Biodiversity and Chemical ecology in Antarctic bryozoans

Biodiversitat i ecologia química de briozous antàrtics

Blanca Figuerola Balañá

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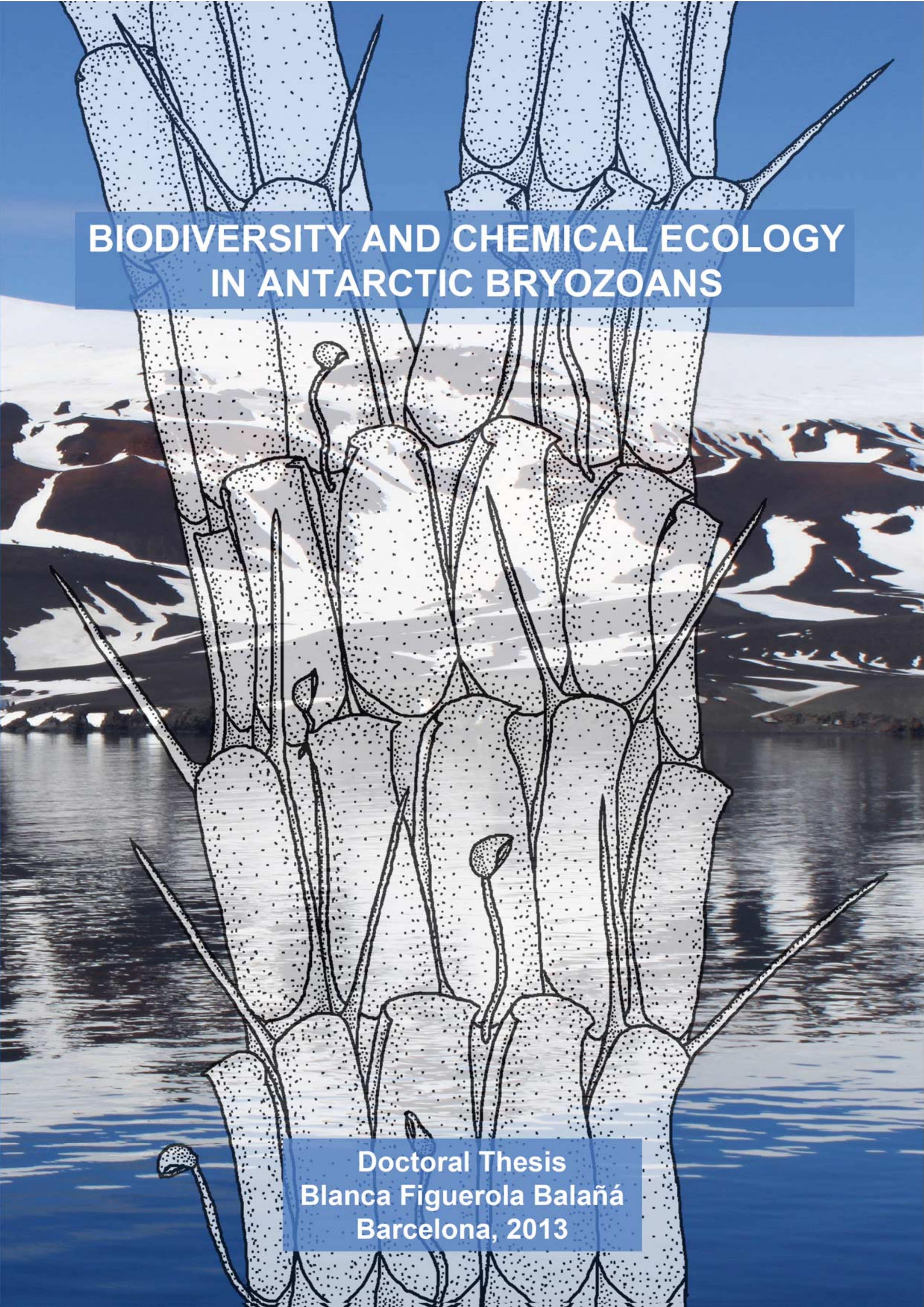

Doctoral Thesis
Blanca Figuerola Balañá
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Programa de doctorat en Biodiversitat Animal
Facultat de Biologia- Departament de Biologia Animal

BIODIVERSITY AND CHEMICAL ECOLOGY IN ANTARCTIC BRYOZOANS

Biodiversitat i ecologia química de briozous antàrtics

Memòria presentada per
Blanca Figuerola Balañá
per optar al grau de
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" If you want to understand the invisible,
look carefully at the visible".
(Talmud)

Photos & illustrations: Blanca Figuerola

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Però també existeixen altres persones que m'han marcat: Oriol (gran company i amic de viatge amb qui hem passat molt bones estones, com la "Full Moon Party" :D), Esther (gran amiga i compi de la carrera! ens ho vam passar bé a Burkina, eh?), Martí (Martini! si n'hem passat de coses! no oblidaré quan et vam venir a "veure" a Xile!), Jordi (sempre optimista i anant amunt i avall... no oblidarem les Penitents! i el millor, sempre portant una dosi d'humor! com era? tenim una boca per...o unes orelles per...?), Míriam (amb caràcter i empenta i una gran artista!), Tina (amb les idees clares amb qui he compartit també bones estones!) i als altres amics "pardals" amb qui he passat grans estones: Jaume, Paloma, Mia, Ernest, Ana, Robin... A tots ells, preguntar-los: "Què és això que se sent... ?". També agraeixo a altra gent que he anat coneixent durant aquests anys que han fet que tingui un bon record d'aquesta època.

Ja a segon de carrera vaig començar a veure què era treballar de biòloga fent pràctiques al Museu de Zoologia de Barcelona amb el Pepe Carrillo (un profesioná! como flipé la primera vez que te subiste en una palmera en un plis plas a buscar pollos de cotorra!). I com em va agradar l'experiència vaig decidir tornar a fer pràctiques l'any següent però aquest cop amb el Grup d'Estudis i Protecció dels Ecosistemes del Camp (GEPEC) on vaig continuar amb ocells i vaig començar a fer els meus primers dibuixos per temes de naturalesa. Gràcies Ramon Ferré, per la teva motivació i per totes les activitats que m'has engrescat a fer! I més que intentaries... ho sé! jaja També he d'agrair als voluntaris, uns cracks i bons naturalistes: David, Eloi, Albert, Adeline, Dídac, Anna... No oblidaré les estones passades com a informadors ambientals!

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No em vull oblidar d'agrair a TOTS els professors de Biologia Animal que he tingut durant la carrera i/o el màster per tot el que he après, sobretot com a persona. Hem passat moltes bones estones i amb molts he fet amistat! També he d'agrair a les "secres" del Departament, les quals m'han ajudat molt en moltes ocasions! No puc oblidar de mencionar a professors d'altres Departaments amb qui també he compartit bons moments, especialment al Xavier Font, Ramon Massalles i Toni Monleón-Getino (qui també m'ha ajudat a solucionar problemes estadístics!).

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Quan acabes la carrera téns la típica "crisi" que no saps per on tirar i tal... D'aquí que parlant amb l'Alberto va sorgir la possibilitat de treballar com a "peixetera", mentre feia el màster de Biodiversitat. Mai oblidarem les campanyes, eh? Moltes gràcies per la teva alegria, generositat i suggeriments científics! Hem passat grans estones! Espero que puguis seguir amb la teva gran passió! També agraeixo a l'Adolf de Sostoa per deixar-me formar part del seu grup de recerca i poder aprendre de peixos de riu!

Fent el màster i el doctorat he conegut també grans persones apassionades pels vertebrats i invertebrats: Laura Cardador (com trobo a faltar les trobades dels "dijous de la White"! haurem de fer un remember algun dia, oi? merci per les bones estones passades!), Francesc (algun dia tornarem a aquell terrat amb bones vistes... :P), las "viejas glorias" del Departament amb qui vaig passar bons moments (Joan, Fran, Raul, Guille, Eva, Rocio, Maria...), Marc López (aquest any és el bo, oi? jeje un encant de persona!), Marc Franch (l'informàtic de vertebrats! merci per les teves ajudes de mapes!), Alex Richter (gràcies per ajudar-me a entendre una mica d'estadística durant el màster), Olatz (mi vasca y compi de "coffees"! y que no falte el humor), Javi (cariñoso y con un humor propio! ;) has llegado muuuyy lejos... hasta las Seychelles! Cuando me invitas???), Manolo (Manolín!! para mí siempre serás el "neeennng" de la facu!), Isa (organitzada i gran dissenyadora! i ara és una gran mare!! merci pels bons consells!), Albert (aquests del barri marítim de Reus...jo crec que et pots considerar mica "juantxi" ja! com trobem a faltar les cerveses al bar!), Matxalen (t'has convertit una experta en ocells! qui ho hauria de dir... ;)), Eloy (cuántos momentos para recordar! puede que algún día encontremos "la foto" por allí :P), Luigi (el italianooo más majo y alegre de todos!), Gemma (com a trànsfuga em superes, havent canviat de dept.! :D), Owen (gran compi de inmersiones en la época que vivía en Barna y un gran amigo!), Mari Carmen (ja fa temps que ens coneixem i sempre recordaré Menorca, ai si en van passar de coses! molta sort per Austràlia!), Mario (lástima que no coincidimos como "peixeterus" pero nos lo pasamos igualmente bien!), Vero i Tere (com va molar tenir-vos de compis uns dies per NZ!), Josep Lluís (et trobem a faltar al dept!), Vera (la próxima doctora serás tu!), Tony (ahora fijo que ya eres experto en R! haha), Moni (gran fotògrafa i molt bona gent!), Guillem, Ximena, Encarna, Mar, Laura Balcells, Irene, Claudia, Urtzi, Inés, Fabi, Alex, José Manuel, Jaime, Francesc, Sandra, Edgar, Eneritz, Raquel, Rubén, Ulla, Debs, Lucía, Susanna, Rocio, Marc Rius, Eli, Oriol, Martí, Víctor, Marcel...i més gent que podria posar!!! Moltes gràcies pels moments compartits!! No puc oblidar tampoc d'esmentar als amics del bar de la "Facu", al José Luís i al Miguel (no hem rigut ni res, eh! sou uns cracks!).

Quan vaig passar d' "Invertebrats a Vertebrats" em vaig ben guanyar el nom de "trànsfuga" que em van adjudicar els becaris. Però per no perdre el costum... un any més tard, quan no sabia què fer amb la meva vida..., vaig tornar a "Invertebrats" al veure un cartell amb un títol molt interessant que hi posava "Vols fer el doctorat en ecologia química d'invertebrats marins antàrtics?". Invertebrats marins?? Antàrtida?? No m'ho vaig pensar gens en anar a picar a la porta del despatx de la Conxita Àvila i sort que hi vaig anar! Moltes gràcies "jefa" per confiar en mi des d'un inici, poder conèixer el món dels briozous antàrtics, ajudar-me a iniciar en el món científic, pels teus consells, per donar-me l'oportunitat de participar en diverses campanyes antàrtiques (una passada!!) i per poder conèixer la "nostra Illa", com dius tu, i sobretot la "família antàrtica",... i per molt més! Aquesta etapa ja s'acaba... però no oblidaré mai tot el que he après i les experiències que he viscut... gràcies a tu!!

Sempre recordaré amb nostàlgia les campanyes antàrtiques d'ACTIQUIM. Qui sap... potser algun dia tornaré. Però com ja saben els que han tingut la sort d'anar-hi varis cops, cada vegada és diferent! Per mi l'Antàrtida està lligada als genials "Conxitos", els quals m'han ajudat, amb qui he après moltes coses durant tot el doctorat i, sobretot, m'han demostrat ser uns grans amics! Començant pel primer projecte, vull agrair al Sergi (Jordisss! el primer "Conxito" que vaig conèixer, responsable, un super tècnic i gran company de campanya! encara no entenc com pots estudiar aquest grup, com li deies? poli... què? que no falti mai el teu bon humor!), Laura (LAU! siempre alegre, creando eventos y con ideas nuevas! tu humor es único! me encanta...también gran compi de viajes: qué bien nos lo pasamos en Koh Tao... ya volvería!) i Jenny (Jennnyyy, es gratis!! una gran companya antàrtica i de lab amb qui hem passat de tot i que ho té tot controlat! ara ja és mig tarragonina però encara li falta trepitjar Reus! i ja saps... "Por mañana!", no???).

En el següent projecte van arribar més "Conxitos", no menys estimats: Juan (ay Juanete! siempre cariñoso y con una sonrisa! un gusto compartir contigo mi última campaña de doctorado!!! eres como mi hermanito menor que nunca tuve!), Carlos (Carlitos! buen compañero y muy optimista! Lástima que no coincidimos mucho de vecinos de mesa!! Pero que no falten los reencuentros escalando. Por cierto, cuál será tu próximo disfraz??) i les noves incorporacions del grup, Maria i Ana (no hem coincidit gaire però, pels moments compartits, sé que també sou genials! a veure si un dia ens retrobem a l'Illa...!).

Però quan penso amb les campanyes, no em puc oblidar tampoc del Javier Cristobo... nuestro gran profesor del "buceo polá" y gran compañero y amigo antártico, siempre ayudando y querido por todos. Nunca olvidaré la primera vez que me estrené contigo como buceadora polar... me diste mucha seguridad y disfruté un montón (y ya sabes, la próxima vez "trae" más mantas!).

A més a més, també formen part d'aquesta gran "família antàrtica" els científics que, sempre que poden, no es perden cap campanya antàrtica: Amós de Gil, Andrés Barbosa, Luismi Peci, Mariano Rodríguez, Manuel Berrocoso, Rafa Benítez, Javier Benayas... I tampoc hi poden faltar els militars de la base de diferents campanyes: Bea, José el cocinero, Juanjo, Manolo, Susana, Carlos, Alex, Andrés, Almudena, Pesqui, Eduardo, Raul, Chema,...), de Las Palmas (Santi, Conde, Jefe de máquinas, Eduardo,...) i de l'Hespérides (Tony,...) que he anat coneixent i que ens han ajudat sempre que han pogut i als tècnics de la UTM: Miki, Hilo, Chris... Un placer compartir con vosotros estos momentos antárticos únicos y por ser grandes amigos/as!

A part de les estades antàrtiques, vaig fer-ne una a Nova Zelanda de la qual tinc molt bons records, sobretot per les persones que vaig conèixer. Vull donar gràcies al Dennis Gordon, sempre atent i amable, qui em va ensenyar molt de taxonomia de briozous. També vull agrair especialment al meu compi de pis, el David (viejo! qué buenos ratos compartidos!), Julio (pescadito, qué fresquita estaba la tarta en mi cara hehe), Juan Carlos (qué crack de las cervezas artesanales), Davide (el italiano y sus abejitas), Meghan (nice to meet you!), Stephen (I enjoyed a lot diving with you), Josue, Anayibi, Mayra, Orlando i Polyana, Tere i Trevor...

Anant cap al món dels briozous, vull agrair especialment al Mikel Zabala qui em va ajudar en els meus inicis en la taxonomia. Encara recordo quan em vas dir que et feia il·lusió que per fi hi hagués algú més interessat en briozous! Moltes gràcies per la teva motivació i espero que tornis a entrar en aquest món fascinant...A part de l'estada, he participat en varis congressos i vull esmentar especialment els que ha organitzat l' "International Bryozoology Association (IBA)". Gràcies al Carlos López-Fe em vaig animar a anar al Congrés Internacional del 2010 on vaig conèixer la "bryozoan family": Aaron O'Dea, Dennis Gordon, Kevin Tilbrook, Piotr Kuklinski, Paul Taylor, Consuelo Sendino, Joanne Porter, Mike Winson, Emanuela Di Martino, Kamil Zágorsek, Carlos López-Fe, Javier Souto, Leandro Vieira, Björn Berning, Thomas Schwaha, Martin Moosbrugger, Andrew Ostrovsky, Oscar Reverter, Julia Cáceres, Phil Bock, Karin Hoch, Abigail Smith, Catherine Reid, Timothy Wood, Hans De Blauwe, Jeremy Jackson, Joachim Scholz, Steve Hageman, Jennifer Loxton, Priska Schäfer, Judith Winston, Teresa Madurell, John Ryland, Judith Fuchs, Masato Hirose, Antoinette Kelso, Urszula Hara, Andrea Waeschenbach, Lais Canabarro, Chiara Lombardi, Rolf Schmidt, Gosia Nowak, Mary Spencer, Antonietta Rosso,... It is an exciting experience to spend time with you! Thank you so much for everything!

Fora de la Facultat, també he d'agrair a molta altra gent durant els anys de Barcelona que han fet que aquests fossin especials. Entre ells, el Pablo, amic de sempre i company de pis durant els anys de carrera i part del doctorat (Pablitoo, ets genial! la convivència ha estat molt fàcil amb tu!), l'Esteve (gran amic! algun dia entendré el teu humor! jaja), la Cei (cariñosa y con una gran voz!), la Pepita (la mama de Barna!), el Paco i l'Eduard (entrar en el "vostre món" és fascinant...hi ha algú que em deu un marsupial...).

I com no, no puc oblidar-me d'agrair als amics de Reus per tots els moments compartits: Àngels (una gran amiga que sempre hi és quan cal!), Joan Albert i Marieta (quina ilu ser la teva padrina de boda!!), Pau i Jurdins (gran artista! la pròxima que es casa...!), Mireia i Bernie (amics de sempre! qui ho diria que aniries a parar a Reus!), Balbina (la gran amiga "Boondd", la reina de la festa amb qui he compartit grans estones), Albert (sempre amb humor!) i Marta (l'amiga Grauu! trobarem a faltar els "cotis" al tren, eh??), Almu (últimament compartint grans estones!), Pep i Mariona (l'artista reflexionador, la gran marassa i la simpàtica Aloma!), Antonio (sempre amb humor i un gran apassionat pels catxés!) i Montse (alegre i sempre apunt per fer coses!), Roger (sempre superant-se) i Alba (dolça i riallera), Toni (el millor venedor i sempre alegre!) i Empar (activa i gran mare!), Marta ("Gnomu", companyes de blubs blubs del camp de Tarragona!), Núria (gran cuinera i sempre apunt per sortir!), Lluís i Esther (el crack de les noves tecnologies, la gran activa i marassa i les seves precioses filles Lluna i Etna), Xavi i Lídia ("Senyor i Senyora Ri", uns motivats de l'escalada!), Nurieta (Subiii, quins vestits que fas!), Carlos ("der Kletterer", motivat number one!), Marta Llorens (una gran artista!), Mònica i Xavi (una genial parella!), Diana, Sita, Maria, Marina, David, Marta i Marcel, Manel i Mireia, Sandra i Humbert, Joan i Gemma, Miquel, Txema, Isa i Biel, Aurora, Marc Micheo, Mireia Fernández, Akane, Iván i Ivet, Isidre, Natàlia, Esther Llansà, i més gent, però no acabaria!

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Entre immersió i immersió, també he compartit moltes experiències durant aquests anys amb molt bona gent del Club d'Immersió de Biologia: Juanan (sempre recordaré la primera immersió al CIB! i el que vas riure, eh?), Ferran (haurem de fer més assajos amb les boies!), Toni Tofa (quan em tornes a portar a veure nudis?), Keko (com va molar fer la secció "Sota a l'aigua amb..."), Pepi (que no faltin els "Fondària"!) i Andreu, Adrià, Vane, Joan, Sandra Pino, Jordi Regàs, Raul i Sandra, Mario, Sandra Manso, Laia, Núria, Enric, Josep, Toni i Roser, Ariadna, Jordi Marturià...

Seguint amb el tema marí, estic molt contenta de formar part d'un grup molt actiu, el "Món Submarí", i haver conegut gent molt maca com el Joan Garcia i la Lucía (un encant de parella, sempre donant-t'ho tot! Quina sort que téns Garbí d'aquests papis!), Tote (sempre alegre i organitzant "eventus"), Pep (el meu profe de navegació, un crack!), Ferran (el creador de Món Submarí!), Josep Maria (gran professional del busseig! quines filmacions submarines que fas!), Gerard (antic amic de música i ara també de busseig!), Eve (les biòlogues que no faltin!), Pili (la trobadora de "nudis" de l'Ametlla!), Jorge, Joan Constantí, Sandra, Héctor, Henry, Irene, Eli, Aurora... Que continuem fent moltes més activitats!!

Continuant parlant de les amistats, no puc oblidar-me dels "cherries": Mireia i Gauthier, Agus, Ventura i Judith, Xavi, Aleix, Neus i Guillem, Paul, Montse, Maria i Jordi, Jordi i Núria, Álvaro i Míriam, Irene i Dani. Ja sabeu que sou com els meus germans, gràcies per tot!

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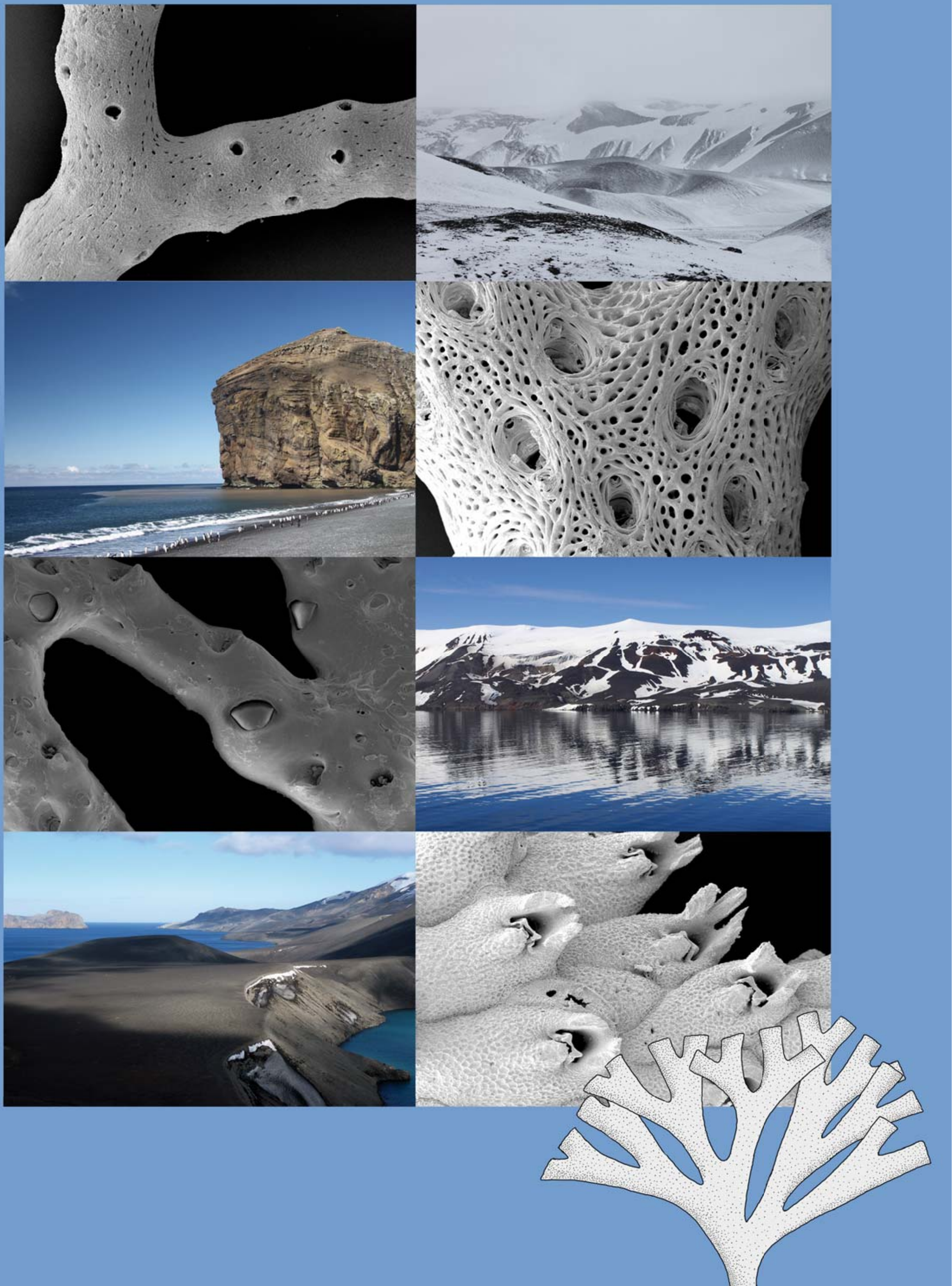
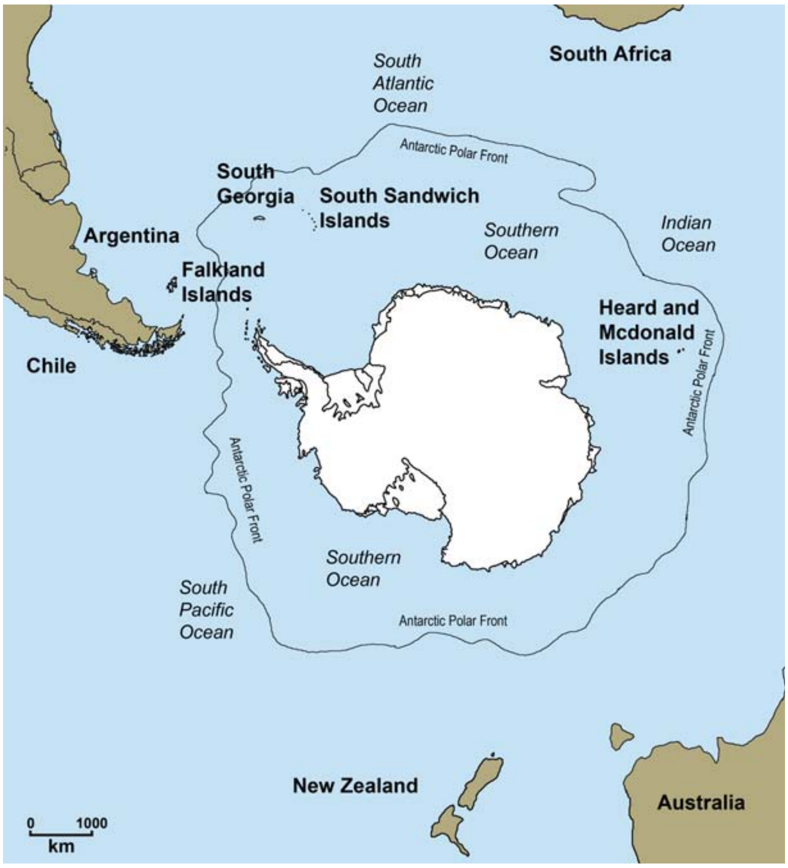


General Introduction

Antarctica: a remote and hostile continent

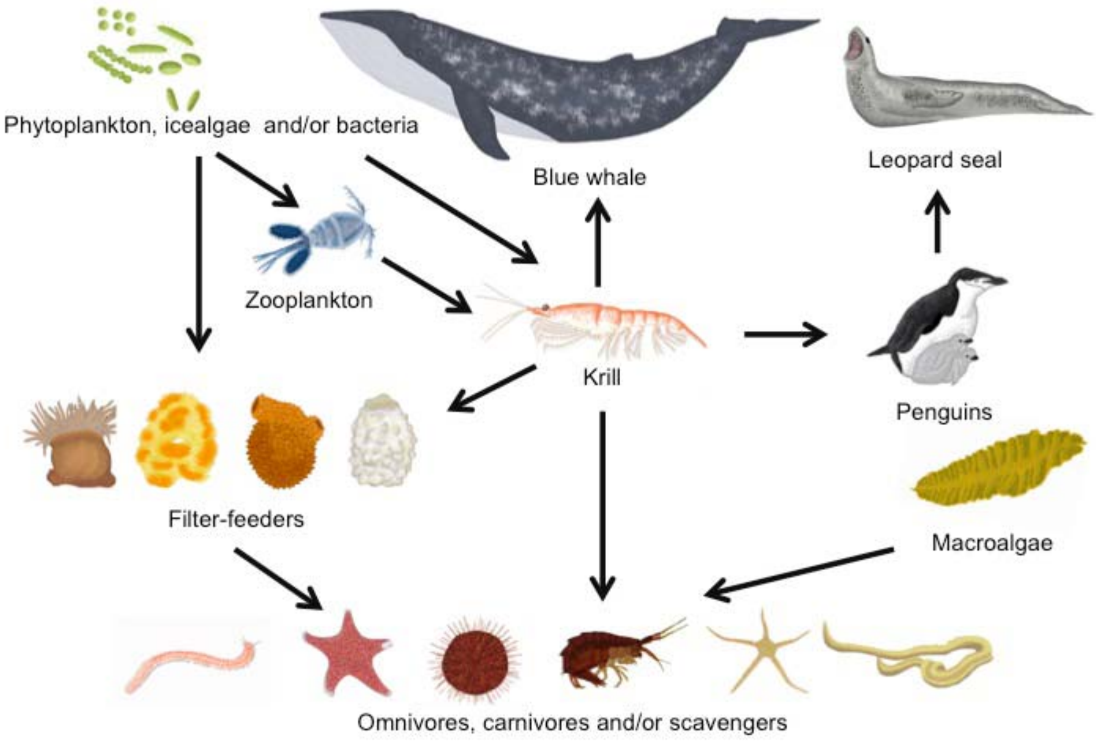
The **Southern Ocean** (SO), covering 35 million km^2 and comprising about 10% of the Earth's oceans, cools and ventilates a large proportion of the global ocean. This is clearly bounded by the Antarctic Continent to the south, and the **Polar Front** (PT, called historically the Antarctic Convergence), one of several fronts within the **Antarctic Circumpolar Current** (ACC), to the north (Fig.1) (Clarke & Johnston 2003; Bargagli 2008). The establishment of the clockwise ACC and the subsequent gradual cooling about 25 Ma ago (Lawver & Gahagan 2003; Upchurch 2008), caused a long biogeographic isolation of Antarctica, which led to a high level of endemism (shelf levels between 50 and 80 %; Arntz *et al.* 1997; Lawver & Gahagan 2003; Scher & Martin 2006; Griffiths *et al.* 2009). All these historical events drove Antarctica to be the coldest, windiest, driest and highest continent, almost entirely covered with ice sheets several kilometers thick (Barnes 2005).

Fig. 1. General map of Antarctica.



Apart of these particular features, surprisingly, the primary production of the SO is nearly 15% of the world total, and thus, plays an important role in sequestering atmospheric CO_2, making the SO one of the major sinks for atmospheric CO_2 (Huntley *et al.* 1991). In particular, polar marine food webs have been described as simpler respect to others elsewhere, with two basic types of primary producers, the pelagic **phytoplankton** and the **ice algae**, which live within the ice (Fig. 2). Especially, in winter, the area covered by sea ice doubles the Antarctic continental surface area (from $3\text{-}4 \times 10^6$ km^2 to $18\text{-}20 \times 10^6$ km^2). Thus, during ice production in the fall, the sea ice decreases the available light for phytoplankton production but it is colonized by sea ice algae, adapted to a low light intensity. In this sense, these sea ice algae are often the unique source of food for a wide variety of protozoan and metazoans, including the **Antarctic krill** (*Euphausia superba*), in seasons and regions where other sources are lacking (Arrigo & Thomas 2004). Conversely, at the retreating pack-ice edge during the short summer time, phytoplankton blooms (dominated by some algal species, typically large diatoms and in some places by unicellular algae of genus *Phaeocystis*) take place in the photic zone and in consequence, high densities of **zooplankton** exist. These maintain abundant communities of diverse pelagic invertebrates, such as salps, copepods, gammarids, and krill. In particular, the trophic web of the Antarctic Ecosystem is centered around this krill species, which are keystone prey items, transferring phytoplankton to a wide diversity of organisms such as fishes, marine mammals (baleen whales, seals…), seabirds (penguins…) and other organisms (Cornejo-Donoso & Antezana 2008; Barnes & Clarke 2011). Bacteria, key components of the **microbial loop** (bacteria, protozoa and viruses), are also a major pathway for carbon flow, using (i) particulate organic material formed through mortality of phyto- and zooplankton and nekton and through the production of faecal pellets, as well as (ii) the dissolved organic matter released by the physiological processes of animal excretion. Among the nanoplankton, zooflagellates are major predators of marine bacteria, being also important components of marine food webs, which transfer bacterial production to higher trophic levels such as copepods and amphipods. In fact, the microbial food web is key for bacterivores, when primary production is absent in winter (Arrigo & Thomas 2004). However, the high nutrient concentrations in the water column demonstrate that only a small portion of nutrients is efficiently consumed, and thus, most part of the primary production is exported via detritus to the bottom. In particular, this organic matter suspended in the water column from phytoplankton, ice algae and fecal material of zooplankton, represents the main energy source for **benthic suspension-feeders**. This allows the establishment of high diversity and biomass of sessile macroinvertebrate communities below areas affected by local disturbances from iceberg scours and anchor ice. These benthic invertebrates have evolved adaptations to these conditions, such as feeding modes to survive in long periods of no food supply. In addition, re-suspension processes of the organic matter deposited on the seafloor enable the availability of food for a long time due to its low degradation rate, explaining the abundance of suspension-feeders, overcoat during winter (Orejas *et al*. 2000; Kaiser & Barnes 2008).

Fig. 2. A diagram of the Antarctic food web.



In the case of the **Antarctic continental shelf**, this benthic fauna is subject to natural impacts related to the seasonal ice formation. In particular, this continental shelf is large, often about 400-500 m depth, but exceeds 1000 m in some places. Hence, it is three-fold higher than that of other continents, resulting of the scour from ice shelves and depression due to the huge ice loading on the continent (Clarke & Johnston 2003). For this reason, the ice scour is an important disturbance in structuring Antarctic benthic communities, at least down to depths of about 500 m, leading to a highly variable faunal distribution in space (e.g. Smale 2008a). In this sense, sites exposed to this intense ice scour are mainly dominated by mobile taxa, such as echinoderms and the isopod *Glyptonotus antarcticus* (Gutt 2007). Additionally, many of the benthic taxa exhibit extended bathymetric ranges as response to glacial-interglacial cycles of shelf ice advance and retreat (Brey *et al.* 1996). During periods of extension of the ice sheet, the intensity of physical disturbance from ice removes invertebrates from shelf, forcing species to migrate out of Antarctica or into deep water, and thus, acting as a refuge for taxa or, in some cases, causing their extinction. During periods of the shelf ice retreats, continental shelf might be re-colonized by slope fauna (Thatje *et al.* 2005b; Brandt *et al.* 2007a, b; Clarke 2008). Moreover, the large Antarctic continental shelf and the isothermal water column (which lacks a thermocline) may also have favored migrations between shelf and the deep sea (Clarke 2003). For this reason, the present benthic fauna comprises a mixture of taxa with different evolutionary histories and biogeographical affinities (Clarke & Crame 1992).

Benthic biodiversity and biogeography of the Southern Ocean: the need of improving management and conservation

More than 7,000 benthic species are estimated to inhabit the SO (De Broyer *et al.* 2011), including about 700 new species of invertebrates from deep waters recently discovered (Brandt *et al.* 2007b). Particularly, bryozoans, sponges and amphipods exhibit high species richness (De Broyer *et al.* 2011). These benthic communities, below areas affected by iceberg scours and anchor ice, evolved in relative stable conditions, thus being likely more sensitive to risk of extinctions caused by anthropogenic impacts. Therefore, the Antarctic ecosystems represent key regions to study biodiversity and climate change. Although the Antarctic ecosystems have been subject to smaller **anthropogenic impacts** respect to the rest of the planet, any potential threat has to be carefully evaluated since this represents a key region to study biodiversity and climate change, where during the early nineteenth century, sealing, whaling and exploration have increased the human presence. Additionally, in the last 50 years, tourism and fishing activities of diverse marine organisms such as krill, fishes and their associated by catch have strongly expanded (Griffiths 2010). Some Antarctic regions are also the most affected by ocean acidification due to the increase of the atmospheric carbon dioxide concentration, consequently reducing calcium carbonate concentration in the water, which forms the skeletons of marine organisms predominant in Antarctica, such as echinoderms and bryozoans (Orr *et al.* 2005). Unfortunately, Antarctic habitats are thus increasingly threatened by overexploitation of living resources, establishment of invasive marine species, anthropogenic pollutants and, in the long-term, climate change, as well as the growing impact of tourism (Bargagli 2008; Tejedo *et al.* 2009). From this perspective, the conservation and management of Antarctic marine biodiversity urgently requires detailed studies of the **biodiversity** and its relationship with environmental conditions (Barnes & Peck 2008).

Antarctic benthic communities are mainly influenced by (i) low temperatures at freezing level (reaching nearly -2°C in winter and barely +2.0°C in summer), (ii) a marked seasonality of the light availability and the primary production and (iii) two major disturbances, the iceberg scouring and the strong currents (e.g. Orejas *et al.* 2000; Gutt 2007; Smale 2007; Clarke 2008; Gerdes *et al.* 2008; Griffiths 2010). Some **adaptive responses** of Antarctic shallow benthic species to these particular environmental features are a slow growth, increased longevity, late maturity, low adult mobility, as well as, in some taxa, gigantism. In fish species, the possession of antifreezing glycoproteins and the lack of blood pigments is essential (Rahmann *et al.* 1984;

Arntz *et al.* 1994; Brandt *et al.* 2007b). Compared with taxa at lower latitudes, some Antarctic groups are widely spread such as amphipods, sponges, bryozoans, isopods and polychaetes, with values of biomass, in some cases, higher than those in equivalent temperate or tropical regions (Dayton *et al.* 1970; Orejas *et al.* 2000; Smale 2007; Cornejo-Donoso & Antezana 2008). Specially sponges, gorgonians and bryozoans, with their three-dimensional forms, create microhabitats for a wide range of associated fauna, representing the richest biomass and the highest faunal diversity in Antarctica (Arntz 1997). In contrast, other groups are certainly underrepresented (decapods, teleostean fishes, bivalves and gastropods) or are completely absent (such as pelagic and benthic top predators e.g. sharks) perhaps due to physiological constraints (Clarke & Johnston 2003; Arntz *et al.* 2005; Thatje *et al.* 2005). It is thought that some species of isopods and amphipods radiated after the extinction of brachyuran decapod crustaceans, probably related to the availability of ecological niches previously occupied by brachyurans (Brandt *et al.* 2012). In consequence, high densities of amphipods, as well as abundant macroinvertebrate generalist predators, which include echinoderms and nemerteans, represent the higher trophic levels. These exercise a strong pressure on sessile organisms, and in the case of the sea stars, as intense as in temperate and tropical regions (McClintock 1994; De Broyer *et al.* 1999, 2007, 2011; Brandt *et al.* 2007a).

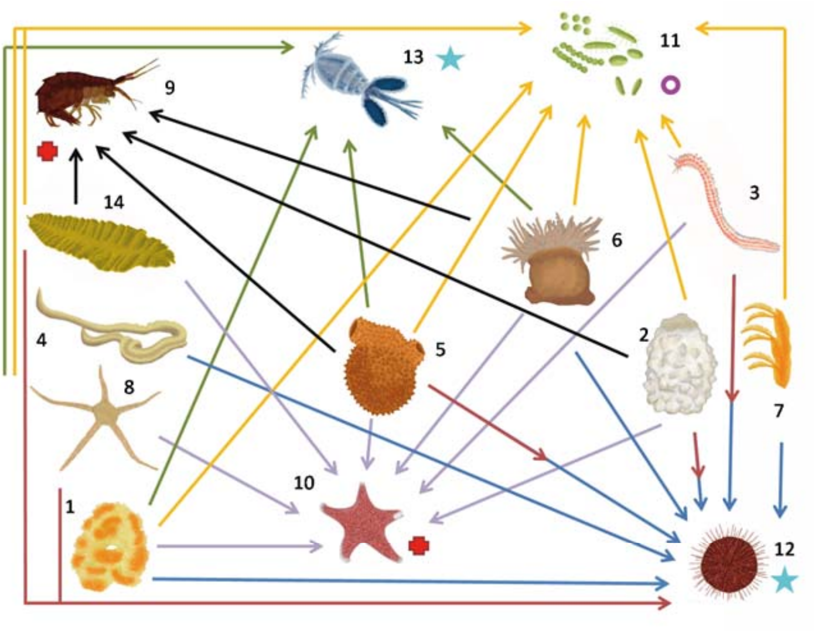
Apart from biodiversity, improving the existing knowledge of **biogeography** is also of fundamental relevance in the conservation and sustainable management of species, especially in Antarctica (Brandt *et al.* 2007a). From this perspective, comparative studies of diversity in the deep sea between Antarctica and the last separated fragments of Gondwana, especially the Patagonian region, are key to understand the evolution of regional communities and their relationships with the fauna outside the PF, as other authors suggest (Clarke *et al.* 2005; Clarke 2008). In particular, Argentine Patagonian (AP) region is one of the most productive large marine ecosystems of the Southern hemisphere due to the convergence of two major wind-driven currents (the cold Falkland/Malvinas and the warm Brazil currents), which promote the establishment of an elevated biomass of benthic invertebrates (Legeckis & Gordon 1982; Acha *et al.* 2004). However, the marine invertebrate diversity in this area has not been well studied so far, especially in some taxa, such as bryozoans, and even less is known in the deep-sea (López Gappa 2000; Acha *et al.* 2004; Miloslavich *et al.* 2011). Additionally, the existence of potential dispersal pathways between this AP region and Antarctica, such as the Malvinas Current, a branch of ACC which flows northwards below 800–1000 m (Hastings 1943; Legeckis & Gordon 1982), and eddies of the ACC (Clarke *et al.* 2005), suggest the existence of a **potential permeability** of the **PF** (e.g. Thatje & Fuentes 2003; Thatje *et al.* 2005a). This hypothesis has never been tested and might be key in explaining regional biogeography.

Antarctic chemo-ecological studies

During evolution, benthic marine organisms, under intense pressure for space, light and food, have developed a wide range of defensive mechanisms ranging from behavioral to physical and chemical strategies (e.g. Paul 1992; Avila *et al.* 2008). In the case of Antarctic ecosystems, these benthic communities, occurring below the influence of ice scouring and anchor ice, seem to be well-structured, in part to due the relatively high level of environmental stability mentioned above (Dayton *et al.* 1974; Arntz *et al.* 1994). This, along with the intense ecological interactions that take place in the Antarctic benthos, explains why these communities are structured mainly by biological factors, such as **predation** and **competition** (Dayton *et al.* 1974), rather than environmental drivers. Consequently, sessile and sluggish invertebrates, highly vulnerable to predators, have developed a wide array of chemical mechanisms using secondary metabolites to avoid predation (Amsler *et al.* 2001; Iken *et al.* 2002; Avila *et al.* 2008; Koplovitz *et al.* 2009; Slattery 2010), competition for space or food (Barnes & Rothery 1996; Bowden *et al.* 2006) and avoiding fouling (Peters *et al.* 2010). In particular, a chemically defended prey may produce metabolites that cause rejection (**unpalatability/repellence**) and/or physiological damage (**toxicity**) to a predator (Pawlik 2012). Contrary to the latitudinal hypothesis (suggesting that the chemical defenses are expected to be more frequent at low latitudes than at the poles; Bakus 1974), recent **feeding repellency**, **toxicity** and **antifouling bioassays** conducted with Antarctic benthic invertebrates have demonstrated that these defenses are comparable to those found in temperate and tropical regions (see Avila *et al.* 2008; McClintock *et al.* 2010). In agreement with this, a previous study of our team proposed the **first ecological model** for describing a wide array of potential chemical ecological relationships (Figuerola *et al.* 2012). This study showed an amazing complex network of interactions between many different benthic organisms, mainly generated to prevent the strong pressure of competition for space and/or food, predation and fouling (Fig. 3). Thus, further studies with larger number of samples are needed to complete and enrich this model and to fill in the existing gaps.

In order to understand the ecological functions of **natural products** isolated from the tissues of marine organisms, a young multidisciplinary field, **marine chemical ecology**, emerged in the 1980s. In this field, polar organisms have been less studied than those from temperate and tropical regions (Paul 1992; Avila *et al.* 2008; Blunt *et al.* 2012). However, recent studies report that Antarctic benthic invertebrates are a rich and diverse source of natural products, with great interest for **ecological** and **pharmacological trends** (e.g. Avila *et al.* 2000; Amsler *et al.* 2001; Iken *et al.* 2002; Reyes *et al.* 2008; Taboada *et al.* 2010; Paul *et al.* 2011; Blunt *et al.* 2012). In particular, several studies demonstrated that some Antarctic species inhabiting shallow areas from McMurdo Sound and the Antarctic Peninsula possess chemical defenses (for review see Avila *et al.* 2008; McClintock *et al.* 2010). However, only in a few cases, the chemistry of the metabolites involved has been fully described and/or their ecological role has been established (e.g. Núñez-Pons *et al.* 2010; Núñez-Pons *et al.* 2012b; Taboada *et al.* 2013).

Fig. 3. Diagram of the proposed model of chemical ecology interactions according to the results obtained in our experiments published in Figuerola *et al.* (2012), where: 1. Bryozoa, 2. Porifera, 3. Annelida, 4. Nemertea, 5. Tunicata, 6. Cnidaria, 7. Hemichordata, 8. Echinodermata; 9. *C. femoratus,* 10. *O. validus,* 11. Bacteria, 12. *S. neumayeri,* 13. Copepoda. 14. Algae. Symbols: Star: toxic activity; Cross: repellency activity; Circle: antifouling activity; Red: cytotoxicity activity; Blue: sperm toxicity. Each group is connected by arrows with the organisms used for each *in vivo* experiment.



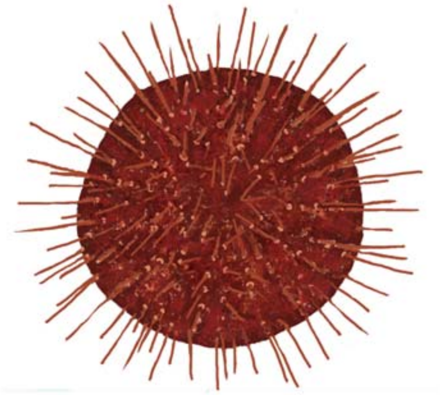
Regarding chemical ecology, we focussed our studies on selected specific chemical defenses of some taxa against common predators, as follows.

Cytotoxic defenses against a common Antarctic sea urchin predator

Echinoderms play a remarkable role in Antarctic benthic ecosystems and control the populations of many benthic organisms (McClintock 1994). Among them, the sea urchin *Sterechinus neumayeri* Meissner, is considered to be one of the most abundant and common species found in Antarctica. In particular, *Sterechinus* is the only genus of Antarctic echinoids, comprising 5 species, being *S. neumayeri* the most abundant (Fig.4; Fell 1976). This species has a circumpolar and wide bathymetric distribution, ranging from a few meters to 810 m deep (Brey & Gutt 1991; Moya *et al.* 2012). In general, grazing by sea urchins severely damages invertebrate colonies and can also negatively affect the recruitment of larvae (McClintock 1994;

Bowden 2005; Bowden *et al.* 2006). Due to the intense predation pressure it exerts, their prey may produce **cytotoxic substances** to prevent the settlement of larvae of this sea urchin, which would ultimately decrease the grazing pressure over them (e.g. McClintock *et al.* 1990). *S. neumayeri* has been used as a model organism in previous studies and its biology is well known (Bosch *et al.* 1987; Brey *et al.* 1995; Tyler *at al.* 2000; Brockington *et al.* 2007), but only a few studies have investigated the indirect chemically mediated interactions against the development of its embryos (McClintock *et al.* 1990; Slattery *et al.* 1995).

Fig.4. The Antarctic sea urchin *Sterechinus neumayeri*



Repellent defenses against Antarctic common generalist predators

Apart from sea urchins, other major Antarctic predators are sea stars and amphipods. Antarctic asteroids occur in the highest trophic levels, replacing fish as major predator and thus, exercising a strong pressure on sessile organisms (McClintock 1994). In particular, predation seems to be largely driven by the common omnivorous opportunistic sea star *Odontaster validus*, which has a wide range of feeding strategies, from suspension-feeding to scavenging and predation on highly diverse assortment of benthic invertebrates and algae (including bryozoans), being therefore, a "keystone species" (Dayton *et al.* 1974; McClintock *et al.* 1994). This asteroid, with a circumpolar distribution, is abundant around the SO down to 2902 m depth (Fig. 5; Dearborn 1977; McClintock *et al.* 1988; see also http://iobis.org/mapper/). Given all these features, we chose this sea star as a model generalist predator (Chapter 5), as it was previously used to conduct several **feeding repellency bioassays** employing different methodologies (McClintock *et al.* 1992, 1993, 1994; Slattery & McClintock 1995; McClintock & Baker 1997; Avila *et al.* 2000; Mahon *et al.* 2003; Peters *et al.* 2009; Núñez-Pons *et al.* 2012a; Taboada *et al.* 2013; see also http://iobis.org/mapper/). Recent experiments demonstrated the presence of feeding repellents in many taxonomical groups of Antarctic invertebrates to avoid sea star predation using this species (Avila *et al.* 2008; McClintock *et al.* 2010; Figuerola *et al.* 2012; Taboada *et al.* 2013).

Fig. 5. The Antarctic sea star *Odontaster validus*

The Amphipoda is also an important Antarctic benthic group, playing a crucial and complex role in organic matter fluxes on the Antarctic sea floor (e.g. Broyer *et al.* 1999; Dauby *et al.* 2001a, b; Brandt *et al.* 2007a). They are also one of the most diverse groups in the SO with an estimated 600+ species (De Broyer *et al.* 2007, 2011). Moreover, they are frequently associated with benthic organisms (Broyer *et al.* 1999). Gammaridean species from the Weddell Sea show a broad diet spectrum on benthic invertebrates, such as bryozoans, cnidarians and sponges, suggesting a lack of prey specialization (Dauby *et al.* 2001b). These benthic invertebrates, apart from representing potential prey for amphipods, can also be potential host biosubstrata, offering a high diversity of microhabitats, and thus potential structural and chemical refuges from predation (Oshel & Steele 1985; De Broyer *et al.* 1999, 2001; Dauby *et al.* 2001a; Huang *et al.* 2008). Specially, *Cheirimedon femoratus* is an opportunistic omnivore circumantarctic lyssianasid gammarid, feeding on different kinds of food (from invertebrates to algae and detritus), with eurybathic distribution (from 0 to 1500 m), and frequently associated with macroinvertebrates (Fig. 6) (Bregazzi 1972; De Broyer *et al.* 2007; Krapp *et al.* 2008). This species has also been used as putative model consumer in previous feeding assays in our group (Núñez-Pons *et al.* 2012b).

Fig. 6. The Antarctic amphipod *Cheirimedon femoratus*

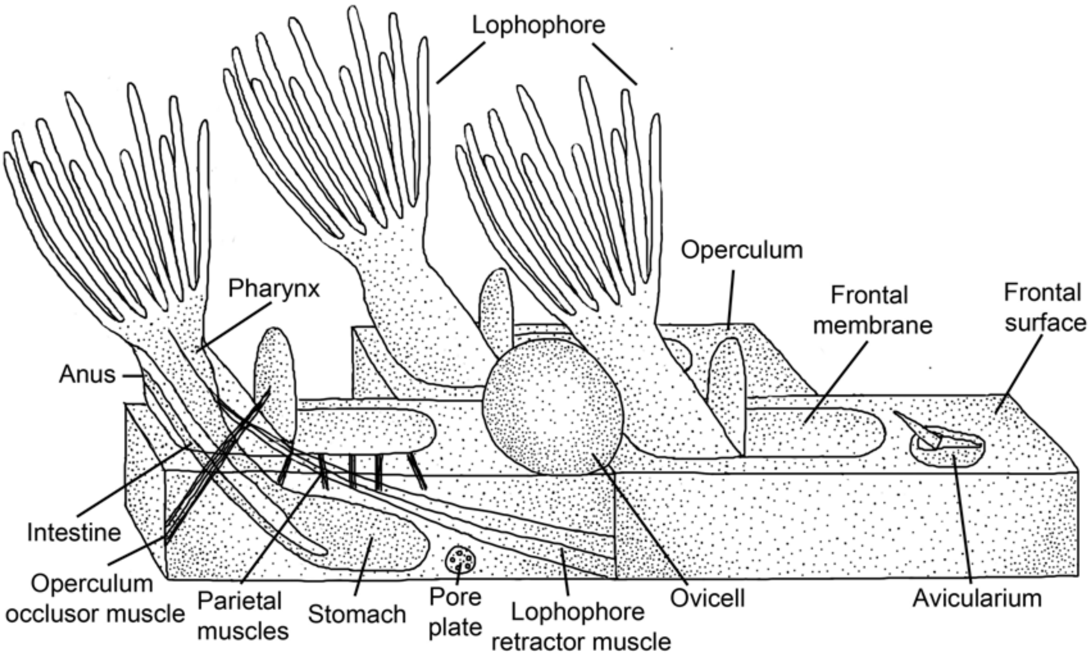
The targeted Antarctic bryozoans of our research: why bryozoans?

General features

Bryozoans (Greek for 'moss animals'), named commonly "sea mats", "moss animals" or "lace corals" for their diverse forms, were previously called **Polyzoa** (Greek for 'many animals'), referring to their colonies of many individuals. Bryozoans are surprisingly abundant aquatic colonial invertebrates, filter-feeders, mainly marine, although they also are common in brackish-water and freshwater. In particular, these colonial invertebrates are almost ubiquitous, inhabiting from the shallow to the abyssal plains. Moreover, they are important components of the fossil record, allowing us to test evolutionary hypotheses and the effects of climate change. Bryozoan colonies are part of the hard substratum epifauna on most rocky shores, and in some habitats, cover considerable areas of suitable rock or, in the case of shallow areas, blades of kelps and algae, and they are important colonizers of artificial substrata, such as boats. Moreover, bryozoan colonies develop a broad spectrum of forms, ranging from encrusting sheets to erect branching chains (cylindrical, laminar...), flexible or rigid, creating in some cases three-dimensional colonies, which provide habitats for abundant motile or encrusting organisms. The individual functional units (modules) of colonies are called **zooids**. The single founding zooid, named **ancestrula**, resulting from the settlement and consequent metamorphosis of a sexually produced larva, originates the rest of the colony. Bryozoan colonies consist of replicated series of genetically and usually morphologically identical units (**autozooids**) separated between them by walls of skeletal material and organic cuticle. These identical units originated, through a non-sexual budding process, from the ancestrula. In particular, each autozooid consists of a **lophophore** (a ring of tentacles whose mouth is situated at the vertex), a gut that opens at an anus found at the base of the lophophore outside the tentacles' ring, reproductive organs and a nerve ganglion situated between the mouth and the anus with nerves radiating to the other parts of zooid. Thus, autozooids are functionally independent units. Frequently, colonies also include specialized polymorphs named **heterozooids**. The lophophore, U-shaped gut and musculature are referred as the **polypide** (little polyp) and the rest of the zooid, including skeleton, are called **cystid**. The eversion of the lophophore into the water to feed on small living and non-living organic particles is realized by a simple hydrostatic system withdrawn by a retractor muscle. A network of tissues of strands, the **funiculus**, is developed around the gut, extending through branches to the communicating pores in the body walls and, thus linking adjacent zooids. This web transports nutrients between zooids and provides food reserves to non-feeding zooids (Fig. 7; Hayward 1995; Hayward & Ryland 1979; Hayward & Ryland 1998; see also www.bryozoa.net).

Bryozoans are classified into three classes: Stenolaemata, Gymnolaemata and Phylactolaemata. The Phylactolaemata is restricted to freshwater, with uncalcified colonies and usually with a horseshoe-shape tentacle crown. Classes Stenolaemata and Gymnolaemata comprise mostly marine bryozoans with a circular tentacle crown and with a calcified skeleton, except for the ctenostomes. On the one hand, the class Stenolaemata, comprising only the living order **Cyclostomatida**, represents colonies with cylindrical zooids and limited polymorphism. On the other hand, the class Gymnolaemata comprises two orders, **Ctenostomatida** and **Cheilostomatida**. The Order Ctenostomatida is mainly characterized by an uncalcified body wall, membranous or gelatinous, heterezooids limited to stolons, spines and **kenozooids** (the simplest type of polymorphism lacking the operculum and lophophore which include diverse types of spines, rhizoids...) and the absence of avicularia. Cheilostomatida, almost exclusively marine, is the most successful living order of bryozoans, with characters such as box-like zooids reinforced with calcium carbonate skeletons, and an **operculum** (membranous lid) protecting the orifice of the frontal wall through which the animal extends its lophopore (Hayward 1995; Hayward & Ryland 1998; see also www.bryozoa.net). In cheilostome bryozoans, the nature and formation of the skeletal components of the body wall is essential for their classification and thus, are divided into two suborders, the **Anasca** and the **Ascophora**. Anascans are characterized by a membranous frontal wall and aschoporans by a calcified frontal shield and an **ascus** (underlying sac which allows the function of the hydrostatic system). Additionally, cheilostome bryozoans are one of the best-represented taxa on the Antarctic shelf (Barnes *et al.* 2009) and a high proportion (56%) is endemic (Hayward 1995; Barnes & De Grave 2000; Clarke & Johnston 2003; Griffiths *et al.* 2009; Griffiths 2010). Most cheilostome bryozoans have developed a high degree of polymorphism in zooids specialized in different tasks for feeding (autozooids), for cleaning and protecting colonies (avicularia, vibracula, spines...) and for reproduction (**ovicell**: globular chamber at the distal end of the zooid in which the embryos are brooded) (Hayward 1995; Winston 2009). **Avicularia**, common among heterozooids, are modified zooids devoid of polypide with a hypertrophied operculum (**mandible**) that opens slowly and closes rapidly by contraction of adductor muscles, discouraging predators or larvae (McKinney 1998). **Vibracula** are modified zooids with a setiform mandible that moves in diverse planes. The morphology, position, and frequency of occurrence of heterezooids, especially of avicularia, are taxonomically significant and used to define species (Hayward 1995).

Fig. 7. Diagram of a portion of a colony of an encrusting bryozoan (adapted from Hayward & Ryland 1998).



Current gaps in knowledge

Two major gaps in our knowledge regarding Antarctic bryozoans, identified after the literature review, are related to (i) its biodiversity and biogeographical patterns, and (ii) their chemical defenses.

The number of taxonomic studies on Antarctic bryozoans has experienced a notable increase in recent years. Until now, near 400 cheilostome bryozoans have been described (Hayward 1995; Gutt *et al.* 2000; López-Fé de la Cuadra & Garcia-Gomez 2000; Barnes & Griffiths 2008; Brandt *et al.* 2012). Even so, the knowledge of the bryozoan species from the SO, their diversity and the environmental conditions in which they live, is still quite unknown (Kuklinski & Barnes 2009). Thus, with the aim of expanding the knowledge of bryozoan species from poorly known regions of SO, our studies (**Chapters 1, 2 and 3**) provide new data on Antarctic bryozoan biodiversity, their spatial patterns and their biogeographic links with neighboring regions. Furthermore, we expected to find new species and new bathymetric and geographical ranges with our data. Our initial hypothesis was to find shared fauna between Antarctica and neighboring regions and thus, bringing into question the extent of Antarctic isolation, as it was suggested for other taxa (Hastings 1943; Moyano 1982, 1999; Barnes & De Grave 2001; Arntz *et al.* 2005; Griffiths *et al.* 2009). In particular, **Chapter 1** explores and describes the bryozoan biodiversity from the slope of the poorly explored AP region. Actually, this study is one of the first studies carried out to evaluate the connectivity of bryozoan populations, mainly from the **slope**, between the AP region and the SO (López Gappa 2000). Additionally, bryozoans are poorly investigated also in some other Antarctic regions, such as the Weddell Sea (Zabala *et al.* 1997; Moyano 2005; Barnes & Kuklinski 2010), giving more value to the study presented in **Chapter 2**. In this Chapter, the bryozoan biodiversity and their spatial patterns from the Southern Ocean are described. Taxonomy is crucial to develop correct biodiversity' studies. Thus, **Chapter 3** provides details about two remarkable aspects: the **description of a new cheilostome species** of *Reteporella* from the Weddell Sea (Antarctica) characterized by the presence of giant vicarious spherical avicularia, and the discussion of the **functional morphology** of these avicularia.

Apart from that, while there is a substantial amount of knowledge on the chemical defenses in some Antarctic taxa, such as sponges, ascidians corals and molluscs, surprisingly, little is known about Antarctic bryozoans (e.g. Avila *et al.* 2008; Figuerola *et al.* 2012; Taboada *et al.* 2013). In fact, some studies found antibiotic and hemolytic activities in Antarctic bryozoan species, but without ecological relevance (Colon- Urban *et al.* 1985; Winston & Bernheimer 1986). However, bryozoans are known to produce natural products such as **alkaloids** and **terpenoids** with different activities, like antifouling and antipredation (Al-Ogily & Knight Jones 1977; Lopanik *et al.* 2004; Gray *et al.* 2005; Lebar *et al.* 2007; Sharp *et al.* 2007). Research in this field continues to be very limited, especially considering that it has been estimated that between 8,700 and 11,100 bryozoan species currently exist in the sea (Appeltans *et al.* 2012). Chemo-ecological studies carried out in our research (**Chapters 4 and 5**) contribute to fill the large gaps of knowledge in the **chemical ecology** of bryozoans in general. Regarding the **nature of the secondary metabolites**, compounds of different polarities have been described among defensive products isolated from diverse taxa (Pawlik *et al.* 1986, Kubanek *et al.* 2000; Pawlik 2012), but only a few ether fractions from Antarctic bryozoans have been tested previously in feeding bioassays against *O. validus* and *C. femoratus* (Figuerola *et al.* 2012; Núñez *et al.* 2012b; Taboada *et al.* 2013), and no butanol fractions have ever been tested before for bryozoans. Thus, our studies take a step forward, testing more extracts, as well as butanol fractions never previously used.

Due of the known activity of some bryozoan natural products, we expected to find new active compounds. Our initial hypothesis was that the chemically defended bryozoans produce metabolites that cause **repellence** and/or **toxicity** to the selected predators. In the case of clonal invertebrates such as bryozoans, the repellence of their compounds may be an advantage versus the toxicity, because a predatory attack on a clonal prey is not usually lethal, also allowing predators to learn to avoid chemically defended prey, and in consequence, favoring the evolution of these chemical defenses. In fact, the production of toxins is linked to a high metabolic cost of the organism, which should prevent the autotoxicity, leading to palatability being the important driving force in the evolution of chemical defenses. However, under the circumstances of prey availability limited to chemically defended species, toxicity could be also advantageous (Pawlik 2012). Thus, here we test these two strategies in the same bryozoan species (**Chapters 4 and 5**). In particular, indirect chemically mediated interactions through cytotoxic compounds have been reported in temperate waters (e.g. Birrell *et al.* 2008; Rasher *et al.* 2011), although they are poorly known in Antarctic ecosystems (Heine *et al.* 1991; McClintock *et al.* 1990, 1992, 1997, 2010), giving high relevance to our study (**Chapter 4**). Additionally, **Chapter 4** contributes to the understanding of the cytotoxic role of bryozoan natural products against a sympatric and widely model organism. In this study, **new adapted protocols** are applied for chemical ecology bioassays using solid gel substrates that simulate the surface of bryozoan colonies and thus, approaching a more ecologically relevant perspective.

Moreover, most studies of chemical defenses in Antarctic benthic invertebrates have been carried out in shallow-water (accessible via scuba diving) from McMurdo Sound and the Western Antarctic Peninsula (Avila *et al.* 2008; McClintock *et al.* 2010). However, recent feeding repellence studies conducted with deep-water benthic species from the Weddell Sea demonstrated high activity in some taxa (Núñez-Pons *et al.* 2012b; Taboada *et al.* 2013). Thus, we decided to focus the chemical studies on **deep-water** bryozoan species, taxon that has received little attention so far, from the Weddell Sea (**Chapters 4 and 5**).

It should also be noted that cheilostome bryozoans possess zooid polymorphs with specific **physical or mechanical defensive functions** against particular small sized predators such as amphipods (Winston 1991), representing convenient model systems for studying physical and chemical defensive strategies. According to this, although predation by smaller zooid-level predators is generally sub-lethal in colonial organisms such as bryozoans, chemical defenses could be frequently developed in Antarctic invertebrates, such as bryozoans (Winston 2009). In our studies, we expected to find repellent compounds to avoid direct or occasional predation, as well as filtration disruptions, stimulating thus chemical defenses against small predators. Regarding predation by echinoderms, bryozoans cannot survive after major injuries of the colonies mainly caused by these large predators (Winston 2009). Therefore, our initial hypothesis is the development of chemical defensive strategies against the sea star *Odontaster validus* (**Chapter 5**). In fact, physical devices like spines and avicularia may not have the same efficacy against small predators, encouraging the evolution of chemical defenses in these cases. For this reason, we contrasted the different potential defensive systems, chemical and physical, in selected Antarctic bryozoan colonies (**Chapters 4 and 5**).

Objectives of this Thesis

The main two goals of this thesis are **to improve our knowledge of the biodiversity and the chemical ecology of Antarctic bryozoans.**

In order to achieve these two main goals, we decided to establish five specific objectives:

1. **To determine bryozoan diversity, their spatial patterns from one of the regions closer to Antarctica (Argentine Patagonian region) and their biogeographic links.** We present new data on bryozoan species richness from the Argentine Patagonian region and their spatial patterns and investigate whether our data agree with the biogeographic regions proposed previously by others, and whether Antarctic isolation exists for cheilostome bryozoans (Chapter 1).

2. **To analyze bryozoan diversity from the Southern Ocean and to describe their spatial patterns.** We identify bryozoan species from the Southern Ocean and, by combining these with existing data on bryozoan distributions in this region; we describe bathymetric and geographic distributions (Chapter 2).

3. **Among the new taxa found, to describe a new species of *Reteporella* from the Weddell Sea (Antarctica).** We describe a new species of *Reteporella* and discuss the functional morphology of avicularia. We also compare the characteristics of all the species of this genus from Antarctica and analyze the intraspecific variability of their main characters (Chapter 3).

4. **To investigate whether Antarctic bryozoans possess chemical defenses that may serve as sperm/embryo cytotoxics and/or as amphipod repellents.** The present study describes new protocols for chemical ecology bioassays using solid gel substrates that simulate the surface of bryozoan colonies in order to approach a reliable ecological perspective. In order to understand the role of bryozoan natural products, we carry out cytotoxicity and substrate preference assays, using sympatric and widely distributed model organisms: the common sea urchin *Sterechinus neumayeri* and the amphipod *Cheirimedon femoratu*s. We also explore the possible existence of an interspecific/intraspecific variability in these defensive traits (Chapter 4).

5. **To evaluate the presence of chemicals that may serve as repellents against relevant Antarctic predators.** In order to establish the role of Antarctic bryozoan chemical products from the Weddell Sea area, the sea star *Odontaster validus* and the amphipod *Cheirimedon femoratus* were selected to conduct feeding repellency assays. We evaluate the presence of natural products that may serve as repellents against these relevant Antarctic predators, and we compare the intra- and interespecific variability in bioactivity, if any. We also contrast the different potential defensive systems, both chemical and physical, in Antarctic bryozoan colonies (Chapter 5).

General structure of the Thesis

This Thesis has been structured in three main parts, which include three and two publications, respectively, already published or submitted for publication, as well as the general discussion and conclusions. A translation into Catalan and Spanish of the General Introduction, each abstract of publications and the General Discussion are included at the end.

Part I "Biodiversity of bryozoans from Antarctic and sub-Antarctic waters" covers the taxonomy and diversity studies, including the first three publications (**Chapters 1, 2 and 3**).

Part II "Chemical ecology in Antarctic bryozoans" presents the study of diverse chemical mechanisms (toxicity, repellence and unpalatability) of some bryozoan species against sympatric organisms, including the other two publications (**Chapters 4 and 5**).

Part III "General discussion and Conclusions "

Supervisor's Report

Dr. Conxita Avila, Director of the PhD thesis entitled "Biodiversity and Chemical Ecology in Antarctic Bryozoans", certifies that the dissertation presented here is the result of the work carried out by Blanca Figuerola Balañá under my guidance and supervision. The contribution of the PhD candidate to each manuscript is detailed below:

Chapter 1. Cheilostome bryozoan diversity from the Southwest Atlantic region: is Antarctica really isolated?
Figuerola B, Gordon DP, Polonio V, Cristobo J, Avila C
Journal of Sea Research (*in press*)
5- Year Impact Factor (2012): 2.287
BF: samples identification, data analysis and statistics, results interpretation, as well as manuscript elaboration

Chapter 2. Spatial patterns and diversity of bryozoan communities from the Southern Ocean: South Shetland Islands, Bouvet Island and Eastern Weddell Sea
Figuerola B, Monleón-Getino T, Ballesteros M, Avila C
Systematics & Biodiversity 10(1): 109–123 (2012)
5- Year Impact Factor (2012): 1.959
BL: samples identification, data analysis and statistics, results interpretation, as well as manuscript elaboration

Chapter 3. Description of a new species of *Reteporella* (Bryozoa: Phidoloporidae) from the Weddell Sea (Antarctica) and the possible functional morphology of avicularia
Figuerola B, Ballesteros M, Avila C
Acta Zoologica 94 (1): 66–73 (2013)
5- Year Impact Factor (2012): 1.284
BL: samples identification, data analysis, results interpretation, as well as manuscript elaboration

Chapter 4. Chemo-ecological interactions in Antarctic bryozoans
Figuerola B, Núñez-Pons L, Monleón-Getino T, Avila C
Submitted

BL: collection and extraction of samples, species identification, designing and performing the experiments, data analysis and statistics, results interpretation, as well as manuscript elaboration

Chapter 5. Feeding deterrence in Antarctic bryozoans
Figuerola B, Núñez-Pons L, Moles J, Avila C
Submitted
BL: collection and extraction of samples, species identification, designing and performing the experiments, data analysis and statistics, results interpretation, as well as manuscript elaboration

From all the co-authors of the different chapters, VP and JM have not been awarded a PhD degree. I hereafter guarantee that none of the information contained in the chapters co-authored by them will be used to elaborate any other part of someone else's PhD theses.

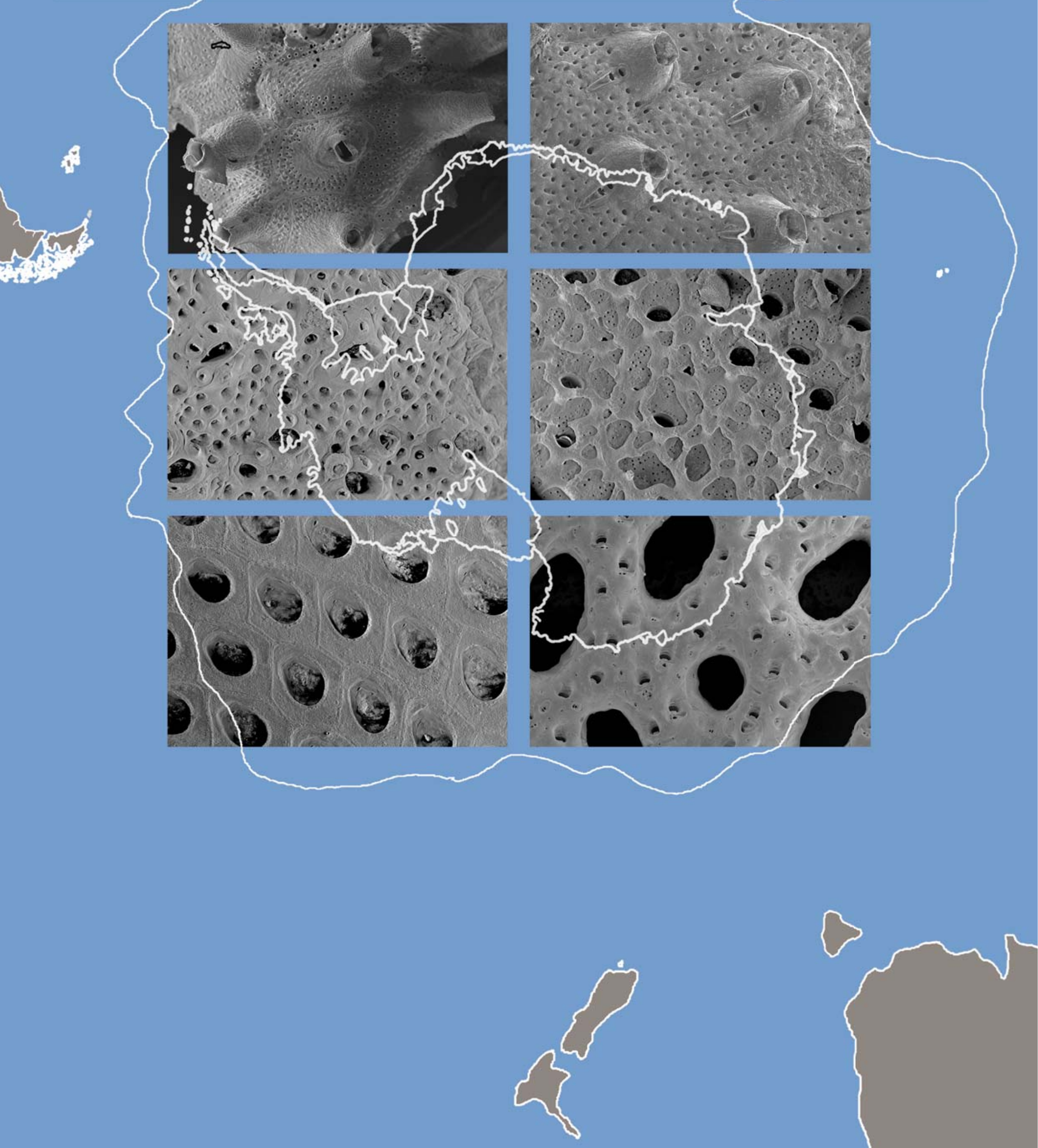
For all of the above, I consider that the contribution of the PhD candidate grants her the right to defend her thesis in front of a scientific committee.

Barcelona, September 3rd 2013.

Dr. **Conxita Avila**

Chapter 1

Cheilostome bryozoan diversity from the southwest Atlantic region: is Antarctica really isolated?



Chapter 1

Cheilostome bryozoan diversity from the southwest Atlantic region: is Antarctica really isolated?

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Accept in: *Journal of Sea research*

Abstract

During the Cenozoic, the break-up of Gondwana was accompanied by a gradual separation of its components and the subsequent establishment of the Antarctic Circumpolar Current, leading to a relative thermal and biogeographic isolation of the Antarctic fauna. However, the zoogeographical affinities of several taxa from South America and Antarctica have been subject to debate, bringing into question the extent of Antarctic isolation. Here we present new data on bryozoan species and their spatial distribution in the Argentine Patagonian (AP) region, as well as an analysis of the bryozoological similarities between deep ranges from Argentina and neighbouring regions. A total of 108 species of cheilostome bryozoans (378 samples), belonging to 59 genera was found. Five new genera and 36 new species were found in the AP region, while 71 species were reported for the first time from Argentina. The bathymetric ranges of 94 species (87 %) were expanded and a high proportion of the identified species (44.4 %) also had an Antarctic distribution. The bryozoological affinities found in the current study between the nearest geographical neighbours are in agreement with the hypothesis of the sequential separation of Gondwana during the Cenozoic. Moreover, a high number of shared species, mainly from the slope, were found in this study between the AP region and Antarctica, thus supporting the idea that the Southern Ocean may have been less isolated over geological time than once thought.

Keywords: Antarctic Polar Front, Falkland/Malvinas Current, Spatial patterns, Species richness, Zoogeography, marine invertebrates

Resum

Durant el Cenozoic, la fragmentació de Gondwana va estar acompanyada per una separació gradual dels seus components i l'establiment posterior del Corrent Circumpolar Antàrtic, portant a un aïllament termal relatiu i biogeogràfic de la fauna antàrtica. Tot i així, les afinitats zoogeogràfiques de diversos taxons procedents d'Amèrica del Sud i de l'Antàrtida han estat subjectes a debat, posant en dubte el grau d' aïllament antàrtic. Aquí es presenten noves dades de les espècies de briozous i la seva distribució espacial a la regió de la Patagònia argentina (PA), així com també una anàlisi de les semblances briozoològiques entre rangs de fondària d'Argentina i de les regions veïnes. Es van trobar un total de 108 espècies (378 mostres), pertanyents a 59 gèneres. A la regió PA, es van trobar cinc gèneres nous i 36 espècies noves, mentre 71 espècies van ser citades per primer cop a l'Argentina. Els rangs batimètrics de 94 espècies es van expandir i es va trobar que una elevada proporció de les espècies identificades (87 %) presentaven també una distribució antàrtica. Les afinitats briozoològiques trobades al present estudi entre les regions geogràfiques més properes estan a favor de la hipòtesi de la separació seqüencial de Gondwana durant el Cenozoic. A més a més, en aquest estudi es va trobar un elevat nombre d'espècies compartides, principalment del talús continental, entre la regió de la PA i l'Antàrtida, recolzant per tant, la idea que l'Oceà Austral ha estat menys aïllat en el temps geològic del què fins ara es pensava.

Paraules clau: Front Polar Antàrtic, Current Falkland/Malvines, Patrons espacials, Riquesa específica, Zoogeografia, invertebrats marins

Introduction

The Patagonian continental shelf and slope, one of the most productive Large Marine Ecosystems (LMEs) of the Southern Hemisphere, extends for about 5649 km along the Atlantic coast of South America (Acha *et al.* 2004; Miloslavich *et al.* 2011). There, two major wind-driven currents coexist: the cold nutrient-rich Falkland/Malvinas and the warm Brazil currents. The Malvinas Current is a branch of the ACC (Antarctic Circumpolar Current), flowing northward along the continental shelf of Argentina to about latitude 30° to 40° S, where it is deflected eastward after meeting the warm southward-flowing Brazil Current (Legeckis & Gordon 1982). At the confluence of these currents, there is high biological production on the continental shelf and slope, promoting elevated biomass of benthic invertebrates (Acha *et al.* 2004). Thus, this region is inhabited by particular species with a wide range of distributions and adaptations to fluctuating conditions resulting from the influence of these subtropical and subantarctic waters (Miloslavich *et al.* 2011).

In recent years, several exotic species have been recorded in the Patagonian region, making it increasingly difficult to establish the original composition of coastal communities, considering that the biodiversity of the southwestern Atlantic is poorly known (Orensanz *et al.* 2002). Since the mid-1700s, the number of new species from South America has increased exponentially and particularly high numbers have been found in Argentina, although marine-invertebrate diversity has not been well studied and even less so in the deep-sea. In particular, the best-known benthic invertebrates in this region are molluscs, echinoderms and cnidarians (Miloslavich *et al.* 2011). In contrast, considering the high bryozoan diversity found with little sampling effort in different areas of the coast, shelf and slope, bryozoan species richness, mainly in northern shelf areas, is still largely underestimated (López Gappa 2000; Moyano 1999). Thus, more studies are needed for this region, mainly at slope and abyssal depths, to evaluate the connectivity of bryozoan populations between the Southern Ocean and South America (Hastings 1943; Moyano 1982, 1999; Barnes & De Grave 2001; Arntz *et al.* 2005; Figuerola *et al.* 2012). In fact, the assessment of biodiversity and biogeography is of particular importance in the conservation and sustainable management of species, especially in Antarctica (Brandt *et al.* 2007a). From this perspective, studies of comparative diversity in the deep sea between Antarctica and the last separated fragments of Gondwana are key to understanding the evolution of regional communities and their relationships with the fauna outside the Polar Front (Clarke *et al.* 2005a; Clarke 2008). Therefore, the abundance and richness of bryozoans, mainly cheilostomes, on temperate and polar continental shelves of the Southern Hemisphere, linked to their strong fossil record, them an ideal taxon for the reconstruction of the paleozoogeographical history of the fragmentation of Gondwana (Barnes & Griffiths 2008).

During the Cenozoic, the break-up of Gondwana, the ancient supercontinent comprising most of the landmasses in today's southern hemisphere, was produced as a sequential separation of their fragments. Before the Antarctic-South American separation, Australia drifted apart from Antarctica. The last shelf links between them were lost, leading to the creation of a seaway, the Hoces Sea (Drake Passage), the subsequent establishment of the clockwise ACC about 25 Ma ago, and a gradual cooling (Lawver & Gahagan 2003; Upchurch 2008). Therefore, the last separated fragments of Gondwana included in particular South America, as well as continental shelves and islands of the Subantarctic Region, located in the Southern Ocean, between the PT and the Subtropical Convergence (between 35º and 45ºS), which served as stepping-stones for the dispersion of shallow fauna. In particular, the Subantarctic Region comprises southern Chile, Patagonia, and New Zealand.

All of these historical events led to relative thermal and biogeographic isolation of the Antarctic fauna, and thus, to high Antarctic endemism (Arntz *et al.* 1997; Lawver & Gahagan 2003; Scher & Martin 2006). In fact, the ACC is the unique global link connecting all major oceans (Atlantic, Pacific, and Indian), being the largest oceanic current system on Earth and promoting the dispersal of marine organisms such as larvae or adults from west to east around Antarctica (Orsi *et al.* 1995; Olbers *et al.* 2004). In contrast, the Antarctic Polar Front (PT), one of several strong fronts within the ACC, is considered a strong hydrographic barrier to free north-south dispersal of shallow benthic fauna. Therefore, the close zoogeographical links between South America and west Antarctica (particularly the Antarctic Peninsula and the Scotia Arc), and less with New Zealand and Australia, may be explained by their associations during the Cenozoic and by faunal exchange through ACC circulation (Zinsmeister 1982; Crame 1999; Moyano 1999, 2005; Clarke 2003; Clarke *et al.* 2005a; Brandt *et al.* 2007a; Downey *et al.* 2012). However, the zoogeographical affinities between South America and Antarctica of diverse taxonomic groups such as bryozoans have been subject to debate in the literature, bringing into question the extent of Antarctic isolation (e.g. Moyano 1982, 1999; Barnes & De Grave 2001; Arntz *et al.* 2005; Griffiths *et al.* 2009; Figuerola *et al.* 2012). In this sense, all the main deep-sea regions are directly connected below 3000 m, and as a result the PT appears to be less of a barrier to the natural north-south migration (Clarke *et al.* 2005a; Brandt *et al.* 2007a). Moreover, the Scotia Arc archipelago proposed by some authors as the only physical link between these two regions, being a potential bryozoan exchange pathway, apart from deep abyssal plains (e.g. Moyano 1999, 2005; Barnes 2005). Other potential dispersal pathways are the Malvinas Current, a branch of the ACC flowing northwards below 800–1000 m (Hastings 1943; Legeckis & Gordon 1982), and eddies of the ACC (Clarke *et al.* 2005a), suggesting the existence of a potential permeability of the PF (e.g. Thatje & Fuentes 2003; Thatje *et al.* 2005a). On the other hand, human dispersal mechanisms have increased faunal exchange and the introduction of new species to Antarctica, by marine debris, ballast water or biofouling of ships' hulls (Barnes 2002; Lewis 2003; Thatje *et al.* 2005a).

In this study, we present new data on species richness and the spatial patterns of bryozoans from the southwestern Atlantic (Argentine Patagonian (AP) region). We also investigate whether our data agree with the biogeographic regions proposed previously by others, and whether Antarctic isolation exists for cheilostome bryozoans. To accomplish this, we analyse bryozoans, one of the best-studied Antarctic taxa, comparing our data with the nearest geographic neighbours from South American, South African, Australian, New Zealand, Subantarctic and Antarctic regions, given the historic links between these regions during the Cenozoic.

Material and Methods

Samples from Patagonia were collected during five cruises (Patagonia 0108, 0209, 0210, 1108 and 1208) of the "ATLANTIS" project carried out by the Instituto Español de Oceanografía, on board the R/V Miguel Oliver. A total of 51 stations were surveyed (2008-2010). Depths of collections ranged from 140 to 1897.67 m, and sampling was done by using rock dredges. Sampling sites were georeferenced by GPS and depth was registered at each station (Fig. 1; Table 1).

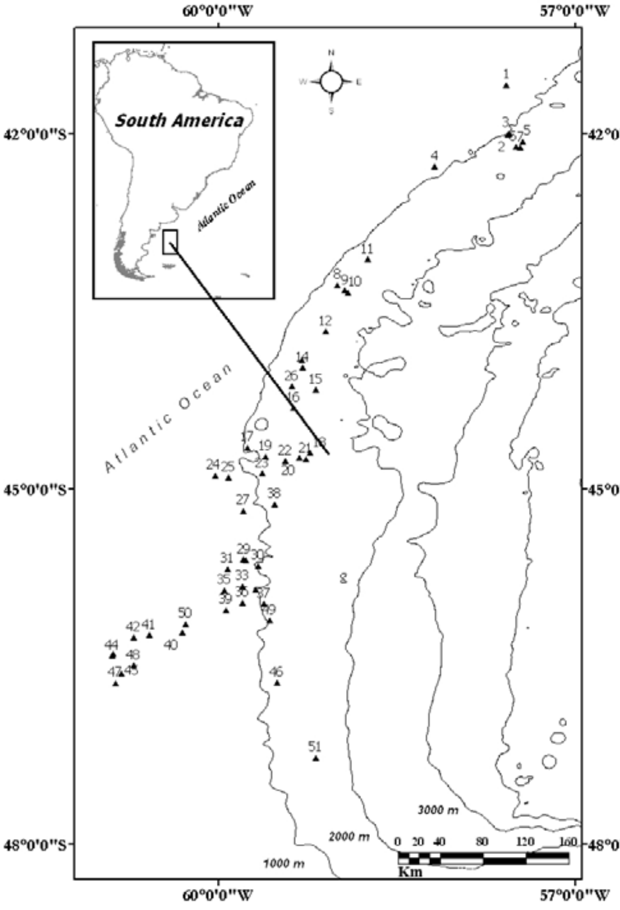
Table 1. Depth and coordinates of the sampling stations.

