Université Libre de Bruxelles (Belgium)

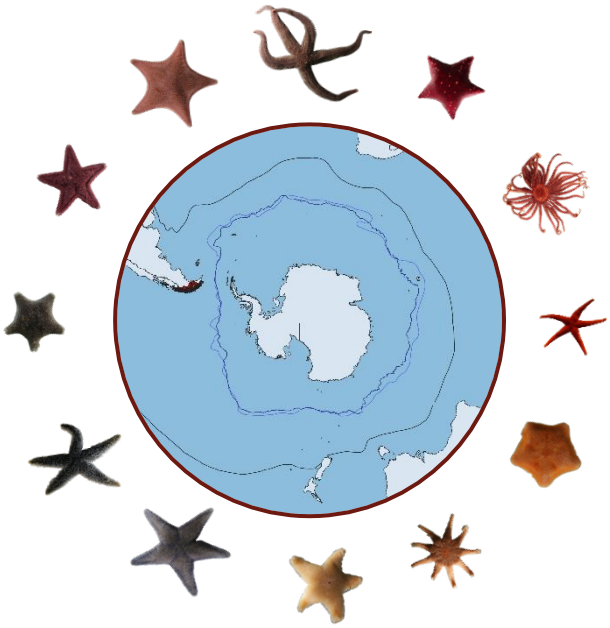
Marine biology laboratory

Sea star (Asteroidea, Echinodermata) diversity in the Magellanic region (South-Chile) and their affinities within the Southern Ocean

Luka Vantomme

Promotor and supervisor:

Dr. Camille Moreau



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No data can be taken out of this work without prior approval of the thesis supervisor

Declaration of authorship

'I hereby declare that that this submitted master's thesis work is a product of my own work and I have listed all the references and resources that have been used.'

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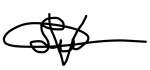

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EXECUTIVE SUMMARY

The Southern Ocean is a complex and dynamic system structured by the presence of fronts acting as latitudinal dispersal barriers and the Antarctic Circumpolar Current facilitating dispersal eastward. Despite sea stars being a diverse and important component of the Southern Ocean benthos, only scarce information is available regarding their diversity and evolution, and taxonomic revisions are required. The Magellanic region (south of Chile) remains under-sampled despite its pivotal location for species distribution being adjacent to three ocean basins. Therefore, we aimed to assess sea star biodiversity in the Magellanic region and evaluate the role of this region in sea star biogeography.

Eight locations were sampled in Chile using various methods (SCUBA diving, snorkelling, and intertidal sampling) and additional specimens from collections at LeMAS were added. We used an integrative taxonomic approach combining morphological identification with DNA barcoding. DNA extractions were performed using a DNeasy Blood & Tissue kit by Qiagen whereafter COI and 16S (for some specimens) were amplified, sequenced by Macrogen Europe, edited, and made available on BOLD. Species were delineated using ASAP and the BIN method in BOLD whereafter additional sequences of those delineated species were mined from BOLD. Genetic diversity indices, haplotype networks and distribution maps were inferred.

We identified 12 species from the Magellanic region and reported *Cycethra frigida*for the first time in this region. Furthermore, we propose to synonymise two pairs of species within the *Anasterias* and *Odontaster* genera. With this study we again emphasised the need to combine morphology and genetics in species identification to unravel taxonomic discrepancies such as synonymous species (morphologically different despite being one genetic entity) or cryptic species (morphologically similar despite being separate genetic entities). Four geographical patterns are observed covering narrow (endemic to the Magellanic region) to broad (circumpolar to bipolar or possible cosmopolitan) distribution ranges. Although developmental mode has been suggested as important in shaping biogeographical patterns, relying on this alone is insufficient and other life history traits, physiological constraints, competition, bathymetrical range, and the possibility of passively rafting on kelp are suggested to be at least equally important.

Future research should focus on sampling more specimens and adding their barcodes to a barcode library to complete the picture of Magellanic biodiversity. Additionally, more regions should be sampled to unravel species' true distribution ranges covering their full intraspecific variation. Using more variable markers would also help to understand recent and ongoing faunal affinities.

ABSTRACT

Sea stars are a diverse and important component of the Southern Ocean benthos. However, only scarce information is available regarding their diversity and evolution, and taxonomic revisions are required. The Magellanic region (south of Chile) remains under-sampled despite its pivotal location for species distribution being adjacent to three ocean basins. Therefore we assessed sea star biodiversity in this region and evaluated its role in sea star biogeography. An integrative approach combining morphological identification with DNA barcoding has been implemented to highlight taxonomic discrepancies such as synonymous species and cryptic species. We identified 12 species from the Magellanic region and reported *Cycethra frigida* there the first time in the Magellanic region. Furthermore we propose to synonymise two species pairs within the *Anasterias* and *Odontaster* genera. Four geographical patterns are observed covering narrow (endemic to the Magellanic region) to broad (circumpolar to bipolar or possible cosmopolitan) distribution ranges. Although developmental mode has been suggested as important in shaping biogeographical patterns, relying on this alone is insufficient and other life history traits, physiological constraints, competition, bathymetrical range, and the possibility of passively rafting on kelp are suggested to be at least equally important.

PLAIN LANGUAGE ABSTRACT

Sea stars are a diverse and important part of the Southern Ocean. However, only scarce information is available regarding their diversity and evolution, and species names need to be checked. The Magellanic region (south of Chile) remains under-sampled even though it is located next to three ocean basins which suggests that it is important for species distribution. Therefore we assessed sea star biodiversity in the Magellanic region and evaluated its role in sea star distribution. We combined morphological identification with genetic identification to discover difference between both methods and indicate misidentifications. We identified 12 species from the Magellanic region and reported the species *Cycethra frigida* for the first time in the Magellanic region. Furthermore we propose that two species pairs within the *Anasterias* and *Odontaster* genus should be considered the same species. Four distribution patterns are observed covering narrow (only present in the Magellanic region) to broad (present within the entire Southern Ocean and even in the Northern hemisphere) distribution ranges. Although reproductive strategy has been suggested to be important in determining distribution patterns, relying on this alone is insufficient and other factors such as temperature tolerance, competition, depth range, and the possibility of passively rafting on kelp are suggested to be at least equally important.

1. INTRODUCTION

1.1 The Southern Ocean

The Southern Ocean (*sensu lato*) comprises all waters south of 45°S and encircles the Antarctic continent. This ocean is a complex and dynamic system structured by the presence of fronts (e.g. the Polar Front) and of the Antarctic Circumpolar Current (ACC). The ACC is the strongest current on earth (Moon et al, 2017) and flows in a clockwise direction around the Antarctic continent. This current acts as a dispersal vector distributing life from west to east in the Southern Ocean, but also forms a barrier for north-south dispersal. Closely linked to the ACC are dynamic front regions characterised by steep gradients in temperature and salinity. They delimit water masses of different densities that undergo limited to no mixing and create one of the strongest natural barriers in the world's oceans (Crame, 1999) limiting north-south dispersal (Clarke et al, 2005; Thornhill et al, 2008; Fraser et al, 2012). This latitudinal frontal system delineates two major zones in the Southern Ocean: the Antarctic and the sub-Antarctic. The Subtropical Front located at around 45°S marks the northern limit of the sub-Antarctic while the Polar Front separates the sub-Antarctic to the north from the Antarctic to the south (Fig 1). The Polar Front marks the location where the cold, less saline Antarctic surface waters with a higher density sink below the warmer, more saline sub-Antarctic waters coming from the north. These fronts are dynamic systems and their latitudinal position (Fig 1) as well as permeability varies with longitude and through time (Park et al, 2014; Soto Àngel & Peña Cantero, 2017; Moreau, 2019). Permeability is enhanced by eddies crossing these fronts. Eddies are whirlpools of water with either a warmer core originating from within the sub-Antarctic or a colder core formed in the Antarctic. Within those eddies, it appears that shallow pelagic species or larvae are able to cross the fronts (Ansorge & Lutjeharms, 2003; Chown et al, 2015; Clarke et al, 2005).

Despite the well-established boundaries of the Antarctic and sub-Antarctic by fronts, the biological affinities within and between those regions are still under discussion (Soto Àngel & Peña Cantero, 2017).

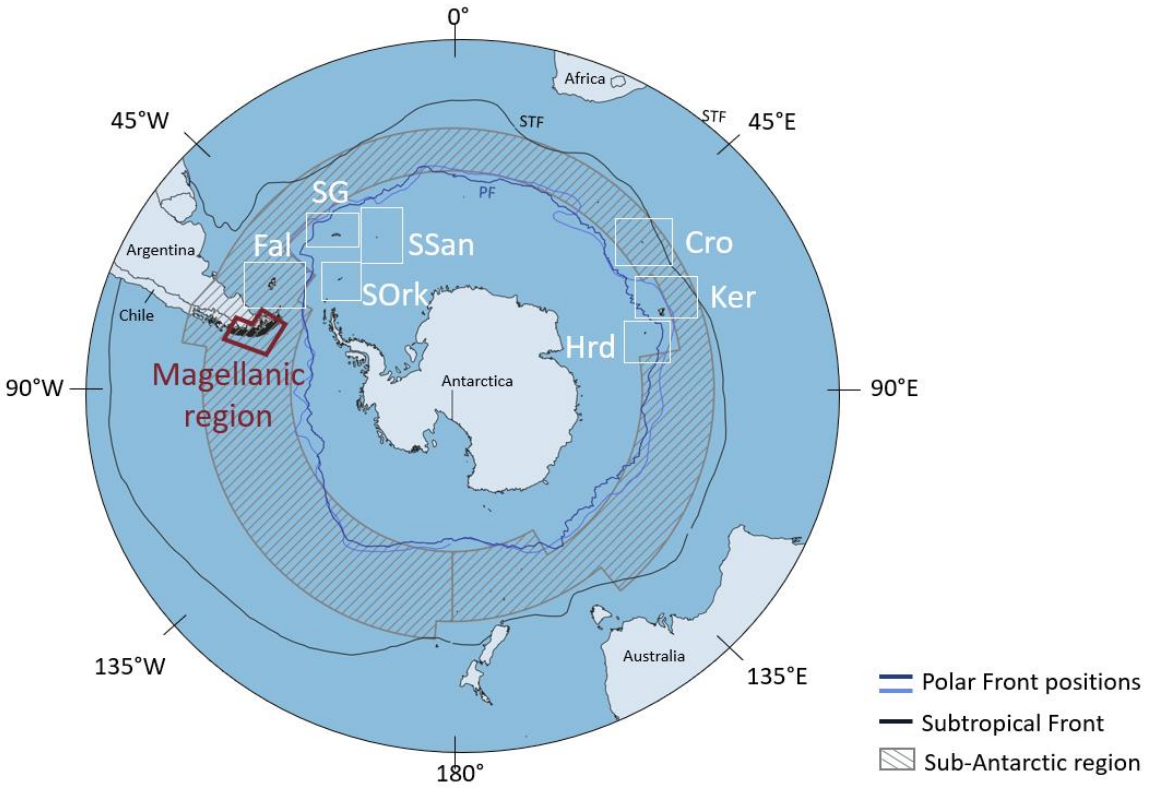

Fig 1| Overview of the major islands in the Southern Ocean and the position of the major fronts: Polar Front variations (lines in the shades of blue, PF) and the Subtropical Front (black line, STF). The sub-Antarctic is indicated in the grey hashed area. Abbreviations: Fal = Falkland Islands, SG = South-Georgia, SOrk = South-Orkney Islands, SSan = South-Sandwich Islands, Cro = Crozet Islands, Ker = Kerguelen, Hrd = Heard Island

1.2 Sea stars in the Southern Ocean

Within these Antarctic and sub-Antarctic waters, echinoderms are one of the most represented benthic macrofaunal phyla in terms of biomass and diversity, encompassing more than 630 accepted species (Griffiths, 2010; Moles et al, 2015; Hibbert, 2016; De Broyer et al, 2022). Among echinoderms, sea stars (Asteroidea) are a major class with 294 accepted species in the Southern Ocean belonging to seven different orders and 38 families (Moreau et al, 2021). They play an important role in structuring marine assemblages (Fraysse et al, 2018). Their relatively large sizes, diverse diets, and presence in nearly all marine environments makes them ecologically important (Rahman et al, 2018). They commonly occupy the highest trophic level, although some variability exists according to habitat and sea star species (Le Bourg, 2020). For example, one of the most common Antarctic sea stars, *Odontaster validus*, has an impact on the surrounding benthic community sometimes far beyond what is predicted by its biomass (Dayton et al, 1974; McClintock et al, 2008). It displays a remarkable array of opportunistic feeding habits including detrital feeding, herbivory, scavenging, and carnivory on sponges and other sea stars increasing its impact on the community (McClintock et al, 2008). *Odontaster validus* has therefore been elevated to keystone species (Dayton et al, 1974; McClintock et al, 2008). Next to their ecological importance, sea stars also are of interest in other domains such as

medical research due to their diverse bioactivities, pharmacological properties, and secondary metabolites (Rahman et al, 2018).

Despite being a diversified and important component of the Southern Ocean benthos, only scarce information is available regarding sea stars' diversity, evolution, or ecological roles (Moreau, 2019). Biodiversity assessments, including species lists, form an important baseline for monitoring and conservation purposes (Griffiths & Waller, 2016) especially in the face of distribution shifts associated to climate change and the increased presence of invasive species. Since the International Polar Year (IPY 2007-2009) and the Census of Antarctic Marine Life (CAML 2005-2010) increasing international efforts have been made to unravel the Southern Ocean biodiversity. Additionally, with the declaration of the international UN decade of Ocean Science (2021-2030), a new interest in ocean science and biodiversity can launch more projects increasing the biodiversity knowledge of the Southern Ocean. Recent large-scale studies highlighted the need for taxonomic revisions and biodiversity reassessments within this entire class and in specific ecosystems and regions (e.g. Jossart et al, 2021; Moreau et al, 2021). Sampling remains one of the major limiting factors in the Southern Ocean (Griffiths, 2010). It varies considerably with geography and bathymetry for multiple reasons (e.g. vicinity of continents and national research stations, high seasonal variation in sea ice cover combined with variable weather conditions, high fuel costs and time spend at sea especially for deep-sea sampling (Griffiths, 2010; Moreau, 2019)), increasing the need to focus more studies on under-sampled regions.

1.3 The Magellanic region

The Magellanic region is one of the under-sampled regions within the Southern Ocean, particularly in coastal waters (Griffiths and Waller, 2016; Moreau et al, 2017). This region (officially called Región de Magallanes y de la Antártica Chilena) is the southernmost, largest of Chile's 16 administrative divisions and is located in the sub-Antarctic (Fig 2). It is one of the most extended fjord regions in the world (Fig 2) and is located at the crossroad between three ocean basins, namely the Pacific to the west, the Atlantic to the east, and the Southern Ocean to the south. This singular geographic position suggests a rich species composition originating from these three distinct ocean basins. A previous study by Pérez-Ruzafa et al. (2013) has indeed shown that different ocean basins host different echinoderm species assemblages. Efforts have been made to assess sea star diversity in this region, but results are inconsistent and cover different geographical areas as well as depth ranges. Pérez-Ruzafa et al. (2013), for example, identified around 50 species from entire Chile while Mutschke & Rios (2017) found 17 species from Chilean fjords north of the Magellanic region. Studies from the Magellanic region found 12 to 24 species (Mutschke & Mah, 2000; de Moura Barboza et al, 2011; Fraysse et al., 2018). This

diversity might, however, be overlooked due to the lower sampling effort and the difficulty of accessing certain locations due to the fjord landscape.

