Benthic marine algae from South Shetland Islands (Antarctica): biodiversity and evolutionary patterns

Rafael Pablo Martín Martín

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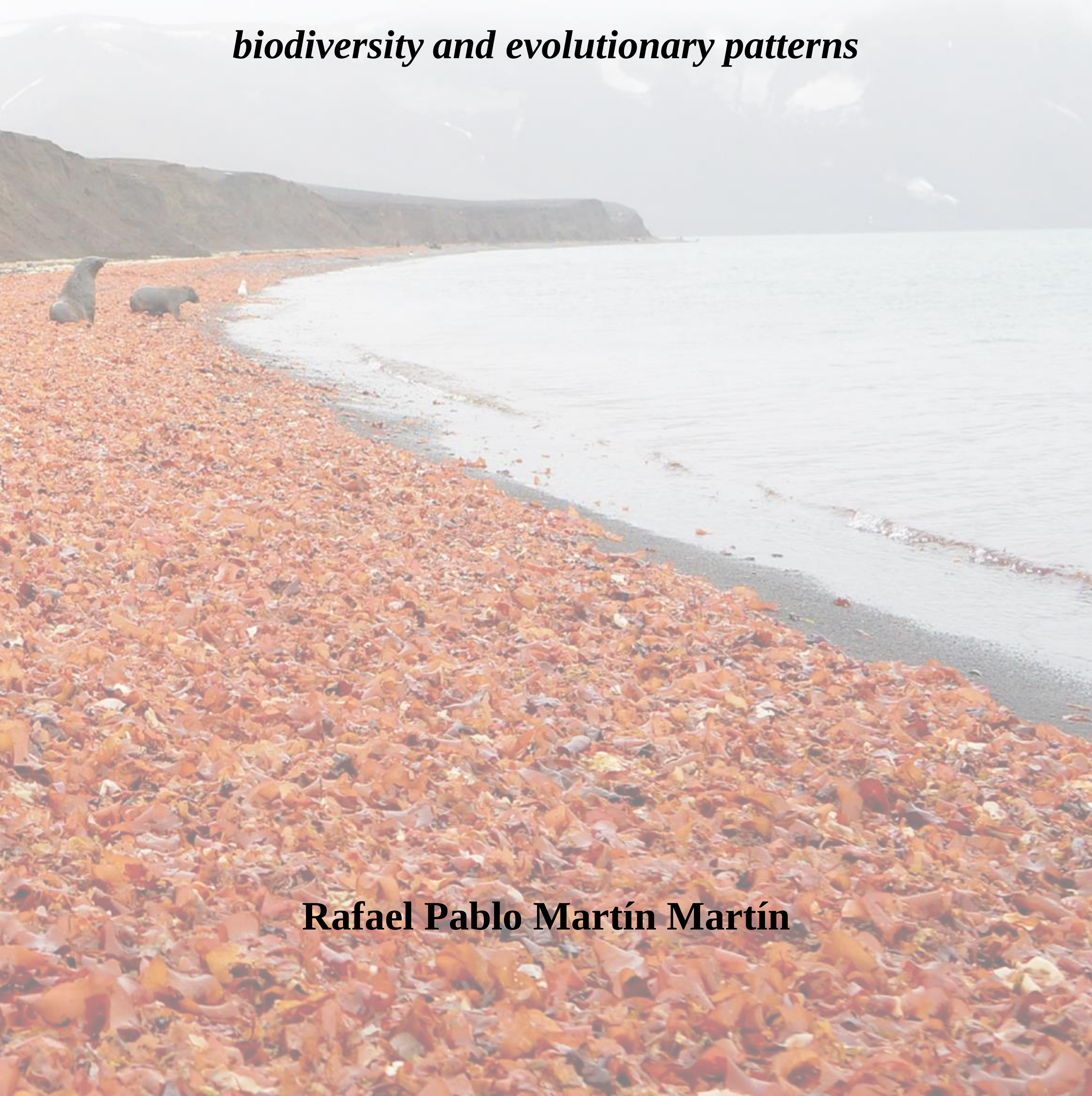

Rafael Pablo Martín Martín

BENTHIC MARINE ALGAE FROM SOUTH SHETLAND ISLANDS (ANTARCTICA): BIODIVERSITY AND EVOLUTIONARY PATTERNS

ALGAS BENTÓNICAS MARINAS DE LAS ISLAS SHETLAND DEL SUR (ANTÁRTIDA): BIODIVERSIDAD Y PATRONES EVOLUTIVOS

PhD program of Vegetal Biodiversity

Laboratory of Botany

Department of Biology, Healthcare and the Environment

Memoria presentada por **Rafael Pablo Martín Martín** para optar al grado de Doctor por la Universidad de Barcelona

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Amelia Gómez Garreta	Rafael Pablo Martín Martín	Jordi Rull Lluch
(Directora)	(Estudiante)	(Director y Tutor)

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ABSTRACT

The present work focuses on different aspects related to the Antarctic seaweed. As the main primary producers of the coastal areas of Antarctica, this group of organisms play a key role in shaping the biological communities and the ecological dynamics of Antarctic shores. However, compared to other groups of Antarctic organisms or the seaweed communities of other regions of the world, Antarctic macroalgae remain less studied. This is especially true in several areas of knowledge as their actual biodiversity, their evolutionary history and patterns, some aspects of their ecology or the total extent of their relations with other Antarctic organisms and their environment. Aiming to address the lack of knowledge of these areas, we aimed to extend it by focusing our work in several key aspects of Antarctic seaweed biology. As so, we tried to respond several specific questions regarding the topics above mentioned. In this context, we studied biodiversity of seaweed communities that are present in the South Shetland Islands. For that, we tried to identify the composition and influencing factors of the seaweed assemblages present in Deception Island. We also studied how seaweed biodiversity affects Antarctic epiphytic diatoms richness, studying the composition of them, both from Deception and Livingston islands. Moreover, we tried to expand the data with evolutionary relevance by analysing the nuclear DNA content and ploidy levels (C values), as well as the nuclear developmental patterning of some common Antarctic seaweed species with no previous data for those characters. This was achieved by using a fluorimetry technique that sets a reference for comparison for future studies on those parameters. Additionally, we condensed the latest knowledge on the chemical ecology of seaweeds and other organisms from Antarctica, and tried to expand it by investigating the potential antimicrobial activity of these seaweeds. For this, we tested their natural extracts against several Antarctic microorganisms and identified several species that were active chemically. Also, we tested the same extracts for antimicrobial action against several microorganisms that are common human pathogenic surrogates and identified potential human interest in these seaweeds on the search for new sources of antibiotics. Finally, we reported our findings on the role seaweed have in the context of climate change in the Antarctic region. Those findings include the identification of non-native kelp rafts that arrived to the maritime Antarctica, and presented several passenger species that have potential invasiveness in the Antarctic. Regarding that, we classified and explored the possible roles that the rafts themselves and the species travelling with them may play in futures scenarios of climate change in Antarctica.

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GENERAL INTRODUCTION

Antarctica and the Southern Ocean

Antarctica, with its special conditions, represents an important factor in regulating global climate (Singh et al., 2016; Budd 1991; Goosse & Fichefet, 1999). It also presents a distinct set of habitats and biological communities, unique to Antarctic conditions (Oliveira et al., 2020; Guidetti et al., 2019; Chown et al., 2015). Even though in recent times, scientific exploration of Antarctica and its biota has grown, the harsh conditions as well as its isolation and distance from the rest of continents makes it a difficult endeavour, and hence, there is still potential undiscovered diversity, ecological processes and potential human applications to be discovered on the continent and the Southern Ocean (SO) (Gómez, 2015; Oliveira et al., 2020; Wiencke & Clayton, 2002). In this work, we tried to extend the knowledge on the above mentioned areas, focusing on the Antarctic marine seaweeds.

Antarctica comprises the most southern land mass of the globe, and together with the SO surrounding it, are considered one of the most isolated regions of the planet. A combination of factors creates special conditions that promote the aforementioned isolation. Maybe the most obvious of these factors is the sheer distance between the Antarctic continent (located over earth geographic South Pole) and other big landmasses (e.g. South America, Africa or Australia). The separation with those other landmasses was promoted by continental drift, which ended around 25-23 million years ago with the opening of the Drake passage and the formation of the Antarctic Circumpolar Current (ACC) (Lyle et al., 2007) (**Fig. 1**). This, and the limited solar radiation received on Antarctica due to its latitude, set the conditions that provoked the total glaciation of the continent and the surrounding waters (Gómez, 2015; Oliveira et al., 2020; Wiencke & Clayton, 2002). The glaciating conditions vary especially during the two Antarctic seasons; the winter, were almost total darkness predominates, oceanic ice shelf forms extensive covers over large coastal areas (e.g. Ross and Weddell Seas) and coastal photosynthetic activity halts; and the summer, were solar radiation reaches 24h, photosynthetic organisms proliferate on the coasts and oceanic ice shelf recedes (Oliveira et al., 2020; Wiencke et al., 2014; Wiencke & Clayton, 2002). Nonetheless, despite these general conditions, the Antarctic region can be divided in several parts. Geographically, the continent is divided in two clear units (**Fig. 1**): East Antarctica, bigger and rounded, with a continental ice dome covering the inland reaching an average depth of almost 2 km (Fretwell et al., 2013); and Western Antarctica, with rougher terrain and an elongated peninsula that reaches latitudes as north as 63ºS (Antarctic Peninsula region). These two units are separated by the Transantarctic Mountain Range, and whereas the East Antarctica is mainly dominated by polar inland desert, the West comprises a mixture of extensive ice sheet covered areas with some glacial coastlines with clear maritime influence (which promotes milder climates compared with the main continental areas). Surrounding the continent, the SO extends to latitudes around 60ºS, wrapping the Antarctic coasts without encountering any other continent. It also presents an abrupt transition with other oceans surrounding it, an oceanographic front which separates the warmer waters from those other water bodies from the colder ones (<4ºC) of the SO (Bargagli, 2008; Clarke & Johnston, 2003; Oliveira et al., 2020). This oceanic front is labelled Antarctic Polar Front (APF), and together with the ACC, they form the largest currents system in the planet (Constantin & Johnson, 2016), flowing clockwise driven by western strong winds produced by the Coriolis force (Lin et al., 2018).

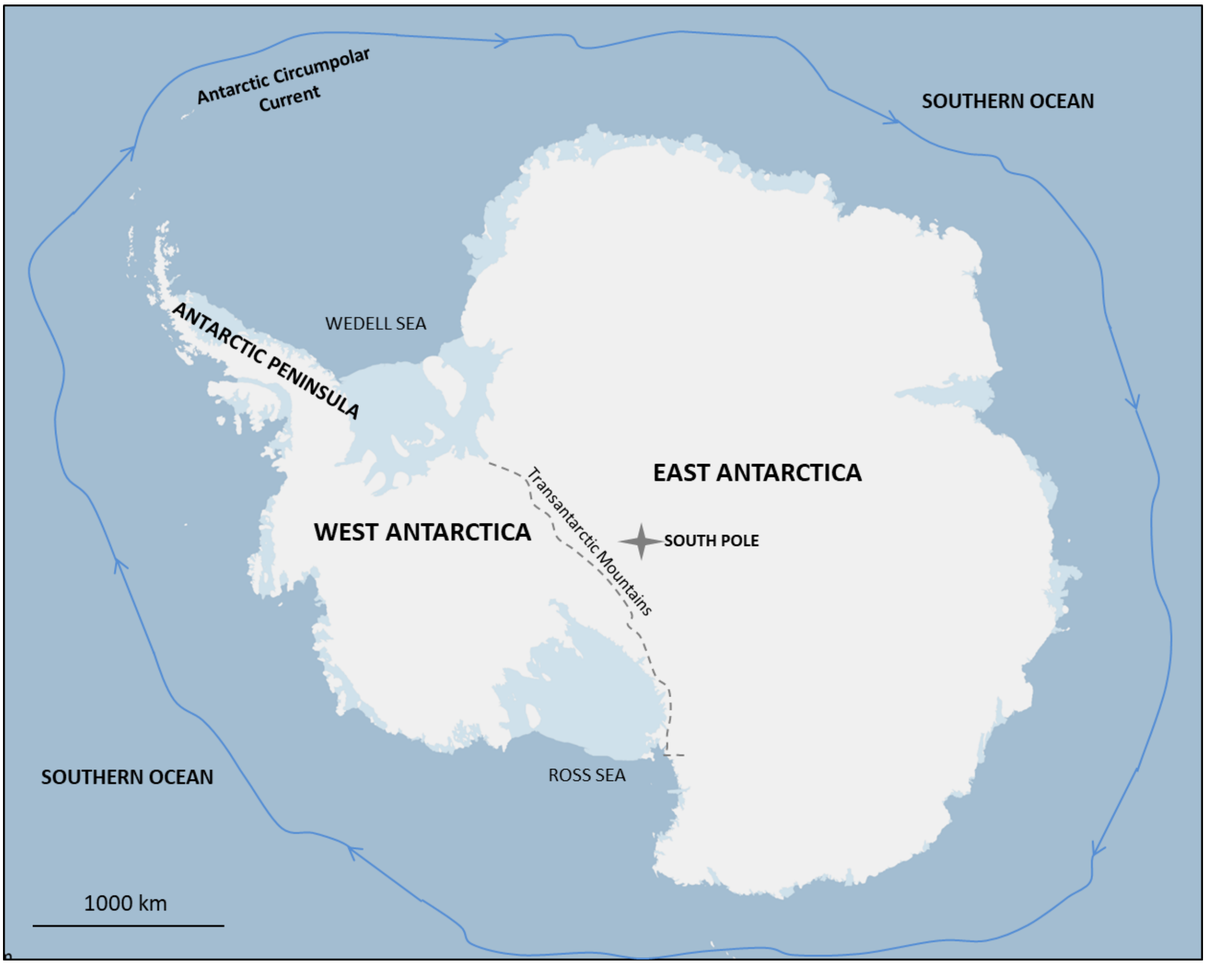

Fig. 1 Antarctica map showing the two main geographic divisions of the continent and several relevant geographic features. Maps have been realized with Q-Gis software and Qantarctica map package.

The SO, the ACC and the APF greatly influence Antarctic conditions, especially in the maritime Antarctica, which comprises the coasts of the continent, and specially the northern areas of the western Antarctica (Antarctic Peninsula region) and all the archipelagos surrounding it. They promoted the thermal isolation of Antarctica and, at least until recent times, have acted as a strong barrier to the crossing of many organisms from other regions (Clarke et al., 2005; Fraser et al., 2020; Kennett, 1977; Lawver & Gahagan, 2003; Macaya et al., 2020). This thermal isolation has dropped the SO temperature (which can reach temperatures as low as -2ºC in the coldest seasons), which in turn, facilitated the drop of temperatures on the Antarctic Continent itself (Sahade et al., 2015; Oliveira et al., 2020; Fraser et al., 2020). Furthermore, this thermal differentiation between the SO and the strong circulation of the ACC quick started the transport of nutrients and phytoplankton-rich surface along the water columns and the coastline, facilitating also the moving of minerals contributed to the water by the melting of coastal glaciers (Griffiths et al., 2009; Oliveira et al., 2020; Wiencke & Clayton, 2002). Altogether, the Antarctic region presents challenging conditions for which Antarctic organisms have been adapting during the course of evolution (generalized polar conditions, extreme variations in light patterns, strong barriers that promote isolation). This, especially on Antarctic seas, has driven the appearance of a distinct biota that presents a high degree of endemism, as well as a plethora of interesting adaptations to cope with those challenging conditions (Griffiths et al., 2011; Oliveira et al., 2020; Pellizzari et al., 2020; Wiencke et al., 2014; Wiencke & Clayton, 2002). For the study of this marine biota, Antarctic Peninsula region and its surrounding islands prove to be a favourable spot. Its higher latitude and maritime influence make working conditions for scientists slightly less hard. Also, the major concentration of research stations of many countries is located here, which facilitate logistics. Furthermore, as we will comment later, the major availability of ice free habitats in this region enhances the presence of benthic communities that can be studied (Teixidó et al., 2002; Oliveira et al., 2020; Barnes & Conlan, 2007; Aronson & Blake, 2001; Clark et al., 2017).

The South Shetland Islands, Deception and Livingston Islands

The South Shetland Islands Archipelago (SSI) (**Fig.2**) has been the main focus area of this work due to the presence of the two Spanish Antarctic Research Stations (BAE - Juan Carlos I, in Livingston Island and BAE - Gabriel de Castilla, in Deception Island). The archipelago is part of the ecoregion 222 (Spalding et al., 2007), which belongs to the aforementioned maritime Antarctica, which are the Antarctic environments whose abiotic conditions are greatly influenced by the SO (e.g. temperature, humidity or precipitations) (Bölteret et al., 2002; Oliveira et al., 2020; Stonehouse, 1989). This maritime influence promotes milder conditions compared with the interior parts of the continent and it is mainly patent along the Antarctic Peninsula and its surrounding Islands. Though the main interior parts of the islands are mainly covered in snow and glaciers, their coasts present a major quantity of ice-free habitats compared with the Antarctic continent. This allows for a greater presence of benthic communities (Teixidó et al., 2002; Oliveira et al., 2020; Barnes & Conlan, 2007; Aronson & Blake, 2001; Clark et al., 2017). Nonetheless, due to their warmer climate, their latitude and greater habitat availability, the Archipelago is one of the main areas vulnerable to abrupt ecological variations derived from climate change (Campana et al., 2020; Knap et al., 1996; Ortiz et al., 2020; Pasotti et al., 2015; Pellizzari et al., 2020). As such, is vital to understand the biological communities of the area, not only for its protection, but also as a potential case study to compare with other climate change impacted zones of the world. In that sense, the primary focus of this work has been two islands of the archipelago: Livingston Island (LI) and Deceptions Island (DI) (**Fig. 2**). This two islands are considered hotspots of biodiversity (Comarci et al., 1992; Sancho et al., 1999; Downie et al., 2000; Pellizzari et al., 2017; Vicente et al., 2007). LI is the second largest island of the archipelago, and is similar in geology and ecology to the rest of the archipelago. It is characterized for being a mountainous island with a long and narrow shape (**Fig. 3)**, wide bays (e.g. South, False and Walker Bays) and several narrow peninsulas (e.g. Hurd, Varna, or Byers, than can reach up to 15 km in length). Nonetheless, due to its proximity, this island has been influenced by the recent volcanism of DI, which has deposited layers of volcanic ash on the ice layers of the glaciers and in the sediments of the coast. The coast itself is mostly comprised of glaciers reaching the sea, some rocky ice-free cliffs, few sandy beaches, and beaches of pebbles and boulders where melting water from the glaciers forms little streams that run to the shore. On the near glacier areas, the ice scouring has eroded most parts of the rocky bottom, but as mentioned earlier, climate change has favoured the retreat of some glaciers, which has exposed several areas of hard bottom (Lagger et al., 2018; Quartino et al., 2013). Those rocky exposed bottoms, the underwater rocky outcrops as well as the pebble bottom coasts is where the habitat presents adequate substrata for benthic communities (specially seaweeds) that reach high levels of biomass (Oliveira et al., 2020; Pellizzari et al., 2020; Quartino et al., 2020; Wiencke & Clayton, 2002). By contrast, DI presents a different geomorphological composition compared to the rest of the SSI. It consists of an active volcano with a diameter of around 15 km, located at the southern part of the archipelago. The central part of this island constitutes the volcano caldera, with an opening to the sea (Neptune Bellows, **Fig. 3**) from which seawater flooded the caldera and formed the inner bay of the island (Port Foster **Fig. 3**).

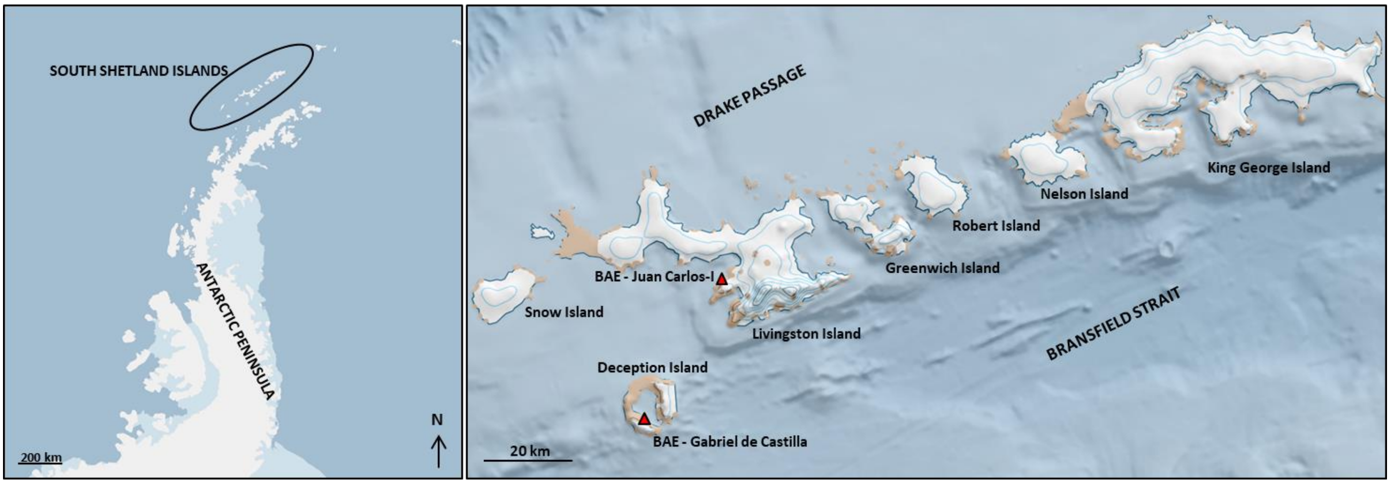

Fig. 2 Left: Map of the Antarctic Peninsula region with the South Shetland Islands location. **Right**: Map of the South Shetland Archipelago with the locations of the two Antarctic Spanish Bases (BAE - Juan Carlos-I and BAE - Gabriel de Castilla). Maps have been done with Q-Gis software and Qantarctica map package.

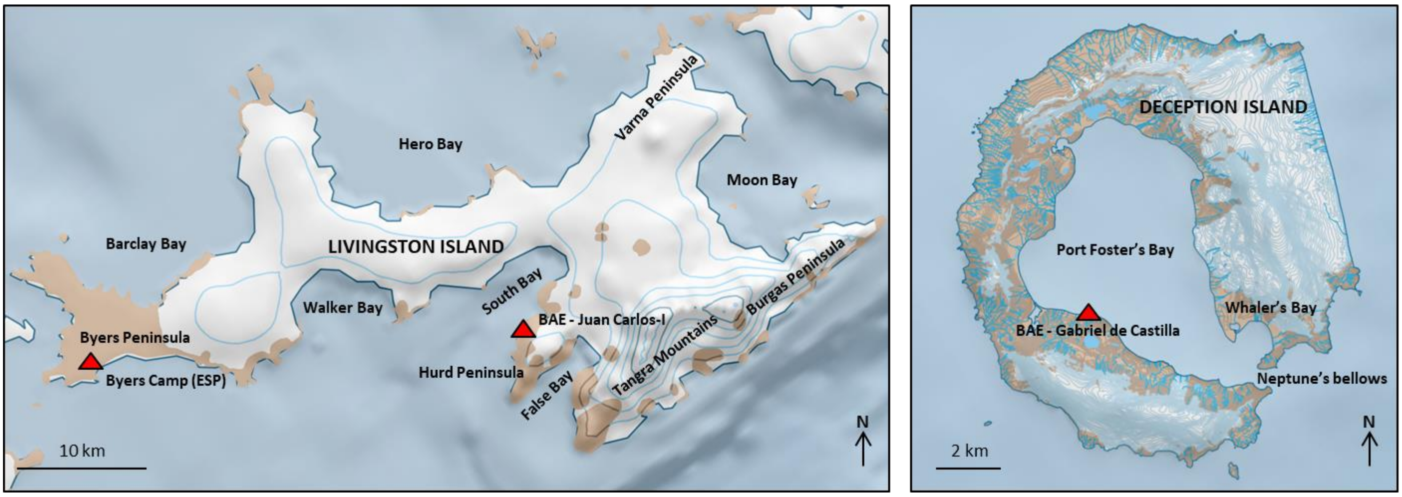

Fig. 3 Left: Map of Livingston Island with the locations on the Spanish Antarctic Base Juan Carlos-I and the Spanish research camp on Byers Peninsula, along with some geographical features of the island. **Right**: Map of Deception Island, indicating the location of with the Spanish Antarctic Base Gabriel de Castilla along with some geographical features of the island. Maps have been done with Q-Gis software and Qantarctica map package.

As an active volcanic area, it possesses some habitats with special physico-chemical properties (especially in Port Foster bay) where waters present warmer temperature, turbidity and volcanic related chemistry (variating pH levels, increased concentration of heavy metals, dissolved sulphuric compounds) which differentiates it from the surrounding Antarctic benthic environments (Elderfield, 1972; Muñoz-Martín et al., 2005; Barnes & Conlan, 2007; Pellizzari et al., 2017). Even though there are some volcanic rocky underwater outcrops and some pebble bottomed coasts, the majority of the benthos of DI coasts is dominated by soft bottoms composed by volcanic ash and lapilli. Those sparse rocky areas are where the greatest benthic communities of macroalgae develop. However, due to the mentioned related volcanic properties of the water and turbidity, the vertical distribution of those communities varies from the ones reported in LI (and other non-volcanic Antarctic benthic environments) (Pellizzari et al., 2017; Pellizzari et al., 2020). For those conditions (specially the thermal ones), DI represents a natural case study that can be analogous to the potential future impacts of climate change on other Antarctic areas. Also, DI is one of the most visited places of Antarctica by tourists, which makes potential impacts derived from human activities an important factor for its biological communities (especially if compared with more typical Antarctic habitats like those of LI).

Antarctic marine flora

Antarctic seaweed communities are still not well understood, especially if compared to other groups of organisms like Antarctic animals (Campana et al., 2020; Chown et al., 2015; Convey et al., 2014; Oliveira et al., 2020; Wiencke & Clayton, 2002). However, several authors have worked during the last decades on many aspects of Antarctic macroalgae. Those studies have provided important knowledge, indicating the distinctiveness of this marine flora compared to the ones of other regions. For example, even though current number of reported seaweed species from Antarctica (around 151 species) is low compared to other areas of the world, Antarctic benthic flora presents one of the greatest degrees of endemism, that can reach up to 33 - 40% (Gómez, 2015; Hommersand et al., 2009; Oliveira et al., 2020; Wiencke & Clayton, 2002). This level of endemism has been favoured by the great amount of time that polar conditions have been present in Antarctica (since around 25-23 million years ago) (Lyle et al., 2007; Fraser et al., 2020). Nonetheless, the evolutionary history and taxonomy of many seaweed groups in Antarctica remains unclear. As example, some works reported the possibility of still to be discovered diversity, in form of previously unrecorded species or new cases of crypsis (Dubrasquet et al., 2018; Hommersand et al., 2009; Oliveira et al., 2020; Wiencke & Clayton, 2002). In this regard, even common Antarctic species may hide greater diversity than expected, especially those species that are shared between floras of Antarctica and South America, Australia or South Africa (Wiencke & Clayton, 2002). As example, the Antarctic populations of species like *Iridaea cordata* (Turner) Bory or *Gigartina skottsbergii* Setchell & N.L.Gardner (that are also present in South America and the Falkland Islands), have been reported to potentially be separate species (Hommersand & Fredericq, 2003). However, and even though several research groups are unravelling aspects using genomic, cytogenetic and population studies (Billard et al., 2015; Dubrasquet et al., 2018; Hommersand et al., 2009; Ocaranza-Barrera et al., 2019; Pellizzari et al., 2017), work on Antarctic marine flora is still to be expanded. Moreover, in available coastal habitats, macroalgae represent the most important primary producers, generating great amounts of biomass that enters Antarctic trophic chains as well as global carbon cycles (Oliveira et al., 2020; Ortiz et al., 2020; Quartino et al., 2020; Rovelli et al., 2019). They are also important ecosystem engineers providing shelter, acting as hatcheries or as a source of food for multitude of other macro-organisms (Gómez, 2015; Huang et al., 2006; Iken, 2012; Momo et al., 2020; Wiencke & Clayton, 2002) as well as presenting complex biological relations with micro-organisms (Amsler, 2008; Amsler et al., 2020; Gaitan-Espitia & Schmid, 2020; Sacristán-Soriano et al., 2017). Part of those interactions involves Antarctic seaweed species and the diatom assemblages that grow epiphytically over the surface of those macroalgae. This relation can carry important consequences for the macroalgal host as well as for the community surrounding it, as aspects related to the photosynthetic performance, degree of epiphytation from other types of organisms or chemical interaction with the environment can be affected by epiphytic diatoms (Pellizzari et al., 2017; Hughes et al., 2020; Montes-Hugo et al., 2009). Even though, those aspects are still not completely understood (Majewska et al., 2013), some studies had tried to understand different aspects of those interactions between seaweed and diatoms from particular points of view (e.g. host perspective, diatom assemblage composition or specific locations) in Antarctica (Al-Handal & Wulff, 2008; Majewska et al., 2016; Totti et al., 2009; Thomas & Jiang, 1986; Majewska et al., 2015). Nonetheless, these previous studies do not combine multiple perspective approaches that are needed to discern greater scale patterns of those types of biological relationships. Apart from the structural and physical interactions with other organisms, there are reports that indicate that many associations between Antarctic seaweeds and other organisms or the harsh Antarctic environment are mediated by chemical activity, usually involving algal secondary metabolites (in functions like photo protection, grazer deterrence, infection protection or antifouling) (Amsler et al., 2020; Flores-Molina et al., 2016; Navarro et al., 2016; Vincent, 2000; Young et al., 2015). Nevertheless, those kind of interactions are complex and knowledge on the chemical ecology of Antarctic seaweeds is still limited compared with other regions (Amsler et al., 2020; Baker et al., 2008; Campana et al., 2020; Wiencke et al., 2007, 2014). Those interactions take special importance if climate change is taken into account. As mentioned above, Western Antarctica is especially vulnerable to climate change. As Antarctic seaweeds play an important role in coastal ecosystems, understanding how they will react to change and how their interactions with the rest of the organisms will be affected is vital for assessment of future climate change scenarios. In that sense, it is also important to take into account the changes in distribution ranges of Antarctic seaweeds and the potential interactions with newly introduced species (due to human transport or climate change). In this context, several events have been reported of macroalgae and other organisms from outside arriving to Antarctica (surpassing the ACC and APF, previously thought to be very strong barriers against species crossings) (Fraser et al., 2018; Lee & Chown, 2007; Lewis et al., 2003; Macaya et al., 2020; Pellizzari et al., 2017). It seems that species capable of surviving long periods on open sea (Fraser et al., 2018; Macaya et al., 2005, 2016) can benefit from the ACC to be dispersed to distant Antarctic coasts. Those events may have been happening during the past, but new climate change conditions may facilitate survival of potential invasive species in the increasingly warmer Antarctica. The effects that such events may represent to the habitats and communities of Antarctica remain to be fully understood. Additionally, the current limited knowledge on Antarctic flora makes it a potential source for human applications. Regarding this, several works investigated the potential of some secondary metabolites from Antarctic seaweeds and reported diverse applications (Amsler et al., 2009; dos Santos et al., 2020; Heiser et al., 2020; Rangel et al., 2019, 2020; Shilling et al., 2019; Young et al., 2015). However, and unlike other organisms in Antarctica (Angulo-Preckler et al., 2015; Avila, 2016; Núñez-Pons et al., 2020; Silva et al., 2021; Rosa et al., 2019; Li et al., 2018) much of Antarctic flora remains unexplored in this respect, and new potential compounds or applications may be waiting to be discovered.

OBJECTIVES

For this work, we focused on expanding the available knowledge on Antarctic seaweeds in various aspects since, as it has been mentioned earlier, it is still less broad as with other Antarctic organisms. In this framework, we divided our efforts in four main objectives of study, for which specific questions were explored on the chapters of this thesis:

1) **Expand the knowledge of seaweed and epiphytic diatom biodiversity on Deception and Livingston Islands**: we aimed to expand the knowledge on the composition of the marine floras of Deception and Livingston Islands and their relation to the rest of communities and factors present on the habitats explored on the islands.
2) **Unravel the nuclear DNA content and evolutionary patterns of Antarctic seaweed**: we studied the DNA contents and nuclear patterning of several Antarctic seaweed species for which there was no previous data. This allowed us to frame these important characters with data from taxonomic relatives from other parts of the world.
3) **Expand the knowledge on the chemical ecology of Antarctic seaweeds**: we reviewed the available knowledge on this field for Antarctic seaweeds. Also, we aimed to study the chemical ecology and potential human applications of natural products from several Antarctic macroalgae species testing against microorganism isolated from Antarctic marine environment and common human pathogenic surrogates.
4) **Understand the role of Seaweeds in the context of climate change in Antarctica**: we identified and studied non-Antarctic seaweeds rafts grounded in Antarctic region. We examined the importance they present for potential events of colonisation/invasion of non-native organisms in Antarctica.

Structure of this thesis

Following the objectives mentioned above, the works have been organized into four main chapters. **Chapter I,** follows the objective one and contains two works. The first explores the biodiversity of benthic communities on DI as well as the potential factors determining their composition on the island. The second, studies the factors and composition that determine the biodiversity of diatoms growing on the macroalgae from both DI and LI. **Chapter II**, focuses on objective two and includes one work which analyses cytogenetic aspects of several Antarctic seaweed; **Chapter III**, which accounts for two studies, were the first one offers a review on the chemical ecology of the SO including seaweed, and the second reports our results of some chemical biointeractions between several Antarctic macroalgae and microorganisms species. Finally, **Chapter IV** comprises one work, which explores some events of hitchhiking organisms on seaweed rafts arriving to Antarctica, and its potential consequences in the future of this region.

SUPERVISORS REPORT

INFORME DE LA DRA. AMELIA GÓMEZ GARRETA Y DEL DR. JORDI RULL LLUCH, DIRECTORES DE LA TESIS DOCTORAL DEL SR. RAFAEL PABLO MARTÍN MARTÍN, SOBRE LA PARTICIPACIÓN QUE EL DOCTORANDO HA TENIDO EN CADA UNO DE LOS ARTÍCULOS Y EL CAPÍTULO DE LIBRO QUE PRESENTA EN SU TESIS Y SOBRE LA CATEGORIZACIÓN DE LAS REVISTAS O LIBROS EN LOS QUE HAN SIDO PUBLICADOS O ESTÁN EN REVISIÓN.

El doctorando Rafael Pablo Martín Martín es el primer autor de dos de las publicaciones sobre algas antárticas incluidas en esta tesis. En estos casos, el doctorando ha sido el responsable del diseño de los trabajos partiendo de su proyecto de tesis. Los restantes trabajos en los que el doctorando no firma como primer autor, son fruto de su participación en los proyectos antárticos DISTANTCOM, BLUEBIO y CHALLANGE, dirigidos por la Dra. Conxita Ávila Escartín y en los que han participado diversos investigadores del campo de la biología marina, incluyendo zoólogos, microbiólogos y botánicos.

A continuación, mostramos un listado de las contribuciones científicas que constituyen el compendio de publicaciones de la tesis de Rafael Pablo Martín Martín, indicando el factor de impacto de la revista correspondiente al año de publicación (o al año 2020 en el caso de publicaciones del año actual) recogido en el *Journal Citation Reports* y la categorización dentro de su ámbito. Asimismo, detallamos la participación del doctorando en cada artículo.

1. Macrobenthic patterns at the shallow marine waters in the caldera of the active volcano of Deception Island, Antarctica.

Autores: Angulo-Preckler, C., Figuerola, B., Núñez-Pons, L., Moles, J., **Martín-Martín, R.**, Rull-Lluch, J., Gómez Garreta, A., Avila, C. (2018).

Revista: *Continental Shelf Research*, 157: 20-31

Participación del doctorando: realización de los muestreos y observaciones, análisis de datos y discusión de los resultados, diseño y redacción del artículo.

Categorización de la revista. Factor de impacto en Scientific Reports (Web of Science): 2,424. Posición: 25/67 (Cuartil 2) en *Oceanography*.

2. Epiphytic diatom community structure and richness is determined by macroalgal host and location in the South Shetland Islands (Antarctica).

Autores: Burfeid-Castellanos, A. M., **Martín-Martín, R. P.**, Kloster, M., Angulo-Preckler, C., Avila, C., Beszteri, B. (2021).

Revista: *PLoS ONE*, 16(4).

Participación del doctorando: realización de los muestreos y observaciones, análisis de datos, discusión de los resultados, diseño y redacción del artículo.

Categorización de la revista. Factor de impacto en Scientific Reports (Web of Science): 2,74. Posición: 27/71 (Cuartil 2) en *Multidisciplinary Sciences.*

3. Nuclear DNA content estimations and nuclear development patterns in Antarctic macroalgae.

Autores: **Martín-Martín, R. P.**, Vilajoliu-Galcerán, E., Rull Lluch, J., Angulo-Preckler, C., Ávila, C., Gómez Garreta, A. (2020).

Revista: *Polar Biology*, 43(9): 1415-1421

Participación del doctorando: recogida y procesado de muestras en la Antártida, participación en la preparación y tinción del material, observación al microscopio y realización de fotografías de los núcleos, análisis de datos, discusión de los resultados, diseño y redacción del artículo.

Categorización de la revista. Factor de impacto en Scientific Reports (Web of Science): 1,728. Posición: 30/58 (Cuartil 3) en *Biodiversity and Conservation*, y posición: 101/169 (Cuartil 3) en *Ecology*.

4. Chemical ecology in the Southern Ocean.

Autores: Angulo-Preckler, C., De Castro-Fernandez, P., **Martin-Martin, R. P.**, Figuerola, B., Avila, C.

Libro: Di Prisco, G., Edwards, H. G. M., Elster, J., Huiskers, A. H. L. (eds.). *Life in Extreme Environments: Insights in Biological Capability*, 251. (Cambridge University Press, 2020)

Participación del doctorando: realización de los muestreos y observaciones, análisis de datos, discusión de los resultados, diseño y redacción del capítulo de libro.

5. Experimental evidence of antimicrobial activity in Antarctic seaweeds: ecological role and antibiotic potential

Autores: **Martín-Martín, R. P.**, Carcedo-Forés, M., Camacho-Bolós, P., García-Aljaro, C., Angulo-Preckler C., Avila, C., Rull Lluch, J., Gómez Garreta, A.

Revista: *Polar Biology* (enviada)

Participación del doctorando: recogida y procesado de muestras en la Antártida, participación en la realización de los antibiogramas, análisis de los datos, discusión de los resultados, diseño y redacción del artículo.

Categorización de la revista. Factor de impacto en Scientific Reports (Web of Science): 1,728. Posición: 30/58 (Cuartil 3) en *Biodiversity and Conservation*, y posición: 101/169 (Cuartil 3) en *Ecology*.

6. Invasive marine species discovered on non–native kelp rafts in the warmest Antarctic island.

Autores: Avila, C., Angulo-Preckler, C., **Martín-Martín, R. P.**, Figuerola, B., Griffiths, H. J., Waller, C. L. (2020).

Revista: *Scientific Reports*, 10(1): 1-9.

Participación del doctorando: realización de los muestreos y observaciones, análisis de datos, discusión de los resultados, diseño y redacción del artículo.

Categorización de la revista. Factor de impacto en Scientific Reports (Web of Science): 3,998. Posición: 17/71 (Cuartil 1) en *Multidisciplinary Sciences.*

Barcelona, 11 de junio de 2021

Dra. Amelia Gómez Garreta

Dr. Jordi Rull Lluch

PUBLICATIONS

Chapter I

Macrobenthic patterns at the shallow marine waters in the caldera of the active volcano of Deception Island, Antarctica

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Macrobenthic patterns at the shallow marine waters in the caldera of the active volcano of Deception Island, Antarctica

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ABSTRACT

Deception Island is an active volcano located at the southern end of the South Shetland Archipelago, in the Antarctic Ocean. After the last eruption in 1970, benthic recolonization took place within the bay, with echinoderms being the dominant epifauna (e.g., the ophiuroid *Ophionotus victoriae*, the echinoid *Sterechinus neumayeri* and the sea star *Odontaster validus*), together with dense infaunal communities (mostly composed by oligochaetes, polychaetes, and bivalves). Here, we aim to describe the actual status of the marine benthic ecosystems inhabiting the shallow subtidal areas of this volcanic island. Benthic species were qualitatively scored as presence versus absence, considering the different sampling effort between localities done over the years. A total of 139 species of macroorganisms, belonging to 16 phyla were found, including fauna and flora, increasing the species richness values previously reported in all sites surveyed within the volcano caldera. Moreover, a dramatic increase in biodiversity was found towards the entrance of the bay. We suggest, however, that recolonization from external waters may not be the only reason for this pattern. In fact, sediment flux rates and substrate instability are common disturbances within the bay, probably being among the major factors determining benthic community assemblages. These processes probably favour deposit feeding communities at the innermost locations of the bay. This study provides a remarkably increased and updated species inventory from previous reports, altogether with a description of the main communities inhabiting the bay and the abiotic factors regulating this, mainly the bottom type.

1. Introduction

Deception Island (DI) is an active volcano at the southwest end of the South Shetland Archipelago. Its central flooded caldera (i.e., Port Foster) connects with the Bransfield Strait through a narrow channel named Neptune's Bellows. This large protected bay ($10 \times 7\ km^2$), has a maximum depth of 160 m (Smith et al., 2003). The characteristics of the surface water masses (< 30 m) of Port Foster are similar to those along the Bransfield Strait. However, deeper areas have been described to be significantly different. Bottom water temperatures in the central and northern sector of Port Foster are about 2–3 °C, suggesting geothermal warming (Ortiz et al., 1992). Indeed, DI harbours one of the warmest sites -albeit patchy- in the Southern Ocean as a result of its volcanic activity (Sturz et al., 2003; Meredith and King, 2005). Methane concentrations also indicate venting of hydrothermal fluids (Tilbrook and Karl, 1994), along with high concentrations of diluted Fe, Mn, and Si (Elderfield, 1972). On seasonal timescales, the water temperatures experience stratification during the summer and full-depth mixture in fall/winter (Lenn et al., 2003). These circumstances suggest that at the sill of Neptune's Bellows there is limited water exchange below 30 m in the summer months (Sturz et al., 2003). Neptune's Bellows is approximately 150 m wide at the narrowest point with a sill depth of 11 m, which minimizes the number of icebergs entering from outside, therefore limiting one of the most important disturbance factors affecting benthic Antarctic communities in other areas, i.e., ice-scouring (Gutt, 2001).

The Antarctic benthos has been categorized as a relatively homogenous biological unit (Barthel and Gutt, 1992; Sarà et al., 1992; Smale 2008; Downey et al., 2012). Many species share the same evolutionary constraints (Clarke and Crame, 1992; Clarke et al., 2004; Arntz et al.,

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2005) and some large-scale ecological conditions are similar around most parts of the continent, i.e., low and relatively stable temperature, seasonality of primary production, and low terrigenous input (Bullivant, 1959; Clarke and Leakey, 1996). Some authors indicate that identical conditions can support similar faunal assemblages in Antarctic sea bottoms (Dayton et al., 1994). However, other studies show that similar faunal assemblages can be found under different physical drivers (Barnes and Conlan, 2007; Angulo-Preckler et al., 2017b). Gutt (2007) proposed two types of core macrobenthos assemblages at community level. The first one would be dominated by sessile and sedentary suspension feeders, while the second one would consist in mobile deposit feeders and infauna. In both communities, this pattern can be overlaid by a second gradient ranging from very high to extremely low abundances.

In Deception island, several studies have documented the impact of eruptions and the posterior recolonization in benthic communities (Gallardo and Castillo, 1968, 1970; Gallardo, 1975; Gallardo et al., 1977, 1999; Retamal et al., 1982; Sáiz-Salinas et al., 1997; Arnaud et al., 1998; Lovell and Trego, 2003; Moya et al., 2003; Cranmer et al., 2003; Barnes et al., 2008; Angulo-Preckler et al., 2017a, 2017b; Pellizzari et al., 2017). Remarkably, an intense repopulation of flora and fauna has been observed in Port Foster since the last eruptions in 1967, 1969, and 1970 (Lovell and Trego, 2003), even though it seems clear from these studies that impacts and their effects on the communities lasted several years. The first surveys revealed that the opportunistic annelid *Echiurus antarcticus* Spengel, 1912 colonised the area in 1972 (Gallardo et al., 1977), although it seems to be absent now (Lovell and Trego, 2003; Barnes et al., 2008). In 1981, the area showed a significant enrichment in polychaetes and cumaceans as the dominant infaunal taxa, while the echinoderms *Ophionotus victoriae* Bell, 1902, *Sterechinus neumayeri* (Meissner, 1900), and *Odontaster validus* Koehler, 1906 became the dominant epifauna within the caldera to the present day (Retamal et al., 1982; Arnaud et al., 1998; Cranmer et al., 2003; Lovell and Trego, 2003; Angulo-Preckler et al., 2017a). Arnaud et al. (1998) considered the benthic system of Port Foster different from the rest of the South Shetland Islands (SSI) and described a lower taxonomic richness (6 species) but a higher biomass. A depth zonation of the dominant benthic fauna was described, with Ascidiacea dominating between 40 and 50 m, Echinoidea from 100 to 150 m, and Ophiuroidea below 150 m (Arnaud et al., 1998). This faunal zonation is dependent on sediment type, from gravels at the shallow stations to sandy and muddy sediments at the deepest areas (Gray et al., 2003). A later study, in which sampling was performed in deeper waters throughout the year, reported higher diversity, with up to 13 phyla, 16 classes, and 68 species (Lovell and Trego, 2003). These authors found a predominance of *O. victoriae*, along with a poor representation of sponges and ascidians in the deeper mid-bay. A scuba diving study reported 10 phyla, 13 classes, and 35 species at the entrance of the bay (Barnes et al., 2008) describing a declining biodiversity gradient. Barnes et al. (2008) described a strong decrease of species richness from the entrance to the internal caldera at subtidal depths, with remarkably low species richness within the bay. In summary, up to 2008, a total of 163 faunal species had been reported for DI (Barnes et al., 2008 and references therein). Moreover, in a recent study of the infauna, we reported the highest densities of organisms found so far for Antarctica, within the sediment of the shallow waters of DI (Angulo-Preckler et al., 2017b). Concerning macroalgae in DI, in a catalogue of benthic algae from the Brandsfield Strait, Gallardo et al. (1999) cited 26 algal species (7 Chlorophyta, 6 Ochrophyta and 13 Rhodophyta), 23 of them were found after the last eruption in 1970. Overall, and despite all these previous studies, the biodiversity of the shallow waters of Port Foster (< 40 m) has never been adequately assessed.

While most studies on shallow-water Antarctic benthic communities focus on the fauna, algal communities have never been included, even if it is well known that synergies between benthic macroalgae and invertebrates are crucial. These effects may be “positive” (richer macroinvertebrate communities or populations with increasing macroalgae) or “negative” (poorer macroinvertebrate communities or populations with increasing macroalgae; Mattson, 2009). Furthermore, DI has been and still is a centre of interest for non-indigenous species (NIS) establishment. DI is one of the Antarctic areas most likely to be colonized by both, natural means due to its close proximity to South America and its relatively mild and locally geothermal influenced climate, and by substantial human activity, such as tourist ships and research activity (IAATO, 2016; COMNAP, 2017). For instance, ship borne transport is probably the main driver for NIS algae arrived at DI (Clayton et al., 1997; Barnes et al., 2008).

The current study aims to provide an updated comprehensive description of the shallow benthic assemblages of DI (fauna and flora), while providing a baseline information for reconstructing the historical community shifts from the entrance to the inside of the caldera to be compared with future studies. Considering the threats of climate change and anthropogenic impacts, an updated description of the current macro- and megabenthos communities and their spatial distributions in this vulnerable area of the planet is essential to assist in building tools to predict potential future environmental changes.

2. Material and methods

2.1. Sampling

Surveys were carried out inside Port Foster's Bay (except for Peter's Pilar) by scuba diving at different depths during the austral summers of 2008–10, 2011–13, and 2015–16 (Table 1, Fig. 1). Samples were randomly collected by scuba diving down to a maximum of 25 m depth, during the projects ACTIQUIM-I, ACTIQUIM-II, and DISTANTCOM. Samples were collected by hand in plastic bottles or bags. Each dive was performed by two-three divers for 25–35 min. Dives on soft-bottoms were done in parallel to the coast line, covering different areas (around 50–100 m length and 5–10 m width), while dives on hard-bottoms covered smaller areas (around 20–30 m length and 10–15 m width). All taxa collected (except macroalgae) were sorted, photographed, and fixed in 10% formalin or 70% ethanol for further taxonomic identification. Macroalgae were frozen until further study, and subsequently pressed and stored in the BCN-Phyc Herbarium (Centre de Documentació de Biodiversitat, University of Barcelona). Samples were identified to the lowest possible taxonomic level using microscopy and comparing morphological structures to the literature available for the different phyla (for example, spicules in sponges, radula/shell in molluscs, etc.).

Stations were selected in order to cover Port Fosters’ inner perimeter, and included sites with distinct physical characteristics. The sampled locations were: Peter's Pilar (PET; outside the bay), Neptune's Bellow (NEP; at the entrance), Whaler's Bay (WHB), Colatinas (COL), Bidones Point (BID), Spanish Antarctic Base (BAE; in front of the Antarctic Base *Gabriel de Castilla*), Fumaroles (FUM), Telephone Bay (TEL; with a lake recently opened to Port Foster), and Pendulum Cove

Table 1

Data of the sampling collections of the ACTIQUIM and DISTANTCOM projects, during the five cruises carried out in Deception Island. ACTIQUIM stands for the projects CGL2007–65453/ANT (ACTIQUIM-I; cruises 1 and 2), and CTM2010–17415 (ACTIQUIM-II; cruises 3 and 4) and DISTANTCOM stands for the project CTM2013–42667/ANT; cruise 1.

Campaign	No. of dives	Years	No. of samples
ACTIQUIM-1	16	2008/2009	165
ACTIQUIM-2	11	2009/2010	178
ACTIQUIM-3	44	2011/2012	336
ACTIQUIM-4	46	2012/2013	233
DISTANTCOM	36	2015/2016	480
Total	**153**		**1413**

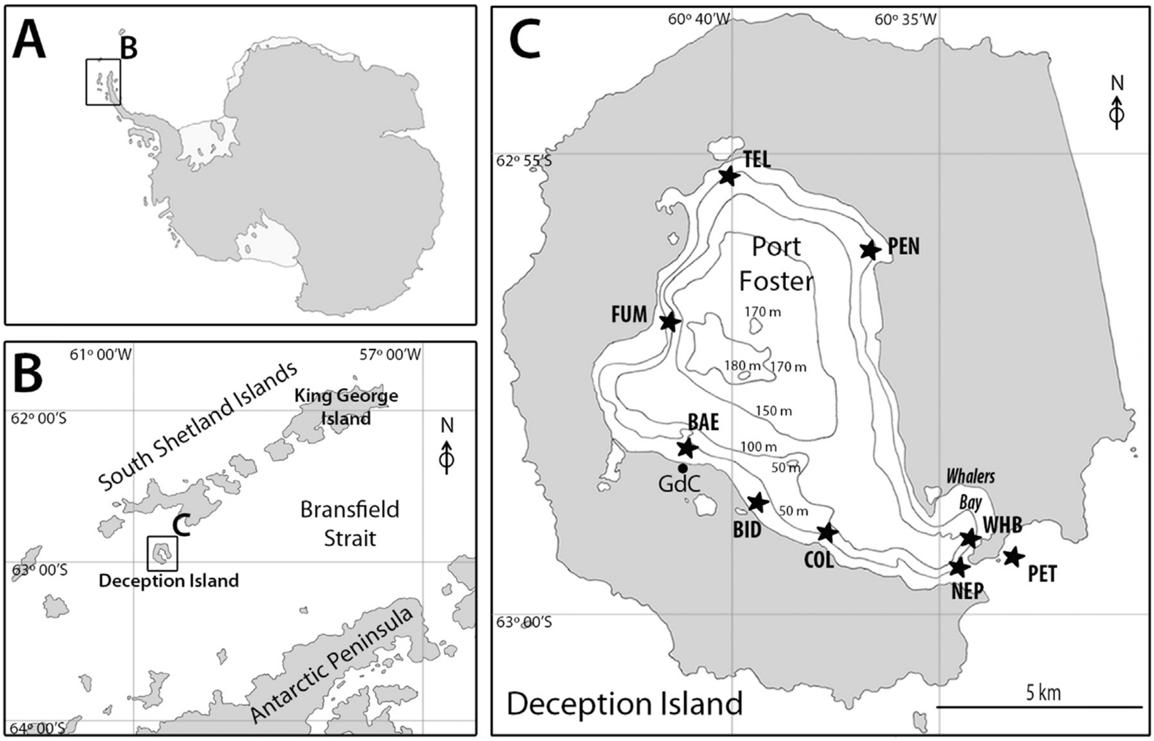

Fig. 1. Sampling area: **(A)** Overview map of Antarctica; **(B)** South Shetland Islands; **(C)** Deception Island (sampling sites marked with a star). **PET**: Peter´s Pilar; **NEP**: Neptune´s Bellows; **WHB**: Whaler´s Bay; **COL**: Colatinas; **BID**: Bidones Point; **BAE**: Antarctic Spanish Base; **FUM**: Fumaroles; **TEL**: Telephone Bay; **PEN**: Pendulum Cove.

