).

4.7.2 SNP datasets

A total of 288 samples were sent for sequencing, consisting of 207 *Charcotia obesa* specimens and 81 *C. amundseni* specimens. Following bioinformatic analyses, several datasets are now available for both *Charcotia* species. The datasets produced with the Stacks v.2 pipeline and the option of *de novo* assembly (no available reference genome of closely related amphipods), were selected for population

genomic analyses. The initial dataset of *C. obesa* included 205 individuals and 100,124 SNPs with on average 54% of missing data; for *C. amundseni,* we obtained ddRAD sequencing data from 81 individuals of 6,830 SNPs with a mean of 45% missing data. Initial filtering for data exploration based on missing data (<40%), minor allele frequency (MAF = 0.05), and Linkage Disequilibrium (LD = 0.2), was performed in R, resulting in datasets of 22,847 and 2,986 SNPs respectively for *C. obesa* and *C. amundseni*. These SNPs comprise both neutral and adaptive variation. The percentage of missing data is relatively high (40%). Encouraging is that according to Nazareno et al. (2017) decreasing this percentage from 50% to 10% or 0% missing data does not significantly alter diversity parameters.

The available datasets are somewhat limited concerning sample size at the population level, geographical coverage, and uneven sampling of different locations, which are common problems in Antarctic research. Because COPE ran during the COVID-19 pandemic, we had to rely mainly on existing samples and recommend complementing our data with additional samples. However, the high number of loci genotyped for each species increases the strength and statistical power of the current study. Previous studies based on simulations, have shown that even a sample size as little as two samples can generate accurate estimates of population genetic parameters with many SNPs (≥1500) (Nazareno et al. 2017) or four to six individuals with ≥1000 SNPs (Willing et al. 2012). Furthermore, it was shown that increasing sample size above four or six individuals has minor impact on the estimates of genetic diversity (Qu et al. 2020; Li et al. 2020). Grouping SNP data according to genetic species identity based on COI did not reduce the number of missing sites, which is why we did not further follow this approach.

4.7.2.1 Population structure of *Charcotia obesa*

After filtering for individuals with <40% missing data, the final dataset included 95 individuals from eight populations (22,847 SNPs). All individuals were sequenced in the same library, so that potential library effects could be excluded. This dataset was split into putatively adaptive SNPs (outliers identified using PCadapt, n=4,745) and a neutral dataset (total dataset after filtering and removal of outliers, n=18,102) to detect drivers of genetic variation. Neutral genetic markers can help investigate processes such as migration, gene flow and dispersal and ultimately connectivity between populations, while adaptive genetic variation (variation under natural selection) has an impact on the fitness of an individual and therefore it's adaptive potential to changes in the environment. Initial data exploration on all SNPs (both neutral and putatively adaptive) showed a geographic separation on PC1 (of the Principal Component Analysis; PCA) between locations around the WAP on the right (in pink and red), locations from the Adélie Coast (south-east) on the left (in blue, green and purple) and two individuals from Breid Bay (on the north side of the continent) in the middle (orange) (Figure 16A). PCA on both neutral and outlier loci shows similar patterns (Figure 16B, C). Genetic differentiation was highest between populations around the WAP and the Adélie Coast with F_{ST} values ranging from 0.035 (between JOI and MDU), 0.074 (between TIP_PEN and MDU) to 0.0.089 (between ADB and MDU) (Figure 16D). Low to moderate population genetic differentiation was observed between amphipods from locations around the WAP and Breid Bay (F_{ST} = 0.0.011 - 0.0.091). Based on the pairwise F_{ST} values, no genetic differentiation occurs between Breid Bay and the Adélie Coast, however a separation is visible on PC1 (Figure 16A, B and D). Interestingly, on PC2 (Figure 16A), the two locations around the WAP showed a separation as well, despite their close geographic proximity. However, this separation is not visible in the neutral dataset (Figure 16B) but is obvious on PC1 in the PCA of the outlier loci (Figure 16C). The F_{ST} value (0.019) between Admiralty Bay (ADB_20) and the tip of the Western Antarctic Peninsula (TIP_PEN_13), indicates limited genetic differentiation between these two populations.

Grouping of *C. obesa* specimens according to genetic species as delimitated by COI did not explain the observed patterns (results not shown).

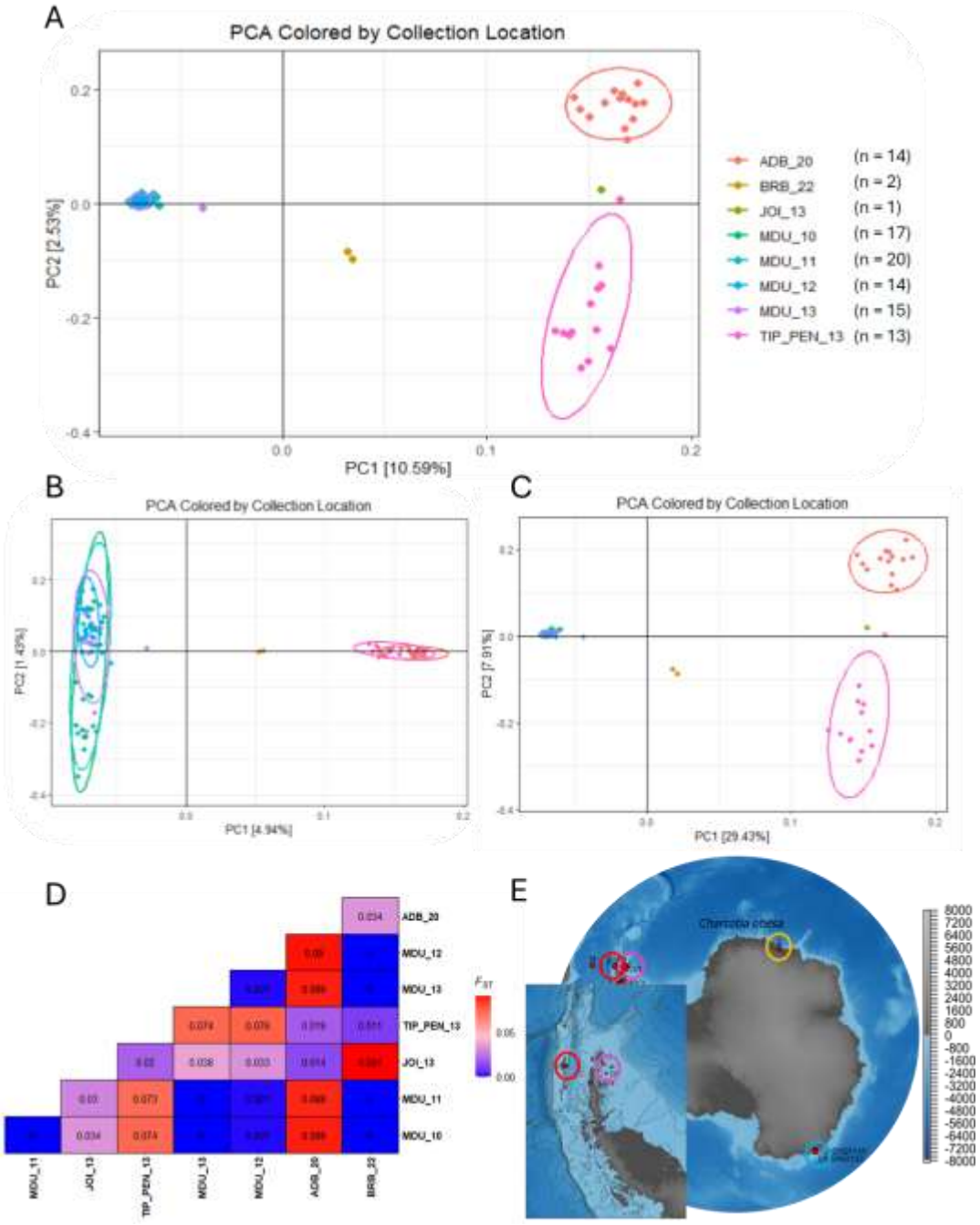


Figure 16. Population genetic differentiation of *Charcotia obesa,* individual samples are coloured by sampling location (red: ADB = Admiralty Bay; orange: BRB = Breid Bay; khaki green: JOI = Joinville Island; green/turquoise/blue/purple: MDU = Dumont D'Urville Sea; pink: TIP_PEN = tip of the West Antarctic Peninsula). A. Principal Component Analysis (PCA) of all SNP loci after initial filtering (LD, MAF and missing data; 22,847 SNPs). B. PCA of neutral loci (18,102 SNPs). C. PCA of outlier loci detected by PCadapt (4,745 SNPs). D. Heatmap showing F_{ST} values of pairwise genetic differentiation among populations of *C. obesa* based on neutral SNPs. E. Map of the SO and zoom of the WAP indicating sampling locations. Colored circles correspond to the colors in the PCAs (A, B, C) for each location.

4.7.2.2 Population structure of *Charcotia amundseni* after filtering

After filtering for individuals with <40% missing data, the final dataset resulted in 2,986 SNPs and 29 individuals from two locations. No library effect influenced the structure of these analyses, since DNA of all individuals were sequenced in the same library. Preliminary results from the PCA do not show any differentiation probably because all individuals (except one) originated from the same location (Figure 17). The 28 individuals from Crown Bay did not all cluster closely together in the PCA, in contrast to the pattern observed in the sister species *C. obesa* (Figure 16, above). Individuals from the two locations of *C. amundseni* clustered separately from each other in the PCA (Figure 17), but additional samples from more locations are required to draw any conclusion on geographic population structuring of *C. amundseni*.

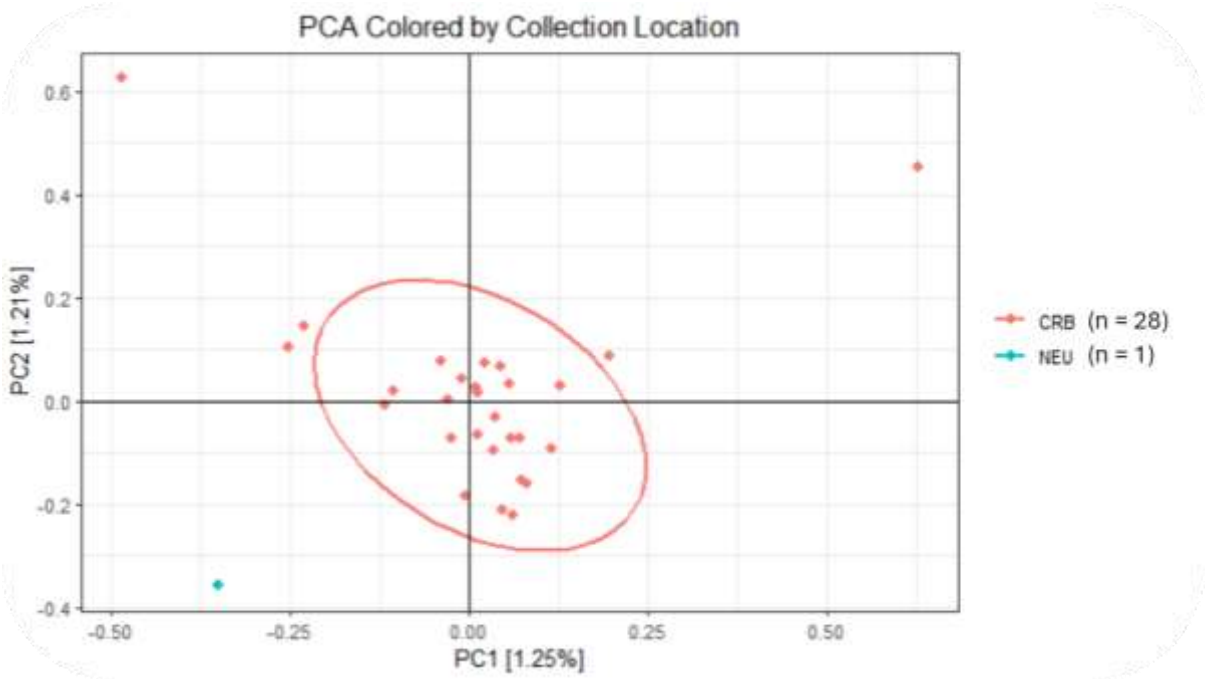


Figure 17. Principal Component Analysis of *Charcotia amundseni* coloured by sampling location (red: CRB = Crown Bay; blue: NEU = Neumayer Station).