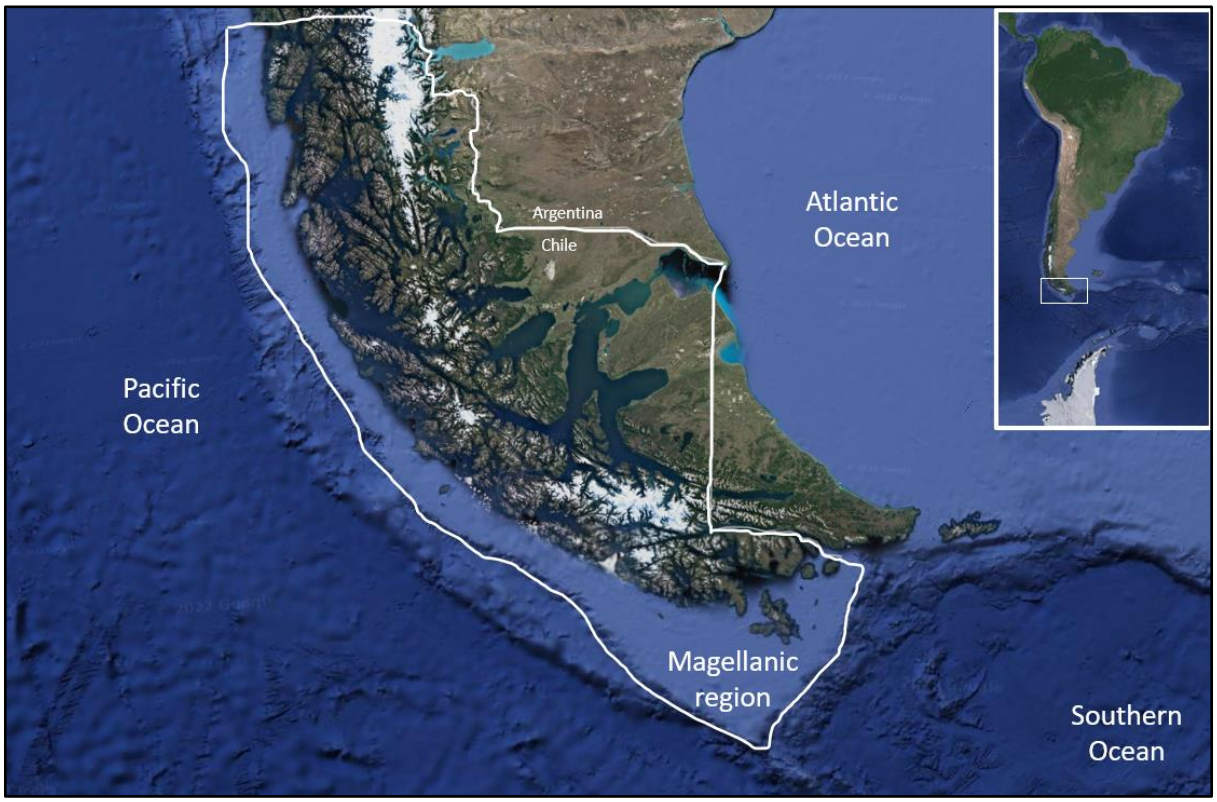

Fig 2| Satellite image of the Magellanic region (bordered in white) showing its extensive fjords and adjacent ocean basins.

1.4 Integrative taxonomy combining morphology and DNA barcoding

To uncover the possibly overlooked sea star diversity, an array of tools are available (e.g. morphological and molecular). Morphology-based identification is the traditional approach to taxonomy. With some level of experience and expertise, observers can quickly identify specimens in the field when clear discriminating morphological characters are known. Studying morphology can also provide information, to some extent, about ecological function and life history traits of the studied species. However, the increase in molecular advances made it evident that this approach comes with some inherent limitations (Hebert et al, 2003a). Taxonomic discrepancies such as synonymous or cryptic species are likely when using only the traditional taxonomic approach. Synonymous species belong to the same genetic entity, but are given two separate species names due to the high morphological variation within this species (e.g. the sea stars *Glabraster antarctica* (Moore et al, 2018) or *Marthasterias* (Wright et al, 2016)) (Hebert et al, 2003a; Ward et al, 2008). Cryptic species on the other hand, belong to two distinct genetic entities, but have a similar morphology that identifies them as one species (e.g. *Henricia* sea stars (Layton et al, 2016; Knott et al, 2018)). An integrative approach is

therefore needed to prevent overestimating biodiversity due to the existence of synonymous species or underestimating biodiversity due to overlooking cryptic species (Jossart et al, 2021). Neither molecular nor morphological taxonomic methods are sufficient on their own (Carstens et al, 2013) and an increasing number of studies implement this integrative approach to identify sea stars (e.g. Layton et al, 2016; Wright et al, 2016; Knott et al, 2018; Peck et al, 2018; Ringvold & Moum, 2019; Jossart et al, 2021), but also other taxa such as brittle stars (e.g. Jossart et al, 2019), holothurians (e.g. Uthicke et al, 2010), fish (e.g. Christiansen et al, 2018), and many more.

DNA barcoding is an approach where a standard DNA sequence is used to assign specimens to known species (Uthicke et al, 2010). It is becoming an important molecular approach alongside morphological taxonomy and its emergence sparked a paradigm shift in biodiversity assessments (Hupalo et al, 2022). It allows an accurate identification of damaged specimens, juveniles, and larvae, which is not always possible using traditional taxonomy (Hebert et al, 2003a; Ward et al, 2008; Uthicke et al, 2010; Janosik et al, 2011; Layton et al, 2016). In animals, the mitochondrial gene COI (cytochrome c oxidase) is most widely used. Previous studies demonstrated the effectiveness of COI in identifying echinoderm species and resolving taxonomic uncertainties, including in Asteroidea (Ward et al, 2008; Layton et al, 2016; Petrov et al, 2016; Wright et al, 2016; Ringvold & Moum (2019)). The effectiveness of this molecular marker lies in its frequent application resulting in the availability of many reference sequences and large numbers of both universal and taxon-specific primers. Additionally, a barcode gap that forms the base of species delineation is shown to be present between most species (Hebert et al, 2003b; Fišer Pečnikar & Buzan, 2014). This barcode gap implies that intraspecific (within species) genetic variation is lower than interspecific (between species) genetic variation (Layton et al, 2016).

Practically, DNA barcoding is based on the comparison of the obtained sequence with reference sequences of specimens with verified identification in a DNA barcode library (e.g. the Barcode Of Life Data system BOLD, GenBank) (Ward et al, 2008). The number of reference sequences is however still low with some taxa that do not have any species barcoded (e.g. Ctenophora) and only 29% of all echinoderm species are barcoded (Gong et al, 2018). The fact that reference sequences rely on morphological identification to verify their taxonomy highlights again the need to combine molecular and morphological approaches.

1.5 Faunal affinities within the Southern Ocean

Faunal affinities refer to present or past connectivity between populations by dispersal events (gene flow). Past affinities resulting from a common origin between species are still reflected in their present morphological, genetic, or behavioural resemblances. Therefore, using biogeographical patterns (i.e. distribution of species) in an integrative approach combined with their associated genetic patterns

allows to infer these past or present faunal affinities. Phylogeography investigates biogeographical patterns within closely related genetical lineages adding a temporal dimension to the equation. Divergence time can be estimated and linked to historical processes (e.g. the opening of the Drake Passage, the onset of the ACC) to form the framework for explaining current species' distribution ranges and untangling their evolutionary history. Estimating faunal affinities by observing long distance dispersal directly is too difficult in marine environments especially at large spatial scales and does not give information about past affinities. Therefore, inferring faunal affinities based on present resemblances, especially genetic resemblances, are an easier alternative (Lowe et al, 2010).

Since our study location, the Magellanic region, is the closest mainland to the Antarctic continent (the Drake Passage, which separates both, is only 800km wide), a pivotal role in connectivity and distribution of life among those regions is suggested. Recent work revealed the faunal affinities between the Magellanic region and the Antarctic continent, notably via stepping stone processes along Scotia Arc islands, and this in both directions (de Moura Barboza et al, 2011; Casares et al, 2017). The Scotia Arc is made up of the islands in between South-America and the Antarctic Peninsula including South-Georgia, the South-Sandwich Islands, and South-Orkney Islands (see Fig 1). Furthermore, it has been demonstrated for several taxa (e.g. echinoids (Diaz et al, 2011), bivalves (Güller et al, 2020), limpets (González-Wevar et al, 2017), sea slugs (Cumming et al, 2014), and also sea stars (Moreau, 2019) that faunal affinities are strong between South-America and the sub-Antarctic islands (e.g. Kerguelen and Crozet). However, generalisations are complicated as connectivity is largely dependent on life history traits, bathymetrical ranges, developmental modes (González-Wevar et al, 2017; Moon et al, 2017), or associations to macroalgae. Affinities are thus taxon dependent with the example of benthic hydroids not showing faunal affinities between South-America and other sub-Antarctic islands (Casares et al, 2017).

1.5.1 Kerguelen and its pivotal position in the sub-Antarctic

Kerguelen is one of the sub-Antarctic islands displaying faunal affinities with the Magellanic region. The Kerguelen archipelago, composed of hundreds of islands, is situated at 49° 20'S and 69° 20'E in the southern part of the Indian Ocean. It is located on the Kerguelen plateau that covers an area of almost 1 250 000km² (Bénard et al, 2010). This plateau is a major obstacle to the ACC as it spans more than 5° of latitude. Shallow water organisms dispersing by kelp rafting or larval drift on the ACC through a Southern Ocean that consists mainly of deep-sea basins do not have many colonising spots within their depth range. Therefore, the Kerguelen plateau is one of these rare colonising spots for those shallow water organisms. Due to the topography of the Kerguelen plateau, the Polar Front passes over the plateau in between the Kerguelen Islands to the north and Heard Island to the south (Fig 3). The Polar

Front has a varying position in this region (spanning nearly 10° in latitude) (Moore et al, 1999; Casares, 2017). While most studies positioned the Polar Front south of Kerguelen (e.g. Park et al, 2014), during some periods the Kerguelen archipelago was situated south of the Polar Front. Kerguelen's pivotal position close to a varying Polar Front and within the ACC, makes it an interesting location to study connectivity with other sub-Antarctic regions as well as with Antarctica.

Over the last decade, a major effort has been made to study marine biodiversity around Kerguelen through several scientific monitoring programs (e.g. Proteker (2011-2021: French polar institute)) as well as the recent expeditions POKER II (POisson KERguelen, 2011) and ACE (Antarctic Circumnavigation Expedition, 2017, Swiss polar institute). A recent biodiversity assessment of Asteroidea in Kerguelen found 37 species validated by genetics (COI) and morphology (Meudec, 2021). Some of those asteroids (e.g. *Glabraster antarctica*) are also reported from the Magellanic region suggesting again that faunal exchanges are present (Moreau et al. 2017). Kerguelen and the Magellanic region are separated by more than 8000km of abyssal plain, raising the question of how species are able to disperse between those regions.

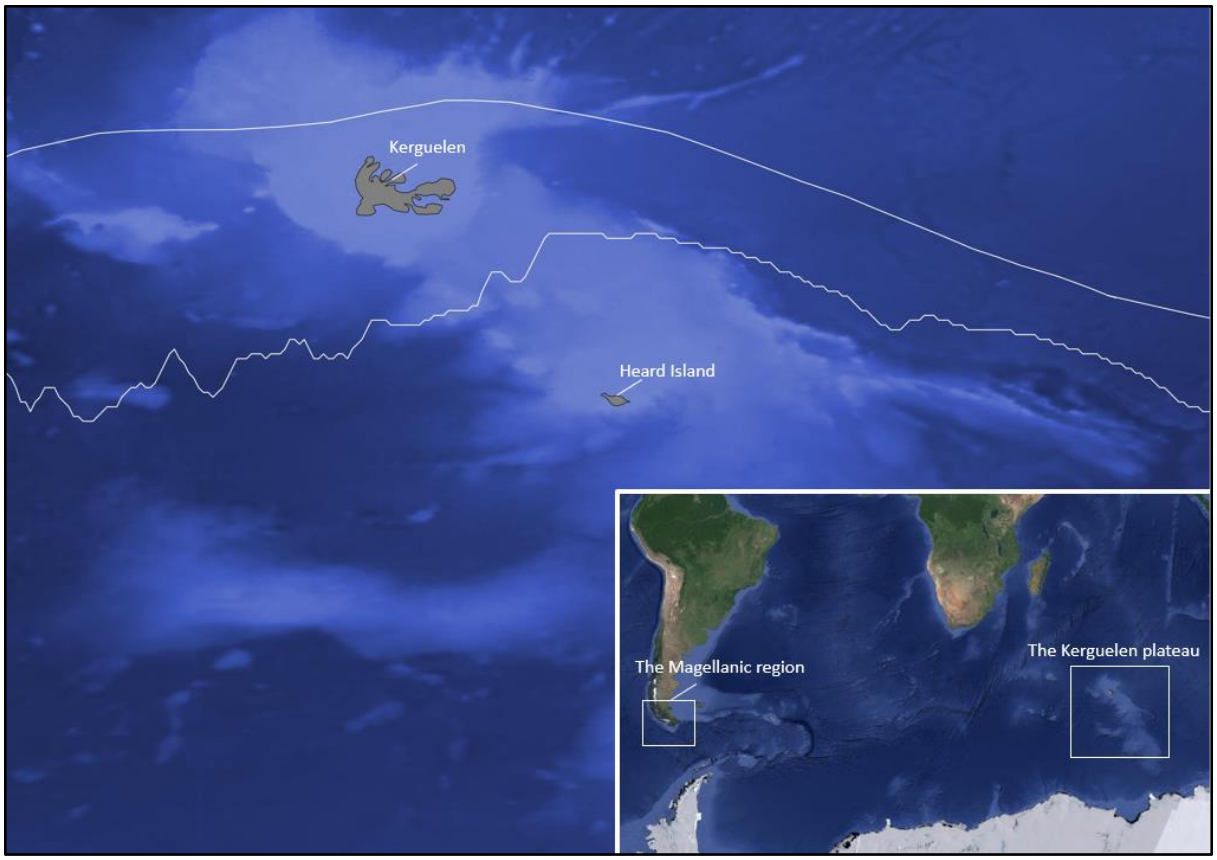

Fig 3| Locations of the Kerguelen archipelago and Heard Island on the Kerguelen plateau. White lines are two positions of the Polar Front. The overview map (bottom right) shows Kerguelen's position relative to the Magellanic region.

1.5.2 The importance of developmental mode in biogeography

As mentioned before, developmental mode and reproductive strategy are important in shaping biogeographical patterns and processes (e.g. dispersal and faunal exchanges). Moreau et al (2017) highlighted that this is especially the case in asteroids. Sea stars have diverse reproductive strategies, the two most common ones being broadcasting and brooding with the latter being more common in colder waters such as polar regions (Mutschke & Mah, 2000; Fraysse et al, 2018). Broadcasters have a pelagic larval stage in contrast to brooders that retain juveniles within or on their body throughout their development. These reproductive strategies are closely linked to their dispersal capacity. Species with a pelagic larval stage (broadcasters) are expected to disperse further by larval drift on ocean currents. However, some species without larvae (brooders) display a wide geographical range suggesting that dispersal ability deduced from reproductive strategy alone is not sufficient (Helmuth et al, 1994; González-Wevar et al, 2021). Recent studies suggested passive rafting on detached floating macroalgae as a dispersal mechanism (Fraser et al, 2011; Cumming et al, 2014; González-Wevar et al, 2021), but direct observations of those dispersals are difficult.

In order to account for the differences in dispersal of both reproductive strategies, we chose both a brooder (*Anasterias antarctica*) and broadcaster (*Glabraster antarctica*) present in both the Magellanic region and Kerguelen to assess the faunal connectivity between those regions.

Taxonomy of *Anasterias antarctica* remains unclear as *A. antarctica* from the Magellanic region and *A. rupicola* from the intertidal in Kerguelen (Moreau et al, 2018; Féral et al, 2019; Meudec, 2021) could be synonymous (Moreau pers. com.). Both are found in very shallow waters and are associated with kelp. Evidence has been found of another species in the same genus, *Anasterias suteri*, rafting on kelp around New Zealand (Waters et al, 2018(b)).

Glabraster antarctica is cosmopolitan in the Southern Ocean and has been found in South-America (Mutschke & Mah, 2000; de Moura Barboza et al, 2011), Kerguelen (Moreau et al, 2018; Féral et al, 2019; Meudec, 2021) as well as in the Antarctic (de Moura Barboza et al, 2011; Moreau et al, 2018) (OBIS, 2022; GBIF.org, 2022). The species is a broadcaster with a planktotrophic larvae displaying lecithotrophic characteristics (Bosch, 1989). Planktotrophic larvae possess the ability to feed (Bosch & Pearse, 1990; Moore et al, 2018). However, contrary to what is expected from this developmental mode, the larvae also possess a large yolk content to use resources from (Bosch, 1989; Fraysse et al, 2018; Moore et al, 2018). The larval duration for the species is reported to last around 60 days (Moore et al, 2018). Additionally, the larvae are highly buoyant, and Ojeda and Santelices (1984) found small individuals of this species associated with *Macrocystis pyrifera* specimens in a kelp forest in Puerto Toro, South Chile. Therefore, the potential of long-distance passive dispersal either through drifting as

a larva on ocean currents or rafting as a juvenile or adult on drifting kelp increases. *Glabraster antarctica* shows a wide morphological variation despite being a single species. It also displays a high genetic diversity with numerous haplotypes. Genetic distances are, however, low enough (lower than 2%) to still be considered as one species (Moore et al, 2018).

2. OBJECTIVES

In this study, we aim to assess the diversity of Asteroidea in the under-sampled coastal waters of the Magellanic region using an integrative approach combining morphological and molecular tools. This work will complement similar efforts carried out around the Kerguelen archipelago (PROTEKER expeditions). Sequences will be publicly available on BOLD adding to the DNA barcode library. We hypothesise that diversity of the coastal Magellanic region is overlooked due to the low sampling efforts despite its pivotal position adjacent to three distinct ocean basins suggesting a rich species diversity.