(PEN; area with high water temperatures due to hydrothermal vents; Somoza et al., 2004). Most sites were characterized by steep slopes, coarse volcanic sands, and muds. The sea bottoms are mostly characterized by a sheltered soft-bottom platform down to ca. 4–6 m depth, and from this platform down the slope increases dramatically, reaching depths ca. 100 m. WHB, NEP, and PET were the only places where rocky walls, boulders or piles of cobbles were present.

2.2. Environmental drivers

Grain size, and organic matter (O.M.) were measured in previous works of our team (Angulo-Preckler et al., 2017a, 2017b). Furthermore, during the austral Summer, between January and February 2017, temperature and pH were recorded, except for PET (due to logistic limitations). Temperature data loggers (Tinytag Aquatic 2, TG-4100) deployed at each site recorded water temperature every 30 min (during 20–25 days). Water samples were collected at each station (15 m depth) by scuba divers in 500 mL Corning brand Pyrex sample bottles and the pH was measured immediately with a Handheld pH meter (WTW315i).

2.3. Statistical analyses

Epibenthic organisms were qualitatively scored as present or absent, mainly due to different sampling efforts undertaken at the different localities and over the years. A presence/absence data matrix, coding `1´ to the presence and `0´ to the absence of each species at each location, was built. In order to assess differences in species composition between sites, a Bray-Curtis similarity matrix was done. Bray-Curtis index is one of the most widely employed indices, being equivalent to the Sörensen index for presence/absence matrices (Legendre and Legendre, 2012). The contributions of the hierarchical agglomerative clustering (using group average method) and non-metric multidimensional scaling (nmMDS) were calculated, allowing us to infer different clusters/groups within the survey. Species richness was calculated for each location and cluster. Furthermore, the frequency of each taxa was calculated using the Dajoz constancy index (CI). According to this index, constant or conspicuous taxa were considered to be those that occurred in more than 50% of the localities; accessory taxa, between 25% and 50% of the localities; and accidental (rare) taxa, up to 25% of the localities.

In order to test whether variation in any of the measured abiotic variables significantly contributed to explain variation in the macro-organisms assemblage structure along the bay (Grain size, organic matter, pH, temperature, distance to the open sea, and bottom type), a distance based redundancy analysis was used (db-RDA; Legendre and Anderson, 1999) Then, a Multivariate multiple regression using the DISTLM routine (Anderson, 2001) tested the significance of these relationships by fitting a linear model based on Bray-Curtis dissimilarities on fourth root transformed abundance data. To retain variables with good explanatory power, as a result of colinearity among variables, the AIC routine was used as a selection criterion (the smaller the value the better the model; Legendre and Anderson, 1999). Analyses were based on a "forward" selection procedure. All multivariate procedures were carried out using PRIMER 6.0 & PERMANOVA+ statistical package (Clarke and Gorley, 2006).

Differences between stations groups (soft-bottoms versus hard-bottoms) were evaluated using the ANOSIM test (analysis of similarities; n = 9999 permutations; Clarke, 1993).

3. Results

A total of 139 species belonging to 16 phyla and 26 classes were collected at shallow waters in more than 150 dives at DI and identified to species level, when possible. Among the macroalgae reported, 31 different species were found, being the rest different invertebrates. Overall, more than one-third of the species recorded are new cites for DI (48/139). New records include 14 molluscs, 10 sponges, 6 echinoderms, 4 bryozoans, 3 cnidarians, 2 nemerteans, 2 tunicates, 1 annelid, 1 platyhelminth, 1 brachiopod, and 4 red macroalgae.

Three of the species were only found outside the bay (PET; the

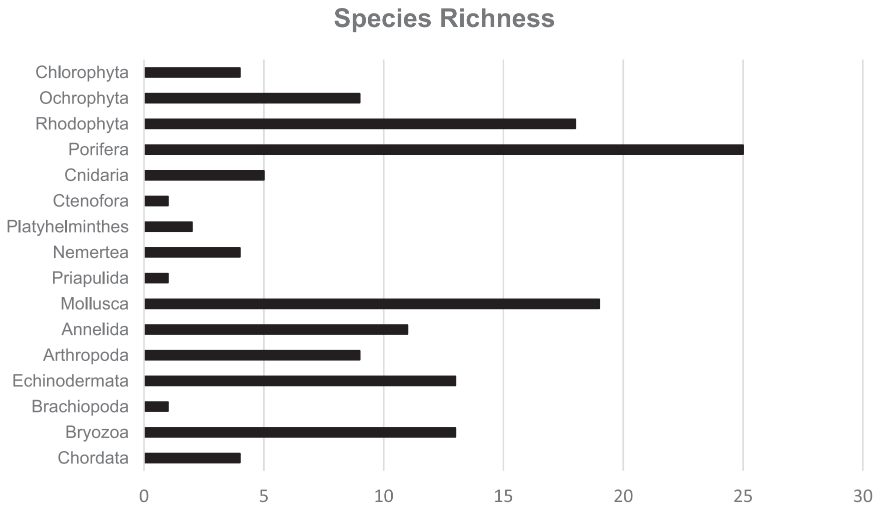

Fig. 2. Species richness per phylum observed in this study at Port Foster (Deception Island).

macroalga *Antarctosaccion applanatum* (Gain) Delépine, 1970, the annelid *Perkinsiana* sp., and the bryozoan *Antarctothoa polystachya* Wright, Hayward and Hughes, 2007), while the rest of taxa were all found within Port Foster (See Supplementary material). The most diverse phyla were Porifera, with 25 species, followed by Mollusca, Rhodophyta, Echinodermata, and Bryozoa with 19, 18, 13, and 13 species respectively (Fig. 2). The species with the widest spatial distributions were the annelid polychaete *Leitoscoloplos kerguelensis* (McIntosh, 1885), the echinoderms *Ophionotus victoriae, Sterechinus neumayeri,* and *Odontaster validus*, the limpet *Nacella concinna* (Strebel, 1908), the nemertean *Parborlasia corrugatus* (McIntosh, 1876), and the alga *Adenocystis utricularis* (Bory de Saint-Vicent) Skottberg, 1907. Only 16 species (11.4%) were characterized as constant or conspicuous taxa, while the remaining 77 (55%) and 46 (33.5%) species were considered as accessory and accidental or rare taxa, respectively (Appendix A).

The nine subtidal sites of DI examined here differed dramatically in species richness (Table 2). Using taxa pooled by locations, three main groups were clearly differentiated in the hierarchical agglomerative cluster. Cluster 1 included the most internal station (Pendulum Cove; PEN). Cluster 2 was represented by several stations (TEL, FUM, BID, BAE, and COL), and was subdivided into three different sub-clusters corresponding with an increasing distance to the open sea. Finally, Cluster 3 included the stations closer to the entrance of the bay (PET, NEP, and WHB; Fig. 3). According to the hierarchical agglomerative cluster and the nmMDS plot analyses of species richness and similarities within community assemblage, these three groups can be further subdivided into five different sub-clusters (Fig. 4).

Cluster 1, composed of a single location (i.e., PEN), was the most divergent cluster. It showed a poor species richness and a community assemblage characterized by only five phyla (Table 3), with a unique algal species (*Hymenocladiopsis prolifera* (Reinsch) M.J. Wynne, 2004), and where opportunistic organisms were predominant (the echinoderms *Ophionotus victoriae* and *Sterechinus neumayeri*, the amphipod *Cheirimedon femoratus* (Pfeffer, 1888) and the widely distributed polychaete *Leitoscoloplos kerguelensis*). Cluster 2 pooled all mid-bay locations discriminating three sub-groups. Sub-cluster 2a (TEL and FUM), despite its poor species richness (S = 22), similar to Cluster 1 (S = 8), differed in the absence of an algal community. Sub-cluster 2b (BAE and BID), was marked by medium species richness (S = 47), sheltering 13 different phyla, and with similar communities of echinoderms and annelids. Sub-cluster 2c (COL), closely related to sub-cluster 2b, with similar species richness (S = 22), but different community assemblage, sheltered an important presence of macroalgae (six species; see Appendix A) besides the common opportunistic species mentioned above. Finally,

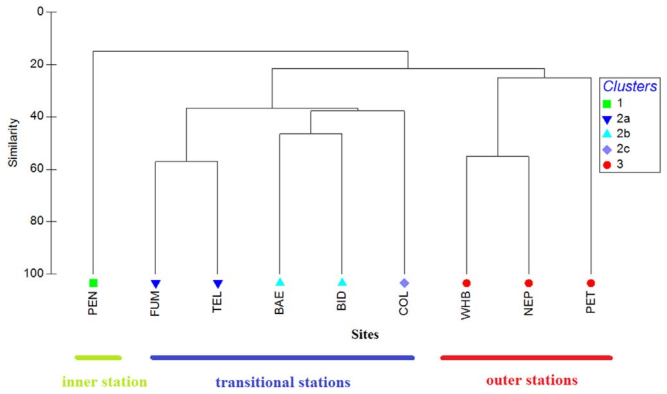

Fig. 3. Hierarchical agglomerative cluster (group average method) based on Bray-Curtis similarities from the presence-absence data matrix. Three main groups of samples (from inner to outer locations) were grouped into five different clusters. Site names as in Fig. 1.

Table 2
Data of the diving points at Deception Island and taxa collected per site. Temperature and pH at each location at Post Foster (mean ± standard deviation).

Dive points	Code	Depth (m)	Number of dives	Species richness	T (°C) ± S.D.[a]	pH ± S.D.[a]	Clusters
Pendulum Cove	PEN	10–20	9	8	2.161 ± 0.42	7.386 ± 0.32	1
Fumarole Bay	FUM	10–20	12	12	1.918 ± 0.29	7.423 ± 0.37	2a
Telephone bay	TEL	10–20	8	17	2.017 ± 0.40	7.741 ± 0.21	2a
Antarctic Spanish Base	BAE	15–20	33	32	2.133 ± 0.34	7.951 ± 0.21	2b
Bidones Point	BID	15–25	23	32	2.278 ± 0.23	7.700 ± 0.08	2b
Colatinas	COL	15–20	5	22	2.369 ± 0.18	7.643 ± 0.10	2c
Whaler's Bay	WHB	15–25	36	95	2.484 ± 0.35	7.980 ± 0.17	3
Neptune's Bellows	NEP	20–25	22	97	2.456 ± 0.27	8.017 ± 0.12	3
Peter's Pilar	PET	10–15	5	33	–	–	3

[a] Mean values with standard deviation calculated from measurements taken in February 2017.

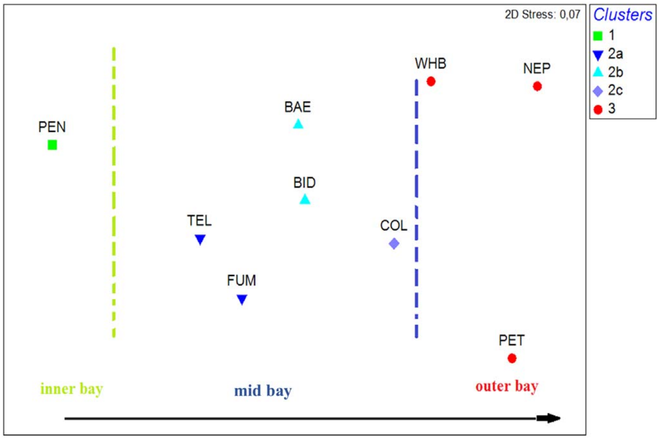

Fig. 4. Non-metric multidimensional scaling ordination of Bray-Curtis similarities from the presence-absence matrix. Stations referred to the clusters defined in Fig. 3. Site abbreviation as listed in Fig. 1.

Table 3
Presence-absence of phyla per cluster.

Phyla	Cluster 1	Cluster 2a	Cluster 2b	Cluster 2c	Cluster 3
Chlorophyta		*	*		*
Ochrophyta		*	*	*	*
Rhodophyta	*	*	*	*	*
Porifera				*	*
Cnidaria			*		*
Ctenofora			*		
Platyhelminthes	*	*			*
Nemertea		*	*	*	*
Priapulida			*		*
Mollusca		*	*	*	*
Annelida	*	*	*	*	*
Arthropoda	*	*	*	*	*
Echinodermata	*	*	*	*	*
Brachiopoda					*
Bryozoa			*		*
Chordata		*	*	*	*
Total	5	10	13	9	15

Cluster 1: PEN. Cluster 2a: TEL, FUM. Cluster 2b: BAE, BID.
Cluster 2c: COL. Cluster 3: WHB, NEP, PET.

Cluster 3 grouped the two richest locations (NEP and WHB), together with the single site outside the bay (PET). This community was characterized by important numbers of macroalgae, sponges, and bryozoans, which were completely absent in most of the inner stations. These groups contribute to the largest richness values in these stations ($S = 127$).

The environmental factors measured during February 2017 showed small variability within locations (Table 2), but very different values respect other localities of the SSI. The average values of temperature and pH for DI at 15 m depth were 2.23 ± 0.21 °C and 7.73 ± 0.24, respectively.

The first two axes from the db-RDA explained 47.5% of the total variation in macrofaunal assemblage structure (Fig. 5). “Bottom type” was significantly and positively correlated with the first axis, which accumulated 33.2% of the total variability of macrofaunal assemblage structure ($p = 0.033$). The second axis of the db-RDA was positively correlated with “Temperature”, but was not significant ($p = 0.159$). The former two variables were those contributing mostly to explain variation in macrofaunal assemblage structure, as a result of strong colinearity among several abiotic variables (such as “pH”, “Grain size” or “Organic Matter” with “Bottom”). Differences between assemblages related to bottom substrate (hard vs. soft) were corroborated by the ANOSIM analysis (Global R = 0.729; *p-value* = 0.036).

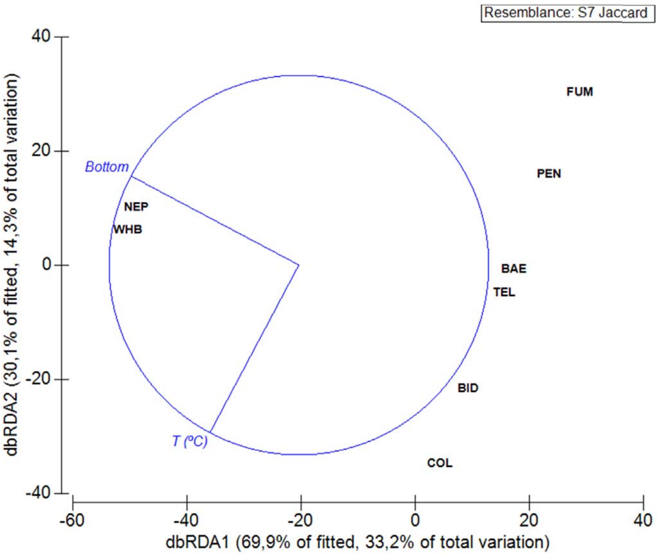

Fig. 5. Main abiotic factors contributing to explain variation in the macrofaunal assemblage structure.

4. Discussion

The shallow waters of Port Foster are characterized by a rich biodiversity, with higher species richness than recorded in previous studies, accounting for a total of 139 species belonging to 16 phyla. This is thus the widest study carried out so far at the shallow waters of DI inner bay. Nonetheless, different methodologies and sampling efforts make the comparison between our data and those of the literature difficult (Cranmer et al., 2003; Lovell and Trego, 2003; Barnes et al., 2008; Pellizzari et al., 2017). The present study has been performed over more than 5 years (153 dives) thus providing an exhaustive coverage of the shallow-water communities within Foster's Bay. For instance, we report here more epibenthic species than previously reported for all sites surveyed at DI by other authors (Lovell and Trego, 2003; Cranmer et al., 2003; Barnes et al., 2008). Similarly to Barnes et al. (2008), a pattern with a dramatic biodiversity decline from the outer (NEP) to the inner sites (PEN) was observed (Fig. 6). We suggest here that this pattern is mainly due to the different substrate typologies, with a high positive correlation between hard substrate and biodiversity. The steep bottom slopes and the instability of the sediment favour the presence of a mobile deposit feeder community (MDC) and an infaunal community (INC) in the soft-bottoms of DI, according to previous studies of our group (Angulo-Preckler et al., 2017b). These areas seem to be occupied by opportunistic detritivores, capable of handling these unfavourable conditions. Furthermore, a community dominated by infauna, with surface and subsurface feeders, is related to a high biogenic sedimentation rate (Ravaioli et al., 1999). In contrast, hard bottoms of DI were populated both by macroalgae and sessile suspension feeder communities (SFC). These communities were composed by large sponges, ascidians, bryozoans, and macroalgae, which provide three-dimensionality to the ecosystem (Dayton and Oliver, 1977). The resulting benthic structure provides a microhabitat for a broad variety of associated fauna, mainly consisting in highly specialized organisms, but also including a few opportunists. The benthic communities at DI, thus, seem to be determined by bottom typology (Fig. 7). The distance to the open sea, and thus to the potential input of either adult or larval species recolonization, is probably another factor favouring this gradient of ecosystem composition.

The presence of most of the newly cited species (48) is not surprising, since some of them are well known from adjacent areas (i.e. Gallardo et al., 1999; Nonato et al., 2000; Pellizzari et al., 2017). Their findings could be related to our more exhaustive sampling surveys, with

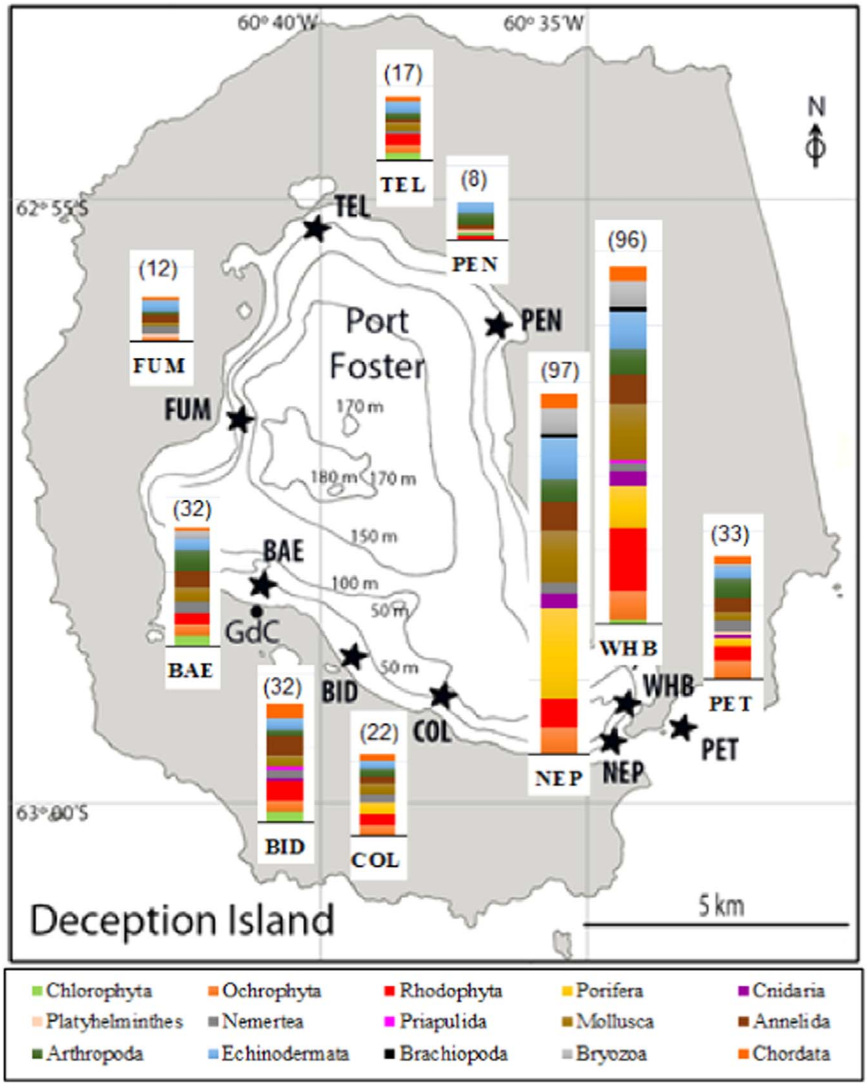

Fig. 6. Species richness by stations (total taxa by site) down to 25 m depth.

respect to previous studies.

An important feature of this study is that it represents the first qualitative analysis of species composition at DI including macroalgae. The inclusion of algae in benthic inventories is key to identifying synergies with invertebrates, and to understand their role in habitat structure, competition for space, or trophic interactions (Mattson, 2009). We observed different communities apart from those recently described by Pellizzari et al. (2017), with a dominance of filamentous green algae in DI shallow waters. Very relevant assemblages of macroalgae (31 species) were observed, mainly dominated by brown and red algae, with dense canopies, restricting the dominance of green algae to intertidal areas. The rocky shores along DI are covered by extensive subtidal macroalgae assemblages, with standing stocks, showing a large variability between localities. Here, it is necessary to remark the importance of the sampling effort performed in our study to cover the distribution of benthic communities along DI and their variability at local scale. In fact, fine-scale variability was shown to be very pronounced in other studies of coastal Antarctic assemblages (Valdivia et al., 2014), suggesting that differences among islands may be lower than those among several locations on the same island.

The shallow waters of DI include some localities among the warmest sites in the Southern Ocean and the caldera is one of the most visited Antarctic places by tourist ships. These factors may have favoured the establishment of invasive NIS species such as the green alga *Ulva intestinalis* Linnaeus, 1753. We found this species growing in dense mats on the intertidal and subtidal rocks at TEL and BID. Although Clayton et al. (1997) collected it as drift specimens for WHB, it now seems to have established on both DI and the rest of the SSI (Pellizzari et al., 2017).

In general, two core benthic communities are easily distinguishable in the shallow-waters of DI, corresponding to those proposed by Gutt (2007) in the ecological classification of shelf inhabiting Antarctic macro-zoobenthic communities: 1) A suspension feeder community (Cluster 3: NEP, PET, and WHB), associated to hard-bottoms, and 2) A mobile deposit feeder community and an infaunal community (Cluster 1: PEN and Cluster 2: TEL, FUM, BAE, BID, and COL) associated to soft-bottoms.

4.1. Suspension feeder community (SFC)

The two richest locations NEP and WHB, the closest to the entrance, are associated with hard substrates (mostly vertical walls). These

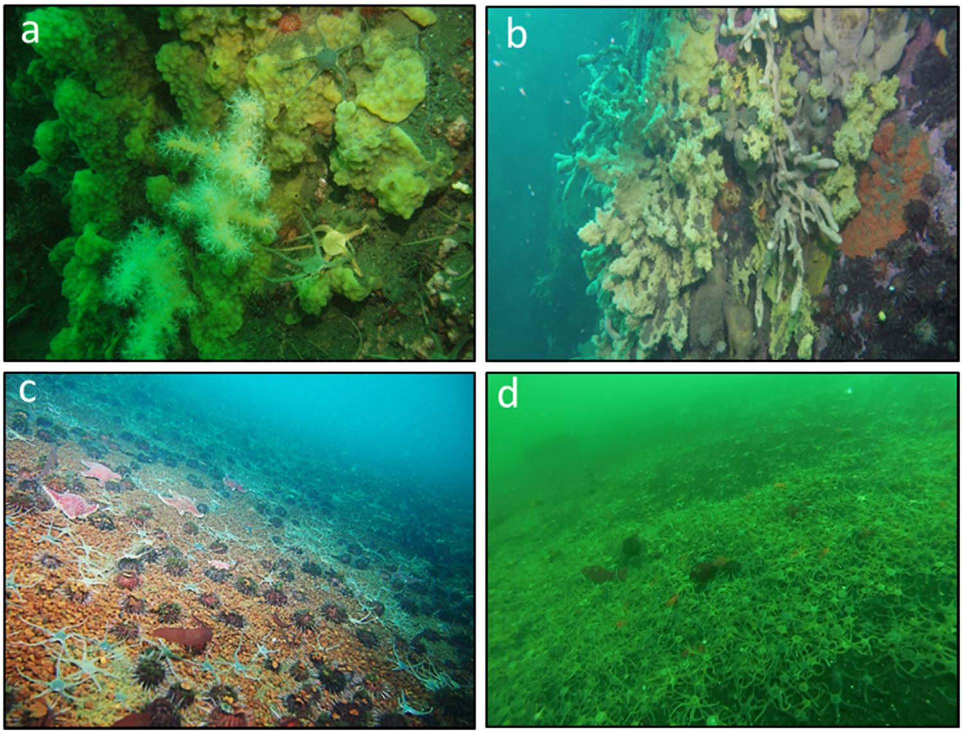

Fig. 7. Underwater photography's showing the two different communities described in this study. **Suspension feeder community**; a) massive sponge *Mycale (Oxymycale) acerata* and the soft-coral *Alcyonium haddoni* at NEP, and b) the sponges *Dendrilla antarctica, Hemigellius pillosus,* the tunicate *Cnemidocarpa verrucosa* at WHB. **Mobile deposit feeder community**; c) the most representative species on the soft-bottoms; *Sterechinus neumayeri*, *Ophionotus victoriae* and *Odontaster validus* at BAE, and d) very high densities of *Ophionotus victoriae* at BID.

cluster together with the single rocky site studied outside the bay PET, all sharing similar community composition with the dominance of sessile suspension feeders. These epibenthic communities are characterized by the predominance of macroalgae, sponges, and bryozoans. Overall, macroalgal assemblages are dominated by canopies of large brown algae (i.e., *Desmarestia* spp., *Himantothallus grandifolius* (A. Gepp and E.S. Gepp) Zinova, 1959, *Cystosphaera jacquinotti* (Montagne) Skottsberg, 1907), at between 15 and 25 m depth when hard bottoms exist. These brown algal stands are accompanied by diverse understory assemblages, mainly composed of red algae (i.e., *Gigartina skottsbergii* Setchell and N.L. Gardner, 1936, *Palmaria decipiens* (Reinsch) R.W. Ricker, 1987, *Georgiella confluens* (Reinsch) Kylin, 1956), and to a lesser extent some green algae (i.e., *Urospora penicilliformis* (Roth) Areschoug, 1866*, Acrosiphonia arcta* (Dillyn) Gain, 1912). Differences in species composition within depth zones are mainly related to substratum type and exposure (Klöser et al., 1994). These taxa are completely absent in inner stations but are instead quite rich and common in other Southern Ocean benthic communities (De Broyer et al., 2014). The two locations at the bay's entrance (WHB and NEP) present the highest species richness, sheltering an hitherto undescribed community of suspension feeders. A dense “animal forest” characterizes the seafloor and vertical walls at these locations, with more than 30 different species of macroalgae and more than 24 sponge species, hosting a huge biomass (personal observations of the authors). The complexity of the animal forests depends on the structuring organisms. Indeed, sponge grounds can form highly complex and diverse three-dimensional structures, influencing hydrodynamics and supplying shelter and food to highly diverse associated fauna (Rossi et al., 2017). Similar findings (i.e. high species richness) were found in molluscs and bryozoans, highlighting the atypical biodiversity hotspot found at the entrance, respect to the rest of the bay. Although these two stations possess very similar assemblages, NEP is defined by the dominance of the massive and fast growing sponges *Mycale (Oxymycale) acerata* Kirkpatrick, 1907 and *Dendrilla antarctica* Topsent, 1905, and canopies of the large brown algae *Desmarestia anceps* Montagne, 1842, *D. menziesii* J. Agardh, 1848 and *Himantothallus grandifolius* (A. Gepp and E.S. Gepp) Zinova, 1959. Instead, WHB shows a higher diversity of algal assemblages (but less abundant), and a sponge assemblage without an apparent dominance of any particular species (the main sponges here are *Hemigellius pilosus* (Kirkpatrick, 1907), *Axinella crinita* Thiele, 1905, *Kirckpatrickia variolosa* (Kirkpatrick, 1907), the ascidia *Cnemidocarpa verrucosa* (Lesson, 1830), and the anemone *Isotealia antarctica* Carlgren, 1899). In this subcommunity, echinoderms and molluscs seem to be the most effective predators that directly or indirectly may control macroalgae and sponge population growth (Moles et al., 2015). We suggest that the absence of physical disturbances, such as ice-scouring and anchor ice inside the bay, could be one of the most important factors allowing for the development of these benthic assemblages here after the last volcanic eruptions.

4.2. Mobile deposit feeder community (MDC) and Infaunal community (INC)

These include all soft-bottom communities, i.e. all the remaining stations (Cluster 1 and 2). They have a relatively homogeneous community with slight variations, with increasing species richness as we approach the entrance of the bay. PEN is the station furthest from the entrance and most divergent within the bay, being home to the most different and poorest benthic community. This could be explained by the geothermally warm waters that characterize this location (Brierley, 1999; authors’ personal observations). Although we did not find significant differences in the main temperature measured (2.161 ± 0.42 °C), with respect to the other localities, PEN showed the lowest pH value (7.386 ± 0.32), indicating venting of hydrothermal fluids. Similar results were obtained in other macro- and megafaunal studies from Angulo-Preckler et al. (2017a, 2017b). FUM and TEL showed similar richness to PEN with some differences in species composition, probably related to the lower number of taxa present in the inner-bay areas. Finally, BAE, BID, and COL hosted a medium level of species richness, but higher than that found in previous surveys, grouping here as a transition zone with benthic communities dominated by mobile deposit feeders. The biodiversity gradient seems to be more defined by the occasional presence of species attached to patchy boulders than to the general composition of the community. However, due to the nature of the data (presence/absence), there could be a statistical bias conferring higher diversity, because rare species play a minor role in the community, especially when these represent a third of the total species (33.5%). This community is mainly composed by the echinoderms *Sterechinus neumayeri*, *Ophionotus victoriae*, and *Odontaster validus* in huge densities (see Angulo-Preckler et al., 2017a), the clam *Laternula elliptica*, and the nemertean *Parborlasia corrugatus*. Besides the more conspicuous epifauna, the infauna in DI is at least as important, with the highest densities found so far for soft-bottom Antarctic macroinvertebrate communities (69 infaunal species and 188,167 ind m^2 in front of the BAE; Angulo-Preckler et al., 2017b) adding 13 new records to DI inventory (see Supplementary material).

4.3. Concluding remarks

It is possible that part of the communities that we now observe in Deception island are, in fact, the remnants of previous well-developed communities, destroyed by the volcanic eruptions more than 40 years ago. Most of these animals are slow-growing species, and their recovery may take decades (e.g. *Laternula elliptica* 36 y maximum age (Abele et al., 2009) or *Sterechinus neumayeri* 40 y maximum age; Brey et al., 1995). The absence of ice-disturbance inside DI, allowed for the development of a rich suspension feeding community associated to hard-bottom substrates, while the sediment instability associated to soft-bottoms favoured a community dominated by infauna and mobile deposit feeders, including huge densities of echinoderms (Angulo-Preckler et al., 2017a, 2017b).

Despite DI being an intensively sampled area in the Southern Ocean, new species are still being recorded, emphasizing the incomplete characterization of the DI biodiversity inventory. Our new data, together with published literature and online database information (OBIS; http://www.iobis.org/ and iATLAS; http://atlas.biodiversity.aq/), indicate that 422 benthic species are now known to be inhabiting DI (see Supplementary material). Although these species values are reliable, one has to be careful with the species names, because most of the oldest studies cited in the literature and databases were conducted before the most recent eruptions, and cannot be confirmed or revised now.

Among our samples, some taxonomical groups are still poorly studied (e.g. amphipods, with more than 20 different unidentified taxa, or nematodes, completely unstudied). Some other taxa that seem to be under-represented at the moment are awaiting further analyses or more comprehensive surveys. Beyond of the aim of this study, limited to epibenthic invertebrates, some new polychaetes were described associated with fresh whale bones experimentally deployed at DI (*Parougia diapason, Osedax deceptionensis, Ophryotrocha clava, O. orensanzi*, and *Cirratulus balaenophilus*; Taboada et al., 2012, 2013, 2015; Glover et al., 2013). Whalebones around DI constitute a relatively common and systematically overlooked hard substrates. These bones are the product of the Norwegian-Chilean whaling factory that operated in the early 20th century (Dibbern, 2010) and are very frequent and conspicuous in the intertidal and subtidal waters of Port Foster.

Differences between species assemblages are related to sediment characteristics. For example in muddy sediments where the infauna and mobile epifauna dominate, or in contrast, when sessile species colonize true hard substrates. Barnes (2005) described the substrate as "…the most important factor influencing community composition…" in the shallow subtidal zone at Signy Island, emphasizing the general importance of sediment for the shallow Antarctic benthos. The shallowest communities of sessile suspension feeders are more strongly defined by physical impacts (ice disturbance, wave action, light penetration, and sediment instability), than by depth, for instance. In the shallowest depths of DI, sediment instability results in a community mainly inhabited by mobile animals, often with only a few dominant species. Therefore, the higher diversity found at DI after the relatively recent eruptions suggests the need for long-term biological and abiotic monitoring in order to establish conservation guidelines across the Antarctic and Sub-Antarctic zones, especially under the threats of increasing tourism and climate change.

Appendix A

See Table A1.

Acknowledgments

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Ethical statement

The research reported here has been conducted in an ethical and responsible manner and comply with all relevant legislation.

1. We have no potential conflict of interest.
2. All procedures performed in this study involving animals were in accordance with the ethical standards of the institution or practice at which the study was conducted.

Contributors

All authors contributed extensively to the work presented in this paper. Design of the work; C.A.P. and C.A. Data collection; C.A.P., B.F., L.N.P., J.M., and C.A. Taxonomical Identifications; B.F., L.N.P., J.M., R.M.M, J.R.L., A.G.G and C.A. Data analysis and interpretation; C.A.P. and C.A. Drafting the article; C.A.P. and C.A.

Table A1
Species checklist from Deception Island by diving surveys at different locations.

	Taxa	BAE	FUM	TEL	PEN	BID	COL	WHB	NEP	PET	CI
Chlorophyta											
Ulvophyceae											
Gomontiaceae	*Monostroma hariotii* Gain, 1911	+						+			A
Ulotrichaceae	*Acrosiphonia arcta* (Dillyn) Gain, 1912	+				+					A
Ulotrichaceae	*Urospora penicilliformis* (Roth) Areschoug, 1866	+		+		+					A
Ulvaceae	*Ulva intestinalis* Linnaeus, 1753			+		+					A
Ochrophyta											
Phaeophyceae											
Adenocystaceae	*Adenocystis utricularis* (Bory de Saint-Vincent) Skottsberg, 1907	+	+	+		+	+	+	+	+	C
Ascoseiraceae	*Ascoseira mirabilis* Skottsberg, 1907							+	+	+	A
Desmarestiaceae	*Desmarestia anceps* Montagne, 1842	+						+	+	+	A
Desmarestiaceae	*Desmarestia antarctica* R.L. Moe and P.C. Silva, 1989	+		+		+		+	+	+	C
Desmarestiaceae	*Desmarestia menziesii* J. Agardh, 1848					+	+	+			A
Desmarestiaceae	*Himantothallus grandifolius* (A. Gepp and E.S. Gepp) Zinova, 1959							+	+		A
Desmarestiaceae	*Phaeurus antarcticus* Skottsberg, 1907						+	+	+		A
Phaeosaccionaceae	*Antarctosaccion applanatum* (Gain) Delépine, 1970									+	R
Seirococcaceae	*Cystosphaera jacquinotti* (Montagne) Skottsberg, 1907							+	+		A
Rhodophyta											
Florideophyceae											
Areschougiaceae	*Notophycus fimbriatus* R.L. Moe, 1986[a]							+	+		A
Bangiaceae	*Porphyra plocamiestris* R.W. Ricker, 1987[a]			+		+		+	+		A
Bonnemaisoniaceae	*Delisea pulchra* (Greville) Montagne, 1844[a]							+			R
Corallinaceae	Corallinaceae	+						+	+	+	A
Delesseriaceae	*Neuroglossum delesseriae* (Reinsch) M.J. Wynne, 1997						+	+	+	+	A
Delesseriaceae	*Paraglossum salicifolium (Reinsch)* -M. Lin, Fredericq and Hommersand, 2012					+		+			A
Delesseriaceae	*Phycodrys antarctica* (Skottsberg) Skottsberg, 1923							+	+		A
Fryeellaceae	*Hymenocladiopsis prolifera* (Reinsch) M.J. Wynne, 2004				+				+		A
Gigartinaceae	*Gigartina skottsbergii* Setchell and N.L. Gardner, 1936							+			R
Gigartinaceae	*Iridaea cordata* (Turner) Bory de Saint-Vincent, 1826	+		+		+		+			A
Gigartinaceae	*Sarcothalia papillosa* (Bory de Saint-Vicent) Leister, 1993							+			R
Palmariaceae	*Palmaria decipiens* (Reinsch) R.W. Ricker, 1987	+		+		+					A
Phyllophoraceae	*Gymnogongrus antarcticus* Skottsberg, 1953							+			R
Phyllophoraceae	*Gymnogongrus turquetii* Hariot, 1907							+			R
Phyllophoraceae	*Phyllophora ahnfeltioides* Skottsberg, 1919							+			R
Plocamiaceae	*Plocamium cartilagineum* (Linnaeus) P.S. Dixon, 1967							+		+	A
Rhodymeniaceae	*Rhodymenia coccocarpa* (Montagne) M.J. Wynne, 2007[a]						+	+	+		A
Wrangeliaceae	*Georgiella confluens* (Reinsch) Kylin, 1956					+	+	+	+	+	C
Porifera											
Demospongiae											
Acarnidae	*Iophon hesperidesi* Rios, Cristobo and Urgorri, 2004[a]								+		R
Axinellidae	*Axinella crinita* Thiele, 1905[a]								+		R
Chalinidae	*Haliclona penicillata* (Topsent, 1908)[a]							+	+		A
Chalinidae	Haliclona sp.1							+	+		A
Chalinidae	Haliclona sp.2								+		R
Chalinidae	Haliclona sp.3								+		R
Darwinellidae	*Dendrilla antarctica* Topsent, 1905[a]							+	+		A
Hymedesmiidae	*Kirkpatrickia variolosa* (Kirkpatrick, 1907)								+		R
Hymedesmiidae	*Phorbas areolatus* Bergquist and Fromont, 1988[a]								+		R
Isodictyidae	*Isodictya kerguelenensis* (Ridley and Dendy, 1886)[a]							+	+		A
Leucettidae	*Leucetta* sp.1								+		R
Leucettidae	*Leucetta* sp.2								+		R
Microcionidae	*Clathria* sp.							+	+		A
Mycalidae	*Mycale (Oximicale) acerata* Kirkpatrick, 1907							+	+		A
Mycalidae	*Mycale* sp.						+		+		A
Myxilidae	*Myxilla (Burtonanchora) asigmata* (Topsent, 1901)[a]								+		R
Myxilidae	*Myxilla (Burtonanchora) lissostyla* Burton, 1938[a]								+		R
Niphatidae	*Hemigellius pilosus* (Kirkpatrick, 1907)[a]							+	+		A
Polymastiidae	*Polymastia invaginata* Kirkpatrick, 1907[a]								+		R
Polymastiidae	*Sphaerotylus antarcticus* Kirkpatrick, 1907							+	+	+	A
Tedaniidae	*Tedania* sp.								+		R
	Haplosclerida sp.1								+		R
	Haplosclerida sp.2						+	+	+		A
	Haplosclerida sp.3							+	+		A
	Poecilosclerida						+	+		+	A
Cnidaria											
Anthozoa											
Alcyoniidae	*Alcyonium haddoni* Wright and Studer, 1889[a]							+	+		A
Edwardsiidae	*Edwardsia meridionalis* Williams, 1981[a]							+		+	A
Actiniidae	*Isotealia antarctica* Carlgren, 1899[a]					+		+	+		A
Hydrozoa											
Symplectoscyphidae	Antarctoscyphus sp.								+		R
Zancleidae	*Zanclea* sp.							+	+		A
Ctenofora											
Tentaculata											

(*continued on next page*)

Table A1 (*continued*)

	Taxa	BAE	FUM	TEL	PEN	BID	COL	WHB	NEP	PET	CI
Bolinopsidae	*Mnemiopsis* sp.	+									R
Platyhelminthes											
Rhabditophora											
Uteriporidae	*Obrimoposthia wandeli* (Hallez, 1906)[a]				+						R
	Unidentified Platyhelminthe		+							+	A
Nemertea											
Anopla											
Lineidae	*Parborlasia corrugatus* (McIntosh, 1876)	+	+	+		+	+	+	+	+	C
Enopla											
Tetrastemmatidae	*Antarctonemertes riesgoae* Taboada et al., 2013[a]	+				+					A
Tetrastemmatidae	*Antarctonemertes valida* (Bürger, 1893)[a]	+	+				+	+	+	+	C
	Nemertea sp.								+	+	A
Priapulida											
Priapulida											
Priapulidae	*Priapulus tuberculatospinosus* (Baird, 1868)					+		+			A
Mollusca											
Bivalvia											
Laternulidae	*Laternula elliptica* (King, 1832)			+		+	+	+	+		C
Limidae	*Limatula hodgsoni* (E. A. Smith, 1907)[a]							+	+		A
Sareptidae	*Aequiyoldia eightsii* (Jay, 1839)					+		+			A
Gastropoda											
Naticidae	*Amauropsis aureolutea* (Strebel, 1908)[a]							+	+		A
Dorididae	*Doris kerguelenensis* (Bergh, 1884)[a]							+	+		A
Charcotiidae	*Charcotia granulosa* Vayssière, 1906[a]							+	+		A
Clionidae	*Clione limacina* (Phipps, 1774)[a]	+						+	+		A
Fionidae	*Cuthona crinita* Minichev, 1972[a]								+		R
Fionidae	*Cuthona modesta* (Eliot, 1907)[a]							+	+		A
Limacinidae	*Limacina antarctica* Woodward, 1854	+						+	+		A
Calliostomatidae	*Margarella antarctica* (Lamy, 1905)[a]							+	+		A
Nacellidae	*Nacella concinna* (Strebel, 1908)	+		+		+	+	+	+	+	C
Naticidae	*Natica* sp.								+		R
Buccinidae	*Parabuccinum bisculptum* (Dell, 1990)[a]						+	+	+		A
Littorinidae	*Pellilitorina pellita* (Martens, 1885)[a]							+			R
Philinorbidae	*Antarctophiline alata* (Thiele, 1912)[a]		+								R
Pneumodermatidae	*Spongiobranchaea australis* d'Orbigny, 1836[a]	+									R
Polyplacophora											
Callochitonidae	*Callochiton steinenii* (Pfeffer, 1886)[a]							+			R
Mopaliidae	*Nuttallochiton mirandus* (Thiele, 1906)[a]							+	+	+	A
Annelida											
Clitellata											
Piscicolidae	Piscicolidae sp.							+	+	+	A
Polychaeta											
Flabelligeridae	*Flabegraviera mundata* (Gravier, 1906)	+				+		+			A
Nephtyidae	*Aglaophamus trissophyllus* (Grube, 1877)	+	+			+	+	+	+	+	C
Nereididae	*Neanthes kerguelenensis* (McIntosh, 1885)								+		R
Orbinidae	*Leitoscoloplos kerguelensis* (McIntosh, 1885)	+	+	+	+	+	+	+	+	+	C
Orbiniidae	*Phylo foetida* (Claparède, 1869)[a]					+					R
Polynoidae	Polynoidae sp.1	+				+		+	+		A
Polynoidae	Polynoidae sp.2							+	+		A
Sabellidae	*Perkinsiana* sp.									+	R
Terebellidae	Terebellidae sp.1							+	+		A
Terebellidae	Terebellidae sp.2							+	+		A
Arthropoda											
Malacostraca											
Caprellidae	Caprellidae sp.							+			R
Chaetiliidae	*Glyptonotus antarcticus* Eights, 1852					+			+		A
Euphausiidae	*Euphausia superba* Dana, 1850	+							+		A
Lysianassidae	*Cheirimedon femoratus* (Pfeffer, 1888)	+			+			+		+	A
Pontogeneiidae	*Gondogeneia antarctica* (Chevreux, 1906)	+		+			+	+	+	+	C
Serolidae	*Ceratoserolis* sp.	+	+	+	+	+	+	+	+	+	C
Serolidae	*Spinoserolis* sp.	+						+			A
	Amphipoda spp.	+			+			+	+	+	C
Pycnogonida											
Nymphonidae	*Nymphon australe* Hodgson, 1902							+	+	+	A
Echinodermata											
Asteroidea											
Asteridae	*Diplasterias brucei* (Koehler, 1908)							+	+	+	A
Asteridae	*Lysasterias* sp.							+	+		A
Ganeriidae	*Perknaster fuscus* Sladen, 1889[a]							+			R
Heliasteridae	*Labidiaster annulatus* Sladen, 1889[a]							+	+		A
Odontasteridae	*Odontaster meridionalis* (E. A. Smith, 1876)							+	+		A
Odontasteridae	*Odontaster validus* Koehler, 1906	+	+	+		+	+	+	+		C
Poraniidae	*Glabraster antarctica* (E. A. Smith, 1876)[a]							+	+		A
Echinoidea											
Echinidae	*Sterechinus neumayeri* (Meissner, 1900)	+	+	+	+	+		+	+		C

(*continued on next page*)

Table A1 (*continued*)

	Taxa	BAE	FUM	TEL	PEN	BID	COL	WHB	NEP	PET	CI
Schizasteridae	*Abatus agassizii* Mortensen, 1910[a]								+		R
Holothuroidea											
Cucumariidae	*Cucumaria* cf. *georgiana* (Lampert, 1886)								+		R
Cucumariidae	*Heterocucumis steineni* (Ludwig, 1898)[a]								+		R
Psolidae	*Psolus granulosus* Vaney, 1906[a]							+		+	A
Ophiuroidea											
Ophiuridae	*Ophionotus victoriae* Bell, 1902	+	+	+	+	+	+	+	+		C
Brachiopoda											
Rhynchonellata											
Terebratulidae	*Liothyrella uva* (Broderip, 1833)[a]							+	+		A
Bryozoa											
Gymnolaemata											
Hippothoidae	*Antarctothoa polystachya* Wright, Hayward and Hughes, 2007[a]									+	R
Arachnopusiidae	*Arachnopusia decipiens* Hayward and Thorpe, 1988[a]								+		R
Beaniidae	*Beania erecta* Waters, 1904							+	+		A
Bugulidae	*Bugula longissima* Busk, 1884[a]							+	+		A
Bugulidae	*Camptoplites angustus* (Kluge, 1914)								+		R
Bugulidae	*Camptoplites giganteus* (Kluge, 1914)							+	+		A
Bugulidae	*Camptoplites latus* (Kluge, 1914)[a]							+	+		A
Flustridae	*Carbasea ovoidea* Busk, 1852								+		R
Chaperiidae	*Chaperiopsis* sp.							+			R
Calloporidae	*Ellisina antarctica* Hastings, 1945							+			R
Electridae	*Harpecia spinosissima* (Calvet, 1904)							+			R
Buffonellodidae	*Hippadenella* cf. *inerma* (Calvet, 1909)	+									R
Inversiuliidae	*Inversiula nutrix* Jullien, 1888	+									R
Chordata											
Ascidiacea											
Ascidiidae	*Ascidia challengeri* Herdman, 1882					+		+	+		A
Corellidae	*Corella eumyota* Traustedt, 1882[a]					+		+	+		A
Styelidae	*Cnemidocarpa verrucosa* (Lesson, 1830)			+		+	+	+	+	+	C
Thaliacea											
Salpidae	*Salpa thompsoni* Foxton, 1961[a]	+	+			+	+	+	+	+	C

[a] New records at Deception Island. Constancy Index (C.I.); C represent constant/conspicuous species, A; accessory species and R; rare/accidental species. The occurrence is indicated (+) along the table.

Appendix B. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.csr.2018.02.005.

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RESEARCH ARTICLE

Epiphytic diatom community structure and richness is determined by macroalgal host and location in the South Shetland Islands (Antarctica)

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Abstract

The marine waters around the South Shetland Islands are paramount in the primary production of this Antarctic ecosystem. With the increasing effects of climate change and the annual retreat of the ice shelf, the importance of macroalgae and their diatom epiphytes in primary production also increases. The relationships and interactions between these organisms have scarcely been studied in Antarctica, and even less in the volcanic ecosystem of Deception Island, which can be seen as a natural proxy of climate change in Antarctica because of its vulcanism, and the open marine system of Livingston Island. In this study we investigated the composition of the diatom communities in the context of their macroalgal hosts and different environmental factors. We used a non-acidic method for diatom digestion, followed by slidescanning and diatom identification by manual annotation through a web-browser-based image annotation platform. Epiphytic diatom species richness was higher on Deception Island as a whole, whereas individual macroalgal specimens harboured richer diatom assemblages on Livingston Island. We hypothesize this a possible result of a higher diversity of ecological niches in the unique volcanic environment of Deception Island. Overall, our study revealed higher species richness and diversity than previous studies of macroalgae-inhabiting diatoms in Antarctica, which could however be the result of the different preparation methodologies used in the different studies, rather than an indication of a higher species richness on Deception Island and Livingston Island than other Antarctic localities.

Introduction

On Antarctic coasts, marine macro- and microalgae in ice and benthos are the main primary producers [1]. When free of shelf-ice, these benthic producers can account for up to 90% of

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the total net primary production of the ecosystem [1]. The surface of macroalgal hosts also serves as habitat for benthic microalgae (mainly diatoms) in the Antarctic and Subantarctic regions. Although macroalgae cannot be interpreted either as synonymous with or part of plants in the systematic sense, these macroalgae-inhabiting diatom assemblages are usually referred to as "epiphytic" in the literature [2–4]. This association provides a basis for complex ecological interactions between diatoms and macroalgal hosts, which are only partially understood [5]. It is known that epiphytic diatoms can facilitate the adherence of other organisms to any substrate [6]. Epiphytic diatoms and other biofouling organisms can also reduce light intensity available to the host algae [7]. Interactions with surface-inhabiting diatoms can influence the performance of macro- and microalgae species to acclimate or adapt to new ecosystem pressures like climate change [8,9] or invasive species from lower latitudes [10,11].

Previous studies on macroalgae- inhabiting diatoms have focussed on the taxonomic composition and ecology of the epiphytic diatom flora on macroalgae around Antarctica [2,3,5,12–15] or terrestrial habitats [16]. Some studies focussed on the floristics and ecology of taxonomically diverse hosts at a single location, constructing a flora of Vestfold Hills [12] and King George's Island Potter Cove [2] respectively. Majewska et al. took a different approach by characterizing the epiphytic diatom ecology and flora of a small number of host taxa [3,13–15] across different locations [15].