4.7.2.3. Conclusions on the population structure and connectivity of *Charcotia* amphipods

The observed population differentiation of *Charcotia obesa* at large geographic scales, for example between populations from the WAP and the distant Adélie Coast or the WAP and the North-Eastern part of the SO (Figure 16 and Figure 17) point to genetic isolation, a signature which was also described from the Southern Ocean octopus *Pareledone turqueti* (Lau et al. 2025). The pattern of genetic differentiation by geographic distance fulfils theoretical expectations because *Charcotia* are brooding species where genetic exchange between sites is expected to be limited. Our results further provide evidence that long-distance rafting (Spencer et al. 2025) does not seem to have facilitated long-distance dispersal of *C. obesa*. We also found evidence for the population structuring of *C. obesa* along the WAP at a much smaller, local scale (see above, Figure 16C; between the South Shetland Islands (Admiralty Bay) and eastern of the tip of the Peninsula close to Marambio base); this pattern implies that the Antarctic Slope Current (Dawson et al. 2023) has not led to genetic exchange between these populations. Only a few other studies on bivalves (Hoffman et al. 20211; Munaz-Ramirez et al. 2020;

Levicoy et al. 2021) reported similar patterns of genetic differentiation at small scales around the Western Antarctic Peninsula and the South Shetland Islands. Likewise, Baird et al. (2011) found evidence for limited gene flow in the amphipod *Eusirus perdentatus* among two closely situated locations along the Eastern coast of Antarctica based on single markers. In greater water depths including the SO, Weston et al. (2022) detected barriers of gene flow for the amphipod *Bathycallisoma schellenbergi*. We did not observe similar patterns for *C. amundseni* occurring at greater depths because of the lack of samples. In any case, the structured population pattern of the benthic *C. obesa* at both small and large scales contrasts with the only RAD sequencing study of Antarctic crustaceans where no genetic population structure was observed in pelagic Antarctic krill on a large scale (Shao et al. 2023; White et al. 2024).

Our results thus show that patterns of gene flow and genetic population differentiation are influenced by life history traits like brooding in the genus *Charcotia*. In addition, the patterns of genetic differentiation and connectivity of *Charcotia* contribute to the delimitation of MPAs.

4.8. Connectivity and local adaptation of *Trematomus* fishes

4.8.1. SNP datasets

Following bioinformatic analyses, several datasets are available for the *Trematomus* species with most samples belonging to the species *T. eulepidotus*, *T. loennbergii/lepidorhinus*, *T. newnesi*, and *T. scotti*. The datasets produced using the Stacks v.2 pipeline and *reference-based* mapping (against *T. bernachii*) were selected for subsequent population genomic analyses. Further filtering on missing data at the loci and individual level, minor allele frequency and maximum heterozygosity resulted in datasets of 27,945-88,240 SNPs. The outlier detection method PCadapt was used on the latter total datasets to identify putatively adaptive SNPs (1,439-17,377 SNPs). The outlier SNPs were removed from the total datasets, which were also filtered for Hardy-Weinberg equilibrium and linkage disequilibrium, to obtain neutral datasets of 8,793-13,438 SNPs.

The available datasets are somewhat limited regarding sample size at the population level, geographical coverage, and uneven sampling of locations, which are common challenges in Antarctic research. However, the high number of loci genotyped for four species increases the strength and statistical power of the current study.

4.8.2. Neutral population structure of *Trematomus* fish

Genetically neutral SNP data sets were analyzed with a variety of statistical methods to test for population differentiation and structure: F_{ST} statistics, Principal Component analysis (PCA) and Discriminant Analysis of Principal Components (DAPC).

4.8.2.1. *Trematomus eulepidotus* (142 individuals; 9,885 SNPs)

The PCA including all individuals morphologically identified as *T. eulepidotus* shows two distinct clusters corresponding to two phylogenetic clades (Figure 18). The first lineage (*T. eulepidotus* 1) is mainly found off the South Orkneys Islands, Filchner West and Filchner Trough. The second lineage (*T. eulepidotus* 2) is present in neighboring stations in Filchner West and Filchner Trough, but also further South and East, in Ronne, Filchner East, Filchner Northeast and Neumayer (Figure 18).

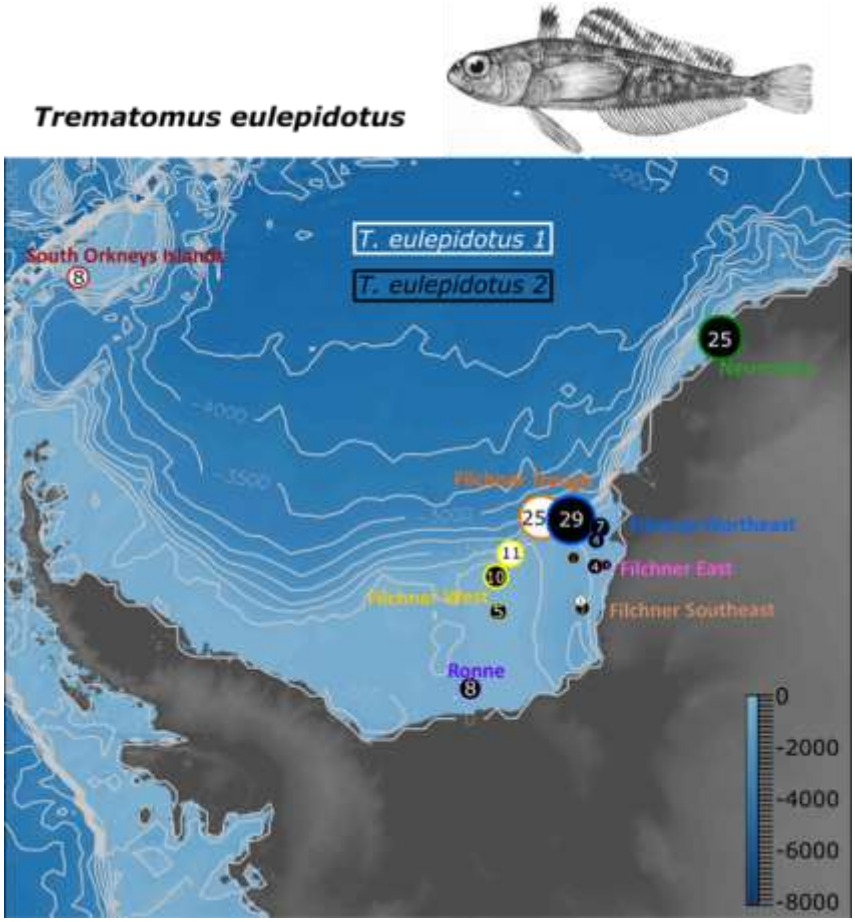


Figure 18. Map of the northern SO showing the sampling site**s** of the *Trematomus eulepidotus* and its two lineages, *T. eulepidotus* 1 (white) and *T. eulepidotus* 2 (black). Points represent sampling stations and are sized and numbered according to the number of individuals sampled. Colors represent different populations (i.e. sampling locations). The 500m isobaths are represented as light grey contour lines.

PCAs and DAPCs of *T. eulepidotus* 1 (84 individuals, 8,849 SNPs) and *T. eulepidotus* 2 indicate genetic structure within the two lineages. In *T. eulepidotus* 2, the Neumayer population is separated from the other populations in the Eastern Weddell Sea in both PCA and DAPC, with significant F_{ST} values of 1.5-1.7 % (Figure 19 A-D). In the DAPC, the Ronne population is also distinct, showing minimal overlap with other populations from the Eastern Weddell Sea (F_{ST} = 0.6-0.7) and significant differentiation with Filchner W further North (Figure 19C). In *T. eulepidotus* 1, the South Orkneys population appears clearly separated from populations in the Eastern Weddell Sea, with significant F_{ST} values of 3.6-3.9 % (Figure 19G). The populations from the Eastern Weddell Sea (Filchner NE, W and Trough) overlap on both PCA and DAPC, showing F_{ST} values of 0.2-0.6 %. However, the Filchner Trough population is significantly differentiated from the two other populations in the Eastern Weddell Sea (Figure 19E-G).

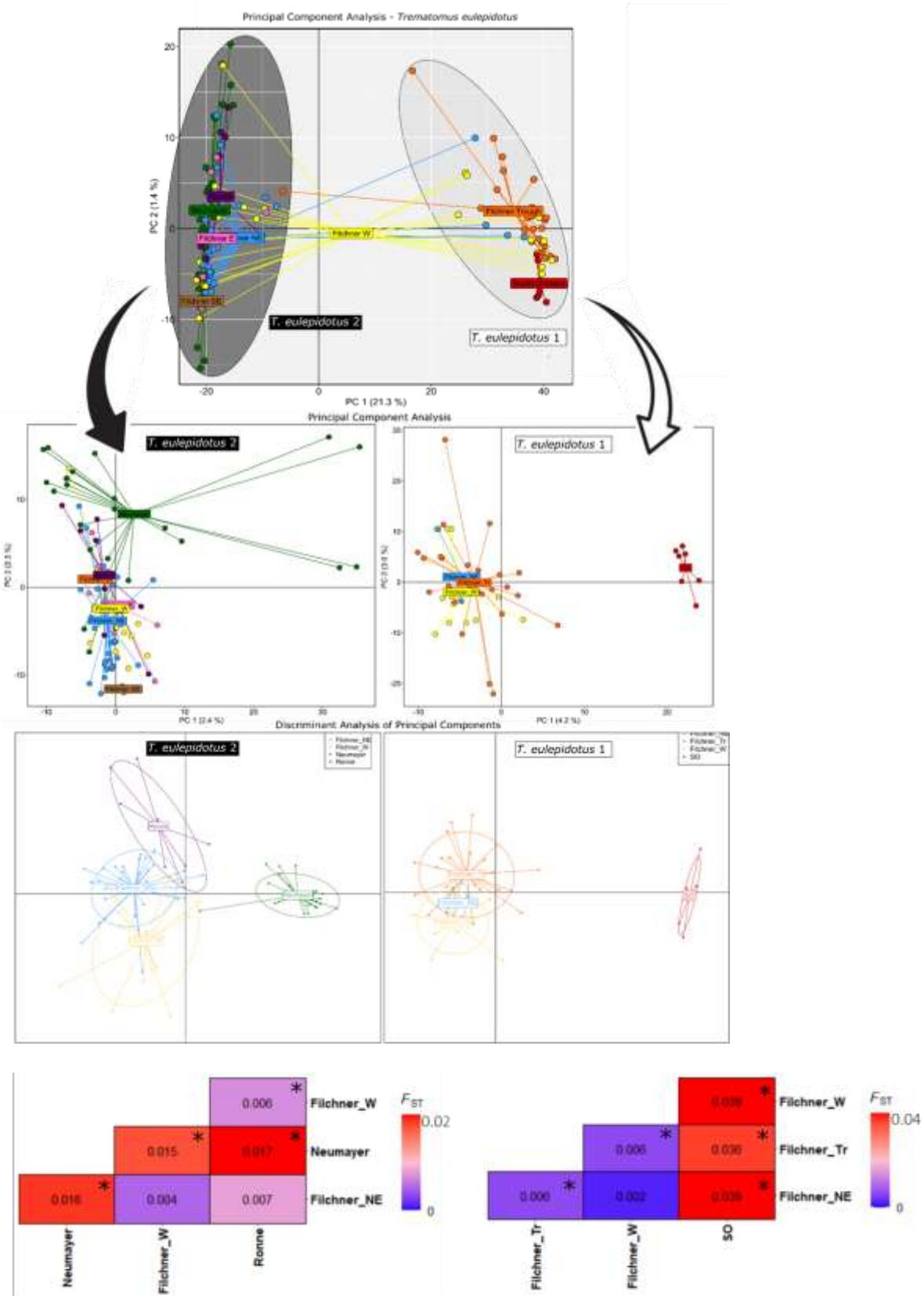


Figure 19. A. PCA of all specimens identified as *Trematomus eulepidotus*. B. PCA of *T. eulepidotus* 2 (black cluster on A.), C. DAPC of *T. eulepidotus* 2, D. Heatmap showing FST values of pairwise comparisons among populations of *T. eulepidotus* 2, E. PCA of *T. eulepidotus* 1 (white cluster on A.), F. DAPC of *T. eulepidotus* 1, G. Heatmap showing FST values of pairwise comparisons among populations of *T. eulepidotus* 1. *significantly differentiated (p-value < 0.05)

4.8.2.2 *Trematomus loennbergii/lepidorhinus* - (116 individuals; 10,898 SNPs)

The PCA including all individuals morphologically identified as *Trematomus loennbergii* and *T. lepidorhinus* shows two distinct clusters corresponding to the two clades (Figure 20). The first lineage (*T. loennbergii* 1) is mainly present in the Ross Sea, Gerlache Strait and Filchner Trough, while some individuals are also found further northeast, in the Riiser-Larsen station. The second lineage (*T. loennbergii/lepidorhinus* 2) is only present in the Eastern Weddell Sea. There, its distribution appears to extend further northeast (Filchner NE, Riiser-Larsen, Neumayer) than the other *T. loennbergii* lineage (Figure 20).