Secondly, we aim to evaluate the role of the southern tip of South-America (the Magellanic region) in sea star biogeography within the Southern Ocean by defining general biogeographical patterns. We want to assess faunal affinities and gene flow using COI and 16S between the Magellanic region and other regions in the Southern Ocean and go into more detail on affinities between the Magellanic region and Kerguelen. The role of developmental mode in the distribution and dispersal of sea stars will be assessed by comparing a brooder (*Anasterias antarctica)* and broadcaster (*Glabraster antarctica*). We hypothesise that distribution pattern is species specific and despite the distance, faunal exchanges are common between the Magellanic region and the Kerguelen islands regardless of the developmental mode.

3. MATERIAL AND METHODS

3.1 Sample collection and morphological identification

A dedicated sampling campaign was carried out to collect sea stars from the Magellanic region, south of Chile, in February - March 2022. Eight locations (Fig 4, Red stars) were investigated using various methods (SCUBA diving, snorkelling, intertidal sampling) (Annex table 1). All sampling events except the intertidal ones, were done within a kelp forest, because the surrounding environment was sandy with no sea stars present. In addition to the aforementioned samples, specimens preserved in 96% ethanol from recent fieldwork carried out by the University of Magellan, Chile (from 2016 to 2019) were included in this study (Fig 4, Blue stars). These latter were collected in an opportunistic way.

All the available specimens were photographed (actinal and abactinal views) to capture their live colour (for fresh samples) and general morphology. Specimens were identified using dedicated scientific literature (e.g. Madsen, 1956; Clark & Downey, 1992; O'Hara, 1998; Mutschke & Mah, 2000; McKnight, 2006; Janosik, 2012; Jossart et al, 2021). All pictures included a ruler for size reference and were made publicly available on BOLD (See '8. Data accessibility'). Specimens were preserved frozen (except for the ones already preserved in 96% ethanol) and stored at the Laboratorio de Ecosistemas Marinos Antárcticos y Subantárctico (LeMAS) at the University of Magellan in Punta Arenas, Chile.

Tissue samples for each specimen were dissected in the form of several podia or a piece of the arm for smaller individuals. These were stored in cold 96% ethanol to ensure good preservation of the DNA before DNA extraction.

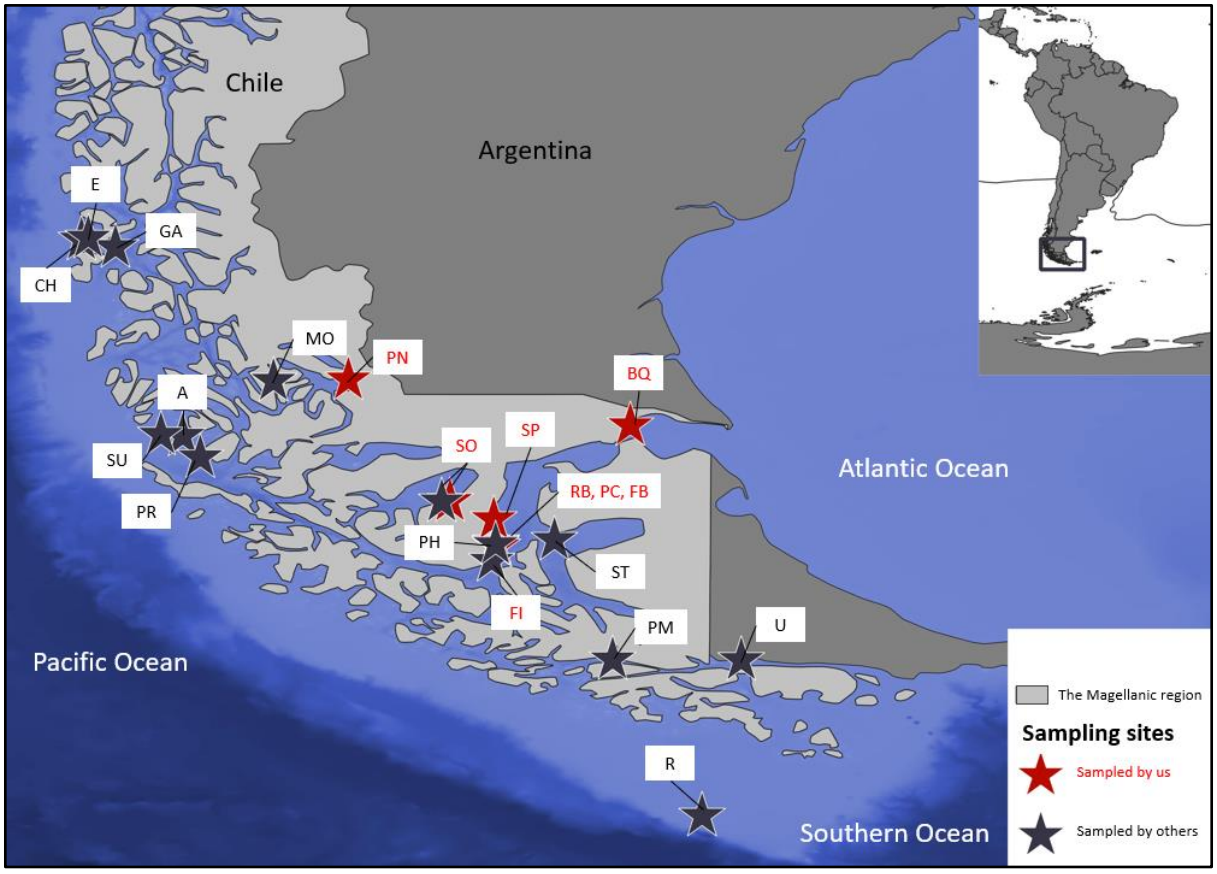

Fig 4| Map of sampling locations in the Magellanic region, Chile (light grey). Blue stars represent samples from the collections at LeMAS while the red stars are locations we sampled during this study. Abbreviations: A = Isla Alta, BQ = Buque Quemado, CH = Seno Copihue, E = Seno Eleuterio, FB = Fuerte Bulnes, FI =Faro san Isidro, GA = Isla García, MO = Canal Montañas, PC = Punta Carreras, PH = Puerto del Hambre, PM = Morrena Pia, PN = Puerto Natales, PR = Isla Parker, R = Diego Ramirez, RB = Rinconada Bulnes, SO = Seno Otway, SP = South of Punta Arenas, ST = Strait of Magellan, SU = Isla Summer, U = Ushuaia.

3.2 COI and 16S amplification

DNA extractions were performed using a DNeasy Blood & Tissue kit by Qiagen available at LeMAS following the instructions of the manufacturer. The success of the DNA extraction was estimated using

a NanoDrop™ 2000 spectrophotometer. For both COI and 16S gene amplification, we used a PCR mix of 6.25µl Accustart Toughmix (including Taq polymerase, buffer, and dNTPs) together with 4.75µl ultrapure water and 0.25µl (10µM) of each primer. A volume of 1.5µl of template DNA was then added for the reaction. The amplification of COI was performed using the forward primer LCOech1aF1 (5'-TTTTTTCTACTAAACACAAGGATATTGG-3': Layton et al, 2016) and reverse primer jgHCO2198 (5'-TAIACYTCIGGRTGICCRAARAAYCA-3': Geller et al, 2013). Amplification of 16S was done using the forward primer R2009 (5'-CGCCTGTTTAYCAAAAACAT-3': Adapted from 16sar-L in Palumbi et al, 1991) and reverse primer F2604 (5-'CGGTCTGAACTCAGATCACG-3': Pothier et al, 2007). All primers were tailed with an M13 tail (forward: 3'-CAGGAAACAGCTATGAC-5', reverse: 3'-TGTAAAACGACGGCCAGT-5') to ease sequencing. The PCR protocol for both COI and 16S consisted of an initial denaturation step of 95°C for 5min followed by 40 cycles of denaturation at 95°C for 45s, primer annealing at 45°C for 45s, and elongation at 72°C for 45s ending with a final elongation at 72°C for 3min. The 16S gene was only amplified in *Anasterias antarctica* and *Glabraster antarctica* specimens.

We assessed the quality of the amplification by performing a gel electrophoresis. We added 2µl of PCR product to 2µl loading dye/GelRed® (10x) mix in equal quantities. Migration was performed in a 1% agarose gel using a voltage of 90V. Samples showing one single bright band at the expected fragment size (~700 bp) were retained. PCR products were then purified by combining 3µl of VWR ExoCleanUp FAST with 7µl of PCR product. This step removes residual primers and single-stranded DNA, and inactivates excess dNTPs by dephosphorylation. One cycle of 5 mins at 37°C to activate the enzymes was followed by 10 min at 80°C to completely deactivate the reagent. Sequencing was finally performed by Macrogen Europe.

3.3 Cleaning and compilation of DNA sequences

Obtained sequences were edited by removing primer sequences and reassigning low quality base pairs using the software Codoncode Aligner v10.0.2. Sequences were then aligned using the Muscle algorithm (Edgar, 2004) implemented in Codoncode Aligner. The absence of stop codons indicating pseudogenes using the echinoderm and flatworm mitochondrial genetic code was also checked. Sequences were blasted in BOLD to assign each species to a BIN (Barcode Index Number indicating a putative species). These BINs are created in BOLD through a clustering algorithm minimizing p-distance between sequences within a BIN while maximizing p-distances between BINs. Since BINs show high concordance with species, this system can be used to verify species identifications (BOLD, 2019). This confirmation was especially needed for juvenile specimens. As for the other metadata (pictures, locations, etc), sequences were added to BOLD (See '8. Data accessibility').

To enlarge the scope of our study and include a maximum of information, we added supplementary DNA sequences for the same identified species from outside the Magellanic region. This was possible from two sources. 1) We sequenced additional samples from other regions that were readily available and curated at the Université Libre de Bruxelles (ULB) in Belgium or at LeMAS in Chile following the protocol mentioned above with the exception that the DNA extraction was performed using a salting out protocol (adapted from Sunnucks et al, 1996) on specimens obtained from the ULB. 2) We mined publicly available sequences within our species' BINs on BOLD.

3.4 Bioinformatics on molecular data

Genetic diversity indexes (nucleotide diversity π, number of haplotypes, and haplotype diversity Hd) were calculated for each species (with the Magellanic region and for their whole geographic range) using the software DnaSP v6.12.03 (Rozas et al, 2017) while intraspecific variation was calculated in MEGA v10.2.0 (Kumar et al, 2018). These indices were only calculated for species with three sequences or more.

To further assess the credibility of the species identified and confirm results obtained through BIN delineation, we ran all sequences through the online species delineation program ASAP (Assemble Species through Automatic Partitioning: https://bioinfo.mnhn.fr/abi/public/asap/). Default settings using the Kimura 2P model were implemented.

Faunal affinities were assessed by mapping the species distribution of the specimens associated to a COI sequence using QGIS v3.10.10. Due to the possibility of an underestimation of species' distributional range attributed to the species not being sequenced covering their full distributional range, we compared found distribution with distributions recorded in OBIS, a publicly available ocean biodiversity platform. OBIS also contains species occurrences based on morphological identification without barcodes. Haplotype networks were generated in Popart (http://popart.otago.ac.nz/) using a TCS network method (Clement et al, 2002) to visualise the genetic structure within each species.

For *Anasterias antarctica* and *Glabraster antarctica*, COI and 16S sequences originating from the Magellanic region or from Kerguelen were kept to also produce haplotype networks, and genetic diversity indices, including only these two regions. Fst values and Φst (using the Kimura 2P nucleotide substitution model) between the two regions were calculated in Arlequin v3.5.2.2 (Excoffier & Lischer, 2010). Their corresponding p-values were obtained in the same program using 999 permutations.

4. RESULTS

4.1 Biodiversity in the Magellanic region

A total of 290 specimens were collected from the Magellanic region belonging to 12 different species within 11 genera, 10 families and 4 different orders (Table 1). COI sequences were obtained for 195 specimens which all resulted in a fragment of 658bp. We were able to assign 11 of the 12 species to a unique BIN in BOLD even though the BIN of *Cosmasterias lurida* is not public (Fig 5, Table 3). The two sequences of *Henricia sp* did not result in a match on BOLD indicating the absence of this species in online databases. ASAP species delineation resulted in the same 12 species (Fig 5).

Anasterias antarctica was sampled and sequenced the most (114 specimens, 79 sequences) while *Pteraster affinis* was found and sequenced only once (Table 1). *Cycethra frigida* was recorded for the first time in this region. We were only able to sequence one of the 15 samples of *Glabraster antarctica* due to the presence of many double peaks after sequencing. Further work is needed to solve this issue. Additional sequences for this species in the Magellanic region were obtained from BOLD. Due to the morphological similarity between both *Cycethra* species, the 22 specimens not yet barcoded, could not be identified to species level and remain '*Cycethra* unidentified' in table 1.

Anasterias antarctica was found in most sampling sites (11) while *Solaster regularis* and *Pteraster affinis* were found in only one site: Fuerte Bulnes (FB) (Table 2). Fuerte Bulnes (FB), Faro san Isidro (FI), and Punta Carreras (PC) were the most diverse sites, each hosting seven different species. No sea star species were observed in Puerto Natales (PN) while seven sites (SP, R, GA, MO, SU, E, ST) only had one species sampled. *Glabraster antarctica* was the only species found in the Strait of Magellan (ST) and this is also the deepest location (deeper than 200m). The shallowest location was South Punta Arenas (SP) which was only sampled in the intertidal zone, leading to only finding *A. antarctica*. Four species (*Labidiaster radiosus, Cycethra frigida, Solaster regularis,* and *Pteraster affinis)* had sequences from the Magellanic region that were only sampled by us (Fig 4 red stars). Those species, except for *C. frigida,* had all been recorded from this region before, but no sequences were available for them to include in this study.

Morphological identification was mostly consistent with genetic identification. However, *Anasterias antarctica* was morphologically divided into two morphospecies based on the colour (either pale yellow or dark green-blue-grey). On the other hand, the two *Cycethra* species were morphologically seen as one species due to the smaller *C. frigida* being very similar to juveniles of *C. verrucosa*. Juveniles

found were often difficult to identify in general, and juveniles of *A. antarctica* and *C. lurida* were sometimes misidentified.

Cycethra verrucosa displayed the highest genetic diversity for all four indexes (N haplotypes = 14, Hd = 0.920, π = 0.0115, and intraspecific distance = 1.15%). Except for the number of haplotypes (N = 2), *Asterina fimbriata* showed the lowest genetic diversity (Hd = 0.286, π = 0.0005, intraspecific distance = 0.05%). Haplotype diversity in *A. fimbriata* was less than half of the second lowest haplotype diversity (Hd = 0.618 in *Labidiaster radiosus*) while the rest stayed within the same range (Hd = 0.618 - 0.920). Intraspecific distance was at least twice as high in *C. verrucosa (*1.15%) and *G. antarctica* (1.14%) compared to the other species (maximum 0.53% in *Cosmasterias lurida*). Further analysis is needed to determine whether or not these species contain overlooked cryptic species. The same pattern was observed in the nucleotide diversity since this value was exactly hundred times lower than intraspecific variation for most species. Two species (*Henricia sp, Pteraster affinis*) only had one haplotype.

Interspecific distances varied greatly due to the presence of species within different orders (Annex fig 1). Highest interspecific distance was recorded between *Henricia sp* and *Pteraster affinis* (30.02%) and lowest interspecific variation was found between the two species within the *Cycethra* genus (6.03%). Interspecific distance was also considerably low (and similar to the congeneric distance between the *Cycethra* species) between *Diplodontias singularis* and *Odontaster penicillatus* (6.99%) who belong to different genera within the same family.