In this study, diatom communities collected from diverse algal hosts belonging to different classes from two islands of the South Shetland archipelago, Deception (DI) and Livingston Islands (LI), were investigated. These islands differ strongly in their geology and geomorphology: DI is an active volcano, with comparatively young coastal ecosystems that undergo thermal disturbance due to volcanic activities on an irregular basis [17]. In contrast, LI harbours relatively undisturbed, pristine coastal locations with very steep inner slope moraines [18]. We attempt to interpret differences in epiphytic diatom compositions in a framework of environmental sorting effects resulting from differences in abiotic environments (island geology / coastline ecology, including depth), biotic interactions with macroalgal hosts (host phylogenetic position and/or gross morphology) and of a presumably low, but perhaps not negligible dispersal limitation in shaping these diatom communities. Although limited sampling in this distant region affects our study and limits the causal interpretability of statistical comparisons, just as it does for similar investigations in general, we attempt to disentangle the correlative contribution of these factors to community differences, while also substantially extending our diatom floristic knowledge of the Antarctic region.

Materials and methods

A total of 36 macroalgal samples from 20 species and 2 macroscopically visible diatom community samples, i.e. diatom blooms attached to a substrate and visible to the naked eye, were taken in three consecutive annual expeditions (2017–2019) to the Antarctic South Shetland Islands, namely Deception (DI) and Livingston Island (LI) (Fig 1). The macroalgal samples were collected by hand from the intertidal range, as well as by snorkelling or by SCUBA divers at subtidal depths (Table 1). Simultaneously, temperature measurements were taken. All sampling permits (CPE-EIA-2013-7, CPE-EIA-2015-7, and CPE-EIA-2017-7) were issued by the Spanish Polar Committee within the Antarctic projects DISTANTCOM and BLUEBIO (CTM2013-42667/ANT and CTM2016-78901).

Macroalgae were obtained simultaneously with other benthic organisms at each sampling spot and pooled together in 1L receptacles, keeping different algal species separated from each other. At the wet lab, the specimens were sorted by *phylum* and identified to lowest taxon, usually species, following literature [19,20]. The species samples of one sampling site and day were

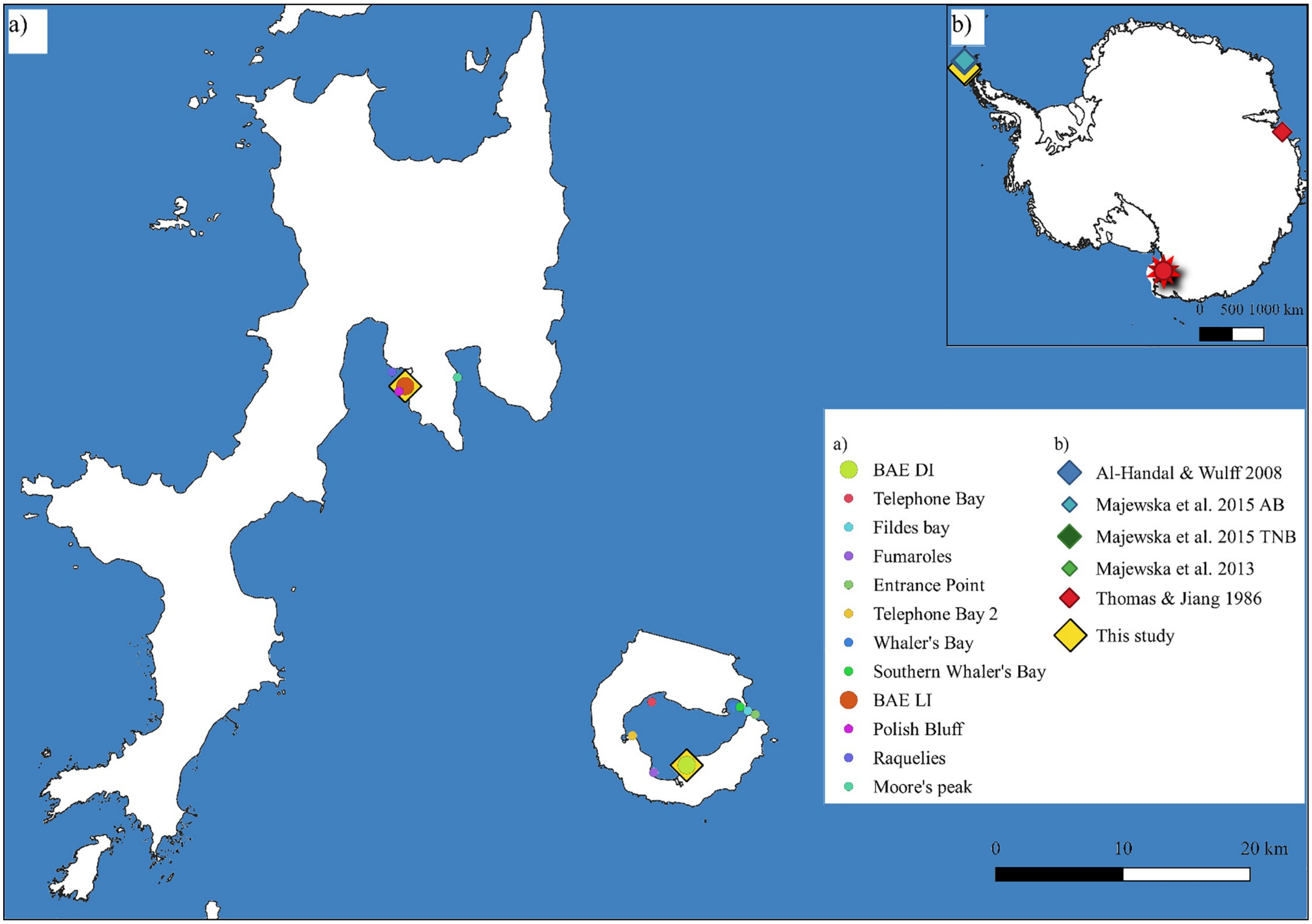

Fig 1. Sampling localities. a) Distribution of sampling sites in Livingston (LI) and Deception Islands (DI). b) sampling sites from previous studies from the literature. The yellow rectangle in b) shows the location of LI and DI. Map constructed with QGIS software.

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kept in separate zip-style bags and frozen at -20˚C until further processing at the University of Barcelona. The macroalgal attributes of branching pattern, based on thallus morphology, and age, meaning the annuality of macroalgae (annual, biannual or pluriannual) of each species, were ascertained according to literature e.g. [19,21,22]. Epiphytic diatoms were extracted under laboratory conditions using a small part of the macroalgae, an overall appraisal of epiphyte incidence was made before scraping the surface into the receptacle with 80–100 ml of water depending on epiphyte density. The algae were also immersed and the samples were centrifuged [e.g. 23]. After this, the macroalgal part was extracted again for further use. Depending on epiphyte concentration, several aliquots were made and fixated using ethanol.

Diatoms were washed in distilled water to reduce remaining salinity. Samples were homogenized and centrifuged at 1000 rpm (Eppendorf centrifuge 5810 R, Eppendorf AG, Germany) for five minutes, and again filled up with deionized water. This procedure was repeated five times. Diatoms were prepared using the Friedrichs' [24] variation of Carr et al.'s method [25], using ten times diluted bleach (Domol Hygiene Reiniger, AGB Rossmann GmbH), based on 5% sodium hypochlorite as the undiluted oxidizing agent, with a treatment period of 30–45 min depending on the amount of organic matter present in the sample. The thus cleaned

Table 1. Sample descriptions: Number of samples, replicates and macroalgal hosts.

	Host class	Host thallus morphology	Host annuality	Number of diatom taxa found (genera)	Number of samples (replicates)	Depth [m]	Year	Island
***Adenocystis utricularis* (Bory) Skottsberg)**	Phaeo	Sac, Unb	A	47 (22)	1 (2)	0	2018	LI
***Ballia callitricha* (C. Agardh) Kützing**	Rhodo	Fil, Bra	A, B	41 (14)	1	22.1	2018	DI
***Berkeleya rutilans* (Trenthepohl ex Roth) Grrunow**	Bacill.	Fil	A	16 (7)	1	4.5	2017	DI
***Cystosphaera jacquinotii* (Montagne) Skottsberg**	Rhodo	Lam, Bra	P	25 (9)	1	27	2017	DI
***Delisea pulchra* (Greville) Montagne**	Rhodo	Fil, Bra	P	50 (16)	4	21–23.4	2018, 2019	LI
***Desmarestia anceps* Montagne**	Phaeo	EBT	P	44 (16)	3	0–22	2017	DI
***D. antarctica* R. L. Moe & P. C. Silva**	Phaeo	EBT	P	57 (24)	2	0–13	2017, 2018	DI, LI
***Desmarestia* sp J. V. Lamouroux**	Phaeo	EBT	P	18 (5)	1	25	2017	DI
***Gigartina skottsbergii* Setchell & N. L. Gardner**	Rhodo	Lam, Bra	A,B	53 (17)	3	5.5–23.4	2017–19	DI, LI
***Gymnogongrus cf. turquettii* Hariot**	Rhodo	Lam, Bra	A, B	47 (11)	2	23–23.4	2018, 2019	LI
***Himantothallus grandifolius* (A. Gepp & E.S. Gepp) Zinova**	Phaeo	Lam, Unb	P	50 (18)	3	23–25	2017, 2018	DI, LI
***Iridaea cordata* (Turner) Bory**	Rhodo	Lam, Unb	A, B	46 (15)	3	0–25	2017, 2018	DI, LI
***Monostroma hariotii* Gain**	Chloro	Lam, Bra	A, B	16 (7)	1	23	2018	LI
***Myriogramme manginii* (Gain) Skottsberg**	Rhodo	Lam, Bra	Pp	32 (11)	1	22.1	2018	LI
***Palmaria decipiens* (Reinsch) R. W. Ricker**	Rhodo	Lam	Pp	55 (23)	3	2–17.5	2017, 2018	DI, LI
***Picconiella plumosa* (Kylin) J. De Toni**	Rhodo	BT	A, B	40 (13)	1	22.1	2018	LI
***Plocamium cartilagineum* (Linnaeus) P.S. Dixon**	Rhodo	EBT	A, B	50 (15)	4	22.1–25	2018, 2019	LI
***P. cf. hookeri* Harvey**	Rhodo	EBT	A, B	23 (5)	1	20	2018	LI
***Pyropia endiviifolia*(A.Gepp & E.Gepp) H.G.Choi & M.S.Hwang**	Rhodo	Lam	A, B	39 (13)	1	23	2018	LI
***Brandinia mosimanniae* L.F. Fernandes & L. K. Procopiak [macroscopic]**	Bacill.	Fil	A	33 (17)	1	8.2	2018	DI
In total				131(85)	38	0–25	2017–2019	DI, LI
In total (macroalgae)				120 (47)	36	0–25	2017–2019	DI, LI

Class names: Phaeo = Phaeophyceae, Rhodo = Rhodophyta, Chloro = Chlorophyta, Bacill = Bacillariophyceae. Sampling sites: LI = Livingston Island, DI = Deception Island. Morphological trends: EBT = Erect Branched Thallus, BT = Branched Thallus, Sac = Saccular Thallus, Fil = Filament, Lam = Laminar Thallus, Bra = Branched, Unb = Unbranched. Annual trends: P = Perennial, Pp = Pseudoperennial, B = Biannual, A = Annual.

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diatom frustules were washed five times following the same procedure as before the bleach treatment. The frustule suspensions were then dripped onto coverslips, left to dry, quality checked and mounted using Norland Optical Adhesive 61 (refraction index = 1.56, Norland Products Inc., Cranbury, New Jersey, US).

Slide scanning methodology was modified after Kloster et al. [26] using a Metafer 4 slide scanner system (MetaSystems, Altlussheim, Germany) attached to an Axio Imager.Z2 microscope (Carl ZEISS AG, Oberkochen, Germany). The scans were made with a 63x objective

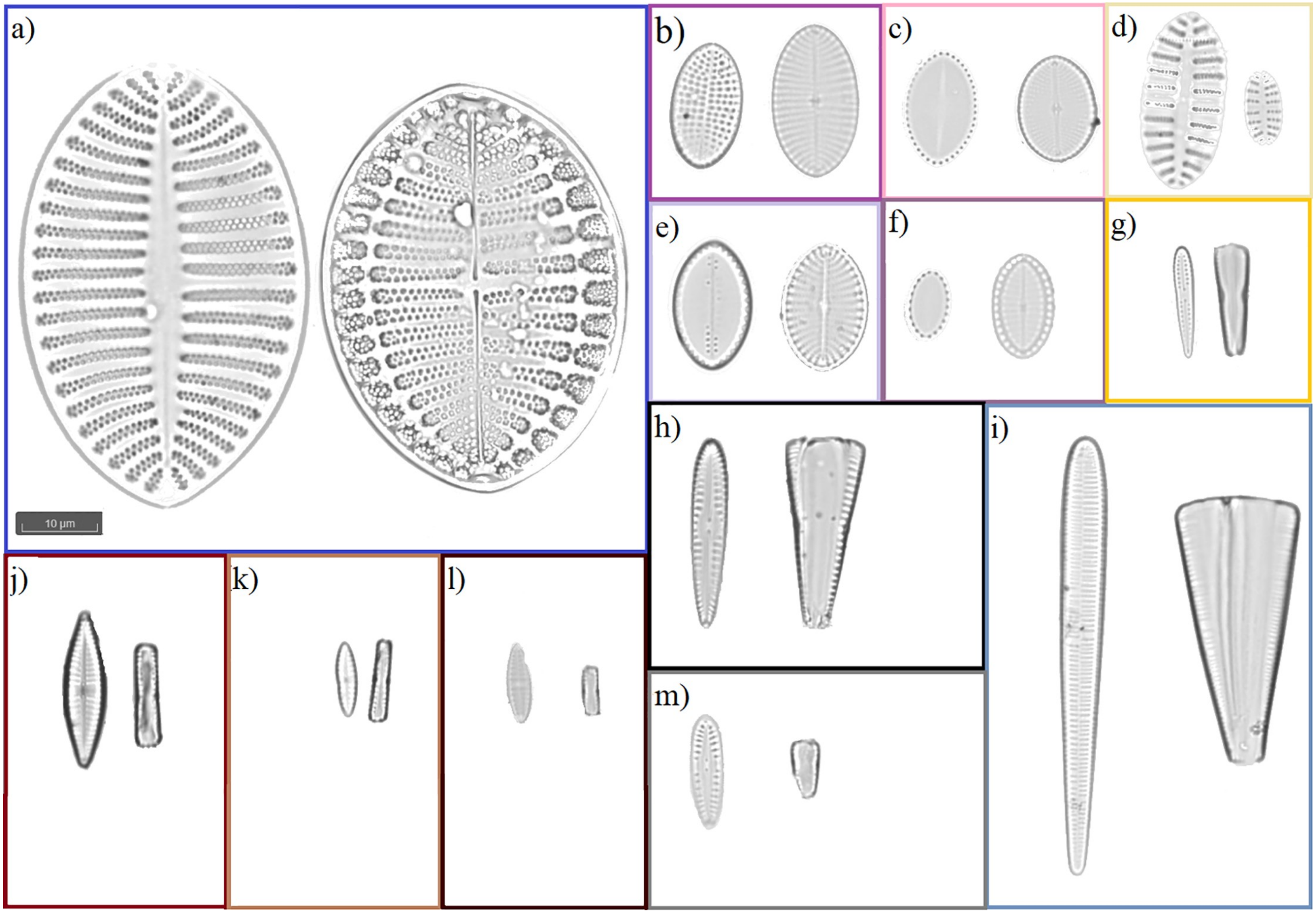

Fig 2. Predominant diatoms found on Antarctic macrophytes. Monoraphid diatoms shown in raphe and raphe-less valve view. Biraphids shown in valvar and pleural view. a) *Cocconeis fasciolata*, b) *Cocconeis californica var. californica*, c) *Cocconeis dallmannii*, d) *Cocconeis sp. 1*, e) *Cocconeis californica var. kerguelensis*, f) *Cocconeis melchioroides*, g) *Gomphonemopsis cf. ligowskii*, h) *Pseudogomphonema kamtschaticum*, i) *Licmophora gracilis*, j) *Navicula glaciei*, k) *Navicula incertata*, l) *Navicula perminuta*, m) *Pseudogomphonema sp. 1*.

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(Zeiss Plan-Apochromat 1.4 with oil immersion) for an area of 54 x 75 visual fields, resulting in 4,050 images per slide, covering an area of 42.5 mm^2. For each field of view, images at 80 different focus levels were taken and combined to extended focus images. For the resulting images 980 pixels equal 100 μm, (e. g. see Fig 2 and images available in PANGAEA: https://doi.pangaea.de/10.1594/PANGAEA.925913.

The 4,050 extended focus depth images depicting each sample slide were stitched together to a virtual slide image using the Fiji image processing software [27]. First, the MIST plug-in [28–30] was used to calculate the exact relative position of the individual field of view-images. Subsequently, the tool MIST converter (Kloster, unpublished) was used to subdivide the slide into 3 segments of not more than 2 GB each and to process the position data for the last step, which utilized Grid / collection stitching ImageJ plug-in [31] for composing the virtual slide images. For each slide, this resulted in three virtual slide images which were then uploaded into the web-based annotation tool BIIGLE 2.0 [32].

In BIIGLE, the “random sampling” function was used to manually examine up to 400 randomly distributed sections of each virtual slide image at high magnification. Diatoms contained within these sections were identified manually until ca. 500 identified specimens were reached for each sample, which mostly was the case during analysing the first of the three virtual slide image segments. In two instances material density on the slide was too low to allow for 500 annotations even after examining all three image segments comprising the sample, but the number of results was deemed sufficient to account for statistical significance. Diatoms were identified using epiphyte specific (e. g. [2,3,13–15]) and general [33,34] taxonomic bibliography to the lowest possible level. For each diatom specimen identified in this procedure, also their position (valvar vs. pleural view) and the presence of teratologic deformations was recorded. Teratologies refer to malformations, i.e. deviations from usual species-specific outline form or valve pattern, that can occur as a result of biotic and abiotic stresses [35].

Once all slides were identified, diatom inventories per virtual slide image were downloaded and, in cases where multiple images for the same slide were annotated, their counts were combined. The resulting inventories were turned into relative abundances (%). We calculated species richness and Shannon diversity [36]. To reduce influence of dominant taxa, relative abundances were square root transformed. Records of epiphytic diatom taxa were collated from previous studies undertaken around Antarctica (Fig 1), namely the South Shetland Islands (King George Island, in Admiralty Bay [15] and Potter Cove [2]), in McMurdo Sound (Terranova Bay [13–15] and Cape Evans [3]) and the Vestfold Hills (Davis Station [12]). Since the methodologies diverged in these studies and Thomas & Jiang did not provide numeric abundances, we used presence-absence data from the identified diatoms in each study. Slide scans and image cut-out of every single specimen identified in our study can be accessed in PANGAEA (https://doi.pangaea.de/10.1594/PANGAEA.925913). Statisical analyses, R Script and data matrices used are available in DRYAD (doi: https://doi.org/10.5061/dryad.ngf1vhhsm:).

Statistical analysis

All statistical analyses were made with R software version 3.6.1 [37] on RStudio version 1.2.5019 [38]. Characterization by host species, thallus morphology, and branching pattern as well as annuality was made using IndVal algorithms [39]. The differential ternary graph showing species distributions of the epiphytic diatoms between three host classes (Phaeophyceae, Rhodophyta and Chlorophyta) was made using the 'ggtern' package [40]. Most multivariate analyses (non-metric dimensional scaling–nMDS-, principal component analysis—PCA) and analysis of similarity (ANOSIM), as well as the richness and diversity measures were calculated using the package 'vegan' [41]. Similarity of percentages (SIMPER) analyses were made using PRIMER software 7.0.13 (Primer-e Quest Research Limited, Auckland, New Zealand). Iterative hierarchical clustering was performed with 'cluster' [42] and 'pvclust' [43] packages. A Mantel test was performed combining the 'geosphere' [44] and 'vegan' packages When significant (P-value < 0.05), these values were further characterized as highly significant (***, $p<0.001$), very significant (** $0.001< p < 0.01$), or significant (*, $0.01 < p <0.05$). The map in Fig 1 was constructed with QGIS software v. 3.16, [45] with the Quantarctica package (ADD_Coastline_res_line_Sliced) [46].

Results

Epiphytic diatom floristics and ecology

A total of 120 species of diatoms of 47 genera (S1 Table) were identified from 36 Antarctic macroalgae (Table 1). All macroalgae studied had varying degrees of epiphytic diatom colonization, with a range of 13 to 56 species per sample. The most frequent and predominant species of diatoms found in association with macroalgae (Fig 2) were generalist diatoms such as *Pseudogomphonema kamtschaticum* (Grunow) Medlin (up to 25% relative abundance in a sample, present in all but one samples) or as yet undescribed species as *Navicula cf. perminuta* Grunow (up to 64% relative abundance in a sample, present in all samples) and *Pseudogomphonema* sp. 1 (up to 59% relative abundance in a sample, present in 29 samples). We recorded 19 diatom species not previously reported from these islands (S1 Table). Teratological frustules accounted for 0 to 2.3% of the counted cells. Diatoms had more teratologies on Rhodophyta (with an incidence of up to 2.4% of the sample and for 57.89% of all samples) than on Phaeophyceae (with an incidence of under 1%, in 32.89% of the samples).

Shannon diversity and diatom species richness (Table 2) did not follow a clear trend with location or depth. Neither host class nor host species was decisive for species richness. However, diatom species composition changed significantly for host class (Mantel statistic $r = 0.45^{***}$). Shannon diversity on Phaeophyceae varied in a wider range (H' = 0.97–3.03) than on Rhodophyta (H' = 1.33–2.64), macroscopically visible diatoms (H' = 0.98–1.38) or Chlorophyta (H' = 1.49). Species richness was also more variable on Phaeophyceae (S = 13–56) than on Rhodophyta (S = 13–42), Bacillariophyceae (S = 16–33) and the Chlorophyta sample (S = 16). For some macroalgae species, different individual samples had similar diversity (such as *Delisea pulchra*, H' = 1.81–2.02) but varying species richness values (S = 19–28), or the other way around, as with *Iridaea cordata* (H' = 1.51–2.35, S = 18–23). In the case of *Himantothallus grandifolius*, diversity had a very wide range (H' = 0.97–2.66) as did species richness (H' = 13–39). All rarefaction curves calculated per host were saturated (S1 Fig) and Rhodophyta had the highest species richness of all macrophytes studied.

The ternary plot illustrates the preferences between the host groups for the predominant species (Fig 3), where only *Pseudogomphonema kamtschaticum* showed no host preference at all (Fig 3). On the other hand, some diatom-macroalgae class relationships are rather specific, as *Licmophora gracilis* was found mostly on the Chlorophyta, *Cocconeis melchioroides* on Rhodophyta, and *Cocconeis fasciolata* on Phaeophyceae. The ternary plot (Fig 3) further shows that most diatom taxa were shared amongst Phaeophyceae and Rhodophyta. Since only one Chlorophyta was sampled, some or all of these might represent host generalist taxa which would also be found on Chlorophyta with more sampling effort.

ANOSIM showed that host class had the highest impact of the macroalgal characteristics on diatom distribution ($R = 0.47^{***}$). Host branching patterns ($R = 0.17^{*}$) and annuality ($R = 0.23^{***}$) also affected the diatom community to varying degrees. As the rarefaction curves (S1 Fig) show, only Phaeophyceae and Rhodophyta arrived at saturation levels with the samples explored. When comparing the diatom communities on these macroalgal classes (Phaeophyceae and Rhodophyta), only Rhodophyta had significant ANOSIM values, e.g. variation inside the class and between species (Table 3). Diatom communities growing on Rhodophyta were found to be substantially shaped by locality ($R = 0.39^{***}$), year ($R = 0.38^{***}$), and annuality of the host ($R = 0.25^{***}$).

The nMDS multivariate analysis (Fig 4) performed on diatom communities showed a small degree of differentiation depending on macroalgal host. A two-dimensional solution was sufficient due to the low stress value recorded (0.16). On the other hand, a SIMPER analysis on predominant diatoms showed very high standard deviation levels (S2 Table). When looking into the most abundantly sampled Rhodophyta and Phaeophyceae (Table 4), the SIMPER analysis results showed that *Navicula perminuta*, *Gomphonemopsis ligowskii* and *Cocconeis melchioroides* were the most significant contributors to the average dissimilarity.

Diatom distribution in Antarctica

Diatoms in the South Shetland Islands. The annual mean temperature of both islands was 2˚C, but the temperature range in DI comprised 0–4˚C (increasing even more towards the

Table 2. Sampling site characterization of depth and temperature (T) and diatom epiphyte richness (S) and diversity (H') found on each macroalgal host.

Locations	Depth (m)	T (˚C)	Macroalgal host	Species diversity (H')	S
Livingston–Raquelies	13	/	*Adenocystis utricularis*	1.76	45
Deception–Antarctic base	4.5	3	*Berkeleya rutilans*	0.98	16
Deception–Fumaroles	8.2	*	*Brandinia sp.*	1.38	33
Livingston–Moore's peak	22.1	2	*Ballia callitricha*	2.58	40
Deception–Whaler's bay	25	2	*Cystosphaera jacquinottii*	1.74	24
Livingston–Polish Bluff	21	3	*Delisea pulchra*	2.02	27
Livingston–Polish Bluff	22.1	1	*Delisea pulchra*	1.81	19
Livingston–Moore's peak	23	2	*Delisea pulchra*	1.93	20
Livingston–Polish Bluff	23.4	2	*Delisea pulchra*	2.02	28
Deception–front of base	0	/	*Desmarestia anceps*	2.17	30
Deception–front of base	0	/	*Desmarestia anceps*	1.51	13
Deception–Fildes bay	22	3	*Desmarestia anceps*	1.33	22
Livingston–Raquelies	13	2	*Desmarestia antactica*	3.03	56
Deception–Antarctic base	0	/	*Desmarestia antarctica*	1.76	17
Deception–Whaler's bay	25	2	*Desmarestia* sp	2.08	17
Deception–Seal colony	5.5	4	*Gigartina skottsbergii*	2.56	42
Livingston–Moore's peak	22.1	2	*Gigartina skottsbergii*	2.18	21
Livingston–Polish Bluff	23.4	2	*Gigartina skottsbergii*	1.34	13
Livingston–Polish Bluff	23.4	2	*Gymnogogrus turquettii*	1.77	32
Livingston–Moore's peak	23	2	*Gymnogongrus turquettii*	2.09	37
Deception–Fildes bay	25	2	*Himantothallus grandifolius*	0.97	13
Deception–Whaler's bay	25	2	*Himantothallus grandifolius*	2.66	39
Livingston–Moore's peak	23	2	*Himantothallus grandifolius*	2.05	23
Deception–front of base	0	/	*Iridaea cordata*	1.51	18
Deception–Whaler's bay	25	2	*Iridaea cordata*	2.35	23
Livingston–Moore's peak	22.1	1	*Iridaea cordata*	2.25	23
Livingston–Moore's peak	23	2	*Monostroma hariotii*	1.49	16
Livingston–Moore's peak	22.1	2	*Myriogramme cf. manguinii*	2.36	31
Deception–Drum	17.5	2	*Palmaria decipiens*	1.96	30
Deception–Telephone bay	14.1	3	*Palmaria decipiens*	1.43	30
Livingston–Antarctic Base	2	2	*Palmaria decipiens*	1.85	26
Livingston–Moore's peak	22.1	2	*Piccionella plumosa*	2.49	39
Livingston–Raquelies	25	2	*Plocamium cartilagineum*	2.18	22
Livingston–Moore's peak	22.1	1	*Plocamium cartilagineum*	2.19	30
Livingston–Moore's peak	23	2	*Plocamium cartilagineum*	2.31	30
Livingston–Polish Bluff	23.4	2	*Plocamium cartilagineum*	1.85	25
Livingston–Moore's peak	20	1	*Plocamium cf. hookerii*	2.01	23
Livingston–Moore's peak	23	2	*Pyropia endiviifolia*	2.64	38

Intertidal temperature was not recorded [/].

* Taken in the fumaroles, temperature estimated between 40–80˚C.

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fumaroles, but not recorded), and 1–3˚C in LI. A total of 15 samples came from DI (diatom epiphyte taxa n = 94) and 23 samples from LI (diatom epiphyte taxa n = 82), and 66 diatom taxa (21 genera) were shared between both islands. Diatom compositions of Rhodophyta and Phaeophyceae from DI and LI clustered together, separated from the Chlorophyta and macroscopic Bacillariophyceae samples, in hierarchical cluster analysis (distance = Euclidean,

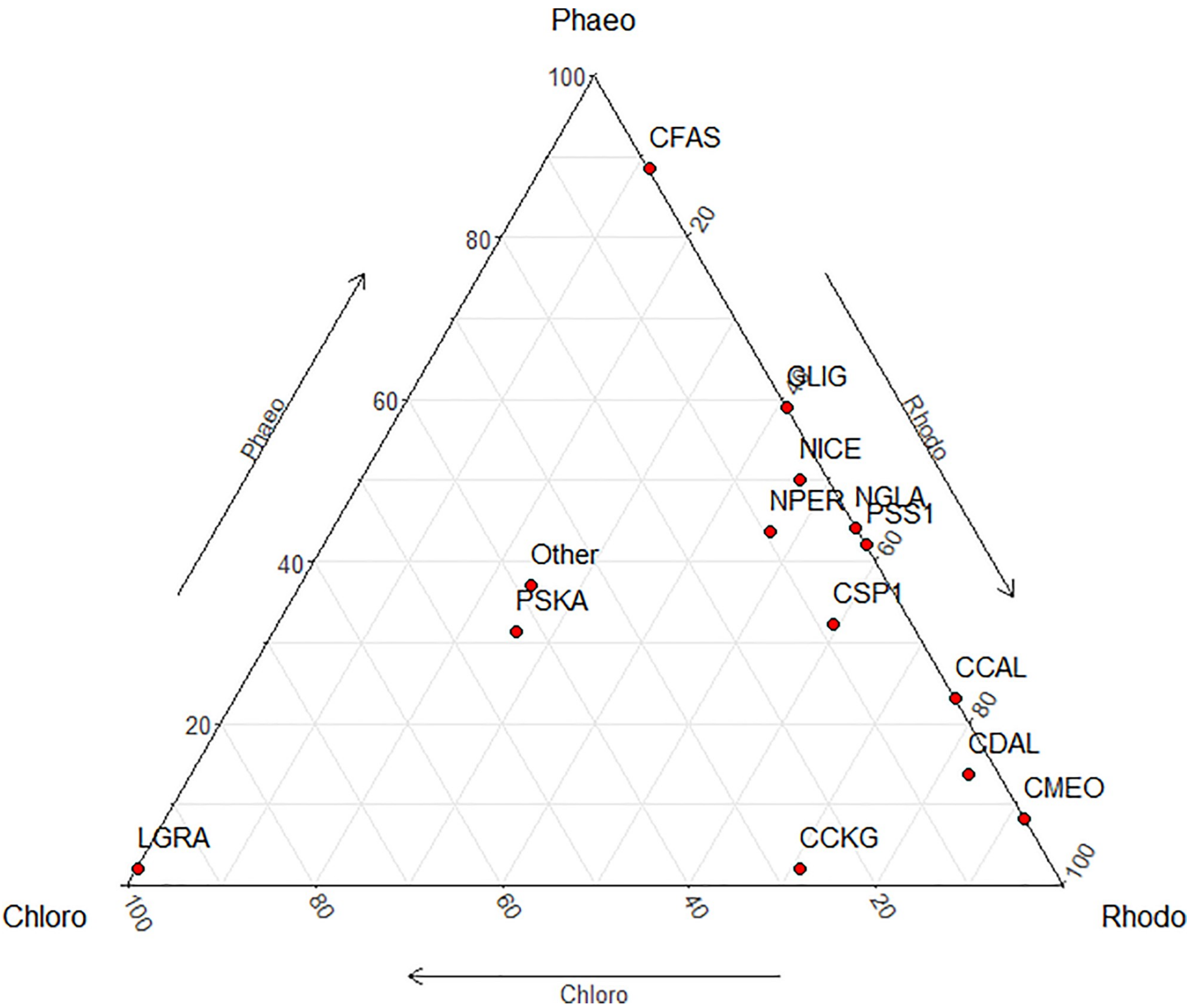

Fig 3. Ternary plot of predominant epiphytic diatoms shared between Rhodophyta (Rhodo), Phaeophyceaes (Phaeo) and Chlorophyta (Chloro). Species codes: CCAL = *Cocconeis californica*, CCKG = *Cocconeis californica var. kerguelensis*, CDAL = *Cocconeis dallmanii*, CFAS = *Cocconeis fasciolata*, CMEO = *Cocconeis melchioroides*, CSP1 = *Cocconeis sp. 1*, GLIG = *Gomphonemopsis ligowskii*, LGRA = *Licmophora gracilis*, NGLA = *Navicula glacialis*, NICE = *Navicula incertata*, NPER = *Navicula perminuta*, PSKA = *Pseudogomphonema kamtschaticum*, PSS1 = *Pseudogomphonema sp 1*, Other = diatom species in under 2% frequency and abundance.

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cluster = average linkage, bootstrap = 94%, not shown). A redundancy analysis (RDA), on the other hand, showed a separation between DI and LI communities (Fig 5). This was corroborated by the results of the Mantel test (geographical distance matrix vs. diatom communities,

Table 3. ANOSIM test results performed on communities from Phaeophyceae (Phae) and Rhodophyta (Rhod) hosts.

	Loc		Depth		Depth Int		Year		Host Morph.		Host Branch		Host Annuality	
	Phae	Rhod	Phae	Rhod	Phae	Rhod	Phae	Rhod	Phae	Rhod	Phae	Rhod	Phae	Rhod
R	0.03	**0.39**	0.19	**0.26**	0.12	**0.36**	0.03	**0.38**	-0.02	0.12	-0.05	**0.29**	0.09	**0.37**
p-val	>0.05	0.002	>0.05	0.03	>0.05	0.01	>0.05	0.0008	>0.05	>0.05	>0.05	0.007	>0.05	0.003

Loc = Location, Depth Int = Depth Interval, Host Morph = Host Morphology, Host Branch = Host Branching pattern, Host Annual = Host Annuality. Significant values are highlighted in bold.

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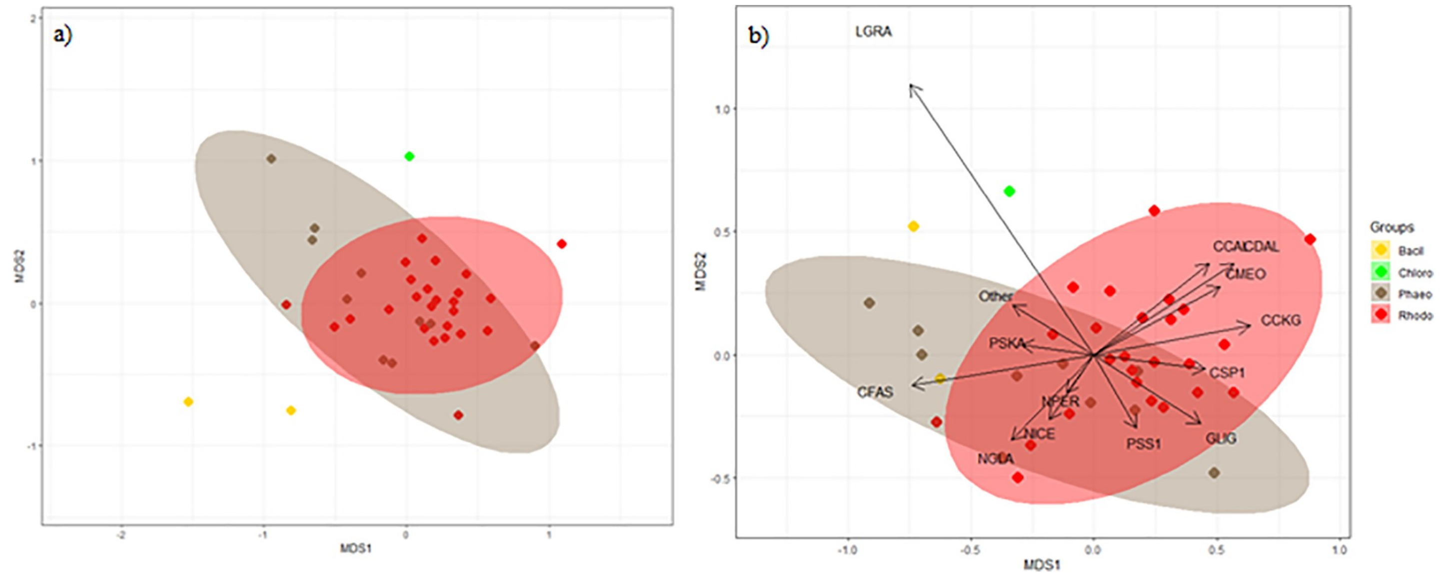

Fig 4. nMDS of the diatom communities. a) complete set and b) predominant diatoms (square root transformed). Bacill = macroscopically visible Bacillariophyceae, Chloro = Chlorophyta, Phaeo = Phaeophyceae, Rhodo = Rhodophyta. Species codes: CCAL = *Cocconeis californica*, CCKG = *Cocconeis californica var. kerguelensis*, CDAL = *Cocconeis dallmanii*, CFAS = *Cocconeis fasciolata*, CMEO = *Cocconeis melchioroides*, CSP1 = *Cocconeis sp. 1*, GLIG = *Gomphonemopsis ligowskii*, LGRA = *Licmophora gracilis*, NGLA = *Navicula glacialis*, NICE = *Navicula incertata*, NPER = *Navicula perminuta*, PSKA = *Pseudogomphonema kamtschaticum*, PSS1 = *Pseudogomphonema sp 1*, Other = diatom species in under 2% frequency and abundance.

https://doi.org/10.1371/journal.pone.0250629.g004

r = 0.299***). The frequency of teratologies found was higher in LI (56.52% of samples had teratological cells, arriving at 2.3% of incidence in a sample) than in DI (66.67% of samples had teratological cells, with an incidence between 0–1% of the samples). However, only samples from Deception island did not arrive to 500 valves due to sparse epiphyte concentration.

Given that we had not enough specimens of macroscopic diatom colonies (n = 2) and Chlorophyta (n = 1), only Rhodophyta and Phaeophyceae samples were considered henceforth. When comparing the depth distribution of predominant diatom taxa (Fig 6), frequent or abundant diatom taxa, or both, differences between the samples from LI and DI became apparent. Larger diatoms, such as *Cocconeis fasciolata* (Ehrenberg) N. E. Brown or *C. antiqua*

Table 4. Average abundance and dissimilarity of diatom communities from Rhodophyta (Rhod) and Phaeophyceae (Phae).

Taxa	Average abundance		Average dissimilarity	SD	Contribution (%)	Cumulated (%)
	Phaes	Rhod				
Navicula perminuta	18.36	15.88	8.48	1.05	11.65	11.65
Gomphonemopsis ligowskii	12.19	8.79	7.42	0.94	10.19	21.84
Cocconeis melchioroides	1.44	13.65	6.48	0.88	8.90	30.73
***Pseudogomphonema* sp. 1**	10.72	12.21	6.44	1.05	8.84	39.58
Cocconeis fasciolata	10.28	0.94	5.13	0.66	7.04	46.61
Pseudogomphonema kamtschaticum	8.80	4.77	4.01	1.09	5.51	52.12
Cocconeis californica var. kerguelensis	0.23	7.38	3.68	0.56	5.05	57.17
Tabularia tabulata	6.80	0.09	3.43	0.32	4.71	61.88
Cocconeis dallmannii	0.92	5.20	2.69	0.62	3.69	65.57
Cocconeis costata	4.94	2.01	2.53	0.73	3.48	69.05
Cocconeis californica	1.21	4.13	2.32	0.47	3.19	72.23

Species ordered in decreasing and cumulated contributions (SIMPER analysis). SD = Standard deviation.

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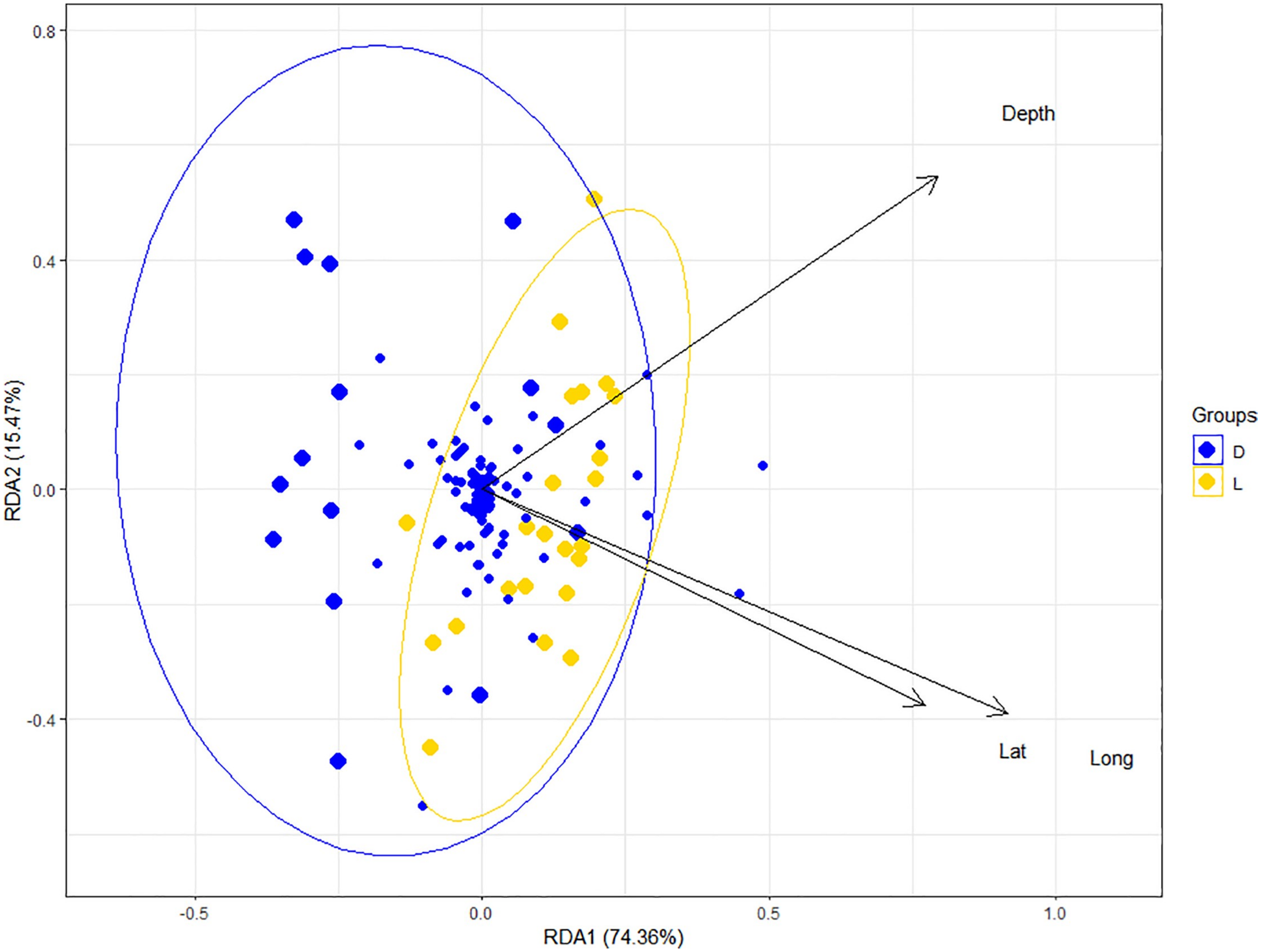

Fig 5. RDA with the first two axes explaining 89.83% of total variance Eigen values of axis 1 = 2.80 and axis 2 = 0.582. DI = Deception island, LI = Livingston island.

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Tempère & Brun, were found on samples located in shallower locations in DI, while smaller diatoms, as *Navicula cf. perminuta* Grunow were mostly found in comparatively deeper samples. On the contrary, in LI, this depth-cell size trend was reversed. The differentiation of diatom communities with respect to sampling depth was significant as well (Mantel statistic r = 0.260***).

ANOSIM showed that the importance of factors determining diatom community composition differed between both islands (Table 5). The predominant factor was host algae species on LI (R = 0.7 ***) and depth on DI (R = 0.54***). Host class was significant in both locations (DI R = 0.38 ***, LI R = 0.4 *). SIMPER analysis (Table 6) further showed an average dissimilarity of 70.71% between islands and an intra-island dissimilarity of 59.51% (LI) and 74.80% (DI). The most characteristic diatom species for DI were *Cocconeis melchioroides*, *Pseudogomphonema* sp. 1 and *Gomphonemopsis ligowskii*. In LI, the predominant diatom was *Navicula perminuta*. Both islands had saturated rarefaction curves (S2 Fig), and DI seemed to have the richest diatom community.

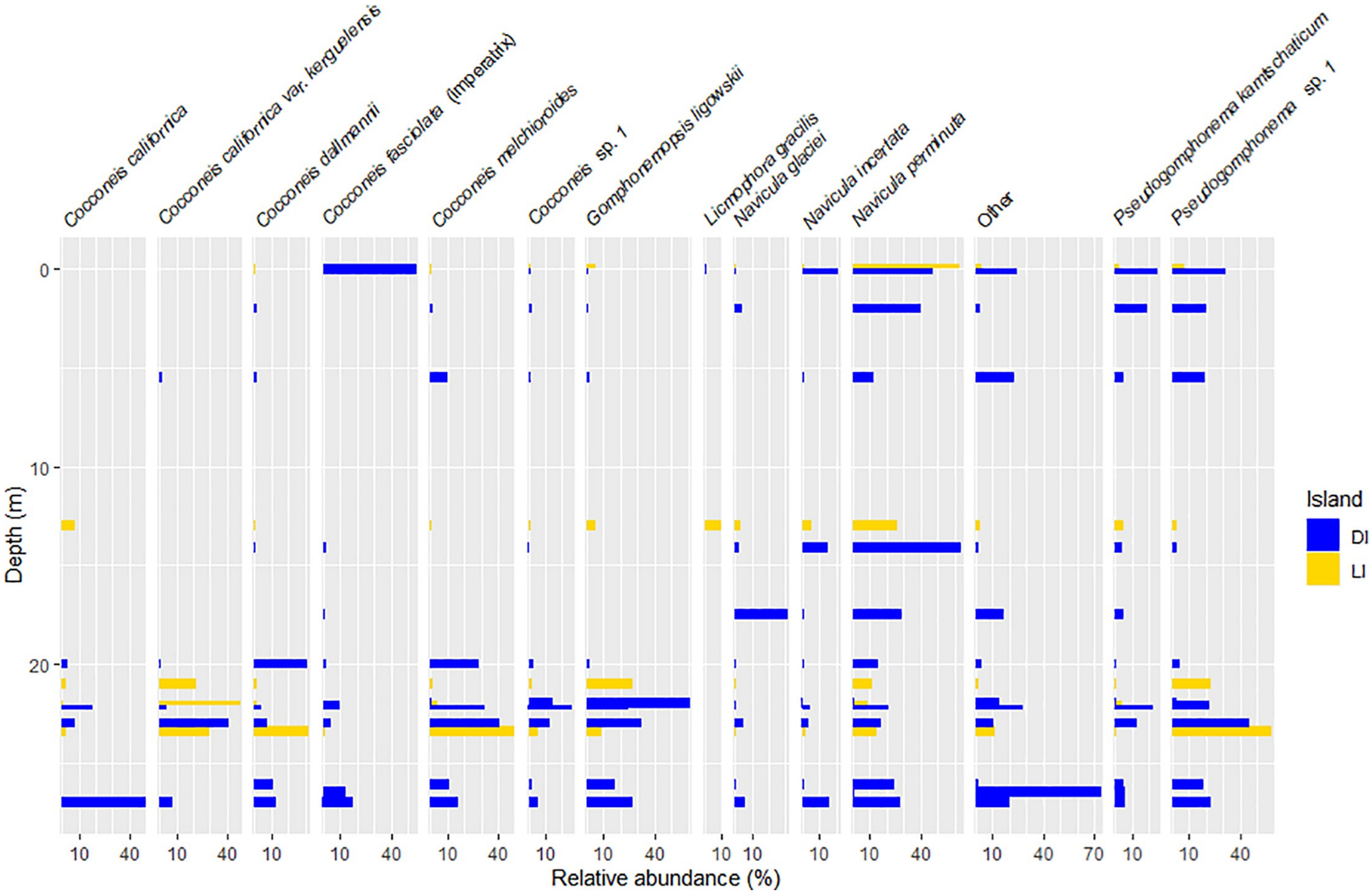

Fig 6. Depth diagram of diatom distribution in Deception and Livingston Island. The rest of diatoms found are summarized in the "Other" panel.

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Antarctic epiphytic diatoms in the literature. A Mantel test on a presence-absence database created from diatom community composition depending on study location showed significance in the richness of the epiphytic diatom communities according to geographical GPS spherical trigonometric distance (Mantel statistic r = 0.4675**). When comparing the diatom composition of the samples, the sample of Vestfold Hills was the most diversified from the rest when comparing epiphytic diatom composition using a hierarchical clustering (Fig 7, S3–S5 Tables). The samples from McMurdo Sound (MS) clustered together, and our samples clustered with the South Shetland Islands (SSI) sample from Potter Cove. The sample from Admiralty Bay, however, clustered with the diatom composition found on macroalgae from MS.

Table 5. ANOSIM test results performed on communities from Deception (DI) and Livingston Island (LI).

	Host algae		Host class		Depth [m]		Year		Host morph.		Host branch		Host annual	
	D	L	D	L	D	L	D	L	D	L	D	L	D	L
R	0.04	**0.70**	**0.38**	**0.40**	**0.54**	0.12	0.26	0.22	0.21	0.13	0.03	0.04	0.15	0.13
p-value	>0.05	0.0001	0.001	0.01	0.008	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Significant values are highlighted in bold.

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Table 6. Breakdown of average dissimilarity between epiphytic diatoms in Deception and Livingston Island locations (SIMPER).

Taxa	Average abundance		Average dissimilarity	SD	Contribution (%)	Cumulated (%)
	Deception	Livingston				
Navicula perminuta	16.89	14.90	8.00	1.01	10.44	10.44
Gomphonemopsis ligowskii	6.11	10.91	6.94	0.98	9.05	19.50
***Pseudogomphonema* sp.1**	8.08	12.51	6.39	0.99	8.34	27.83
Cocconeis melchioroides	2.71	13.09	6.37	0.87	8.31	36.14
Cocconeis fasciolata	7.64	0.97	3.91	0.56	5.11	41.25
Cocconeis californica var. kerguelensis	0.83	7.36	3.78	0.57	4.94	46.19
Pseudogomphonema kamtschaticum	6.19	5.54	3.42	1.00	4.46	50.65
Cocconeis californica	3.64	2.48	2.79	0.47	3.64	54.29
Cocconeis dallmannii	0.96	5.22	2.79	0.63	3.64	57.92
Berkeleya rutilans	5.18	0.00	2.60	0.27	3.39	61.31
Tabularia tabulata	5.00	0.09	2.54	0.27	3.31	64.62
Brandinia	4.96	0.01	2.49	0.28	3.25	67.87
Navicula incertata	4.49	1.78	2.24	0.73	2.92	70.79

Species ordered in decreasing and cumulated contributions (SIMPER analysis). SD = standard deviation.