Ref.	Station	Latitude (S)	Longitude (W)	Depth (m)
1	PAT0210DR04	41° 35' 34.8"	57° 34' 54.12"	436
2	PAT0210DR06	42° 0' 43.92"	57° 34' 7.32"	485
3	PAT0210DR05	42° 0' 5.76"	57° 33' 16.92"	586
4	PAT0210DR07	42° 16' 51.96"	58° 11' 11.04"	1096
5	PAT0210DR09	42° 4' 8.76"	57° 26' 29.76"	1048
6	PAT0210DR08	42° 6' 55.08"	57° 29' 55.68"	1090
7	PAT0210DR11	42° 7' 15.6"	57° 27' 58.68"	1148
8	PAT0209DR15	43° 17' 5.78"	59° 0' 3.25"	1244
9	PAT0209DR14	43° 19' 45.76"	58° 56' 24.15"	1553
10	PAT0209DR13	43° 20' 46.93"	58° 54' 37.43"	1472
11	PAT0209DR16	43° 3' 58.75"	58° 44' 37.50"	1529
12	PAT0209DR12	43° 40' 33.52"	59° 6' 5.87"	1635
13	PAT0209DR01	43° 55' 0.86"	59° 18' 3.75"	1393
14	PAT0209DR11	43° 58' 52.70"	59° 17' 25.8"	1500
15	PAT0209DR10	44° 10' 3.55"	59° 10' 58.66"	1581
16	PAT0209DR08	44° 19' 8.65"	59° 22' 22.08"	1478

Table 1. (Continued)

Ref.	Station	Latitude (S)	Longitude (W)	Depth (m)
17	PAT0209DR05	44° 39' 19.74"	59° 45' 21.27"	991
18	PAT0209DR06	44° 42' 2.81"	59° 13' 54.36"	1898
19	PAT0209DR02	44° 43' 54.78"	59° 36' 28.43"	1248
20	PAT0209DR07	44° 44' 35.55"	59° 19' 17.45"	1620
21	PAT0209DR04	44° 45' 4.05"	59° 16' 0.49"	1577
22	PAT0209DR03	44° 46' 7.74"	59° 26' 15.60"	1513
23	PAT1108DR11	44° 52' 19.38"	59° 38' 7.50"	1248
24	PAT1108DR01	44° 53' 23.23"	60° 1' 41.63"	650
25	PAT1108DR02	44° 54' 30.42"	59º 55' 10.73"	659
26	PAT0209DR09	44° 8' 23.63"	59° 23' 0.20"	1629
27	PAT1108DR09	45° 11' 25.59"	59° 47' 42.16"	941
28	PAT1108DR10	45° 35' 53.84"	59° 47' 23.25"	1051
29	PAT1108DR10R	45° 36' 20.99"	59° 46' 24.24"	1232
30	PAT1208DR05	45° 39' 17.40"	59° 39' 52.14"	1263
31	PAT1208DR07	45° 40' 56.46"	59° 55' 39"	839
32	PAT1208DR03	45° 49' 34.20"	59° 47' 54.26"	924
33	PAT1208DR04	45° 49' 37.08"	59° 47' 59.28"	973
34	PAT1208DR09	45° 51' 20.10"	59° 41' 19.62"	1038
35	PAT1208DR16	45° 51' 40.079"	59° 57' 4.5"	761
36	PAT1208DR14	45° 57' 51.00"	59° 47' 53.28"	854
37	PAT1208DR11	45° 58' 29.94"	59° 37' 9.72"	1088
38	PAT0108DR14	45° 8' 5.72"	59° 31' 47.60"	1180
39	PAT1208DR06	46° 1' 37.51"	59° 56' 23.81"	748
40	PAT1208DR17	46° 13' 2.10"	60° 18' 31.80"	158
41	PAT1208DR15	46° 14' 24.95"	60° 35' 7.24"	146
42	PAT1208DR01	46° 15' 33.36"	60° 42' 50.82"	825
43	PAT1208DR12	46° 23' 54.90"	60° 53' 8.7"	138
44	PAT1208DR13	46° 24' 46.14"	60° 53' 49.14"	140
45	PAT1108DR08	46° 33' 38.33"	60° 48' 54.11"	145
46	PAT1108DR03	46° 38' 22.62"	59° 30' 23.22"	1061
47	PAT1108DR07	46° 38' 40.53"	60° 51' 57.58"	148
48	PAT1108DR04	46° 57' 43.19"	59° 21' 49.08"	1242
49	PAT1208DR10	46° 6' 54.60"	59° 34' 15.63"	959
50	PAT1208DR02	46° 8' 43.32"	60° 16' 43.56"	846
51	PAT1108DR05	47° 16' 26.17"	59° 10' 49.90"	1399

Fig. 1. Map of the sampling stations from AP shelf and slope.



Species identification and literature data

Once on board, the colonies of bryozoans were preserved in 70% ethanol for taxonomic identification using the existing literature: d'Orbigny (1842), Busk (1884), Waters (1904), Hastings (1943), López Gappa (1982), Gordon (1984, 1986, 1989), López Gappa & Lichtschein (1990), Hayward (1995), López de la Cuadra & García-Gómez (2000), Branch & Hayward (2005), Hayward & Winston (2011) and Ramalho *et al.* (2011).

Some literature data regarding bathymetric ranges and biogeographic distribution of the studied species were obtained from Busk (1884), Hastings (1943), López Gappa (1982, 2000), López Gappa & Lichtschein (1990), Branch & Hayward (2005), Hayward & Winston (2011) and Ramalho *et al.* (2011), as well as from the SCAR's Marine Biodiversity Information database (SCAR-MarBIN; http://www.scarmarbin.be/) and the Global Biodiversity Information Facility database (GBIF; www.gbif.org); (Table 2; Appendix S2).

Table 2. Bathymetric ranges and biogeographic distributions of the species studied using data from the present study, the literature, and the GBIF and SCAR databases (see also www.bryozoa.net and invertebrates.si.edu/antiz). *New bathymetric range described in this study, **First record for Argentina. Localities: AN = Antarctica; AR = Argentina; AT = Atlantic; AU = Australia; B = Brazil; BI = Bouvet Island; CH = Chile; CI = Cook Islands; FI = Falkland Islands (Malvinas); FR = France; G = Greece; H = Hungary; HI/MI = Heard Island and McDonald Islands; M = Morocco; N = Norway; NA = North Atlantic; NZ = New Zealand; SAF = South Africa; SAM = South America; SAT = South Atlantic; SE = Seychelles; SG/SS = South Georgia and the South Sandwich Islands; SH = Saint Helena; SI = South Indian Ocean; SO = Somalia; U =Uruguay.

Species	**Bathymetric range (m)**	**Biogeographic distribution**	**References**
Adelascopora secunda Hayward & Thorpe, 1988	45–1635.33* (previously 903)	AN, AR, CH, SG/SS	GBIF and SCAR databases
Adeonella sp.	485*	AR**	This study
Aimulosia australis Jullien, 1888	18–1500* (previously 250)	AN, AR, CI, CH, SAT, SG/SS	López Gappa (2000); GBIF and SCAR databases
Amastigia benemunita (Busk, 1884)	50–586.33* (previously 341)	AR, CH, F, SH	López Gappa (2000); GBIF and SCAR databases
Amastigia crassimarginata (Busk, 1884)	368–990.67* (previously 463)	AR	López Gappa (2000); GBIF and SCAR databases
Amastigia gaussi (Kluge, 1914)	5–1586	AN, AR**, FI, SG/SS	López Gappa (2000); GBIF and SCAR databases
Amastigia sp. 1	157.67*	AR**	This study
Amastigia sp. 2	145*–146*	AR**	This study
Amphiblestrum familiaris Hayward & Thorpe, 1989	40–2032	AN, AR**	GBIF and SCAR databases

Table 2. (Continued)

Species	**Bathymetric range (m)**	**Biogeographic distribution**	**References**
Apiophragma hyalina (Waters, 1904)	100–1538	AN, AR**	GBIF and SCAR databases
Arachnopusia sp.	1478.33*–1635.33*	AR**	This study
Aspericreta favulosa Hayward & Thorpe, 1989	286–1247.67* (previously 1208)	AN, AR**	GBIF and SCAR databases
Aspericreta sp.	1629*	AR**	This study
Aspidostoma coronatum (Thornely, 1924)	104–1581.33* (previously 975)	AN, AR**, SG/SS	GBIF and SCAR databases
Aspidostoma giganteum (Busk, 1854)	186–1893	AN, AR, CH, FI, SAT	López Gappa (2000); GBIF and SCAR databases
Austroflustra australis López Gappa, 1982	140*–1241.67* (previously 272-535)	AR, FI, SAT	López Gappa (2000); GBIF and SCAR databases
Austroflustra gerlachi López Gappa, 1982	272–1897.67* (previously 535)	AR, FI, SAT	López Gappa (2000); GBIF and SCAR databases
Buffonellodes rimosa Jullien, 1888	0–846.03* (previously 121)	AN, AR**, AT, FI, G, H, H, NZ	López Gappa (2000); GBIF and SCAR databases
Buffonellodes sp.	1241.67*-1620*	AR**	This study
Cabarea darwinii var. *guntheri* Hastings, 1943	336–1096* (previously 463)	AR	López Gappa (2000); Hastings (1943); GBIF and SCAR databases

Table 2. (Continued)

Species	**Bathymetric range (m)**	**Biogeographic distribution**	**References**
Caberea darwinii Busk, 1884	5–1513* (previously 697)	AN, AR, AU, B, CH, FI, HI/MI, N, NZ, SAF, SG/SS, SH	López Gappa (2000); GBIF and SCAR databases
Caberea sp.	761.33*	AR**	This study
Camptoplites bicornis var. *quadriavicularis* Hastings, 1943	336–1399* (previously 341)	AR, SAT	López Gappa (2000); GBIF and SCAR databases
Cellaria clavata (Busk, 1884)	0–3442	AN, AR, FR, CH, FI, HI/MI, SAT, SG/SS, SI	López Gappa (2000); GBIF and SCAR databases
Cellaria malvinensis (Busk, 1852)	0–846.03* (previously 548)	AN, AR, CH, FI, M, NZ, SAF, SAT, SG/SS, SI	GBIF and SCAR databases
Cellarinella dubia Waters, 1904	0–516	AN, AR, AU, CH, FI	López Gappa (2000); GBIF and SCAR databases
Cellarinella sp.	140*–854*	AR**	This study
Chaperiopsis patulosa (Waters, 1904)	15–838.67* (previously 500)	AN, AR, FI	López Gappa (2000); GBIF and SCAR databases
Chaperiopsis sp.	1472.33*–1635.33*	AR**	This study
Chartella notialis Hayward & Winston, 1994	272–1148.33* (previously 511)	AN, AR	GBIF and SCAR databases
Chronocerastes sp.		AR**	This study

Table 2. (Continued)

Species	**Bathymetric range (m)**	**Biogeographic distribution**	**References**
Columnella magna (Busk, 1884)	748*–-5340 (previously 890)	AN, AR, NZ, SAF, SE, SG/SS, SO	López Gappa (2000); GBIF and SCAR databases
Cornucopina pectogemma (Goldstein, 1882)	80–3501	AN, AR**, NA, NZ, SAF, SAT, SG/SS, SH, SI	GBIF and SCAR databases
Cornucopina sp.	1061.5*	AR**	This study
Ellisina sp.	1635.33*	AR**	This study
Escharella sp.	1478.33*	AR**	This study
Euthyroides sp.	1247.67*	AR**	This study
Exochella hymanae (Rogick, 1956)	15–1629.00* (previously 1162)	AN, AR**, SG/SS	GBIF and SCAR databases
Fenestrulina antarctica Hayward & Thorpe, 1990	18–1528.67* (previously 579)	AN, AR**	GBIF and SCAR databases
Fenestrulina crystallina Hayward & Ryland, 1990	93–1620* (previously 522)	AN, AR**	GBIF and SCAR databases
Fenestrulina fritilla Hayward & Ryland, 1990	132–1528.67* (previously 1150)	AN, AR, FI, SG/SS	López Gappa (2000); GBIF and SCAR databases
Fenestrulina sp.	990.67*	AR**	This study
Figularia sp.	1148.33*–1635.33*	AR**	This study

Table 2. (Continued)

Species	**Bathymetric range (m)**	**Biogeographic distribution**	**References**
Foveolaria elliptica Busk, 1884	384–987	AR, AU, CH, NZ	López Gappa (2000); GBIF and SCAR databases
Foveolaria terrifica (Hincks, 1881)	145–1629* (previously 297)	AR, FI	López Gappa (2000); GBIF and SCAR databases
Galeopsis bullatus Hayward, 1993	177–761.33* (previously 177)	AN, AR**, HI/MI, SG/SS	GBIF and SCAR databases
Gigantopora spathula Hayward & Winston, 2011	384–990.67* (previously 494)	AN, AR**	Hayward & Winston (2011)
Himantozoum obtusum Hastings, 1943	40–511	AN, AR, FI, SAT	López Gappa (2000); GBIF and SCAR databases
Himantozoum sp.	1399*	AR**	This study
Hippomonavella ramosae López de la Cuadra & García-Gómez, 2000	212–1472.33* (previously 231)	AR**, SAT, SG/SS	GBIF and SCAR databases
Ichthyaria oculata Busk, 1884	145*–297 (previously 272)	AR, FI, U	López Gappa (2000); GBIF and SCAR databases
Ipsibuffonella sp.	1513*–1629*	AR**	This study
Jolietina latimarginata (Busk, 1884)	73–854* (previously 73)	AN, AR, FI, SAM	López Gappa (2000); GBIF and SCAR databases
Lageneschara peristomata Hayward & Winston, 2011	124–1513* (previously 128)	AN, AR**	Hayward & Winston (2011)

Table 2. (Continued)

Species	**Bathymetric range (m)**	**Biogeographic distribution**	**References**
Lageneschara sp.	973.33*	AR**	This study
Malakosaria sp.	1051.33*–1247.67*	AR**	This study
Melicerita blancoae López Gappa, 1981	74–1247.67* (previously 621)	AN, AR, FI, SAT	López Gappa (2000); GBIF and SCAR databases
Melicerita sp.	145*–1577*	AR**	This study
Membranicellaria sp.	1263*–1577*	AR**	This study
Menipea flagellifera Busk, 1884	59–825.17* (previously 697)	AN, AR, FI, SAF, SAT, SI	López Gappa (2000); GBIF and SCAR databases
Micropora brevissima Waters, 1904	5–1577* (previously 1162)	AN, AR, CH, FI, SAF, AN, SG/SS	López Gappa (2000); GBIF and SCAR databases
Microporella crustula Hayward & Winston, 2011	119–825.17* (previously 124)	AN, AR**	Hayward & Winston (2011)
Microporella hyadesi (Jullien, 1888)	49*–903*	AR, AT, CH, FI, NZ, SG/SS	López Gappa (2000); GBIF and SCAR databases
Microporella sp.	990.67*–1581.33*	AR**	This study
Notoplites antarcticus Waters, 1904	104–990.67* (previously 634)	AN, AR**	GBIF and SCAR databases

Table 2. (Continued)

Species	**Bathymetric range (m)**	**Biogeographic distribution**	**References**
Ogivalia elegans (d'Orbigny, 1842)	73–1244.33* (previously 866)	AR, CH, FI, SAT	López Gappa (2000); GBIF and SCAR databases
Ogivalia sagittirostra Hayward & Winston, 1994	384–973.33* (previously 494)	AN, AR, CH, FI, SG/SS	GBIF and SCAR databases
Orthoporidra brachyrhyncha Moyano, 1985	104–1635.33* (previously 975)	AN, AR	GBIF and SCAR databases
Orthoporidra compacta (Waters, 1904)	5–2010	AN, AR**, NZ, SG/SS	GBIF and SCAR databases
Orthoporidra stenorhyncha Moyano, 1985	120–1289	AN, AR**	GBIF and SCAR databases
Osthimosia sp.	138.33*	AR**	This study
Osthimosia bicornis (Busk, 1881)	0–2000	AN, AR, BI, CH, FI, HI/MI, NZ, SAF, SG/SS	López Gappa (2000); GBIF and SCAR databases
Osthimosia clavata Waters, 1904	15–1629* (previously 1414)	AN, AR	GBIF and SCAR databases
Osthimosia malingae Hayward, 1992	61–761.33* (previously 247)	AN, AR**, SAT	GBIF and SCAR databases
Osthimosia notialis Hayward, 1992	18–846.03* (previously 622)	AN, AR, SAT, SG/SS	GBIF and SCAR databases
Paracellaria elephantina Hayward & Thorpe, 1989	485–1478.33* (previously 610)	AN, AR**, SAT	GBIF and SCAR databases
*Paracellaria elizabetha*e Branch & Hayward, 2005	360–1478.33* (previously 376)	AR**, SAF	Branch & Hayward (2005)

Table 2. (Continued)

Species	**Bathymetric range (m)**	**Biogeographic distribution**	**References**
Parasmittina dubitata Hayward, 1980	6–1248* (previously 144)	AR, CH, FI, SAT	López Gappa (2000); GBIF and SCAR databases
Reteporella antennata Ramalho, Muricy & Taylor, 2011	341–485* (previously 341)	AR**, B	Ramalho, Muricy & Taylor (2011)
Reteporella gigantea (Busk, 1884)	360–1513* (previously 914.4)	SAF	Branch & Hayward (2005); Busk (1884)
Reteporella longichila Hayward, 1993	7–748* (previously 634)	AN, AR**	GBIF and SCAR databases
Reteporella magellensis (Busk, 1884)	91–1393.33* (previously 1097)	AR, FI, SAT	Hayward & Winston (2011)
Reteporella sp. 1	973.33*–1528.67*	AR**	This study
Reteporella sp. 2	990.67*–1577*	AR**	This study
Reteporella sp. 3	1247.67*–1629*	AR**	This study
Reteporella sp. 4	854*–973.33*	AR**	This study
Reteporella sp. 5	854*	AR**	This study
Reteporella sp. 6	973.33*–1393.33*	AR**	This study
Reteporella sulcula Hayward & Winston, 2011	384–940.67* (previously 494)	AN, AR**	Hayward & Winston (2011)
Reteporella tortuosa Hayward & Winston, 2011	384–1472.33* (previously 494)	AN, AR**	Hayward & Winston (2011)

Table 2. (Continued)

Species	**Bathymetric range (m)**	**Biogeographic distribution**	**References**
Smittina anecdota Hayward & Thorpe, 1990	170–1635.33* (previously 1489)	AN, AR**, HI/MI	GBIF and SCAR databases
Smittina rogickae Hayward & Taylor, 1984	0–1635.33* (previously 1497)	AN, AR**	GBIF and SCAR databases
Smittina sp. 1	1513*	AR**	This study
Smittina sp. 2	1393.33*–1629*	AR**	This study
Smittinella sp. Canu & Bassler, in Bassler, 1934	761.33*	AR**	This study
Smittoidea malleata Hayward & Thorpe, 1989	73–1897.67* (previously 1150)	AN, AR**	GBIF and SCAR databases
Smittoidea sp. 1	1513*–1635.33*	AR**	This study
Smittoidea sp. 2	138.33*	AR**	This study
Spigaleos sp. 1	748*–1620*	AR**	This study
Spigaleos sp. 2	1244.33*	AR**	This study
Talivittaticella frigida (Waters, 1904)	145–1577* (previously 600)	AN, AR, SAF	Branch & Hayward (2005); López Gappa (2000)
Turritigera cribrata Hayward, 1993	73–1581.33* (previously 628)	AN, AR**, AU, SG/SS	GBIF and SCAR databases
New genus 1	1399*–1629*	AR**	This study
New genus 2	1472.33*	AR**	This study

Table 2. (Continued)

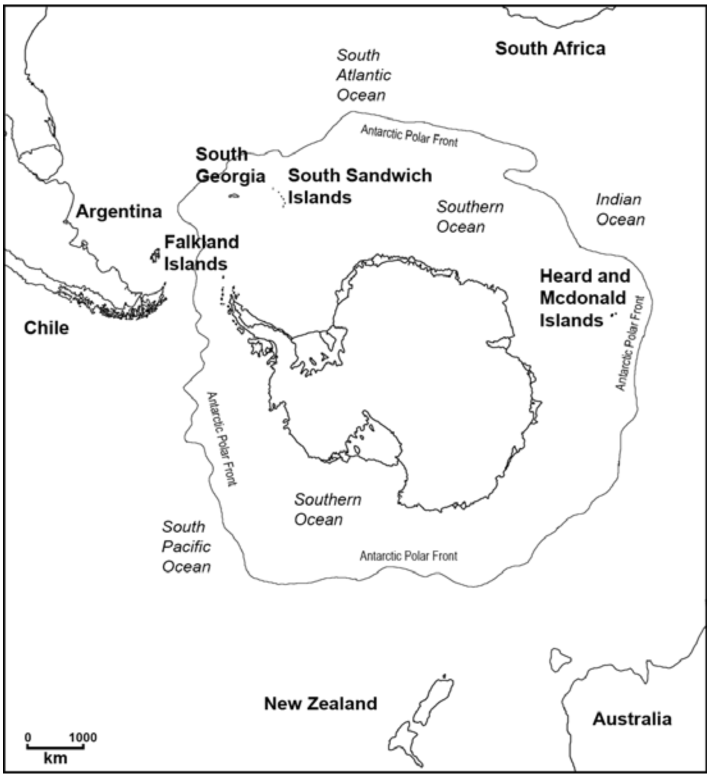
Species	**Bathymetric range (m)**	**Biogeographic distribution**	**References**
New genus 3	1096*–1553*	AR**	This study
New genus 4	1577*	AR**	This study
New genus 5	1635.33*	AR**	This study

Statistics

Relative abundance (N), relative species richness (S, number of species present) and three diversity indices (Margalef, Shannon-Wiener and Simpson's) were calculated for each slope region and latitude sampled in the AP region, in order to provide new information about community composition of cheilostome bryozoans. The Margalef (D_{MG}) index is based on the number of species (species richness), while the others are indices of proportional abundances of species. The Shannon-Wiener (H') index is strongly influenced by the occurrence of rare species and Simpson's (1- Lambda') index by the importance of the most dominant species. An expected species accumulation curve was also computed, and Chao1 and Jacknife1 methods were used to estimate the theoretical number of expected species in this region. With the purpose of knowing if a relationship exists between the number of bryozoan species and the depth or latitude from the AP region, non-parametric correlations were calculated (Kendall's tau; Sokal & Rohlf 1981).

In order to better represent the spatial patterns of bryozoan communities from the entire Argentine region along depth ranges and compared to other neighboring regions, our new data from Patagonia were analysed together with previous data on depth ranges and biogeographic regions from Argentina (Fig. 2).

Fig. 2. General map of neighboring regions of Antarctica.



Presence/absence data were used to perform similarity matrices using the Bray-Curtis similarity index. Bray-Curtis index was chosen, as one of the most widely employed indices, being equivalent to the Sörensen index for presence–absence matrices (Clarke *et al.* 2006; Legendre & Legendre 2012). The resulting similarity matrices were analyzed by cluster analysis (single-linkage clustering method) and multi-dimensional scaling (MDS). The cluster was then plotted to evaluate the similarities in species composition between the different regions. The MDS analysis was used to evaluate the similarities between ranges of depth for the species because it assumes no shape between variables (Legendre & Legendre 2012). In order to categorize the continuous variable depth and to represent it in the MDS analysis, it was divided into 100 m interval categories (e.g. 100 m category includes depths of 0–100 m). The first two dimensions were plotted and the distance between dots denotes their similarity measured by the stress value. A stress value of less than 0.1 indicates that the plot accurately represents similarities, while a stress value greater than 0.3 indicates that the points are close to being randomly placed (Clarke 1993). Bathymetric ranges and biogeographic regions for each species found in the current study and species from Argentina are detailed in Table 1 and Appendix S2, respectively.

In order to verify that the defined groups were statistically supported, we performed an analysis of similarity (ANOSIM), Global R statistic, which does not require normal distributional data. The ANOSIM randomization test compares within- and between-group similarity of elements measured by the Bray-Curtis index and calculates a global R statitistic. The resulting *R*-value ranges between 0 and 1, with high values indicating a large degree of discrimination among groups (Clarke & Green 1988). To identify the taxa that better explain the differences between the various depth zones, SIMPER analyses were carried out using Bray-Curtis similarity matrices (Appendix S1). Low contributions were set at 60%. All statistic analyses were performed using Vegan software (R version 2.15.2).

Results

A total of 108 species of cheilostome bryozoans (378 samples), belonging to 34 families and 59 genera, were found at depths between 138.33 and 1897.67 m within an area of the southwestern Atlantic between 42º and 47º S, and between 57º and 60º W (Table 1 and 2; Fig. 1). The list of identified samples includes 5 new genera and 36 new species which will be described in further studies. Also, we found a new, second species of the genus *Membranicellaria* and a new, fourth species of the genus *Malakosaria,* as well as the second record in this region for seven other species. Furthermore, a total of 71 out of 108 species (65.7%) were reported for the first time for Argentina. Therefore, an expansion of their known geographical distribution is reported here as well (Table 2). Remarkably, a high percentage of the identified species (48 of 108, 44.4

%) had been reported to have an Antarctic distribution previously, while only two species found here were reported to be endemic to Argentina (Table 2).

The most common family was Smittinidae with 57 samples (15 %), followed by Phidoloporidae (42 samples, 11.1 %), Aspidostomatidae (41, 10.8%), Cellariidae (31, 8.2 %), Celleporidae (24, 6.3%), Microporellidae (22, 5.8 %), Buffonellodidae (19, 5%), Flustridae and Lekythoporidae (16, 4.2 %) and Foveolariidae (15, 3.9 %). The new species belong to the genera *Adeonella*, *Amastigia*, *Arachnopusia*, *Aspericreta*, *Buffonellodes*, *Caberea*, *Cellarinella*, *Cornucopina*, *Ellisina*, *Escharella*, *Euthyroides*, *Fenestrulina*, *Figularia*, *Himantozoum*, *Ipsibuffonella*, *Lageneschara*, *Malakosaria*, *Melicerita*, *Membranicellaria*, *Microporella*, *Orthoporidra*, *Osthimosia*, *Reteporella*, *Smittina*, *Smittinella*, *Smitoidea* and *Spigaleos*. *Smittina* and *Aspidostoma*, with 47 and 41 samples respectively, were the dominant genera. The most abundant species was *Aspidostoma giganteum*, which represented 10.8 % of the total specimens collected, and *Smittina rogickae* (6.8 %).

Species richness and diversity indices

The highest values of species richness and of the three diversity indices were found around latitude 44° S (Table 3; % S = 57; D_{MG} = 11.617, H’ = 3.699 and 1-Lambda’ = 0.963) with *Aspidostoma giganteum*, *Smittina anecdota* and *S. rogickae* as dominant species. In contrast, the values were low at latitudes 41° and 47° S. In reference to depth, the middle slope had the highest values of species richness and diversity (Table 4; % S = 77.78; D_{MG} = 14.94, H’ = 3.89 and 1-Lambda’ = 0.964), with *A. giganteum* as the dominant species.

The expected species accumulation curve has still to reach the asymptote (Fig. 3). Species richness estimates (Chao1 and Jack1) indicated that the theoretical number of expected species would be between 162 and 155, respectively.

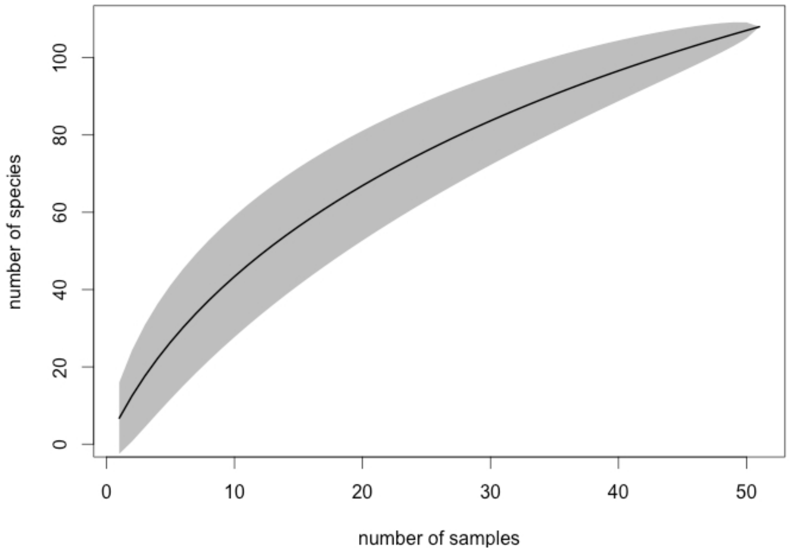
Table 3. Species distribution and latitude. Number of stations, dominant species found in the sample, total number of individuals found (no. ind), percentage of relative species richness (% S) , Margalef index (D_{Mg}), Shannon–Wiener diversity index, H’ (base $\log_e$) and Simpson’s Index (1 – Lambda’) for each latitude zone.

Latitude (S)	no. stations	Dominant species	no. ind	%S	D_{MG}	H’	1 – Lambda’
41º	1	*A. coronatum*, *C. notialis*, *F. terrifica*, *M. blancoae*, *O. brachyrhyncha*	5	5	2.485	1.609	0.8
42º	6	*A. secunda*, *A. giganteum*, *C. clavata*	28	20	5.701	2.88	0.936
43º	7	*A. giganteum*, *S. anecdota*, *S. rogickae*	72	36	8.183	3.353	0.956
44º	12	*A. giganteum*, *S. anecdota*, *S. rogickae*	124	57	11.617	3.699	0.963
45º	12	*A. giganteum*, *Spigaleos* sp.	71	38	8.679	3.39	0.955
46º	12	*A. vulgaris*, *C. clavata*	71	37	8.445	3.379	0.957
47º	1	*A. giganteum*	7	6	2.569	1.747	0.816

Table 4. Species distribution and depth. Number of sampled stations, dominant species found in the sample, total number of individuals found (nº ind), Margalef index (D_{Mg}), Shannon–Wiener diversity index, H’ (base $\log_e$) and Simpson’s Index (1 – Lambda’) at different depth ranges.

Depth range	no. stations	Dominant species	no. ind	%S	D_{MG}	H’	1 – Lambda’
continental shelf (0–200)	6	*C. clavata*	39	21.3	6.01	2.968	0.939
upper slope (200–800)	7	*C. clavata*, *M. blancoae*	44	27.78	7.66	3.229	0.95
middle slope (800–1600)	34	*A. giganteum*	259	77.78	14.936	3.895	0.964
lower slope (1600–1900)	4	*A. giganteum*, *S. rogickae*	36	22.22	6.418	3.015	0.941

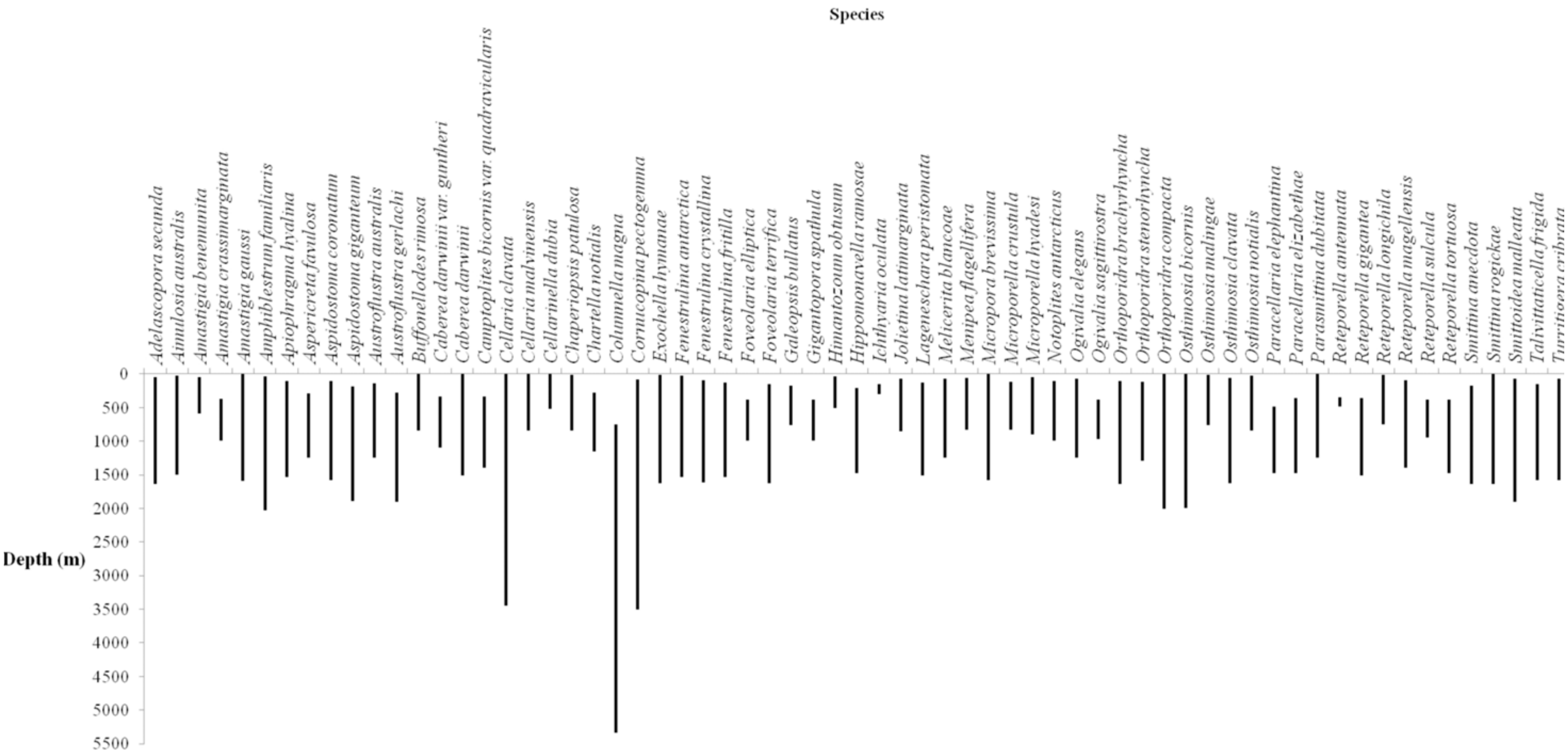
Fig. 3. Expected species accumulation curve (black solid line) with 95% confidence interval (grey area) based on data from 51 stations for bryozoans from the AP region.



Bathymetric distribution

The new data from this study were analysed jointly with data from the literature and existing databases, revealing new bathymetric ranges in 94 species (87 % of the species found in our study). *Columnella magna*, *Cornucopina pectogemma* and *Cellaria clavata* were the only species found in deeper waters (5340, 3501 and 3442 m, respectively) and showed the widest bathymetric ranges. The majority of species (72.2 %) were present at depths between 0 and 1600 m. Of a total of 108 species analysed from the literature and our new data, no species were restricted to the continental shelf (128–200 m as defined by Portela *et al.* 2012; Fig. 4).

Fig. 4. New bathymetric ranges of the bryozoan species found in the present study, including our own data, the literature and the GBIF and SCAR databases.



The correlations between species number and depth (tau = 0.7914, p = 0.4287; Fig. 5) and latitude (tau = -0.7997, p = 0.4239; Fig. 6) from the AP region were not significant.

Fig. 5. Number of bryozoan species from the AP region for each depth range. The correlation between species number and depth (tau = 0.7914, p = 0.4287) from the AP region was not significant.

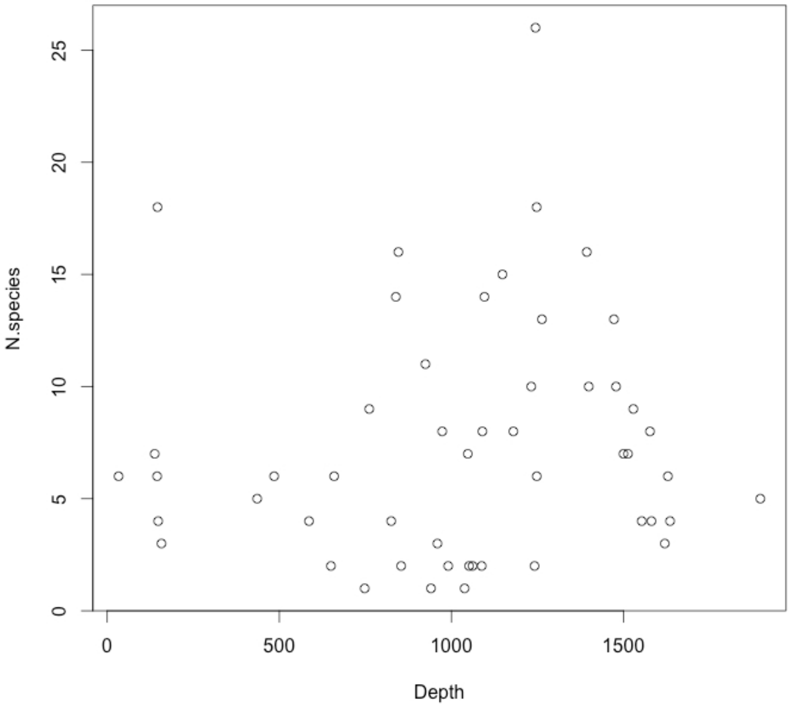
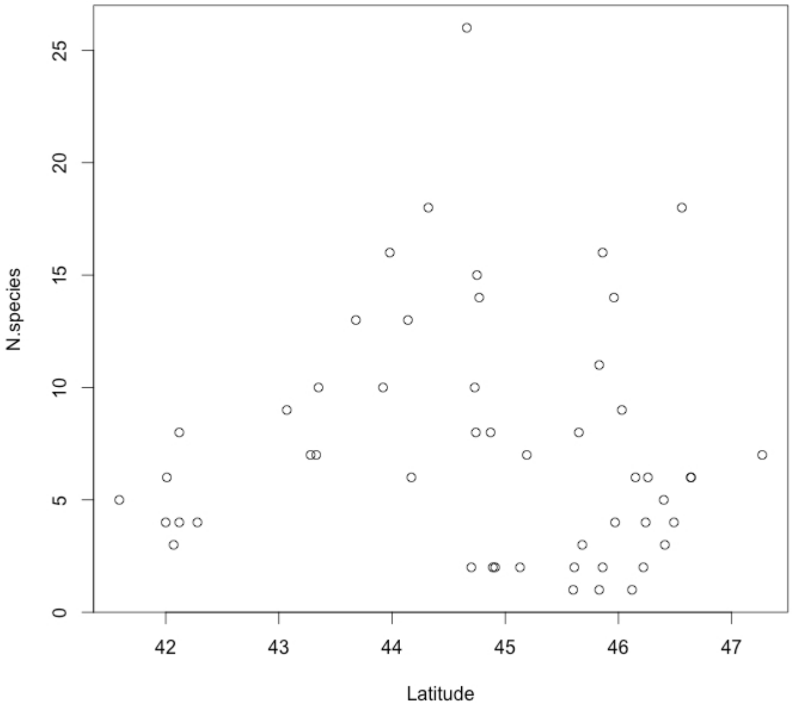
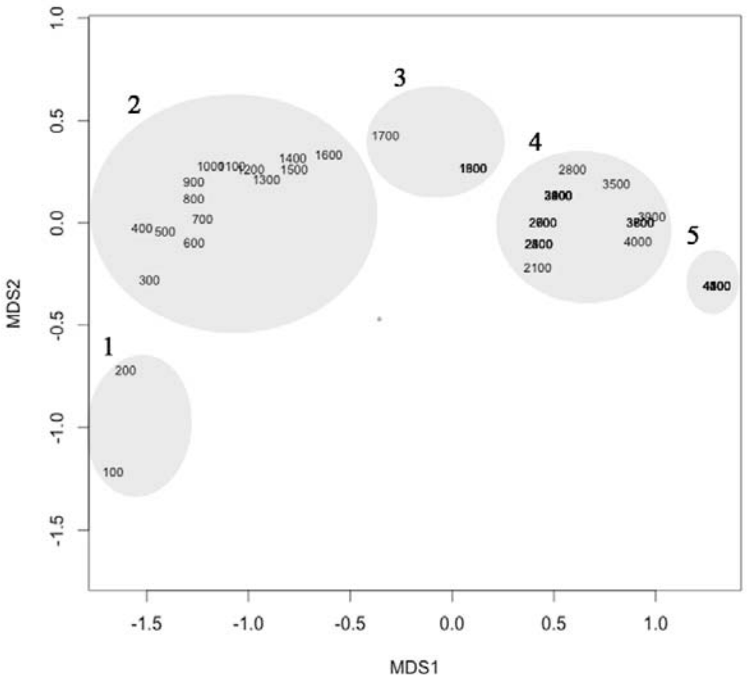


Fig. 6. Number of bryozoan species from the AP region at different latitudes. The correlation between species number and latitude (tau = -0.7997, p = 0.4239) from the AP region was not significant.



Low MDS stress values (0.03) indicate good representation in the 2-dimensional ordination (Clarke 1993). Five depth zones were discriminated by the MDS analysis in the bathymetric distribution of the species from Argentina (Fig. 7): (1) a zone between 0 and 200 m with the presence of 125 species, (2) a zone between 200 and 1600 m characterized by the presence of 85 species, (3) a zone between 1600 and 1900 m with the presence of 14 species; (4) a zone between 1900 and 3900 m with the presence of 8 species and (5) a zone between 3900 and 4500 m, with the presence of 6 species. A significant difference in species composition between groups was found (ANOSIM Global R = 0.9306, p= 0.001). A high number of species (71, 54, 52 and 53, respectively) accounted for about 60 % of the dissimilarity between group 1 and groups 2, 3, 4 and 5 (Appendix S1). Twenty-six species contribute the most to these differences between groups 2 and 3, and between 2 and 4, and 27 species to the differences between 2 and 5. *Aspidostoma giganteum*, *Austroflustra gerlachi*, *Smittoidea malleata* and *Talivittacella problematica* contributed most in explaining the differences between groups 3 and 4. In the case of group 3 versus group 5, the differences were mainly explained by *Cellaria clavata*, *Formosocellaria magnifica*, *Smittoidea malleata* and *Columnella gracilis*. The highest contribution in the differences between groups 4 and 5 was due to *Cellaria clavata*, *Formosocellaria magnifica*, *Columnella gracilis* and *C. magna armata* (to know which groups these particular species are associated with, see Appendix A and B).

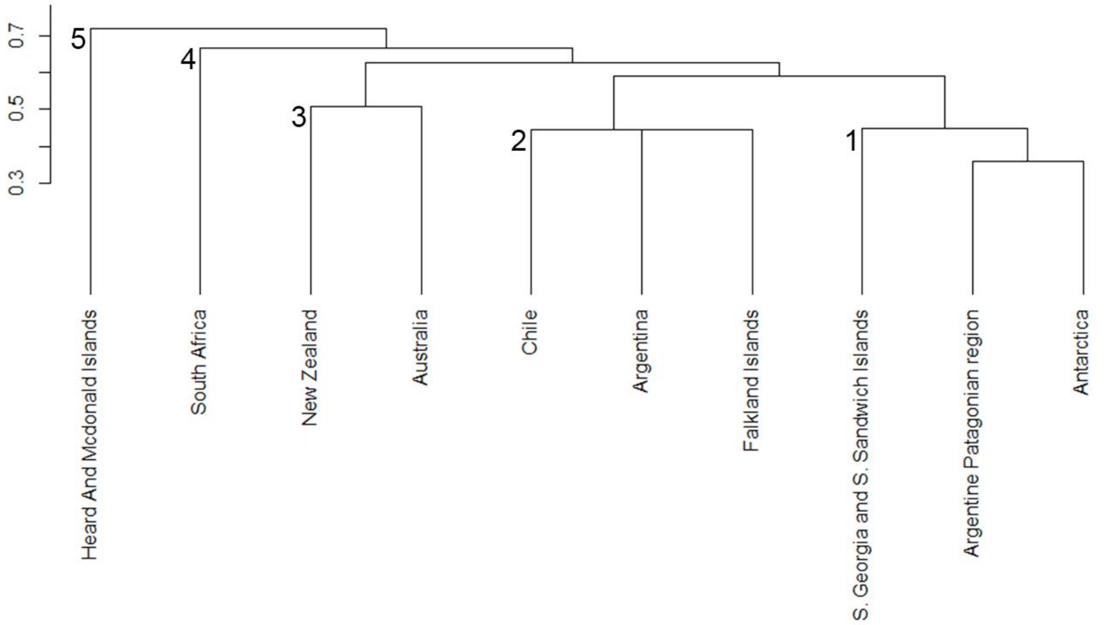
Fig. 7. Multidimensional scaling ordination (MDS) of the different species in relation to depth using data from the present study, the literature and the GBIF and SCAR databases. Points numbered 100–4500 correspond to different depth ranges (stress = 0.03). Group 1: 0–200 m; group 2: 200–1600 m; group 3: 1600–1900 m; group 4: 1900–4000 m and group 5: 4000–4500 m.



Biogeographic distribution

Cluster analyses suggested five principal groups of similar faunal composition between regions (Figs 2 and 8). The first group (1) was represented by the Antarctic and AP regions (this study), with 46 species in common, and subantarctic South Georgia and the Sandwich Islands with 18 shared species. Other South American regions (Argentina, Chile and Falkland Islands), all clustered together (2), and were notably close to the first group, sharing 39 species between them. The temperate Australian and New Zealand regions were more dispersed, both forming the same cluster (3), with 17 species in common. Finally, South Africa (4) and the isolated Heard and McDonald Islands (Kerguelen Plateau) (5) were strong outliers, characterized by a lower number of species. The ANOSIM tests showed that these groupings were statistically strong (ANOSIM Global R = 0.992, p= 0.001).

Fig. 8. Dendrogram of the hierarchical clustering (single linkage) of the bryozoan fauna using Bray-Curtis distance. Additional data have been obtained from the literature and from GBIF and SCAR databases.



Discussion

Cheilostome bryozoans from the AP continental shelf and slope exhibit a high range of eurybathy, with new bathymetric ranges in 94 species (87 % of the studied species). Remarkably, 43 bryozoan species have been recorded here deeper than 1000 m, the most eurybathic species being *Columnella magna*, with a depth range of 748–5340 m. Thus, an expansion in the known geographic distribution of most the species has been reported here. In agreement with this, few bryozoan species have been reported deeper than the shelf break and most samples come from less than 500 m (López Gappa 2000). Considering that 44.4 % of the bryozoan species found here were also previously reported in Antarctica, the bathymetric ranges determined by our study are in agreement with other studies establishing wide depth ranges for most Antarctic cheilostome bryozoans (Barnes & Kuklinski 2010; Figuerola *et al.* 2012). Moreover, recent studies reported a lower proportion of Antarctic cheilostome bryozoan species to be endemic, supporting the idea that the Southern Ocean may have been less isolated over geological time than once thought (56 %, Barnes & Griffiths 2008; 55 %, Figuerola *et al.* 2012). The low endemicity and wide eurybathy of bryozan species found in this study also demonstrate their capacity to live in regions with a wide range of conditions (e.g. temperature and salinity), as Barnes & Griffiths (2008) suggested.

High diversity was found in the AP region, with 28 % of the analysed samples (378) belonging to different species, including several new genera and new species of the genus *Membranicellaria*. The highest values of percentage relative species richness (% S = 57) and of the three diversity indices (D_{MG} = 11.617, H' = 3.699 and 1-Lambda' = 0.963) were found at about 44º latitude. These values are in good agreement with the large biological production found on the continental shelf and slope at latitudes 30º to 46º S, which clearly allows for the establishment of diverse benthic invertebrate assemblages. This high diversity is probably related to the existence of the confluence of two major wind-driven currents, the subantarctic nutrient-rich Falkland/Malvinas and the subtropical Brazil currents (López Gappa & Lichtschein 1989; Acha *et al.* 2004; Miloslavich *et al.* 2011). Furthermore, the richest stations from Patagonia occurred on the middle slope (% S = 77.78; D_{MG} =14.94, H' = 3.89 and 1-Lambda' = 0.964), coinciding with the zone of the Falkland/Malvinas Current (below 800–1000 m), where there is upwelling of nutrient-rich waters and associated plankton blooms (Muñoz *et al.* 2012). Similar to our results, a high density of cold-water corals with associated fauna were found on the AP continental slope (Muñoz *et al.* 2012). Cold-water coral ecosystems, with their complex three-dimensional habitat structures, are among the richest biodiversity hotspots in the deep sea, providing food and a multitude of microniches, shelter and nursery areas for a number of associated species, including bryozoans (Van den Hove & Moreau 2007). However, the interpretation of these results must be treated with some caution because of the scarce sampling effort in the middle slope.

Remarkably, 65.7 % of the species analysed were here reported for the first time in Argentina, demonstrating that this region has been poorly explored so far (Hastings 1943; López Gappa & Lichtschein 1990; López Gappa 2000). Indeed, the expected species-accumulation curve showed no sign of approaching an asymptote. One hundred and eight species have been found to date, but the species richness statistics (Chao1 and Jack1) predict that between 162 and 155 species will be found in this region as more samples are collected.

To the best of our knowledge, the existence of horizontal and vertical variability in bryozoan communities found in the current study provides new information on the structure of these communities in Argentina. MDS analysis shows that bryozoans are distributed in zones or depth bands. The AP continental shelf is generally down to 100 m depth (128-200 m; Portela *et al.* 2012), while the upper slope descends to depths from 128–200 m to 250–750 m, and the mid-continental slope from 250–750 to about 1600 m (Muñoz *et al.* 2012). The bryozoan distribution found in the current study fits quite well with these proposed limits: the species composition of the continental shelf (0–200 m) differs from that of the upper and middle continental slope (200–1600 m), the lower slope (1600–4000 m) and deep water (4000–4500 m). Moreover, MDS discriminates two regions in the lower slope (1600–3900 m and 3900–4000 m). These differences in species composition could be explained by the presence of a variable habitat of the slope characterised by both depositional and erosive elements (e.g. terraces, moats and channels), which progressively connect the continental shelf with the abyssal plain between 43º and 49º S (Hernandez-Molina *et al.* 2010; Muñoz *et al.* 2012). SIMPER analyses showed that the differences in species' composition between the continental shelf, upper, middle and lower slope regions were explained by a number of bryozoan species, suggesting that the structure of the bryozoan communities was diverse and complex. In contrast, few species contribute most to explaining the differences in communities between slope and deep waters. This fact may be related to the general bathymetric trend of a decrease in species numbers with increasing depth towards the abyssal basins, which can be related to the decreasing availability of food (e.g. Linse *et al.* 2007). However, the bryozoan species richness value is still largely underestimated in the deeper waters of this region, as discussed above.

Cluster analyses of regional bryozoan species composition support the hypothesis of the sequential separation of Gondwana during the Cenozoic. The Antarctic and AP regions in the current study were clustered together, with 46 species in common, suggesting closer faunal affinities. The regions of Argentina, Chile and the Falkland Islands, with strong geographical links, were notably clustered closer to the first group, while subantarctic South Georgia and the Sandwich Islands occupied an intermediate position between Antarctica and South America. Our results are in agreement with previous studies reporting the occurrence of several shared marine species between Antarctica and South America (e.g. Moyano 1982, 1999; Barnes & De Grave 2001; Arntz *et al.* 2005; Ramos-Espla *et al.* 2005; Barnes & Griffiths 2008; Figuerola *et al.* 2012). Interestingly, some authors suggested a strong similarity between these two regions for molluscs, ascidians and bryozoans (Linse 2002; Primo & Vázquez 2007; Griffiths *et al.* 2009). Thus, these similar compositions could be related to the fact that these were the last fragments drifting apart during the break-up of Gondwana (Clarke 2003; Clarke *et al.* 2005; Lawver & Gahagan 2003; Upchurch 2008).

On the other hand, dispersal mechanisms play an essential role in the distributional patterns (Downey *et al.* 2012). In fact, marine organisms can freely migrate in and out of the Polar Front via the deep abyssal plains (above approximately 3000 m), where the Southern Ocean is connected to the other oceans. A strong deep-sea faunal exchange exists, but it requires some degree of eurybathy in potentially colonizing species (Brandt *et al.* 2007a). In this sense, most bryozoan species in the current study came from depths over 900 m and are eurybathic. Thus, the dispersal pathway could partly explain the high percentage of Antarctic bryozoan species found in the AP region. Supporting this, some authors have suggested that migrations of the Antarctic fauna to South America via the deep ocean occur during glacial maxima, in relation to extinction avoidance, when the continental ice sheets extend to the edge of the continental shelf in many Antarctic areas (e.g. Thatje *et al.* 2005b; Brandt *et al.* 2007a; Clarke 2008). This fact could help to understand some bryozoogeographical links found in this study between neighboring regions. However, we have to keep in mind that nearly all bryozoans possess low larval-dispersal potential (most cheilostomes release lecithotrophic larvae of short pelagic duration), unlike other taxa, and only highly dispersive taxa usually show strong links to other oceans (Brandt *et al.* 2007b; Barnes & Griffiths 2008). In any case, currents can distribute lecithotrophic larvae at considerable distances (Brandt *et al.* 2007a). On the other hand, bryozoans can use diverse potential dispersal mechanisms, such as fouling on a variety of different floating substrata including macroalgae (Watts *et al.* 1998). In fact, bryozoans are commonly effective colonizers of surfaces and are frequently reported as rafters (Thiel & Gutow 2005; Key *et al.* 2012). Moreover, the epibiosis of bryozoan colonies on motile marine animals such as crabs, isopods and pycnogonids can improve gamete dispersal and increase their geographic ranges. In particular, a considerable percentage of Antarctic pycnogonids (26% of 115 pycnogonids belonging to 9 species) were reported to be fouled by Antarctic cheilostome bryozoans (Key *et al.* 2013). Additionally, ten cheilostome bryozoans were found on the abundant giant Antarctic marine isopod *Glyptonotus antarcticus*, and one of the reported species was *Smittina rogickae* (Key & Barnes 1999), frequently found in our study . Furthermore, bryozoans may colonize distant locations via island archipelagos, like those in the Scotia arc, that act as stepping stones.

On the other hand, subantarctic South Georgia and the Sandwich Islands (part of the Scotia Arc archipelagos) are the tips of a subsurface mountain chain linking the Andes and the Antarctic Peninsula, and therefore, they are geographically in an intermediate position. Our results are in accordance with this too. Other studies on different taxa also demonstrated that South Georgia and the Sandwich Islands are transitional regions, thus supporting the role of the Scotia Arc archipelagos as physical links between Antarctica and South America (Arntz *et al.* 2005; Barnes 2005; Primo & Vázquez 2007), with, in some cases, a gradient of similarity between

Patagonia, the Scotia Arc and the Antarctic Peninsula (e.g. Ramos-Espla *et al.* 2005). In agreement with this, the Scotia Arc showed a shelf fauna predominantly Magellanic on the northern branch, whereas on the southern branch it was predominantly Antarctic (e.g. Arntz & Brey 2003).

Brandt *et al*. (2007a) suggested that wider dispersal is produced by the distribution of water masses in the world oceans. The high percentage of bryozoan species from the AP region with an Antarctic distribution found in the current study may suggest that the PT appears to be less of a barrier for this group, as other authors have also debated (e.g. Clarke *et al.* 2005a; Brandt *et al.* 2007a). Recent studies also suggest the existence of a potential permeability of the PF, facilitating the explanation of some zoogeographical links and demonstrating that the barriers to biological invasion are primarily physiological rather than geographical (e.g. Thatje & Fuentes 2003; Thatje *et al.* 2005a). The presence of some common bryozoan species between these regions may also be in part explained by the potential passive northwards transport of larvae (or perhaps even adults) via the Falkland/Malvinas Current to considerable distances (Hastings 1943; Legeckis & Gordon 1982; Brandt *et al.* 2007a). Thus, the influence of subantarctic water in low latitudes leads to conditions favourable to the establishment of cold-water organisms in these regions. Other dispersal pathways, like eddies of ACC, may increase the bryozoan connexion found between these regions, transporting bryozoan colonies attached to driftwood and other marine debris (Clarke *et al.* 2005a). On the other hand, the increase of human dispersal mechanisms (e.g. ballast water and marine debris of anthropogenic origin such as floating plastic) in and out of Antarctica has favoured faunal exchange and the introduction of alien species, by-passing oceanographic barriers (Barnes 2002; Thatje *et al.* 2005a; Aronson *et al.* 2007). Fouling allows very rapid dispersion over scales from kilometers to global level. Interestingly, many bryozoans are important components of biofouling, able to travel on vessel hulls and human/marine debris (Watts *et al.* 1998). Accordingly, most of the exotic bryozoans found on the Argentine coast were components of the fouling community restricted to commercial harbors, such as that of Mar del Plata (Orensanz *et al.* 2002). However, part of the difficulty for potential colonizers is the drop in temperature across the PF. In any case, climate change will probably allow the establishing of new taxa in Antarctica, overcoming the current marine barriers.

Our study also showed that the Australasian region (New Zealand and Australia) tends to cluster separately, supporting the idea that this region was separated long before the Antarctic-South American separation. Finally, the regions of South Africa and the Antarctic Heard and McDonald Islands represent the most separated groups. In agreement with the low values of similarity of South Africa with other regions, the first break across Gondwana was probably initiated in the mid-Jurassic, when East Gondwana, comprising Antarctica, Madagascar, India and Australia, began to separate from Africa (Upchurch 2008). In support, "hotspots" of cheilostome regional endemisms have been found in South Africa (Barnes & Griffiths 2008; Griffiths *et al.* 2009). On the other hand, the remote Heard and McDonald Islands (53° S), situated in the south Indian Ocean, within the PF between South Africa and Australia, present low affinity to the other regions, as showed by the cluster analysis. The eastwards flow of the ACC, carrying bryozoan species attached to drifting natural (e.g. macrophytes) or artificial substrata, could explain the long-distance dispersal of some bryozoan species (Barnes 2002). Barnes (2002) reported Bryozoa as one of the most abundant taxa on marine debris. Also, Watts *et al.* (1998) argued that rafting was a key element in the biogeography of cheilostome bryozoans.

In agreement with this, Moyano (1999) also found a mixture of Magellanic and Antarctic bryozoan species in other remote subantarctic regions of the Indian Ocean influenced by the ACC (Prince Edward, Crozet and Kerguelen). In fact, Heard Island and other subantarctic islands clustered together in a previous bryozoan diversity study (Barnes & Griffiths 2008). Moreover, Griffiths *et al.* (2009) reported that the majority of species found at Heard Island were also recorded at Kerguelen Island, suggesting that this similarity is related to the fact that, in the past, both islands (which share the Kerguelen Plateau) were on the same side of the PF. Considering that the greatest outliers in the analyses were these Antarctic islands, and that they have not been adequately sampled bryozoologically, their dissimilarity could perhaps be related to undersampling and to the large distances between Antarctica and other regions of the southern hemisphere, as well as to their isolation and the long period of separation of the land masses.

Summary and conclusions

A high diversity of bryozoans was found in the AP region, probably related to the existence of the confluence of two major wind-driven currents, the subantarctic nutrient-rich Falkland/Malvinas and the subtropical Brazil currents. Moreover, new genera and species were discovered and 65.7 % of the species were reported for the first time in Argentina, confirming that this region has been poorly explored so far. Thus, more studies on the biodiversity and biogeography are needed in this region, in order to know the taxonomic composition, the biogeographic and bathymetric ranges of their species and, consequently, to detect future changes caused by anthropogenic perturbations. In addition, the diversity patterns found in this study are partly influenced by the different sampling effort in the region and the number of stations without bryozoans may be overestimated. In fact, future explorations, mainly of slope and deep-water bryozoan faunas of South American and Antarctic margins and basins, could show a higher number of shared species than reported here. There was evidence of bryozoological affinities in the current study between Argentina and the nearest geographical neighbours, mainly Antarctica, supporting the hypothesis of sequential separation of Gondwana during the Cenozoic. Moreover, other potential pathways may explain the high similarity found in the bryozoan communities, mainly from slope depths in the AP region, thus overcoming the oceanic barrier of the PF. Therefore, the high number AP species shared with Antarctica in our study, linked to the lower proportion of endemic Antarctic cheilostome bryozoans reported in recent studies (closer to 50%), support the idea that the Southern Ocean may have been less isolated over geological time than once thought.

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Appendix S1. Results of the SIMPER analysis showing the species that contribute most to the dissimilarity between depth zones of the MDS. Contrib. %: percentage contribution of the species to the total dissimilarity; Cum. %: cumulative contribution to the total dissimilarity.

	Species	**Contrib. %**	**Cum. %**		**Species**	**Contrib. %**	**Cum. %**
1 vs 2	*Adeonella patagonica*	0.79	1.09	**1 vs 5**	*Adeonella patagonica*	10.74	1.07
	Amastigia nuda	0.79	2.06		*Amastigia benemunita*	10.74	2.15
	Amphiblestrum novella	0.79	3.08		*Amastigia nuda*	10.74	3.44
	Andreella uncifera	0.79	4.11		*Amphiblestrum novella*	10.74	4.29
	Arachnopusia globosa	0.79	5.14		*Andreella uncifera*	10.74	5.37
	Austroflustra gerlachi	0.79	6.17		*Arachnopusia globosa*	10.74	6.44
	Beania costata	0.79	7.2		*Arachnopusia monoceros*	10.74	7.52
	Beania inermis	0.79	8.23		*Beania costata*	10.74	8.59
	Beania maxilla	0.79	9.26		*Beania inermis*	10.74	9.66
	Beania inermis unicornis	0.79	10.29		*Beania magellanica*	10.74	10.74
	Calloporina patagonica	0.79	11.31		*Beania maxilla*	10.74	11.81
	Cellaria scoresbyi	0.79	12.34		*Beania inermis unicornis*	10.74	12.88
	Cellaria variabilis	0.79	13.37		*Buffonellodes glabra*	10.74	13.96
	Cellarinella dubia	0.79	14.4		*Buffonellodes rimosa*	10.74	15.032
	Chaperiopsis propinqua	0.79	15.43		*Caberea darwinii*	10.74	16.1
	Ellisina antarctica	0.79	16.46		*Calloporina patagonica*	10.74	17.18
	Exochella longirostris	0.79	17.49		*Camptoplites reticulatus*	10.74	18.25
	Fenestrulina dupla	0.79	18.51		*Carbasea ovoidea*	10.74	19.33
	Foveolaria terrifica	0.79	19.54		*Cellaria clavata*	10.74	20.4
	Lacerna hosteensis	0.79	20.57		*Cellaria malvinensis*	10.74	21.47
	Menipea patagonica	0.79	21.6		*Cellaria ornata*	10.74	22.56
	Scruparia ambigua	0.79	22.63		*Cellaria scoresbyi*	10.74	23.62
	Smittina jacobensis	0.79	23.66		*Cellaria variabilis*	10.74	24.7

Appendix S1. (Continued)

	Species	Contrib. %	Cum. %		Species	Contrib. %	Cum. %
1 vs 2	*Smittina jullieni*	0.79	24.69	**1 vs 5**	*Cellarinella dubia*	1.07	25.77
	Smittina lebruni	0.79	25.71		*Celleporina bicostata*	1.07	26.84
	Smittina leptodentata	0.79	26.74		*Chaperiopsis patulosa*	1.07	27.92
	Smittina monacha	0.79	27.77		*Chaperiopsis propinqua*	1.07	28.99
	Talivittaticella frigida	0.79	28.8		*Ellisina antarctica*	1.07	30.06
	Villicharixa strigosa	0.79	29.83		*Ellisina incrustans*	1.07	31.14
	Adelascopora secunda	7.42	30.79		*Exochella longirostris*	1.07	32.21
	Beania magellanica	7.42	31.75		*Fenestrulina dupla*	1.07	33.28
	Buffonellodes glabra	7.42	32.72		*Formosocellaria magnifica*	1.07	34.36
	Celleporina bicostata	7.42	33.68		*Himantozoum obtusum*	1.07	35.43
	Ellisina incrustans	7.42	34.64		*Lacerna hosteensis*	1.07	36.5
	Reteporella gigantea	7.42	35.6		*Melicerita blancoae*	1.07	37.58
	Arachnopusia monoceros	6.916	36.5		*Menipea flagellifera*	1.07	38.65
	Cellaria ornata	6.916	37.4		*Menipea patagonica*	1.07	39.73
	Notoplites elongatus	6.916	38.29		*Micropora brevissima*	1.07	40.7
	Osthimosia eatonensis	6.916	39.19		*Notoplites elongatus*	1.07	41.87
	Smittoidea sigillata	6.916	40.09		*Ogivalia elegans*	1.07	42.95
	Tricellaria aculeata	6.916	40.99		*Osthimosia bicornis*	1.07	44.02
	Reteporella tortuosa	6.27	41.8		*Osthimosia eatonensis*	1.07	45.1
	Camptoplites bicornis quadravicularis	6.16	42.6		*Parasmittina dubitata*	1.07	46.17
	Austroflustra australis	6.03	43.39		*Scruparia ambigua*	1.07	47.24
	Amastigia benemunita	5.85	44.14		*Smittina jacobensis*	1.07	48.32
	Aspidostoma giganteum	5.85	44.9		*Smittina jullieni*	1.07	49.39
	Himantozoum obtusum	5.85	45.66		*Smittina lebruni*	1.07	50.46
	Smittina anecdota	5.85	46.41		*Smittina leptodentata*	1.07	51.54

Appendix S1. (Continued)

	Species	Contrib. %	Cum. %
1 vs 2	*Chartella notialis*	5.43	47.12
	Carbasea ovoidea	5.3	47.81
	Osthimosia clavata	5.3	48.5
	Caberea darwinii guntheri	4.83	49.12
	Turritigera stellata	4.83	49.75
	Aspidostoma coronatum	4.76	50.36
	Fenestrulina antarctica	4.76	50.98
	Fenestrulina crystallina	4.76	51.6
	Chaperiopsis erecta	4.32	52.16
	Amastigia crassimarginata	4.23	52.71
	Buffonellodes rimosa	4.23	53.26
	Camptoplites reticulatus	4.23	53.81
	Cellaria malvinensis	4.23	54.36
	Chaperiopsis patulosa	4.23	54.9
	Menipea flagellifera	4.23	55.45
	Paracellaria elephantina	4.1	55.98
	Arachnopusia admiranda	4.09	56.51
	Bracebridgua subsulcata	4.09	57.05
	Carbasea elegans	4.09	57.58
	Catadysis immersum	4.09	58.11
	Chiastosella watersi	4.09	58.64
	Chondriovelum angustilobatum	4.09	59.17
	Fenestrulina incusa	4.09	59.7
1 vs 3	*Adeonella patagonica*	1.05	1.08
	Amastigia benemunita	1.05	2.15
1 vs 5	*Smittina monacha*	1.07	52.61
	Smittina smittiana	1.07	53.68
	Smittoidea sigillata	1.07	54.76
	Tricellaria aculeata	1.07	55.83
	Villicharixa strigosa	1.07	56.9
2 vs 3	*Caberea darwinii*	2.25	2.98
	Fenestrulina fritilla	2.25	5.95
	Lageneschara peristomata	2.25	8.93
	Micropora brevissima	2.25	11.90
	Talivittacella frigida	2.25	14.98
	Reteporella gigantea	2.14	14.88
	Osthimosia bicornis	2.01	17.7
	Reteporella magellensis	1.82	22.78
	Smittoidea malleata	1.82	25.15
	Reteporella tortuosa	1.78	27.5
	Camptoplites bicornis quadravicularis	1.7	29.74
	Foveolaria terrifica	1.6	31.86
	Orthoporidra brachyrhyncha	1.6	33.97
	Smittina rogickae	1.6	36.09
	Austroflustra australis	1.6	38.19
	Melicerita blancoae	1.6	40.3
	Ogivalia elegans	1.6	42.41
	Orthoporidra compacta	1.6	44.52
	Parasmittina dubitata	1.6	46.63
	Adelascopora secunda	1.56	48.68

Appendix S1. (Continued)

	Species	**Contrib. %**	**Cum. %**		**Species**	**Contrib. %**	**Cum. %**
1 vs 3	*Amastigia nuda*	1.05	3.23	**2 vs 3**	*Aspidostoma coronatum*	1.47	50.62
	Amphiblestrum novella	1.05	4.3		*Fenestrulina antarctica*	1.47	52.56
	Andreella uncifera	1.05	5.38		*Chartella notialis*	1.41	54.42
	Arachnopusia globosa	1.05	6.45		*Smittina anecdota*	1.4	56.27
	Arachnopusia monoceros	1.05	7.53		*Osthimosia clavata*	1.34	58.04
	Aspidostoma giganteum	1.05	8.6		*Fenestrulina crystallina*	1.29	59.74
	Austroflustra gerlachi	1.05	9.68	**2 vs 4**	*Austroflustra gerlachi*	2.52	2.71
	Beania costata	1.05	10.75		*Caberea darwinii*	2.52	5.43
	Beania inermis	1.05	11.83		*Fenestrulina fritilla*	2.52	8.14
	Beania magellanica	1.05	12.9		*Foveolaria terrifica*	2.52	10.85
	Beania maxilla	1.05	13.98		*Lageneschara peristomata*	2.52	13.56
	Beania inermis unicornis	1.05	15.05		*Micropora brevissima*	2.52	16.28
	Buffonellodes glabra	1.05	16.13		*Orthoporidra brachyrhyncha*	2.52	18.99
	Buffonellodes rimosa	1.05	17.2		*Smittina rogickae*	2.52	21.7
	Caberea darwinii	1.05	18.29		*Talivittacella frigida*	2.52	24.42
	Calloporina patagonica	1.05	19.35		*Adelascopora secunda*	2.4	26.99
	Camptoplites reticulatus	1.05	20.43		*Reteporella gigantea*	2.4	29.57
	Carbasea ovoidea	1.05	21.51		*Osthimosia bicornis*	2.24	31.98
	Cellaria malvinensis	1.05	22.58		*Reteporella magellensis*	2.01	34.14
	Cellaria ornata	1.05	23.66		*Reteporella tortuosa*	1.98	36.28
	Cellaria scoresbyi	1.05	24.73		*Aspidostoma giganteum*	1.97	38.4
	Cellaria variabilis	1.05	25.81		*Smittina anecdota*	1.97	40.53
	Cellarinella dubia	1.05	26.88		*Camptoplites bicornis quadravicularis*	1.89	42.56
	Celleporina bicostata	1.05	27.96		*Osthimosia clavata*	1.82	44.52
	Chaperiopsis patulosa	1.05	29.03		*Austroflustra australis*	1.76	46.41

Appendix S1. (Continued)

	Species	Contrib. %	Cum. %
1 vs 3	*Chaperiopsis propinqua*	1.05	30.11
	Ellisina antarctica	1.05	31.18
	Ellisina incrustans	1.05	32.26
	Exochella longirostris	1.05	33.33
	Fenestrulina dupla	1.05	34.41
	Himantozoum obtusum	1.05	35.49
	Lacerna hosteensis	1.05	36.56
	Melicerita blancoae	1.05	37.64
	Menipea flagellifera	1.05	38.71
	Menipea patagonica	1.05	39.79
	Micropora brevissima	1.05	40.86
	Notoplites elongatus	1.05	41.94
	Ogivalia elegans	1.05	43.01
	Osthimosia bicornis	1.05	44.09
	Osthimosia eatonensis	1.05	45.16
	Parasmittina dubitata	1.05	46.24
	Scruparia ambigua	1.05	47.31
	Smittina jacobensis	1.05	48.39
	Smittina jullieni	1.05	49.46
	Smittina lebruni	1.05	50.54
	Smittina leptodentata	1.05	51.61
	Smittina monacha	1.05	52.69
	Smittina smittiana	1.05	53.76
	Smittoidea malleata	1.05	54.84
	Smittoidea sigillata	1.05	55.91
2 vs 4	*Melicerita blancoae*	1.76	48.32
	Ogivalia elegans	1.76	50.2
	Orthoporidra compacta	1.76	52.1
	Parasmittina dubitata	1.76	53.99
	Aspidostoma coronatum	1.66	55.79
	Fenestrulina antarctica	1.66	57.58
	Fenestrulina crystallina	1.66	59.37
2 vs 5	*Austroflustra gerlachi*	2.39	2.46
	Caberea darwinii	2.39	4.91
	Cellaria clavata	2.39	7.37
	Fenestrulina fritilla	2.39	9.83
	Formosocellaria magnifica	2.39	12.29
	Foveolaria terrifica	2.39	14.74
	Lageneschara peristomata	2.39	17.2
	Micropora brevissima	2.39	19.66
	Orthoporidra brachyrhyncha	2.39	22.11
	Smittina rogickae	2.39	24.57
	Talivittacella frigida	2.39	27.02
	Adelascopora secunda	2.26	29.36
	Reteporella gigantea	2.26	31.69
	Osthimosia bicornis	2.12	33.88
	Columnella gracilis	1.95	35.89
	Columnella magna armata	1.95	37.9
	Cookinella flustroides	1.95	39.9
	Domosclerus corrugatus	1.95	41.92

Appendix S1. (Continued)

	Species	Contrib. %	Cum. %
1 vs 3	*Tricellaria aculeata*	1.05	56.99
	Villicharixa strigosa	1.05	58.07
1 vs 4	*Adeonella patagonica*	1.09	1.12
	Amastigia benemunita	1.09	2.24
	Amastigia nuda	1.09	3.35
	Amphiblestrum novella	1.09	4.47
	Andreella uncifera	1.09	5.59
	Arachnopusia globosa	1.09	6.71
	Arachnopusia monoceros	1.09	7.83
	Beania costata	1.09	8.95
	Beania inermis	1.09	10.06
	Beania magellanica	1.09	11.18
	Beania maxilla	1.09	12.3
	Beania inermis unicornis	1.09	13.42
	Buffonellodes glabra	1.09	14.54
	Buffonellodes rimosa	1.09	15.66
	Caberea darwinii	1.09	16.77
	Calloporina patagonica	1.09	17.89
	Camptoplites reticulatus	1.09	19.01
	Carbasea ovoidea	1.09	20.13
	Cellaria malvinensis	1.09	21.25
	Cellaria ornata	1.09	22.37
	Cellaria scoresbyi	1.09	23.48
	Cellaria variabilis	1.09	24.6
	Cellarinella dubia	1.09	25.72
2 vs 5	*Reteporella magellensis*	1.92	43.89
	Reteporella tortuosa	1.88	45.82
	Aspidostoma giganteum	1.86	47.74
	Smittina anecdota	1.86	49.66
	Camptoplites bicornis quadravicularis	1.79	51.5
	Osthimosia clavata	1.71	53.26
	Austroflustra australis	1.68	54.99
	Melicerita blancoae	1.68	56.72
	Ogivalia elegans	1.68	58.45
3 vs 4	*Aspidostoma giganteum*	10.17	16.05
	Austroflustra gerlachi	10.17	32.09
	Smittoidea malleata	10.17	4814
	Talivittacella problematica	5.9	57.46
3 vs 5	*Cellaria clavata*	8.37	30.18
	Formosocellaria magnifica	8.37	40.23
	Smittoidea malleata	8.37	50.29
	Columnella gracilis	6.44	58.03
4 vs 5	*Cellaria clavata*	9.99	14.4
	Formosocellaria magnifica	9.54	28.16
	Columnella gracilis	9.14	41.34
	Columnella magna armata	9.14	54.52

Appendix S1. (Continued)

	Species	**Contrib. %**	**Cum. %**
1 vs 4	*Celleporina bicostata*	1.09	26.84
	Chaperiopsis patulosa	1.09	27.96
	Chaperiopsis propinqua	1.09	29.08
	Ellisina antarctica	1.09	30.19
	Ellisina incrustans	1.09	31.31
	Exochella longirostris	1.09	32.43
	Fenestrulina dupla	1.09	33.55
	Himantozoum obtusum	1.09	34.67
	Lacerna hosteensis	1.09	35.79
	Melicerita blancoae	1.09	36.91
	Menipea flagellifera	1.09	38.02
	Menipea patagonica	1.09	39.14
	Micropora brevissima	1.09	40.26
	Notoplites elongatus	1.09	41.38
	Ogivalia elegans	1.09	42.5
	Osthimosia bicornis	1.09	43.62
	Osthimosia eatonensis	1.09	44.73
	Parasmittina dubitata	1.09	45.85
	Scruparia ambigua	1.09	46.97
	Smittina jacobensis	1.09	48.1
	Smittina jullieni	1.09	49.21
	Smittina lebruni	1.09	50.33
	Smittina leptodentata	1.09	52.45
	Smittina monacha	1.09	52.56
	Smittina smittiana	1.09	53.68

Appendix S1. (Continued)

	Species	Contrib. %	Cum. %	Species	Contrib. %	Cum. %
1 vs 4	*Smittoidea sigillata*	1.09	54.8			
	Tricellaria aculeata	1.09	55.92			
	Villicharixa strigosa	1.09	57.04			
	Talivittaticella problematica	0.66	57.71			

Appendix S2. Bathymetric ranges and biogeographic distributions of the species studied. Pulled data from the present study, the literature and the GBIF and SCAR databases. Localities: AN = Antarctica; AR = Argentina; AU = Australia; CH = Chile; FI = Falkland Islands (Malvinas); HI/MI = Heard Island and McDonald Islands; NZ = New Zealand; SAF = South Africa; SG/SS = South Georgia and the South Sandwich Islands. -: unknown.