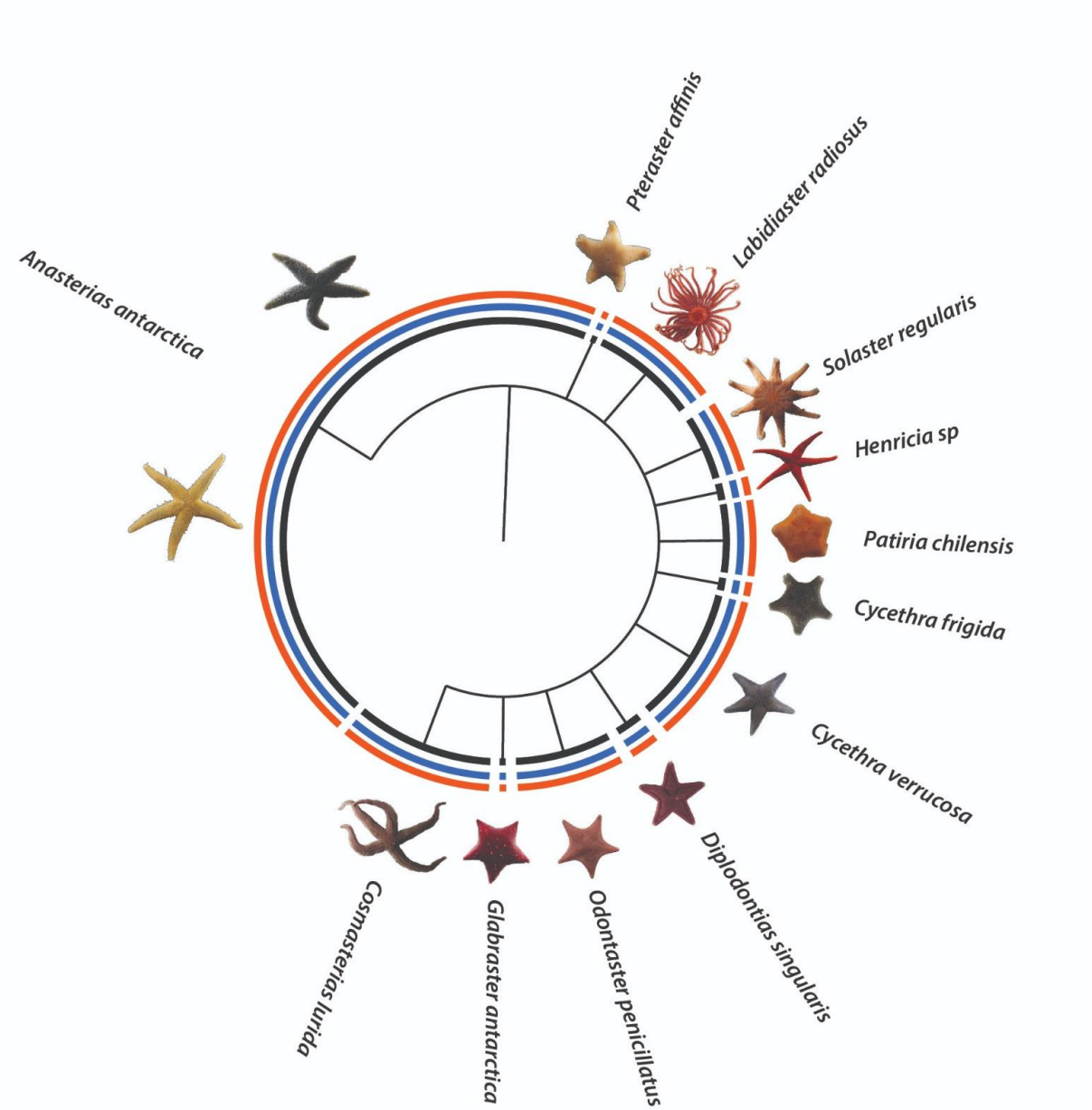

Fig 5| Visualisation of the 12 species present in the Magellanic region. Red lines indicate the species delineation through ASAP while blue lines indicate the separation into different BINs in BOLD.

Table 1| Taxonomic table of specimens from the Magellanic region indicating the number of specimens, number of sequences and the four genetic diversity indices. Abbreviations: Hd = haplotype diversity, π = nucleotide diversity.

Order	Family	Genus	Species	N specimen	N sequences	N haplotypes	Hd	π	Intraspecific distance (%)
Forcipulatida	Asteriidae	*Anasterias*	***Anasterias antarctica***	114	79	13	0.776	0.0047	0.47
	Heliasteridae	*Labidiaster*	***Labidiaster radiosus***	11	11	5	0.618	0.0014	0.14
	Stichasteridae	*Cosmasteris*	***Cosmasterias lurida***	21	16	9	0.900	0.0052	0.53
Spinulosida	Echinasteridae	*Henricia*	***Henricia sp.***	2	2	1	-	-	-
Valvatida	Asterinidae	*Asterina*	***Asterina fimbriata***	18	7	2	0.286	0.0005	0.05
	Ganeriidae	*Cycethra*	*Cycethra* unidentified	22					
			Cycethra verrucosa	25	25	14	0.920	0.0115	1.15
			Cycethra frigida	3	3	2	0.667	0.0020	0.20
	Odontasteridae	*Diplodontias*	***Diplodontias singularis***	8	7	4	0.810	0.0029	0.29
		Odontaster	***Odontaster penicillatus***	16	16	10	0.892	0.00.30	0.30
	Poraniidae	*Glabraster*	***Glabraster antarctica***	32	21	8	0.829	0.0112	1.14
	Solasteridae	*Solaster*	***Solaster regularis***	7	7	3	0.714	0.0013	0.13
Velatida	Pterasteridae	*Pteraster*	***Pteraster affinis***	1	1	1	-	-	-
			TOTAL	**290**	**195**				

Table 2| Species presence per sampling site. Abbreviations: BQ = Buque Quemado, FB = Fuerte Bulnes, FI = Faro san Isidro, PC = Punta Carreras, RB = Rinconada Bulnes, SO = Seno Otway, SP = South of Punta Arenas, CH =Seno Copihue, R = Diego Ramirez, PM = Pia Morrena, PH = Puerto del Hambre, U = Ushuaia, A = Isla Alta, PR = Isla Parker, GA = Isla García, MO = Montañas, SU = Isla Summer, E = Seno Eleuterio, ST = Strait of Magellan, PN = Puerto Natales.

Species	BQ	FB	FI	PC	RB	SO	SP	CH	R	PM	PH	U	A	PR	GA	MO	SU	E	ST	PN
Anasterias antarctica	X		X	X	X	X	X	X	X	X	X	X								
Labidiaster radiosus		X	X	X																
Cosmasterias lurida		X	X	X	X	X							X	X						
Henricia sp.		X											X							
Asterina fimbriata	X			X							X				X					
Cycethra verrucosa	X		X	X							X		X	X		X				
Cycethra frigida	X				X															
Diplodontias singularis			X	X		X		X		X							X			
Odontaster penicillatus		X	X							X	X		X					X		
Glabraster antarctica		X	X	X	X														X	
Solaster regularis		X																		
Pteraster affinis		X																		

4.2 DNA barcode library

The DNA barcode library for the species identified in this work yielded 485 additional sequences from outside the Magellanic region resulting in a total of 675 sequences (Table 3). In the final dataset, most sequences belonged to *Glabraster antarctica* (382) while only two sequences were available for *Henricia sp.* No extra sequences were found for four species (*Cosmasterias lurida, Cycethra verrucosa, Diplodontias singularis,* and *Henricia sp)* compared to 361 additional sequences for *G. antarctica*. This large number of additional sequences led to an increase in the number of haplotypes of 141 in *G. antarctica*. Intraspecific variation did not change much (<0.10%) for most species except for a doubling in *Labidiaster radiosus, Odontaster penicillatus,* and *Solaster regularis* and a four times increase in *Asterina fimbriata.* Intraspecific variation was not higher in either reproductive strategy as both the highest and lowest intraspecific variation are recorded in broadcasters. Changes in haplotype diversity ranged from a decrease of 0.108 in *A. antarctica* to a doubling in *A. fimbriata*.

Table 3| Breakdown of the number of sequences compiled into the DNA barcode library with calculation of genetic diversity indices per species and the BIN in BOLD they belong to. Mag = sequences from the Magellanic region (same as in Table 2), extra = extra sequences from outside of the Magellanic region, Total = all sequences combined. * changes compared to initial calculation on specimens only from the Magellanic region are indicated between brackets

Species name	N Mag	N extra	N Total	π	N haplotypes *	Hd*	Intraspecific variation *	BOLD BIN
Anasterias antarctica	79	28	107	0.0042	14 (+1)	0.668 (-0.108)	0.41% (-0.06)	BOLD:AAA8344
Cosmasterias lurida	16	0	16	0.0052	9 (+0)	0.900 (+0)	0.53% (+0.00)	Private
Cycethra verrucosa	25	0	25	0.0115	14 (+0)	0.920 (+0)	1.15% (+0.00)	BOLD:AAR5363
Cycethra frigida	3	12	15	0.0017	5 (+3)	0.695 (+0.028)	0.17% (-0.03)	BOLD:ADG2622
Diplodontias singularis	7	0	7	0.0026	4 (+0)	0.810 (+0)	0.29% (+0.00)	BOLD:AEH4090
Glabraster antarctica	21	361	382	0.0182	149 (+141)	0.980 (+0.151)	1.71% (+0.57)	BOLD:AAB6633
Henricia sp	2	0	2	0.0000	1	NA	0.00% (+0.00)	No match
Labidiaster radiosus	11	2	13	0.0042	6 (+1)	0.718 (+0.100)	0.43% (+0.29)	BOLD:ACB6572
Odontaster penicillatus	16	46	62	0.0062	27 (+17)	0.928 (+0.036)	0.63% (+0.33)	BOLD:ABW1983
Asterina fimbriata	7	5	12	0.0025	6 (+4)	0.682 (+0.396)	0.25% (+0.20)	BOLD:ACI1273
Solaster regularis	7	5	12	0.0026	5 (+2)	0.818 (+0.104)	0.26% (+0.13)	BOLD:AAM2777
Pteraster affinis	1	21	22	0.0020	10 (+9)	0.749 (NA)	0.20% (NA)	BOLD:AAC7424
TOTAL	**195**	**485**	**675**					

4.3 Faunal affinities and biogeographical patterns

4.3.1 Within the entire Southern Ocean

Four biogeographical patterns were found. Four of the 12 species (*Cycethra verrucosa, Cosmasterias lurida, Diplodontias singularis,* and *Henricia sp*) were only present in the Magellanic region (Table 4, example in Fig 6, all species in Annex fig 2). A second biogeographical pattern was formed by species only present within the sub-Antarctic, north of the Polar Front. Four species displayed this pattern: *Anasterias antarctica, Solaster regularis, Cycethra frigida,* and *Asterina fimbriata*. Two species (*Labidiaster radiosus* and *Odontaster penicillatus*) had distributions mainly in the sub-Antarctic, but each had a single sequence originating from the Antarctic (the Antarctic Peninsula and South-Georgia respectively). The last two species had distributions throughout the Southern Ocean: *Pteraster affinis* and *G. antarctica* with the latter being found in most locations.

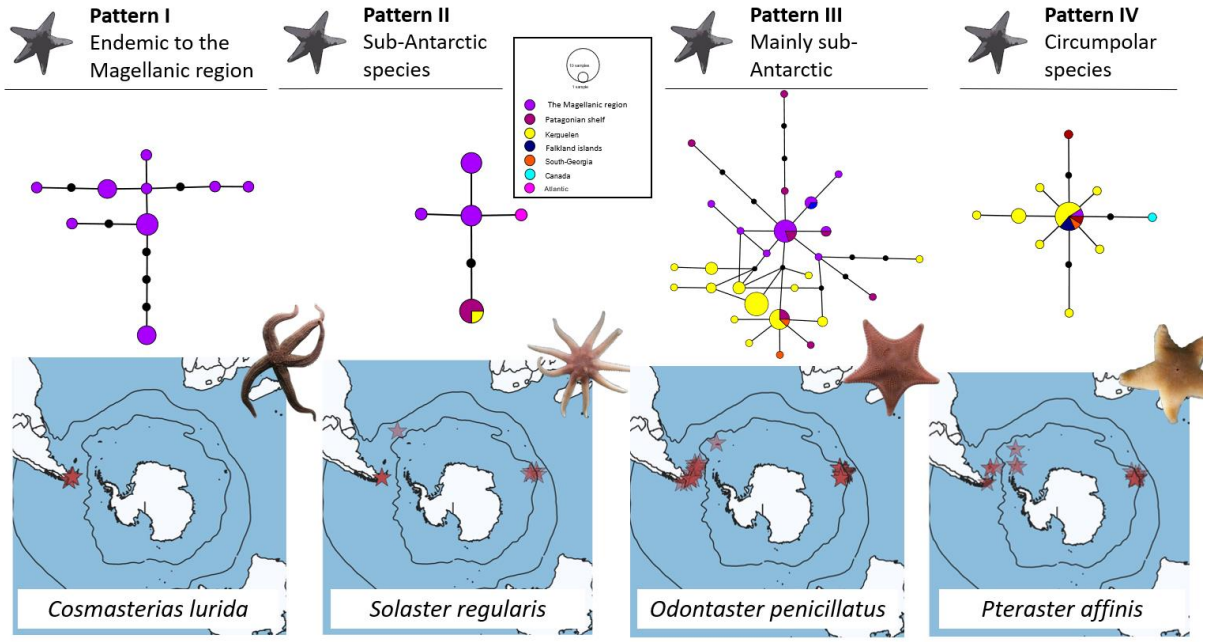

Fig 6| Examples for each geographical pattern showing species distribution as well as their associated haplotype network.

Table 4| Presence of specimens associated to COI sequences in the different regions and their subsequent distribution type. Abbreviations: Mag =the Magellanic region (coastal), PaS = Patagonian shelf, Fal : Falkland Islands, Atl = Atlantic, Cro = Crozet, Ker = Kerguelen, SG = South-Georgia, SSan = South-Sandwich Islands, SOrk = South-Orkney Islands, Hrd = Heard Island, Ant = Antarctica, Can = Canada. References: (1) Fraysse et al, 2018; (2) Mutschke & Mah, 2000; (3) Bosch & Pearse, 1990

			Sub-Antarctic						Antarctic					
Species	Biogeo-graphical pattern	Developmental mode	Mag	PaS	Fal	Atl	Cro	Ker	SG	SSan	SOrk	Hrd	Ant	Can
Anasterias antarctica	II	Brooder[1,2]	X		X		X	X						
Cosmasterias lurida	I	Broadcaster[1]	X											
Cycethra verrucosa	I	Unknown	X											
Cycethra frigida	II	Broadcaster[2]	X		X			X						
Diplodontias singularis	I	Unknown	X											
Glabraster antarctica	IV	Broadcaster[1,3]	X	X	X			X	X	X	X	X	X	
Henricia sp	I	Unknown	X											
Labidiaster radiosus	III	Unknown	X					X					X	
Odontaster penicillatus	III	Broadcaster[1,3]	X	X	X			X	X					
Asterina fimbriata	II	Brooder[1]	X	X	X									
Solaster regularis	II	Unknown	X	X		X		X						
Pteraster affinis	IV	Brooder[1]	X	X	X			X	X		X			X

4.3.2 Affinities between the Magellanic region and Kerguelen

For the analysis of the faunal affinities between the Magellanic region and Kerguelen 21 COI sequences from the Magellanic region and 49 from Kerguelen were obtained for *Glabraster antarctica* and 19 and 79 for *Anasterias antarctica* respectively.

We sequenced 16S in 27 specimens of *A. antarctica* and 23 of *G. antarctica* which resulted in fragments of 671bp and 597bp respectively (Table 5). This gene was less variable than COI as it had a nucleotide diversity of 0.0022 for *A. antarctica* compared to 0.0043 for the COI. A similar result was found for *G. antarctica* as 16S had a nucleotide diversity of 0.0021 while COI was almost seven times higher (0.0146). Five 16S haplotypes were found for *A. antarctica* and seven for *G. antarctica*. More haplotypes were obtained for COI (13 for *A. antarctica* and 31 for *G. antarctica*).

Haplotype networks (Fig 7) showed a mix of shared and non-shared haplotypes between the Magellanic region and Kerguelen.

Highly significant Fst and Φst values for COI between populations in both regions were found for both *A. antarctica* (Φst = 0.27, p<0.001; Fst = 0.23, p<0.001) and *G. antarctica* (Φst = 0.33, p<0.001; Fst = 0.09, p<0.001). Based on 16S data, Fst for *A. antarctica* was significant (Fst = 0.24, p = 0.01) while Φst was not (Φst = 0.05, p = 0.17). Φst for 16S in *G. antarctica* was not significant (Φst = 0.10, p = 0.07) in contrast to Fst being highly significant (Fst = 0.31, p = 0.001).