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ANOSIM showed significant differences in diatom composition by study after controlling for geographic effect (R = 0.78***), after controlling for geographic effects study made out (R = 0.81***). For further characterization, a SIMPER analysis was used following the distribution around Antarctica. Samples from SSI, MS, and Vestfold Hills (VH) showed significant differences among each other, with MS and VH having the highest average dissimilarity (99.51), followed by South Shetland Islands and VH (98.89), and SSI and MS being the lowest (80.54). MS was characterised by the most frequent taxa *Fragilariopsis nana*, *Cocconeis fasciolata* and *Pseudogomphonema kamtschaticum*. In VH only *Nitzschia lecointei* seemed to be characteristic. The most frequent taxa in SSI, which includes the diatoms of our study, were *Navicula perminuta* and *Cocconeis melchioroides*. The comparison of diversity (S6 Table) showed that the DI samples had the highest species richness overall (S = 94) and a relatively high Shannon diversity (H' = 3.16) compared to the other SSI samples (H' = 2.63–2.90). SSI and MS sites had similar values of diversity (H' = 2.63–3.87) and richness (S = 45–118).

Discussion

The total number of taxa identified in this study, 129 species and 44 genera, exceeds the number of taxa in previous Antarctic epiphytic diatom studies. Even after eliminating the diatom samples from the dataset, a total of 120 species and 42 genera of epiphytic diatoms were identified on macroalgal samples, still surpassing the diversity found in previous studies. This could be an effect of a broader sampling along the depth gradient, of a high richness of macroalgal species investigated in this study, or the gentle preparation method used. A partial explanation of high diatom species richness in Antarctic-Subantarctic marine benthos might be the unusually high nutrient concentrations (especially of nitrate) surrounding the Antarctic peninsula [22] in combination with higher iron levels [47]. The high richness of macroalgal species investigated in this study in combination with the ecological niche diversity is, however, probably more important. Majewska et al [3,15] studies were only based on three Rhodophyta taxa. The study on epiphytic diatoms in Vestfold Hills [12] had 17 host species, but epiphytic diatom species numbers remained low as the authors only reported diatoms commonly found on different types of macroalgae and sea ice. The most comparable study would be Al-Handall et al.

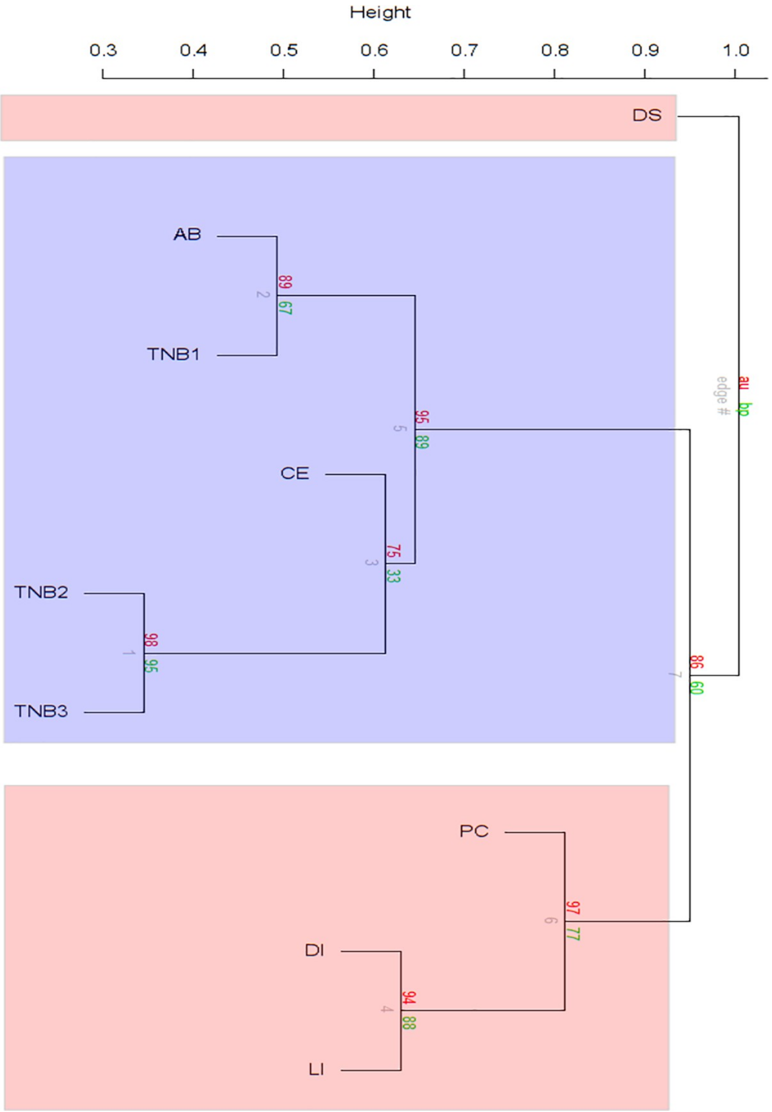

Fig 7. Hierarchical clustering calculated with 10,000 permutations with the studies of Davis Station (DS), Terra Nova Bay (TNB1, 2, 3), and South Shetland Islands (Deception [DI], Livingston [LI], and King George Island, divided into Potter Cove [PC] and Admiralty Bay [AB]) with presence-absence data aggregated at the study level (n total = 192).

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[2] (19 host species and individual samples), which listed 50 species, compared to our total of 20 macrophyte taxa (and 38 samples).

In spite of previous studies, 20 diatom species were recorded for the first time in DI and LI (S1 Table, bold). Most of them pertained to the *Cocconeis* Ehrenberg genus, a monoraphid and mostly epiphytic diatom [48]. This genus was also predominant in previous studies [3,13–15]. One frequent diatom taxon was identified as an unknown species. *Pseudogomphonema* sp. 1 was smaller than *Pseudogomphonema plinskii* Witkowski, Metzelin & Lange-Bertalot and the endophytic diatom found inside the macroalgal genus *Neoabbottiella* [49] and could be yet undescribed. In contrast with the epiphytic diatom studies, usual proxies for sea-ice as *Fragilariopsis curta* [50] and *Thalassiosira antarctica* [51] were not found in as much predominance as, for instance, in Majewska et al. [3].

Epiphytic diatom floristics and ecology

Seaweeds respond to changes in several ways, including by secreting secondary metabolites with antibiotic or antifouling activities on surfaces susceptible to epiphytic invasion [19–21,52]. This could activate the acclimatisation mechanisms of epiphytic diatoms and co-specialization could be prompted. In our study however, the difference between both islands could reflect different taxonomic coverages of sampling, rather than genuine biogeographic signal: whereas most of the samples from LI were Rhodophyta (>82%), in DI the proportions were 40% Rhodophyta and 46% Phaeophyceae. Therefore, the DI samples contained more information on the effect of host at the higher taxonomic level and could be better compared with Al-Handal and Wulff [2], while LI samples more information at lower taxonomic level within red algae, showing more similarities to the Majewska results [3].

Apart from taxonomic identity, also branching pattern and annuality of the host have previously been found to shape epiphyte communities [53]. We found such associations, too, like some species of *Cocconeis* occurring only on branched Rhodophyta hosts (e.g. *Desmarestia* or *Plocamium*), which coincides with findings in other Antarctic and worldwide marine epiphytic diatom studies [3,54–57]. In our study, the effect of branching pattern and annuality was only significant on Rhodophyta and not on Phaeophyceae hosts. As the host age increases, so does the colonization by a mature biofilm community [58]. Maturity of the biofilm, and thus organism position inside a polysaccharide casing, could also protect the community living in it, as shown in several heavy metal studies [59,60]. This might partially explain the annuality effect.

It is instrumental to compare study designs across Antarctic-Subantarctic epiphyte diatom studies. Whereas most sampling campaigns in these distant regions are opportunistic by necessity [2,12], Majewska and collaborators [3,13–15] deliberately focused on three macroalgal host species, systematically capturing epiphyte variability on these selected hosts. In contrast, the present study sampled non-selectively, but the so far broadest diversity of host taxa, and at least some of them repeatedly. Comparing results from both types of approaches, it becomes clear that a systematic and repeated sampling of a broader range of host species will be required for a final clarification of the specificity of host-epiphyte associations. With respect to host-trait effects on epiphyte communities, it would be interesting to more systematically compare branched vs unbranched red and brown seaweed host taxa (for instance the Rhodophyta *Plocamium cartilagineum* vs. *Iridaea chordata*, and the Phaeophyceae *Desmarestia antarctica* vs. *Hymantothallus grandifolius*).

Geographical distribution of diatoms in Antarctica

The total species richness found in DI (93) far exceeded the expectations for an extreme environment, being lower in LI (82). In LI a higher variability in light impact could have been expected because of glacier inputs [61]. Better micronutrient supply due to the volcanic exudations on DI might have increased the number of species [47]. On the other hand, substrate consistency could also have an effect on diatom colonization. The fine (lapilli) consistency in DI causes quickly changing light intensities, since the substrate can quickly redeposit itself after being moved. In contrast, the light influx of LI does not depend on movement of lapilli, but varies due to the input from time constrained glacier melt [62], thus providing more stable irradiation for macroalgae and epiphytic diatoms during the sampled Antarctic summer.

Deception island (DI) is a quiescent volcano, with a semi-submerged cone. The caldera is only 180 m deep [63] and has active hydrothermal vent activity [64]. This has been found to increase the bioavailability of trace elements [65], and also to increase colloid suspension because of the fine sediment or lapilli [64]. The existence of active fumaroles also increases the temperature range measured in the water and substrate [66], thus further segregating potential ecological niches in the ecosystem. Another important difference between the two islands is that LI has a slightly higher tide amplitude and narrower range of water temperature than Deception island [67].

The difference in diatom taxa of both islands was smaller than expected and also smaller than the host effect. Host species that were sampled in DI and LI once or more were compared between and within locations (*Desmarestia antarctica*, *Gigartina skottsbergii*, *Hymantothallus grandifolius*, *Iridaea cordata* and *Palmaria decipiens*) and showed that the dissimilarity among DI samples was greater than in LI or in comparison between DI and LI. This might point to the environmental variability inside the DI caldera creating more ecological niches for diatom species to fill [68], but unfortunately, physicochemical and light intensity measurements which would be needed to substantiate this are not available.

On a broader geographic scale, similarity of diatom communities around Antarctica was strongly dependent on study. Using presently available data, it is not possible to separate geographic differences from environmental effects and possible effects of methodological differences among studies (further discussed in Closing methodological remarks). It is of course to be seen as a tentative comparison of epiphytic diatom distribution around Antarctica, since other variables concerning seasonality and physicochemical composition of the waters in each of the studies was mostly unavailable and further, synchronous studies should be made to answer the question of epiphytic diatom biogeography around Antarctica. This study would be a first approach, but as discussed, new and more standardized / synchronised efforts should be made in the future to obtain a clear picture on the ecological variations of epiphytic diatoms along the Antarctic coastline.

Closing methodological remarks

One of the most striking observations of our study was the strong effect of study upon epiphytic diatom communities. As discussed above, the exact cause of this study effect is difficult to pin down based on presently available data, but preparation method might be part of it. The dehydration method used by Majewska and collaborators [3,5,13–15] permitted a quantitative in situ observation but could potentially lead to overlooking taxa growing in lower layers of the established biofilm on the host algae. As previously discussed by Majewska et al [3], the reduced diatom species richness in Al-Handal & Wulff [2] and Thomas & Jiang [12] could be an effect of dissolution of lightly silicified frustules. Although silicate is known to dissolve faster in alkaline than in acidic milieu, Carr [25] and Friedrichs [24] found that a short-time bleach treatment, as used in this study, is more gentle to diatom frustules than commonly applied harsh oxidizing acid treatments which was used by previous ones. Parallel preparations from the same sample using both types of approaches in the future would be useful to test whether the effect of preparation treatment is indeed the dominant cause of study effect. Once this has been clarified, a clearer recommendation for standardizing the methodology of epiphytic diatom preparation can be given which will be important to improve the comparability of results among different studies.

Another methodological difference of our study from previous ones was the use of virtual slide microscopy and web-based manual taxonomic annotation. We did not systematically test this effect in these studies, but checking individual samples both on the light microscope and in the slide scans indicates that this is not causing a major bias for observing taxonomic composition (a study systematically comparing this effect is presently in preparation). We think that this methodology has some potential advantages for the future. For instance, a digital image of every single frustule identified in this study is available in PANGAEA (doi: https://doi.pangaea.de/10.1594/PANGAEA.925913). Future studies making literature comparisons, like attempted also above, will thus not only have presence-absence records, but also every one of these images, making it even possible to re-identify any or all frustules as deemed necessary. This can, in the long run, when such data sets accumulate, contribute a lot to transparency and comparability among different studies.

In conclusion, in this study we compared epiphytic diatom communities living on several macroalgae in Deception and Livingston Island. We found that the number of species in DI samples exceeded those from LI and from previous studies. The former observation may point to a higher proportion of niches found on the volcanic island. The second one would be explained by a gentler preparation method, though this needs a clear causal confirmation in the future.

Supporting information

S1 Fig. Rarefaction curves of macrophytes hosts. Rhodo = Rhodophyta, phaeo = Phaeophyceae, bacil = Bacillariophyceae, chloro = Chlorophyta.
(TIF)

S2 Fig. Rarefaction curves of sample location in this study. Dec = Deception island, Liv = Livingston island.
(TIF)

S1 Table. Epiphytic diatom composition and frequency on Antarctic macroalgae, specific proportion and occurrence on Deception (DI) and Livingston Island (LI). Pheaophyceae (Phaeo, n = 10), Rhodophyta (Rhodo, n = 25), Bacillariophyceae (Bac, n = 2) and Chlorophytas (Chlo, n = 1) have been investigated. Macroalgal host: Au = *Adenocystis utricularis*, Bc = *Ballia callitricha*, Cj = *Cystosphaera jacquinotii*, Dp = *Delisea pulchra*, Dan = *Desmarestia anceps*, Dant = *Desmarestia antarctica*, Ds = *Desmarestia sp.*, Gs = *Gigartina skottsbergii*, Gt = *Gymnogongrus turquettii*, Hg = *Himantothallus grandifolius*, Ic = *Iridaea cordata*, Mh = *Monostroma hariotii*, Mm = *Myriogramme cf. manginii*, Pd = *Palmaria decipiens*, Pp = *Piccionella plumosa*, Pc = *Plocamium cartilagineum*, Ph = *Plocamium cf. hookeri*, Pe = *Pyropia endiviifolia*. Bold text shows first records in DI and LI.
(DOCX)

S2 Table. SIMPER analysis of comparison of predominant diatom species in LI and DI.
(DOCX)

S3 Table. SIMPER analysis of comparison of epiphytic diatom communities in the South Shetland Islands (SSI, n = 4) and Vestfold Hills (VH, n = 1).
(DOCX)

S4 Table. SIMPER analysis of comparison of diatom communities in the South Shetland Islands (SSI, n = 4) and MacMurdo Sound (MMS, n = 4).
(DOCX)

S5 Table. SIMPER analysis of comparison of epiphytic diatom communities in the Vestfold Hills (VH, n = 1) and MacMurdo Sound (MMS, n = 4).
(DOCX)

S6 Table. Diversity/Entropy indices of the compared studies. * = contains only macroalgal information, not Bacillariophyceae. | = total data of the study.
(DOCX)

S7 Table. Mantel result comparison of parameters and diatom abundance.
(DOCX)

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Chapter II

Nuclear DNA content estimations and nuclear development patterns in Antarctic macroalgae

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SHORT NOTE

Nuclear DNA content estimations and nuclear development patterns in Antarctic macroalgae

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Abstract

Nuclear DNA content of marine macroalgae is known for only about a 3% of the globally known taxa, and so far, the only available data concerning marine Antarctic macroalgae refer to the Phaeophyceae *Ascoseira mirabilis* and *Desmarestia antarctica*. As these data can be useful for species delimitation when combined with other taxonomical information, we aimed to expand the available number of measurements in the understudied Antarctic seaweed flora. To address this, we measured the nuclear DNA content of 12 Antarctic marine algae (seven Rhodophyceae and five Phaeophyceae). Nuclear DNA analyses were carried out by spectrofluorimetry and image analysis from samples conserved in Carnoy, using DAPI as DNA marker. For ten of these taxa, our values represent the first estimations to date. The nuclear DNA content estimates obtained for the Antarctic red algae examined vary between 2C = 0.38 pg in *Gigartina skottsbergii* and 2C = 1.63 pg in *Neuroglossum delesseriae*. In brown algae, the values range from 2C = 0.18 pg in both *Desmarestia antarctica* and *Desmarestia menziesii* to 2C = 0.96 pg in *Phaeurus antarcticus*. Furthermore, this study allowed us to identify nuclear developmental patterns for the first time in two Antarctic seaweeds (*Ballia callitricha* and *Neuroglossum delesseriae*).

Keywords Nuclear DNA content · Nuclear patterns · Antarctica · Seaweeds

Introduction

The nuclear DNA content of a species, expressed as C–value, is the total quantity of not replicated nuclear DNA of a gamete, and it is constant and independent from the level of ploidy of the individuals (Swift 1950; Greilhuber et al. 2005). C–values are used in a wide range of biological fields for its fundamental biological meaning (Goff & Coleman 1990; Bennett et al. 2000; Salvador et al. 2009; Bennett & Leitch 2011), and it is used in industrial, taxonomic, and phylogenetic studies, as well as biogeographic analysis (Bennett & Leitch 2001, 2005a,b; Pellicer et al. 2010). C–values define ploidy level and the genome size of species of interest, both parameters of great importance to complement evolutionary, taxonomic, and ecological information (Kapraun 2005; Chénais et al. 2012 and Husband et al. 2013). As an example, C–value has been related to ecological parameters in many taxonomic groups correlating the amount of DNA and the thermal regime, i.e., latitude and altitude. However, there is some controversy about this, as many studies point to a larger genome size in species that live in extreme environments

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(Arctic ecosystems or high mountain), while other studies found the contrary (Bennett & Leitch 2001, 2005a,b; Pellicer et al. 2010). Other studies used this parameter to describe important developmental aspects of seaweed, as major nuclear patterns of multinucleate or endopolyploid uninucleate cells in the development of the Florideophyceae (Goff & Coleman 1990).

Nonetheless, nuclear DNA content of marine macroalgae has been studied only for about a 3% of the globally known taxa (Kapraun 2005, 2007; Gómez Garreta et al. 2010; Ribera Siguan et al. 2011; Phillips et al. 2011; Kapraun & Freshwater 2012; Salvador–Soler et al. 2016) and so far, the only available data concerning Antarctic macroalgae refer to *Ascoseira mirabilis* Skottsberg and *Desmarestia antarctica* R.L.Moe & P.C.Silva. In the same way, the nuclear development patterns in algae have been only rarely observed since they were established (Goff & Coleman 1990) and they have not been investigated in Antarctic algae yet. For these reasons, our goal here has been to provide further data on nuclear DNA content and development patterns, to be used in future studies on the flora of the Antarctic regions.

Materials and methods

Species selection

12 Antarctic algal species were selected for this study comprising 7 Rhodophyceae and 5 Phaeophyceae (Table 1). The selection criteria were mainly the lack of data of nuclear DNA content at genus or family levels, as well as abundance in the sampling area.

Sampling

Sampling was performed in the South Shetland Islands and the Antarctic Peninsula during the austral summers of 2009–2010, and 2012–2013 (in the frame of ACTIQUIM

Table 1 Sampling data: studied algal species, locality, demographic information, and collecting date

Phylum	Class	Family	Species	Locality	Latitude	Longitude	Sampling date
Rhodophyta	Florideophyceae	Balliaceae	*Ballia callitricha* (C.Agardh) Kützing	Kopaitic Is. (Antarctic Peninsula)	63°18′46.23"S	57°54′34.24"W	25/12/2012
Rhodophyta	Florideophyceae	Gigartinaceae	*Gigartina skottsbergii* Setchell & N.L.Gardner	Vera Is. (Antarctic Peninsula)	63°18′41.95"S	57°55′7.66"W	26/12/2012
Rhodophyta	Florideophyceae	Kallymeniaceae	*Callophyllis* sp.	Sapo Is. (Antarctic Peninsula)	63°19′2.87"S	57°55′23.46"W	27/12/2012
Rhodophyta	Florideophyceae	Delesseriaceae	*Neuroglossum delesseriae* (Reinsch) M.J.Wynne	Sapo Is. (Antarctic Peninsula)	63°19′2.87"S	57°55′23.46"W	27/12/2012
Rhodophyta	Florideophyceae	Rhodomelaceae	*Picconiella plumosa* (Kylin) J.De Toni	Sapo Is. (Antarctic Peninsula)	63°19′2.87"S	57°55′23.46"W	27/12/2012
Rhodophyta	Florideophyceae	Rhodymeniaceae	*Rhodymenia coccocarpa* (Montagne) M.J.Wynne	Colatinas (Deception Is.)	62°59′22.43"S	60°37′17.50"W	11/01/2010
Rhodophyta	Florideophyceae	Fryeellaceae	*Hymenocladiopsis prolifera* (Reinsch) M.J.Wynne	Whaler´s Bay (Deception Is.)	62°58′51.85"S	60°33′41.28"W	11/01/2010
Ocrophyta	Phaeophyceae	Desmarestiaceae	*Himantothallus grandifolius* (A.Gepp & E.S.Gepp) Zinova	Fildes Point (Deception Is.)	62°59′33.22"S	60°33′25.22"W	06/02/2013
Ocrophyta	Phaeophyceae	Desmarestiaceae	*Desmarestia antarctica* R.L.Moe & P.C.Silva	Antarctic Spanish Base Beach (Deception Is.)	62°58′35.25"S	60°40′31.97"W	12/12/2012
Ocrophyta	Phaeophyceae	Desmarestiaceae	*Desmarestia menziesii* J.Agardh	Whaler´s Bay (Deception Is.)	62°58′51.85"S	60°33′41.28"W	14/12/2012
Ocrophyta	Phaeophyceae	Desmarestiaceae	*Phaeurus antarcticus* Skottsberg	Whaler´s Bay (Deception Is.)	62°58′51.85"S	60°33′41.28"W	11/01/2010
Ocrophyta	Phaeophyceae	Ascoseiraceae	*Ascoseira mirabilis* Skottsberg	False Bay (Livingston Is.)	62°41′44.49"S	60°20′10.13"W	15/12/2012

research projects). Details on the sampling localities information are available in Table 1. Specimens were collected from intertidal and subtidal ranges, down to 25 m of depth, either by snorkeling or scuba diving.

Sample conservation

Samples were frozen (− 20 °C) after collection in situ and stored until their arrival to the laboratory at the University of Barcelona. Once there, the specimens were defrosted to confirm identification of species and life phase of each individual (Table 2). For nuclear DNA quantification, we followed a modification of Kapraun (2005) and Goff & Coleman (1990) protocols, as our previous experience with this kind of measurements determined that defrosting does not affect measures compared to Carnoy fixation in situ. For this, several fragments of each individual (ca. 0.5 mm^2) were taken and conserved separately as replicates. These fragments were fixed with Carnoy solution (3:1 of 95% ethanol–glacial acetic acid) during 24 h and stored in 70% ethanol at 4°C during at least 24 h for later nuclear DNA content analysis.

DNA quantification

After 70% ethanol storage, samples were rehydrated in distilled water and softened in 5% w/v EDTA (Goff & Coleman 1990) for 12–48 h. The samples were subsequently squashed with rugged slides and then transferred to coverslips previously soaked with subbing solution. The coverslips with the samples and the subbing were left air dried and then stained with 0.5 µg/mL 4′,6–diamidino–2–phenylindole (DAPI; Sigma Chemical Co. St. Louis, MO 63,178) and mounted in microscopy preparations following literature methods (Goff & Coleman 1990; Kapraun & Nguyen 1994). Nuclear DNA contents were measured using fluorimetry and image analysis, following a procedure modified from Kapraun & Dunwoody (2002) and Choi et al. (1994). The images obtained were then analyzed using MetaMorph software (Molecular Devices, Toronto, Canada). The nuclear DNA content was obtained by comparison of fluorescence intensity of the nuclei with a standard with constant nuclear DNA amount. Following Kapraun & Nguyen (1994) and Kapraun & Dunwoody (2002), we used *Gallus gallus* (Linnaeus) erythrocytes with constant nuclear DNA content of 2.4 pg (Clowes et al. 1983) as standard. For statistical reasons, we tried to reach a minimum of 100 measured nuclei per species for analyses of ploidy peaks. Nonetheless, in

Table 2 Measurements of nuclear DNA content in picograms (mean ± s.d.) for the studied Antarctic algae, number of nuclei, and life phase

Taxa	Nº individuals	Nº nuclei	Life phase	1C	2C	4C	8C	16C	32C
Ballia callitricha (C.Agardh) Kützing	1	123	Sporophyte	–	0.93 ± 0.25	–	4.08 ± 1.06	6.77 ± 1.25	14.98 ± 1.24
Gigartina skottsbergii Setchell & N.L.Gardner	2	209	Sporophyte	–	0.38 ± 0.11	0.89 ± 0.08	–	–	–
Callophyllis sp.	1	93	Gametophyte	0.22 ± 0.07	0.44 ± 0.09	–	–	–	–
Neuroglossum delesseriae (Reinsch) M.J.Wynne	2	289	Gametophyte	0.88 ± 0.26	1.63 ± 0.23	–	–	–	–
Picconiella plumosa (Kylin) J.De Toni	1	50	Gametophyte	0.72 ± 0.20	1.28 ± 0.14	–	–	–	–
Rhodymenia coccocarpa (Montagne) M.J.Wynne	2	246	Gametophyte	0.22 ± 0.08	0.54 ± 0.10	–	–	–	–
Hymenocladiopsis prolifera (Reinsch) M.J.Wynne	1	93	Gametophyte	–	0.43 ± 0.03	–	–	–	–
Himantothallus grandifolius (A.Gepp & E.S.Gepp) Zinova	1	124	Sporophyte	–	0.36 ± 0.15	0.92 ± 0.18	–	–	–
Desmarestia antarctica R.L.Moe & P.C.Silva	1	161	Sporophyte	–	0.18 ± 0.01	0.39 ± 0.02	–	–	–
Desmarestia menziesii J.Agardh	2	180	Sporophyte	–	0.18 ± 0.01	0.38 ± 0.01	–	–	–
Phaeurus antarcticus Skottsberg	1	167	Sporophyte	–	–	1.92 ± 0.26	–	–	–
Ascoseira mirabilis Skottsberg	1	60	Gametophyte	–	0.33 ± 0.08	0.67 ± 0.05	–	–	–

some species, this was not possible because some nuclei were not properly stained.

Nuclei measurement results were then analyzed for each species to identify ploidy levels as peak classes of nuclei in the same range of nuclear DNA quantity (picograms, pg). Nuclei not belonging to peak classes (those classes with low number of nuclei, i.e., between 0 and 10) in the histograms were discarded for calculations of ploidy peaks. For each peak class, nuclear DNA quantity mean and standard deviation were calculated. Also, ploidy level to each peak class was assigned tentatively as in Kapraun (2005), taking into account the life phase of the individuals sampled (whenever possible) and/or by comparing to ploidy levels assignations of closely related taxa in the Kew Royal Botanic Gardens C–value database when available. All the results obtained here will be incorporated into the C–values algae database of the Kew Royal Botanic Garden (https://cvalues.science.kew.org/).

Results and discussion

Except for *A. mirabilis* and *D. antarctica* (Phillips et al. 2011), our measures are the first values of nuclear DNA content measured for Antarctic algae so far. Relative to the nuclear DNA content values, these vary between 2C = 0.38 pg of *Gigartina skottsbergii* Setchell & N.L.Gardner and 2C = 1.63 pg of *Neuroglossum delesseriae* (Reinsch) M.J.Wynne for the red seaweeds, as well as 2C = 0.18 pg for both *Desmarestia* species studied and 2C = 0.96 pg of *Phaeurus antarcticus* Skottsberg (deduced from 50% of the 4C value assigned) for the brown ones (Table 2).

For most Phaeophyceae, the 2C and 4C ploidy levels (except in *Phaeurus* in which we only identified a peak corresponding with 4C) were assigned taking into account the life phase of the material (all except *A. mirabilis* were sporophytes), and, as mentioned before, by comparing to the ploidy levels of the closest taxa present in the Kew database. In the case of *A. mirabilis*, 2C and 4C ploidy levels were assigned to the material measured, even though our sample was not a fertile individual. This was deduced because we know that the cells measured were of a diploid individual in phases G1 and G2, as *A. mirablis* is described as having a diploid monogenetic life cycle (thus having a diploid gametophyte).

The same criterion was applied to the seven Rhodophytes analysed. Two of the species were sporophytes (*Ballia challitricha* (C.Agardh) Kützing and *Gigartina skottsbergii* Setchell & N.L.Gardner), and the other five species were in gametophytic phase. For the two sporophytes, we determined that the ploidy peaks observed corresponded to 2C and 4C levels. Surprisingly, *B. callitricha* showed several additional peaks with increasing number of ploidy levels, reaching up to what appears to be 32C (14.98 pg). The measures of those two species constitute the second measures of nuclear DNA inside the family Gigartinaceae and the first estimates for the whole order Balliales, respectively. For the gametophytes, we were able to assign 1C and 2C values for all five species, with the exception of *Hymenocladiopsis prolifera* (Reinsch) M.J.Wynne, for which we only identified a ploidy level of 2C. Also, it is worth noting that the values measured on *Callophyllis* sp. are the first ones for the Kallymeniaceae family.

The values of nuclear DNA content obtained in this study for *D. antarctica* are four times lower than those provided by Phillips et al. (2011) for the same species. Something similar happens if we compare our values for this species (and those of *D. menziesii*) with those of the other two non-Antarctic *Desmarestia* species included in the Kew database. An explanation could be that the individuals considered in the database were polyploid. Even though more data for this genus would be necessary to further explain our observations, our measures could also suggest no relation between higher ploidy levels and extreme environments for these taxa. Nevertheless, further knowledge of the chromosome complement of the Antarctic species would be of great relevance to better understand this phenomenon in this genus. Similarly, further measurements and wider samplings for Antarctic seaweed species would prove vital to confirm observations like these and also to increase our knowledge in the previously unknown families.

Taking into account the number of nuclei studied, we consider our nuclear DNA content measurements and ploidy level assignation to be fairly accurate, as our measures show consistency with previously measured close taxa available (Kapraun 2005; Kapraun et al. 2007; Gómez Garreta et al. 2010 and Phillips et al. 2011) and Kew C–value database. The only samples studied that may raise some concern could be *Picconiella plumosa* (Kylin) J.De Toni and *A. mirabilis* due to the lower number of nuclei we were able to measure. Those lower numbers may be related with cell wall permeability or secondary metabolites of those particular species, perhaps interfering with the staining process. Further studies and measures for those species will shed light in what may cause this. Nonetheless, in those two cases, our measures are consistent with those previously published for other Rhodomelaceae and for *A. mirabilis* in the Kew database.

Another important finding of our study is the identification of two nuclear patterns for *B. callitricha* and *N. delesseriae*. Those are the first observations of the kind in any Antarctic seaweed, as nuclear patterns have been described by Goff & Coleman (1990) only in non-polar macroalgae. This was possible by observing some stained not disaggregated tissues in the samples of *B. callitricha* and *N. delesseriae* (Fig. 1a, b). *Ballia callitricha* (Fig. 1c,e) showed a pattern

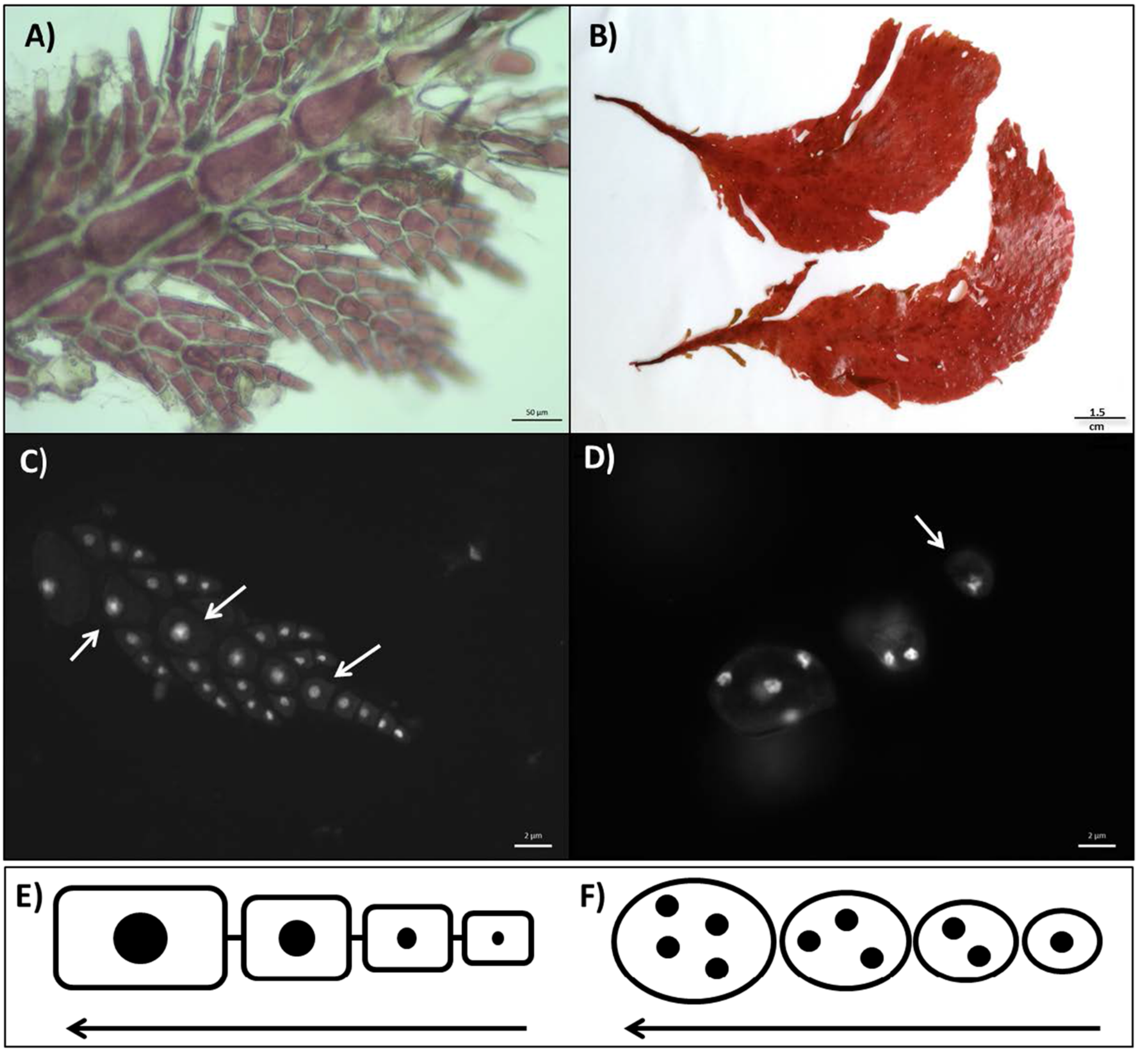

Fig. 1 **a** Picture of *Ballia callitricha* under the microscope; **b** Picture of *Neuroglossum delesseriae*; **c** Fluorescence image with increased contrast, showing non-disaggregated material of *B. callitrica* with stained nuclei (bright spheres inside cells) of increasing ploidy of axial derived cells (marked with arrows); **d** Fluorescence image with increased contrast showing *N. delesseriae* material, presenting cortical cells with one nuclei (arrow) and medullar cells with increasing number on nuclei (middle and bottom cells); **e** Nuclear patterning scheme for *B. callitricha* of uninucleated non-polyploid apical cell deriving in uninucleated highly polyploid axial cells (modified from Goff & Colemann 1990); **f** Nuclear model scheme for N. delesseriae, depicting the model of uninucleated non-polyploid cortical cells deriving in multinucleated non-polyploid medullar cells (modified from Goff & Colemann 1990)

consisting of an apical uninucleated non-polyploid cell (2C = 0.93 pg) deriving in an axial sequence of uninucleated cells of increasing ploidy (8C = 4.08 pg, 16C = 6.77 pg and 32C = 14.98 pg) as shown in Fig. 1e. On the other hand, *N. delesseriae* (Fig. 2d, f), possesses a pattern of cortical, uninucleated, non-polypoid cells (1C = 0.88 pg or 2C = 1.63 pg), deriving towards the medulla in polynucleated (with up to 5–12 non-polyploid nuclei) larger cells. In this case, the pattern is equal to what has been observed in other Delesseriaceae by Goff & Coleman (1990). For *B. callitricha*, the increasing ploidy of the axial cells would provide an explanation for our nuclear DNA measurements of increasing ploidy peaks (up to 32C). According to Goff & Coleman (1990), these two patterns of increasing ploidy level in derived cells represent two different strategies to maintain the correlation between the nuclear DNA content and the cytoplasmic volume when the cell size increases during development.

Similar to land plants, seaweeds present mechanisms of genome size variation (Šmarda & Bureš 2010; Leitch & Leitch 2013; Sjotun et al. 2017). Those mechanisms may produce polyploidy events (Bennetzen et al. 2005; Bothwell et al. 2010; Garbary & Clarke 2002) that lead to the apparition of autopolyploids in the populations and thus act as driving speciation phenomena (Tayalé & Parisod 2013; De Strome et al. 2014; Sjotun 2017). As the Antarctic seaweed flora has a high percentage of endemic species (Wiencke et al. 2002, 2014), understanding the processes promoting speciation is vital to unveil the history and evolution of these communities.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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Chapter III

Chemical ecology in the Southern Ocean

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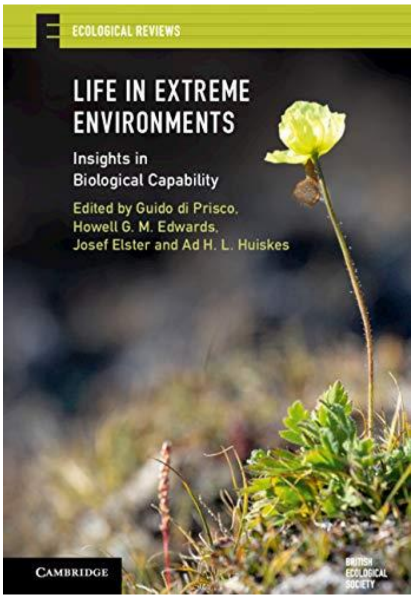

CHAPTER ELEVEN

Chemical ecology in the Southern Ocean

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11.1 Introduction

This chapter aims to review the most recent findings regarding chemical ecology in Antarctic marine macroorganisms, provide some insights into how environmental changes may affect the production of natural compounds, and how species may adapt (or not) to new scenarios related to climate change. The ecological significance of bioactive compounds in the marine environment remains as one of the most understudied topics of recent years. Even if many compounds have been described from marine organisms (Blunt et al., 2018, and previous reports), only a few have been investigated for their role in the environment where the organisms actually live (Puglisi et al., 2019 and previous reports, Puglisi & Becerro, 2018), and this is especially significant for antarctic areas (Avila et al., 2008; Núñez-Pons & Avila, 2015; Principe & Fisher, 2018). In fact, many organisms' interactions in the marine benthos may be mediated by chemicals that are currently unknown or undescribed. Recent reviews have covered the ca. 600 natural compounds described from antarctic marine benthic organisms (Lebar et al., 2007; Soldatou & Baker, 2017; Tian et al., 2017), but only a few have reported the ecological role of their compounds and/or extracts (Avila et al., 2008; McClintock et al., 2010; Núñez-Pons & Avila, 2015; Avila 2016a; Angulo-Preckler et al., 2018; Núñez-Pons et al., 2018; von Salm et al., 2018). Thus, antarctic marine benthos is still an

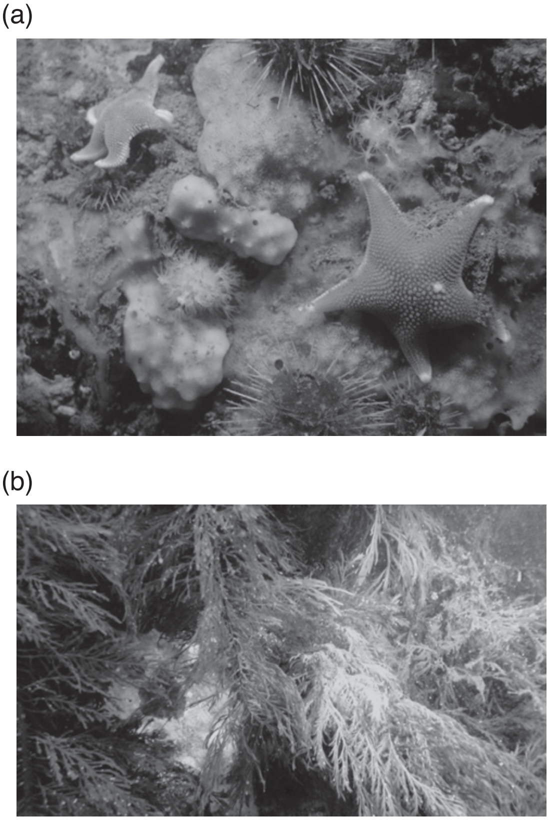

Figure 11.1 Antarctic benthic communities photographed at shallow waters in Deception Is. (Antarctica). (a) Typical invertebrate associations in a hard-bottom substrate (18-m depth). (b) Common algal communities on a rocky wall (15-m depth). (A black and white version of this figure will appear in some formats. For the colour version, please refer to the plate section.)

unexplored source of interesting natural products yet to be discovered. Southern Ocean ecosystems hold a huge amount of biodiversity, much higher than ever thought before, with many cryptic species being recently described, and thus chemical diversity is expected to be very high too (Downey et al., 2012; Wilson et al., 2013; De Broyer et al., 2014; Avila 2016a, 2016b). How antarctic organisms use these compounds is the subject of this review, along with the possible changes that we could expect in relation to environmental changes, particularly climate change (Figure 11.1).

Anthropogenic environmental change is a global phenomenon, with strong impact on biodiversity all around the planet (IPCC 2014). Polar regions are suffering the fastest rates of warming, with a loss of sea ice and the retreat of coastal glaciers and ice shelves too (IPCC 2014). The Antarctic Peninsula, in particular, is one of the areas with the fastest change over the last 50 years (Turner et al., 2009; Ducklow et al., 2013). Polar benthic marine species are exposed to major challenges due to environmental changes, mainly consisting in higher temperatures, ocean acidification, increasing UV radiation, and altered levels of sea ice and iceberg scouring (Núñez-Pons et al., 2018; Peck, 2018), although other potentially important stressors have also been reported, such as salinity and hypoxia (Clark & Peck, 2009a, 2009b; Tremblay and Abele 2016). Antarctic organisms are thus very vulnerable to environmental changes (Peck, 2018). Their unique characteristics and evolution under extreme physical and biological conditions make them very interesting examples to test possible changes in our planet. In fact, Peck (2018) pointed out recently that there is a very urgent need to do more research in polar areas because they are the fastest changing regions in the planet due to climate change impacts, and contain faunas that are possibly the least capable of resisting change globally.

The effects of climate change on population and community ecology currently receives a lot of attention in research, and many articles describe either observed or potential changes in marine invertebrate distribution and population dynamics (e.g. Fabry et al., 2008; Wang, 2014; Griffiths et al., 2017). However, very few studies deal with possible changes in the chemical ecology of the organisms (Campbell et al., 2011). Thus, the assessment of how climate change may affect the synthesis of natural products in marine organisms appears to be a relatively uncharted field of research. Some studies, however, suggest the possible influence of environmental factors, such as seasonal changes, depth and light on the biosynthesis of natural compounds in non-polar species (Turon et al., 1996; Swearingen & Pawlik, 1998; Duckworth & Battershill, 2001; Peters et al., 2004; Ferretti et al., 2009), most of them with the aim of finding suitable conditions for culturing marine invertebrates, e.g. sponges, to obtain compounds with pharmaceutical or biotechnological properties (Ferretti et al., 2009).

Marine natural products comprise mainly secondary metabolites that regulate the biology, coexistence and coevolution of the species, without directly participating in their primary metabolism (i.e. growth, development and reproduction; Torssel, 1983). Natural products often play important roles in predator–prey interactions, but also in symbiosis, competition, antifouling, reproduction, larval settlement and other relationships (Amsler et al., 2001; Figuerola et al., 2012b; Puglisi et al., 2019). One of the most studied roles of natural compounds in antarctic communities is the antipredatory activity, with many protected species already described in areas such as the Ross Sea, the Western Antarctic Peninsula, the Eastern Weddell Sea and Bouvet Island (Amsler et al., 2001, 2014; Avila et al., 2008; McClintock et al., 2010; Figuerola et al., 2013a; Taboada et al., 2013; Núñez-Pons & Avila, 2014a, 2014b). Not surprisingly, these areas are the closest to scientific research stations, while vast unstudied areas remain to be investigated. Other ecological activities have been less studied so far. We review here the data on chemical ecology of selected antarctic organisms from 2000 to 2018.

11.2 Types of molecules

A characteristic of secondary metabolites is their limited phylogenetic distribution; while primary metabolites, such as the common amino acids, carbohydrates and nucleosides, are chemically identical in virtually all organisms, both simple and complex secondary metabolites are generally limited to a given species, genus or family, or even a species chemotype (Torssel, 1983; Blunt et al., 2018). There are a number of classes of natural products, recognised on the basis of their biosynthetic origin, such as polyketides, terpenes and alkaloids (Torssel, 1983). Chemical studies on selected antarctic phyla are briefly discussed here.

Even though the number of described metabolites from macroalgae has increased since the mid-twentieth century, the proportion of antarctic seaweed species studied in this field is still smaller than in other geographical areas (Wiencke & Clayton, 2002; Wiencke et al., 2014; von Salm et al., 2018). Antarctic macroalgae possess a relatively rich diversity of molecules with different roles, which are ecologically important because they are key players in antarctic shorelines, structuring their communities (Wiencke & Clayton, 2002; Wiencke et al., 2014). An important part of the metabolites described from algae are halogenated, but each group (Rhodophyta, Chlorophyta and Ochrophyta) tends to produce its own unique metabolites. Most compounds are isoprenoids (terpenes, carotenoids, cystones, meroterpenoids, cystodiones and steroids), but polyketides and shikimates (mostly aromatic products such as quinones, prenylated hydroquinones and tannins) are also abundant (Young et al., 2007; Blunt et al., 2018). Only 17 antarctic green seaweed taxa have been described so far (Wiencke & Clayton, 2002; Amsler & Fairhead, 2006; Wiencke et al., 2014). Most molecules described from chlorophytes are terpenoids, but contrary to other seaweed groups, such as red algae, they do not have a high level of halogenation (Blunt et al., 2007; Amsler et al., 2008; Young et al., 2015; von Salm et al., 2018; Blunt et al., 2018). Most of them produce volatile halogenated organic (VHO) compounds (Laturnus et al., 1996), and also some UVR-absorbing pigments (Núñez-Pons et al., 2018). Compounds produced by antarctic Ochrophyta include mostly phlorotanins (polyphenols), and also diterpenes and acetogenins (Blunt et al., 2007; Amsler et al., 2008, 2009; von Salm et al., 2018). Red algae (ca. 80 spp.) are the most diverse group of macroalgae both in species number and metabolites described, possessing a wide variety of chemical structures (Wiencke & Clayton, 2002; Amsler & Fairhead, 2006; Amsler et al., 2008; Wiencke et al., 2014). Remarkably, in contrast with brown algae, they lack phlorotannins (Blunt et al., 2018; Núñez-Pons et al., 2018).

Sponges are dominant components of the antarctic benthos and they play an important role in the structure and dynamics of benthic communities (Dayton et al., 1974; McClintock, 1987; Dayton, 1989). In the past century, 15 species of antarctic sponges, belonging to 14 genera, were chemically investigated (reviewed in Avila et al., 2008). A variety of structural types and new metabolites were isolated from *Latrunculia* (Ford & Capon, 2000; Furrow et al., 2003; Li et al., 2018), *Isodictya* (Moon et al., 2000; Vankayala et al., 2017), *Crella* (Ma et al., 2009) and hexactinellid sponges (Núñez-Pons et al., 2012a; Carbone et al., 2014), among others (Table 11.1). Suberitane derivatives from the sponge genus *Suberites* have been proposed as compounds with high taxonomic relevance (Díaz-Marrero et al., 2003, 2004). However, in a recent study, we reported very close analogues from another antarctic sponge, *Phorbas areolatus*, thus adding *Phorbas* as another genus of interest for the discovery of novel sesterterpenoids (Solanki et al., 2018). A literature review pointed out many other interesting sesterterpenoids from other species of the genus *Phorbas* in other geographical areas (Daoust et al., 2013; Wang et al., 2016). In light of these findings, we suggested a taxonomical reinvestigation of the species of the genera *Suberites* and *Phorbas* (Solanki et al., 2018).

Soft corals are sessile organisms often without physical and/or behavioural defences. This has led to a great development of chemical defences. Thus, the vast majority of natural products described from cnidarians are from Anthozoa, the largest of the four cnidarian classes, and particularly from Octocorallia, with >80% of the compounds identified (Harper et al., 2001; Blunt et al., 2018). Here, the typical defensive chemicals are terpenoids and steroids (Paul, 1992; von Salm et al., 2014, and reviewed in Núñez-Pons & Avila, 2015), although they may also include potent toxins (Slattery & McClintock, 1995; Jouiaei et al., 2015). In recent years, some new natural products, mainly terpenoids, have been isolated from antarctic octocorals. *Alcyonium antarcticum* (*A. paessleri*) has been found to possess several terpenoids, including paesslerins A and B (Rodríguez Brasco et al., 2001), alcyopterosins (Palermo et al., 2000; Carbone et al., 2009), alcyonicene and deacetoxy-alcyonicene, and some other sesquiterpenes (Manzo et al., 2009). Alcyopterosins are illudalane sesquiterpenoids also described for other species of the same genus, namely *A. grandis*, *A. haddoni*, *A. paucilobulatum* and *A. roseum* (Núñez-Pons & Avila, 2015). The gorgonian *Dasystenella acanthina* also produces sesquiterpenes (Gavagnin et al., 2003). In addition to terpenoids, new steroids have also been described in *Anthomastus bathyproctus* (Mellado et al., 2005).