Species	Bathymetric range (m)	Geographic distr.
Adelascopora secunda Hayward & Thorpe, 1988	400–1700	AN, AR, CH, SG/SS
Adeonella patagonica Hayward, 1988	0–100	AN, CH
Aetea anguina (Linnaeus, 1758)	0–200	AN, AR, AU, CH, FI, NZ, SAF
Aetea australis Jullien, 1888	0–100	AR, AU, NZ
Aetea curta Jullien, 1888	0–101	AR, AU
Aetea ligulata Busk, 1852	0–102	AR, FI, NZ
Aetea sica (Couch, 1844)	0–103	AR
Aimulosia australis Jullien, 1888	140–1500	AN, AR, CH, SG/SS
Amastigia benemunita (Busk, 1884)	0–600	AR, CH
Amastigia crassimarginata (Busk, 1884)	300–1000	AR
Amastigia gaussi (Kluge, 1914)	200–500	AN, AR**, FI, SG/SS
Amastigia nuda Busk, 1852	0–00	AR, FI, NZ
Amastigia vibraculifera Hastings, 1943	0–00	AR, FI
Amphiblestrum familiaris Hayward & Thorpe, 1989	1100–1200	AN, AR**

Appendix S2. (Continued)

Species	Bathymetric range (m)	Geographic distr.
Amphiblestrum novella Hayward & Thorpe, 1989	0–00	AR, FI
Apiophragma hyalina (Waters, 1904)	1400–1500	AN, AR**
Andreella patagonica López Gappa, 1981	0–00	AR
Andreella uncifera (Busk, 1884)	0–200	AN, AR, CH, FI
Arachnopusia admiranda Moyano, 1982	100–200	AR
Arachnopusia globosa Hayward & Thorpe, 1988	0–200	AR, FI
Arachnopusia monoceros (Busk, 1854)	0–400	AN, AR, AU, CH, FI, SAF, SG/SS
Arachnopusia valligera Hayward & Thorpe, 1988	0–100	AR, NZ
Aspericreta favulosa Hayward & Thorpe, 1989	1200–1300	AN, AR**
Aspidostoma coronatum (Thornely, 1924)	900–1600	AN, AR**, SG/SS
Aspidostoma giganteum (Busk, 1854)	700–1900	AN, AR, CH, FI
Austroflustra australis López-Gappa, 1982	200–1300	AR, FI
Austroflustra gerlachi López Gappa, 1982	200–1900	AR, FI
Austrothoa yagana Moyano & Gordon, 1980	—	AR, CH
Beania costata (Busk, 1876)	0–170	AN, AR, AU, FI, SAF
Beania fragilis Ridley, 1881	0–100	AR, CH
Beania inermis (Busk, 1852)	0–200	AN, AR, AU, CH, FI, NZ
Beania magellanica (Busk, 1852)	0–300	AR, AU, CH, FI, NZ, SAF
Beania maxilla (Jullien, 1888)	0–200	AR, CH, FI
Beania unicornis Hastings, 1943	0–200	AR
Bracebridgia subsulcata (Smitt, 1873)	0–200	AR
Buffonellodes glabra Hayward, 1991	0–300	AR, CH, FI
Buffonellodes rimosa Jullien, 1888	0–900	AN, AR**, FI, NZ
Bugula flabellata Thompson *in* Gray, 1848	0–100	AR, AU, NZ
Bugula hyadesi Jullien, 1888	0–100	AR

Appendix S2. (Continued)

Species	Bathymetric range (m)	Geographic distr.
Bugula multiserialis (d'Orbigny, 1847)	—	AR
Bugula neritina (Linnaeus, 1758)	0–100	AN, AR, AU, NZ
Bugula simplex Hincks, 1886	—	AR
Bugula stolonifera Ryland, 1960	0–100	AR, AU, CH, FI, NZ
Caberea darwinii Busk, 1884	0–100	AN, AR, AU, CH, FI, HI/MI, NZ, SAF, SG/SS
Caberea darwinii guntheri Hastings, 1943	0–400	AR
Callopora deseadensis López Gappa, 1981	0–100	AR, CH
Calloporina patagonica Hayward & Ryland, 1990	0–200	AR
Camptoplites asymmetricus Hastings, 1943	300–500	AR, NZ
Camptoplites atlanticus Hastings, 1943	100–300	AR, FI
Camptoplites bicornis (Busk, 1884)	0–100	AN, AR, NZ
Camptoplites bicornis var. *quadriavicularis* Hastings, 1943	200–1400	AR
Camptoplites reticulatus (Busk, 1881)	1000–4500	AR, NZ
Carbasea elegans Busk, 1852	100–200	AR, AN
Carbasea ovoidea Busk, 1852	0–700	AN, AR, CH, HI/MI, SG/SS
Catadysis immersum (Busk, 1884)	100–200	AR, FI
Cellaria clavata (Busk, 1884)	0–3500	AN, AR, CH, FI, HI/MI, SG/SS
Cellaria dubia (Busk, 1884)	1000–1100	AN, AR
Cellaria malvinensis (Busk, 1852)	0–900	AN, AR, CH, FI, NZ, SAF, SG/SS
Cellaria ornata (d'Orbigny, 1847)	0–400	AR
Cellaria scoresbyi Hastings, 1946	0–200	AR, CH, FI, NZ, SAF
Cellaria variabilis (Busk, 1884)	0–200	AR, CH, FI
Cellarinella dubia Waters, 1904	0–200	AN, AR, AU, CH, FI
Celleporella alia Hayward, 1993	100–700	AN, AR

Appendix S2. (Continued)

Species	Bathymetric range (m)	Geographic distr.
Celleporella bougainvillei (d'Orbigny, 1847)	0–100	AN, AR, SG/SS
Celleporella chiloensis Moyano, 1982	—	AR, CH
Celleporella discreta (Busk, 1854)	—	AR, CH
Celleporella hyalina Linnaeus, 1767	0–100	AN, AR, AU, CH, NZ, SAF, SG/SS
Celleporella patagonica Busk, 1852	0–101	AR
Celleporella tehuelcha López Gappa, 1985	0–102	AR
Celleporina bicostata Hayward, 1980	0–100	AR, FI
Chaperia acanthina (Lamouroux, 1825)	—	AR, NZ
Chaperiopsis erecta (Busk, 1884)	300–1100	AR, SG/SS
Chaperiopsis galeata (Busk, 1854)	0–100	AN, AR, CH, FI, SG/SS, HI/MI
Chaperiopsis indefensa Hayward & Thorpe, 1988	0–100	AR
Chaperiopsis orbiculata Hayward & Thorpe, 1988	100–500	AN, AR, SG/SS
Chaperiopsis patulosa (Waters, 1904)	0–900	AN, AR, FI
Chaperiopsis propinqua Hayward & Thorpe, 1988	0–200	AR, CH, FI
Chartella notialis Hayward & Winston, 1994	200–1200	AN, AR
Chiastosella watersi Stach, 1937	100–200	AR, NZ
Chondriovelum angustilobatum Moyano, 1974	100–200	AR, CH
Chorizopora brongniartii (Audouin, 1826)	0–100	AR, NZ
Codonellina galeata (Busk, 1854)	0–100	AR
Columnella cribraria Busk, 1884	3400–3500	AR
Columnella gracilis (Busk, 1884)	4400–4500	AR
Columnella magna (Busk, 1884)	700–1100	AN, AR, NZ, SAF, SG/SS
Columnella magna var. *armata* (Busk, 1884)	4400–4500	AR
Conopeum reticulum (Linnaeus, 1767)	—	AR
Cookinella flustroides d'Hondt, 1981	4300–4500	AR

Appendix S2. (Continued)

Species	Bathymetric range (m)	Geographic distr.
Cornucopina ovalis versa Hastings, 1943	200–500	AR, FI
Cornucopina pectogemma (Goldstein, 1882)	100–1000	AN, AR**, NZ, SAF, SG/SS
Crepidacantha crinispina Levinsen, 1909	—	AR, AU, NZ
Cryptostomaria cylindrica (Harmer, 1926)	3800–3900	AR
Cryptosula pallasiana (Moll, 1803)	0–100	AR, AU, NZ
Discoporella depressa (Conrad, 1841)	0–100	AR
Domosclerus corrugatus (Busk, 1884)	4300–4500	AR, NZ, SAF
Electra longispina (Calvet, 1904)	0–100	AR
Electra monostachys (Busk, 1854)	0–100	AR
Ellisina antarctica Hastings, 1945	0–200	AN, AR, CH, NZ
Ellisina incrustans (Waters, 1898)	0–300	AN, AR, CH, FI
Euginoma biseriata d'Hondt, 1981	2700–2800	AR
Euginoma cavalieri Lagaaij, 1963	2000–2100	AR
Exochella discors Hayward, 1991	0–100	AR
Exochella hymanae (Rogick, 1956)	1500–1700	AN, AR**, SG/SS
Exochella longirostris Jullien, 1888	0–200	AN, AR, CH, FI
Fenestrulina antarctica Hayward & Thorpe, 1990	900–1600	AN, AR**
Fenestrulina crystallina Hayward & Ryland, 1990	900–1700	AN, AR**
Fenestrulina dupla Hayward & Ryland, 1990	0–200	AR
Fenestrulina fritilla Hayward & Ryland, 1990	100–1600	AN, AR**, FI, SG/SS
Fenestrulina incusa Hayward & Ryland, 1990	100–200	AR
Fenestrulina majuscula Hayward, 1980	0–100	AR, CH, FI
Fenestrulina malusii Audouin, 1826	0–100	AN, AR, AU, CH, SG/SS, NZ
Flustrapora magellanica Moyano, 1970	100–200	AN, AR
Formosocellaria magnifica (Busk, 1884)	3400–4500	AR

Appendix S2. (Continued)

Species	Bathymetric range (m)	Geographic distr.
Foveolaria elliptica Busk, 1884	300–900	AR, AU, CH, NZ
Foveolaria cyclops (Busk, 1854)	—	AR, NZ
Foveolaria terrifica (Hincks, 1881)	200–1700	AR, FI
Galeopsis bullatus Hayward, 1993	700–800	AN, AR**, HI/MI, SG/SS
Galeopsis patagonicus Hayward, 1993	100–200	AR, FI
Galeopsis pentagonus (d'Orbigny, 1847)	100–200	AR, FI, NZ
Gigantopora spathula Hayward & Winston, 2011	300–1000	AN, AR**
Gregarinidra variabilis (Moyano, 1974)	100–200	AR, FI
Hemismittoidea hexaspinosa (Uttley & Bullivant, 1972)	—	AR, NZ
Himantozoum margaritiferum (Busk, 1884)	—	AR
Himantozoum obtusum Hastings, 1943	0–600	AN, AR, FI
Hippadenella falklandensis Hayward, 1991	0–100	AR, FI
Hippadenella margaritifera (Quoy & Gaimard, 1824)	—	AR
Hippadenella rouzaudi (Calvet, 1904)	0–100	AR, CH, FI
Hippomonavella ramosae López de la Cuadra & Garcia-Gomez, 2000	1400–1500	AR**, SG/SS
Hippoporina aulacomyae López Gappa, 1981	—	AR
Hippothoa divaricata Lamouroux, 1821	—	AR, CH
Hippothoa flagellum Manzoni, 1870	—	AN, AR, AU, FI, NZ, SG/SS
Ichthyaria oculata Busk, 1884	100–300	AR, FI
Ichthyaria profunda d'Hondt, 1981	2700–2800	AR
Inversiula nutrix Jullien, 1888	100–200	AN, AR, SG/SS
Inversiula patagonica Hayward & Ryland, 1991	100–200	AR
Jolietina latimarginata (Busk, 1884)	700–900	AN, AR, FI
Lacerna eatoni (Busk, 1876)	0–100	AN, AR, CH
Lacerna hosteensis Jullien, 1888	0–200	AN, AR, CH, FI, SG/SS

Appendix S2. (Continued)

Species	Bathymetric range (m)	Geographic distr.
Lageneschara peristomata Hayward & Winston, 2011	100–1600	AN, AR**
Melicerita atlantica Busk, 1884	—	AR
Melicerita blancoae López Gappa, 1981	0–1300	AN, AR, FI
Melicerita temaukeli Moyano, 1997	—	AR, CH
Membranicellaria dubia (Busk, 1884)	—	AR
Membranipora membranacea (Linnaeus, 1767)	—	AU, AR, CH, NZ, SAF
Menipea flagellifera Busk, 1884	0–900	AN, AR, FI, SAF
Menipea patagonica Busk, 1852	0–200	AR, CH, FI
Micropora brevissima Waters, 1904	0–1600	AN, AR, CH, FI, SAF, SG/SS
Microporella crustula Hayward & Winston, 2011	100–900	AN, AR**
Micropora notialis Hayward & Ryland, 1993	0–100	AR, AN, CH, NZ, SG/SS
Microporella diademata (Lamouroux, 1825)	0–200	AR, AU, NZ
Microporella hyadesi (Jullien, 1888)	0–200	AR, CH, FI, NZ, SG/SS
Neoflustra dimorphica López Gappa, 1982	—	AR
Notoplites antarcticus (Waters, 1904)	900–1000	AN, AR**
Notoplites crateriformis (Busk, 1884)	3400–3500	AR
Notoplites elongatus (Busk, 1884)	0–400	AR, AN, FI, SAF
Odontoporella adpressa (Busk, 1854)	0–100	AR, FI
Ogivalia elegans (d'Orbigny, 1847)	0–1300	AR, CH, FI
Ogivalia sagittirostra Hayward & Winston, 1994	300–1000	AN, AR, CH, FI, SG/SS
Orthoporidra brachyrhyncha Moyano, 1985	100–1700	AN, AR
Orthoporidra compacta (Waters, 1904)	100–1300	AN, NZ, SG/SS
Orthoporidra petiolata (Waters, 1905)	—	AR, AN
Orthoporidra stenorhyncha Moyano, 1985	100–200	AN, AR**
Orthoporidroides erectus (Waters, 1888)	200–300	AR, CH

Appendix S2. (Continued)

Species	Bathymetric range (m)	Geographic distr.
Osthimosia bicornis (Busk, 1881)	0–1500	AN, AR, CH, FI, HI/MI, NZ, SAF, SG/SS
Osthimosia clavata Waters, 1904	700–1700	AN, AR
Osthimosia eatonensis (Busk, 1881)	0–300	AN, AR, CH, FI, SG/SS, NZ
Osthimosia malingae Hayward, 1992	100–800	AN, AR**
Osthimosia magna Moyano, 1974	0–100	AR, FI
Osthimosia notialis Hayward, 1992	800–900	AN, AR, SG/SS
Osthimosia rudis (Busk, 1881)	1000–1100	AR
Paracellaria cellarioides Hayward & Thorpe, 1989	200–500	AR, FI, SG/SS
Paracellaria elephantina Hayward & Thorpe, 1989	800–1500	AN, AR**
*Paracellaria elizabetha*e Branch & Hayward, 2005	1400–1500	AR**, SAF
Parafigularia magellanica (Calvet, 1904)	—	AR, CH
Parasmittina dubitata Hayward, 1980	0–1300	AR, CH, FI
Phonicosia jousseaumei Jullien, 1888	—	AR, AU, NZ
Platychelyna planulata Hayward, 1980	200–300	AR, FI
Plesiothoa australis Moyano & Gordon, 1980	—	AR, NZ
Porella hyadesi Jullien, 1888	—	AN, AR
Reteporella spatulifera (Waters, 1905)	100–200	AR
Reteporella antennata Ramalho, Muricy & Taylor, 2011	400–500	AR**
Reteporella gigantea (Busk, 1884)	300–1600	SAF
Reteporella longichila Hayward, 1993	700–800	AN, AR**
Reteporella magellensis (Busk, 1884)	100–1400	AR, FI
Reteporella sulcula Hayward & Winston, 2011	400–1000	AN, AR**
Reteporella tortuosa Hayward & Winston, 2011	400–1500	AN, AR**
Reteporellina moyanoi d'Hondt, 1981	—	AR
Romancheina labiosa (Busk, 1854)	0–100	AR, CH

Appendix S2. (Continued)

Species	Bathymetric range (m)	Geographic distr.
Sclerodomus denticulatus Busk, 1884	—	AR
Scruparia ambigua (d'Orbigny, 1847)	0–200	AR, CH, NZ
Scrupocellaria puelcha (d'Orbigny, 1847)	100–200	AR
Securiflustra bifoliata d'Hondt, 1981	200–600	AR, FI
Smittina anecdota Hayward & Thorpe, 1990	600–1700	AN, AR**, HI/MI
Smittina insulata Hayward & Thorpe, 1990	100–200	AR, FI
Smittina jacobensis (Busk, 1884)	0–200	AR, SAF
Smittina jullieni Moyano, 1983	0–200	AR, CH, FI
Smittina lebruni (Waters, 1905)	0–200	AR, CH
Smittina leptodentata Hayward & Thorpe, 1990	0–200	AR, CH, FI
Smittina marionensis (Busk, 1854)	—	AN, AR
Smittina monacha Jullien, 1888	0–200	AR, CH, FI
Smittina pliofistulata Hayward & Thorpe, 1990	200–400	AR, FI
Smittina portiuscula Hayward & Thorpe, 1990	0–100	AR
Smittina rogickae Hayward & Taylor, 1984	100–1700	AN, AR**
Smittina smittiana (Busk, 1884)	0–1100	A, AR, CH, FI
Smittina stigmatophora (Busk, 1884)	0–100	AR
Smittina uruguayensis d'Hondt, 1981	1600–1700	AR
Smittoidea cribrooecia Hayward & Thorpe, 1990	100–200	AR, FI
Smittoidea malleata Hayward & Thorpe, 1989	1500–1900	AN, AR**
Smittoidea pachydermata Hayward & Thorpe, 1990	100–200	AR, CH, FI
Smittoidea rhynchota Hayward & Thorpe, 1990	100–200	AN, AR, FI, SG/SS
Smittoidea sigillata (Jullien, 1888)	0–400	AN, AR, CH, FI
Sphaerulobryozoon pedunculatum d'Hondt, 1981	2000–2500	AR
Stephanollona longispinata (Busk, 1884)	—	AR, NZ

Appendix S2. (Continued)

Species	Bathymetric range (m)	Geographic distr.
Stomhypselosaria watersi Hayward & Thorpe, 1989	100–700	AN, AR, FI, SG/SS
Talivittaticella frigida (Waters, 1904)	200–1600	AN, AR, SAF
Talivittaticella problematica (d'Hondt, 1981)	2700–3900	AR, NZ
Tricellaria aculeata (d'Orbigny, 1847)	0–400	AR, CH, FI, NZ
Turbicellepora patagonica Hayward, 1992	0–100	AR
Turritigera cribrata Hayward, 1993	1400–1600	AN, AR**, AU, SG/SS
Turritigera stellata Busk, 1884	200–1100	AN, AR
Umbonula alvareziana (d'Orbigny, 1847)	—	AR

Chapter 2

Spatial patterns and diversity of bryozoan communities from the Southern Ocean: South Shetland Islands, Bouvet Island and Eastern Weddell Sea

Chapter 2

Spatial patterns and diversity of bryozoan communities from the Southern Ocean: South Shetland Islands, Bouvet Island and Eastern Weddell Sea

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Abstract

In this study we report new data on the biodiversity, the geographic and the bathymetric distribution of bryozoans collected during the ANT XXI/2 cruise (November 2003 to January 2004) in the Eastern Weddell Sea and Bouvet Island, and during the Spanish Antarctic expedition ECOQUIM (January 2006) in the South Shetland Islands. Our data on distribution were analysed together with previous studies carried out in the same regions. A total of 54 species of Antarctic bryozoans (206 samples), including a new species of the genus *Reteporella* were found. Two species were reported for the first time from Bouvet Island, one from the Weddell Sea and one from Spiess Seamount. 55% of all species identified were endemic to Antarctica. In the Weddell Sea, the regions of Austasen and Kapp Norvegia exhibit the highest relative species richnesses, followed by the Vestkapp region. Multivariate and cluster analyses revealed small-scale spatial variability in the community structure along depth and between localities.

Keywords: Bryozoans, Multi-dimensional scaling, Antarctica, Geographic distribution, Bathymetric distribution

Resum

En aquest estudi es presenten noves dades de biodiversitat i de distribució geogràfica i batimètrica de briozous recol·lectats durant la campanya ANT XXI/2 (Novembre 2003-Gener 2004) a l' Est del Mar de Weddell i a l' Illa de Bouvet, i durant l'expedició antàrtica espanyola ECOQUIM (Gener 2006) a les Illes Shetland del Sud. Les nostres dades han estat analitzades juntament amb estudis previs duts a terme a les mateixes regions. En el nostre estudi es van trobar un total de 54 espècies de briozous antàrtics (206 mostres), incloent una espècie nova del gènere *Reteporella*. Es van citar dues espècies per primer cop a l'Illa de Bouvet, una al Mar de Weddell i una a la muntanya submarina de Spiess. El 55% de totes les espècies eren endèmiques de l'Antàrtida. Al Mar de Weddell, les regions d'Austasen i Kapp Norvegia exhibien la riquesa específica relativa més alta, seguida de la regió Vestkapp. Les anàlisis multivariants i de clúster revelaven, a petita escala, variabilitat espacial en l'estructura de les comunitats en profunditat i entre localitats.

Paraules clau: Briozous, Escalament multidimensional, Antàrtida, Distribució geogràfica, Distribució batimètrica

Introduction

The conservation and management of marine biodiversity requires detailed studies of the biodiversity and its relationship with environmental conditions (e.g. de Voogd *et al.* 2009). Although they seem to be under less intense pressures when compared to other ecosystems globally, Antarctic habitats are threatened by overexploitation of living resources, establishment of invasive marine species and climate change, as well as the growing impact of tourism (e.g. Tejedo *et al.* 2009). The Antarctic fauna has evolved in stable conditions, thus it is likely to be more sensible and, for this reason, the risk of extinctions caused by anthropic impacts in these ecosystems makes it essential to intensify research on Antarctic biodiversity (Barnes & Peck 2008).

Knowledge of the bryozoan species from the Southern Ocean, its diversity, and the environmental conditions in which they live, are still very poor (e.g. Kuklinski & Barnes 2009), largely determined by the relative inaccessibility of the region. An understanding of how and why similarities and differences exist between benthic communities inhabiting Antarctic ecosystems may provide information about the physical and biological factors that influence bryozoan distributions.

More than 700 new species of invertebrates from deep Antarctic waters have been recently discovered, with bryozoans, sponges and amphipods exhibiting high species richness (Brandt *et al.* 2007). Therefore, recent studies of Antarctic biodiversity in the region have described a rich and varied fauna (e.g. Hayward & Winston 2011). In general, the Antarctic shelf and slope are known to be able to support biomass levels of macrobenthos far higher than those in equivalent habitats in boreal and subtropical regions of equal depth (Arntz *et al.* 1994).

In recent years, the number of taxonomic studies on Antarctic bryozoans has experienced a notable increase (e.g. Hayward 1995; López-Fé de la Cuadra & García-Gómez 2000; Gutt *et al.* 2000). Since the scientific results of the Belgian Antarctic Expedition in 1897-99 (Waters 1904), over 300 species have been described and new descriptions continue to appear (Clarke & Johnston 2003; Gontar 2008; López & Liuzzi 2008; Kuklinski & Barnes 2009; Griffiths 2010; Figuerola *et al.* 2013). Cheilostomatid bryozoans are one of the best represented taxa on the Antarctic shelf (Barnes *et al.* 2009) and a high proportion (56%) are endemic (Hayward 1995; Barnes & De Grave 2000; Clarke & Johnston 2003; Griffiths *et al.* 2009; Griffiths 2010). Many bryozoan species have been reported from the Antarctic Peninsula or the Ross Sea (Hayward 1995). However, bryozoans are poorly investigated in some other Antarctic regions, such as the Weddell Sea (Zabala *et al.* 1997; Moyano 2005, Barnes *et al.* 2010). High levels of biodiversity, with more than 400 species and subspecies of Bryozoa in 32 stations, were found in the first collection from this area during the ANT XIII/3 Expedition (1996) with Polarstern (Arntz *et al.*

1997), and in recent sampling expeditions in the deep Weddell sea (Arntz *et al*. 2005a; Barnes & Kuklinski 2010).

In polar waters, benthic assemblages are characterized by both bathymetric and horizontal variability (e.g. Cummings *et al.* 2006; Smale 2008). Diversity of Antarctic species is determined by a synergy of physical (depth, substratum, iceberg scouring…) and biotic factors (e.g. community type) (e.g. Smale 2008; Starmans *et al*. 1999; Griffiths 2010), and in the eastern Weddell Sea shelf, differences in currents cause heterogeneity. Iceberg scouring is the major disturbance affecting the benthos of this continental shelf because it disrupts large areas of the seafloor above 300 m. All these factors play a key role in structuring recent Antarctic shelf benthic communities (Brandt *et al*. 2007; Gutt & Piepenburg 2003; Thatje *et al*. 2005).

The objectives of this paper are: (1) to present species-level information on new samples analysed for this study, and (2) by combining these with existing data on bryozoan distributions in the region, to describe patterns of distribution in relation to depth and spatial location.

Material and Methods

Collection methods

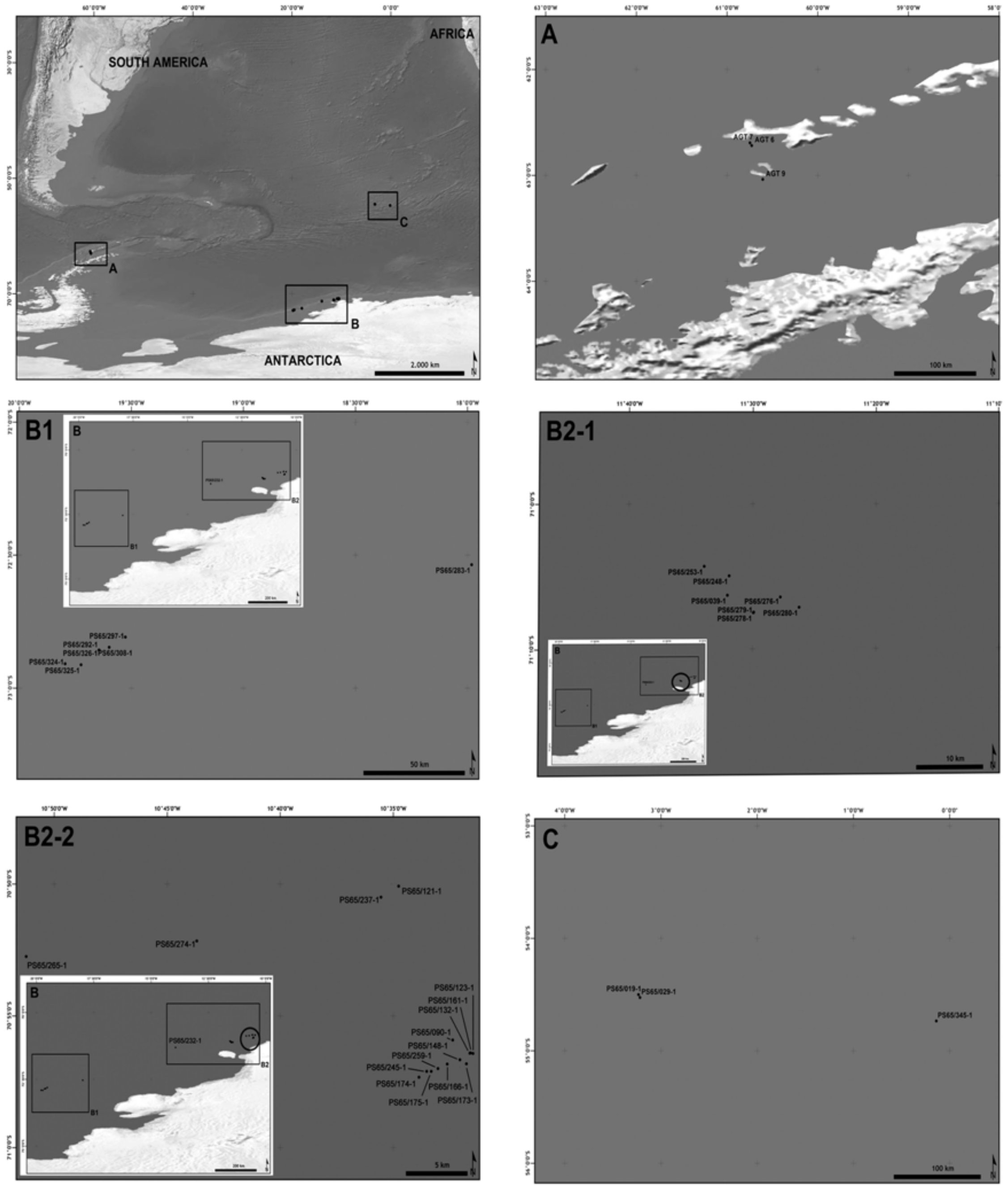
Samples from the Weddell Sea and Bouvet Island were collected during the Antarctic cruise ANT XXI/2 (from November 2003 to January 2004) of R/V Polarstern (AWI, Bremerhaven, Germany) at 56 stations surveyed. Samples from the South Shetland Islands were collected at 3 stations (Fig. 1) from the BIO *Hespérides* in January 2006 during the ECOQUIM cruise. Depths of collections ranged from 27 to 910 m, using Bottom trawl, Agassiz trawl, Rauschert dredge, Epibenthic sledge and Giant box corer in the Weddell Sea and Bouvet Island. In the South Shetland Islands, an Agassiz trawl and a Rock dredge were used instead. Sampling sites were georeferenciated and depth was registered at each point (Table 1).

After taking pictures of the living animals, the colonies of bryozoans were preserved in 70 % ethanol for further taxonomic identification. We classified most of the samples at species level using Hayward (1995).

Literature data

Some data of sampling stations and their characteristics (Zabala *et al*. 1997; Barnes & Kuklinski 2010) and some data of bathymetric ranges and biogeographic distribution of the species studied (Hayward 1995; Zabala *et al*. 1997; Gontar & Zabala 2000; Arntz *et al*. 2006; Barnes *et al*. 2008; Barnes & Kuklinski 2010) came from the literature and Global Biodiversity Information Facility database (GBIF; www.gbif.org).

Fig. 1. Map of the regions of the Weddell sea, Bouvet Island, Spiess seamount and the Shetland Islands. 1. Map of all regions; A: Shetland Islands; B: Area of Weddell sea; B1: region of Vestkapp; B2: regions of Kapp Norvegia and Austasen; B2-1, B2-2: region of Austasen; C: vicinities of Bouvet Island.



Additional data from South America, New Zealand and South Africa have been obtained from Moyano (1982, 1999), Gordon (1984, 1986), Florence *et al.* (2007); see also www.bryozoa.net. Data of Antarctic endemicity came from Hayward (1995), the SCAR's Marine Biodiversity Information database (SCAR-MarBIN; http://www.scarmarbin.be/) and the Global Biodiversity Information Facility database (GBIF; www.gbif.org).

Data analysis

In order to obtain representative numbers of individuals and species for the analysis, some data of the same species of bryozoans found in previous cruises in these regions (at 35 stations) were extracted from the literature, and analysed together with our new data from the ANT XXI/2 cruise (collected at 59 stations). In total we analysed data from 94 sampling stations.

Cluster and non-metric multidimensional scaling (MDS) ordination analyses were performed in order to assess similarities of samples. Due to unequal sampling efforts, binary data (presence/absence) was preferred to make the distance matrix using the Sørensen coefficient. The cluster was then plotted using the single linkage clustering technique to evaluate the similarities in species composition between regions. In order to evaluate the significant differences between regions, a test for binomial proportions was used ($p<0.05$).

The MDS analysis was used to evaluate the similarities between ranges of depth for the genera because it assumes no shape between variables (Legendre *et al.*, 1998). In order to categorize the continuous variable depth and to represent it in the MDS analysis, it was divided into 100 m interval categories (e.g. 100 m category includes depths from 0 to 100 m). The first two dimensions were plotted and the distance between dots denotes their similarity measured by the stress value. A stress value of less than 0.1 indicates that the plot accurately represents similarities, while a stress value greater than 0.3 indicates that the points are close to being randomly placed (Clarke 1993). Bathymetric ranges for each genus sampled in the Weddell Sea are detailed in Table 1.

In order to determine whether different assemblages do exist between ranges of depth and neighboring sites, relative abundance (N) and relative species richness (S, number of species present) and p-value (test for binomial proportions) were calculated for each depth range and area. A sample-based rarefaction curve was also computed. Chao2, Jacknife1 and Jacknife2 methods were used to estimate the theoretical number of expected species within each area (Colwell & Coddington 1994). Chao2 is an abundance-based nonparametric estimator of species richness that works by examining the number of species in a sample observed more than once relative to the number of species that is observed just once. In the absence of complete inventories, these nonparametric estimators have been shown to perform better than most other methods, such as observed species richness (Krebs 1999).

Diversity indices are commonly used to provide more information about community composition than simply species richness, such as the rarity and commonness of species and they also take the relative abundances of different species into account. The Margalef index is based on the number of species (species richness), while the others are indices of proportional abundances of the species. The Shannon-Wiener index is strongly influenced by the occurrence of rare species and Simpson's index by the importance of the more dominant species. Pielou's (evenness) and Berger-Parker indices calculate the relationship between the observed diversity and the maximum diversity, as well as between the number of the individuals of the most abundant species and the total number of individuals in the sample, respectively (Gray, 2000). Diversity indices are used to assess the impact of disturbances on the marine environment. In this aspect, the Shannon–Wiener index is more sensitive (high values mean an improvement in the environmental state) (Gray 2000). In the case of the Simpson and Berger-Parker indices, higher values correspond to a lower diversity (Salas *et al.* 2004; Marqués *et al.* 2009). The values of diversity indices calculated from the data of the present study of stations sampled with Agassiz trawl (AT), Bottom trawl (BT) and Rauschert dredge (RD) did not show significant differences (bootstrap confidence interval were overlapping). For this reason, five alpha diversity indices were calculated for each region only in these stations: Margalef (D_{Mg}), Shannon–Wiener (H'), Simpson's (1 – Lambda'), Pielou's (J') and Berger–Parker (B–P). The diversity indices of Kapp Norvegia were not calculated due to the absence of samples collected with any of these trawls in the present study.

Statistical significance was established at $p<0.05$. Ordination analyses were performed using VegAna software (v.1.6.0, De Cáceres 2003). Diversity analyses (relative abundance and species richness and diversity indices) were carried out with Past (Hammer *et al.* 2001) and the bootstrap method was used to obtain a more robust non-parametric estimate of the confidence intervals (95%) (Briggs *et al.* 1997). The test for binomial proportions was performed with Minitab Statistical Software. The SPSS (version 14.0, SPSS Inc, Chicago, Illinois, USA) package was used for the rest of data analysis.

Table 1. Sampling stations and their characteristics from this study and from the literature. AT: Agassiz Trawl, RD: Rauschert dredge, BT: BottomTrawl, GBC: Giant box corer, ES: Epibenthic sledge, R: Rock dredge, BP: bentopelagic trawl, MG: Multibox corer and GK: large box corer

Station	Date	Latitude (S)	Longitude (W)	Depth (m)	Gear	References
PS65/345-1	11/01/04	54º 44. 12’	00º 08. 31'	629.4	RD	This study
PS65/019-1	24/11/03	54° 30. 01'	03° 13. 97'	259.7	AT	This study
PS65/029-1	25/11/03	54° 31. 59'	03° 13. 05'	376.8	AT	This study
AGT 7	06/01/06	62° 41. 575'	60° 44. 827'	27.9	RD	This study
AGT 6	06/01/06	62° 43. 117'	60° 43. 683'	94.9	RD	This study
AGT 9	07/01/06	63° 02. 292'	60° 36. 355'	110.3	AT	This study
PS67/102-11	06/03/05	65° 35. 40'	36° 29. 00'	4794	AT	Barnes & Kuklinski (2010)
30	01/03/96	70° 05. 30'	08° 20. 00'	2315	AG	Zabala *et al.* (1997)
30	01/03/96	70° 05. 30'	08° 20. 00'	2315	DR	Zabala *et al.* (1997)

Table 1. (Continued)

Station	Date	Latitude (S)	Longitude (W)	Depth (m)	Gear	References
PS65/069-1	07/12/03	70° 25. 87'	08° 37. 43'	413.6	RD	Barnes & Kuklinski (2010)
32	04/03/96	70° 28. 90'	08° 15. 10'	286	DR	Zabala *et al.* (1997)
31	02/03/96	70° 30. 90'	10° 44. 20'	1586	DR	Zabala *et al.* (1997)
PS65/121-1	11/12/03	70° 50. 08'	10° 34.76'	274	AT	This study
PS65/121-1	11/12/03	70° 50. 08'	10° 35. 54'	268	AT	Barnes & Kuklinski (2010)
PS65/237-1	22/12/03	70° 50. 50'	10° 35. 54'	264.4	BT	This study
PS65/336-1	05/01/04	70º 50. 75’	10º 28. 01’	281.2	AT	This study
PS65/339-1	05/01/04	70º 50. 78’	10º 28. 51’	273.6	RD	This study
PS65/274-1	28/12/03	70° 52. 16'	10° 43. 69'	290.8	BT	This study
PS65/265-1	27/12/03	70° 52. 75'	10° 51. 24'	294.8	BT	This study
PS65/090-1	09/12/03	70° 55. 92'	10° 32. 37'	288	AT	This study
PS65/123-1	11/12/03	70° 56. 41'	10° 31. 58'	283.2	GBC	This study
PS65/132-1	12/12/03	70° 56. 42'	10° 31. 61'	284.4	BT	This study
PS65/161-1	15/12/03	70° 56. 43'	10° 31. 47'	279.6	AT	This study
PS65/148-1	13/12/03	70° 56. 67'	10° 32. 05'	302.4	BT	This study
PS65/173-1	16/12/03	70° 56. 82'	10° 31. 76'	296.4	AT	This study
PS65/166-1	15/12/03	70° 56. 83'	10° 32. 61'	338	BT	This study
PS65/259-1	24/12/03	70° 57. 00'	10° 33. 02'	332.8	BT	This study
PS65/175-1	16/12/03	70° 57. 11'	10° 33. 32'	337.2	BT	This study
PS65/245-1	22/12/03	70° 57. 11'	10° 33. 52'	337.2	BT	This study
PS65/174-1	16/12/03	70° 57. 33'	10° 33. 86'	351.6	BT	This study
1	05/02/96	71° 03. 10'	11° 25. 50'	462	BT	Zabala et al. (1997)
PS65/253-1	23/12/03	71° 04. 30'	11° 33. 92'	308.8	BT	This study
PS65/248-1	23/12/2003	71° 04. 96'	11° 31. 90'	286.8	BT	This study
PS65/039-1	05/12/03	71° 06. 30'	11° 32. 04'	175.2	AT	This study
PS65/276-1	28/12/03	71° 06. 44'	11° 27. 76'	277.2	AT	This study
PS65/280-1	29/12/03	71° 07. 15'	11° 26. 23'	228.4	AT	This study
PS65/279-0	29/12/03	71° 07. 43'	11° 29. 83'	119.2	AT	Barnes & Kuklinski (2010)
PS65/279-1	29/12/03	71° 07. 48'	11° 29. 91'	119.6	AT	This study
PS65/278-1	29/12/03	71° 07. 51'	11° 29. 94'	120	AT	Barnes & Kuklinski (2010)
24	21/02/96	71° 08. 15'	11° 32. 25'	123	AG	Zabala *et al.* (1997)
24	21/02/96	71° 08. 30'	11° 32. 40'	119	GK	Zabala *et al.* (1997)
2	22/02/96	71° 18. 60'	12° 25. 40'	181	MG	Zabala *et al.* (1997)
PS67/078-9	21/02/05	71° 09. 39'	13° 59. 30'	2156	ES	Barnes & Kuklinski (2010)
PS67/078-11	21/02/05	71° 09. 39'	13° 59. 33'	2157	AT	Barnes & Kuklinski (2010)
PS67/074-6	20/02/05	71° 18. 35'	13° 57. 71'	1030	ES	Barnes & Kuklinski (2010)
PS65/232-1	21/12/03	71° 18. 61'	13° 56. 12'	910	ES	This study
2	09/02/96	71° 18. 70'	12° 17. 10'	170	AG	Zabala *et al.* (1997)
2	22/02/96	71° 19. 10'	12° 22. 80'	159	MG	Zabala *et al.* (1997)
2	22/02/96	71° 19. 20'	12° 27. 00'	253	MG	Zabala *et al.* (1997)
25	23/02/96	71° 22. 90'	14° 19. 20'	622	AG	Zabala *et al.* (1997)
25	23/02/96	71° 22. 90'	14° 19. 20'	622	DR	Zabala *et al.* (1997)
25	23/02/96	71° 23. 10'	14° 19. 80'	634	AG	Zabala *et al.* (1997)

Table 1. (Continued)

Station	Date	Latitude (S)	Longitude (W)	Depth (m)	Gear	References
25	23/02/96	71° 23. 10'	14° 19. 70'	621	GK	Zabala *et al.* (1997)
25	23/02/96	71° 23. 10'	14° 19. 70'	628	GK	Zabala *et al.* (1997)
21	18/02/96	71° 26. 50'	21° 10. 50'	253	BP	Zabala *et al.* (1997)
7	08/02/96	71° 26. 80'	13° 44. 00'	215	GK	Zabala *et al.* (1997)
6	11/02/96	71° 27. 40'	13° 43. 30'	212	AG	Zabala *et al.* (1997)
26	24/02/96	71° 29. 30'	14° 18. 60'	216	DR	Zabala *et al.* (1997)
26	24/02/96	71° 29. 30'	14° 19. 50'	210	DR	Zabala *et al.* (1997)
29	29/02/96	71° 30. 70'	12° 26. 40'	494	GK	Zabala *et al.* (1997)
29	28/02/96	71° 31. 50'	12° 25. 50'	504	BP	Zabala *et al.* (1997)
6	08/02/96	71° 31. 80'	13° 34. 50'	254	AG	Zabala *et al.* (1997)
6	25/02/96	71° 32. 10'	13° 44. 10'	362	AG	Zabala *et al.* (1997)
9	26/02/96	71° 32. 60'	12° 26. 30'	570	AG	Zabala *et al.* (1997)
9	10/02/96	71° 34. 00'	12° 25. 80'	604	BT	Zabala *et al.* (1997)
9	26/02/96	71° 34. 70'	12° 26. 60'	560	AG	Zabala *et al.* (1997)
9	26/02/96	71° 34. 70'	12° 26. 60'	560	DR	Zabala *et al.* (1997)
3	26/02/96	71° 39. 30'	12° 05. 10'	209	GK	Zabala *et al.* (1997)
5	06/02/96	71° 39. 75'	12° 41. 00'	255	MG	Zabala *et al.* (1997)
5	06/02/96	71° 40. 49'	12° 41. 70'	254	EB	Zabala *et al.* (1997)
5	07/02/96	71° 41. 10'	12° 44. 30'	227	BT	Zabala *et al.* (1997)
4	20/02/96	71° 41. 20'	12° 30. 80'	438	MG	Zabala *et al.* (1997)
4	20/02/96	71° 41. 50'	12° 31. 70'	436	GK	Zabala *et al.* (1997)
4	20/02/96	71° 41. 60'	12° 29. 40'	440	AG	Zabala *et al.* (1997)
PS65/283-1	30/12/03	72° 32. 16'	17° 58. 88'	585.2	ES	This study
PS65/297-1	01/01/04	72° 48. 50'	19° 31. 60'	668	RD	This study
PS65/308-1	02/01/04	72° 50. 18'	19° 35. 94'	622	RD	This study
20	18/02/96	72° 50. 50'	19° 26. 00'	428	BP	Zabala *et al.* (1997)
PS65/292-1	31/12/03	72° 51. 43'	19° 38. 62'	597.6	BT	This study
PS65/326-1	03/01/04	72° 51, 43'	19° 38. 67'	616	RD	This study
PS65/326-1	03/01/04	72° 51. 70'	19° 39. 22'	605.2	RD	Barnes & Kuklinski (2010)
PS65/324-1	03/01/04	72° 54. 52'	19° 47. 74'	693.6	RD	This study
PS65/324-1	03/01/04	72° 54. 55'	19° 47. 30'	647.2	RD	Barnes & Kuklinski (2010)
PS65/325-1	03/01/04	72° 54. 76'	19° 43. 48'	457.6	RD	This study
18	16/02/96	73° 15. 40'	21° 27. 60'	1704	AG	Zabala *et al.* (1997)
18	16/02/96	73° 16. 70'	21° 25. 50'	1538	AG	Zabala *et al.* (1997)
17	16/02/96	73° 18. 00'	21° 09. 90'	468	BT	Zabala *et al.* (1997)
12	13/02/96	73° 18. 10'	21° 10. 10'	459	BT	Zabala *et al.* (1997)
11	13/02/96	73° 22. 60'	21° 10. 60'	338	BT	Zabala *et al.* (1997)
21	18/02/96	73° 22. 90'	21° 10. 00'	283	BP	Zabala *et al.* (1997)
14	14/02/96	73° 36. 10'	22° 35. 70'	850	BT	Zabala *et al.* (1997)
13	14/02/96	73° 36. 30'	22° 19. 10'	620	BT	Zabala *et al.* (1997)
15	15/02/96	73° 42. 00'	22° 30. 50'	446	BT	Zabala *et al.* (1997)
16	15/02/96	73° 53. 40'	22° 26. 90'	246	BT	Zabala *et al.* (1997)

Results

A total of 54 species of Antarctic bryozoans (206 samples), belonging to 12 families and 27 genera, were found with different trawls, from depths between 27 and 910 m in the studied areas (Table 2). The list includes a newly described species, *Reteporella rosjoarum* (Figuerola *et al*. 2013). Furthermore, two species were reported for the first time from Bouvet Island, one from the Weddell Sea and one from Spiess Seamount. Eight of the species were identified only to genus level. The most diverse Infraorder was Lepraliomorpha with 18 species (33%). 55% of the species found were endemic to the Southern Ocean (see Hayward 1995, SCAR-MarBIN and GBIF databases), with a total of 49 species. *Reteporella* with 6 species was the dominant genus. Most of the species found were *Bostrychopora dentata*, which represented 9.7% of the total specimens collected, and *Nematoflustra flagellata* (6.7%). These were followed by *Austroflustra vulgaris, Alcyonidium sp., Carbasea curva, Cellarinella nutti* and *Osthimosia curtioscula*. *Austroflustra vulgaris* was the only species found in the three studied areas from the Weddell Sea.

Data from recent cruises reported in the literature and the GBIF database together with our own data were jointly analysed, revealing that four species have been found far from their known distribution range in the Weddell Sea. Therefore, an expansion in their known geographical distribution is reported here (Table 2).

Bathymetric ranges

From a total of twenty-seven genera analysed on the different cruises, 50.2 % (16 genera) were restricted to the continental shelf (18 species), and above 900 m. *Camptolites, Melicerita* and *Cellaria* were the only genera found in deeper waters (5900, 4802 and 4531 m, respectively) and showed the widest bathymetric ranges (Fig. 2). Seven genera showed large bathymetric ranges: *Carbasea*, (31-2846m), *Austroflustra* and *Cornucopina* (5-2700m), *Cellarinella* (5-2334m), *Isosecuriflustra* (22-2315m), *Kymella* (0-2157m) and *Nematoflustra* (0-2100m). Four genera (15 species) were present at depths between 0 and 700 m.

Table 2. Bathymetric ranges and biogeographic distribution of the species studied using data from the present study, the literature and the GBIF database.

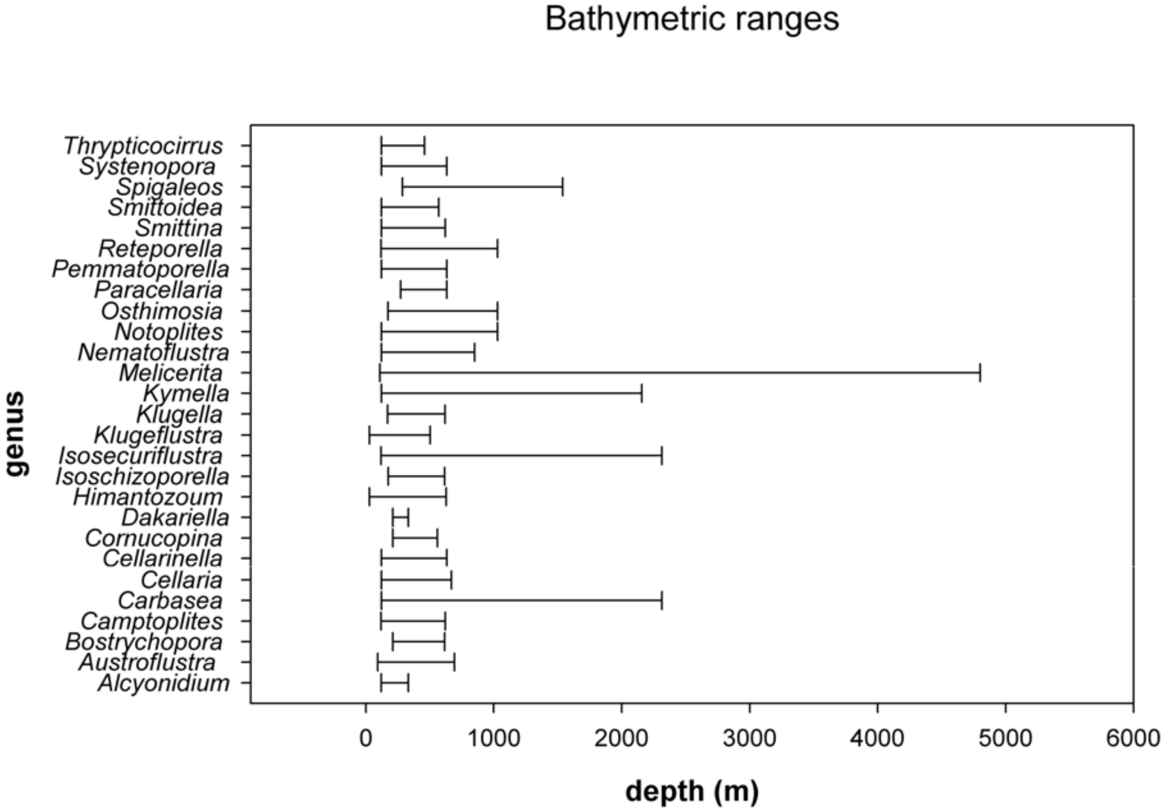
* New bathymetric range described in this study, ** First record for Bouvet Island, *** First record for Spiess Seamount, **** First record for the Weddell Sea

Species	**Present study**	**Bathymetric distr (m)**	**Geographic distr**	**New records for species**	**References**
Carbasea curva Kluge, 1914	X	31-2846	Weddell sea		Zabala *et al.* (1997); Gontar & Zabala (2000)
Klugeflustra antarctica Hastings, 1943	X	5-732	Livingston		Hayward (1995)
Isosecuriflustra angusta Kluge, 1914		31-2315	Weddell sea		Zabala *et al.* (1997); Gontar & Zabala (2000)
Isosecuriflustra tenuis Kluge, 1914	X	22- 639* (previously 634)	Weddell sea		Zabala *et al.* (1997); Gontar & Zabala (2000)
Austroflustra vulgaris Kluge, 1914	X	5-2700	Bouvet Island*, Livingston*, Weddell sea		Arntz *et al.* (2006); Hayward (1995); Zabala *et al.* (1997); Gontar & Zalaba (2000)
Nematoflustra flagellata Waters, 1904		0-2100	Weddell sea		Zabala *et al.* (1997); Gontar & Zabala (2000)
Camptoplites angustus Kluge, 1914	X	5-720	Weddell sea		Zabala *et al.* (1997); Gontar & Zabala (2000)
Camptoplites bicornis Busk, 1884		5-5900	Weddell sea		Zabala *et al.* (1997); Gontar & Zabala (2000)
Camptoplites giganteus Kluge, 1914	X	20-294* (previously 293)	Weddell sea		Zabala *et al.* (1997); Gontar & Zabala (2000)
Camptoplites tricornis Waters, 1904		5-2000	Weddell sea		Zabala *et al.* (1997); Gontar & Zabala (2000)
Cornucopina polymorpha Kluge, 1914	X	5-2700	Bouvet Island	**	Arntz *et al.* (2006)
Himantozoum antarcticum Calvet, 1909	X	5-1517	Weddell sea, Livingston		Zabala *et al.* (1997); Gontar & Zabala (2000); Hayward (1995)
Klugella echinata Kluge, 1914		170-640	Weddell sea		Zabala *et al.* (1997); Gontar & Zabala (2000)
Notoplites antarcticus Waters, 1904		104-634	Weddell sea		Zabala *et al.* (1997); Gontar & Zabala (2000)
Notoplites drygalskii Kluge, 1914		123-1030	Weddell sea		Zabala *et al.* (1997); Gontar & Zabala (2000); Barnes & Kuklinski (2010)
Cellaria aurorae Livingstone, 1928		5-2334	Weddell sea		Zabala *et al.* (1997); Gontar & Zabala (2000) Barnes & Kuklinski (2010)
Cellaria diversa Livingstone, 1928	X	5-3545	Weddell sea		Zabala *et al.* (1997); Gontar & Zabala (2000)

Table 2. (Continued)

Osthimosia curtioscula Hayward, 1992	X	104-1150	Weddell sea		Zabala *et al.* (1997); Gontar & Zabala (2000); Barnes & Kuklinski (2010)
Spigaleos horneroides Waters, 1904	X	283-1543	Spiess Seamount	***	
Reteporella antarctica Waters, 1904		61-622	Weddell sea		Zabala *et al.* (1997); Gontar & Zabala (2000)
Reteporella erugata Hayward, 1992	X	86-1030	Bouvet Island*		Arntz *et al.* (2006); Barnes & Kuklinski (2010)
Reteporella frigida Waters, 1904		5-923	Weddell sea		Zabala *et al.* (1997); Gontar & Zabala (2000) Barnes & Kuklinski (2010)
Reteporella hippocrepis Waters, 1904	X	61-634	Bouvet Island, Weddell sea	**	Arntz *et al.* (2006); Zabala *et al.* (1997); Gontar & Zabala (2000)
Reteporella lepralioides Waters, 1904		61-634	Weddell sea		Zabala *et al.* (1997); Gontar & Zabala (2000)
Reteporella sp. n. *Figuerola, Ballesteros & Avila, in press*	X	264*	Weddell sea*	****	Zabala *et al.* (1997); Gontar & Zabala (2000)
Alcyonidium unidentified species *Kirkpatrick, 1902*	X	73-655	Weddell sea		Zabala *et al.* (1997); Gontar & Zabala (2000)

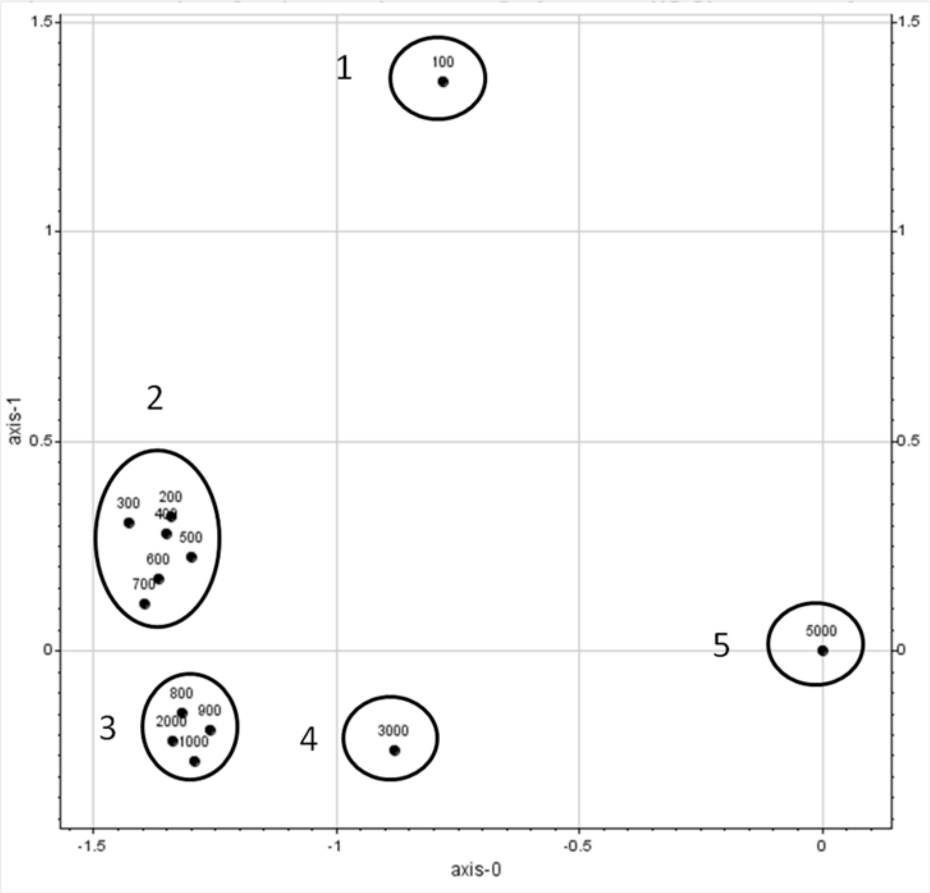
Fig. 2. New bathymetric ranges of bryozoans genera from the Southern Ocean found in the present study both from our own data and the literature and the GBIF database. Additional data have been obtained from Hayward (1995), Zabala *et al.* (1997), Gontar & Zabala (2000), Arntz *et al.* (2006) and Barnes & Kuklinski (2010).



Bathymetric distribution

Low stress values (0.03) of the MDS indicate a good representation in the 2-dimensional ordination (Clarke 1993). Five depth zones were discriminated by the multidimensional scaling analysis in bathymetric distribution (Fig. 3): 1) a zone between 0 and 100 m with the presence of three genus, 2) a zone between 100 and 700 m characterized by the presence of all of the genera, a similar composition at each depth (77.9% of the genera appear in each 100 m of depth) and the presence of the genus *Dakariella* only in one depth interval (200-300 m), 3) a zone between 700 and 2000 m with a high similarity of generic composition but with fewer (nine) genera, 4) a zone between 2000 and 3000 m with the presence of four genera, and 5) another zone between 3000 and 5000 m characterized by the presence of *M. obliqua* only.

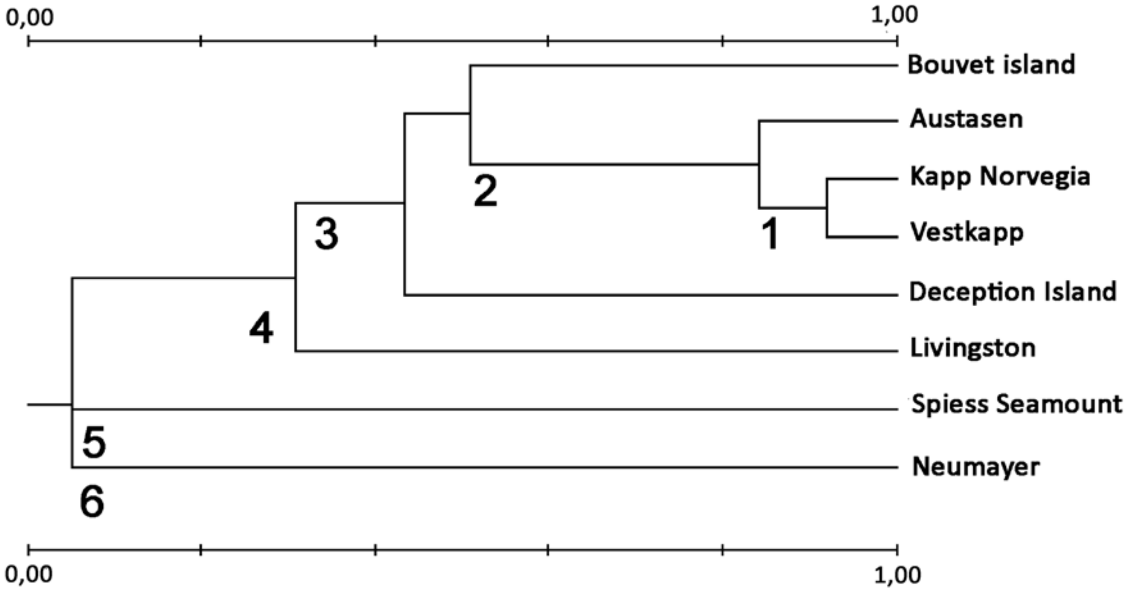
Fig. 3. Plot of the multidimensional scaling ordination (MDS) of the different genera in relation to depth. Points numbered 100-5000 correspond to different depth ranges (stress= 0.03). Additional data have been obtained from Hayward (1981, 1995), Zabala *et al.* (1997), Gontar & Zabala (2000), Arntz *et al.* (2006) and Barnes & Kuklinski (2010). Group 1: 0-100m; group 2: 100-700m; group 3: 700-2000m; group 4: 2000-3000m and group 5: 3000-5000m.



Geographic distribution

Cluster analyses suggested six principal groups of similar faunal composition (Fig. 4). The first group (1) is represented by the region of Eastern Weddell Sea comprised of the sub-regions of Kapp Norvegia (30 stations), Vestkapp (20 stations) and Austasen (32 stations) with the same number of species but of different composition. The islands of groups 3 and 4, the region of the Spiess Seamount (5) and the region of Neumayer (6) were represented by more separated groups with a lower number of species. In the results of test for binomial proportions, the sub-regions of Kapp Norvegia with Austasen and Vestkapp showed no significant differences ($p<0.05$). Deception Island, Livingston Island, Spiess Seamount and the Neumayer region showed significant differences with other regions ($p<0.05$). Bouvet Island exhibited significant differences with respect to all other regions ($p<0.05$).

Fig. 4. Dendrogram from hierarchical clustering (single linkage) of the bryozoan fauna from the Southern oceans using Sørensen distance (Pearson cophenetic index 0.97). Additional data have been obtained from Hayward (1981, 1995), Zabala *et al.* (1997), Gontar & Zabala (2000), Arntz *et al.* (2006), Barnes *et al.* (2008) and Barnes & Kuklinski (2010).



Species richness and diversity indices

Relative species richness (S) was low at depths between 0 and 100 m and from 800 to 5000 m, with significant differences between ranges from 100 to 700 m (test for binomial proportions, $p<0.01$). The highest value was found between 300 and 400 m (Table 3; Fig. 5). The regions of Austasen, Kapp Norvegia and Vestkapp (with the same number of species but of different composition) had the highest species richness, followed by Bouvet and Deception islands (Fig. 6).

Table 3. Number of species (nº spp), % of relative species richness (S) and p-value (P) for each depth range in the Eastern Weddell Sea. Additional data have been obtained from Hayward (1995), Zabala *et al.* (1997), Gontar & Zabala (2000), Arntz *et al.* (2006) and Barnes & Kuklinski (2010).

depth (m)	nº spp	% S	P
100	3	11.11	-
200	23	85.19	0.000
300	27	100.00	0.000
400	26	96.30	0.000
500	25	92.59	0.000
600	24	88.89	0.000
700	21	77.78	0.000
800	9	33.33	0.099
900	9	33.33	p>0.1
1000	8	29.63	p>0.1
2000	8	29.63	p>0.1
3000	4	14.81	p>0.1
5000	1	3.70	p>0.1

Fig. 5. Number of species (nº spp) and % of relative species richness (S) related to depth ranges in the Eastern Weddell Sea and the Antarctic Peninsula. Additional data have been obtained from Hayward (1995), Zabala *et al.* (1997), Gontar & Zabala (2000), Arntz *et al.* (2006) and Barnes & Kuklinski (2010).

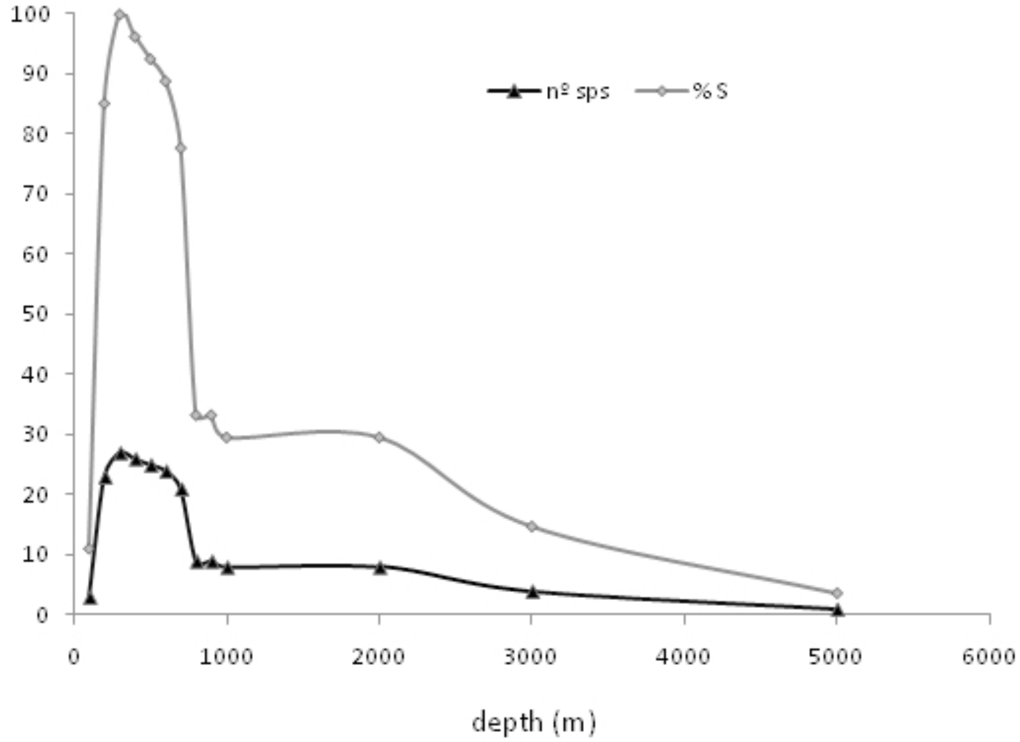
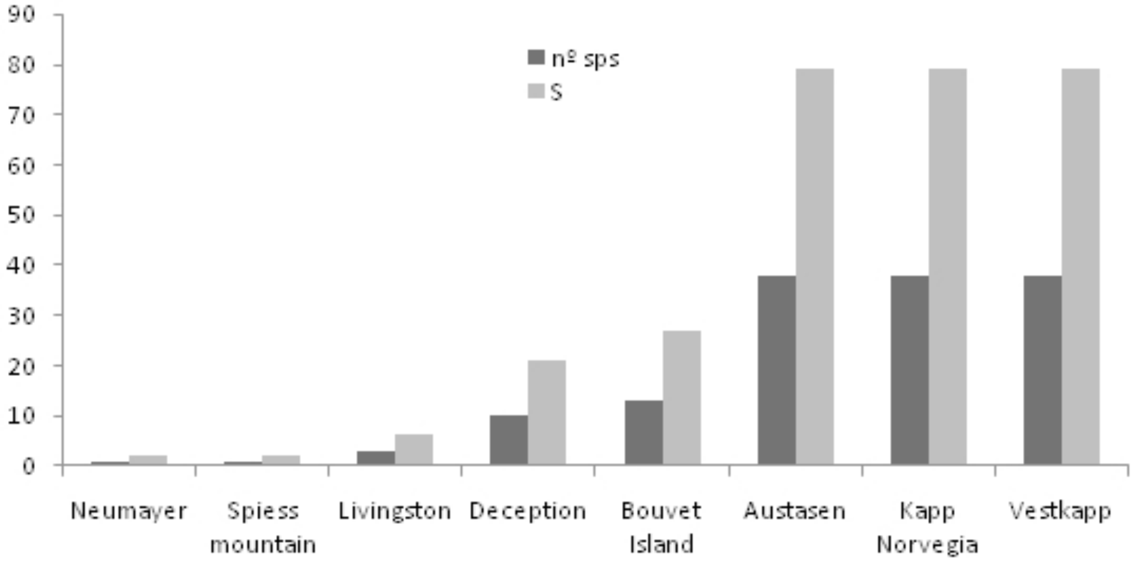
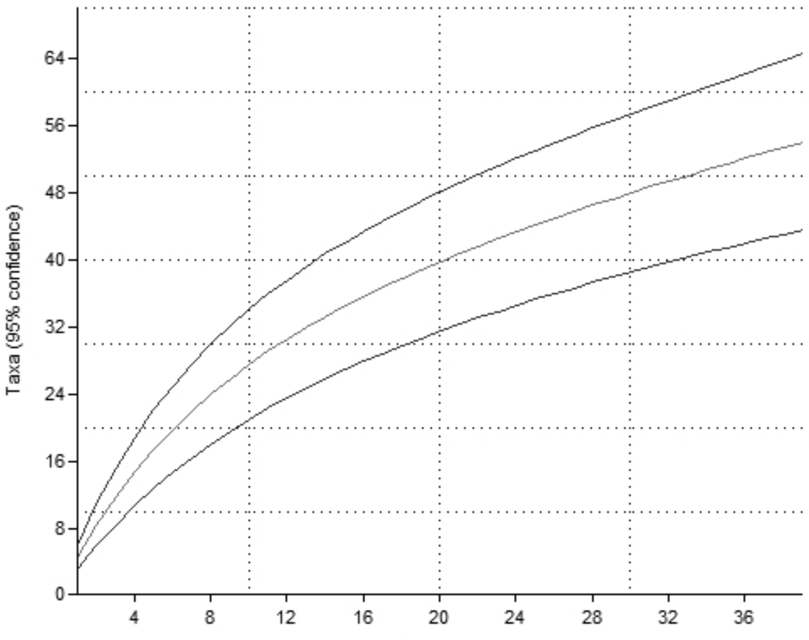


Fig. 6. Number of species (nº spp) and % of relative species richness (S) in different areas of the Eastern Weddell Sea and the Antarctic Peninsula. Additional data have been obtained from Hayward (1981, 1995), Zabala *et al.* (1997), Gontar & Zabala (2000), Arntz *et al.* (2006), Barnes *et al.* (2008) and Barnes & Kuklinski (2010).



The accumulation curve has still to reach the asymptote: 54 species have been found, but up to 90 (Chao2) can be expected as more samples are collected (Fig. 7). Jacknife1 and Jacknife2 methods estimated the theoretical number of expected species. These values are 82 and 97, respectively. However, in our case (absence of complete inventories), Chao2 has been shown to perform better than most other methods (Krebs 1999).

Fig. 7. Sample-based rarefaction curve. Expected species richness value was computed with 95% confidence interval.



Five alpha diversity indices were calculated for each region only for the stations sampled with Agassiz trawl (AT), Bottom trawl (BT) and Rauschert dredge (RD) since they did not show significant differences (Table 4). The Shannon-Wiener and Margalef indices changed between regions with the highest value of indices and number of species in the region of Austasen (H'= 3.445; D_{Mg}= 8.64), followed by Vestkapp (H'= 2.844, D_{Mg}= 5,498), while Bouvet Island and Livingston Island showed low values (Table 5). The diversity indices for Kapp Norvegia could not be calculated due to the unavailability of samples collected using these methods. Samples from Deception Island and Spiess seamount contained only one species.

Table 4. Diversity indexes for the three types of sampling (AT: Agassiz Trawl,, BT: BottomTrawland RD: Rauschert dredge) from the present study with 95% confidence intervals using Bootstrap method: Margalef index (D_{Mg}), Shannon–Wiener diversity index, H' (base log e), Simpson's Index (1 – Lambda'), Pielou's index (J') and Berger–Parker index (B–P).

	D_{Mg}		**H'**		**1-Lambda'**		**J'**		**BP**	
	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
AT	5.87	8.21	3.01	3.40	0.93	0.96	0.72	0.87	0.07	0.17
BT	6.16	8.44	3.05	3.41	0.94	0.96	0.71	0.85	0.08	0.16
RD	5.11	7.67	2.82	3.27	0.92	0.96	0.74	0.90	0.08	0.20

Table 5. Characteristics of the regions sampled with the three dominant types of sampling (AT, BT and RD). For each region: dominant species found in the sample, total number of species found (N spp), Margalef index (D_{Mg}), Shannon–Wiener diversity index, H' (base log e), Simpson's Index (1 – Lambda'), Pielou's index (J') and Berger–Parker index (B–P)

Site	**Dominant species found in the sample**	**N spp**	**D_{Mg}**	**H'**	**1-Lambda'**	**J'**	**B-P**
Bouvet Island	*Osthimosia curtioscula*	13	1.559	1.525	0.7692	0.9188	0.3077
Austasen	*Bostrychopora dentata*	145	8.64	3.445	0.9557	0.7124	0.1241
Vestkapp	*Carbasea curva* *Nematoflustra flagellata*	38	5.498	2.844	0.9294	0.8186	0.1316
Spiess Seamount	*Spigaleos horneroides*	1	0	0	0	1	1
Livingston Island	*Austroflustra vulgaris* *Himantozoum antarcticum* *Klugeflustra antarctica*	3	1.82	1.099	0.6667	1	0.3333
Deception Island	*Melicerita latilaminata*	1	0	0	0	1	1

Similarity with other regions

In our study, the Antarctic region is connected by some genera shared with South America (55.5%), New Zealand (48.15%) and South Africa (37.04%). In fact, the South Shetland Islands had a composition slightly more similar to South America (43.75 %) than to Antarctica (29.63 %) (Table 6).

Table 6. Genera found in this study in Antarctica and Scotia Arc. Additional data from South America, New Zealand and South Africa have been obtained from Moyano (1982, 1999), Gordon (1984, 1986), Florence *et al.* (2007); see also www.bryozoa.net.

Genera	**Antarctica**	**South America**	**New Zealand**	**South Africa**	**Scotia Arc**
Carbasea	x	x	x	x	
Klugeflustra	x			x	x
Isosecuriflustra	x				
Austroflustra	x	x	x		x
Nematoflustra	x				
Camptoplites	x	x	x		x
Cornucopina	x	x	x		x
Himantozoum	x	x	x	x	x
Klugella	x				
Notoplites	x	x	x	x	
Cellaria	x	x	x	x	x
Paracellaria	x	x			x
Melicerita	x	x	x		x
Cellarinella	x	x			
Systenopora	x				
Isoschizoporella	x				
Dakariella	x				
Kymella	x				
Smittina	x	x	x	x	
Smittoidea	x	x	x	x	
Thrypticocirrus	x				
Pemmatoporella	x				
Bostrychopora	x				
Osthimosia	x	x	x	x	
Spigaleos	x				
Reteporella	x	x	x	x	
Alcyonidium	x	x	x	x	

Discussion

The benthic fauna of the continental shelf of the Eastern Weddell Sea, as described for some other areas, is dominated by suspension feeders, such as bryozoans, and variations in their abundance are critical to the organization of the whole community (Teixidó *et al.* 2002, 2004). This shelf reaches great depths, with the shelf break at about 900–1.000 m (e.g. Linse *et al.* 2006). Few bryozoan species have been reported from below the shelf break (Barnes & Kuklinski 2010) and most benthic samples come from depths of less than 500 m (Griffiths 2010).

Antarctic bryozoans analyzed here exhibit a high range of eurybathy. Bathymetric distributions of Antarctic fauna reported in the literature demonstrate that some species extend over large depth ranges (e.g. Brey *et al.* 1996; Soler-Membrives *et al.* 2009). 27 bryozoan species of this study have been recorded in the Southern Ocean deeper than 1000 m. The case of the genus *Camptolites* is even more amazing, showing a depth range of 0-5900 m. The existence of eurybathic species has been explained by the evolutionary history of the Southern Ocean fauna (e.g. Munilla 2001). Thatje & colleagues (2005) suggested that the impact of the grounded ice sheets on most of the Antarctic continental shelf during Cenozoic glacial periods affected the benthic communities. Therefore, the continental shelf was further recolonized by deep-water organisms with wide bathymetric tolerances and thus, depth seems to be a less important factor in controlling the distribution of communities compared to other areas. Changes in Antarctic biodiversity have been found to be associated with the movement of taxa between shelf and the deep sea (Arntz *et al.* 1997; Brey *et al.* 1996; Brandt & Hilbig 2004). However, this possibility has not been proved so far (Thatje *et al.* 2005). The possibility should also be considered that some deep occurrences (>1000m) are due to transport off the shelf by currents, as shown for bryozoans from elsewhere, such as New Zealand (e.g. Lagaaij 1973; Hayward 1981; Taylor *et al.* 2004).

Horizontal and vertical variability in Antarctic bryozoan distributions does exist. For some benthic species, horizontal and bathymetric distribution patterns have been described, but, in the case of most bryozoan species from this area, their horizontal distribution and bathymetric ranges are relatively unknown. Multidimensional scaling analysis in our study showed that bryozoans were distributed in zones or depth bands. Clarke *et al.* (2003) reported that in Antarctica the continental shelf lies at depths between 500 m and 700 m and in some places depths exceeds 1000 m, while the continental slope is found at 1000-3000 m and the deep sea at over 3000 m. For example, Prydz Bay is considered to be Antarctic shelf with the deepest areas about 1200m (O'Brien *et al.* 2007).The bryozoan distribution found in our study fits well with these proposed limits: the species composition of continental shelf (0-700m or 800-1000m) differs from that of the continental slope and of the deep sea (>3000m). However, the sample effort banding may have influenced these results. In agreement with this, Kaiser & colleagues (2011) found that the shelf, and abyssal bryozoans were clearly separated in the Weddell Sea.

Some studies have demonstrated that Antarctic megafaunal density generally decreases with depth (Arntz *et al*. 1994; Thatje & Mutschke 1999; Rex *et al*. 2006; Linse *et al*. 2007), which can be related to the decreasing availability of food with depth. However, other factors could be correlated with this, such as a limited availability of substratum for encrusting species at depth. Decrease in organic matter is considered to be the main limiting factor for the Antarctic benthos (Lampitt *et al*. 2001; Saiz *et al*. 2008; Arntz *et al*. 1994). Barnes & Kuklinski (2010) also reported that the bryozoan species richness decreases rapidly with depth. Nevertheless, abundances are very variable at depths of 1000–3500m and some authors have suggested the existence of patchy distribution patterns (e.g. Brandt *et al*. 2005). In agreement with this, slope richness of some taxa and of some areas was larger than that of the shelf or abyss zones (Kaiser *et al*. 2011). In contrast, other findings suggested that abundance increases with depth in some areas of the Weddell Sea and decreases with depth in other areas, such as Kapp Norvegia (Linse *et al*. 2002). In addition, there are other factors we must take into account, such as biological factors (e.g. food availability and predation), which may have more influence at small spatial scales and depths greater than 20 m, where physical disturbance by ice is less frequent. With this regard, Smale (2008) found high variability in the distribution of species in these conditions.

The result of our cluster analyses indicated a spatial pattern in the distribution of species of bryozoans, and the different regions observed agree with the different zoogeographical zones of diversity suggested by previous authors (Barnes & De Grave 2000; Barnes & Kuklinski 2010): the Subantarctic islands (Bouvet Island), East Antarctica (eastern Weddell Sea), West Antarctica and the Scotia Arc (Deception and Livingston Islands). At a smaller scale, we observed a horizontal variability in assemblage composition between some regions. The regions of Kapp Norvegia (30 stations), Vestkapp (20 stations) and Austasen (32 stations) showed the same values of relative species richness. However, the regions of Kapp Norvegia and Vestkapp are more similar (the subgroup of cluster 1 has the highest similarity, 92%), indicating an even greater similarity in species composition. The reason for this similarity could be their proximity. Moreover, Gerdes *et al*. (2008) proposed that the shelf off Austasen has to be considered as a patchwork of disturbed areas and this could be the reason for its higher diversity (higher values of D_{Mg}, H' and 1-Lambda' indices) compared with Vestkapp. In contrast, the value of the Berger-Parker and Pielou's indices were lower because there were many individuals of one species (*B. dentata*). However, this area shows the same value of species richness as the region of Kapp Norvegia (84% similarity).

The regions of Neumayer, the Spiess Seamount and the islands of Bouvet, Deception and Livingston are separated geographically, and exhibit the lowest species richness and diversity because they are distant from other regions and scarcely sampled. Many new records of known or unknown species can be expected to be found in the future. Also, the rarefaction curve showed no sign of approaching an asymptote. Fifty four species have been found, but total numbers estimated by species richness statistics (Chao2) suggest that at least 90 species of bryozoans will be found in the studied area as more samples are collected.

Some studies have reported that Bouvet Island shows similarity with the region of the Weddell Sea and has a similar taxonomic richness (Barnes 2006; Gutt *et al*. 2006) and our results for bryozoans are in agreement with that. This could be due to the existence of a permanent import of species by dispersion of marine benthic animals (Pielou 1975). Other studies have demonstrated that the general composition and diversity of Bouvet Island were not lower compared to the Patagonian shelf and only moderately lower than the Antarctic continental shelf (Arntz *et al*. 2006; Gutt *et al*. 2006).

Bouvet Island and the region of Spiess Seamount are located at present a particular position relative to the Antarctic Circumpolar Current and may be in a potential zone of faunal exchange among the various regions and across the Polar Front (Linse 2006). Larvae of different invertebrates from the Scotia Arc could reach Bouvet Island with the Circumpolar Current or from the Weddell Sea with the Weddell Gyre (Barnes 2006). One hypothesis is that Bouvet Island could have acted as supply sources to the Weddell Sea during glacial maximum, when this island was not covered by ice and adult of species could travel on kelp or pumice with currents of the Weddell Sea Gyre (Barnes & Kuklinski 2010). The benthos of the Spiess Seamount is characterized by being extremely poor (Arntz *et al*. 2006). However, the cluster analyses showed Deception Island to be more separated than the other islands (0% of similarity). San Vicente *et al*. (1997) suggested that the reduced number of species at Deception Island was probably related to the last volcanic episode and to the present acidity in the surface sediment. This could also explain the low bryozoans diversity. Also, the availability of hard substrates limits the abundance and diversity of bryozoans (Hughes 2001). Also, many filter feeders have a preference for an elevated position which may enhance prey capture (Wildish & Kristmanson 1997). Deception Island has few hard substrates and this could affect bryozoan diversity. However, Barnes *et al*. (2008) reported that the undersurfaces of boulders from Deception Island are dominated by bryozoans (cryptofauna).

The presence of a common bryozoan fauna between South America and the Western Antarctica can be explained by their proximity during the Tertiary (Zinsmeister 1979) and by the relatively similar environmental conditions related to the Antarctic Circumpolar Current (Moyano 1982).

Various studies support the role of the Scotia Arc as the link between Antarctica and South

America (e.g. Arntz *et al.* 2005b). In our study, the South Shetland Islands showed a balanced composition between these two regions, thus supporting this hypothesis. Although Antarctic endemism is very high, zoogeographically, there are clear relationships between the fauna of Antarctica and those of South America, New Zealand and South Africa. These similarities could be traced back to the time where continents were part of Gondwana. Also, in the Oligocene, a paleobiogeographic connection between New Zealand and Patagonia may have existed, as shown by the presence of common taxa, through the West Antarctic Rift System (Casadío *et al.* 2010).

In Antarctica, a clear latitudinal cline in diversity, oriented north to south along the western Antarctic Peninsula, has been reported also for macroalgae and molluscs (Moe & deLaca 1976; Schiaparelli *et al.* 2006). The existence of a similar cline in bryozoan diversity has been found in this study, with a higher richness at 70-73ºS (Austasen, Kapp Norvegia and Vestkap) than at 54-70º S (Bouvet, Livingston and Deception Islands, and Neumayer). However, the interpretation of these results must be treated with some caution because they are based on the frequency of occurrence rather than the abundance of species.

Concluding remarks

During the past two decades, research of the basic descriptive taxonomy and benthic ecology from the Southern Ocean has improved greatly, demonstrating that this area is quite rich and diverse. However, some almost inaccessible regions, such as some parts of Antarctica, are difficult to sample and the research on biodiversity is limited by the lack of richness data for some groups, such as the bryozoans. Although the results of the analyses performed here from new data on bryozoan biodiversity increase our knowledge of species geographical ranges, they are still limited because samples were collected from only a few areas. The scales of the latitudinal and the bathymetric gradients are large and the majority of marine studies have only sampled small areas. This causes an underestimation of diversity because it has been demonstrated that species richness varies with increasing sampled area (Gray 2000). The main limitation of this study is the use of data from different methods of sampling. However, the bathymetric and geographical distributions of the studied species contribute to a better understanding of Antarctic bryozoan diversity and distributions and it is relevant in the establishment of biogeographical patterns. More intensive sampling of bryozoans along a wider geographical range is needed for the Weddell Sea and other Antarctic areas.

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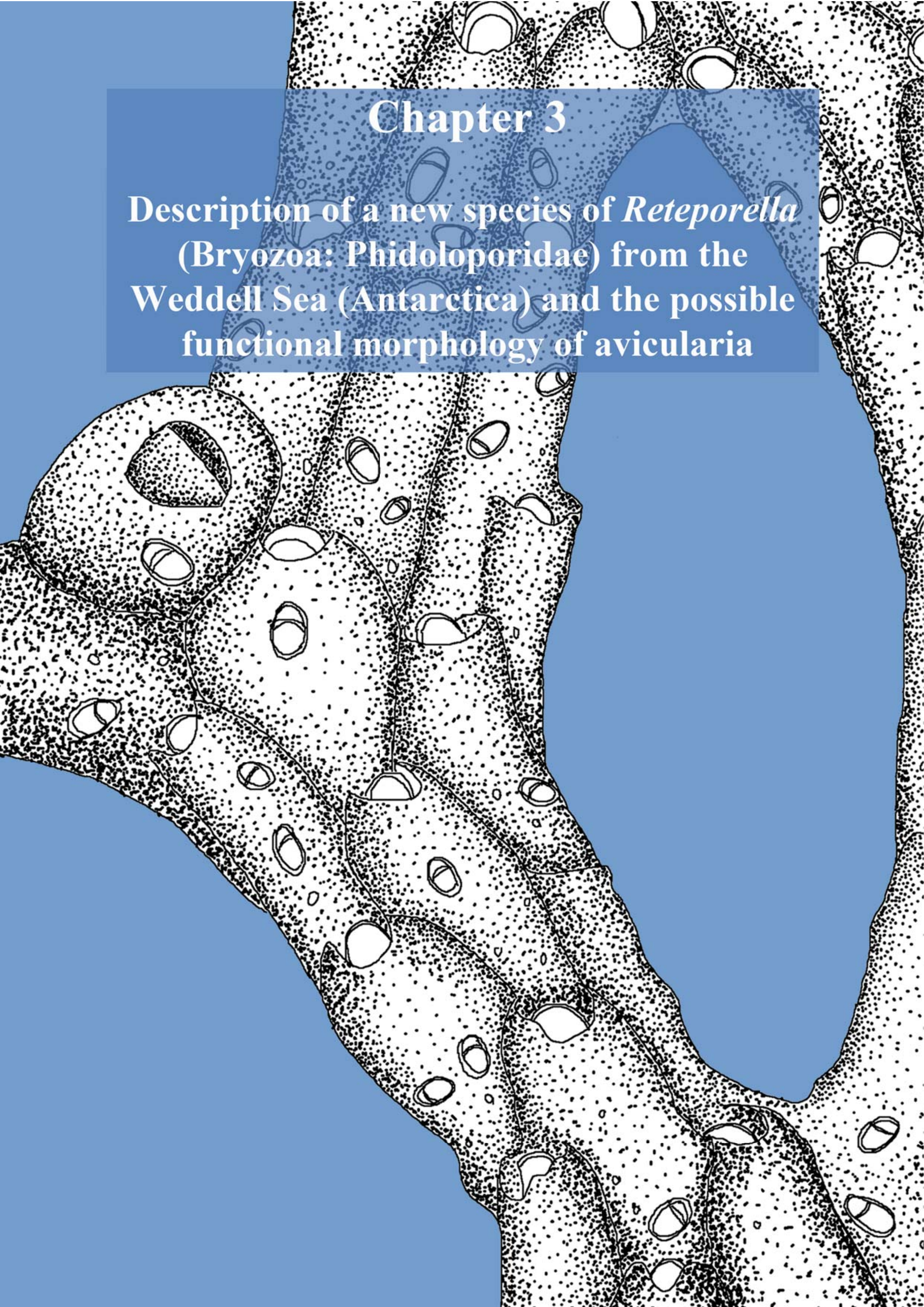
Chapter 3

Description of a new species of *Reteporella* (Bryozoa: Phidoloporidae) from the Weddell Sea (Antarctica) and the possible functional morphology of avicularia



Chapter 3

Description of a new species of *Reteporella* (Bryozoa: Phidoloporidae) from the Weddell Sea (Antarctica) and the possible functional morphology of avicularia

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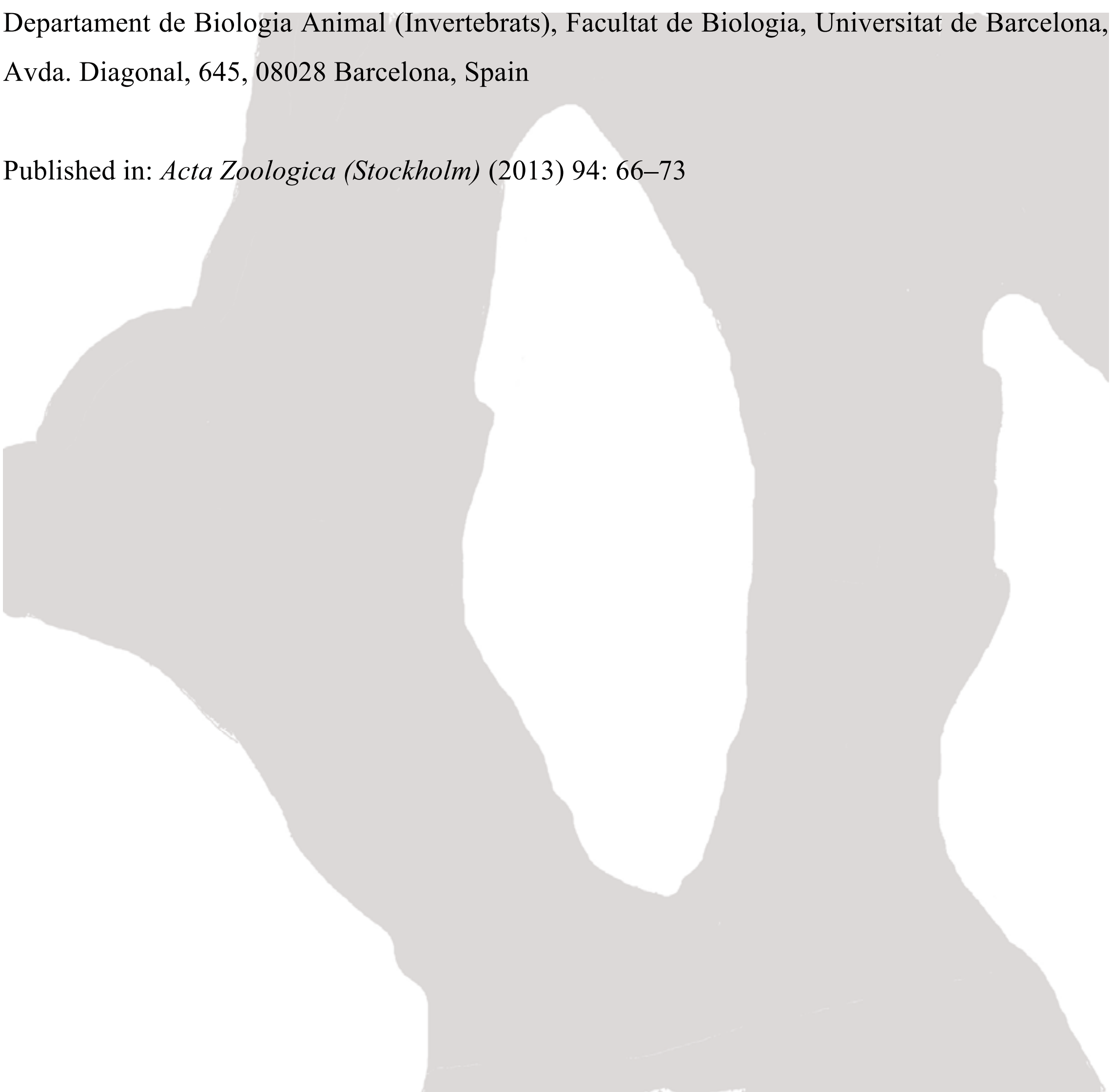


Abstract

A new species of cheilostome bryozoan, *Reteporella rosjoarum* sp. n., belonging to the family of Phidoloporidae is described from the Weddell Sea (Antarctica) and illustrated with binocular microscope and SEM micrographs. SEM has been used to observe the essential characters to describe the new species and to compare it to similar species. Three samples from three different stations were collected at 332–597 m depth during the ANT XXI/2 cruise of the R/V Polarstern (AWI, Bremerhaven, Germany) using a bottom trawl. The new species is characterized by the presence of giant vicarious spherical avicularia. The distinctive morphology of these avicularia discriminates this species from Antarctica congeners. The variability in avicularian morphometrics demonstrates that intraspecific variation between localities (Austasen and Drescher Inlet) may exist. It is also interesting to relate the morphology of the avicularia with the possible functions in Cheilostomata.

