Deep-Sea Stylasterid Corals in the Antarctic, Sub-Antarctic and Patagonian Benthos: Biogeography, Phylogenetics, Connectivity and Conservation

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Declaration

I declare that the work presented in this thesis is, to the best of my knowledge and belief, original and my own work, except as acknowledged in the text, and that material has not been submitted, either in whole or in part, for a degree at this or any other university.

Narissa Bax

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Statement of Co-Authorship

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The author N. N. Bax was responsible for the overall study, interpretation biogeographic patterns, and the writing and final revision of the manuscript (>85% of work). However, this work was assisted by the co-author as outlined below.

S. D. Cairns assisted in idea formulation, and provided editorial revisions on the manuscript.

I the undersigned agree with the above stated author contributions for the published manuscript contributing to this thesis.

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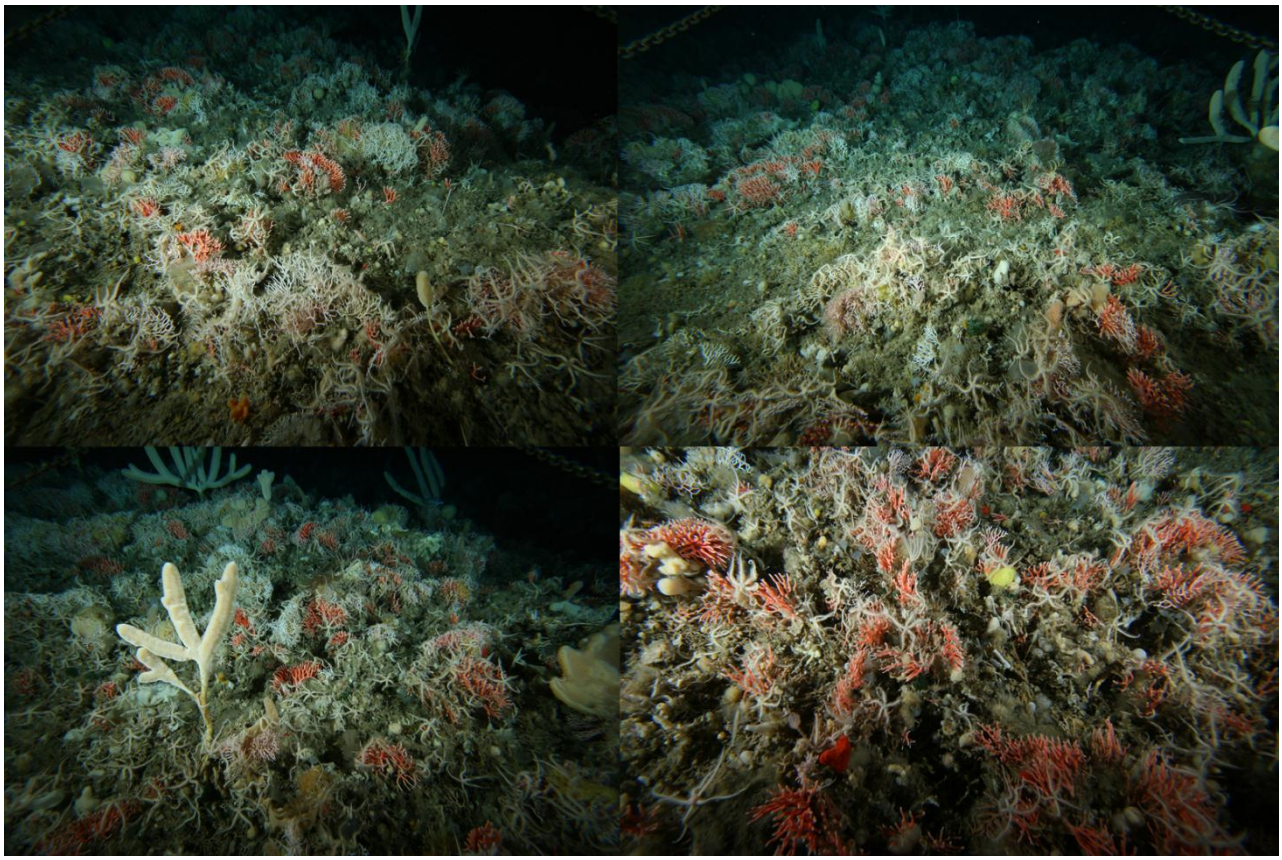
List of Abbreviations

ABW Adélie Bottom Water
ACC Antarctic Circumpolar Current
ASH Aragonite Saturation Horizon
BOLD Barcode of Life
$CaCO_3$ Calcium Carbonate
CAML Census of Antarctic Marine Life
CCAMLR Committee for the Conservation of Antarctic Marine Living Resources
CEAMARC Collaborative East Antarctic Marine Census
CO_2 Carbon dioxide
CO1 Cytochrome c oxidase subunit 1
CSH Calcite Saturation Horizon
DNA Deoxyribonucleic acid
EEZ Exclusive Economic Zone
IMAS Institute for Marine and Antarctic Studies
IPCC Intergovernmental Panel on Climate Change
ITS Internal Transcribed Spacer
NIWA National Institute for Water and Atmospheric Research
NBP Nathaniel B Palmer
MEGA Molecular Evolutionary Genetic Analysis
PCR Polymerase Chain Reaction
ROV Remotely Operated Vehicle
SCAR Scientific Committee on Antarctic Research
SCAR-marBIN Scientific Committee on Antarctic Research, Marine Biodiversity Network
VME Vulnerable Marine Ecosystem
WoRMS World Register of Marine Species
16S Mitochondrial ribosomal subunit

Thesis Statement

Deep-sea mount and shelf locations are defined as Vulnerable Marine Ecosystems (VMEs); isolated areas of high biodiversity and productivity. Corals are one of the main habitat-forming taxa on seamounts providing the ecological framework upon which the ecosystem is based. The recent discovery of field-like aggregations of deep-sea stylasterid coral reefs in the Antarctic benthos highlights their conservation importance, and VME classification under the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) recognises that the conservation of these reefs is crucial to the maintenance of biodiversity.



Images of coral fields of *Errina* spp. in the Dumont d'Urville Sea discovered during the CEAMARC research cruise in 2007/2008. This area is listed as a VME by CCAMLR. Image AAD ©.

Abstract

Large aggregations of sylasterid corals have been identified throughout the offshore waters of the Antarctic, Sub-Antarctic and South America. These biodiverse regions are interspersed by deep trenches, channels, sedimentary plains and isolated rocky habitat, which may facilitate or inhibit dispersal over evolutionary and ecological time scales. Deep-sea sampling has increased exponentially, across these benthic habitats, due to collaborative projects such as the Census of Antarctic Marine Life (CAML). Consequently, it is now possible to attempt to combine genetic and taxonomic expertise, explore evolutionary relationships and assess this data in relation to environmental change – both past and future.

The biogeographic distribution of stylasterid corals is representative of population isolation, based on the discovery of dissimilar species aggregations throughout sampled regions. To further investigate this biogeographic pattern, I sampled all 33 of the known stylasterid species documented from the Antarctic, Sub-Antarctic, South West Atlantic and Patagonian fiord regions across depths (~10 m - > 2000 m), geographic spatial scales (~10 km – 10, 000 km), and habitat types (shelf, slope, seamount and fiords). Genetic relationships were investigated using DNA sequence data from multiple gene regions including: The mitochondrial ribosomal subunit (16S), cytochrome c oxidase subunit 1 (CO1), and the nuclear Internal Transcribed Spacer (ITS). This data was assigned to four research components to determine 1) the biogeographic distribution of Antarctic and Sub-Antarctic stylasterids (n = 33 species, 14 genera). 2) Phylogenetic relationships based on morphology and genetics (n = 12 species, 8 genera). 3) Phylogenetic relationships incorporating the fossil record, to assess the evolutionary history of stylasterid populations in the Drake Passage (n = 7 species, 6 genera), and lastly, 4) genetic and demographic connectivity between populations to inform conservation management regimes (n = 7 species, 4 genera).

Morphological taxonomy combined with mitochondrial DNA sequence data produced a well aligned phylogenetic cladogram. The genetic variability seen in stylasterid 16S and CO1 sequences was comparatively higher than other coral and hydrozoan studies, offering potential for these gene regions in DNA barcoding. This has practical implications including the discovery of new species, cataloguing of Antarctic biodiversity and identification of specimens that are impossible to determine by taxonomic means. However, phylogenetic and taxonomic alignment was only achieved through the incorporation of systematic expertise in species identification, and inter-species relationships remain unresolved when compared to

the nuclear ITS gene region. Therefore, the incorporation of more gene regions for study, and the use of molecular taxonomy as a complementary tool, rather than a replacement for traditional systematics is recommended for future studies.

When the mitochondrial phylogeny was calibrated with the fossil record, phylogenetic topology represented an evolutionary scenario in which stylasterid ancestors' speciated in the Drake Passage during the Eocene/Oligocene transition boundary from calcite to aragonite sea conditions (~ 34 MYA). The phylogeny also suggests that skeletal bi-mineralogy may have played a central role in the speciation process. The presence of calcite in some genera and literature on the utility of either calcite or aragonite through oceanic time suggest a successional progression toward aragonite mineralogy in response to modern oceanic conditions (Oligocene => modern). Further research in this area may lead to the identification of acclimation states in stylasterid corals, and information on their ability to buffer impending ocean acidification, as the chemical state of the Southern Ocean shifts towards calcite sea conditions in the near future.

When investigating genetic population connectivity in the Sub-Antarctic, and across the Polar Front into South America, estimates demonstrate limited to no gene-flow across spatial scales of 300 - > 1000 km. Large scale comparisons were clearly subdivided, and genetic subdivision was evident both among populations either side of, and north of the Polar Front based on CO1 data. However, disparate gene-flow estimates derieved from 16S signify that populations were connected through evolutionary linkages, and connectivity south of the Polar Front may be amplified by the presence of the Antarctic Circumpolar Current (ACC). For fine scale comparision, local estimates of connectivity (~ 200 km) between two *Errina* spp. fiord populations in Patagonia, Chile, showed no evidence of genetic subdivision (F_{ST} = 0, p = 0.6). Similarly, *Errina* spp in East Antarctica also showed no evidence of genetic subdivision (ITS-1 F_{ST} = 0.03 P = 0.165 and ITS-2 F_{ST} = 0.002, P = 0.27). However, despite a lack of genetic differentiation in ITS *Errina* population comparisons, haplotype networks typify a pattern of adaptive radiation from a common ancestor, and upon comparing nucleotide polymorphism in CO1 (π =0.012 – 0.11), 16S (π =0 – 0.05), ITS-1 (π 0 - 0.002) and ITS-2 (π 0.02 – 0.03) it was determined that relative variability in 16S and ITS represented historic connections, whilst CO1 being more variable, may also be more recent.

Taken together, results suggest that a multitude of factors influence stylasterid coral populations, and temporal variation is particularly important in the context of this study. It is recommended that researchers focus on contemporary measures of connectivity, preserve specimens with genetic research in mind (> 90% ethanol preservation at the time of collec-

tion), and incorporate more loci to test connectivity across multiple spatial scales and species. The potential use of CO1 or 16S as barcoding genes will help in this process. However, until funding towards more deep-sea Antarctic sampling and molecular information emerges, the data presented in this thesis has ascribed a measure of localised geographic segregation, historic isolation and a limited capacity to recover following benthic disturbance. Substantiating that stylasterid corals congregate in diminutive and isolated populations. Therefore, to preempt anthropogenic damage to coral ecosystems, patterns of geographic isolation need to be incorporated into the design of Antarctic Marine Protected Areas (MPAs) - to preserve essential habitat, buffer climate change, mitigate the effects of ocean acidification, and combat localised impacts such as destructive fisheries which pose a direct threat to coral populations, and their associated taxa.

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Thesis Introduction

The Southern Ocean covers an area of 34.8 million km^2 and the deepest parts of it possess unique environmental features including a deep continental shelf and the formation of abyssal currents (Brandt *et al*., 2007). These features provide habitat for an extraordinary biota which has evolved over the last 100 million years in the coldest, most isolated region of the ocean (Clarke & Johnston, 2003). This isolation, combined with evolutionary drivers such as glaciation, has led to the evolution of rare genomic, physiological and ecological life history traits (Thatje, 2012). Antarctic species may be connected to surrounding deep oceans, but limited in their dispersal capacity. Hence, there is a high prevalence of endemics and species show unique biogeographic distributions (De Broyer *et al*., 2014a). These irreplaceable species and habitats are highly threatened, so much so that their demise may occur before science and conservation managers can identify and ascertain how to protect them (Kaiser *et al*., 2013).

1.1 Antarctic Deep-Sea

Further investigation, documentation and conservation of the Antarctic deep-sea has become vital in light of recent evidence on the vulnerability of deep-sea ecosystems (UNEP, 2006) combined with current estimates on the susceptibility of the Antarctic benthos to rapid climate change (IPCC, 2013). Such environments are often associated with strong ocean currents and are dominated by filter feeders (de Forges & Koslow, 2000). These organisms, such as corals and their associated fauna, are typically long-lived and slow growing (Adkins *et al*., 2004) with apparent low reproductive capacities and limited dispersal (Samadi *et al*., 2006). Such a life-history strategy conveys high susceptibility to anthropogenic disturbance, particularly for endemics (McClain, 2007). If isolated deep-sea environments are in fact composed mainly of endemics (UNEP, 2006), a lack of external recruitment could significantly reduce their potential to recover following disturbance.

The Antarctic Benthic Environment

The Southern Ocean contains unique species assemblages with comparatively high diversity and richness estimates (Arntz & Rios, 1999; Clarke & Johnston, 2003). Speciation occurs when a species cohort becomes isolated either geographically or by some event that reduces the gene flow of a species. The benthic environment is thermally isolated from other oceans by the Antarctic Circumpolar Current (ACC). The ACC formed after the break-up of

Gondwana (Boger, 2011), and subsequent cooling ~28 – 41 MYA (Lawver & Gahagan 2003). A number of studies have linked this isolation with the presence of rare species and evidence of cryptic speciation (e.g., Matschiner *et al*., 2009; De Broyer & Danis, 2011) and unique phenomena such as gigantism (Brandt *et al.*, 2007) and dwarfism (Ramirez-Llodra *et al*., 2010) in Antarctic waters. A faunal isolation hypothesis provides a theory to describe Antarctic biodiversity (Brandt, 2007), and states that oceanic barriers limit the dispersal and migration potential of larvae. This results in structured populations with high levels of endemism, local radiation and adaptation (Hunter & Halanych, 2008; Thornhill *et al.*, 2008). However, despite evidence confirming these trends, there are studies which dispute them (see Thatje, 2012 for a current summary). Ultimately, the majority of the Antarctic benthos is poorly understood. Often the mere existence of a species is undocumented.

Information on benthic structure and biological processes operating in Antarctic marine ecosystems has only been reported in the last decade (Kaiser *et al*., 2013). For the majority of described species, data are limited to presence/absence records which do not provide information regarding ecological processes such as connectivity, genetic diversity, or population structure (Grant & Linse, 2009; Griffiths, 2010). Nor do these data give us any idea of basic biology e.g., reproduction, growth, and species interactions such as predation and competition, etc. The cold temperatures and seasonal fluctuation in food supply tend to favour A selected life history characteristics, as outlined by Greenslade (1983), as a strategy for organisms adapted to severe, but stable and predictable, environments such as the polar regions. Key A selected traits in the Southern Ocean include; seasonal breeding, longevity, reproduction through brooded larvae over extended periods of time and low fecundity. A number of Antarctic species illustrate these traits (Thatje, 2012). However, the extent to which these traits are selected for in the Southern Ocean remains unknown for the majority of fauna.

To address our lack of knowledge of faunal assemblages, their distribution, abundance and the life history characteristics of Antarctic marine life, a long-term, collaborative study was undertaken between 2005/06 - 2011/12 to produce an inventory of life in the Southern Ocean under the umbrella of the Census of Marine Life (CoML). The Antarctic component, the Census of Antarctic Marine Life (CAML) was a collaboration involving multiple countries, scientists and institutes, and aimed to establish base-line information on diversity to preserve the Antarctic environment in a time of rapid climate change (De Broyer & Danis, 2011). This exploration exponentially increased Antarctic sampling and, to date, over

one million distribution records have been collected. The current Registry of Antarctic Marine Species (RAMS) lists 99,956 taxa (as of 26/10/14) (De Broyer *et al*., 2014b). Organisms that epitomise the typical A selected life-history traits associated with the Southern Ocean, and have been described in comparative detail following CAML sampling expeditions, include echinoderms (Hunter & Halanych, 2008), pycnogonids (Arango *et al*., 2011), amphipods (Baird *et al*., 2011, 2012), and cephalopods (Strugnell *et al*., 2008), to name a few. However, key information is still lacking in relation to the taxonomy of a number of groups and the majority of collected specimens await description due to a lack of taxonomic expertise, identification tools for shipboard identification, and out-dated (or non-existent) reference material, especially in relation to deep-sea species (Griffiths, 2010). Nevertheless, our level of knowledge on the Antarctic benthos has increased and it has been stated that science is now, through the advent of molecular tools, in a position to address important questions relating to species evolution, population connectivity and genetic adaptation (Clarke & Johnston, 2003).

Antarctic Benthic Population Connectivity and Isolation and Endemism

The Antarctic benthos is composed of multiple habitat types extending to a maximum depth of 4000 m. These habitats include; the Antarctic continental shelf which extends to ~ 800 m, and is a dominant bathymetric feature surrounding the continent and deep-sea mounts such as the Scotia Arc Sea mount and island chain (Brandt *et al*., 2007), and the Larsen ice shelf (Fig 1). Multiple ecosystems have been discovered in these habitats comprised of deep-sea reefs (Post *et al*., 2010), sponge gardens (Janussen & Tendal, 2007), bryozoan fields (Barnes, 2004), brittle star cities (Hunter & Halanych, 2008), polycheate dominated mud habitats and thriving isopod communities (Brandt *et al*., 2007). Areas of high biodiversity are interspersed by deep troughs, basins, sedimentary plains and rocky outcrops, which may facilitate or inhibit dispersal. In this manner, the deep-sea is composed of interconnected localised meta-populations (McClain, 2007). The extent to which dispersal occurs between populations has serious implications for species re-colonisation following disturbance (Gutt *et al*., 2011). Therefore, a greater knowledge of connectivity is vital in order to successfully conserve marine biodiversity in the Antarctic deep-sea.

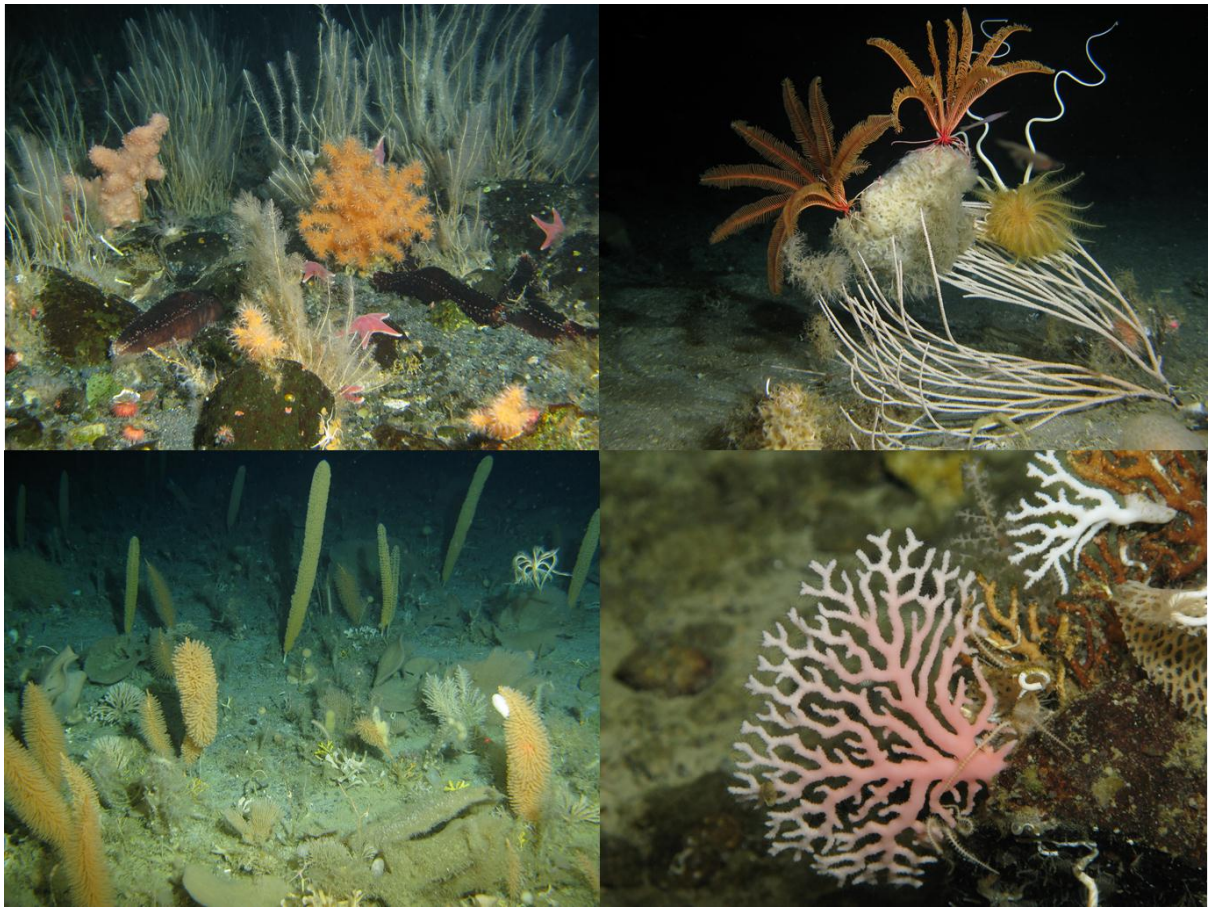

Figure 1. Selection of images from the Larsen Ice shelf illustrating high biodiversity habitats in the deep Southern Ocean (image © Laura Fillinger AWI).

Deep-sea mount and shelf locations are defined as Vulnerable Marine Ecosystems (VME); characteristically, isolated areas of high biodiversity and productivity (Le Goff-Vitry *et al*., 2004; Samadi *et al.,* 2006). Connectivity between deep-sea mount and shelf populations may operate over varying spatial and temporal scales, with varying levels of dispersal and recruitment between regions (Cowen *et al*., 2000). Until recently, the Southern Ocean, with the present-day ACC, was regarded as a type of conveyer belt for species dispersal. The clockwise circulation of the ACC around the Antarctic continent has the potential to transport larvae across great distances (Nowlin & Klinck, 1986; Clarke & Johnston, 2003), but panmixia (the capacity for random mating between populations), or homogenous species assemblages appears to be low or lacking (Thornhill *et al*., 2008). Thatje (2012) discusses early life history as the predominant factor defining the distribution of Southern Ocean benthic invertebrates, citing a direct relationship between dispersal capacity and speciation rate (inferring a negative correlation between endemism and dispersal). Dispersal potential is unknown for the majority of Antarctic deep-sea fauna, and the ability to disperse between meta-populations will potentially buffer the effects of disturbance and maintain genetic diversity between iso-

lated populations (de Forges *et al*., 2000). If dispersal is limited, as it appears to be, extinction in a changing environment is probable for a number of endemic invertebrates (Barnes *et al*., 2009).

High levels of regional endemism and diversity are frequently discussed in the Antarctic literature (Linse *et al*., 2006; Brandt *et al*., 2007; Strugnell *et al*., 2009). For example endemism is particularly high in a number of invertebrates including: amphipods (Baird *et al*, 2012), pycnogonids (Munilla & Soler Membrives, 2009), isopods (Brandt *et al*., 2007), and certain echinoderm classes (Piepenburg, 2005). However, the mechanisms promoting endemism leading to speciation and demographic connectivity patterns in the Southern Ocean are rarely tested and remain hypothetical. Research into understanding connectivity on a regional scale in Antarctica is urgently needed (CAML *scientific statement*), and Marine Protected Area (MPA) proposals require estimates of demographic exchange between populations (Palumbi, 2003). However, only two studies have been able to assess connectivity at small spatial scales in the Antarctic benthic environment (< 10 - 100 Km) (Baird *et al*., 2012; Leese *et al*, 2010). At broader spatial scales (> 500 km), the majority of connectivity research has focused on near shore species from the Scotia Arc and Antarctic Peninsula (Hunter & Halanych, 2008; Wilson *et al*., 2007; 2009). These studies, although informative, do not incorporate vast areas of the sea floor, and have found unique and often conflicting results.

For example, demographic connectivity estimates across local spatial scales, of < 10 km in the East Antarctic near shore benthos, suggest genetic structure exists between populations of the brooding amphipod *Orchomenella franklini* (Baird *et al*., 2012). Whilst, Hunter and Halanych (2008) found significant genetic connectivity across a broad geographic scale (> 500 km) in brooding brittle star populations of *Astrotoma agassizii* from the Antarctic Peninsula, indicating that neither reproductive mode nor geographic distance present a dispersal barrier for this species. In contrast, Wilson *et al.* (2007) conducted a study in the same region as Hunter and Halanych (2008), with some of the same sample locations, and found significant genetic structure between populations of the free spawning crinoid *Promachocrinus kerguelensis*. These results were somewhat unexpected based on the species' reproductive biology (Wilson *et al*., 2007; Hunter & Halanych, 2008) and limited geographic barriers to dispersal (Baird *et al*., 2012), illustrating that dispersal is often species or regionally specific and further research on multiple species within and between areas is needed to more accurately assign a measure of demographic connectivity in Antarctica.