Bryozoans are particularly well represented sessile suspension feeders in the antarctic benthic communities (ca. 390 spp.) (De Broyer & Danis, 2011)

Table 11.1 *Chemicals from marine benthic macroorganisms from Antarctica reported from 2000 to 2018*

Natural products	Phylum	Taxa	Location	References
Cystos phaerol	Ochrophyta	*Cystosphaera jacquinotii*	WAP, South Shetland Islands	Ankisetty et al., 2004
Bromoform		*Desmarestia anceps*	WAP, South Shetland Islands	Ankisetty et al., 2004
Plastoquinones		*Desmarestia menziesii*	WAP, South Shetland Islands	Ankisetty et al., 2004
Phlorotanins, Acetogenins, Diterpenes		*Desmarestia menziesii*, *D. anceps*, *D. antarctica*, *Himantothallus grandifolius*, *Ascoseira mirabilis*, *Cystosphaera jacquinotii*	WAP, South Shetland Islands	Ankisetty et al., 2004
Halogenated furanones, Pulchralides, Fimbrolides, Acetoxyfimbrolide, Hydroxyfimbrolide	Rhodophyta	*Delisea pulchra*	WAP, South Shetland Islands	Ankisetty et al., 2004
Halogenated organic compounds		*Delisea pulchra*, *Plocamium cartilagineum*	WAP, South Shetland Islands	Maschek & Baker, 2008
Halogenated terpenes		*Delisea pulchra*, *Plocamium cartilagineum, Pantoneura plocamioides*	WAP, South Shetland Islands	Argandona et al., 2002
Sulfated polysaccharides		*Iridaea cordata*	WAP, South Shetland Islands	Kim et al., 2017

P-hydroxybenzaldehyde, P-methoxyphenol		*Myriogramme smithii*	WAP, South Shetland Islands	Ankisetty et al., 2004
Epi-plocameneD		*Plocamium cartilagineum*	WAP, South Shetland Islands	Ankisetty et al., 2004
Keto-steroid	Porifera	*Anoxycalyx ijimai*, *A. joubini*, *Rossella antarctica*, *R. fibulata*, *R. nuda*, *R. racovitzae*, *R. villosa*	Weddell Sea	Núñez-Pons et al., 2012a; Núñez-Pons & Avila, 2014a
Glassponsine		*Anoxycalyx joubini*	Weddell Sea	Carbone et al., 2014
Norselic acids A-E		*Crella* sp.	Anvers island	Ma et al., 2009
Darwinolide, Membranolide B, C, and D, Dihydrogracilin A		*Dendrilla membranosa*	Anvers Island	Ankisetty et al., 2004; Witowski, 2015; von Salm et al., 2016; Ciaglia et al., 2017
Antifreeze peptide		*Homaxinella balfourensis*	McMurdo Sound	Wilkins et al., 2002
Erebusinone, 3-Hydroxykyrunenine, Methyl 3-Hydroxyanthranilate		*Isodyctia erinacea*	Ross Island	Moon et al., 2000; Vankayala et al., 2017
Organohalogens		*Kirkpatrickia variolosa*, *Artemisina apollinis*, *Phorbas glaberrima*, *Halichondria* sp., *Leucetta antarctica*	King George Island	Vetter & Janussen, 2005
Discorhabdin G		*Latrunculia apicalis*	McMurdo Sound	Furrow et al., 2003
Discorhabdins, Tsitsikamma mines		*Latrunculia biformis*	Weddell Sea	Li et al., 2018
Discorhabdin R		*Latrunculia* sp.	Prydz Bay	Ford & Capon, 2000
Flabellone		*Lissodendoryx (Lissodendoryx) flabellata*	Terranova Bay	Cutignano et al., 2012

Table 11.1 (*cont.*)

Natural products	Phylum	Taxa	Location	References
Suberitenones A and B, Oxapyrosuberiterone, Isosuberiterone B, 19-episuberiterone B, Isoxaspirosuberiterone		*Phorbas areolatus*	Deception Island	Solanki et al., 2018
Caminatal, Oxaspirosuberitenone, 19-episuberiterone B, Suberiterone B		*Suberites caminatus*	King George Island	Díaz-Marrero et al., 2003, 2004
Suberitenones C and D, Suberiphenol		*Suberites* sp.	King George Island	Lee et al., 2004
Linderazulene, Ketolactone, C-16-Azulenoid	Cnidaria	*Acanthogorgia laxa*	South Shetland Islands	Patino Cano et al., 2018
Ainigmaptilone A and B		*Ainigmaptilon antarcticus*	Weddell Sea	Iken & Baker, 2003
Illudalane sesquiterpenes		*Alcyonium antarcticum*, *A. grandis*, *A. haddoni, A. paucilobulatum, A. roseum*	Weddell Sea, Deception Is.	Carbone et al., 2009; Nunez-Pons et al., 2013
Alcyonicene, deacetoxy-alcyonicene, and other sesqui terpenes		*Alcyonium antarcticum*	Terra Nova Bay	Manzo et al., 2009
Paesslerins A and B		*Alcyonium antarcticum* (*A. paessleri*)	South Georgia Islands	Rodriguez Brasco et al., 2001
Alcyopterosins A-O		*Alcyonium antarcticum* (*A. paessleri*)	South Georgia Islands	Palermo et al., 2000
Steroids		*Anthomastus bathyproctus*	South Shetland Island	Mellado et al., 2005

Trans-beta-farnesene, Isofuranodiene, Furanoeudesmane		*Dasystenella acanthina*	Terra Nova Bay	Gavagnin et al., 2003
Hodgsonal	Mollusca	*Bathydoris hodgsoni*	Weddell Sea	Iken et al., 1998; Avila et al., 2000
Granuloside		*Charcotia granulosa*	Deception Is., Livingston Is.	Cutignano et al., 2015; Moles et al., 2016
Diterpene glycerides		*Doris kerguelenensis*	Weddell Sea, Ross Sea and Antarctic Peninsula	Iken et al., 2002; Cutignano et al., 2011; Maschek et al., 2012; Wilson et al., 2013
Tambjamine A	Bryozoa	*Bugula longissima*	Antarctica (unknown locality)	Lebar et al., 2007
Asterosaponins and steroids	Echinodermata	*Diplasterias brucei*	Terra Nova Bay	Ivanchina et al., 2006, 2011
Disulphated polyhydroxysteroids		*Gorgonocephalus chilensis*	Antarctica (unknown locality)	Maier et al., 2000
Liouvilloside A and B		*Staurocucumis liouvillei*	Antarctica (unknown locality)	Maier et al., 2001
Triterpene glycosides		*Staurocucumis liouvillei*, *S. turqueti*, *Achlionice violaecuspidata*	Weddell Sea	Antonov et al., 2008, 2009, 2011; Silchenko et al., 2013
Rossinones	Tunicata	*A. falklandicum*, *A. fuegiense*, *A. meridianum*, *A. millari*, *Synoicum adareanum*	Weddell Sea	Nunez-Pons et al., 2010; 2012b
Aplicyanins A-F		*Aplidium cyaneum*	Weddell Sea	Reyes et al., 2008
Meridianins		*Aplidium falklandicum*, *A. meridianum*	Weddell Sea	Nunez-Pons et al., 2015
Rossinone B and others		*Aplidium fuegiense*	Weddell Sea	Carbone et al., 2012; Nunez-Pons et al., 2012b

Table 11.1 (*cont.*)

Natural products	Phylum	Taxa	Location	References
Meridianins		*Aplidium meridianum*, *Synoicum* sp.	South Georgia Is., Anvers Island	Lebar & Baker, 2010
Rossinone A and B		*Aplidium* sp.	Ross Sea	Appleton et al., 2009
Palmerolide A, Hyousterones A–D and Abeohyousterone, Palmerolides D-G		*Synoicum adareanum*	Anvers Island	Diyabalanage, 2006; Miyata et al., 2007 Noguez et al., 2011

WAP: Western Antarctic Peninsula.

and some species show circumpolar distributions and broad bathymetrical ranges (Figuerola et al., 2012a). This relevant worldwide benthic group is a source of pharmacologically interesting substances such as alkaloids and terpenoids with various ecological defensive activities from antifouling to antipredation (Lebar et al., 2007; Sharp et al., 2007). A remarkable number of antarctic species has been proven to be bioactive (Angulo-Preckler et al., 2015; Figuerola et al., 2012b, 2013a, 2013b, 2014a, 2014b, 2017; Taboada et al., 2013), although only one metabolite, the alkaloid tambjamine A from *Bugula longissima,* has been identified so far (Lebar et al., 2007).

Tunicata (Chordata) are exclusively marine animals, sessile in adult stages, and protected by a more or less tough tunic. They have developed a great variety of defensive mechanisms to avoid predation and overgrowth, including physical protection, but mostly chemistry-based defences. These include accumulation of heavy metals or acids within their tissues and the use of bioactive compounds (Núñez-Pons et al., 2012b). Different strategies do exist, while colonial ascidians tend to produce antifouling or repellent chemicals, some solitary ascidians instead tend to be overgrown by epibionts to hide from possible predators (Stoecker, 1980; Bryan et al., 2003; Lambert, 2005). Ascidians mostly possess nitrogenated compounds, particularly aromatic heterocycles, like peptides, alkaloids and amino acid derived metabolites (Blunt et al., 2018). Also, in smaller amounts, they present some non-nitrogenous compounds, such as lactones, terpenoids or quinones (Blunt et al., 2018). Antarctic ascidians coming from shallow and deep bottoms present bioactive natural products such as palmerolide A, a group of ecdysteroids, meridianins, aplicyanins and rossinones (Diyabalanage et al., 2006; Miyata et al., 2007; Seldes et al., 2007; Appleton et al., 2009). It is often unclear whether the tunicates are the true producers of the molecules or if associated microbes may play a role in their chemical ecology (Núñez-Pons et al., 2012b).

Natural products from antarctic molluscs have very recently been reviewed in Avila et al. (2018). Very few new studies have dealt with other groups, such as echinoderms or other minor groups (Table 11.1). Overall, secondary metabolites in antarctic marine organisms are critical for structuring marine benthic communities (Avila et al., 2008; Figuerola et al., 2012b; von Salm et al., 2018). Chemical marine natural products display unique carbon skeletons and functional groups (Table 11.1), terpenoids, acetogenins and compounds of mixed biosynthesis being the major classes of compounds found. The total number of antarctic benthic macroorganisms chemically studied from 2000 to 2018 was 45, being 12 macroalgae (6 Ochrophyta and 6 Rhodophyta), 14 Porifera, 9 Cnidaria, 2 Mollusca, 3 Echinodermata and 5 Tunicata. The number and diversity of natural products being found is quickly increasing, and the next question is how these compounds function in nature.

11.3 Ecological activity

Polar regions are more difficult to access than other areas in the planet, and thus scientific progress has been slower there. Nevertheless, the extreme and often unique marine environments surrounding Antarctica, as well as the many unusual trophic interactions in antarctic marine communities, may be expected to favour the development of new natural products and/or for finding novel biological roles for them (Amsler et al., 2001; Avila et al., 2008). A number of new secondary metabolites with various activities, such as unpalatability, antibacterial, antitumor, cytotoxicity and others, has been reported from antarctic organisms, including sponges, cnidarians, bryozoans, molluscs, echinoderms, tunicates, microorganisms and symbiotic microorganisms, such as sponge-associated microbes (Avila et al., 2008; Papaleo et al., 2012; Núñez-Pons et al., 2012c; Núñez-Pons & Avila, 2015; von Salm et al., 2018). A complete list of the ecological activities of identified molecules from antarctic marine benthic macroorganisms recently described (from 2000 to 2018) is shown in Table 11.2. In many cases it is not clear whether compounds are produced through *de novo* biosynthesis by the organism itself or whether they are acquired through diet or have microbial symbiont origin (Avila et al., 2008; von Salm et al., 2018). Ecological studies on selected antarctic natural products are briefly discussed here.

Isolated compounds from macroalgae are few, but chemical extracts of macroalgae showed several remarkable activities, including predator deterrence, antibiotic and UVR protection (McClintock & Karentz, 1997; Schnitzler et al., 2001; Amsler et al., 2005, 2009; Fairhead et al., 2005; Erickson et al., 2006; Rhimou et al., 2010; Figuerola et al., 2012b). A specific review on UV-protecting compounds from antarctic organisms has been published recently (Núñez-Pons et al., 2018), and therefore we will not discuss these here. Deterrency has been described for extracts and tissue of brown algae species against sympatric herbivores (Ankisetty et al., 2004; Amsler et al., 2005, 2008, 2009, 2014; Huang et al., 2006), such as in the genus *Desmarestia* (*D. antarctica, D. anceps* and *D. menziesii*), as well as in *Himantothallus grandifolius*, *Cystosphaera jacquinotii* and *Ascoseira mirabilis*. Brown seaweeds are very important in terms of biomass in Antarctica, as well as in species diversity (ca. 27 spp.) and grade of endemism (12 spp.) (Wiencke & Clayton, 2002; Amsler & Fairhead, 2006; Wiencke et al., 2014). Therefore, this group and their chemical interactions are a very relevant component of antarctic benthic communities (Amsler et al., 2009). Furthermore, an interesting example of allopathic activity has been described in *D. menziesii* in which plastoquinones act against herbivores, affect fertility in sea urchins, deter seastars, and prevent proliferation of some bacteria (Rivera, 1996; Ankisetty et al., 2004).

Some examples of highly active molecules from red algae are halogenated furanones, such as pulchralide, fimbrolide, acetoxyfimbrolide and hydroxyfimbrolide, the last two with a strong antimicrobial activity (Ankisetty et al., 2004). One of the most well-known red algae from Antarctica is *Plocamium cartilagineum*, which shows a great profusion of compounds both to deter predators and to control microbial proliferation, ranging from epiplocamene, pyranoids, to cyclic and acyclic halogenated monoterpenes (Fries, 2016; von Salm et al., 2018).

As mentioned above, examples of ecologically relevant chemically mediated relationships include repellent substances from a range of antarctic macroalgae (Amsler et al., 2005; Aumack et al., 2010; Bucolo et al., 2011), and defensive molecules from diverse invertebrates, such as the sponges *Latrunculia apicalis* (Furrow et al., 2003), *Rossella* spp. (Núñez-Pons et al., 2012a), *Phorbas areolatus* (Solanki et al., 2018) and several other antarctic sponges (Peters et al., 2009; Núñez-Pons et al., 2012a; Angulo-Preckler et al., 2018); the cnidaria *Alcyonium* spp. (Carbone et al., 2009; Núñez-Pons et al., 2013) and three other soft corals (Slattery & McClintock, 1995); the brachiopod *Liothyrella uva* (McClintock et al., 1993; Mahon et al., 2003); the nudibranch molluscs *Bathydoris hodgsoni* (Avila et al., 2000) and *Doris kerguelenensis* (Iken et al., 2002); the ascidians *Distaplia cylindrica* (McClintock et al., 2004), *Cnemidocarpa verrucosa* (McClintock et al., 1991), *Aplidium* spp., *Synoicum* spp. (Núñez-Pons et al., 2010, 2012b) and several other ascidians (Koplovitz et al., 2009); as well as eggs, embryos and larvae of a range of invertebrate species (McClintock & Baker, 1997; Moles et al., 2017). Activity in molluscs is reviewed in Avila et al. (2018). Furthermore, in several multispecies studies, many antarctic marine species were found to contain lipophilic fractions that repelled the starfish *Odontaster validus* and the amphipod *Cheirimedon femoratus* (Taboada et al., 2013; Núñez-Pons & Avila, 2014b; Moles et al., 2015). A more recent study with 20 antarctic sponges evaluated repellence against seastar and antimicrobial activity against sympatric bacteria revealing a striking antimicrobial activity (100%) and 22% repellency (Angulo-Preckler et al., 2018).

Some alcyopterosins are also feeding deterrents against the omnivorous antarctic seastar *Odontaster validus* (Carbone et al., 2009). No ecological activity has been described for the rest of *Alcyonium* spp. natural products, except for moderate cytotoxicity of paesslerins A and B (Rodríguez Brasco et al., 2001). Ainigmaptilone A, isolated from the gorgonian coral *Ainigmaptilon antarcticus*, also showed repellence towards *O. validus*, along with antifouling properties, while ainigmaptilone B did not show any of these activities (Iken & Baker, 2003). Pigments and pigment derivatives may also be used for defensive purposes. Two sesquiterpenoids from *Acanthogorgia laxa* present antifouling activity against a wide array of microorganisms (Patiño Cano et al., 2018). Moreover, the seven steroids from the octocoral *Anthomastus bathyproctus* displayed weak cytotoxicity (Mellado et al., 2005). Even if compounds have not been identified yet, extracts from many other cnidarian species have proven to be repellent to *O. validus* too (Avila, 2016a). Further studies should be directed to identify the chemicals behind these ecological activities.

Interestingly, a variety of chemical defensive strategies are common in several antarctic bryozoan species against microorganisms and abundant and ubiquitous sympatric predators with circumpolar and eurybathic distributions. This evidences that their natural products are used for a wide array of ecological roles. In particular, several experiments showed antibacterial activity against a range of antarctic bacteria (Figuerola et al., 2014b, 2017), while the same and/or other species displayed repellent activities against the seastar *Odontaster validus* and the amphipod *Cheirimedon femoratus* (Taboada et al., 2013; Figuerola et al., 2017), and cytotoxic activities against the sea urchin *Sterechinus neumayeri*, reducing its reproductive success (Figuerola et al., 2013b, 2014a). These studies also demonstrated the presence of lipophilic and/or hydrophilic bioactive compounds in different antarctic bryozoan species, and these activities sometimes differed within populations of the same species (Figuerola et al., 2013a, 2017). In particular, some species exhibited antibacterial, cytotoxic and/or repellent activities of different nature (lipophilic or hydrophilic) at different sites (e.g. *Notoplites drygalskii*) or in the same area at similar (e.g. *Cornucopina pectogemma*) or different depths (e.g. *Camptoplites angustus* and *C. tricornis*; Figuerola et al., 2013a, 2014b, 2017). These inter- and intraspecific variabilities found in bryozoan species could be attributed to environmental-induced responses, genetic variability among populations and/or bacterial symbiotic associations (Davidson & Haygood, 1999; Morris & Prinsep, 1999; McGovern & Hellberg, 2003; Pawlik, 2012). However, no clear prove of these has been provided so far for these species.

Chemical defences might be more prevalent in particular species with the lack of apparent physical defensive structures. The best group to test this hypothesis is the ctenostome bryozoans, which are not protected by calcarious skeletons. In fact, the antarctic ctenostome *Alcyonidium flabeliforme* showed cytotoxic activity against the sperm of the sea urchin and significant repellency towards *O. validus* (Figuerola et al., 2013b; Taboada et al., 2013). In agreement, other species of the same genus from diverse regions produce bioactive compounds (Sharp et al., 2007). Several species with lightly calcified frontal walls like the antarctic *Melicerita obliqua* seem to compensate their vulnerability to predation with chemical defences (Figuerola et al., 2013a). Other species did not show chemical defences, suggesting the presence of alternative chemical and/or physical defensive mechanisms. Indeed, it is well known that cheilostome bryozoans exhibit rigid exoskeletons and/or a wide variety of skeletal structures, including spines, avicularia and vibracula, with protective function (Winston 2010). For example, defensive chemicals against the seastar *O. validus* were not detected in *Dakariella dabrowni*, which possess a rigid well-calcified skeleton (Figuerola et al., 2013a). Also, some authors suggest encrusting species are more capable of resisting damage by predators and effectively repair from grazing injuries, compared to erect colonies (Best & Winston, 1984). Therefore, chemical defences might be more common in erect and flexible forms as suggested for the antarctic species *Klugella echinata* and *N. drygalskii* (Figuerola et al., 2013a). Contrary to this hypothesis, the erect and flexible *C. pectogemma*, *C. polymorpha* and *Nematoflustra flagellata* did not show repellence to the seastar *O. validus* (Figuerola et al., 2013a, 2017) although both species appeared to be defended against the amphipod *C. femoratus*. Feeding repellent responses of a wide range of flexible and non-flexible bryozoans were also more frequent in the assays with *C. femoratus* than towards the seastars (Figuerola et al., 2013a). These suggest that amphipods might exert a higher localised pressure in bryozoans than seastars. In addition, these species possess defensive physical devices such as avicularia and vibracula. Although there is evidence of trade-offs between chemical and physical defensive mechanisms in antarctic bryozoans, previous studies showed a general trend in combining both defensive strategies, suggesting complementary traits. Further isolation and characterisation of the metabolites involved in these chemoecological interactions should be conducted.

Remarkably, a strong antifouling activity was reported *in situ* for different antarctic species, including the hydroid *Eudendrium* sp., the sponges *Phorbas glaberrima* and an *Hadromerida* sp., the tunicate *Synoicum adareanum* (Angulo-Preckler et al., 2015). Recently, the crude extracts of *Mycale tylotornota* (sponge) and *Cornucopina pectogemma* (bryozoan) avoided *in situ* fouling by eukaryotic organisms in field experiments, showing that invertebrates may also modulate the attachment of different microbial communities, either by natural products of the invertebrate itself or by natural products produced by the microbial community, resulting in different levels of fouling.

Although the ecological function of many metabolites from most tunicates remains undetermined, it is known that at least some of them are used as predator deterrents (Núñez-Pons et al., 2010) as well as antifoulants (Davis & Bremner, 1999). Antarctic examples are reported in Table 11.2. Most compounds come from the genera *Aplidium* and *Synoicum*. Cytotoxicity has been reported for aplycianins, rossinones and palmerolide A, while feeding repellence has been described for meridianins, rossinones and ecdysteroids.

In general, most authors keep looking at predator deterrence as the main ecological assay, although in recent years we observe an increasing number of studies widening their scope to different ecological roles, such as antimicrobial inhibition and cytotoxic effects (Table 11.2).

11.4 Adaptation to climate change?

According to the available information, climate change may already be affecting many antarctic organisms in different ways (Constable et al., 2014; Turner

Table 11.2 *Ecological activity of identified molecules from Antarctic marine benthic macroorganisms from 2000 to 2018*

		Feeding deterrence			Bioactivity				
Phylum	Taxa	Macropredator	Mesopredator	Antimicrobial activity	Cytotoxicity	Antifouling	Antifreezing	Growth inhibition	Allelopathy
Ochrophyta	*Ascoseira mirabilis*	*	*	*					
	Cystosphaera jacquinotii	*	*	*					
	Desmarestia anceps	*	*	*					
	Desmarestia antarctica	*	*	*					
	Desmarestia menziesii	*	*	*					
	Himantothallus grandifolius	*	*	*	*				
Rhodophyta	*Delisea pulchra*		*	*					
	Iridaea cordata	*							
	Myriogramme smithii		*						
	Palmaria decipiens	*							
	Pantoneura plocamioides		*						
	Plocamium cartilagineum		*	*					
Porifera	*Artemisina apollinis*								
	Crella sp.		*	*					*
	Dendrilla membranosa			*	*	*			
	Halichondria sp.								*
	Homaxinella balfourensis						*		
	Isodictya erinacea	*	*					*	
	Kirkpatrick ia variolosa			*	*				*
	Latrunculia apicalis	*			*				
	Latrunculia biformis				*				

	Latrunculia sp.			*				
	Lissodendoryx flabellata				*			
	Phorbas areolatus	*		*	*			
	Phorbas glaberrima							*
	Suberites caminatus				*			
Cnidaria	*Acanthogorgia laxa*							
	Ainigmaptilon antarcticus	*						
	Alcyonium antarcticum	*			*			
	(A. paessleri)							
	Alcyonium grandis	*						
	Alcyonium haddoni	*						
	Alcyonium paucilobulatum	*						
	Alcyonium roseum	*						
	Anthomastus bathyproctus				*			
	Dasystenella acanthina	*				*		
Mollusca	*Bathydoris hodgsoni*	*				*		
	Doris k erguelenensis	*			*			
Echinodermata	*Diplasterias brucei*				*			
	Gorgonocephalus chilensis				*			
	Staurocucumis liouvillei			*	*			
Tunicata	*Aplidium cyaneum*				*			
	Aplidium falklandicum	*	*		*			
	Aplidium fuegiense	*	*		*			
	Aplidium meridianum	*	*		*			
	Synoicum adareanum	*	*		*		*	

et al., 2014; Poloczanska et al., 2016; Ashton et al., 2017; Griffiths et al., 2017). For example, penguins are affected by many more parasites (Díaz et al., 2017), while some benthic species may experience changes in their distribution ranges (Barnes et al., 2009; Gutt et al., 2011; Fillinger et al., 2013; Pasotti et al., 2015), or even reduce some of their interspecific relationships (Barnes et al., 2014). Since Antarctica plays an important role in the Earth's climate regulation system, knowledge of climate-change related processes is vital to understand and predict future scenarios. From this perspective, information on how Antarctic organisms relate with one another and with other components of the communities via natural products is key to advance in our understanding of a climate-changing world.

The ability to physiologically respond to temperature stress has been studied in many different antarctic taxa over many years (reviewed by Peck, 2018). Results showed poor survival capacities, but there seems to be some variation at species level (Ashton et al., 2017). Regarding natural products, we do not know yet whether changes in temperature, pH, calcification and others, may affect the production and/or use of chemicals by marine benthic organisms in Antarctica. The potential calcification problems in groups like bryozoans (see above) could dramatically affect the trade-offs between chemical and physical defences in these species, and thus their survival in the years to come. Metabolites related to unpalatability, as halogenated monoterpenes (like anverene and epi-plocamene) which define relations between macroalgae and sympatric herbivores, may vary upon environmental conditions, and therefore, trophic relationships in the antarctic ecosystems could be strongly affected by climate change. The macroalgae *Desmarestia menziesii*, for instance, increases phlorotannin production when exposed to acidification (Schoenrock et al., 2015). Another example could perhaps be the potential stress-induced compounds found in the nudibranch *D. kerguelenensis*, austrodoral and austrodoric acid (Gavagnin et al., 2003), among the highly diverse chemical arsenal of this mollusc (Avila et al., 2018), although this requires further investigation. Some variation in chemicals has also been cited for the macroalgae *Plocamium cartilagineum* from different localities (Young et al., 2013, 2015), the sponge *Dendrilla membranosa* (Witowski, 2015) and some bryozoan species (see above), which could be related to changes related to habitat or geographical specificity. Whether this could be related to adaptation to environmental change remains to be further investigated.

As an *a priori* assumption, one could think that, for instance, increasing water temperature would be a stressful factor that could induce organisms to stop producing secondary metabolites, since this is an expensive strategy. On the contrary, for some species, a higher temperature could lead to an increase in chemical defences as a reaction to gather protection against the same stress. Our preliminary experiments suggest that this could be a species-specific trend (unpublished data from the authors) and that it deserves further experimentation and analysis. For example, it is not the same to study a nudibranch mollusc that biosynthesises its own chemical defences than a sponge that contains a very rich microbiome, which may in fact be the producer of the bioactive compounds. How to test the effects of climate change in natural compounds production may therefore be a challenging task that should consider not only the different variables associated to environmental change but also the different strategies of the benthic organisms studied.

To conclude, we believe that even if advances have been made recently in understanding the ecological role of natural products in antarctic marine benthos, there is still a lot to be done to clarify the potential use of chemicals by cold-water organisms. Among other priorities, it is essential that further studies address the topic of compound production related to climate change and how this may affect species survival, before it is too late.

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Experimental evidence of antimicrobial activity in Antarctic seaweeds: ecological role and antibiotic potential

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EXPERIMENTAL EVIDENCE OF ANTIMICROBIAL ACTIVITY IN ANTARCTIC SEAWEEDS: ECOLOGICAL ROLE AND ANTIBIOTIC POTENTIAL

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Abstract

Seaweeds display a wide range of secondary metabolites which serve multiple functions, including chemical and ecological mediation with microorganisms. Moreover, they also showed potential for human use due to their diverse bioactivities, like antibiotic properties. Nonetheless, seaweeds from all regions are not equally understood in chemical ecology terms. Hence, Antarctic seaweeds are from the lesser studied groups. Aiming to strengthen the current understanding of the chemical ecology and potential bioactivity of Antarctic seaweeds, we performed an antibiotic activity screening using crude extracts from 22 Antarctic macroalgae species. Extractions were performed separating lipophilic and hydrophilic fractions at natural concentrations. Antimicrobial activity assays were performed by the disk diffusion method against seven Antarctic bacteria and seven human pathogenic surrogates. Our results showed that red seaweeds (especially *Delisea pulchra*) inhibited larger numbers of microorganisms compared to brown ones, and that lipophilic fractions were more active than hydrophilic ones. Both types of bacteria tested (Gram-negative and Gram-positive) were inhibited, suggesting a trend of non-specific chemical defense. However, Gram-negative bacteria along with one pathogenic fungus showed greater resistance. Our study defines the chemical interactions between previously not screened sympatric Antarctic seaweeds and microorganisms, as well as the potential of the seaweed extracts for pharmacological applications.

Keywords: Antarctica, antibiotic activity, Rhodophyta, Phaeophyceae, chemical ecology.

Introduction

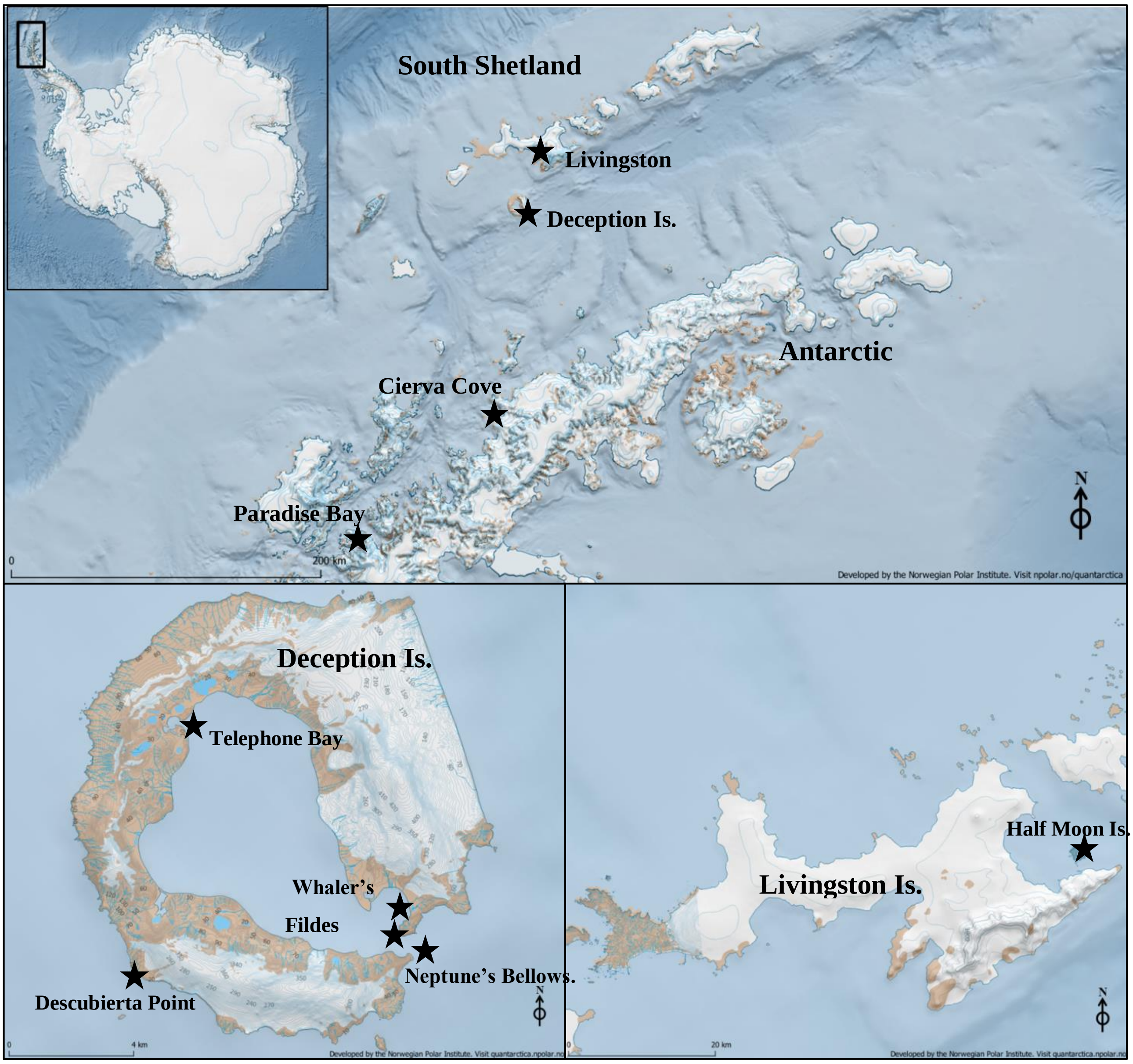
Antarctica presents a high degree of endemic seaweed species (Oliveira et al., 2020; Christian Wiencke et al., 2002), mainly favored by the prolonged isolation (Oliveira et al., 2020; Franciane Pellizzari et al., 2020; Christian Wiencke et al., 2002) and extreme conditions of seasonal light variation and low temperatures (Clark et al., 2017; Fraser et al., 2020; Ivan Gómez, 2015; Christian Wiencke et al., 2002). Seaweeds dominate the communities on shallow areas of the Antarctic coasts (down to 40 m) that are ice free, performing multiple ecosystem functions and biotic interactions (Charles D. Amsler et al., 2020; Gaitan-Espitia & Schmid, 2020; Iván Gómez & Huovinen, 2020b; Momo et al., 2020; Christian Wiencke et al., 2002). As part of those interactions, Antarctic seaweeds are exposed to a wide range of microorganisms present in the surrounding water, like bacteria, fungi, and microalgae (Gaitan-Espitia & Schmid, 2020; Singh et al., 2016). Those microorganisms are present in high concentrations in the marine water column (Bej et al., 2010; Jenkins et al., 1998; Zdanowski, 1995). It is known that microorganisms may interact with macroalgae in different ways (Alvarado et al., 2018; C. D. D. Amsler, 2008; Gaitan-Espitia & Schmid, 2020). Even though it is still not fully understood, several studies suggest that seaweeds regulate those interactions chemically (Gaitan-Espitia & Schmid, 2020), using secondary metabolites (as phlorotannins, isoterpenoids, and volatile halogenated organic compounds). Several studies have screened seaweed species (including some Antarctic species) in search of potential bioactivity, pointing out that algal chemodiversity is indeed very high (Benites Guardia, 2019; Bernardi et al., 2016; Carroll et al., 2020; von Salm et al., 2018; Ryan M. Young et al., 2015). Nevertheless, macroalgae remain less studied than benthic Antarctic fauna, especially if we compare them to groups like sponges and other macroinvertebrates (C. Angulo-Preckler et al., 2018; C. Avila et al., 2008; Solanki et al., 2018; Vankayala et al., 2017). Furthermore, the difficulties of performing research in Polar Regions keep the number of studies on chemical activity of native seaweed species in low numbers, hiding the full ecological implications of this activity. Aside from the ecological implications, the diversity of those compounds represents high potential to find useful bioactive molecules, which could be vital for some fields like medicine. As example, even though antibiotic-resistant microorganisms have been studied since the appearance of the first antibiotic treatments (D'Costa et al., 2011), the number of highly resistant human pathogenic microorganisms has rapidly raised during recent years (Aslam et al., 2018; Ben et al., 2019; Berendonk et al., 2015; Lerminiaux & Cameron, 2019; Ventola, 2015; Zaman et al., 2017). In some cases this increasing trend has led to serious health risks, becoming nowadays a public health concern. Even though bioprospection of natural sources with antibiotic potential has grown recently (Andersson et al., 2020; Pang et al., 2019; Ragheb et al., 2019), new sources of potentially useful compounds is needed as new resistant pathogenic strains are identified each year. For this, lesser studied organisms, like Antarctic seaweeds, represent an opportunity to unravel new ecological interactions in Antarctic ecosystems, as well as new sources of potentially useful compounds. In this context, the two main objectives of our study were to elucidate the chemical interactions between Antarctic seaweeds and sympatric microorganisms and to evaluate the antibiotic potential of those macroalgae extracts against surrogates of common human pathogens. Additionally, we also tried to determine whether there were significant differences in the chemical activity between sampled seaweed with strict Antarctic distribution and wider distributed ones.

Materials and methods

Species selection for the antimicrobial assay was done by taking into account the lack of previous information on their bioactivity, as well as their potentially interesting chemistry, when there was any previous report on specific taxa. The species selected for the bioassay were 22 Antarctic macroalgae (**Table 1**), fourteen of which were Rhodophyta and eight Phaeophyceae (Ochrophyta). Seaweed sampling was performed during several Antarctic cruises (along the west Antarctic Peninsula and South Shetland Islands archipelago) in the framework of the ACTIQUIM and DISTANTCOM projects, during the austral summers of 2012, 2013, 2016, and 2017 (**Figure 1, Table 1**). Collection of the samples was made by scuba diving for subtidal specimens and by hand for intertidal species. The samples were immediately frozen (-20^{o}C) until the chemical study was performed. Once in the laboratory, and previous to chemical extractions, fragments of the frozen samples were used to perform taxonomic identification at species level following Wienke & Clayton (2002) keys along with specific taxonomic monographies for each species to avoid misidentification. For this, morphology and microscopic anatomy of the sampled species was studied with histologic preparations and observation of optical microscopy of the fragments taken.

Chemical extractions were made following a modified protocol (C. Angulo-Preckler et al., 2015), which was based on previous studies (C. Avila et al., 2000; Bhosale et al., 2002; K. Iken et al., 2002; Murugan & Ramasamy, 2003). According to this procedure, in order to establish the polarity of the bioactive molecules, lipophilic and hydrophilic extracts were obtained separately. To that end, the algae material was first cleaned of epiphyte organisms, and then weighted to obtain values of wet weight. After that, the samples were fragmented for homogenization before grinding with acetone. The resulted mixture was then filtered with filter paper and treated with ultrasonic waves (during 5 min) to increase the breakup of cells initiated by the acetone. This step was repeated three times per sample, obtaining a liquid solution with some algal solid residue, which was left to dry and weighted. The acetone was then evaporated *in vacuo* in a rotary evaporator. After this, fractionation of extracts by polarity was achieved using specific solvents for each type of extract. For lipophilic compounds, diethyl ether 100% (Et_2O) was used and the process was repeated three times. The resulting ethereal solution was evaporated again *in vacuo* and transferred to pre-weighted vials alongside Et_2O. The content of the vials was again evaporated and weighted to obtain the ethereal extract weight. After that, they were stored frozen (-20^oC) until used for the antimicrobial assays. As for the separation of hydrophilic compounds, it was performed by using butanol 100% (BuOH). In this case, it was done twice. After this, similarly to the lipophilic compounds, the butanolic solution containing hydrophilic fractions was transferred to the pre-weighted vials, adding trichloromethane in this case, lyophilized and weighted again before stored frozen.

Figure 1. Maps of the sampling locations and stations listed on **Table 1** (marked with stars). A) Antarctic continent. B) Antarctic Peninsula region and South Shetland archipelago. C) Deception Island. D) Livingston Island. Map constructed with QGIS software (v. 3.16) with Quantarctica package



Also, all the algal solid residues were weighted to obtain the Total Dry Weight (DW_T, adding the weights of algal solid residue, dry Et_2O crude extracts, dry weight of BuOH, and dry weight of aqueous residue). This is necessary to calculate the extract natural concentration (**Table 1)**, which will be further used in the microbial experiments in order to simulate the real concentration in nature.

Antimicrobial activity inhibition was performed by using the crude extracts with the agar disk diffusion method on isolated cultures of a variety of microorganisms, as described in (Acar, 1980; Álvarez, 1990; C Angulo-Preckler et al., 2017; Figuerola et al., 2014, 2017; Salvador, N. et al., 2007). The microorganisms used in the tests consisted in 13 different bacteria (seven Antarctic isolates and six strains used as pathogen surrogates obtained from culture collections) and one pathogenic fungus (**Table 2**). Antarctic isolates included *Psychrobacter* sp., *Paracoccus* sp., *Oceanobacillus* sp., *Bacillus aquamaris*, *Micrococcus* sp. and two strains of *Arthrobacter* sp., previously isolated from different Antarctic organisms and substrata (**Table 2**). The other six bacteria strains used here were *Vibrio cholerae* CECT 657, *Escherichia coli* O157:H7, ATCC 43888, *Pseudomonas aeruginosa* NCTC 10332T, *Escherichia coli* CECT 515, *Bacillus cereus* CECT 4014, *Staphylococcus aureus* CECT 59, and the fungus *Candida albicans* CECT 1001. The Antarctic bacteria were incubated for 48h on marine agar medium at 20^{o}C while the pathogenic representatives were incubated at 37^{o}C for 24h in Muller-Hinton medium. To achieve a turbidity of 0.5 McFarland, the microorganisms were transferred to a dilution medium with different NaCl concentrations (1.5% for the Antarctic strains and 0.85% for the pathogenic ones). After that, microorganisms were distributed homogeneously in separated testing plates with the corresponding agar medium to perform the agar disk diffusion tests. For each microbial strain and fraction three replicates of the bioassay were performed.

Extracts at natural concentration equivalents were inoculated on the diffusion paper disks (6 mm, PRAT DUMMAS *France*) using Hamilton syringes avoiding saturation of the disks. To this, methanol was used as solvent to infuse the two different types of extracts in the disks due to its rapid evaporation. Furthermore, for each test three controls were used for each replicate: one positive (impregnated with chloramphenicol) and two negatives, one consisting of a blank disk and another soaked only with the corresponding solvent used for the extractions (diethyl ether for the ethereal extracts and methanol for the butanolic ones). The prepared disks were then placed on the testing plates with the different microorganisms for the inhibition test. The inoculated plates with the disks were incubated for 48h at 20-22^{o}C for Antarctic bacteria, and for 24h at 37^{o}C for the pathogenic microorganisms. After incubation, the inhibition halii surrounding the paper disk were measured to assign the antimicrobial activity for each extract. Antibacterial activity was established following (Mahon et al., 2003) criteria to establish inhibition categories. These criteria classify weak inhibition between 0.1-1 mm (+), moderate inhibition 1.1-1.9 mm halii (++), and strong inhibition >2.0 mm (+++). Additionally, as some of our extracts exceeded measures of 4 mm, we classified them as very strong inhibition (++++).

Table 1. Seaweed sampling and extraction data

Seaweed species	Location	Station	Latitude (S)	Longitude (W)	Dry weight (g)	Natural concentration (mg/g of dry weight)	
Rhodophyta						**Ethereal**	**Butanolic**
Neuroglossum delesseriae	Deception Is.	Neptune's Bellows	63° 00' 00"	60° 34' 00"	15.73	12.51	5.32
Iridaea cordata	Livingston Is.	Half Moon Is.	62° 35' 44"	59° 54' 12"	3.05	0.45	19.94
Palmaria decipiens	Ant. Peninsula	Cierva Cove	64° 09' 03"	60° 57' 04"	1.06	22.57	46.79
Curdiea racovitzae	Ant. Peninsula	Cierva Cove	64° 09' 03"	60° 57' 04"	2.49	28.57	15.88
Phyllophora ahnfeltioides	Deception Is.	Fildes Point	62° 59' 28.55"	60° 33' 39.6"	0.47	28.17	12.16
Pantoneura plocamioides	Deception Is.	Whaler's Bay	62°58'53,91"	60°33'44,46"	1.98	0.05	0.03
Hymenocladiopsis prolifera	Deception Is.	Whaler's Bay	62°58'53,91"	60°33'44,46"	5.07	45.14	3.54
Leniea lubrica	Deception Is.	Whaler's Bay	62°58'53,91"	60°33'44,46"	9.25	70.77	2.85
Delisea pulchra	Deception Is.	Whaler's Bay	62°58'53,91"	60°33'44,46"	0.35	47.89	3.10
Sarcothalia papillosa	Deception Is.	Whaler's Bay	62°58'53,91"	60°33'44,46"	9.92	10.75	3.51
Phycodrys austrogeorgica	Deception Is.	Whaler's Bay	62°58'53,91"	60°33'44,46"	0.27	16.96	30.84
Rhodymenia subantarctica	Deception Is.	Whaler's Bay	62°58'53,91"	60°33'44,46"	0.52	283.61	14.18
Gigartina skottsbergii	Deception Is.	Whaler's Bay	62°58'53,91"	60°33'44,46"	14.14	11.04	0.24
Plocamium cartilagineum	Ant. Peninsula	Paradise Bay	64° 51' 57"	62° 51' 55"	0.46	11.11	1.75
Phaeophyceae							
Desmarestia menziesii	Ant. Peninsula	Cierva Cove	64° 09' 03"	60° 57' 04"	0.29	47.99	55.06
Desmarestia antarctica	Deception Is.	Fildes Point	62° 59' 28,55"	60° 33' 39,6"	0.49	205.15	80.48
Desmarestia anceps	Deception Is.	Fildes Point	62° 59' 28,55"	60° 33' 39,6"	0.77	66.15	44.10
Cystosphaera jacquinotii	Deception Is.	Whaler's Bay	62°58'53,91"	60°33'44,46"	10.59	28.95	1.12
Himantothallus grandifolius	Deception Is.	Fildes Point	62° 59' 27"	60° 33' 19"	13.54	92.68	2.59
Ascoseira mirabilis	Deception Is.	Descubierta Point	62° 59' 45"	60° 43' 31"	3.70	59.55	14.03
Phaeurus antarcticus	Deception Is.	Whaler's Bay	62°58'53,91"	60°33'44,46"	9.15	40.73	3.05
Adenocystis utricularis	Deception Is.	Telephone Bay	62° 55' 12"	60° 39' 37"	0.14	262.94	53.59

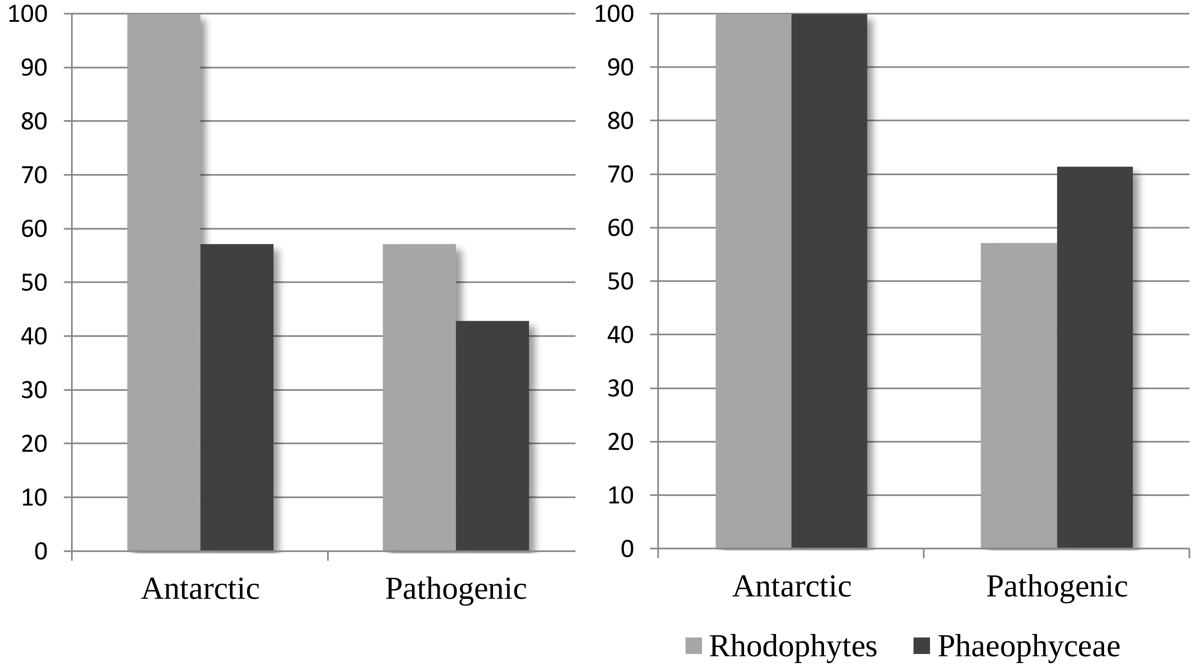
Table 2. List of tested microorganisms: strain code, species name, source, classification and cell wall type

Strain code	Microorganism	Isolated from	Taxa	Gram staining
	Antarctic			
A	*Psychrobacter* sp.	*Dendrilla antarctica* (sponge)	Bacteria	-
B	*Paracoccus* sp.	Stone	Bacteria	-
C	*Arthrobacter* sp. Isolate 1	*Axinella crinita* (sponge)	Bacteria	+
D	*Arthrobacter* sp. Isolate 2	*Axinella crinita* (sponge)	Bacteria	+
E	*Oceanobacillus* sp.	*Haliclona* sp. (sponge)	Bacteria	+
F	*Bacillus aquamaris* sp.	Sediment	Bacteria	+
G	*Micrococcus* sp.	Sediment	Bacteria	+
	Pathogenic			
H	*Vibrio cholerae* CECT 657	Culture collection	Bacteria	-
I	*Escherichia coli* O157:H7, ATCC 43888	Culture collection	Bacteria	-
J	*Pseudomonas aeruginosa* NCTC 10332T	Culture collection	Bacteria	-
K	*Escherichia coli* CECT 515	Culture collection	Bacteria	-
L	*Bacillus cereus* CECT 4014	Culture collection	Bacteria	+
M	*Staphylococcus aureus* CECT 59	Culture collection	Bacteria	+
N	*Candida albicans*	Culture collection	Fungi	N/A

Results

Our results showed differences in the percentage of inhibition depending on type of seaweed, microorganisms and chemical fraction of the extracts. As a summary, for the seaweed type, more Rhodophyta where active compared to the Phaeophyceae tested. Also, Antarctic microorganisms were more inhibited than pathogenic surrogates. Concerning the type of chemical fraction, the lipophilic extractions showed greater percentage of inhibition compared to hydrophilic ones (see **Figure 2**). In that sense, it is also worth noting that the natural concentration (mg/g of dry weight) of the different chemical extractions of our samples showed that lipophilic fractions (ethereal extracts) were in higher concentrations than hydrophilic ones for both, Rhodophyta and Phaeophyceae (see natural concentrations in **Table 1**). This was specially the case for Phaeophyceae, as ethereal extracts showed more than three times more concentrated than butanolic ones.

Figure 2. Percentages of inhibition of Antarctic or pathogenic microorganisms by butanolic (left) and ethereal (right) extractions of Rhodophytes and Phaeophiceae



Of the 22 macroalgae species studied here (14 Rhodophyta and eight Phaeophyceae), a total of 44 extracts (22 hydrophilic and 22 lipophilic) were tested against 14 microbial strains (**Table 3** and **4**). Lipophilic extracts showed stronger inhibition effects (52% of all the lipophilic extracts showed some antimicrobial activity) and higher inhibition values than the hydrophilic ones (30% of hydrophilic extracts were active). Thus, for the lipophilic extracts, nearly 30% of the active ones exhibited strong inhibitions (+++), in front of 13% of the hydrophilic ones. Nonetheless, the taxa with greater activity in the lipophilic extracts also displayed greater levels of inhibition in the hydrophilic fractions (*i.e. Delisea pulchra* and *Desmarestia antarctica*). Variability between the three replicates generally was very low (with overall mean differences between samples <0.5 mm in the halii). Nonetheless, some individual replicates showed higher variation in the tests with *Desmarestia menziesii* and *Phyllophora ahnfeltioides* against *Psychrobacter* sp. and *Delisea pulchra* against *Vibrio cholerae* (greater than 1mm, compared with the other two replicates of the same tests). However, as the rest of the replicates for the other tests and species showed no major variability, for those individual cases, these replicates were not included in the study.

A total of 14 of the 22 macroalgae studied showed antimicrobial activity against, at least, one microbial strain. In proportion, the 71% of Rhodophyta tested (10 out of 14) presented antimicrobial activity, whereas the 50% (4 out of 8) of Phaeophyceae were active, being the red algae the group with higher number of species chemically active in our tests. The species with the largest number of microorganism strains inhibited was the red algae *D. pulchra*. This species inhibited 11 of the 14 microorganism strains tested (around 79%) with both lipophilic and hydrophilic extracts. *D. pulchra* was also the only tested algae that showed inhibition against the fungus *Candida albicans*. Morover, *D. pulchra* presented the greater halii inhibition size, having the highest inhibition value (18.7%) with the lipophilic test against *V. cholerae*. For the brown seaweeds, the most active taxon was *D. antarctica*, which inhibited around 70% of the bacterial strains. This species presented the most intense activity with the lipophilic extracts against *Psychrobacter* sp. In contrast to those algae, several species showed no activity, among which, four brown algae (*Cystosphaera jacquinotii, Himantothallus grandifolius, Ascoseira mirabilis* and *Phaeurus antarcticus*) and four red algae (*Hymenocladiopsis prolifera, Sarcothalia papillosa, Leniea lubrica* and *Plocamium cartilagineum*).