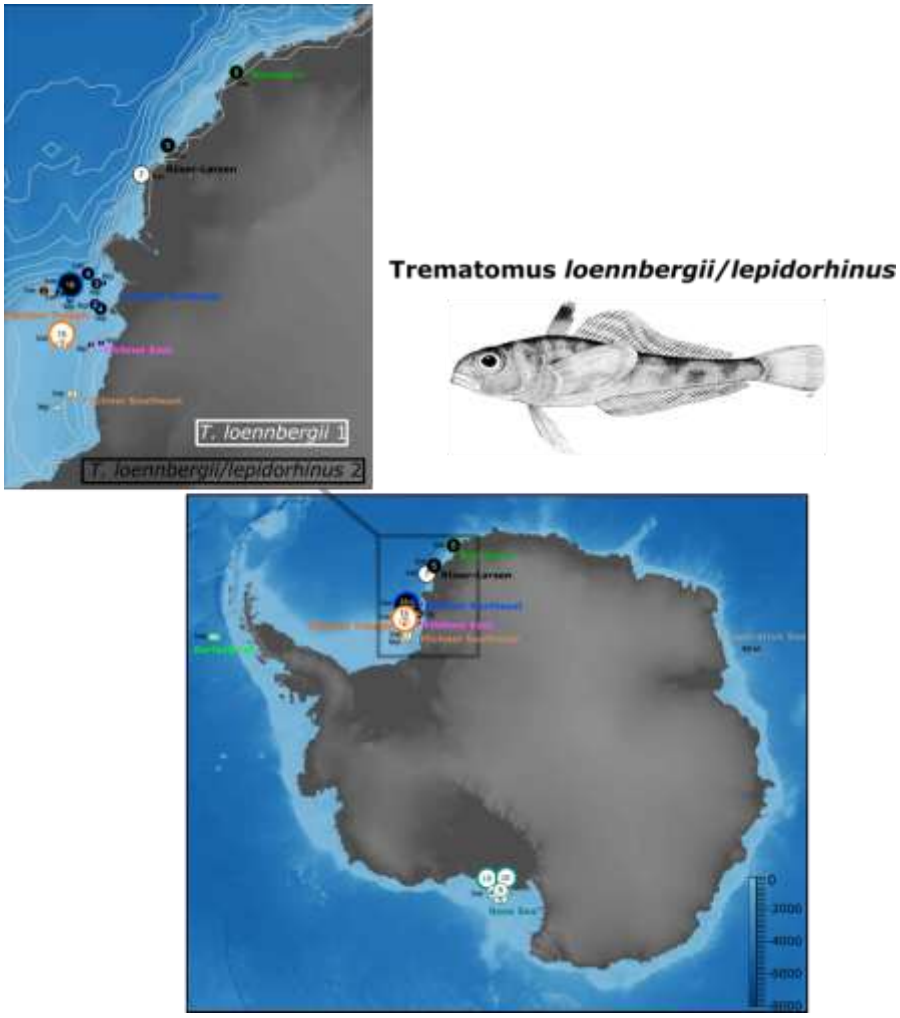


Figure 20. Map of the SO and northern SO (insert) showing thesampling sites of *Trematomus loennbergii/lepidorhinus* samples and the two lineages, *T. loennbergii* 1 (white) and *T. loennbergii/lepidorhinus* 2 (black). Points represent stations and are sized and numbered according to the number of individuals sampled. Colors represent populations (i.e. sampling locations). The 500 m isobaths are represented as light grey contour lines (top left figure).

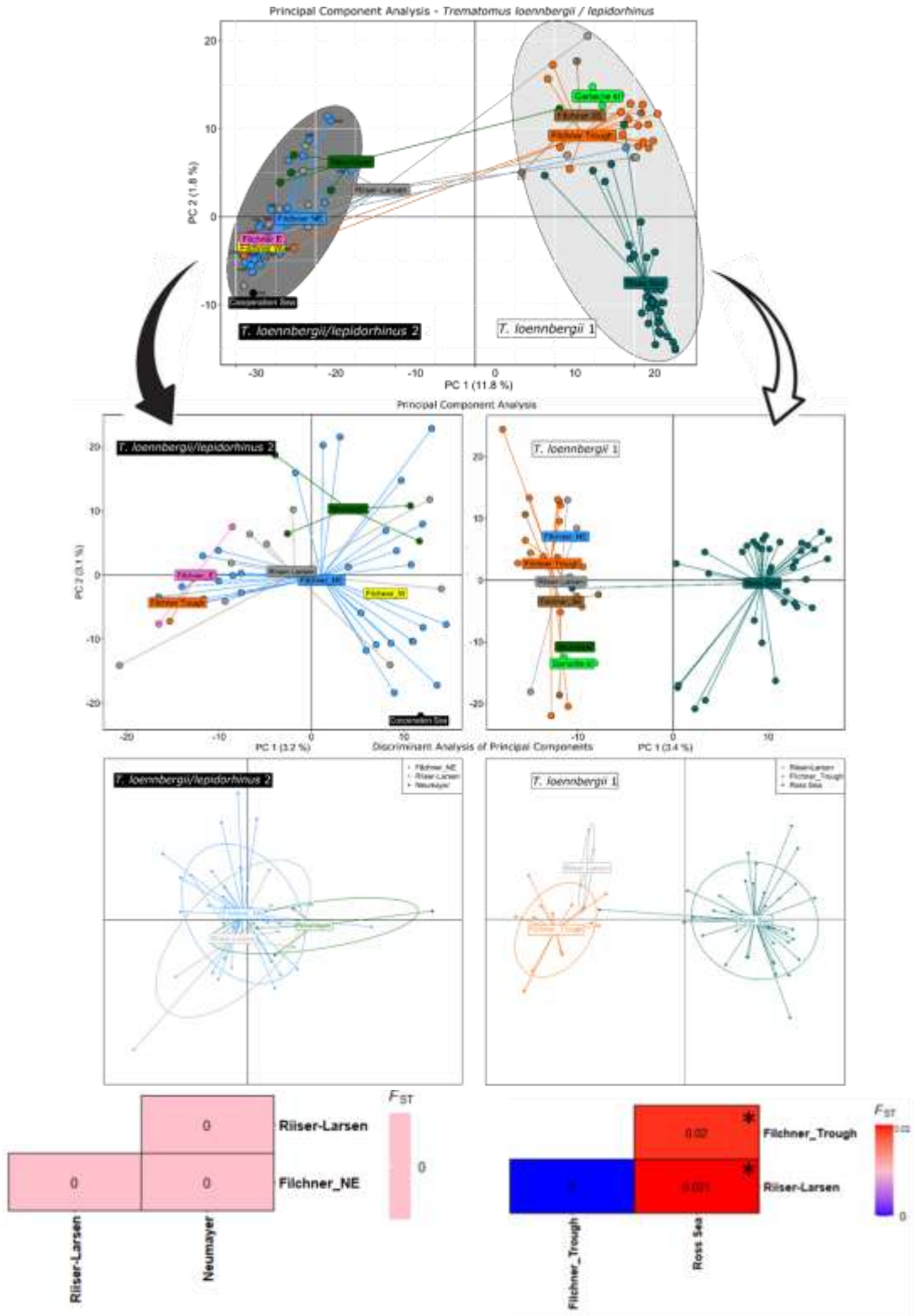


Figure 21. A. PCA of all specimens identified as *Trematomus loennbergii/lepidorhinus*. B. PCA of *T. loennbergii/lepidorhinus* 2 (black cluster on A.), C. DAPC of *T. loennbergii/lepidorhinus* 2. D. Heatmap illustrating FST values of pairwise comparisons among populations of *T. loennbergii/lepidorhinus* 2. E. PCA of *T. loennbergii* 1 (white cluster on A.) F. DAPC of *T. loennbergii* 1, G. Heatmap showing FST values of pairwise comparisons among populations of *T. loennbergii* 1. *significantly differentiated (p-value < 0.05).

Within species *T. loennbergii/lepidorhinus* 2, no genetic structure is detected among the sampled populations, which are all from the Eastern Weddell Sea. They largely overlap on both PCA and DAPC (Figure 21B-C) and they show no population differentiation (F_{ST} = 0%; Figure 21D). PCAs and DAPCs both indicate genetic structure within *T. loennbergii* 1 (Figure 21E-F). The Ross Sea population appears clearly separated from the Eastern Weddell Sea populations, also showing significant F_{ST} values of 2 % (Figure 21G). The Eastern Weddell Sea populations overlap in both PCA and DAPC, (Figure 21E-F) and their differentiation is not significant (F_{ST} = 0%; Figure 21G).

4.8.2.3. ***Trematomus scotti*** - (72 individuals; 12,966 SNPs)

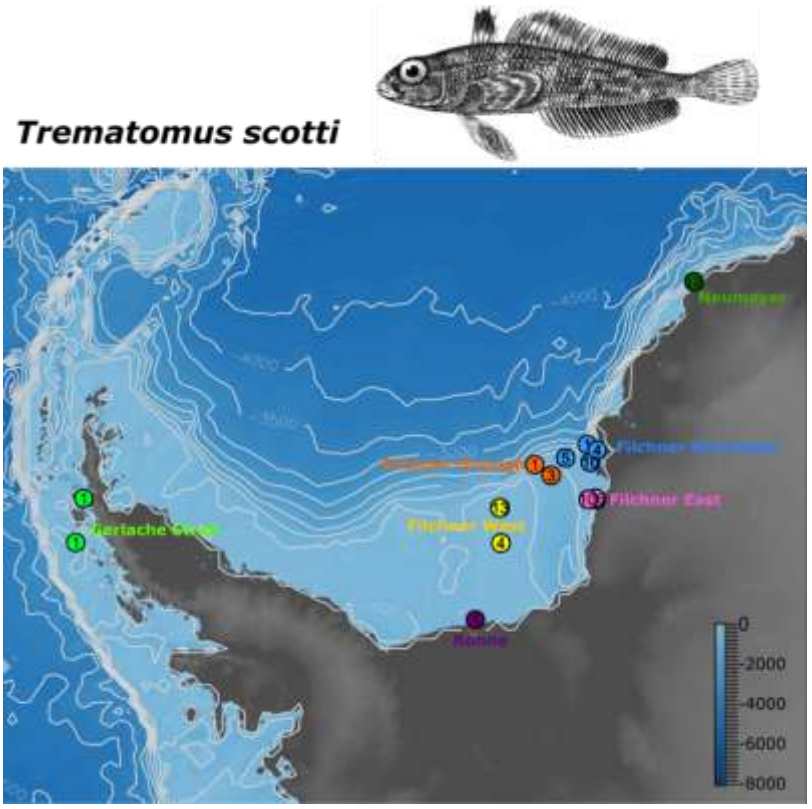


Figure 22. Map of the northern SO showing the sampling sites of *Trematomus scotti* specimens. Points represent stations and are colored according to population (i.e. sampling locations). Points are numbered according to the number of individuals sampled in each station. The 500 m isobaths are represented as light grey contour lines on the map.