1.2 Antarctic Deep-Sea Stylasterid Corals

Corals are one of the main habitat-forming taxa in the Antarctic deep-sea, and specimens have been known from depths of > 494 m in Antarctica since 1841 when the Erebus expedition sampled the Ross Sea (Cairns, 1983). However, since this time very few attempts to describe the diversity of Antarctic coral fauna have been made (Cairns, 1982; 1983). Stylasterid corals are a species-rich taxonomic group with over 247 known species world-wide (Cairns, 2011). Twenty nine morphological species of Stylasteridae coral are described from the Antarctic and Sub-Antarctic. Eighteen of these are found near the Antarctic continent with 33 – 54.5% listed as endemic (Cairns, 1983). As a result of the recent CAML studies, stylasterid coral collections are available in a number of invertebrate collections world-wide but, until now these collections have remained unsorted, unidentified and unpublished. This means there remain many gaps in our understanding of abundance, distribution and population structure. There are no reproductive data available for any Antarctic Stylasterid species and very little morphological data have been formally documented since the 1980's when Dr Stephen Cairns identified and compiled museum specimens (Cairns, 1983). Cairns (1992) in his review of stylasterid distributions suggests that they may be sensitive to fluctuating salinity, high sediment, competition (e.g., from the other stony coral group Scleractinia) and nutrient levels due to sediment build-up in polyps. He further states that substrate type may be the key limiting factor, as stylasterids require a hard substrate to settle, and show a preference for vertical surfaces.

Stylasterid Coral Fields

Research using stylasterid corals is particularly informative for population level analysis as they are abundant, widespread, and a species rich taxonomic group (Cairns, 2011). Deep-sea reefs are usually dominated by a single structural genus, such as *Lophelia* (e.g., Flot *et al*., 2013), *Desmophyllum* (e.g., Miller *et al*., 2011) and *Errina* (e.g., Häussermann & Försterra, 2007) and tend to have a more extensive geographic range than shallow water corals (Freiwald, 2002), which may be because shallow reef environments are composed of several species occupying various niches that relate to light (Veron, 1983).

The stylasterid genus *Errina* has been identified as a key structural habitat-forming coral in numerous locations including New Zealand and Chile's fiords (Miller *et al*., 2004; Häussermann & Försterra, 2007), and East Antarctica (Post *et al*., 2010). The recent discovery of field-like aggregations of *Errina* on the Antarctic continental shelf indicates that deep-

sea stylasterid coral populations form important ecosystems in the Southern Ocean. Consequently, *Errina* spp. aggregations in the Dumont d'Urville Sea have been listed as a Vulnerable Marine Ecosystem (VME), and *Errina* spp. are listed as VME indicator taxa through The Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) (Post *et al*, 2010 thesis statement image). This status provides a frame work through which these ecosystems are given conservation significance and protection from anthropogenic threats.

Post *et al*., (2010) outlined contrasting distribution patterns of the stylasterid coral *Errina* spp. near the George V continental slope in the Dumont d'Urville Sea. However, while they suggest that they may not have fully captured their distribution, they speculate that distributions may be related to a number of physical factors such as salinity, depth and nutrient availability and that *Errina* may not occur above 430 m. The majority of *Errina* spp. in the Dumont d'Urville Sea are distributed between 570 - 950 m, which is below the region of ice scour. However, during a recent German voyage at the Larsen B ice shelf east of the Antarctic Peninsula, a shallow community was discovered with 14 - 26 colonies m^{-2} at 160 and 240 m depth, respectively (Gutt pers.com.). In addition, benthic distributions in fiord regions extend from 10 m depth (Häussermann & Försterra, 2007), suggesting that *Errina* spp. has a eurybathic distribution. Therefore, research on stylasterids allows for study across broad geographic and bathymetric ranges.

Errina antarctica has been found in vast field-like aggregations in shallow waters (10 – 30 m) off the southern Chilean fiords between the Central Patagonian Zone (48°S) and Tierra del Fuego (55°S). This morphotype of *E. antarctica* is characterised by large, erect branching colonies inhabiting rocky substrate, characteristic of the Patagonian fiord system. The extensive abundance (±80% coverage) in this region (Häussermann & Försterra, 2007), combined with the video footage estimating similar abundance in the Southern Ocean (D Bowden, pers.com), confirms the importance of *Errina* spp. corals to the Antarctic and Patagonian ecosystems.

1.3 Potential Threats to Antarctic Deep-sea Coral Ecosystems

The existence of high biodiversity stylasterid coral fields emphasises the conservation importance of the Antarctic benthos, under the jurisdiction of the Antarctic treaty system, managed by CCAMLR (Cordonnery, 1998), in combination with their Protocol on Environ-

mental Protection to the Antarctic Treaty (The Madrid Protocol) (Grant *et al*., 2012 Fig 2). Under article II of the Madrid Protocol, the area of land and sea south of 60° S is dedicated in the interests of all people as a natural reserve, devoted to peace and science. However, the reality of the modern era, dictates that such a designation is idealistic. In a time of rapid environmental change, decreasing global resource availability and increasing social and economic interest in Antarctica, detrimental impacts to sensitive and vulnerable ecosystems are increasing (De Boyer *et al*., 2014a). To mitigate these impacts, it is vital that scientifically accurate data are incorporated into conservation management regimes. Corals possess a number of life history characteristics (e.g., long lived, slow growing, limited dispersal etc.) that make them particularly vulnerable to extinction (Miller *et al.*, 2004), and there are many immediate, direct anthropogenic and natural threats to Antarctic corals including, but not limited to, the following:

Grounded Icebergs (Ice Scours)

Natural sources of coral mortality include iceberg scours, from iceberg transport along the benthos, which is reported to cause damage to 400 m depth (Massom *et al*., 2009). An ice scour is the grinding of the seabed by the bottom of icebergs broken from the sea ice. This grinding can cause gouges in the benthos similar to the effect of a glacier carving out a valley on land (Gutt & Piepenburg, 2003). Ice scours pose a localised threat to deep-sea reefs and sponge gardens nearest to the Antarctic continent (Beaman & Harris, 2005). This type of disturbance regulates the diversity of the Antarctic benthos (Gutt & Piepenburg, 2003). However, global warming is expected to increase the number and frequency of ice berg scours (Beaman & Harris, 2005). We have observed this increase in recent years with the collapse of the Larson ice shelf in 1995 (Larsen A) (Rott *et al*., 1996), 2002 (Larsen B) (Scambos *et al*., 2003) on the Antarctic Peninsula, and in East Antarctica with the Mertz glacier calving in 2010 (Tamura *et al*., 2012). Increased ice-berg scour, if combined with other anthropogenic influences, threatens to remove or damage fragile coral skeletons, eliminate substrate suitable for larval settlement, and allow more competitive taxa to colonise the available space, thus, out competing stylasterids and substantially altering ecosystem structure.

Deep-Sea Bottom Trawling

Deep-sea trawling has a significant negative, wide-reaching impact on the entire benthic ecosystem (documented globally UNEP, 2006). Bottom trawling and deep-sea fishing activities are regulated by CCAMLR who have prohibited bottom trawling in the Southern

Ocean, due to documented impacts to non-target species, including deep-sea corals (UNEP, 2006). As a result, CCAMLR conservation measure 22-06 (2008) and conservation measure 22-07 (2009a), pertaining to VMEs (CCAMLR, 2012), only allow mid-water trawls, and long line fisheries to < 550 m (CCAMLR, 2009b). While occasional exceptions are made for scientific collection (Hosie *et al*., 2011), bottom trawling is unlikely to pose a significant threat to sea mount and shelf locations in CCAMLR regulated territory. Nonetheless, bottom trawling was used for the mackerel ice fishery (*Champsocephalus gunnari*) at Herd Island (Kock 1991), up until the 1980's, and the state of the trawled region has not been assessed. It is unlikely to have recovered to a healthy state; in the decades since trawling began recovery has not been documented anywhere in the world (Clark & O'Driscoll, 2000; Collie *et al*., 2000). Furthermore, while there are currently no reports of illegal bottom trawling in the Southern Ocean, nor a legal trawl fishery in the region, global fish stocks are decreasing (Clark, 2009), so it should be noted as a substantial threat. CCAMLR fisheries regulations are open to discussion which means they may change in the future and this could mean devastation for deep-sea coral ecosystems in the Southern Ocean.

Long Line Fisheries

Long line fisheries use a long mainline set in the water, with many baited hooks, and heavy weights. These weights cause the line to drag along the benthos creating a significant disturbance and a considerable amount of by-catch, including corals, which are dragged to the surface when a line is retrieved (Pauly, 2008). There are regulated and Illegal, Unreported and Unregulated (IUU) long line fisheries operating in Antarctica (Fabra & Gascón, 2008). Long line fishing for the two Antarctic toothfish species (*Dissostichus eleginoides* and/or *D. mawsoni*) is regulated by CCAMLR and approved by the Marine Stewardship Council (MSC) as a sustainable fishery. The regulated fishery in the Ross Sea collects scientific data, has a fisheries observer program and records and preserves by-catch (Parker & Bowden, 2009). CCAMLR regulated tooth fisheries also operate at South Georgia, South Sandwich Islands, Heard and McDonald Islands, Macquarie Island, Crozet Islands and Kerguelen Islands (Fig 2). CCAMLR fisheries adhere to catch restrictions and the move-on rule, where fishing is halted and prohibited within a 1nm radius of a VME area if VME taxa e.g., corals, sea pens, sponges and bryozoans, are encountered (CCAMLR, 2009a). Argentina and Chile regulate fisheries within their respective Exclusive Economic Zones (EEZ), and within the CCAMLR boundary (between 45°S and 60°S). There are no published data on the impact of these fisheries on benthic habitat. However, even regulated fisheries that operate over an ex-

tensive area will likely affect fragile benthic fauna such as corals (Roberts *et al*., 2006), more so if combined with impact from IUU fisheries.

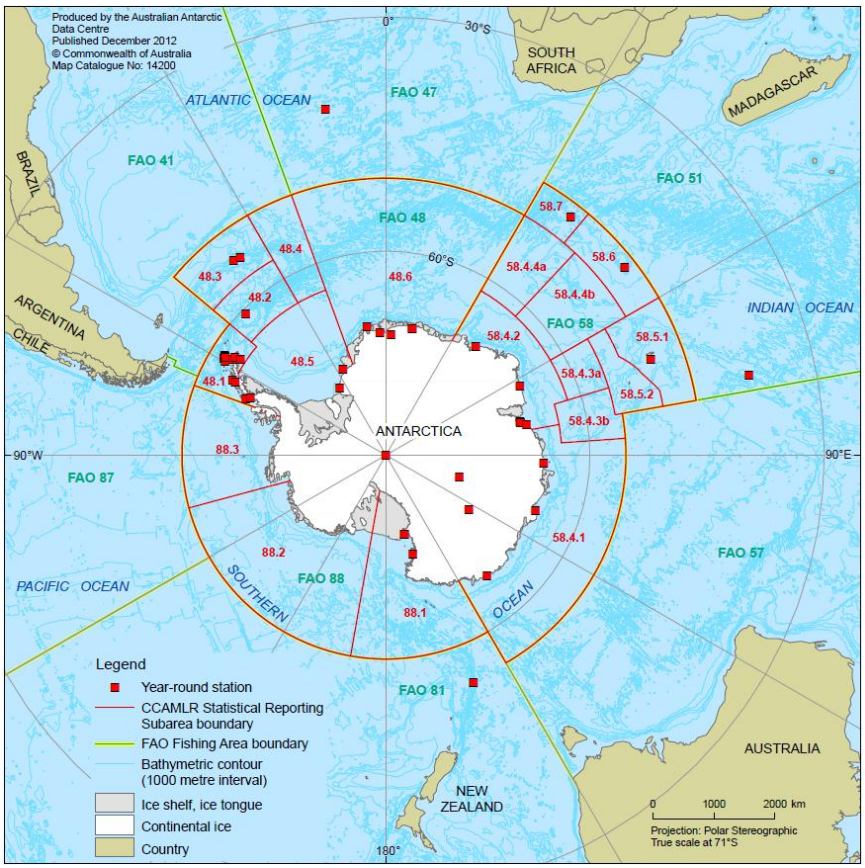

Figure 2. Southern Ocean FAO fishing area boundary regulated by CCAMLR. Image © CCAMLR.

Ocean Acidification

Deep-sea calcifying organisms such as corals face a significant, global threat known as ocean acidification (Guinotte & Fabry, 2008). The latest IPCC assessment on climate change states; " while the effects of observed ocean acidification on the marine biosphere are as yet undocumented, the progressive acidification of oceans, is expected to have negative impacts on marine shell-forming organisms (e.g., corals) and their dependant species" (IPCC, 2013). Scleractinian corals form the majority of the deep-sea reef structure and rely on aragonite to deposit their calcium carbonate ($CaCO_3$) skeletons. Organisms that utilise aragonite are thought to be the most vulnerable to changes in oceanic pH (Feely *et al*., 2004). Correspondingly, nearly 70% of the world's known deep-sea coral reefs are predicted to fall below the Aragonite Saturation Horizon (ASH), the region in the water column where aragonite calcification may no longer occur, by 2100. 15% of scleractinian corals already subsist below

the ASH (Guinotte *et al*., 2006). Due to the chemistry of CO_2 in cold water, Antarctica is predicted to be the region of the world where the effects of ocean acidification are most prevalent (Guinotte & Fabry, 2008).

Current estimates suggest that over one third of the total CO_2 emitted into the atmosphere has been absorbed by the ocean (Sabine *et al.,* 2004). Around 40% of the global inventory of anthropogenic CO_2 can be found in the Southern Ocean. This equates to a total pH decrease of 0.14 in the Southern Ocean since the industrial era (Feely *et al*., 2004). The long-term consequences of ocean acidification are unknown. As oceanic pH decreases, the depth of the saturation horizon in the water column becomes shallower, changing the range and composition of deep-sea ecosystems (Orr *et al.,* 2005). Guinotte & Fabry (2008) suggest the ASH, which is currently estimated at ~ 1000 m (Feely *et al*., 2004), may rise to surface waters in the Southern Ocean by 2100. Experimental results indicate that calcifying organisms do not readily acclimatise to decreasing carbonate saturation states (Orr *et al*., 2005; Guinotte & Fabry, 2008; Hall-Spencer *et al.,* 2008). If this holds true under natural conditions, the geographical range of some coral species may be reduced while others could become extinct. Stylasterid skeletons have been shown to contain both aragonite and calcite (Cairns & MacIntyre, 1992). The Calcite Saturation Horizon (CSH) is much deeper (> 2000 m deeper in some parts of the Southern Ocean) than the ASH (Barnes & Peck, 2008). Therefore, a calcifier which has the ability to utilise both aragonite and calcite may have a greater capacity to acclimate to changing oceanic pH than purely aragonite calcifiers. This makes the study of stylasterid corals all the more relevant to the maintenance of Antarctic biodiversity, as they may survive predicted climate change scenarios.

1.4 Thesis Objectives and Outline

This study aims to clarify relationships among deep-sea populations in the Antarctic, using stylasterid corals as an ecological model to investigate patterns of biodiversity, and ecology on deep-sea reefs. Stylasterid population structure, species relationships and diversity are unknown, due in part to the expense and inaccessibility of their habitat. As a result of recent Antarctic expeditions and the CAML initiative, coral collections are available for study in a number of invertebrate collections world-wide. Until now these collections have remained unsorted, unidentified and unpublished. Correspondingly, this is the first study to investigate stylasterid species molecular relationships in Antarctica and incorporates the following four research chapters and their corresponding aims:

Chapter 2: The Census of Antarctic Marine Life (CAML) Scientific Committee of Antarctic Research and Marine Benthic Information Network (SCAR-MarBIN) compiled a Biogeographic Atlas of the Southern Ocean to combine data collected during the CAML 2007/08 - 2012/13 time period. The Stylasteridae (Coelenterata: Hydrozoa) form a chapter within this publication. This paper aims to summarise the current state of knowledge of the biogeography of stylasterid corals in Antarctica and the Sub-Antarctic.

Bax N. N., Cairns, S. D., (2014) Stylasteridae (Cnidaria; Hydrozoa). In: De Broyer C., Koubbi P., Griffisths H. J., Raymond B., Biogeographic Atlas of the Southern Ocean. *Cambridge Press:* SCAR, 107-112.

Chapter 3: A multi-disciplinary analysis of Antarctic and Sub-Antarctic stylasterid coral species relationships: do molecular and taxonomic relationships align?

This chapter aims to clarify the inter- and intra-species relationships of Antarctic stylasterid species using genetic and morphological identification to delineate phylogenetic connections.

Chapter 4: Evolutionary relationships of Drake Passage Stylasterid corals.

This chapter combines mitochondrial phylogenetic data with the ancient fossil record (~65 - 50 MYA) to investigate evolutionary relationships and determine the patterns and processes that may have shaped speciation in Antarctic and Sub-Antarctic stylasterids, using the Drake Passage as a case study.

Chapter 5: Connectivity and Conservation of Stylasterid corals in the Antarctic and Sub-Antarctic.

This chapter aims to determine the level of genetic exchange between stylasterid coral populations in Antarctica by comparing intra-specific variation within (local scale connectivity) and between (large scale connectivity) regions, and resolve Circum-Antarctic patterns of population connectivity, focusing on seven relatively common species; *Errina fissurata* Gray, 1872, *Errina laterorifa* Eguchi, 1964, *Errina antarctica* (Gray, 1872), *Errinopsis fenestrata* Cairn, 1983, *Stylaster densicaulis* Moseley, 1879, *Cheiloporidion pulvinatum* Cairns, 1983 and *Conopora verrucosa* (Studer, 1878).

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The Census of Antarctic Marine Life SCAR-Marine Biodiversity Information Network Biogeographic Atlas of the Southern Ocean: Stylasteridae (Cnidaria, Hydrozoa)

The Census of Antarctic Marine Life SCAR-Marine Biodiversity Information Network Biogeographic Atlas of the Southern Ocean was published in 2014 to synthesise Antarctic Biogeographic information to date. The following statement is provided as an abstract to give background to this publication, to which the Stylasteridae (cnidarian, Hydrozoa) forms a component:

'The "Biogeographic Atlas of the Southern Ocean" is a legacy of the International Polar Year 2007-2009 (www.ipy.org) and of the Census of Marine Life 2000-2010 (www.coml.org), contributed by the Census of Antarctic Marine Life (www.caml.aq) and the SCAR Marine Biodiversity Information Network (www.scarmarbin.be; www.biodiversity.aq). The scope of the Biogeographic Atlas of the Southern Ocean is to present a concise synopsis of the present state of knowledge of the distributional patterns of the major benthic and pelagic taxa and of the key communities, in the light of biotic and abiotic factors operating within an evolutionary framework. Each chapter has been written by the most pertinent experts in their field, relying on vastly improved occurrence datasets from recent decades, as well as on new insights provided by molecular and phylogeographic approaches, and new methods of analysis, visualisation, modelling and prediction of biogeographic distributions.'

Bax N. N., Cairns, S. D., (2014) Stylasteridae (Cnidaria; Hydrozoa). In: De Broyer C., Koubbi P., Griffisths H. J., Raymond B., Biogeographic Atlas of the Southern Ocean. Cambridge Press: SCAR, 107-112.

A Multi-Disciplinary Analysis of Antarctic and Sub-Antarctic Stylasterid Coral Species Relationships: Do Molecular and Taxonomic Relationships Align?

This study uses a multi-disciplinary taxonomic approach, combining genetics and morphology, to assess species relationships among stylasterid corals in the Antarctic and Sub-Antarctic. DNA sequences of the mitochondrial ribosomal subunit (16S), cytochrome c oxidase subunit 1 (CO1) and the nuclear Internal Transcribed Spacer (ITS) were obtained from 131 stylasterid corals collected from the waters of Antarctica, the Sub-Antarctic, Patagonia and the South West Atlantic. The nuclear ITS gene region was included for some taxa as a more variable gene region for comparison, and showed markedly different intra-species relationships compared to mitochondrial data for *Errina* and *Errinopsis* species. Mitochondrial trees suggest congruence between morphology and genetics for CO1 and 16S gene regions and phylogenetic clade compositions indicate that generic and species level morphological complexity correlate well with genetic similarity for *Errina*, *Errinopsis*, and *Cheiloporidion* species. However, based on current morphological delineations, in order to most accurately align genetics and morphology, a re-examination of the derived character state is suggested to resolve discrepancies between phylogenetic topology. The basal clade in CO1 and 16S comparions was *Conopora verrucosa*, a species identified morphologically by its cyclosystem and lack of gastrostyle. The gastrostyle is considered a basal morphological character, and the comparable phylogenetic placement of *Stellapora echinata*, *Sporadopora dichotoma* and *Stylaster densicaulis* can be attributed to this synapomorphy. Therefore, it is recommended, based on these genetic data sets, that the cyclosystem be attributed as a basal character state and the gastrostyle be attributed as more derived in future studies. Furthermore, CO1 and 16S mitochondrial markers may be useful DNA barcoding genes for stylasteridae, and where taxonomic expertise is not available, genetic data may substitute traditional species taxonomy to facilitate the quantification of stylasterid diversity in the Antarctic and Sub-Antarctic benthos.

Introduction

Studies on deep-sea corals, some of the dominant taxa on seamount and shelf locations globally, are often limited by inadequate taxonomic data. This hinders the understanding of biodiversity in these important ecosystems (Baco & Cairns, 2012). Quantification of diversity is particularly important in isolated geographic locations such as the understudied deep Antarctic Ocean, where the majority of benthic life is either undescribed or undiscovered. Corals, broadly defined as cnidarian sessile benthic invertebrates formed from a colony of polyps 'having continuous or discontinuous calcium carbonate or horn-like skeletal elements'(Cairns, 2007), are ubiquitous throughout the world's deep oceans (Freiwald *et al*., 2004; Cairns, 2011). Morphological characters are traditionally used to classify coral species, but morphological characters are not always informative at the species level of classification (Knowlton, 2000). Coral species identification is complicated by a number of factors. Corals can be polymorphic in form, have overlapping morphologies, few diagnostic characters, can utilise different reproductive strategies even within species, and often occur within close proximity to other closely related individuals. As a result, species boundaries are often unclear (Veron, 1995; Willis *et al*., 1997; Forsman *et al*., 2009).

Coral Species Relationships – from Tropical Corals to Deep-sea Corals

Coral species are typically separated based on morphology, reproductive behaviour, and/or genetics (e.g., *Orbicella annularis* species complex in Fukami *et al*., 2008, Budd *et al*., 2012, *Acropora* species complex in Van Oppen *et al*., 2001). However, such characteristics can be conflicting (Miller & Babcock, 1997; Miller & Benzie, 1997). Coral skeletal morphology is often associated with factors separate to reproductive isolation or evolutionary divergence, such as phenotypic plasticity, hybridisation and incomplete lineage sorting (van Oppen *et al*., 2001). Hence, many aspects of coral species relationships remain unclear despite the application of molecular and morphological methods (Medina & Szmant, 1999; Aguilar & Sanchez, 2007). This means workable descriptions for most coral species are lacking (e.g., the *Pocillopora* spp. review in Schmidt-Roach, 2012; 2013), as is the applicability of genetic initiatives, such as DNA barcoding - where DNA identification is used either in place of, or as a complementary tool to morphological taxonomy (DeSalle *et al*., 2005). Further, the majority of genetic studies focus on reef-building, shallow water, zooxanthellate (with photosynthetic algal symbionts) corals, principally within the order Scleractinia (Kitahara *et al*., 2010). The literature on deep-sea or cold water azooxanthallate (lacking photosyn-

thetic algal symbionts) coral species is increasing (Miller *et al*., 2010; McFadden *et al*., 2011). However, until the last decade, they have been largely ignored outside of the North Atlantic (Freiwald *et al*., 2004), due to their comparative inaccessibility and the high cost of conducting research. The exception is the gorgonian corals, the principal octocoral group in the deep-sea (Watling *et al*., 2011), for which a reasonable amount of molecular literature exists (McFadden *et al*., 2006; 2011; Watling *et al*., 2011).

It is surmised that the total area of reef structure in the deep ocean could surpass known shallow water coral distribution, and coral reef structure provides the heterogeneous environment that many organisms rely upon (Guinotte & Fabry 2008). In the deep-sea the lack of available hard substrate for recruitment is critical to population persistence (Davies & Guinotte, 2011; Yesson *et al*., 2012). However, competition for light, an important limiting resource on tropical coral reefs (Mundy & Babcock, 2000), is absent in the deep-sea. The photic zone ends at ~ 200 m, and corals are known from depths below 6000 m (Keller, 1976). The deep-sea is considered a seasonally stable environment; where salinity, pressure and temperature rarely fluctuate, and nutrient input is cyclic (Kiriakoulakis *et al*., 2009). This means generation times are likely to be long, which could result in limited selective pressure. The literature on deep-sea corals indicates that deeper sea species may be more easily differentiated from one another by molecular methods than their shallow water relatives (Kitahara *et al*., 2010; Miller *et al*., 2011), but exceptions remain e.g., *Narella* spp. (Baco & Cairns, 2012). If low intra-specific genetic variability exists in deep-sea corals, it may be easier to determine independent morphological relationships and their comparative genetic taxonomy.