Antarctic microorganisms were more inhibited than the pathogenic strains. Specifically, all seven Antarctic microorganism strains were inhibited at least by one seaweed species both by the hydrophilic and lipophilic extracts. Regarding the pathogenic strains, four out of seven (57%) were inhibited by at least one seaweed species when hydrophilic extracts were tested, and six out of seven (85%) showed inhibition for the lipophilic extracts. Furthermore, the results showed that Gram-negative bacteria were more resistant than Gram-positive ones to macroalgal extracts, as less strains of this group were inhibited (83% of the Gram-negative strains were inhibited by at least one extract). For this group of bacteria, therefore, only 26% of the 44 extracts tested (22 lipophilic and 22 hydrophilic) presented activity. On the other hand, Gram-positive bacteria were all inhibited by at least some algal extracts. In this case, the proportion of active extracts was 30%. The only strain that showed no inhibition at all by any of the macroalgal extracts tested was *Pseudomonas aeruginosa*, which belongs to the Gram-negative bacteria. It is worth mentioning also that in the tests with hydrophilic extracts, there were three strains that showed no inhibition, which were again *P. aeruginosa* and the two strains of *E. coli*. Apart from that, as mentioned before, the fungus *Candida albicans* was only inhibited by the two fractions of *D. pulchra.*

Table 3. Antibiotic activity of butanolic extracts of seaweed species against microorganism strains. Data expressed as in Mahon et al. (2003)

Species	Antarctic strains*							Pathogenic strains*							∑ Inhibited strains
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	
Rhodophyta															
Neuroglossum delesseriae	-	-	++	++	++	+	+++	-	-	-	-	-	-	-	5
Iridaea cordata	-	-	-	-	-	-	-	++	-	-	-	-	-	-	1
Palmaria decipiens	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Curdiea recovitzae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Phyllophora ahnfeltioides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Pantoneura plocamioides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Hymenocladiopsis prolifera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Leniea lubrica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Delisea pulchra	++	+++	++++	++++	++++	++++	++++	++++	-	-	-	+++	++++	+++	11
Sarcothalia papillosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Phycodrys austrogeorgica	-	-	+	-	-	-	-	-	-	-	-	-	-	-	1
Rhodymenia subantarctica	-	-	-	++	-	-	-	-	-	-	-	-	-	-	1
Gigartina skottsbergii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Plocamium cartilagineum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Phaeophyceae															
Desmarestia menziesi	-	-	++	+	-	+	-	-	-	-	-	+	-	-	4
Desmarestia antarctica	-	-	++	++	-	+	++	+++	-	-	-	+	+++	-	7
Desmarestia anceps	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Cystosphaera jacquinotii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Himantothallus grandifolius	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Ascoseira mirabilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Phaeurus antarcticus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Adenocystis utricularis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Total active extracts	**1**	**1**	**5**	**5**	**2**	**4**	**3**	**3**	**0**	**0**	**0**	**3**	**2**	**1**	

*Antarctic and pathogenic microorganism strains from A to N correspond to strain codes in **Table 2**.

Table 4. Antibiotic activity of ethereal extracts of seaweed species against microorganism strains. Data is expressed following Mahon et al. (2003)

Species	**Antarctic strains***							**Pathogenic strains***							**∑ Inhibited strains**
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	
Rhodophyta															
Neuroglossum delesseriae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Iridaea cordata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Palmaria decipiens	++	-	-	+	-	-	-	-	-	-	-	-	-	-	2
Curdiea recovitzae	+	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Phyllophora ahnfeltioides	+++	++	-	-	-	-	-	-	-	-	-	-	-	-	2
Pantoneura plocamioides	+	+	++	+++	-	+	+++	-	-	-	-	-	-	-	5
Hymenocladiopsis prolifera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Leniea lubrica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Delisea pulchra	++	++++	++++	++++	++++	++++	++++	++++	-	-	-	+++	++++	++++	11
Sarcothalia papillosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Phycodrys austrogeorgica	-	-	++	-	-	-	+	-	-	-	-	-	-	-	2
Rhodymenia subantarctica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Gigartina skottsbergii	-	-	-	-	+	-	+	+	-	-	-	-	-	-	3
Plocamium cartilagineum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Phaeophyceae															
Desmarestia menziesi	+++	+++	++	++	++	-	+	-	-	-	-	-	-	-	6
Desmarestia antarctica	+++	-	++	+++	+	+	+++	-	++	-	+++	++	+++	-	10
Desmarestia anceps	++	-	+	-	-	+	-	-	-	-	-	-	-	-	2
Cystosphaera jacquinotii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Himantothallus grandifolius	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Ascoseira mirabilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Phaeurus antarcticus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Adenocystis utricularis	-	-	-	-	-	-	-	+++	-	-	-	-	-	-	1
Total active extracts	**8**	**4**	**7**	**6**	**5**	**5**	**7**	**4**	**1**	**0**	**1**	**3**	**3**	**1**	

*Antarctic and pathogenic microorganism strains from A to N correspond to strain codes in **Table 2**.

Discussion

Most extracts of Antarctic macroalgae tested here showed antimicrobial activity. In terms of intensity of the different extractions, inhibition tests showed stronger effect of lipophilic extracts than hydrophilic ones. These coincides with previous studies that used other algal species (Aguila et al., 2012; Charles D. Amsler et al., 2009; Charles D. Amsler & Fairhead, 2006; Baker et al., 2008; M. J. Pérez et al., 2016; Rajauria et al., 2013; Salvador, N. et al., 2007; Shannon & Abu-Ghannam, 2016; Ryan M. Young et al., 2015). Several reasons could explain this. For example, it could be partially attributed to greater lipophilic concentration in the extracts (**Table 1**) compared to the hydrophilic ones. It is known that lipophilic compounds are present in seaweed cell walls in large amounts compared to hydrophilic ones (Iván Gómez & Huovinen, 2020a; C. Wiencke et al., 2014; Christian Wiencke et al., 2002), and this could also explain the larger natural concentration in extracts. On the other hand, some studies (Charles D. Amsler et al., 2009; Baker et al., 2008; Carroll et al., 2020; von Salm et al., 2018; E. B. Young et al., 2007) linked inhibition activity to types of lipophilic molecules that could be behind the activity observed in our samples. Those molecules range from isoprenoids (like terpenoids, diterpenes, meroterpenoids, carotenoids, and steroids), to aromatic products (like tannins), polyphenols (like phlorotannins) and acetogenins. Moreover, previous studies reported inhibitory activity from secondary metabolites of lipophilic nature in other types of Antarctic organisms (C. Angulo-Preckler et al., 2015; C Angulo-Preckler et al., 2017; Figuerola et al., 2014, 2017; Sacristán-Soriano et al., 2017), which would reinforce the possible main role of these lipophilic compounds in chemical interactions.

Regarding the lower inhibitory activity observed in hydrophilic compounds, it could be explained by two main reasons: as polar compounds, they may easily dilute in seawater, leading to a lower concentration in the extracts compared to lipophilic compounds (Sotka et al., 2009), or they could be sensitive to temperature changes during the extraction process (Cox et al., 2012). In addition, another plausible factor would be related to the above mentioned larger amounts of lipophilic compounds in cell surfaces and walls compared to the hydrophilic ones, suggesting that the later are less available to participate in the activity.

Concerning the microorganisms used, Antarctic bacteria resulted more sensitive than the pathogenic strains (all the Antarctic strains inhibited by at least one extract, compared to 86% of pathogenic microorganisms), and in general, Gram-negative bacteria seem to be more resistant to macroalgae than Gram-positive ones. Those results are in line with former works on Atlantic macroalgae (Freile-Pelegrín & Morales, 2004) as well as other Antarctic organisms (C. D. D. Amsler, 2008; Charles D. Amsler et al., 2009; Figuerola et al., 2014, 2017; Solanki et al., 2018; von Salm et al., 2018; Ryan M. Young et al., 2015). Antarctic bacteria sensitivity could be explained by coevolution of Antarctic seaweeds with those bacteria species. The shared evolutionary history has led to the developing of seaweed chemical interaction with bacteria, for example, to prevent infections or fouling (Aguila et al., 2012; M. J. Pérez et al., 2016; Shannon & Abu-Ghannam, 2016).

For the pathogenic surrogates tested, *Pseudomonas* sp. was not inhibited by any seaweed. This diverges from previous tests with *Pseudomonas* where activity was reported by non-Antarctic seaweeds, including Rhodophyta and Phaeophyceae (M. J. Pérez et al., 2016; Shannon & Abu-Ghannam, 2016). Specificity in bacteria or seaweed species used in the studies may be behind this difference. In contrast, our *Psychrobacter* sp. was inhibited by similar number of lipophilic extracts from three brown seaweeds (*Desmarestia*) and five Rhodophyta (**Table 4**). This is quite interesting if we compare to previous studies like (Salvador, N. et al., 2007) where Gram-negative bacteria tested against macroalgae from the Iberian Peninsula were mainly inhibited by Rhodophyta instead of brown algae. A larger number of Iberian Ochrophytes having inhibitory activity against Gram-positive microorganisms was also observed there. In our case though, red and brown seaweeds seem to affect mainly Gram-positive bacteria, which raises the need of further experimentation with Antarctic species to fully understand this.

With respect to the pathogenic fungus *Candida albicans*, it was inhibited only by one macroalgal species with the two different extracts (*D. pulchra*). This is similar to that reported by (M. J. Pérez et al., 2016; Shannon & Abu-Ghannam, 2016) for the fungus *Saccharomyces*, which was more difficult to inhibit than other fungi (e.g. *Cryptococcus neoformans*). These results seem to reinforce the idea that unicellular fungi (at least non-Antarctic ones) are more resistant than bacteria to seaweed chemical activity. Further tests with Antarctic marine unicellular fungi may shed light into the specific relationship that may occur between these two groups of organisms.

About the brown seaweed species tested**,** *Desmarestia* species showed a wide range of activity (similar to red seaweeds), in both types of extracts. Our results coincide with previous records of antimicrobial activity on *Desmarestia*, as those from some previous works (Hornsey & Hide, 1974) performed on *Desmarestia aculeata* (Linnaeus) J.V.Lamouroux and *Desmarestia ligulata* (Stackhouse) J.V.Lamouroux and other studies (Benites Guardia, 2019) with *Desmarestia confervoides (Bory)* M.E.Ramírez & A.F.Peters. Nonetheless, on that last study, antimicrobial activity was found on the desmarestial *Himantothallus grandifolius*, a species that showed no antimicrobial activity in our tests. This difference may be due to a combination of several factors as organism variability (e.g. the specific bacteria strains used). Our results also differ from the study of (K. Iken et al., 2011) which found activity in both types of extracts in *Desmarestia anceps*. Contrastingly, our results did not show any hydrophilic antimicrobial activity in this species. The main reason for this could be the major dilution of the hydrophilic molecules compared to lipophilic ones (Sotka et al., 2009), although other factors may be also involved. Also unlike (K. Iken et al., 2011), we found that *Desmarestia antarctica* was one the most active brown algae. *Adenocystis utricularis* also showed antimicrobial activity, even if it acted only against *Vibrio cholerae* (with a strong inhibitory action). Antiviral activity in *A. utricularis* has been linked to fucoidans (Ponce et al., 2003), which opens an interesting field of study on the chemistry of this particular species.

A part from the differences mentioned, our findings on Phaeophyceae are, in general, well in line with what is known about brown seaweed chemistry, especially in terms of the greater lipophilic activity compared to hydrophilic extracts, as seen previously to this study (Golan et al., 2011). Even though our study focuses in the activity of natural extracts, as mentioned earlier, several specific compounds from brown macroalgae are known to present chemical activity similar to those we observed. Taking this in to account, we can suppose that some of those molecules would be, at least in part, responsible for the results we observed. Examples of those compounds would be phenolic molecules and phlorotannins (Charles D. Amsler & Fairhead, 2006; Benites Guardia, 2019; Boland et al., 1982; Fairhead et al., 2005a; M. J. Pérez et al., 2016; Rivera et al., 1990). Additionally, Phaeophyceae are rich in polysaccharides as alginates, laminarine, and fucoidin that have been also linked to antimicrobial activity (Baba et al., 1988; Hamrun et al., 2020; Kadam et al., 2015; Vieira et al., 2017).

Regarding the Rhodophyta tested, their greater level of activity compared to brown seaweeds is similar to the results of previous studies (Bouhlal et al., 2013; Caccamese et al., 1980, 1981). As an example, species of the order Bonnemaisoniales, like *Bonnemaisonia asparagoides* (Woodward) C.Agardh, *Bonnemaisonia hamifera* Hariot, *Asparagopsis armata* Harvey, which live in warmer latitudes, have shown antimicrobial activity (Paul et al., 2006; Salvador, N. et al., 2007). Previous studies are only available for two of the red seaweeds tested here, *Delisea pulchra* and *Plocamium cartilagineum*. For the former (which is also a Bonnemaisonial), antifouling properties have been reported (Hentzer & Givskov, 2003; Maximilien et al., 1998; Ren et al., 2002, 2004). These results are consistent with the great inhibitory activity our samples showed, reinforcing the idea that *D. pulchra* has a rich diversity of chemical interactions with Antarctic microorganisms. However, is worth mentioning that contrary to what (Hentzer & Givskov, 2003) reported on this species, our samples produced inhibition on the pathogenic surrogate of *Pseudomonas aeruginosa*. This dissimilarity could be attributed to factors like chemical variability of certain secondary metabolites on the species, as reported by previous works (Fairhead et al., 2005b; S. R. Longford et al., 2007). For the other previously studied species, *Plocamium cartilagineum,* several compounds with antimicrobial activity were found previously (Cueto et al., 1991; Harden et al., 2009; Rovirosa et al., 1990). However, in our study, *P. cartilagineum* extracts showed no antimicrobial activity. One of the main reasons for this may be that former studies were performed using *P. cartilagineum* from warmer latitudes. Antarctic populations of *P. cartilagineum* have been suggested to be different from those populations outside Antarctica, even giving the possibility that they belong to a totally non-described species of the genus (Christian Wiencke et al., 2002). If that is the case, chemical differences between species of the genus from different habitats could explain the contrast in the results. About the compounds behind the activity in red seaweed natural extracts, previous works reported several active secondary metabolites that could be good candidates. Examples include halogenated isoprenoids (like monoterpenes and carotenoids) which proved active(C. D. D. Amsler, 2008; Charles D. Amsler et al., 2009; Carroll et al., 2020; R. M. Young et al., 2013; Ryan M. Young et al., 2015) in several Rhodophyta families with Antarctic representatives (like Plocamiales, Rhodomelaceae, Rhizophyllidaceae, and Delesseriaceae). As example some furanones showed to modulate bacterial activity (Fairhead et al., 2005b; S. R. Longford et al., 2007; Ren et al., 2002, 2004). In addition, there are red seaweed polysaccharide extracts (like carragenans, sulfacted galactans and galactofucans) with established antimicrobial activity (Harden et al., 2009).

Symbiotic relationships between macroalgae and microorganisms may also play an important role in explaining the chemical activity observed in our study. Previous authors explored interactions where hosts (macroalgae) may obtain benefit from the presence and production of bioactive compounds by their microbiome causing competence or inhibition that prevents harmful microorganisms to set foot on the seaweeds (Gaitan-Espitia & Schmid, 2020; Maurya et al., 2015). Here, we looked at the macroalgae as the holobionts, thus, analyzing the bioactivity as a whole, and therefore we cannot dismiss the possibility that at least in part the chemical responses of the seaweeds could be originated from their symbiotic community. Further studies exploring the composition and chemical activity of the macroalgal microbiomes may unveil new interesting interactions and chemical compounds with potential applications.

Conclusions

As antibiotic-resistant bacteria pose a global threat to human health, our results provide new insights on the role of macroalgae as a source of bioactive compounds potentially useful for humans. In addition, our results contribute to a better understanding of chemical ecology in Antarctic macroalgae. It seems clear that Antarctic macroalgae are as bioactive as seaweeds from other parts of the world, even though studies on their chemical activity are scarcer than in other geographical areas. A combination of evolutionary history and ecological factors determine how algae species respond and use natural products. In that sense, further analyses of very active species, like *D. pulchra* and the genus *Desmarestia*, will prove vital to comprehend their chemical activity to a full extent. Also, further studies combining seaweed microbiome and chemical activity will be of great interest for future research in the ecology of the Antarctic communities, and the potential discovery of new applications.

Declarations

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Conflict of interests

The authors declare that they have no conflict of interest.

Availability of data and material

All herbarium vouchers from the samples studied are available for consulting in the seaweed collection of the Centre de Documentació de Biodiversitat Vegetal (CedocBiv; https://crai.ub.edu/en/about-crai/CeDocBiV) from the Centre de Recursos per a l'Aprenentatge i la Investigació (CRAI) of the University of Barcelona. Chemical data and measurements are available from the corresponding author.

Code availability

Not applicable

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

Author Contribution

CGA CAP, CA, JRL and AGG conceived and designed research. RPMM, MCF and PCB conducted experiments and analysed the data. RPMM wrote the manuscript. CA received the funding. All authors read and approved the manuscript.

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Chapter IV

Invasive marine species discovered on non–native kelp rafts in the warmest Antarctic island

Conxita Avila, Carlos Angulo-Preckler, Rafael P. Martín-Martín, Blanca Figuerola, Huw James Griffiths & Catherine Louise Waller

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Invasive marine species discovered on non–native kelp rafts in the warmest Antarctic island

Conxita Avila[1*], Carlos Angulo-Preckler[1], Rafael P. Martín-Martín[2], Blanca Figuerola[3], Huw James Griffiths[4] & Catherine Louise Waller[5]

Antarctic shallow coastal marine communities were long thought to be isolated from their nearest neighbours by hundreds of kilometres of deep ocean and the Antarctic Circumpolar Current. The discovery of non–native kelp washed up on Antarctic beaches led us to question the permeability of these barriers to species dispersal. According to the literature, over 70 million kelp rafts are afloat in the Southern Ocean at any one time. These living, floating islands can play host to a range of passenger species from both their original coastal location and those picked in the open ocean. Driven by winds, currents and storms towards the coast of the continent, these rafts are often cited as theoretical vectors for the introduction of new species into Antarctica and the sub-Antarctic islands. We found non-native kelps, with a wide range of "hitchhiking" passenger organisms, on an Antarctic beach inside the flooded caldera of an active volcanic island. This is the first evidence of non-native species reaching the Antarctic continent alive on kelp rafts. One passenger species, the bryozoan *Membranipora membranacea*, is found to be an invasive and ecologically harmful species in some cold-water regions, and this is its first record from Antarctica. The caldera of Deception Island provides considerably milder conditions than the frigid surrounding waters and it could be an ideal location for newly introduced species to become established. These findings may help to explain many of the biogeographic patterns and connections we currently see in the Southern Ocean. However, with the impacts of climate change in the region we may see an increase in the range and number of organisms capable of surviving both the long journey and becoming successfully established.

Human activity and shipping have long been considered the principal threats to the "biosecurity" of the remote and isolated shallow marine ecosystems of Antarctica[1]. However, recent work has shown that the Southern Ocean's (SO) strong, circumpolar winds, currents and fronts may not be a barrier to natural colonization from the north[2–4]. Floating kelp is a potential vector for distributing species across the vast oceanic distances between the sub-Antarctic islands. It has been estimated that there may be over 70 million kelp rafts afloat at any one time in the Sub-Antarctic, 94% of which are *Durvillaea antarctica*[5]. The remote archipelagos distributed between 45 and 60° S are key locations for dispersal either side of the Polar Front (PF) and across[2–4,6]. The discovery of the non-Antarctic bull kelp, *D. antarctica* on Antarctic beaches, coupled with oceanographic models, demonstrate a non-anthropogenic mechanism for species introduction into Antarctica[4]. Genomic analyses revealed that the kelp specimens originated in the sub-Antarctic (Kerguelen Island and South Georgia) and dispersed thousands of kilometres to reach the Antarctic coast[4]. The only epibionts found on these specimens were goose barnacles (*Lepas australis*), and this epipelagic species is likely to have colonised the kelp during its time drifting in the open ocean[4].

Deception Island (DI) is an active volcano in the South Shetland Islands, located off the West Antarctic Peninsula. The flooded caldera of DI is species poor in comparison with neighbouring islands due to recent

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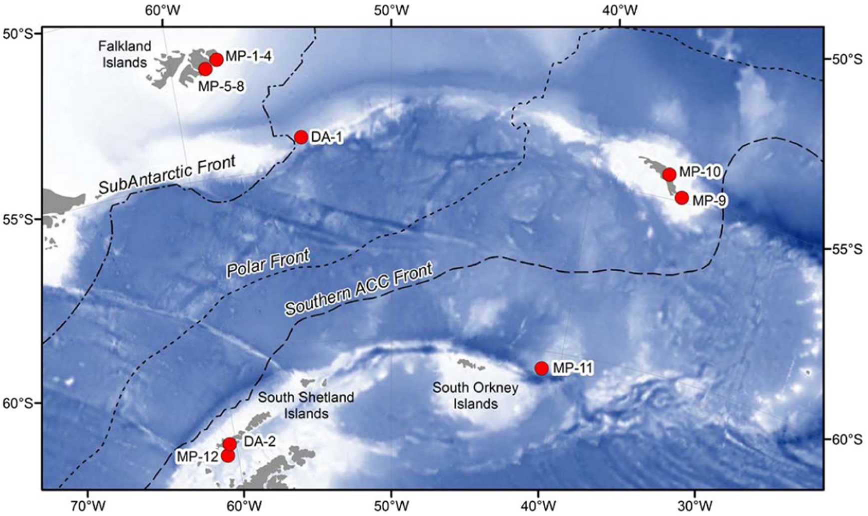

Figure 1. Map of the collecting localities showing the Polar Front (dotted line) and sampling points (in red). DA (*Durvillaea antarctica*), MP (*Macrocystis pyrifera*). MP-1–4: Falkland Islands (North), MP-5-8: Mare Harbour (Falkland Islands), DA-1: South of Falkland Islands (Drake passage), MP-9: South Georgia Islands (South), MP-10: South Georgia Islands (North), MP-11: South Sandwich Islands, and MP-12: Deception Island (South Shetland Islands), DA-2: Livingston Island (South Shetland Islands).

Code	Species	Place	Lat (S)	Lon (W)	Polar Front	Date (mm/yy)
MP-1	*Macrocystis pyrifera*	Falkland Islands	−51,690	−57,865	North	02/16
MP-2	*Macrocystis pyrifera*	Falkland Islands	−51,690	−57,865	North	02/16
MP-3	*Macrocystis pyrifera*	Falkland Islands	−51,690	−57,865	North	02/16
MP-4	*Macrocystis pyrifera*	Falkland Islands	−51,690	−57,865	North	02/16
MP-5	*Macrocystis pyrifera*	Mare Harbour (Falklank Is.)	−51,903	−58,423	North	03/16
MP-6	*Macrocystis pyrifera*	Mare Harbour (Falklank Is.)	−51,903	−58,423	North	03/16
MP-7	*Macrocystis pyrifera*	Mare Harbour (Falklank Is.)	−51,903	−58,423	North	03/16
MP-8	*Macrocystis pyrifera*	Mare Harbour (Falklank Is.)	−51,903	−58,423	North	03/16
DA-1	*Durvillaea antarctica*	South Falkland Islands	−54,110	−54,340	North	02/16
MP-9	*Macrocystis pyrifera*	South Georgia	−54,880	−35,514	South	03/16
MP-10	*Macrocystis pyrifera*	South Georgia	−54,326	−36,382	South	03/16
MP-11	*Macrocystis pyrifera*	South Sandwich Islands	−60,52	−41,04	South	03/16
MP-12	*Macrocystis pyrifera*	Deception Island	−62,9789	−60,657	South	02/17
DA-2	*Durvillaea antarctica*	Livingston Island	−62,661	−60,398	South	02/19

Table 1. Rafting kelp collected in this study. MP: *Macrocystis pyrifera*. DA: *Durvillaea antarctica*.

eruptions (1970) and ongoing volcanic activity[7]. Recent work shows an increasing biodiversity gradient towards the entrance of the bay[7,8]. The geothermal and morphological nature of the caldera provides a relatively calm and warm-water habitat, with bottom water temperatures of about 2–3 °C, protected from ice disturbances (ice scouring, anchor ice, etc), perhaps offering favourable habitat for potential invasive species entering Antarctica.

Macroalgal rafting has been suggested to explain similarities in species composition and low genetic differentiation of intertidal marine communities across the sub-Antarctic[9–11]. This hypothesis implies some degree of successful colonization or mixing of the transported species with native sub-Antarctic species. However, all the possible natural pathways at both sides and across the PF result in a low probability that an individual raft will ever make landfall at a site with suitable characteristics for colonisation, given the vastness of the SO and the small size of most of the islands[12]. If a species succeeds to establish a local population, however, it may face little competition for resources and space, and may thrive[13]. In this context, thus, DI could represent a proxy for what may happen in other parts of Antarctica.

Marine species may reach Antarctic waters by a number of different dispersal mechanisms. Rafting on floating macroalgae is likely to be the biggest vector for natural dispersal into Antarctic waters. In a similar passive way, plastics have also been reported to carry a variety of epibionts in Antarctic waters[14]. Bryozoans are effective colonizers of surfaces and one of the most important components of biofouling assemblages[15,16]. Five bryozoan species were found attached to a plastic debris collected on Adelaide Island (Antarctic Peninsula)[14]. All of these species were endemic to the Antarctic and it was estimated that debris had been in the water for at least 1 yr. Most colonies were reproductively active, having the possibility of releasing larvae during transportation. In fact, the

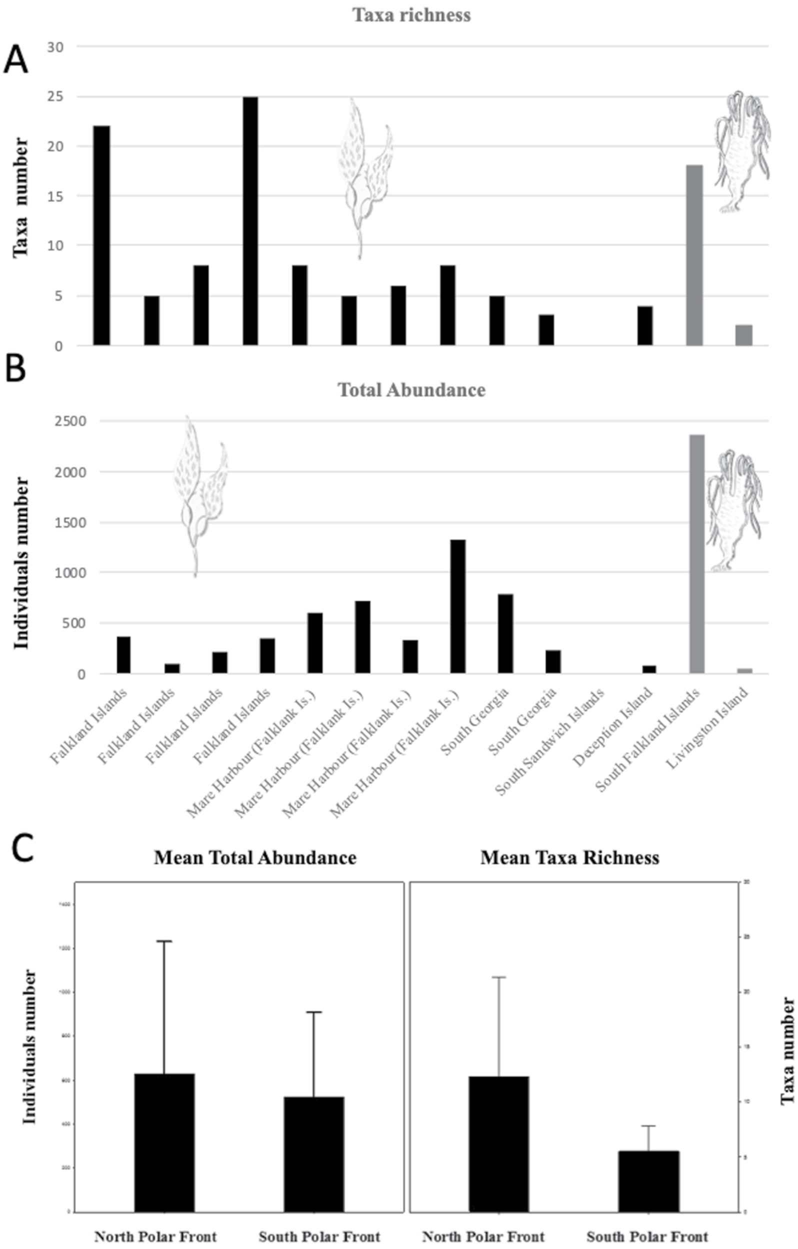

Figure 2. Abundance and taxa richness of epibionts in the rafting algae studied here. Taxa richness (**A**). Total abundance (**B**). Black bars: *Macrocystis pyrifera*, Grey bars: *Durvillaea antarctica*. Means of total abundance and taxa richness (**C**) at North and South of the Polar Front (PF) (± s.d.).

cyphonaute larvae of the bryozoan *M. membranacea* have been found in ballast water[17], and their colonies can raft on kelp, such as *Macrocystis* spp and *Nereocystis* spp, as well as on plastic debris[18]. Fraser *et al.*[19] reported 10 invertebrate species rafting on algae for at least 400 km, during several weeks, between New Zealand and the neighbouring sub-Antarctic islands.

The recent discovery of fresh specimens of the non-native giant kelps (*Macrocystis pyrifera* and *D. antarctica*) with a range of epibiotic animals and algae as passengers, washed up on the shores of Deception and Livingston Islands, provides a unique opportunity to study a potential colonisation event. Here we present the first evidence of non-native shallow water epibiotic organisms reaching Antarctica by long-term rafting. By identifying the species found living on the kelp and examining their distributions we assess the potential impacts of these species becoming established.

Methods

Samples were collected from the sub-Antarctic to Antarctic islands (Fig. 1, Table 1). Twelve rafting floating kelps were collected on both sides of the PF during the Antarctic expedition of the RRS James Clark Ross in 2016. Two more kelps were collected South of the PF. *M. pyrifera* was collected on the beach in DI (South Shetland Islands) during the Distantcom-2 Antarctic cruise in February, 2017. *D. antarctica* fragments were collected on the beach

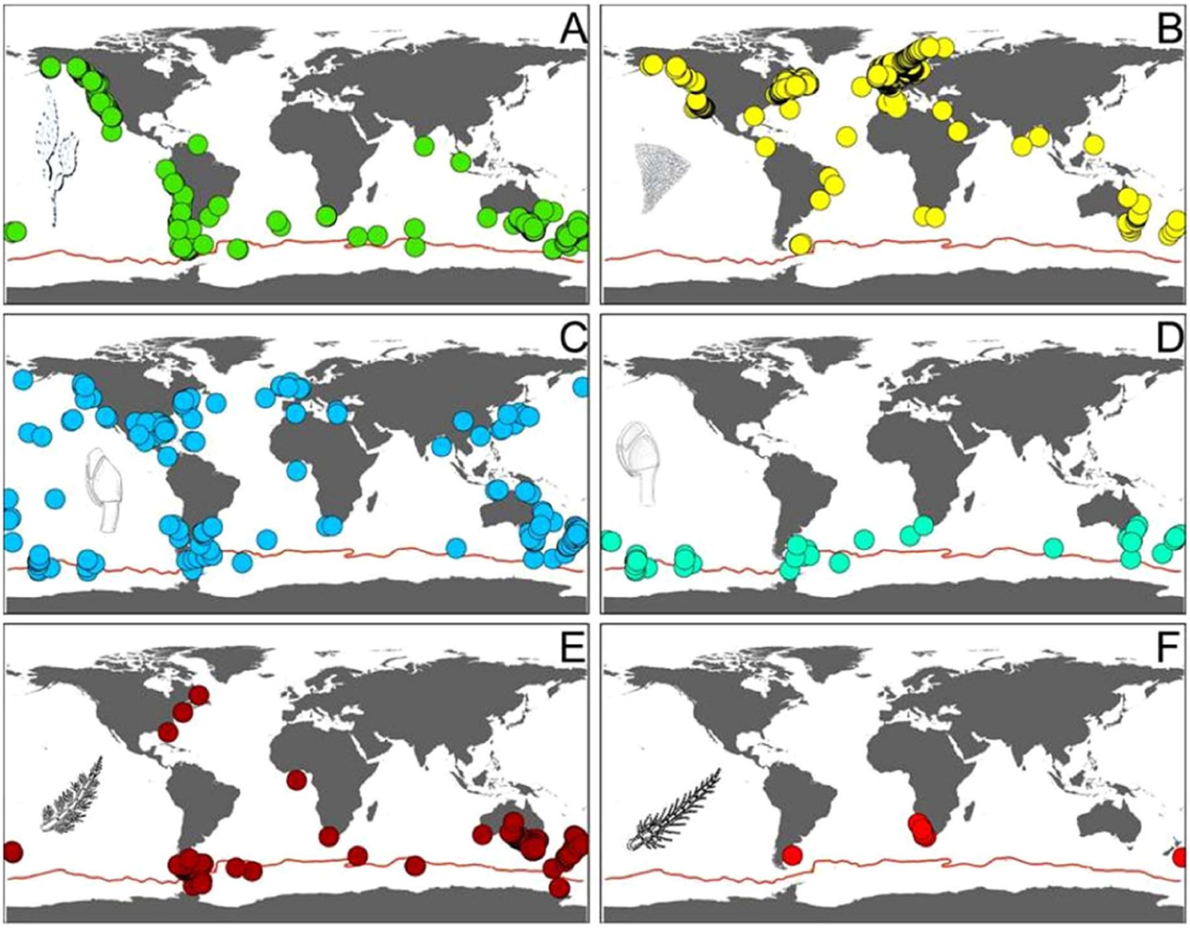

Figure 3. Known distributions of the epibiotic species found associated with *Macrocystis pyrifera* (**A**: Distribution of *M. pyrifera*) on the South Shetland Islands: *Membranipora membranacea* (**B**); *Lepas anatifera* (**C**); *Lepas australis* (**D**); *Ballia callitricha* (**E**) and *Ballia sertularioides* (**F**). Data from GBIF[45].

in front of the Spanish station in Livingston Island in February, 2019 during the Bluebio-2 cruise. Samples were photographed and frozen for further identification of the seaweeds and their epibionts. Samples of rafting kelp ranged from 0.5 to 18.1 kg wet weight. The passenger species traveling upon the kelps reached a total of 7534 specimens (538 ± 637 passengers/kelp, within a range from 0 to 2362 per kelp) and were identified to the lowest possible taxonomic level in the laboratory. The entire rafts were sampled for fauna. Identification of seaweed samples was achieved by studying morphological features, as well as histological examination of the thallus.

Passengers into the cold. Abundance and taxa richness of epibionts found on floating macroalgae in the Southern Ocean vary between the species of kelp *(M. pyrifera* and *D. antarctica*) and the individual rafts (Fig. 2). Other rafts, including those formed by *D. antarctica*, were observed at DI but were not sampled for fauna. Among the four passenger species found alive on *M. pyrifera* in DI, the most significant in terms of potential ecological impact, other than the non-native kelp itself, is the cheilostome bryozoan *Membranipora membranacea*. This is a well-known encrusting species with a proven ability to colonise new environments and cause significant damage to ecosystems by limiting the ability of seaweeds to reproduce and grow[20]. This bryozoan is widely distributed in temperate oceans with distinct populations in the Pacific (North Pacific, Chile, Australia and New Zealand) and Atlantic oceans (North East Atlantic and South Africa) (Fig. 3). *M. membranacea* has become an established invasive species in the North West Atlantic along the coast of North America and has caused extensive losses of kelp canopy through a process of defoliation[21]. Although the species is recorded as far north as northern Scandinavia in the Arctic, it has never been previously reported from south of the PF, but it is likely to already be well adapted to cold water conditions, therefore posing more than a hypothetical risk for Antarctic waters.

The combination of having a long-lived planktonic larva (from 2 weeks to 2 months), sexual (hermaphroditic zooids) and asexual reproduction, fast growth rates, effective food acquisition in a wide range of flow rates, ability to form large colonies and to colonize kelps make *M. membranacea* a successful disperser, colonizer, and invasive species[22–24]. Potentially, these kelp can be transported much farther than bryozoan larvae[25–31]. Furthermore, their heavy encrustations may have a negative impact on marine ecosystems by increasing the brittleness of kelp blades, followed by extensive losses of kelp canopy[21], and by limiting the ability of the seaweeds to reproduce and grow, specifically interfering with spore release from the kelp blade[20]. It has also been shown that other species of the same genus may block nutrient uptake and photosynthesis[32,33].

Phyllum	Taxa	MP-1	MP-2	MP-3	MP-4	MP-5	MP-6	MP-7	MP-8	DA-1	MP-9	MP-10	MP-11	MP-12	DA-2
Rhodophyta	*Ballia callitricha*													1	
Rhodophyta	*Ballia sertularioides*													1	
Porifera	Porifera				2	2									
Cnidaria	Anthozoa					3									
Cnidaria	Hydrozoa	1				13				3		5	7		
Bryozoa	Bryozoa	1	1	1	20	18	5	3		17	27	5	120		
Bryozoa	Cyclostomatidae			2											
Bryozoa	*Membranipora membranacea*													1	
Entoprocta	Entoprocta						100								
Mollusca	Mollusca				1										
Mollusca	Bivalvia			4	2		500			27	71	3	39		
Mollusca	Gastropoda				2					2					
Mollusca	*Kidderia* sp.					2									
Mollusca	*Scurria scurra*					1									
Mollusca	*Gaimardia trapesina*	97					76	30							
Mollusca	Nudibranchia						2								
Mollusca	*Nacella*	2													
Mollusca	*Nacella mytilina*	2													
Mollusca	Fissurellidae				1										
Platyhelminthes	Platyhelminthes				1	1									
Annelida	Nemertea									1			2		
Annelida	Polychaeta									3	601	5	1005		
Annelida	Polynoidae	1			8	1									
Annelida	Cirratulidae				2										
Annelida	Serpulidae	101	100	100	105		100	200							
Annelida	Syllidae	6			2										
Annelida	Terebellidae	7			8										
Annelida	Nereidae	3			4										
Annelida	Capitellidae	5													
Annelida	*Torodrilus* sp.														1
Annelida	Sabellidae	1													
Sipuncula	Sipuncula				3										
Arthropoda	Insecta	1													
Arthropoda	Haplocheira	78													
Arthropoda	Harpacticoida	40													
Arthropoda	Calanoida	1													
Arthropoda	Pedunculata					2230									
Arthropoda	*Joeropsis curvicornis*					1									
Arthropoda	Caprellidae					1									
Arthropoda	Pantopoda					1									
Arthropoda	Ostracoda					25									
Arthropoda	Cucumariidae				15										
Arthropoda	Eusiridae			13	25	1									
Arthropoda	Isopoda									56	10	31	33		
Arthropoda	Munnidae					1									
Arthropoda	Amphipoda		2	50	42	55				493	20	280	115		
Arthropoda	Corophiidae		2	50	65										
Arthropoda	Ischyroceridae				3	5									
Arthropoda	*Ischyromene eatoni*	1													
Arthropoda	*Halicarcinus planatus*	5			19										
Arthropoda	*Plakarthrium punctatissimum*	1													
Arthropoda	*Peltariom spinulosum*	1													
Arthropoda	*Exosphaeroma lanceolatum*	1													
Arthropoda	*Lepas australis*													76	
Arthropoda	*Lepas anatifera*														50
Echinodermata	Echinoidea				3										
Echinodermata	Asteroidea				9										
Continued															

Phyllum	Taxa	MP-1	MP-2	MP-3	MP-4	MP-5	MP-6	MP-7	MP-8	DA-1	MP-9	MP-10	MP-11	MP-12	DA-2
Echinodermata	Ophiuroidea				3										
Echinodermata	Apodida				1										
Chordata	Actinopteri					1									
	Seaweed	4	1	1	2							5	5		

Table 2. Organisms found as passengers on the kelp raft in this study (numbers indicate counts). MP: *Macrocystis pyrifera*. DA: *Durvillaea antarctica*.

The other three species found alive on the kelp in DI have all been previously reported south of the PF. *Ballia callitricha* and *B. sertularioides* are shallow water red algae with a general Southern Hemisphere distribution that includes previous records from the Ross Sea, Antarctica (Fig. 3), but not from DI or West Antarctica[8]. Juveniles and adults of the southern goose barnacle *Lepas australis*, were also found. This species, commonly found attached to floating substrata such as macroalgae, volcanic pumice, and plastics in the Southern Ocean (Fig. 3), was the only species recently reported on a specimen of giant bull kelp, *D. antarctica*, found on King George Island, also part to the South Shetland Islands group[4].

Using growth rates cited by Fraser *et al.*[4,19] we estimate an age of approximately 30 days for the barnacles, *L. australis*, found at DI, suggesting that colonization happened in the open sea. Alternative, faster transportation mechanisms may also exist (e.g. shipping vectors and heavy storms). In fact, Lewis *et al.*[34] suggested hull-fouling is likely to be the most important vector for transporting species to Antarctica as ships create novel pathways, moving across currents and often visiting many locations over short periods of time. The increasing ship activity appears to be a very important factor increasing the probability of non–native marine species establishing within the Antarctic region in the coming decades (over 180 ships were active around Antarctica and the sub-Antarctic islands in 2017–2018, on potentially more than 500 voyages)[35]. The presence of small–sized specimens of *L. anatifera* in the kelp found at Livingston Island could also indicate a short-term rafting for this species. Abundant, alive *L. anatifera* specimens found on *D. antarctica* fragments in South bay, Livingston Island, represent, in fact, the first Antarctic report for the species, which was described in tropical and subtropical waters of South America[36]. The potential effects of barnacle colonization in Antarctica are unknown, but in fact, being pelagic rafting species, they seem unlikely to pose any real threat to the shallow water ecosystems, especially as *L. australis* is already commonly found on rafts and litter in the Southern Ocean. However, their heavy growth could sink the kelp, thus facilitating access to the seafloor for other benthic passengers.

The other floating and beached kelp samples (*M. pyrifera* and *D. antarctica*) collected from either side of the PF were found to be carrying organisms within 12 different phyla as passengers (Tables 1 and 2). Each kelp raft examined represented a different, although sometimes overlapping, subset of organisms usually found inhabiting shallow marine habitats. Only one of the floating specimens, MP-11, an example of the non-Antarctic *M. pyrifera* found near the South Orkney Islands, had no passengers at all. The most commonly found taxa included amphipod crustaceans, polychaete worms, molluscs, and bryozoans (Table 2). The DI floating kelp was the only specimen collected south of the PF carrying *M. membranacea*, although this bryozoan was frequently found at the Falkland Islands, a potential source of kelp rafts in that region[3]. Although more studies are needed to know if *M. membranacea* has become established in the SO, the potential for this species to impact Antarctic ecosystems could be high, not only in DI, as macroalgal substrates are widespread and colder temperatures are not preventing its spread. For example, a recent study based on a baseline data on presence/absence and abundance of this bryozoan near its current northern range limit suggests that the available algal substrate may be more important than temperature in limiting the spread and abundance of *M. membranacea*[37]. On the other hand, MP-5, collected from the open ocean north of the PF, was heavily encrusted with thousands of adult and juvenile goose barnacles. This specimen was also host to a rich and varied community of other organisms that are likely to have been associated with the raft before it became dislodged (Table 2).

Other significant findings included the brachyuran crabs *Halicarcinus planatus* and *Peltariom spinulosum* in the *M. pyrifera* fragments washed up on the shore at the Falkland Islands. *H. planatus* was first recorded in Antarctica at the shores of the South Orkney Islands in 1903[38]. It was reported again by Aronson *et al.*[39] at the external side of the caldera of DI, supporting the hypothesis that DI could be the entrance gate for non-native species. *H. planatus* is a widely-distributed species in temperate waters, found from New Zealand to the Falklands and southern South America, as far north as Peru and Argentina[40,41]. *H. planatus* has also been found alive on floating kelp[42]. Although *H. planatus* was not found in our previous studies at Deception and Livingston Islands[8], we did find it on *M. pyrifera* washed up on the shore of the Falkland Islands (pers data 2016, SO-AntEco expedition), which could easily be re-floated by high tides or rough weather. The impact of these crabs on local species is not known but could potentially be devastating due to the absence of durophagous fauna in Antarctic shallow benthic ecosystems[43,44].

Rafting to the south. The transport of organisms on ships' hulls or in ballast water can take less than 4% of the time it would take to reach the same destination by rafting[11]. Although this significant reduction in time taken to reach Antarctica might allow a wider range of species to reach the continent alive, they would still need to be capable of surviving the conditions at their destination in order to become established. As such, our observation of a species with a documented track record of invasive and negative ecological impacts, such as *Membranipora membranacea*, in an active volcano (DI), with warmer, more favourable conditions, is very significant. The species

Figure 4. Rafting kelp and passengers. *Macrocystis pyrifera* (**A**) with passengers found at Deception Island, and *Durvillaea antarctica* (**B**) with cirripeda from Livingston Island, South Shetland Islands, Antarctica. (**C**–**F**) Passengers found on *M. pyrifera* at DI: the red alga *Ballia callitricha* (**C**); the bryozoan *Membranipora membranacea* (**D**); the cirripeda *Lepas australis* (**E**); and the cirripeda *L. anatifera* (**F**) on *D. antarctica* from Livingston Island.

reported here are common and well-distributed organisms and thus have the potential to persist or even thrive in the milder conditions of the caldera of DI (Fig. 4). It could only be a matter of time before some of these species acclimatize to the Antarctic environment and spread. These findings are even more relevant in the current context of global change, which could facilitate the survival of these species in other Antarctic environments once settled in favourable areas, such as DI, further reaching other places around the Antarctic peninsula. Therefore, these species may be useful indicators of climate change in Antarctic habitats and should be carefully monitored during the next years.

Conclusions

Non-native, non-Antarctic kelp is reaching Antarctica now and again, particularly at Deception and Livingston Islands. DI is a key location for first colonisation of Antarctica due to its strategic location and the higher temperature of seawater compared to adjacent areas. The presence of passengers on the kelp, especially *Membranipora membranacea* and *Lepas anatifera* (as well as *Halicarcinus planatus* in the water outside the DI caldera) demonstrate that natural colonisation, or invasion, can happen at any time. Actually, *M. membranacea* has already become an invasive species in many places outside of Antarctica, and it is believed to have a potentially negative impact on marine ecosystems. Effects of passengers in Antarctic ecosystems are largely unknown, and therefore, we believe that monitoring these potentially invasive species in the frame of global change is crucial in the coming years.

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Author contributions

C.A., C.A.P., R.P.M.M., B.F., H.J.G. and C.L.W. collected and identified samples in Antarctica and in the lab. C.A., C.A.P., R.P.M.M. and H.J.G. performed data analysis and interpretation, and all authors contributed to the final version of the manuscript.

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The authors declare no competing interests.

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GENERAL DISCUSSION

This thesis has been focused on the study of the Antarctic seaweeds. For this purpose, the different knowledge areas and aspects explored in this work have been divided in the four chapters presented below. **Chapter I** exposed the observations made on the benthic communities, that included the biodiversity of seaweeds on the benthic communities from DI, as well as the diversity epiphytic diatoms of macroalgae from LI and DI. **Chapter II** described the results of the analyses on the cytogenetic characteristics of several Antarctic seaweed species. In **Chapter III** we explored the current knowledge on chemical ecology on Antarctic seaweeds and also, reported our results on chemical ecology (specifically on antimicrobial activity) for different Antarctic algae studied. Lastly, **Chapter IV** described our findings on the potential dispersion and invasiveness of several rafting kelps, and the organisms travelling associated with them. Altogether, this thesis has served to emphasize the need for additional research in the different areas related to the Antarctic seaweed communities. As aforementioned, the knowledge on many aspects of Antarctic macroalgae is lacking compared to what is known for other groups of Antarctic organisms and marine floras from other regions. There is still undiscovered biodiversity in specific seaweed taxa, as well as in communities that have not been fully explored (for example, in areas were scientific prospections of seaweeds have been scarce, like some parts of the eastern Antarctica). Moreover, the evolutionary characteristics and patters that composed the actual diversity of Antarctic macroalgae are still unknown since few Antarctic species have been investigated in terms of cytogenetics or genomically. Also, there is still great unexplored potential for human applications (both in terms of nature protection and biotechnological discoveries) that will benefit from further study of marine Antarctic macroalgae. Last but not least, the complete functioning and dynamics of Antarctic seaweed communities remains to be fully understood, especially in the context of new climate change related situations (e.g. range variations, invasions, competition and adaptation) as well as in terms of inter-organism relationships (especially with Antarctic microorganisms). Following this, I will comment some of the most remarkable achievements on the knowledge areas explored in this thesis.

Chapter I: Biodiversity of seaweeds from Deception Island and epiphytic Diatom biodiversity from Deception and Livingston Islands

As commented in the introduction of the work here presented, DI represents one of the most human frequented areas in Antarctica, also possessing distinct features regarding its recent and still active volcanism. All this combined, makes DI a natural laboratory where possible alterations related to climate change and human presence on the Antarctic environment can be studied on native populations of different species (e.g. effects of rise in ocean temperature, acidification of waters or contamination). Nonetheless, despite being a reasonably studied area, our work reveals that there were several seaweed species non-recorded previously on the island (*Notophycus fimbriatus, Porphyra plocamiestris, Delisea pulchra, Rhodymenia coccocarpa*), which indicates that biodiversity patterns and composition on this area are still to be fully studied. Concerning the macroalgae assemblages observed, there is a clear pattern of distribution inside Port Foster bay, showing that substrata availability (rocky formations and ice-scouring free habitats), as well as proximity to hydrothermal sources play a role affecting the composition and distribution of the marine flora (Pellizzari et al., 2017; Pellizzari et al., 2020). Parameters like those of DI represent a possible scenario of climate change for the rest of Antarctic coasts and, as some works also suggest, the observations we present can be an example of how Antarctic communities may shift if altered by climate change or increased human presence (Hughes & Ashton, 2017; McCarthy et al., 2019; Hughes et al., 2020). To better understand this, we tried to discern the biodiversity present on different spots of the island with different conditions, with our observations covering a gradient from the island bay entrance to the inner part. Our results show the presence of, at least, three main types of seaweed communities in the island. The first one presents the highest biodiversity, with a well-developed three-dimensional structure, composed by canopy-forming species (like *Desmarestia* spp, *Cystosphaera jackinotii* and *Himantothallus grandifolius*) and under-canopy taxa (with species like *Neuroglossum delesseriae*, *Phycodrys antarctica*, *Plocamium cartilagineum*, *Georgiella confluens* or *Sarcothalia papillosa*). As mentioned in our work, the abundance of rocky substrata on this area is greater than in other zones, but also ice-scouring by glacier icebergs is less frequent compared with other habitats of the rest of the SSI. Also, even though the points near the bay entrance are influenced by the open sea, they are still affected by the volcanic conditions that dominate the inner parts of the bay. In this regard, those spots present a slightly warmer temperature and higher concentration of volcanic compounds (like sulphuric compounds and heavy metals) than those of the open sea and other places of the archipelago, but not as intense as in the innermost parts of the bay (Elderfield, 1972; Muñoz-Martín et al., 2005; Bartolini et al., 2014). The second type of seaweed community is distributed along the intermediate locations between the bay entrance and the innermost parts of it. It presents an intermediate level of biodiversity compared to the other two main communities of the island. Regarding the factors influencing it, this transitional community is more distant to the influence of open sea due to its distance to the bay entrance; in addition, hydrothermal vents are more common near the spots that comprise this community than the previous one. Also, though rocky outcrops are not as common as in the bay entrance, there are still some formations that help to sustain some sessile organisms. In that sense, this community is characterized by a decreasing gradient of seaweed biodiversity as distance to the bay entrance increases. Nevertheless, in the spots near the entrance, it presents canopy and under canopy-forming seaweeds that form developed assemblages similar to those of the first community. Finally, the third type of seaweed community we observed is characterized by being present in soft bottom areas, which are the dominant habitat in the inner parts of the bay. As exposed, those habitats present few surfaces were seaweeds can attach, as the majority of the bottoms are composed by volcanic ash and lapilli. This factor limits the macroalgal and animal biodiversity by limiting the presence of sessile organisms, as some authors also suggested (Clarke et al., 2005; Barnes, 2005; Cranmer et al., 2003; Pellizzari et al., 2017). Adding to that, hydrothermal vents are frequent in this area which, as mentioned before, maintain warmer water temperatures and increase the concentrations of solved volcanic compounds compared with other Antarctic marine habitats. Volcanic activity of the island may produce events of sudden peaks in those two parameters on the hydrothermal vents, which can act as a limiting factor in the establishment of more complex communities, promoting only the presence of organisms that can deal with those kinds of variations. Canopy-forming seaweed species (e.g., *H. grandifolius*, *C. jackinotii*, or *Desmarestia* species) cannot cope with conditions like these, and hence, species of algae are nearly absent here (e.g. the only subtidal species of Pendulum Cove was *Hymenocladiopsis prolifera*). Apart from that, it is worth noting that on intertidal areas of the bay coast and the spots closer to glaciers where rocky formations were available, some seaweed also appeared, with species with small habit or filamentous morphology and tolerant to environmental fluctuations like ice scouring, air exposure and radiation light and salinity variations (e.g. Chlorophytes like *Acrosiphonia arcta* or *Urospora penicilliformis* and some generalist red seaweed species like *Palmaria decipiens*). Overall, our observations state that the biodiversity on DI, despite recent eruption events, is higher than previously reported, which raises the need to further understand the composition and dynamics of the benthic communities present in the island, as it can be an important example for the marking off conservation guidelines and climate change mitigation efforts in the area, as well as the rest of Antarctica. Adding to that, the ship traffic that enters the island bay (research vessels and touristic ferries) passes near the points of those richest communities, and represent an opportunity for species not from the island to enter the bay and find suitable spots to colonize due to the island especial characteristics. That reinforces the idea that factors like human activity and the milder environmental conditions, combined, make the island seaweed assemblages a proxy to what can happen in other Antarctic regions if climate change promotes the appearing of new, ice-free habitats, where suitable substrata becomes available, water temperatures rises and new species have the opportunity to arrive (Hughes et al., 2020; Macaya et al., 2020; Pellizzari et al., 2017; Pellizzari et al., 2020). Being this the case, the distribution of some species could be expanded, and the pre-existing communities typical of heavily glaciated areas (like those dominated by mobile bottom feeder animals and the seaweeds capable of surviving the ice scouring from genera like *Litothamnion*, *Mesophyllum*, *Urospora* *Ulothrix*, *Palmaria* or *Monostroma* that are common in many regions of Antarctica) may be disrupted, shifting towards assemblages dominated by sessile filter feeders and big canopies of Antarctic primary producers (e.g. *C. jackinotii, H. grandifolius*, *Ascoseira mirabilis* or *Desmarestia* species), as well as new potentially introduced species from warmer latitudes (Fraser et al., 2020; Macaya et al., 2020; Fraser et al., 2018; Valdivia, 2020; Huovinen & Gómez, 2020). This would cause alterations in the biodiversity and ecology, and maybe even generating negative feedback loops of impacts along Antarctic coasts which, in turn and due to the importance of the Antarctic region in the world's ecology, may end up affecting other parts of the glove with unknown effects.