For *T. scotti*, F_{ST} values between five Eastern Weddell Sea populations (Filchner W, Trough, NE, E and Neumayer) are very low, ranging between 0 to 0.4 % (Figure 23A). Only the Filchner Trough population is significantly differentiated from Filchner NE and E (F_{ST} =0.3-0.4%, and the latter two populations are significantly differentiated from each other (F_{ST} =0.3%). Matching this low level of population differentiation, the DAPC reveals extensive overlap between all populations (Figure 23B).

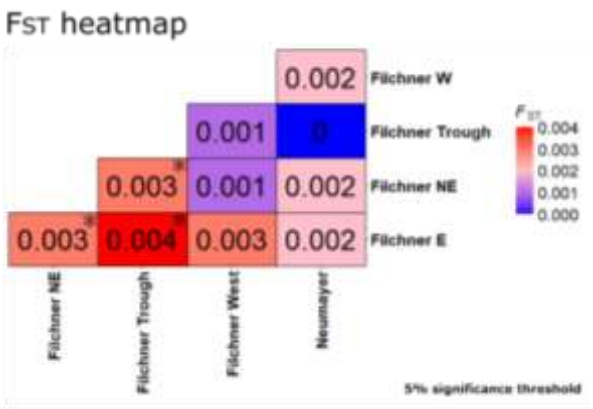


Figure 23A. F_{ST} heatmap between populations of *Trematomus scotti*. *significantly differentiated (p-value < 0.05)

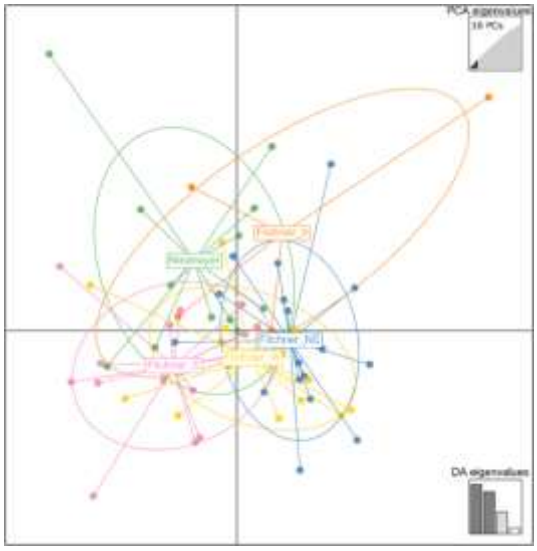


Figure 23B. PCA and DAPC of *Trematomus scotti* populations.

4.8.2.4. ***Trematomus newnesi*** - (96 individuals; 13,438 loci)

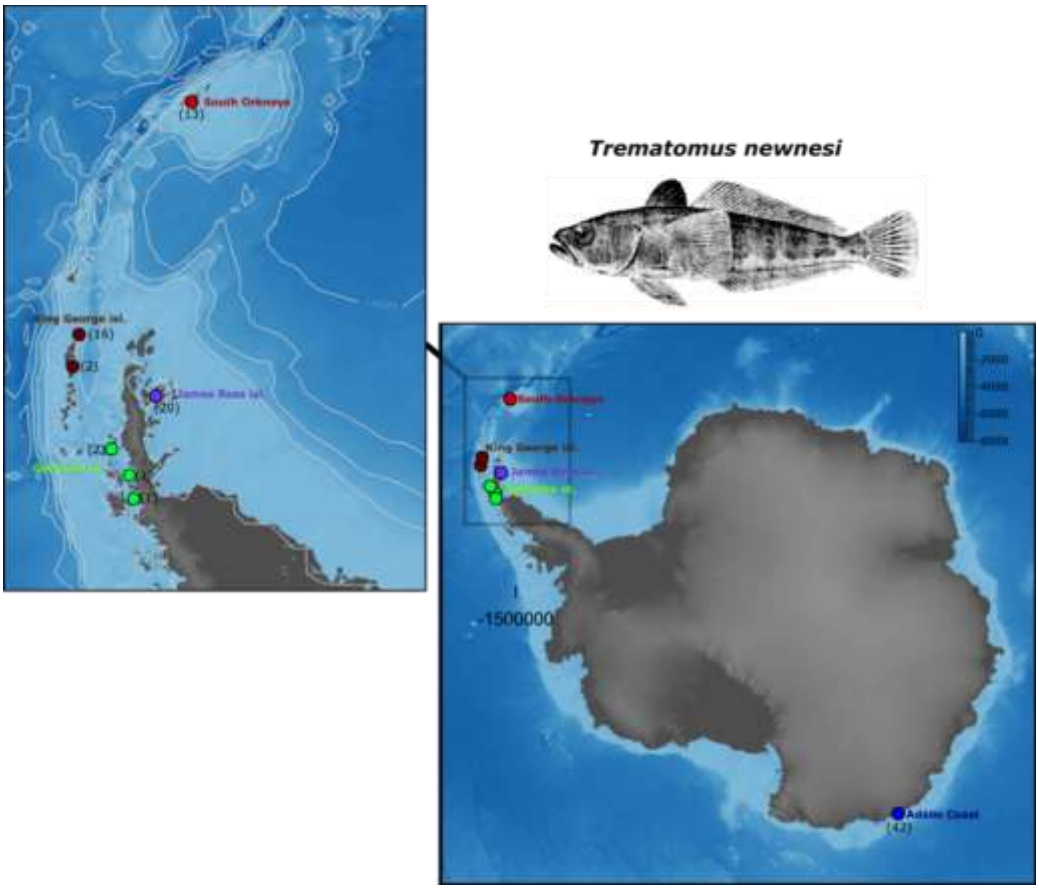


Figure 24. Map of the SO and Antarctic Peninsula (insert) showing the sampling sites of *Trematomus newnesi* specimens. Points represent stations and are colored according to population (i.e. sampling locations). Numbers beside the circles represent the number of individuals sampled in each station. The 500m isobaths are represented as light grey contour lines on the map (top left part of the figure).

For *T. newnesi*, F_{ST} values between four locations in the Peninsula area (Gerlache Strait, James Ross Island, King George Island and South Orkneys) range between 0.8 % (James Ross Island vs both King George Island and South Orkneys) and 2.2 % (Gerlache Strait vs King George Island). James Ross Island and King George Island are significantly differentiated (F_{ST} 0.8%), while differentiation with South Orkneys is not significant (although with similar F_{ST} values of 0.8-1.1%). Similar significant F_{ST} values are found between Gerlache Strait and other Peninsula populations than between the very distant Adélie Coast and the Peninsula populations (about 2%; Figure 25A). The DAPC shows that the Terre Adélie and South Orkneys populations are genetically distinct from all others. The populations around the Western Antarctic Peninsula overlap extensively, with Gerlache Strait showing some separation (though with a very low number of individuals; Figure 25B).

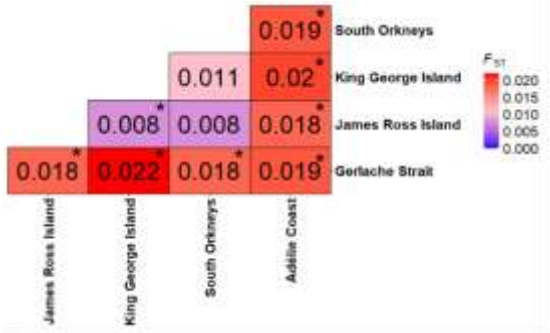


Figure 25A. F_{ST} heatmap between populations of *Trematomus newnesi.* *significantly differentiated (p-value <0.05)

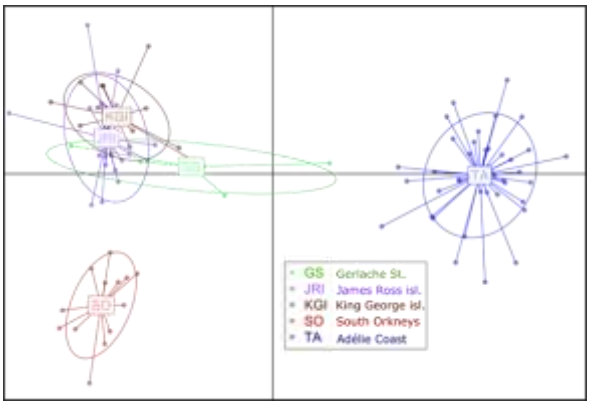


Figure 25B. DAPC of *Trematomus newnesi* populations.

4.8.2.4. Conclusions on neutral population structure and connectivity of *Trematomus* fishes

The six *Trematomus* species generally show significant genetic structure. Genetic differentiation is distinct between distant regions, such as the Eastern Weddell Sea and the Ross Sea (for *T. loennbergii* 1), or the Peninsula area and the Adélie Coast (for *T. newnesi*), but also for some species also between geographically closer areas, such as the South Orkneys islands and the tip of the Peninsula (for *T. eulepidotus* 1), or even between localities within the Eastern Weddell Sea (for *T. eulepidotus* 1, 2 and *T. scotti*). Other fish species show significant differentiation over regional scales, such as *Harpagifer antarcticus* over the Western Antarctic Peninsula (Bernal-Duràn et al. 2024). In contrast, some species show a striking lack of differentiation on a circum-Antarctic scale, such as the commercially exploited Antarctic toothfish *Dissotichus mawsoni* (Choi et al. 2021; Ceballos et al. 2021; Maschette et al. 2023) or the mesopelagic fish *Electrona antarctica* (Van de Putte et al. 2012).

These results emphasize the need to unravel population structure and connectivity for individual species as there are profound differences even among closely related species. Similarly, as described above for the amphipod *C. obesa*, we found evidence for population differentiation in certain *Trematomus* species over large geographic distances but also at local scales. The contrasting connectivity patterns observed among species likely originate from the interplay between species-specific life-history traits—particularly those influencing larval dispersal—and ocean currents, which shape the population structure of Antarctic fishes.

For instance, our study shows that different *Trematomus* species show contrasting population structure across the Eastern Weddell Sea area. While some species display little to no genetic

differentiation along the eastern Weddell Sea coast (*T. scotti*, *T. loennbergii* 1 and *T. loennbergii/lepidorhinus* 2), others present a marked structure among localities (T*. eulepidotus* 1). These contrasting patterns cannot be readily attributed to habitat differences. *Trematomus loennbergii* is considered as an epibenthic species predominantly feeding on benthic prey (La Mesa et al. 2015), while *T. scotti* is strictly benthic (La Mesa et al. 2019). *Trematomus eulepidotus* appears more associated with the pelagic environment, as an epibenthic species that is predominantly zooplanktivorous (Brenner et al. 2001). Species which are more present in the pelagic habitat seem to show more genetic structure among localities compared to the predominantly benthic ones, which appears counterintuitive. Larval duration does not explain such differences either, as *T. eulepidotus* appears to have a longer larval duration (>10 months; Kellerman 1990) than *T. loennbergii* and *T. scotti* (about 5-6 months; La Mesa et al. 2015). These species have different spawning periods, in summer for *T. eulepidotus* and autumn for *T. loennbergii*, *T. lepidorhinus* and *T. scotti* (La Mesa et al. 2008, 2015). Exploring the dispersal of larvae in relation to ocean currents at different times of the year, as well as adult mobility and migration behaviour, would be interesting perspectives to further explore the putative drivers of the observed genetic connectivity patterns.