Under this hypothesis a coral species recruiting into a new deep benthic habitat (establishing a founder population) may maintain the same morphology as its parental population and genetic divergence or differentiation may not occur among isolated populations, due to a lack of selective pressure. To date, some studies substantiate this theory of low genetic and morphological variation. For example Miller *et al*., (2011) quantify minor (but still statistically significant) morphological variation in the coral *Desmophyllum dianthus* across the entire Southern Hemisphere despite marked genetic differences related to geography and depth. In the Northern Hemisphere, Addamo *et al*., (2012) document the opposite result, with marked morphological variation, but genetically *Desmophyllum dianthus* and *Lophelia pertusa* were nearly indistinguishable. Flot *et al*., (2013) found close to no genetic difference

across ~ 7500 km in *Lophelia pertusa*, and studies on *Madrepora oculata* (Lin *et al*., 2012) and *Paragorgia aborea* (Herrera *et al*., 2010; 2012) corroborate findings of lower than expected genetic variation across broad spatial scales. These studies benefit from the cosmopolitan distribution of their chosen coral taxa, but are limited by the conundrum that such studies must address – do these species constitute one species with a wide geographic distribution, or are there obscure levels of speciation? Given that marine speciation is a complex process with numerous drivers, the division is often unclear (Knowlton, 2000). Furthermore, the term cosmopolitan species does not apply to the majority of benthic fauna (McClain, 2007). In the deep-sea, singletons (a solo sample per species) and endemics are common, baseline levels of variability are lacking and the application of molecular techniques to differentiate deep-sea coral species is an emerging and important field of research.

Molecular Tools Provide Information on Species Relationships

The development of new molecular techniques provides mechanisms to investigate deep-sea coral relationships. Molecular data from related, widely distributed species at multiple rapidly evolving loci can be used to approximate species evolutionary history by providing phylogenetic reconstruction of species relationships (McCook *et al.*, 2009; Steneck *et al.*, 2009). Due to increased deep-sea sampling and advances in molecular genetic tools recent studies have obtained genetic data for many deep-sea coral groups; Sclearactinia (Kitahara *et al*., 2010), Octocorallia (McFadden *et al*., 2006; 2011; Baco & Cairns, 2012), Antipatharia (Brugler & France, 2007), and stylasterid corals (Lindner *et al*., 2008). Of these, stylasterids remain the least studied (Table 1), despite their abundance in deep-sea collections, their ecological importance in benthic habitats, the availability of morphological taxonomic literature (Cairns, 1983), and a relatively good fossil record for the group (Lindner *et al*., 2008).

Table 1. Genetic literature available per coral group, illustrating that stylasterid corals are the least studied of the deep-sea corals. Number of publications sourced from Web of Science based on topic searches including publications post-1945 (as of August 2014). *Antipatharians are not listed from Antarctica.

Coral Group	Publications	Genetic Publications	Antarctic Publications	Antarctic Genetic Publications
Scleractinia (Order)	202	28	20	0
Gorgonacea (order)	219	19	11	1
Antipatharia* (order)	104	12	-	-
Stylasteridae (family)	37	2	4	0

Stylasterid Corals

Stylasterids are calcified hydrozoans and, as such, are unique amongst deep-sea corals. Three hundred and twenty five species are described world-wide, the majority from the Southern Hemisphere (Cairns, 2011). Stylasterids preferentially select insular distributions on deep rocky habitat with pristine high-flow water masses (Cairns, 1992). A predominantly deep living family (only 10% of species live shallower than 50m Cairns, 1992), they are entirely azooxanthallate (Cairns, 2011), and considered monophyletic within Hydrozoa (Cartwright *et al*., 2008). The only molecular phylogenetic analysis available for comparison is Lindner *et al*., (2008), which include shallow and deep-sea stylasterid corals from temperate and tropical oceans. There are no genetic data available for stylasterids from Antarctica. Only two Sub-Antarctic specimens were used in the study by Lindner *et al*., (2008).

There are a number of morphological revisions available for stylasterids world-wide (see Cairns, 2011 for a summary). The last morphology-based geographic study in the Antarctic and Sub-Antarctic was Cairns (1983), in which species are well described based on increasing morphological complexity, from most derived to most complex. Recent deep-sea sampling in the Southern Ocean, developed through collaborative projects such as the Census of Antarctic Marine Life (CAML), have increased the availability of Antarctic specimens for study (Grant & Linse, 2009; De Broyer *et al*., 2014). Consequently, it is now possible to attempt to combine genetic and taxonomic expertise to delineate relationships within this understudied family of corals.

This study employs a multidisciplinary approach to the reconstruction of species relationships, combining genetic data with morphologically identified species from multiple locations. The taxonomic groupings for Southern Hemisphere stylasterid corals are well documented in Cairns (1983; 1991). However, the addition of multiple samples over the past ~ 30 years from a wide geographic range, combined with evidence that a number of species occur in sympatry with closely related species and genera (Bax & Cairns, 2014), allows for a detailed investigation into their species relationships. Molecular identification of specimens combined with morphological taxonomic delineations will enable us to determine if groups identified based on genetic data agree with groups identified based on morphological data. This is often in disagreement in corals (e.g., Octocorals McFadden *et al*., 2011). Similar discrepancies may exist within Stylasteridae, as illustrated in the phylogeny presented in Lindner *et al*., (2008), which shows unrelated morphological genera within clades from the tropical and temperate regions. Comparable disagreements may exist in Antarctic and Sub-Antarctic species.

Closely related species may be genetically dissimilar, but morphologically the same (Addamo *et al*., 2012), and vice versa (Baco & Cairns, 2012; Flot *et al*., 2011). Either may be the case in stylasterid corals. For example Bax and Cairns (2014) outline the co-occurrence of *Errina fissuarata*, *Errina laterorifa*, *Errina gracilis* and *Inferiolabiata labiata* in field-like aggregations. These species have very few morphological characters to differentiate them from one another without detailed microscopic examination, and even then some confusion can remain depending on the quality of the preserved sample (pers. obs.). Further, the closely related genera of *Errinopsis* and *Errina* are thought to form a genus complex based on their mineralogy and shared derived characteristics (synapomorphies) (Cairns & MacIntyre, 1992). With these species and generic associations in mind, this study aims to determine a consensus phylogenetic tree based on congruence between the morphological and genetic divergence of Antarctic and Sub-Antarctic stylasterid corals. In order to substantiate such a consensus, three focus questions are addressed 1) Is there a difference between species groupings based on morphology and genetics? 2) Are genetic differences among species equivalent across different gene regions? And consequently, 3) is there potential for DNA barcoding as a tool for the unambiguous identification of stylasterid species?

Methods

Study Area, Collection and Samples

Tissue from deep-sea stylasterid coral samples was obtained in four ways: 1) through existing research collections from; the Smithsonian National Museum of Natural History (NMNH), British Natural History Museum (NHM), the Museum National d'Histoire Naturelle (MNHN), National Institute for Water and Atmospheric Research (NIWA) and, Australian Antarctic Division (AAD) and through the Instituto Español de Oceanografía, Centro Oceanográfico de Gijón. 2) By personal or collaborative collection at sea during recent Antarctic research voyages from; three *Nathaniel B Palmer* research voyages; NBP11-03, NBP 11-05 and NBP 08-05 to the Antarctic Peninsula and Drake Passage, and a voyage through the Collaborative East Antarctic Marine Census (CEAMARC) to the Dumont d'Urville Sea associated with the Census for Antarctic Marine Life (CAML). 3) from by-catch through cooperation with the New Zealand Ministry of Fisheries (MFish), MFish Observers and Observer Program staff under MFish Projects ANT200801, ANT200901 and ANT201001. Samples from voyages were obtained either by beam trawl or epibenthic sled from depths of 130 m to a maximum sampling depth of 2149 m. In addition, *Errina antarctica* fiord morphotypes were collected by SCUBA, and remotely operated vehicle (ROV) from the Chilean fiords in collaboration with the Alfred Wegener Institute (AWI) on the Explorador II. Sample number, replicate, location, latitude, longitude, morphological identification and voyage were recorded for all study specimens (Appendix Table 1). Specimens were collected from a geographic range spanning from 200 - > 8000 km encompassing the offshore waters of Antarctica, the Sub-Antarctic and South America including the South West Atlantic and Patagonia (Fig 1).

Morphological Identification of Species

Samples were identified to species based on morphology. A dissecting microscope was used to examine skeletal structures and digital photographs were taken of each individual. An assessment of morphology was made based on available keys and literature (Cairns, 1983; Cairns, 2011). Morphological characters including the gastrostyle, dactylostyle and cyclosystem were visualised with Scanning Electron Microscopy (SEM) and compared to type material where possible. SEM photographs were used as a reference to identify fine scale morphological characters for a subset of specimens. Skeletal samples were bleached to re-

move coral tissue, rinsed in water and air dried before coating in bronze for visualisation under SEM.

Figure 1. Antarctic Map of the geographic regions where stylasterid corals were collected for this study: Ross Sea and Dumont d'Urville Sea in East Antarctica. The Drake Passage and Scotia Arc island chain in the Sub-Antarctic. The range of some Sub-Antarctic species extended into the South West Atlantic, and Patagonia, these species are also included in the study (Appendix Table 1). Map edited from the AAD data centre.

Molecular Protocols

For the recent collections, material was preserved as close to the time of collection as possible in > 90% ethanol specifically for genetic analysis. DNA extraction and sequencing was attempted for all recently collected specimens. DNA extraction and amplification was trialled for some older samples from museum collections during the course of this study but they proved unsuitable for genetic analysis due to either their lack of quality (i.e., degraded sample) or preservation method (i.e., formalin). Specimens which showed evidence of contamination, poor-identification or questionable morphological accuracy were removed from analysis. Genomic DNA was extracted from coral specimens following the standard extraction procedure using the Qiagen DNeasy protocol for the purification of total DNA from an-

imal tissues (QIAGEN). The procedure was modified to include an overnight incubation at 56ºC to completely lyse coral tissue.

Two mitochondrial regions, Cytochrome c oxidase subunit I (CO1) and the 16S Ribosomal Subunit (16S rDNA) were targeted. The CO1 mitochondrial DNA is the most commonly used gene region for DNA barcoding through initiatives such as the Barcode of Life (BOLD) (www.barcodeoflife.org). CO1 has proved highly effective in identifying a number of animal groups (Grant & Linse, 2009), including some corals (Kitahara *et al*., 2010). However, it is often considered problematic for cnidarian species due to its slow rate of evolution in lower metazoans (Geller *et al*., 2013). This is the first study to test CO1 on Antarctic stylasterid corals, thus it was hoped that CO1 amplification would provide a conservative measure of stylasterid coral species relationships. 16S is the large subunit ribosomal RNA gene in the mitochondrial DNA. This region was chosen for three reasons: 1) it is considered to be relatively conserved, evolving at a slower rate than the mitochondrial genome as a whole; 2) it is a relatively large fragment (> 500 bp); and 3) it has proven useful in a number of phylogenetic studies on shallow and deep-sea corals (Romano & Palumbi 1997; Le Goff-Vitry *et al*., 2004), to identify species level differences in Stylasteridae (Lindner *et al*., 2008) and to reveal variability between populations that have been isolated for long periods (Benzie, 1999).

A third marker, the Internal Transcribed Spacer (ITS) nuclear region is a non-coding portion of RNA, with a high copy rate, extensive intra-specific variation, and a rapid rate of substitution (Vollmer & Palumbi, 2004). ITS is one of the most commonly used molecular marker for shallow water Scleractinian corals (Medina & Szmant, 1999; Vollmer & Palumbi 2004; Forsman *et al*., 2006) and it has been successfully utilised to infer relationships at or below the species level of identification (e.g., cryptic species) (Forsman *et al*, 2006). This is the first study to apply ITS primers to stylasterid coral species, and the hyper-variability of the marker may reveal hidden differences not apparent in the more conservative 16S and CO1 gene regions.

Extracted DNA was used as a template in Polymerase Chain Reactions (PCR). Hydrozoan 16S, universal ITS primers, and Metazoan specific CO1 primers were used in this study (summarised in Table 2). Each 12.5 µl Polymerase Chain Reaction (PCR) reaction contained Promega GoTaq® Green Master Mix, 0.5µl of a 10 µM solution of forward and re-

verse primer pairs, < 250 ng of DNA template and nuclease-free water. The thermal cycling profile varied by gene region. For ITS and CO1 this consisted of an initial denaturation at 95ºC for 2 minutes, then 40 cycles of a three step program 95ºC for 30 sec, 40-60ºC for 45 sec (annealing temperatures varied Table 2) and 72ºC; for one minute, with a final extension at 72ºC, this was modified for CO1 to include 40 cycles. The 16S protocol was taken from Lindner *et al*., (2008). A positive control of *E. fissurata* amplified by Bax (2009) was used to identify the validity of PCR reactions. Sufficient master mix was retained for each PCR trial to be used as a negative control which contained all listed reagents, but no template DNA in order to control for contamination and the amplification of non-target DNA. Successful PCR reactions were purified using the Qiagen MinElute purification kit designed to produce high end-concentrations of DNA. To elute the DNA, 30 µl of milliQ was used in place of a buffer. To increase the overall DNA yield prior to sequencing, two PCR reactions were purified for each sample. The two purified PCRs were then combined into one micro-centrifuge sample to make a total concentration 3-6 ng/uL for sequencing. Samples were sequenced in both directions at the Australian Genome Research Facility (AGRF) in Brisbane using the ABI platform.

Table 2. Details of PCR primers and annealing temperatures used to amplify DNA from eleven species of deep-sea stylasterid coral. The resulting, or expected amplicon size (in base pairs) is indicated.

DNA Region & Primers	Direction	Primer Sequence	Annealing Temp (°C)	Fragment Size (bp)	Reference
16S					
SHA	5'-3'	TCGACTGTTTACCAAAAACATAGC	35-60	~600	Cunningham & Buss, 1993
SHB	3'-5'	ACGGAATGAACTCAAATCATGTAAG	35-61	~600	
ITS					
ITS-5	5'-3'	GGAAGTAAAAGTCGTAACAAGG	56	~600	White *et al.*, 1990
ITS-4	3'-5'	TCCTCCGCTTATTGATATGC	56	~600	
CO1					
jgHCO2198-1	5'-3'	TABACYTCBGGRTGBCCRAARAAYCA	50-60	~200	Geller *et al.*, 2013
jgLCO1490-1	3'-5'	TBTCBACBAAYCAYAARGAYATTGG	50-61	~200	

Data Analysis

Sequences for each species were edited and checked for errors using the software program Molecular Evolutionary Genetic Analysis (MEGA) 5.0 (Tamura *et al*., 2011). Consensus sequences were generated for each specimen using forward (5'–3') and reverse (3'–5') primer sequences (Table 1). Occasionally it was not possible to obtain sequences in both directions. In these cases, sequence data were only used if the sequences were clean and reliable. All sequences generated in this study will be lodged with GenBank (###).

Basic Local Alignment Search Tool (BLAST; NCBI; www.ncbi.nlm.nih.gov) searches were performed on each sequence to confirm that each sequence was in fact a stylasterid coral, and the appropriate DNA region had been amplified. Additionally, multiple alignments across all samples were used to identify outliers within the data set; these outliers may have been the result of contamination and were removed prior to analysis. Sequence quality varied among samples; some sequences were longer than others, and some groups of sequences had a greater number of comparative base pair differences. Hence it was appropriate to select the most parsimonious data set that optimised both sequence length and species comparison.

Phylogenetic Analysis

Initial tree topology was determined in MEGA using Neighbour Joining and Minimum Evolution phylogenetic alignments to determine bootstrap support and topologies. Final analyses were conducted in Mr Bayes using the following models determined in jModelTest (Possada, 2008); a GTR + G + I model for 16S data, and a HKY + G+ I model for CO1 data, the concatenated data set was run with a partition under a GTR + G + I model with state frequencies set to account for maximum variability. ITS was run under a HKY model. DNA evolutions (the estimated change in sequence composition over time) ran over 100,000,000 generations or until split frequency distribution was below < 0.002. Bayesian probability values were comparative to bootstrap values calculated in MEGA based on maximum likelihood. Dendrograms were edited in Fig Tree 1.4.0 (Rambaut, 2006). The final trees combined concatenated and the most parsimonious individual alignments for each gene region in the following five data sets: 1) mitochondrial CO1 DNA sequence data from 47 specimens, 8 species and 289 base pairs; 2) Mitochondrial 16S DNA sequence data from 72 specimens, 12 species and 137 base pairs, to align the maximum number of species available for study; 3) Mitochondrial 16S DNA sequence data from 57 specimens, 9 species and 293base pairs, to align the maximum sequence overlap; 4) Concatenated partition analysis of mitochondrial

CO1 and 16S DNA sequence data from 33 specimens, 7 species and 872 base pairs, to align maximum parsimony in tree topology; and 5) Ribosomal ITS-2 DNA sequence data from 45 specimens, 6 species and 142 base pairs. A DNA sequence of *Hydricthella epigorgia* (Ptilocodiidae) was used as an outgroup species. This species is in the superfamily Hydractinoidea, which also contains Stylasteridae, and the species has been shown to occupy a lineage basal to Stylasteridae (Cartwright, 2008).

To further assess the relative genetic difference between species by gene region, haplotype networks were generated to compare shared and unshared ITS, CO1 and 16S haplotypes (GenAlEx v6.501) (Peakall & Smouse, 2006), to obtain a graphical representation of relationships using the program Network v4.5.1.6 (www.fluxus-technology.com). Parameters defined the median-joining algorithm with default settings including gaps and indels and a 95% plausible connection limit (Bandelt *et al*., 1999). This analysis tested inter-genetic species relationships in CO1 (n = 17, 289bp), 16S (n = 24, 293bp) and ITS (n= 44, 142bp) in species sequenced for all three gene regions in *Errina* and *Errinopsis* species. Data sets were used to determine the correlations and/or discrepancies which may exist between morphological and genetic delineations in Antarctic and Sub-Antarctic stylasteridae.

Results

Phylogenetic Relationships Inferred from CO1 and 16S Data

All 33 known stylasterid taxa from Antarctica and the Sub-Antarctic were collected during the course of this study (see Bax & Cairns, 2014). Twelve of the most common Southern Ocean species amplified successfully; *Errina fissurata* Gray, 1872, *Errina laterorifa* Eguchi, 1964, *Errina antarctica* (Gray, 1872), *Errina gracilis* von Marenzeller, 1903 *Errinopsis fenestrata* Cairns, 1983, *Errinopsis reticulum* Broch, 1951, *Inferiolabiata labiata* (Moseley, 1879), *Sporadopora dichotoma* (Moseley, 1876), *Stylaster densicaulis* Moseley, 1879, *Stellapora echinata* (Moseley, 1879), *Cheiloporidion pulvinatum* Cairns, 1983 and *Conopora verrucosa* (Studer, 1878). The final sample set spanned from the South West Atlantic to East Antarctica (> 7500 km) (Fig 1) (Appendix Table 1). Sequence quality was variable by gene region, and the potential data set of > 700 individuals was greatly reduced to 119 mtDNA sequences in total. 16S had the highest amplification success (n = 72), for twelve species. CO1 sequenced less reliably (n = 47), for nine species. There was suffi-

cient overlap between the 16S and CO1 data to analyse a concatenated data set (for 7 species) as well as for each gene region independently.

Genetic groupings are generally consistent with species groupings based on morphological delineations. Both CO1 and 16S gene regions differentiate well between genera, as clades are distinct with well supported Bayesian probability values (~ 100%) (Figs 3 - 6). The genetic variability between species, illustrated in haplotype networks (Fig 7), is higher in CO1 comparisons than in 16S comparisons, and phylogenetic clades are more distinct (Fig 3). Cairns (1983) described primitive to derived species listings which place *Sporadopora* as the least derived genus and *Stylaster* and *Conopora* as the most derived. In contrast, the genetic data suggests that *Conopora* is the least derived genus along with *Sporadopora* (Figs 3 – 5). Therefore, the overall magnitude of genetic divergence does not correlate entirely with what we might expect based on linear morphological assessments from Cairns (1983).

The genetic phylogenies presented here are morphologically aligned for some species but not others. Both CO1 and 16S phylogenies suggest that *Errina* and *Errinopsis* are closely related in well supported separate clades (Figs 3 - 6). These two genera have a number of synapomorphies, and are described as a genus complex (Cairns & McIntyre, 1992). Therefore, the pairings of *Errinopsis reticulum* and *Errinopsis fenestrata,* and the *Errina* spp. (Figs 3 - 6) correspond well with their morphologies (Cairns, 1983). However, molecular level relationships differ for some morphology-based relationships (Cairns, 1983). For example; *Cheiloporidion pulvinatum* is most closely related to the *Errina* species (Figs 3 - 6). This relationship is potentially more resolved in the concatenated data set (Fig 6), where *C. pulvinatum* is the basal clade; nevertheless, more gross morphological similarities exist between *E. reticulum* and *C. pulvinatum*. Both genera have fenestrate branching morphology and specimens of the three species (*E. fenestrata, E. reticulum, C. pulvinatum*) are so similar that without differences in colouring (white, orange and pink respectively) they could be superficially confused (pers. obs.). Further, *Inferiolabiata labiata* was expected to fall close to the *Errina* clade, but it is more closely related to *Stylaster densicaulis* (Fig 4). *Stellapora echinata* is an expected basal group (Fig 4), however, 16S, CO1 and concatenated phylogenetic alignment place *S. echinata* as most closely related to *S. densicaulis* (Figs 3, 5 & 6). Alignments also show *S. dichotoma* and *C. verrucosa* as most divergent to the other clades (Figs 3 - 5). These pairings are counter to synapomorphies, and based on morphological delineations *C. verrucosa* should align with *S. densicaulis*, and *S. dichotoma* and *S. echinata* share more

resemblances (Fig 2). With these discrepancies in mind, morphological similarity does not appear to be in alignment with genetic similarity based on traditional taxonomic views for all species (see Cairns 1983, 1987; Cairns & McIntyre, 1992), and a reanalysis of the derived character state may be needed to explain genetic associations.

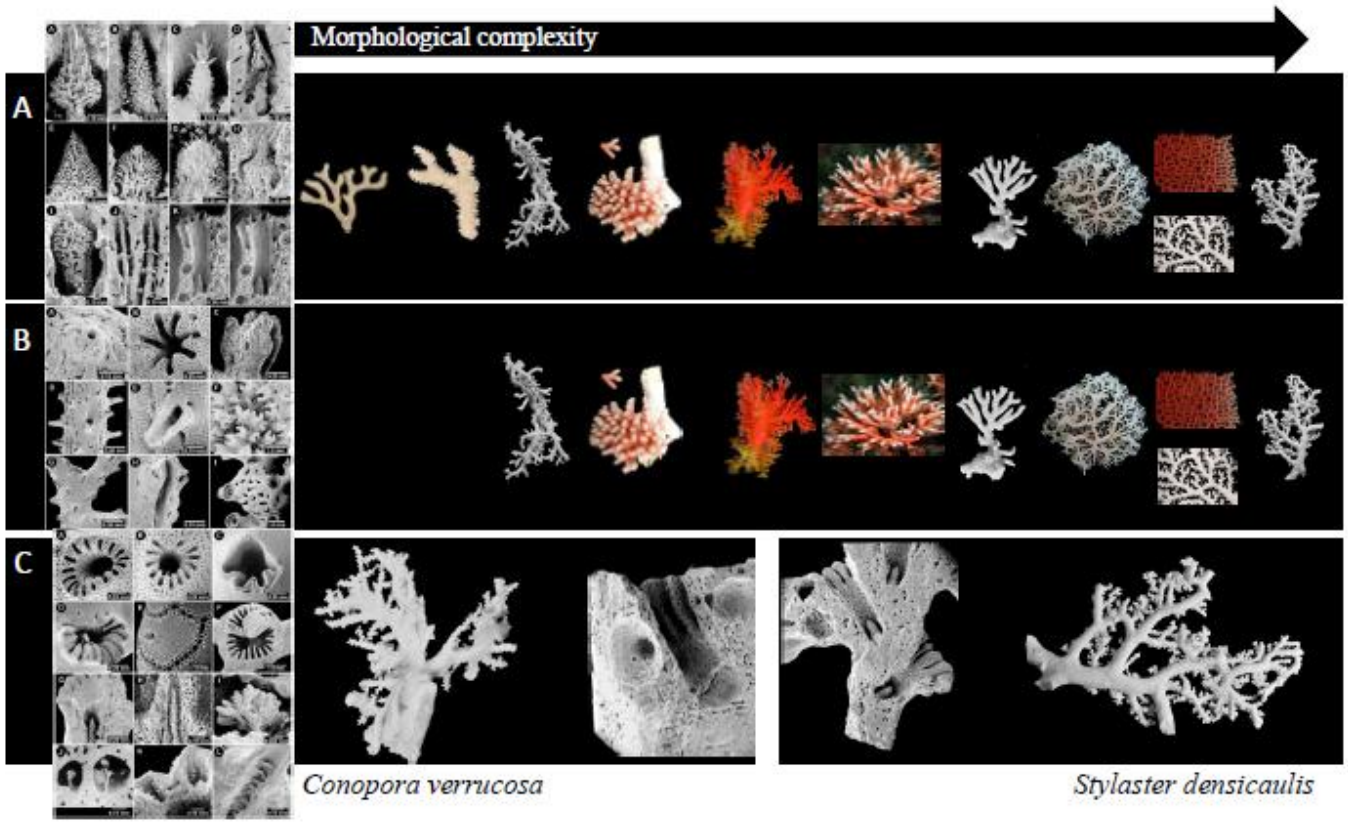

Figure 2. Morphological variation from primitive to derived character states (Cairns, 1983). *Sporadopora* is considered to be the least complex, and *Stylaster* is most derived, above *C. verrucosa*, which lacks a gastrostyle (A), and dactylostyles (B). SEM images are taken from Cairns, (2011) to show the fine scale diversity of (A) gastrostyles, (B) dactylopores and (C) cyclosystems. Species listed from left to right in row (A) include; *Sporadopora dichotoma*, *Stellapora echinata*, *Inferiolabiata labiata*, *Errina fissurata*, *Errina laterorifa*, *Errina antarctica*, *Errina gracilis*, *Cheiloporidion pulvinatum*, *Errinopsis reticulum*, *Errinopsis fenestrata* and *Stylaster densicaulis*. The gastrostyle is shared across 11 species, the dactylopore spine is shared by nine species (B), and the cyclosystem, the most derived character (C), is only found in *S. densicaulis* and *C. verrucosa*. Images also referenced in cladograms (Fig 3 - 7). *E. antarctica* (© Mathias Hune), *E. laterorifa* and *E. reticulum* (© Greg Rouse), other images are the authors own.