Keywords: taxonomy, Antarctica, Bryozoa, Phidoloporidae, Weddell Sea

Resum

Una espècie nova de briozou queilostòmat, *Reteporella rosjoarum* n. sp., pertanyent a la família de Phidoloporidae i procedent del Mar de Weddell (Antàrtida) ha estat descrita i il·lustrada mitjançant l'ús de la lupa binocular i amb imatges obtingudes amb el MER (Microscopi Electrònic de Rastreig). Es va utilitzar el MER per observar els caràcters essencials per descriure la nova espècie i per comparar-la amb espècies similars. Es van recol·lectar tres mostres de tres estacions diferents a 332-597 m de profunditat durant la campanya ANT XXI/2 a bord del Polarstern R/V (AWI, Bremerhaven, Alemània) utilitzant una xarxa d'arrossegament. La nova espècie es caracteritza per la presència d'aviculàries vicariants gegants i esfèriques. La morfologia distintiva d'aquestes aviculàries discrimina aquesta espècie dels seus congèneres antàrtics. La variabilitat morfomètrica de l'aviculària demostra que pot existir una variació intraespecífica entre localitats (Austasen i Drescher Inlet). També es discuteix la morfologia de l'aviculària en relació a les seves possibles funcions en l'ordre Cheilostomata.

Paraules clau: taxonomia, Antàrtica, Bryozoa, Phidoloporidae, mar de Weddell

Introduction

In recent years, the number of taxonomic studies of Antarctic bryozoans has increased notably (e.g. Hayward 1995; Gutt *et al.* 2000; López-Fé de la Cuadra & García-Gómez 2000). Since the scientific results of the Belgian Antarctic Expedition (Waters 1904), over 300 species have been described and new descriptions continue to appear (Clarke & Johnston 2003; Gontar 2008; López & Liuzzi 2008; Kuklinski & Barnes 2009). Cheilostome diversity is very rich in Antarctica, and cheilostomes comprise a major component of Antarctic benthos over extensive areas (Figuerola *et al.* 2012a). Moreover, a high proportion of cheilostomes (56%) are endemic (Hayward 1995; Barnes & De Grave 2000; Clarke & Johnston 2003; Griffiths 2010). New species of bryozoans continue to be described, especially in some regions that are not well known (Moyano 2005), such as the Weddell Sea, and are sensitive to environmental changes (Weller *et al.* 1992; Barnes *et al.* 2006; Winston 2009). Global climate change may lead to range contraction in many species, and documentation of new taxa and their distribution is important to determine future impacts.

Zooid polymorphism is characteristic of cheilostomes, constituting division of labor in these organisms. The avicularium is a modified zooid with a hypertrophied operculum (mandible) that lacks a feeding function. The mandible is opened slowly by contraction of abductor muscles and is closed rapidly by contraction of adductor muscles (McKinney 1998). Avicularia have a wide diversity of shapes and sizes, and this diversity probably relates to variations in their function and ontogeny (e.g. Winston 1984). Their morphology, position, and frequency of occurrence are taxonomically significant and used to define species (e.g. Hayward 1995; Kuklinski & Barnes 2009). Vicarious avicularia take the place of an ordinary autozooid and are the same size or larger than regular zooids in the colony (Hyman 1959).

Among the bryozoan families present in Antarctica, the family Phidoloporidae Gabb & Horn, 1862, is moderately well represented (10 or more species). Popularly referred to as lace corals, they occur in shelf and coastal benthic environments throughout the world, including polar seas (Hayward 1999).

This family is mostly characterized by a branching, reticulate, or encrusting colony, a few marginal pores on the autozooid frontal wall, a sinuate or rounded primary orifice, the presence of a beaded distal rim in the primary orifice, and a variety of adventitious avicularia. In this family, most Antarctic species belong to the genus *Reteporella* Busk, 1884, which presents either a tree-like colony architecture, a reticulate cup, or has fenestrate sheets folded and anastomosing to develop complex three-dimensional colonies. The genus is widely distributed in Antarctica, with the majority of species living in Antarctic shelf areas (Hayward 1995). *Reteporella* includes most of the species originally assigned to the genus *Retepora* (Lamarck 1801). Eleven Antarctic species have been described in the genus so far (Table 1). In this work, we describe a new species of *Reteporella* and discuss the functional morphology of avicularia. We also compare the characteristics of all the species of this genus from Antarctica. Finally, because the three samples of this species show biometrical differences, the intraspecific variability has been analyzed.

Materials and Methods

Bryozoans were collected during the Antarctic cruise ANT XXI⁄2 (December 2003–January 2004) of R⁄V Polarstern (Alfred Wegener Institute, Bremerhaven, Germany). Samples were obtained from three stations in the eastern Weddell Sea area. A bottom trawl was used to collect the samples, which occurred at depths of 332–598 m. Bryozoan colonies were sorted on deck and preserved in 70% ethanol. For studying skeletal morphology, the samples were cleansed in an ultra- sonic bath or bleached with NaClO. Specimens were coated with gold and examined using SEM at the Scientific-Technical Services of the University of Barcelona.

Morphological characters of the three samples were measured by binocular microscope (n = 10).

All variables were tested for normality (Kolmogorov–Smirnov's test) and for homogeneity of variance (Levene, F-test). Significant effects were further explored with one-way analysis of variance (ANOVA) followed by Games–Howell post hoc tests (GH tests). The GH post hoc multiple comparison test is one of the most powerful and robust for unequal variances (Day and Quinn 1989). SPSS was used for the analyses.

The holotype and two paratypes described in this study were deposited at the 'Centre de Recursos i Biodiversitat Animal' (CRBA) of the University of Barcelona, Barcelona, Spain.

Table 1 Main characters of all known Antarctic species of *Reteporella* and their distributions. LF: length of fenestrules; SA: serial autozooids; MP: marginal pores; OS: oral spines, N: number of avicularia, F avicularia: shape of avicularia, L avicularia: length of avicularia

Species	LF (mm)	SA	MP	OS	N avicularia	F avicularia	L avicularia (mm)	Shape of ovicell	Locality
R. antarctica (Waters, 1904)	1-1.5	03-jun		no	>= 1	oval	0.25	oval	Queen Mary, Ross Sea, Bellingshausen Sea, S. Georgia
					variably	triangular	-	scaphoid	
R. dorsoporata (Liu & Hu, 1991)	-	06-oct	03-abr	2	-	triangular, hooked	-	globose	Weddell Sea
					-	elliptical	-		
R.erugata (Hayward, 1993)	1.5-2 x 0.75-1	04-ago	02-mar	no	numerous	oval	0.1-0.15	globular, broader	S. Georgia, Palmer Archipelago, Ross Sea
					>= 1 within each fenestrula	triangular, sharply hooked, giant	0.3-0.35		
R. frigida (Waters, 1904)	1.5-2 x 0.5-1	04-ago	02-mar	2	numerous	oval, lacking columella, extensive palate	0.1-0.25	flattened, median fissure	S. Shetlands, Palmer Archipelago, Ross Sea, Bellingshausen Sea
					variably, also within fenestrulae	triangular, sharply hooked	0.5		
R. frigidoidea (Liu & Hu, 1991)	-	04-ago	-	2	variably	triangular, hooked	-	globose	Weddell Sea
					variably	triangular, smaller	-		
R. gelida (Waters, 1904)	1-1.5 x 0.75	03-jun	02-mar	2	numerous	crossbar, lacking columella, small rounded palatal foramen	0.1-0.15	globose, median fissure	Palmer Archipelago, Bellingshausen Sea, Ross Sea
					numerous, within fenestrulae	elongate triangular	>0.3		
R. hippocrepis (Waters, 1904)	1.5-2.5 x 0.5-1	03-jun	few	no	numerous	oval, stout crossbar lacking a columella	0.1	oval, short frontal fissure	numerous localities, common in Ross Sea
					often within fenestrulae	triangular, hooked rostrum	> 0.4		
R. jepralioides (Waters, 1904)	01-feb	03-jun	few	2	>= 1	oval, crossbar, lacking columella	0.1-0.15	globose, median foramen	Bellingshausen Sea, few localities in the Ross Sea
					-	triangular, abruptly hooked	0.25		

Table 1. (Continued)

Species	LF (mm)	SA	MP	OS	N avicularia	F avicularia	L avicularia (mm)	Shape of ovicell	Locality
R. longichila (Hayward, 1993)	2-2.5 x 1	03-may	03-abr	3	numerous	oval, stout crossbar, lacking columella, extensive palate	0.1-0.15	-	Palmer Archipelago, 6 stations in the Ross Sea
					inner edges of the fenestrulae	columella on the crossbar, short median fissure	>0.4		
R. parva (Hayward, 1993)	1-1.5 x 0.5-0.75	02-abr	02-abr	3	infrequent	crossbar bearing a knob-like columella, rostrum hooked	0.35	-	single locality of Ross Sea
					-	oval	-		
R. protecta (Waters, 1904)	0.75-1.5 x about 0.5	03-ago	03-may	3	numerous	oval, semicircular mandible, proximal to peristome	0.05-0.1	elongate oval, frontal fissure	S.Georgia, S. Shetland Isles, Palmer Archipelago, Bellingshausen Sea
					numerous, also within fenestrulae	triangular, large	0.2-0.25		
R. rosjoarum sp.n.	2.25-3.45 x 0.75-1.8	03-jun	02-mar	no	numerous	giant with cystid, globular, semicircular mandible	0.61-0.7	globose, median foramen	Weddell Sea
					numerous	oval, crossbar, lacking columella	0.13-0.16		

Results

Systematics

Suborder Ascophorina Levinsen, 1909 Infraorder Lepraliomorpha Gordon, 1989
Superfamily Celleporoidea Johnston, 1838
Family Phidoloporidae Gabb & Horn, 1862
Genus *Reteporella* Busk, 1884
Reteporella rosjoarum sp. n. (Figs 1–2)

Material examined

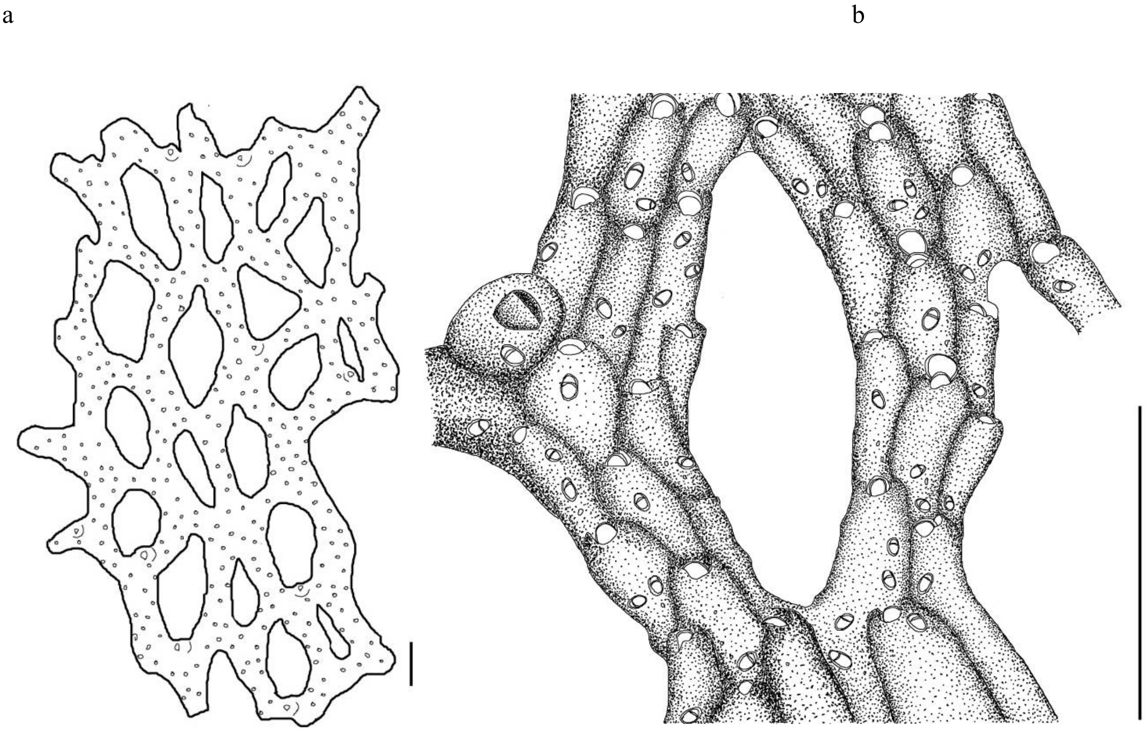
Holotype: One fragment of a colony (#1172: CRBA-4260) preserved in 70% ethanol; collected by C. Avila & M. Ballesteros. Region of Drescher Inlet (Weddell Sea, Antarctica), 72°51.43'S, 19°38.62'W (597.6 m).

Paratypes: Two fragments of different colonies (#560: CRBA-4258 and #864: CRBA-4259) preserved in 70% ethanol; collected by C. Avila & M. Ballesteros. Region of Austasen (Weddell Sea, Antarctica), 70°57.33'S, 010°33.86'W (351.6 m) and 70°57.00'S, 10°33.02'W (332.8 m), respectively.

Diagnosis

Reteporella with colony formed from reticulate sheets; autozooids nearly rectangular, centrally convex, depressed laterally, separated by indistinct sutures (Figs 1A,B and 2A–D). One or more adventitious avicularia present on each autozooid: oval, smaller than autozooids, with varying orientation. Giant avicularia frequent, spherical, vicarious, larger than autozooids, mandible semicircular, normal to frontal plane of colony, with varying orientation (Fig. 2E–L). Ovicell hyperstomial, globose, flattened frontally; frontal longitudinal fissure present (Fig. 2M–N).

Fig. 1 *Reteporella rosjoarum* sp. n. **a.** Colony (Scale bar: 1mm), top: proximal, bottom: distal. **b.** Zooids (Scale bar: 2mm).



Description

Colony heavily calcified, comprising folded reticulate sheets; maximum size and architecture unknown. Fenestrules commonly 2.25–3.45 x 0.75–1.8 mm, but with different sizes in different parts of the colony; their shape usually irregular: oval, elliptical, round-triangular and usually elongated and oval. Trabeculae variable in width, usually consisting of 3–6 longitudinal autozooidal series (Figs 1A,B and 2A,B). Frontal side of the colony formed by autozooids, avicularia, and gonozooids. Dorsal side of colony divided by boundary walls into very large areas, only presenting pores and one kind of avicularia. Autozooids situated on frontal side of colony, nearly rectangular, 0.67–1.01 x 0.22–0.45 mm, centrally convex, depressed laterally, separated by indistinct sutures. Zooidal frontal shield smooth, without tuberculation, perforated by 2–3 small, inconspicuous marginal pores. Primary orifice subcircular, usually longer than wide, distolateral rim finely denticulated (Fig. 2C,D). Without oral spines. Operculum subcircular, longer than wide. Peristome developed simply as a projecting angular lobe on one side of proximal edge of orifice, which remains partly visible in frontal view. Two kinds of avicularia present on frontal side. One or more adventitious avicularia present on each autozooid: oval, 0.13–0.16 mm long, smaller than autozooids, normal to frontal plane, with varying orientation, crossbar stout, lacking a columella. Giant avicularia frequent, spherical, vicarious, 0.61–0.7 x 0.52– 0.65 mm, larger than autozooids, mandible semicircular, normal to frontal plane of colony, with varying orientation; fenestral avicularia few in number, usually one or more within a fenestra; the orientation of the mandible dependent on the position of the avicularia in a fenestra. Dorsal side with only adventitious avicularia (Fig. 2E–L). Ovicell hyperstomial, prominent, globose, imperforate, flattened frontally, longer than wide, or equal in length and width; frontal longitudinal fissure present, typical of species of *Reteporella* (Fig. 2M,N).

Etymology

Reteporella rosjoarum sp. n. is dedicated to the parents of the first author, Roser Balaña and Joan Figuerola, for giving her the opportunity to study biology.

Remarks

This species differs from all other Antarctic and Subantarctic species of *Reteporella* by the presence of giant spherical avicularia and the absence of elongate triangular avicularia (Fig. 2E–H). The new species resembles only *Reteporella erugata* in the general form of its autozooids and peristome. Morphological characters are similar in the three samples and only differ significantly ($P < 0.05$) in five measurements: mean number of the length of primary orifice of the autozooid, width of the small avicularia, width of the giant avicularia, and length and width of the mandible of the giant avicularia (Table 2–3).

Discussion

Bryozoans are filter-feeding animals and mostly sessile. For this reason, they require physical and chemical defenses (Figuerola *et al.* 2012b). Although the functions of the variety of avicularia are still largely unknown, morphological and behavioral studies suggest physical protection, cleaning, and defensive functions against predators or grazers (Busk 1854; Harmer 1909; Canu & Bassler 1929; Hyman 1959; Cook 1963; Winston 1984, 1991).

The morphological diversity of avicularia is likely to be of considerable ecological importance, representing different responses to various selective pressures in the environment, and it may indicate a variety of biological roles (Carter 2008). Stable environments, such as Antarctic environment, seem to be favorable to the evolution of complex systems of polymorphism in cheilostome bryozoans (Venit 2007). The presence of spherical avicularia may indicate that this unusual form has adaptive value in this area (Winston 2009). Several observations have demonstrated the ability of certain avicularia to capture small organisms. The trapped organisms are generally too large to be consumed by autozooids, but over time, they may die and decay, attracting ciliates and bacteria upon which bryozoans are known to feed (Kaufmann 1971; Winston 1984, 1991). The giant size of avicularia may be more advantageous to capture relatively large parts of microinvertebrates. Additionally, Silén (1977) suggested that certain species possess more than one type effective against different predator types. The peculiar form of the spherical avicularia may be a specialization to capture a particular predator. Also avicularia of different sizes can coexist in one colony and could capture a wider spectrum of predators (Carter *et al.* 2010b).

Fig. 2 *Reteporella rosjoarum* sp. n. **a** and **b.** Part of the colony. **c** Denticulations of autozooids . **d.** Detail of the primary orifice. **e.** Group of giant spherical avicularia **f.** Giant avicularium without mandible. **g.** Giant avicularium . **h.** Orifice in the palate of the avicularium. **i.** Small avicularium. **j.** Group of small avicularia. **k** and **l.** Small avicularia of dorsal side of the colony. **m.** Denticulations of an ovicell. **n.** Detail of an ovicell showing the longitudinal fissure**.** (scanning electron microscope images); (**a-e, g-l**, holotype, CRBA-4260; **f** paratype, CRBA-4258, both from the Weddell Sea).

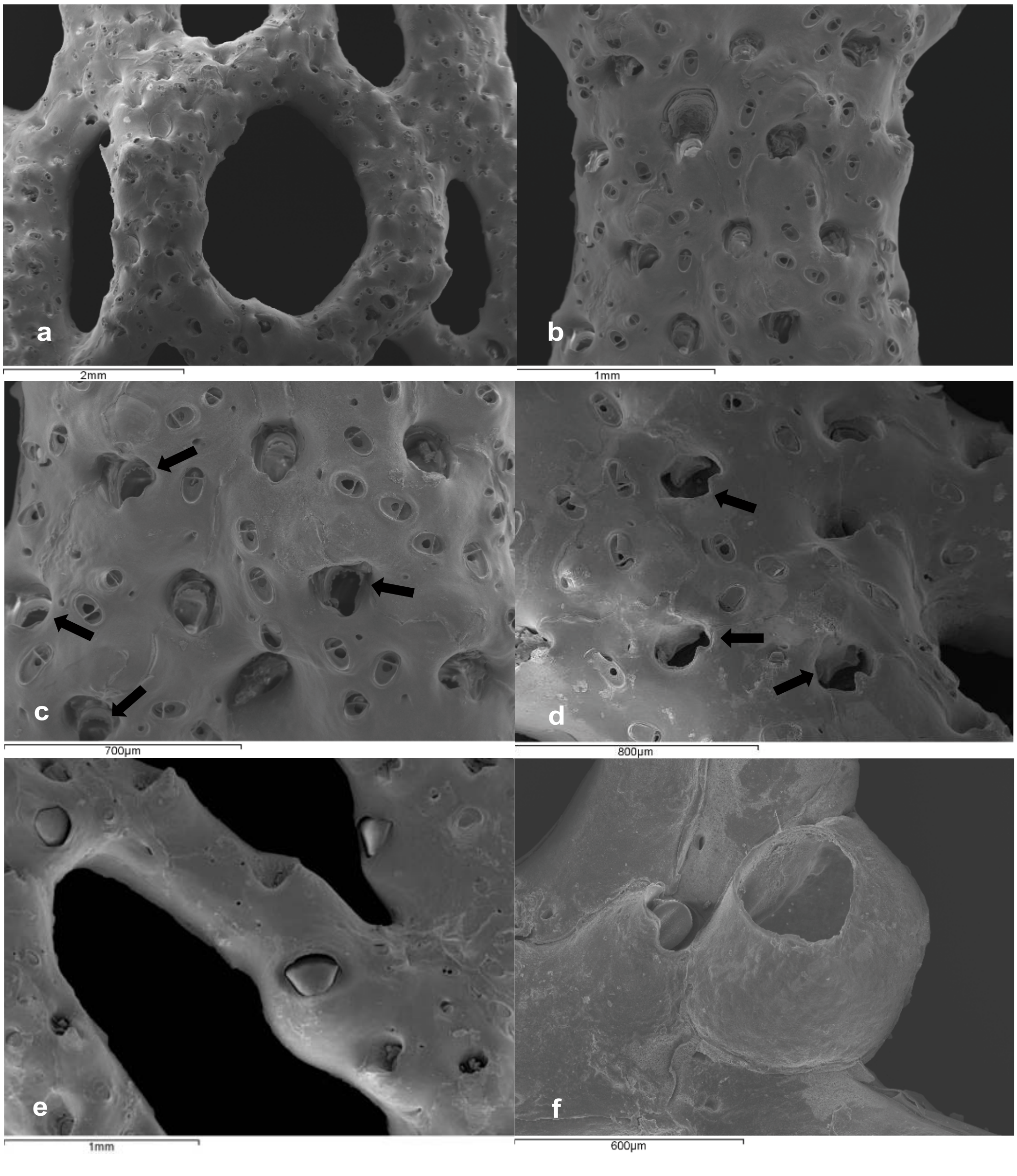


Fig. 2. (Continued)

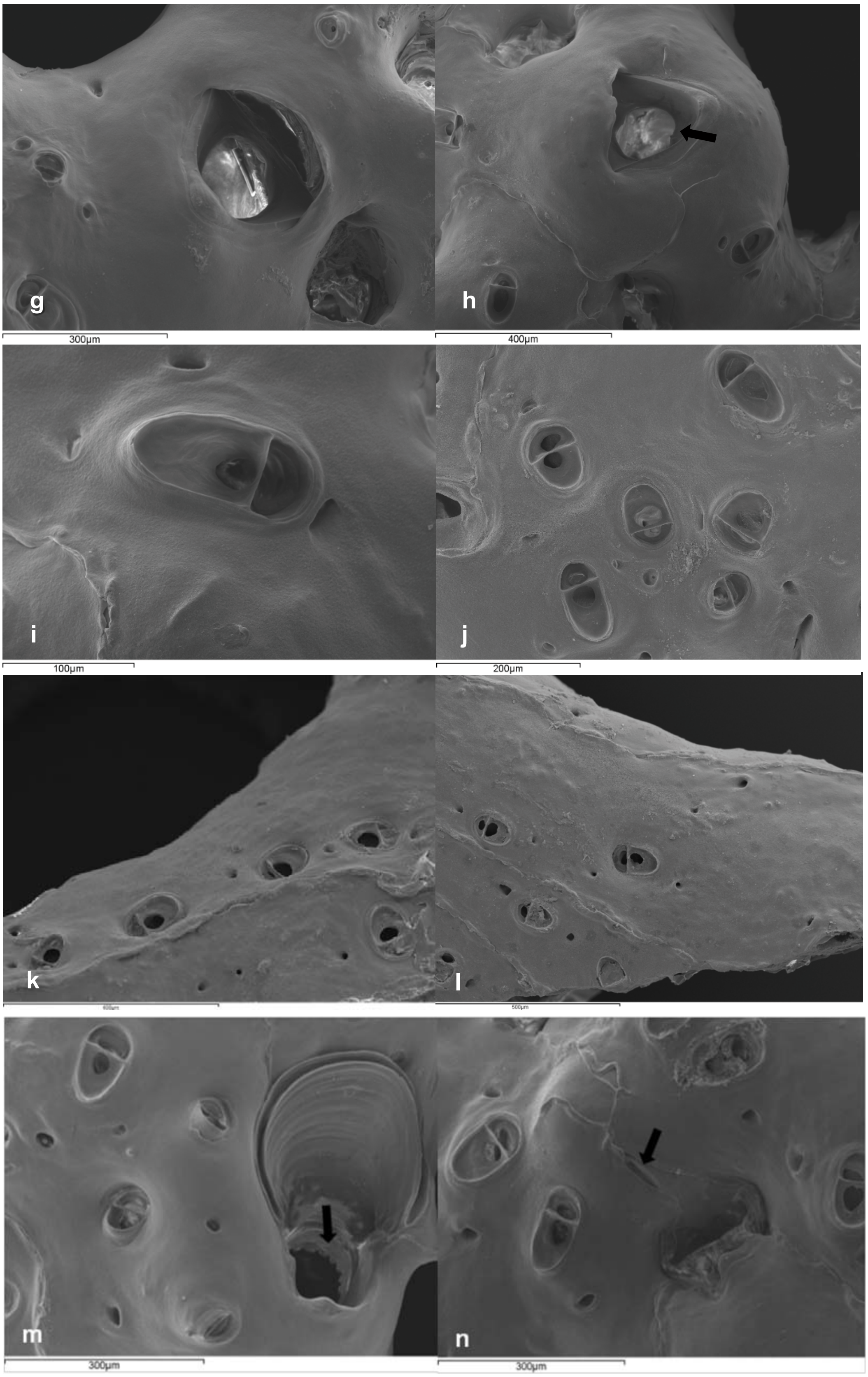


Table 2 Means of the measurements of characters between the three samples of *Reteporella rosjoarum* sp. n. Measurements in mm, with an average of 10 measurements for each structure. * Means differ significantly using the Games-Howell Post Hoc test *(P< 0.05*)

	sample #560			sample #864			sample #1172		
	n	Mean (mm)	SD	n	Mean (mm)	SD	n	Mean (mm)	SD
primary orifice of autozooid, length	10	0,1388*	0,0282	10	0,1594	0,0203	10	0,1875*	0,028
primary orifice of autozooid, width	10	0,1463	0,0328	10	0,1556	0,0266	10	0,1594	0,0182
primary orifice of ovicelled zooid, length	1	0,1313	-	10	0,1425	0,0131	10	0,1781	0,0408
primary orifice of ovicelled zooid, width	1	0,1313	-	10	0,195	0,0268	10	0,1706	0,0138
ovicell length	1	0,2625	-	10	0,3806	0,0251	10	0,3338	0,0373
ovicell width	1	0,2625	-	10	0,3375	0,0492	10	0,3469	0,047
small avicularia length	10	0,1556	0,028	10	0,1406	0,0283	10	0,1294	0,0272
small avicularia width	10	0,0881*	0,0127	10	0,0675*	0,0158	10	0,0788	0,0172
giant avicularia length	10	0,6056	0,1492	3	0,7	0,0573	10	0,615	0,1189
giant avicularia width	10	0,5213*	0,1182	3	0,65*	0,0286	10	0,57	0,1384
mandible of giant avicularia length	10	0,1819*	0,0127	3	0,2438*	0	10	0,1969*	0,0345
mandible of giant avicularia width	10	0,2644*	0,0488	3	0,3188*	0	10	0,2738*	0,0469

Table 3 Values of the differences of means of the three samples of *Reteporella rosjoarum*. * Means differ significantly using the Tukey test *(P<0.05*). L: length, P: primary, W: width.

samples	Difference of means (I-J)	L P.orifice autozooid	Sig.	W small avicularia	Sig.	W giant avicularia	Sig.	L mandible of giant avicularia	Sig.	W mandible of giant avicularia	Sig.
#864	1172	-0,02813(*)	0,05	-0,01125	0,30	0,08	0,25	0,04688(*)	0,01	0,04500(*)	0,03
	560	0,020625	0,18	-0,02062(*)	0,01	0,12875(*)	0,02	0,06188(*)	0,00	0,05437(*)	0,02
#1172	864	0,02813(*)	0,05	0,01125	0,30	-0,08	0,25	-0,04688(*)	0,01	-0,04500(*)	0,03
	560	0,04875(*)	0,00	-0,009375	0,37	0,04875	0,68	0,015	0,43	0,009375	0,90
#560	864	-0,020625	0,18	0,02062(*)	0,01	-0,12875(*)	0,02	-0,06188(*)	0,00	-0,05437(*)	0,02
	1172	-0,04875(*)	0,00	0,009375	0,37	-0,04875	0,68	-0,015	0,43	-0,009375	0,90

Observations on living colonies have shown that sessile avicularia capture organisms belonging to groups capable of predation on bryozoans, including syllid polychaetes, amphipods, isopods, nudibranchs, pycnogonids, and nematodes (Winston 1986, 1991; Carter *et al.* 2010a), some of which are abundant in Antarctica.

Hastings (1945) suggested that the large avicularia of *Crassimarginatella exilimargo*, which have a vestigial polypide with tentacles and functional gut, could have the capacity to feed and to distribute nutrients. The funicular system of the parent autozooid maintains this miniature polypide. This might be the case in the large avicularium *R. rosjoarum* too.

The function of avicularia in chemical defense has been relatively understudied. The avicularia of *Synnotum aegyptiacum* have very large glands, whereas autozooids do not (Marcus 1939). Consequently, they seem be important in the function of the avicularia. Winston (1984) suggested that some avicularia may contain secretory compounds. Lutaud (1964) found bacteria in the glands of some avicularia. The large capacity of the spherical avicularia in our species could indicate also the presence of large glands, although further studies are needed to test this.

Also avicularia could be actively involved in deterring settlement of epibionts, as well as preventing other organisms from removing food particles otherwise intended for autozooids (Harmer 1909). Giant avicularia are situated around the fenestrae, and Harmer (1909) demonstrated that, in these areas of *Reteporella*, the colony is completely free from encrusting organisms and the larvae of conspecifics do not appear to settle.

A more reasonable hypothesis for the presence of avicularia with a large internal capacity could be the additional function as a storage reserve for nutrients and to distribute resources during unfavorable conditions (Winston 1984). *Reteporella rosjoarum* sp. n. differs from the 11 Antarctic species described of the same genus mainly by the presence of giant vicarious, spherical avicularia. The distinctive morphology, position, and frequency of occurrence of these avicularia are taxonomically important, and these characters are frequently used to define species. Variability in avicularian morphometrics is common in bryozoans and demonstrates the existence of intraspecific variation between localities. The absence of a typical elongate triangular avicularia also discriminates this species from Antarctica congeners. However, the general form of its autozooids and peristome is similar to another species, *Reteporella erugata*.

Mean values vary significantly in five characters of samples examined of the new species (Table 3). However, all other morphological characters are similar in the three colonies (Table 2), and therefore, these differences could be due to variation in environmental factors (Kuklinski & Barnes 2009) at the three stations (e.g. sedimentation and temperature) (Moore 1977).

The presence of the giant avicularia in the three specimens indicates that they belong to the same species and that intraspecific variation between localities (Austasen and Drescher Inlet) may exist. Moreover, intraspecific variability in avicularian morphometrics is common (Carter *et al.* 2010b), and the length of avicularia is a significant contributor to morphometric variability (Carter *et al.* 2008).

Further studies, as well as more samples from the Weddell Sea, are needed to establish the general shape of the colony and the distributional range of this species.

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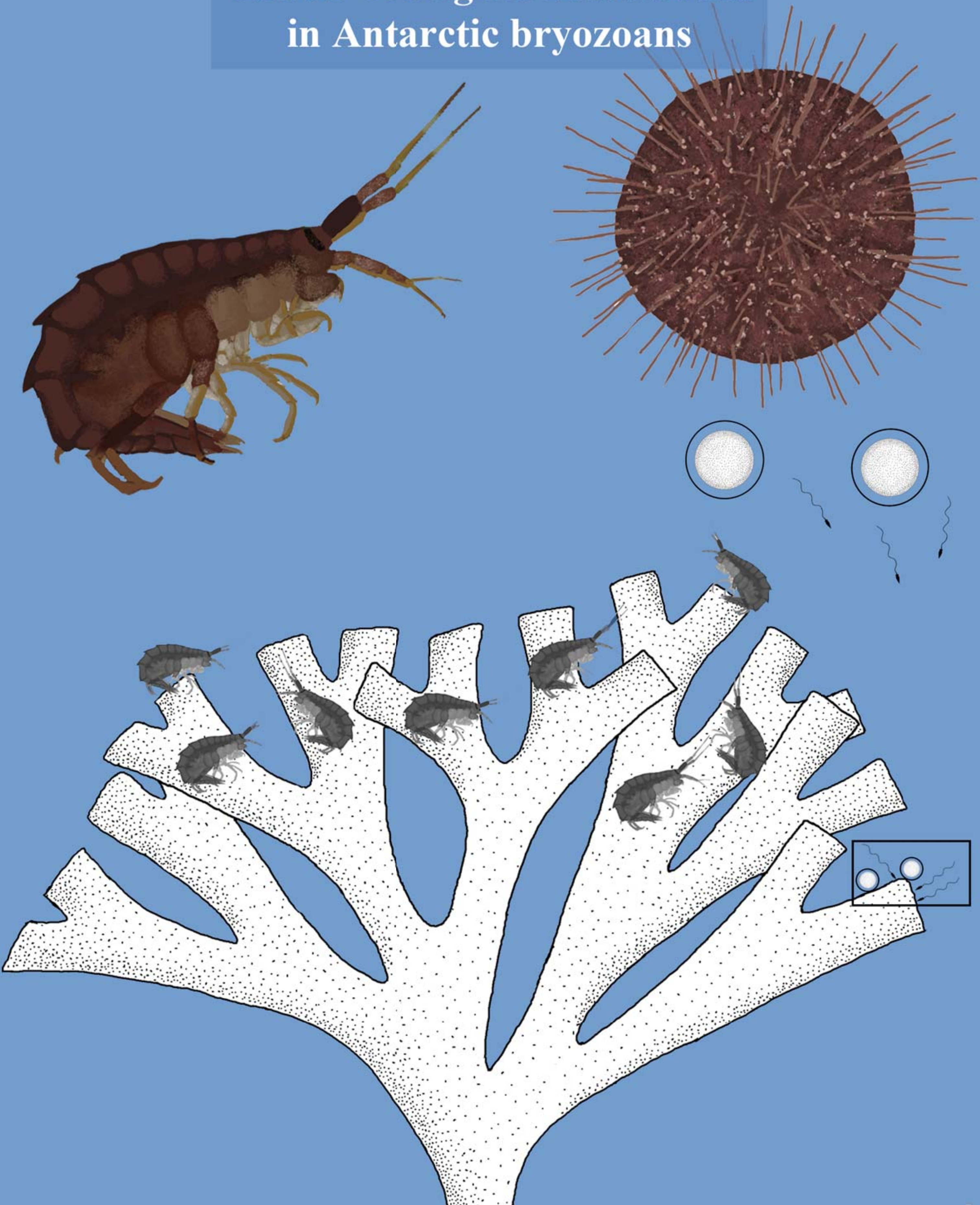
Chapter 4

Chemo-ecological interactions in Antarctic bryozoans



Chapter 4

Chemo-ecological interactions in Antarctic bryozoans

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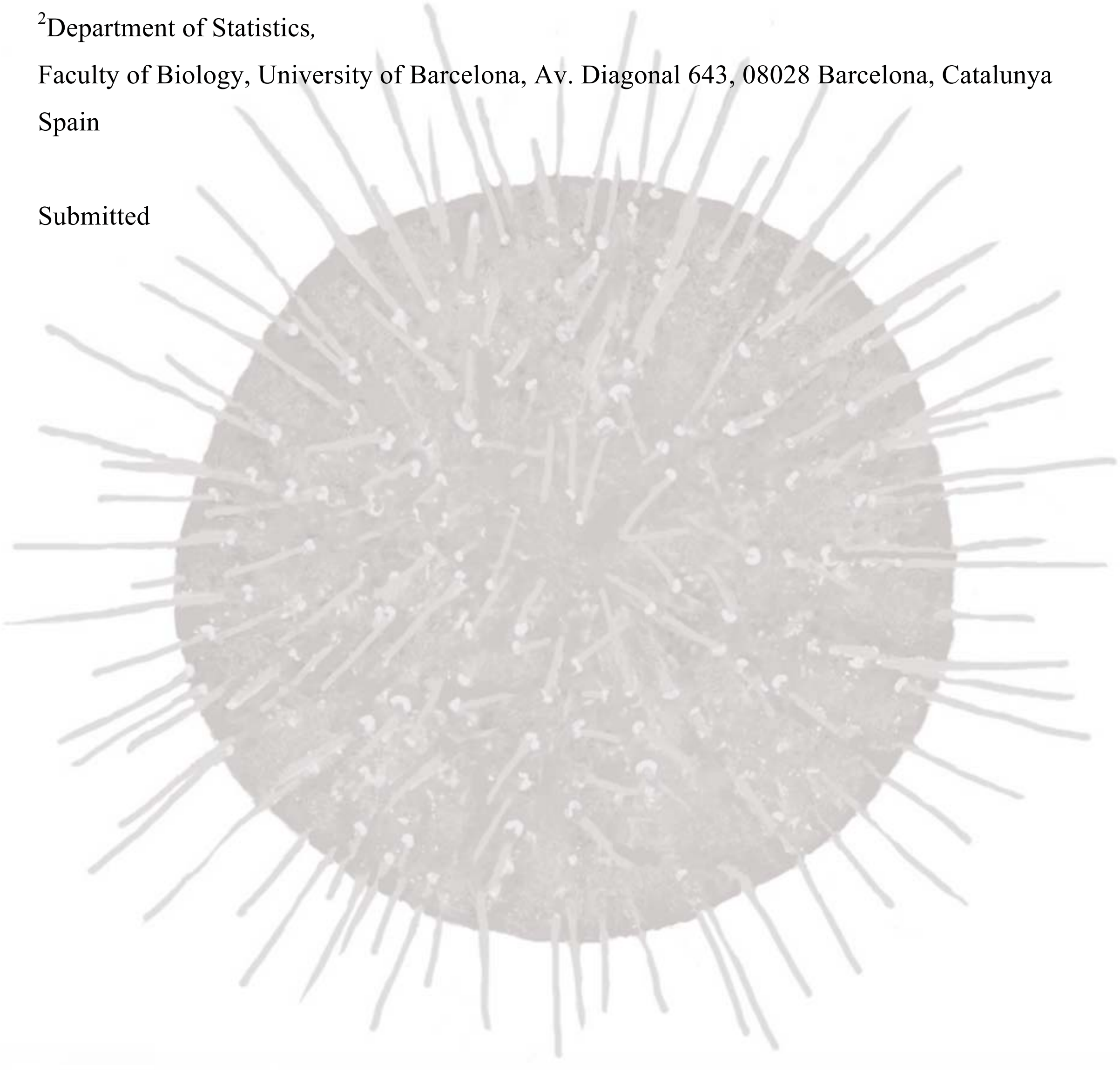


Abstract

The role of bioactive metabolites in ecological interactions involving Antarctic bryozoans has been scarcely studied so far. Bryozoans are one of the most abundant and diverse members of the Antarctic benthos, and are preyed up by diverse kinds of predators, from zooidal to colonials' levels. They seem to be casual food items of the common Antarctic sea urchin *Sterechinus neumayeri* and the ubiquitous omnivorous amphipod *Cheirimedon femoratus*. In this study, the cytotoxic activity against embryos and sperm of the sea urchin *S. neumayeri,* and the substrate preferences of the amphipod *C. femoratus* were assessed using organic extracts from Antarctic bryozoans, in order to determine the presence of chemical defenses. New adapted protocols were designed using a solidifying gel, commonly employed in antifouling studies, for simulating bryozoans' surface. We analyzed 32 organic extracts from 16 samples that belonged to 13 different bryozoan species. No cytotoxicity was detected against embryos of *S. neumayeri*, while 12 of the 13 bryozoan species were cytotoxic to sperm at natural concentrations. In the substrate preference assays, the amphipod *C. femoratus* was repelled by 10 species. The variable bioactivities found in both types of organic partitions of extracts (ether and butanol) indicated the presence of both lipophilic and hydrophilic defenses. Inter- and intraspecific variability of chemical defenses was detected also, suggesting environmental induced responses, symbiotic production, and/or genetic variability. Possible alternative defensive mechanisms are also discussed for species with low or no chemical defenses. Our results clearly support the fact that chemically-mediated bioactivity in Antarctic bryozoans is common, and there is a likely ecological role of cytotoxic and repellent compounds for their protection.

Key words: *Cheirimedon femoratus,* Cytotoxicity, *Sterechinus neumayeri,* Substrate preference, Repellence, marine invertebrates, benthos, polar ecology

Resum

El rol dels metabòlits bioactius dels briozous antàrtics en les interaccions ecològiques ha estat poc estudiat. Els briozous són uns dels més abundants i diversos membres del bentos antàrtic i són la presa de diversos tipus de depredadors des de nivells zooidals a colonials. Semblen ser aliment casual de l'eriçó de mar antàrtic comú *Sterechinus neumayeri* i de l'amfípode omnívor *Cheirimedon femoratus*. En aquest estudi, l'activitat citotòxica contra embrions i esperma de l'eriçó de mar *S. neumayeri* i les preferències de substrat de l'amfípode *C. femoratus* han sigut avaluades utilitzant extractes orgànics de briozous antàrtics per tal de determinar la presència de defenses químiques. Es van dissenyar nous protocols adaptats, utilitzant un gel solidificant, freqüentment empleat en estudis antirecobriment, per simular químicament la superfície dels briozous. Es van analitzar 32 extractes orgànics procedents de 16 mostres que pertanyien a 13 espècies diferents. No es va detectar citotoxicitat contra els embrions de l'eriçó de mar *S. neumayeri*, mentre que 12 de les 13 espècies de briozous provades eren citotòxiques contra l'esperma a concentracions naturals. En els bioassajos de preferència de substrat, l'amfípode *C. femoratus* era repel·lit per 10 espècies. La bioactivitat variable trobada en els dos tipus de particions dels extractes (etèrica i butanòlica) indicava la presència de defenses lipofíliques i hidrofíliques. També es va detectar variabilitat intra- i interespecífica de defenses químiques, suggerint respostes ambientals induïdes, la presència de simbionts i/o variabilitat genètica. Es discuteixen també mecanismes de defensa alternativa en espècies amb poca o sense defensa química. Els nostres resultats recolzen clarament el fet que la bioactivitat és comuna en briozous antàrtics i que hi ha un paper ecològic dels compostos citotòxics i repel·lents en la seva protecció.

Paraules clau: *Cheirimedon femoratus,* Citotoxicitat, *Sterechinus neumayeri,* Preferència de substrat, Repel·lència, invertebrats marins, bentos, ecologia polar

Introduction

The continental shelves of Antarctica are home to a high diversity and abundance of benthic suspension-feeding organisms. The Antarctic Circumpolar Current, since it's origin in the Eocene (Barker & Thomas 2004), has led to the biogeographic isolation of Antarctica and a high level of environmental stability (Walton & Doake 1987; Clarke 1992; Dayton et al. 1994). This, along with the diverse ecological interactions that take place in Antarctic benthos, explains why these communities are considered to be structured mainly by biological factors, such as predation and competition (Dayton *et al.* 1974) rather than environmental drivers. Indeed, numerous members of the Antarctic benthic communities have developed a wide array of chemical mechanisms to avoid predation (Amsler *et al.* 2001; Koplovitz *et al.* 2009; Slattery 2010), competition for space or food (Barnes & Rothery 1996; Bowden *et al.* 2006) and fouling (Peters *et al.* 2010; and reviewed in Avila *et al.* 2008 and McClintock *et al.* 2010). Surprisingly, though, there are few studies on the chemical ecology of one major component of the Antarctic benthos: the Bryozoa (e.g. Dayton *et al.* 1974; Lebar *et al.* 2007; Figuerola *et al.* 2012b).

The bryozoan fauna of the Eastern Weddell Sea is rich and varied, and new species are still being discovered at a considerable high rate, suggesting greater and yet unknown richness (Kuklinski & Barnes 2009; Hayward & Winston 2011; Figuerola *et al.* 2013). Cheilostome bryozoans are particularly well represented, with many types of colony forms among species and morphological defensive devices to avoid predation, from zooidal to colonial levels (Winston 1986, 2010). Within colonies, there are three principal polymorphic structures which provide protection from small predators including isopods, amphipods, polychaetes and larvae of other benthic organisms looking for somewhere to settle: (1) calcified spines that protect the frontal membranous surface of zooids from grazing (Hayward 1995). (2) Avicularia, which are modified zooids. They possess a hypertrophied operculum (mandible) that opens slowly and closes rapidly by contraction of adductor muscles (McKinney 1998; Winston 1991), and have even been observed to capture prey. (3) Vibracula, modified zooids with a usually extended setiform mandible that moves in diverse planes, used to 'whip' colony surface, cleaning and discouraging settling epibionts (O'Dea 2009).

Echinoids are important members of the Antarctic benthic fauna. Their diet is quite varied and bryozoans are considered to be one of their common food items (Jacob *et al.* 2003). Sea urchin grazing severely damages living colonies and can also negatively affect the recruitment of larvae (McClintock 1994; Bowden 2005; Bowden *et al.* 2006). In this sense, we predict that some Antarctic bryozoans may produce cytotoxic substances to prevent the settlement of embryos and larvae of this sea urchin, ultimately decreasing the ecological pressure over them, as has been similarly proposed by other authors in the past (e.g. McClintock *et al.* 1990). These kinds of indirect chemically mediated interactions have been reported in temperate waters (e.g. Birrell *et al.* 2008; Rasher *et al.* 2011), although they are poorly known in Antarctic ecosystems (Heine *et al.* 1991; McClintock *et al.* 1990, 1992, 1997, 2010).

In the Antarctic benthic system, Amphipoda are a major component that plays an important and complex role in organic matter fluxes (e.g. De Broyer *et al.* 1999; Dauby *et al.* 2001a, b). Amphipods are one of the most diverse groups with an estimated 600+ species (De Broyer *et al.* 1999, 2007, 2011). Their success can be related to the wide range of feeding strategies acquired, ranging from suspension-feeding to scavenging, herbivory, necrophagy and carnivory (Dauby *et al.* 2001b). The broad-spectrum diet of Antarctic amphipods include invertebrates such as sponges, bryozoans and cnidarians, which have been found in the digestive tracts of different gammaridean species from the Weddell Sea (Dauby *et al.* 2001b). This non-selectivity in prey items could be the result of a marked seasonal cycle in the Antarctic Ocean primary productivity. During the long winter, the relative scarcity of new organic matter might lead to opportunistic feeding behaviors amongst vagile consumers, with a progressive change from predation to scavenging, depending on food availability (De Broyer *et al.* 2007). Opportunistic feeding habits appear to be related with habitat preferences. Dense populations of amphipods, for instance, live associated with a wide range of biosubstrata in the Eastern shelf of the Weddell Sea (De Broyer *et al.* 2007). Their potential living hosts include bryozoans, hydrozoans, macroalgae, octocorals, ascidians, sponges, which offer structural and/or chemical refuge from predation, as well as possible sources of direct or indirect (accidental) nutrition (Oshel & Steele 1985; De Broyer *et al.* 1999, 2001, Dauby *et al.* 2001a; Huang *et al.* 2008).

In this study, we explore the chemical nature of ecological interactions between Antarctic bryozoans and two common benthic grazers: the common sea urchin *Sterechinus neumayeri* and the amphipod *Cheirimedon femoratu*s. Bryozoans' chemical agents that may serve as sperm/embryo cytotoxics and/or as amphipod repellents were examined through different experimental approaches. Interspecific and intraspecific variability in their defensive traits were examined, also analyzing the relationship between chemical defensive mechanisms and morphological defenses.

Methods and materials

Selection and sampling of the model experimental organisms

The sea urchin *Sterechinus neumayeri* and the lyssianasid amphipod *Cheirimedon femoratus* were selected as model competitors for their abundance and ubiquitous eurybathic distributions, as well as for the ecological interactions potentially occurring between these and the bryozoan species analysed. *Sterechinus neumayeri* is one of the most common echinoid species (Fell 1997), with a circumpolar and wide bathymetric distribution, ranging from a few meters to 810 m depth (Brey & Gutt 1991; Moya *et al.* 2012). *Cheirimedon femoratus* is a circumantarctic, opportunistic, omnivorous lyssianasid amphipod, found from 0 to 1500 m, frequently associated with biosubstrata (De Broyer *et al.* 2007; Jazdzewski *et al.* 1991; Dauby *et al.* 2001b; Bregazzi 1972a).

Experiments were performed during the Antarctic cruise ACTIQUIM-3 in January 2012, at the Antarctic Spanish Base (BAE) 'Gabriel de Castilla', Deception Island (62° 59.369' S, 60° 33.424'W), where these two species are abundant and easily collected. Specimens of the sea urchin *S. neumayeri* were collected by SCUBA diving at Whalers and Bidones Bays at 10-15 m depth. Hundreds of individuals of the amphipod *C. femoratus* were captured between 2 and 7 m depth by SCUBA diving using nets, along the shoreline of the BAE. Once captured, amphipods and sea urchins were immediately transported to the laboratory and kept in large aquariums with fresh seawater. Upon completion of the experiments all organisms were returned to the sea.

Sample collection and identification

Sixteen Antarctic bryozoan samples belonging to 13 different species were collected between 273.6 m and 351.6 m depth during the ANT XXI/2 (November 2003-January 2004) cruise on board the *R/V Polarstern* (Alfred Wegener Institute for Polar and Marine Research Bremerhaven, Germany) using Agassiz Trawl and Bottom Trawl in the Eastern Weddell Sea (Antarctica) (Fig. 1). The 13 bryozoan species represented a wide array of morphotypes that included: different levels of calcification from lightly to more calcified; different colony forms including encrusting broad sheet and erect branched, frondose and dense bushy; and different types of intracolonial polymorphism and structures (spines, avicularia, vibracula etc.), that offer a range of potential microhabitats for amphipods (Fig. 2). The density of the polymorphs (spines, avicularia and/or vibracula) per cm^2 was counted for the 13 species. With the purpose of knowing if a relationship exists between the number of physical defenses (number of polymorphs: spines, avicularia and vibracula) and the level of the activity (number of the active extracts; ranging from 0 active extracts to 4), a Pearson correlation coefficient was calculated.

Each bryozoan sample comprised several colonies from a single collecting station. Samples were sorted on deck, photographed, and a voucher portion of colonies of each sample was fixed in 70% ethanol for further taxonomical identification at the Faculty of Biology (University of Barcelona). Identifications were done following Hayward (1995). Sampling material for chemical ecology studies was conserved at -20°C until further examination.

Fig.1. Main sampling areas of study (A, B and C) at the Weddell Sea, Antarctica.

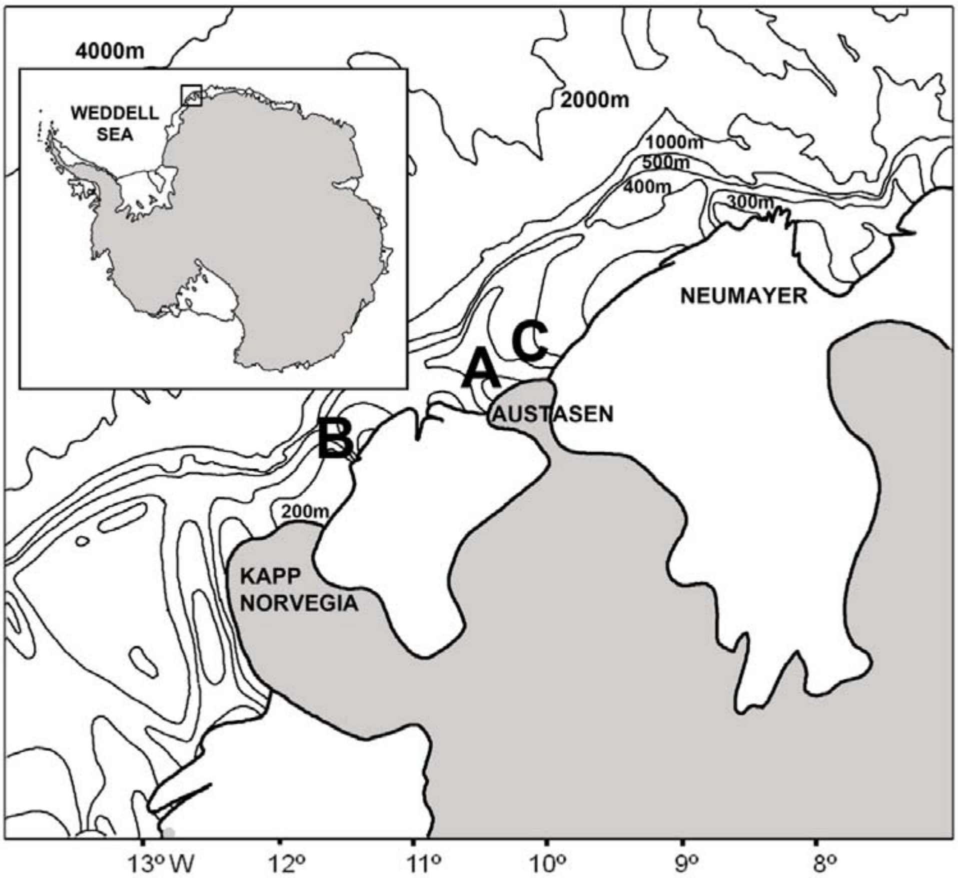
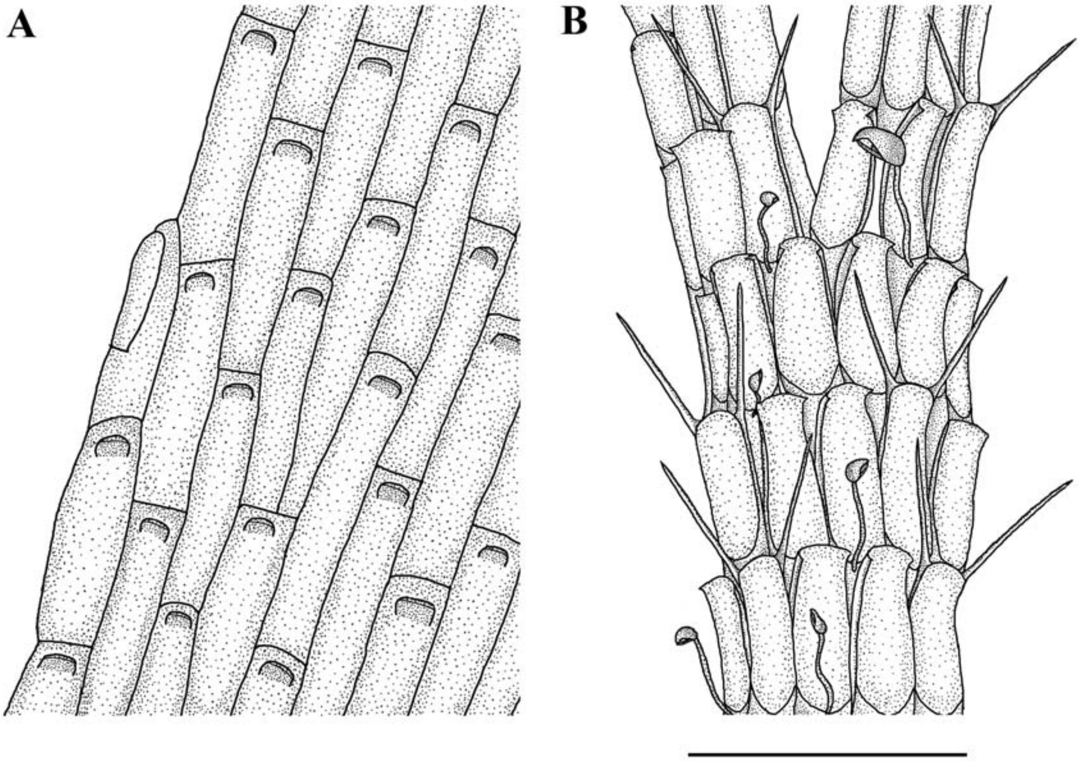


Fig. 2. Physical defensive structures of some Antarctic bryozoan species. A) Portion of a colony of *Isosecuriflustra tenuis*, with autozooids and one avicularium with a semielliptical mandible at the branch margin; B) Portion of a colony of *Camptoplites angustus*, with autozooids and distinct sizes of bird's head avicularia supported by a very long and mobile stalk. Scale bar: 1 mm.



Chemical extraction and fractionation of bryozoan samples

Bryozoan samples were extracted with acetone at room temperature. Ether and butanol partitions were obtained through sequential fractionation of the water residues using diethyl ether and butanol solvents respectively. All extraction steps were repeated 3 times, except for the butanol partition, which was performed once. Organic solvents were evaporated using a rotary evaporator resulting in dry crude fractions. Ether (comprising typically the most apolar lipophilic metabolites) and butanol (polar hydrophilic metabolites) partitions were used in the bioassays at their corresponding natural concentrations, according to dry weight calculations (see below). A final water residue was obtained and preserved for further studies.

Preparation of artificial substrates for bioassays

A new adapted protocol was used to carry out the ecological assays, using gels made of Phytagel^{TM} (Sigma Chemical Co.), a gelifying ingredient previously employed in other chemical ecology studies (Henrikson & Pawlik 1995; Clavico *et al.* 2006; Zamzow *et al.* 2010). An advantage of this gel, when prepared including crude extracts from marine samples, is the slow diffusion of the compounds into the water. Metabolites apparently diffuse in a similar way to what occurs in nature, thus simulating the surface of chemically defended living organisms (Henrikson & Pawlik 1995).

Sterechinus neumayeri is a free-spawning sea urchin. Masculine gametes (sperm) and fertilized eggs are released into the water column as planktonic early-life history stages, floating around and getting in contact with nearby biosurfaces. *Cheirimedon femoratus* is a bottom-dwelling amphipod, frequently associated with living substrata in a non-specific way. Our intention here is to recreate natural circumstances for testing cytotoxic and repellent activities. This was approached through the performance of bioassays, observing the reactions of these two potentially competing species (*S. neumayeri* and *C. femoratus*) when contacting Phytagel forms containing organic extracts from our bryozoan samples.

Bryozoan crude extracts were included into the Phytagel^{TM} substrates at tissue concentrations (here after referred to as the 'natural concentration'). The natural concentration was calculated as in previous studies of our group (e.g. Núñez-Pons *et al.* 2012), by dividing dry ether or butanol partitions (EE or BE) by the total dry weight (TDW = DW + EE + BE, where DW is the dry weight of the solid remains of the extracted sample). We chose dry weight, because it avoids deviations of weight parameters related to water content in aquatic samples.

The prepared artificial gel substrates consisted on extract-containing gels (treatments) and gels containing only solvent or distilled water (two controls). The gels were placed at the bottom of experimental tubes/chambers/vessels to chemically simulate the surface of bryozoans in embryo cytotoxicity / sperm cytotoxicity / substrate preference assays, respectively. The relative quantity of extract needed for the experiments was then calculated with respect to the total dry weight of the gel substrate required to produce a whole set of extract-treated gel forms for a single experiment (15 replicates).

For each treatment, sets of 15.6 mg of gel substrate, Phytagel^{TM}, were dissolved in 1 ml of distilled water and mixed for 5s. The gel mixture was heated in a microwave. Later, 0.3 ml portions of the Phytagel^{TM} solution were poured into each of the tubes and chambers, whereas 1ml portions were poured into larger vessels. After cooling down to 60ºC, the crude extracts previously dissolved in methanol were diluted into the gel solution and vigorously mixed for even distribution of the extract in the gel form. Finally, tubes, chambers and/or vessels were stored at 4ºC to allow homogeneous diffusion of the extract components throughout the gel forms. Control gel substrates were prepared in the same way, but adding only methanol or distilled water into the gel.

Cytotoxicity bioassays with sea urchins

Collected sea urchins were acclimatized for three days in fresh seawater tanks. Subsequently, they were induced to spawn by injecting 1ml of 0.5 M KCl solution into the internal cavity through the peristome. Gamete release in *S. neumayeri* was induced to at least 9 individuals in order to obtain a minimum of 3 males and 3 females with sufficient gamete emission.

a) Sperm cytotoxicity procedure

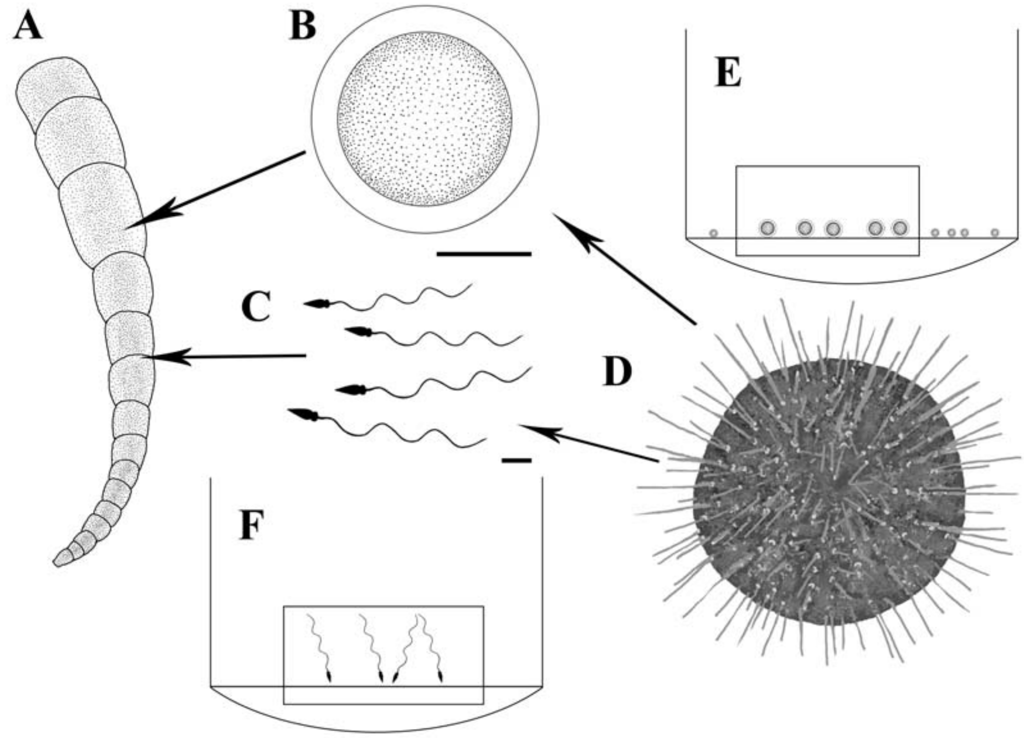
This test was developed following previously published procedures, using the common sea urchin *Sterechinus neumayeri* (Heine *et al.* 1991; McClintock *et al.* 1992). Our procedure introduces some specific modifications, in particular, the inclusion of organic extracts in the gels in order to approach a more ecologically relevant perspective, instead of dissolving them into the water.

An aliquot of 0.1 ml of *S. neumayeri* sperm dissolved in filtered seawater (0.2 µm) was added to the chambers containing the corresponding gel substrates and 1.9 ml of filtered seawater, achieving a total volume of liquid of 2 ml (Fig. 3). Sperm tend to sink on the chambers surface (pers. obs.). Two control sets of experiments were run simultaneously: the first negative control chambers contained gel substrates prepared with no solvent, while the other set of controls possessed gel substrates prepared with solvent alone (methanol). Treatment tests consisted of chambers with gel substrates containing bryozoan extracts at natural concentration. Five replicates of each test and five replicates for each one of the two controls were run simultaneously. The percentage of mobile sperm cells (25%, 50%, 75% or 100%) was checked under light microscope (40x) 20 min after the sperm solution was added into each chamber. Extracts were considered toxic when >25% of the sperm was inactive.

b) Embryo cytotoxicity procedure

This test was developed as a modified protocol of that previously proposed by Volpi Ghirardini *et al.* (2005) for the Mediterranean Sea urchin, *Paracentrotus lividus*. The present method introduces some modifications in order to adapt the assays to an Antarctic species with much longer developmental cycles, the sea urchin *Sterechinus neumayeri*. This species takes about 2-3 days to reach the blastula stage (Bosch *et al.* 1987), in contrast to *P. lividus*, in which the blastula develops in about 6-12 h (Russo *et al.* 2003). Therefore, the new protocol performed here is adapted to the much slower developmental timings of *S. neumayeri*. For this, a higher volume of seawater was required, in order to acquire longer experimental times of exposure in the assays. Moreover, the introduction of artificial gel substrata releasing the extracts in the water, and simulating chemically bryozoans surface, adds ecological significance, and allows the use of smaller amounts of extracts (Volpi Ghirardini *et al.* 2005) (Fig. 3).

Fig. 3. General scheme of the cytotoxicity bioassays against sperm and embryos of the sea urchin *Sterechinus neumayeri,* with gels simulating biosubstrata. (A) Bryozoan rod form; (B) Ovule (Scale bar: 50μm); (C) Sperm (Scale bar: 5μm); (D) Adult urchin; Fertilized eggs (E) and sperm (F) on the artificial substrata (gel) covering the bottom of experimental laboratory glass tubes or chambers, respectively.



The obtained sperm and eggs were separately transferred to 50 or 100 ml beakers with filtered seawater (0.22 μm filter), resulting in sperm and eggs suspensions. The egg suspension was diluted to a final concentration of 1000 eggs·ml^{-1}. After that, 0.1 ml of the sperm suspension was transferred to incubation chambers with 9 ml of filtered seawater. After one hour, 1 ml of egg suspension was added directly to the sperm solution in the incubation chambers (100 eggs·ml^{-1}). Fertilization success in the solution was verified through microscope observations by the presence of the fertilization membrane. When a minimum of 95% of the eggs were fertilized, they were filtered (20 μm) and 0.5 ml of eggs solution (about 50 eggs) was then added to 24 ml testing tubes (controls and treatments) containing the corresponding gel substrates on the bottom. For each daily cytotoxic test, five replicate controls of each type (prepared with both distilled water and with solvent alone) and five replicate treatment assays were conducted. The solvent-free control substrates consisted of 22 ml of 0.5 μm filtered seawater included in the gel; the solvent control substrates consisted of 22 ml of 0.5 μm filtered sea water with solvent alone (methanol) included in the gel. Finally, treatment substrates consisted of 22 ml of 0.5 µm filtered seawater with the bryozoan extract diluted into the solvent incorporated into the gel. Experiments were performed in a cold room at regular seawater temperature, 0ºC. The percentage of blastula in each treatment was determined by counting eggs under the microscope (10x) using a plankton counting camera. For a period of five days, the progress of embryonic development in the sea urchin was monitored, in both controls and treatments, every 12-16 hours.

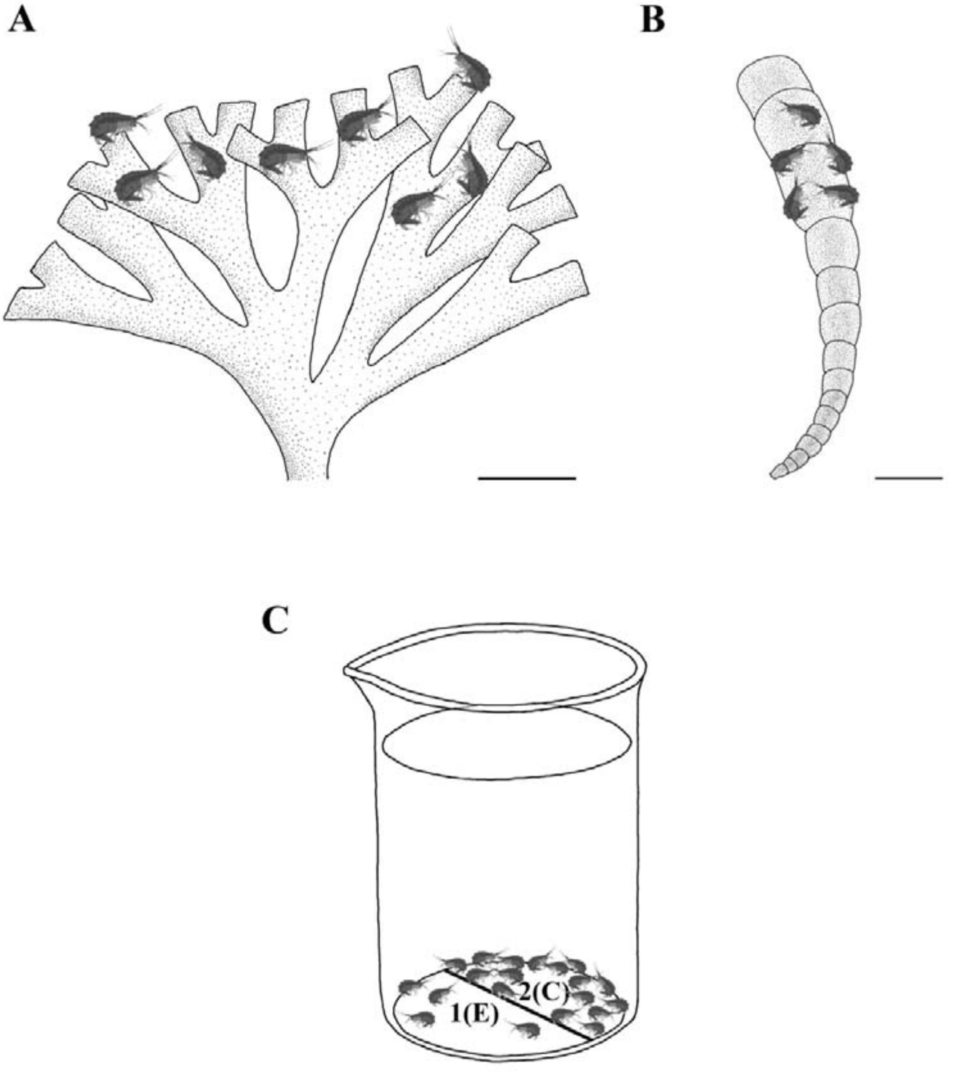
Cytotoxicity bioassays were statistically evaluated by the two-sample Mann-Whitney-Wilcoxon (MW) test using Stats package (R version 2.15.2). The MW non-parametric rank-based test does not assume that the difference between the samples is normally distributed. Resulting p-values were corrected for multiple testing by the Benjamini-Hochberg method, which reduces the risk of false positives and controls for the rate of false negatives (Benjamini & Hochberg 1995).

Substrate preference bioassays with amphipods

Each assay consisted on 15 replicate vessels filled with 100 ml of seawater, each containing two equally sized areas of different gel (Phytagel^{TM}) substrates (treatment gels containing extract at the natural concentration and extract-free control gels) and 20 amphipods of the species *Cheirimedon femoratus* (Fig. 4). Both substrates had a semicircular shape and together occupied the whole area of the bottom of each vessel. Amphipods' preference over the two substrates was monitored. Previous trials to establish the methodology indicated that all individuals of *C. femoratus* immediately occupied substrates, and that the number of substrate changes decreased to about 30% after 1 h. Therefore, based on the previous experimental timings we decided to run the experiments for 2h. The amphipods present in each type of substrate were then counted.

Each of the 15 replicate vessels was thus represented by a paired result, yielding two sets of data: a number of amphipods on the treatment substrate, and a number of amphipods on the control substrate. Since assumptions of normality and homogeneity of variances were not met, the two sets of data were compared using the non-parametric Exact Wilcoxon test using R version 2.15.2.

Fig. 4. General scheme of two laminar colonies used by amphipods as biosubstrata (A and B), and general scheme of the substrate preference bioassay (C). Laminar forms of flexible branched (A) and rigid sabre-like rod (B) colonies respectively; (C) Vessel filled with 100 ml of seawater, containing two equally sized areas of different gel substrates (treatment gels containing extract (represented by 1E) and extract-free control gel (2C) and 20 amphipods. Scale bar: 15 mm.

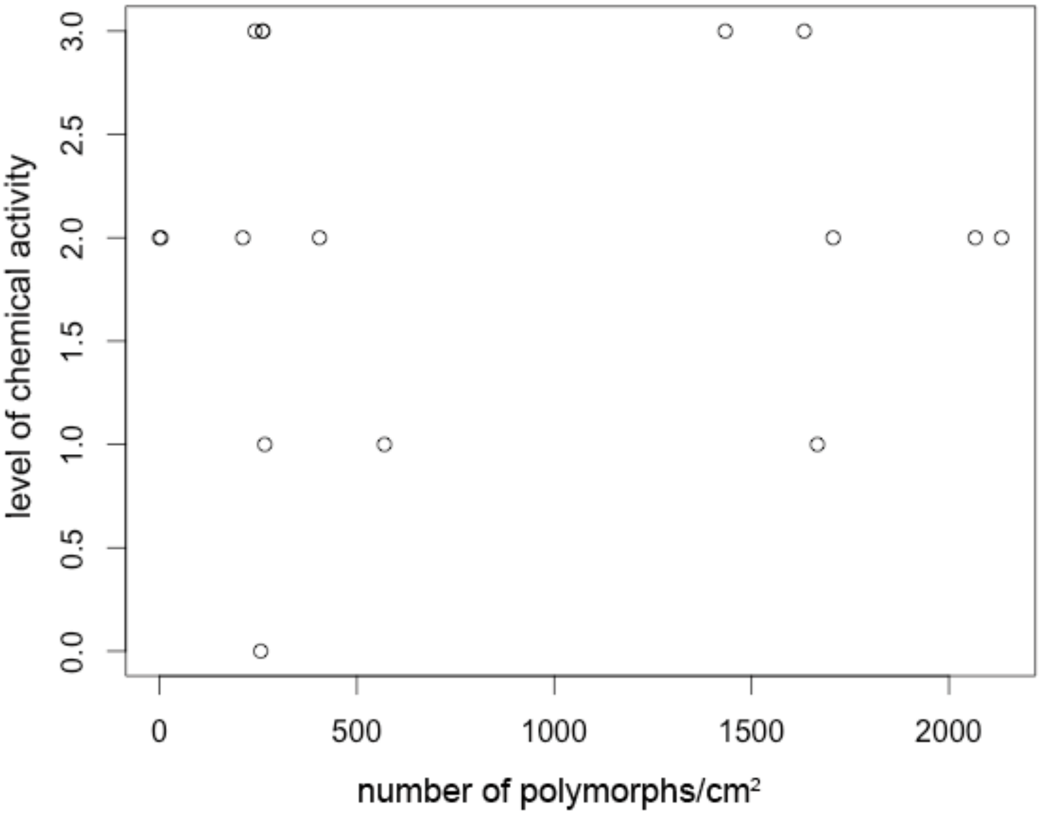


Results

Physical bryozoan defensive strategies and potential biosubstrata

The morphology of the colonies and of the defensive structures of the studied species has been described (Fig. 2, Table 1). Overall, six species possess hard calcified skeletons, ten are flexible and all but one possess avicularia, vibracula or spines. In reference to the morphological form of the colonies, nine exhibit laminar sheets/branches and three and four possess biserial and multiserial branches, respectively. No correlation exists between the number of polymorphs (spines, avicularia and vibracula) and the chemical defenses (r = 0.077, p= 0.77; Fig. 5).

Fig.5. Relationship between physical (spines, avicularia and/or vibracula) and chemical defenses. No correlation exists.



Cytotoxicity bioassays with sea urchins

The bryozoan samples displayed activity against sea urchin sperm but did not exhibit any cytotoxicity against the development of its embryos (MW, p>0.005). Eighteen extracts out of 32 (56.2 %) were toxic to sperm at natural concentration (MW, p<0.005; Table 1). Activity was detected in both partitions of four species (*Camptoplites angustus* (1), *Dakariella dabrowni, Isoschizoporella secunda* and *Smittina antarctica*). Intra- and interespecific variabilities were detected in the genus *Camptoplites*. Both partitions from *C. angustus* (1) were active, contrarily to the extracts from *C. angustus* (2), which did not display activity. *C. bicornis* showed activity only in the butanol partition. Contrarily, in the genus *Notoplites* activity was found in the two species tested.

Some extracts instead were considered toxic in this study, although they showed a mild activity (25% of immobile sperm cells). In particular, ether partitions of *Bostrychopora dentata*, *C. angustus* (1), *C. tricornis* (2), *I. secunda*, *N. drygalskii* (2) and *Smittina antarctica* displayed activity causing 50% of sperm motility inhibition, and *Melicerita obliqua* and *N. drygalskii* (1), 25% of immobile sperm cells. Butanol partitions of *Klugella echinata* produced a 25% sperm motility inhibition.

Substrate preference bioassays with amphipods

Non-parametric paired tests revealed significant differences in substrate preference (Table 1) in 13 extracts (Fig. 4 and 6), while in other tested extracts amphipods were evenly distributed over the substrates. Butanol partitions (56.2%) were more active than ether partitions (25%). Both extracts (ether and butanol) from the species *C. tricornis* (1) and *S. contracta* were repellent. Intra- and interespecific differences were detected in the genus *Camptoplites*. The butanol partition from *C. angustus* (1) was active, contrarily to the same extract from *C. angustus* (2). *C. tricornis* (1) displayed activity in both extracts, but the conspecific sample 2 was only active in the butanol partition. Instead, no activity was found in *C. bicornis.* Moreover, intraspecific variation was found in the species *N. drygalskii*, with activity recorded in the ether partition in sample 1, but instead, with activity in the butanol partition in the conspecific sample 2.

Table 1. Collection data, morphological characters and bioactivity recorded from the different assays performed with ether and butanol partitions obtained from Antarctic bryozoans. Level of significant differences (Mann-Whitney-Wilcoxon and Exact Wilcoxon tests, respectively): * p<0.05; ** p<0.005; *** p<0.0001. Data of characters and defensive structures came from Hayward (1995) and from this study. Zones A, B and C: sampling areas as marked in figure 1.

							Sperm cytotoxicity		**Repellent activity**	
Species	**Latitude (S)**	**Longitude (W)**	**Zone**	**Depth (m)**	**Morphological description of colony**	**Physical defenses**	**EE**	**EB**	**EE**	**EB**
Bostrychopora dentata (Waters, 1904)	70° 57.00’	10° 31.61’	A	284.4	rigid, broad laminar sheets, folded-erect	sessile avicularia	**	-	-	-
Camptoplites angustus (Kluge, 1914 (1))	70° 50.75’	10° 28.01’	C	281.2	flexible, multiserial branches, erect, bushy	bird's head avicularia, pediculate; autozooids with 2 spines	**	**	-	**
Camptoplites angustus (Kluge, 1914 (2))	70° 50.78’	10° 28.51’	C	273.6	flexible, multiserial branches, erect, bushy	bird's head avicularia, pediculate; autozooids with 2 spines	-	-	-	-
Camptoplites bicornis (Busk, 1884)	70° 56.83'	10° 32.61'	A	338	flexible, biserial branches, erect, bushy	bird's head avicularia, pediculate; autozooids with 2 spines	-	**	-	-
Camptoplites tricornis (Waters, 1904 (1))	70° 56.67'	10° 32.05'	A	302.4	flexible, multiserial branches, erect, bushy	bird's head avicularia, pediculate; autozooids with 3 spines	**	-	**	**
Camptoplites tricornis (Waters, 1904 (2))	70° 57.33'	10° 33.86'	A	351.6	flexible, multiserial branches, erect, bushy	bird's head avicularia, pediculate; autozooids with 3 spines	**	-	-	*
Dakariella dabrowni (Rogick, 1956d)	70° 57.00'	10° 33.02'	A	332.8	rigid, broad laminar sheets, encrusting or erect	sessile avicularia	**	**	-	-

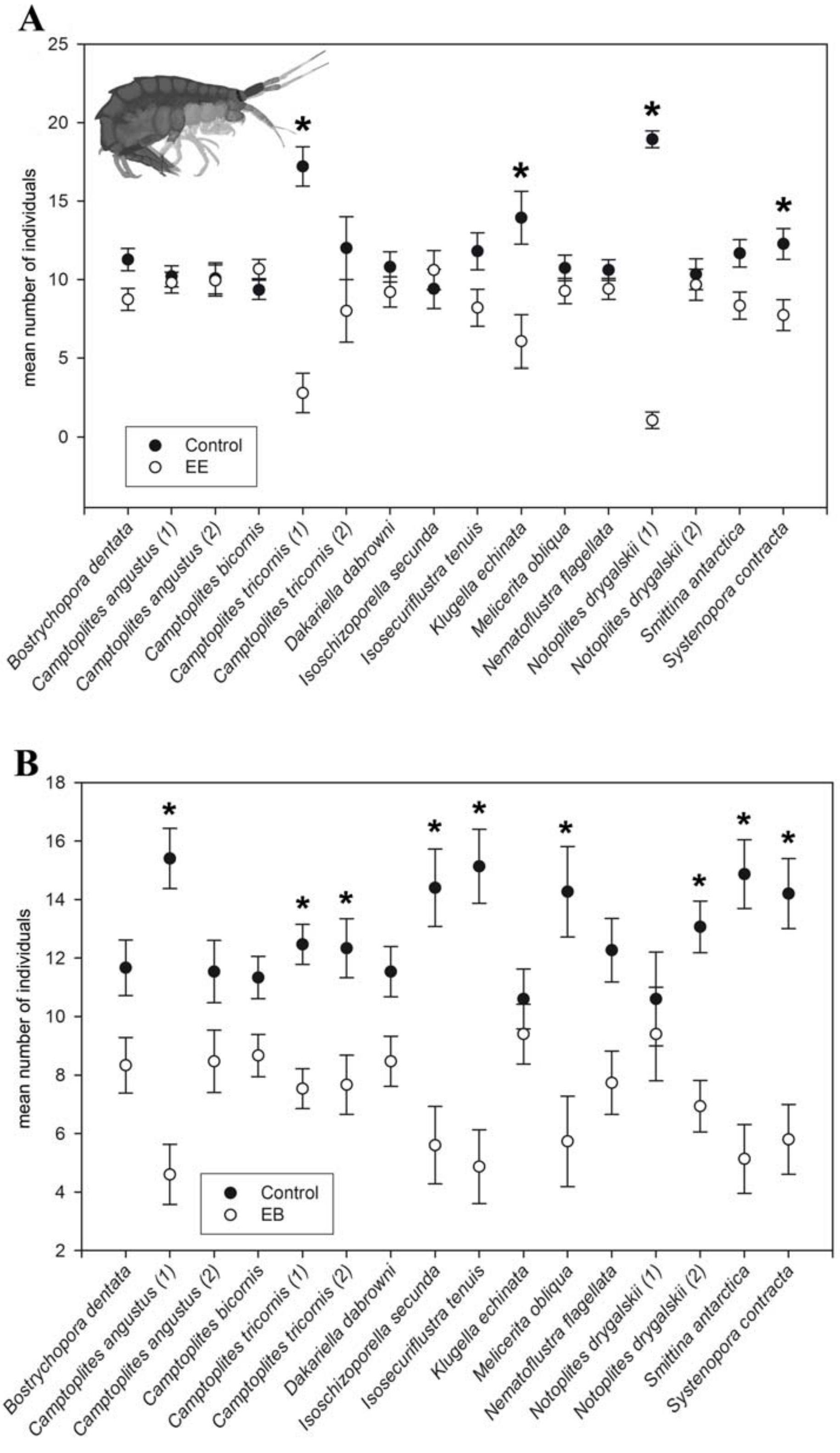
Table 1. (Continued)

Species	**Latitude (S)**	**Longitude (W)**	**Zone**	**Depth (m)**	**Morphological description of colony**	**General physical defenses**	**Sperm cytotoxicity**		**Repellent activity**	
							EE	**EB**	**EE**	**EB**
Isoschizoporella secunda Hayward & Taylor, 1984	71° 06.44'	11° 27.76'	B	277.2	rigid, broad laminar sheets, folded-erect	sessile avicularia	**	**	-	**
Isosecuriflustra tenuis (Kluge, 1914)	70° 52.75'	10° 51.24'	A	294.8	flexible, laminar branches, erect, broad fronds	sessile avicularia	-	**	-	**
Klugella echinata (Kluge, 1914)	70° 56.83'	10° 32.61'	A	338	flexible, laminar branches, erect, broad fronds	sessile avicularia; autozooids with 3 to 9 spines	-	**	*	-
Melicerita obliqua (Thornely, 1924)	71° 06.44'	11° 27.76'	B	277.2	rigid, laminar, erect, sabre-like rod	-	**	-	-	*
Nematoflustra flagellata (Waters, 1904)	70° 56.42'	10° 31.61'	A	284.4	flexible, laminar branches, erect, dense bushy tufts	autozooids with vibracula	-	-	-	-
Notoplites drygalskii (Kluge, 1914) (1)	70° 57.11'	10° 33.32'	A	337.2	flexible, biserial branched, erect, bushy	sessile avicularia; spines at bifurcation of colony	**	-	***	-
Notoplites drygalskii (Kluge, 1914) (2)	71° 04.30'	11° 33.92'	B	308.8	flexible, biserial branches, erect, bushy	sessile avicularia; spines at bifurcation of colony	**	-	-	*

Table 1. (Continued)

Species	**Latitude (S)**	**Longitude (W)**	**Zone**	**Depth (m)**	**Morphological description of colony**	**General physical defenses**	**Sperm cytotoxicity**		**Repellent activity**	
							EE	**EB**	**EE**	**EB**
Smittina antarctica (Waters, 1904)	71° 06.44'	11° 27.76'	B	277.2	rigid, curved laminar branches, erect, irregular three-dimensional structure	sessile avicularia	**	**	-	**
Systenopora contracta Waters, 1904	71° 06.44'	11° 27.76'	B	277.2	rigid, laminar branches, erect	sessile avicularia	**	-	*	*

Fig. 6. Substrate preference bioassays with the amphipod *Cheirimedon femoratus* performed with gel substrates treated with (A) ether (EE) and (B) butanol (EB) partitions from Antarctic bryozoans extracts. The diagrams show the paired results of control and extract-treated substrate, representing the mean number of amphipod individuals on each substrate for each experiment out of the 15 replicate tests, and the correlated standard error bars. (*): Control substrate significantly preferred, significant differences (p<0.05) according to Exact Wilcoxon Tests.