Regarding the diversity of epiphytic diatoms studied on the seaweed of DI and LI, our work combines several perspectives that are useful to better understand which aspects may be behind the observed patterns of composition and distribution of these microorganisms. The range of seaweed samples analysed included 20 seaweed species with representatives from different seaweed types (specifically Rhodophyta and Phaeophyceae) morphologies and life cycle. This included small species with filamentous or globular morphology (like *Ballia callitricha* or *Adenocistis utricularis*), midsize under-canopy taxa with laminar or ramificated habit (like *Palmaria decipiens*, *Gigartina skottsbergi* or *Iridaea cordata*), and big canopy-forming species (like *Desmarestia anceps* or *Himantothallus grandifolius*). The biodiversity of diatoms we found in these seaweed samples, has increased number of species that previous studies reported for the region, even citing 20 new diatom taxa for the first time in DI and LI (mainly from the genus *Cocconeis*). As stated in the chapter, we cannot discards that this diversity may be related to the wider sampling performed (in terms of bathymetry, seaweed host, and area) compared to more localized sampling areas of previous studies on SSI region (Al-Handal & Wulff, 2008; Majewska et al., 2016), environmental factors like the high seawater micronutrient concentrations on the area (Zacher et al., 2010), the diversity of seaweed communities of the sampling sites (Oliveira et al., 2020), or the diversity of niches related to the abiotic conditions on the studied spots. Nonetheless, as we have seen, the major factor determining epiphytic diatoms seems to be the related to the host type (Phaeophyceae, Rhodophyta or Chlorophyta), which in some cases accounted for a considerable deal of the variance observed between the samples. In that sense, and despite some variance within some seaweed species, the richness of epiphytic diatoms was highest in the red seaweeds, indicating that, in general, they may represent a better host for many diatom species. Morphology of the host (branching pattern) and annularity had no conclusive influence on the diatom diversity as those aspects only appear significant in red seaweed. In that regard, we can't discard that chemical ecology of the potential seaweed host may play a significant role on preselecting diatom species over the morphology. Also, as mentioned before, our work combines the information of the diatom communities found on a greater diversity of seaweed in comparison with previous studies, which normally examined fewer macroalgal host species (Majewska, et al., 2016; Majewska et al., 2015; Thomas & Jiang, 1986; Al-Handal & Wulff, 2008). However, due to differences regarding the seaweed host sampling distribution between the two studied islands, we cannot discard that the taxonomic variance observed between the DI and LI are reflect of the sampling coverages. Nonetheless, the samples from DI included a more balanced sampling of red and brown seaweeds, which allowed for a more precise view of the previously mentioned host effect on the recorded diatom species. Also, despite the sampling considerations, we observed a richer overall diversity on DI compared to LI, which could be related to volcanic aspects of the island, like micronutrient enrichment of the waters, water temperature and turbidity (Bendia et al., 2018), which may create greater diversity of niches, an thus, boost diatom diversity. On the other hand, some individual samples from LI had richer individual diversity, possibly due to factors related to less turbidity of the water (Isla et al., 2001), which may increase the local photosynthetic performance, and hence the potential diversity, of both, hosts and diatoms. Those questions raise the need to perform further studies that help to clarify how some characteristics of the environment affect the communities of diatoms from seaweeds in Antarctica, which at the same time, will help to increase the knowledge on the overall dynamics of the Antarctic ecology.

Chapter II: Evolutionary patterns of Antarctic seaweeds, with focus on cytogenetics

Marine macroalgae are, in general, one of the marine organisms' groups with less proportion of studies on cytogenetics (except for the few seaweeds species currently cultured and with commercial use). Nonetheless, as many studies suggested, they are crucial components of coastal ecosystems all over the world (Gómez & Huovinen, 2020; Kirkman & Kendrick, 1997; Ware et al., 2019). As mentioned earlier in this chapter, lack of data is especially notable for the Antarctic marine flora in many aspects and, yet, cytogenetic measurements are especially useful on understanding population dynamics, complement evolutionary information of genomic studies, and comprehend life cycles of species (which is especially important for ecology and for those species with potential human use) (Kapraun, 2005; Bennett et al., 2000; Čertnerová & Škaloud, 2020; Gregory et al., 2007; Ribera Siguan et al., 2011). Our results in this regard provide new cytogenetic data for 12 previously not studied species, contextualizing them to records of related species, and also identified the nuclear patters of the species *Neuroglossum delesseriae* and *Ballia callitricha*. Our study included common representative species of the current known marine flora of Antarctica, and sets a baseline of records for comparison in further research on Antarctic seaweeds. These data also provide a useful step on understanding some still unclear aspects of important biological parameters, specifically the C-value. The different groups of macroalgae show variable patterns on the values of nuclear DNA content across taxa, adding complexity to the puzzling question of the C-value Paradox (Gregory, 2005; Moore, 1984). This paradox, that revolves around the apparent lack of connection between organism complexity and genome size on eukaryotes, has been explored by several authors that provided possible explanations and clues on how to explain it (Eddy, 2012; Freeling et al., 2015). One of those possible explaining factors involves organism exposure to stressful conditions or very selective environments along evolutionary history (Berman, 2016; Scholes & Paige, 2015); however, different studies found different trends on their observations (Cavalier-Smith, 1978; Kubátová et al., 2008; Sjøtun et al., 2017). As so, there is no clear interpretation on the possible effects of ambient selectiveness and genome size trends during evolution. In that context, comparative data from organisms exposed to different environmental ranges and conditions are useful to discern any possible patterns related to this idea on nuclear DNA content. Our measurements, being from a flora that has evolved during a considerable time in extreme conditions, represent a valuable source to shed light on this issue. Thus, some of our studied species showed no significant variations compared to data from close relative taxa (of the same family, if available). Nonetheless, as the results of this chapter noted, some of our samples (namely *Desmarestia* species) presented values lower than previously measured for the same genus. Taking this into account, our evidence seems to discard a correlation between greater levels of ploidy and extreme environmental pressures; notwithstanding, further studies on Antarctic species would prove vital to confirm our findings. Nevertheless, assuming that the environment is not a determining factor for genome size, it could be theorized that other biological functions (specific of the taxa) may be the responsible for its determination. As example, despite *Desmarestia*, our samples show trends in line with those of their respective families, which may indicate that taxonomic aspects related to biological traits specific of each species may be behind the trends. In that sense, genome size may be linked to genomic requirements related to aspects like life cycle (different ploidy levels required for reproduction in different life phases), developmental growth (increased necessity of expression of particular genes), secondary metabolism (overexpression of genes to boost production of compounds) or special cell dynamics (e.g. lack of cyclosis in red seaweeds) at order or family level to name some (Goff & Coleman, 1990). As mentioned above, the data on parameters like C-value on algae are low compared, for example, with land plants (Gregory et al., 2007), so additional works including genomic data like ours would help to identify those possible trends at taxonomic levels. Also, our observations of nuclear patterning on the two previously non-studied species complement the still incomplete taxonomic information available for those taxa, and help to understand how those possible factors related to genome size influence the values measured for these species, as they showed differences related to growth and nuclear division. Information on this aspect is relevant to understand the developmental biology and life cycles (Kapraun, 2005; Goff & Coleman, 1990; Ribera Siguan et al., 2011; Gómez Garreta et al., 2010) of those two species, and our work provides source of comparison for further studies in these regards. In the same manner, we used fluorimetry analysis for our measurements and observations, which represents a fairly easy method to produce comparable data on future studies on Antarctic marine macroalgae. Though there are alternative methods to identify ploidy levels such as genome sequencing, chromosome counting, Fuelgen densitometry or nuclei release for flow cytometry (Goff & Coleman, 1990; Gregory et al., 2007; Gall et al., 1993), our technique presents some advantages in terms of simplicity, with comparable reliability. As example, the method used requires less time and sample processing in determining the DNA content, and it is particularly a better option for algae, as some of the mentioned alternative techniques present difficulties when dealing with the chemistry or cell structures of this type of organisms. As example, it is difficult to determine the phase or number of chromosomes at early stages of development (Deshmukhe & Tatewaki, 1993) and, contrary to techniques as cytometry or sequencing, fluorimetry enables to observe and measure algal tissue and individual cells independently allowing identification of phenomena like ploidy cell distribution patterns or endoreduplications (Goff & Coleman, 1990; Kapraun, 2005; Katagiri et al., 2016). Overall, the work presented in this chapter represents a step forward in terms of new data and methodology for further strengthening the knowledge on evolutionary tendencies of Antarctic seaweed species.

Chapter III: Chemical ecology of Antarctic seaweeds and potential antibiotic applications

Knowledge of the chemical ecology of Antarctic seaweed is not as wide as is the case for other groups of organisms (Amsler, 2008; Young et al., 2015). To better frame this situation, our work provides a baseline of understanding by gathering general knowledge about Antarctic chemical ecology, including the seaweeds, generated during recent years, as well as the possible implications and applications of the findings for the different organism groups. Following this topic, and using it as a guide to interpret our findings, in the second part of this chapter we provided our results on the chemical ecology of 22 species of Antarctic macroalgae. As stated in the work, the relationships between Antarctic seaweed species and the Antarctic microorganisms present in the SO are still not fully known. Complex dynamics have been reported between seaweeds and microorganism from other regions (Longford et al., 2019; Saha & Weinberger, 2019; Egan et al., 2013), demonstrating the ecological importance of those biological interactions, both for the seaweed species and for the rest of the coastal communities they dominate. In this context, our results with Antarctic seaweed taxa showed potential antimicrobial activity derived from natural extracts at natural concentration. The growth inhibition tested in the extracts of the seaweeds allowed us to discern which of the seaweed species possess strong protection against co-occurring microorganisms (which was especially evident for the genus *Desmarestia*, and the species *Delisea pulchra*). This information can help to better understand the framework in which biotic interactions develop, especially when combined with available reports on ecological parameters like epiphytation degree, microorganism fouling or life cycle dynamics. However, several molecules (e.g. halogenated compounds like phenolic molecules and phlorotannins, or some polysaccharides like certain alginates) can be responsible of the activities observed, as there are reports of certain compounds from macroalgae that showed similar biochemical activities in Rhodophyta as well as phaeophyceae (Pérez, et al., 2016; Caccamese et al., 1981; Young et al., 2015; Amsler, 2008). In that aspect, as we extracted hydrophilic and lipophilic fractions separately, some groups of molecules can be considered for further research and confirmation. In that framework, our results coincide with the general trend of observations in that lipophilic molecules present a greater range of activity against microorganisms (Kadam et al., 2015; Pérez et al., 2016; Salvador et al., 2007), thus making the possible molecules present in this fraction the better candidate for future analyses (e.g. aromatic compounds as quinones and tannins, or isoprenoids like certain terpenes and carotenoids). It is also well known that seaweed respond chemically to stress and changes to environmental conditions (Young et al., 2015; Gómez & Huovinen, 2020; Amsler, 2008), and that some important Antarctic seaweed species are very sensitive to climate change related phenomena like increased temperature changes, increased radiation levels and changes in salinity, to name some (Schoenrock et al., 2015; Pellizzari et al., 2020; Navarro et al., 2020; Wiencke et al., 2009; Schoenrock et al., 2016). Those effects have the potential to disrupt the biological interactions of marine macroalgae with Antarctic marine microorganisms, which may lead to detrimental changes in biodiversity of Antarctic coastal communities. Our results represent important evidence to comprehend how Antarctic communities are structured in a fine scale level, and provide useful data for future studies on how those interactions may shift due to impacts related to climate change. Additionally, our work represents a starting point on the search of potential human chemical applications on the 22 particular species of macroalgae tested. The natural extractions we tested were more active in lipophilic fractions, and Gram-positive bacteria were the most inhibited. It is worth noting that the seaweed *D. pulchra* showed activity even against the fungus *Candida albicans*, which showed to be resistant to all other extracts. As explained in the chapter, the need for new antibiotic sources has grown in recent years due to appearance of new resistant human pathogens that pose a serious threat to human health (Gonzalez-Villoria & Valverde-Garduno, 2016; Torres et al., 2015; Pachori et al., 2019; Aslam et al., 2018). Despite bioprospection has grown following this need (Ferreira et al., 2021; Anjali et al., 2019; Cushnie et al., 2020), Antarctic seaweeds are not between the most studied groups. In this regard, our assay adds to the evidence that Antarctic species (and particularly the aforementioned *D. pulchra* and *Desmarestia* species) are potential sources of new antibiotic compounds. Furthermore, results like these would help guide future research to find what types of compounds might be behind certain activities and against what type of microorganisms they would be more effective.

Chapter IV: Dispersal of non-native macroalgae on Antarctic waters and potential for bioinvasions

The SO presents several aspects that have been considered strong barriers to sea travelling organisms (Fraser et al., 2020; Fraser et al., 2018; Guillemin et al., 2020; Macaya et al., 2020). The main phenomena associated to this conception are related to the strength and patterns of currents along the ACC, the abrupt change in physic-chemical characteristics of seawater (pH, salinity, temperature) along the APF, the glacial conditions of the Antarctic coasts and the extreme light regimes derived from Antarctic latitudes (Oliveira et al., 2020; Pellizzari et al., 2020). However, increasing evidence is appearing on the permeability of those barriers, with evidence of possible organisms arriving to Antarctica from warmer latitudes, not only by human action, but also by passive travelling (Fraser et al., 2018; Macaya et al., 2020; Fraser et al., 2020; López et al., 2018; Tala et al., 2019). Our work in **Chapter IV** represents new and impacting evidence of this permeability derived from seaweed rafts (composed of the species of kelp *Durvillaea antarctica* and *Macrocystis pyrifera*) enduring the passing to the Antarctic region afloat. As stated in our results, a not negligible amount of animal phyla as well as two seaweed species (*Ballia callitricha* and *Ballia sertularioides*) were alive on board the identified rafts. The rafts themselves need to be considered potential candidates for bioinvasions and not only as biological ferries. The two kelp species collected have a sub-Antarctic distribution and possess many adaptations that make them suited to cope with long periods of time afloat along the SO. There, the ACC can disperse the rafts great distances, ending up in suitable places like the ones in which we found them. Additionally, they possess adaptations to somewhat cold environments (like those of Patagonia or the sub-Antarctic islands where they are native), which enables them to survive in the Antarctic if it reaches the values of some climate change scenarios (Fraser et al., 2018; Macaya et al., 2020; Tala et al., 2016). Additionally, rafting species are able to acclimate and present reproductive potential after crossings to Antarctica depending on specific physiological requirements of particular species and local environmental variations derived from climate change (Fraser et al., 2018; López et al., 2018; Macaya et al., 2005, 2020), which represents a serious possibility of colonisation by newly arrived species. Moreover, some of the animals found on the kelp rafts are known to present potential for invasiveness (*Membranipora membranacea* and *Lepas anatifera*), which combined with the fact that some rafts were found in one of the warmest places in Antarctica, consolidates our report as important reference to justify Antarctic monitoring of possible colonisations of foreign species. As we commented in **Chapter IV**, if we consider the raw number of rafts estimated to be afloat on the SO at any time (around over 70 million rafts, mainly *Durvillaea antarctica*), the influx of arrivals of potential invasive species appears to be greater than expected (Fraser et al., 2020, 2018; Macaya et al., 2020; Tala et al., 2019), raising the importance of reports like ours to prevent and monitor the potential events of colonisation derived from future climate change conditions.

CONCLUSIONS

The different studies on Antarctic seaweeds of the four chapters comprised in this work have served to refine the knowledge on the different topics treated. Below, the final conclusions of each chapter are summarized, following the main research topics covered:

Chapter I

1) The presence and composition of Antarctic seaweed communities within DI show to be mainly related to substrate type and availability, and there are at least three distinct types of macroalgal assemblages in Port Foster bay: the first, with greater algal biodiversity thanks to the greater availability of rocky substrata located near the bay entrance; the second, with a gradient of algal biodiversity, due to an intermediate degree of rocky areas and soft bottoms distributed at mid distance from the bay entrance; and the third, with lesser algae biodiversity, dominated by soft bottoms and comprising the innermost parts of the bay.

2) The more diverse algae assemblages found in the parts of DI closest to the entrance to Port Foster bay, are dominated by a mix of canopy forming species and sub canopy taxa, and are associated with animal communities dominated by suspension feeders.

3) The intermediate localities with an intermediate level of algae biodiversity, present some canopy-forming species in the rocky outcrops similar to those of the entrance, and some less complex points with fewer and smaller seaweed species.

4) Less complex seaweed communities are present in the inner parts of Port Foster's bay at DI, where rocky substrates are less common, and labile bottoms of ash and lapilli dominate and affect water turbidity to a greater extent. The scarce algal species here coexist alongside animal communities dominated by mobile deposit feeders as well as infaunal communities characteristic of soft bottoms.

5) Seaweed host type (Rhodophyte or Phaeophyceae) seems explain a significant amount of variation of epiphytic diatom diversity on seaweeds from DI and LI, which could indicate host selectiveness. Though seaweed branching and anuality had some significance on Rhodophyte hosts, no clear evidence was found for Phaeophyceae, in which case, chemical ecology of the host may be related.

6) Seaweed epiphytic diatom's biodiversity seems to be overall richer in DI than in LI, maybe due to greater diversity of niches created by the volcanic conditions the islands Port Foster's bay. On the other hand, some individual seaweed samples showed richer diatom communities, which may be related to better light conditions on the waters of LI

Chapter II

7) Nuclear DNA content measured by fluorimetry represents straight forward technique that can facilitate the production of new data for Antarctic seaweeds due to its simplicity and reliability compared with more complex and time-consuming protocols. As Antarctic seaweed species are lacking in this type of data, rapid production may benefit the knowledge on evolutionary parameters like this.

8) In general, genome size measurements expressed as C-value for measured Antarctic seaweed species is consistent with the trends that close taxa from other regions possess and, hence, there is no apparent relation between environmental selective pressures and genome size. This indicates that C-values for Antarctic seaweed may be more influenced by the species' life cycle and taxonomic evolutionary history.

9) *Desmarestia* species measured showed remarkable lower C-values than previous measurements for this genus from other regions, further contradicting the theory that relates greater ploidy levels with strong environmental selective pressures. Even though previous measurements on *Desmarestia* may be derived from polyploid material, further investigation is needed to clarify this difference with Antarctic material.

10) Nuclear patterning for the species *Ballia callitricha* and *Neuroglossum delesseriae* have been identified for the first time. Those patterns correspond to a model of uninucleated non-polyploid apical cell deriving in uninucleated highly polyploid axial cells, and uninucleated non-polyploid cortical cells deriving in multinucleated non-polyploid medullar cells respectively.

Chapter III

11) Some natural extracts from Antarctic seaweed possess antibiotic activity against Antarctic microorganisms, indicating possible defensive interactions against some types of bacteria. This activity seems more common against Gram-positive bacteria and in lipophilic fractions. The species that showed greater activity were *Delisea pulchra*, *Neuroglossum delesseriae* and *Pantoneura plocamioides* from Rhodophyta, and *Desmarestia anceps*, *Desmarestia antarctica* and *Desmarestia menziesii* from Ochrophyta.

12) Some natural extracts from Antarctic seaweed also showed antibiotic activity against common human pathogen surrogates, which indicates a possible application of Antarctic seaweeds as source of new antibiotic compounds. The species that showed greater antibiotic potential were *Delisea pulchra* (Rhodophyta) and *Desmarestia* spp (Ochrophyta). As with Antarctic microorganisms, the activity observed was more common against Gram-positive bacteria and in lipophilic fractions.

13) Previous reports about molecules extracted from several seaweed species, indicate that some components of the secondary metabolism may be behind several types of activities. Some of those compounds are widespread on related groups of macroalgae, making those chemicals potentially responsible (at least in part) for the types of activity we observed in our natural products tests (e.g. isoprenoids, terpenoids, aromatic quinones, or phlorotannins). Nonetheless, species-specific variations in these common compounds, and undescribed chemicals unique to the species analysed, may also play a part of this activity.

Chapter IV

14) Non-native kelp species (*Durvillaea antarctica* and *Macrocystis pyrifera*) reach the Antarctic coasts as rafts, crossing the Antarctic Polar Front and then dispersed by the Antarctic circumpolar currents. These rafts represent potential candidates for colonization, and possible future changes in the Antarctic climate may cause the establishment of populations of these kelps species on the Antarctic shores.

15) The non-native kelp rafts arriving to Antarctica also act as ferries for non-Antarctic animals from other regions that also present potential colonisation and invasion capabilities (like *Membranipora membranacea* and *Lepas anatifera*). This fact makes these events plausible sources of bioinvasions in future scenarios of climate change throughout Antarctica.

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Cover: Palmaria decipiens, Iridaea cordata and *Gigartina skottsbergii* carpet on Deception Island shores by Rafael Pablo Martín Martín

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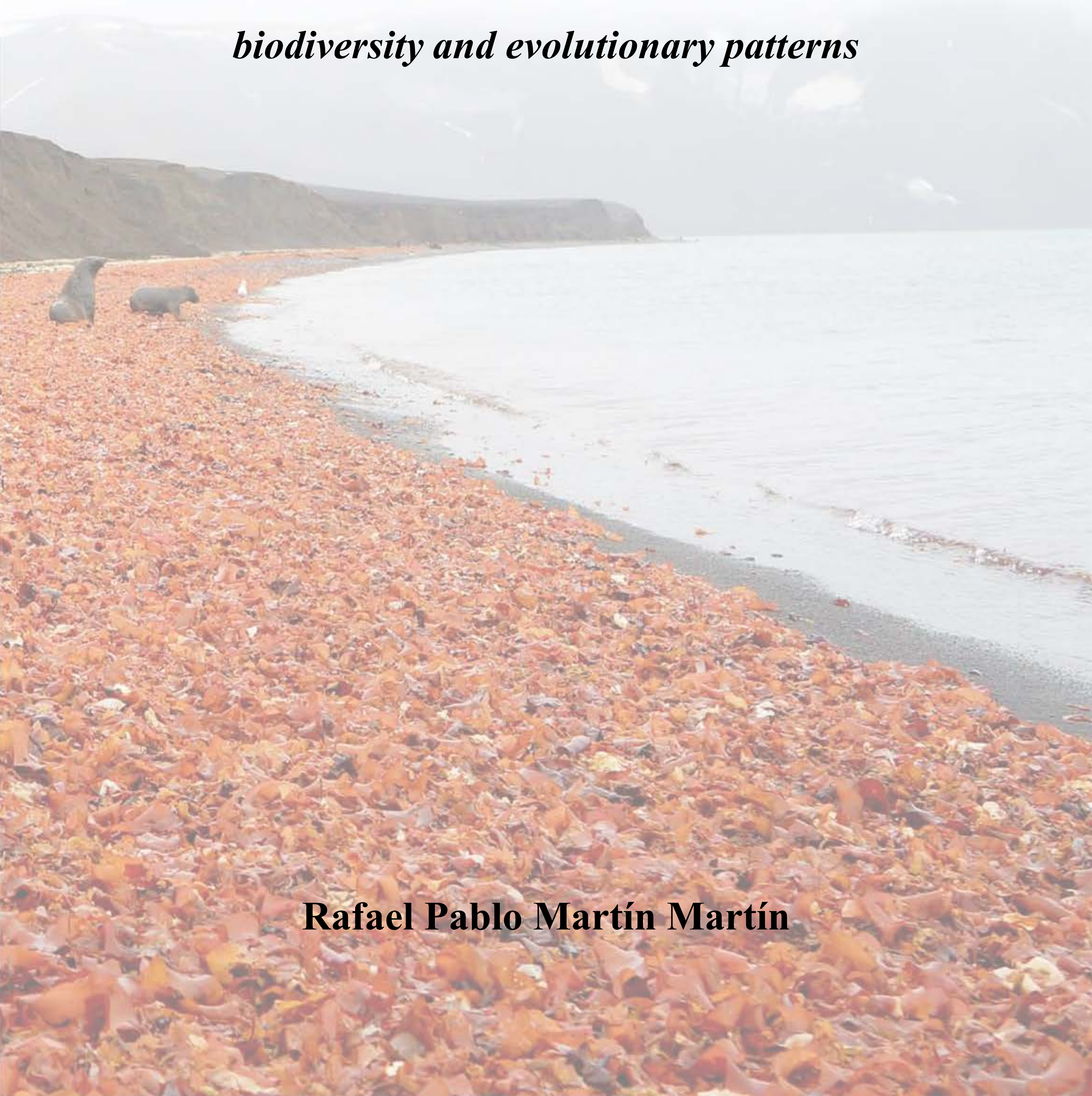
Benthic marine algae from South Shetland Islands (Antarctica):

biodiversity and evolutionary patterns



Rafael Pablo Martín Martín

BENTHIC MARINE ALGAE FROM SOUTH SHETLAND ISLANDS (ANTARCTICA): BIODIVERSITY AND EVOLUTIONARY PATTERNS

ALGAS BENTÓNICAS MARINAS DE LAS ISLAS SHETLAND DEL SUR (ANTÁRTIDA): BIODIVERSIDAD Y PATRONES EVOLUTIVOS

PhD program of Vegetal Biodiversity

Laboratory of Botany

Department of Biology, Healthcare and the Environment

UNIVERSITAT DE BARCELONA

Memoria presentada por **Rafael Pablo Martín Martín** para optar al grado de Doctor por la Universidad de Barcelona

Junio 2021

Amelia Gómez Garreta	Rafael Pablo Martín Martín	Jordi Rull Lluch
(Directora)	(Estudiante)	(Director y Tutor)

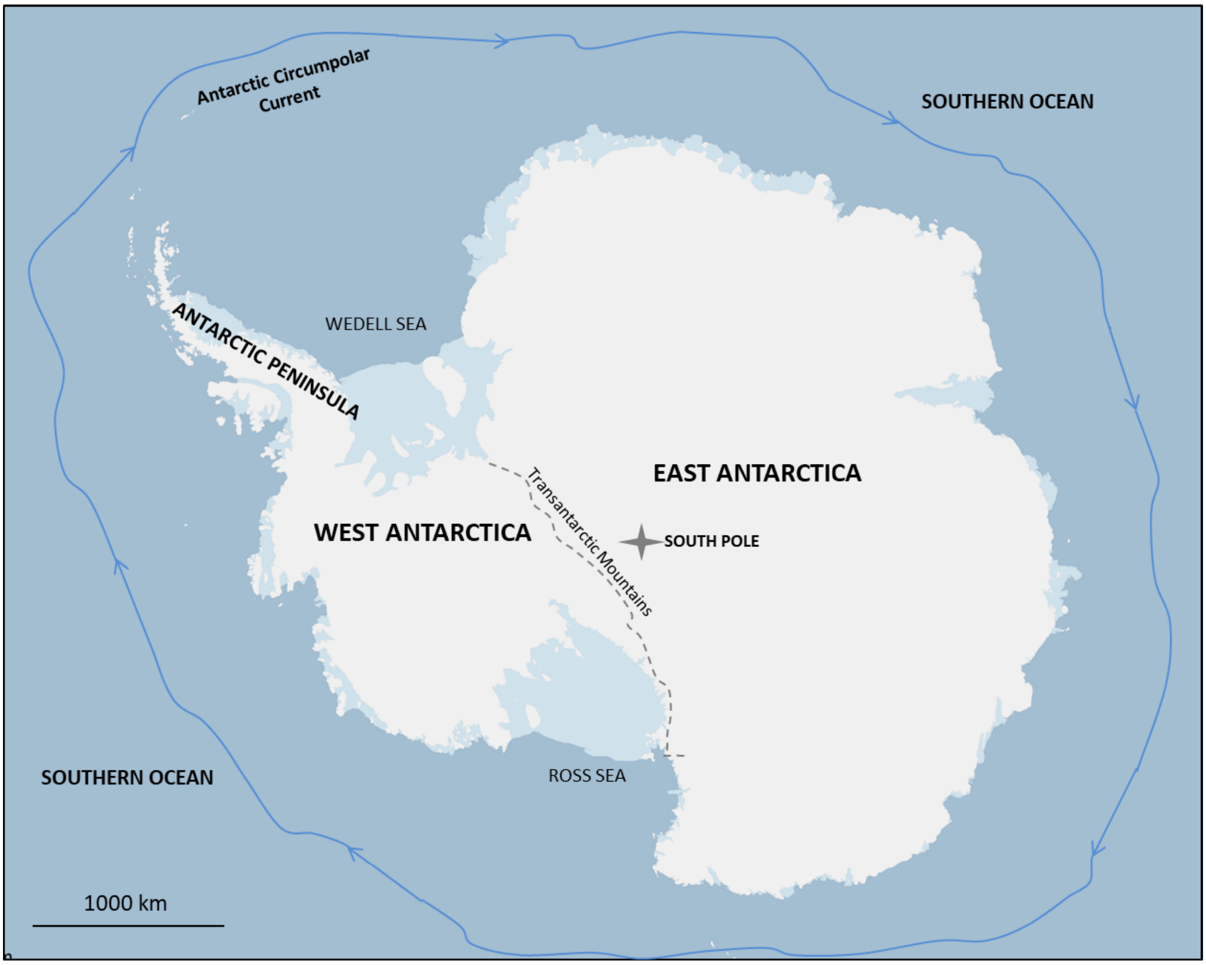


Fig. 1 Antarctica map showing the two main geographic divisions of the continent and several relevant geographic features. Maps have been realized with Q-Gis software and Qantarctica map package.

The SO, the ACC and the APF greatly influence Antarctic conditions, especially in the maritime Antarctica, which comprises the coasts of the continent, and specially the northern areas of the western Antarctica (Antarctic Peninsula region) and all the archipelagos surrounding it. They promoted the thermal isolation of Antarctica and, at least until recent times, have acted as a strong barrier to the crossing of many organisms from other regions (Clarke et al., 2005; Fraser et al., 2020; Kennett, 1977; Lawver & Gahagan, 2003; Macaya et al., 2020). This thermal isolation has dropped the SO temperature (which can reach temperatures as low as -2ºC in the coldest seasons), which in turn, facilitated the drop of temperatures on the Antarctic Continent itself (Sahade et al., 2015; Oliveira et al., 2020; Fraser et al., 2020). Furthermore, this thermal differentiation between the SO and the strong circulation of the ACC quick started the transport of nutrients and phytoplankton-rich surface along the water columns and the coastline, facilitating also the moving of minerals contributed to the water by the melting of coastal glaciers (Griffiths et al., 2009; Oliveira et al., 2020; Wiencke & Clayton, 2002). Altogether, the Antarctic region presents challenging conditions for which Antarctic organisms have been adapting during the course of evolution (generalized polar conditions, extreme variations in light patterns, strong barriers that promote isolation). This, especially on Antarctic seas, has driven the appearance of a distinct biota that presents a high degree of endemism, as well as a plethora of interesting adaptations to cope with those challenging conditions (Griffiths et al., 2011; Oliveira et al., 2020; Pellizzari et al., 2020; Wiencke et al., 2014; Wiencke & Clayton, 2002). For the study of this marine biota, Antarctic Peninsula region and its caldera, with an opening to the sea (Neptune Bellows, **Fig. 3**) from which seawater flooded the caldera and formed the inner bay of the island (Port Foster **Fig. 3**).

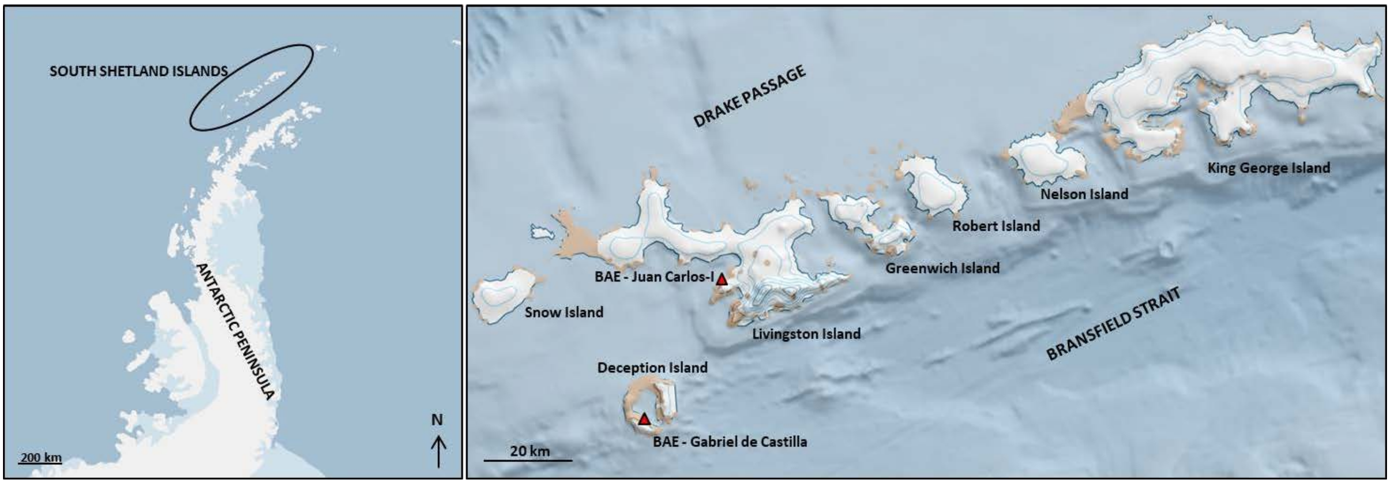


Fig. 2 Left: Map of the Antarctic Peninsula region with the South Shetland Islands location. **Right**: Map of the South Shetland Archipelago with the locations of the two Antarctic Spanish Bases (BAE - Juan Carlos-I and BAE - Gabriel de Castilla). Maps have been done with Q-Gis software and Qantarctica map package.

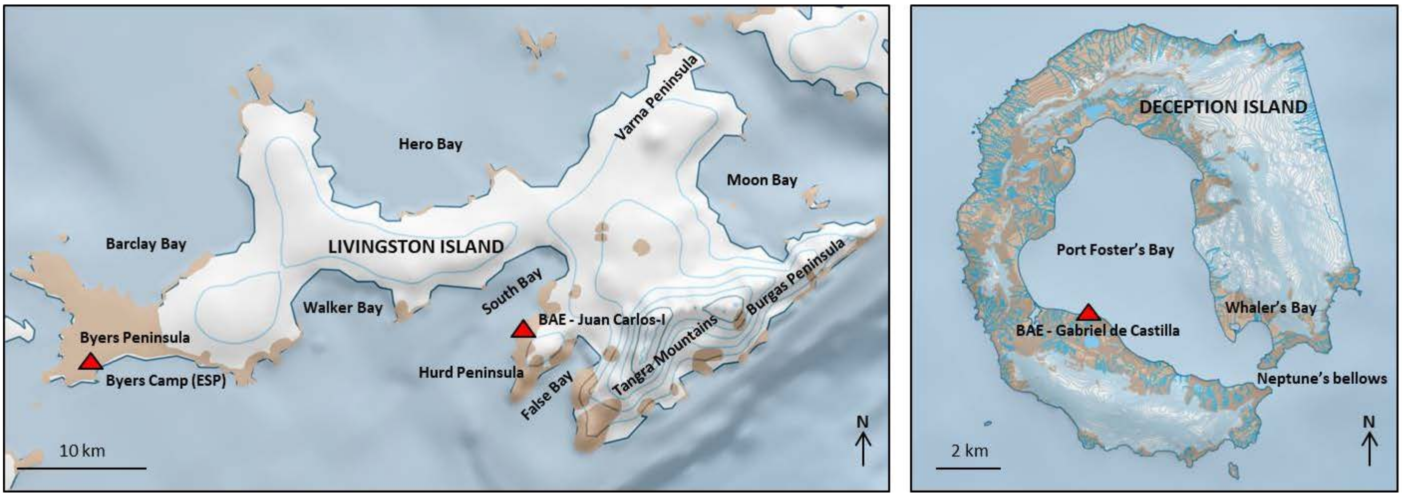


Fig. 3 Left: Map of Livingston Island with the locations on the Spanish Antarctic Base Juan Carlos-I and the Spanish research camp on Byers Peninsula, along with some geographical features of the island. **Right**: Map of Deception Island, indicating the location of with the Spanish Antarctic Base Gabriel de Castilla along with some geographical features of the island. Maps have been done with Q-Gis software and Qantarctica map package.

As an active volcanic area, it possesses some habitats with special physico-chemical properties (especially in Port Foster bay) where waters present warmer temperature, turbidity and volcanic related chemistry (variating pH levels, increased concentration of heavy metals, dissolved sulphuric compounds) which differentiates it from the surrounding Antarctic benthic environments (Elderfield, 1972; Muñoz-Martín et al., 2005; Barnes & Conlan, 2007; Pellizzari et al., 2017). Even though there are some volcanic rocky underwater outcrops and some pebble bottomed coasts, the majority of the benthos of DI coasts is dominated by soft bottoms composed by volcanic ash and lapilli. Those sparse rocky areas are where the greatest benthic communities of macroalgae develop. However, due to the mentioned related volcanic properties of the water and turbidity, the vertical distribution of those communities varies from the

Participación del doctorando: recogida y procesado de muestras en la Antártida, participación en la preparación y tinción del material, observación al microscopio y realización de fotografías de los núcleos, análisis de datos, discusión de los resultados, diseño y redacción del artículo.

Categorización de la revista. Factor de impacto en Scientific Reports (Web of Science): 1,728. Posición: 30/58 (Cuartil 3) en *Biodiversity and Conservation,* y posición: 101/169 (Cuartil 3) en *Ecology.*

4. Chemical ecology in the Southern Ocean.

Autores: Angulo-Preckler, C., De Castro-Fernandez, P., **Martin-Martin, R. P.**, Figuerola, B., Avila, C.

Libro: Di Prisco, G., Edwards, H. G. M., Elster, J., Huiskers, A. H. L. (eds.). *Life in Extreme Environments: Insights in Biological Capability*, 251. (Cambridge University Press, 2020)

Participación del doctorando: realización de los muestreos y observaciones, análisis de datos, discusión de los resultados, diseño y redacción del capítulo de libro.

5. Experimental evidence of antimicrobial activity in Antarctic seaweeds: ecological role and antibiotic potential

Autores: **Martín-Martín, R. P.**, Carcedo-Forés, M., Camacho-Bolós, P., García-Aljaro, C., Angulo-Preckler C., Avila, C., Rull Lluch, J., Gómez Garreta, A.

Revista: *Polar Biology* (enviada)

Participación del doctorando: recogida y procesado de muestras en la Antártida, participación en la realización de los antibiogramas, análisis de los datos, discusión de los resultados, diseño y redacción del artículo.

Categorización de la revista. Factor de impacto en Scientific Reports (Web of Science): 1,728. Posición: 30/58 (Cuartil 3) en *Biodiversity and Conservation,* y posición: 101/169 (Cuartil 3) en *Ecology.*

6. Invasive marine species discovered on non–native kelp rafts in the warmest Antarctic island.

Autores: Avila, C., Angulo-Preckler, C., **Martín-Martín, R. P.**, Figuerola, B., Griffiths, H. J., Waller, C. L. (2020).

Revista: *Scientific Reports*, 10(1): 1-9.

Participación del doctorando: realización de los muestreos y observaciones, análisis de datos, discusión de los resultados, diseño y redacción del artículo.

Categorización de la revista. Factor de impacto en Scientific Reports (Web of Science): 3,998. Posición: 17/71 (Cuartil 1) en *Multidisciplinary Sciences.*

Barcelona, 11 de junio de 2021

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SHORT NOTE

Nuclear DNA content estimations and nuclear development patterns in Antarctic macroalgae

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Abstract

Nuclear DNA content of marine macroalgae is known for only about a 3% of the globally known taxa, and so far, the only available data concerning marine Antarctic macroalgae refer to the Phaeophyceae *Ascoseira mirabilis* and *Desmarestia antarctica*. As these data can be useful for species delimitation when combined with other taxonomical information, we aimed to expand the available number of measurements in the understudied Antarctic seaweed flora. To address this, we measured the nuclear DNA content of 12 Antarctic marine algae (seven Rhodophyceae and five Phaeophyceae). Nuclear DNA analyses were carried out by spectrofluorimetry and image analysis from samples conserved in Carnoy, using DAPI as DNA marker. For ten of these taxa, our values represent the first estimations to date. The nuclear DNA content estimates obtained for the Antarctic red algae examined vary between $2C = 0.38$ pg in *Gigartina skottsbergii* and $2C = 1.63$ pg in *Neuroglossum delesseriae*. In brown algae, the values range from $2C = 0.18$ pg in both *Desmarestia antarctica* and *Desmarestia menziesii* to $2C = 0.96$ pg in *Phaeurus antarcticus*. Furthermore, this study allowed us to identify nuclear developmental patterns for the first time in two Antarctic seaweeds (*Ballia callitricha* and *Neuroglossum delesseriae*).

Keywords Nuclear DNA content · Nuclear patterns · Antarctica · Seaweeds

Introduction

The nuclear DNA content of a species, expressed as C–value, is the total quantity of not replicated nuclear DNA of a gamete, and it is constant and independent from the level of ploidy of the individuals (Swift 1950; Greilhuber et al. 2005). C–values are used in a wide range of biological fields for its fundamental biological meaning (Goff & Coleman 1990; Bennett et al. 2000; Salvador et al. 2009; Bennett & Leitch 2011), and it is used in industrial, taxonomic, and phylogenetic studies, as well as biogeographic analysis (Bennett & Leitch 2001, 2005a,b; Pellicer et al. 2010). C–values define ploidy level and the genome size of species of interest, both parameters of great importance to complement evolutionary, taxonomic, and ecological information (Kapraun 2005; Chénais et al. 2012 and Husband et al. 2013). As an example, C–value has been related to ecological parameters in many taxonomic groups correlating the amount of DNA and the thermal regime, i.e., latitude and altitude. However, there is some controversy about this, as many studies point to a larger genome size in species that live in extreme environments

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(Arctic ecosystems or high mountain), while other studies found the contrary (Bennett & Leitch 2001, 2005a,b; Pellicer et al. 2010). Other studies used this parameter to describe important developmental aspects of seaweed, as major nuclear patterns of multinucleate or endopolyploid uninucleate cells in the development of the Florideophyceae (Goff & Coleman 1990).

Nonetheless, nuclear DNA content of marine macroalgae has been studied only for about a 3% of the globally known taxa (Kapraun 2005, 2007; Gómez Garreta et al. 2010; Ribera Siguan et al. 2011; Phillips et al. 2011; Kapraun & Freshwater 2012; Salvador–Soler et al. 2016) and so far, the only available data concerning Antarctic macroalgae refer to *Ascoseira mirabilis* Skottsberg and *Desmarestia antarctica* R.L.Moe & P.C.Silva. In the same way, the nuclear development patterns in algae have been only rarely observed since they were established (Goff & Coleman 1990) and they have not been investigated in Antarctic algae yet. For these reasons, our goal here has been to provide further data on nuclear DNA content and development patterns, to be used in future studies on the flora of the Antarctic regions.

Materials and methods

Species selection

12 Antarctic algal species were selected for this study comprising 7 Rhodophyceae and 5 Phaeophyceae (Table 1). The selection criteria were mainly the lack of data of nuclear DNA content at genus or family levels, as well as abundance in the sampling area.

Sampling

Sampling was performed in the South Shetland Islands and the Antarctic Peninsula during the austral summers of 2009–2010, and 2012–2013 (in the frame of ACTIQUIM

Table 1 Sampling data: studied algal species, locality, demographic information, and collecting date

Phylum	Class	Family	Species	Locality	Latitude	Longitude	Sampling date
Rhodophyta	Florideophyceae	Balliaceae	*Ballia callitricha* (C.Agardh) Kützing	Kopaitic Is. (Antarctic Peninsula)	63°18′46.23"S	57°54′34.24"W	25/12/2012
Rhodophyta	Florideophyceae	Gigartinaceae	*Gigartina skottsbergii* Setchell & N.L.Gardner	Vera Is. (Antarctic Peninsula)	63°18′41.95"S	57°55′7.66"W	26/12/2012
Rhodophyta	Florideophyceae	Kallymeniaceae	*Callophyllis* sp.	Sapo Is. (Antarctic Peninsula)	63°19′2.87"S	57°55′23.46"W	27/12/2012
Rhodophyta	Florideophyceae	Delesseriaceae	*Neuroglossum delesseriae* (Reinsch) M.J.Wynne	Sapo Is. (Antarctic Peninsula)	63°19′2.87"S	57°55′23.46"W	27/12/2012
Rhodophyta	Florideophyceae	Rhodomelaceae	*Picconiella plumosa* (Kylin) J.De Toni	Sapo Is. (Antarctic Peninsula)	63°19′2.87"S	57°55′23.46"W	27/12/2012
Rhodophyta	Florideophyceae	Rhodymeniaceae	*Rhodymenia coccocarpa* (Montagne) M.J.Wynne	Colatinas (Deception Is.)	62°59′22.43"S	60°37′17.50"W	11/01/2010
Rhodophyta	Florideophyceae	Fryeellaceae	*Hymenocladiopsis prolifera* (Reinsch) M.J.Wynne	Whaler´s Bay (Deception Is.)	62°58′51.85"S	60°33′41.28"W	11/01/2010
Ocrophyta	Phaeophyceae	Desmarestiaceae	*Himantothallus grandifolius* (A.Gepp & E.S.Gepp) Zinova	Fildes Point (Deception Is.)	62°59′33.22"S	60°33′25.22"W	06/02/2013
Ocrophyta	Phaeophyceae	Desmarestiaceae	*Desmarestia antarctica* R.L.Moe & P.C.Silva	Antarctic Spanish Base Beach (Deception Is.)	62°58′35.25"S	60°40′31.97"W	12/12/2012
Ocrophyta	Phaeophyceae	Desmarestiaceae	*Desmarestia menziesii* J.Agardh	Whaler´s Bay (Deception Is.)	62°58′51.85"S	60°33′41.28"W	14/12/2012
Ocrophyta	Phaeophyceae	Desmarestiaceae	*Phaeurus antarcticus* Skottsberg	Whaler´s Bay (Deception Is.)	62°58′51.85"S	60°33′41.28"W	11/01/2010
Ocrophyta	Phaeophyceae	Ascoseiraceae	*Ascoseira mirabilis* Skottsberg	False Bay (Livingston Is.)	62°41′44.49"S	60°20′10.13"W	15/12/2012

research projects). Details on the sampling localities information are available in Table 1. Specimens were collected from intertidal and subtidal ranges, down to 25 m of depth, either by snorkeling or scuba diving.

Sample conservation

Samples were frozen (− 20 °C) after collection in situ and stored until their arrival to the laboratory at the University of Barcelona. Once there, the specimens were defrosted to confirm identification of species and life phase of each individual (Table 2). For nuclear DNA quantification, we followed a modification of Kapraun (2005) and Goff & Coleman (1990) protocols, as our previous experience with this kind of measurements determined that defrosting does not affect measures compared to Carnoy fixation in situ. For this, several fragments of each individual (ca. 0.5 mm^2) were taken and conserved separately as replicates. These fragments were fixed with Carnoy solution (3:1 of 95% ethanol–glacial acetic acid) during 24 h and stored in 70% ethanol at 4ºC during at least 24 h for later nuclear DNA content analysis.

DNA quantification

After 70% ethanol storage, samples were rehydrated in distilled water and softened in 5% w/v EDTA (Goff & Coleman 1990) for 12–48 h. The samples were subsequently squashed with rugged slides and then transferred to coverslips previously soaked with subbing solution. The coverslips with the samples and the subbing were left air dried and then stained with 0.5 µg/mL 4′,6–diamidino–2–phenylindole (DAPI; Sigma Chemical Co. St. Louis, MO 63,178) and mounted in microscopy preparations following literature methods (Goff & Coleman 1990; Kapraun & Nguyen 1994). Nuclear DNA contents were measured using fluorimetry and image analysis, following a procedure modified from Kapraun & Dunwoody (2002) and Choi et al. (1994). The images obtained were then analyzed using MetaMorph software (Molecular Devices, Toronto, Canada). The nuclear DNA content was obtained by comparison of fluorescence intensity of the nuclei with a standard with constant nuclear DNA amount. Following Kapraun & Nguyen (1994) and Kapraun & Dunwoody (2002), we used *Gallus gallus* (Linnaeus) erythrocytes with constant nuclear DNA content of 2.4 pg (Clowes et al. 1983) as standard. For statistical reasons, we tried to reach a minimum of 100 measured nuclei per species for analyses of ploidy peaks. Nonetheless, in

Table 2 Measurements of nuclear DNA content in picograms (mean ± s.d.) for the studied Antarctic algae, number of nuclei, and life phase

Taxa	Nº individuals	Nº nuclei	Life phase	1C	2C	4C	8C	16C	32C
Ballia callitricha (C.Agardh) Kützing	1	123	Sporophyte	–	0.93 ± 0.25	–	4.08 ± 1.06	6.77 ± 1.25	14.98 ± 1.24
Gigartina skottsbergii Setchell & N.L.Gardner	2	209	Sporophyte	–	0.38 ± 0.11	0.89 ± 0.08	–	–	–
Callophyllis sp.	1	93	Gametophyte	0.22 ± 0.07	0.44 ± 0.09	–	–	–	–
Neuroglossum delesseriae (Reinsch) M.J.Wynne	2	289	Gametophyte	0.88 ± 0.26	1.63 ± 0.23	–	–	–	–
Picconiella plumosa (Kylin) J.De Toni	1	50	Gametophyte	0.72 ± 0.20	1.28 ± 0.14	–	–	–	–
Rhodymenia coccocarpa (Montagne) M.J.Wynne	2	246	Gametophyte	0.22 ± 0.08	0.54 ± 0.10	–	–	–	–
Hymenocladiopsis prolifera (Reinsch) M.J.Wynne	1	93	Gametophyte	–	0.43 ± 0.03	–	–	–	–
Himantothallus grandifolius (A.Gepp & E.S.Gepp) Zinova	1	124	Sporophyte	–	0.36 ± 0.15	0.92 ± 0.18	–	–	–
Desmarestia antarctica R.L.Moe & P.C.Silva	1	161	Sporophyte	–	0.18 ± 0.01	0.39 ± 0.02	–	–	–
Desmarestia menziesii J.Agardh	2	180	Sporophyte	–	0.18 ± 0.01	0.38 ± 0.01	–	–	–
Phaeurus antarcticus Skottsberg	1	167	Sporophyte	–	–	1.92 ± 0.26	–	–	–
Ascoseira mirabilis Skottsberg	1	60	Gametophyte	–	0.33 ± 0.08	0.67 ± 0.05	–	–	–

some species, this was not possible because some nuclei were not properly stained.