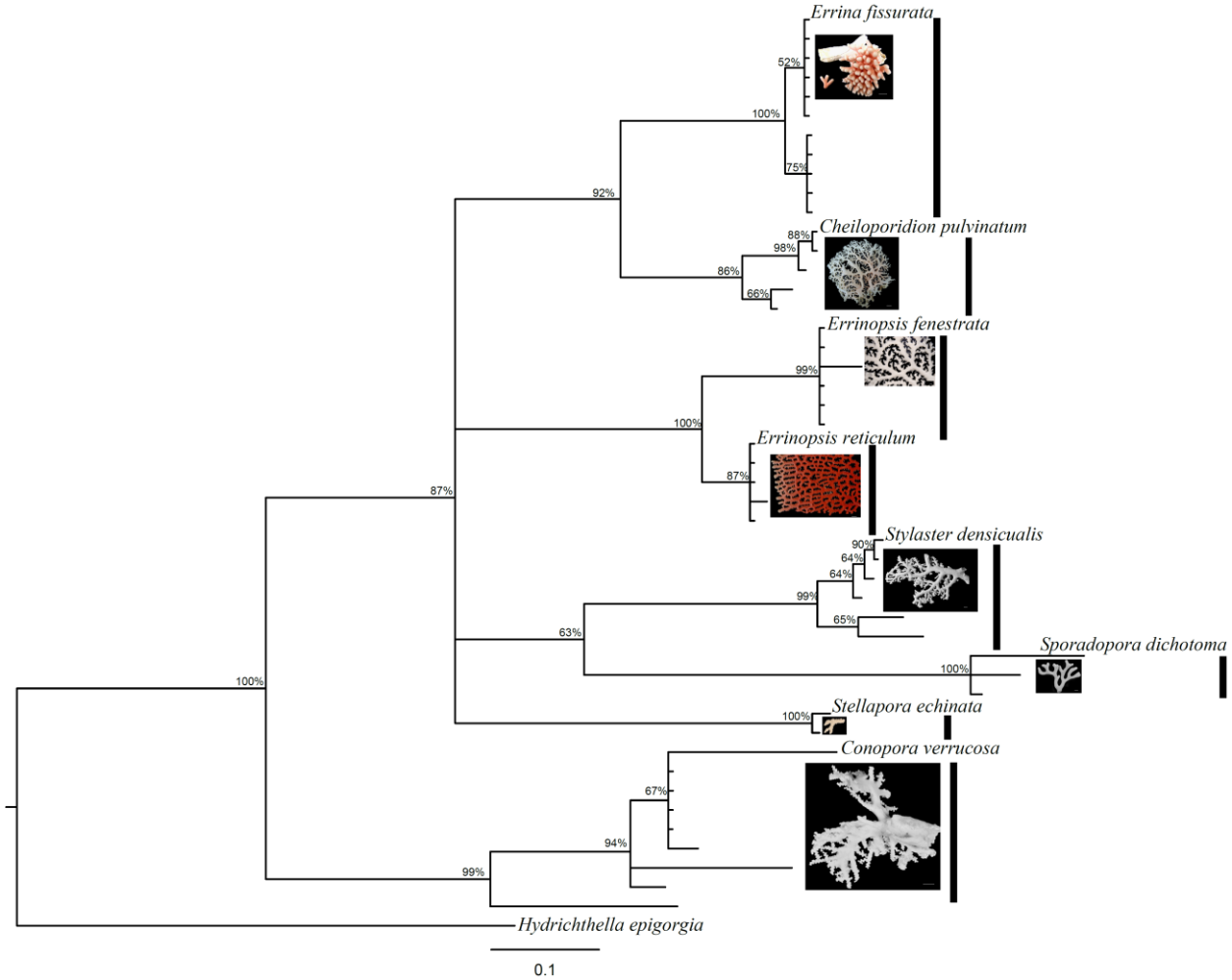

Figure 3. Phylogenetic relationships of stylasterid corals based on mitochondrial CO1 DNA sequence data (total alignment length = 289 bp). Percent values are Bayesian posterior probabilities.

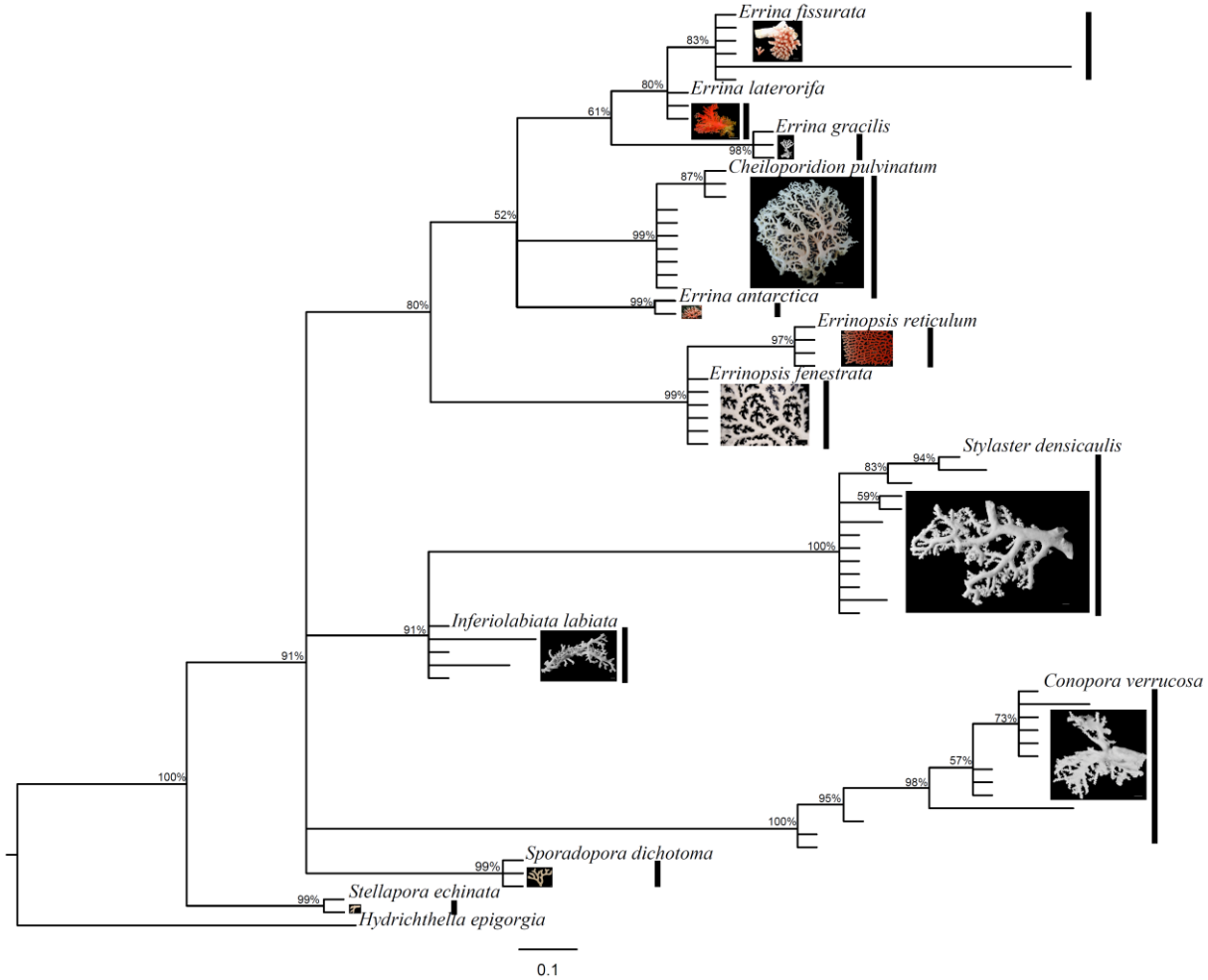

Figure 4. Phylogenetic relationships of stylasterid corals based on mitochondrial 16S DNA sequence data (total alignment length = 137 bp). Percent values are Bayesian posterior probabilities.

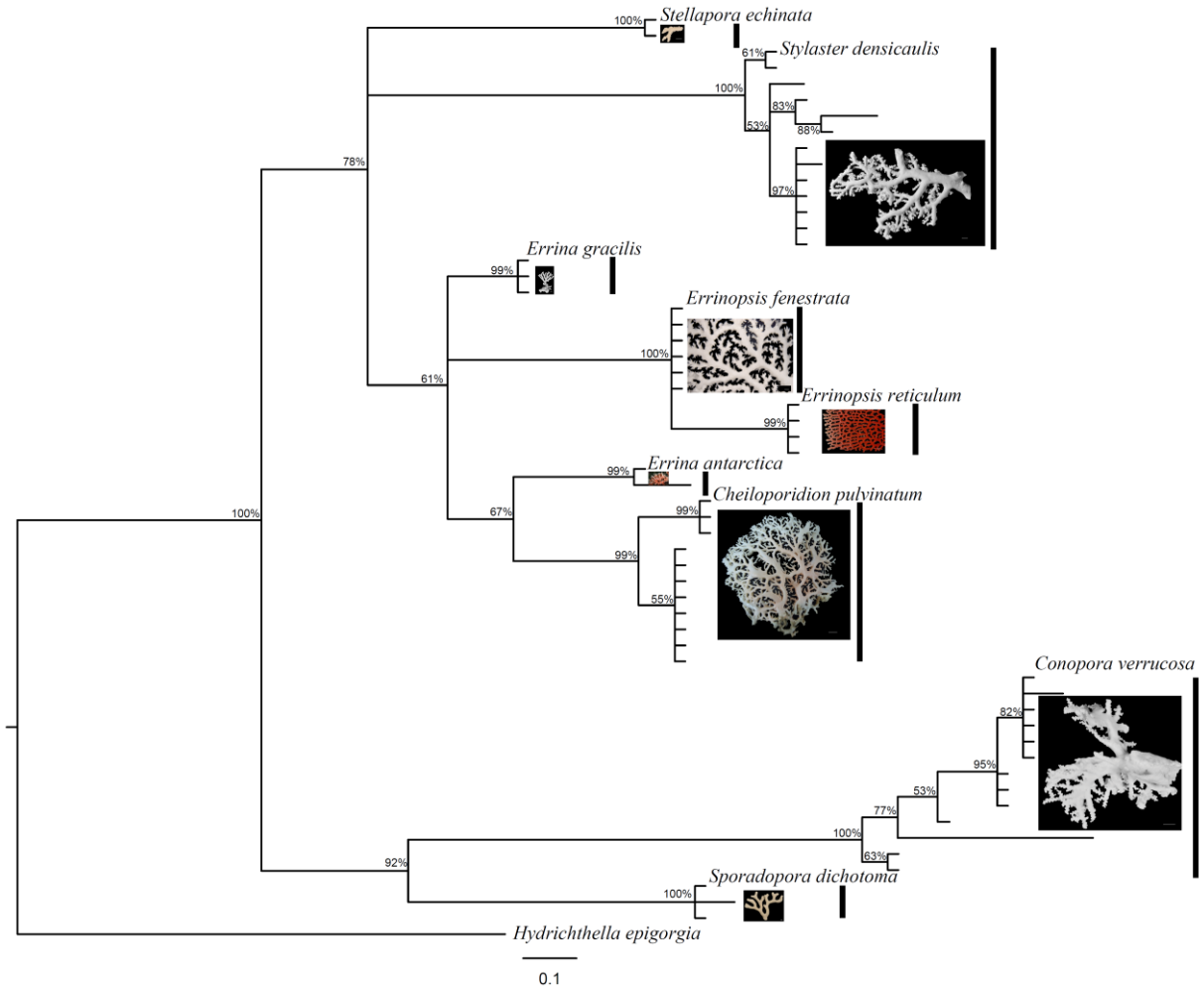

Figure 5. Phylogenetic relationships of stylasterid corals based on mitochondrial 16S DNA sequence data (total alignment length = 293bp). Percent values are Bayesian posterior probabilities.

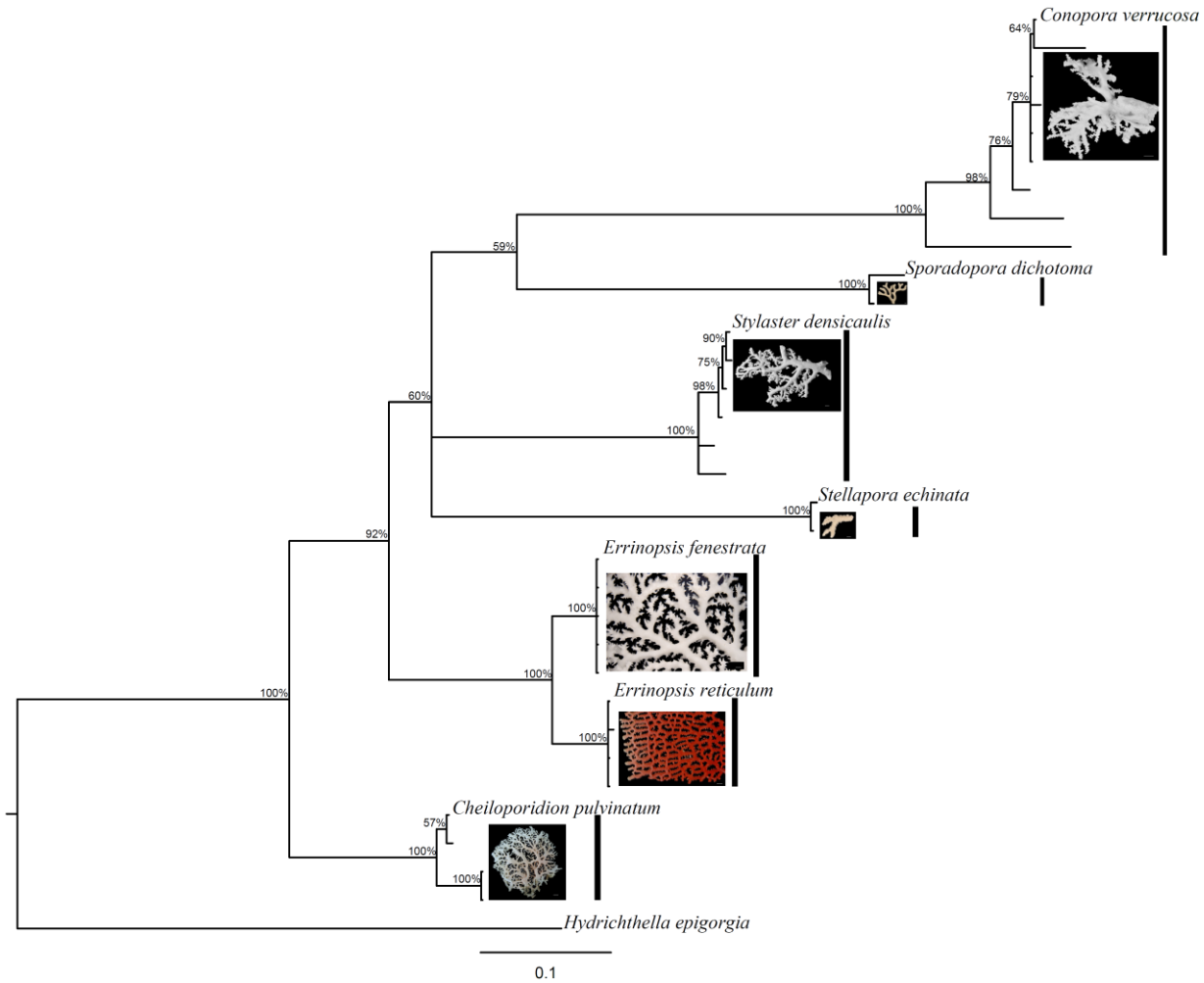

Figure 6. Phylogenetic relationships of stylasterid corals based on concatenated mitochondrial CO1 and 16S DNA sequence data (total alignment length = 872 bp). Values are percent Bayesian posterior probabilities.

Species Relationships in the *Errina*/*Errinopsis* Complex Inferred from ITS Data

ITS phylogenetic topology was markedly different to 16S and CO1 in regards to species arrangement (Fig 7). As with 16S and CO1 all 33 stylasterid taxa from Antarctica and the Sub-Antarctic were trialled during the course of this study. However, due to sequencing issues associated with the nuclear ITS region amplification success was low (n=44), and it was only possible to obtain portions (18S, ITS-1, 5.8S, ITS-2, 28S) rather than the complete 670bp of the ITS region. The final ITS tree is based on mainly ITS-2, the region with the highest variability and included the following species: *Errina fissurata*, *Errina laterorifa*, *Errina antarctica*, c.f *Errina gracilis* n.sp, *Errinopsis fenestrata* and *Errinopsis reticulum.* Species clades are well supported, with minimal within-species discrepancy, except in the case of the *E. antarctica* clade which includes cf *E. gracilis* n.sp, and *E. fenestrata* (Fig 7). These three species were collected from different geographic regions. *Errina antarctica* is from the Patagonian fiords in Chile, *E. fenestrata* is from the Scotia Arc, in the Sub-Antarctic, and cf *Errina gracilis* n.sp. is from the Shackleton Ice Shelf in East Antarctica. These locations are separated by 1000s of km and, despite synampomorphies, clear species-specific morphological structure is evident. Contamination is unlikely as specimens were collected on different voyages and sequenced in different laboratories. Therefore, the genetic similarity of these three species implies nuclear ITS relationships are not as resolved as the mitochondrial phylogenies based on 16S and CO1.

Due to the different levels of variation apparent in 16S, CO1 and ITS comparisons, the three gene regions were compared in a haplotype network including all available *Errina* spp. and *Errinopsis* spp. sequence data. 16S and CO1 haplotype networks do not corroborate with ITS network connections, and place *Errina* and *Errinopsis* in groupings reflective of their morphological relationships (Fig 8). In the ITS network, similarly to the ITS phylogenetic tree (Fig 7), *Errina laterorifa* is extremely divergent to *E. fissurata* (Fig 8). These two taxa are described as sister species (Cairns, 1983), but inter-specific variation is high enough to consider either a synonymization of the genera *Errina* and *Errinopsis*, or the separation of *E. fissurata* and *E. laterorifa* to different genera (Fig 7), based on comparative levels of variation within ITS in the coral literature (Table 3).

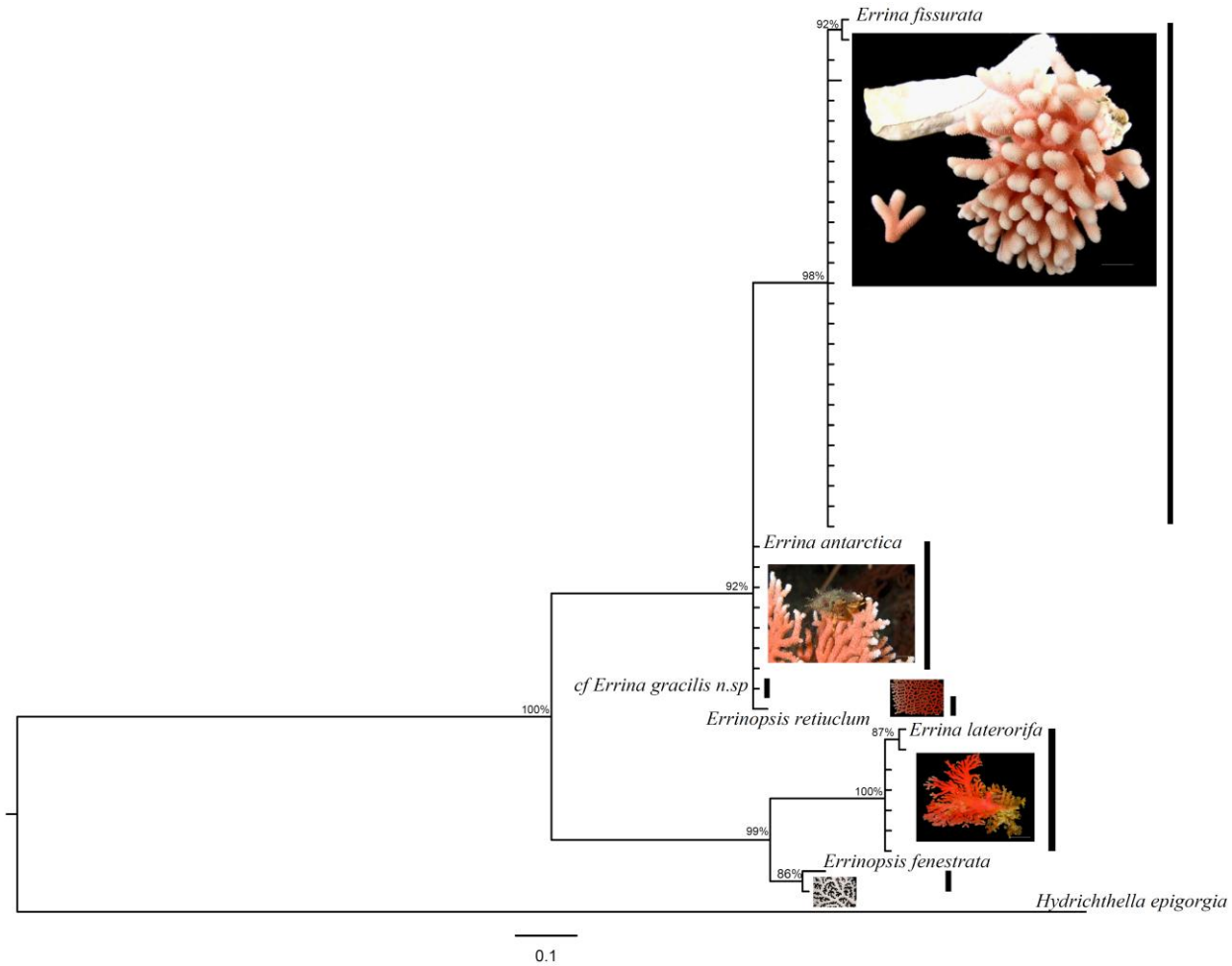

Figure 7. Phylogenetic relationships of stylasterid corals based on ribosomal ITS DNA sequence data from (total alignment length = 142 bp, mostly ITS-2). Values are percent Bayesian posterior probabilities.

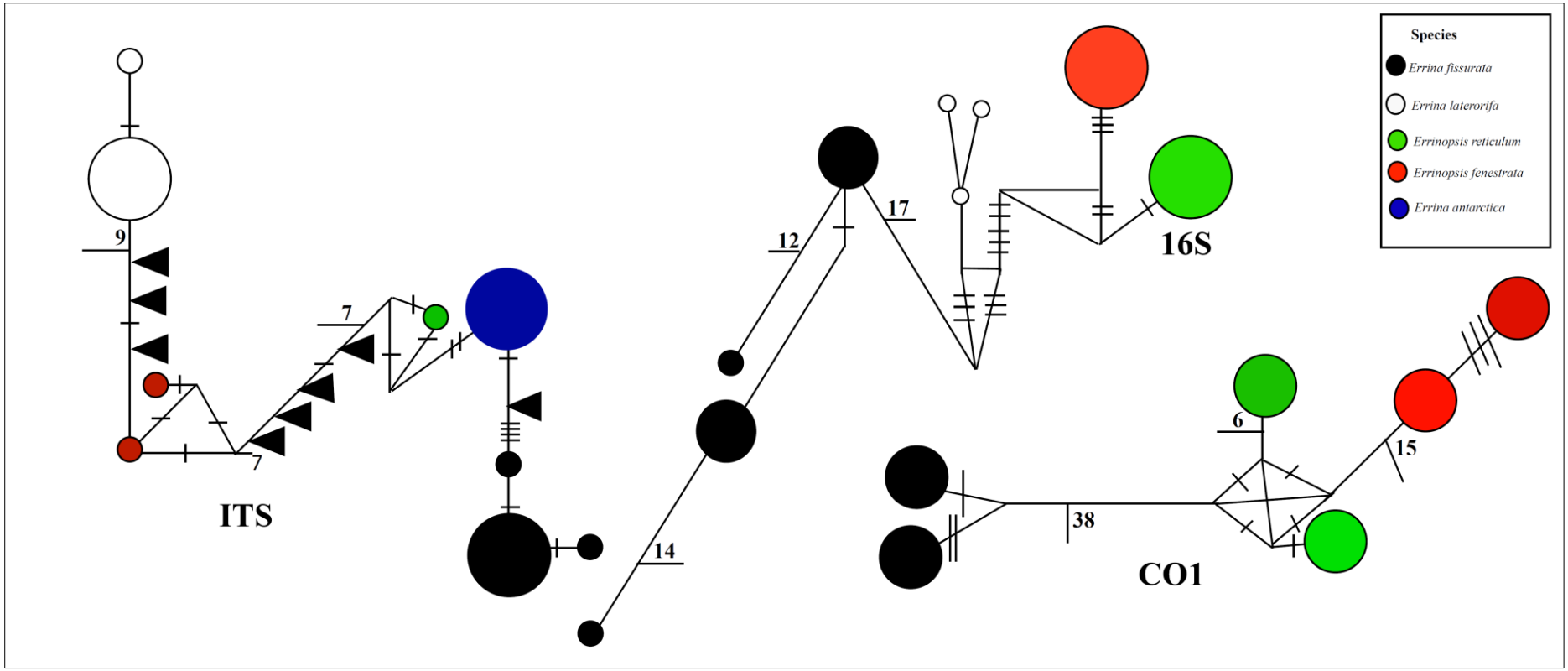

Figure 8. Haplotype networks based on ITS rDNA (n = 44, 142bp), 16S mtDNA (n = 24, 293bp), CO1 mtDNA (n = 17, 289bp) sequence data. Each node at a branch joining point represents a parallel mutation between a median vector (a triangle or square configuration in the network), each node on a branch represents a base pair mutation (represented numerically where there are >5bp mutations). Triangles represent indels. Haplotypes are sized according to abundance.