Discussion

Our results indicate that most bryozoan species tested here may display cytotoxicity and/or repellent activity as ecologically relevant defensive strategies. This supports the hypothesis that chemical mechanisms are of great importance in the ecology of Antarctic benthic communities (Avila *et al.* 2008; McClintock *et al.* 2010). The present study also demonstrates the presence of both lipophilic and hydrophilic bioactive compounds in different Antarctic bryozoan species that play a role in mediating ecological interactions with sea urchins and amphipods. Most known marine repellents are lipid-soluble (Sotka *et al.* 2009), but compounds of different polarities have previously been described among defensive products (Pawlik *et al.* 1986; Kubanek *et al.* 2000; Pawlik 2012). In a previous study, Núñez-Pons *et al.* (2012) found that the ether portion of a sample of the erect/encrusting bryozoan *Isoschizoporella secunda* showed no feeding repellent activity against the amphipod *Cheirimedon femoratus.* Our results now detected a strong repellent activity against the amphipod only in the butanol partition, suggesting that the bioactive chemicals in this species are likely of hydrophilic nature.

We observed intraspecific variability regarding bioactive agents in bryozoan samples from distant locations. *Notoplites drygalskii* exhibited bioactivity of different nature (lipophilic or hydrophilic) depending on sampling site (zone A or B). Conversely, only one of the two partitions from *Camptoplites angustus* was active, demonstrating variability in the same area (C) at different depths, both in sperm cytotoxicity and in substrate preference bioassays. Similarly, *C. tricornis* showed bioactive chemicals of different nature in the same geographic location (A) and different depth range, lacking activity only in the ether partition of sample 2 in the substrate preference bioassay. In contrast, both samples (1 and 2) of this species exhibited activity in the ether partitions in the sperm cytotoxicity bioassay. Our findings therefore suggest intraspecific variability in chemistry and bioactivity between sites and depth, probably as an adaptive response to diverse abiotic and biotic factors and/or genetic variability, as happens in other marine invertebrate populations (e.g. Page *et al.* 2005; Noyer *et al.* 2011; Pawlik 2012). The bryozoan species studied here have wide bathymetric ranges in the Eastern Weddell Sea (Figuerola *et al.* 2012a), with the widest range recorded in *C. bicornis* and *Melicerita obliqua.* Thus, the variability of natural products in these species could be related to them living in different habitats. Additionally, variations in bryozoan chemical composition could be related to bacterial symbiotic associations, which in turn may have distinct environmental controls. One well-studied example of this is the bryozoan bacterial symbiont, *Endobugula sertula,* producer of chemical compounds that confer resistance to predation (bryostatins). This microbial symbiont is present in all life stages of *Bugula neritina* (Lopanik *et al.* 2004) but the symbiosis and the chemical metabolites produced also differ geographically, even with some northern forms of this species lacking bacteria (McGovern & Hellberg 2003). Moreover, the different bioactivities within species could be caused by induced or activated responses, such as post-attack defenses, and therefore their concentrations may depend upon the level of predation, as has been demonstrated in other marine benthic invertebrates, including corals and sponges (Teeyapant & Proksch 1993; Pawlik *et al.* 1995; Hoover *et al.* 2008). This, however, has yet to be demonstrated in Antarctic invertebrates.

Physical defenses in bryozoans

The absence of chemical defenses in some of the species of our study could be related to the presence of alternative defensive mechanisms. Antarctic cheilostome bryozoans exhibit a remarkable variety of hard structures with apparent protective function (Hayward 1995), such as rigid exoskeletons, as well as diverse characteristic structures including spines, avicularia and vibracula (Best & Winston 1984). In agreement with this, most colonies from our collection were observed to possess such devices. *Dakariella dabrowni* for instance, contained no detectable defensive chemicals, yet protection could be achieved by it's rigid well calcified skeleton and capacity for an encrusting horizontal growth, which enables effective repair from grazing injuries. Contrarily, the lack of apparent physical defensive structures in *Melicerita obliqua* is likely compensated by effective chemical defense, suggesting evidence of potential resource trade-offs. This species has lightly calcified frontal walls (as an anascan cheilostome), thus being less resistant to grazing by predators (McKinney *et al.* 2003). Erect bryozoans appear to be more vulnerable to destruction by colony-level predators (Jackson & McKinney 1990). Moreover, erect bryozoans tend to adopt a wide range of colony forms (from coral-like to bush-like forms; Ryland & Hayward 1991) providing more potential refuges for settling amphipods. These facts drive to the hypothesis that chemical defenses might be more prevalent in erect forms. We found that the erect and flexible bryozoans *Klugella echinata* and *Notoplites drygalskii* did possess both strategies of chemical defenses, as well as they exhibited hardened spines. Spiny structures overarching the vulnerable frontal membranes of some species like *K. echinata* are actually apparently less effective in affording protection respect to other mechanisms, including the heavy calcification of colonies in aschoporan cheilostomes, such as *Bostrychopora dentata* (McKinney *et al.* 2003).

Physical defenses in bryozoans comprise also particular structures, like bird's head avicularia, that have a mandible, which snaps shut upon narrow objects such as the appendages of gammarid amphipods (Forbes 1938; Kaufmann 1971; Winston 2010). The bryozoans of the genus *Camptoplites* are the only representatives in Antarctica to possess large pedunculate bird's head avicularia (Hayward 1995). Interestingly, we detected no chemical activity against amphipods settlement in neither, *Camptoplites angustus* (2) nor *C. bicornis*. This drives us to pressume that defense in these two species may already be achieved through physical means by the possession of three and four kinds of bird's head avicularia, respectively, including a gigantic and globular form and two short spines in *C. bicornis* (Hayward 1995). These would make chemical defenses apparently unnecessary (Hayward 1995). Contrastingly, in the species *C. tricornis*, both chemical and physical defenses (four types of avicularia and three spines) do co-occur. We detected no chemical defenses in *Bostrychopora dentata* and this may be due to the species' highly calcified skeleton and high density of sessile avicularia. *Nematoflustra flagellata* is weakly calcified but also showed no chemical defenses. In this case, the vibracula zooids in their colonies could be effective enough for its overall protection (Hayward 1995). In general, our results show a general trend to the possession of a combination of both chemical and physical defensive mechanisms in most species, without a relationship between the density of the polymorphs and the level of chemical activity. These likely suggest complementary traits and low incidence of defense redundant strategies (see Fig. 2 and 5).

Cytotoxic activity bioassays with sea urchins

To the best our knowledge, few experiments of cytotoxic activity against sperm and early life stages of the common Antarctic sea urchin *Sterechinus neumayeri* have been performed to date using extracts of sympatric organisms (e.g. McClintock *et al.* 1990; Figuerola *et al.* 2012b). In this study with Antarctic bryozoans, cytotoxicity against embryos was not detected. Nevertheless, there is evidence in the literature of the presence of cytotoxic compounds in bryozoans, as is the case of the circumpolar bryozoan *Flustra foliacea*. This species, extensively studied, possesses bioactive alkaloids that display *in vitro* cytotoxicity against the human colon cancer cell line HCT-116 (Lysek *et al.* 2002). Also, the species *Carbasea curva* was reported to show moderate haemolytic activity in the laboratory (Winston & Bernheimer 1986). These reported bioactivities however, have no ecological significance.

In contrast to the absence of embryo cytotoxicity found in our samples, many of our bryozoan species (68 %) did reveal toxic activities against sea urchin sperm. Four species (*Camptoplites angustus* (1), *Dakariella dabrowni, Isoschizoporella secunda* and *Smittina antarctica*) revealed to possess cytotoxic compounds in both, ether and butanol partitions. Echinoids tend to consume bryozoans incidentally whilst pursuing other invertebrates (Lidgard 2008; Winston 2009) and are reported to be among the common food items of *S. neumayeri* (Jacob *et al.* 2003). Therefore, the grazing activities (direct and incidental) of this sea urchin are described to be responsible for significant mortality of settling larvae and juveniles of benthic invertebrates (Bowden 2005; Bowden *et al.* 2006). The presence of cytotoxic compounds that may reduce reproduction success, such as those found in this study (*i.e.*, affecting sperm viability), could reduce recruitment and, consequently, the grazing and ecological pressure caused by this sea urchin (e.g. McClintock *et al.* 1990; Pawlik 1992; Bowden *et al.* 2006).

Substrate preference bioassays with amphipods

Most bryozoan species analyzed here (85%) possess chemicals that cause substrate avoidance towards the common Antarctic amphipod *Cheirimedon femoratus*. In addition, two species (*Camptoplites tricornis* (1) and *Systenopora contracta*) seem to combine several repellent compounds of different polarities. The main predators of bryozoans at the zooidal level are: amphipods, copepods, nudibranchs and pycnogonids. Some small crustaceans belonging to the Order Amphipoda are considered specialist feeders of bryozoan autozooids, like the Antarctic species *Gnathiphimedia mandibularis* (Coleman 1989; Dauby *et al.* 2001b; Lidgard 2008). Predators may feed directly on bryozoans or on their epibiota, which tend to concentrate profiting their feeding currents (Amsler *et al.* 2009; De Broyer *et al.* 2001). *C. femoratus* is a scavenger amphipod described as a generalist feeder eating, from invertebrates to algae and detritus (Bregazzi 1972b), being bryozoans a potential prey. Thus, the presence of repellent metabolites on the surface of bryozoans may prevent the settlement of amphipods, and consequently avoid predation and any secondary detrimental effects, such as reduced rates in filter feeding caused by disruption of flow by dense populations of attached amphipods. Like many bottom-dwellers, *C. femoratus* has limited swimming ability and usually associates ephemerally with living substrata, using them both as habitat and potential prey. Recent studies using gel shapes that simulated algal species demonstrated the capacity of some Antarctic amphipods to choose habitat, based on the three-dimensional structure (Zamzow *et al.* 2010). However, our study is the first one to use gels as substrates to evaluate the habitat choice in relation to chemical compositions (without influence of shapes). Several authors have proposed the use of invertebrates as nurseries, or as structural and/or chemical refuges from predation, *i.e.*, Amsler & colleagues (2009) with sponges. Therefore, the high structural complexity of some bryozoan species (e.g. bushy colonies in *Camptoplites*, frondose colonies in *Isosecuriflustra tenuis* and *Klugella echinata*, folded forms in *Bostrychopora dentata* and *Isoschizoporella secunda* and irregular three-dimensional structures in *Smittina antarctica*) could similarly provide small crustaceans like amphipods, *refugia* from predation from the Antarctic common fish *Notothenia coriiceps* for example, or others (Daniels 1982). Actually, because of the colony diversity shapes and structures, many bryozoans provide potential substrates for many Antarctic amphipods (De Broyer *et al.* 2001). Carter (2008) observed that colonies of *Bugula flabellata* provided habitat for various organisms, including gammarid amphipods. In this sense, *B. dentata* and *N. flagellata* could provide a suitable potential substrate since they produce structurally-complex colonies of folded broad sheets and frondose colonies respectively. Simultaneously, they lack repellent defenses against this amphipod.

New adapted chemical ecology protocols

The new adapted protocols described using gel substrates aimed to evaluate cytotoxic and substrate avoidance activities in bryozoans, and demonstrated to provide several advantages: 1) the prepared artificial gel substrates (previously reported to release extracts slowly), simulated chemically protected invertebrate surfaces, conferring more realistic ecological conditions respect to previous studies; 2) the Antarctic organisms used as models (the urchin *Sterechinus neumayeri* and the amphipod *Cheirimedon femoratus*) are ubiquitous, common and easy to collect; 3) small quantities of extract could be used while applying natural concentrations; and 4) the short timings of sperm cytotoxicity and substrate preference assays (20 min and 2h, respectively) allow the performance of more experiments during the commonly short Antarctic cruises.

Conclusions

Our study demonstrates that cytotoxic and repellent compounds are present in Antarctic bryozoans, and that they could play a major defensive role. This defense is used in conjunction with a variety of physical protective mechanisms. Chemical defenses are found to be prevalent in a number of bryozoans, deterring amphipods and reducing the reproductive success of sea urchin. Amphipods are considered deleterious to bryozoans for their direct and indirect predation and the burden of commensality. By reducing sea urchin sperm survival, metabolites produced by bryozoans may regulate adult grazing on a local scale, although further work is required to confirm this hypothesis. Intra- and inter-specific variability in the activity of chemical compounds, at local and regional spatial scales, suggests phenotypic plasticity in compound production driven by biotic or abiotic factors. Similar hypotheses have been suggested for some authors for nudibranchs and sponges (McClintock et al. 2010) and require further examination in the highly understudied bryozoans.

Some bryozoan species have demonstrated to be potential sources of pharmacologically promising substances, such as cytotoxic alkaloids (Prinsep *et al.* 2004; Sharp *et al.* 2007). However, research on chemical ecology and the role of the implicated metabolites continues to be limited (Sharp *et al.* 2007; Blunt *et al.* 2012), especially considering that between 8,700 and 11,100 bryozoan species are currently estimated to exist (Appeltans *et al.* 2012). Thus, chemical and ecological investigations of bryozoans should be further developed, in order to comprehend ecology of this so understudied but worldwide relevant marine benthic group.

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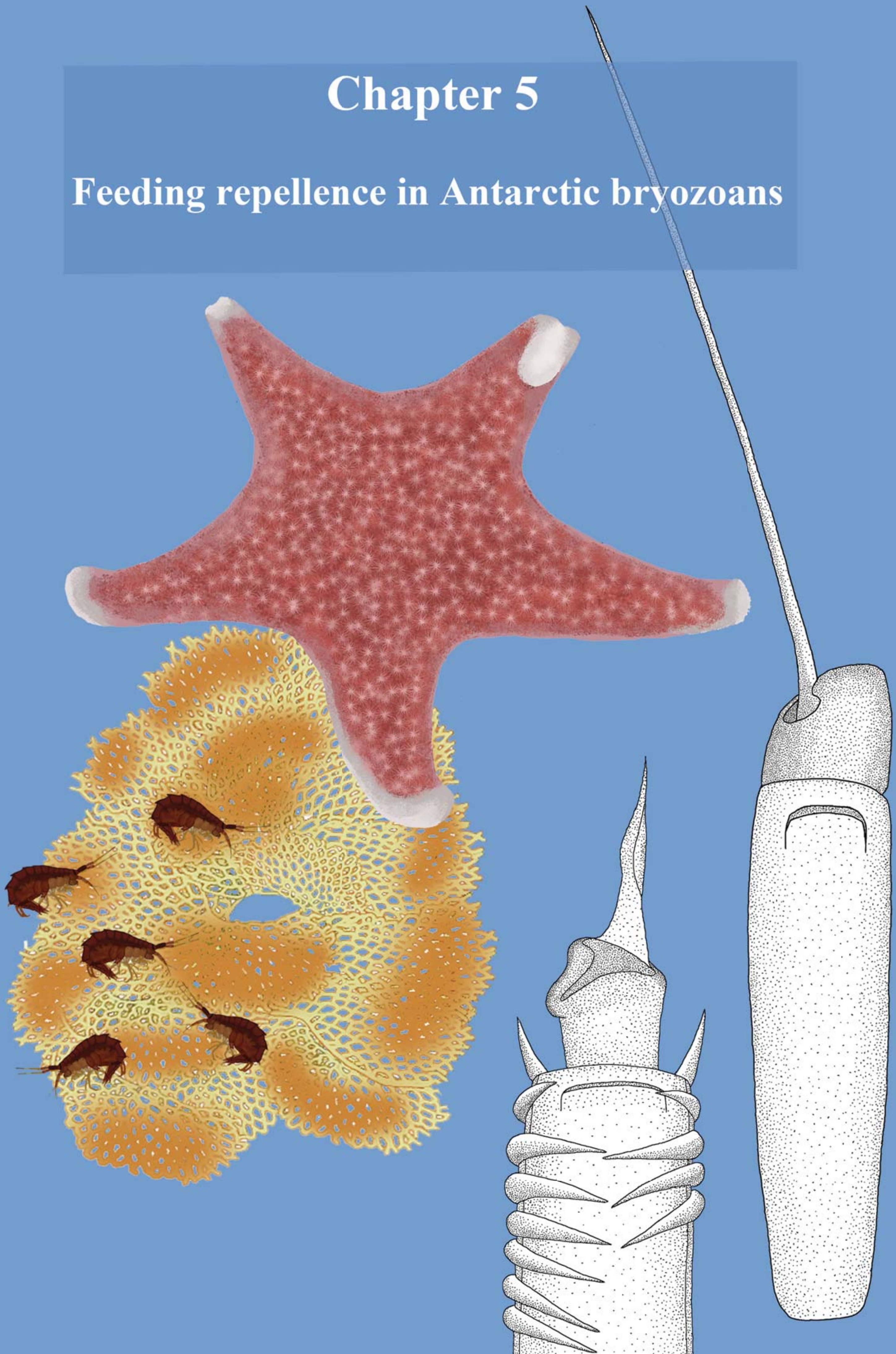
Chapter 5

Feeding repellence in Antarctic bryozoans



Chapter 5

Feeding repellence in Antarctic bryozoans

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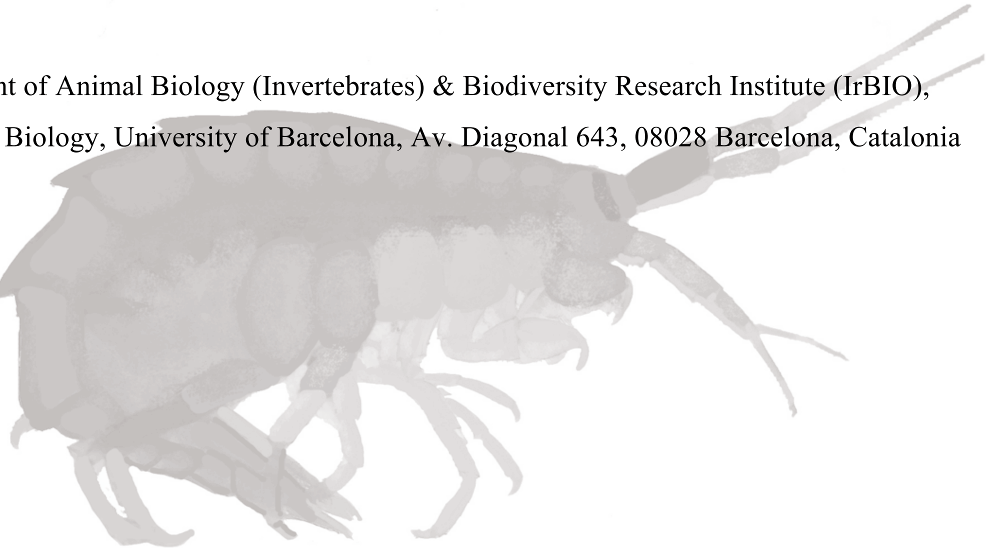


Abstract

The Antarctic sea star *Odontaster validus* and the amphipod *Cheirimedon femoratus* are relevant predators in benthic communities. Some bryozoans are part of the diet of this asteroid, and, as other sessile macroinvertebrates, bryozoans represent both potential host biosubstrata and prey for the omnivorous lysianassid amphipod *C. femoratus*. In response to such ecological pressure, bryozoans develop strategies to deter potential predators, ranging from physical (spines, hard skeleton) to chemical mechanisms (natural products). However, Antarctic bryozoans' chemical ecology has been scarcely studied. In this study we evaluated the presence of defenses against predation in selected species of Antarctic bryozoans. The sympatric omnivorous consumers *O. validus* and *C. femoratus* were selected to perform feeding assays with 16 ether and 16 butanol extracts obtained from 16 samples that belonged to 13 different bryozoan species. Most species (9) were active (12 ether and 1 butanol extracts) in sea star bioassays. Only one butanol extract displayed repellence, indicating that repellents against the sea star are mainly lipophilic. Repellence towards *C. femoratus* was found in all species in different extracts (10 ether and 12 butanol), suggesting that defenses against the amphipod might be both of lipophilic and hydrophilic nature. Inter- and intraspecific variability of bioactivities was occasionally detected, suggesting possible environmental inductive responses, symbiotic associations, and/or genetic variability. Multivariate analysis revealed similarities among species in relation to bioactivities of ether and/or butanol extracts. These findings support the hypothesis that, while in some cases alternative chemical or physical mechanisms may also provide protection, repellent compounds do play an important role in Antarctic bryozoans as defenses against sympatric predators.

Key words: *Odontaster validus*, *Cheirimedon femoratus,* Chemical ecology, Chemical defense, Deception Island

Resum

L'estrella de mar antàrtica *Odontaster validus* i l'amfípode *Cheirimedon femoratus* són depredadors rellevants en les comunitats bentòniques antàrtiques. Alguns briozous són part de la dieta d'aquest asteroid i, com altres macroinvertebrats sèssils, representen un biosubstrat potencial i una presa per l'amfípode omnívor *C. femoratus*. En resposta a la pressió ecològica, els briozous desenvolupen estratègies per dissuadir als predadors potencials, que abasten des de mecanismes físics (espines, esquelet) a mecanismes químics (productes naturals). Tot i així, l'ecologia química dels briozous antàrtics ha estat poc estudiada fins ara. En aquest estudi s'avaluava la presència de defenses contra la depredació en espècies seleccionades de briozous antàrtics. Les espècies simpàtriques i omnívores, *O. validus* i *C. femoratus*, van ser seleccionades per dur a terme assaigs d' alimentació amb 16 extractes etèrics i 16 butanòlics obtinguts de 16 mostres que pertanyien a 13 espècies de briozous diferents. La majoria d'espècies (9) eren actives (12 extractes etèrics i 1 butanòlic) en els bioassaigs amb l' estrella de mar, malgrat que només un extracte butanòlic mostrava repel·lència, indicant que els compostos repel·lents són principalment lipofílics. La repel·lència contra *C. femoratus* es trobava en totes les espècies en diferents extractes (10 etèrics i 12 butanòlics), suggerint que les defenses podrien ser de natura lipofílica i/o hidrofílica. Es va detectar ocasionalment variabilitat inter- i intraespecífica en les bioactivitats, suggerint respostes inductives ambientals, associacions simbiòtiques i/o variabilitat genètica. Els anàlisis multivariants revelaven similituds entre espècies en relació a la bioactivitat dels extractes etèrics i /o butanòlics. Aquests resultats suporten la hipòtesi que, mentre altres mecanismes químics o físics alternatius poden també proporcionar protecció en alguns casos, els compostos repel·lents juguen un paper molt important en briozous antàrtics com a defenses contra depredadors simpàtrics.

Paraula clau: *Odontaster validus*, *Cheirimedon femoratus,* Ecologia química, Defensa química, Illa Decepció

Introduction

The continental shelf of the eastern Weddell Sea and other Antarctic regions is characterized by presenting diverse, well-structured benthic communities, dominated by eurybathic suspension feeders such as sponges, gorgonians, bryozoans, and ascidians (Dayton *et al.* 1974; Teixidó *et al.* 2002; Figuerola *et al.* 2012a). The Antarctic Circumpolar Current led to the isolation of the Antarctic continent, approximately 20 million years ago. This allowed benthic species to co-evolve sharing similar ecological conditions, characterized by low and relatively stable temperatures and extreme seasonality of primary production (Clarke 1992; Arntz *et al.* 1997; Clarke *et al.* 2004; Dayton *et al.* 1994). Antarctic marine benthic communities below the effects of anchor ice and ice scour (<30 m depth) are principally influenced by intense biotic interactions (Dayton *et al.* 1974; Orejas *et al.* 2000), which constitute important driving forces in controlling population structure (Pawlik 2012). Such ecological relationships propitiate the evolution of chemical defenses to avoid predation, competition (either for food or space), and/or fouling, drawing a map of complex chemically mediated ecological interactions (Avila *et al.* 2008; McClintock *et al.* 2010; Figuerola *et al.* 2012b).

Generalist echinoderm and crustacean predators occupy higher trophic levels in Southern Ocean communities (Dearborn *et al.* 1983; Huang *et al.* 2007). Antarctic echinoderms are the dominant mobile megafaunal taxa regarding abundance and diversity, showing a higher diversity along the shelf, slope, and on the deep-sea plains (Brandt *et al.* 2007). In particular, the common omnivorous sea star *Odontaster validus* (Koehler 1906), which presents an extensive diet, drives intense predation events (Dayton *et al.* 1974; McClintock *et al.* 2010). This ubiquitous eurybathic sea star, found all around the Southern Pole down to 2902 m depth, has been chosen previously as a model generalist predator to conduct feeding repellence bioassays using different methodologies (McClintock *et al.* 1992, 1993, 1994; Slattery & McClintock 1995; McClintock & Baker 1997; Avila *et al.* 2000; Mahon *et al.* 2003; Peters *et al.* 2009; Núñez-Pons *et al.* 2012a; Taboada *et al.* 2013; see also http://iobis.org/mapper/). According to the high predation pressure exerted by this keystone asteroid, recent studies demonstrated the presence of feeding repellents in crude organic extracts of most taxonomical groups of Antarctic invertebrates (Avila *et al.* 2008; McClintock *et al.* 2010; Figuerola *et al.* 2012b; Taboada *et al.* 2013).

High diversity (more than 600 species estimated) and huge densities of opportunistic crustacean amphipods are found on the bottoms of the eastern shelf of the Weddell Sea, while the diversity of scavenger species in the continental slope (1000–3000 m) appears to be even richer than elsewhere in the world (Dauby *et al.* 2001b; Brandt *et al.* 2007; Huang *et al.* 2007; De Broyer *et al.* 2011). One of the causes of their abundance and success is related to their ability to switch feeding strategies, ranging from suspension-feeding to scavenging, depending on food availability (Dauby *et al.* 2001b). Many gammaridean species from the Weddell Sea show a broad diet spectrum on benthic invertebrates, such as bryozoans, cnidarians and sponges, suggesting a lack of prey specialization (Dauby *et al.* 2001b). These benthic sessile invertebrates, besides representing potential prey for amphipods, can also be potential host biosubstrata, offering a high diversity of microhabitats, and thus available structural and chemical refuges from predation (Osheland Steele 1985; De Broyer *et al.* 1999, 2001; Dauby *et al.* 2001a; Huang *et al.* 2008). *Cheirimedon femoratus* (Pfeffer 1888) (Gammaridea: Lysianassidae) is an opportunistic omnivore with circumantarctic and eurybathic distribution (from 0 to 1500 m depth), frequently associated to macroinvertebrates (De Broyer *et al.* 2007; Krapp *et al.* 2008). This scavenger amphipod is described as a generalist feeder, preying upon invertebrates, algae and detritus (Bregazzi 1972b), and it has been recently used as a putative model consumer in feeding repellence assays (Núñez-Pons *et al.* 2012b).

In the Southern Ocean, Bryozoa comprehend a rich and varied faunal group, with many new species described in recent years (Kuklinski & Barnes 2009; Hayward & Winston 2011; Figuerola *et al.* 2013). Antarctic bryozoans are often characterized for having circumpolar distributions, a broad bathymetric range, and for presenting higher specific richness respect to tropical areas, e.g. Hawai'i (Hayward 1995; Clarke & Johnston 2003; Figuerola *et al.* 2012a). The most successful living order of bryozoans is the Cheilostomatida, presenting a high proportion of endemism (56%) in Antarctic waters (Barnes *et al.* 2009; Griffiths 2010). Cheilostomatida are morphologically characterized for possessing box-like zooids, reinforced with calcium carbonate skeletons, and an operculum protecting the orifice through which the animal extends its ring of tentacles (lophophore) (Hayward 1995). Most cheilostomes have developed a high degree of polymorphism in zooids, being these specialized in different tasks including feeding (autozooids), cleaning and protecting colonies (e.g. avicularia and vibracula) and reproduction (gonozooids) (Winston 2009). Moreover, bryozoans are known to produce natural products such as alkaloids and terpenoids (both from hydrophilic and lipophilic origin, respectively) with deterrent activities, like antifouling and antipredation (Al-Ogily & Knight Jones 1977; Lopanik *et al.* 2004; Gray *et al.* 2005; Sharp *et al.* 2007). Thus, bryozoan colonies represent convenient model systems for studying diverse defensive strategies. Appeltans and colleagues (2012) estimate the world bryozoan species richness from 8,700 to 11,100; however, their role in the benthic communities specially focused on their natural products is understudied (Sharp *et al.* 2007; Blunt *et al.* 2012). In particular, Antarctic bryozoan chemical ecology remains poorly studied, although being a rich and diverse phylum (Winston & Bernheimer 1986; Lebar *et al.* 2007; Figuerola *et al.* 2012b; Taboada *et al.* 2013; unpublished results from the authors).

In order to establish the role of Antarctic bryozoan natural products from the Weddell Sea, the sea star *Odontaster validus* and the amphipod *Cheirimedon femoratus* were selected as model predators to conduct feeding repellence assays in order to: 1) evaluate the presence of natural products that may serve as repellents against these relevant Antarctic predators, and 2) compare the intra- and interespecific variability of bioactivity in selected species. Moreover, we compared the possible protection provided by the different potential defensive systems, both chemical and physical, in Antarctic bryozoan colonies.

Material and methods

Collection and identification of bryozoan samples

Sixteen Antarctic bryozoan samples belonging to 13 different species were collected between 273,6 m and 351,6 m depth during the ANT XXI/2 (November 2003–January 2004) cruise on board R/V Polarstern, from the Alfred Wegener Institute for Polar and Marine Research (Bremerhaven, Germany). Samples were collected using Agassiz Trawl and Bottom Trawl in the eastern Weddell Sea (Antarctica) (Table 1). Each bryozoan sample comprised several colonies obtained from each collecting station. Bryozoan colonies were sorted on deck, photographed, and a voucher portion of each was fixed in 70% ethanol for further taxonomical studies at the Faculty of Biology (University of Barcelona). Hayward's (1995) guide was used for species identification, and a detailed description of structural defensive devices was done by microscopical observations. All colonies were stored at -20°C for further chemical ecology experiments.

Collection of experimental sea star and amphipod predators

Alive individuals of the voracious eurybathic Antarctic sea star *Odontaster validus* were sampled at Port Foster Bay in Deception Island, South Shetland Islands (62º 59.369' S, 60º 33.424' W), during three campaigns: ACTIQUIM–1 (December 2008–January 2009), ACTIQUIM–2 (January 2010) and ACTIQUIM–3 (January–February 2012). Hundreds of sea stars were collected by scuba diving on sea bottoms ranging from 3 to 15 m depth. Hundreds of amphipods of the circumpolar scavenger *Cheirimedon femoratus* were captured between 2 to 7 m depth by scuba diving using fishing nets, along the coastline of the Antarctic Spanish Base (BAE) during the ACTIQUIM–3 campaign (January 2012).

Chemical extractions of bryozoan samples

Bryozoan colonies were exhaustively extracted with acetone at room temperature by using a mortar and pestle, and then sequentially partitioned into ether and butanol extracts. A water residue was also obtained and conserved frozen for further studies. All extraction steps were repeated 3 times, except for the butanol, which was done once. Organic solvents were evaporated under reduced pressure, resulting in dry ether and butanol extracts, and aqueous residues. Ether (comprising the most apolar lipophilic metabolites) and butanol (polar hydrophilic metabolites) extracts were used for bioassays at their corresponding natural concentrations, according to dry weight calculations (see below).

Physical defenses versus chemical defenses

The number of polymorphs (spines, avicularia and/or vibracula) per cm^2 was counted in three different portions of colonies for 13 species and it is shown as a mean. With the purpose of knowing if a relationship exists between the density of physical defenses and the level of the repellent activity of each species for the two experiments, the Pearson correlation coefficient was calculated. The repellent activity was here referred as the number of active extracts in the two experiments; ranging from 0 active extracts to 4 (ether and butanol extracts in the two experiments).

Feeding repellence assays with the asteroid Odontaster validus

The common omnivorous sea star *O. validus* has been already used in previous feeding repellence studies of our group as a generalist model of Antarctic predator (e.g. Avila *et al.* 2000; Núñez-Pons *et al.* 2010, 2012a; Taboada *et al.* 2013). Sea stars, ranging 6.5–10.5 cm diameter, were acclimated for 5 days in large tanks, with seawater pumped directly from the sea, before being used in the feeding repellence assays. Ether and butanol extracts from Antarctic bryozoans were diluted into diethyl ether and methanol, respectively, and then coated uniformly into shrimp-based food items until the solvent was evaporated, following the methodology previously described (Avila *et al.* 2000; Taboada *et al.* 2013). Sea stars were individually accommodated in 2.5 L tanks, and later shrimp cubes (5×5×5 mm, 13.09 ± 3.43 mg of dry weight) with or without extract were offered (10 replicates each test). Control feeding shrimp cubes were only treated with solvent. The natural concentration was calculated as in previous studies of our group (e.g. Núñez-Pons *et al.* 2012), by dividing dry ether or butanol partitions (EE or BE) by the total dry weight (TDW = DW + EE + BE, where DW is the dry weight of the solid remains of the extracted sample). We used dry weight for our calculations because this parameter eliminates deviations related to water content, which is very variable in aquatic samples. After 24 hours the number of eaten food units was recorded for each test, and uneaten items were frozen. Later, Thin Layer Chromatography (TLC) of the remaining food cubes demonstrated the permanence of the compounds on the shrimp cubes. Feeding repellence was statistically evaluated by using Fisher’s Exact test for each experiment referred to the simultaneous control (Sokal & Rohlf 1995). After the experiments the sea stars were returned alive to the sea.

Table 1. Sample collection data, skeleton, defensive structures, and bioactivity of ether and butanol extracts from Antarctic bryozoans. Results of three ether extracts (EE) of *B. dentata*, *I. secunda* and *I. tenuis* come from Taboada *et al.* (2013). Colonies were defined here as flexible (weakly calcified: capable of being bent) or rigid (heavily calcified: resistant to the deformation). Significance: + p<0.05; ++ p<0.005; +++ p<0.0001; -: not significant. EE: ether extract, BE: butanol extract. Feeding repellence: tests using *Odontaster validus*. Feeding preference: tests using *Cheirimedon femoratus*.

Species	**Latitude (S)**	**Longitude (W)**	**Depth (m)**	**Skeleton**	**Defensive structures**	**Feeding repellence**		**Feeding preference**	
						EE	**BE**	**EE**	**BE**
Bostrychopora dentata Waters, 1904	70° 57.00’	10° 31.61’	284.4	Rigid	Paired, lateral oral avicularia; one or more avicularia on frontal wall	++	-	+	++
Camptoplites angustus Kluge, 1914 (1)	70° 50.75’	10° 28.01’	281.2	Flexible	Three kinds of bird’s head avicularia, pediculate; autozooid with two large spines	++	-	-	++
Camptoplites angustus Kluge, 1914 (2)	70° 50.78’	10° 28.51’	273.6	Flexible	Three kinds of bird’s head avicularia, pediculate, autozooid with two large spines	+++	+	-	+++
Camptoplites bicornis Busk, 1884	70° 56.83'	10° 32.61'	338	Flexible	Four kinds of bird’s head avicularia, pediculate; autozooid with two short spines	++	-	-	++
Camptoplites tricornis Waters, 1904 (1)	70° 56.67'	10° 32.05'	302.4	Flexible	Four kinds of bird’s head avicularia, pediculate; autozooid with three large spines	+++	-	-	+++
Camptoplites tricornis Waters, 1904 (2)	70° 57.33'	10° 33.86'	351.6	Flexible	Four kinds of bird’s head avicularia, pediculate; autozooid with three large spines	+	-	++	-

Table 1. (Continued)

Species	Latitude (S)	Longitude (W)	Depth (m)	Skeleton	Defensive structures	Feeding repellence		Feeding preference	
						EE	BE	EE	BE
Isoschizoporella secunda Hayward & Taylor, 1984	71° 06.44'	11° 27.76'	277.2	Rigid	Suboral avicularium	+	-	-	++
Isosecuriflustra tenuis Kluge, 1914	70° 52.75'	10° 51.24'	294.8	Flexible	Avicularia along branch margins	+	-	-	+
Klugella echinata Kluge, 1914	70° 56.83'	10° 32.61'	338	Flexible	Avicularia at proximal end of autozooid; autozooid with three to nine pairs of spines	++	-	+++	-
Melicerita obliqua Thornely, 1924	71° 06.44'	11° 27.76'	277.2	Rigid	-	+	-	+	++
Nematoflustra flagellata Waters, 1904	70° 56.42'	10° 31.61'	284.4	Flexible	Vibraculum at the distal end of autozooid	-	-	++	++
Notoplites drygalskii Kluge, 1914 (1)	70° 57.11'	10° 33.32'	337.2	Flexible	Two kinds of avicularia; autozooid with spine at distal end only to bifurcation of colony	+	-	++	++
Notoplites drygalskii Kluge, 1914 (2)	71° 04.30'	11° 33.92'	308.8	Flexible	Two kinds of avicularia; autozooid with spine at distal end only to bifurcation of colony	+	-	++	+
Smittina antarctica Waters, 1904	71° 06.44'	11° 27.76'	277.2	Rigid	Suboral avicularium	-	-	++	-
Systenopora contracta Waters, 1904	71° 06.44'	11° 27.76'	277.2	Rigid	Two kinds of avicularia	-	-	+++	++

Feeding preference assays with the amphipod Cheirimedon femoratus

The ubiquitous and opportunistic amphipod, *C. femoratus* (Bregazzi 1972; De Broyer *et al.* 2007), was chosen for our experiments, following the protocol recently described by Núñez-Pons and coauthors (2012b). Artificial caviar-textured food items were prepared with 10 mg/mL alginate aqueous solution containing 66.7 mg/mL of a concentrated dried feeding stimulant (Phytoplan®). The powdered food was mixed into the cold alginate solution with a drop of green or red food coloring (see below), and introduced into a syringe without needle. The mixture was then added drop-wise into an aqueous 0.09 M (1%) $CaCl_2$ solution, where it polymerized into spheroid pellets, approximately 2.5 mm in diameter. For treatment pearls, extracts were dissolved in a minimum volume of extract to totally wet the powdered food and the solvent was evaporated, resulting in a uniform coating of extract prior to being added into the alginate aqueous mixture. The relative quantity of each extract was calculated according to the natural concentration in a dry weight basis attending to the explanations exposed above. Control pellets were prepared similarly but with solvent alone. Alive organisms were maintained in large 8 L aquariums and were starved for 3–5 days. Each assay consisted on 15 replicate containers filled with 500 mL of sea water and 15 amphipods each, which were offered a simultaneous choice of 10 treatment and 10 control extract-free pellets of different colorations (20 food pearls in total: 10 control and 10 extract-treated), green or red easily distinguished. The colors for treatment or control pearls were randomly switched throughout the experiment, and, moreover, previous trials confirmed the null effect of the different colorations in feeding preferences (p=0.47, n.s.). The assays ended when approximately half or more of either food types had been consumed, or 4 h after food presentation, and amphipods were never re-used. The number of consumed and not consumed pearls of each color (control or treatment) was recorded for each replicate container, considering that a food pearl was eaten when it was ingested up to at least 1/8 its original size. Finally, statistics were calculated to determine feeding preference of extract-treated pearls respect to the paired extract-free controls to consequently establish repellent activities. Each replicate was represented by a paired result yielding two sets of data (treatments and controls). Since assumption of normality and homogeneity of variances were not met, our data were compared by non-parametric procedures by applying the Exact Wilcoxon test with R-command software. Uneaten treatment food pearls were preserved for extraction and analyzed by TLC to check for possible alterations after testing. No major changes were observed. Once testing was over, amphipods were brought back to the sea.

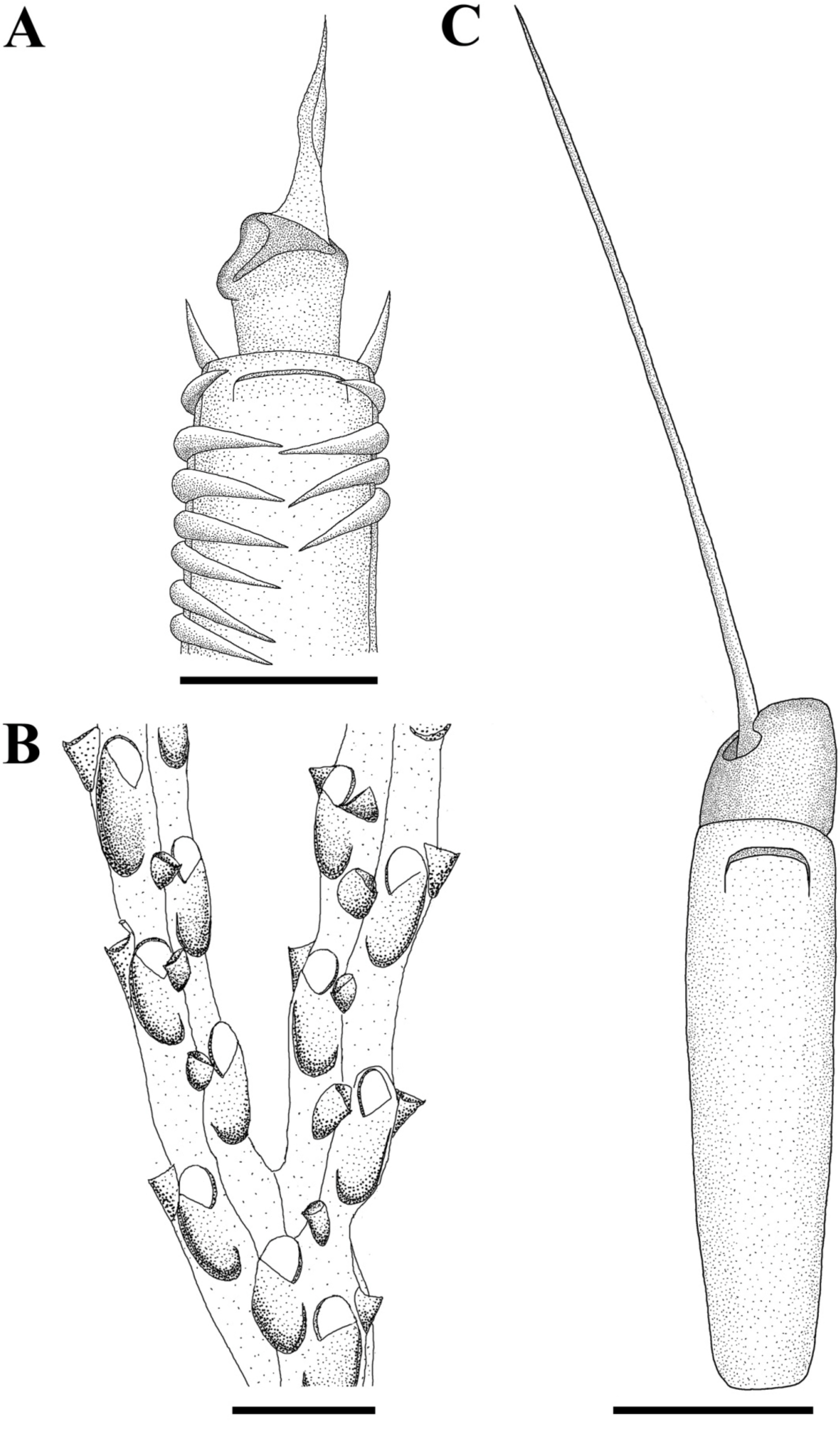
Activity’ relationships between species

Nonmetric multidimensional scaling (NMDS) ordination analyses were performed using Vegan software (R version 2.15.2) in order to assess the similarities of bryozoan species in relation to: a) the type of active extract (ether and/or butanol) and b) the type of antifeeding repellence (sea star and/or amphipod). Binary data (active/inactive) was used to make the distance matrix using the Bray-Curtis similarity index. Bray-Curtis index was chosen to be among the most widely employed indices, being equivalent to the Sörensen index for presence/absence matrices (Clarke *et al.* 2006; Legendre & Legendre 2012). The resulting similarity matrix was analyzed by NMDS, where the first two dimensions were plotted. The distance between dots indicates their similarity measured by stress values. A stress value of less than 0.1 indicates that the plot accurately represents similarities, while a stress value greater than 0.3 indicates that the points are close to being randomly placed (Clarke 1993). In order to verify statistical support in the defined groups, we performed an analysis of similarity (ANOSIM, Global R statistic), which does not require normal distribution data. The ANOSIM randomization test compares the within and between group similarity of elements measured by the Bray-Curtis index and calculates a global R statitistic. The resulting *R*-value ranges between 0 and 1, with high values indicating a large degree of discrimination among groups (Clarke & Green 1988).

Results

Bryozoan species collected showed different levels of skeleton calcification, diversity in zooid density and zooid types (spines, avicularia and vibracula; Fig.1). Colonies were defined here as flexible (weakly calcified, e.g. *Klugella echinata*) or rigid (heavily calcified, e.g. *Bostrychopora dentata*). A total of 16 ether and 16 butanol extracts obtained from 16 bryozoan samples, representing 13 species with rigid or flexible skeletons and several defensive structures, were tested at their natural concentration in both types of feeding assays. Our study demonstrates that all species tested exhibit some kind of repellent activity against the sea star *Odontaster validus* and/or the amphipod *Cheirimedon femoratus* (Table 1). All species of the genera *Camptoplites* and *Notoplites* presented repellent compounds against both predators. Their repellent defenses were found in both ether and butanol extracts, indicating the presence of defensive compounds in both lipophilic and hydrophilic extracts. However, some species did not show activity in all extracts (ether and butanol). In the case of *Camptoplites tricornis* and *Klugella echinata* only ether extracts displayed activity in both experiments.

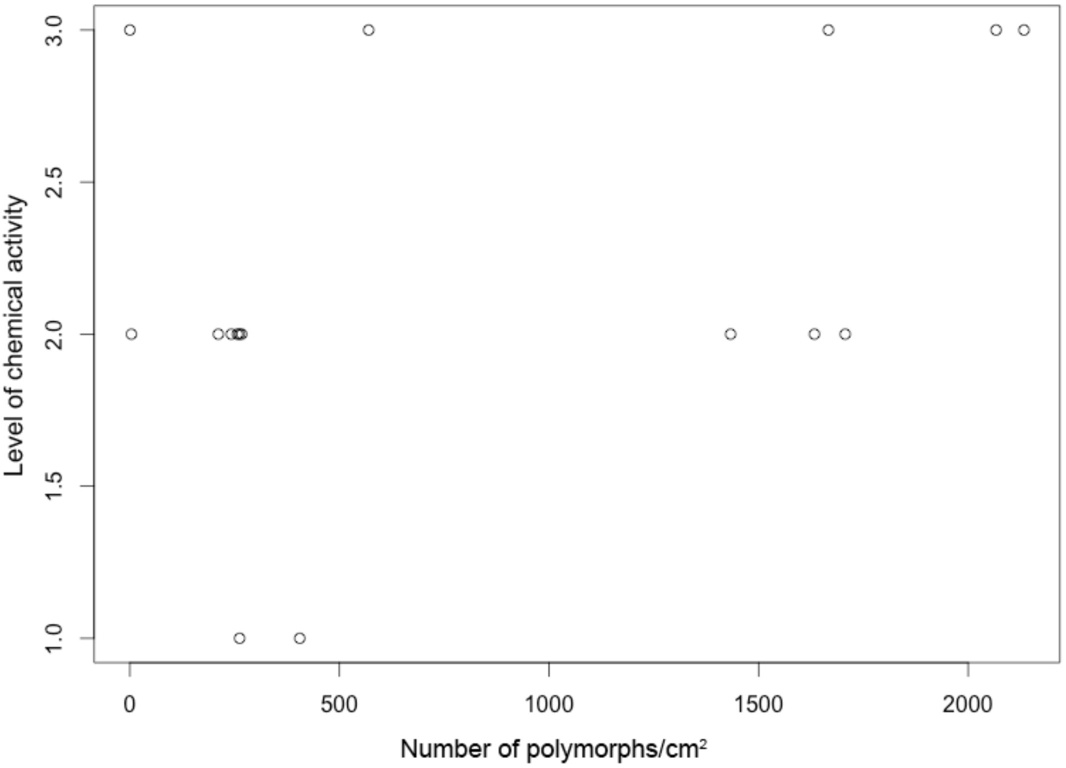
Fig. 1. Defensive structures of some Antarctic bryozoans. A) Autozooid of *Klugella echinata* with frontal membrane overarched by a variable number of marginal spines, and with columnar avicularium situated at the proximal end; B) Sessile avicularia, with sharply hooked mandible, in a part of *Notoplies drygalskii'* branched colony; C) Avicularium with a whip like seta (vibraculum) situated at the distal end of autozooid of *Nematoflustra flagellata.* Scale bar: 0.5 mm.



Physical defenses versus chemical defenses

Bryozoan colonies with diverse levels of flexibility and/or defensive structures show different mechanical and/or chemical defensive strategies (Table 1). In this sense, most of the species tested may employ a variety of defensive mechanisms. *Dakariella dabrowni*, *Smittina antarctica,* and *Systenopora contracta,* with rigid well calcified skeleton, did not show deterrent activity towards the asteroid *O. validu*s. *Melicerita obliqua*, a species without physical defensive structures and lightly calcified frontal walls (anascan cheilostome), showed repellent activity in both experiments. However, considering all the studied species, no correlation existed between the number (density) of polymorphs (spines, avicularia and vibracula) and the chemical defenses (r = 0.427, p= 0.099; Fig. 2).

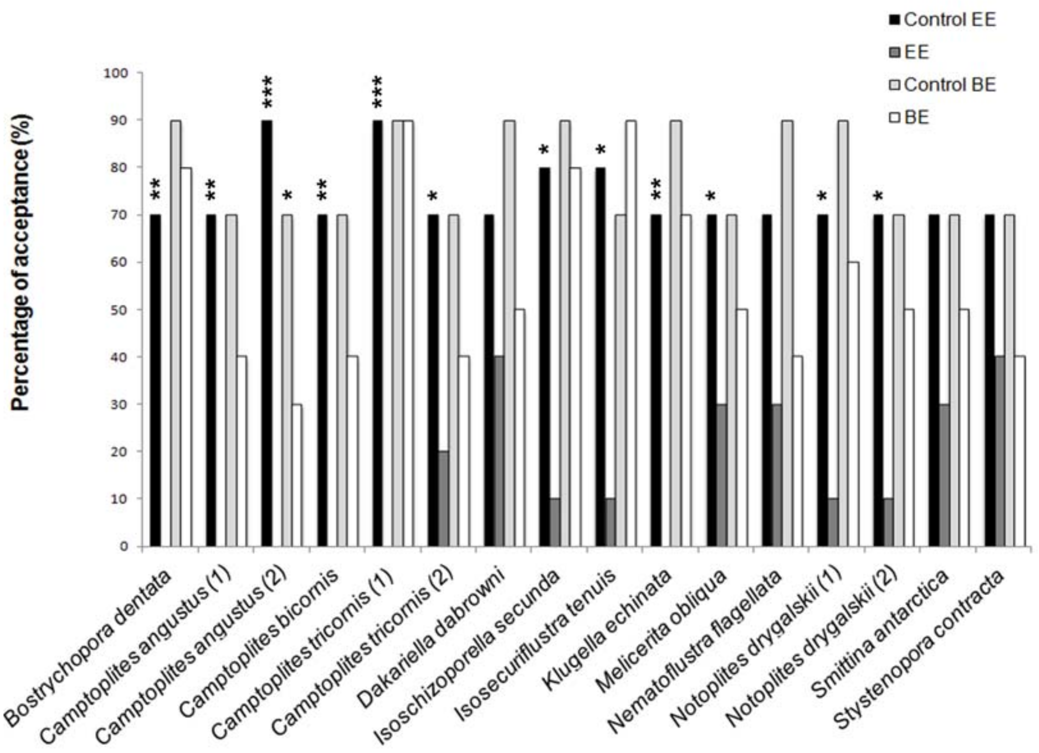
Fig.2. Relationship between physical (spines, avicularia and/or vibracula) and level of chemical defenses (referred to active extracts of each sample for the two experiments) for 16 samples (r = 0.427, p= 0.099).



Feeding repellence assays with the asteroid Odontaster validus

Odontaster validus rejected 9 of the 13 bryozoan species (69%) that were presented to them as ether extracts (EE), indicating the presence of lipophilic defensive metabolites (Fig. 3). In contrast, only one butanol extract (BE) from *Camptoplites angustus* (2) resulted repellent to the sea star. The flexible bryozoan *Nematoflustra flagellata* and the rigid bryozoans *Dakariella dabrowni*, *Smittina antarctica* and *Systenopora contracta* did not show any activity. Intra- and interespecific variability was found in the genus *Camptoplites*, even when the samples came from relatively close areas and similar depths.

Fig 3. Feeding repellence assays, with ether (EE) and butanol (BE) extracts from Antarctic bryozoans against the sea star *Odontaster validus*, showing the paired results of control and extract treated shrimp cubes for each test (10 replicates each test), expressed as the percentage of acceptance. Significant differences: p<0.05*, p<0.005**, p<0.0001*** with control as the preferred food (Fisher's Exact test). The results of the ether extracts of *B. dentata*, *I. secunda* and *I. tenuis* come from Taboada *et al.* (2013).



Feeding preference assays with the amphipod Cheirimedon femoratus

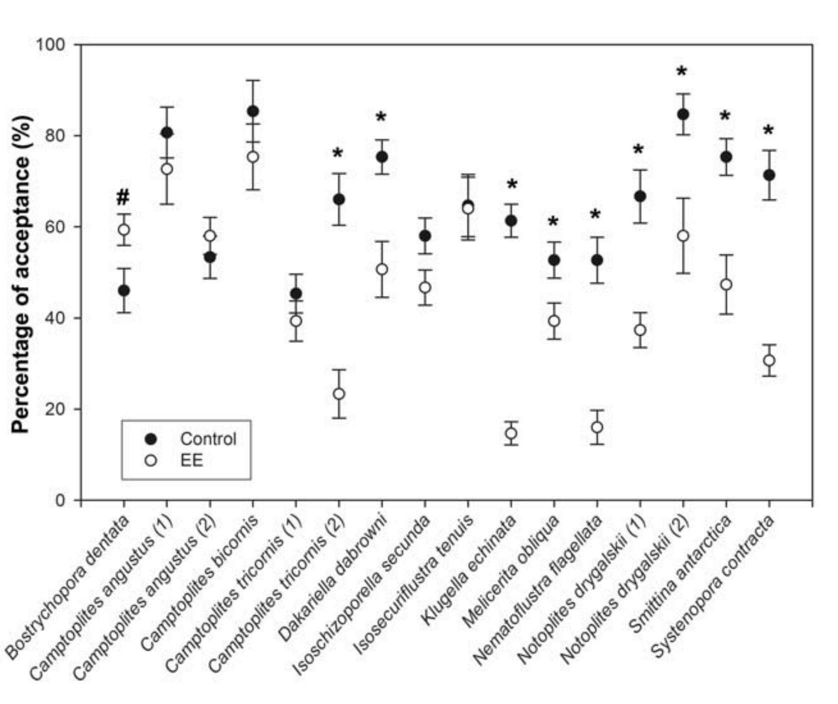
All bryozoan species tested caused repellence to the amphipod, either with both (ether and butanol) extracts, or with one of them (Fig. 4). Actually, most of the lipophilic (62%) and hydrophilic extracts (75%) repelled *Cheirimedon femoratus*, and six species displayed activity in both extracts. Interespecific variability was found again in the genus *Camptoplites*, whereas intraspecific differences were detected in *C. angustus*, *C. tricornis* and *Notoplites drygalski.* Colonies of the genus *Camptoplites* showed repellence only in the hydrophilic extracts except for *C. tricornis* (2).

Activity' relationships between species

Comparing both tests, four species were the most active: *B. dentata*, *C. angustus* (2), *M. obliqua*, *N. drygalskii* (1) and (2). These species displayed activity in three experiments: ether extracts and both extracts in the feeding repellence and preference bioassays, respectively, except in the case of *C angustus* (2), where both extracts in the feeding repellence and the butanol extract in the preference bioassay were active. Eight species showed activity in two extracts (*C. angustus* (1), *C. bicornis*, *C. tricornis* (1), *I. secunda,* and *I. tenuis*, in ether extract towards sea star and butanol extract towards amphipod; *C. tricornis* (2) and *K. echinata* in both ether extracts, *N. flagellata* and *S. contracta* in both extracts towards amphipod) and two species (*D. dabrownii* and *S. antarctica*) only displayed activity in one (ether extract towards amphipod; Table 1).

Low stress values (<0.03) of the MDS indicated a good representation in the 2-dimensional ordination (Clarke, 1993). Five groups were discriminated by the MDS analysis in relation to the bioactivity of bryozoan species of both experiments (Fig. 5): Group–1 with five species (*Camptoplites angustus* (1) and (2), *C. bicornis*, *C. tricornis* (1), *Isoschizoporella secunda,* and *Isosecuriflustra tenuis*) characterized for displaying activity in the ether extract (and in the butanol extract in the case of *C. angustus* (2)) against the starfish and in the butanol extract towards the amphipod; Group–2 with three species (*Bostrychopora dentata*, *Notoplites drygalskii* (1) and (2), and *M. obliqua*) displaying activity in ether extracts against the starfish and all extracts in preference bioassays; Group–3 including two species (*C. tricornis* (2) and *Klugella echinata*) showing activity only in ether extracts; Group–4 represented by two species (*Nematoflustra flagellata* and *Systenopora contracta*) that reveal activity only towards the amphipod in both extracts; finally, Group–5 comprising two species (*Dakariella dabrownii* and *Smittina antarctica*) that displayed repellence only in preference bioassays (ether extract). A significant difference in activity between groups was found (ANOSIM Global R=0.92, p=0.001).

Fig 4. Feeding preference bioassays against the amphipod *Cheirimedon femoratus* with ether (EE) and butanol (BE) extracts from Antarctic bryozoans, showing the paired results of control and extract treated foods (15 replicates each test) with the mean percentage of acceptance and standard error bars. Significant differences (*): control pearls as food preferred; (#): treated pearls as preferred food.



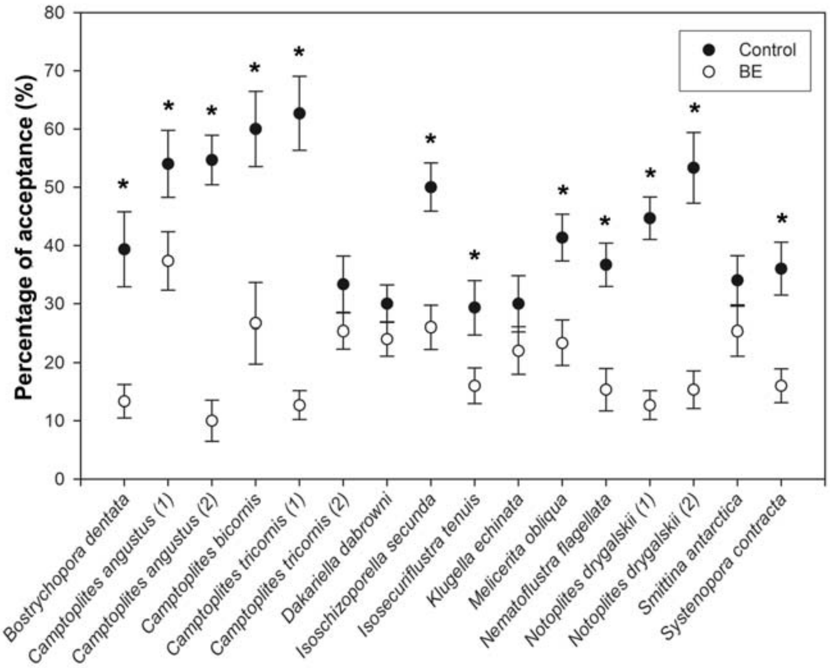
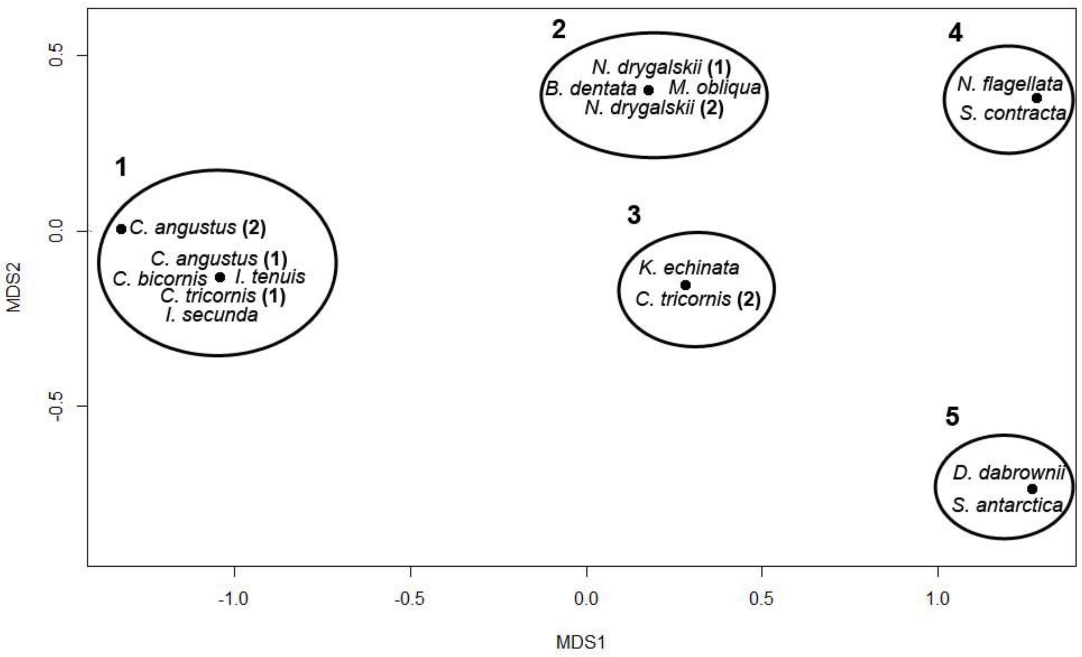


Fig 5. Multidimensional scaling ordination (MDS) plot of different bryozoan species in relation to the number of their active ether and butanol extracts in the two experiments of repellent defense. Circles correspond to grouped species (Groups 1─5) in relation to number and type of active extracts in both bioassays (stress< 0.03).



Discussion

Feeding repellence against generalist predators

The species of our study appear to be readily defended against at least one of the two abundant predators, the sea star *Odontaster validus* and the amphipod *Cheirimedon femoratus*, thus indicating the importance of chemical defenses against predation in Antarctic bryozoans. In Antarctica, sessile organisms are subjected to an intense predation pressure, which has driven to the development of chemical defenses in a high percentage of species (Lebar *et al.* 2007; Avila *et al.* 2008; McClintock *et al.* 2010). Nonetheless, bryozoans have been poorly investigated in terms of their chemical ecology so far (Avila *et al.* 2008). To the best of our knowledge, only 17 ether extracts have been tested previously in feeding bioassays against *O. validus* and only one against *C. femoratus* (Figuerola *et al.* 2012b; Núñez-Pons *et al.* 2012b; Taboada *et al.* 2013), and no butanol extracts have ever been tested before our study.

Feeding repellent responses were more frequent in the assays with *Cheirimedon femoratus* respect to the sea star tests, suggesting that bryozoans might require more protection against host opportunistic amphipods, which may exert a higher localized pressure (Núñez-Pons *et al.* 2012a). Unpublished results from our group (Núñez-Pons *et al.*) also propose that the amphipods' test seem to be more discriminative for feeding repellence when comparing both assays. Moreover, the implicated metabolites responsible for the activity appear to be present in both the hydrophilic as well as the lipophilic extracts. An extended activity recorded in our hydrophilic extracts contrasts to previous studies with different species of amphipods, which were more (or only) susceptible to lipidic defenses (Amsler *et al.* 2009; Koplovitz *et al.* 2009). Regarding the chemistry of cold-water bryozoans, alkaloids and terpenes were isolated from the circumpolar bryozoan *Flustra foliacea,* being responsible for ecological activities, including discouraging predators, a typical function of these compounds (Paul 1992; Lebar *et al.* 2007; Sharp *et al.* 2007). In addition, one alkaloid, tambjamine, was isolated from the Antarctic bryozoan *Bugula longissima* (Lebar *et al.* 2007). For the species studied here, chemical analyses are in progress in order to identify the natural products responsible for these activities.

Our results suggest interespecific and intraspecific variability in the genera *Camptoplites* and *Notoplites*, although this variability could exist also for other genera not tested yet at this level. Geographic and/or temporal variability in the production of secondary metabolites induced by local environmental pressures specific to the location, or genetic variation between populations could be the origin of this differences, as it happens in other marine taxa such as sponges (Duckworth & Battershill 2003; Page *et al.* 2005), soft corals (Puglisi *et al.* 2000), gorgonians (Roussis *et al.* 2000), and molluscs (Cutignano *et al.* 2011). However, more studies in these species are needed to confirm this. Likewise, different geographical chemotypes have been detected in the bryozoan *Amathia wilsoni* (Blackman & Matthews 1985; Morris & Prinsep 1999). Other kinds of tambjamines, similar to those previously mentioned, were originally isolated from various marine sources including bryozoans, ascidians, and their nudibranch predators, and also bacteria (Carte & Faulkner 1983; Lindquist & Fenical 1991; Blackman & Li 1994). The similarity of bryozoan natural products with other metabolites found in marine and terrestrial environments, supports the idea of a broad evolutionary retention of these types of compounds, or perhaps that some compounds isolated from Bryozoa might have a symbiotic origin (Sharp *et al.* 2007). Symbiotic bacteria have been proposed to be responsible for the production of numerous natural products found in invertebrates (e.g. Piel *et al.* 2005), although this has been only seldom demonstrated. For instance, the bryozoan *Bugula neritina* harbors different strains of bacteria responsible for producing bryostatins in a single specific geographic location (Davidson & Haygood 1999). Thus, geographic and seasonal variations of associated bacterial communities may also cause the intra- and interespecific differences in chemical profiles (Anderson *et al.* 2010).

The omnivorous sea star *Odontaster validus* feeds on a highly diverse assortment of benthic invertebrates and algae (McClintock *et al.* 1994), including bryozoans as part of its regular diet (Dayton *et al.* 1974). Although bryozoans are clonal organisms, with extensive regenerative potential, they cannot survive after major injuries of the colonies caused mainly by large predators (e.g. sea stars, echinoids) (Winston 2009). Thus, defensive strategies against such predators are expected to be developed. Our results showed that *Camptoplites angustus* (2) seems to combine feeding repellents of different nature towards *O. validus* appearing in both ether and butanol extracts. Instead, other bryozoan samples, which cause significant levels of feeding repellence, are mostly defended by the presence of deterrents in their lipophilic extracts. In previous studies with Antarctic sponges, lipophilic extracts were also reported to show higher tube-foot retraction responses in the spongivorous sea star *Perknaster fuscus* compared to hidrophylic extracts (McClintock *et al.* 2000). In recent studies, we demonstrated the existence of repellent compounds against *O. validus* in the ether extracts of 10 out of 17 Antarctic bryozoans (mostly different species to our study, except the ether extracts of *B. dentata*, *I. secunda* and *I. tenuis*), representing a very active phylum with extended deterrent activities (Figuerola *et al.* 2012b; Taboada *et al.* 2013).

The generalist amphipod *Cheirimedon femoratus* was deterred by all bryozoan species tested here. In previous studies, a single bryozoan ether extract was tested from the species *Isoschizoporella secunda* resulting suitable (Núñez-Pons *et al.* 2012b). Accordingly to our results, only the butanol extract of this species displayed repellence, which could confirm that in this case the active compound is of hydrophilic nature. Although predation by smaller zooid-level predators, such as amphipods, is generally sub-lethal in colonial organisms (Winston 2009), chemical defenses are frequently developed in clonal organisms to avoid future attacks. In addition, deterrents against amphipods are frequently reported in other Antarctic sessile invertebrates (Núñez Pons *et al.* 2012). Certain amphipod species are considered specialist bryozoan predators at the zooid level, feeding on individual autozooids (Lidgard 2008). For instance, the amphipod *Gnathiphimedia mandibularis* has adapted its mandibles for crushing hard items such as the skeletons of some bryozoan species, which are its principal food source (Coleman 1989). Therefore, repellent activities reported here could be useful to prevent the settlement of transient organisms, like amphipods, which feed directly or occasionally on bryozoan colonies, and which may also disrupt feeding currents (De Broyer *et al.* 2001). Similar consequences are caused by amphipod populations associating with sponges, as suggested by Amsler and colleagues (2009).

Diverse bryozoan shapes and structures provide many potential substrates for amphipods to be used as nurseries or as physical or chemical refuges from predation (Winston 2009). In fact, benthic amphipod species from the eastern Weddell Sea are often found on bryozoan colonies (De Broyer *et al.* 2001). Among the most frequent bryozoan morphologies found, there are those erect rigid or flexible laminar (e.g. *Nematoflustra flagellata*, *Isosecuriflustra tenuis* and *Isoschizoporella secunda*) and bush forms (e.g. species of *Camptoplites* and *Notoplites drygalsky*). While laminar species grow in a two-dimensional fashion, the bush forms provide three-dimensional substrates (e.g. genera *Bugula* and *Camptoplites*) with internal space where small crustaceans may hide from predators and feed on debris, diatoms, and other small organisms (Carter 2008). *C. femoratus*, as many other benthic amphipods, is a bottom-dweller with reduced swimming capacity, found regularly associated with living substrata, mainly macroalgae but also invertebrates (Zamzow *et al.* 2010; Amsler *et al.* 2009). Thus, we propose that the use of repellent compounds to avoid settlement of transient species such as *C. femoratus* in sessile organisms like bryozoans may be useful to avoid direct or occasional predation, as well as filtration disruptions, as discussed above.

Relationships between deterrent activity and physical defenses

Most bryozoan species seem to show a combination of chemical and physical defenses (spines, avicularia and/or vibracula) without a clear relationship between the density of the polymorphs and the level of chemical activity, suggesting either complementary mechanisms of protection, or redundant defenses. For example, although *Klugella echinata* and *Notoplites drygalskii* possess both chemical strategies and physical defenses (avicularia and spines), these species appear more vulnerable for having membranous frontal membranes (McKinney *et al.* 2003). Furthermore, the lack of chemical defenses to avoid *O. validus* in *Dakariella dabrowni*, *Smittina antarctica,* and *Systenopora contracta* could be related to the presence of other chemical or likely physical defensive mechanisms, such as their hard skeletons, proposed to be comparable with echinoid spines or bivalve shells (Hayward 1995). In fact, several studies have demonstrated trade-offs between physical and chemical defenses in some benthic invertebrates, leading to an optimization of energy addressed to primary biological functions: growth, reproduction (Stachowicz & Linsquist 2000; Hines & Pawlik 2012). In addition, encrusting cheilostome bryozoans such as *D. dabrowni* may form hard aggregates capable of resisting damage by predators (Best & Winston 1984; Winston 1986). These Antarctic bryozoan species also show abundant and diverse forms and sizes of avicularia, with an apparent protective function, as discussed in the recent description of a new Antarctic species (Figuerola *et al.* 2013). Avicularia are specialized zooids with an hypertrophied operculum (mandible), lacking a feeding role. Although the functions of various avicularia are still unknown, morphological and behavioral studies suggest physical protection, cleaning, and defensive functions against predators or grazers (Winston 1986, 1991). In particular, their mandibles are slowly opened by abduction and rapidly closed by adduction, discouraging potential predators (Winston 1986). Additionally, avicularia may have different forms and sizes to capture particular predators (Silén 1977). A highly specialized form is the often-polymorphic so-called “bird’s-head” avicularia, which can only be found in Antarctica in the genus *Camptoplites*, represented in the present study by three species: *C. angustus*, *C. bicornis* and *C. tricornis* (Hayward 1995). Several observations on living colonies show that the mandible may capture items, like arthropod appendages of potential small-sized predators, including amphipods (Winston 1986, 1991, 2009; Carter *et al.* 2010). Likewise, other studies document the capture of gammarid amphipods by the bird's-head form of *Bugula* (Forbes 1938; Kaufmann 1971). These trapped organisms may end up dying and, over time, decaying, attracting bacteria, thus providing food sources for bryozoans (Winston 1991). In addition, the long slender peduncles of the avicularian *Camptoplites* sway slowly back and forth across the frontal surface of the colony's branches, carrying the organism toward the edge of the colony (Winston 2010). Another advantage of the three species of *Camptoplites* studied here is the presence of three or four kinds of avicularia and spines. Even that, flexible *Camptoplites* species showed repellent activity against both the sea star and the amphipod. In this case, the combination of physical and chemical defenses may increase their defensive efficiency against the amphipod. Instead, *Nematoflustra flagellata* is weakly calcified but it possesses vibracula, modified zooids with an operculum in form of long seta, which act as potential physical deterrents against predators (Hayward 1995). The vibration of small organisms presumably triggers a wave provoking the movement of setae over the colony's surface carrying settling organisms outside the branches (Winston 2010). Although *N. flagellata* possess vibracula, this species also showed repellent activity against the amphipod.

In summary, the presence of physical defenses, such as avicularia, spines, and vibracula, together with their regeneration potential, and to the presence of deterrent metabolites may significantly increase both protection against predation as well as ecological success, respect to that offered by each defensive strategy separately. Similarly, hydroids combine the nematocysts with chemical defenses (Stachowicz & Lindquist 2000). There are cases though, such as *Melicerita obliqua,* where the lack of apparent physical defensive structures and the lightly calcified frontal walls, providing less resistance to grazing predators (McKinney *et al.* 2003), is likely compensated by effective chemical defenses. Actually, this species showed significant repellent activity in the assays towards both sea star and amphipods.

Similarities between species in relation to their bioactivities

Most species of *Camptoplites, Isoschizoporella secunda,* and *Isosecuriflustra tenuis* had similar deterrent bioactivity against the two generalist predators, as suggested by our MDS analysis. These species, except for *I. secunda*, exhibit a flexible skeleton, being apparently physically defenseless to predators (Hayward 1995), and thus requiring chemical defenses. Additionally, the active compounds of these species were of different nature, either lipophilic or hydrophilic. *Bostrychopora dentata*, *Notoplites drygalskii* (1) and (2), and *Melicerita obliqua* formed a group characterized for displaying all feeding repellence strategies in most extracts. In particular, *N. drygalskii* (1) and (2) also possess flexible colonies, and therefore, the use of chemical protection is presumably needed. Conversely, *M. obliqua* has a rigid skeleton but lacks other defensive structures, and accordingly possesses effective chemical defenses. The other rigid species, *B. dentata*, has diverse sessile avicularia; however, these are small and could possibly not be effective enough to discourage the predators of this study. In some of these cases, diverse strategies co-exist, suggesting that the combination of lipophilic and hydrophilic chemical defenses, or of these with physical defenses, may be required for defensive effectiveness. The flexible colonies of *C. tricornis* (2) and *Klugella echinata* were grouped together, since both caused chemical deterrence against the two predators, thus compensating their lack of rigidity. In contrast, *Nematoflustra flagellata* and *Systenopora contracta* displayed activity in both extracts only against the amphipod. Finally, *Dakariella dabrowni* and *Smittina antarctica* were grouped together for being rejected by *C. femoratus* in only one extract, but these species posses a hard skeleton, which likely may constitute an important physical defense against predation, such as that exerted by *O. validus*.

Concluding remarks

The diversity and high frequency of defensive activity against two sympatric predators showed in our study, along with the high number of morphological defensive structures, demonstrates the importance of defenses against common predators in Antarctic bryozoans. In this sense, understanding prey-predator interactions in Antarctica is a key aspect in these stable ecosystems, where biological factors are the main driver structuring benthic communities (Dayton *et al.* 1974). Our study suggests that chemical defenses are as common as physical defenses among Antarctic bryozoans, indicating that two or more different strategies may significantly increase protection in this group of invertebrates. Moreover, some species showed only chemical or physical defenses, suggesting potential resource trade-offs. To date, research in marine natural products and their role in an ecological context focused mostly on phyla such as Porifera, Cnidaria, and Mollusca (Appeltans *et al.* 2012; Blunt *et al.* 2012). In the case of Antarctic bryozoans, there are very few chemical ecology studies (Avila *et al.* 2008), although some cold-water bryozoans were reported to harbor pharmacologically interesting bioactive products (Lebar *et al.* 2007). This study has proven to increase the knowledge on Antarctic chemical ecology, particularly in bryozoans. However, further studies are needed to better understand the complexity of chemically mediated Antarctic ecological interactions involving this phylum and to identify their natural products.

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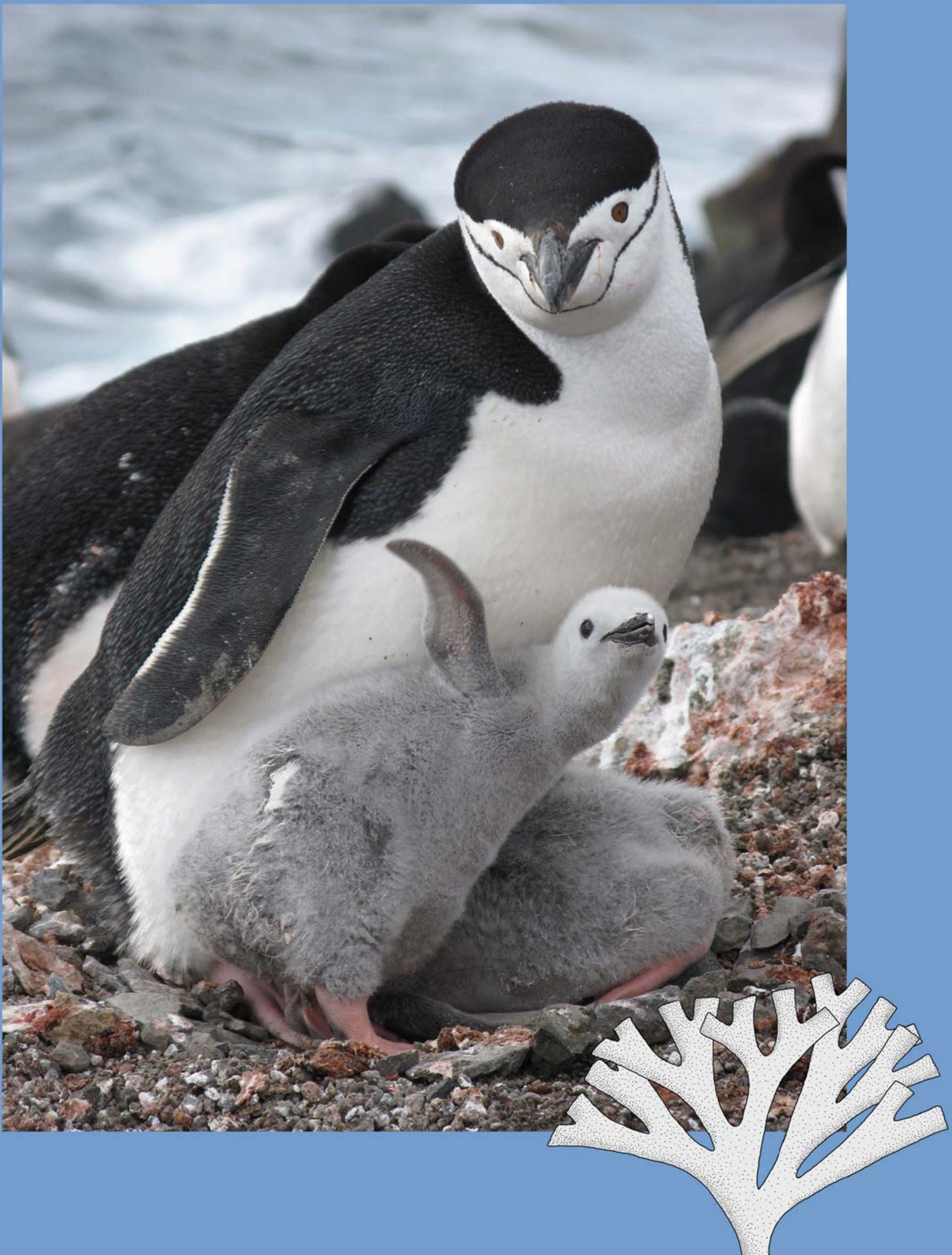
General Discussion and Conclusions



General Discussion and Conclusions

General Discussion

The current thesis covers two important and poorly known aspects of Antarctic bryozoans: **biodiversity** and **chemical ecology**. Our work highlights the need of gathering more information on biogeography, diversity and taxonomy of the scarcely known Antarctic bryozoans, in order to better understand their community' structure through depth and diverse spatial regions, and to determine how to conserve them (Chapters 1, 2 and 3). Additionally, our studies on bryozoan biodiversity provide insights into the potential physical and biotic factors, which influence the structure of Antarctic bryozoan communities (Chapters 1 and 2). Moreover, our biogeographical data lead to the discussion of the **extent of Antarctic isolation**, demonstrating the high value of our research on deep-sea fauna (Chapter 1). Among the new species found, a **new bryozoan species** has been also described (Chapter 3) and more remain still undescribed, requiring further studies. Since the studied bryozoan communities, below areas affected by local disturbances (iceberg scours and anchor ice), are mainly subject to biotic factors such as competence and predation, the evolution has favoured the development of **chemical mechanisms** in benthic organisms, which have also been investigated here (Chapters 4 and 5). In order to study these chemical interactions, **new adapted protocols** were designed using sympatric and abundant predators. Here, the most important findings are discussed in two sections (diversity and chemical ecology), providing a general view of the results and reviewing how the research findings help to fill in the knowledge gaps highlighted. Finally, investigation's limitations and recommendations for further research are also considered.

Antarctic bryozoan biodiversity (Chapters 1, 2 and 3)

Biogeography

The **comparative analyses** of diversity carried out here (Chapters 1 and 2) between Antarctica and the last separated fragments of Gondwana, especially the Patagonian region, and islands which serve as stepping-stones for shallow fauna, help to determine the **evolution** of regional communities and their relationships with the fauna outside the PF, as other authors suggest (Clarke *et al.* 2005; Clarke 2008). Thus, these studies provide a baseline to establish **biogeographical patterns** of cheilostome bryozoans. From this perspective, our results support the **hypothesis of the sequential separation of Gondwana** during the Cenozoic, with coincident biogeographic regions as proposed previously by other authors (e.g. Moyano 1982; Barnes & De Grave 2001; Arntz *et al.* 2005; Barnes & Griffiths 2008). In this sense, in our study (Chapter 1), the Antarctic and AP regions are clustered together, suggesting closer faunal affinities. Thus, these similar compositions could be related to the fact that these were the last fragments drifting apart during the break-up of Gondwana (Clarke 2003; Clarke *et al.* 2005; Lawver & Gahagan 2003; Upchurch 2008). In contrast, the Australasian region (New Zealand and Australia) tends to cluster separately, supporting the idea that this region was separated long before the Antarctic-South American separation. In the case of the SO, our results (Chapter 2) are in agreement with the different zoogeographical zones of diversity suggested by previous authors (Barnes & De Grave 2000; Barnes & Kuklinski 2010): the Sub-Antarctic islands (Bouvet Island), East Antarctica (eastern Weddell Sea), West Antarctica and the Scotia Arc (Deception and Livingston Islands). In particular, Bouvet Island shows a high similarity with the region of the Weddell Sea in our study and others (Barnes 2006; Gutt *et al.* 2006). Bouvet Island is located at a particular position relative to the ACC and may be in a potential zone of faunal exchange among the various regions and across the PF (Linse 2006). Moreover, we discuss in Chapter 1 that the high number of AP species shared with Antarctica, linked to the lower proportion of endemic Antarctic cheilostome bryozoans found, support the idea that over geological time the SO may have been less isolated than once thought (Barnes & Griffiths 2008). Therefore, our results question the real **extent of Antarctic isolation** for cheilostome bryozoans. We suggest that the PF appears to be less of a barrier for this group, as other authors have also debated (e.g. Thatje & Fuentes 2003; Clarke *et al.* 2005; Thatje *et al.* 2005a; Brandt *et al.* 2007a). Another important aspect to consider is the role of the **Scotia Arc** as a potential physical link between Antarctica and South America in bryozoans (Arntz *et al.* 2005; Barnes 2005). Dispersal mechanisms are also known to play an essential role in the distributional patterns. In the case of bryozoans, which are commonly effective **rafters**, rafting is a key element in the biogeography of cheilostome bryozoans, allowing for a very fast dispersion over scales from km to global level (Watts *et al.* 1998; Key *et al.* 2013). We suggest that the presence of some common bryozoan species between these regions may also be, in part, explained by the potential **passive northwards** transport of larvae (or perhaps even adults) to considerable distances via the branch of the ACC flowing northward along the continental shelf of Argentina, the Falkland/Malvinas Current (Brandt *et al.* 2007a). Other dispersal pathways of organisms, like **eddies** of ACC, may increase the bryozoan connection found between these regions, transporting bryozoan colonies attached to driftwood and other marine debris (Clarke *et al.* 2005). On the other hand, the increase of **human dispersal mechanisms** (e.g. ballast water and marine debris of anthropogenic origin such as floating plastic) in and out of Antarctica may favour the faunal exchange and the introduction of alien species, by-passing oceanographic barriers, as suggested by other authors too (Barnes 2002; Thatje *et al.* 2005a). An important issue is the **free migration** of marine organisms in and out of the PF via the deep abyssal plains, where the SO is connected to the other oceans, being relevant in our case, where the majority of species from the AP region come from the continental slope (e.g. Thatje *et al.* 2005b; Brandt *et al.* 2007a; Clarke 2008).