Table 5| Number of sequences of 16S and COI with genetic diversity indices as well as Φst and Fst values. ** indicates a very significant p-value (<0.01), and *** a highly significant p-value (<0.001)

Gene	Species name	N sequences	π	N haplotypes	Hd	Intraspecific variation	Φst	Fst
16S	***Anasterias antarctica***	27	0.0022	5	0.618	0.22%	0.05 (p=0.17)	0.24** (p=0.01)
	Glabraster antarctica	23	0.0021	7	0.791	0.21%	0.10 (p=0.07)	0.31*** (p=0.001)
COI	***Anasterias antarctica***	98	0.0042	13	0.691	0.43%	0.27*** (p<0.001)	0.23*** (p<0.001)
	Glabraster antarctica	70	0.0147	31	0.925	1.50%	0.33*** (p<0.001)	0.09*** (p<0.001)

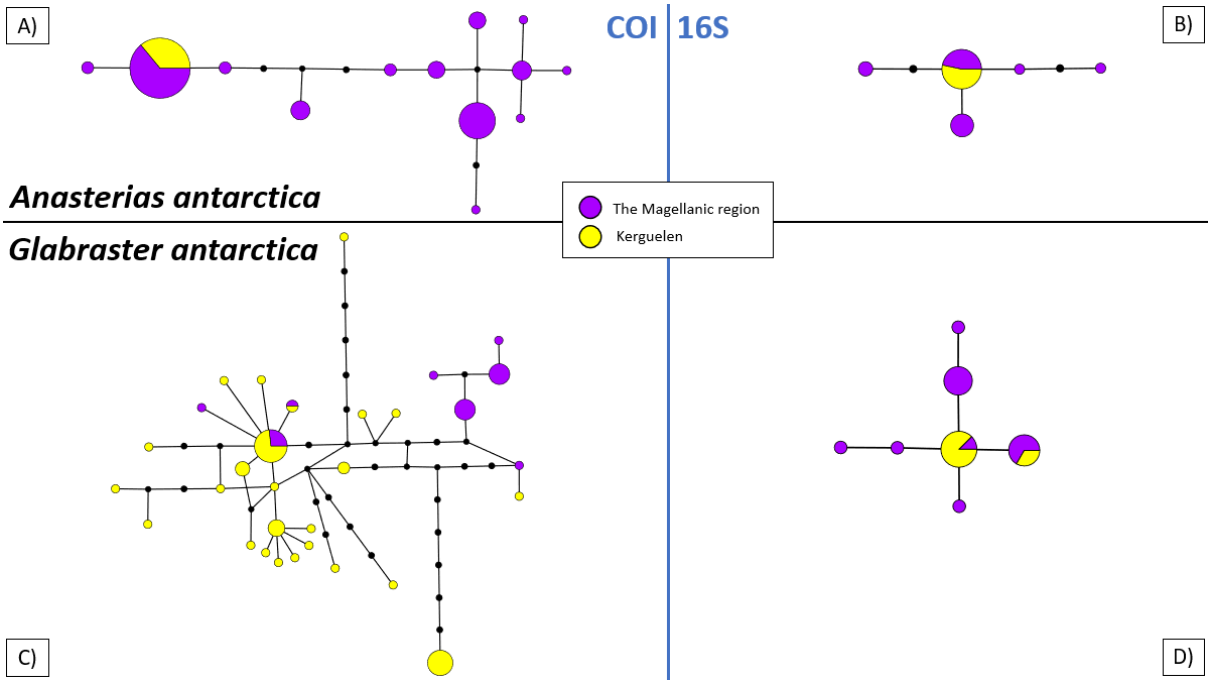

Fig 7| Haplotype networks for *Anasterias antarctica* (A depicts haplotype networks using COI, B uses 16S) and *Glabraster antarctica* (C depicts haplotype networks using COI, D uses 16S).

5. DISCUSSION

5.1 Biodiversity in the Magellanic region

5.1.1 Species richness

In the present study we recorded 12 sea star species in the coastal waters of the Magellanic region (south of Chile). We obtained congruent results using both ASAP and BIN methods to delineate species based on their genetic signatures. Genetic identification through DNA barcoding was mostly in line with morphological identification (but see '5.1.2 Taxonomic discrepancies highlighting the importance of an integrative approach'). Previous studies reported a higher species richness with up to 24 species (Mutschke & Mah, 2000; de Moura Barboza et al, 2011; Fraysse et al, 2018). Differences between this study and previous works are most likely due to different geographical ranges (the Magellanic region or only Tierra del Fuego), different sampling sites, the fact that our sampling was restricted to 20m, and/or observer bias. This study is preliminary and more extensive work on unravelling Magellanic sea star diversity should follow. We reported *Cycethra frigida* for the first time in the Magellanic region extending its range beyond Kerguelen and the Macquarie Islands where it has been previously recorded.

5.1.2 Taxonomic discrepancies highlighting the importance of an integrative approach

In this study we used an integrative taxonomic approach by combining morphological identification with genetic identification as suggested in previous works (e.g. Layton et al, 2016; Wright et al, 2016; Ringvold & Moum, 2019; Jossart et al, 2021). Using morphology, most species could be identified quickly and the more time consuming DNA barcoding was not necessary. However, some differences were found between the two methods.

Firstly, morphological identification could not separate the two *Cycethra* species and *Cycethra frigida* was subsequently overlooked. This species has previously been recorded from Kerguelen (e.g. Meudec, 2021) and the Macquarie Islands (O'Hara, 1998). *Cycethra frigida* may have been overlooked due to its morphological similarities to juveniles of its sister species *Cycethra verrucosa* that was also present in the Magellanic region (see Annex fig 3 for a morphological comparison). O'Hara (1998) noted that the species can be distinguished by a greater number of adambulacral spinelets (four to eight) in *C. verrucosa* compared to only three to four in *C. frigida* (Clark & Downey, 1992). Due to *C. frigida* not being reported within the Magellanic region prior to sampling, the number of adambulacral spinelets was not immediately checked using a stereomicroscope and counting later on was not accurately

possible from the pictures taken. Therefore, a more detailed morphological examination is suggested when identifying those species in the future. If *C. frigida* has been confused with its sister species in this study, this species has likely been overlooked in other regions that also report *C. verrucosa*. BOLD only reported the presence of *C. verrucosa* in the Magellanic region. However, occurrence databases (OBIS and GBIF), largely based on morphological identifications only, showed a wider distribution of *C. verrucosa*, including the Magellanic region, the Scotia Arc, Bouvet Island, Heard Island, and large parts of Antarctica. *Cycethra frigida* had not been recorded from those regions. Since occurrences from OBIS or GBIF can often not be checked with DNA barcoding nor pictures, caution needs to be taken when using these datasets. Occurrences of *C. verrucosa* outside of the Magellanic region should be verified by DNA barcoding alongside detailed morphological analysis, and checked for the possible presence of *C. frigida*. The Magellanic region is so far the only region where both species are reported together. This example illustrates the importance of combining morphology with molecular approaches to not underestimate biodiversity as has already been highlighted in previous studies by Janosik et al (2011), Layton et al (2016), and Knott et al (2018) as well.

On the other hand, taxonomic discrepancies can lead to an overestimation of biodiversity as already demonstrated in Wright et al (2016) and Moore et al (2018). Species within the genus *Anasterias* have been named *A. rupicola* when found in the intertidal of Kerguelen, but *A. antarctica* when found in South-America. Genetics in this study demonstrated that it is the same species occurring in the two regions and that its status should be revised. We found the same discrepancy for *Odontaster meridionalis* (Kerguelen) and *O. penicillatus* (South-America) forming a similar genetic entity. This contrasts with Janosik et al (2011) who do separated both species in their study. However, Moreau et al (2021) indicated that their *O. meridionalis* sequences are misidentifications of *Asterina fimbriata*. Additionally, two other *Odontaster* species, *Odontaster roseus* and *Odontaster pearsei,* have been repeatedly misidentified as *O. meridionalis* which further questions the existence of a true genetic entity *O. meridionalis* (Guzzi et al, submitted). This leads us to believe that due to the increase in the number of reference sequences during the last decade, the species *O. penicillatus* has now become clear while the existence of a genetic entity called *O. meridionalis* should be further investigated.

Even within the small number of species recorded, two synonymous species and one overlooked species were discovered suggesting that taxonomic discrepancies such as these are common. Therefore, integrative taxonomic approaches should be more frequently implemented and more extensive biodiversity assessments are needed. Increasing the barcoding effort will result in an increase in reference sequences in the DNA barcode library, which will lead to more accurate identifications in the future.

5.2 Additions to the DNA barcode library

The use of a DNA barcode library is only efficient if it contains reference sequences with correct associated species identifications. BOLD contains more than 11 million sequences covering animal, plant, as well as fungus species. Nearly 11 000 of those sequences belong to sea stars, covering seven orders and making up 577 BINs from all oceans. The World Asteroidea Database (Mah, 2022) however indicates the existence of 1925 asteroid species showing that the number of barcoded species is still low compared to the total number of accepted species (Gong et al, 2018; Jossart et al, 2021). Additionally, under-representation of geographical coverage within a species, may lead to an incomplete picture of its intraspecific genetic diversity (Huemer & Mutanen, 2022). Due to recent advances in sequencing technology and computational software, the implementation of DNA barcoding and barcode-based studies such as eDNA and metabarcoding are increasing (Gostel & Kress, 2022). DNA barcode libraries do not only aid in species identification, but are also implemented in other domains such as population genetics, phylogenetics, and community-based studies (Hajibabei et al, 2007). Therefore, the need for a solid DNA barcode library covering a wide range of species is fundamental. In this study, we contributed with almost 200 new sequences belonging to 12 species to the DNA barcode library in BOLD including sequences of two species that did not have publicly available sequences. This work also builds a preliminary baseline for future work on diversity in the Magellanic region.

5.2.1 Genetic distances

The average intraspecific variation values found (0.17-1.71%) are in line with Ward et al (2008) who reported intraspecific variation within 37 species of asteroids ranging from 0% to 1.85%. For most species, intraspecific genetic diversity of specimens within the Magellanic region reflected their intraspecific diversity throughout the entire Southern Ocean. This could suggest an overall panmixia within the Southern Ocean. Magellanic sequences from *Asterina fimbriata,* however, displayed only a quarter of this species' intraspecific variation and half of its haplotype diversity. This indicates that intraspecific genetic diversity can still be underestimated when sequences do not cover the full geographical coverage of a species.

Interspecific variation (6-30%) showed a wide range due to the presence of phylogenetically distant species belonging to different orders (Annex fig 1). Congeneric distance between the *Cycethra* species was averaged 6.03% in this study, but according to Ward et al. (2008) even congeneric distances have a wide range from 2.17% to 22.85%. Distance distribution histograms obtained through ASAP showed no overlap between intraspecific and congeneric distances meaning that the barcoding gap is present and species delineation of the sequences obtained in this study based on genetic methods is reliable.

5.3 Faunal affinities and biogeographical patterns

5.3.1 Four biogeographical patterns in the Southern Ocean

Although we expected to find species originating from the three adjacent oceans, we only found species with a distribution within the Southern Ocean (sensu lato) with a single specimen of the species *Pteraster affinis* from the Northern hemisphere as exception. Pérez-Ruzafa et al (2013) mentioned a gradient of faunas from the Atlantic and Pacific through the Magellanic region, but we did not observe a change in sea star composition based on longitude.

With the exception of a single *Pteraster affinis* specimen from Canada (Layton et al, 2016), all specimens were found south of the Subtropical Front. Oceanic fronts have shown to form a barrier for epipelagic and benthic species (Clarke et al, 2005; Thornhill et al, 2008; Fraser et al, 2012) and crossing is thus not possible for every species. Even if species manage to cross an oceanic front, the most difficult part is tolerating the sudden change in temperature. Many shallow water echinoderms are stenothermic (Thandar, 1989) meaning that they tolerate only a narrow temperature range. Additionally, other factors such as competition, ecological niche, evolutionary history, and life history traits play a role in explaining the near absence of these species beyond the Southern Ocean.

We could generalise four geographical patterns within the Magellanic sea stars. The first pattern was formed by species endemic to the Magellanic region. Out of the four species found with this geographical pattern, only *Diplodontias singularis* had a similar distribution recorded in OBIS. Not much is known about the reproduction strategy of *Diplondontias*, but its shallow depth range (up to 84m) could be one of the reasons limiting the colonisation of other regions in the Southern Ocean as more than 70% is deeper than 2000m. Species of the genus *Henricia* are known to be difficult to differentiate morphologically (Knott et al, 2018), which also translated when using DNA barcode libraries. This genus has been acknowledged as a complex by many authors (e.g. Ringvold & Stien, 2001 and references therein; Knott et al, 2018) and the suggestion that species within this genus hybridise makes taxonomy even more complicated (Ringvold & Stien, 2001). Therefore *Henricia sp.* could not be identified to species level and its complete distribution remains unclear. *Henricia obesa* has been found previously in the Magellanic region, but the single *H. obesa* sequence present on BOLD did not match with a high similarity with our sequences. Another species found in the Magellanic region is *Henricia studeri*, but there is no reference sequence for this species on BOLD. We added two more sequences for *Henricia* species in the reference database in the hope that the taxonomic issues in this genus might get resolved in the future. Two other species with a distribution endemic to the Magellanic region also had records on OBIS in Antarctica (*Cosmasterias lurida)* as well as covering the whole Scotia Arc and Kerguelen (*Cycethra verrucosa)*. As species identification on OBIS cannot be verified, it remains unclear

whether or not those two species truly have distributions outside the Magellanic region as suggested by OBIS.

The second geographical pattern included species with distributions only in the sub-Antarctic, north of the Polar Front. Four of our species belonged to this type (*Anasterias antarctica, Solaster regularis, Cycethra frigida,* and *Asterina fimbriata*). Distributions on OBIS showed a wider distribution for *A. antarctica/A. rupicola* also including records south of the Polar Front (e.g. the Antarctic Peninsula). Even wider distributions were recorded for *S. regularis* on OBIS covering the entire Southern Ocean north and south of the Polar Front. Efforts in barcoding specimens originating from south of the Polar Front should confirm these species' distribution patterns. The two other species had distributions on OBIS confirming their sub-Antarctic "only" distribution with *A. fimbriata* being restricted to the Patagonian shelf (South-America and the Falklands) except for one observation from south of New Zealand.

The third geographical pattern was formed by species that are mainly found within the sub-Antarctic, but that did have a single observation south of the Polar Front. *Labidiaster radiosus* had one observation from the Antarctic Peninsula while *O. penicillatus/O. meridionalis* had one observation from South-Georgia. On OBIS, both species had a distribution covering the entire Southern Ocean suggesting a wider distribution of these species with a lack of sequences covering their full distribution. However, recent studies evaluating *Odontaster* taxonomy in the Southern Ocean (e.g. Janosik et al, 2011; Peck et al, 2018; Guzzi et al, submitted) indicated that misidentifications within the *Odontaster* genus are common and that this species is probably not found in the Antarctic. The strict separation between the Antarctic and the sub-Antarctic based on faunal compositions is however not clear and species dependent (Soto Àngel & Peña Cantero, 2017). South-Georgia seems to be a mix of the two regions explaining the presence of *O. penicillatus* around this island (Soto Àngel & Peña Cantero, 2017 and references therein). Therefore this geographical pattern is likely not a species' true distribution but rather an underestimation of a circumpolar pattern as a result of lack of sequences covering its full distribution (as might be the case for *Labidiaster radiosus*) or an overestimation of a sub-Antarctic pattern as a result of the lack of clear separation between the Antarctic and sub-Antarctic (as is probably the case for *O. penicillatus*).

The last geographical pattern included circumpolar species. *Glabraster antarctica* has been recorded nearly everywhere in the Southern Ocean. High dispersal capability is suggested by its wide bathymetrical range, the presence of a pelagic larval stage, and its association to floating macroalgae. Crossing the Polar Front coincides with a sudden drop or increase in temperature. Especially Antarctic species have a low tolerance to temperature increases (Clarke et al, 2005) suggesting that those

species are less likely to colonise the sub-Antarctic contrary to sub-Antarctic species colonising the Antarctic. *Pteraster affinis* had fewer records, but had one specimen originating from Canada (Layton et al, 2016). Despite Layton et al (2016) identifying this specimen as *Pteraster miliaris,* we found that *P. affinis* is its closest match on BOLD. *Pteraster affinis* has been acknowledged to be one of the few species shared between Northern and Southern hemispheres (Jossart et al, 2021). Very few shallow marine organisms have a true cosmopolitan distribution as sea surface temperature varies greatly throughout the world's oceans with sea surface temperature going up to more than 25°C in the tropics, reaching a maximum of 12°C in the sub-Antarctic while even decreasing below zero in the Antarctic. When reporting cosmopolitism authors, however, often do not include polar regions in their cosmopolitan concept (e.g. Martínez, 2008). Deep-sea species are more often found to have a cosmopolitan distribution (Costello et al, 2017). The presence of one *P. affinis* specimen from the Northern hemisphere might indicate recent migration between hemispheres. As physiological constraints would lead to the death of individuals crossing the warm surface layers in the tropics, deep-sea migration is suggested to explain dispersal between hemispheres (Jossart et al, 2021). Pterasteridae mainly occur in deep, cold waters confirming the possibility of deep-sea dispersal (Jossart et al, 2021). Either this species has a bipolar distribution or it has a cosmopolitan distribution with occurrences at higher depths outside the polar regions that are not yet sampled.

Those four geographical patterns only took sequenced individuals into account. Even though the species names cannot be confirmed on OBIS, the wider distributional range on OBIS for most species suggests that species have not been sequenced covering their full distributional range. Therefore this preliminary study encourages the increase in barcoding effort throughout the entire Southern Ocean.