The connectivity between the tip of the Peninsula and the South Orkneys Islands was non-significant for *T. newnesi* (F_{ST} =0.8-1.1 %), except with Gerlache Strait, but the latter population included only four individuals. *Trematomus newnesi* is a semipelagic to cryopelagic species (Eastman & DeVries 1982), and its dispersal might therefore be facilitated by ice drift. The Antarctic Slope Current splits as it nears the South Scotia Ridge and the eastward branch flows cyclonically around the Powell Basin towards the South Orkney Plateau. Modelling krill transport showed that particles released on the eastern coast of the Peninsula may reach the South Orkney Plateau, a tendency that is increased when sea-ice associated behaviour is considered (Young et al. 2024). Similarly, no significant differentiation was found for the icefish *Chionodraco rastrospinosus,* suggesting ongoing gene flow between the Northwest Antarctic Peninsula and the South Orkneys Islands (Papetti et al. 2012)

In our results, a genetic break is observed at the level of the Filchner Trough for several species. First, individuals collected in the Filchner Trough were significantly differentiated east (*T. scotti)* and west (*T. eulepidotus* 1) of the through. Secondly, the two lineages found within both *T. eulepidotus* and the *T. loennbergii/lepidorhinus* group have contrasting geographical distributions: in both cases, one lineage is primarily found in the Filchner Trough and Western Antarctic locations, and the other is predominantly present in the Eastern Weddell Sea, outside the trough. Such congruent results suggest a genetic connection between the Filchner Trough and the western side of the Weddell Sea. The pelagic silverfish (*Pleurogramma antarctica*) showed similar patterns of genetic connectivity between the Filchner Trough and the western Weddell Sea (Ashford et al. 2017; Caccavo et al. 2019), while populations of the Antarctic toothfish were not yet studied from the Filchner Trough.

For the Antarctic silverfish, a network of local, connected populations associated with trough systems along East Antarctica is suggested, with the westward-flowing Antarctic Slope Current (ASC) providing a mechanism for the exchange of individuals among these populations (Caccavo et al. 2018). In the Eastern Weddell Sea, a current system indeed provides westward transport along the shelf and slope. The Antarctic Coastal Current (AACC) transports water westward between glacial trough systems along the inner shelf. Similarly, the Antarctic Slope Current (ASC) transports water westward over the continental slope, forms the southern branch of the Weddell Gyre, and then reaches waters north of the Antarctic Peninsula. The ASC is thought to be continuous from the Amundsen Sea along the Ross

Sea continental shelf and East Antarctica (Whitworth et al. 1998; Heywood et al. 2004; Dawson et al. 2023). For Antarctic silverfish, it has been hypothesized that fish entrained in the trough outflow are carried offshore towards the continental shelf-break, where mixing with inflowing water masses carries a proportion back inshore. Fish reaching the continental shelf-break become exposed to currents along the continental slope, which transport them westwards (Ashford et al. 2017; Caccavo et al. 2019). The connectivity patterns observed here for *T. eulepidotus* and *T. loennbergii/lepidorhinus* are similar and thus support this hypothesis (Figures 26 and 27).

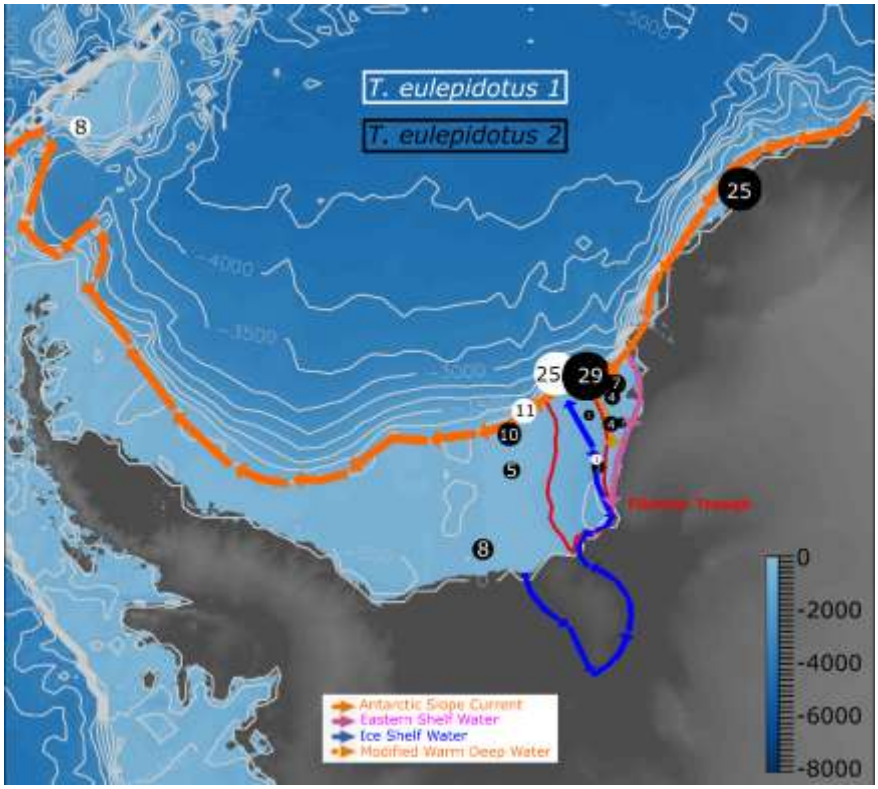


Figure 26. Map of the northern SO showing the geographic distribution of the *Trematomus eulepidotus* and its two lineages, *T. eulepidotus* 1 (white) and *T. eulepidotus* 2 (black) with a zoom into the Western Antarctic Peninsula and the Wedell Sea. The current system is overlayed and represented by coloured arrows. Points represent sampling stations and are sized and numbered according to the number of individuals sampled. The 500 m isobaths are represented as light grey contour lines.

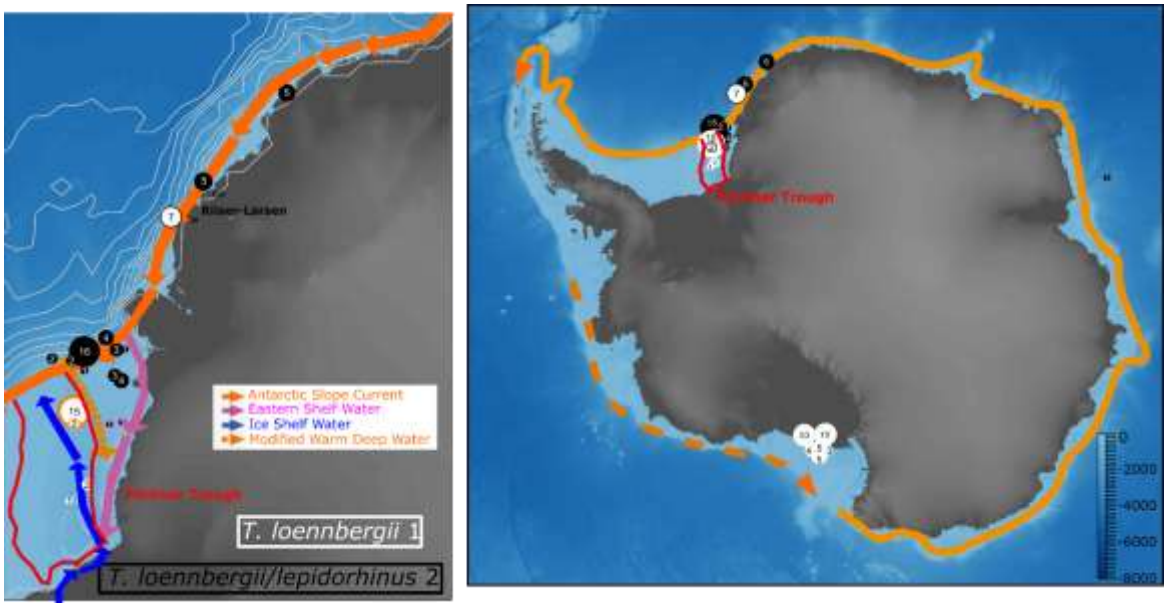
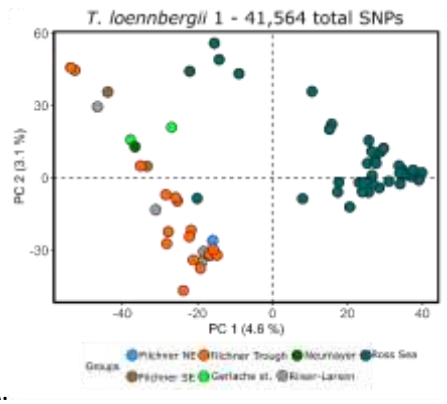


Figure 27. Map of part of the SO showing the geographic distribution of *Trematomus loennbergii/lepidorhinus* samples and the two lineages, *T. loennbergii* 1 (white) and *T. loennbergii/lepidorhinus* 2 (black). The current system is overlayed and represented by coloured arrows. Points represent stations and are sized and numbered according to the number of individuals sampled. The 500 m isobaths are represented as light grey contour lines. The left part of the figure provides a zoom into the Eastern part of the Wedell Sea.

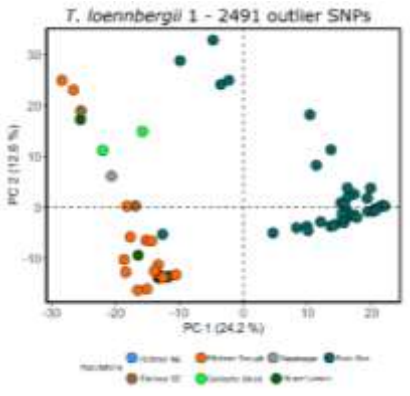
4.8.3. Evidence for adaptation in *Trematomus* fish species

All datasets (unfiltered for linkage disequilibrium and Hardy-Weinberg equilibrium) for all six *Trematomus* species were split into their putatively adaptive (outliers of SNPs as identified with PCadapt) and neutral (filtered for linkage disequilibrium, Hardy-Weinberg equilibrium and SNP outliers removed) components, in order to explore drivers of genetic variation.

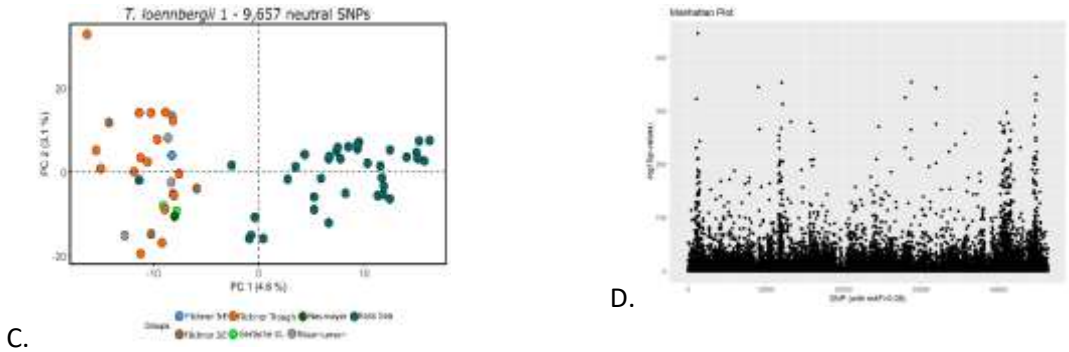
In *T. loennbergii* 1, all datasets showed a genetic differentiation between populations from the Ross Sea and other regions (Eastern Weddell Sea and Gerlache Strait). The PCAs of the total and outlier datasets showed more spread of individuals on PC2 than the PCA of the neutral dataset (Figure 28A, B and C). The Manhattan plot of –log10 p-values for each SNP indicated genomic regions with a higher density of outlier SNPs (Figure 28D), which could be important for adaptation.



A.