Table 3. Summary of ITS sequence variation reported in coral taxa. Edited from Miller *et al*., (2006), and updated based on literature to 2014.

Taxonomic group	Sequence length	Among genera	Among congeneric species	Within species	References
Paracyanthus steamsii	275 (ITS-1)			<1%	Beauchamp & Powers, 1996
Balanaphyllia elegans	279 (ITS-1)			<5%	Beauchamp & Powers, 1996
Madracis spp.	~613 (Full ITS)		~6%	3.3 - 3.5%	Diekmann *et al*., 2001
Platygyra spp.	539 (Full ITS)	~17%	~14%	<1.2%	Lam & Moreton., 2003
Scleractinians	104-369 (ITS-2)	>60%		1.3 - 23%	Lam & Moreton., 2003
Montastrea annularis, M. fraksi and M. faveolata	704 (ITS-1 - ITS-2)		1%		Lopez & Knowlton., 1997
Montastraea annularis	665 (Full ITS)			<3%	Medina *et al*., 1999
Stichopathes spp.	670 (Full ITS)		13.60%	~1%	Miller *et al*., 2006
Parantipathes spp.	710 (Full ITS)		12.8	2.50%	Miller *et al*., 2006
Solenosmila spp.	393 (ITS-1) 325 (ITS-2)			nil	Miller *et al*., 2006
Madrepora spp.	330 (ITS-1) 270 (ITS-2)			12-15% 30%	Miller *et al*., 2006
Stephanocyathus spp.	653 (Full ITS)		12.50%	2.30%	Miller *et al*., 2006
Acropora valida	ITS-1			29%	Odorico & Miller., 1997

Plesiastrea versipora	409 (Full ITS)			<4%	Rodriguez-Lanetty., 2001
Acropora longicyanthus	490 (ITS-1 + 5.8S)			25% & 11% (ITS-1 only)	Takabayashi *et al.*, 1998
Goniopora tenuidens	810 (ITS-1 + 5.8S)			15% (ITS-1 only)	Takabayashi *et al.*, 1998
Heliogungia actiniformis	740 (ITS-1 + 5.8S)			2% (ITS-1 only)	Takabayashi *et al.*, 1998
Stylophora pistillata	850 (ITS + 5.8S)			31% (ITS-1 only)	Takabayashi *et al.*, 1998
	142 (ITS-2)	32%	52%	3.6 -5%	This study
Errina laterorifa	142 (ITS-2)	32%	52%	0.72%	This study
Errina antarctica	142 (ITS-2)	31%	52%	<1%	This study
Errinopsis fenestrata	142 (ITS-2)	14%	20%	10%	This study
Errinopsis reticulum	142 (ITS-2)	26%	20%	0	This study
Acropora spp	405 (Full ITS)		0- 5.2%		Van Oppen *et al.*, 2000
Pseudopterogorgia elisa-bethae	ITS 562bp			7.30%	Gutiérrez-Rodríguez *et al*., 2009
Lophelia pertusa	603 (ITS2)			0%	Flot *et al*., 2013
Goniastrea spp.			0 - 098%		Huang *et al*., 2014

Discussion

Genetic differentiation among morphological stylasterid species was well defined, though morphological disagreement regarding inter-species clade arrangement exists. If characters are assessed from primitive to derived as outlined in Cairns (1983, 1984) (Fig 2), *Sporadopora* or *Stellapora* would be the most basal clade and *Conopora* or *Stylaster* would be the most derived, or most closely related, and they are not (Figs 3 - 6). Such discrepancies are well documented in corals (Flot *et al*., 2011; Baco & Cairns, 2012), speciation is not a linear process (Clarke & Johnson, 1996; 2003), and 16S and CO1 loci are known to be conservative in cnidarians (Forsman *et al*., 2006; 2010). Thus, it is to be expected that relationships are not aligned in totality and a more variable gene region may clarify fine scale intra-species level differences not apparent within mitochondrial data. For this reason the nuclear ITS was used as a comparison, and showed differentiation between species which contradict the mitochondrial cladograms (Fig 7). Only *Errina* and *Errinopsis* species amplified successfully for comparison (Fig 8). A number of the other available genetic markers; 54kDa (SRP54 Bax, 2009), the mitochondrial displacement loop (D-Loop Bax, 2009), and calmodulin (Lindner *et al*., 2008) have been trialled on the group with varying levels of success. To date the markers used in this study show the highest amplification success and appear to be the most informative available.

Is there a difference between stylasterid morphology and genetics?

I propose that stylasterid genetic and morphological relationships are evolutionarily sound based on synapomorphies. However, the configuration of synapomorphies differs from the character states described in morphological literature (see Cairns, 1983) (Fig 2). Key to this hypothesis is the gastrostyle, a calcareous structure thought to aid in the protection of the polyp. The gastrostyle may be more recently derived than stated in morphological delineations (Cairns, 2011). Six of the studied genera (*Errina*, *Errinopsis*, *Cheiloporidion*, *Inferiolabiata*, *Stellapora* and *Stylaster*) contain gastrostyles, have overlapping distributional records and a similar spine structure (Cairns, 1983) (Fig 2). If a lack of morphological complexity is to correlate with the basal (ancient) state the least morphologically complex coral *Sporadopora dichotoma* would fall out at the base of the phylogenetic tree (Cairns, 1983), and it did for most alignments (Figs 3 - 5). However, note that *S. dichotoma* aligned with *C. verrucosa* at the base of cladograms (Figs 3 - 5), an unexpected result based on *C. verrucosa* as

one of the most morphologically complex and *S. dichotoma* as least morphologically complex amongst the species included here (Fig 2).

In the CO1 alignment (Fig 3), *S. dichotoma* shows a similar branch length to *Stylaster densicaulis*. *Stylaster* is one of the more morphologically complex and speciose genera (110 described species world-wide, compared to 9 species of *Sporadopora* (WoRMS, 2012). *Stylaster* species complexity is largely attributed to the morphological structure called a cyclosystem. Cairns (2011) described the cyclosystem as a 'well-coordinated functional unit' potentially developed to improve feeding efficiency as well as polyp/larval protection and defence. *Stylaster densicaulis* and *C. verrucosa* are the only species included herein with cyclosystems (Fig 2). The presence of *C. verrucosa* as basal in the CO1 and 16S phylogenies (Figs 3 - 5), a species which lacks a gastrostyle, adds significant evidence to support a re-examination of these species relationships, and the definition of the cyclosystem as a derived character may need to be reconsidered.

Cyclosystems have a distinct skeletal structure, and form an important basis of current phylogenetic delineations (see Cairns, 1983 and 2011 for a summary). Both cyclosystemate and non-cyclosytemate fossil forms co-occur throughout the paleo-record (Cairns, 2011, Lindner *et al*., 2008). The phylogenies presented here indicate that cylosystems evolved separately (either once with two losses Fig 4 or twice Fig 6). Cairns (2011) suggested that phylogeny presented in Lindner *et al*., (2008), is composed of three distinct clades. Clade 1) contains double-chambered cyclosystemate species, including *Conopora* spp. which lack a gastrostyle (Fig 2), *Plibothrus* spp. with no observed gastrostyle (Cairns, 1983) and *Lepidopora microstylus*, which in contrast to the other two genera has a rudimentary gastrostyle (Lindner *et al*., 2008). Clade 2) contains *Stylaster* spp. (with both cyclosystems and gastrostyles). Clade 3) contains the other stylasterid taxa (some cyclosystemate and some not). This suggests that the common ancestor to all stylasterid clades had cyclosystems. The molecular phylogenies presented here support this hypothesis.

The oldest known stylasterid appears to be *Conopora* (Jell *et al*., 2011), therefore its placement as the basal clade (Figs 3 - 5), rather than the derived clade (Fig 7), is a parsimonious conclusion and a review of the literature on cyclosystems confirms the importance of this morphological character trait in stylasterid evolution. The skeletal arrangement of cyclosystems often varies by species (e.g., sympodial *vs.* even arrangement in *Stylaster* sp. (see

Cairns, 1983), and cyclosystems are known to regenerate rapidly on broken colony branches (i.e. *Stylantheca californicus* (surmised in Brook & Stone, 2007). In *Conopora* species, reproductive ampullae are associated with cyclosystems (Cairns, 1983). Further, some stylasterid species (e.g., *Errinopora*) have pseudocyclosystems, defined as cyclosystem-like structures with a gastropore surrounded by dactylopores, lacking the same level of structural fusion (Cairns & Lindner, 2011). This indicates that structural compartmentalisation of essential characters is a common adaptive strategy in stylasterids. Therefore, the variations seen in cyclosystem arrangement and complexity may have been selected for independently in multiple species through evolutionary time.

Is there a difference between the results from ITS, CO1 and 16S gene regions?

ITS is traditionally the most variable region available for coral studies (Forsman *et al*., 2006). However, the multiple issues associated with ITS such as homoplasy (Nei & Kumar, 2000), incomplete lineage sorting (or reticulate evolution) and introgressive hybridisation (Veron, 1995) often limit the utility of ITS data in coral phylogenetics (Vollmer & Palumbi, 2004). These factors lead to high intra-individual variability in ITS sequence data, and variability is known to vary between species depending upon species specific mutation rates (summarised in Table 3). Sample amplification was unsuccessful across all three gene regions for the majority of samples, and it was impractical to compare nuclear and mitochondrial data within a concatenated phylogeny. Based on the comparative variability found in this study between ITS, CO1 and I6S, it is likely that nuclear mitochondrial discordance would limit the applicability of concatenation even if sufficient data were available for comparison.

Network connections show that ITS variation at the species level is evident in distinct haplotype groupings of *Errina fissurata*, *Errina laterorifa*, *Errinopsis fenestrata* and *Errinopsis reticulum* (Fig 8). However, species relationships differ markedly from what we might expect based on morphology (Cairns, 1983), and 16S and CO1 phylogenetic relationships, which place *E. fissurata* as either separate from *Errinoposis* spp, or sister to other *Errina* species (Figs 3 - 6). Counterintuitively, of the six species included in the ITS parsimony network, *E. laterorifa* and *E.fissurata* are the most genetically divergent. When investigating the levels of intra- and inter-specific sequence variation in *Errina* spp. the amount of variation apparent between *E. fissurata* and *E. laterorifa* sequences suggests they are different

genera (~32% Table 3), rather than congeneric species as described in Cairns (1983), (we expect ~ 0.5 - 14% variation Table 3), and suggests a synonymisation of the genus *Errinopsis* under the senior synonym of *Errina*, or the separation of *Errina* species into different genera. It is unexpected to have such a large amount of genetic difference between morphologically described similar species, and morphological characteristics show a higher support for 16S and CO1 genetic relationships (Cairns, 1983). Therefore, the relationships presented herein can be considered reliable for 16S and CO1 based on significant genetic differentiation, whilst inter-species relationships inferred from ITS are questionable in comparison.

How do results compare to Antarctic hydrozoan and coral groups and is there potential for DNA barcoding in stylasterids?

The cnidarian mitochondrial genome is considered stable in comparison to other metazoans and is characterized by low rates of evolution that make it often impossible to distinguish species using mtDNA sequences (Van Oppen *et al*., 2000; 2001). For example, in the deep sea sclearactinian coral *Madrepora oculata* intra-specific differences were so low that when the genome of the species was examined in full only the arrangement of genes differed by geographic clade, whilst the sequence alignments were the same (Lin *et al*., 2012). It is intriguing to find clear segregation between stylasterid genera and in most cases species for the CO1 and 16S gene regions. Given that mitochondrial differentiation was much higher than expected, this may have important implications regarding our understanding of the evolution and biodiversity of the Antarctic benthos, with specific applications to coral genomics. For example, mitochondrial markers are more advantageous than nuclear markers for a number of reasons; haploid markers are cheaper, easier to sequence and there are no heterozygosity issues. This is particularly helpful when studying smaller populations as mitochondrial markers coalesce faster, alleviating problems associated with shared ancestral polymorphism, and we can assume a genetic asymptote of selection (Flot *et al*., 2013).

CO1 is the most commonly used DNA barcoding gene (DeSalle *et al*., 2005), and proved more reliable than 16S and ITS at differentiating fine scale relationships here, with a higher variability (Fig 8). For example CO1 showed a clear differentiation between two clades of *E. fissurata* (Fig 3), whereas posterior probability values in 16S show the lowest support when differentiating between the closely related *E. fissurata*, *E. laterorifa* and *E. gracilis* (Fig 4). Similarly, *C. pulvinatum* and *S. densicaulis* inter-species clades show a higher differentiation in CO1 than 16S (Fig 3 *vs.* Fig 4 & 5). Very few hydrozoan studies use

CO1 (Govindarajan *et al*., 2005; Geller *et al*., 2013), as standard primers are known to have amplification difficulties (Peña Cantero *et al*., 2009). The primers used in this study were recently developed by Geller *et al*., (2013), and offer potential for future studies, but sequencing issues remain. Despite multiple attempts, a smaller subset of samples amplified here for CO1 than 16S, and although less variable than CO1, 16S also successfully differentiates between genera and species. Therefore, 16S may prove to be a better barcoding gene for stylasterids. Studies on other hydrozoan groups substantiate its utility at genera level for some taxa (Govindarajan *et al*., 2005), including Antarctic genera (Peña Cantero *et al*., 2009).

Conclusion

Stylasterids are an understudied family of corals, with little to no genetic data available for many species. This study presents a general congruence between morphology and genetics based on mitochondrial DNA and the reassignment of synapomorphies. ITS relationships are unfavourable at the inter-species level when compared to mitochondrial topography, and more research is needed to determine the applicability of the region to stylasterids. However, intra-species overlap is highly conserved, and the possibility remains that ITS variability is phylogenetically informative. The genetic differences between described sister species was so substantial, and comparatively so justifiably explained by 16S and CO1 data that a synonymisation of *Errina* spp. and *Errinopsis* spp., or the separation of *Errina fissurata* and *Errina laterorifa* as separate genera is not recommended without further evidence.

The differentiation apparent in CO1 and 16S is considered substantial in comparison to other Anthozoan and Hydrozoan groups. Therefore, there is potential for DNA barcoding in stylasterids. This has a number of practical implications - the discovery of new species, cataloguing of Antarctic marine life and identification of specimens that cannot be determined by taxonomic means. However, the results of this study were only achieved through the incorporation of taxonomic expertise in species identification. Intra and inter-species relationships remain unresolved across the three gene regions, CO1 amplification success was low, and 16S inter-species relationships were the least resolved where closely related species are concerned. Therefore, the incorporation of molecular taxonomy as a complementary tool, rather than the elimination of traditional species taxonomy is

recommended to future and ongoing collaborative initiatives such as the CAML and BOLD to most accurately quantify stylasterid diversity in the Antarctic and Sub-Antarctic benthos.

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Evolutionary Relationships of Drake Passage Stylasterid Corals

Stylasterid corals are widely distributed in field-like abundance throughout the Drake Passage. These coral fields are predominantly deep (> 500 m), found on rocky substrate in isolated patches off seamount and shelf locations, and their ability to predominate in select habitats is likely driven by a number of evolutionary and ecological factors. A Bayesian mitochondrial phylogeny (CO1 and 16S) was combined with the fossil record and mineralogy data to investigate stylasterid evolution in the Drake Passage, and to test if selection towards either calcite or aragonite carbonate mineralogy has a phylogenetic component. The estimation of divergence dates based on a Bayesian relaxed clock and fossil calibration provide evidence that stylasterids may have radiated within the Drake Passage following de-acidification of the world's oceans linked to the Eocene/Oligocene boundary (~ 34 MYA). Subsequently their speciation may correspond to adaptations linked to skeletal mineralogy. Aragonite is the predominant and likely ancestral calcium carbonate mineralogy of Drake Passage stylasterids, whilst the two most derived genera, *Errinopsis* and *Cheiloporidion*, have predominately calcite skeletons, at some locations and depths. The evolutionary scenarios that may have led to calcite in some genera and the utility of either calcite or aragonite are discussed in the context of both paleo-environmental and future climate change. This information provides valuable insight into the evolution of stylasterids in the Antarctic benthos, and offers a basis for future research of this understudied group of diverse and ecologically important bi-mineralic corals.

Introduction

Evolution in the Antarctic and Sub-Antarctic Environment

Benthic communities throughout the Antarctic and Sub-Antarctic waters are highly biodiverse (Rogers *et al*., 2007), and the Antarctic region has a remarkable history characterised by tectonic shifts, fluctuating glacial cycles and high species richness in patchily distributed habitats (Clarke, 2003). Species richness among taxonomic groups likely arises due to a balance between factors such as species formation, the rate of extinction, and evolutionary time (Thatje *et al*., 2012). Evolutionary processes include speciation e.g., vicariance (geography related species formation), dispersion, immigration, isolation, selection, adaptation, extinction and climate change (Mora *et al*., 2006; Glor, 2010; Rogers *et al*., 2012). Genetic studies reveal that isolation and adaptive radiation are common (Wilson *et al*., 2007; Baird *et al*., 2011; Strugnell *et al*., 2011), and vicariant speciation associated with tectonic transformations is well documented (e.g., Patarnello *et al*., 1996; Crame, 1999; Matschiner *et al*., 2011).

Adaptive radiation in the Antarctic benthos is often linked to the history of the region, and geography has important implications for the evolution of marine benthic fauna. At the end of the Jurassic era (157 – 130 MYA) the supercontinent Gondwana began to separate (Boger, 2011). By the Eocene (~ 50 MYA) a rift had formed between Antarctica and Australasia, and Antarctica and South America were divided by ocean (Livermore *et al*., 2007). At the end of the Eocene (~ 35 MYA) the Drake Passage had opened and Antarctica became isolated by the formation of the Antarctic Circumpolar Current (ACC) (Aronson *et al*., 2007). Following the formation of the ACC extreme climate forcing produced the Antarctic ice sheet, and climatic fluctuations resulted in periodic glacial and inter-glacial cycling (see Clarke & Crame, 1992).

Glacial formation and retreat is a frequently cited driver of speciation in Antarctica (Wilson *et al*., 2009; Griffiths *et al*., 2010). Glacial-interglacial (cold-warm) cycles are thought to contribute to the exceptionally high incidence of cryptic speciation and adaptive radiations in Antarctica (Wilson *et al*., 2007; 2009 Baird *et al*., 2011; 2012). This has been seen in recent studies of the biota of the Ross and Weddell Sea shelves, which were completely iced over during the last glacial maximum 22-18 thousand years ago (Denton & Hughes, 2002). These areas were more recently colonised by benthic fauna (e.g., Wilson *et*

al., 2007; Hunter & Halanych, 2010), potentially allowing for niche specialisation. Currently, the Southern Ocean is considered stable within seasons (winter/summer), in an extended inter-glacial period. However, the apparent stability we see today has only been in place for the last 10,000 years, and the climate has undergone substantial geological and oceanic change prior to this time. Furthermore, the Antarctic climate is predicted to change dramatically in the coming decades (IPCC, 2013), and understanding how ecosystems have responded to past climate events may help us understand what will happen in the future.

Ocean Acidification Threatens Deep-Sea Antarctic Corals

One of the most important discoveries in recent years is the climate association phenomenon of ocean acidification (Guinotte & Fabry, 2008). The chemistry of the oceans is changing at an unprecedented rate due to the influx of anthropogenic carbon dioxide (CO_2) into the atmosphere (Feely *et al*., 2004). Current estimates suggest that over one third of the total CO_2 emitted into the atmosphere, around 118 million tonnes of CO_2, has been absorbed by the ocean. Acidification of ocean waters due to the influx of carbon dioxide from the atmosphere penetrates slowly to the deep-sea over time scales of several centuries (Guinotte *et al*., 2006). Surface pH (< 300 m) is largely buffered by the high ambient concentrations of carbonate ions. In deeper waters (> 500 m), respiratory CO_2 eliminates available carbonate, thereby reducing the buffering capacity of those waters. pCO_2 (carbon dioxide partial pressure) is highest in cold water (Aronson *et al*., 2011), and it is predicted that deep-sea corals will be the first to experience the detrimental effects of ocean acidification (Turley *et al*., 2007; Guinotte & Fabry, 2008). Around 40% of the global inventory of anthropogenic CO_2 can be found in the Southern Ocean. This equates to a total pH decrease of 0.14 in the Southern Ocean since the beginning of the industrial era (Feely *et al*., 2004), and laboratory and *in situ* coral studies indicate that pH decreases may induce sub-lethal effects by slowing metabolic processes, growth rate, and calcification (e.g., Orr *et al*., 2005; Kleypas *et al*., 2006; Kline *et al*., 2012).

The saturation horizon in the deep-sea is defined as the point below which calcification is inhibited due to the unavailability of essential carbonate ions (Feely *et al*., 2004). The level of the saturation horizon differs depending upon the type of carbonate mineral calcifying organisms utilise (aragonite or calcite) to deposit their skeletons. For the majority of deep-sea corals this mineral is aragonite although some coral species have been shown to produce skeletons comprised both of aragonite and calcite (Cairns & MacIntyre,

1992; Thresher *et al*., 2011). The saturation horizon for calcite (CSH) is much deeper (> 3000 m in some parts of the Southern Ocean) than the aragonite saturation horizon (ASH) (~ 1000 m). Therefore, corals that utilise calcite may be able to occupy deeper habitats than aragonite calcifiers, and better adjust to undersaturation.

Paleo-Environmental Change and Coral Calcification

On paleo-oceanic time scales, calcifiers have either predominated or perished in response to changes in calcite or aragonite saturation states through geological time (Honisch *et al*., 2012). This environmental variation in the paleo-Antarctic is extremely difficult to assess. However, despite limitations, a substantial literature has emerged on the broad oceanic processes driving calcite and aragonite fluctuations through geological time (summarised in Porter, 2010; Ries, 2010), thus inferring paleo-Antarctic patterns from what is known about the global ocean through time. Much of this literature stems from the Stanley and Hardie (1998) model, which outlines how calcite and aragonite dominant seas have fluctuated from ~ 550 MYA through to the modern era. In their model five time periods are defined to explain when oceanic conditions were either most favourable for aragonite (aragonite seas I, II, III), or calcite (calcite seas I, II). In many cases, evolutionary succession defined as the process by which a species assemblage successively advances toward an evolutionary stable climax, is in concert with these paleo-oceanographic transitions from calcite to aragonite seas (Honisch *et al*., 2012; Porter *et al*., 2010).

The predominance of the aragonite coral order Scleractinia during aragonite seas, and the rudist bivalves during calcite seas provides a good example of this type of succession. The fossil record, and what is known about the calcification strategies of these two calcifying families, shows that during the Triassic ~ 240 MYA (aragonite sea II), scleractinians were abundant throughout the world oceans. The progression from aragonite to calcite sea conditions during the Cretaceous ~175 MYA (calcite sea II), signalled scleractinian population declines (Simpson *et al*., 2011). This decline in Scleractinia aligns with an increase in the rudist bivalves. When oceanic chemistry reverted to an aragonite sea in the Oligocene (aragonite sea III), the rudist bivalves became extinct (Steuber *et al*., 2002), and Scleractinians diversified. This renewed benthic dominance is evident on modern oceanic coral reefs (Perrin & Bosellini, 2012). Stanley and Hardie (1998) explain this pattern of calcification and succession through ontogeny. In this way calcifying animals either predominate or decline based on the favourable conditions in which they arose, as opposed to

those in which they currently live (see Reis, 2010; Porter, 2010). Therefore, as the oceans change in our current era of ocean acidification, succession may cause population declines in aragonite corals as they may not have the capacity to adapt their mode of calcification to changing saturation states (Porter, 2010), predicted to be more similar to Eocene conditions by 2100 (IPCC, 2013).

There is evidence to suggest that corals may have adjusted to decreasing saturation states in the paleo-ocean. Some coral lineages are non-calcifying (see Lin *et al*., 2014), with ancient representatives which may have existed in an anemone state without calcified skeletons during the Cretaceous (see Fine & Tchernov 2007; Fautin *et al*., 2009). Some Antarctic coral families have bi-mineralic representatives (Stylasteridae Cairns & MacIntyre, 1992; Isididae Thresher *et al*., 2010), which may be able to alternate their calcification strategy in response to ocean chemistry (Reis *et al*., 2010). Therefore patterns of calcification and succession linked to changing saturation states may hold true on broad taxonomic, temporal and geographic scales (as outlined by Honisch *et al*., 2012), but not at finer scales, such as within and between Antarctic coral species assemblages.

The calcification responses of Antarctic corals are currently unknown for all species, in fact there is very little data on Antarctic coral species beyond taxonomic descriptions (see Cairns, 1982; 1983). The only exception being the cosmopolitan aragonite scleractinian coral *Desmophyllum dianthus*, for which a reasonable literature relating to ocean acidification exists (see Miller *et al*., 2011; Jantz *et al*., 2013; Fillinger & Ritcher, 2013). Most of these studies are based on extant *D. dianthus* populations outside of Antarctica, predominantly within the Chilean fiords. Only one study by Margolin *et al*., (2014) provides any historic information on this species in Antarctica, and only within the last ~100,000 years, in the Drake Passage. Margolin *et al*., (2014) did not address ocean acidification specifically; however they did find evidence of unique environmental requirements liked to productivity, oxygen concentration and carbonate saturation state. Paleo-oceanographic studies often find synergistic interactions between common variables - e.g., oxygen, carbon, and nitrogen (Coggon *et al*., 2010). Therefore, our ability to predict adaptation, acclimation or past ecosystem succession in response to decreasing saturation states is hampered by an inability to tease apart the inter-relational factors linked to calcification.