Diversity and spatial patterns

Considering that Antarctic and Patagonian habitats are threatened by overexploitation of living resources, establishment of invasive marine species including bryozoans, climate change and the growing impact of tourism, their conservation is essential. Our research on taxonomy, diversity and spatial patterns of bryozoan communities (Chapters 1, 2 and 3) is the **baseline** needed to manage the regional biodiversity and to implement **conservation strategies** in these poorly known regions (López Gappa 2000; Brandt *et al.* 2007a; Barnes & Peck 2008; Tejedo *et al.* 2009; Barnes & Kuklinski 2010). However, the implementation of conservation actions has to take into consideration several current limiting factors, such as the scarce sampling done in these regions, and also the financial and political constraints, among others. Therefore, further taxonomic and long-term studies are highly recommended, so that future changes caused by anthropogenic perturbations can be detected.

Our studies (Chapters 1 and 2) are also among the first characterizations of the bryozoan communities, mainly at the **slope**, from the **AP region** (Hastings 1943; López Gappa & Lichtschein 1990; López Gappa 2000), and from the SO, specially the **Weddell Sea** (Zabala *et al.* 1997; Moyano 2005, Barnes & Kuklinski 2010). In particular, few bryozoan species from the AP region and the SO were previously reported from below the shelf break and most samples from the AP region studied so far come from less than 500 m (López Gappa 2000; Barnes & Kuklinski 2010). In the case of the SO, bryozoans are poorly known, mainly due to the relative inaccessibility of this region (Kuklinski & Barnes 2009).

Moreover, the studies of this Thesis provide an overall picture of cheilostome bryozoan biodiversity from the AP and the SO regions, demonstrating that it is both rich and abundant. In particular, the highest values of species richness from the AP region found around latitude 44º S (%S = 57) and at middle slope (%S = 77.78) seem to be explained by the confluence of the **subantarctic nutrient-rich Falkland/Malvinas** and the **subtropical Brazil currents**, where upwelling of nutrient-rich waters and associated plankton blooms occur, playing an important role in sustaining this great diversity (Acha *et al.* 2004; Miloslavich *et al.* 2011; Muñoz *et al.* 2012). In the case of the SO (South Shetland Islands, Bouvet Island and Eastern Weddell Sea), the higher richness found at 70–73º S (%S = 79.17; Eastern Weddell Sea) respect to the islands could be mainly explained by the **large distances** between the Eastern Weddell Sea and these islands, as well as by their **isolation**. Moreover, the highest value of species richness found at continental shelf (%S = 100) could be due to the **decreasing availability of food with depth** (e.g. Arntz *et al.* 1994; Linse *et al.* 2007). However, more intensive and uniform sampling is needed for these regions and the deep sea to confirm this.

The new data analyzed here also improve our understanding of the **spatial patterns** of bryozoan communities from the AP and the SO regions. Interestingly, our research also shows an **expansion in the known distribution** of a high portion (87 %) of bryozoan species from the AP region. Also, four species have been found far from their known distribution range in the Weddell Sea. Moreover, a synergy of physical (depth, substratum, iceberg scouring...) and biotic factors (e.g. food availability) play a key role in structuring Antarctic communities, which are characterized by both **bathymetric** and **horizontal variability**, as found in this and other studies (Gutt & Piepenburg 2003; Thatje *et al.* 2005; Cummings *et al.* 2006; Brandt *et al.* 2007b; Smale 2008b; Griffiths 2010). Despite the existence of some bathymetric variability in bryozoan communities, many bryozoan species analyzed here present a high level of **eurybathy**, being probably explained by the evolutionary history of the SO fauna during Cenozoic glacial periods, as mentioned earlier (Thatje *et al.* 2005).

Taxonomy

As mentioned above, apart from better understanding biogeographical and diversity patterns, improving our knowledge on **taxonomy** is essential to implement management strategies. Our results stress the importance of **taxonomical studies** in these scarcely explored regions, reporting a high number of **new genera** and **species** and **new records**. In the case of bryozoans, the morphology, position, and frequency of occurrence of avicularia are taxonomically significant and used to define species (e.g., Hayward 1995; Kuklinski & Barnes 2009). The variability in avicularian morphometrics found in our study is frequent, demonstrating the existence of **intraspecific variation** between localities (Kuklinski & Barnes 2009; Carter *et al.* 2010b). Moreover, the study of avicularian characteristics helps to understand their potential roles, as discussed below. In general, the shape of avicularia questions their **functional morphology**, which is still largely unknown and it is also discussed in our study. Different functions are suggested for avicularia, such as **physical protection, cleaning, and defensive roles** against predators or grazers, as commented in the Introduction. These potential functions might be quite useful in bryozoans, which are filter-feeding animals and mostly sessile (Busk 1854; Harmer 1909; Canu & Bassler 1929; Hyman 1959; Cook 1963; Winston 1984, 1991). Among the new species found in our study, a bryozoan of the genus of *Reteporella* characterized by **rare giant spherical avicularia** is described, leading to discuss which are the potential roles of the avicularia. Stable environments such as Antarctic regions, below areas affected by local disturbances, tend to favor the evolution of complex systems of polymorphism in cheilostome bryozoans (Venit 2007). Thus, the strange avicularia shape of this new species may indicate an adaptive value in this Antarctic region. The best reasonable hypothesis for the presence of avicularia with a large internal capacity could be the additional function as a storage reserve for nutrients and to distribute resources during unfavorable conditions, as suggested by Winston (1984). Remarkably, the giant size of the avicularia found may likewise be more advantageous than small avicularia to capture relatively large parts of microinvertebrates, as shown on living colonies, where sessile avicularia may capture predators of bryozoans, such as nudibranchs, polychaetes, amphipods and pycnogonids (Winston 1991; Carter *et al.* 2010a). Thus, this shape of the avicularia might be a specialization to capture particular Antarctic predators (Silén 1977). Additionally, the giant avicularia described here could be actively involved in deterring settlement of epibionts (Harmer 1909). All these, thus, demonstrates that the functional morphology of avicularia is **complex** and still largely unknown. Further studies, as well as more samples from the Weddell Sea, are needed to establish the general shape of the colonies, the distributional range, and the intraspecific variability of this new species.

Chemical ecology in Antarctic bryozoans (Chapters 4 and 5)

Besides the influence of physical factors, Antarctic bryozoan communities below areas affected by local disturbances are also subjected to biotic factors. Our initial hypothesis was that, during evolution, this high pressure generated by the biotic factors led to the development of **chemical mechanisms** of defense and protection, as stated in the Introduction. Considering that Antarctic bryozoans have been scarcely studied in terms of their chemical ecology, our studies are among the first reports on chemical ecology of Antarctic bryozoans (to our knowledge only three other peer-reviewed papers and one book chapter are available: Winston and Bernheimer 1986; Lebar *et al.* 2007; Figuerola *et al.* 2012; Taboada *et al.* 2013). Moreover, some cold-water bryozoans harbor pharmacologically interesting bioactive products (Lebar *et al.* 2007; Sharp *et al.* 2007). Therefore, our research also represents a **first baseline** to the research of new potential bioactive compounds from Antarctic bryozoans.

Our findings demonstrate the importance of diverse **chemical ecology mechanisms against competence and predation** in Antarctic bryozoans. Diverse Antarctic taxa exhibit different chemical mechanisms (Avila *et al.* 2008; McClintock *et al.* 2010). Considering that most of the reported effective repellent secondary metabolites from invertebrates are lipid-soluble, previous studies mainly focused on interactions with lipophilic extracts (Sotka *et al.* 2009). However, compounds of different polarities have been previously described among defensive products (Pawlik *et al.* 1986; Kubanek *et al.* 2000; Pawlik 2012) and our results confirm this. Surprisingly, our research shows the presence of both **lipophilic** and **hydrophilic bioactive compounds** in different Antarctic bryozoan species.

Also, we find **intra-** and **interspecific variability** in the extract's activity of some bryozoan species, suggesting environmental induced responses, presence of diverse bacterial symbiotic associations, and/or genetic variability, as it happens in other marine taxa (Puglisi *et al.* 2000; Page *et al.* 2005; Cutignano *et al.* 2011). However, more studies are needed in these bryozoan species, so that the structure and the origin of the active compounds can be determined.

Physical defensive strategies

Potential physical defensive mechanisms such as avicularia, spines, and vibracula, for species with little or no chemical defense are also discussed here. Our results show a general trend to possess a combination of chemical and physical mechanisms, suggesting the existence of **complementary traits**. However, the absence of chemical defenses in some species could be related to the presence of these physical defensive mechanisms. In our study, there are some evidences of **potential resource trade-offs** between physical and chemical defenses. A trade-off between strategies leads to an optimization of energy that may be addressed to primary biological functions, such as growth or reproduction, as other authors suggest in diverse marine invertebrates (Stachowicz & Linsquist 2000; Hines & Pawlik 2012). Therefore, the presence of physical defenses, together with their regeneration potential, and to the presence of repellent metabolites may significantly increase protection against predation respect to that offered by each defensive strategy separately.

Cytotoxicity and repellence to substrates

To test cytotoxic and repellent activities, the common and abundant sea urchin *Sterechinus neumayeri* and the amphipod *Cheirimedon femoratus* were selected, respectively. Sea urchins prey frequently on some bryozoans (Jacob *et al.* 2003), and grazing by *S. neumayeri* severely damages bryozoan colonies (McClintock 1994; Bowden 2005; Bowden *et al.* 2006). Moreover, sea urchins are free-spawning species and thus, masculine gametes (sperm) and eggs, when released, may contact frequently with nearby living surfaces. On the other hand, *C. femoratus* is a bottom-dweller, frequently associated with many living substrata (Oshel & Steele 1985; De Broyer *et al.* 1999, 2001; Dauby *et al.* 2001a; Huang *et al.* 2008). The **new adapted protocols** used here to carry out these bioassays (Chapter 4) seem to be an adequate method to discriminate active extracts. Our study is the first to use a gel, previously employed in other chemical ecology studies (Henrikson & Pawlik 1995; Clavico *et al.* 2006; Zamzow *et al.* 2010), as a substrate to evaluate the habitat choice exclusively related to the chemical composition of the gel (without influence of shapes) in bryozoans. As mentioned in Chapter 4, these methods provide several advantages, such as 1) artificial gel substrates, simulating chemically alive invertebrate surfaces, and thus conferring more realistic ecological conditions respect to previous studies, 2) the use as models of ubiquitous, common and easy to collect Antarctic organisms, 3) the employment of small quantities of extract at natural concentrations and 4) short timings of sperm cytotoxicity and substrate preference assays, useful during the commonly short Antarctic cruises. Thus, this chemical ecology method recreates natural circumstances for testing cytotoxic and repellent activities against both organisms (*S. neumayeri* and *C. femoratus*).

In the case of *S. neumayeri*, this new adapted protocol design is a modified protocol of that commonly used as standard, proposed by Volpi Ghirardini *et al.* (2005) for the Mediterranean sea urchin, *Paracentrotus lividus*. Our method introduces some modifications in order to adapt the assays to an Antarctic species with much longer developmental cycles, the sea urchin *S. neumayeri*. This species takes about 2-3 days to reach the blastula stage (Bosch *et al.* 1987), in contrast to *P. lividus*, in which the blastula develops in about 6-12 hours (Russo *et al.* 2003). The ideal bioassay is to test cytotoxicity against different stages of early development until pluteus stage, which develops in about 21 days. However, this is difficult to do during the commonly short Antarctic cruises. Thus, longer campaigns are recommended. Moreover, in the future, these results should ideally be validated with *in situ* experiments with living bryozoans, which could confirm the ecological relevance of the cytotoxic compounds of diverse invertebrates against embryo and sperm.

Most bryozoan species tested here display **cytotoxicity** (cell dead) and/or **repellent activity** (rejection) against the sea urchin *Sterechinus neumayeri* and the amphipod *Cheirimedon femoratus*, respectively. To the best our knowledge, few experiments of cytotoxic activity against sperm and early life stages of the common Antarctic sea urchin *S. neumayeri* have been performed to date using extracts of sympatric organisms (e.g. McClintock *et al.* 1990; Figuerola *et al.* 2012), adding value to our study. Cytotoxicity against embryos was not detected in our study, although there is evidences in the literature of the presence of cytotoxic compounds in laboratory, as is the case of the circumpolar bryozoan *Flustra foliacea*, with bioactive alkaloids that display cytotoxicity against the human colon cancer cell line HCT-116 (Lysek *et al.* 2002), and the species *Carbasea curva* which was reported to show moderate haemolytic activity (Winston & Bernheimer 1986). Contrarily to the absence of embryo cytotoxicity found in our samples, some bryozoan species reveal toxic activities against sea urchin sperm (Fig. 8). In this sense, the grazing activities of echinoids, particularly of *S. neumayeri*, are considered to be responsible for significant mortality of settling larvae and juveniles of benthic invertebrates (Bowden 2005; Bowden *et al.* 2006) and they tend to consume bryozoans incidentally whilst pursuing other invertebrates (Lidgard 2008; Winston 2009). Therefore, the presence of cytotoxic compounds against sperm found in this study might play an important indirect role at small scales, representing a mechanism of protection/competence, reducing the recruitment of this sea urchin and, consequently, the grazing pressure and colonization on bryozoan surfaces (e.g. McClintock *et al.* 1990; Pawlik 1992; Bowden *et al.* 2006).

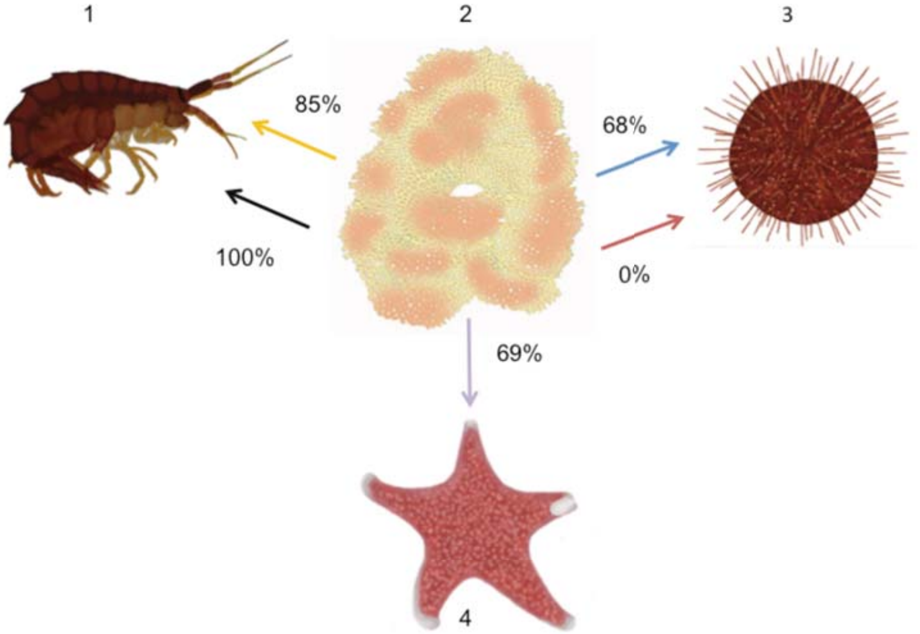
Otherwise, the main predators of bryozoans at zooid level are amphipods, copepods, nudibranchs and pycnogonids, which may feed directly on bryozoans (source of nutrition) or profiting their fouling microbiota, concentrated frequently by the feeding currents (Amsler *et al.* 2009; De Broyer *et al.* 2001). Our results also suggest that most bryozoan species analyzed here possess chemicals that cause substrate avoidance for the common amphipod *Cheirimedon femoratus*, being presumably useful to prevent the settlement of this omnivorous amphipod species (Bregazzi 1972), and consequently avoid predation and any other secondary detrimental effect, such as reduced rates in filter feeding caused by disruption of flow by dense populations of amphipods (Fig. 8). In agreement with our hypothesis, *C. femoratus* has limited swimming ability and usually associates ephemerally with living substrata, using them both as habitat and as potential prey.

Feeding repellence

Although bryozoan samples of our study come from shallow and deep sea, the species tested in these chemo-ecological studies are common and eurybathic, being thus, more easy for us to select an adequate co-existing predator. When selecting an Antarctic predator for the experiments, one should take into account that some species exhibit a marked seasonal variation in metabolic rate and feeding activity, such as *Sterechinus neumayeri* (Brockington & Clarke 2001). **Generalist predators** are ideal for feeding repellency assays since they ingest a wide range of species, and potentially inducing the acquisition of defenses in their prey. Thus, we selected appropriate, co-occurring generalist predators. In particular, given their abundance and their omnivory, and their ubiquitous and eurybathic distributions, as well as their likely ecological interactions with the bryozoan species analyzed, the macropredator sea star *Odontaster validus* and the mesograzer amphipod *Cheirimedon femoratus* were selected as model organisms in our bioassays (Chapter 5). Since only these two predators are used, experiments testing a wider array of predators in the future are recommended. The high incidence of repellence found in Antarctic invertebrates in other studies by our group demonstrates that these are appropriate predators (Núñez-Pons *et al.* 2012b; Taboada *et al.* 2013). Moreover, **comparative studies** testing **macropredators** and **micropredators** (between 0.2 to 20 mm; McClintock & Baker 2001) help us to ascertain whether there are differences in the induction of chemical defenses in bryozoans. Especially, macropredators such as *O. validus* cause major injuries on bryozoan colonies, after which bryozoans cannot survive despite being clonal organisms with extensive regenerative potential (Winston 2009). Contrastingly, micropredators such as *C. femoratus* occur in high densities, which may be a potential threat to their prey, although predation by smaller zooid-level predators is generally sub-lethal in colonial organisms (Winston 2009).

As mentioned earlier, Antarctic sessile organisms are subjected to an intense **predation pressure**, which has driven to the development of chemical defenses in a high percentage of species (Lebar *et al.* 2007; Avila *et al.* 2008; McClintock *et al.* 2010). Our results demonstrate that bryozoans seem to be readily defended against at least one of the two abundant predators, *O. validus* and *C. femoratus* (Fig. 8). As expected, most bryozoan species studied here show chemical defenses against either the sea star or the amphipod, or both. Also, our results agree with recent studies where we demonstrated the existence of repellent compounds against *O. validus* in the ether extracts of 10 out of 17 Antarctic bryozoans (Figuerola *et al.* 2012; Taboada *et al.* 2013). In the case of *C. femoratus*, this amphipod was repelled by all bryozoan species tested in our study. As discussed above (Chapter 4), this feeding repellence activity could be useful to prevent the settlement of the amphipod, which feeds directly or occasionally on bryozoan colonies, and which may also disrupt feeding currents (De Broyer *et al.* 2001). Especially, feeding repellent responses are more frequent in the assays with *C. femoratus* compared with those with the sea star, suggesting that bryozoans might require more protection against amphipods, which exert a localized pressure. In general, these results lead to the conclusion that this phylum is very active with extended repellent activities. Thus, based on the general and complex model previously proposed by our team describing a wide array of possible chemical ecology relationships (Figuerola *et al.* 2012), we now add new pieces in this complex puzzle still to solve (Fig. 8).

Fig. 8. Diagram of chemo-ecological interactions in thirteen Antarctic bryozoan species based in the general model proposed by our team (Figuerola *et al.* 2012), where: 1. *C. femoratus*, 2. Bryozoa, 3. *S. neumayeri*, 4. *O. validus*. Black: Feeding repellence activity; Yellow: Repellence for substrate; Red: embryos cytotoxic activity; Blue: sperm toxicity; Violet: Feeding repellence activity. The Phylum Bryozoa is connected by arrows with the organisms used for each *in vivo* experiment with the percentage of active bryozoan species indicated in numbers.



Although some bryozoan species are potential sources of pharmacologically promising substances, including cytotoxic alkaloids (Prinsep *et al.* 2004; Sharp *et al.* 2007), our chemo-ecological studies are the **first step** to find **bioactive compounds** involved in the ecological interactions of this phylum. The high activity found against the diverse predators used in this study suggests that a better exploration may lead to the possible use of bryozoan compounds as potential drugs with pharmacological applications. However, so far it has not been possible for us to identify the natural compounds or testing possible pharmacological activities, due to the small amount of samples. Further studies should deal with these interesting aspects too.

To sum up

Above all, this thesis wants to highlight the urgent need to better study Antarctic bryozoan biodiversity. These species should be protected because of their role in these rare and fragile Antarctic ecosystems, apart from their potential pharmacological use. We can only protect what we know and understand how it functions. We provided new key information on bryozoan biodiversity, spatial distribution, taxonomy and chemical ecology. We hope that this research fills in some of the existing gaps and creates a baseline for future work on this amazing group of animals.

“If Antarctica were music it would be Mozart.
Art, and it would be Michelangelo.
Literature, and it would be Shakespeare.
And yet it is something even greater; the only place on earth that is still as it should be.
May we never tame it."

Andrew Denton

Final Conclusions

The final conclusions of this PhD Thesis are summarized below, separated in the two main research topics:

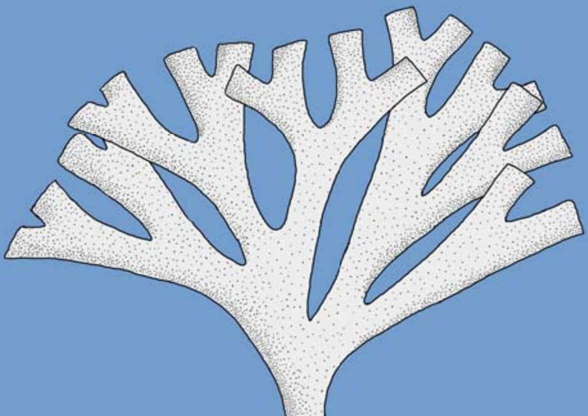
Biodiversity of bryozoans from Antarctic and sub-Antarctic waters

1. The bryozoological affinities found between Antarctica and the last separated fragments of Gondwana, especially the Patagonian region, and the islands which serve as stepping-stones for shallow fauna, are in agreement with the hypothesis of the sequential separation of Gondwana during the Cenozoic
2. The high number of AP species shared with Antarctica found in our study question the real extent of Antarctic isolation for cheilostome bryozoans
3. The presence of shared common bryozoan species between these regions may also be explained by the free migration of marine organisms in and out of the PF via the deep abyssal plains and the potential passive northwards transport of larvae (or perhaps even adults) to considerable distances, via the branch of the ACC flowing northward along the continental shelf of Argentina, the Falkland/Malvinas Current
4. The role of the Scotia Arc and other dispersal pathways, like eddies of ACC, and human dispersal mechanisms, may increase the bryozoan connection found between the Antarctica and the AP region
5. Cheilostome bryozoans from the AP region and the SO, mainly of deep-sea, are rich in diversity, abundant and eurybathic, but still scarcely known
6. The high diversity found around latitude 44º S and at middle slope from the AP region is probably related to the existence of the subantarctic Falkland/Malvinas and the subtropical Brazil currents, where upwelling of nutrient-rich waters occur
7. The higher richness found at the Eastern Weddell Sea respect to the islands, and in particular, at the continental shelf, is probably related to the large distances between the continent and these islands, as well as to their isolation, and to the decreasing availability of food with depth, respectively
8. The bathymetric distribution from the the AP region and the SO found in our studies fits well with the limits of the continental shelf, the slope and the deep sea
9. The new species of cheilostome bryozoan, *R. rosjoarum* n. sp., is characterized by the presence of unusual giant spherical avicularia, which may indicate a variety of biological roles, demonstrating that avicularian functions are complex and still largely unknown

Chemical ecology in Antarctic bryozoans

10. A large incidence of cytotoxicity and/or repellence to substrates of bryozoans against the sea urchin *S. neumayeri* and the amphipod *C. femoratus* was reported, supporting the hypothesis that chemical mechanisms are of great importance to avoid predation and competence
11. The bryozoans species tested here showed feeding repellence activity against at least one of the two abundant predators considered, the sea star *O. validus* and the amphipod *C. femoratus*, indicating the importance of chemical defenses against predation in Antarctic bryozoans
12. Feeding repellent responses were more frequent in the assays with *C. femoratus* compared with those with *O. validus*, suggesting that bryozoans might require more protection against amphipods, which exert a more localized pressure
13. The intra- and interspecific variability in bioactivity suggest an adaptive response to diverse abiotic and biotic factors, presence of microorganisms and/or genetic variability
14. The general trend in our study indicates the presence of a combination of both chemical and physical defensive mechanisms in most bryozoan species, suggesting complementary traits
15. The new adapted protocols using gel substrates described here aim at evaluating cytotoxic and substrate avoidance activities in bryozoans, and demonstrate many advantages over previous methods

Catalan Summary



Introducció General

L'Antàrtida: un continent remot i hostil

L'**Oceà Austral** (OA), que cobreix 35 milions de km^2 i comprèn aproximadament el 10% dels oceans de la Terra, refreda i ventila una gran part dels oceans del món. Està clarament limitat al sud pel continent antàrtic, i al nord pel **Front Polar** (FP, anomenat històricament la Convergència Antàrtica), un dels diversos fronts dins del **Corrent Circumpolar Antàrtic** (Fig.1) (CCA; Clarke & Johnston 2003; Bargagli 2008). Fa aproximadament 25 Ma, l'establiment del CCA i el gradual refredament posterior (Lawver & Gahagan 2003; Upchurch 2008), va provocar un aïllament biogeogràfic de l'Antàrtida, portant a un alt nivell d'endemisme (nivells de la plataforma continental entre 50 i 80%; Arntz *et al.* 1997; Lawver & Gahagan 2003; Scher & Martin 2006; Griffiths *et al.* 2009). Tots aquests esdeveniments històrics van portar l'Antàrtida a ser el continent més fred, més ventós, més sec i més alt del planeta, gairebé íntegrament cobert amb capes de gel de diversos quilòmetres de gruix (Barnes 2005).

A part d'aquestes característiques particulars, la producció primària de l'OA és sorprenentment al voltant del 15% del total mundial i, per tant, juga un paper important en el segrest del CO_2 de l'atmosfera, fent de l'OA un dels principals embornals de CO_2 atmosfèric (Huntley *et al.* 1991). En particular, les xarxes tròfiques polars s'han descrit com a més simples respecte a altres, d'altres parts del món, amb dos tipus bàsics de productors primaris, el **fitoplàncton** pelàgic i les **algues del gel** (Fig. 2). Sobretot a l'hivern, l'àrea coberta pel gel marí duplica la superfície continental de l'Antàrtida (de 3-4x10^6 km^2 a 18-20x10^6 km^2). Així, durant la producció del gel a la tardor, el gel marí fa disminuir la llum disponible necessària per a la producció del fitoplàncton, però és colonitzat per algues, adaptades a una baixa intensitat de llum. En aquest sentit, aquestes algues del gel marí són sovint l'única font d'aliment, en les estacions i regions on no hi ha altres fonts, per a una gran varietat de protozous i metazous, incloent el **krill antàrtic** (*Euphausia superba*) (Arrigo & Thomas 2004). Per contra, quan el gel es retira durant el temps curt d'estiu, a la zona fòtica es produeixen "blooms" de fitoplàncton (dominats per algunes espècies d'algues, en general grans diatomees i en alguns llocs per les algues unicel·lulars del gènere *Phaeocystis*) i en conseqüència, apareixen altes densitats de **zooplàncton**. Aquest zooplàncton manté abundants comunitats de diversos invertebrats pelàgics, com salpes, copèpodes, gammàrids, i krill. En particular, la xarxa tròfica de l'ecosistema antàrtic se centra en aquesta espècie de krill que és la presa clau, transferint el fitoplàncton a una gran diversitat d'organismes com peixos, mamífers marins (balenes, foques...), aus (pingüins...) i altres organismes (Cornejo-Donoso & Antezana 2008; Barnes & Clarke 2011). Els bacteris, els components clau del **bucle microbià** (bacteris, protozous i virus), són també una important via del flux de carboni, utilitzant (i) la matèria orgànica particulada formada per fito/zooplàncton i nècton mort i per pèl·lets fecals, així com (ii) la matèria orgànica dissolta alliberada pels processos fisiològics de l'excreció dels animals. Entre el nanoplàncton, els zooflagel·lats són els principals depredadors de bacteris marins, sent també importants components de les xarxes tròfiques marines, ja que transfereixen la producció bacteriana a nivells tròfics superiors, com els copèpodes i els amfípodes. De fet, la xarxa tròfica microbiana és clau pels bacterívors a l'hivern, quan la producció primària és inexistent (Arrigo & Thomas 2004). No obstant això, les altes concentracions de nutrients a la columna d'aigua demostren que només una petita part dels nutrients és consumida de manera eficient, i per tant, gran part de la producció primària és exportada a través del detritus al fons marí. En particular, aquesta matèria orgànica en suspensió a la columna d'aigua procedent del fitoplàncton, les algues del gel i la matèria fecal del zooplàncton, representa la font principal d'energia dels **suspensívors bentònics**. Aquest fet permet l'establiment d'una alta diversitat i biomassa de comunitats de macroinvertebrats sèssils per sota de les àrees afectades per l'erosió del fons marí produïda pels icebergs. Aquests invertebrats bentònics han desenvolupat adaptacions a aquestes condicions, com ara formes d'alimentació per sobreviure sense aliment durant llargs períodes. A més, els processos de resuspensió de la matèria orgànica dipositada al fons marí permeten la disponibilitat d'aliments durant molt de temps, per la seva baixa taxa de degradació, fet que explica l'abundància de suspensívors, sobretot durant l'hivern (Orejas *et al.* 2000; Kaiser & Barnes 2008).

En el cas de la **plataforma continental antàrtica**, aquesta fauna bentònica està subjecta a impactes naturals relacionats amb la formació del gel estacional. En particular, aquesta plataforma continental és profunda, sovint al voltant de 400-500 m de profunditat, però excedeix de 1.000 m en alguns llocs. Per tant, és tres vegades més gran que la d'altres continents, com a resultat de l'erosió de les plataformes de gel i la depressió causada per la immensa massa de gel sobre el continent (Clarke & Johnston 2003). Per aquesta raó, els icebergs són una pertorbació important en l'estructuració de les comunitats bentòniques antàrtiques, almenys fins a profunditats d'uns 500 m, fet que porta a una distribució faunística molt variable en l'espai (p. ex. Smale 2008a). En aquest sentit, els llocs exposats a aquesta erosió intensa causada pel gel estan dominats principalment per taxons mòbils, com equinoderms i l'isòpode *Glyptonotus antarcticus* (Gutt 2007). A més, molts dels tàxons bentònics mostren amplis rangs batimètrics com a resposta als cicles glacials-interglacials d'avançament i retrocés de la plataforma del gel (Brey *et al.* 1996). Durant els períodes d'extensió de la capa de gel, la intensitat de les pertorbacions físiques del gel elimina invertebrats de la plataforma, obligant a les espècies a migrar fora de l'Antàrtida o a aigües més profundes, i per tant, actuant com un refugi per als tàxons o, en alguns casos, causant la seva extinció. Durant els períodes de retrocés de la plataforma de gel, la plataforma continental pot ser re-colonitzada per la fauna del talús continental (Thatje *et al.* 2005b; Brandt *et al.* 2007a, b; Clarke 2008). A més a més, la gran plataforma continental antàrtica i la columna d'aigua isotèrmica (la qual no té una termoclina) també poden haver afavorit migracions entre la plataforma i la plana abissal (Clarke 2003). Per aquesta raó, aquesta fauna bentònica comprèn una barreja de tàxons amb diferents històries evolutives i afinitats biogeogràfiques (Clarke & Crame 1992).

Biodiversitat bentònica i biogeografia de l'Oceà Austral: la necessitat d'una millora en la gestió i conservació

S'estima que més de 7.000 espècies bentòniques habiten l'OA (De Broyer *et al.* 2011), incloent prop de 700 noves espècies, recentment descobertes, d'invertebrats d'aigües profundes (Brandt *et al.* 2007b). En particular, briozous, esponges i amfípodes presenten una gran riquesa específica (De Broyer *et al.* 2011). Aquestes comunitats bentòniques, per sota de les zones afectades per l'erosió dels icebergs, es van desenvolupar en condicions de relativa estabilitat, sent així probablement més sensibles al risc d'extincions causades pels impactes antropogènics. Per tant, els ecosistemes antàrtics representen regions clau per estudiar la biodiversitat i el canvi climàtic. Tot i que aquests ecosistemes han estat objecte d'un **impacte antropogènic** menor en comparació amb la resta del planeta, qualsevol amenaça potencial ha de ser avaluada acuradament, ja que representa una regió clau per a l'estudi de la biodiversitat i el canvi climàtic, on a principis del segle XIX, la caça de foques i balenes i l'exploració han impulsat l'augment de la presència humana a l'Antàrtida. A més, en els últims 50 anys, el turisme i les activitats de pesca de diversos organismes marins com el krill, peixos i altres organismes capturats amb la pesca han augmentat fortament (Griffiths 2010). Algunes regions antàrtiques també són de les més afectades per l'acidificació de l'oceà causada per l'augment de la concentració de diòxid de carboni atmosfèric. En conseqüència, hi ha una reducció de la concentració de carbonat de calci a l'aigua, que és el que forma els esquelets d'organismes marins predominants a l'Antàrtida, com ara equinoderms i briozous (Orr *et al.* 2005). Per desgràcia els hàbitats antàrtics estan, per tant, cada vegada més amenaçats per la sobreexplotació dels recursos vius, l'establiment d'espècies invasores marines, els contaminants antropogènics i, a llarg termini, pel canvi climàtic, així com pel creixent impacte del turisme. Des d'aquesta perspectiva, la conservació i la gestió de la biodiversitat marina antàrtica requereix urgentment estudis detallats sobre la **biodiversitat** i la seva relació amb les condicions ambientals (Barnes & Peck 2008).

Les comunitats bentòniques antàrtiques estan influenciades principalment per (i) una baixa temperatura en el nivell de congelació (arribant a gairebé -2° C a l'hivern i al voltant de 2° C a l'estiu), (ii) una marcada estacionalitat de la disponibilitat de llum i de la producció primària i (iii) dos grans pertorbacions, l'abrasió dels icebergs i els forts corrents (p. ex. Orejas *et al.* 2000; Gutt 2007; Smale 2007; Clarke 2008; Gerdes *et al.* 2008; Griffiths 2010). Algunes **respostes adaptatives** de les espècies bentòniques antàrtiques superficials a aquestes característiques ambientals particulars són un lent creixement, l'augment de la longevitat, una maduresa tardana, una baixa mobilitat dels adults, així com en alguns tàxons, gegantisme. En les espècies de peixos, és essencial la possessió de glicoproteïnes anticongelants i la manca de pigments sanguinis (Rahmann *et al.* 1984; Arntz *et al.* 1994; Brandt *et al.* 2007b). En comparació amb els tàxons de latituds més baixes, alguns grups de l'Antàrtida es troben àmpliament distribuïts com els amfípodes, esponges, briozous, isòpodes i poliquets, amb valors de biomassa, en alguns casos, superiors als de regions temperades o tropicals (Dayton *et al.* 1970; Orejas *et al.* 2000; Smale 2007; Cornejo-Donoso & Antezana 2008). Especialment, les esponges, les gorgònies i els briozous, amb les seves formes tridimensionals, creen microhàbitats per a un ampli rang de fauna associada, representant la biomassa més rica i la diversitat faunal més alta de l'Antàrtida (Arntz 1997). Per contra, altres grups són certament poc representats (decàpodes, peixos teleostis, bivalves i gasteròpodes) o estan completament absents (com depredadors pelàgics i bentònics, per exemple, els taurons), segurament a causa de les limitacions fisiològiques (Clarke & Johnston 2003; Arntz *et al.* 2005; Thatje *et al.* 2005). Es creu que algunes espècies d'isòpodes i amfípodes van irradiar després de l'extinció dels crustacis decàpodes braquiürs, fet probablement relacionat amb la disponibilitat de nínxols ecològics ocupats prèviament pels braquiürs (Brandt *et al.* 2012). En conseqüència, les densitats elevades d'amfípodes, així com els macroinvertebrats depredadors abundants i generalistes, que inclouen equinoderms i nemertins, representen els nivells tròfics superiors. Aquests exerceixen una forta pressió sobre els organismes sèssils, i en el cas de les estrelles de mar, tan intensa com en regions temperades i tropicals (McClintock 1994; De Broyer *et al.* 1999, 2007, 2011; Brandt *et al.* 2007a).

A més de la biodiversitat, la millora dels coneixements existents sobre la **biogeografia** és també de rellevància fonamental en la conservació i la gestió sostenible de les espècies, especialment a l'Antàrtida (Brandt *et al.* 2007a). Des d'aquesta perspectiva, els estudis comparatius de la diversitat en zones profundes entre l'Antàrtida i els últims fragments que es van separar de Gondwana, especialment la regió de la Patagònia, són clau per entendre l'evolució de les comunitats regionals i les seves relacions amb la fauna externa al FP, com altres autors suggereixen (Clarke *et al.* 2005; Clarke 2008). En particular, la regió de la Patagònia argentina (PA) és un dels grans ecosistemes marins més productius de l'hemisferi sud a causa de la convergència de dos dels corrents més importants impulsats pel vent (el corrent fred de les Malvines i el càlid de Brasil), que promou l'establiment d'una elevada biomassa d'invertebrats bentònics (Legeckis & Gordon 1982; Acha *et al.* 2004). No obstant això, la diversitat d'invertebrats marins en aquesta àrea no ha estat ben estudiada, especialment tàxons com els briozous, i molt menys els que provenen de major profunditat (López Gappa 2000; Acha *et al.* 2004; Miloslavich *et al.* 2011). A més, l'existència de possibles vies de dispersió entre aquesta regió de la PA i l'Antàrtida, com el corrent de Malvines, una branca del CCA, que flueix cap al nord per sota dels 800-1000 m (Hastings 1943; Legeckis & Gordon 1982), i els remolins del CCA (Clarke *et al.* 2005), suggereixen l'existència d'una **permeabilitat potencial** del **FP** (p. ex. Thatje & Fuentes 2003; Thatje *et al.* 2005a). Aquesta hipòtesi no ha estat provada i podria ser clau per explicar la biogeografia regional.

Estudis antàrtics químico-ecològics

Durant l'evolució, els organismes marins bentònics, sota una intensa pressió per l'espai, la llum i els aliments, han desenvolupat un ampli rang de mecanismes de defensa comportamentals, físics i/o químics (p. ex. Paul 1992; Avila *et al.* 2008). En el cas dels ecosistemes antàrtics, aquestes comunitats bentòniques, que es troben sota la influència de l'erosió del gel, semblen estar ben estructurades, en part a causa del relatiu alt nivell d'estabilitat del medi ambient esmentat anteriorment (Dayton *et al.* 1974; Arntz *et al.* 1994). Aquest fet, juntament amb les interaccions ecològiques intenses que ocorren al bentos de l'Antàrtida, explica per què aquestes comunitats s'estructuren principalment per factors biològics, com la **depredació** i la **competència** (Dayton *et al.* 1974), enlloc dels factors ambientals. En conseqüència, els invertebrats sèssils i lents, molt vulnerables als depredadors, han desenvolupat una àmplia gamma de mecanismes químics mediats per metabòlits secundaris per evitar la depredació (Amsler *et al.* 2001; Iken *et al.* 2002; Avila *et al.* 2008; Koplovitz *et al.* 2009; Slattery 2010), la competència per l'espai o l'aliment (Barnes & Rothery 1996; Bowden *et al.* 2006) i el recobriment (Peters *et al.* 2010). En particular, una preda defensada químicament pot produir metabòlits que causen rebuig (**inapetència/repel·lència**) i/o danys fisiològics (**toxicitat**) a un depredador (Pawlik 2012). Al contrari de la hipòtesi latitudinal (la qual suggereix que s'espera que les defenses químiques siguin més freqüents a latituds baixes que als pols; Bakus 1974), recents bioassajos de **repel·lència alimentària**, de **toxicitat** i **recobriment** duts a terme amb invertebrats bentònics antàrtics han demostrat que aquestes defenses són comparables a les que es troben en regions temperades i tropicals (vegeu Avila *et al.* 2008; McClintock *et al.* 2010). D'acord amb això, un estudi previ del nostre equip va proposar el **primer model ecològic** per descriure una àmplia gamma de possibles relacions ecològiques químiques (Figuerola *et al.* 2012). Aquest estudi va mostrar una increïble i complexa xarxa d'interaccions entre molts diferents organismes bentònics, generada principalment per evitar la forta pressió de la competència per l'espai i/o aliments, la depredació i el recobriment (Fig. 3). Per tant, es necessiten més estudis amb un major nombre de mostres per completar i enriquir aquest model i per omplir els buits existents.

Per entendre les funcions ecològiques dels **productes naturals** aïllats a partir dels teixits dels organismes marins, a la dècada de 1980 va sorgir un jove camp multidisciplinari, anomenat **ecologia química marina**. En aquest camp, els organismes polars han estat menys estudiats que els de les regions temperades i tropicals (Paul 1992; Avila *et al.* 2008; Blunt *et al.* 2012). No obstant això, estudis recents demostren que els invertebrats bentònics antàrtics són una font molt rica i diversa de productes naturals, amb gran interès **ecològic** i **farmacològic** (p. ex. Avila *et al.* 2000; Amsler *et al.* 2001; Iken *et al.* 2002; Reyes *et al.* 2008; Taboada *et al.* 2010; Paul *et al.* 2011; Blunt *et al.* 2012). En particular, diversos estudis han demostrat que algunes espècies antàrtiques que habiten en zones poc profundes de McMurdo Sound i la Península Antàrtica posseeixen defenses químiques (veure revisions d'Avila *et al.* 2008; McClintock *et al.* 2010). Malgrat això, només en uns pocs casos, la química dels metabòlits involucrats ha estat completament descrita i/o el seu paper ecològic establert (p. ex. Núñez-Pons *et al.* 2010; Núñez-Pons *et al.* 2012b; Taboada *et al.* 2013).

Pel que fa a l'ecologia química, els nostres estudis es van centrar en defenses químiques específiques d'alguns tàxons contra depredadors comuns, com es detalla continuació.

Defenses citotòxiques contra un depredador antàrtic comú, l'eriçó de mar

Els equinoderms tenen un paper notable en els ecosistemes bentònics antàrtics i en el control de les poblacions de molts organismes bentònics (McClintock 1994). Entre els equinoderms, l'espècie d'eriçó de mar *Sterechinus neumayeri* Meissner, és considerada com una de les més abundants i comunes de l'Antàrtida. En particular, *Sterechinus* és l'únic gènere dels equinoideus antàrtics, i comprèn 5 espècies, sent *S. neumayeri* la més abundant (Fig.4; Fell 1976). Aquesta espècie és circumpolar i té una distribució batimètrica àmplia, que va des d'uns pocs metres fins a 810 metres de profunditat (Brey & Gutt 1991; Moya *et al.* 2012). En general, els eriçons, quan brostegen, danyen greument les colònies de diversos tàxons, incloent briozous, i també poden afectar negativament el reclutament de larves (McClintock 1994; Bowden 2005; Bowden *et al.* 2006). A causa de la pressió intensa de depredació exercida per aquest eriçó de mar, les seves preses poden produir **substàncies citotòxiques** que impedeixen l'assentament de les seves larves, el que en última instància, pot reduir la pressió de brosteig sobre aquestes preses (p. ex. McClintock *et al.* 1990). *S. neumayeri* s'ha utilitzat com un organisme model en estudis anteriors i la seva biologia és ben coneguda (Bosch *et al.* 1987; Brey *et al.* 1995; Tyler *et al.* 2000; Brockington *et al.* 2007), però només uns pocs estudis han investigat aquestes interaccions químiques indirectes contra el desenvolupament d'embrions d'aquesta espècie (McClintock *et al.* 1990; Slattery *et al.* 1995).

Defenses repel·lents contra depredadors antàrtics comuns i generalistes

A part dels eriçons de mar, uns altres grans depredadors antàrtics són les estrelles de mar i els amfípodes. Els asteroides antàrtics es troben en els nivells tròfics més alts, substituint als peixos com a principals depredadors i, per tant, exercint una forta pressió sobre els organismes sèssils (McClintock 1994). En particular, la depredació sembla estar impulsada en gran mesura per l'estrella de mar comuna, oportunista i omnívora *Odontaster validus*, que té una àmplia gamma d'estratègies d'alimentació, des de suspensívora a la depredació d'una gran varietat d'invertebrats bentònics i algues (incloent briozous), sent, per tant, una "espècie clau" (Dayton *et al.* 1974; McClintock *et al.* 1994). Aquest asteroide, amb una distribució circumpolar descrita fins als 2902 m de profunditat, és abundant en tot l'OA (Fig. 5; Dearborn 1977; McClintock *et al.* 1988; vegeu també http://iobis.org/mapper/). Donades totes aquestes característiques, es va optar per aquesta estrella de mar com a depredador generalista model (Capítol 5), utilitzada anteriorment per dur a terme diversos assajos biològics de **repel·lència alimentària** utilitzant diferents metodologies (McClintock *et al.* 1992, 1993, 1994; Slattery & McClintock 1995; McClintock & Baker 1997; Avila *et al.* 2000; Mahon *et al.* 2003; Peters *et al.* 2009; Núñez-Pons *et al.* 2012a; Taboada *et al.* 2013; vegeu també http://iobis.org/mapper/). Experiments recents han demostrat la repel·lència de les fraccions lipofíliques en molts grups taxonòmics d'invertebrats antàrtics per evitar la depredació d'aquesta estrella de mar (Avila *et al.* 2008; McClintock *et al.* 2010; Figuerola *et al.* 2012; Taboada *et al.* 2013).

Els amfípodes també són un important grup bentònic antàrtic, jugant un paper important i complex en els fluxos de matèria orgànica en el fons marí de l'Antàrtida (p. ex. Broyer *et al.* 1999; Dauby *et al.* 2001a, b; Brandt *et al.* 2007a). També són un dels grups més diversos a l'OA amb un nombre estimat d'espècies de més de 600 (De Broyer *et al.* 2007, 2011). D'altra banda, s'associen sovint amb organismes bentònics (Broyer *et al.* 1999). Els gammàrids del Mar de Weddell mostren una dieta amb un ampli espectre d'invertebrats bentònics, com els cnidaris, briozous i esponges, el que suggereix una manca d'especialització per un sol tipus de presa (Dauby *et al.* 2001b). Aquests invertebrats bentònics, a més de representar una presa potencial pels amfípodes, també poden ser possibles biosubstrats, oferint una gran diversitat de microhàbitats, i, per tant, refugis potencials estructurals i químics enfront la depredació (Oshel & Steele 1985; De Broyer *et al.* 1999, 2001; Dauby *et al.* 2001a; Huang *et al.* 2008). Especialment, *Cheirimedon femoratus* és un gammàrid lyssianàsid circumantàrtic, omnívor i oportunista, alimentant-se des d'invertebrats fins a algues i detritus, amb una distribució euribàtica (de 0 a 1500 m), freqüentment associat a macroinvertebrats (Fig. 6) (Bregazzi 1972; De Broyer *et al.* 2007; Krapp *et al.* 2008) i utilitzat com a model en assajos previs d'alimentació (Núñez-Pons *et al.* 2012b).

Els briozous antàrtics com a objectiu de la nostra recerca: per què briozous?

Característiques generals

Els briozous (en grec "animals molsa"), anomenats comunament "estores del mar" o "animals molsa" per les seves diverses formes, s'anomenaven **Polyzoa** (en grec "molts animals"), en referència a les seves colònies formades per molts individus. Sorprenentment, són abundants invertebrats colonials aquàtics, filtradors, principalment marins, encara que també són comuns en aigües salobres i en aigua dolça. En particular, aquests invertebrats colonials són gairebé ubics, habitant des d'aigües poc profundes fins a les planes abissals. A més, són components importants dels registres fòssils, el que permet provar hipòtesis evolutives i els efectes del canvi climàtic. Les colònies de briozous són part de l'epifauna del substrat dur en la majoria de les zones rocoses, i en alguns hàbitats, cobreixen àrees considerables de roca o, en el cas de les zones poc profundes, fulles de "kelp" i algues, i són colonitzadors importants de substrats artificials, com vaixells. D'altra banda, les colònies de briozous desenvolupen un ampli espectre de formes, des de làmines incrustants a branques erectes ramificades (cilíndriques, laminars...), flexibles o rígides, creant en alguns casos colònies en tres dimensions, que proporcionen hàbitats per abundants organismes incrustants o mòbils. Les unitats individuals funcionals (mòduls) de les colònies s'anomenen **zooides**. El zooide fundador, anomenat **ancèstrula**, resultat de l'assentament i la conseqüent metamorfosi d'una larva sexual, origina la resta de la colònia. Les colònies de briozous es componen d'una sèrie d'unitats replicades, genèticament i en general morfològicament idèntiques (**autozooides**), separades entre si per parets de material esquelètic i de cutícula orgànica. Aquestes unitats idèntiques són originades a partir de l'ancèstrula, de forma asexual per gemmació. En particular, cada autozooide consisteix d'un **lofòfor** (un anell de tentacles, en el qual la boca es troba en el vèrtex), un intestí que s'obre a l'anus situat a la base del lofòfor, a l'exterior de l'anell de tentacles, uns òrgans reproductors i un gangli nerviós situat entre la boca i l'anus amb nervis que irradien a les altres parts del zooide. Per tant, els autozooids són unitats funcionalment independents. Amb freqüència, les colònies també inclouen polimorfs especialitzats anomenats **heterozooides**. El lofòfor, l'intestí en forma d'U i la musculatura formen el **polípid** (pòlip petit) i la resta del zooide, incloent l'esquelet, es diu **cístid**. L'eversió del lofòfor dins l'aigua per alimentar-se de petites partícules orgàniques vives o no vives es realitza per un simple sistema hidrostàtic. Una xarxa de teixits de filaments, el **funicle**, es desenvolupa al voltant de l'intestí, estenent-se a través de branques cap als porus comunicants de les parets del cos i, vinculant així els zooides adjacents. Aquesta xarxa transporta nutrients entre zooides i proporciona reserves d'aliment per a zooides sense funció alimentària (Fig. 7; Hayward 1995; Hayward & Ryland 1979; Hayward & Ryland 1998; vegeu també www.bryozoa.net).

Els briozous es classifiquen en tres classes: Stenolaemata, Gymnolaemata i Phylactolaemata. La classe Phylactolaemata està restringida a l'aigua dolça, amb colònies no calcificades i en general amb una corona de tentacles en forma de ferradura. Les classes Stenolaemata i Gymnolaemata comprenen principalment briozous marins amb una corona de tentacles circular i un esquelet calcificat, a excepció dels ctenòstoms. D'una banda, la classe Stenolaemata, que comprèn només l'ordre **Cyclostomatida**, presenta colònies amb zooides cilíndrics i un polimorfisme limitat. D'altra banda, la classe Gymnolaemata comprèn dos ordres, **Ctenostomatida** i **Cheilostomatida**. L'Ordre Ctenostomatida es caracteritza principalment per una paret no calcificada, membranosa o gelatinosa, heterezooides limitats a estolons, espines i **quenozooides** (el tipus més simple de polimorfisme que no té opercle ni lofòfor i que inclou diversos tipus d'espines, rizoides...) i absència d'aviculària. L'ordre Cheilostomatida, gairebé exclusivament marí, presenta caràcters com ara zooides en forma de caixa reforçada amb esquelets de carbonat de calci, i un **opercle** (tapa membranosa) per protegir l'orifici de la paret frontal, a través del qual l'animal estén el seu lofòfor (Hayward 1995; Hayward & Ryland 1998; vegeu també www.bryozoa.net). En briozous queilostòmats, la natura i la formació dels components de l'esquelet de la paret del cos és essencial per a la seva classificació i, per tant, es divideixen en dos subordres, **Anasca** i **Ascophora**. L'ordre Anasca es caracteritza per una paret frontal membranosa i l'ordre Ascophora per un escut frontal calcificat i un **asc** (sac subjacent que permet la funció del sistema hidrostàtic). A més, els briozous queilostòmats són un dels tàxons millor representats a la plataforma de l'Antàrtida (Barnes *et al.* 2009) i una alta proporció (56%) són endèmics (Hayward 1995; Barnes & De Grave 2000; Clarke & Johnston 2003; Griffiths *et al.* 2009; Griffiths 2010). La majoria dels briozous queilostòmats han desenvolupat un alt grau de polimorfisme en zooides especialitzades en diferents tasques per a l'alimentació (autozooides), per a la neteja i la protecció de colònies (aviculàries, vibràcules, espines...) i per a la reproducció (**ovicel·la**: càmera globular a l'extrem distal del zooide on els embrions són incubats) (Hayward 1995; Winston 2009). L'**aviculària**, comú entre els heterozooides, és un zooide modificat mancat de polípid amb un opercle hipertrofiat (**mandíbula**) que s'obre lentament i es tanca ràpidament per la contracció dels músculs adductors, descoratjant depredadors o larves (McKinney 1998). La **vibràcula** és un zooide modificat amb una mandíbula setiforme que es mou en diversos plans. La morfologia, la posició, i la freqüència d'ocurrència dels heterezooides, especialment de les aviculàries, és taxonòmicament important i s'utilitza per definir espècies (Hayward 1995).

Llacunes actuals en el coneixement

Es van identificar dos grans llacunes en el coneixement dels briozous antàrtics, després de revisar la literatura, relacionades amb (i) la biodiversitat i els seus patrons biogeogràfics i (ii) l'ecologia química.

El nombre d'estudis taxonòmics en briozous antàrtics ha experimentat un notable increment en els últims anys. Fins ara, s'han descrit prop de 400 briozous queilostòmats (Hayward 1995; Gutt *et al.* 2000; López-Fé de la Cuadra & García-Gómez 2000; Barnes & Griffiths 2008; Brandt *et al.* 2012). Tanmateix, el coneixement de les espècies de briozous de l'OA, la seva diversitat i les condicions ambientals on viuen, és encara molt pobre (Kuklinski & Barnes 2009). Així, amb l'objectiu d'ampliar el coneixement de les espècies de briozous de regions poc conegudes de l'OA, els nostres estudis (**Capítols 1, 2 i 3**) proporcionen noves dades sobre biodiversitat dels briozous antàrtics, els seus patrons espacials i els seus vincles biogeogràfics amb les regions veïnes. En aquest aspecte, s'espera trobar noves espècies i nous rangs batimètrics i geogràfics. D'altra banda, la nostra hipòtesi inicial era que es trobaria fauna compartida i, per tant, qüestionaria el grau d'aïllament de l'Antàrtida, com se suggereix en altres tàxons (Hastings 1943; Moyano 1982, 1999; Barnes & De Grave 2001; Arntz *et al.* 2005; Griffiths *et al.* 2009). En particular, el **Capítol 1** explora i descriu la biodiversitat de briozous procedents del talús continental de la regió de la PA, pobrament explorada fins ara. Actualment, aquest estudi és un dels primers en avaluar la connectivitat de les poblacions de briozous, principalment del **talús**, entre la regió de la PA i l'OA (López Gappa 2000). Addicionalment, els briozous han estat poc investigats en altres regions antàrtiques, com el Mar de Weddell (Zabala *et al.* 1997; Moyano 2005; Barnes & Kuklinski 2010), donant més valor a l'estudi presentat en el **Capítol 2**. En aquest Capítol, es descriu la biodiversitat i els patrons espacials dels briozous de l'OA. La taxonomia és necessària pel desenvolupament correcte dels estudis sobre biodiversitat. Per tant, la informació del **Capítol 3** proporciona detalls de dos aspectes notables: la **descripció d'una nova espècie de queilostomat** de *Reteporella* del Mar de Weddell (Antàrtida) caracteritzada per la presència d'una aviculària vicariant gegant i esfèrica, i la discussió de la **morfologia funcional** d'aquesta aviculària.

A part de la diversitat, si bé hi ha una quantitat considerable de coneixements sobre les defenses químiques d'alguns tàxons antàrtics, com les esponges, ascidis, corals i mol·luscs, sorprenentment, poc se sap sobre els briozous antàrtics (p. ex. Avila *et al.* 2008; Figuerola *et al.* 2012; Taboada *et al.* 2013). De fet, alguns estudis han trobat activitats antibiòtiques i hemolítiques en espècies de briozous antàrtics, però sense rellevància ecològica (Colon- Urban *et al.* 1985; Winston & Bernheimer 1986). No obstant això, els briozous són coneguts per produir productes naturals, com ara alcaloïds i **terpens**, amb diferents activitats, com antiincrustants i antidepredació (Al-Ogily & Knight Jones 1977; Lopanik *et al.* 2004; Gray *et al.*

2005; Lebar *et al.* 2007; Sharp *et al.* 2007). La recerca en aquest camp segueix sent molt limitada, sobretot tenint en compte que es calcula que hi ha actualment entre 8.700 i 11.100 espècies de briozous al mar (Appeltans *et al.* 2012). Els estudis químico-ecològics duts a terme en la nostra recerca (**Capítols 4 i 5**) contribueixen a omplir les grans llacunes de coneixement en l'**ecologia química** de briozous en general. En quant a la **naturalesa dels metabòlits secundaris**, s'han descrit compostos de diferents polaritats entre els productes defensius aïllats a partir de diversos tàxons (Pawlik *et al.* 1986, Kubanek *et al.* 2000; Pawlik 2012) però poques fraccions d'èter procedents de briozous antàrtics han estat provades prèviament en bioassajos de repel·lència alimentària contra *O. validus* i *C. femoratus* (Figuerola *et al.* 2012; Núñez *et al.* 2012b; Taboada *et al.* 2013) i cap fracció butanòlica de briozous ha estat provada anteriorment. Per tant, els nostres estudis donen un pas cap endavant, provant més extractes, com també fraccions hidrofíliques mai utilitzades prèviament.

A causa de l'activitat coneguda d'alguns productes naturals de briozous, inicialment esperàvem trobar nous compostos actius. La nostra hipòtesi inicial era que els briozous defensats químicament produeixen metabòlits que causen **repel·lència** i/o **toxicitat** contra els depredadors seleccionats. En el cas dels invertebrats clonals, com ara els briozous, la repel·lència dels seus compostos pot ser un avantatge enfront de la toxicitat, ja que un atac del depredador en aquestes preses clonals no és normalment letal, permetent al depredador aprendre a evitar la presa defensada químicament i en conseqüència, afavorint l'evolució d'aquestes defenses químiques. De fet, la producció de toxines està vinculada a un alt cost metabòlic de l'organisme, qui hauria de prevenir l'autotoxicitat, conduint-nos a pensar que la palatabilitat és la força impulsora més important en l'evolució de les defenses químiques. No obstant això, sota les circumstàncies d'una disponibilitat de preses limitada a espècies químicament defensades, la toxicitat podria ser avantatjosa (Pawlik 2012). Per tant, aquí avaluem aquestes dues estratègies en les mateixes espècies de briozous (**Capítols 4 i 5**). En particular, els tipus d'interaccions químiques indirectes a través dels compostos citotòxics han estat investigades en aigües temperades (p. ex. Birrell *et al.* 2008; Rasher *et al.* 2011), tot i que són poc conegudes en els ecosistemes antàrtics (Heine *et al.* 1991; McClintock *et al.* 1990, 1992, 1997, 2010), donant una alta rellevància al nostre estudi (**Capítol 4**). A més, el **Capítol 4** contribueix a la comprensió de la funció citotòxica de productes naturals de briozous contra un organisme simpàtric. En aquest estudi s'apliquen **nous protocols adaptats** per bioassajos d'ecologia química, utilitzant un gel sòlid com a substrat que simula la superfície de les colònies de briozous i, per tant, aproximant-se més a una perspectiva ecològicament rellevant.

D'altra banda, la majoria dels estudis de defenses químiques en invertebrats bentònics antàrtics s'han dut a terme en aigües poc profundes (accessibles a través de busseig amb escafandra) de McMurdo Sound i de la Península Antàrtica Occidental (Avila *et al.* 2008; McClintock *et al.* 2010). No obstant això, els últims estudis de repel·lència alimentària realitzats amb espècies bentòniques d'aigües profundes del Mar de Weddell demostraven una elevada activitat principalment en alguns tàxons (Núñez-Pons *et al.* 2012b; Taboada *et al.* 2013). Per tant, vam decidir enfocar els estudis químics en espècies de briozous d'**aigües profundes** del Mar de Weddell, ja que han rebut molt poca atenció fins al moment (**Capítols 4 i 5**).

També cal assenyalar que els briozous queilostòmats posseeixen zooides polimorfs amb **funcions defensives físiques o mecàniques** específiques contra depredadors particulars de petita mida, com ara els amfípodes (Winston 1991), representant sistemes model molt convenients per a l'estudi d'ambdues estratègies defensives, físiques i químiques. D'acord amb això, tot i que la depredació a nivell de zooide per depredadors més petits és generalment sub-letal en organismes colonials com els briozous, freqüentment es desenvolupen defenses químiques (Winston 2009). En els nostres estudis, s'espera trobar compostos repel·lents per evitar la depredació directa o ocasional, així com les interrupcions de la filtració, estimulant així les defenses químiques contra petits depredadors. Pel que fa a la depredació per part dels equinoderms, els briozous no poden sobreviure després de lesions importants en les seves colònies, principalment causades per aquests grans depredadors (Winston 2009). Per tant, la nostra hipòtesi de partida és el desenvolupament d'estratègies de defensa química contra macrodepredadors, com l'estrella de mar *Odontaster validus* (**Capítol 5**). De fet, les defenses físiques com espines i aviculàries no poden tenir la mateixa eficàcia en comparació amb els petits depredadors, fomentant l'evolució de les defenses químiques en aquests casos. Per aquesta raó, hem contrastat els diferents sistemes defensius potencials, químics i físics, en colònies seleccionades de briozous antàrtics (**Capítols 4 i 5**).

Objectius d'aquesta Tesi

Els dos principals objectius d'aquesta tesi són **millorar el coneixement de la biodiversitat i l'ecologia química de briozous antàrtics**.

Per aconseguir aquests dos objectius principals, vam decidir establir cinc objectius específics:

1. **Determinar la diversitat de briozous i els seus patrons espacials en una de les regions més pròximes a l'Antàrtida (regió de la Patagònia argentina) i els seus vincles biogeogràfics.** Es presenten noves dades sobre la riquesa específica de briozous de la regió de la Patagònia argentina i els seus patrons espacials i s'investiga si les nostres dades estan d'acord amb les regions biogeogràfiques proposades anteriorment per altres autors i grups zoològics, així com si existeix aïllament antàrtic en briozous queilostòmats (Capítol 1).

2. **Analitzar la diversitat de briozous l'Oceà Austral i descriure els seus patrons espacials.** S'identifiquen les espècies de briozous procedents de l'Oceà Austral i, mitjançant la combinació d'aquests amb les dades existents sobre la distribució de briozous en aquesta regió, es descriuen les distribucions batimètriques i geogràfiques (Capítol 2).

3. **Descriure, entre els nous tàxons trobats, una nova espècie de *Reteporella* del Mar de Weddell (Antàrtida).** Es descriu una nova espècie de *Reteporella* i es discuteix la morfologia funcional de l'aviculària. També es comparen les característiques de totes les espècies d'aquest gènere de l'Antàrtida i s'analitza la variabilitat intraespecífica dels seus principals caràcters (Capítol 3).

4. **Investigar si els briozous antàrtics posseeixen defenses químiques citotòxiques contra l'esperma/embrions d'eriçons i/o repel·lents d'amfípodes.** El present estudi descriu nous protocols per bioassaigs d'ecologia química utilitzant substrats de gel sòlid que simulen la superfície de les colònies de briozous per tal d'acostar-se a una perspectiva ecològica més fiable respecte a assaigs anteriors. Per entendre el paper dels productes naturals dels briozous, es realitzen assaigs de citotoxicitat i de preferència de substrat, utilitzant organismes simpàtrics model àmpliament distribuïts: l'eriçó de mar comú *Sterechinus neumayeri* i l'amfípode *Cheirimedon femoratus*. També s'explora en

aquests trets defensius la possible existència de variabilitat interespecífica/intraespecífica (Capítol 4).

5. **Avaluar la presència de productes naturals repel·lents contra depredadors antàrtics rellevants.** Per tal d'establir el paper dels productes naturals de briozous de la zona del Mar de Weddell, l'estrella de mar *Odontaster validus* i l'amfípode *Cheirimedon femoratus* s'han seleccionat per dur a terme assaigs de repel·lència alimentària. S'avalua la presència de productes naturals que puguin servir com a repel·lents contra aquests depredadors antàrtics, i es compara la variabilitat intra- i interespecífica en la bioactivitat, si s'escau. També es contrasten els diferents sistemes de defensa potencials, tant químics com físics, en colònies de briozous antàrtics (Capítol 5).

Estructura general de la Tesi

Aquesta tesi s'ha estructurat en tres parts principals, que inclouen tres i dos articles, respectivament, ja publicats o enviats per a la seva publicació, i la discussió general i conclusions. Una traducció en català i castellà de la Introducció General, de cada resum de les publicacions i de la Discussió General han estat incloses a la part final.

Part I "Biodiversitat de briozous d'aigües antàrtiques i sub-antàrtiques" cobreix estudis de taxonomia i diversitat, incloent les tres primeres publicacions (**Capítols 1, 2 i 3**).

Part II "Ecologia química de briozous antàrtics" presenta l'estudi de diversos mecanismes químics (toxicitat i repel·lència) d'algunes espècies de briozous contra organismes simpàtrics, incloent les altres dues publicacions (**Capítols 4 i 5**).

Part III "Discussió general i Conclusions"

Resultats

A continuació es resumeixen de forma clara i concisa, els principals resultats obtinguts en cada un dels articles científics que formen aquesta Tesi.

Capítol 1. Diversitat de briozous queilostòmats procedents de la regió sud-oest atlàntica: està realment aïllada l'Antàrtida?

Durant el Cenozoic, la fragmentació de Gondwana va estar acompanyada per una separació gradual dels seus components i l'establiment posterior del Corrent Circumpolar Antàrtic, portant a un aïllament termal relatiu i biogeogràfic de la fauna antàrtica. Tot i així, les afinitats zoogeogràfiques de diversos taxons procedents d'Amèrica del Sud i de l'Antàrtida han estat subjectes a debat, posant en dubte el grau d' aïllament antàrtic. Aquí es presenten noves dades de les espècies de briozous i la seva distribució espacial a la regió de la Patagònia argentina (PA), així com també una anàlisi de les semblances briozoològiques entre rangs de fondària d'Argentina i de les regions veïnes. Es van trobar un total de 108 espècies (378 mostres), pertanyents a 59 gèneres. A la regió PA, es van trobar cinc gèneres nous i 36 espècies noves, mentre 71 espècies van ser citades per primer cop a l'Argentina. Els rangs batimètrics de 94 espècies es van expandir i es va trobar que una elevada proporció de les espècies identificades (87 %) presentaven també una distribució antàrtica. Les afinitats briozoològiques trobades al present estudi entre les regions geogràfiques més properes estan a favor de la hipòtesi de la separació seqüencial de Gondwana durant el Cenozoic. A més a més, en aquest estudi es va trobar un elevat nombre d'espècies compartides, principalment del talús continental, entre la regió de la PA i l'Antàrtida, recolzant per tant, la idea que l'Oceà Austral ha estat menys aïllat en el temps geològic del què fins ara es pensava.

Capítol 2. Patrons espacials i diversitat de comunitats de briozous de l'Oceà Austral: Illes Shetland del Sud, Illa de Bouvet i Est del Mar de Weddell

En aquest estudi es presenten noves dades de biodiversitat i de distribució geogràfica i batimètrica de briozous recol·lectats durant la campanya ANT XXI/2 (Novembre 2003-Gener 2004) a l' Est del Mar de Weddell i a l' Illa de Bouvet, i durant l'expedició antàrtica espanyola ECOQUIM (Gener 2006) a les Illes Shetland del Sud. Les nostres dades han estat analitzades juntament amb estudis previs duts a terme a les mateixes regions. En el nostre estudi es van trobar un total de 54 espècies de briozous antàrtics (206 mostres), incloent una espècie nova del gènere *Reteporella*. Es van citar dues espècies per primer cop a l'Illa de Bouvet, una al Mar de Weddell i una a la muntanya submarina de Spiess. El 55% de totes les espècies eren endèmiques de l'Antàrtida. Al Mar de Weddell, les regions d'Austasen i Kapp Norvegia exhibien la riquesa específica relativa més alta, seguida de la regió de Vestkapp. Les anàlisis multivariants i de clúster revelaven, a petita escala, variabilitat espacial en l'estructura de les comunitats en profunditat i entre localitats.

Capítol 3. Descripció d'una nova espècie de *Reteporella* (Bryozoa: Phidoloporidae) del Mar de Weddell (Antàrtica) i la possible morfologia funcional de l'aviculària

Una espècie nova de briozou queilostòmat, *Reteporella rosjoarum* n. sp., pertanyent a la família de Phidoloporidae i procedent del Mar de Weddell (Antàrtida) ha estat descrita i il·lustrada mitjançant l'ús de la lupa binocular i amb imatges obtingudes amb el MER (Microscopi Electrònic de Rastreig). Es va utilitzar el MER per observar els caràcters essencials per descriure la nova espècie i per comparar-la amb espècies similars. Es van recol·lectar tres mostres de tres estacions diferents a 332-597 m de profunditat durant la campanya ANT XXI/2 a bord del Polarstern R/V (AWI, Bremerhaven, Alemània) utilitzant una xarxa d'arrossegament. La nova espècie es caracteritza per la presència d'aviculàries vicariants gegants i esfèriques. La morfologia distintiva d'aquestes aviculàries discrimina aquesta espècie dels seus congèneres antàrtics. La variabilitat morfomètrica de l'aviculària demostra que pot existir una variació intraespecífica entre localitats (Austasen i Drescher Inlet). També es discuteix la morfologia de l'aviculària en relació a les seves possibles funcions en l'ordre Cheilostomata.

Capítol 4. Interaccions químico-ecològiques en briozous antàrtics

El rol dels metabòlits bioactius dels briozous antàrtics en les interaccions ecològiques ha estat poc estudiat. Els briozous són uns dels més abundants i diversos membres del bentos antàrtic i són la presa de diversos tipus de depredadors des de nivells zooidals a colonials. Semblen ser aliment casual de l'eriçó de mar antàrtic comú *Sterechinus neumayeri* i de l'amfípode omnívor *Cheirimedon femoratus*. En aquest estudi, l'activitat citotòxica contra embrions i esperma de l'eriçó de mar *S. neumayeri* i les preferències de substrat de l'amfípode *C. femoratus* han sigut avaluades utilitzant extractes orgànics de briozous antàrtics per tal de determinar la presència de defenses químiques. Es van dissenyar nous protocols adaptats, utilitzant un gel solidificant, freqüentment empleat en estudis antirecobriment, per simular químicament la superfície dels briozous. Es van analitzar 32 extractes orgànics procedents de 16 mostres que pertanyien a 13 espècies diferents. No es va detectar citotoxicitat contra els embrions de l'eriçó de mar *S. neumayeri*, mentre que 12 de les 13 espècies de briozous provades eren citotòxiques contra l'esperma a concentracions naturals. En els bioassajos de preferència de substrat, l'amfípode *C. femoratus* era repel·lit per 10 espècies. La bioactivitat variable trobada en els dos tipus de particions dels extractes (etèrica i butanòlica) indicava la presència de defenses lipofíliques i hidrofíliques. També es va detectar variabilitat intra- i interespecífica de defenses químiques, suggerint respostes ambientals induïdes, la presència de simbionts i/o variabilitat genètica. Es discuteixen també mecanismes de defensa alternativa en espècies amb poca o sense defensa química. Els nostres resultats recolzen clarament el fet que la bioactivitat és comuna en briozous antàrtics i que hi ha un paper ecològic dels compostos citotòxics i repel·lents en la seva protecció.

Capítol 5. Estratègies defensives contra la depredació en briozous antàrtics

L'estrella de mar antàrtica *Odontaster validus* i l'amfípode *Cheirimedon femoratus* són depredadors rellevants en les comunitats bentòniques antàrtiques. Alguns briozous són part de la dieta d'aquest asteroid i, com altres macroinvertebrats sèssils, representen un biosubstrat potencial i una presa per l'amfípode omnívor *C. femoratus*. En resposta a la pressió ecològica, els briozous desenvolupen estratègies per dissuadir als predadors potencials, que abasten des de mecanismes físics (espines, esquelet) a mecanismes químics (productes naturals). Tot i així, l'ecologia química dels briozous antàrtics ha estat poc estudiada fins ara. En aquest estudi s'avaluava la presència de defenses contra la depredació en espècies seleccionades de briozous antàrtics. Les espècies simpàtriques i omnívores, *O. validus* i *C. femoratus*, van ser seleccionades per dur a terme assaigs d' alimentació amb 16 extractes etèrics i 16 butanòlics obtinguts de 16 mostres que pertanyien a 13 espècies de briozous diferents. La majoria d'espècies (9) eren actives (12 extractes etèrics i 1 butanòlic) en els bioassaigs amb l' estrella de mar, malgrat que només un extracte butanòlic mostrava repel·lència, indicant que els compostos repel·lents són principalment lipofílics. La repel·lència contra *C. femoratus* es trobava en totes les espècies en diferents extractes (10 etèrics i 12 butanòlics), suggerint que les defenses podrien ser de natura lipofílica i/o hidrofílica. Es va detectar ocasionalment variabilitat inter- i intraespecífica en la bioactivitat, suggerint respostes inductives ambientals, associacions simbiòtiques i/o variabilitat genètica. Els anàlisis multivariants revelaven similituds entre espècies en relació a la bioactivitat dels extractes etèrics i /o butanòlics. Aquests resultats recolzen la hipòtesi que, mentre altres mecanismes químics o físics alternatius poden també proporcionar protecció en alguns casos, els compostos repel·lents juguen un paper molt important en briozous antàrtics com a defenses contra depredadors simpàtrics.

Discussió General

Aquesta tesi cobreix dos aspectes importants i poc coneguts dels briozous antàrtics: la **biodiversitat** i l'**ecologia química**. El nostre treball destaca la importància de reunir més informació sobre la biogeografia, diversitat i taxonomia dels briozous antàrtics, poc coneguts fins ara, amb l'objectiu d'entendre millor les variacions espacials i batimètriques en l'estructura de la comunitat i establir la millor manera de conservar-los (Capítols 1, 2 i 3). A més, els nostres estudis sobre la biodiversitat dels briozous donen una idea dels possibles factors físics i biòtics, que influeixen en l'estructura de les comunitats dels briozous antàrtics (Capítols 1 i 2). D'altra banda, les nostres dades biogeogràfics qüestionen el **grau** d'**aïllament** de l'**Antàrtida**, el que demostra l'elevat valor de la nostra investigació sobre la fauna d'aigües profundes (Capítol 1). Entre les noves espècies trobades, també es descriu una **nova espècie** de **briozou** (Capítol 3) i es fa palès que cal fer més estudis per les espècies que queden encara per descriure. Com les comunitats estudiades de briozous, per sota de les zones afectades per pertorbacions locals (erosió per icebergs), estan subjectes principalment a factors biòtics com la competència i la depredació, l'evolució ha afavorit el desenvolupament de **mecanismes químics** de protecció (Capítols 4 i 5). Per tal d'estudiar les interaccions químiques, es van dissenyar **nous protocols adaptats** utilitzant depredadors simpàtrics i abundants. Aquí, les troballes més importants es discuteixen en dues seccions (biodiversitat i ecologia química), proporcionant una visió general dels resultats i revisant com els resultats de la nostra recerca ajuden a omplir els buits existents en el coneixement actual. Finalment, també es consideren les limitacions de la recerca i es proposen recomanacions per a una futura investigació.

Biodiversitat de briozous antàrtics (Capítols 1, 2 i 3)

Biogeografia

L'**anàlisi comparatiu** de diversitat realitzat aquí (Capítol 1 i 2) entre l'Antàrtida i els últims fragments que es van separar de Gondwana, especialment la regió de la Patagònia, i les illes que serveixen de connexió per a la fauna de poca profunditat, ajuda a determinar l'**evolució** de les comunitats regionals i les seves relacions amb la fauna de fora del FP, com altres autors suggereixen (Clarke *et al.* 2005; Clarke 2008). Els nostres estudis permeten establir els **patrons biogeogràfics** de briozous queilostòmats. Des d'aquesta perspectiva, els nostres resultats recolzen la **hipòtesi de la separació seqüencial de Gondwana** durant el Cenozoic, amb les regions biogeogràfiques coincidents proposades anteriorment per altres autors (p. ex. Moyano 1982; Barnes & De Grave 2001; Arntz *et al.* 2005; Barnes & Griffiths 2008). En aquest sentit, en el nostre estudi (Capítol 1), les regions antàrtiques i de la PA estan agrupades, el que suggereix afinitats faunístiques més properes. Per tant, aquestes composicions similars podrien estar relacionades amb el fet que aquests eren els últims fragments que es van distanciar durant la fragmentació de Gondwana (Clarke 2003; Clarke *et al.* 2005; Lawver & Gahagan 2003; Upchurch 2008). En contrast, la regió d'Australàsia (Nova Zelanda i Austràlia) tendeix a agrupar-se separadament, recolzant la idea que aquesta regió es va distanciar molt abans de separar-se Amèrica del Sud i l'Antàrtida. En el cas de l'OA, els nostres resultats (Capítol 2) estan d'acord amb les diferents zones de diversitat zoogeogràfiques suggerides per altres autors (Barnes & De Greu 2000; Barnes & Kuklinski 2010): les illes subantàrtiques (Illa Bouvet), est de l'Antàrtida (est del Mar de Weddell), oest de l'Antàrtida i l'Arc d'Escòcia (Illes Decepció i Livingston). En particular, l'Illa de Bouvet mostra similitud amb la regió del Mar de Weddell, en el nostre estudi i altres (Barnes 2006; Gutt *et al.* 2006). L'Illa de Bouvet es troba en una posició particular respecte a la CCA i pot estar en una zona d'intercanvi potencial de fauna entre les diverses regions i a través del FP (Linse 2006). D'altra banda, es discuteix en el Capítol 1 que l'elevat nombre d'espècies de la PA compartides amb l'Antàrtida, vinculat a la menor proporció d'espècies endèmiques de briozous queilostòmats trobada, donen suport a la idea que l'OA, a través del temps geològic, pot haver estat menys aïllat del què fins ara es pensava (Barnes & Griffiths 2008). Per tant, els nostres resultats qüestionen el **grau real** d'**aïllament** de l'**Antàrtida** en briozous queilostòmats. Es suggereix que el FP sembla no ser realment una barrera per a aquest grup, cosa que altres autors també han debatut (p. ex. Thatje & Fuentes 2003; Clarke *et al.* 2005; Thatje *et al.* 2005a; Brandt *et al.* 2007a). Un altre aspecte important a considerar és el paper de l'**Arc d'Escòcia** com un possible vincle físic entre l'Antàrtida i Amèrica del Sud en briozous (Arntz *et al.* 2005; Barnes 2005). També se sap que els mecanismes de dispersió juguen un paper essencial en els patrons de distribució. En el cas dels briozous, el "ràfting" és un element clau en la biogeografia dels queilostòmats a causa de la seva capacitat de ràpida dispersió des d'una escala de quilòmetres a una escala global (Watts *et al.* 1998; Key *et al.* 2013). També es suggereix que la presència d'algunes espècies de briozous comunes entre aquestes regions pot ser explicada en part pel **transport potencial passiu** de larves (o potser fins i tot d'adults) a distàncies considerables a través de la branca de l'CCA que flueix cap al nord al llarg de la plataforma continental d'Argentina, el corrent de les Falkland/Malvines (Brandt *et al.* 2007a). Altres vies de dispersió dels organismes, com els **remolins** del CCA, poden augmentar la connexió entre aquestes regions, transportant colònies de briozous fixats a trossos de fusta i altres deixalles marines (Clarke *et al.* 2005). D'altra banda, es discuteix que l'augment dels **mecanismes de dispersió humans** (p. ex. l'aigua de llast i les deixalles marines d'origen antropogènic com el plàstic flotant) cap a dins i fora de l'Antàrtida podrien afavorir l'intercanvi de fauna i la introducció d'espècies exòtiques, superant les barreres oceanogràfiques, com suggereixen altres autors (Barnes 2002; Thatje *et al.* 2005a). Un tema important discutit també aquí és la **lliure migració** d'organismes marins a dins i fora del FP a través de les planes abissals, on l'OA està connectat als altres oceans, sent rellevant en el nostre cas, on la majoria de les espècies de la regió de la PA provenen del talús continental (p. ex. Thatje *et al.* 2005b; Brandt *et al.* 2007a; Clarke 2008).

Diversitat i patrons espacials

Tenint en compte que els hàbitats de l'Antàrtida i la Patagònia es troben amenaçats per la sobreexplotació dels recursos vius, l'establiment d'espècies marines invasores com els briozous, el canvi climàtic i el creixent impacte del turisme, la seva conservació és essencial. La nostra investigació sobre la taxonomia, la diversitat i els patrons espacials de les comunitats de briozous (Capítols 1, 2 i 3) és una **base** necessària per a la gestió de la biodiversitat regional i per posar en pràctica **estratègies de conservació** en aquestes regions poc conegudes (López Gappa 2000; Brandt *et al.* 2007a; Barnes & Peck 2008; Tejedo *et al.* 2009; Barnes & Kuklinski 2010). No obstant això, la implementació d'accions de conservació ha de prendre en consideració diversos factors limitants, com són la falta de mostres d'aquestes regions i les limitacions financeres i polítiques, entre d'altres. Per tant, es recomanen futurs estudis taxonòmics i a llarg termini perquè es puguin detectar els futurs canvis causats per pertorbacions antropogèniques.

Els nostres estudis (Capítols 1 i 2) són també una de les primeres caracteritzacions de les comunitats de briozous, principalment del **talús**, de la **regió de la PA** (Hastings 1943; López Gappa & Lichtschein 1990; López Gappa 2000), i de l'OA, especialment el **Mar de Weddell** (Zabala *et al.* 1997; Moyano 2005, Barnes & Kuklinski 2010). En particular, poques espècies de briozous de la regió de la PA i de l'OA s'han citat prèviament per sota de la plataforma continental, i la majoria de les mostres estudiades fins al moment de la regió de la PA provenen de menys de 500 m (López Gappa 2000; Barnes & Kuklinski 2010). En el cas de l'OA, els briozous són poc coneguts, principalment a causa de la inaccessibilitat relativa d'aquesta regió (Kuklinski & Barnes 2009).

A més, els estudis d'aquesta Tesi proporcionen una visió global de la biodiversitat dels briozous queilostòmats de la regió de la PA i de l'OA, demostrant que és rica i abundant. En particular, els valors més alts de riquesa d'específica trobats al voltant de la latitud 44º S (S = 57) i en el talús mig (S = 77.78) semblen ser explicats per la confluència dels **corrents subantàrtic Falkland/Malvines**, ric en nutrients, i el **subtropical de Brasil**, on la surgència d'aigües riques en nutrients i els "blooms" de plàncton associats, juguen un paper important en el manteniment d'aquesta gran diversitat (Acha *et al.* 2004; Miloslavich *et al.* 2011; Muñoz *et al.* 2012). En el cas de l'OA (Illes Shetland del Sud, Illa de Bouvet i est del Mar de Weddell), la major riquesa específica trobada a la latitud 70-73º S (%S = 79.17; est del Mar de Weddell) respecte a les illes podria ser explicada principalment per les **grans distàncies** entre l'est del Mar de Weddell i aquestes illes, així com pel seu **aïllament**. D'altra banda, el valor més alt de riquesa específica trobat a la plataforma continental (%S = 100) podria ser degut a la disminució de la **disponibilitat dels aliments en augmentar la profunditat** (p. ex. Arntz *et al.* 1994; Linse *et al.* 2007). No obstant això, cal un mostreig més intensiu i equitatiu en aquestes regions i en zones profundes per confirmar-ho.