5.3.2 The role of developmental mode

The three brooding species (*Anasterias antarctica, Asterina fimbriata,* and *Pteraster affinis*) did not all show a limited distribution when compared to broadcasters. For example, *A. antarctica* was present in the Magellanic region and Kerguelen while *Pteraster affinis* was also recorded in islands of the Scotia Arc and even in the Northern hemisphere. Biogeographical distribution based on reproductive strategy alone is thus not enough. Pérez-Ruzafa et al (2013) previously mentioned that environmental factors are responsible for species' distribution in addition to developmental modes. Water temperature and specifically minimum temperature would be the most important factor on global scale while competition, disease, food availability, and substrate are more important on small scales (Pérez-Ruzafa et al, 2013). Therefore environmental factors combined with species' physiological constraints as well as other life history traits and biological competition should be considered in explaining species' distribution range.

5.3.3 Affinities between the Magellanic region and Kerguelen

To specifically assess connectivity between the Magellanic region and Kerguelen, 16S was sequenced additionally to COI. This gene was less variable with two to eight times lower nucleotide diversity compared to COI. Even though both genes are located on the mitochondrial DNA, 16S has been reported to be more conservative and to have a slower mutation rate than COI (Iuri et al, 2007; Janosik et al, 2011).

Both the brooder *Anasterias antarctica* and the broadcaster *Glabraster antarctica,* shared haplotypes between the Magellanic region and Kerguelen (COI and 16S data). Based on 16S data, only Fst values were significant, but due to the low underlying genetic diversity of this marker, these Fst and Φst values might be biased (Mehta et al, 2019). Based on COI data, both significant Fst and Φst values were found between these regions. This pattern might indicate a limited gene flow between these two regions, without excluding some long-distance dispersal events. According to the clockwise circulation of the ACC, this dispersal is more likely to occur from South-America to Kerguelen than the other way around. Further investigations of gene flow patterns based on other molecular markers with higher mutation rates are required to differentiate past and ongoing gene flow (see 'Limits and perspectives').

Glabraster antarctica was present in both the Magellanic region and Kerguelen and showed the broadest distribution covering the entire Southern Ocean. Haplotype networks showed shared haplotypes between those regions implying that this species can disperse between them. This species also has the highest depth range up to 3200m, possibly connecting populations from South-America with Kerguelen, Antarctica, and other sub-Antarctic islands via the abyss (Díaz et al, 2011). This broadcasting species has a larval stage that can stay in the water column for 60 days (Bosch, 1989). Based on Argo float trajectories (https://fleetmonitoring.euro-argo.eu/dashboard) and particle trajectory simulations (Fraser et al, 2018), the drift of 8000km from the Magellanic region to Kerguelen was estimated to take 200-400 days. This is more than 40 times the duration of the reported larval stage in *G. antarctica*. Both regions are separated by abyssal plains deeper than 4000m which is outside this species' depth range. Therefore the possibility for this species to disperse from the Magellanic region to Kerguelen via its larvae is unlikely.

Anasterias antarctica in turn also showed affinities between the Magellanic region and Kerguelen, but does not have a wide depth range (up to 11m) and is a brooder without a pelagic larval stage. This is why for both species, passive rafting on kelp is suggested to explain their wide dispersal as both species have been found in association with kelp (Ojeda & Santelices, 1984; O'Hara, 1998; Waters et al, 2018(a and b)).

5.3.4 Kelp rafting as suggested dispersal mechanism

The Antarctic Polar Front has long been considered an impenetrable barrier for epipelagic and benthic species limiting species' distribution within the Southern Ocean (Clarke et al, 2005; Thornhill et al, 2008; Fraser et al, 2012). Exceptions are large marine mammals and deep-sea species since the Polar Front is less of a barrier deeper down (Clarke et al, 2005; Dìaz et al, 2011; Fraser et al, 2012). However, during the previous decades, more studies have shown evidence of species crossing the Polar Front (Helmuth et al, 1994; references in Clarke et al, 2005; Fraser et al, 2011; Janosik et al, 2011; Chown et al, 2015; Moon et al, 2017; González-Wevar et al, 2021). Several ways of crossing have been proposed such as shallow pelagic species or larvae crossing the front within mesoscale eddies (Ansorge & Lutjeharms, 2003; Chown et al, 2015; Clarke et al, 2005). These eddies are whirlpools of water running in the opposite direction of the main current (here the ACC). They either have a cold core originating from the Antarctic and swirl north of the Polar Front or they are warm core sub-Antarctic eddies swirling south of the Polar Front. Another way species can cross would be to passively drift on floating substrates such as driftwood, plastic litter, ship hulls, and buoyant kelp. Within the echinoderms, passive rafting on buoyant kelp is most often suggested as a dispersal agent (Fraser et al, 2011; González-Wevar et al, 2021), but direct observation of those dispersals is difficult. Therefore, most studies assessing such dispersal events are done using molecular tools. However, it is not always clear if found affinities are the result of ongoing gene flow or remanences of past affinities. These past affinities could date back to interglacial periods changing and weakening oceanic features like the Polar Front making dispersal between the Antarctic and sub-Antarctic easier (Dìaz et al, 2011). To exclude those past affinities, a marker with a fast mutation rate is needed. Other studies assessed dispersal by analysing biodiversity and origin of a found kelp raft (e.g. Fraser et al, 2011; Waters et al, 2018(a)). Methods to determine the origin and destination (when found floating) have only recently started to be developed and implemented, but show great potential for future research. Smiths (2002) estimated that, at any time, 70 million floating kelp rafts are drifting in the Southern ocean north of the Polar Front while Fraser et al. (2018) revealed large numbers of these kelp rafts also being able to cross the Polar Front. However, when epifauna is found on floating rafts it is still unsure whether the raft will hit land soon enough so that the species can survive the trip and if it will wash ashore in a suitable habitat for the species to settle. Despite these uncertainties, the large number of rafts suggests that at least some will be successful each year facilitating colonisation by its epifauna. Most of the species recorded on those rafts are brooding species (Gibson et al, 2005). Brooding would allow their offspring to remain on the raft for multiple generations while broadcasters would not be able to fertilize externally due to the too low concentration of gametes (Gibson et al, 2005; Fraser et al, 2018).

6. LIMITS AND PERSPECTIVES

Despite the preliminary results obtained, there are some limitations in this work that need to be addressed.

Firstly, due to the fjord landscape in the Magellanic region, accessing sampling locations by car is difficult. Diving from a boat is the best option for less accessible locations, but requires more planning and is expensive. In addition, we wanted to sample in a region with kelp forests since these harbour a lot of life contrary to the bare sand in the surroundings. Since only few samplings have been done in this region before, we relied on the knowledge of a local diver and researcher (Dr. Karin Gérard) to find sampling locations. Therefore, less known and less accessible regions were not sampled possibly overlooking biodiversity. Previously taken samples available at LeMAS filled parts of these geographical gaps, but there is still a lot to discover.

In this study we assessed faunal affinities using the COI and 16S gene. These genes however have a relatively slow mutation rate and low intraspecific variability. Detailed insight in genetic structuring and current gene flow is not possible this way. We tried amplifying and sequencing two internally transcribed spacers (ITS1 and ITS2) in the nuclear genome, but we failed to find a protocol that consistently succeeded. Moreover, the few sequences we did obtain showed very low intra and interspecific variability. Future research should focus on using nuclear markers with higher mutation rates such as microsatellites or SNPs to address recent these population dynamics.

Morphological identification was done quickly due to time constraints when in Chile and revised after DNA barcoding using the pictures taken. Looking at morphology of certain species like the two *Cycethra* species and *Henricia* in more detail will help identification without the need for genetics and will increase the number of correctly identified specimens and thus sequences on BOLD.

Evaluating the role of developmental mode in species' geographical pattern would be more accurate when comparing a brooder and broadcaster that have similar depth ranges. Bathymetrical range could be important in determining colonisation in the Southern Ocean that consists largely of deep-sea. In this study, our broadcaster *Glabraster antarctica* had a much larger bathymetrical range compared to our brooder *Anasterias antarctica* possibly acting as a confounding factor.

We recommend future research to sample more locations within the Magellanic region to obtain a more complete picture of the Magellanic sea star biodiversity. More samples from the geographically close Falkland Islands would also be interesting. Certain species showed a restricted distribution on

BOLD compared to other biodiversity platforms (e.g. OBIS). Sequencing specimens covering species' full geographical distribution will shed a light on the true distribution of each species and aid in linking biogeographical patterns and processes.

7. CONCLUSION

We recorded a sea star diversity of 12 species from the coastal Magellanic region. This is less than in previous studies, but we recorded *Cycethra frigida* for the first time in this region. This is only the beginning of unravelling the sea star biodiversity in this region as more locations and less accessible regions could still host an undiscovered diversity. With this study we again emphasised the need to combine morphology and genetics in species identification to gain the most accurate estimate of biodiversity. We synonymised two species pairs within the *Anasterias* and *Odontaster* genera. We generalised four different geographical patterns in the Magellanic sea stars from endemic species to circumpolar to one species that even occurs in both hemispheres. Although developmental mode has been suggested to determine species distribution, relying on this alone is insufficient and other life history traits such as bathymetrical range and the possibility of passively rafting on kelp are suggested to be at least equally important. In order to gain a more detailed look into ongoing faunal affinities, the use of more variable markers like microsatellites or SNPs are recommended for future research.

8. DATA ACCESSIBILITY

All specimen metadata (sample location, picture, species name), and sequences (if sequenced) can be found on BOLD in projects LVPAT (the Magellanic region) and ASSOG (South-Georgia).

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10. REFERENCES

Ansorge, I. J., & Lutjeharms, J. R. E. (2003). Eddies originating at the south-west Indian ridge. *Journal of Marine Systems, 39*(1-2), 1-18.

Bénard, F., Callot, J. P., Vially, R., Schmitz, J., Roest, W., Patriat, M., Loubrieu, B. & The ExtraPlac Team (2010). The Kerguelen plateau: Records from a long-living/composite microcontinent. *Marine and Petroleum Geology*, *27*(3), 633-649.

BOLD (2019). barcode of Life Data Systems Handbook. Available at https://www.boldsystems.org/libhtml_v3/static/BOLD4_Documentation_Draft1.pdf

Bosch, I. (1989). Contrasting modes of reproduction in two Antarctic asteroids of the genus *Porania*, with a description of unusual feeding and non-feeding larval types. *The Biological Bulletin, 177*(1), 77-82.

Bosch, I., & Pearse, J. S. (1990). Developmental types of shallow-water asteroids of McMurdo Sound, Antarctica. *Marine Biology, 104*(1), 41-46.

Carstens, B. C., Pelletier, T. A., Reid, N. M., & Satler, J. D. (2013). How to fail at species delimitation. *Molecular ecology, 22*(17), 4369-4383.

Casares, B. M., Àngel, J. J. S., & Cantero, Á. L. P. (2017). Towards a better understanding of Southern Ocean biogeography: new evidence from benthic hydroids. *Polar Biology, 40*(10), 1975-1988.

Chown, S. L., Clarke, A., Fraser, C. I., Cary, S. C., Moon, K. L., & McGeoch, M. A. (2015). The changing form of Antarctic biodiversity. *Nature*, *522*(7557), 431-438.

Christiansen, H., Dettai, A., Heindler, F. M., Collins, M. A., Duhamel, G., Hautecoeur, M., Steinke, D., Volckaert, F. A. M. & Van de Putte, A. P. (2018). Diversity of mesopelagic fishes in the Southern Ocean-a phylogeographic perspective using DNA barcoding. *Frontiers in Ecology and Evolution, 120*.

Clark, A. M., & Downey, M. E. (1992). *Starfishes of the Atlantic*.

Clarke, A., Barnes, D. K., & Hodgson, D. A. (2005). How isolated is Antarctica? *Trends in Ecology & Evolution, 20*(1), 1-3.

Clement, M., Snell, Q., Walker, P., Posada, D., & Crandall, K. (2002). TCS: Estimating gene genealogies. Parallel and Distributed Processing Symposium, *International Proceedings, 2,* 184.

Costello, M. J., Tsai, P., Wong, P. S., Cheung, A. K. L., Basher, Z., & Chaudhary, C. (2017). Marine biogeographic realms and species endemicity. *Nature communications, 8*(1), 1-10.

Crame, J. A. (1999). An evolutionary perspective on marine faunal connections between southernmost South America and Antarctica. *Scientia Marina, 63*, 1-14.

Cumming, R. A., Nikula, R., Spencer, H. G., & Waters, J. M. (2014). Transoceanic genetic similarities of kelp-associated sea slug populations: long-distance dispersal via rafting? *Journal of Biogeography, 41*(12), 2357-2370.

Dayton, P. K., Robilliard, G. A., Paine, R. T., & Dayton, L. B. (1974). Biological accommodation in the benthic community at McMurdo Sound, Antarctica. *Ecological Monographs*, *44*(1), 105-128.

De Broyer, C., Clarke, A.; Koubbi, P., Pakhomov, E., Scott, F., Vanden Berghe, E. & Danis, B. (Eds.) (2022). Register of Antarctic Marine Species. Accessed at https://www.marinespecies.org/rams

de Moura Barboza, C. A., de Moura, R. B., Lanna, A. M., Oackes, T., & Campos, L. S. (2011). Echinoderms as clues to Antarctic~ South American connectivity. *Oecologia Australis, 15*(1), 86-110.

Díaz, A., Féral, J. P., David, B., Saucède, T., & Poulin, E. (2011). Evolutionary pathways among shallow and deep-sea echinoids of the genus *Sterechinus* in the Southern Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography, 58*(1-2), 205-211.

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic acids research, 32*(5), 1792-1797.

Excoffier, L. & Lischer, H.E. L. (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources. 10*: 564-567

Féral, J. P., Poulin, E., De Ridder, C., & Saucède, T. (2019). A field guide to coastal echinoderms of the Kerguelen Islands. *Zoosymposia, 15*, 33-43.

Fišer Pečnikar, Ž., & Buzan, E. V. (2014). 20 years since the introduction of DNA barcoding: from theory to application. *Journal of applied genetics*, *55*(1), 43-52.

Fraser, C. I., Nikula, R., Ruzzante, D. E., & Waters, J. M. (2012). Poleward bound: biological impacts of Southern Hemisphere glaciation. *Trends in ecology & evolution, 27*(8), 462-471.

Fraser, C. I., Morrison, A. K., Hogg, A. M., Macaya, E. C., van Sebille, E., Ryan, P. G., ... & Waters, J. M. (2018). Antarctica's ecological isolation will be broken by storm-driven dispersal and warming. *Nature climate change*, *8*(8), 704-708.

Fraysse, C., Calcagno, J., & Pérez, A. F. (2018). Asteroidea of the southern tip of South America, including Namuncurá Marine Protected Area at Burdwood Bank and Tierra del Fuego Province, Argentina. *Polar Biology, 41*(12), 2423-2433.

GBIF.org (2022), GBIF Home Page. Available from: https://www.gbif.org.

Geller, J., Meyer, C., Parker, M., & Hawk, H. (2013). Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular ecology resources, 13*(5), 851-861.

Gibson, R. N., Atkinson, R. J. A., & Gordon, J. D. M. (2005). The ecology of rafting in the marine environment. II. The rafting organisms and community. *Oceanography and Marine Biology: An Annual Review*, *43*, 279-418.

Gong, S., Ding, Y., Wang, Y., Jiang, G., & Zhu, C. (2018). Advances in DNA barcoding of toxic marine organisms. *International journal of molecular sciences*, *19*(10), 2931.

González-Wevar, C. A., Hüne, M., Segovia, N. I., Nakano, T., Spencer, H. G., Chown, S. L., Saucède, T., Johnstone, G., Mansilla, A. & Poulin, E. (2017). Following the Antarctic Circumpolar Current: patterns and processes in the biogeography of the limpet *Nacella* (Mollusca: Patellogastropoda) across the Southern Ocean. *Journal of biogeography, 44*(4), 861-874.

González-Wevar, C. A., Segovia, N. I., Rosenfeld, S., Noll, D., Maturana, C. S., Hüne, M., Naretto, J., Gérard, K., Díaz, A., Spencer, H. G., Saucède, T., Féral, J.-P., Marley, S.A., Brickle, P., Wilson, N.G. & Poulin, E. (2021). Contrasting biogeographical patterns in *Margarella* (Gastropoda: Calliostomatidae: Margarellinae) across the Antarctic Polar Front. *Molecular Phylogenetics and Evolution, 156,* 107039.

Gostel, M. R., & Kress, W. J. (2022). The Expanding Role of DNA Barcodes: Indispensable Tools for Ecology, Evolution, and Conservation. *Diversity, 14*(3), 213.

Griffiths, H. J. (2010). Antarctic marine biodiversity–what do we know about the distribution of life in the Southern Ocean? *PloS one, 5*(8), e11683.