Ocean Acidification and Deep-sea Coral Mineralogy

Due to the vulnerability of deep-sea calcifiers, especially habitat forming species such as deep-sea corals (Orr *et al*., 2005; Guinotte *et al*.,2006; Turley *et al*., 2007), recent literature has focused on carbonate mineralogy to determine the capacity of corals to deal with ocean acidification (Thresher *et al*., 2011; Thiagarajan *et al*., 2013; Margolin *et al*., 2014). This is based, in part, upon the hypothesis that corals which utilise calcite may be less susceptible to decreases in pH, as they can potentially persist in deeper habitats below the ASH (~ 1000 m), and above the CSH (~ 3000 m) (Feely *et al*., 2004). However, substantiating such a hypothesis, especially in the understudied deep Antarctic Ocean is extremely challenging. Geographic and bathymetric distribution patterns are limited and due to collection protocols (e.g., mainly trawling) depth data are usually non-specific. Variability in saturation states within and between geographic regions on geological time scales is not well understood. If biogeographic data is available (see de Broyer *et al*., 2014 for a current synthesis), corresponding coral mineralogy data is often lacking (Cairns & MacIntyre, 1992 provide the only reference for Antarctica to date), and oceanographic information is either regionally restricted or inferred from broad scale oceanographic data sets which lack fine scale resolution applicable to the Antarctic benthos. For example, saturation horizon data is only available in East Antarctica (Poisson *et al*., 1987; Moy *et al*., 2009), or inferred from global data sets (Feely *et al*., 2004). When investigating these processes from a paleo-oceanic perspective, studies are further restricted as information is sparse in spatial and temporal coverage, and fine-scale biological data are lacking and only available by proxy (e.g., ice cores, fossils etc.) (IPCC, 2013).

Case study: Stylasterid coral mineralogy and phylogenetic relationships in the Drake Passage

The Drake Passage benthic environment is characterised by geographic and oceanic change over millions of years. The heterogeneity of seamount, shelf and slope habitats through time may have increased skeletal mineral plasticity in certain stylasterid taxa, leading to a fixed functional mineralogy and/or a certain calcification strategy. The Drake Passage and Scotia Arc region is described as the most species rich locality for stylasterids within the Antarctic region. Of the 33 known Antarctic stylasterids, 12 genera are found in this vicinity (16 species) (Bax & Cairns, 2014). According to Cairns and MacIntyre (1992) two genera, *Errinopsis* and *Errinopora*, have a predominantly calcite skeleton and nine genera, including *Adelopora*, *Crypthelia*, *Inferiolabiata*, *Lepidopora*, *Stylaster*, *Stellapora*, *Cheiloporidion*,

Sporadopora and *Conopora*, have predominantly aragonite skeletons. One genus, *Errina* is described as bi-mineralic: whereby some species have mostly aragonite and some species have primarily calcite skeletons. This combination of high stylasterid diversity and variety of calcium carbonate skeletal form, suggests that the Drake Passage may be an epicenter of diversity for Stylasteridae, and that calcium carbonate polymorphism may have been important in the evolution of some genera. This claim is further substantiated by the distribution of either aragonite or calcite polymorphs within stylasterid $CaCO_3$ skeletons, in alignment with their morphological species relationships (Cairns & MacIntyre, 1992). Thus, Cairns and MacIntyre (1992) predict phylogenetic carbonate determinism.

The Eocene/Oligocene boundary was the last paleo-oceanic transition from calcite to aragonite seas, following a time of historically high acidification and warm temperatures termed the Paleocene-Eocene Thermal Maximum (PEMT) (Honisch *et al*., 2012). If stylasterid mineralogy is phylogenetically determined, then we might expect species that arose during periods of differing ocean chemistry to have evolved contrasting mineralogies. For example, Stanley and Hardie (1998) hypothesise that either side of the Eocene/Oligocene boundary different calcification strategies were more favourable. Before ~ 34 MYA calcite calcification was favoured (termed a calcite sea). After ~ 34 MYA aragonite was favoured (termed an aragonite sea). This time period 34 MYA is also linked to a rapid de-acidification of the world's oceans, and deepening of the CCD from a paleo-depth of ~ 3000 m (Pusz *et al*., 2011), to its modern depth of ~ 4000 m (Broecker, 2008). During this time the ASH (~ 1000 m) and CSH (~ 3000) were correspondingly set to their modern depths (Broecker, 2008), potentially opening up new deeper habits for benthic calcifiers and facilitating diversification. To best assess this evolutionary scenario in stylasterids, phylogenetic relationships are considered alongside skeletal composition of species to investigate if changes in mineralogy correlate with the Eocene/Oligocene period of evolutionary divergence ~ 34 MYA. In addition, phylogenetic relationships are overlain with the available depth and location data to examine links between calcite mineralogy and environmental conditions. Evidence that stylasterids have adapted to changing environmental conditions in the past will have important implications for long-term survival through future changes in ocean acidification and chemistry (IPCC).

Methods

Study Area, Collection and Samples

Coral samples were collected mainly by Hein and Blake trawl, and in some cases in dredge and core samples (Waller & Robinson NPB1103 cruise report, 2011). Samples were obtained from depths of 130 m to a maximum sampling depth of 2,149 m. Sample number, replicate, location, latitude, longitude, morphological identification and voyage were recorded for all study specimens (Appendix Table 2). Samples are housed at the Darling Harbour Marine Lab in Maine, USA and a reference collection will be deposited at the Tasmanian Museum and Art Gallery (TMAG), Hobart, Tasmania.

Tissues from deep-sea stylasterid coral samples were obtained by collaborative collection at sea during three recent Antarctic research voyages on the *Nathaniel B Palmer*; NBP11-03, NBP 11-05 and NBP 08-05 to the Antarctic Peninsula and Drake Passage. Material was preserved as close to the time of collection as possible in > 90% ethanol specifically for genetic analysis. Coral species identification based on morphology was determined based on the available keys and literature (Cairns, 1983), and Scanning Electron Microscopy (SEM) was used in some cases to examine fine scale structure. Seven stylasterid coral taxa where chosen for this study due to an established phylogenetic congruence between their morphology and genetic relationships based on two mitochondrial DNA gene regions (CO1 and 16S) (out-lined in chapter 2), their abundance in the Drake Passage across a diverse number of habitat types and depths (Waller *et al*., 2011), and comparative information on their mineralogy and age based on fossil studies (Stolarski, 1998, Lindner *et al*., 2008 Table 2, Emily Ciscato unpublished data). These seven species are: *Errinopsis fenestrata* Cairns, 1983, *Errinopsis reticulum* Broch, 1951, *Sporadopora dichotoma* (Moseley, 1876), *Stylaster densicaulis* Moseley, 1879, *Stellapora echinata* (Moseley, 1879), *Cheiloporidion pulvinatum* Cairns, 1983 and *Conopora verrucosa* (Studer, 1878). The final sample set spanned six sites within the Drake Passage; depth distribution was compared to scleractinian records in the following regions from video assays outlined in Waller *et al*., (2011) and scleractinian collections from Margolin *et al*., (2014).

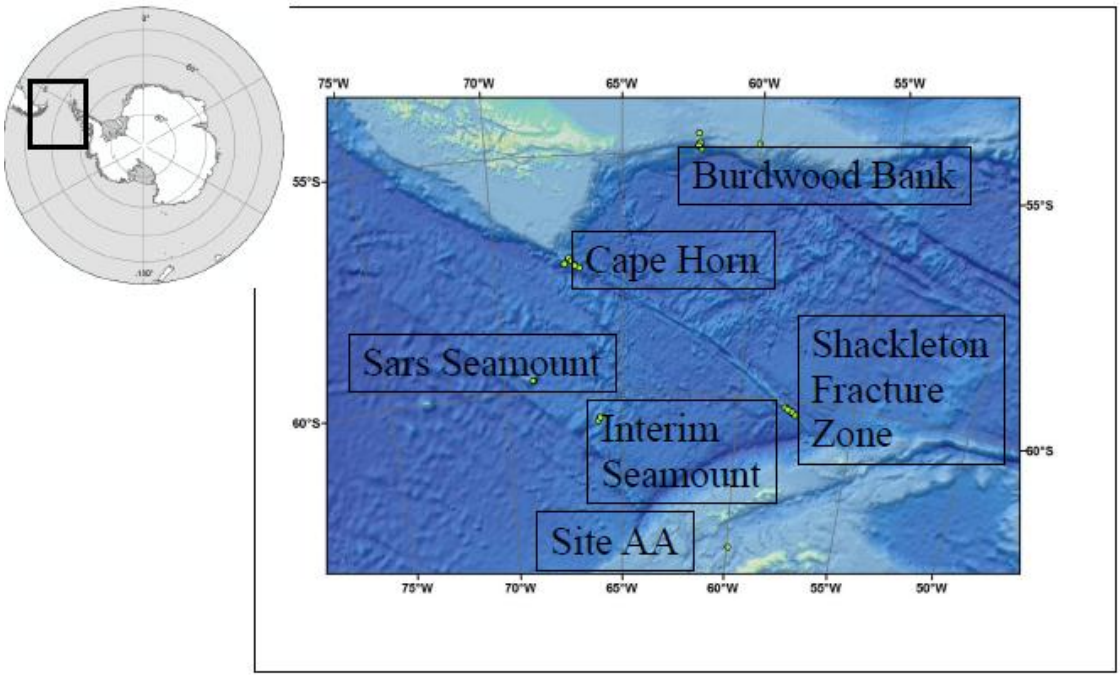

Figure 1. Map of the geographic locations where stylasterid corals were collected for this study in the Drake Passage. High densities of scleractinian corals have also been sampled from these locations (Waller *et al*., 2011, Margolin *et al*, 2014), and biogeographic data are available for stylasterid corals (Bax & Cairns, 2014). Map provided by Kathryn Scanlon WHOI.

Drake Passage Locations

Burdwood Bank

The Burdwood Bank is a continental plateau connected to the South American shelf within the Argentine Exclusive Economic Zone (EEZ) (Cusminsky & Whatley, 2000). An aqueous boundary between the Antarctic Intermediate Water (AAIW) and Upper Circumpolar Deep Water (UCDW) at 1000 m was identified by Margolin *et al*., (2014), along with a substantial drop in oxygen at 500 m. Scleractinian solitary corals have been collected from 120 m – 1879 m from sediment habitat (Margolin *et al*., 2014), and large aggregations of the habitat forming stylasterid coral *Errinopsis reticulum* have been collected in this region (Bax & Cairns, 2014).

Cape Horn

Cape Horn is a rocky substratum at the tip of South America within both the Chilean and Argentine EEZs. At Cape Horn the AAIW occurs above 1000 m and the UCDW flows

below 1000m (Margolin *et al*., 2014). Stylasterid corals have been recorded in great abundance from 500 m – 1400 m on rocky outcrops where octocorals were also present (Waller & Robinson, 2011, Fig 2), and solitary scleractinians were present on sediment locations.

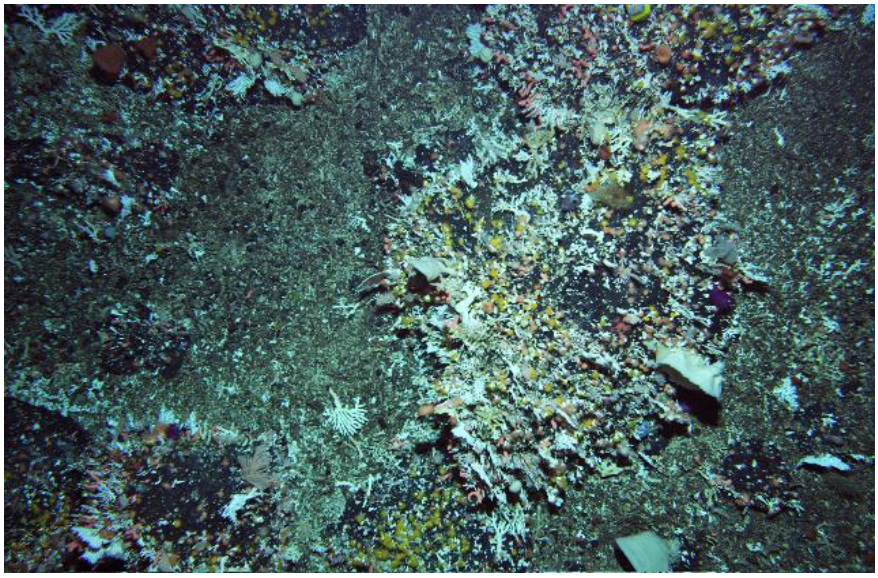

Figure 2. Stylasterid corals recorded in high density at Cape Horn during the NBP-1103 research voyage in 2011 (image from Waller & Robinson, 2011). Based on subsequent identifications these corals are specimens of either *Stylaster densicaulis* or *Conopora verrucosa* (pers. identification).

Sars Seamount

Sars Seamount lies within the Chilean EEZ south of the Polar Front and consists of predominantly gravel habitat. Video image surveys conducted by Waller and Robinson (2011) from 490 – 610 m, document stylasterids as a dominant component of the benthic fauna (present in 96% of images), second only to sponges (99% of images). Scleractinians are comparatively rare, and were found in only 6% of images from 600 m – 1900 m with the majority of specimens above 1000 m (Margolin *et al*., 2014).

Interim Seamount

Interim seamount is composed of three peaks along an extended bathymetric track where deeper depths vary from 1030 m – 1175 m with mainly gravel habitat interspersed with bedrock and very little sedimentation (Waller *et al*., 2011). Scleractinian corals have been collected within a restricted depth range from 1000 – 1300 m (Margolin *et al*., 2014),

and primnoid octocorals predominate over stylasterids in image surveys (95% *vs.* 31% of images) (Waller *et al*., 2011).

Shackleton Fracture Zone

The Shackleton Fracture Zone (SFZ) is an oceanic transverse ridge. The ACC is more restricted at the SFZ and semi-enclosed eddies exist within the ACC constraining Antarctic Bottom Water (ABW) (Livermore *et al*., 2004). Habitat is predominantly gravel, but rocks and sediment are also present. Stylasterids and octocorals seem to share an equally high abundance in the region (~ 86% of images from Waller *et al*., 2011), and scleractinian corals have been collected from 700 m – 2295 m (Margolin *et al*., 2014).

West Antarctic Peninsula – site AA

Site AA is located on the West Antarctic Peninsula (WAP) shelf; the WAP is a sediment slope with an average depth of ~ 450 m, with numerous deviations and a steep drop off. Only one stylasterid sample was recovered from this site; scleractinians were recovered from 500 m – 600 m. However, very few biological samples are documented, and the only reference for this site is the NBP-1103 cruise report consequently there is very little information for this location.

Molecular Protocols

DNA was extracted following the standard procedure using the Qiagen DNeasy protocol for the purification of total DNA from animal tissues (QIAGEN), modified to include an overnight incubation at 56ºC to completely lyse the tissue. Two mitochondrial regions, Cytochrome c oxidase subunit I (CO1) and the 16S Ribosomal Subunit (16S rDNA) were used for phylogenetic analysis. Extracted DNA was used as a template in Polymerase Chain Reactions (PCR). Hydrozoan primers were used for 16S, metazoan-specific primers were designed by Geller *et al*., (2013) for CO1 (summarised in Table 1.). Each 12.5 µL PCR reaction contained Promega GoTaq® Green Master Mix, 10 µM solution of forward and reverse primer pairs, < 250 ng of DNA template and nuclease-free water. The thermal cycling profile varied by gene region. For CO1 this consisted of an initial denaturation at 95ºC for 2 minutes, then 40 cycles of a three step program 95ºC for 30 sec, 40 - 60ºC for 45 sec (annealing temperatures varied Table 1.) and 72ºC for one minute, with a final extension at 72ºC for five minutes. The 16S protocol was taken from Lindner *et al* (2008). A positive control of *E. fissurata* amplified by Bax (2009) was used to confirm PCR success. Sufficient

master mix was retained for each PCR trial to be used as a negative control which contained all listed reagents, but no template DNA to control for contamination and the amplification of non-target DNA. Successful PCR reactions were purified using the Qiagen MiniElute purification kit designed to produce high end-concentrations of DNA. To elute the DNA, 30 μL of milliQ was used in place of a buffer. To increase the overall DNA yield prior to sequencing, two PCR reactions were purified for each sample. The two purified PCRs were then combined into one microcentrifuge sample to make a total concentration 3 - 6 ng/μL which was prepared for sequencing. Samples were sequenced in both directions at the Australian Genome Research Facility (AGRF) in Brisbane using the ABI platform.

Table 1. Details of PCR primers and annealing temperatures used to amplify two mitochondrial DNA regions from eleven species of deep-sea stylasterid coral. The resulting, or expected amplicon size (in base pairs) is indicated.

DNA Region & Primers	Direction	Primer Sequence	Annealing Temp (°C)	Fragment Size (bp)	Reference
16S					
SHA	5'-3'	TCGACTGTTTACCAAAAACATAGC	35-60	~600	Cunningham & Buss, 1993
SHB	3'-5'	ACGGAATGAACTCAAATCATGTAAG	35-61	~600	
CO1					
jgHCO2198-1	5'-3'	TABACYTCBGGRTGBCCRAARAAYCA	50-60	~200	Geller *et al*., 2013
jgLCO1490-1	3'-5'	TBTCBACBAAYCAYAARGAYATTGG	50-61	~200	

Phylogenetic Analysis and Estimation of Divergence Time

The final data set included CO1 and 16S concatenated alignments run in Mr Bayes using a Gamma + HKY model (Hasegawa, Kishino & Yano, 1985) chosen as the most appropriate model in Jmodeltest (Bazinet *et al*., 2014). A DNA sequence of *Hydricthella epigorgia* (Ptilocodiidae) was used as an outgroup. This species is in the superfamily Hydractinoidea, which also contains stylasteridae, and the species has been shown to occupy a lineage basal to Stylasteridae (Cartwright, 2008).

The 872 base pair alignment of a majority rule consensus of 33 16S and CO1 DNA sequence alignments (from Mr Bayes alignments in chapter 3) were analysed in BEAST (Bayesian Evolutionary Analysis Sampling Trees) v1.8 (Drummond & Rambaut, 2012). The xml files for BEAST were created in BEAUTi (Bayesian Evolutionary Analysis Utility) v1.8

(Drummond *et al*., 2012). The Yule birth-rate process was chosen as the appropriate prior, where extinction and speciation are equally likely under a normal distribution (with 10% of standard error), with a lognormal relaxed clock among lineages. The HKY model (Hasegawa, Kishino, Yano 85 model) was selected for molecular clock estimation over sixty million generations, saved every 6,000 generations to calculate phylogenetic relationships and posterior probabilities (the first 6,000 were discarded as burn-in). Chain convergence, node ages and highest posterior density (HPD) intervals were visualised in TRACER v1.6 (Rambaut & Drummond *et al*., 2013).

Fossil Record, Mineralogy and Age Estimates

The fossil record for the Stylasteridae was tabulated in Lindner *et al*., (2008). Stylasterid fossils can be traced back to the late Cretaceous 65 million years ago and the earliest Antarctic fossil (*Conopora mariae*) was dated to the Eocene ~ 50 million years ago (Stolarski, 1998). BEAST analysis calibrated the oldest stylasterid fossil dated to ~ 65 MYA to allow for maximum divergence time. Node constraints were assigned a normal prior distribution with an absolute upper bound of 65 MYA at the clade root to signify the most recent common ancestor (MRCA) to Stylasteridae. Additional priors were set as default, and where required a normal distribution was assigned to allow for maximum uncertainty in the calibration estimates (Ho, 2012). The uncorrelated relaxed clock model (Ho *et al*., 2005) was estimated based on a minimum value of 1.0 substitutions per site per million years due to an unknown rate of evolution in stylasterid corals, and cnidarians generally (see Shearer *et al*., 2002 for a summary in Anthozoans).

Carbon dated fossil age estimates and mineralogy (calcite/aragonite) were taken from the available literature (Lindner *et al*., 2008; Cairns & MacIntyre, 1992), and skeletal composition estimates from the Drake Passage were made available by Emily Ciscato and Dr. Laura Robinson (unpublished data) using established methods (Burke *et al*., 2010; Margolin *et al*., 2014) for six of the seven stylasterid species collected from the same sample locations as the extant species used here (Ciscato *et al*., in prep.). This information is also used to indirectly assess if there is any evidence to suggest coral calcification may be limited below the Aragonite Saturation Horizon (ASH).

Results

Three key patterns emerge from phylogenetic analysis, when overlaid with mineralogy, depth and location data from Drake Passage stylasterid corals:

1) Aragonite is likely to be the ancestral and predominant skeletal composition of stylasterids.

The placement of *Conopora verrucosa* as the basal ancestor is consistent with the fossil record (Fig 3). This conclusion is based on the Lindner *et al*., (2008) calibration, dating *Conopora arborescens* from the Faske formation to ~ 65 MYA. The oldest and only stylasterid fossil from Antarctica is also a congeneric species, *Conopora mariae*, dated to ~ 50 million years (Stolarski, 1998). To account for a margin of error associated with *Conopora* fossil estimates, a range of ~ 10 million years is shown in the phylogeny, placing the MRCA to Drake Passage stylasterids at 70 – 60 million years (Fig 3). This time frame also allows for the possibility that stylasterid ancestors evolved outside of Antarctica and is further substantiated by the presence of all six studied genera prior to the formation of the ACC that isolated Antarctica from the rest of the southern ocean areas around 35 MYA (Livermore *et al*., 2005).

Differential carbonate composition and phylogenetic tree topology suggests that ancestral stylasterids were predominantly aragonite (from > 90 - ~ 40 MYA), and species producing calcite skeletons arose more recently (~ 40 - 30 MYA) (Fig 3). Therefore, the common ancestor to Drake Passage stylasterids most likely had an aragonite skeleton. The presence of aragonite as the dominant polymorph in four of the six sampled genera, including the oldest genus, substantiates this monophyletic ancestral state (plesiomorphy) in *Stylaster*, *Conopora*, *Stellapora* and *Sporadopora*.

2) Radiation within the Drake Passage stylasteridae does not appear to be linked to major geological events, but oceanic chemistry may have influenced radiation within stylasterids ~ 40 - 30 MYA.

Four of the six genera studied here (*Errinopsis*, *Cheiloporidion*, *Stylaster* and *Sporadopora*) likely diverged during the latter half of the Eocene, around 40MYA. This time

period is after the initiation of the Drake Passage (50 MYA) and the PETM (55 MYA) (Fig 3) suggesting these two events had little influence on speciation in Drake Passage stylasterids. Divergence dates align more closely with substantial paleo-oceanic change in the Drake Passage within the suggested time of ACC initiation (~ 28 - 41 MYA Lawver & Gahagan 2003), strongly associated with oceanic de-acidification during the Eocene/Oligocene epoch boundary, the lowering of the CCD by ~1km ~34 MYA (Coxall *et al*., 2005; Rea & Lyle, 2005; Merico *et al*., 2008), and the most recent switch from calcite to aragonite seas (Stanley & Hardy, 1998) (Fig 3). This temporal correlation suggests that oceanic change had the most substantial effect on stylastrid populations. However, divergence dates have a large margin of error (~ 10 million years). Consequently, skeletal composition linked to oceanic chemistry may have been instrumental in the speciation process, but more research would be needed to pin point divergence times, and link divergence to paleo-chemical drivers.

3) The two most derived genera; *Errinopsis* and *Cheiloporidion* were characterized by calcite forms. Two phylogenetic interpretations exist for when these calcite forms likely arose:

Scenario 1): Eocene - an evolutionary character state

Calcite may have arisen once as a carbonate character state during the latter half of the Eocene, when the MRCA to *Cheiloporidion* and *Errinopsis* first diverged from *Stellapora* and which coincides with a calcite sea (Fig 3). Subsequently in both genera there has been a switch back to the ancestral aragonite condition in some species/locations during subsequent aragonitic sea conditions, possibly reflecting phenotypic plasticity. This interpretation would be the most parsimonious in that calcitic forms are monophyletic (i.e., calcite arose once) and it supports the ontongenetic calcification hypothesis (Spencer & Hardie, 1998) in that the evolution of species with different skeletal mineralogy can be linked to ocean chemistry.

Scenario 2): Miocene - a recent character state

An alternative interpretation of calcite acquisition in *Cheiloporidion* and *Errinopsis* is that calcite acquisition evolved recently, and arose independently in these two genera during the Miocene (Fig 3). This explanation is less parsimonious in the context that calcite arose twice (i.e. is paraphyletic) and there is no link to ocean chemistry as conditions during the

Miocene reflected an aragonitic sea. In this instance, it is possible that other environmental conditions lead to the evolution of calcite forms, for example geography or bathymetry. Divergence of the two forms of *C. pulvinatum* occurred around ~ 10 MYA which was during a period of geographic change across the Scotia Arc (Fig 3), Thus, it is possible that the isolation of the Burwood Bank (calcitic forms) from the Sars Seamount (aragonite forms) led to the divergence in *C. pulvinatum* mineralogy. However, there was no equivalent pattern found in *E. fenestrata*, which occurs both at Burdwood Bank and Sars Seamont, where mineralogy is consistent in both locations, although the low replication across locations limits geographic inference.