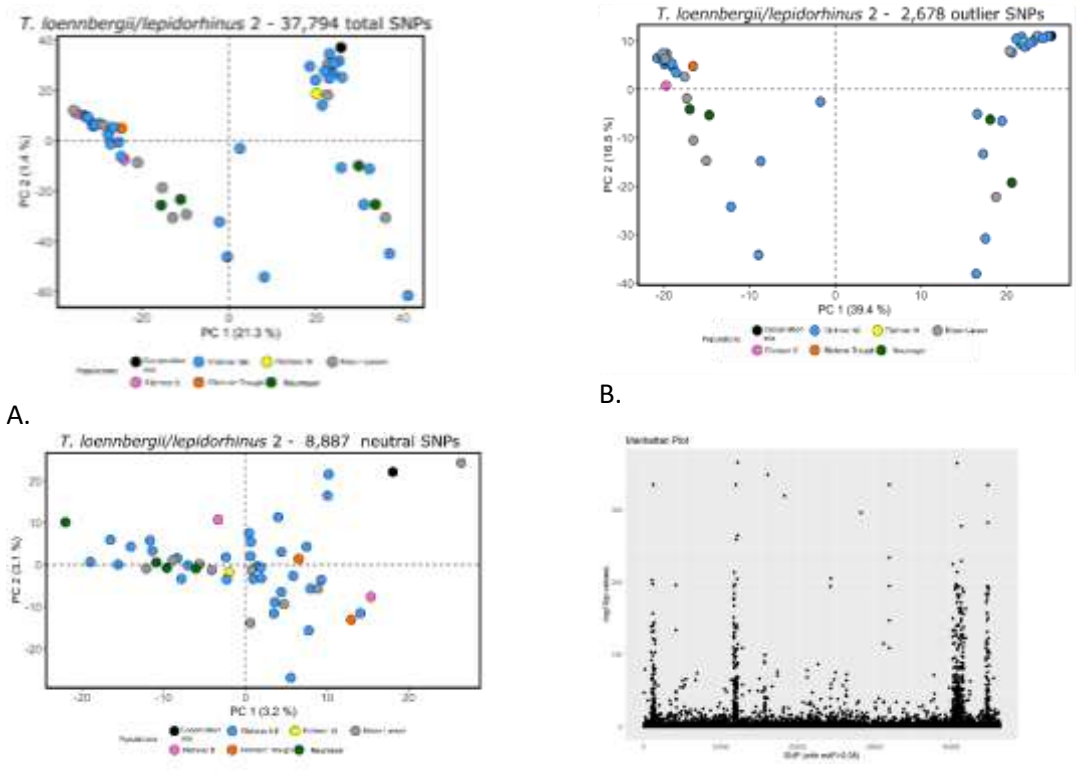
B.



C. D.

Figure 28. **PCA of *Trematomus loennbergii* 1** based on A. the total dataset, B. the putatively adaptive dataset of outliers and C. the neutral dataset (without outliers). D. Manhattan plot of –log10 p-values associated with all SNPs resulting from outlier detection analysis with PCadapt.

In *T. loennbergii/lepidorhinus* 2, the PCAs based on the total and outlier datasets showed similar patterns of two band-like groups revealing no geographical structure, with one individual positioned in between (Figure 29A and B). This pattern disappeared on the PCA based on the neutral dataset, which showed no clear groups, and no geographical structure (Figure 29C). The Manhattan plot of –log10 p-values for each SNP clearly indicated that outlier SNPs are concentrated in certain genomic regions (Figure 29D).

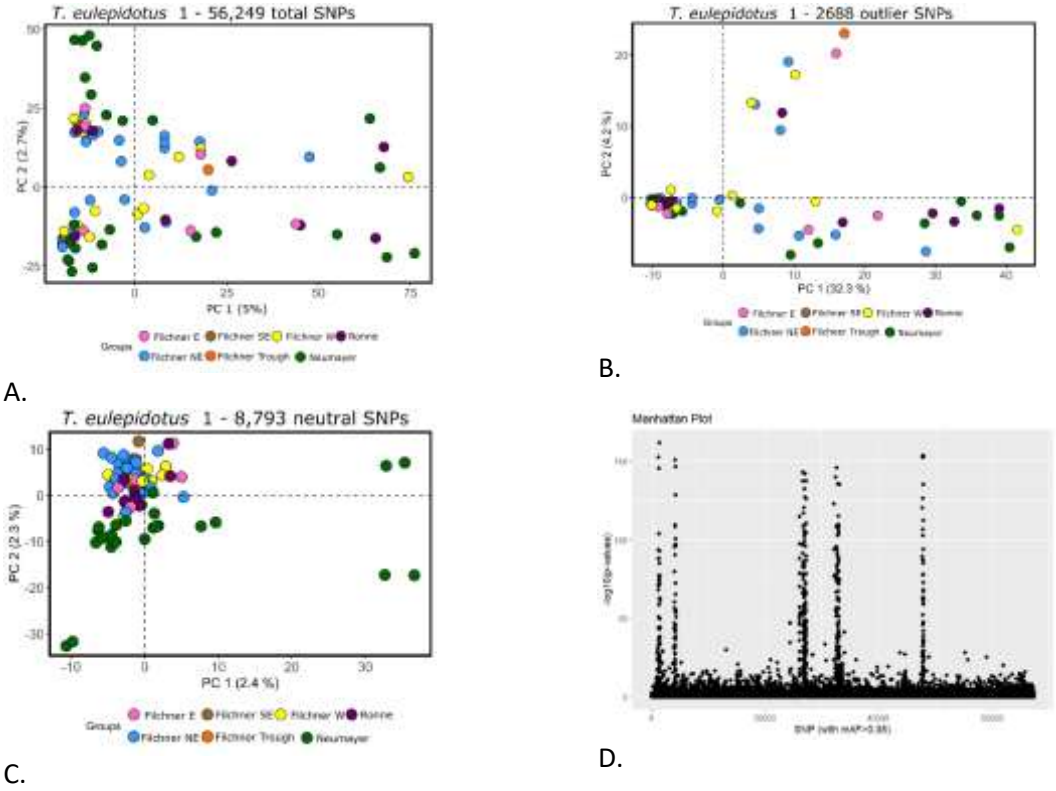


A. B. C. D.

Figure 29. **PCA of *Trematomus loennbergii/lepidorhinus* 2** based on A. the total dataset, B. the putatively adaptive dataset of outliers and C. the neutral dataset (without outliers). D. Manhattan plot of –log10 p-values associated with all SNPs resulting from outlier detection analysis with PCadapt.

In *T. eulepidotus* 1, the PCAs based on the total and outlier datasets showed band-like patterns with no geographical structure (Figure 30A and B). Such pattern disappeared on the PCA based on the neutral dataset, which showed genetic differentiation of the Neumayer population from locations in

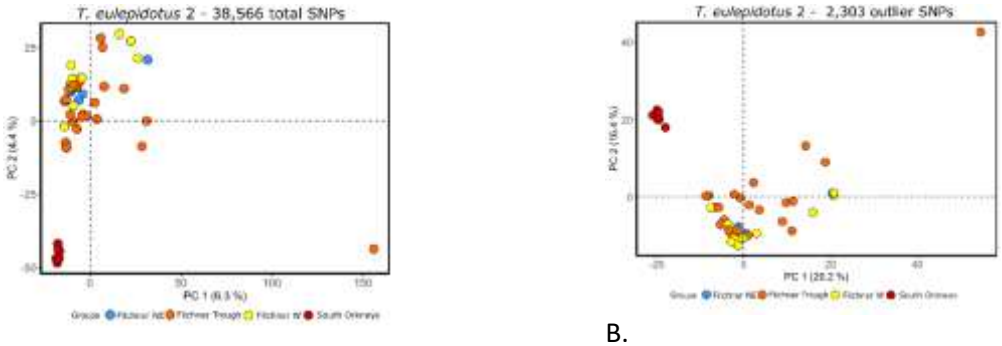
the other Eastern Weddell Sea (Figure 30C). The Manhattan plot of –log10 p-values for each SNP clearly indicated that outlier SNPs were concentrated in some genomic regions (Figure 30D).



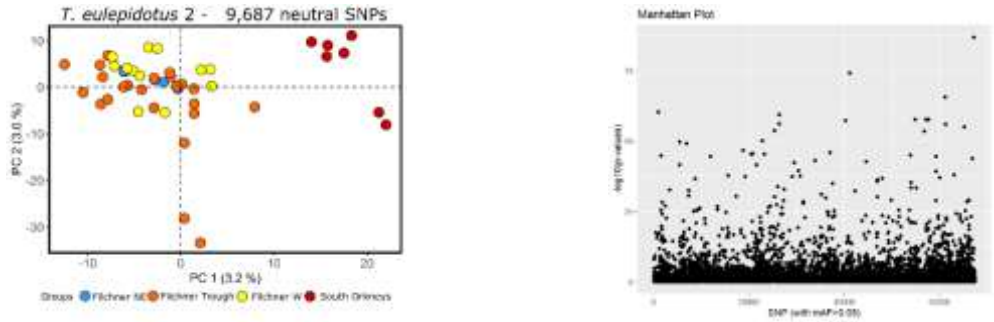
A. B. C. D.

Figure 30. **PCA of *Trematomus eulepidotus* 1** based on A. the total SNP dataset, B. the putatively adaptive dataset of outliers and C. the neutral dataset (without outliers). D. Manhattan plot of –log10 p-values associated with all SNPs resulting from outlier detection analysis with PCadapt.

In *T. eulepidotus 2*, all PCAs showed the differentiation of the South Orkneys and the Eastern Weddell Sea population. While all Eastern Weddell Sea populations overlapped, the Filchner Trough population appeared partly differentiated (Figure 31A, B, C). One individual from the Filchner Trough clustered separately from all others on the PCAs of the total and outlier datasets (Figure 31A, B), but not on the PCA of the neutral dataset (Figure 31C). The Manhattan plot of –log10 p-values for each SNP showed no specific pattern in the genomic location of outlier SNPs (Figure 31D).



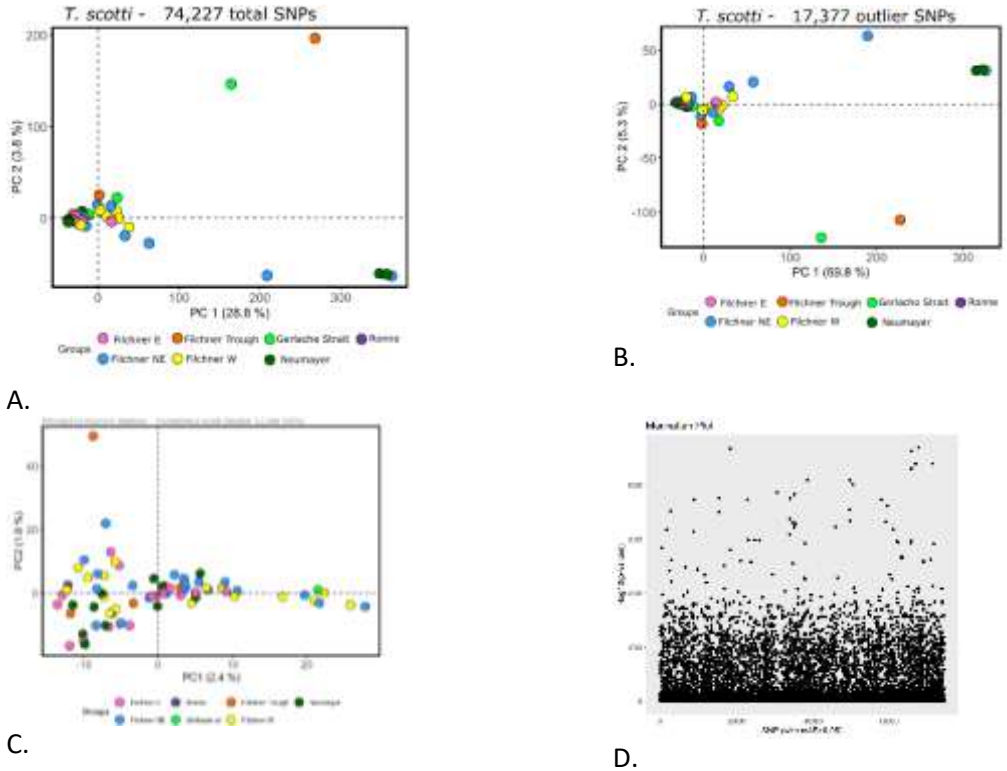
A. B.



C. D.

Figure 31. **PCA of *Trematomus eulepidotus* 2** based on A. the total SNP dataset, B. the putatively adaptive dataset of outliers and C. the neutral dataset (without outliers). D. Manhattan plot of –log10 p-values associated with all SNPs resulting from outlier detection analysis with PCadapt.