Griffiths, H. J., & Waller, C. L. (2016). The first comprehensive description of the biodiversity and biogeography of Antarctic and Sub-Antarctic intertidal communities. *Journal of biogeography, 43*(6), 1143-1155.

Güller, M., Puccinelli, E., & Zelaya, D. G. (2020). The Antarctic Circumpolar Current as a dispersive agent in the Southern Ocean: evidence from bivalves. *Marine Biology, 167*(10), 1-13.

Guzzi, A., Alvaro, M.C., Danis, B., Moreau, C. & Schiaparelli, S. (2022 submitted). Not all that glitters is gold: barcoding effort reveals taxonomic incongruences in iconic Ross Sea seastars. *Diversity* 2021, *13*.

Hajibabaei, M., Singer, G. A., Hebert, P. D., & Hickey, D. A. (2007). DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *TRENDS in Genetics, 23*(4), 167-172.

Hebert, P. D., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 270*(1512), 313-321.

Hebert, P. D., Ratnasingham, S., & De Waard, J. R. (2003b). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(suppl_1), S96-S99.

Helmuth, B. S., Veit, R. R., & Holberton, R. (1994). Dispersal of benthic invertebrates in the Scotia Arc by kelp rafting. *Antarctic journal - Review,* 145-147.

Hibberd, T. (2016). Describing and predicting the spatial distribution of benthic biodiversity in the sub-Antarctic and Antarctic [Doctoral dissertation, University of Tasmania].

Huemer, P., & Mutanen, M. (2022). An Incomplete European Barcode Library Has a Strong Impact on the Identification Success of Lepidoptera from Greece. *Diversity, 14*(2), 118.

Hupalo, K., Copilaș-Ciocianu, D., Leese, F., & Weiss, M. (2022). COI is not enough: integrative taxonomy reveals striking overestimation of species diversity in a Mediterranean freshwater amphipod.

Iuri, V., Patti, F. P., & Procaccini, G. (2007). Phylogeography of the sea urchin *Paracentrotus lividus* (Lamarck)(Echinodermata: Echinoidea): first insights from the South Tyrrhenian Sea. In *Biodiversity in Enclosed Seas and Artificial Marine Habitats* (pp. 77-84). Springer, Dordrecht.

Janosik, A. M., Mahon, A. R., & Halanych, K. M. (2011). Evolutionary history of Southern Ocean *Odontaster* sea star species (Odontasteridae; Asteroidea). *Polar biology, 34*(4), 575-586.

Janosik, A. M. (2012). Seeing stars: a molecular and morphological investigation of the *Odontasteridae* (Asteroidea) [Doctoral dissertation, Auburn University].

Jossart, Q., Sands, C. J., & Sewell, M. A. (2019). Dwarf brooder versus giant broadcaster: combining genetic and reproductive data to unravel cryptic diversity in an Antarctic brittle star. *Heredity, 123*(5), 622-633.

Jossart, Q., Kochzius, M., Danis, B., Saucède, T., & Moreau, C. V. (2021). Diversity of the Pterasteridae (Asteroidea) in the Southern Ocean: a molecular and morphological approach. *Zoological Journal of the Linnean Society, 192*(1), 105-116.

Knott, K. E., Ringvold, H., & Blicher, M. E. (2018). Morphological and molecular analysis of *Henricia* Gray, 1840 (Asteroidea: Echinodermata) from the Northern Atlantic Ocean. *Zoological Journal of the Linnean Society, 182*(4), 791-807.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K.(2018). MEGA: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 1547-1549.

Layton, K. K., Corstorphine, E. A., & Hebert, P. D. (2016). Exploring Canadian echinoderm diversity through DNA barcodes. *PloS one, 11*(11), e0166118.

Le Bourg, B. (2020). Trophic ecology of Southern Ocean sea stars: Influence of environmental drivers on trophic diversity [Doctoral dissertation, Université de Liège].

Lowe, W. H., & Allendorf, F. W. (2010). What can genetics tell us about population connectivity? *Molecular ecology, 19*(15), 3038-3051.

Madsen, F. J. (1956). *Asteroidea: With a survey of the Asteroidea of the Chilean shelf*. CWK Gleerup.

Mah, C.L. (2022). World Asteroidea Database. Accessed at https://www.marinespecies.org/asteroidea

Martínez, S. (2008). Shallow water Asteroidea and Ophiuroidea of Uruguay: composition and biogeography. *Revista de Biología Tropical, 56*(3), 205-214.

McClintock, J. B., Angus, R. A., Ho, C., Amsler, C. D., & Baker, B. J. (2008). A laboratory study of behavioral interactions of the Antarctic keystone sea star *Odontaster validus* with three sympatric predatory sea stars. *Marine biology*, *154*(6), 1077-1084.

McKnight, D. G. (2006). Echinodermata: Asteroidea (Seastars). 3. *Orders Velatida, Spinulosida, Forcipulatida, Brisingida with adenda to Paxillosida, Valvatida. Auckland, New Zealand: National Institute of Water and Atmospheric Research*.

Mehta, R. S., Feder, A. F., Boca, S. M., & Rosenberg, N. A. (2019). The relationship between haplotype-based F ST and haplotype length. *Genetics, 213*(1), 281-295.

Meudec, L. (2021). Analyse de la diversité des astéries du Plateau des Kerguelen par approches génétique et morphologique, et modélisation des habitats. [Master's thesis, Université libre de Bruxelles].

Moles, J., Figuerola, B., Campanya-Llovet, N., Monleon-Getino, T., Taboada, S., & Avila, C. (2015). Distribution patterns in Antarctic and Subantarctic echinoderms. *Polar Biology, 38*(6), 799-813.

Moon, K. L., Chown, S. L., & Fraser, C. I. (2017). Reconsidering connectivity in the sub-Antarctic. *Biological Reviews, 92*(4), 2164-2181.

Moore, J. K., Abbott, M. R., & Richman, J. G. (1999). Location and dynamics of the Antarctic Polar Front from satellite sea surface temperature data. *Journal of Geophysical Research: Oceans*, *104*(C2), 3059-3073.

Moore, J. M., Carvajal, J. I., Rouse, G. W., & Wilson, N. G. (2018). The Antarctic Circumpolar Current isolates and connects: Structured circumpolarity in the sea star *Glabraster antarctica*. *Ecology and evolution, 8*(21), 10621-10633.

Moreau, C., Saucede, T., Jossart, Q., Agüera, A., Brayard, A., & Danis, B. (2017). Reproductive strategy as a piece of the biogeographic puzzle: a case study using Antarctic sea stars (Echinodermata, Asteroidea). *Journal of Biogeography*, *44*(4), 848-860.

Moreau, C., Mah, C., Agüera, A., Améziane, N., Barnes, D., Crokaert, G., Eléaume, M., Griffiths, H., Guillaumot, C., Hemery, L.G., Jażdżewska, A., Jossart, Q., Laptikhovsky, V., Linse, K., Neill, K., Sands, C., Saucède, T., Schiaparelli, S., Siciński, J., Vasset, N. & Danis, B. (2018). Antarctic and sub-Antarctic Asteroidea database. *ZooKeys,* (747), 141.

Moreau, C. (2019). Diversity and phylogeography of Southern Ocean sea stars (Asteroidea) [Doctoral dissertation, Université Bourgogne Franche-Comté; Université libre de Bruxelles].

Moreau, C., Jossart, Q., Danis, B., Eléaume, M., Christiansen, H., Guillaumot, C., Downey, R. & Saucède, T. (2021). The high diversity of Southern Ocean sea stars (Asteroidea) reveals original evolutionary pathways. *Progress in Oceanography, 190*, 102472.

Mutschke, E., & Mah, C. (2009). Asteroidea–Starfish. *Marine Benthic Fauna of Chilean Patagonia. Nature in Focus, Santiago*.

Mutschke, E., Gerdes, D., & Ríos, C. (2017). Distribution and abundance patterns of echinoderms in the fjord and channel complex from a subantarctic north Patagonian Ice field, Magellan region. *Revista de Biología Tropical, 65*(1-1), S60-S72.

OBIS (2022) Ocean Biodiversity Information System. Intergovernmental Oceanographic Commission of UNESCO. www.obis.org.

O'hara, T. (1998). Origin of Macquarie Island echinoderms. *Polar Biology*, *20*(2), 143-151.

Ojeda, F. P., & Santelices, B. (1984). Invertebrate communities in holdfasts of the kelp *Macrocystis pyrifera* from southern Chile. *Marine ecology progress series. Oldendorf, 16*(1), 65-73.

Palumbi, S. (1991). Simple fool's guide to PCR.

Park, Y. H., Durand, I., Kestenare, E., Rougier, G., Zhou, M., d'Ovidio, F., Cotté, C. & Lee, J. H. (2014). Polar Front around the Kerguelen Islands: An up-to-date determination and associated circulation of surface/subsurface waters. *Journal of Geophysical Research: Oceans, 119*(10), 6575-6592.

Peck, L. S., Clark, M. S., & Dunn, N. I. (2018). Morphological variation in taxonomic characters of the Antarctic starfish *Odontaster validus*. *Polar Biology, 41*(10), 2159-2165.

Pérez-Ruzafa, A., Alvarado, J. J., Solís-Marín, F. A., Hernández, J. C., Morata, A., Marcos, C., … & Williams, S. M. (2013). Latin America echinoderm biodiversity and biogeography: Patterns and affinities. In *Echinoderm research and diversity in Latin America* (pp. 511-542). Springer, Berlin, Heidelberg.

Petrov, N. B., Vladychenskaya, I. P., Drozdov, A. L., & Kedrova, O. S. (2016). Molecular genetic markers of intra-and interspecific divergence within starfish and sea urchins (Echinodermata). *Biochemistry (Moscow), 81*(9), 972-980.

Pothier, J. F., Wisniewski-Dye, F., Weiss-Gayet, M., Moenne-Loccoz, Y., & Prigent-Combaret, C. (2007). Promoter-trap identification of wheat seed extract-induced genes in the plant-growth-promoting rhizobacterium *Azospirillum brasilense* Sp245. *Microbiology, 153*(10), 3608-3622.

Rahman, M. A., Molla, M. H. R., Megwalu, F. O., Asare, O. E., Tchoundi, A., & Shaikh, M. M. (2018). The sea stars (Echinodermata: Asteroidea): Their biology, ecology, evolution and utilization. *SF J Biotechnol Biomed Eng*. 2018; 1 (2), 1007.

Ringvold, H., & Moum, T. (2020). On the genus *Crossaster* (Echinodermata: Asteroidea) and its distribution. *PLoS ONE, 1*5(1), e0227223.

Ringvold, H., & Stien, J. (2001). Biochemical differentiation of two groups within the species-complex Henricia Gray, 1840 (Echinodermata, Asteroidea) using starch-gel electrophoresis. *Hydrobiologia*, *459*(1), 57-59.

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., & Sánchez-Gracia, A. (2017). DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular biology and evolution, 34*(12), 3299-3302.

Smith, S. D. (2002). Kelp rafts in the Southern Ocean. *Global Ecology and Biogeography*, *11*(1), 67-69.

Soto Àngel, J. J., & Peña Cantero, Á. L. (2017). A new piece in the puzzle of the Antarctic Biogeography: What do benthic hydroids tell us about the Scotia Arc affinities? *Polar Biology, 40*(4), 863-872.

Sunnucks, P., & Hales, D. F. (1996). Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus Sitobion (Hemiptera: Aphididae). *Molecular biology and evolution*, *13*(3), 510-524.

Thandar, A. S. (1989). Zoogeography of the southern African echinoderm fauna. *African Zoology, 24*(4), 311-318.

Thornhill, D. J., Mahon, A. R., Norenburg, J. L., & Halanych, K. M. (2008). Open-ocean barriers to dispersal: A test case with the Antarctic Polar Front and the ribbon worm *Parborlasia corrugatus* (Nemertea: Lineidae). *Molecular ecology*, *17*(23), 5104-5117.

Uthicke, S., Byrne, M., & Conand, C. (2010). Genetic barcoding of commercial Bêche-de-mer species (Echinodermata: Holothuroidea). *Molecular Ecology Resources, 10*(4), 634-646.

Ward, R. D., Holmes, B. H., & O'HARA, T. D. (2008). DNA barcoding discriminates echinoderm species. *Molecular Ecology Resources, 8*(6), 1202-1211.

Waters, J. M., King, T. M., Fraser, C. I., & Craw, D. (2018a). An integrated ecological, genetic and geological assessment of long-distance dispersal by invertebrates on kelp rafts. *Frontiers of Biogeography, 10*(3-4).

Waters, J. M., King, T. M., Fraser, C. I., & Garden, C. (2018b). Rafting dispersal in a brooding southern sea star (Asteroidea: *Anasterias*). *Invertebrate Systematics, 32*(2), 253-258.

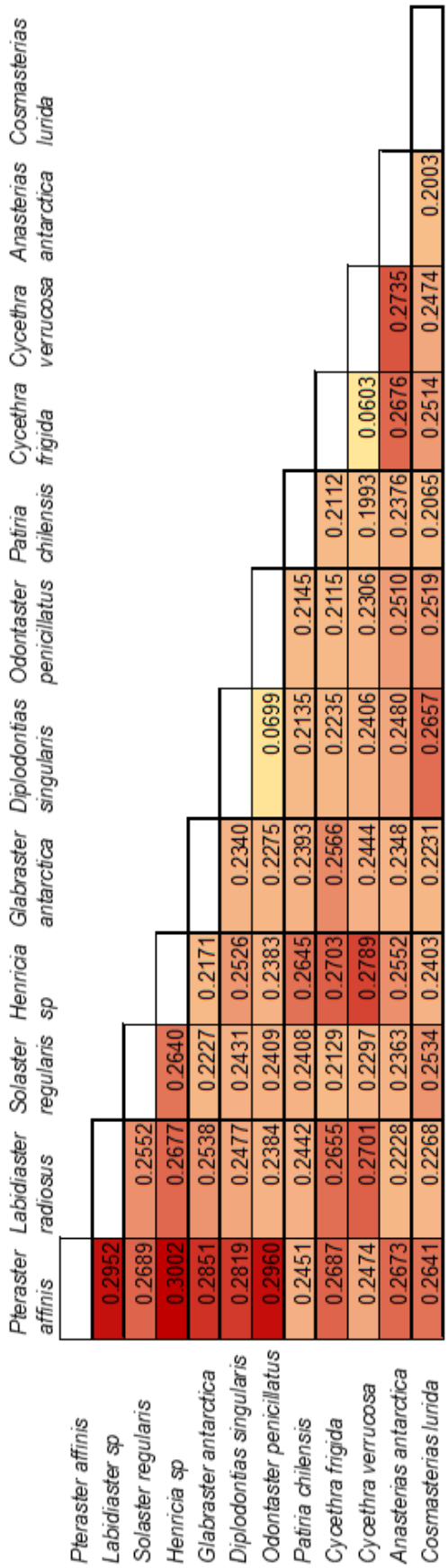
Wright, A. G., Pérez-Portela, R., & Griffiths, C. L. (2016). Determining the correct identity of South African *Marthasterias* (Echinodermata: Asteroidea). *African journal of marine science, 38*(3), 443-455.