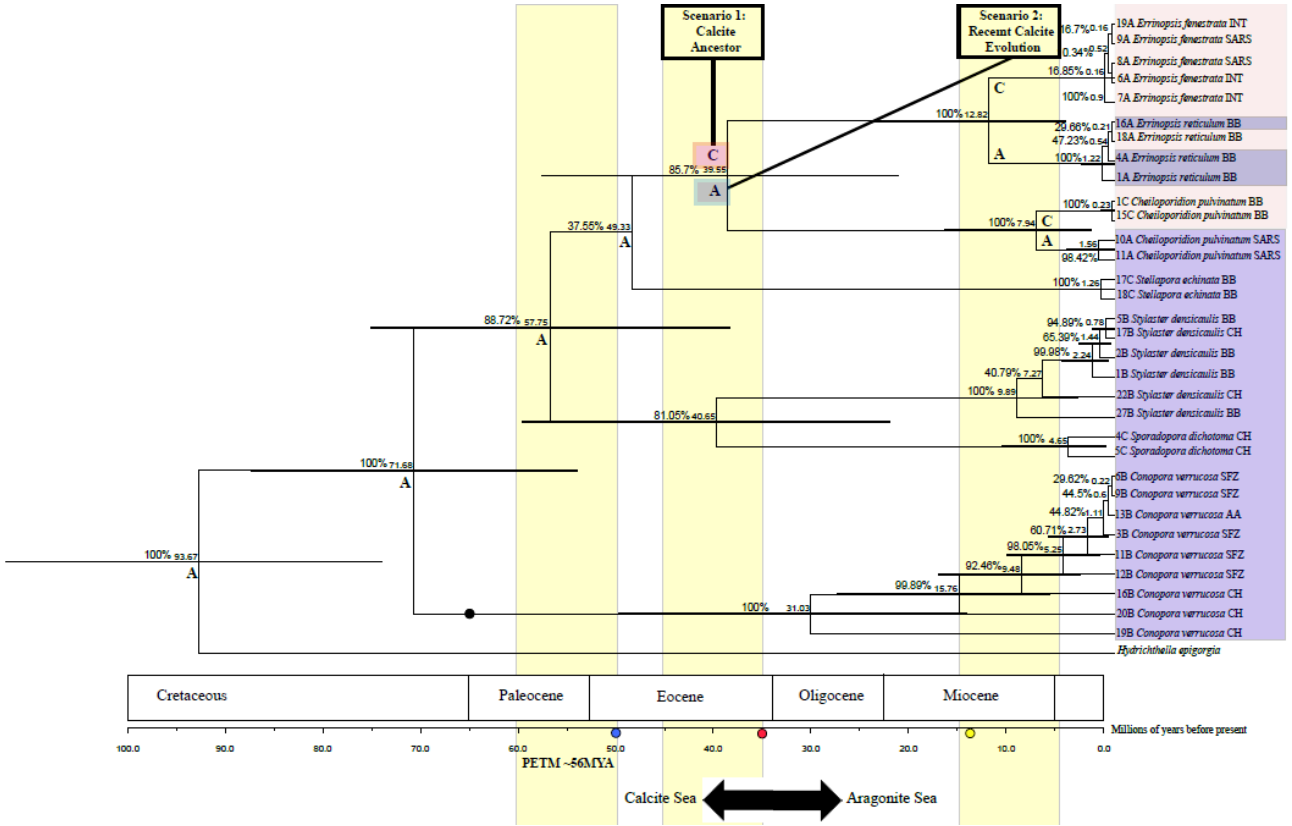

Figure 3. Bayesian phylogenetic tree for CO1 and 16S compiled in BEAST (Rambaut & Drummond, 2007), under the HKY85 model of sequence evolution. Scale is in millions of years from 0 (present) to 100 million years with the most recent common ancestor estimated at 65 MYA and represented by (•) on the cladogram. To illustrate fossil calibrated divergence between stylasterid species, the margin of error (~ 10 million years, shown in yellow) is stated along the time line starting with 1) the thermal isolation of Antarctica and initiation of the Drake Passage region ~ 50 MYA (•), 2) ACC formation (~35MYA) and 3) the lowering of the CCD of the Southern Ocean ~34MYA, and 4) the switch from calcite to aragonite seas (based on the Spencer & Hardie (1998) model of calcite/aragonite seas (•), and 5) geological shifts across the Scotia Arc (~15MYA) (based on Strugnell *et al*., 2008) (•). The PETM, a time frame linked to the reformation of calcite and aragonite in surface waters world-wide is also indicated at 55 MYA. Calcite = C (pink), or Aragonite = A (blue) is indicated at clade nodes. In the Eocene calcite (Scenario 1) or aragonite (Scenario 2) is indicated at the node ~40MYA.

Discussion

Calcium Carbonate Composition of the Drake Passage Stylasteridae

This study has shown that the common $CaCO_3$ polymorph ancestor to stylasterids was aragonite, and that the predominant calcium carbonate polymorph in modern Drake Passage stylasterids is also aragonite. The majority of stylasterid species have maintained their ancestral aragonite skeletal form, inherited from the MRCA to the basal clade of *Conopora verrucosa* (Fig 3). All records suggest that this genus is aragonite in both modern and fossil forms (Cairns & MacIntyre, 1992; Stolarski, 1998; Jell *et al*., 2011). The closest calcifying hydrozoan relatives to Stylasteridae are also aragonite, and aragonite is the predominant and ancestral bio-mineral in stylasterids studied in Cairns and MacIntyre (1992). Furthermore, Cairns & MacIntyre (1992) present information on genera not included in this analysis, and describe aragonite skeletal mineralogy in four of the six Sub-Antarctic genera, confirming that Drake Passage Stylasterids conform to the mineralogy common of stylasteridae world-wide. Given that these conclusions are well supported from genetic and morphological data, the existence of an atypical calcite skeletal minerology in the most derived phylogenetic clade, containing *Errinopsis* and *Cheiloporidion* ~ 40 – 30 MYA, is surprising (Fig 3). Two possible evolutionary explanations of this unusual pattern are considered here. 1) The use of calcite arose during the Eocene when the MRCA of these two genera arose, or 2) evolution of the use of calcite is a more recent adaptation, seen in multiple lineages during the Miocene.

Radiation in Drake Passage Stylasteridae during the Eocene/Oligocene Boundary

The closest paleo-environmental event to the estimated period of divergence of most of the extant stylasterid genera recorded in the Drake Passage is the Eocene/Oligocene boundary ~ 34 MYA (e.g., Rea & Lyle 2005; Coxall *et al*., 2005; Merico *et al*., 2008). There was a transition from calcite to aragonite seas during this time period (Stanley & Hardie, 1998; Porter, 2010; Ries, 2010). In contrast, there is a comparative lack of divergence in stylasterids during other geological events such as the PETM (~ 55 MYA) (Zubarev *et al*., 2013), and the opening of the Drake Passage (> 50 MYA) (Livermore *et al*., 2005), suggesting that stylasterid radiation was most influenced by oceanic change ~ 34 MYA (Fig 3). Strugnell *et al*., (2008b) suggest a lineage of deep-sea octopus arose in the Drake Passage during the Eocene (33 MYA), reinforcing that this time period may have been instrumental in the evolution of Antarctic invertebrate groups, and the opening of new deep habitat. However, divergence estimates in Strugnell *et al*., (2008b) place the height of octopus

radiation at ~ 15 MYA. Comparatively, minimal radiation is documented in stylasterids during this time (Fig 3). Strugnell *et al*., (2008 a,b,c) provide the only evolutionary phylogenetic study for comparison pre 15 MYA in the Drake Passage, and it is likely that sessile calcifying benthic fauna, such as corals, were differentially affected compared to pelagic molluscs.

In the Eocene, 56 to 34 MYA, oceanic conditions were more acidic than the modern ocean (Coxall *et al*., 2005), the oceanic state was more favourable for calcite skeletal forms (Porter, 2010) and consequently I propose that *Errinopsis* and *Cheiloporidion*, which appeared during this time, are calcitic as a consequence of the oceanic state in which they arose (Scenario 1 Fig 3). However, *Stylaster densicaulis* and *Sporadopora distichotoma* also diverged from their MRCA within the Eocene (Fig 3), but these species retained the ancestral condition of aragonite skeletons hence it appears that oceanic chemistry affected stylasterid lineages in different ways.

Of interest is that both *Errinopsis* and *Cheiloporidion* have more recently switched their mineralogy from the hypothesized natal calcite to the ancestral aragonite, which may reflect the oceanic state of the Oligocene to the present day (aragonite). The presence of both skeletal minerologies within a single species is uncommon compared to most invertebrates studied to date (although see literature on bi-mineralic shells in modern molluscs in Feely *et al*., 2004, and reviews in Porter, 2010; Honisch *et al*., 2012). However, e*x-situ* experiments on modern scleractinian species have shown that such a switch in mineralogy was possible in the Cretaceous (Ries, 2010; Higuchi *et al*., 2014). Laboratory experiments on the shallow water corals, *Acropora tenuis* (Higuchi *et al*., 2014), *Acropora cervicornis*, *Montipora digitata* and *Porites cylindrical* (Ries, 2010), showed that skeletal growth is faster in aragonite favourable conditions, whilst growth (at a lower rate) was also documented during calcite favorable conditions, along with the precipitation of calcite to form bi-mineralic skeletons. Ries (2010) suggests this bi-mineralogy may have been a beneficial calcification strategy, enabling scleractinian corals to survive through the Cretaceous (calcite sea), and then prosper in the Oligocene (aragonite sea). This may also be the case in stylasterid corals, which are bi-mineralic across most species (Cairns & MacIntyre, 1992), and represented by fossil forms in the Cretaceous (Lindner *et al*., 2008). However, the adaptive benefit of bi-mineralogy remains to be tested on stylasterids, and studies suggest an evolutionary constraint on mineralogy adaptation, potentially relating to energy cost (Reis, 2010; Higuchi

et al., 2014). Such a constraint further supports the hypothesis that calcite in *Errinopsis* and *Cheiloporidion* is an evolutionary character state, with subsequent divergence toward aragonite as the most plausible explanation for the patterns observed here (i.e. scenario 1, Fig 3).

The *Errina*, *Errinopsis*, *Errinopora* + *Cheiloporidion* Genus Complex

The phylogeny of Cairns and MacIntyre (1992) reported a switch to calcite mineralogy in the clade including *Errina*, *Errinopisis*, *Errinopora* and *Cheiloporidion*. The timing of this switch was previously unknown, and is estimated here from genetic data at ~ 45 – 35 MYA (Fig 3). In the morphological cladogram of Cairns and MacIntyre (1992), this switch followed divergence from *Stellapora* (based on morphological similarity), and the genetic data here agrees with their conclusion (Fig 3). Three genera, *Errinopsis*, *Errinopora* and *Errina*, are described by Cairns and MacIntyre (1992) as a genus complex based on divergence both in morphology and mineralogy. Based on genetic (Chapter 3), morphological (Cairns, 1983), and mineral similarity (Cairns & MacIntyre, 1992) *C. pulvinatum* qualifies for inclusion within the *Errina/Errinopsis/Errinopora* complex. Due to sequencing and sampling limitations mtDNA data were unavailable for *Errinopora* and only 16S DNA was available for *Errina*, negating incorporation into the final BEAST phylogeny. However, despite limited comparative data, 16S phylogenetic alignment shows that *Errina* species are closely related to *Errinopsis* and *Cheiloporidion* as would be expected based on morphology (Chapter 3). Therefore, shared characteristics unique to these genera, such as mineralogy, may be maintained as either an evolutionary by-product, or due to some adaptive advantage.

The dominant field-forming Antarctic genus is *Errina* (Post *et al*., 2010; Bax & Cairns, 2014). *Errina* species have been identified as bi-mineralic by Cairns & MacIntyre (1992), with some species containing mostly calcite, while some contain mostly aragonite. Most of the field-forming species in Antarctica have a calcite mineralogy (Cairns & MacIntyre, 1992; Riddle pers. com.), as do other field-forming *Errina* species outside of Antarctica; for example, the three species of *Errina* which form aggregations in the New Zealand fiords (Cairns & MacIntyre, 1992). In contrast, some of the *Errina* spp. known to contain aragonite are listed as having a limited distribution, and do not occur in high abundance (e.g., *Errina kerguelensis* Bax & Cairns, 2014). *Errinopsis* is also documented as a field forming genus, identified in field-like abundance at Burdwood Bank (Bax & Cairns,

2014). Similarly, *Cheiloporidion pulvinatum* is a habitat-forming species in the South West Atlantic (Bax *et al*., unpublished data). Therefore, calcite mineralogy may be instrumental in allowing species within this genus complex to predominate in selected habitats, leading to high coral coverage.

However, counter to this hypothesis, *Errinopora* is a rare and endemic coral that does not, to our knowledge, form field-like aggregations (Bax & Cairns, 2014). In addition, *Errina antarctica* forms dense aggregations in the Patagonian fiords (Häussermann & Försterra, 2007), but has an aragonite mineralogy (Cairns & MacIntyre, 1992). Furthermore, aragonite corals have also been recorded in large aggregations (e.g., *Stylaster densicaulis/Conopora verrucosa* pers. identification from Waller & Robinson, 2011 Fig 2). Therefore, calcite mineralogy may or may not contribute to the ability of stylasterids to form field-like aggregations in select habitats, and further research will be needed to substantiate the utility of mineralogy in the *Errina/Errinopsis/Errinopora* + *Cheiloporidion* genus complex.

Location and Depth Related Mineralogy Differences

The alternate explanation for the existance of aragonite and calcite skeletal minerology in *Cheiloporidion* and *Errinopsis* is that this character state arose more recently (during the Meiocene) and independently in both genera (Scenario 2, Figure 3). However, other data from this study do not provide strong support for this alternate hypothesis. Recent evolution of calcite forms could be linked to modern day environmental conditions. For example, *Cheiloporidion pulvinatum* samples from Burdwood Bank are distinct from those at Sars Seamount based on minerology and genetics, and this may signify isolation and subsequent divergence through localised adaptation (Fig 3). However, there was no equivalent genetic or minerology differences between *E. fenestrata* populations sampled across a similar geographic range, providing little support for this hypothesis.

Lastly, it was expected that aragonite would be favoured in shallow waters above the ASH (~ 1000 m), and that calcite would be favoured in deeper habitats below the ASH (Feely *et al*., 2004). This hypothesis of calcite minerology at deeper depths is unsupported in *Errinopsis* and *Cheiloporidion*, which appear to be eurybathic either side of the ASH (300 m - > 2000 m appendix). Furthermore, *Stylaster* and *Sporadopora* (which occur across similar depths as *Errinopsis* and *Cheiloporidion*) diverged from their MRCA around the same time

period as the calcite genera, but both retain aragonite skeletons below the predicted depth of the ASH (Fig 3, appendix). In combination, these results are counterintuitive to any evidence of calcite selection in deeper waters, negating an adaptive ability for stylasterids to exist below the ASH, or out-compete aragonite calcifiers in the Drake Passage.

Conclusion

Evolution is a complex process in any group of organisms, and there is unlikely to be a single driver for such a diverse fauna as the Antarctic benthos (Clarke *et al*., 2004). For corals, the combined effect of oceanographic chemistry and productivity during climatic events likely had a substantial effect on their biogeography (Margolin *et al*., 2014). In stylasterids this has resulted in adaptations linked to morphology and mineralogy (Cairns & MacIntyre, 1992). The main period of phylogenetic radiation in Drake Passage stylasterids correlates with oceanic de-acidification during the Eocene/Miocene transition boundary from calcite to aragonite seas. Future acidification conditions may lead to an inverse pattern of divergence as the oceans shift from aragonite seas to calcite sea conditions (e.g., Parker *et al*., 2010; Pandolfi *et al*., 2011; Ries, 2011), culminating in potential extinctions within Antarctic populations. This could shift the competitive advantage towards non-calcifiers (Guinotte *et al*., 2006), or as outlined here – calcifiers that can adapt their mineralogy. However, optimism in this regard will need to be substantiated in future studies, and the unprecedented rates of change in IPCC (2013) predictions do not bode well given the slow rate of evolution in these species. Furthermore, ocean acidification not only poses a significant threat to deep-sea corals, but all associated fauna, and studies on a variety of taxa across multiple habitats are needed to predict ecosystem succession both in the past, and of increasing relevance, under future climate scenarios. Until this time, conservation of coral habitats, and their dependent ecosystems should be assigned the highest priority.

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Estimates of Deep-Sea Stylasterid Coral Connectivity

Stylasterid corals are an important, abundant and diverse component of sessile benthic fauna in the deep-sea, with a eurybathic depth range (~ 10 – 2000 m +) in fiord, seamount and shelf locations. Connectivity between coral habitats is maintained through gene flow, which counteracts the effects of disturbance through the maintenance of genetic diversity and incoming recruitment, ensuring population survival. However, genetic studies of shallow water stylasterids corals have revealed considerable structure, indicating restricted gene flow and isolation or selection, and this has important consequences for conservation and management of coral populations generally. There are no data on gene flow among deep water populations of stylasterids and consequently we do not know if similar patterns of isolation exist at depth. Levels of connectivity were indirectly measured in deep-sea stylasterids using DNA sequence data of the mitochondrial ribosomal subunit (16S), cytochrome c oxidase subunit 1 (CO1) and Internal Transcribed Spacer (ITS) among circum-polar, Sub-Antarctic, Patagonian and South-West Atlantic Ocean populations of seven species. Circum-Antarctic connectivity estimates were statistically limited due to low sample size. To account for this, population connectivity was assessed in two main geographic regions in 1) Antarctic Peninsula and South America, and 2) East Antarctica.

Both regions were investigated across different spatial scales: among geographic locations (1000s of km), and within geographic locations (10 - 100s of km). Genetic separation among populations within the Drake Passage, where benthic habitats are separated by up to 500 km, and between the Drake Passage and South America where populations are separated by > 2000 km. was found for three species: *Conopora verrucosa*, *Errina laterorifa* and *Cheiloporidion pulvinatum*. When investigating dispersal estimates across the Polar Front, the species *Stylaster densicaulis* and *Errinopsis fenestrata* showed no genetic differentiation based on 16S haplotypes, whist CO1 data showed genetic subdivision among Drake Passage populations. In contrast, little to no genetic subdivision was evident in the *Errina* species at any spatial scale in the Ross Sea and Patagonian fiords based on ITS DNA sequence data.

The results presented here demonstrate that inference of subdivision and gene flow between populations vary by species, molecular marker, study region and spatial scale, suggesting that a multitude of factors influence population connectivity within stylasterid coral populations. Identifying that some regions and populations are isolated whilst others

may be effectively panmictic is critical to the effectual management of these deep-sea populations.

Introduction

Connectivity in the Ocean

Connectivity (and the inverse, isolation) has become a central topic in marine ecology and connectivity research has developed to incorporate the scale and mechanism by which gene flow operates within and between geographically separated populations. Gene flow, defined as genetically effective migration (Bohonak, 1999), helps counter the effects of inbreeding and genetic drift, and contributes to the maintenance of genomic diversity (Carlon, 1999; Ayre & Hughes, 2000; Almany *et al*., 2009).

For benthic marine invertebrates, such as corals, gene flow and connectivity are achieved through larval dispersal. Ocean currents may link areas of high productivity (e.g., nutrient upwelling around seamount and continental shelves), and enable dispersal and maintain gene flow, if larvae have the capacity for pelagic dispersal (Botsford *et al*., 2009; Saenz-Agudelo *et al*., 2009). Larvae of benthic marine invertebrates can have a pelagic phase during which they feed (planktotrophic) allowing them to remain in the water column for days, weeks, or even months (Siegel *et al*., 2003; Sale *et al*., 2005), with the potential to disperse across large distances during this time (1000s of km, Kinlan & Gaines, 2003). However, some species have only a provisional amount of food from their parents (lecithotrophic) and can only remain in the water column until their natal food supply is exhausted (Siegal *et al*, 2003), while some benthic marine species lack a pelagic larval phase altogether (Thatje *et al*., 2012).

The dispersal capacity of a species is often correlated with reproductive mode, particularly pelagic larval duration (PLD) whereby the longer a larva spends in the water column, the greater the likelihood and distance of dispersal (Hellberg, 2009). In contrast, marine species that lack a planktonic phase are thought to have limited dispersal capabilities, often recruiting within their natal population (Carlon & Olson, 1993). However, there are a number of cases where the PLD (Bowen *et al*., 2006) or reproductive type (Miller & Ayre, 2008) do not function as good predictors of population structure. Weersing and Toonan

(2009) reviewed the current literature on the subject and found that PLD alone does not correlate well with genetic structuring between populations, to the extent that the authors question if there is a relationship at all. Hence, conservation managers cannot rely on assumptions based on a species reproductive mode, and a multidisciplinary approach is needed to assess connectivity in the marine environment to effectively manage marine ecosystems.

A good example of a multi-disciplinary approach to connectivity is the field of seascape genetics (Galindo *et al.*, 2006), whereby population genetic techniques typically applied to terrestrial environments (landscape genetics) are used to measure marine connectivity. This approach incorporates a species' ecology (i.e. life history), genetic population structure, and physical barriers to dispersal (i.e. geographic features such as ridges or land masses, oceanographic features such as fronts and eddies). In general, isolation tends to increase with distance in oceanic environments (Slatkin, 1993), with a greater differentiation between populations on a larger scale (> 2000 km) (Richards, 2007; Cowen & Sponaugle, 2009). Local scale genetic connectivity patterns are often characterised by 'chaotic genetic patchiness' (Toonon & Grosberg, 2010), because over small spatial scales (10s of km), levels of gene flow are often confounded by spatial and temporal variation in recruitment (Magalon *et al*., 2005; Hunter & Halanych, 2008). Only a few studies to date have found fine scale genetic structure (< 100 m Miller & Ayre, 2008; Miller *et al*., 2009), although most studies do not consider such small spatial scales. Using the seascape genetics approach Selkoe *et al*., (2010) were able to determine a spatial marine management strategy for a 2135 km area of Southern California, illustrating that through collaboration between scientific disciplines (such as population genetics, oceanographic modelling and ecology) the seascape genetics approach may elucidate local scale connectivity and effectively aid in the designation of Marine Protected Areas (MPAs).

Connectivity and Conservation Management

Population persistence is dependent upon stock (established adult population) and supply (recruitment of juveniles from within and outside of the population) to maintain genetic diversity (Almany *et al*., 2009). Interconnected populations are more likely to recover following a disturbance as incoming recruits recolonise the region and re-establish populations (Steneck *et al*., 2009). In contrast, isolated or closed populations that rely on internal recruitment are more vulnerable to reduced genetic diversity and genetic drift which

can lead to extinction if their populations become depleted (Botsford, 2009). Depleted populations may experience a decrease in fitness at low densities, termed the Allee effect (Stephens *et al*., 1999). The premise of the Allee effect is often applied in conservation genetics to avert ecosystem collapse, assess MPAs and identify sites of conservation importance; as the ability of a population to adapt to change is highly dependent upon genetic diversity, determined by population size and the inflow of allelic diversity.

The marine environment is exposed to an array of anthropogenic pressures, including fisheries, resource mining and exploration, climate change, pollution, introduced species and habitat destruction/modification (see Costello *et al*., 2010; Ramirez-Llodra *et al*., 2010). With so many pressures and potential threats to consider, understanding and maintaining the ecological processes, including connectivity, are especially important for conservation management. Sustainable resource use should be, and in a most cases is, the goal of management regimes. The implementation of MPAs to ensure prolonged ecosystem survival is one such strategy employed to maintain connectivity in species rich shallow water environments world-wide (e.g., tropical coral reefs Botsford *et al*., 2009), and more recently in deep-sea ecosystems in the Atlantic, Mediterranean, Southern Indian Ocean and the Southern Ocean (© FAO 2007-2014).

MPA implementation is particularly challenging in the deep-sea due to limitations regarding accessibility, high research cost, ease of scientific study and where fisheries is concerned - a lack of defined oceanographic boundaries, and international resource competition. Coined 'the tragedy of the commons' (Hardin, 1968), international fisheries compete for the same limited resource, often a single desired fish species (e.g., the Orange Roughy *Hoplostethus atlanticus* (Clark & Driscoll, 2000; Morato & Watson, 2004; Clark & Rowden, 2009) and subsequently deplete its availability rather than fish sustainably. Such competition for resources in deep-sea ecosystems and the use of destructive fishing technologies such as benthic trawling (Koslow *et al*., 2001, Hall-Spencer & Fossa, 2002), has resulted in ecosystem damage and population declines on seamounts world-wide. For example Tasmania (Koslow *et al*., 2001), the UK and Ireland (Grehan *et al*., 2005, Wheeler *et al*., 2005), Alaska (Stone, 2006), Florida (Reed *et al*., 2007), and New Zealand (Probert *et al*., 1997) among others (Koslow *et al*., 2000).

Southern Ocean fisheries are subject to regulations under the Committee for the Conservation of Antarctic Marine Living Resources (CCAMLR 2008 – 2012 summarised in Table 1), and to date all regulated deep-sea bottom fishing involves long line fisheries, not benthic trawling (Gianni, 2004). However, there remains a considerable threat from illegal, unreported and unregulated (IUU) fishers. By-catch, including benthic invertebrates such as corals, is associated with long line fisheries and trawling restrictions are open to discussion in the future (Parker & Bowden, 2009, CCAMLR, 2008). With decreasing fish stocks world-wide pressure and investment in Southern Ocean fisheries is expected to increase (Collins *et al*., 2010). Therefore, understanding benthic ecosystems, before fisheries impact, is of the utmost conservation importance. In order to prevent fisheries impact, the precautionary approach is advocated under CCAMLR through the classification of high biodiversity locations as Vulnerable Marine Ecosystems (VMEs). These areas are protected from fisheries and VME indicator species, including taxonomic groups such as corals, sponges, bryozoans echinoderms and molluscs (currently 27 taxon, CCAMLR, 2009), within these regions are classified as VME indicator taxa.