Les noves dades analitzades aquí també milloren la nostra comprensió dels **patrons espacials** de les comunitats de briozous de les regions de la PA i de l'OA. Curiosament, la nostra recerca també mostra una **expansió en la distribució coneguda** d'una proporció molt elevada (87%) de les espècies de briozous de la regió de la PA. D'altra banda, una sinergia de factors físics (profunditat, substrat, abrasió per icebergs...) i biòtics (per exemple, la disponibilitat d'aliment) juguen un paper clau en l'estructuració de les comunitats antàrtiques, que es caracteritzen, per tant, per una **variabilitat batimètrica** i **horitzontal**, com es veu en aquest i altres estudis (Gutt & Piepenburg 2003; Thatje *et al.* 2005; Cummings *et al.* 2006; Brandt *et al.* 2007b; Smale 2008b; Griffiths 2010). Tot i l'existència d'una variabilitat batimètrica en les comunitats de briozous, moltes espècies de briozous analitzades aquí presenten un alt grau d'**euribàtia**, sent explicat probablement per la història evolutiva de la fauna de l'OA durant els períodes glacials del Cenozoic, com s'ha esmentat anteriorment (Thatje *et al.* 2005).

Taxonomia

A més d'una millor comprensió biogeogràfica i dels patrons de diversitat, millorar el nostre coneixement sobre la **taxonomia** és essencial per implementar estratègies de gestió, com abans s'ha comentat. Els nostres resultats remarquen la importància dels **estudis taxonòmics** en aquestes regions escassament explorades, incloent un gran nombre de **nous gèneres** i **espècies,** i **noves cites**. En el cas dels briozous, la morfologia, la posició, i la freqüència d'ocurrència de les aviculàries són taxonòmicament importants i s'utilitzen per definir espècies (p. ex. Hayward 1995; Kuklinski & Barnes 2009). La freqüent variabilitat trobada en el nostre estudi en la morfometria de l'aviculària, demostra l'existència de **variació intraespecífica** entre localitats (Kuklinski & Barnes 2009; Carter *et al.* 2010b). D'altra banda, l'estudi de les característiques de l'aviculària ajuda a entendre les seves possibles funcions, com es discuteix a continuació. En general, la forma de les aviculàries qüestiona la seva **morfologia funcional**, que segueix sent en gran part desconeguda. Es suggereixen diverses funcions de les aviculàries com ara la **protecció física**, la **neteja**, i la **defensa** contra els predadors o herbívors, com s'ha comentat en la Introducció. Aquestes funcions potencials podrien ser útils en els briozous, ja que són animals filtradors, i la majoria sèssils (Busk 1854; Harmer 1909; Canu & Bassler 1929; Hyman 1959;

Cook 1963; Winston 1984, 1991). Entre les noves espècies trobades en el nostre estudi, es descriu un briozou del gènere *Reteporella* caracteritzat per una **rara aviculària esfèrica i gegant**, portant-nos a reconsiderar quines són les possibles funcions de l'aviculària. Els ambients estables com les regions antàrtiques, per sota les àrees afectades per les pertorbacions locals, tendeixen a afavorir l'evolució de sistemes complexos de polimorfisme en queilostòmats (Venit 2007). Per tant, la forma estranya de l'aviculària d'aquesta nova espècie pot indicar un nivell d'adaptació en aquesta regió antàrtica. La millor hipòtesi raonable per a la presència d'aviculàries amb una gran capacitat interna podria ser la seva funció addicional com a emmagatzematge de nutrients i la distribució d'aquests recursos en condicions desfavorables, com suggereix Winston (1984). Sorprenentment, també es comenta, que la mida gegant de la aviculària podria ser més avantatjosa que les aviculàries petites, al poder capturar parts relativament grans de microinvertebrats, com s'ha demostrat en colònies vives, on les aviculàries sèssils capturen depredadors de briozous, com nudibranquis, poliquets, amfípodes i picnogònids (Winston 1991; Carter *et al.* 2010a). Per tant, aquesta forma de l'aviculària podria ser una especialització per capturar determinats depredadors antàrtics (Silén 1977). A més, l'aviculària gegant podria participar activament en evitar l'assentament d'epibionts (Harmer 1909). Tots aquests motius, per tant, demostren que la morfologia funcional de l'aviculària és **complexa** i encara molt desconeguda. Cal fer més estudis, així com obtenir més mostres del Mar de Weddell, per establir la forma general de la colònia, l'àrea de distribució, i la variabilitat intraespecífica d'aquesta nova espècie.

Ecologia química en briozous antàrtics (Capítols 4 i 5)

A més de la influència dels factors físics, les comunitats de briozous antàrtics, sota les àrees afectades per pertorbacions locals, també estan sotmeses a factors biòtics. La nostra hipòtesi inicial era que durant l'evolució, l'elevada pressió generada pels factors biòtics ha conduit al desenvolupament de **mecanismes químics** de defensa i competència, com s'ha comentat a la Introducció. Tenint en compte que els briozous antàrtics han estat poc estudiats en el camp de l'ecologia química, els nostres estudis són dels primers en aquest camp en briozous antàrtics (fins on es coneix, només existeixen tres publicacions i un capítol de llibre: Winston & Bernheimer 1986; Lebar *et al.* 2007; Figuerola *et al.* 2012; Taboada *et al.* 2013). D'altra banda, alguns briozous d'aigua freda presenten productes bioactius d'interès farmacològic (Lebar *et al.* 2007; Sharp *et al.* 2007). Per tant, la nostra investigació també representa una **primera base** per a la recerca de nous compostos potencialment bioactius de briozous antàrtics.

Els nostres resultats demostren la importància de diversos **mecanismes d'ecologia química contra la competència i la depredació** en briozous antàrtics.

Diversos tàxons antàrtics presenten diferents mecanismes químics (Avila *et al.* 2008; McClintock *et al.* 2010). Tenint en compte que la major part dels metabòlits secundaris repel·lents i efectius citats en invertebrats són liposolubles, els estudis anteriors es van centrar principalment en les interaccions mediades per extractes lipofílics (Sotka *et al.* 2009). No obstant això, s'han descrit anteriorment compostos de diferents polaritats entre els productes defensius (Pawlik *et al.* 1986; Kubanek *et al.* 2000; Pawlik 2012) i els nostres resultats confirmen aquest fet. Sorprenentment, la nostra investigació mostra la presència de **compostos bioactius** tant **lipofílics** com **hidrofílics** en diferents espècies de briozous antàrtics.

A més, trobem **variabilitat intra- i interespecífica** en l'activitat dels extractes d'algunes espècies de briozous, fet que suggereix respostes ambientals induïdes, la presència de diverses associacions simbiòtiques microbianes, i/o variabilitat genètica, com passa en altres tàxons marins (Puglisi *et al.* 2000; Page *et al.* 2005; Cutignano *et al.* 2011). No obstant això, es necessiten més estudis en aquestes espècies per tal que es pugui determinar l'origen d'aquestes diferències.

Estratègies físiques defensives

Els possibles mecanismes defensius físics o morfològics com ara les aviculàries, espines i vibràcules, en espècies amb poques o sense defenses químiques també són discutits en el nostre estudi. Els nostres resultats mostren una tendència general a posseir una combinació de mecanismes físics i químics, i això suggereix l'existència de **estratègies complementàries**. Malgrat això, l'absència de defenses químiques en algunes espècies podria estar relacionada amb la presència d'aquests mecanismes de defensa físics. Hi ha algunes evidències de **recursos potencials "trade-offs"** entre les defenses físiques i químiques. Un equilibri entre les estratègies condueix a una optimització de l'energia que pot ser destinada a funcions biològiques primàries, com el creixement o la reproducció, com altres autors suggereixen en diversos invertebrats marins (Stachowicz & Linsquist 2000; Hines & Pawlik 2012). Per tant, la presència de defenses físiques, juntament amb el seu potencial de regeneració, i la presència de metabòlits de dissuasió pot augmentar significativament la protecció contra la depredació respecte a la que ofereix cada estratègia defensiva per separat.

Citotoxicitat i repel·lència a substrats

Per realitzar els bioassajos citotòxics i repel·lents, es van seleccionar l'eriçó de mar *Sterechinus neumayeri* i l'amfípode *Cheirimedon femoratus*, ambdós comuns i abundants. D'una banda, *S. neumayeri*, brostejant, danya greument les colònies de briozous (McClintock 1994; Bowden 2005; Bowden *et al.* 2006) de les que s'alimenta freqüentment (Jacob *et al.* 2003). A més, els eriçons de mar són espècies amb fecundació externa i, per tant, els gàmetes masculins (espermatozoides) i els òvuls, quan s'alliberen, poden posar-se en contacte freqüentment amb superfícies properes. D'altra banda, *C. femoratus* és bentònic i es troba freqüentment associat amb substrats vius (Oshel & Steele 1985; De Broyer *et al.* 1999, 2001; Dauby *et al.* 2001a; Huang *et al.* 2008). Els **nous protocols adaptats** utilitzats per dur a terme aquests bioassaigs (Capítol 4) semblen ser un mètode molt adient per discriminar els extractes actius. El nostre estudi és el primer a utilitzar un gel, utilitzat anteriorment en altres estudis d'ecologia química (Henrikson & Pawlik 1995; Clavico *et al.* 2006; Zamzow *et al.* 2010), com a substrat per avaluar l'elecció de l'hàbitat exclusivament relacionada amb la composició química del gel (sense influència de formes) en briozous. Com s'ha esmentat en el Capítol 4, aquests mètodes ofereixen una sèrie d'avantatges com ara 1) el gel com a substrat artificial, simulant químicament superfícies d'invertebrats vius, i així conferint condicions ecològiques més realistes respecte als estudis anteriors, 2) l'ús d'organismes antàrtics model ubics, comuns i fàcils de capturar, 3) l'ús de petites quantitats d'extracte a concentracions naturals i 4) duracions curtes dels assajos de citotoxicitat d'esperma i de preferència de substrat, molt útil durant les campanyes antàrtiques, normalment curtes. Per tant, aquest mètode d'ecologia química recrea circumstàncies més naturals per provar les activitats citotòxiques i repel·lents contra els dos organismes (*S. neumayeri* i *C. femoratus*).

En el cas del mètode utilitzat en *S. neumayeri*, aquest nou disseny de protocol adaptat a l'eriçó antàrtic és una modificació d'un mètode estàndard utilitzat comunament i proposat per Volpi Ghirardini *et al.* (2005) per a l'eriçó de mar Mediterrani, *Paracentrotus lividus*. El nostre mètode introdueix algunes modificacions per tal d'adaptar els assajos a una espècie antàrtica, l'eriçó de mar *S. neumayeri*, amb cicles molt més llargs de desenvolupament. Aquesta espècie triga uns 2-3 dies per arribar a la fase de blàstula (Bosch *et al.* 1987), en contrast amb *P. lividus*, en què la blàstula es desenvolupa en aproximadament 6-12 h (Russo *et al.* 2003). El bioassaig ideal seria provar la citotoxicitat contra els diferents estadis de desenvolupament inicials fins a l'estadi de plúteus, que es desenvolupa en aproximadament 21 dies. Tanmateix, això és difícil de dur a terme durant les campanyes antàrtiques, normalment curtes. Per tant, es recomanen campanyes més llargues. D'altra banda, en el futur, aquests resultats haurien de ser idealment validats amb experiments *in situ* amb briozous vius, fet que podria confirmar la rellevància ecològica dels compostos citotòxics de diversos invertebrats contra embrions i esperma.

La majoria d'espècies de briozous provades mostren **citotoxicitat** (cèl·lules mortes) i/o **activitat repel·lent** (rebuig) en contra de l'eriçó de mar *Sterechinus neumayeri* i l'amfípode *Cheirimedon femoratus*, respectivament. En la mesura que sabem, pocs experiments d'activitat citotòxica contra els espermatozoides i els estadis inicials del cicle de vida de l'eriçó comú antàrtics *S. neumayeri* s'han realitzat fins a la data, utilitzant extractes d'organismes simpàtrics (p. ex. McClintock *et al.* 1990; Figuerola *et al.* 2012), afegint valor al nostre estudi. No s'ha detectat citotoxicitat contra embrions en el nostre estudi, tot i que hi ha evidències en la literatura de la presència de compostos citotòxics en laboratori, com és el cas del briozou circumpolar *Flustra foliacea*, amb alcaloides bioactius que mostren citotoxicitat contra la línia cel·lular HCT-116 de càncer de còlon humà (Lysek *et al.* 2002), i l'espècie *Carbasea curva* que mostra activitat hemolítica moderada (Winston & Bernheimer 1986). Contràriament a l'absència de citotoxicitat contra embrions a les nostres mostres, algunes espècies de briozous revelen activitats tòxiques contra l'esperma d'eriçó de mar. En aquest sentit, les activitats de brosteig d'equinoids, particularment de *S. neumayeri*, es consideren responsables d'una mortalitat significativa de larves i juvenils assentats d'invertebrats bentònics (Bowden 2005; Bowden *et al.* 2006) i es creu que tendeixen a consumir briozous incidentalment mentre persegueixen a altres invertebrats (Lidgard 2008; Winston 2009). Per tant, la presència de compostos citotòxics contra l'esperma trobats en aquest estudi, podria tenir un paper indirecte important a petita escala, representant un mecanisme de protecció/competència, reduint el reclutament d'aquest eriçó de mar i, en conseqüència, la pressió de brosteig i la colonització de superfícies de briozous (p. ex. McClintock *et al.* 1990; Pawlik 1992; Bowden *et al.* 2006).

En contrast, els principals depredadors de briozous a nivell de zooide són amfípodes, copèpodes, nudibranquis i picnogònids, que poden alimentar-se directament d'ells o de la seva microbiota, concentrada amb freqüència pels corrents d'alimentació (Amsler *et al.* 2009; De Broyer *et al.* 2001). Els nostres resultats també suggereixen que la majoria de les espècies de briozous analitzats aquí posseeixen substàncies químiques que provoquen rebuig a l'amfípode comú *Cheirimedon femoratus*, sent presumiblement útils per prevenir l'assentament d'aquesta espècie d'amfípode omnívor (Bregazzi 1972), i per tant, evitar la depredació. D'altra banda, les poblacions denses d'aquests amfípodes podrien causar efectes secundaris perjudicials pels briozous, com la interrupció del flux i, per tant, la reducció de les taxes de filtració (Fig. 8). D'acord amb la nostra hipòtesi, *C. femoratus* amb una limitada capacitat natatòria, en general s'associa amb substrats vius, utilitzant-los tant com a hàbitat i com a preses potencials.

Repel·lència alimentària

Encara que les mostres de briozous del nostre estudi procedeixen d'aigües poc profundes i també de profundes, les espècies provades en aquests estudis químico-ecològics són comunes i euribàtiques, sent per tant, més fàcil seleccionar un adequat depredador simpàtric coexistent. En considerar els experiments, s'ha de tenir en compte, quan es selecciona un depredador antàrtic, que algunes espècies mostren una marcada variació estacional en la taxa metabòlica i en l'activitat d'alimentació, com *Sterechinus neumayeri* (Brockington & Clarke 2001). Els **depredadors generalistes** són ideals per assajos de repel·lència alimentària, en ingerir una àmplia gamma d'espècies, i, en conseqüència, induint l'adquisició de les defenses en les seves preses. Per tant, pels experiments es van seleccionar, depredadors generalistes simpàtriques, segons el que va suggerir Pawlik (2012). En particular, donada la seva abundància, omnivorisme, distribució ubíqua i euribàtica, i les seves possibles interaccions ecològiques amb les espècies de briozous analitzats, l'estrella de mar macrodepredadora *Odontaster validus* i l'amfípode microdepredador *Cheirimedon femoratus* van ser seleccionats com a organismes model en els bioassaigs (Capítol 5). No obstant això, només hem utilitzat aquests dos depredadors en la nostra investigació, i per tant, es recomana en futurs experiments provar una gamma més àmplia de depredadors. L'elevada incidència de repel·lència en invertebrats antàrtics contra aquests depredadors descrita en altres estudis demostra que aquests són depredadors apropiats per als nostres estudis (Núñez-Pons *et al.* 2012b; Taboada *et al.* 2013). D'altra banda, aquests **estudis comparatius** provant **macrodepredadors** i **microdepredadors** (entre 0.2 i 20 mm; McClintock & Baker 2001) ajuden a determinar si existeixen diferències en la inducció de defenses químiques en briozous. Especialment, els macrodepredadors com *O. validus* causen lesions importants en les colònies de briozous, després de les quals els briozous no poden sobreviure, malgrat ser organismes clonals amb un ampli potencial regeneratiu (Winston 2009). També, els microdepredadors com ara *C. femoratus* es troben en altes densitats, podent ser una amenaça potencial per les seves preses, encara que la depredació per depredadors petits a nivell de zooide és generalment subletal en els organismes colonials (Winston 2009).

Com s'ha dit anteriorment, els organismes sèssils antàrtics es veuen sotmesos a una pressió de depredació intensa, fet que ha portat al desenvolupament de defenses químiques en un elevat percentatge d'espècies (Lebar *et al.* 2007; Avila *et al.* 2008; McClintock *et al.* 2010). Els nostres resultats demostren que els briozous semblen estar defensats contra almenys un dels dos depredadors més abundants, *O. validus* i/o *C. femoratus*. Com era d'esperar, la majoria de les espècies de briozous estudiades presenten defenses químiques contra l'estrella de mar o l'amfípode, o ambdós. A més, els nostres resultats estan d'acord amb estudis recents en què es demostrava l'existència de compostos repel·lents contra *O. validus* en 10 de 17 extractes etèrics de briozous antàrtics (Figuerola *et al.* 2012; Taboada *et al.* 2013). En el cas de *C. femoratus*, aquest amfípode és dissuadit per totes les espècies de briozous provats en el nostre estudi. Com s'ha discutit en l'assaig de preferència de substrat (Capítol 4), aquesta activitat de repel·lència alimentària podria ser útil per prevenir l'assentament d'aquest amfípode, que s'alimenta directament o ocasionalment de les colònies de briozous, i que pot pertorbar els corrents usats pels briozous per alimentar-se (De Broyer *et al.* 2001). Les respostes repel·lents són especialment freqüents en els assajos amb *C. femoratus* en comparació amb aquells realitzats amb l'estrella de mar, fet que suggereix que els briozous poden requerir més protecció contra els amfípodes, els quals exerceixen una pressió més localitzada. En general, aquests resultats porten a la conclusió que aquest fílum és molt actiu químicament, amb àmplies activitats repel·lents. Per tant, basant-nos en el model general i complex, prèviament proposat pel nostre equip, descrivint una àmplia gamma de possibles relacions d'ecologia química (Figuerola *et al.* 2012), afegim ara noves peces en aquest complex trencaclosques encara per resoldre.

Malgrat que algunes espècies de briozous han demostrat ser fonts potencials de substàncies farmacològicament prometedores, incloent alcaloïdes citotòxics (Prinsep *et al.* 2004; Sharp *et al.* 2007), els nostres estudis químico-ecològics són un **primer pas** per trobar **compostos bioactius** que participen en les interaccions ecològiques d'aquest fílum. L'elevada activitat trobada en aquest estudi enfront diversos depredadors suggereix que una millor exploració en aquest camp pot donar lloc a la possible utilització d'aquests extractes de briozous, com a potencials fàrmacs amb aplicacions farmacològiques. No obstant això, fins ara no ha estat possible identificar els compostos naturals en aquest estudi ni establir possibles activitats farmacològiques a causa de les petites quantitats de mostres obtingudes. Els nous estudis també s'haurien d'ocupar d'aquests aspectes interessants.

En resum

Per sobre de tot, aquesta tesi vol remarcar la necessitat d'un millor estudi de la biodiversitat i l'ecologia dels briozous antàrtics. Aquestes espècies han de ser protegides pel seu paper en els rars i fràgils ecosistemes antàrtics, a més del seu potencial ús farmacèutic. Només podem protegir el que sabem que existeix i entenem com funciona. Hem proporcionat nova informació clau sobre la seva diversitat biològica, la distribució espacial, la seva taxonomia i la seva ecologia química. Esperem que aquesta investigació ompli algunes de les llacunes existents i creï una bona base per a futurs treballs sobre el tema.

Conclusions finals

Les conclusions finals de la present tesi es resumeixen a continuació, separades en els dos temes de recerca principals:

Biodiversitat dels briozous en aigües antàrtiques i sub-antàrtiques

1. Les afinitats briozoològiques trobades entre l'Antàrtida i els últims fragments que es van separar de Gondwana, especialment la regió de la Patagònia, i les illes que serveixen de vincle entre la fauna de poca profunditat, coincideixen amb la hipòtesi de la separació seqüencial de Gondwana durant el Cenozoic
2. L'elevat nombre d'espècies de la regió de la PA compartit amb l'Antàrtida qüestiona el grau real d'aïllament de l'Antàrtida en briozous queilostòmats
3. La presència d'algunes espècies de briozous comunes entre aquestes regions també es pot explicar per la lliure migració d'organismes marins dins i fora del FP, a través de les planes abissals profundes, i pel potencial transport passiu de larves (o potser fins i tot d'adults), a distàncies considerables a través de la branca del CCA que flueix cap al nord al llarg de la plataforma continental de l'Argentina, el Corrent Falkland / Malvines
4. El paper de l'Arc d'Escòcia i altres vies de dispersió dels organismes, com els remolins del CCA i els mecanismes de dispersió humans, pot augmentar la connexió trobada entre l'Antàrtida i la regió de la PA
5. Els briozous queilostòmats de la regió de la PA i de l'OA, principalment de profunditat, són rics, abundants i euribàtics però encara poc coneguts
6. La gran diversitat que es troba a la regió de la PA està probablement relacionada amb l'existència dels corrents sub-antàrtic de Falkland/Malvines i el subtropical de Brasil, on existeix una surgència d'aigües riques en nutrients i blooms de plàncton associats
7. La major riquesa trobada a l'est del Mar de Weddell respecte a les illes i a la plataforma continental està probablement relacionada amb les grans distàncies entre el continent i les illes, així com al seu aïllament, i a la menor disponibilitat d'aliments enaugmentar la profunditat, respectivament
8. La distribució batimètrica trobada en el nostre estudi s'ajusta bé als límits de la plataforma continental, el talús i la plana abissal
9. La nova espècie de briozou queilostòmat trobada, *R. rosjoarum* n. sp., es caracteritza per la presència d'una inusual aviculària esfèrica gegant, fet què pot indicar una varietat de funcions biològiques, mostrant que les seves funcions són complexes i encara molt desconegudes

Ecologia química en briozous antàrtics

10. La majoria de les espècies de briozous mostraven activitat citotòxica i/o repel·lent contra l'eriçó de mar *Sterechinus neumayeri* i l'amfípode *Cheirimedon femoratus*, recolzant la hipòtesi que els mecanismes químics són de gran importància per evitar la depredació i la competència
11. Totes les espècies de briozous estudiats mostraven activitat de repel·lència alimentària contra almenys un dels dos depredadors abundants considerats, l'estrella de mar *Odontaster validus* i l'amfípode *Cheirimedon femoratus*, indicant la importància de les defenses químiques contra la depredació en briozous antàrtics
12. Les respostes de repel·lència alimentària eren més freqüents en els assajos amb *C. femoratus* en comparació amb aquells realitzats amb *O. validus*, fet que suggereix que els briozous poden requerir més protecció contra els amfípodes, els quals exerceixen una pressió més localitzada
13. La variabilitat intraespecífica en la bioactivitat trobada entre diferents llocs i profunditats suggereix una resposta d'adaptació a diversos factors tant abiòtics com biòtics i/o variabilitat genètica
14. La tendència general en el nostre estudi indica la possessió d'una combinació de mecanismes físics i químics en la majoria de les espècies, fet que suggereix estratègies complementàries
15. Amb l'objectiu d'avaluar les activitats citotòxiques i de repel·lència al substrat, es proposen i descriuen nous protocols adaptats que utilitzen substrats de gel, i demostren tenir diversos avantatges sobre altres mètodes utilitzats anteriorment

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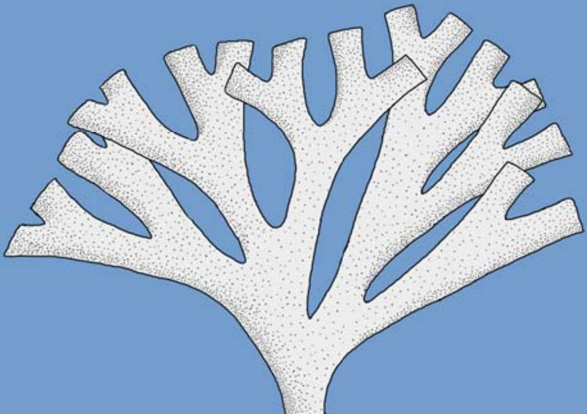

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Appendices

Appendix I

A copy of each of the two chapters of this thesis already published are included below in their original format.

Systematics and Biodiversity (2012), **10(1)**: 109–123

Taylor & Francis
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Research Article

Spatial patterns and diversity of bryozoan communities from the Southern Ocean: South Shetland Islands, Bouvet Island and Eastern Weddell Sea

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In this study, we report new data on the biodiversity and the geographic and bathymetric distribution of bryozoans collected during the ANT XXI/2 cruise (November 2003 to January 2004) in the Eastern Weddell Sea and Bouvet Island, and during the Spanish Antarctic expedition ECOQUIM (January 2006) in the South Shetland Islands. Our data on distribution were analysed together with previous studies carried out in the same regions. A total of 54 species of Antarctic bryozoans (206 samples), including a new species of the genus *Reteporella* were found. Two species were reported for the first time from Bouvet Island, one from the Weddell Sea and one from Spiess Seamount. Fifty-five per cent of all species identified were endemic to Antarctica. In the Weddell Sea, the regions of Austasen and Kapp Norvegia exhibit the highest relative species richness, followed by the Vestkapp region. Multivariate and cluster analyses revealed small-scale spatial variability in the community structure along depth and between localities.

Key words: Antarctica, bathymetric distribution, bryozoans, geographic distribution, multidimensional scaling

Introduction

The conservation and management of marine biodiversity requires detailed studies of the biodiversity and its relationship with environmental conditions (de Voogd *et al*., 2009). Although they seem to be under less intense pressures when compared with other ecosystems globally, Antarctic habitats are threatened by overexploitation of living resources, establishment of invasive marine species and climate change, as well as the growing impact of tourism (Tejedo *et al*., 2009). The Antarctic fauna has evolved in stable conditions, thus it is likely to be more sensitive and, for this reason, the risk of extinctions caused by anthropogenic impacts in these ecosystems makes it essential to intensify research on Antarctic biodiversity (Barnes & Peck, 2008).

Knowledge of the bryozoan species from the Southern Ocean, their diversity and the environmental conditions in which they live, is still very poor (Kuklinski & Barnes, 2009), which is largely determined by the relative inaccessibility of the region. An understanding of how and why similarities and differences exist between benthic communities inhabiting Antarctic ecosystems may provide information about the physical and biological factors that influence bryozoan distributions.

More than 700 new species of invertebrates from deep Antarctic waters have been recently discovered, with bryozoans, sponges and amphipods exhibiting high species richness (Brandt *et al*., 2007). Therefore, recent studies of Antarctic biodiversity in the region have described a rich and varied fauna (Hayward & Winston, 2011). In general, the Antarctic shelf and slope are known to be able to support biomass levels of macrobenthos far higher than those in equivalent habitats in boreal and subtropical regions of equal depth (Arntz *et al.*, 1994).

In recent years, the number of taxonomic studies on Antarctic bryozoans has experienced a notable increase (Hayward, 1995; Gutt *et al*., 2000; López-Fé de la Cuadra & García-Gómez, 2000). Since the scientific results of the Belgian Antarctic Expedition in 1897–99 (Waters, 1904), over 300 species have been described and new descriptions continue to appear (Clarke & Johnston, 2003; Gontar, 2008; López & Liuzzi, 2008; Kuklinski & Barnes, 2009; Griffiths, 2010; Figuerola *et al*., 2012). Cheilostomatid bryozoans are one of the best-represented taxa on the

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Antarctic shelf (Barnes *et al*., 2009) and a high proportion (56%) are endemic (Hayward, 1995; Barnes & De Grave, 2000; Clarke & Johnston, 2003; Griffiths *et al*., 2009; Griffiths, 2010). Many bryozoan species have been reported from the Antarctic Peninsula or the Ross Sea (Hayward, 1995). However, bryozoans are poorly investigated in some other Antarctic regions, such as the Weddell Sea (Zabala *et al*., 1997; Moyano, 2005, Barnes & Kuklinski, 2010). High levels of biodiversity, with more than 400 species and subspecies of Bryozoa in 32 stations, were found in the first collection from this area during the ANT XIII/3 Expedition (1996) with Polarstern (Arntz & Brey, 2005), and in recent sampling expeditions in the deep Weddell Sea (Arntz & Brey, 2005; Barnes & Kuklinski, 2010).

In polar waters, benthic assemblages are characterized by both bathymetric and horizontal variability (Cummings *et al.*, 2006; Smale, 2008). Diversity of Antarctic species is determined by a synergy of physical (depth, substratum, iceberg scouring . . .) and biotic factors (e.g. community type) (Starmans *et al*., 1999; Smale, 2008; Griffiths, 2010), and in the eastern Weddell Sea shelf, differences in currents cause heterogeneity. Iceberg scouring is the major disturbance affecting the benthos of this continental shelf because it disrupts large areas of the seafloor above 300 m. All of these factors play a key role in structuring recent Antarctic shelf benthic communities (Gutt & Piepenburg, 2003; Thatje *et al*., 2005; Brandt *et al*., 2007).

The objectives of this research were: (1) to present species-level information on new samples analysed for this study and (2) by combining these with existing data on bryozoan distributions in the region, to describe patterns of distribution in relation to depth and spatial location.

Materials and methods

Collection methods

Samples from the Weddell Sea and Bouvet Island were collected during the Antarctic cruise ANT XXI/2 (from November 2003 to January 2004) of R/V Polarstern (AWI, Bremerhaven, Germany) at 56 stations surveyed. Samples from the South Shetland Islands were collected at three stations (Fig. 1) from the BIO *Hespérides* in January 2006 during the ECOQUIM cruise. Depths of collections ranged from 27 to 910 m, using Bottom trawl, Agassiz trawl, Rauschert dredge, Epibenthic sledge and Giant box corer in the Weddell Sea and Bouvet Island. In the South Shetland Islands, an Agassiz trawl and a Rock dredge were used instead. Sampling sites were georeferenced and depth was registered at each point (Table 1).

After taking pictures of the living animals, the colonies of bryozoans were preserved in 70% ethanol for further taxonomic identification. We classified most of the samples at species level using Hayward (1995).

Literature data

Some data of sampling stations and their characteristics (Zabala *et al*., 1997; Barnes & Kuklinski, 2010) and some data of bathymetric ranges and biogeographic distribution of the species studied (Hayward, 1995; Zabala *et al*., 1997; Gontar & Zabala, 2000; Arntz *et al*., 2006; Barnes *et al*., 2008; Barnes & Kuklinski, 2010) came from the literature and Global Biodiversity Information Facility database (GBIF; www.gbif.org). Additional data from South America, New Zealand and South Africa have been obtained from Moyano (1982, 1999), Gordon (1984, 1986) and Florence *et al.* (2007); see also www.bryozoa.net.

Data of Antarctic endemicity came from Hayward (1995), the SCAR's Marine Biodiversity Information database (SCAR-MarBIN; http://www.scarmarbin.be/) and the Global Biodiversity Information Facility database (GBIF; www.gbif.org).

Data analysis

In order to obtain representative numbers of individuals and species for the analysis, some data of the same species of bryozoans found in previous cruises in these regions (at 35 stations) were extracted from the literature, and analysed together with our new data from the ANT XXI/2 and ECOQUIM cruises (collected at 59 stations). In total we analysed data from 94 sampling stations.

Cluster and non-metric multidimensional scaling (MDS) ordination analyses were performed in order to assess similarities of samples. Due to unequal sampling efforts, binary data (presence/absence) was preferred to make the distance matrix using the Sørensen coefficient. The cluster was then plotted using the single linkage clustering technique to evaluate the similarities in species composition between regions. In order to evaluate the significant differences between regions, a test for binomial proportions was used ($P < 0.05$).

The MDS analysis was used to evaluate the similarities between ranges of depth for the genera because it assumes no shape between variables (Legendre & Legendre, 1998). In order to categorize the continuous variable depth and to represent it in the MDS analysis, it was divided into 100 m interval categories (e.g. 100 m category includes depths from 0–100 m). The first two dimensions were plotted and the distance between dots denotes their similarity measured by the stress value. A stress value of less than 0.1 indicates that the plot accurately represents similarities, while a stress value greater than 0.3 indicates that the points are close to being randomly placed (Clarke, 1993). Bathymetric ranges for each genus sampled in the Weddell Sea are detailed in Table 1.

In order to determine whether different assemblages do exist between ranges of depth and neighbouring sites, relative abundance (N) and relative species richness (S, number

Table 1. Sampling stations and their characteristics from this study and from the literature. AT: Agassiz trawl, RD: Rauschert dredge, BT: Bottom trawl, GBC: Giant box corer, ES: Epibenthic sledge, R: Rock dredge, BP: bentopelagic trawl, MG: Multibox corer and GK: large box corer.

Location	Station	Date	Latitude (S)	Longitude (W)	Depth (m)	Gear	References
Spiess Seamount	PS65/345-1	11/01/2004	54° 44. 12′	00° 08. 31′	629.4	RD	This study
Bouvet Island	PS65/019-1	24/11/2003	54° 30. 01′	03° 13. 97′	259.7	AT	This study
Bouvet Island	PS65/029-1	25/11/2003	54° 31. 59′	03° 13. 05′	376.8	AT	This study
Livingston	AGT 7	06/01/2006	62° 41. 575′	60° 44. 827′	27.9	RD	This study
Livingston	AGT 6	06/01/2006	62° 43. 117′	60° 43. 683′	94.9	RD	This study
Deception	AGT 9	07/01/2006	63° 02. 292′	60° 36. 355′	110.3	AT	This study
Weddell Sea	PS67/102-11	06/03/2005	65° 35. 40′	36° 29. 00′	4794	AT	Barnes *et al*. (2010)
Neumayer	30	01/03/1996	70° 05. 30′	08° 20. 00′	2315	AG	Zabala *et al*. (1997),
Neumayer	30	01/03/1996	70° 05. 30′	08° 20. 00′	2315	DR	Zabala *et al*. (1997)
Neumayer	PS65/069-1	07/12/2003	70° 25. 87′	08° 37. 43′	413.6	RD	Barnes *et al*. (2010)
Neumayer	32	04/03/1996	70° 28. 90′	08° 15. 10′	286	DR	Zabala *et al*. (1997)
Neumayer	31	02/03/1996	70° 30. 90′	10° 44. 20′	1586	DR	Zabala *et al*. (1997)
Austasen	PS65/121-1	11/12/2003	70° 50. 08′	10° 34.76′	274	AT	This study
Austasen	PS65/121-1	11/12/2003	70° 50. 08′	10° 35. 54′	268	AT	Barnes *et al*. (2010)
Austasen	PS65/237-1	22/12/2003	70° 50. 50′	10° 35. 54′	264.4	BT	This study
Austasen	PS65/336-1	05/01/2004	70° 50. 75′	10° 28. 01′	281.2	AT	This study
Austasen	PS65/339-1	05/01/2004	70° 50. 78′	10° 28. 51′	273.6	RD	This study
Austasen	PS65/274-1	28/12/2003	70° 52. 16′	10° 43. 69′	290.8	BT	This study
Austasen	PS65/265-1	27/12/2003	70° 52. 75′	10° 51. 24′	294.8	BT	This study
Austasen	PS65/090-1	09/12/2003	70° 55. 92′	10° 32. 37′	288	AT	This study
Austasen	PS65/123-1	11/12/2003	70° 56. 41′	10° 31. 58′	283.2	GBC	This study
Austasen	PS65/132-1	12/12/2003	70° 56. 42′	10° 31. 61′	284.4	BT	This study
Austasen	PS65/161-1	15/12/2003	70° 56. 43′	10° 31. 47′	279.6	AT	This study
Austasen	PS65/148-1	13/12/2003	70° 56. 67′	10° 32. 05′	302.4	BT	This study
Austasen	PS65/173-1	16/12/2003	70° 56. 82′	10° 31. 76′	296.4	AT	This study
Austasen	PS65/166-1	15/12/2003	70° 56. 83′	10° 32. 61′	338	BT	This study
Austasen	PS65/259-1	24/12/2003	70° 57. 00′	10° 33. 02′	332.8	BT	This study
Austasen	PS65/175-1	16/12/2003	70° 57. 11′	10° 33. 32′	337.2	BT	This study
Austasen	PS65/245-1	22/12/2003	70° 57. 11′	10° 33. 52′	337.2	BT	This study
Austasen	PS65/174-1	16/12/2003	70° 57. 33′	10° 33. 86′	351.6	BT	This study
Austasen	1	05/02/1996	71° 03. 10′	11° 25. 50′	462	BT	Zabala *et al*. (1997)
Austasen	PS65/253-1	23/12/2003	71° 04. 30′	11° 33. 92′	308.8	BT	This study
Austasen	PS65/248-1	23/12/2003	71° 04. 96′	11° 31. 90′	286.8	BT	This study
Austasen	PS65/039-1	05/12/2003	71° 06. 30′	11° 32. 04′	175.2	AT	This study
Austasen	PS65/276-1	28/12/2003	71° 06. 44′	11° 27. 76′	277.2	AT	This study
Austasen	PS65/280-1	29/12/2003	71° 07. 15′	11° 26. 23′	228.4	AT	This study
Austasen	PS65/279-0	29/12/2003	71° 07. 43′	11° 29. 83′	119.2	AT	Barnes *et al*. (2010)
Austasen	PS65/279-1	29/12/2003	71° 07. 48′	11° 29. 91′	119.6	AT	This study
Austasen	PS65/278-1	29/12/2003	71° 07. 51′	11° 29. 94′	120	AT	Barnes *et al*. (2010)
Austasen	24	21/02/1996	71° 08. 15′	11° 32. 25′	123	AG	Zabala *et al*. (1997)
Austasen	24	21/02/1996	71° 08. 30′	11° 32. 40′	119	GK	Zabala *et al*. (1997)
Austasen	2	22/02/1996	71° 18. 60′	12° 25. 40′	181	MG	Zabala *et al*. (1997)
Austasen	PS67/078-9	21/02/2005	71° 09. 39′	13° 59. 30′	2156	ES	Barnes *et al*. (2010)
Austasen	PS67/078-11	21/02/2005	71° 09. 39′	13° 59. 33′	2157	AT	Barnes *et al*. (2010)
Kapp Norvegia	PS67/074-6	20/02/2005	71° 18. 35′	13° 57. 71′	1030	ES	Barnes *et al*. (2010)
Kapp Norvegia	PS65/232-1	21/12/2003	71° 18. 61′	13° 56. 12′	910	ES	This study
Kapp Norvegia	2	09/02/1996	71° 18. 70′	12° 17. 10′	170	AG	Zabala *et al*. (1997)
Kapp Norvegia	2	22/02/1996	71° 19. 10′	12° 22. 80′	159	MG	Zabala *et al*. (1997)
Kapp Norvegia	2	22/02/1996	71° 19. 20′	12° 27. 00′	253	MG	Zabala *et al*. (1997)
Kapp Norvegia	25	23/02/1996	71° 22. 90′	14° 19. 20′	622	AG	Zabala *et al*. (1997)
Kapp Norvegia	25	23/02/1996	71° 22. 90′	14° 19. 20′	622	DR	Zabala *et al*. (1997)
Kapp Norvegia	25	23/02/1996	71° 23. 10′	14° 19. 80′	634	AG	Zabala *et al*. (1997)
Kapp Norvegia	25	23/02/1996	71° 23. 10′	14° 19. 70′	621	GK	Zabala *et al*. (1997)
Kapp Norvegia	25	23/02/1996	71° 23. 10′	14° 19. 70′	628	GK	Zabala *et al*. (1997)
Kapp Norvegia	21	18/02/1996	71° 26. 50′	21° 10. 50′	253	BP	Zabala *et al*. (1997)
Kapp Norvegia	7	08/02/1996	71° 26. 80′	13° 44. 00′	215	GK	Zabala *et al*. (1997)
Kapp Norvegia	6	11/02/1996	71° 27. 40′	13° 43. 30′	212	AG	Zabala *et al*. (1997)
Kapp Norvegia	26	24/02/1996	71° 29. 30′	14° 18. 60′	216	DR	Zabala *et al*. (1997)
Kapp Norvegia	26	24/02/1996	71° 29. 30′	14° 19. 50′	210	DR	Zabala *et al*. (1997)

(Continued on next page)

Table 1. (Continued)

Location	Station	Date	Latitude (S)	Longitude (W)	Depth (m)	Gear	References
Kapp Norvegia	29	29/02/1996	71° 30. 70′	12° 26. 40′	494	GK	Zabala *et al*. (1997)
Kapp Norvegia	29	28/02/1996	71° 31. 50′	12° 25. 50′	504	BP	Zabala *et al*. (1997)
Kapp Norvegia	6	08/02/1996	71° 31. 80′	13° 34. 50′	254	AG	Zabala *et al*. (1997)
Kapp Norvegia	6	25/02/1996	71° 32. 10′	13° 44. 10′	362	AG	Zabala *et al*. (1997)
Kapp Norvegia	9	26/02/1996	71° 32. 60′	12° 26. 30′	570	AG	Zabala *et al*. (1997)
Kapp Norvegia	9	10/02/1996	71° 34. 00′	12° 25. 80′	604	BT	Zabala *et al*. (1997)
Kapp Norvegia	9	26/02/1996	71° 34. 70′	12° 26. 60′	560	AG	Zabala *et al*. (1997)
Kapp Norvegia	9	26/02/1996	71° 34. 70′	12° 26. 60′	560	DR	Zabala *et al*. (1997)
Kapp Norvegia	3	26/02/1996	71° 39. 30′	12° 05. 10′	209	GK	Zabala *et al*. (1997)
Kapp Norvegia	5	06/02/1996	71° 39. 75′	12° 41. 00′	255	MG	Zabala *et al*. (1997)
Kapp Norvegia	5	06/02/1996	71° 40. 49′	12° 41. 70′	254	EB	Zabala *et al*. (1997)
Kapp Norvegia	5	07/02/1996	71° 41. 10′	12° 44. 30′	227	BT	Zabala *et al*. (1997)
Kapp Norvegia	4	20/02/1996	71° 41. 20′	12° 30. 80′	438	MG	Zabala *et al*. (1997)
Kapp Norvegia	4	20/02/1996	71° 41. 50′	12° 31. 70′	436	GK	Zabala *et al*. (1997)
Kapp Norvegia	4	20/02/1996	71° 41. 60′	12° 29. 40′	440	AG	Zabala *et al*. (1997)
Vestkapp	PS65/283-1	30/12/2003	72° 32. 16′	17° 58. 88′	585.2	ES	This study
Vestkapp	PS65/297-1	01/01/2004	72° 48. 50′	19° 31. 60′	668	RD	This study
Vestkapp	PS65/308-1	02/01/2004	72° 50. 18′	19° 35. 94′	622	RD	This study
Vestkapp	20	18/02/1996	72° 50. 50′	19° 26. 00′	428	BP	Zabala *et al*. (1997)
Vestkapp	PS65/292-1	31/12/2003	72° 51. 43′	19° 38. 62′	597.6	BT	This study
Vestkapp	PS65/326-1	03/01/2004	72° 51, 43′	19° 38. 67′	616	RD	This study
Vestkapp	PS65/326-1	03/01/2004	72° 51. 70′	19° 39. 22′	605.2	RD	Barnes & Kuklinski (2010)
Vestkapp	PS65/324-1	03/01/2004	72° 54. 52′	19° 47. 74′	693.6	RD	This study
Vestkapp	PS65/324-1	03/01/2004	72° 54. 55′	19° 47. 30′	647.2	RD	Barnes & Kuklinski (2010)
Vestkapp	PS65/325-1	03/01/2004	72° 54. 76′	19° 43. 48′	457.6	RD	This study
Vestkapp	18	16/02/1996	73° 15. 40′	21° 27. 60′	1704	AG	Zabala *et al*. (1997)
Vestkapp	18	16/02/1996	73° 16. 70′	21° 25. 50′	1538	AG	Zabala *et al*. (1997)
Vestkapp	17	16/02/1996	73° 18. 00′	21° 09. 90′	468	BT	Zabala *et al*. (1997)
Vestkapp	12	13/02/1996	73° 18. 10′	21° 10. 10′	459	BT	Zabala *et al*. (1997)
Vestkapp	11	13/02/1996	73° 22. 60′	21° 10. 60′	338	BT	Zabala *et al*. (1997)
Vestkapp	21	18/02/1996	73° 22. 90′	21° 10. 00′	283	BP	Zabala *et al*. (1997)
Vestkapp	14	14/02/1996	73° 36. 10′	22° 35. 70′	850	BT	Zabala *et al*. (1997)
Vestkapp	13	14/02/1996	73° 36. 30′	22° 19. 10′	620	BT	Zabala *et al*. (1997)
Vestkapp	15	15/02/1996	73° 42. 00′	22° 30. 50′	446	BT	Zabala *et al*. (1997)
Vestkapp	16	15/02/1996	73° 53. 40′	22° 26. 90′	246	BT	Zabala *et al*. (1997)

of species present) and *P* value (test for binomial proportions) were calculated for each depth range and area. A sample-based rarefaction curve was also computed. Chao2, Jacknife1 and Jacknife2 methods were used to estimate the theoretical number of expected species within each area (Colwell & Coddington, 1994). Chao2 is an abundance-based non-parametric estimator of species richness that works by examining the number of species in a sample observed more than once relative to the number of species that is observed just once. In the absence of complete inventories, these non-parametric estimators have been shown to perform better than most other methods, such as observed species richness (Krebs, 1999).

Diversity indices are commonly used to provide more information about community composition than simply species richness, such as the rarity and commonness of species and they also take the relative abundances of different species into account. The Margalef index is based on the number of species (species richness), while the others are indices of proportional abundances of the species. The Shannon–Wiener index is strongly influenced by the occurrence of rare species and Simpson's index by the importance of the more dominant species. Pielou's (evenness) and Berger–Parker indices calculate the relationship between the observed diversity and the maximum diversity, as well as between the number of the individuals of the most abundant species and the total number of individuals in the sample, respectively (Gray, 2000). Diversity indices are used to assess the impact of disturbances on the marine environment. In this aspect, the Shannon–Wiener index is more sensitive (high values mean an improvement in the environmental state) (Gray, 2000). In the case of the Simpson and Berger–Parker indices, higher values correspond to a lower diversity (Salas *et al*., 2004; Marqués *et al*., 2009). The values of diversity indices calculated from the data of the present study of stations sampled with Agassiz trawl (AT), Bottom trawl (BT) and Rauschert dredge (RD) did not show significant differences (bootstrap confidence

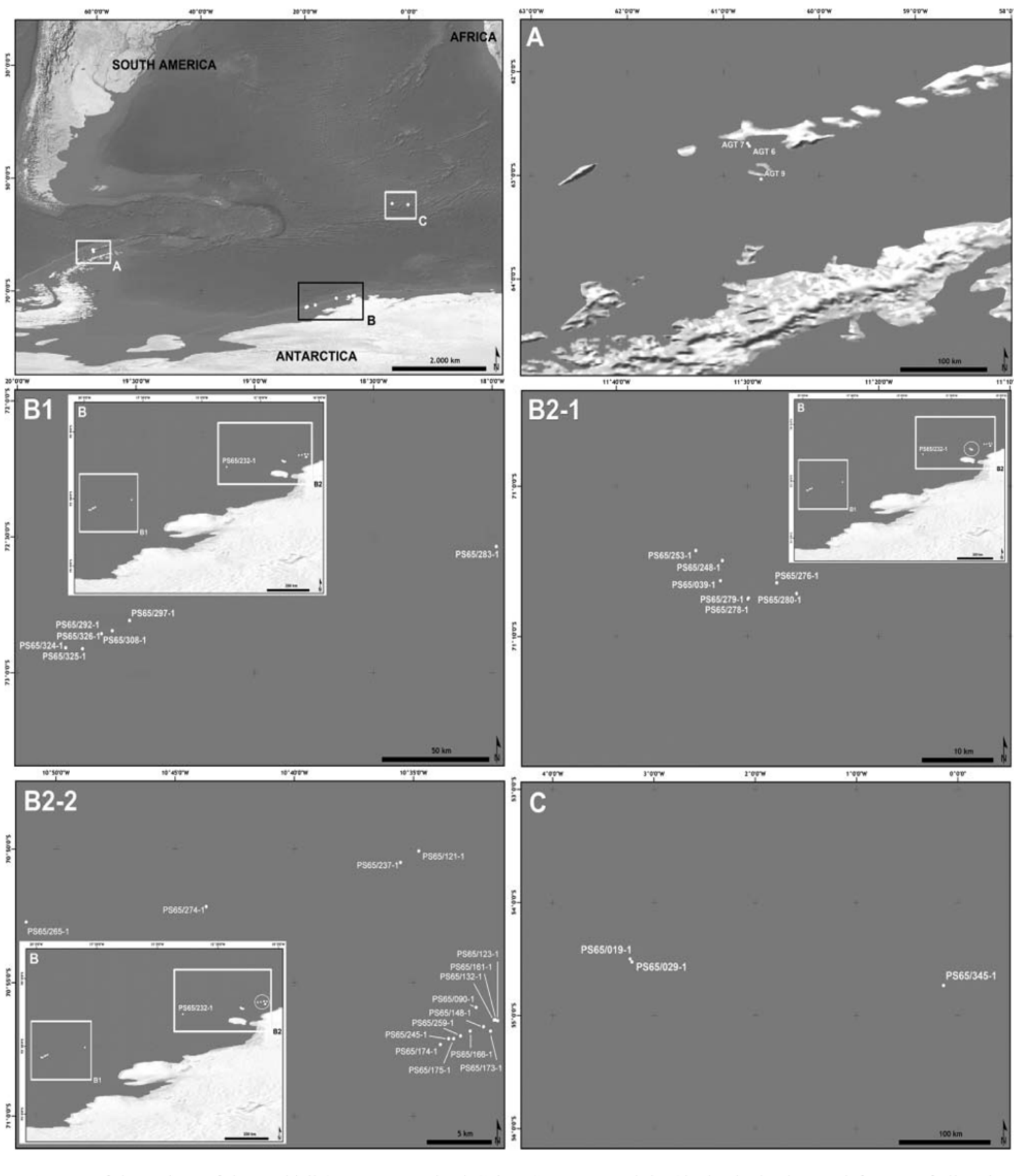

Fig. 1. Map of the regions of the Weddell Sea, Bouvet Island, Spiess Seamount and the Shetland Islands. Top left: map of all regions; A: Shetland Islands; B: area of Weddell sea; B1: region of Vestkapp; B2: regions of Kapp Norvegia and Austasen; B2-1, B2-2: region of Austasen; C: vicinities of Bouvet Island.

interval were overlapping). For this reason, five alpha diversity indices were calculated for each region only in these stations: Margalef (D_{Mg}), Shannon–Wiener (H'), Simpson's (1 – Lambda'), Pielou's (J') and Berger–Parker (B–P). The diversity indices of Kapp Norvegia were not calculated due to the absence of samples collected with any of these trawls in the present study.

Statistical significance was established at $P < 0.05$. Ordination analyses were performed using VegAna software (v.1.6.0; De Cáceres *et al*., 2003). Diversity analyses (relative abundance and species richness and diversity indices) were carried out with Past (Hammer *et al*., 2001) and the bootstrap method was used to obtain a more robust non-parametric estimate of the confidence intervals (95%)

(Briggs *et al*., 1997). The test for binomial proportions was performed with Minitab Statistical Software. The SPSS (version 14.0, SPSS Inc, Chicago, Illinois, USA) package was used for the rest of the data analysis.

Results

A total of 54 species of Antarctic bryozoans (206 samples), belonging to 12 families and 27 genera, were found with different trawls, from depths between 27 and 910 m in the studied areas (Table 2). The list includes a newly described species, *Reteporella rosjoarum* (Figuerola *et al*., 2011). Furthermore, two species were reported for the first time from Bouvet Island, one from the Weddell Sea and one from Spiess Seamount. Eight of the species were identified only to genus level. The most diverse Infraorder was Lepraliomorpha with 18 species (33%). Fifty-five per cent of the species found were endemic to the Southern Ocean (see Hayward, 1995, SCAR-MarBIN and GBIF databases), with a total of 49 species. *Reteporella* with six species was the dominant genus. Most of the species found were *Bostrychopora dentata*, which represented 9.7% of the total specimens collected, and *Nematoflustra flagellata* (6.7%). These were followed by *Austroflustra vulgaris*, *Alcyonidium* sp., *Carbasea curva*, *Cellarinella nutti* and *Osthimosia curtioscula*. *Austroflustra vulgaris* was the only species found in the three studied areas from the Weddell Sea.

Data from recent cruises reported in the literature and the GBIF database together with our own data were jointly analysed, revealing that four species have been found far from their known distribution range in the Weddell Sea. Therefore, an expansion in their known geographical distribution is reported here (Table 2).

Bathymetric ranges

From a total of 27 genera analysed on the different cruises, 50.2% (16 genera) were restricted to the continental shelf (18 species) and above 900 m. *Camptoplites*, *Melicerita* and *Cellaria* were the only genera found in deeper waters (5900, 4802 and 4531 m, respectively) and showed the widest bathymetric ranges (Fig. 2). Seven genera showed large bathymetric ranges: *Carbasea* (31–2846 m), *Austroflustra* and *Cornucopina* (5–2700 m), *Cellarinella* (5–2334 m), *Isosecuriflustra* (22–2315 m), *Kymella* (0–2157 m) and *Nematoflustra* (0–2100 m). Four genera (15 species) were present at depths between 0 and 700 m.

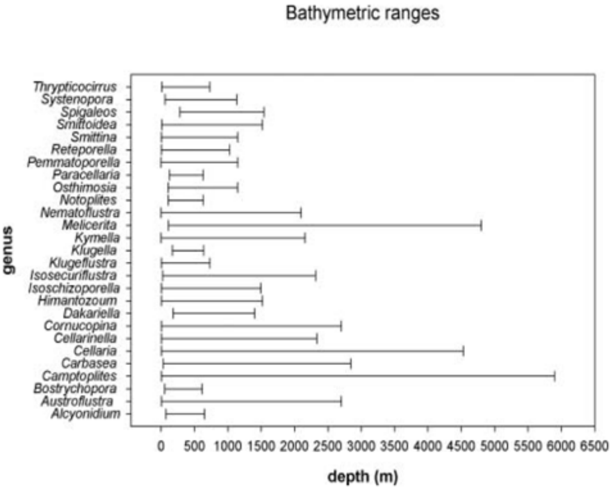

Fig. 2. New bathymetric ranges of bryozoans genera from the Southern Ocean found in the present study both from our own data and the literature and the GBIF database. Additional data have been obtained from Hayward (1995), Zabala *et al.* (1997), Gontar & Zabala (2000), Arntz *et al.* (2006) and Barnes & Kuklinski (2010).

Bathymetric distribution

Low stress values (0.03) of the MDS indicate a good representation in the 2-dimensional ordination (Clarke, 1993). Five depth zones were discriminated by the multidimensional scaling analysis in bathymetric distribution (Fig. 3): (1) a zone between 0 and 100 m with the presence of three genera, (2) a zone between 100 and 700 m characterized by the presence of all of the genera, a similar composition at each depth (77.9% of the genera appear in each 100 m of depth) and the presence of the genus *Dakariella* only in

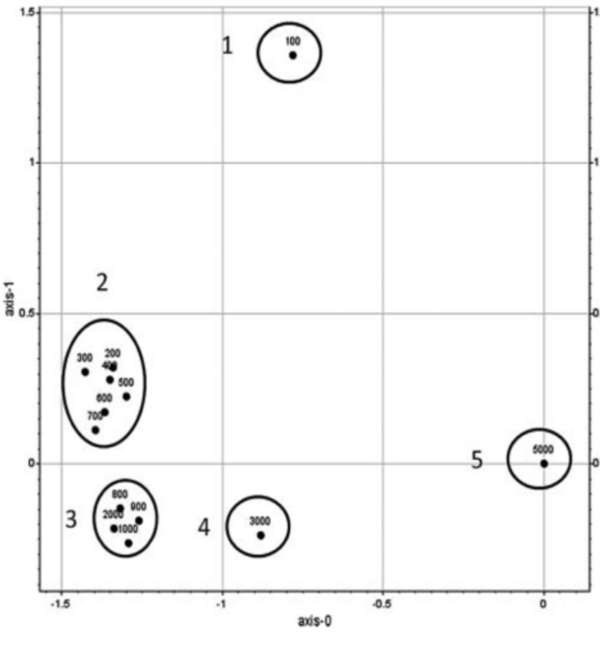

Fig. 3. Plot of the multidimensional scaling ordination (MDS) of the different genera in relation to depth. Points numbered 100–5000 correspond to different depth ranges (stress = 0.03). Additional data have been obtained from Hayward (1981, 1995), Zabala *et al.* (1997), Gontar & Zabala (2000), Arntz *et al.* (2006) and Barnes & Kuklinski (2010). Group 1: 0–100 m; group 2: 100–700 m; group 3: 700–2000 m; group 4: 2000–3000 m and group 5: 3000–5000 m.

Table 2. Bathymetric ranges and biogeographic distribution of the species studied using data from the present study, the literature and the GBIF database.

Species	Present study	Bathymetric distr. (m)	Geographic distr.	New records for species	References
Carbasea curva Kluge, 1914	X	31–2846	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Klugeflustra antarctica Hastings, 1943	X	5–732	Livingston		Hayward (1995)
Isosecuriflustra angusta Kluge, 1914		31–2315	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Isosecuriflustra tenuis Kluge, 1914	X	22–639* (previously 634)	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Austroflustra vulgaris Kluge, 1914	X	5–2700	Bouvet Island, Livingston, Weddell Sea		Arntz *et al*. (2006); Hayward (1995); Zabala *et al*. (1997); Gontar & Zalaba (2000)
Nematoflustra flagellata Waters, 1904		0–2100	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Camptoplites angustus Kluge, 1914	X	5–720	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Camptoplites bicornis Busk, 1884		5–5900	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Camptoplites giganteus Kluge, 1914	X	20–294* (previously 293)	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Camptoplites tricornis Waters, 1904		5–2000	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Cornucopina polymorpha Kluge, 1914	X	5–2700	Bouvet Island	**	Arntz *et al*. (2006)
Himantozoum antarcticum Calvet, 1909	X	5–1517	Weddell Sea, Livingston		Zabala *et al*. (1997); Gontar & Zabala (2000); Hayward (1995)
Klugella echinata Kluge, 1914		170–640	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Notoplites antarcticus Waters, 1904		104–634	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Notoplites drygalskii Kluge, 1914		123–1030	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000); Barnes & Kuklinski (2010)
Cellaria aurorae Livingstone, 1928		5–2334	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000); Barnes *et al*. (2010)
Cellaria diversa Livingstone, 1928	X	5–3545	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Cellaria moniliorata Rogick, 1956d		10–4531	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Cellaria incula Hayward and Ryland, 1993	X	123–668* (previously 634)	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Paracellaria wandeli Calvet, 1909		5–2700	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Melicerita latilaminata Rogick, 1956d	X	5–528	Deception		Barnes *et al*. (2008)
Melicerita obliqua Thornely, 1924		0–4802	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000); Barnes & Kuklinski (2010)
Cellarinella nodulata Waters, 1904		118–1133	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Cellarinella nutti Rogick, 1956d		11–2334	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Cellarinella rogickae Moyano, 1965		5–1517	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000))
Cellarinella watersi Calvet, 1909		5–1517	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Systenopora contracta Waters, 1904		61–1133	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Isoschizoporella secunda Hayward and Taylor, 1984	X	18–1495	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Isoschizoporella tricuspis Calvet, 1909		5–759	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Dakariella dabrowni Rogick, 1956d		181–1404	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Kymella polaris Waters, 1904		0–2157	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000); Barnes & Kuklinski (2010)
Smittina antarctica Waters, 1904		5–1150	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Smittoidea albula Hayward and Taylor, 1984		35–628	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)

(Continued on next page)

Table 2. (Continued)

Species	Present study	Bathymetric distr. (m)	Geographic distr.	New records for species	References
Smittoidea ornatipectoralis Rogick, 1956d	X	10–1517	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Thrypticocirrus contortuplicata Calvet, 1909		10–732	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Pemmatoporella marginata Calvet, 1909		0–1150	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Bostrychopora dentata Waters, 1904	X	56–616* (previously 567)	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Osthimosia curtioscula Hayward, 1992	X	104–1150	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000); Barnes & Kuklinski (2010)
Spigaleos horneroides Waters, 1904	X	283–1543	Spiess Seamount	***	
Reteporella antarctica Waters, 1904		61–622	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Reteporella erugata Hayward, 1992	X	86–1030	Bouvet Island		Arntz *et al*. (2006); Barnes *et al*. (2010)
Reteporella frigida Waters, 1904		5–923	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000); Barnes & Kuklinski (2010)
Reteporella hippocrepis Waters, 1904	X	61–634	Bouvet Island, Weddell Sea	**	Arntz *et al*. (2006); Zabala *et al*. (1997); Gontar & Zabala (2000)
Reteporella lepralioides Waters, 1904		61–634	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)
Reteporella sp. nov. Figuerola, Ballesteros and Avila 2012	X	264*	Weddell Sea	****	Zabala *et al*. (1997); Gontar & Zabala (2000)
Alcyonidium unidentified species Kirkpatrick, 1902	X	73–655	Weddell Sea		Zabala *et al*. (1997); Gontar & Zabala (2000)

*New bathymetric range described in this study, **First record for Bouvet Island, ***First record for Spiess Seamount, ****First record for the Weddell Sea.

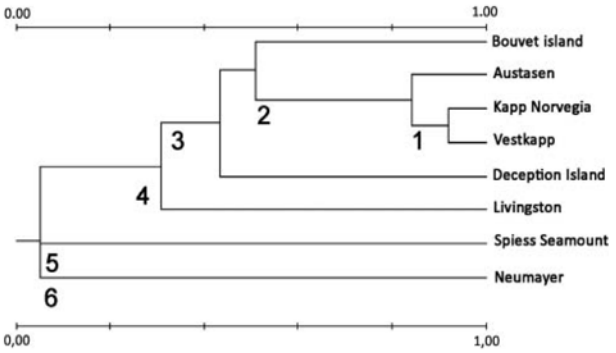

Fig. 4. Dendrogram from hierarchical clustering (single linkage) of the bryozoan fauna from the Southern oceans using Sørensen distance (Pearson cophenetic index 0.97). Additional data have been obtained from Hayward (1981, 1995), Zabala *et al.* (1997), Gontar & Zabala (2000), Arntz *et al.* (2006), Barnes *et al.* (2008) and Barnes & Kuklinski (2010).

one depth interval (200–300 m), (3) a zone between 700 and 2000 m with a high similarity of generic composition but with fewer (nine) genera, (4) a zone between 2000 and 3000 m with the presence of four genera, and (5) another zone between 3000 and 5000 m characterized by the presence of *M. obliqua* only.

Geographic distribution

Cluster analyses suggested six principal groups of similar faunal composition (Fig. 4). The first group (1) is represented by the region of Eastern Weddell Sea comprised of the subregions of Kapp Norvegia (30 stations), Vestkapp (20 stations) and Austasen (32 stations) with the same number of species but of different composition. The islands of groups 3 and 4, the region of the Spiess Seamount (5) and the region of Neumayer (6) were represented by more separated groups with a lower number of species. In the results of tests for binomial proportions, the subregions of Kapp Norvegia with Austasen and Vestkapp showed no significant differences ($P < 0.05$). Deception Island, Livingston Island, Spiess Seamount and the Neumayer region showed significant differences with other regions ($P < 0.05$). Bouvet Island exhibited significant differences with respect to all other regions ($P < 0.05$).

Species richness and diversity indices

Relative species richness (S) was low at depths between 0 and 100 m and from 800 to 5000 m, with significant differences between ranges from 100 to 700 m (test for binomial proportions, $P < 0.01$). The highest value was found between 300 and 400 m (Table 3; Fig. 5). The regions of Austasen, Kapp Norvegia and Vestkapp (with the same number of species but of different composition) had the highest species richness, followed by Bouvet and Deception Islands (Fig. 6).

Table 3. Number of species (nº sps), per cent of relative species richness (% S) and *P* value (*P*) for each depth range in the Eastern Weddell Sea. Additional data have been obtained from Hayward (1995), Zabala *et al*. (1997), Gontar & Zabala (2000), Arntz *et al*. (2006) and Barnes & Kuklinski (2010).

Depth (m)	nº sps	% S	*P*
100	3	11.11	–
200	23	85.19	0.000
300	27	100.00	0.000
400	26	96.30	0.000
500	25	92.59	0.000
600	24	88.89	0.000
700	21	77.78	0.000
800	9	33.33	0.099
900	9	33.33	$P > 0.1$
1000	8	29.63	$P > 0.1$
2000	8	29.63	$P > 0.1$
3000	4	14.81	$P > 0.1$
5000	1	3.70	$P > 0.1$

The accumulation curve has still to reach the asymptote: 54 species have been found, but up to 90 (Chao2) can be expected as more samples are collected (Fig. 7). Jacknife1 and Jacknife2 methods estimated the theoretical number of expected species. These values are 82 and 97, respectively. However, in our case (absence of complete inventories), Chao2 has been shown to perform better than most other methods (Krebs, 1999).

Five alpha diversity indices were calculated for each region only for the stations sampled with Agassiz trawl (AT), Bottom trawl (BT) and Rauschert dredge (RD) since they did not show significant differences (Table 4). The Shannon–Wiener and Margalef indices changed between regions with the highest value of indices and number of species in the region of Austasen ($H' = 3.445$; $D_{Mg} = 8.64$), followed by Vestkapp ($H' = 2.844$, $D_{Mg} = 5{,}498$), while Bouvet Island and Livingston Island showed low values

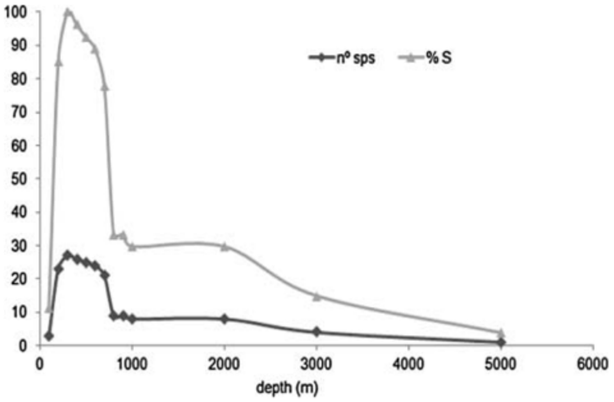

Fig. 5. Number of species (nº sps) and per cent of relative species richness (% S) related to depth ranges in the Eastern Weddell Sea and the Antarctic Peninsula. Additional data have been obtained from Hayward (1995), Zabala *et al.* (1997), Gontar & Zabala (2000), Arntz *et al.* (2006) and Barnes & Kuklinski (2010)

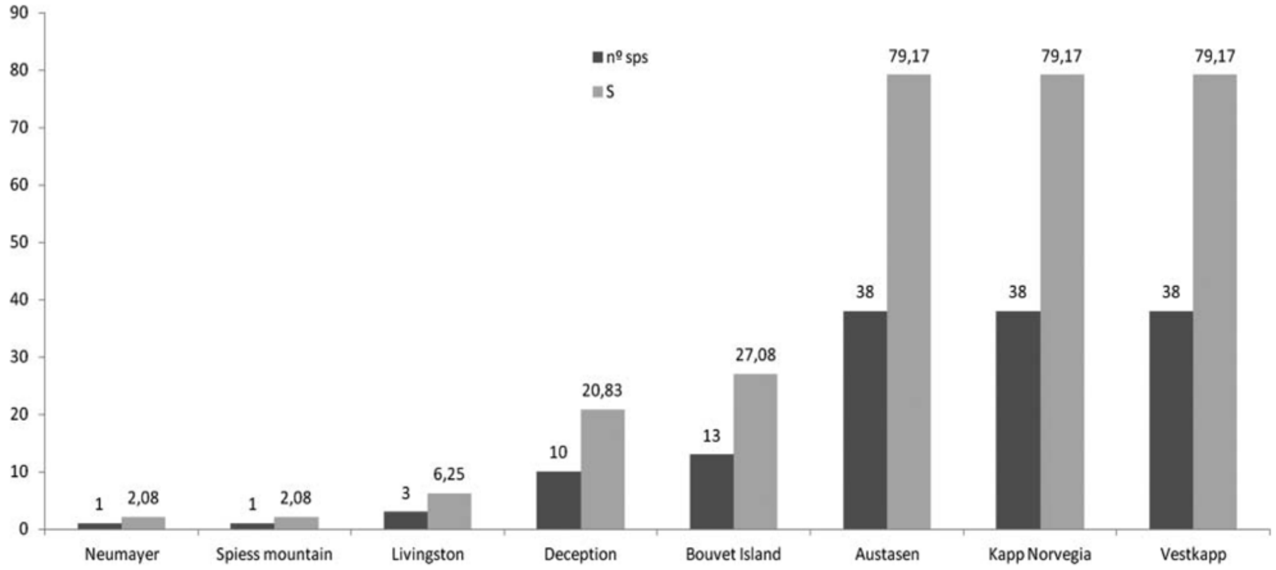

Fig. 6. Number of species (nº sps) and per cent of relative species richness (S) in different areas of the Eastern Weddell Sea and the Antarctic Peninsula. Additional data have been obtained from Hayward (1981, 1995), Zabala *et al.* (1997), Gontar & Zabala (2000), Arntz *et al.* (2006), Barnes *et al.* (2008) and Barnes & Kuklinski (2010).

(Table 5). The diversity indices for Kapp Norvegia could not be calculated due to the unavailability of samples collected using these methods. Samples from Deception Island and Spiess Seamount contained only one species.

Similarity with other regions

In our study, the Antarctic region is connected by some genera shared with South America (55.5%), New Zealand (48.15%) and South Africa (37.04%). In fact, the South Shetland Islands had a composition slightly more similar to South America (43.75%) than to Antarctica (29.63%) (Table 6).

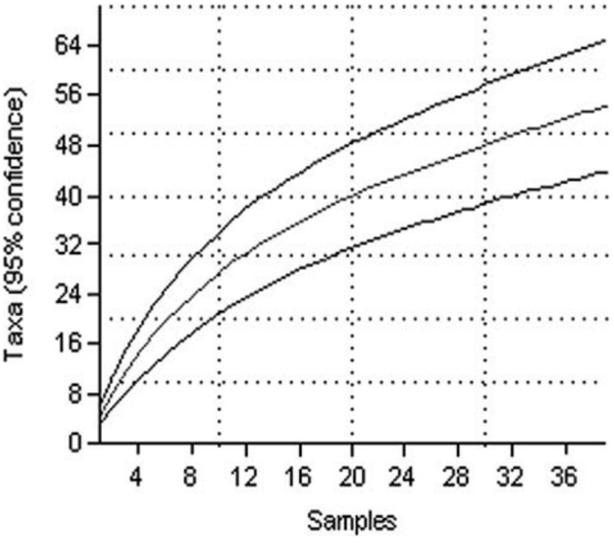

Fig. 7. Sample-based rarefaction curve. Expected species richness value was computed with 95% confidence interval.

Discussion

The benthic fauna of the continental shelf of the Eastern Weddell Sea, as described for some other areas, is dominated by suspension feeders, such as bryozoans, and variations in their abundance are critical to the organization of the whole community (Teixidó *et al*., 2002, 2004). This shelf reaches great depths, with the shelf break at about 900–1000 m (Linse *et al*., 2006). Few bryozoan species have been reported from below the shelf break (Barnes & Kuklinski, 2010) and most benthic samples come from depths of less than 500 m (Griffiths, 2010).

Antarctic bryozoans analysed here exhibit a high range of eurybathy. Bathymetric distributions of Antarctic fauna reported in the literature demonstrate that some species extend over large depth ranges (Brey *et al*., 1996; Soler i Membrives *et al*., 2009). Twenty-seven bryozoan species of this study have been recorded in the Southern Ocean deeper than 1000 m. The case of the genus *Camptoplites* is even more amazing, showing a depth range of 0–5900 m. The existence of eurybathic species has been explained by the evolutionary history of the Southern Ocean fauna (Munilla, 2001). Thatje and colleagues (2005) suggested that the impact of the grounded ice sheets on most of the Antarctic continental shelf during Cenozoic glacial periods affected the benthic communities. Therefore, the continental shelf was further recolonized by deep-water organisms with wide bathymetric tolerances and thus, depth seems to be a less important factor in controlling the

Table 4. Diversity indices for the three types of sampling (AT: Agassiz trawl, BT: Bottom trawl and RD: Rauschert dredge) from the present study with 95% confidence intervals using Bootstrap method: Margalef index (D_{Mg}), Shannon–Wiener diversity index, H' (base log e), Simpson's Index (1 – Lambda'), Pielou's index (J') and Berger–Parker index (B–P).

	D_{Mg}		H′		1-Lambda′		J′		BP	
	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
AT	5.87	8.21	3.01	3.40	0.93	0.96	0.72	0.87	0.07	0.17
BT	6.16	8.44	3.05	3.41	0.94	0.96	0.71	0.85	0.08	0.16
RD	5.11	7.67	2.82	3.27	0.92	0.96	0.74	0.90	0.08	0.20

distribution of communities compared with other areas. Changes in Antarctic biodiversity have been found to be associated with the movement of taxa between the shelf and the deep sea (Brey *et al*., 1996; Arntz *et al*., 1997; Brandt & Hilbig, 2004). However, this possibility has not been proved so far (Thatje *et al*., 2005). The possibility should also be considered that some deep occurrences (>1000 m) are due to transport off the shelf by currents, as shown for bryozoans from elsewhere, such as New Zealand (Lagaaij, 1973, Hayward, 1981; Taylor *et al*., 2004).

Horizontal and vertical variability in Antarctic bryozoan distributions does exist. For some benthic species, horizontal and bathymetric distribution patterns have been described, but, in the case of most bryozoan species from this area, their horizontal distribution and bathymetric ranges are relatively unknown. Multidimensional scaling analysis in our study showed that bryozoans were distributed in zones or depth bands. Clarke *et al*. (2003) reported that in Antarctica the continental shelf lies at depths between 500 m and 700 m and in some places depths exceed 1000 m, while the continental slope is found at 1000–3000 m and the deep sea at over 3000 m. For example, Prydz Bay is considered to be Antarctic shelf with the deepest areas about 1200 m (O'Brien *et al*., 2007). The bryozoan distribution found in our study fits well with these proposed limits: the species composition of continental shelf (0–700 m or 800–1000 m) differs from that of the continental slope and of the deep sea (>3000 m). However, the sample effort banding may have influenced these results. In agreement with this, Kaiser and colleagues (2011) found that the shelf and abyssal bryozoans were clearly separated in the Weddell Sea.

Some studies have demonstrated that Antarctic megafaunal density generally decreases with depth (Arntz *et al*., 1994; Thatje & Mutschke, 1999; Rex *et al*., 2006; Linse *et al*., 2007), which can be related to the decreasing availability of food with depth. However, other factors could be correlated with this, such as a limited availability of substratum for encrusting species at depth. Decrease in organic matter is considered to be the main limiting factor for the Antarctic benthos (Arntz *et al*., 1994; Lampitt *et al*., 2001; Saiz-Salinas *et al*., 2008). Barnes & Kuklinski (2010) also reported that the bryozoan species richness decreases rapidly with depth. Nevertheless, abundances are very variable at depths of 1000–3500 m and some authors have suggested the existence of patchy distribution patterns (Brandt *et al*., 2005). In agreement with this, slope richness of some taxa and of some areas was larger than that of the shelf or abyss zones (Kaiser *et al*., 2011). In contrast, other findings suggested that abundance increases with depth in some areas of the Weddell Sea and decreases with depth in other areas, such as Kapp Norvegia (Linse *et al*., 2002). In addition, there are other factors we must take into account, such as biological factors (e.g. food availability and predation), which may have more influence at small spatial scales and depths greater than 20 m, where physical distur-

Table 5. Characteristics of the regions sampled with the three dominant types of sampling (AT, BT and RD). For each region: dominant species found in the sample, total number of species found (nº sps), Margalef index (D_{Mg}), Shannon–Wiener diversity index, H' (base log e), Simpson's Index (1 – Lambda'), Pielou's index (J') and Berger–Parker index (B–P).

Site	Dominant species found in the sample	nº sps	D_{Mg}	H′	1-Lambda′	J′	B-P
Bouvet Island	*Osthimosia curtioscula*	13	1.559	1.525	0.7692	0.9188	0.3077
Austasen	*Bostrychopora dentata*	145	8.64	3.445	0.9557	0.7124	0.1241
Vestkapp	*Carbasea curva* *Nematoflustra flagellata*	38	5.498	2.844	0.9294	0.8186	0.1316
Spiess Seamount	*Spigaleos horneroides*	1	0	0	0	1	1
Livingston Island	*Austroflustra vulgaris* *Himantozoum antarcticum* *Klugeflustra antarctica*	3	1.82	1.099	0.6667	1	0.3333
Deception Island	*Melicerita latilaminata*	1	0	0	0	1	1

Table 6. Genera found in this study in Antarctica and Scotia Arc. Additional data from South America, New Zealand and South Africa have been obtained from Moyano (1982, 1999), Gordon (1984, 1986), Florence *et al*. (2007); see also www.bryozoa.net.

Genera	Antarctica	South America	New Zealand	South Africa	Scotia Arc
Carbasea	x	x	x	x	
Klugeflustra	x			x	x
Isosecuriflustra	x				
Austroflustra	x	x	x		x
Nematoflustra	x				
Camptoplites	x	x	x		x
Cornucopina	x	x	x		x
Himantozoum	x	x	x	x	x
Klugella	x				
Notoplites	x	x	x	x	
Cellaria	x	x	x	x	x
Paracellaria	x	x			x
Melicerita	x	x	x		x
Cellarinella	x	x			
Systenopora	x				
Isoschizoporella	x				
Dakariella	x				
Kymella	x				
Smittina	x	x	x	x	
Smittoidea	x	x	x	x	
Thrypticocirrus	x				
Pemmatoporella	x				
Bostrychopora	x				
Osthimosia	x	x	x	x	
Spigaleos	x				
Reteporella	x	x	x	x	
Alcyonidium	x	x	x	x	

bance by ice is less frequent. With this regard, Smale (2008) found high variability in the distribution of species in these conditions.

The result of our cluster analyses indicated a spatial pattern in the distribution of species of bryozoans, and the different regions observed agree with the different zoogeographical zones of diversity suggested by previous authors (Barnes & De Grave, 2000; Barnes & Kuklinski, 2010): the Sub-Antarctic islands (Bouvet Island), East Antarctica (eastern Weddell Sea), West Antarctica and the Scotia Arc (Deception and Livingston Islands). At a smaller scale, we observed a horizontal variability in assemblage composition between some regions. The regions of Kapp Norvegia (30 stations), Vestkapp (20 stations) and Austasen (32 stations) showed the same values of relative species richness. However, the regions of Kapp Norvegia and Vestkapp are more similar (the subgroup of cluster 1 has the highest similarity, 92%), indicating an even greater similarity in species composition. The reason for this similarity could be their proximity. Moreover, Gerdes *et al*. (2008) proposed that the shelf off Austasen has to be considered as a patchwork of disturbed areas and this could be the reason for its higher diversity (higher values of D_{Mg}, H' and 1-Lambda' indices) compared with Vestkapp. In contrast, the value of the Berger–Parker and Pielou's indices were lower because there were many individuals of one species (*B. dentata*). However, this area shows the same value of species richness as the region of Kapp Norvegia (84% similarity).

The regions of Neumayer, the Spiess Seamount and the islands of Bouvet, Deception and Livingston are separated geographically, and exhibit the lowest species richness and diversity because they are distant from other regions and scarcely sampled. Many new records of known or unknown species can be expected to be found in the future. Also, the rarefaction curve showed no sign of approaching an asymptote. Fifty-four species have been found, but total numbers estimated by species richness statistics (Chao2) suggest that at least 90 species of bryozoans will be found in the studied area as more samples are collected.

Some studies have reported that Bouvet Island shows similarity with the region of the Weddell Sea and has a similar taxonomic richness (Barnes, 2006; Gutt *et al*., 2006) and our results for bryozoans are in agreement with that. This could be due to the existence of a permanent import of species by dispersion of marine benthic animals (Pielou, 1975). Other studies have demonstrated that the general composition and diversity of Bouvet Island were not lower compared with the Patagonian shelf and only moderately lower than the Antarctic continental shelf (Arntz *et al*., 2006; Gutt *et al*., 2006).

Bouvet Island and the region of Spiess Seamount are located at a particular position relative to the Antarctic Circumpolar Current and may be in a potential zone of faunal exchange among the various regions and across the Polar Front (Linse, 2006). Larvae of different invertebrates from the Scotia Arc could reach Bouvet Island with the Circumpolar Current or from the Weddell Sea with the Weddell Gyre (Barnes, 2006). One hypothesis is that Bouvet Island could have acted as a supply source to the Weddell Sea during the glacial maximum, when this island was not covered by ice and adults of species could travel on kelp or pumice with currents of the Weddell Sea Gyre (Barnes & Kuklinski, 2010). The benthos of the Spiess Seamount is characterized by being extremely poor (Arntz *et al*., 2006). However, the cluster analyses showed Deception Island to be more separated than the other islands (0% of similarity). San Vicente *et al*. (1997) suggested that the reduced number of species at Deception Island was probably related to the last volcanic episode and to the present acidity in the surface sediment. This could also explain the low bryozoan diversity. Also, the availability of hard substrates limits the abundance and diversity of bryozoans (Hughes, 2001). Many filter feeders have a preference for an elevated position which may enhance prey capture (Wildish & Kristmanson, 1997). Deception Island has few hard substrates and this could affect bryozoan diversity. However, Barnes *et al*. (2008) reported that the undersurfaces of boulders from Deception Island are dominated by bryozoans (cryptofauna).

The presence of a common bryozoan fauna between South America and the Western Antarctica can be explained by their proximity during the Tertiary (Zinsmeister, 1979) and by the relatively similar environmental conditions related to the Antarctic Circumpolar Current (Moyano, 1982).

Various studies support the role of the Scotia Arc as the link between Antarctica and South America (e.g. Arntz *et al*., 2005). In our study, the South Shetland Islands showed a balanced composition between these two regions, thus supporting this hypothesis. Although Antarctic endemism is very high, zoogeographically, there are clear relationships between the fauna of Antarctica and those of South America, New Zealand and South Africa. These similarities could be traced back to the time when continents were part of Gondwana. Also, in the Oligocene, a palaeobiogeographic connection between New Zealand and Patagonia may have existed, as shown by the presence of common taxa, through the West Antarctic Rift System (Casadío *et al*., 2010).

In Antarctica, a clear latitudinal cline in diversity, oriented north to south along the western Antarctic Peninsula, has been reported also for macroalgae and molluscs (Moe & deLaca, 1976; Schiaparelli *et al*., 2006). The existence of a similar cline in bryozoan diversity has been found in this study, with a higher richness at 70–73°S (Austasen, Kapp Norvegia and Vestkap) than at 54–70°S (Bouvet, Livingston and Deception Islands, and Neumayer). However, the interpretation of these results must be treated with some caution because they are based on the frequency of occurrence rather than the abundance of species.

Conclusions

During the past two decades, research of the basic descriptive taxonomy and benthic ecology from the Southern Ocean has improved greatly, demonstrating that this area is quite rich and diverse. However, some almost inaccessible regions, such as some parts of Antarctica, are difficult to sample and the research on biodiversity is limited by the lack of richness data for some groups, such as the bryozoans. Although the results of the analyses performed here from new data on bryozoan biodiversity increase our knowledge of species' geographical ranges, they are still limited because samples were collected from only a few areas. The scales of the latitudinal and the bathymetric gradients are large and the majority of marine studies have only sampled small areas. This causes an underestimation of diversity because it has been demonstrated that species richness varies with increasing sampled area (Gray, 2000). The main limitation of this study is the use of data from different methods of sampling. However, the bathymetric and geographical distributions of the studied species contribute to a better understanding of Antarctic bryozoan diversity and distributions and it is relevant in the establishment of biogeographical patterns. More intensive sampling of bryozoans along a wider geographical range is needed for the Weddell Sea and other Antarctic areas.

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Description of a new species of *Reteporella* (Bryozoa: Phidoloporidae) from the Weddell Sea (Antarctica) and the possible functional morphology of avicularia

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Abstract

Figuerola, B., Ballesteros, M. and Avila, C. 2013. Description of a new species of *Reteporella* (Bryozoa: Phidoloporidae) from the Weddell Sea (Antarctica) and the possible functional morphology of avicularia. —*Acta Zoologica* (Stockholm) **94**: 66–73.

A new species of cheilostome bryozoan, *Reteporella rosjoarum* sp. n., belonging to the family of Phidoloporidae is described from the Weddell Sea (Antarctica) and illustrated with binocular microscope and SEM micrographs. SEM has been used to observe the essential characters to describe the new species and to compare it to similar species. Three samples from three different stations were collected at 332–597 m depth during the ANT XXI/2 cruise of the R/V Polarstern (AWI, Bremerhaven, Germany) using a bottom trawl. The new species is characterized by the presence of giant vicarious spherical avicularia. The distinctive morphology of these avicularia discriminates this species from Antarctica congeners. The variability in avicularian morphometrics demonstrates that intraspecific variation between localities (Austasen and Drescher Inlet) may exist. It is also interesting to relate the morphology of the avicularia with the possible functions in Cheilostomata.