11. ANNEXES

Annex table 1| Sample method and depth per sample site

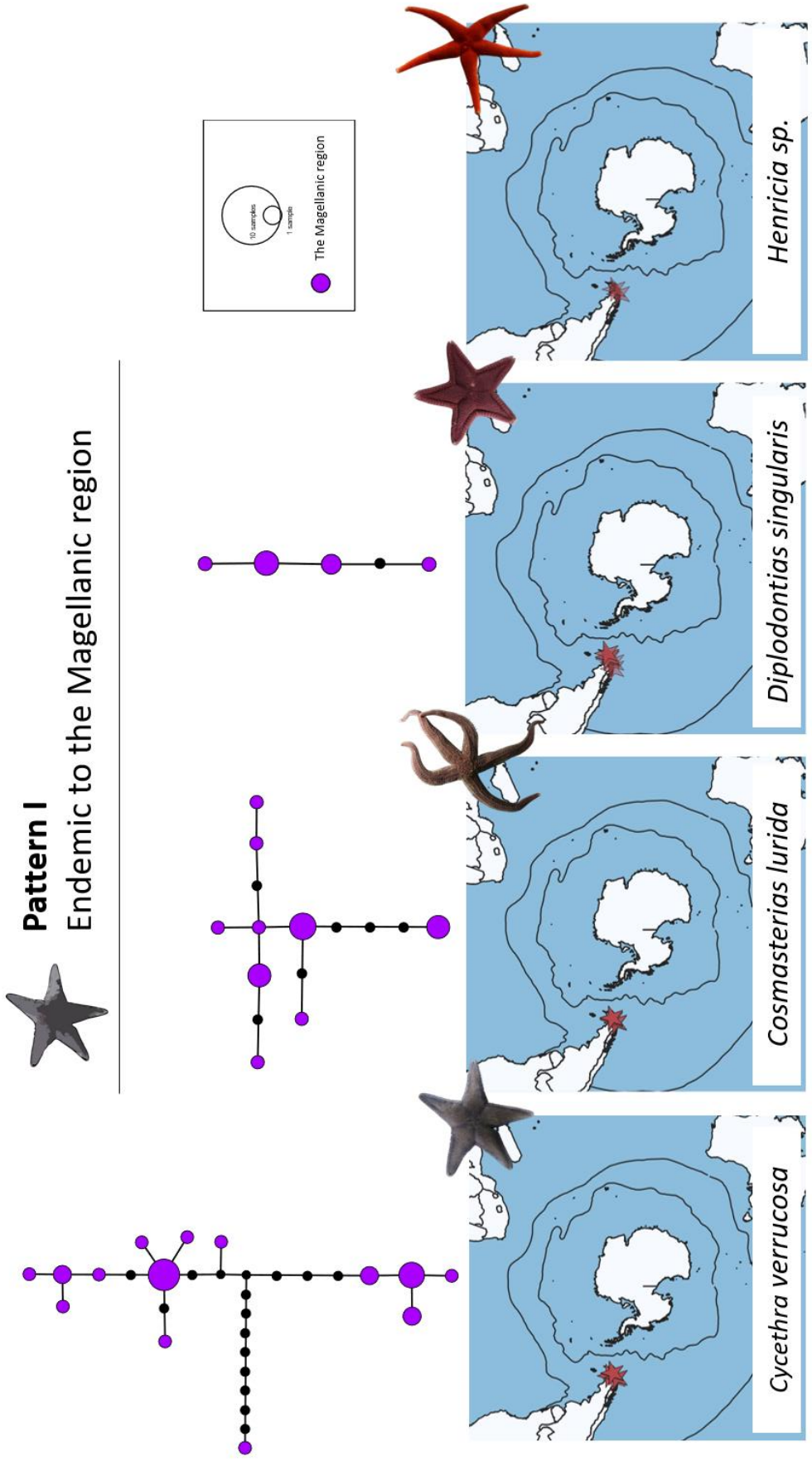
Abbreviation sample site	Sample site	Sample method	Depth
BQ	**Buque Quemado**	Intertidal	<1m
FB	**Fuerte Bulnes**	SCUBA diving	7-20m
FI	**Faro san Isidro**	SCUBA diving	6-14m
PC	**Punta Carreras**	SCUBA diving	3-8m
RB	**Rinconada Bulnes**	Snorkelling + intertidal	0-5m
SO	**Seno Otway**	Snorkelling + Intertidal	0-5m
SP	**South Punta Arenas**	Intertidal	<1m
CH	**Seno Copihue**	SCUBA diving	0-15m
R	**Diego Ramirez**	SCUBA diving	0-15m
PM	**Pia Morrena**	SCUBA diving	0-15m
PH	**Puerto del Hambre**	SCUBA diving	7-20m
U	**Ushuaia**	Intertidal	<1m
A	**Isla Alta**	SCUBA diving	0-15m
PR	**Isla Parker**	SCUBA diving	0-15m
GA	**Isla Garcia**	SCUBA diving	0-15m
MO	**Montañas**	SCUBA diving	0-15m
E	**Seno Eleuterio**	SCUBA diving	0-15m
ST	**Strait of Magellan**	SCUBA diving	0-15m
PN	**Puerto Natales**	Diving	2-5m

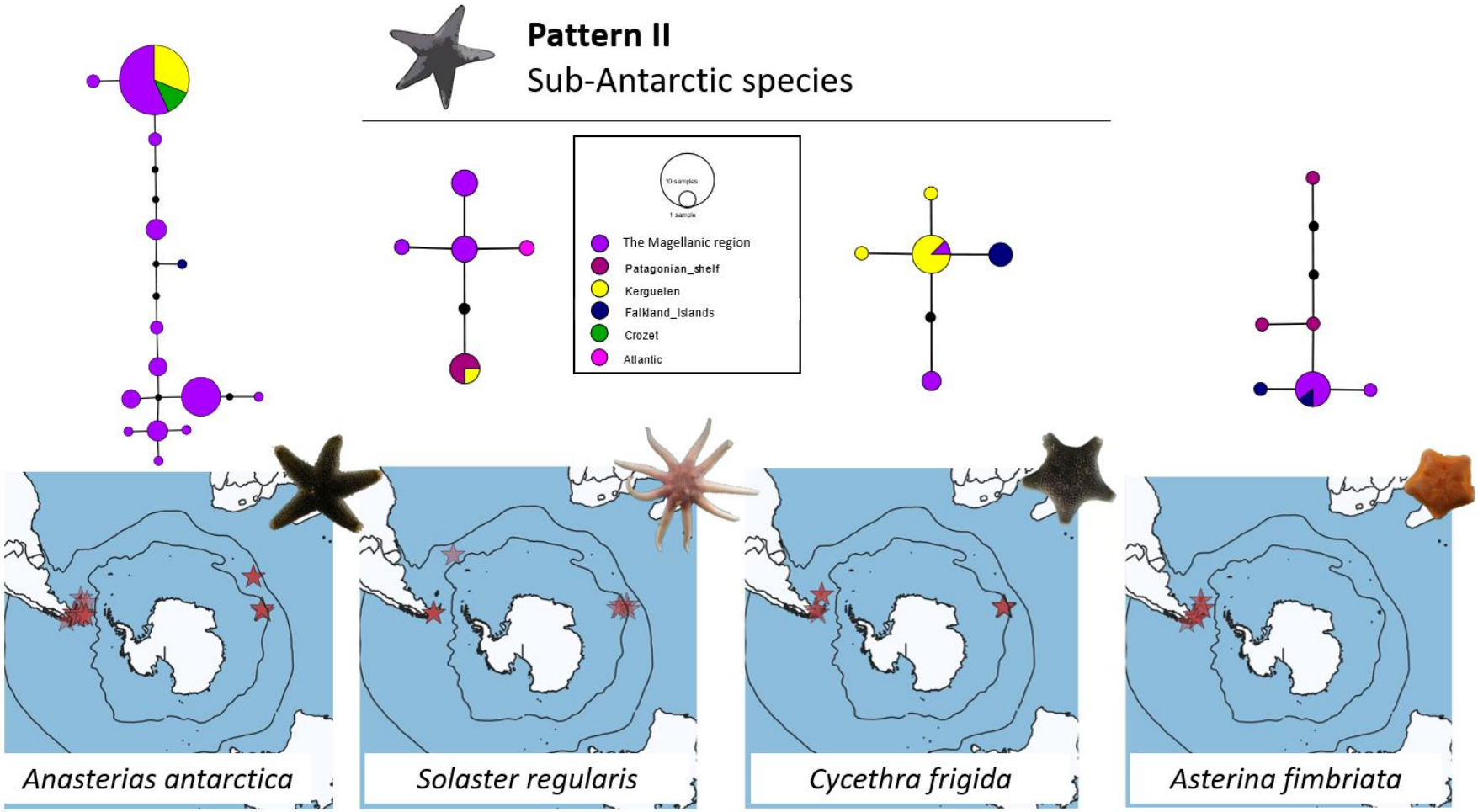
Annex fig 1| Interspecific distances colored by value. Lighter yellow indicates lower interspecific distances, red indicates higher interspecific distances.

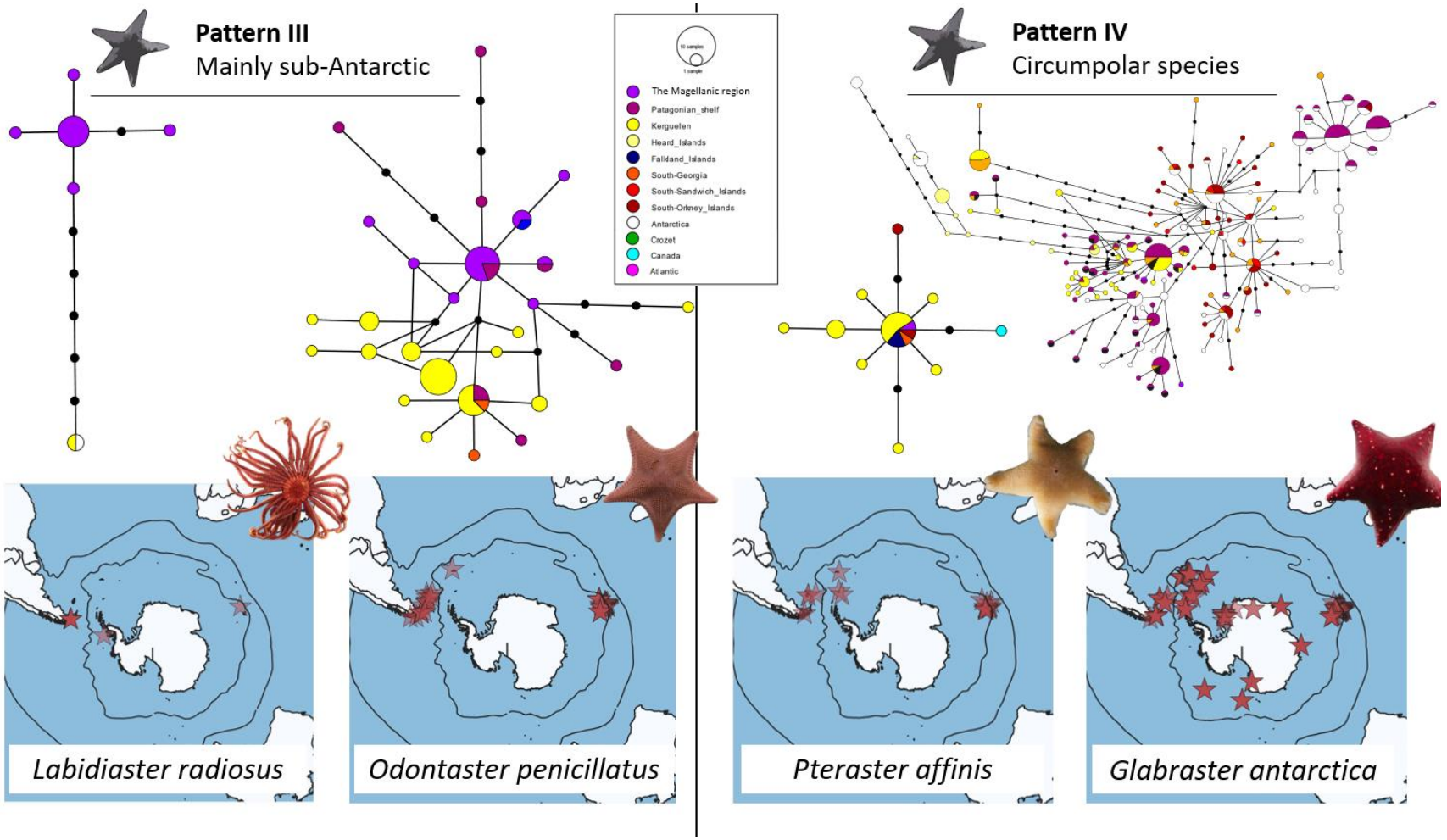


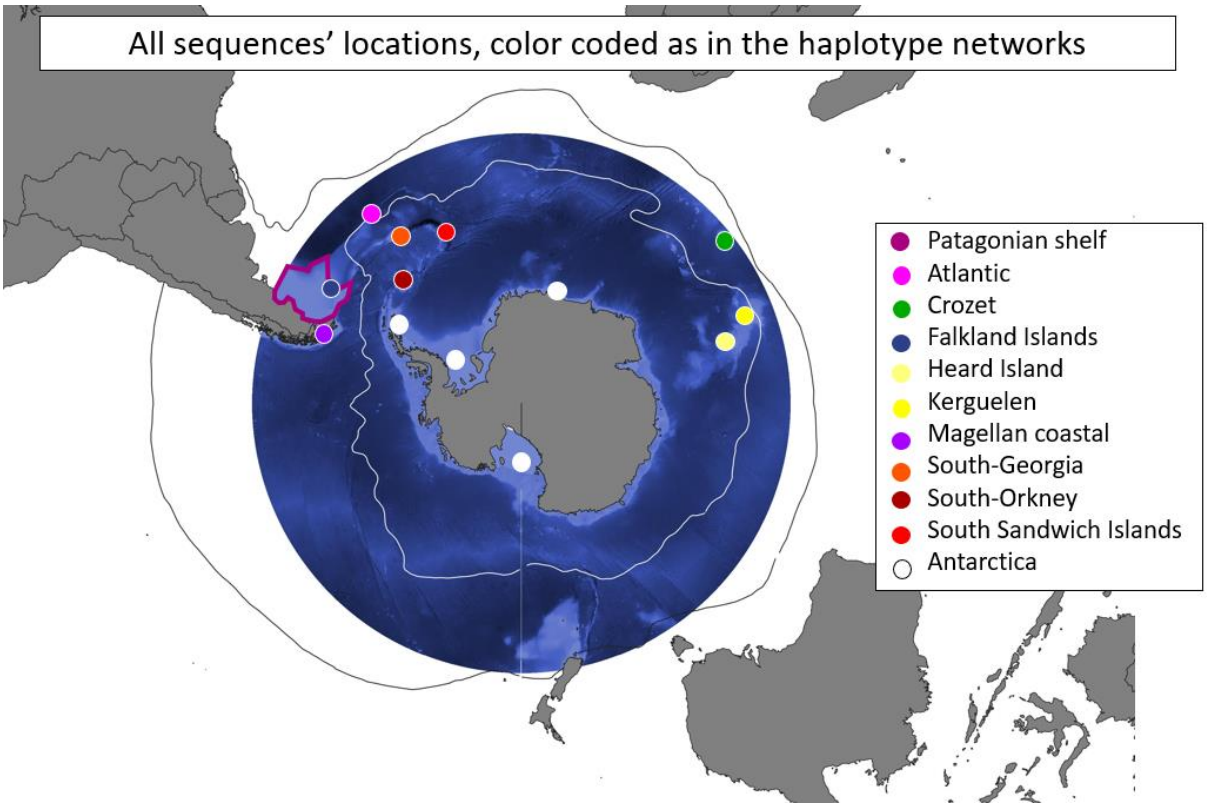
	Pteraster affinis	*Labidiaster radiosus*	*Solaster regularis*	*Henricia sp*	*Glabraster antarctica*	*Diplodontias singularis*	*Odontaster penicillatus*	*Patiria chilensis*	*Cycethra frigida*	*Cycethra verrucosa*	*Anasterias antarctica*	*Cosmasterias lurida*
Pteraster affinis												
Labidiaster sp	0.2952											
Solaster regularis	0.2689	0.2552										
Henricia sp	0.3002	0.2677	0.2640									
Glabraster antarctica	0.2851	0.2538	0.2227	0.2171								
Diplodontias singularis	0.2819	0.2477	0.2431	0.2526	0.2340							
Odontaster penicillatus	0.2960	0.2384	0.2409	0.2383	0.2275	0.0699						
Patiria chilensis	0.2451	0.2442	0.2408	0.2645	0.2393	0.2135	0.2145					
Cycethra frigida	0.2687	0.2655	0.2129	0.2703	0.2566	0.2235	0.2115	0.2112				
Cycethra verrucosa	0.2474	0.2701	0.2297	0.2789	0.2444	0.2406	0.2306	0.1993	0.0603			
Anasterias antarctica	0.2673	0.2228	0.2363	0.2552	0.2348	0.2480	0.2510	0.2376	0.2676	0.2735		
Cosmasterias lurida	0.2641	0.2268	0.2534	0.2403	0.2231	0.2657	0.2519	0.2065	0.2514	0.2474	0.2003	

Annex fig 2| Distribution of species with sequences and their associated haplotype network




Pattern II
Sub-Antarctic species
10 samples
1 sample
The Magellanic region
Patagonian_shelf
Kerguelen
Falkland_Islands
Crozet
Atlantic
Anasterias antarctica
Solaster regularis
Cycethra frigida
Asterina fimbriata


Pattern III
Mainly sub-Antarctic
Pattern IV
Circumpolar species
10 samples
1 sample
The Magellanic region
Patagonian_shelf
Kerguelen
Heard_Islands
Falkland_Islands
South-Georgia
South-Sandwich_Islands
South-Orkney_Islands
Antarctica
Crozet
Canada
Atlantic
Labidiaster radiosus
Odontaster penicillatus
Pteraster affinis
Glabraster antarctica


All sequences' locations, color coded as in the haplotype networks
Patagonian shelf
Atlantic
Crozet
Falkland Islands
Heard Island
Kerguelen
Magellan coastal
South-Georgia
South-Orkney
South Sandwich Islands
Antarctica

Annex fig 3| Morphological comparison of *Cycethra* species