In *T. scotti*, the PCAs based on the total and outlier datasets revealed similar patterns of one main group and a few individuals being separating along both axes (Figure 32A, B). This pattern disapeared on the PCA based on the neutral dataset, which showed no clear groupings and no geographical structure (Figure 32C). The Manhattan plot of –log10 p-values for each SNP did not indicate any specific pattern in the genomic location of outlier SNPs (Figure 32D).



A. B.

C. D.

Figure 32. **PCA of *Trematomus scotti*** based on A. the total SNP dataset, B. the putatively adaptive dataset of outliers and C. the neutral dataset (without outliers). D. Manhattan plot of –log10 p-values associated with all SNPs resulting from outlier detection analysis with PCadapt.

In *T. newnesi*, the PCA based on the total dataset showed band-like patterns, separating the Adélie Coast population on PC1 (Figure 33A). The PCA based on the outlier dataset showed two groups with no geographical structure, and one specimen from King George Island cluster separately on PC2. This pattern disappeared on the PCA based on the neutral dataset, which revealed genetic the differentiation of the Adélie Coast population, and of a few specimens from the Gerlache Strait (Figure

33C). The Manhattan plot of –log10 p-values for each SNP clearly shows indicated that outlier SNPs were concentrated in certain genomic regions (Figure 33D).

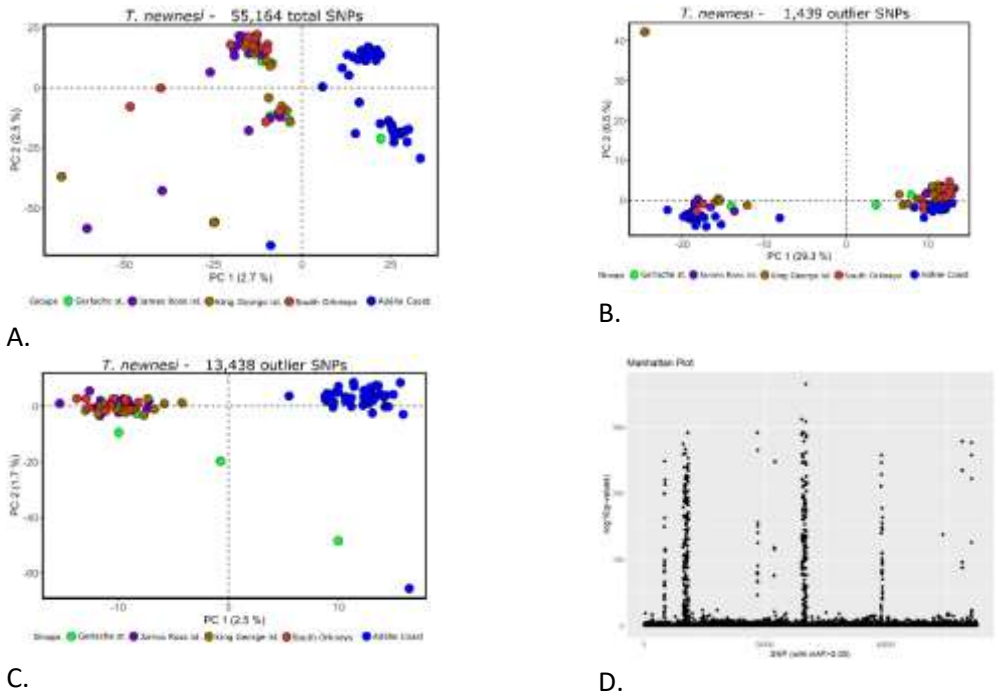


Figure 33. **PCA of *Trematomus newnesi*** based on A. the total SNP dataset, B. the putatively adaptive dataset of outliers and C. the neutral dataset (without outliers). D. Manhattan plot of –log10 p-values associated with all SNPs resulting from outlier detection analysis with PCadapt.

In most *Trematomus* species, we observed genetic adaptive clustering that did not correspond to geographical structure on the PCAs of the total datasets. Such pattern differences appeared to be driven exclusively by putatively adaptive SNPs, as they disappear when these loci are excluded from the analysis. Since these outlier SNPs often seem to be clustered in specific genomic regions, they might be located in regions of reduced recombination, possibly due to chromosomal rearrangements. Low recombination within chromosomal rearrangements may lead to independent evolution of the affected genomic regions despite high gene flow in the rest of the genome (Faria & Navarro 2010; Wellenreuther et al. 2019), which allows the expression of specialized phenotypes associated with local adaptation (Berg et al. 2017; Mérot et al. 2018). Chromosomal rearrangements involve structural changes such as inversion, fusion, fission, and translocation and have been shown to be frequent in *Trematomus* (Auvinet et al. 2018, 2020). While the adaptive role of such chromosomal rearrangements remains to be explored in *Trematomus* species, they may be a critical driver of local adaptation in the genus. Chromosomal rearrangements have been hypothesized to drive local adaptation in many fish species, such as the capelin *Mallotus villosus (*Cayuela et al., Preprint), the polar cod *Boreogadus saida* (Hoff et al. 2024), the Atlantic cod *Gadus morhua* (Berg et al. 2017) and the nine-spined stickleback *Pungitius pungitius* (Natri et al. 2019).

4.9 Overall recommendations

The COPE project ran from 2019 to 2025 including the COVID-19 pandemic and associated lockdowns, which had detrimental effects on certain aspects of the project. For example, there were no Antarctic cruises in 2020 in which COPE researchers could participate and only one cruise in 2021. Also, scientific

visits were impossible in 2020 and 2021. Consequently, we acquired a very limited number of new samples and had to mainly rely on opportunistic analyses using existing samples from the two COPE partner institutions and samples that were already on loan from other institutions. Still, the COPE consortium managed to achieve all planned tasks.

The COPE project successfully produced classic species distribution models (SDMs) for two species of *Charcotia* amphipods and two species of *Trematomus* fish based on existing occurrence data and environmental layers. We also assessed the quality of these SDMs statistically as recommended by (Guillaumot et al. (2019, 2020a, 2021). While we developed models SO wide statistically supported SDMs for *C. amundseni,* T*. eulepidotus* and *T. loennbergii*, this was impossible for *C. obesa*, a species distributed at shallower water depths. Still, for the first three species, our SDMs are useful to direct future sampling efforts. Even when aiming at local SDMs of the Western Antarctic Peninsula, we could not obtain meaningful occurrence probabilities for *C. obesa*. Likewise, SDMs did not provide high occurrence probabilities of *T. eulepidotus* and *T. loennbergii* in the Filchner area although we know that both species occur there and that this is an important region from a ecological point of view (see below). It is obvious that additional occurrence data are required to provide statistically well-supported SDMs for a wide range of Antarctic taxa. This issue is typical for SDMs of understudied areas like the Southern Ocean (SO). Although SDMs can be powerful tools especially to predict future distributions under climate change scenarios (Guillaumot et al. 2020b), yet the potential of this tool can currently not be fully utilized. Antarctic databases of occurrence data need to be expanded significantly, including for those regions in the Southern Ocean which are not routinely investigated. Likewise, environmental layers need to be updated to allow the inclusion of more recent years with rising temperatures for the construction of SDMs. Because of these issues, we did not construct SDMs taking genetic structuring into account here, but we recommend doing this in the future with more extensive datasets.

Our stable isotope findings indicate that even sister species of scavenging amphipods differ in trophic level and realized ecological niche, and that there are also regional intraspecific differences. Given the strong influence of sea ice dynamics on benthic food webs (Rossi et al. 2019) and the current record of low sea ice coverage (Purch & Doddridge 2023), we expect that global change will affect the trophic ecology of many, even closely related species, ultimately disturbing food webs and increasing the vulnerability of the SO to biodiversity loss. We recommend that trophic ecology of a wide range of benthic and pelagic taxa of the SO is investigated as soon as possible to capture these changes in Antarctic food webs with stable isotope and fatty acid analyses.

By successfully applying molecular approaches, the COPE project could contribute to more realistic assessments of endemic diversity in Antarctic ecosystems. In both groups of target organisms of the project, we detected additional cryptic diversity. We found up to six genetic additional species in the morphospecies *C. amundseni*, significantly increasing the known diversity of this genus. For the investigated *Trematomus* fish, we identified two cryptic lineages in two morphospecies each. In each case, the two lineages presented contrasting geographical distributions, with a genetic break at the level of the Filchner Trough, likely caused by oceanographic conditions in the region. The two lineages in both *T. eulepidotus* and the *T. loennbergii/lepidorhinus* group might still be hybridizing. Hybridization is increasingly recognized as an important eco-evolutionary mechanism as it can potentially promote biological novelty and change ecological interactions (Porretta & Canestrelli

2023). Furthermore, we addressed the long-standing puzzle on species identity of T*. loennbergii* and *T*. *lepidorhinus,* which are not reciprocally monophyletic and should be synonymized.
COPE generated the first estimates of genome sizes in 16 species of Antarctic amphipods. We confirmed a wide range of sizes and the existence of giant genomes in certain species. There were no clear differences in genome size according to depth distribution or body size, but differences between pelagic and benthic amphipods was observed with larger genome size occurring in benthic species. Information on genome size is essential to design full genome studies, which will be possible soon.

Making full use of genome-wide estimates of genetic diversity with ddRAD sequencing, COPE provides important novel insights into the connectivity patterns of amphipods and fish species with contrasting dispersal strategies. We found two kinds of genetic connectivity in both taxa, geographical isolation as well as population structuring at small scales. Putative adaptive genetic variation is observed between locations around the WAP, which surprisingly fall within the same benthic bioregion (Douglas et al. 2014). Both patterns have also been described for other benthic Antarctic taxa (Hoffman et al. 2021; Munaz-Ramirez et al. 2020; Levicoy et al. 2021; Lau et al. 2024). Such strong evidence from multiple species allows to identify areas of shared concern for connectivity across regions (Gates et al. 2024) and is thus highly relevant for the delimitation of MPAs as it indicates barriers to gene flow at different scales. Localized population structuring suggests lack of connectivity and lower resilience and requires special protection (Leiva et al. 2022), which can be accomplished with the inclusion of adjacent bioregions as it is planned for CCAMLR's Domain 1 Marine Protected Area (D1MPA) proposal around the West Antarctic Peninsula. Population differentiation at a large-scale calls for the establishment of multiple MPAs in the SO as they have been planned by CCAMLR for nine domains allowing for stepping-stone continuity (Assis et al. 2021) and creating networks of connected MPAs (Leiva et al. 2022). We also identified a key area with a stepping stone role for *Trematomus* fish, the Filchner Trough, which is connected to the western Weddell Sea through the Antarctic Slope Current. Connectivity is expected to drastically change under climate change (Gerber et al. 2014), especially for populations with source-sink dynamics (Silva et al. 2021) making the delimitations of multiple MPAs in the SO as networks an urgent matter.

5. DISSEMINATION AND VALORISATION

PROJECT MEETINGS

There were countless internal meetings for the COPE project between members of the two teams at the RBINS and the KU Leuven including the promotors and the Early Career Researchers; meeting details are not provided here but these meetings occurred at least monthly throughout the entire project, either online (during COVID-19 restrictions) or in person.

Kick-off meeting with the follow-up committee:
19.8.2020 – online because of COVID-19 restrictions

Annual project meetings with the follow-up committee

19.11.21 – online because of COVID-19restrictions.

28.11.22 - the Belgian project scientists were physically present, the members of the follow-up committee who could participate (Agnes Dettai and Emma Young) joined the meeting online.

19.12.23 – the Belgian scientists of the project were present physically, the members of the follow-up committee who could participate (Agnes Dettai, Emma Young and Bruno Danis) joined the meeting online.

Final project meeting

04.03.2025 at the KU Leuven – 19 participants - 16 present at the conference venue plus three participants online. Three members of the follow-up committee participated: Emma Young, Agnes Dettai and Nils Vanstappen.