Nuclei measurement results were then analyzed for each species to identify ploidy levels as peak classes of nuclei in the same range of nuclear DNA quantity (picograms, pg). Nuclei not belonging to peak classes (those classes with low number of nuclei, i.e., between 0 and 10) in the histograms were discarded for calculations of ploidy peaks. For each peak class, nuclear DNA quantity mean and standard deviation were calculated. Also, ploidy level to each peak class was assigned tentatively as in Kapraun (2005), taking into account the life phase of the individuals sampled (whenever possible) and/or by comparing to ploidy levels assignations of closely related taxa in the Kew Royal Botanic Gardens C–value database when available. All the results obtained here will be incorporated into the C–values algae database of the Kew Royal Botanic Garden (https://cvalues.science.kew.org/).

Results and discussion

Except for *A. mirabilis* and *D. antarctica* (Phillips et al. 2011), our measures are the first values of nuclear DNA content measured for Antarctic algae so far. Relative to the nuclear DNA content values, these vary between 2C = 0.38 pg of *Gigartina skottsbergii* Setchell & N.L.Gardner and 2C = 1.63 pg of *Neuroglossum delesseriae* (Reinsch) M.J.Wynne for the red seaweeds, as well as 2C = 0.18 pg for both *Desmarestia* species studied and 2C = 0.96 pg of *Phaeurus antarcticus* Skottsberg (deduced from 50% of the 4C value assigned) for the brown ones (Table 2).

For most Phaeophyceae, the 2C and 4C ploidy levels (except in *Phaeurus* in which we only identified a peak corresponding with 4C) were assigned taking into account the life phase of the material (all except *A. mirabilis* were sporophytes), and, as mentioned before, by comparing to the ploidy levels of the closest taxa present in the Kew database. In the case of *A. mirabilis*, 2C and 4C ploidy levels were assigned to the material measured, even though our sample was not a fertile individual. This was deduced because we know that the cells measured were of a diploid individual in phases G1 and G2, as *A. mirablis* is described as having a diploid monogenetic life cycle (thus having a diploid gametophyte).

The same criterion was applied to the seven Rhodophytes analysed. Two of the species were sporophytes (*Ballia challitricha* (C.Agardh) Kützing and *Gigartina skottsbergii* Setchell & N.L.Gardner), and the other five species were in gametophytic phase. For the two sporophytes, we determined that the ploidy peaks observed corresponded to 2C and 4C levels. Surprisingly, *B. callitricha* showed several additional peaks with increasing number of ploidy levels, reaching up to what appears to be 32C (14.98 pg). The measures of those two species constitute the second measures of nuclear DNA inside the family Gigartinaceae and the first estimates for the whole order Balliales, respectively. For the gametophytes, we were able to assign 1C and 2C values for all five species, with the exception of *Hymenocladiopsis prolifera* (Reinsch) M.J.Wynne, for which we only identified a ploidy level of 2C. Also, it is worth noting that the values measured on *Callophyllis* sp. are the first ones for the Kallymeniaceae family.

The values of nuclear DNA content obtained in this study for *D. antarctica* are four times lower than those provided by Phillips et al. (2011) for the same species. Something similar happens if we compare our values for this species (and those of *D. menziesii*) with those of the other two non-Antarctic *Desmarestia* species included in the Kew database. An explanation could be that the individuals considered in the database were polyploid. Even though more data for this genus would be necessary to further explain our observations, our measures could also suggest no relation between higher ploidy levels and extreme environments for these taxa. Nevertheless, further knowledge of the chromosome complement of the Antarctic species would be of great relevance to better understand this phenomenon in this genus. Similarly, further measurements and wider samplings for Antarctic seaweed species would prove vital to confirm observations like these and also to increase our knowledge in the previously unknown families.

Taking into account the number of nuclei studied, we consider our nuclear DNA content measurements and ploidy level assignation to be fairly accurate, as our measures show consistency with previously measured close taxa available (Kapraun 2005; Kapraun et al. 2007; Gómez Garreta et al. 2010 and Phillips et al. 2011) and Kew C–value database. The only samples studied that may raise some concern could be *Picconiella plumosa* (Kylin) J.De Toni and *A. mirabilis* due to the lower number of nuclei we were able to measure. Those lower numbers may be related with cell wall permeability or secondary metabolites of those particular species, perhaps interfering with the staining process. Further studies and measures for those species will shed light in what may cause this. Nonetheless, in those two cases, our measures are consistent with those previously published for other Rhodomelaceae and for *A. mirabilis* in the Kew database.

Another important finding of our study is the identification of two nuclear patterns for *B. callitricha* and *N. delesseriae*. Those are the first observations of the kind in any Antarctic seaweed, as nuclear patterns have been described by Goff & Coleman (1990) only in non-polar macroalgae. This was possible by observing some stained not disaggregated tissues in the samples of *B. callitricha* and *N. delesseriae* (Fig. 1a, b). *Ballia callitricha* (Fig. 1c,e) showed a pattern

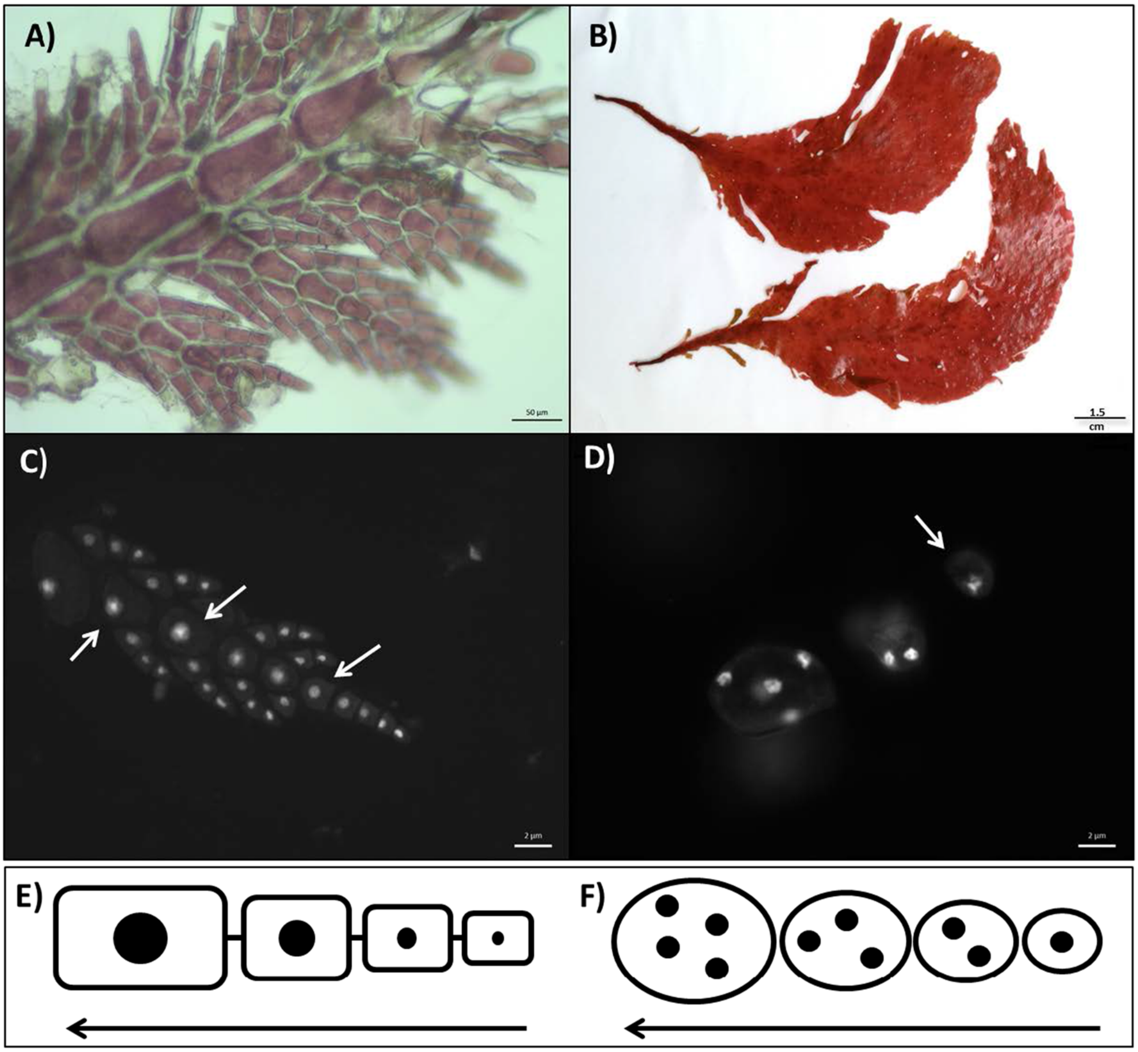

Fig. 1 **a** Picture of *Ballia callitricha* under the microscope; **b** Picture of *Neuroglossum delesseriae*; **c** Fluorescence image with increased contrast, showing non-disaggregated material of *B. callitrica* with stained nuclei (bright spheres inside cells) of increasing ploidy of axial derived cells (marked with arrows); **d** Fluorescence image with increased contrast showing *N. delesseriae* material, presenting cortical cells with one nuclei (arrow) and medullar cells with increasing number on nuclei (middle and bottom cells); **e** Nuclear patterning scheme for *B. callitricha* of uninucleated non-polyploid apical cell deriving in uninucleated highly polyploid axial cells (modified from Goff & Colemann 1990); **f** Nuclear model scheme for N. delesseriae, depicting the model of uninucleated non-polyploid cortical cells deriving in multinucleated non-polyploid medullar cells (modified from Goff & Colemann 1990)

consisting of an apical uninucleated non-polyploid cell (2C = 0.93 pg) deriving in an axial sequence of uninucleated cells of increasing ploidy (8C = 4.08 pg, 16C = 6.77 pg and 32C = 14.98 pg) as shown in Fig. 1e. On the other hand, *N. delesseriae* (Fig. 2d, f), possesses a pattern of cortical, uninucleated, non-polypoid cells (1C = 0.88 pg or 2C = 1.63 pg), deriving towards the medulla in polynucleated (with up to 5–12 non-polyploid nuclei) larger cells. In this case, the pattern is equal to what has been observed in other Delesseriaceae by Goff & Coleman (1990). For *B. callitricha*, the increasing ploidy of the axial cells would provide an explanation for our nuclear DNA measurements of increasing ploidy peaks (up to 32C). According to Goff & Coleman (1990), these two patterns of increasing ploidy level in derived cells represent two different strategies to maintain the correlation between the nuclear DNA content and the cytoplasmic volume when the cell size increases during development.

Similar to land plants, seaweeds present mechanisms of genome size variation (Šmarda & Bureš 2010; Leitch & Leitch 2013; Sjotun et al. 2017). Those mechanisms may produce polyploidy events (Bennetzen et al. 2005; Bothwell et al. 2010; Garbary & Clarke 2002) that lead to the apparition of autopolyploids in the populations and thus act as driving speciation phenomena (Tayalé & Parisod 2013; De Strome et al. 2014; Sjotun 2017). As the Antarctic seaweed flora has a high percentage of endemic species (Wiencke et al. 2002, 2014), understanding the processes promoting speciation is vital to unveil the history and evolution of these communities.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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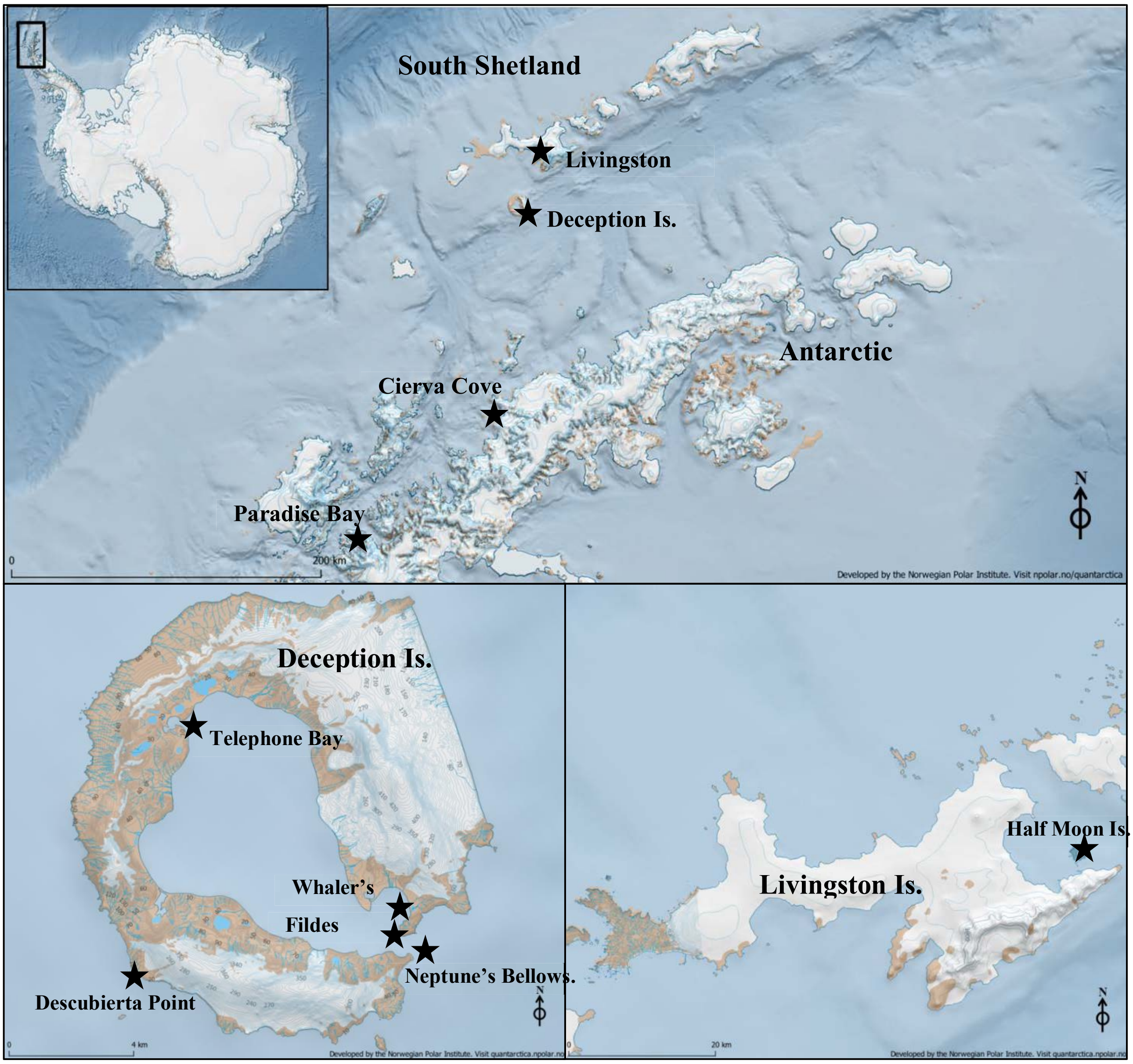
each type of extract. For lipophilic compounds, diethyl ether 100% (Et_2O) was used and the process was repeated three times. The resulting ethereal solution was evaporated again *in vacuo* and transferred to pre-weighted vials alongside Et_2O. The content of the vials was again evaporated and weighted to obtain the ethereal extract weight. After that, they were stored frozen (-20^{o}C) until used for the antimicrobial assays. As for the separation of hydrophilic compounds, it was performed by using butanol 100% (BuOH). In this case, it was done twice. After this, similarly to the lipophilic compounds, the butanolic solution containing hydrophilic fractions was transferred to the pre-weighted vials, adding trichloromethane in this case, lyophilized and weighted again before stored frozen.

Figure 1. Maps of the sampling locations and stations listed on **Table 1** (marked with stars). A) Antarctic continent. B) Antarctic Peninsula region and South Shetland archipelago. C) Deception Island. D) Livingston Island. Map constructed with QGIS software (v. 3.16) with Quantarctica package



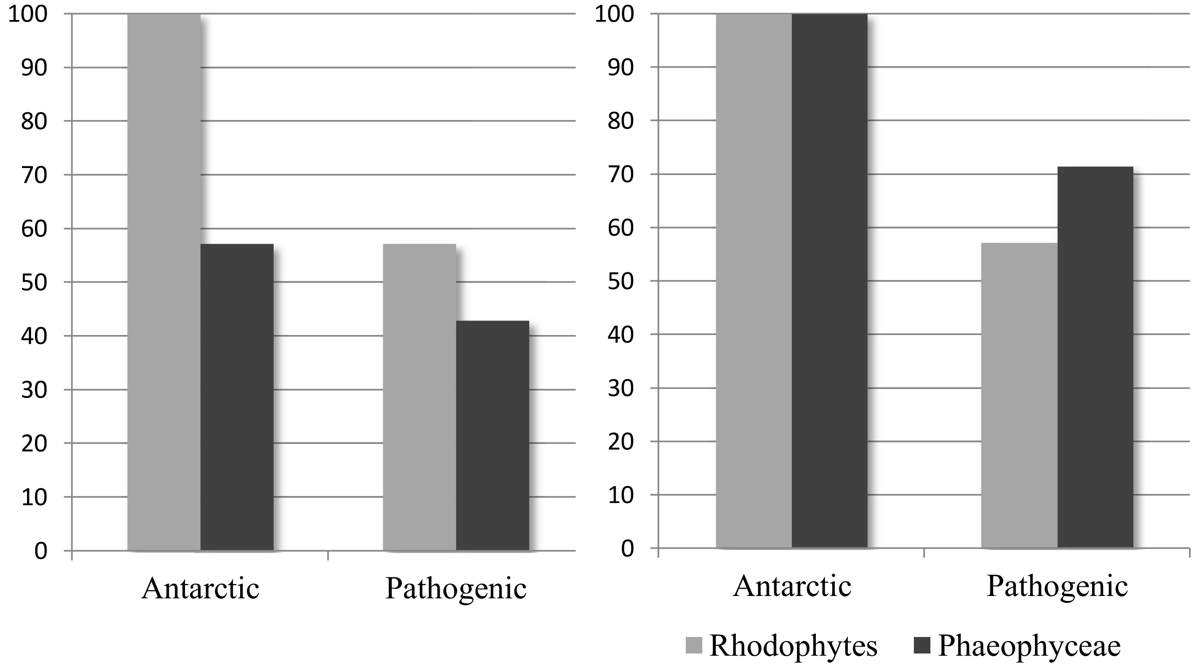
Also, all the algal solid residues were weighted to obtain the Total Dry Weight (DW_T, adding the weights of algal solid residue, dry Et_2O crude extracts, dry weight of BuOH, and dry weight of aqueous residue). This is necessary to calculate the extract natural concentration (**Table 1)**, which will be further used in the microbial experiments in order to simulate the real concentration in nature.

Antimicrobial activity inhibition was performed by using the crude extracts with the agar disk diffusion method on isolated cultures of a variety of microorganisms, as described in (Acar, 1980; Álvarez, 1990; C Angulo-Preckler et al.,

Results

Our results showed differences in the percentage of inhibition depending on type of seaweed, microorganisms and chemical fraction of the extracts. As a summary, for the seaweed type, more Rhodophyta where active compared to the Phaeophyceae tested. Also, Antarctic microorganisms were more inhibited than pathogenic surrogates. Concerning the type of chemical fraction, the lipophilic extractions showed greater percentage of inhibition compared to hydrophilic ones (see **Figure 2**). In that sense, it is also worth noting that the natural concentration (mg/g of dry weight) of the different chemical extractions of our samples showed that lipophilic fractions (ethereal extracts) were in higher concentrations than hydrophilic ones for both, Rhodophyta and Phaeophyceae (see natural concentrations in **Table 1**). This was specially the case for Phaeophyceae, as ethereal extracts showed more than three times more concentrated than butanolic ones.

Figure 2. Percentages of inhibition of Antarctic or pathogenic microorganisms by butanolic (left) and ethereal (right) extractions of Rhodophytes and Phaeophiceae



Of the 22 macroalgae species studied here (14 Rhodophyta and eight Phaeophyceae), a total of 44 extracts (22 hydrophilic and 22 lipophilic) were tested against 14 microbial strains (**Table 3** and **4**). Lipophilic extracts showed stronger inhibition effects (52% of all the lipophilic extracts showed some antimicrobial activity) and higher inhibition values than the hydrophilic ones (30% of hydrophilic extracts were active). Thus, for the lipophilic extracts, nearly 30% of the active ones exhibited strong inhibitions (+++), in front of 13% of the hydrophilic ones. Nonetheless, the taxa with greater activity in the lipophilic extracts also displayed greater levels of inhibition in the hydrophilic fractions (*i.e. Delisea pulchra* and *Desmarestia antarctica*). Variability between the three replicates generally was very low (with overall mean differences between samples <0.5 mm in the halii). Nonetheless, some individual replicates showed higher variation in the tests with *Desmarestia menziesii* and *Phyllophora ahnfeltioides* against *Psychrobacter* sp. and *Delisea pulchra* against *Vibrio cholerae* (greater than 1mm, compared with the other two replicates of the same tests). However, as the rest of the replicates for the other tests and species showed no major variability, for those individual cases, these replicates were not included in the study.

A total of 14 of the 22 macroalgae studied showed antimicrobial activity against, at least, one microbial strain. In proportion, the 71% of Rhodophyta tested (10 out of 14) presented antimicrobial activity, whereas the 50% (4 out of 8) of Phaeophyceae were active, being the red algae the group with higher number of species chemically active in our tests. The species with the largest number of microorganism strains inhibited was the red algae *D. pulchra.* This species inhibited 11 of the 14 microorganism strains tested (around 79%) with both lipophilic and hydrophilic extracts. *D. pulchra* was also the only tested algae that showed inhibition against the fungus *Candida albicans*. Morover, *D. pulchra* presented the greater halii inhibition size, having the highest inhibition value (18.7%) with the

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Invasive marine species discovered on non–native kelp rafts in the warmest Antarctic island

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Antarctic shallow coastal marine communities were long thought to be isolated from their nearest neighbours by hundreds of kilometres of deep ocean and the Antarctic Circumpolar Current. The discovery of non–native kelp washed up on Antarctic beaches led us to question the permeability of these barriers to species dispersal. According to the literature, over 70 million kelp rafts are afloat in the Southern Ocean at any one time. These living, floating islands can play host to a range of passenger species from both their original coastal location and those picked in the open ocean. Driven by winds, currents and storms towards the coast of the continent, these rafts are often cited as theoretical vectors for the introduction of new species into Antarctica and the sub-Antarctic islands. We found non-native kelps, with a wide range of "hitchhiking" passenger organisms, on an Antarctic beach inside the flooded caldera of an active volcanic island. This is the first evidence of non-native species reaching the Antarctic continent alive on kelp rafts. One passenger species, the bryozoan *Membranipora membranacea*, is found to be an invasive and ecologically harmful species in some cold-water regions, and this is its first record from Antarctica. The caldera of Deception Island provides considerably milder conditions than the frigid surrounding waters and it could be an ideal location for newly introduced species to become established. These findings may help to explain many of the biogeographic patterns and connections we currently see in the Southern Ocean. However, with the impacts of climate change in the region we may see an increase in the range and number of organisms capable of surviving both the long journey and becoming successfully established.

Human activity and shipping have long been considered the principal threats to the "biosecurity" of the remote and isolated shallow marine ecosystems of Antarctica[1]. However, recent work has shown that the Southern Ocean's (SO) strong, circumpolar winds, currents and fronts may not be a barrier to natural colonization from the north[2–4]. Floating kelp is a potential vector for distributing species across the vast oceanic distances between the sub-Antarctic islands. It has been estimated that there may be over 70 million kelp rafts afloat at any one time in the Sub-Antarctic, 94% of which are *Durvillaea antarctica*[5]. The remote archipelagos distributed between 45 and 60° S are key locations for dispersal either side of the Polar Front (PF) and across[2–4,6]. The discovery of the non-Antarctic bull kelp, *D. antarctica* on Antarctic beaches, coupled with oceanographic models, demonstrate a non-anthropogenic mechanism for species introduction into Antarctica[4]. Genomic analyses revealed that the kelp specimens originated in the sub-Antarctic (Kerguelen Island and South Georgia) and dispersed thousands of kilometres to reach the Antarctic coast[4]. The only epibionts found on these specimens were goose barnacles (*Lepas australis*), and this epipelagic species is likely to have colonised the kelp during its time drifting in the open ocean[4].

Deception Island (DI) is an active volcano in the South Shetland Islands, located off the West Antarctic Peninsula. The flooded caldera of DI is species poor in comparison with neighbouring islands due to recent

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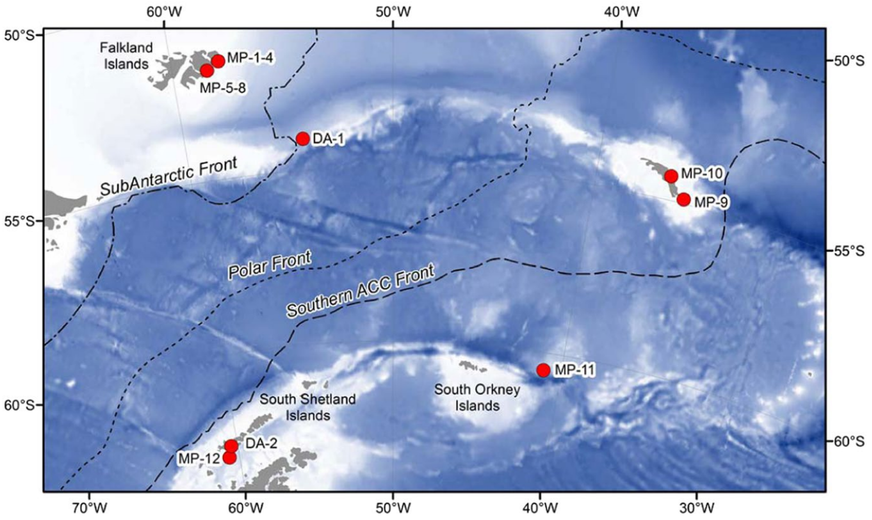

Figure 1. Map of the collecting localities showing the Polar Front (dotted line) and sampling points (in red). DA (*Durvillaea antarctica*), MP (*Macrocystis pyrifera*). MP-1–4: Falkland Islands (North), MP-5-8: Mare Harbour (Falkland Islands), DA-1: South of Falkland Islands (Drake passage), MP-9: South Georgia Islands (South), MP-10: South Georgia Islands (North), MP-11: South Sandwich Islands, and MP-12: Deception Island (South Shetland Islands), DA-2: Livingston Island (South Shetland Islands).

Code	Species	Place	Lat (S)	Lon (W)	Polar Front	Date (mm/yy)
MP-1	*Macrocystis pyrifera*	Falkland Islands	−51,690	−57,865	North	02/16
MP-2	*Macrocystis pyrifera*	Falkland Islands	−51,690	−57,865	North	02/16
MP-3	*Macrocystis pyrifera*	Falkland Islands	−51,690	−57,865	North	02/16
MP-4	*Macrocystis pyrifera*	Falkland Islands	−51,690	−57,865	North	02/16
MP-5	*Macrocystis pyrifera*	Mare Harbour (Falklank Is.)	−51,903	−58,423	North	03/16
MP-6	*Macrocystis pyrifera*	Mare Harbour (Falklank Is.)	−51,903	−58,423	North	03/16
MP-7	*Macrocystis pyrifera*	Mare Harbour (Falklank Is.)	−51,903	−58,423	North	03/16
MP-8	*Macrocystis pyrifera*	Mare Harbour (Falklank Is.)	−51,903	−58,423	North	03/16
DA-1	*Durvillaea antarctica*	South Falkland Islands	−54,110	−54,340	North	02/16
MP-9	*Macrocystis pyrifera*	South Georgia	−54,880	−35,514	South	03/16
MP-10	*Macrocystis pyrifera*	South Georgia	−54,326	−36,382	South	03/16
MP-11	*Macrocystis pyrifera*	South Sandwich Islands	−60,52	−41,04	South	03/16
MP-12	*Macrocystis pyrifera*	Deception Island	−62,9789	−60,657	South	02/17
DA-2	*Durvillaea antarctica*	Livingston Island	−62,661	−60,398	South	02/19

Table 1. Rafting kelp collected in this study. MP: *Macrocystis pyrifera*. DA: *Durvillaea antarctica*.

eruptions (1970) and ongoing volcanic activity[7]. Recent work shows an increasing biodiversity gradient towards the entrance of the bay[7,8]. The geothermal and morphological nature of the caldera provides a relatively calm and warm-water habitat, with bottom water temperatures of about 2–3 °C, protected from ice disturbances (ice scouring, anchor ice, etc), perhaps offering favourable habitat for potential invasive species entering Antarctica.

Macroalgal rafting has been suggested to explain similarities in species composition and low genetic differentiation of intertidal marine communities across the sub-Antarctic[9–11]. This hypothesis implies some degree of successful colonization or mixing of the transported species with native sub-Antarctic species. However, all the possible natural pathways at both sides and across the PF result in a low probability that an individual raft will ever make landfall at a site with suitable characteristics for colonisation, given the vastness of the SO and the small size of most of the islands[12]. If a species succeeds to establish a local population, however, it may face little competition for resources and space, and may thrive[13]. In this context, thus, DI could represent a proxy for what may happen in other parts of Antarctica.

Marine species may reach Antarctic waters by a number of different dispersal mechanisms. Rafting on floating macroalgae is likely to be the biggest vector for natural dispersal into Antarctic waters. In a similar passive way, plastics have also been reported to carry a variety of epibionts in Antarctic waters[14]. Bryozoans are effective colonizers of surfaces and one of the most important components of biofouling assemblages[15,16]. Five bryozoan species were found attached to a plastic debris collected on Adelaide Island (Antarctic Peninsula)[14]. All of these species were endemic to the Antarctic and it was estimated that debris had been in the water for at least 1 yr. Most colonies were reproductively active, having the possibility of releasing larvae during transportation. In fact, the

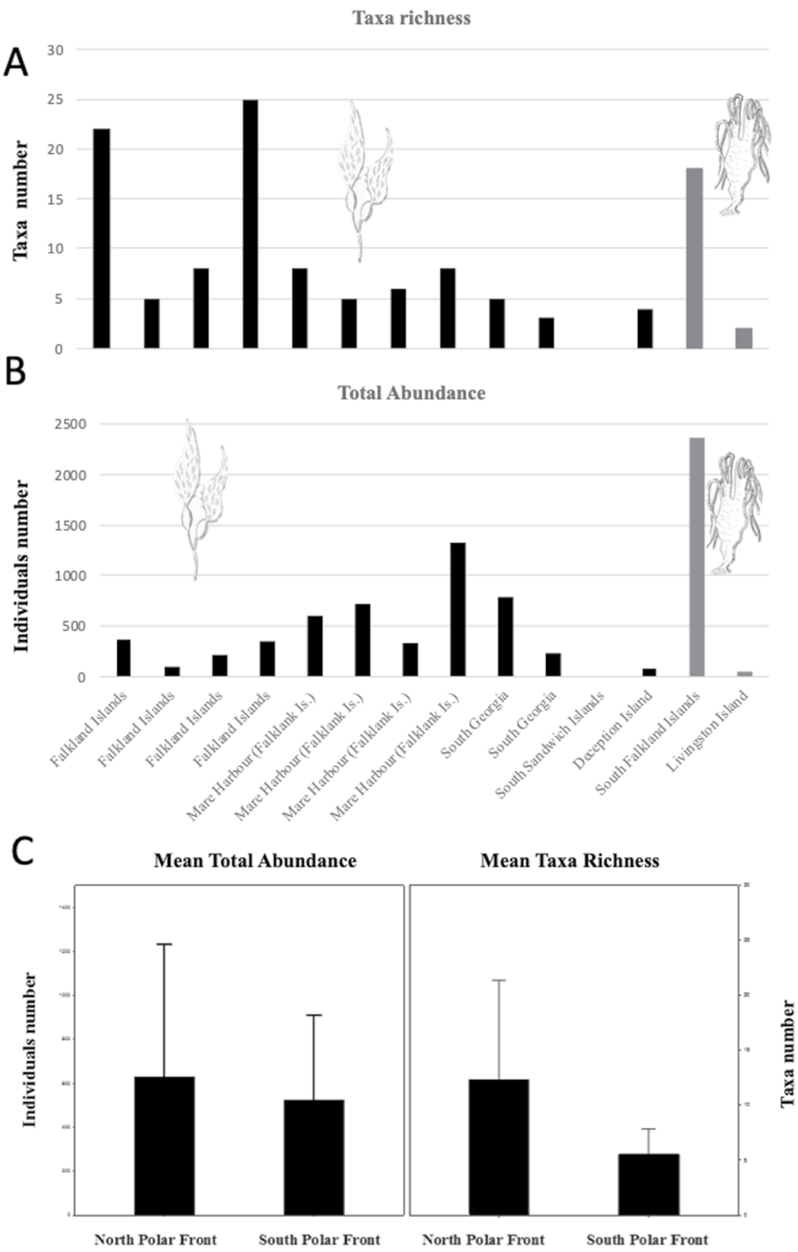

Figure 2. Abundance and taxa richness of epibionts in the rafting algae studied here. Taxa richness (**A**). Total abundance (**B**). Black bars: *Macrocystis pyrifera*, Grey bars: *Durvillaea antarctica*. Means of total abundance and taxa richness (**C**) at North and South of the Polar Front (PF) (± s.d.).

cyphonaute larvae of the bryozoan *M. membranacea* have been found in ballast water[17], and their colonies can raft on kelp, such as *Macrocystis* spp and *Nereocystis* spp, as well as on plastic debris[18]. Fraser *et al.*[19] reported 10 invertebrate species rafting on algae for at least 400 km, during several weeks, between New Zealand and the neighbouring sub-Antarctic islands.

The recent discovery of fresh specimens of the non-native giant kelps (*Macrocystis pyrifera* and *D. antarctica*) with a range of epibiotic animals and algae as passengers, washed up on the shores of Deception and Livingston Islands, provides a unique opportunity to study a potential colonisation event. Here we present the first evidence of non-native shallow water epibiotic organisms reaching Antarctica by long-term rafting. By identifying the species found living on the kelp and examining their distributions we assess the potential impacts of these species becoming established.

Methods

Samples were collected from the sub-Antarctic to Antarctic islands (Fig. 1, Table 1). Twelve rafting floating kelps were collected on both sides of the PF during the Antarctic expedition of the RRS James Clark Ross in 2016. Two more kelps were collected South of the PF. *M. pyrifera* was collected on the beach in DI (South Shetland Islands) during the Distantcom-2 Antarctic cruise in February, 2017. *D. antarctica* fragments were collected on the beach

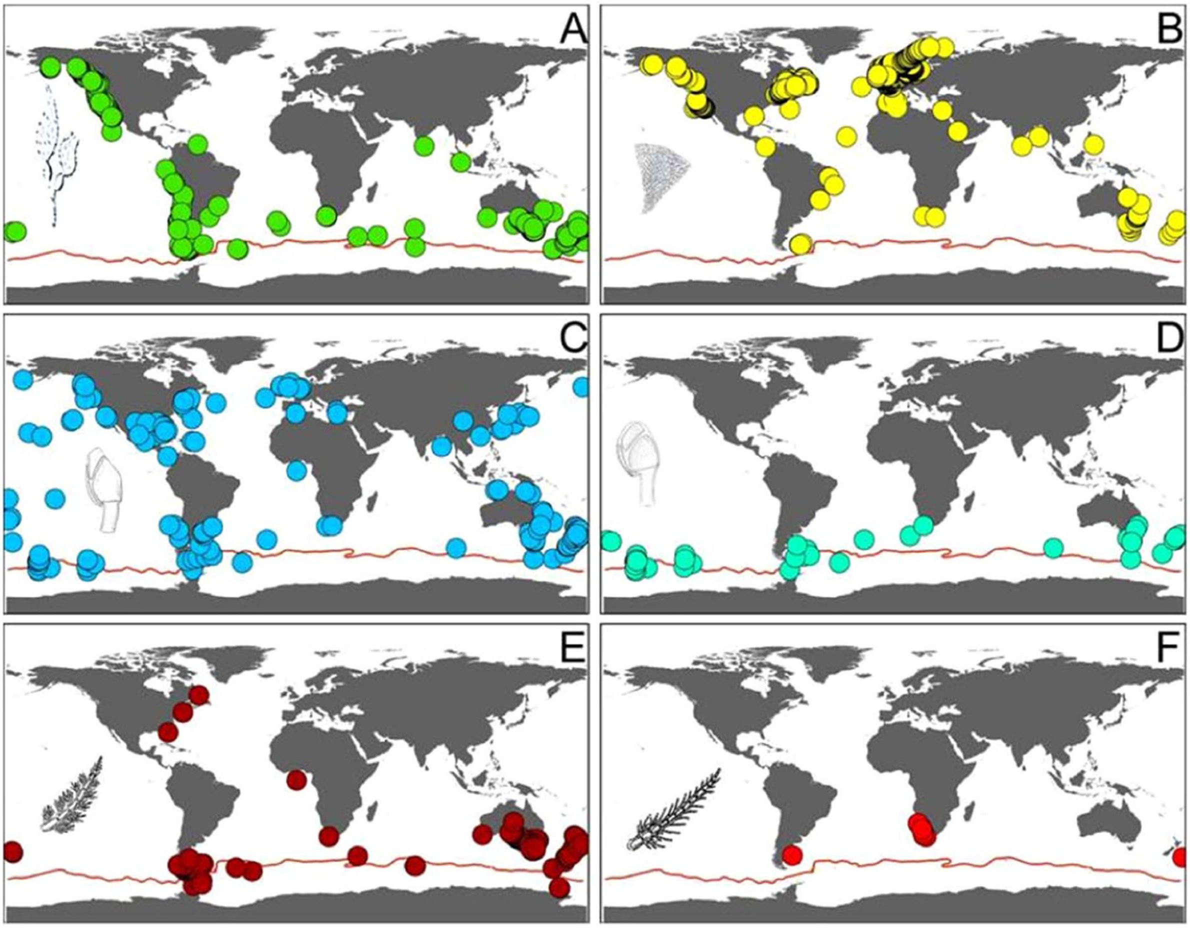

Figure 3. Known distributions of the epibiotic species found associated with *Macrocystis pyrifera* (**A**: Distribution of *M. pyrifera*) on the South Shetland Islands: *Membranipora membranacea* (**B**); *Lepas anatifera* (**C**); *Lepas australis* (**D**); *Ballia callitricha* (**E**) and *Ballia sertularioides* (**F**). Data from GBIF[45].

in front of the Spanish station in Livingston Island in February, 2019 during the Bluebio-2 cruise. Samples were photographed and frozen for further identification of the seaweeds and their epibionts. Samples of rafting kelp ranged from 0.5 to 18.1 kg wet weight. The passenger species traveling upon the kelps reached a total of 7534 specimens (538 ± 637 passengers/kelp, within a range from 0 to 2362 per kelp) and were identified to the lowest possible taxonomic level in the laboratory. The entire rafts were sampled for fauna. Identification of seaweed samples was achieved by studying morphological features, as well as histological examination of the thallus.

Passengers into the cold. Abundance and taxa richness of epibionts found on floating macroalgae in the Southern Ocean vary between the species of kelp *(M. pyrifera* and *D. antarctica*) and the individual rafts (Fig. 2). Other rafts, including those formed by *D. antarctica*, were observed at DI but were not sampled for fauna. Among the four passenger species found alive on *M. pyrifera* in DI, the most significant in terms of potential ecological impact, other than the non-native kelp itself, is the cheilostome bryozoan *Membranipora membranacea*. This is a well-known encrusting species with a proven ability to colonise new environments and cause significant damage to ecosystems by limiting the ability of seaweeds to reproduce and grow[20]. This bryozoan is widely distributed in temperate oceans with distinct populations in the Pacific (North Pacific, Chile, Australia and New Zealand) and Atlantic oceans (North East Atlantic and South Africa) (Fig. 3). *M. membranacea* has become an established invasive species in the North West Atlantic along the coast of North America and has caused extensive losses of kelp canopy through a process of defoliation[21]. Although the species is recorded as far north as northern Scandinavia in the Arctic, it has never been previously reported from south of the PF, but it is likely to already be well adapted to cold water conditions, therefore posing more than a hypothetical risk for Antarctic waters.

The combination of having a long-lived planktonic larva (from 2 weeks to 2 months), sexual (hermaphroditic zooids) and asexual reproduction, fast growth rates, effective food acquisition in a wide range of flow rates, ability to form large colonies and to colonize kelps make *M. membranacea* a successful disperser, colonizer, and invasive species[22–24]. Potentially, these kelp can be transported much farther than bryozoan larvae[25–31]. Furthermore, their heavy encrustations may have a negative impact on marine ecosystems by increasing the brittleness of kelp blades, followed by extensive losses of kelp canopy[21], and by limiting the ability of the seaweeds to reproduce and grow, specifically interfering with spore release from the kelp blade[20]. It has also been shown that other species of the same genus may block nutrient uptake and photosynthesis[32,33].

Phyllum	Taxa	MP-1	MP-2	MP-3	MP-4	MP-5	MP-6	MP-7	MP-8	DA-1	MP-9	MP-10	MP-11	MP-12	DA-2
Rhodophyta	*Ballia callitricha*													1	
Rhodophyta	*Ballia sertularioides*													1	
Porifera	Porifera				2	2									
Cnidaria	Anthozoa					3									
Cnidaria	Hydrozoa	1				13				3		5	7		
Bryozoa	Bryozoa	1	1	1	20	18	5	3		17	27	5	120		
Bryozoa	Cyclostomatidae			2											
Bryozoa	*Membranipora membranacea*													1	
Entoprocta	Entoprocta						100								
Mollusca	Mollusca				1										
Mollusca	Bivalvia			4	2		500			27	71	3	39		
Mollusca	Gastropoda				2					2					
Mollusca	*Kidderia* sp.					2									
Mollusca	*Scurria scurra*					1									
Mollusca	*Gaimardia trapesina*	97					76	30							
Mollusca	Nudibranchia						2								
Mollusca	*Nacella*	2													
Mollusca	*Nacella mytilina*	2													
Mollusca	Fissurellidae				1										
Platyhelminthes	Platyhelminthes				1	1									
Annelida	Nemertea									1			2		
Annelida	Polychaeta									3	601	5	1005		
Annelida	Polynoidae	1			8	1									
Annelida	Cirratulidae				2										
Annelida	Serpulidae	101	100	100	105		100	200							
Annelida	Syllidae	6			2										
Annelida	Terebellidae	7			8										
Annelida	Nereidae	3			4										
Annelida	Capitellidae	5													
Annelida	*Torodrilus* sp.														1
Annelida	Sabellidae	1													
Sipuncula	Sipuncula				3										
Arthropoda	Insecta	1													
Arthropoda	Haplocheira	78													
Arthropoda	Harpacticoida	40													
Arthropoda	Calanoida	1													
Arthropoda	Pedunculata					2230									
Arthropoda	*Joeropsis curvicornis*					1									
Arthropoda	Caprellidae					1									
Arthropoda	Pantopoda					1									
Arthropoda	Ostracoda					25									
Arthropoda	Cucumariidae				15										
Arthropoda	Eusiridae			13	25	1									
Arthropoda	Isopoda									56	10	31	33		
Arthropoda	Munnidae					1									
Arthropoda	Amphipoda		2	50	42	55				493	20	280	115		
Arthropoda	Corophiidae		2	50	65										
Arthropoda	Ischyroceridae				3	5									
Arthropoda	*Ischyromene eatoni*	1													
Arthropoda	*Halicarcinus planatus*	5			19										
Arthropoda	*Plakarthrium punctatissimum*	1													
Arthropoda	*Peltariom spinulosum*	1													
Arthropoda	*Exosphaeroma lanceolatum*	1													
Arthropoda	*Lepas australis*													76	
Arthropoda	*Lepas anatifera*														50
Echinodermata	Echinoidea				3										
Echinodermata	Asteroidea				9										

Continued

Phyllum	Taxa	MP-1	MP-2	MP-3	MP-4	MP-5	MP-6	MP-7	MP-8	DA-1	MP-9	MP-10	MP-11	MP-12	DA-2
Echinodermata	Ophiuroidea				3										
Echinodermata	Apodida				1										
Chordata	Actinopteri					1									
	Seaweed	4	1	1	2							5	5		

Table 2. Organisms found as passengers on the kelp raft in this study (numbers indicate counts). MP: *Macrocystis pyrifera*. DA: *Durvillaea antarctica*.

The other three species found alive on the kelp in DI have all been previously reported south of the PF. *Ballia callitricha* and *B. sertularioides* are shallow water red algae with a general Southern Hemisphere distribution that includes previous records from the Ross Sea, Antarctica (Fig. 3), but not from DI or West Antarctica[8]. Juveniles and adults of the southern goose barnacle *Lepas australis*, were also found. This species, commonly found attached to floating substrata such as macroalgae, volcanic pumice, and plastics in the Southern Ocean (Fig. 3), was the only species recently reported on a specimen of giant bull kelp, *D. antarctica*, found on King George Island, also part to the South Shetland Islands group[4].

Using growth rates cited by Fraser *et al.*[4,19] we estimate an age of approximately 30 days for the barnacles, *L. australis*, found at DI, suggesting that colonization happened in the open sea. Alternative, faster transportation mechanisms may also exist (e.g. shipping vectors and heavy storms). In fact, Lewis *et al.*[34] suggested hull-fouling is likely to be the most important vector for transporting species to Antarctica as ships create novel pathways, moving across currents and often visiting many locations over short periods of time. The increasing ship activity appears to be a very important factor increasing the probability of non–native marine species establishing within the Antarctic region in the coming decades (over 180 ships were active around Antarctica and the sub-Antarctic islands in 2017–2018, on potentially more than 500 voyages)[35]. The presence of small–sized specimens of *L. anatifera* in the kelp found at Livingston Island could also indicate a short-term rafting for this species. Abundant, alive *L. anatifera* specimens found on *D. antarctica* fragments in South bay, Livingston Island, represent, in fact, the first Antarctic report for the species, which was described in tropical and subtropical waters of South America[36]. The potential effects of barnacle colonization in Antarctica are unknown, but in fact, being pelagic rafting species, they seem unlikely to pose any real threat to the shallow water ecosystems, especially as *L. australis* is already commonly found on rafts and litter in the Southern Ocean. However, their heavy growth could sink the kelp, thus facilitating access to the seafloor for other benthic passengers.

The other floating and beached kelp samples (*M. pyrifera* and *D. antarctica*) collected from either side of the PF were found to be carrying organisms within 12 different phyla as passengers (Tables 1 and 2). Each kelp raft examined represented a different, although sometimes overlapping, subset of organisms usually found inhabiting shallow marine habitats. Only one of the floating specimens, MP-11, an example of the non-Antarctic *M. pyrifera* found near the South Orkney Islands, had no passengers at all. The most commonly found taxa included amphipod crustaceans, polychaete worms, molluscs, and bryozoans (Table 2). The DI floating kelp was the only specimen collected south of the PF carrying *M. membranacea*, although this bryozoan was frequently found at the Falkland Islands, a potential source of kelp rafts in that region[3]. Although more studies are needed to know if *M. membranacea* has become established in the SO, the potential for this species to impact Antarctic ecosystems could be high, not only in DI, as macroalgal substrates are widespread and colder temperatures are not preventing its spread. For example, a recent study based on a baseline data on presence/absence and abundance of this bryozoan near its current northern range limit suggests that the available algal substrate may be more important than temperature in limiting the spread and abundance of *M. membranacea*[37]. On the other hand, MP-5, collected from the open ocean north of the PF, was heavily encrusted with thousands of adult and juvenile goose barnacles. This specimen was also host to a rich and varied community of other organisms that are likely to have been associated with the raft before it became dislodged (Table 2).

Other significant findings included the brachyuran crabs *Halicarcinus planatus* and *Peltariom spinulosum* in the *M. pyrifera* fragments washed up on the shore at the Falkland Islands. *H. planatus* was first recorded in Antarctica at the shores of the South Orkney Islands in 1903[38]. It was reported again by Aronson *et al.*[39] at the external side of the caldera of DI, supporting the hypothesis that DI could be the entrance gate for non-native species. *H. planatus* is a widely-distributed species in temperate waters, found from New Zealand to the Falklands and southern South America, as far north as Peru and Argentina[40,41]. *H. planatus* has also been found alive on floating kelp[42]. Although *H. planatus* was not found in our previous studies at Deception and Livingston Islands[8], we did find it on *M. pyrifera* washed up on the shore of the Falkland Islands (pers data 2016, SO-AntEco expedition), which could easily be re-floated by high tides or rough weather. The impact of these crabs on local species is not known but could potentially be devastating due to the absence of durophagous fauna in Antarctic shallow benthic ecosystems[43,44].

Rafting to the south. The transport of organisms on ships' hulls or in ballast water can take less than 4% of the time it would take to reach the same destination by rafting[11]. Although this significant reduction in time taken to reach Antarctica might allow a wider range of species to reach the continent alive, they would still need to be capable of surviving the conditions at their destination in order to become established. As such, our observation of a species with a documented track record of invasive and negative ecological impacts, such as *Membranipora membranacea*, in an active volcano (DI), with warmer, more favourable conditions, is very significant. The species

Figure 4. Rafting kelp and passengers. *Macrocystis pyrifera* (**A**) with passengers found at Deception Island, and *Durvillaea antarctica* (**B**) with cirripeda from Livingston Island, South Shetland Islands, Antarctica. (**C**–**F**) Passengers found on *M. pyrifera* at DI: the red alga *Ballia callitricha* (**C**); the bryozoan *Membranipora membranacea* (**D**); the cirripeda *Lepas australis* (**E**); and the cirripeda *L. anatifera* (**F**) on *D. antarctica* from Livingston Island.

reported here are common and well-distributed organisms and thus have the potential to persist or even thrive in the milder conditions of the caldera of DI (Fig. 4). It could only be a matter of time before some of these species acclimatize to the Antarctic environment and spread. These findings are even more relevant in the current context of global change, which could facilitate the survival of these species in other Antarctic environments once settled in favourable areas, such as DI, further reaching other places around the Antarctic peninsula. Therefore, these species may be useful indicators of climate change in Antarctic habitats and should be carefully monitored during the next years.

Conclusions

Non-native, non-Antarctic kelp is reaching Antarctica now and again, particularly at Deception and Livingston Islands. DI is a key location for first colonisation of Antarctica due to its strategic location and the higher temperature of seawater compared to adjacent areas. The presence of passengers on the kelp, especially *Membranipora membranacea* and *Lepas anatifera* (as well as *Halicarcinus planatus* in the water outside the DI caldera) demonstrate that natural colonisation, or invasion, can happen at any time. Actually, *M. membranacea* has already become an invasive species in many places outside of Antarctica, and it is believed to have a potentially negative impact on marine ecosystems. Effects of passengers in Antarctic ecosystems are largely unknown, and therefore, we believe that monitoring these potentially invasive species in the frame of global change is crucial in the coming years.

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Author contributions

C.A., C.A.P., R.P.M.M., B.F., H.J.G. and C.L.W. collected and identified samples in Antarctica and in the lab. C.A., C.A.P., R.P.M.M. and H.J.G. performed data analysis and interpretation, and all authors contributed to the final version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Cover: Palmaria decipiens, Iridaea cordata and *Gigartina skottsbergii* carpet on Deception Island shores by Rafael Pablo Martín Martín