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Introduction

In recent years, the number of taxonomic studies of Antarctic bryozoans has increased notably (e.g., Hayward 1995; Gutt *et al.* 2000; López-Fé de la Cuadra and Garcia-Gomez 2000). Since the scientific results of the Belgian Antarctic Expedition (Waters 1904), over 300 species have been described and new descriptions continue to appear (Clarke and Johnston 2003; Gontar 2008; López and Liuzzi 2008; Kuklinski and Barnes 2009). Cheilostome diversity is very rich in Antarctica, and cheilostomes comprise a major component of Antarctic benthos over extensive areas (Figuerola *et al.* 2012a). Moreover, a high proportion of cheilostomes (56%) are endemic (Hayward 1995; Barnes and De Grave 2000; Clarke and Johnston 2003; Griffiths 2010). New species of bryozoans continue to be described, especially in some regions that are not well known (Moyano 2005), such as the Weddell Sea, and are sensitive to environmental changes (Weller *et al.* 1992; Barnes *et al.* 2006; Winston 2009). Global climate change may lead to range contraction in many species, and documentation of new taxa and their distribution is important to determine future impacts.

Zooid polymorphism is characteristic of cheilostomes, constituting division of labor in these organisms. The avicularium is a modified zooid with a hypertrophied operculum (mandible) that lacks a feeding function. The mandible is opened slowly by contraction of abductor muscles and is closed rapidly by contraction of adductor muscles (McKinney 1998). Avicularia have a wide diversity of shapes and sizes, and this diversity probably relates to variations in their function and ontogeny (e.g., Winston 1984). Their morphology, position, and frequency of occurrence are taxonomically significant and used to define species (e.g., Hayward 1995; Kuklinski and Barnes 2009). Vicarious avicularia take the place of an ordinary autozooid and are the same size or larger than regular zooids in the colony (Hyman 1959).

Among the bryozoan families present in Antarctica, the family Phidoloporidae Gabb and Horn, 1862, is moderately well represented (10 or more species). Popularly referred to as lace corals, they occur in shelf and coastal benthic environments throughout the world, including polar seas (Hayward 1999).

This family is mostly characterized by a branching, reticulate, or encrusting colony, a few marginal pores on the autozooid frontal wall, a sinuate or rounded primary orifice, the presence of a beaded distal rim in the primary orifice, and a variety of adventitious avicularia. In this family, most Antarctic species belong to the genus *Reteporella* Busk, 1884, which presents either a tree-like colony architecture, a reticulate cup, or has fenestrate sheets folded and anastomosing to develop complex three-dimensional colonies. The genus is widely distributed in Antarctica, with the majority of species living in Antarctic shelf areas (Hayward 1995). *Reteporella* includes most of the species originally assigned to the genus *Retepora* (Lamarck, 1801). Eleven Antarctic species have been described in the genus so far (Table 1). In this work, we describe a new species of *Reteporella* and discuss the functional morphology of avicularia. We also compare the characteristics of all the species of this genus from Antarctica. Finally, because the three samples of this species show biometrical differences, the intraspecific variability has been analyzed.

Materials and Methods

Bryozoans were collected during the Antarctic cruise ANT XXI/2 (December 2003–January 2004) of R/V Polarstern (Alfred Wegener Institute, Bremerhaven, Germany). Samples were obtained from three stations in the eastern Weddell Sea area. A bottom trawl was used to collect the samples, which occurred at depths of 332–598 m. Bryozoan colonies were sorted on deck and preserved in 70% ethanol. For studying skeletal morphology, the samples were cleansed in an ultrasonic bath or bleached with NaClO. Specimens were coated with gold and examined using SEM at the Scientific-Technical Services of the University of Barcelona.

Morphological characters of the three samples were measured by binocular microscope ($n = 10$).

All variables were tested for normality (Kolmogorov–Smirnov's test) and for homogeneity of variance (Levene, *F*-test). Significant effects were further explored with one-way analysis of variance (ANOVA) followed by Games–Howell *post hoc* tests (GH tests). The GH *post hoc* multiple comparison test is one of the most powerful and robust for unequal variances (Day and Quinn 1989). SPSS was used for the analyses.

The holotype and two paratypes described in this study were deposited at the 'Centre de Recursos i Biodiversitat Animal' (CRBA) of the University of Barcelona, Barcelona, Spain.

Results

Systematics

Suborder Ascophorina Levinsen, 1909
Infraorder Lepraliomorpha Gordon, 1989
Superfamily Celleporoidea Johnston, 1838
Family Phidoloporidae Gabb & Horn, 1862
Genus *Reteporella* Busk, 1884
Reteporella rosjoarum sp. n. (Figs 1–2)

Material examined

Holotype: One fragment of a colony (#1172: CRBA-4260) preserved in 70% ethanol; collected by C. Avila and M. Ballesteros. Region of Drescher Inlet (Weddell Sea, Antarctica), 72°51.43′S, 19°38.62′W (597.6 m).

Paratypes: Two fragments of different colonies (#560: CRBA-4258 and #864: CRBA-4259) preserved in 70% ethanol; collected by C. Avila and M. Ballesteros. Region of Austasen (Weddell Sea, Antarctica), 70°57.33′S, 010°33.86′W (351.6 m) and 70°57.00′S, 10°33.02′W (332.8 m), respectively.

Diagnosis

Reteporella with colony formed from reticulate sheets; autozooids nearly rectangular, centrally convex, depressed laterally, separated by indistinct sutures (Figs 1A,B and 2A–D). One or more adventitious avicularia present on each autozooid: oval, smaller than autozooids, with varying orientation. Giant avicularia frequent, spherical, vicarious, larger than autozooids, mandible semicircular, normal to frontal plane of colony, with varying orientation (Fig. 2E–L). Ovicell hyperstomial, globose, flattened frontally; frontal longitudinal fissure present (Fig. 2M–N).

Description

Colony heavily calcified, comprising folded reticulate sheets; maximum size and architecture unknown. Fenestrules commonly 2.25–3.45 × 0.75–1.8 mm, but with different sizes in different parts of the colony; their shape usually irregular: oval, elliptical, round-triangular and usually elongated and oval. Trabeculae variable in width, usually consisting of 3–6 longitudinal autozooidal series (Figs 1A,B and 2A,B). Frontal side of the colony formed by autozooids, avicularia, and gonozooids. Dorsal side of colony divided by boundary walls into very large areas, only presenting pores and one kind of avicularia. Autozooids situated on frontal side of colony, nearly rectangular, 0.67–1.01 × 0.22–0.45 mm, centrally convex, depressed laterally, separated by indistinct sutures. Zooidal frontal shield smooth, without tuberculation, perforated by 2–3 small, inconspicuous marginal pores. Primary orifice subcircular, usually longer than wide, distolateral rim finely denticulated (Fig. 2C,D). Without oral spines. Operculum subcircular, longer than wide. Peristome developed simply as a projecting angular lobe on one side of proximal edge of orifice, which remains partly visible in frontal view. Two kinds of avicularia present on frontal side. One or more adventitious

Table 1 Main characters of all known Antarctic species of *Reteporella* and their distributions

Species	LF (mm)	SA	MP	OS	N avicularia	F avicularia	L avicularia (mm)	Shape of ovicell	Locality
R. antarctica (Waters 1904)	1–1.5	3–6		No	≥1	Oval	0.25	Oval	Queen Mary, Ross Sea, Bellingshausen Sea, S. Georgia
					Variably	Triangular	–	Scaphoid	
R. dorsoporata (Liu and Hu 1991)	–	6–10	3–4	2	–	Triangular, hooked	–	Globose	Weddell Sea
					–	Elliptical	–		
R. erugata (Hayward 1993)	1.5–2 × 0.75–1	4–8	2–3	No	Numerous	Oval	0.1–0.15	Globular, broader	S. Georgia, Palmer Archipelago, Ross Sea
					≥1 within each fenestrula	Triangular, sharply hooked, giant	0.3–0.35		
R. frigida (Waters 1904)	1.5–2 × 0.5–1	4–8	2–3	2	Numerous	Oval, lacking columella, extensive palate	0.1–0.25	Flattened, median fissure	S. Shetlands, Palmer Archipelago, Ross Sea, Bellingshausen Sea
					Variably, also within fenestrulae	Triangular, sharply hooked	0.5		
R. frigidoide a (Liu and Hu 1991)	–	4–8	–	2	Variably	Triangular, hooked	–	Globose	Weddell Sea
					Variably	Triangular, smaller	–		
R. gelida (Waters 1904)	1–1.5 × 0.75	3–6	2–3	2	Numerous	Crossbar, lacking columella, small rounded palatal foramen	0.1–0.15	Globose, median fissure	Palmer Archipelago, Bellingshausen Sea, Ross Sea
					Numerous, within fenestrulae	Elongate triangular	>0.3		
R. hippocrepis (Waters 1904)	1.5–2.5 × 0.5–1	3–6	Few	No	Numerous	Oval, stout crossbar lacking a columella	0.1	Oval, short frontal fissure	Numerous localities, common in Ross Sea
					Often within fenestrulae	Triangular, hooked rostrum	>0.4		
R. lepralioides (Waters 1904)	1–2	3–6	Few	2	≥1	Oval, crossbar, lacking columella	0.1–0.15	Globose, median foramen	Bellingshausen Sea, few localities in the Ross Sea
					–	Triangular, abruptly hooked	0.25		
R. longichila (Hayward 1995)	2–2.5 × 1	3–5	3–4	3	Numerous	Oval, stout crossbar, lacking columella, extensive palate	0.1–0.15	–	Palmer Archipelago, 6 stations in the Ross Sea
					Inner edges of the fenestrulae	Columella on the crossbar, short median fissure	>0.4		
R. parva (Hayward 1995)	1–1.5 × 0.5–0.75	2–4	2–4	3	Infrequent	Crossbar bearing a knob-like columella, rostrum hooked	0.35	–	Single locality of Ross Sea
					–	Oval	–		
R. protecta (Waters 1904)	0.75–1.5 × about 0.5	3–8	3–5	3	Numerous	Oval, semicircular mandible, proximal to peristome	0.05–0.1	Elongate oval, frontal fissure	S.Georgia, S. Shetland Isles, Palmer Archipelago, Bellingshausen Sea
					Numerous, also within fenestrulae	Triangular, large	0.2–0.25		
R. rosjoarum sp.n.	2.25–3.45 × 0.75–1.8	3–6	2–3	No	Numerous	Giant with cystid, globular, semicircular mandible	0.61–0.7	Globose, median foramen	Weddell Sea
					Numerous	Oval, crossbar, lacking columella	0.13–0.16		

LF, length of fenestrules; SA, serial autozooids; MP, marginal pores; OS, oral spines; N, number of avicularia; F avicularia, shape of avicularia; L avicularia, length of avicularia.

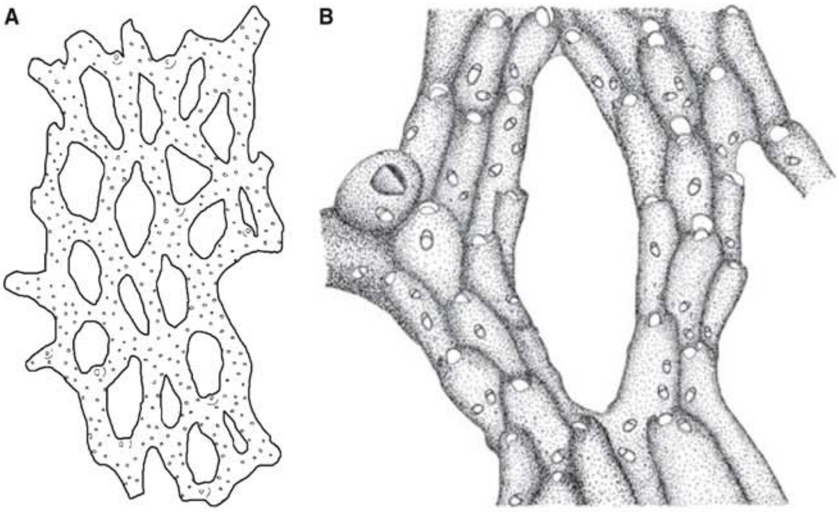


Fig. 1—*Reteporella rosjoarum* sp. n. —**A.** Colony (scale bar: 1 mm), top: proximal, bottom: distal. —**B**. Zooids (scale bar: 2 mm).

avicularia present on each autozooid: oval, 0.13–0.16 mm long, smaller than autozooids, normal to frontal plane, with varying orientation, crossbar stout, lacking a columella. Giant avicularia frequent, spherical, vicarious, 0.61–0.7 × 0.52–0.65 mm, larger than autozooids, mandible semicircular, normal to frontal plane of colony, with varying orientation; fenestral avicularia few in number, usually one or more within a fenestra; the orientation of the mandible dependent on the position of the avicularia in a fenestra. Dorsal side with only adventitious avicularia (Fig. 2E–L). Ovicell hyperstomial, prominent, globose, imperforate, flattened frontally, longer than wide, or equal in length and width; frontal longitudinal fissure present, typical of species of *Reteporella* (Fig. 2M,N).

Etymology

Reteporella rosjoarum sp. n. is dedicated to the parents of the first author, Roser Balañá and Joan Figuerola, for giving her the opportunity to study biology.

Remarks

This species differs from all other Antarctic and Subantarctic species of *Reteporella* by the presence of giant spherical avicularia and the absence of elongate triangular avicularia (Fig. 2E–H). The new species resembles only *Reteporella erugata* in the general form of its autozooids and peristome. Morphological characters are similar in the three samples and only differ significantly ($P < 0.05$) in five measurements: mean number of the length of primary orifice of the autozooid, width of the small avicularia, width of the giant avicularia, and length and width of the mandible of the giant avicularia (Table 2–3).

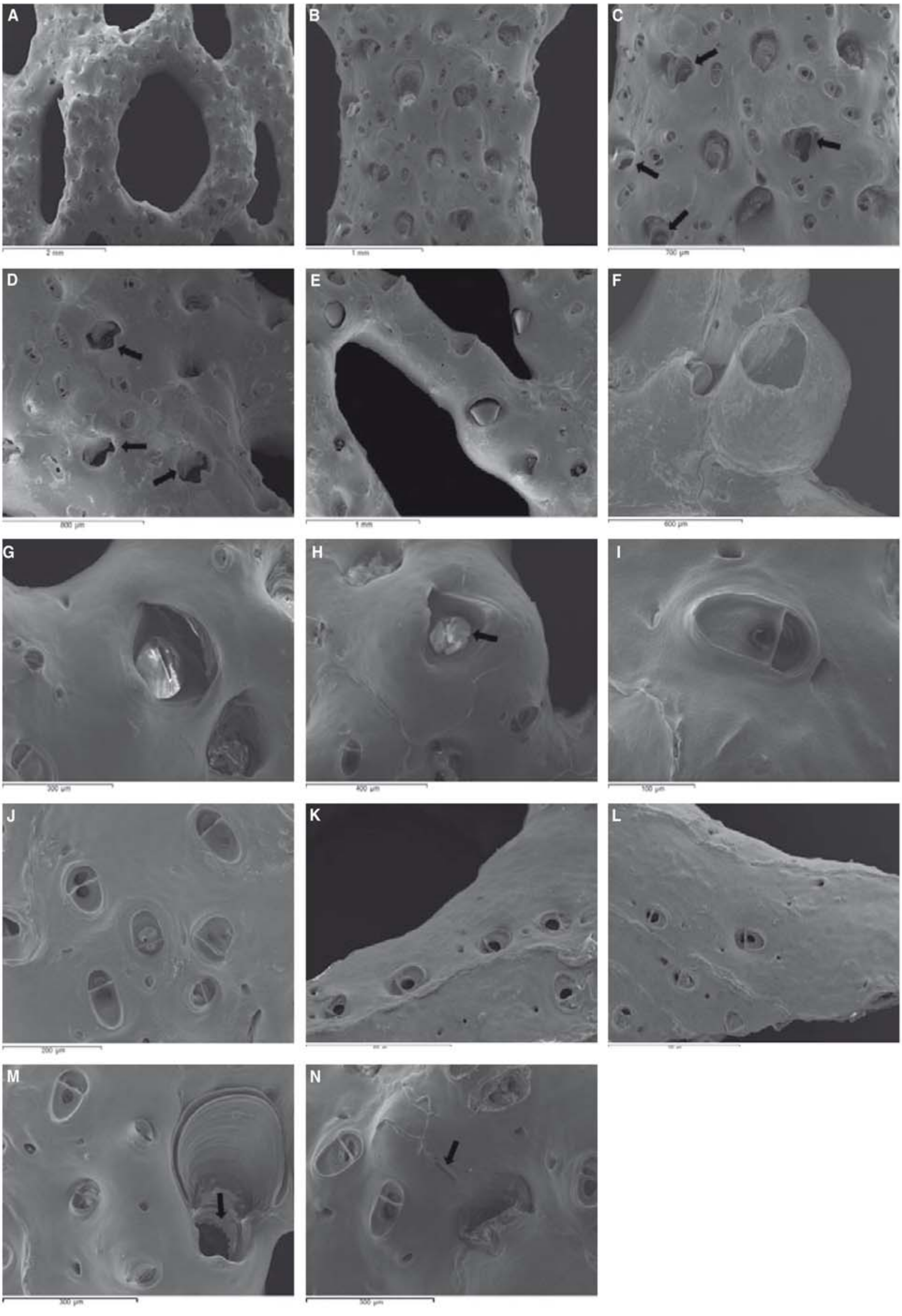
Discussion

Bryozoans are filter-feeding animals and mostly sessile. For this reason, they require physical and chemical defenses (Figuerola *et al.* 2012b). Although the functions of the variety of avicularia are still largely unknown, morphological and behavioral studies suggest physical protection, cleaning, and defensive functions against predators or grazers (Busk 1854; Harmer 1909; Canu and Bassler 1929; Hyman 1959; Cook 1963; Winston 1984, 1991).

The morphological diversity of avicularia is likely to be of considerable ecological importance, representing different responses to various selective pressures in the environment, and it may indicate a variety of biological roles (Carter 2008). Stable environments, such as Antarctic environment, seem to be favorable to the evolution of complex systems of polymorphism in cheilostome bryozoans (Venit 2007). The presence of spherical avicularia may indicate that this unusual form has adaptive value in this area (Winston 2009). Several observations have demonstrated the ability of certain avicularia to capture small organisms. The trapped organisms are generally too large to be consumed by autozooids, but over time, they may die and decay, attracting ciliates and bacteria upon which bryozoans are known to feed (Kaufmann 1971; Winston 1984, 1991). The giant size of avicularia may be more advantageous to capture relatively large parts of microinvertebrates. Additionally, Silén (1977) suggested that certain species possess more than one type effective against different predator types. The peculiar form of the spherical avicularia may be a specialization to capture a particular predator. Also avicularia of different sizes can coexist in one colony and could capture a wider spectrum of predators (Carter *et al.* 2010b).

Observations on living colonies have shown that sessile avicularia capture organisms belonging to groups capable of predation on bryozoans, including syllid polychaetes, amphipods, isopods, nudibranchs, pycnogonids, and nematodes (Winston 1986, 1991; Carter *et al.* 2010a), some of which are abundant in Antarctica.

Hastings (1945) suggested that the large avicularia of *Crassimarginatella exilimargo,* which have a vestigial polypide with tentacles and functional gut, could have the capacity to


A
B
C
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F
G
H
I
J
K
L
M
N
2 mm
1 mm
700 μm
800 μm
1 mm
600 μm
300 μm
400 μm
100 μm
200 μm
300 μm
500 μm

Table 2 Means of the measurements of characters between the three samples of *Reteporella rosjoarum* sp. n. Measurements in millimeters, with an average of 10 measurements for each structure

	Sample #560			Sample #864			Sample #1172		
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Primary orifice of autozooid, length	10	0.1388*	0.0282	10	0.1594	0.0203	10	0.1875*	0.028
Primary orifice of autozooid, width	10	0.1463	0.0328	10	0.1556	0.0266	10	0.1594	0.0182
Primary orifice of ovicelled zooid, length	1	0.1313	–	10	0.1425	0.0131	10	0.1781	0.0408
Primary orifice of ovicelled zooid, width	1	0.1313	–	10	0.195	0.0268	10	0.1706	0.0138
Ovicell length	1	0.2625	–	10	0.3806	0.0251	10	0.3338	0.0373
Ovicell width	1	0.2625	–	10	0.3375	0.0492	10	0.3469	0.047
Small avicularia length	10	0.1556	0.028	10	0.1406	0.0283	10	0.1294	0.0272
Small avicularia width	10	0.0881*	0.0127	10	0.0675*	0.0158	10	0.0788	0.0172
Giant avicularia length	10	0.6056	0.1492	3	0.7	0.0573	10	0.615	0.1189
Giant avicularia width	10	0.5213*	0.1182	3	0.65*	0.0286	10	0.57	0.1384
Mandible of giant avicularia length	10	0.1819*	0.0127	3	0.2438*	0	10	0.1969*	0.0345
Mandible of giant avicularia width	10	0.2644*	0.0488	3	0.3188*	0	10	0.2738*	0.0469

*Means differ significantly using the Games–Howell *post hoc* test ($P < 0.05$)

Table 3 Values of the differences of means of the three samples of *Reteporella rosjoarum*

Samples	Difference of means (I-J)	L of P.orifice autozooid	Sig.	W of small avicularia	Sig.	W of giant avicularia	Sig.	L of mandible of giant avicularia	Sig.	W mandible of giant avicularia	Sig.
#864	1172	−0.02813*	0.05	−0.01125	0.30	0.08	0.25	0.04688*	0.01	0.04500*	0.03
	560	0.020625	0.18	−0.02062*	0.01	0.12875*	0.02	0.06188*	0.00	0.05437*	0.02
#1172	864	0.02813*	0.05	0.01125	0.30	−0.08	0.25	−0.04688*	0.01	−0.04500*	0.03
	560	0.04875*	0.00	−0.009375	0.37	0.04875	0.68	0.015	0.43	0.009375	0.90
#560	864	−0.020625	0.18	0.02062*	0.01	−0.12875*	0.02	−0.06188*	0.00	−0.05437*	0.02
	1172	−0.04875*	0.00	0.009375	0.37	−0.04875	0.68	−0.015	0.43	−0.009375	0.90

*Means differ significantly using the Tukey test ($P < 0.05$). L, length; P, primary; W, width.

feed and to distribute nutrients. The funicular system of the parent autozooid maintains this miniature polypide. This might be the case in the large avicularium *R. rosjoarum* too.

The function of avicularia in chemical defense has been relatively understudied. The avicularia of *Synnotum aegyptiacum* have very large glands, whereas autozooids do not (Marcus 1939). Consequently, they seem be important in the function of the avicularia. Winston (1984) suggested that some avicularia may contain secretory compounds. Lutaud (1964) found bacteria in the glands of some avicularia. The large capacity of the spherical avicularia in our species could indicate also the presence of large glands, although further studies are needed to test this.

Also avicularia could be actively involved in deterring settlement of epibionts, as well as preventing other organisms from removing food particles otherwise intended for autozooids (Harmer 1909). Giant avicularia are situated around the fenestrae, and Harmer (1909) demonstrated that, in these areas of *Reteporella*, the colony is completely free from encrusting organisms and the larvae of conspecifics do not appear to settle.

A more reasonable hypothesis for the presence of avicularia with a large internal capacity could be the additional function as a storage reserve for nutrients and to distribute resources during unfavorable conditions (Winston 1984). *Reteporella rosjoarum* sp. n. differs from the 11 Antarctic species described of the same genus mainly by the presence of giant vicarious, spherical avicularia. The distinctive morphology, position, and frequency of occurrence of these avicularia are taxonomically important, and these characters are frequently used to define species. Variability in avicularian morphometrics is common in bryozoans and demonstrates the existence of

Fig. 2—*Reteporella rosjoarum* sp. n. —**A** and **B**. Part of the colony. —**C.** Denticulations of autozooids. —**D**. Detail of the primary orifice. —**E.** Group of giant spherical avicularia. —**F**. Giant avicularium without mandible. —**G**. Giant avicularium. —**H.** Orifice in the palate of the avicularium. —**I.** Small avicularium. —**J**. Group of small avicularia. —**K** and **L**. Small avicularia of dorsal side of the colony. —**M**. Denticulations of an ovicell. —**N**. Detail of an ovicell showing the longitudinal fissure. Scanning electron microscope images: (**A–E, G–L)** holotype, CRBA-4260; (**F**) paratype, CRBA-4258, both from the Weddell Sea).

intraspecific variation between localities. The absence of a typical elongate triangular avicularia also discriminates this species from Antarctica congeners. However, the general form of its autozooids and peristome is similar to another species, *Reteporella erugata*.

Mean values vary significantly in five characters of samples examined of the new species (Table 3). However, all other morphological characters are similar in the three colonies (Table 2), and therefore, these differences could be due to variation in environmental factors (Kuklinski and Barnes 2009) at the three stations (e.g., sedimentation and temperature) (Moore 1977).

The presence of the giant avicularia in the three specimens indicates that they belong to the same species and that intraspecific variation between localities (Austasen and Drescher Inlet) may exist. Moreover, intraspecific variability in avicularian morphometrics is common (Carter *et al.* 2010b), and the length of avicularia is a significant contributor to morphometric variability (Carter *et al.* 2008).

Further studies, as well as more samples from the Weddell Sea, are needed to establish the general shape of the colony and the distributional range of this species.

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Appendix II

List of other articles published or under review related to this thesis (*the published chapter is included below in their original format):

Figuerola B, Taboada S, Monleón A, Vázquez J, Avila C (*in press*) Cytotoxic activity of Antarctic benthic organisms against the common sea urchin *Sterechinus neumayeri*. *Oceanography: open access*

Xavier JC, Barbosa A, Agustí S, Alonso-Sáez L, Alvito P, Ameneiro J, Avila C, Baeta A, Canário J, Carmona R, Catry P, Ceia F, Clark MS, Cristobo FJ, Cruz B, Duarte CM, **Figuerola B**, Gili JM, Gonçalves AR, Gordillo FJL, Granadeiro JP, Guerreiro M, Isla E, Jiménez C, López-González PJ, Lourenço S, Marques JC, Moreira E, Mota AM, Nogueira M, Núñez-Pons L, Orejas C, Paiva VH, Palanques A, Pearson GA, Pedrós-Alió C, Peña Cantero AL, Power DM, Ramos JA, Rossi S, Seco J, Sañé E, Serrão EA, Taboada S, Tavares S, Teixidó N, Vaqué D, Valente T, Vázquez E, Vieira RP, Viñegla B (*in press*) Polar marine biology science in Portugal and Spain: Recent advances and future perspectives. *Journal of Sea Research*

***Figuerola B**, Núñez-Pons L, Vázquez J, Taboada S, Cristobo FJ, Ballesteros M, Avila C (2012) Chemical interactions in Antarctic marine benthic ecosystems. In: Cruzado A (eds) *Marine ecosystems.* In-Tech, Rijeka, Croatia, pp 105–126

Figuerola B, Vázquez J, Montes MJ, Mercadé E, Blanch AR, Avila C (*submitted*) Antibacterial activity in Antarctic bryozoans

Moles J, Campanyà- Llovet N, **Figuerola B**, Monleón-Getino, Avila C (*submitted*) Distribution patterns and biodiversity in Antarctic sea stars, brittle stars and sea cucumbers (Echinodermata; Asteroidea, Ophiuroidea and Holothuroidea)

5

Chemical Interactions in Antarctic Marine Benthic Ecosystems

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1. Introduction

Antarctic marine ecosystems are immersed in an isolated, relatively constant environment where the organisms inhabiting their benthos are mainly sessile suspension feeders. For these reasons, physical and chemical biotic interactions play an essential role in structuring these marine benthic communities (Dayton et al., 1974; Orejas et al., 2000). These interactions may include diverse strategies to avoid predation (e.g. Iken et al., 2002), competition for space or food (e.g. Bowden et al., 2006) and avoiding fouling (e.g. Rittschof, 2001; Peters et al., 2010). For instance, in the marine benthos, one of the most extended effective strategies among sessile soft-bodied organisms is chemical defense, mediated by several bioactive natural products mostly considered secondary metabolites (e.g. Paul et al., 2011). The study of the "chemical network" (chemical ecology interactions) structuring the communities provides information about the ecology and biology of the involved species, the function and the structure of the community and, simultaneously, it may lead to the discovery of new compounds useful to humans for their pharmacological potential (e.g. Avila, 1995; Bhakuni, 1998; Munro et al., 1999; Faulkner, 2000; Lebar et al., 2007; Avila et al., 2008). In the last three decades, the study of marine chemical ecology has experienced great progress, thanks to the new technological advances for collecting and studying marine samples, and the possibility of identification of molecules with smaller amounts of compounds (e.g. Paul et al., 2006, 2011; Blunt et al., 2011).

Polar organisms have been less studied, compared with their temperate and tropical counterparts (Paul, 1992; Blunt et al., 2009). However, recent studies report that Antarctic benthic invertebrates are a rich and diverse source of natural products, with great interest from both the ecological and the pharmacological point of view (e.g. Avila et al., 2000, 2008; Amsler et al., 2001; Iken et al., 2002; Lebar et al., 2007; Reyes et al., 2008; Taboada et al., 2010; Paul et al., 2011). Moreover, several researches have demonstrated that some Antarctic species inhabiting shallow areas from McMurdo Sound and the Antarctic Peninsula possess chemical defenses (for review see Avila et al., 2008; McClintock et al., 2010), even if only in few cases the chemistry of the metabolites involved has been fully described and/or their ecological role has been established (e.g. Núñez-Pons et al., 2010; Núñez-Pons et al., in prep).

In the last years our research group has been studying the ecological activity of marine natural products obtained from Antarctic benthic organisms by using *in situ* experiments. Furthermore, as part of our investigations, previously unknown species for science have been described (Ballesteros & Avila, 2006; Ríos & Cristobo, 2006; Figuerola et al., in press), and new compounds have been isolated and described too (e.g. Antonov et al., 2008, 2009, 2011; Reyes et al., 2008; Carbone et al., 2009; Carbone et al., in prep). Also, we have extended the range of species from our previous analysis by studying Antarctic macroalgae, which are known to be prolific producers of secondary metabolites with pharmaceutical applications (e.g. Hoyer et al., 2002; Ankisetty et al., 2004). As a general objective our aim here is to integrate all the experimental data obtained from the assays conducted with different taxonomical groups in order to establish a preliminary ecological model of the chemically-mediated interactions in the Antarctic benthos. This model will, for the first time, consider the mechanisms that regulate the chemical interactions among the different Antarctic benthic organisms studied. Our specific objectives are trying to determine the a) feeding-deterrence activities towards sympatric predators, including a macropredator (*Odontaster validus* Koehler) and a mesograzer, *Cheirimedon femoratus* Pfeffer, b) toxicity potential against a copepod, *Metridia gerlachei* Giesbrecht, c) cytotoxicity against embryos and sperm of the Antarctic sea urchin *Sterechinus neumayeri* Meissner and d) antifouling activity against microbial biofilms.

2. Material and methods

2.1 Samples collection and identification

Marine benthic invertebrates and algal samples were collected in the Southern Ocean in four Antarctic campaigns: two in the Eastern Weddell Sea (Antarctica) and vicinities of Bouvet island (Sub-Antarctica) on board the R/V Polarstern, from the Alfred Wegener Institute for Polar and Marine Research (AWI Bremenhaven, Germany) during the ANT XV/3 (January-March 1998) and ANT XXI/2 cruises (November 2003-January 2004); a third one on board the BIO Hespérides during the ECOQUIM-2 cruise (January 2006) around the South Shetland Islands; and finally, the ACTIQUIM-1 cruise at Deception Island mainly by scuba-diving, although other sampling methods were used as well (December 2008-January 2009). Sample collection took place between 0 m and 1524 m depth by using various trawling devices: bottom trawl, Agassiz trawl, Rauschert dredge and epibenthic sledge, and also, as said, by scuba diving (0-15m). Samples were sorted and photographed on deck, frozen at -20 °C, and a voucher portion of each sample or, in some cases, whole individuals, were fixed in 10% formalin or 70% ethanol and stored at the Dept. of Animal Biology (Invertebrates), University of Barcelona (Spain), for taxonomical identification.

Individuals of the sea star *Odontaster validus*, the sea urchin *Sterechinus neumayeri*, the amphipod *Cheirimedon femoratus* and the copepod *Metridia gerlachei* were collected for *in situ* ecological experiments in Deception Island by scuba diving at Port Foster Bay (Deception Island: 62º 59,369' S, 60º 33,424' W) from 0-15 m depth (December 2008 - January 2009 and January 2010). After experimentation, these invertebrates were brought back alive to the sea.

2.2 Chemical extractions

Chemical extractions were done in the laboratories from the Faculty of Biology (University of Barcelona). Frozen animals were carefully dissected into different sections when possible, in order to locate the compounds within the body of the organisms (although this is not discussed here). The different sections were made according to the taxonomic group (e.g. internal/external, apical/basal parts in sponges, echinoderms and tunicates; poliparium/axis in cnidarian octocorals; mantle/foot in opistobranch molluscs; gill slits in ascidians; tentacles in holoturian echinoderms…). These body sections were extracted separately, and thus the total number of extracts is larger than the total number of species tested. Samples were extracted with acetone, and sequentially partitioned into diethyl ether and butanol fractions. All steps were repeated three times, except for the butanol which was done once. Organic solvents were then evaporated under reduced pressure, resulting in dry diethyl ether and butanolic extracts, and an aqueous residue. An aliquot of all the diethyl ether extracts (lipophilic fraction) was used for the bioassays at different concentrations for the different experiments. The detailed description of the extraction procedure has been reported elsewhere (Avila et al., 2000; Iken et al., 2002). Butanolic extracts and water residues were kept aside for future investigations.

2.3 Experiments of chemical ecology and statistical treatment

All experiments of chemical ecology took place in the Spanish Antarctic Base “Gabriel de Castilla” in Deception Island (South Shetland Islands, Antarctica) during the Austral Summers of 2008-2009 and 2009-2010.

2.3.1 Feeding experiments with a macropredator, the seastar *Odontaster validus*, and a mesograzer, the amphipod *Cheirimedon femoratus*

The omnivorous sea star *O. validus* occupies the top predator position that fish occupy in temperate and tropical areas (McClintock, 1994). For this reason, this ubiquitous sea star is used as putative macropredator in feeding-deterrence experiments to test the presence of chemical defenses in selected marine invertebrates and algae (e.g. Avila et al., 2000, Iken et al., 2002). The amphipod *Cheirimedon femoratus* was chosen as mesograzer consumer in feeding-preference assays because this voracious, omnivorous-scavanger crustacean is found in notably high densities in Antarctica exerting remarkable, localized ecological pressures, often underestimated (Huang et al., 2007).

The sea star experiments were carried out over 24 h. Extracts, fractions and/or isolated compounds were dissolved in the solvent carrier (diethyl ether) and slowly pipetted at their natural dry weight concentration (mg extract g^{-1} dry wt tissue) onto shrimp pieces, and the solvent was left to totally evaporate under the hood, resulting in a uniform coating of extract. Normalization of natural concentrations based on biomass using wet or dry weight are appropriate when ingredients are homogeneously distributed, and also when using biting and not-biting predators. Moreover, dry weight has been proven to be the most constant parameter for avoiding the variability caused by the water content. Control shrimp pieces were treated with solvent only. Feeding-deterrence experiments are described in detail in precedent investigations (e.g. Avila et al. 2000). The bioassays consisted on 10 replicates in which the sea stars were individually transferred into 2.5 l-buckets filled with fresh seawater (1±0.5ºC), and they were offered a treatment or a control diet, respectively, by putting a shrimp piece in the centre of the bucket and the asteroid on top. A food item was considered rejected when *Odontaster validus* lost physical contact with it, and it was considered eaten when the food was ingested completely after the testing period (Fig. 1). Afterwards, eaten and uneaten shrimp pieces were counted for statistical analysis. Feeding repellence was evaluated as a contingency table 2x2, and since the number of replicates was small (n=10) by using Fisher's Exact tests for each experiment using extract-treated shrimp pieces referred to the control run simultaneously (Sokal & Rohlf, 1995).

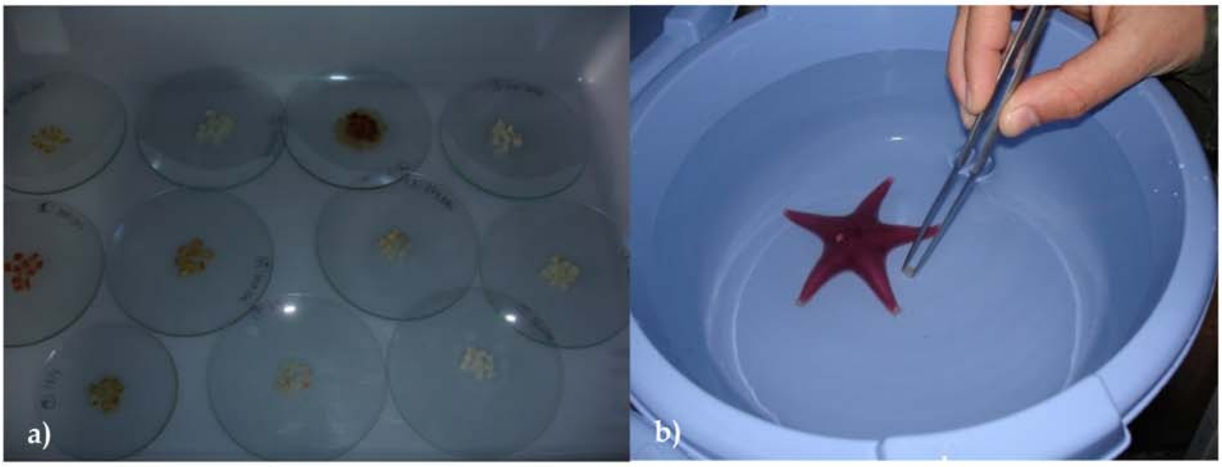

Fig. 1. *Odontaster validus* feeding-repellence experiments. a) Shrimp pieces being prepared with extract coatings for the tests; b) A sea star being offered a shrimp piece.

The generalist amphipod *Cheirimedon femoratus* was used as a potential mesograzer predator. It was presented to a simultaneous choice of two different food types, consisting of a control (extract-free) diet (which the predator readily consumed) and a treatment diet, where the extracts were included at natural concentration according to a dry weight basis (see above). Both diets consisted on alginate-based artificial foods containing a powdered commercial aquarium diet as a food attractant. Control food was prepared with only solvent, which was left to evaporate onto the food powder prior to being gelified into food pellets. For treatment diets, extracts were added into the food mixture dissolved in the carrier solvent (which was similarly evaporated). Groups of 15-20 amphipods were transferred into 1L-bottles filled with sea water, and were presented to a choice of extract-free control and extract-treated diets. The assays ran until either food type had been consumed up to one-half or more. At the end of the experiment, the consumed food was calculated for statistical analysis and determination of feeding preferences of extract-treated foods from the paired simultaneous controls to consequently establish repellent activities. The two food types were presented together, and therefore we measured separately for each replicate container and each food type the quantity of ingested food, and calculated the differences for each experimental unit (replicate). The changes in the two food types held in the same container are not independent and possess correlated errors, making it impossible to analyze them separately. Each replicate is represented by a paired result yielding two sets of data (treatments and controls), which can be compared, since assumption of normality and homogeneity of variances are not met, by non-parametric procedures, that is by applying the Exact Wilcoxon test, which was calculated using R-command software.

2.3.2 Toxicity activity against the copepod *Metridia gerlachei*

Metridia gerlachei is a common omnivorous copepod frequently found in the waters of Port Foster (King & LaCasella, 2003). For this experiments, we used plates with 2 ml seawater where 10-15 copepods were placed. Each experiment consisted of 5 replicates with the ethereal extract to be tested at natural concentration, 5 negative control assays (only filtered sea water), and 5 solvent assays (filtered seawater with solvent). During experimentation, copepods were observed over time for survival. Extracts were considered toxic when, considering the 5 replicates for each test, >50% of the copepods died.

2.3.3 Cytotoxicity activity against embryo and sperm in the Antarctic sea urchin *Sterechinus neumayeri*

Sessile organisms may prevent the settlement of sympatric organisms by displaying cytotoxic activities that may act against embryos and larvae of other invertebrates, in their attempt to colonize the surface of sessile invertebrates, such as sponges, ascidians, bryozoans and polychaetes (e.g. Heine et al., 1991; McClintock et al., 1990). In the Antarctic marine benthic environment, *Sterechinus neumayeri* is one of the most abundant and common species of sea urchin, and its biology is well known (*e.g.* Bosch et al., 1987; Brey et al., 1995). For these reasons, this species was chosen for our bioassays.

After acclimatization, sea urchins were induced to spawn by injecting 1ml of 0.5 M KCl solution into the coelomic cavity through the peristome. The cytotoxicity test was developed according to the protocol proposed by Volpi Ghirardini and collaborators (2005) for the Mediterranean sea urchin *Paracentrotus lividus*. Some modifications were introduced in the original procedure, mainly focused on the volume of sea water used and the time that embryos were exposure to extracts, in order to adapt it to the characteristics of *S. neumayeri*. Details of this modified method are described in a paper that is being prepared (Figuerola et al., in prep) (Fig. 2). The percentage of blastula stage in each treatment was determined for statistical analysis. A S regression model ($Y = \exp(0.702 + 124{,}928/X)$, $R^2 = 0.6125$) was calculated between % of the number of the blastula (Y) and the initial concentration of eggs

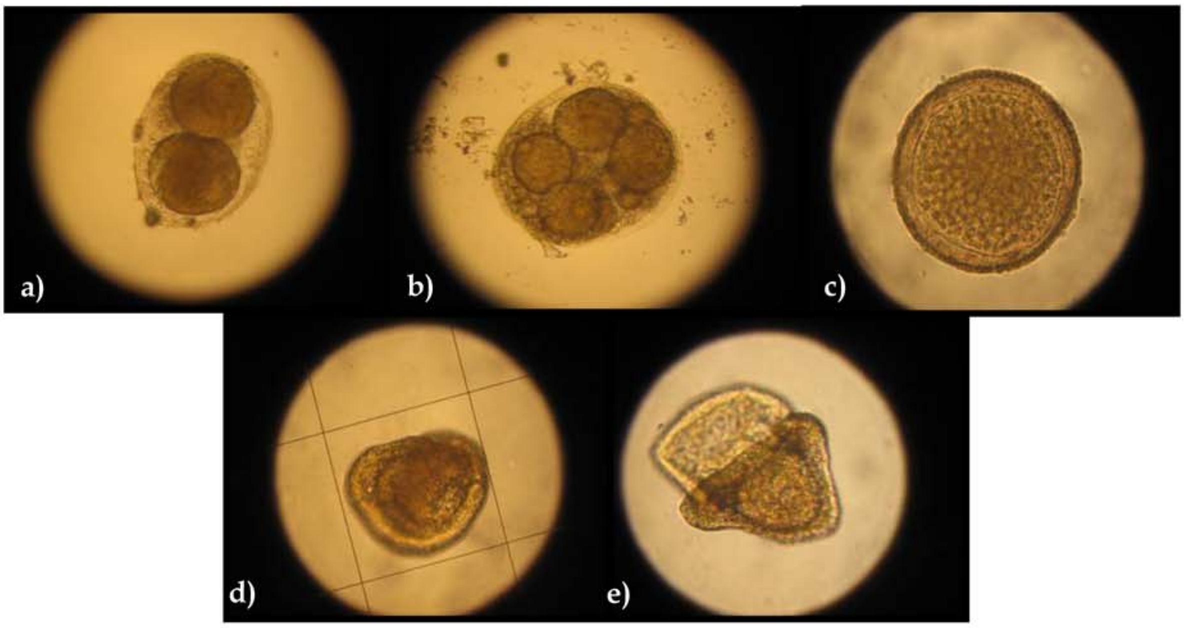

Fig. 2. Different stages of embryonic development of the sea urchin *Sterechinus neumayeri*.
a) Stage of 2 cells; b) Stage of 4 cells; c) Morula stage; d) Dipleurula larva; e) Pluteus larva.

(X) only using the data from the control group and solvent to obtain a representation of the normal behavior (without the influence of the extracts) of the experimental conditions. Also, confidence intervals (CI) of prediction (upper and lower: UCL and LCL) of 95% coverage were calculated to detect extract samples outside CI.

The sperm test was developed following the procedure of similar experiments conducted in the past using *S. neumayeri* (Heine et al., 1991; McClintock et al., 1992). Previous sperm assays utilized 25-ml volumes of test solutions in 25 x 150 mm glass test tubes. The refined method used here was based on 0.25 ml test volumes. The use of smaller wells also allowed an increase in the number of samples to be tested at one time. *S. neumayeri* sperm was obtained as described above for the cytotoxicity tests. Every day that a sperm toxicity test was conducted, a blank control (sperm with filtered sea water) and a positive control (sperm in filtered sea water with ethereal extract) were run simultaneously. Ten replicates using extracts at different concentrations were tested for each of the samples. Sperm mobility was checked under a light microscope (40x) 20 min after the sperm solution was added to each well. Extracts were considered toxic when <25% of the sperm was active. Moreover, a binomial test of proportions ($p<0,001$) was calculated in order to evaluate the effects of different concentrations of extracts.

2.3.4 Antifouling activity

Different marine organisms such as bacteria, algae and invertebrates colonize surfaces underwater. However, many sessile marine invertebrates possess chemical or physical defenses to prevent the settlement of epibionts (e.g. Kelly et al., 2003; Sivaperumal et al., 2010). The presence of different kinds of compounds may influence the growth of other species which could settle near or over marine invertebrates. We evaluated the antibacterial activity of different extracts using the methods described in the literature with Antarctic bacteria (e.g. Jayatilake et al., 1996; De Marino et al., 1997, Mahon et al., 2003). Selected bacteria from the sea water were collected during the campaign, cultured on marine agar Difco brand (DMA 2216), and later sent to specialists for further identification. Filter paper discs impregnated with 20 µL of solution were placed on the surface of inoculated plates. Each test consisted in one disc without any additive (negative control), one disc with chloramphenicol (positive control), one disc impregnated with the solvent (diethyl ether, negative control) and one disc impregnated with the extract at natural concentration. Each culture of microorganisms was inoculated for triplicate on the surface of marine agar with the paper discs. Diffusion methods were based on the homogeneous distribution of the extract on solid culture media. The amount of the extract, as the number of bacteria (inoculum), was carefully controlled. After incubation, we measured the diameters of the inhibition halos and the results were interpreted using cut points as established internationally. Zones of growth inhibition larger than 2 mm were considered active.

3. Results

3.1 Feeding experiments with a macropredator, the seastar *Odontaster validus,* and a mesograzer, the amphipod *Cheirimedon femoratus*

In feeding-deterrence experiments using the seastar *O. validus*, 160 extracts (139 species) were tested belonging to different Phyla: Porifera (43 species), Cnidaria (17), Tunicata (15), Bryozoa (17), Echinodermata (5), Annelida (7), Algae (8), and other groups (11). A total of 76 deterrent extracts (66 species) were found, revealing significant differences in food consumption between simultaneous control and treatment tests (p<0.05), with control as the preferred food (Fisher's exact test). The deterrent extracts were thus 48,2% of the tested species, from organisms belonging to the taxa Porifera (22), Cnidaria (10), Tunicata (11), Bryozoa (10), Echinodermata (3), Annelida (4) and Algae (1), and others (5) (Avila et al. in prep) (Fig. 3).

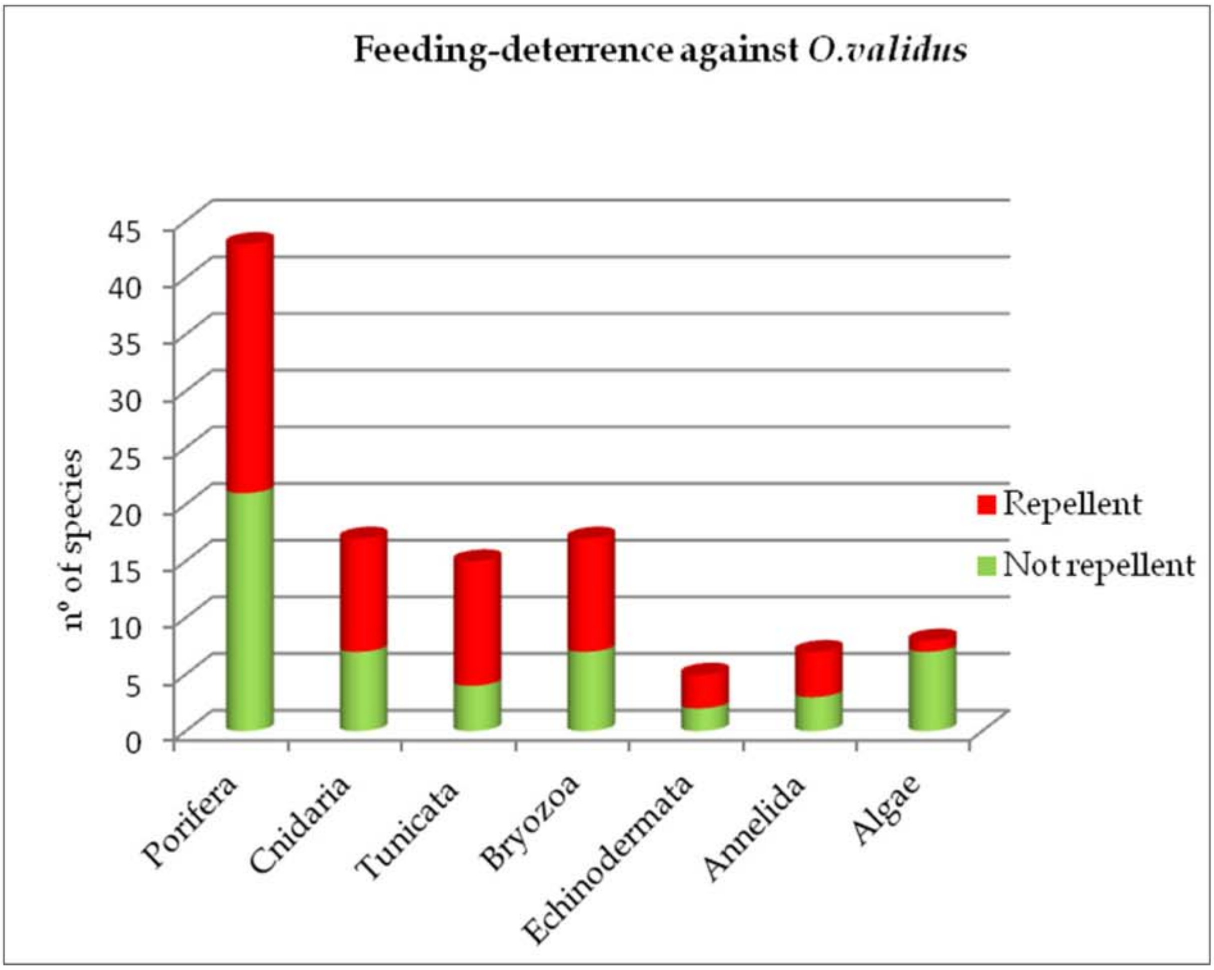

Fig. 3. Feeding-deterrence activity results against the seastar *Odontaster validus* in the different phyla tested.

In the experiment of feeding-preference using *C. femoratus*, 52 extracts were tested from Porifera (15), Cnidaria (14), Tunicata (12), Bryozoa (1), Echinodermata (1) and several extracts from macroalgae (8) (Núñez-Pons et al., in prep) A total of 36 extracts (33 species) out the 52 tested (40 species) were active (88,8% of the tested species) against the amphipod, revealing significant differences in food ingestion (p<0.05), being the control food preferred respect to the paired, unpreferred extract-treated, diet (Exact Wilcoxon test). These extracts corresponded to the taxa Porifera (7), Cnidaria (12), Tunicata (8) and Algae (6) (Fig.4).

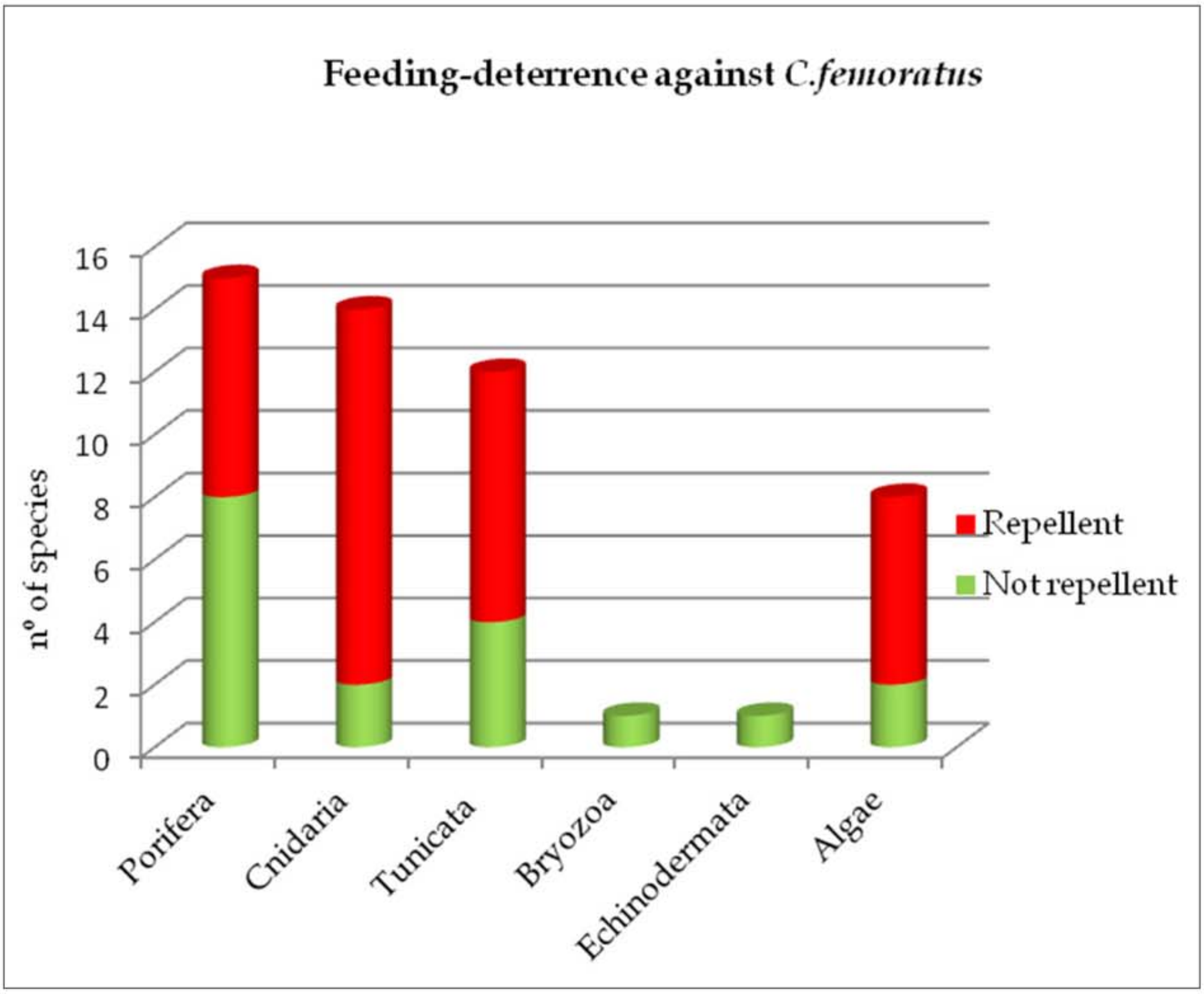

Fig. 4. Feeding-preference towards the amphipod *Cheirimedon femoratus* in the different Phyla tested.

3.2 Toxicity activity against the copepod *Metridia gerlachei*

We tested 24 species (32 extracts) belonging to the taxa Porifera (9), Cnidaria (3), Tunicata (1), Bryozoa (6), Echinodermata (4), and Hemichordata (1) and 14 of them (58, 3%) were toxic against copepods. A total of 14 active extracts (12) were detected (50% of the tested species) from organisms belonging to the Phyla Porifera (4), Cnidaria (1), Tunicata (1), Bryozoa (2), Echinodermata (4) (Fig. 5).

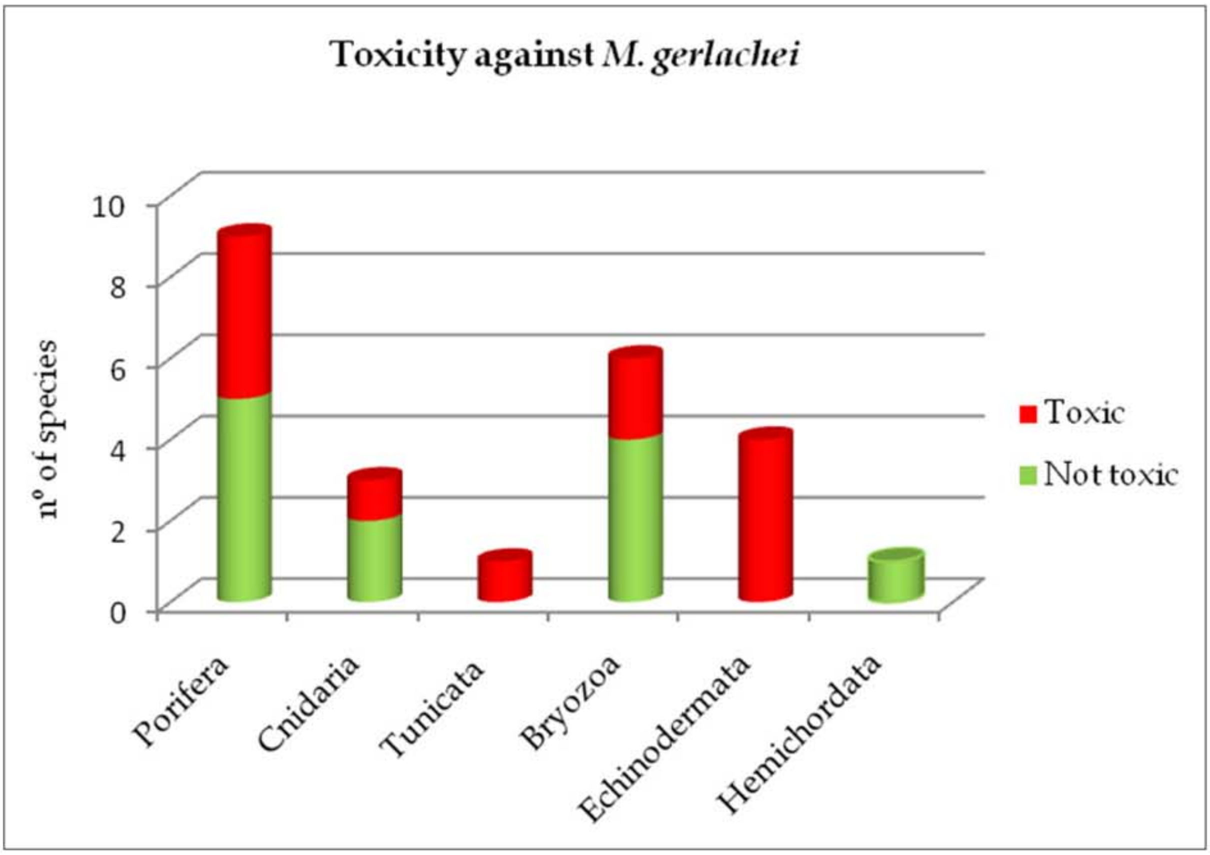

Fig. 5. Toxicity activity against *M. gerlachei* in different taxa.

3.3 Cytotoxicity against sea urchin embryos

A total of 17 species were tested, belonging to the Phyla Porifera (9), Cnidaria (1), Tunicata (2), Bryozoa (2), Annelida (1), Nemertea (1) and Algae (1). The toxic extracts (extracts outside confidence intervals described above) belong to Porifera (4), Cnidaria (1), Tunicata (2), Annelida (1), Nemertea (1) and Algae (1) (Fig. 6).

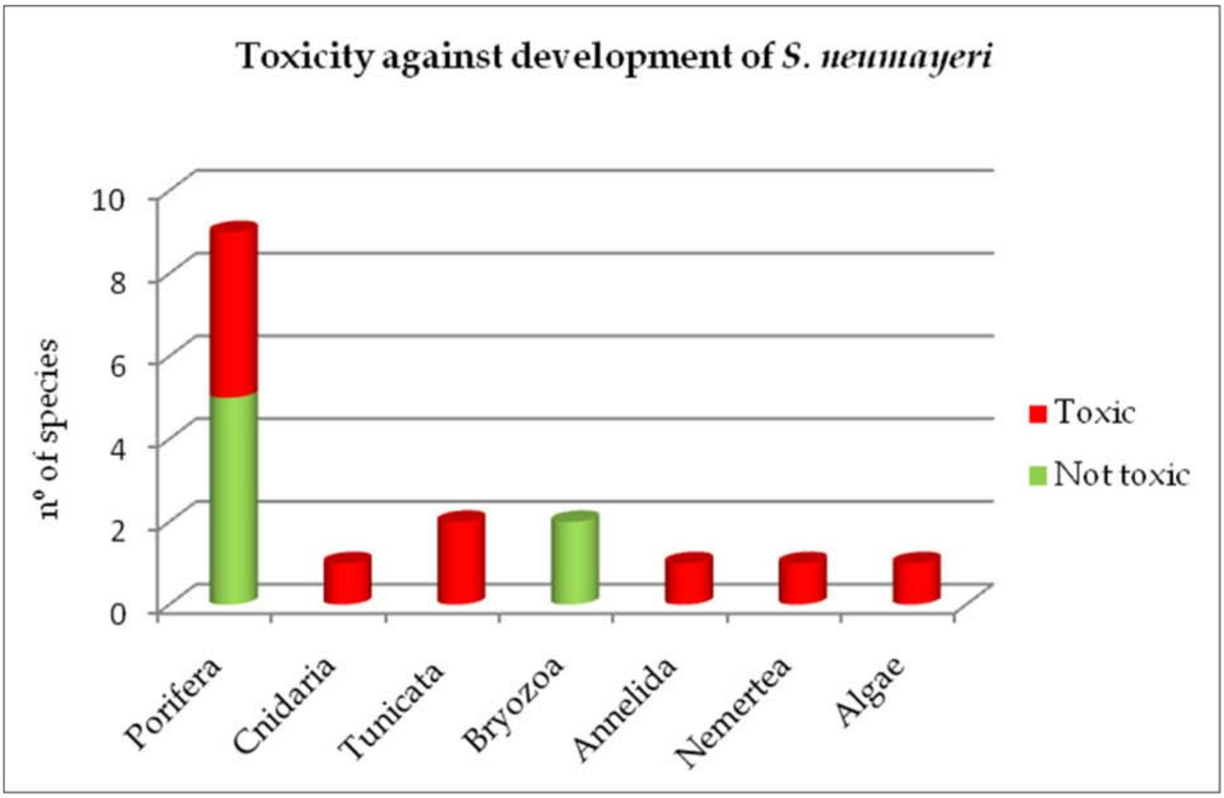

Fig. 6. Toxicity against development of *Sterechinus neumayeri* in different Phyla.

3.4 Cytotoxicity against sea urchin sperm

A total of 20 species (24 extracts) were tested. All the extracts except one were toxic to sperm (Fig.7) (<25% of the sperm was active) at the maximum concentration (1 mg ml^{-1}) and about 90% of the samples tested were active at the intermediate concentration. These extracts belonged to the taxa Porifera (4), Cnidaria (3), Bryozoa (2), Echinodermata (2), Annelida (6), Nemertea (1) and Hemichordata (3). Finally, 13 (15 extracts) out the 20 tested species (65%) were toxic to sperm at the lowest concentration.

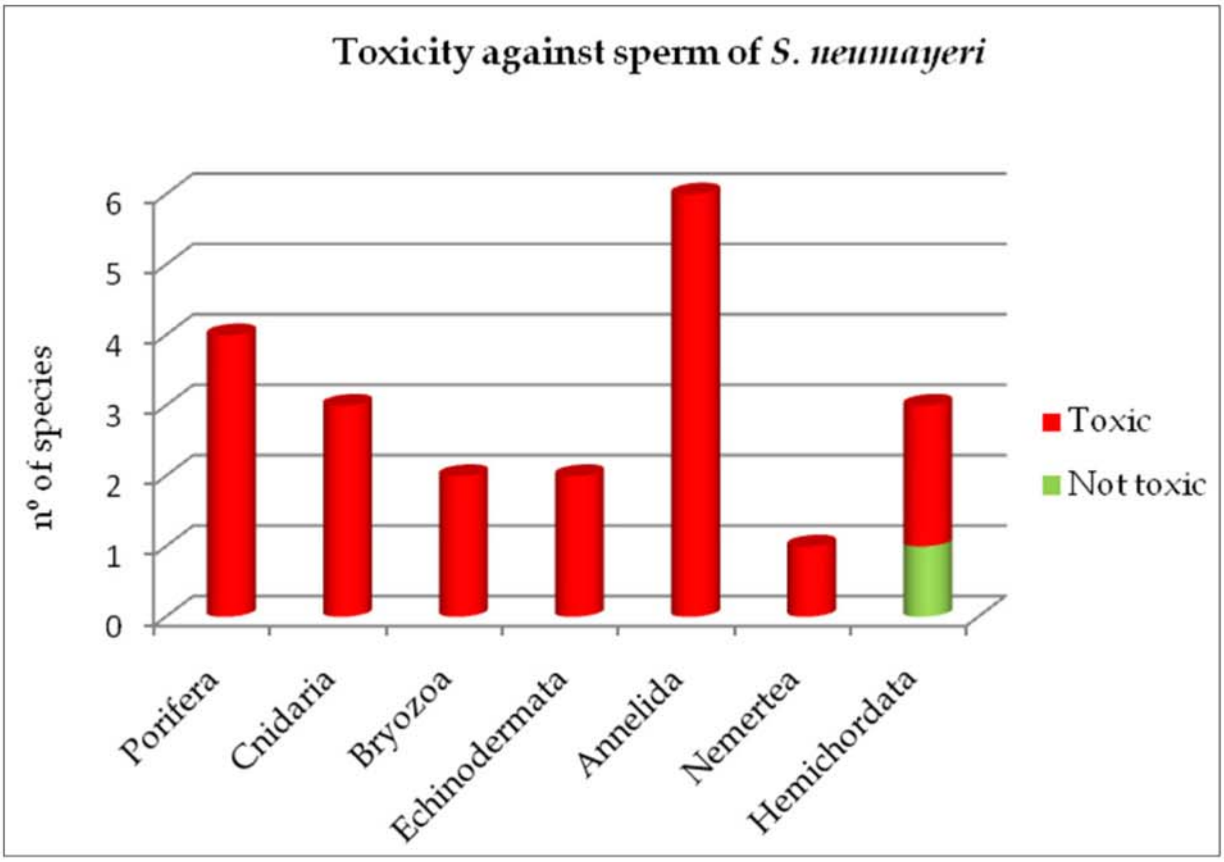

Fig. 7. Toxicity against sperm of *Sterechinus neumayeri* in different taxa.

3.5 Antifouling activity

We evaluated 130 extracts (70 species) from Porifera (22), Cnidaria (7), Tunicata (4), Bryozoa (14), Echinodermata (3), Annelida (6), Nemertea (1), Hemichordata (4), Algae (8) and others (1). A total of 28 extracts (24) were active (30.8%) from Porifera (5), Cnidaria (3) Tunicata (1), Bryozoa (3), Echinodermata(2), Annelida (3), Hemichordata (1) and Algae (2) (Fig. 8). This means all these active extracts produced zones of growth inhibition larger than 2 mm.

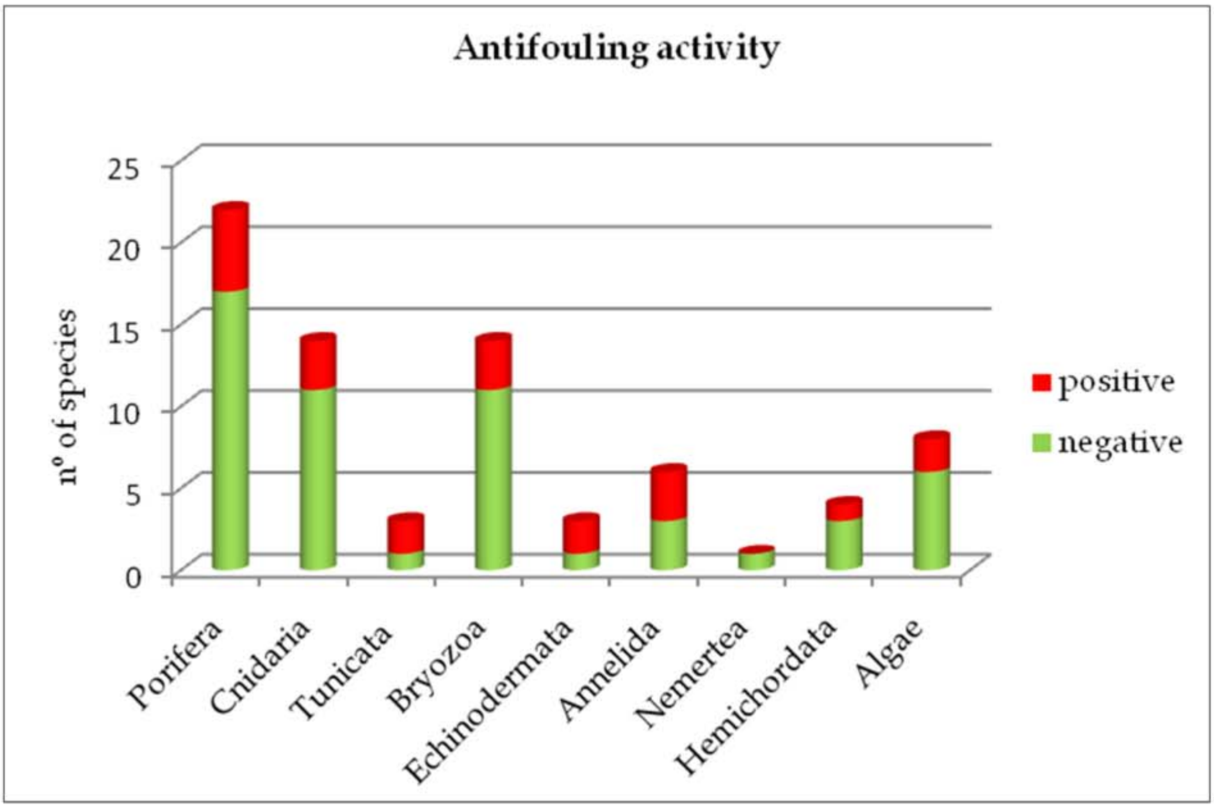

Fig. 8. Antifouling activity in the different taxa.

4. Discussion and conclusions

The Antarctic benthos appears to be greatly regulated by chemical interactions, mainly interfering with competence and predation (Fig. 9). Predation seems to be largely driven by the omnivorous sea star *O. validus,* known to have a noteworthy and extensive diet (McClintock et al., 2010). According to the high predation pressure described for this asteroid (Dayton et al., 1974), our results showed that repellence to avoid sea star predation is present in almost all the zoological groups of Antarctic invertebrates. This is demonstrated by the fact that more than 50% of the studied species of the main taxonomical groups exhibited significant deterrent activities. In agreement with these findings, previous experiments already demonstrated strong feeding deterrency towards this sea star in the opisthobrach molluscs *Austrodoris kerguelenensis* (Gavagnin et al., 2000; Iken et al., 2002) and *Bathydoris hodgsoni* (Avila et al., 2000). In the present survey, the tunicates exhibited the highest repellent activity (73%). Not surprisingly, the tunicate *Aplidium falklandicum* was recently found to possess particular alkaloid metabolites, the meridianins (A-G), responsible for this deterrent activity when tested isolated (Núñez-Pons et al., 2010). The phylum Porifera resulted to be also a quite active group (51%) and, in fact, other studies sustain this strong feeding deterrence reported for Antarctic sponges (21 species active out of the 27 species tested; Peters et al., 2009). Our study also found that 60% of the echinoderm samples were unsuitable for *O. validus*, although this species is known to feed on another seastars, namely *Acodontaster conspicuus* (Dayton et al., 1974). Cnidarians have already demonstrated the presence of chemical defenses, like the gorgonian coral *Ainigmaptilon antarcticus* (Iken & Baker 2003), and our results support this with more than 50% of the studied species (58%)

being active. The bryozoans displayed a similar deterrence as the cnidarians (58%), and also the polychaetes (57%), although this group was much less represented in number of samples tested. In fact, bryozoans have also been reported to be part of the diet of *O. validus* (Dayton et al., 1974). Finally, the algae seem also a potential food for this asteroid. Dearborn (1977) found diatoms, as well as red algae, in the stomach contents of *O. validus*. However the lower activity (14% of species) found in our study for this group may indicate a carnivorous preference of the star, despite being described as an opportunistic omnivorous consumer. All these results support the idea that many species from most of the phyla of Antarctic marine benthic invertebrates studied contain chemical defences against this voracious generalist sea star.

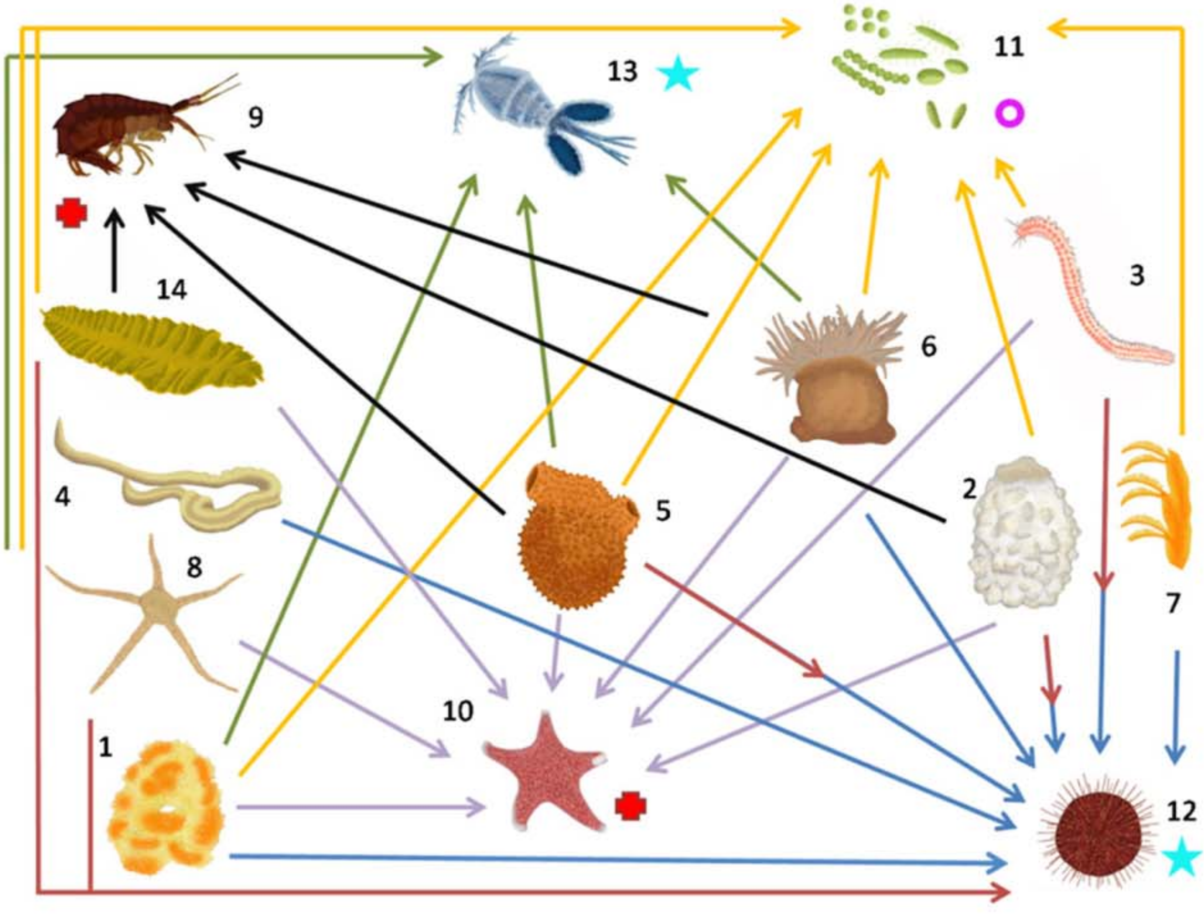

Fig. 9. Diagram of the proposed model of chemical ecology interactions according to the results obtained in our experiments, where: 1. Bryozoa, 2. Porifera, 3. Annelida, 4. Nemertea, 5. Tunicata, 6. Cnidaria, 7. Hemichordata, 8. Echinodermata; 9. *C. femoratus*, 10. *O. validus*, 11. Bacteria, 12. *S. neumayeri,* 13. Copepoda. 14. Algae. Symbols: Star: toxic activity; Cross: repellency activity; Circle: antifouling activity; Red: cytotoxicity activity; Blue: sperm toxicity. Each group is connected by arrows with the organisms used for each *in vivo* experiment.

Feeding preferences tested towards the amphipod *C. femoratus* revealed a high repellent activity in both benthic invertebrates and algae. Among the four majorly represented groups, the cnidarians displayed the highest incidence of feeding deterrent activities (85%), followed by the macroalgae (75%), the tunicates (66%), and finally the sponges (46%). Previous experiments with different species of Antarctic gammarid amphipods suggested that many macroalgae had feeding deterrent properties (Huang et al., 2006). It is worth to note that the amphipod used here, *C. femoratus,* has never been used previously as putative consumer in feeding assays. However, its voracious scavenger-omnivorous habits turn this mesograzer into a very suitable organism to test chemical defense. Actually, Bregazzi (1972) reported a large variety of food items (from algae to copepods and euphausiid larvae) in the stomach contents of this species. The impressive unpalatable activities recorded in our samples could be explained by the fact that, as many benthic amphipods, *C. femoratus* may use sessile organisms (mainly algae and sponges, but also others) both as host (biosubstrata) as well as a potential prey. This exerts a localized, constant pressure, which could be more intense than that caused by wandering mobile predators, such as sea stars or fish (Toth et al., 2007). Another species used as a model in previous Antarctic bioassays is the sympatric omnivorous amphipod *Gondogeneia antarctica*. This species, however, has repeatedly shown the problem of finding artificial foods too attractive, causing a phagostimulatory effect on the crustacean, and hence making the results obtained hard to interpret (Amsler et al., 2005, 2009a and b; Iken et al., 2009; Koplovitz, et al., 2009). Instead, the amphipod *C. femoratus* seems to possess a quite discriminatory potential to detect unpalatabilities, as observed in our results. Only bryozoans and echinoderms did not seem to produce a repellent effect against the mesograzer, but very few species were tested compared to other groups and, therefore, more samples have to be assayed before establishing any general conclusion for these groups.

Regarding the toxic activity against the copepod *M. gerlachei,* all groups except Hemichordata had some active species. Echinoderms showed, surprisingly, the highest activity (100%). Why this happens remains unknown at the moment, since *M. gerlachei* is omnivorous, feeding on phytoplankton, copepod eggs and small metazoans (Metz & Schnack-Schiel, 1995). Toxicity against the copepod could be a very useful mechanism to avoid the competition for phytoplankton food in benthic filter feeders, such as tunicates, sponges and bryozoans, and this is probably what our results reflect. No comparable results are currently available in the literature.

Few experiments have tested the activity from extracts of Antarctic benthic invertebrates against sperm and early life stages of the common Antarctic echinoid *S. neumayeri* (e.g. McClintock et al., 1990, 1992; Heine et al., 1991; Slattery et al., 1995) and, therefore, our contribution may give a wider idea of these cytotoxic mechanisms. In our tests, the sperm toxicity against *S. neumayeri* was evident in all species (100%) except in the group of Hemichordata (66% of species), similarly to what happened with the copepod test. Cytotoxicity against the development of this sea urchin was 100% in almost all groups, except sponges (44%) and bryozoans (0%), although we tested more samples of sponges than other groups. Pearse & Giese (1966) reported that *S. neumayeri* fed mostly on diatoms. Some studies have reported that the grazing by this sea urchin could be the responsible for significant mortality of settling larvae and juveniles of benthic invertebrates (Bowden, 2005; Bowden et al., 2006). Moreover, the settlement of pelagic larvae on or near their prey is frequent in marine predator invertebrates (Pawlik, 1992). Therefore, the presence of cytotoxic compounds found in this study for so many species may play an important role as a mechanism of defence/competence, reducing the recruitment of this sea urchin and, consequently, the grazing pressure and the colonization of the surface (e.g. McClintock et al., 1990; Bowden et al., 2006).

Different marine organisms such as bacteria, algae and invertebrates colonize submerged surfaces. The bacteria are metabolically versatile organisms capable of colonizing multiple surfaces, so it is not surprising that most organisms and, especially, the filter-feeders that are likely to be in trouble if the fouling is intensive, produce defenses against bacterial colonization. In the past, only a few studies were carried out to test the antifouling activity of Antarctic invertebrates against sympatric bacteria. Peters and colleagues (2010) tested the antifouling activity of extracts from Antarctic demosponges isolating bacteria from the surface of them and the majority of extracts did not display an inhibition activity. The reason could be that these bacteria were resistant to the substances from these sponges and, therefore, they were growing on their surface. Contrary to these results, the antifouling activity found in our tests is quite apparent in representatives of most taxa, including sponges (22%). In our case, bacteria were isolated from the water and we tested different species. This could be the cause of a higher activity in our results. Moreover, echinoderms (66%) and polychaetes (50%) were the most actives, followed by the cnidarians (42%). This is in agreement with previous results reporting antimicrobial activity in the soft corals *Alcyonium paessleri* and *Gersemia antarctica* (Slattery et al., 1995) . Tunicates, pterobranchs and algae had the same percentage of active species (25%). This is quite high if compared to the antimicrobial activity detected in only one (*Distaplia colligans*) out of 14 tunicate species tested previously (Koplovitz et al., 2011). The fact that the species tested are different could be the reason for these discrepancies. Compared with other taxa, bryozoans exhibited less activity (21%) and nemertins did not show any activity, although the number of species tested in these groups was too low to allow further considerations.

In summary, our results show that many different benthic organisms showed different strategies of defense, protection and/or competition (Fig. 9). However, it is important to emphasize that, not all phyla were equally tested, and therefore conclusions have to be considered cautiously. In many cases we found different defensive mechanisms in the same organism. This was previously reported for the Antarctic soft corals *Alcyonium paessleri* and *Gersemia antarctica,* which possess compounds with feeding-deterrence, antifouling and toxicity properties (e.g. Slattery & McClintock, 1995, 1997; Slattery et al., 1995). In our case, many species of Porifera and Cnidaria were active in all experiments (frequently over 40% or more of the tested species) and most Tunicata species quite as well (over 50% of species). In contrast, the Antarctic tunicates of the genus *Aplidium* displayed notable repellent activity in the feeding experiments (Núñez-Pons et al., 2010), but not in those of antibacterial nor antifouling.

Not surprisingly, other taxonomical groups were active in just one or a few tests, thus indicating the presence of one or only a few defensive lines (Fig. 9). For example, the phylum Bryozoa exhibited more activity in the experiment of feeding-deterrence against *O. validus* (58% of species) in relation to the antifouling experiment (21%) and no activity was found in the cytotoxicity experiment. The reason of these differences may be the presence in species of this phylum of physical defenses, such as avicularia, used for different roles, such as the prevention of the settlement of epibionts or larvae (e.g. Harmer, 1909). Moreover, in some groups, such as Annelida, only some species were active, and possibly, they have other types of defences no tested here, or physical protection strategies, such as living in their own bio-constructed tubes.

To our knowledge, this is the first ecological model proposed for describing the interactions in the Antarctic marine benthos, considering a wide array of possible chemical ecology relationships. We believe that these interactions are mainly generated to prevent the strong pressure of competition for space and/or food, predation and fouling to which Antarctic organisms are exposed. This general model shows an amazingly complex network of interactions between Antarctic organisms (Fig. 9). Further studies with larger number of samples are needed to complete and enrich this model and to bring some light to the existing gaps of knowledge. Nowadays, the research in marine chemical ecology in Antarctica continues to grow and new data will help to further advance in our knowledge on the role of chemical compounds in the Antarctic benthos. In order to successfully accomplish this task, the close collaboration among ecologists, chemists and microbiologists is essential. Also, further studies, such as those regarding antifouling and cytotoxicity activities, are needed to determine the ecological relevance of these mechanisms in Antarctic environments. Moreover, the bulk of the research in chemical ecology has been done on the phylum Porifera (Paul et al., 2011) compared to the few studies carried out in other phyla, such as Bryozoa, Annelida, Nemertea and Hemichordata, which are quite understudied. To fully understand this "chemical network", we will expand our studies to more types of experiments and more organisms during the development of our current project, ACTIQUIM-II.

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