The programme included a welcome by the local organiser (Filip Volckaert), followed by an introduction to the project by the coordinator (Isa Schön) and oral presentations by the Early Career Researchers (Dorien Aerts and Marie Verheye) on the project results. A second session included presentations from other Belgian Early Career Researchers and two members of the follow-up committee who were present, Emma Young from BAS and Agnès Dettaï from the Museum of Natural History in Paris. A third session was devoted to Science Policy where Anton Van De Putte introduced the Biodiversa+ project WOBEC followed by an extensive open discussion on the relevance of the scientific results of COPE for CCAMLR and how to communicate them.

Presentations

Invited speaker

Christiansen, H. (2020) Unravelling connectivity and adaptation in Southern Ocean fish using reduced genome representation data. Berlin Center for Genomics in Biodiversity Research Seminar Series, Online, 11 September 2020. Invited speaker and oral presentation.

Oral presentations

1. Aerts, D.N. (2023) The amazing diversity of a taxon; Genome sizes of twenty Antarctic amphipod species using different methods. Zoology conference in Leiden, 30-31 May 2023.

2. Christiansen, H. (2020) Biodiversity.aq and POLA3R portal use case: DNA metabarcoding of the prey and microbiome of museum specimens of Antarctic fishes. LifeWatch.be Users & Stakeholder Meeting, Online, 1 October 2020.
3. Van de Putte A. (2020) The COPE project: Conservation management of polar ecosystems using genomic approaches to study connectivity across spatial and functional scales. Poster presentations at the SCAR Open Science online conference 2020.
4. Verheye M.L. (2022) Antarctica as an evolutionary incubator? Phylogenetic comparative study of the amphipod family Iphimediidae on the Antarctic shelf. Zoology, Kortrijk, Belgium, 22-23 September 2022.
5. Maes S.M.*, Verheye M.L.* (2023) Population genomics of polar cod (Boreogadus saida) in a vanishing Arctic seascape. Fishbase/sealifebase symposium, Royal Museum for Central Africa, Tervuren, Belgium, 4 September 2023. *co-first authors.
6. Verheye M.L. (2024) Integration of genetic connectivity and local adaptation in the design of marine protected areas in the Southern Ocean: a case study of *Trematomus* fishes. I-MarCo 2024, 7th International conference on Marine Connectivity, Montpellier, France, 27-31 May 2024.

Poster presentations

1. Aerts D. (2021) Conservation management of polar ecosystems (COPE): genomic approaches to study connectivity across spatial and functional scales. Abstracts of the iMarCo conference, Paris, France, 6-8 December 2021.
2. Aerts D. (2022) Phylogeography and cryptic diversity of *Charcotia* amphipods in the Southern Ocean. Abstracts of the Zoology conference, Kortrijk, Belgium, 22-23 September 2022; and at the VLIZ Marine Science Day (Online), 2 March 2022.
3. Verheye M.L. (2023) Connectivity and adaptation of *Trematomus* fishes across the Antarctic continental shelf. Abstracts of the ICES - SEA-UNICORN symposium Human Impacts on Marine Functional Connectivity, Sesimbra, Portugal, 22-25 May 2023.
4. Verheye M.L. (2023) Connectivity and adaptation of *Trematomus* fishes across the Antarctic continental shelf. Abstracts of the Zoology conference in Leiden, the Netherlands, 30-31 May 2023.
5. Verheye M.L. (2024) COnservation management of Polar Ecosystems (COPE project): using genomic approaches to study connectivity in Antarctic fishes and amphipods, across spatial and functional scales. I-MarCo 2024, 7th International conference on Marine Connectivity, Montpellier, France, 27-31 May 2024.

TEACHING AT EXTERNAL WORKSHOPS

Teaching at the Genetics Training School workshop of COST ACTION SEA-UNICORN (20-24 March 2023), including Seascape genomics theory (Volckaert, F.A.M.) and computer lab with R (Verheye, M.L.) on 24th March 2023. Organisors: Volckaert, F.A.M. and Andrello, M.

SUPPORT TO DECISION MAKING

Anton Van de Putte participated in the online 2020 meeting of the CCAMLR Scientific Committee (SC) and Commission. Due to the COVID-19 pandemic, the meeting working groups of the SC were not formed and the meeting had a reduced agenda. In 2022, Anton Van de Putte represented Belgium in Belgian (COORMULTI), EU (EU-coordination), and CCAMLR Scientific Committee Bureau Meetings

(online). He also attended the CCAMLR Scientific Committee Symposium on February 8-10, 2022. Anton van de Putte participated actively in several online meetings. He represented Belgium at the October 2023 CCAMLR meeting in Hobart, Australia. In his role as the Alternate Representative for the Commission, Belgian representative for the Scientific Committee and Chair for the Data Services Advisory Group, A. Van de Putte actively participated in COORMULTI and EU-coordination meetings in 2023, as well as the online Scientific Committee Bureau Meetings in preparation for the October 2023 meetings. Additionally, he contributed to two key documents in 2023: an update on the SCAR Antarctic Biodiversity Portal and the Summary for Policy Makers from the first Marine Ecosystem Assessment for the Southern Ocean (MEASO).

Dorien Aerts joined the second week of WG-EMM of CCAMLR in July 2024 in Leeuwarden (NL) to follow discussions relevant to ecosystem monitoring. She was selected as intern for the international CCAMLR internship 2024. She was trained for three months from October to December 2024 at the CCAMLR office in Hobart (Australia) and also attended the CCAMLR meeting of 2024.

6. PUBLICATIONS

A1 PUBLICATIONS

1. Maes, S.M. *, **Verheye, M.L**. *, Bouchard, C., Geslain, E., **Hellemans, B**., Johansen, T., Lucassen, M., Mark, F.C., Ólafsdóttir, A.H., Snoeijs-Leijonmalm, P., Zelenina, D., MOSAiC Team Eco‡,. **Volckaert, F.A.M., Christiansen, H**.*, Flores, H.* (2025) Genome-wide variant analysis detects trans-Arctic connectivity and local adaptation in polar cod (*Boreogadus saida*), Molecular Ecology, in press. *co-first and last authors.

2. **Aerts D**., Kolder A., Lepoint G., N. Michel L.N. & **Schön I**. (under review). Divergent feeding habits of sister species of the Antarctic amphipod genus *Charcotia*. Submitted to Belgian Journal of Zoology.
3. Caccavo J.A., **Christiansen H**., Constable A.J., Ghigliotti L., Trebilco R., Brooks C.M., Cotte C., Desvignes T., Dornan T., Jones C.D., Koubbi P., Saunders R.A., Strobel A., Vacchi M., **Van de Putte A**., Walters A., Waluda C.M., Woods B.L. & Xavier J.C. (2021) Productivity and change in fish and squid in the Southern Ocean. Frontiers in Ecology and Evolution 9, 624918, doi:10.3389/fevo.2021.624918.
4. Maes S.M., **Christiansen H**., Mark, F.C., Lucassen, M., **Van de Putte A.P**., **Volckaert F.A.M**. & Flores H. (2021) High gene flow in polar cod (*Boreogadus saida*) from West-Svalbard and the Eurasian Basin. Journal of Fish Biology 99, 49-60, doi:10.1111/jfb.14697.
5. Frédérich B., Heindler F.M., **Christiansen H**., Dettai A., **Van de Putte A.P**., **Volckaert F.A.M**. & Lepoint G. (2022) Repeated morphological diversification in endemic Antarctic fishes of the genus *Trematomus*. Belgian Journal of Zoology 152, 55-73, doi:10.26496/bjz.2022.99

PLANNED A1 PUBLICATIONS

1. **Aerts, D.**, Tawfeeq, M., Monten, A-L., Greeve, C., Flot, J-F., **Volckaert, F.A.M**., **Van de Putte, A**. & **Schön, I.** (2025) The amazing diversity of a taxon: Genome sizes of twenty Antarctic amphipod species. To be submitted to *Genome*
2. **Aerts, D.**, Kolder, A., Morren, L., **Verheye, M**., Geslain, E., **Hellemans, B**., **Van de Putte, A**., **Volckaert, F.A.M**. & **Schön, I**. (2025) Cryptic diversity of *Charcotia* amphipods in the Southern Ocean. To be submitted to *Hydrobiologia*
3. **Aerts, D.**, **Verheye, M**., Geslain, E., **Christiansen, H**., **Hellemans, B**., **Van de Putte, A**., **Volckaert, F.A.M**.* & **Schön, I**.* (2025) Southern Ocean amphipod population structure and connectivity. To be submitted to *Ecology and Evolution* *co-last
4. Canals O., Baillie, S., Dahle G., Rodriguez-Ezpeleta, N., **Volckaert, F.A.M**. Illuminating the ocean's twilight zone with a genetic flashlight. To be submitted to *ICES journal of Marine Science.*
5. **Verheye, M**., **Van de Putte, A., Schön, I**., **Hellemans, B**., Geslain, E., **Aerts, D**., **Christiansen, H.*, Volckaert, F.A.M**.*co-last. Genetic connectivity and local adaptation in four *Trematomus* species of the Antarctic shelf.

ABSTRACTS

1. **Aerts, D., Christiansen, H., Van de Putte, A., Volckaert, F. Schön, I**. (2022) Phylogeography and cryptic diversity of Charcotia amphipods in the Southern Ocean. Abstracts of the Zoology conference, Kortrijk, Belgium, 22-23 September 2022; and at the VLIZ Marine Science Day (Online), 2 March 2022.

2. **Aerts, D.N., Verheye, M.L., Christiansen, H., Van de Putte, A., Volckaert, F.A.M., Schön, I**. (2023) The amazing diversity of a taxon; Genome sizes of twenty Antarctic amphipod species using different methods. Abstracts of the Zoology conference in Leiden, 30-31 May 2023.
3. (2020) Marine Ecosystem Assessment for the Southern Ocean (MEASO): Productivity and change in fish and squid in the Southern Ocean. Abstracts of the SCAR Open Science conference 2020 (online).
4. **Christiansen H., Van de Putte A.P**., Guillaumot C., Barrera-Oro E., **Volckaert F.A.M**. & Young E.F. (2020) Large scale connectivity of the marbled rockcod *Notothenia rossii* revealed through population genomics and modelling. Abstracts of the SCAR Open Science conference 2020 (online).
5. **Van de Putte A., Christiansen H., Volckaert F., Schön I. & Aerts D**. (2020) The COPE project: Conservation management of polar ecosystems using genomic approaches to study connectivity across spatial and functional scales. Abstracts of the SCAR Open Science conference 2020 (online).
6. **Verheye M.L**., **Christiansen H**., Heindler F.M., Geslain E., Bista I., Dettai A., **Hellemans B., Van de Putte A., Schön I., Volckaert F.A.M**. (2023) Connectivity and adaptation of *Trematomus* fishes across the Antarctic continental shelf. Abstracts of the ICES - SEA-UNICORN symposium Human Impacts on Marine Functional Connectivity, Sesimbra, Portugal, 22-25 May 2023.
7. **Verheye M.L., Christiansen H**., Heindler F.M., Geslain E., Bista I., Dettai A., **Hellemans B., Van de Putte A., Schön I., Volckaert F.A.M.** (2023) Connectivity and adaptation of *Trematomus* fishes across the Antarctic continental shelf. Abstracts of the Zoology conference in Leiden, the Netherlands, 30-31 May 2023.
8. **Verheye M.L.*, Christiansen H.*, Van de Putte A., Schön I**., Geslain, E., **Hellemans B**., Mueter F., **Volckaert F.A.M**. (2024) Integration of genetic connectivity and local adaptation in the design of marine protected areas in the Southern Ocean: a case study of *Trematomus* fishes. Abstracts of I-MarCo 2024, 7th International conference on Marine Connectivity, Montpellier, France, 27-31 May 2024. *co-first authors.
9. **Verheye M.L.*, Aerts D.*, Christiansen H., Van de Putt, A**., Geslain E., **Hellemans B., Schön I**. *, **Volckaert F.A.M.*** (2024) COnservation management of Polar Ecosystems (COPE project): using genomic approaches to study connectivity in Antarctic fishes and amphipods, across spatial and functional scales. Abstracts of I-MarCo 2024, 7th International conference on Marine Connectivity, Montpellier, France, 27-31 May 2024. *co-first and last authors.

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ANNEXES

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