If a VME indicator taxon, such as a coral, is caught on a fisheries long line whilst fishing within a legal fishing area the move on rule is put into effect and a one nautical mile radius is declared a no take zone (Parker *et al*., 2009). Fourty six VMEs are presently listed under CCAMLR conservation measure 22-06 and 22-07 (Table 1) and for the management of these multiple VMEs it is important to consider that demographic change, such as replenishing a population following fisheries impact (Botsford 2003), requires recruitment through connectivity to maintain population fitness (Burridge *et al*., 2012). Recruitment can occur either from within or external to an impacted population (Botsford, 2003). If connectivity exists between VME networks, populations are more resilient to change, than isolated populations without external recruitment. Therefore, understanding connectivity processes will be central to future management and conservation of these important ecosystems, and would need to be taken into account when forming management plans for Antarctic VMEs.

Table 1. CCAMLR Conservation measures implemented to mitigate the impact of bottom fishing in the Southern Ocean.

Conservation Measure	Regulation
22 – 05 (CCAMLR, 2008)	Prohibits bottom trawling south of the CCAMLR boundary at 60°S
22 – 06 (CCAMLR, 2008)	Requires CCAMLR members to report evidence of VME areas to prevent fisheries impact on VMEs.
22 – 07 (CCAMLR, 2009)	Requires CCAMLR members to report VME taxa caught as by-catch to prevent fisheries impact on VMEs. Includes the move on rule, and the designation of a 1nm VME conservation area where VME taxa are encountered as by-catch.
22 – 08 (CCAMLR, 2009)	Prohibits exploratory bottom fishing in waters less than 550m.
22 – 09 (CCAMLR, 2012)	Outlines measures for the registration and protection of VMEs identified within the CCAMLR boundary.

Connectivity in Antarctica and the Sub-Antarctic

Antarctic benthic marine invertebrate communities are dominated by brooders that arguably do not have a pelagic larval phase (Thatje *et al*., 2012). Therefore, the mechanisms that maintain circum-Antarctic populations of many species are unclear and the scale of larval dispersal and gene flow is in need of further study. The Antarctic Circumpolar Current (ACC) is thought to act as a 'conveyer belt' facilitating dispersal of invertebrate larvae in an Easterly direction (Nikula *et al*., 2010). The inshore Antarctic Coastal Current flows mainly westward, and contains a number of eddies and gyres associated with continental bathymetry not subject to a unitary flow like the ACC (Moffat *et al*., 2008). However, despite the more localised flow of the inshore Antarctic Counter Current, gene flow is generally claimed to be homogenous among populations of Antarctic fauna (Griffiths, 2010). There are a number of Antarctic invertebrate species which show this pattern of circum-polar connectivity e.g., the brittle star *Astrotoma agassizii* (Hunter & Halanych, 2008), and the nemertean worm *Parborlasia corrugatus* (Thornhill *et al*., 2008), among others (summarised in Thatje *et al*., 2012). Therefore, despite the heterogeneous benthic environment, Antarctic invertebrates continue to be defined as a somewhat homogenous unit (e.g., Hedgpeth 1969; Dayton 1990). This homogenous nature is based on three pervading assumptions in the literature: 1) that strong circumpolar currents promote the distribution of juveniles, 2) that ecological conditions are favourably similar along the Antarctic coast, and 3) that most invertebrate species have a circumpolar distribution (see Gutt, 2013). However, more recent efforts to

understand benthic biodiversity support a more sub-divided bio-geographic pattern (Brandt *et al*., 2007; Clarke, 2008).

Recent studies refute the over-simplification of circum-Antarctic connectivity (Wilson *et al*, 2009; Baird *et al*., 2011). Studies have emerged suggesting local scale isolation of Antarctic invertebrate populations (~ 100 m Baird *et al*., 2012), independent of life history mode in some cases (e.g., Wilson *et al*., 2007). Presenting congruence with a brooding or spawning dispersal strategy in some (e.g., Hart *et al*., 2006), and the opposite of what might be expected by reproductive mode in others (see Thatje *et al*., 2012). Additional variables, including; iceberg scour (Griffiths *et al*., 2010), nutrient supply (Post *et al*., 2010), historic glaciation (Leese *et al*., 2008; Baird *et al*., 2011) and reproductive mode (Brandt *et al*., 2007, Arango *et al*., 2011, Baird *et al*, 2012) have all been linked to genetic diversity and population structure. Further, cryptic speciation, described as reproductively isolated populations within a species (see Held, 2003), appears to be extremely common in the Southern Ocean (Wilson *et al*, 2007; Baird *et al*, 2011). These factors have led many to argue for a revision of the Antarctic paradigm of circumpolar connectivity linked to the ACC – largely due to genetic research which suggests connectivity estimates are often specific to species or geographic locality (e.g., Wilson *et al*, 2007; 2009; Miller *et al*, 2010; Baird *et al*, 2011; 2012).

Most connectivity studies in Antarctica have been focused primarily on large scale hydrodynamic features, and have been centred on the relatively accessible areas of the Antarctic Peninsula (Wilson *et al*., 2009). Southern Ocean populations are often viewed as independent of those in surrounding regions such as Patagonia, the Drake Passage, Scotia Arc, South America and South West Atlantic Ocean (Brandt *et al*., 2007). This biogeographic separation is attributed to the presence of the Polar Front (also cited as the Antarctic Convergence, Eastman 1993), which provides a strong physical and thermal barrier (a 3- 4º C temperature differential) to dispersal (Barnes & Peck, 2008). However, studies show that dispersal does occur across the Polar Front between Antarctica and the sub-Antarctic, South America, and some sub-Antarctic islands (e.g., Wilson *et al*., 2007; Jörger *et al*., 2014). Therefore, the ACC both enables dispersal for some species and acts as a barrier to dispersal to others on either side of the Polar Front (e.g., Antarctic near shore fauna) (Clarke *et al*., 2005; Wilson *et al*., 2007; Hunter & Halanych, 2008). Therefore, the ACC may not be the predominant factor in defining species connectivity.

Connectivity and Conservation of Antarctic and Sub-Antarctic Deep-sea Corals

The recent discovery of field-like aggregations of stylasterid corals and a high abundance of associated fauna such as brittle stars, polycheates and crinoids in East Antarctica (Post *et al*., 2010), vividly illustrates ecosystem diversity in the Antarctic benthos. These coral fields are now documented in a number of locations (Bax & Cairns, 2014). The Antarctic and Sub-Antarctic benthic environment is composed of a myriad of habitat types extending to a maximum depth of 4000 m (Clarke & Johnson, 2003), and oceanic links and taxonomic affinities have been documented in surrounding regions such as Patagonia (Häussermann & Försterra, 2007), and the South West Atlantic (Spalding *et al*., 2007). However, sampling has been generally restricted to depths above 500 m due to limitations on infrastructure limitations, accessibility and research funding (Wilson *et al*., 2007), presenting a number of gaps in our knowledge on species distribution and connectivity in the deep Southern Ocean.

Although connectivity in shallow water corals has been studied extensively using modern genetic methods, our understanding of the spatial scale of gene flow is adequate for only a few species (see Jones *et al*., 2009 for the most recent review). There are few genetic studies on the connectivity of deep-sea coral populations (Le Goff-Vitry *et al*., 2004, Miller *et al*., 2010; 2011), and none on Antarctic species. The genetic data we have suggests local recruitment of larvae, and limited gene flow in deep-sea corals generally (e.g., Le Goff-Vitry *et al*., 2004). Within the Sub-Antarctic region the only published genetic study on deep-sea corals examined scleractinian coral populations and found genetic differentiation across depths, consistent with the stratification of the Sub-Antarctic Mode Water, Antarctic Intermediate Water, the Circumpolar Deep and North Pacific Deep Waters in the Southern Ocean (Miller *et al*., 2011) indicating vertical stratification in larval dispersal, and bathymetric isolation. However, without imperical testing across coral groups and geographic regions, patterns of connectivity between Antarctic coral populations remain hypothetical.

Stylasterids: A Model for Understanding Connectivity

Stylasterid corals occur in great abundance from Antarctica (CCAMLR, 2009) to the Patagonian fiords (Häussermann & Försterra, 2007), the South West Atlantic (Bax & Cairns, 2014) and the Drake Passage (Waller & Robinson, 2011). Depths of occurrence vary between 10 m (fiord populations only) (Häussermann & Försterra, 2007) and 2000 + m (throughout the South West Atlantic, Drake Passage and Southern Ocean regions) (Cairns, 2011). They have been recorded in dense aggregations (Post *et al*., 2010) that support

biodiversity equivalent to that found in tropical reef systems (e.g., Cairns & Lindner, 2011) and occupy diverse habitats including sea mounts, shelf and fiord locations. Recent biodiversity discovery efforts such as the Census of Antarctic Marine Life (CAML) have started to reveal the importance of stylasterid corals in Antarctic and Southern Ocean benthic ecosystems (Post *et al*., 2010; Hosie *et al*., 2011; Kaiser *et al*., 2013).

Stylasterid corals are a species-rich taxonomic group with over 247 known species world-wide (Cairns, 2011), of which 13% are described from the Sothern Ocean (Bax & Cairns, 2014). Little is known about their ecology and life history. We only have a handful of publications on reproduction in deep-sea coral species world-wide (Waller & Tyler, 2005; Flint *et al*., 2006; Brook & Stone, 2007), and only one publication for deep-sea Antarctic species (Waller *et al*., 2008). Reproductive mode is known for only three scleractinians in the sub-Antarctic (*Flabellum thouarsii, F. curvatum, F impensum*), and all are brooding species (Waller *et al*., 2008). However, there are no data on reproduction for Antarctic octocoral or stylasterid species, and no studies on dispersal in these species. It is theorised by Stratford (2002) that the most likely method of reproduction in Antarctic/deep-sea stylasterids would be brooding larvae that settle close to adult colonies. The limited existing data on stylasterids suggest an insular dispersal capacity. Like many Antarctic species, stylasterids appear to brood their larvae. One of the earliest studies on stylasterid reproduction was for *Allopora californica* which has a brooded larva with a short lecithotrophic stage (Ostarello, 1973). Brook and Stone (2007) summarised information about all the stylasterid species studied to date, and found most of them were classified as gonochoristic (separate sexes) brooders (one species *S. roseus* may be hermaphroditic (Goedbloed, 1962) and recent investigations on Brazillian species of *Errina* have found the same (Cordeiro pers. com.). *Errina novaezealandiae* broods larvae which crawl from cavities in parent colony branches and settle within 24 hours and less than one meter from parent colonies (Stratford, 2004). Stratford (2002) also documented the presence of microscopic mucus covered hairs covering the larval body. He speculated that these mucus hairs may function like parachutes in strong currents, enabling larvae to transport themselves away from parent colonies via current-aided dispersion. However, subsequent genetic studies have shown that *E. novaezelandiae* has highly subdivided populations throughout the New Zealand fiords, consistent with the production of larvae that settle close to parental colonies (Miller *et al*., 2004). Therefore, the known reproductive modes exhibited in stylasterid corals are indicative of limited dispersal potential often associated with highly structured, patchily distributed, isolated populations,

common of the Antarctic benthos (Clarke & Johnston, 2003; Thatje, 2012). Given their apparent circum-Antarctic distributions and likely limited dispersal capacity, stylasterids represent an excellent model organism with which to test widely held predictions of circum-Antarctic connectivity.

This study incorporates DNA sequence data from seven morphologically diverse stylasterid coral species to indirectly measure connectivity among benthic invertebrate populations in the waters of the Antarctic, sub-Antarctic and South America. Connectivity is assessed within the Antarctic Peninsula and South American regions to investigate dispersal across the Polar Front, and in East Antarctica and the Patagonian fiords to assess connectivity between field-like aggregations of *Errina* spp. Regions are assessed at two scales 1) across large spatial scales > 1000 km (i.e. between regions) and 2) across local spatial scales ~ 10 – 300 km (i.e. within locations). It is predicted that genetic connectivity will decrease with increasing geographic distance (i.e. connectivity between seas < within seas). The results of this study will be used to inform conservation managers of the connectivity in deep-sea coral populations across multiple habitats (e.g., seamounts, shelf and fiords ecosystems) and seas.

Methods

Study Area, Collection and Samples

Antarctic benthic samples were collected either by beam trawl, epibenthic sled or as by-catch in regulated fisheries. Samples were obtained from depths of 130 m to a maximum sampling depth of 2149 m. Sample number, replicate, location, latitude, longitude, morphological identification and voyage were recorded for all study specimens (Appendix tables 3 & 3.1).

Tissue from deep-sea stylasterid coral samples was obtained in four ways; 1) Through existing research collections including; the Smithsonian National Museum of Natural History (NMNH), British Natural History Museum (NHM), the Museum National d'Histoire Naturelle (MNHN), National Institute for Water and Atmospheric Research (NIWA) and, Australian Antarctic Division (AAD) and through the Instituto Español de Oceanografía, Centro Oceanográfico de Gijón. 2) By personal or collaborative collection at sea during recent Antarctic research voyages including; three *Nathaniel B Palmer* research

voyages; NBP11-03, NBP 11-05 and NBP 08-05 to the Antarctic Peninsula and Drake Passage, and a voyage through the Collaborative East Antarctic Marine Census (CEAMARC) to the Dumont d'Urville Sea associated with the Census for Antarctic Marine Life (CAML). 3) through cooperation with the New Zealand Ministry of Fisheries (MFish), MFish Observers and Observer Programme staff under MFish Projects ANT200801, ANT200901 and ANT201001 and 4) *Errina antarctica* was collected by SCUBA and remote operated vehicle from the Chilean fiords in collaboration with the Alfred Wagner Institute (AWI) on the Explorador II.

For the recent collections, material was preserved as close to the time of collection as possible in > 90% ethanol to facilitate genetic analysis. Study species were chosen based on their ecological relevance and abundance in collections. All specimens are known, or speculated, to be field-forming species based on their abundance in Antarctic collections and video/photographic images. Some stylasterid species are considered highly insular in their distribution (e.g., *Errinopsis fenestrata* (Cairns, 1983) and samples included here were replicated across as wide a geographic range as possible. However, due to both their insular distribution in the Southern Ocean and the difficulties associated with Antarctic sampling geographic comparisons were limited for some species/regions. In total seven species were included in this study; *Errina fissurata* Gray, 1872, *Errina laterorifa* Eguchi, 1964, *Errina antarctica* (Gray, 1872), *Errinopsis fenestrata* Cairn, 1983, *Stylaster densicaulis* Moseley, 1879, *Cheiloporidion pulvinatum* Cairns, 1983 and *Conopora verrucosa* (Studer, 1878). Specimens were either from sea mounts (Drake Passage), Antarctic shelf locations (East Antarctica, Bransfield Strait on the Peninsula), fiords (Chilean Patagonia), or the South American plateau (Burdwood Bank), enabling comparison across a broad range of habitats. The final sample set spanned from the South West Atlantic to East Antarctica (> 7500 km) (Fig 1).

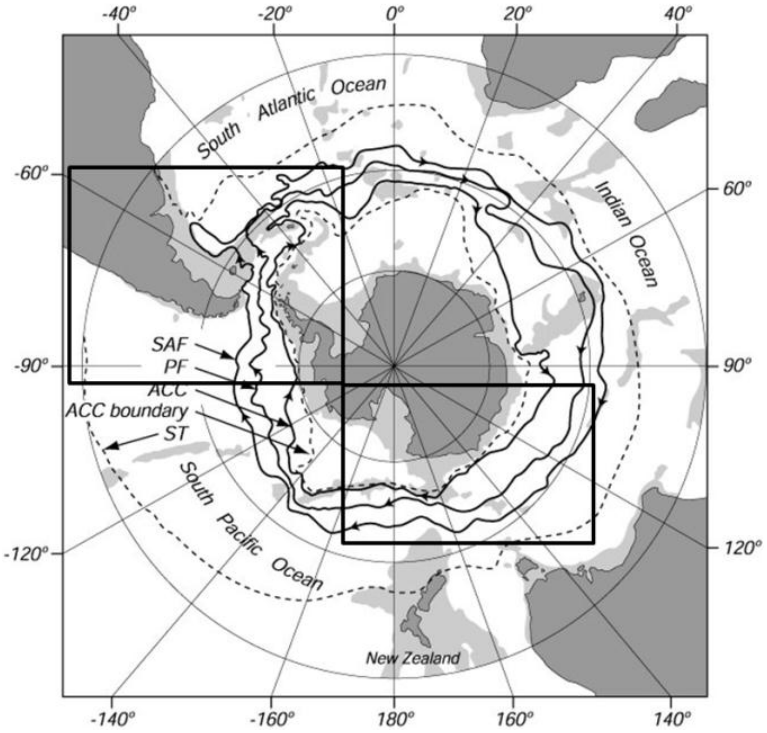


Figure 1a. Map of the distribution of the Sub-Antarctic Front (SAF), ACC boundary, the Southern Temperate boundary (ST) and the Polar Front (PF). The two main geographic regions where stylasterid corals were collected for this study are highlighted, 1) The Sub-Antarctic and South America, and 2) East Antarctica. Within these regions genetic connectivity was independently assessed within the Drake Passage, Ross Sea (Fig 1b) and Patagonia (Fig 1c). (Appendix Table 3 & 3.1). Map edited from Orsi (1995).

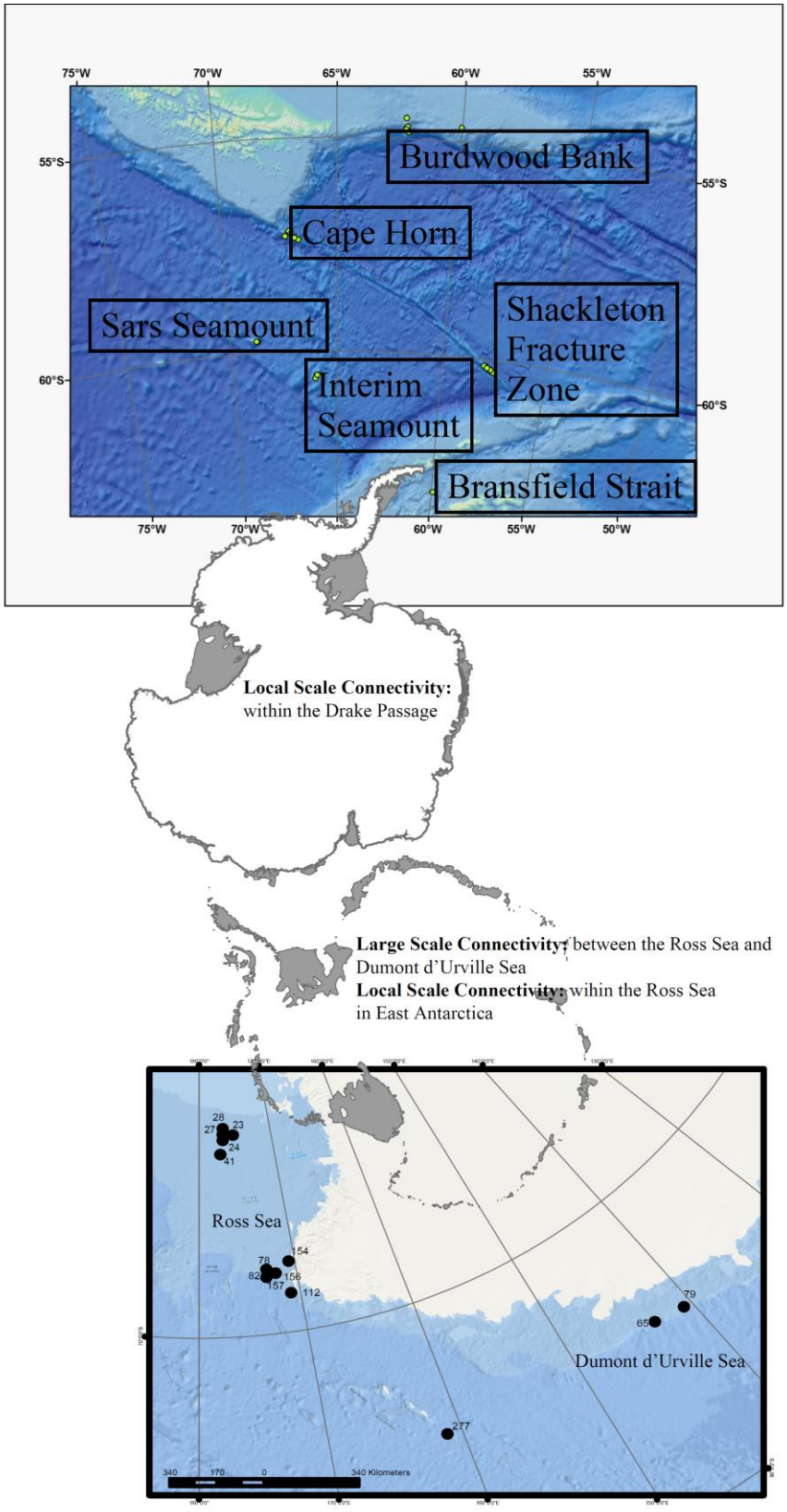
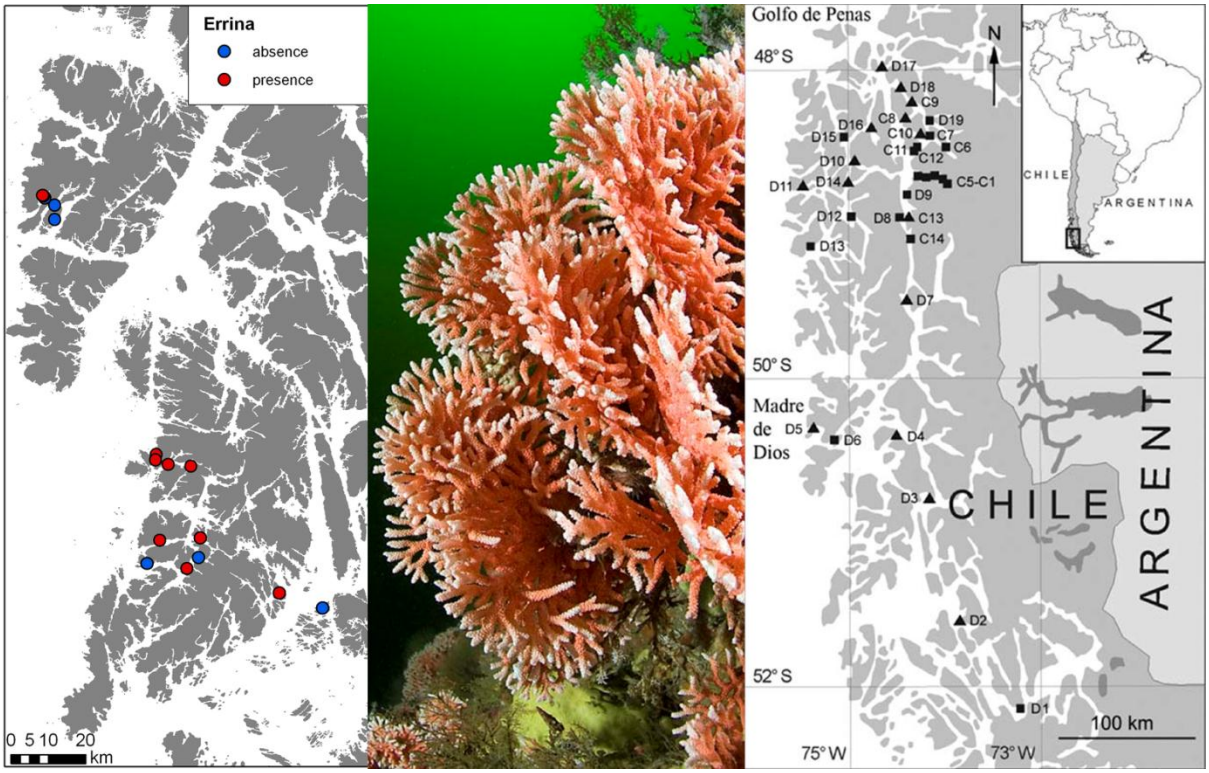


Figure 1b. Locations in the Drake Passage, Ross and Dumont d'Urville Sea. Drake Passage locations include six sites between ~ 300 – 500 km apart. In the Ross Sea there are 13 stations in total, distances between stations vary from 10 – 73km. The Ross Sea and Dumont d'Urville Sea are separated by > 2000 km. Sample depths spanned 103 – 1930 m (Appendix Table 3 & 3.1). Drake Passage map provided by Kathryn Scanlon (WHOI). East Antarctica map edited from the SCAR database.



A **B** **C**

Figure 1c. Local scale connectivity was assessed among fiords in the Chilean Region of Patagonia based on the A) presence/absence of *E. antarctica* (© Laura Fillinger), which occurs in large aggregations along vertical walls in the fiords B) *E. antarctica* coral at 18 m (© Mathias Hune). C) Map of study sites in the central Patagonian zone. Study sites: C1-C14 (2005) and D1-D19 from Häussermann & Försterra, 2007, triangles show where *E. antarctica* was found. Sample sites Canal Copihue D11 and Grupo Dacres D2 designate the two sampling sites compared by AMOVA in this study (~ 200 km apart). Note these sites were all approximately 10 - 30 m deep, due to Deep Water Emergence (DWE).

Molecular Protocols

Genomic DNA was extracted from coral specimens following the standard DNA extraction procedure using the Qiagen DNeasy protocol for the purification of total DNA from animal tissues (QIAGEN), the procedure was modified to include an overnight incubation at 56ºC to completely lyse the tissue. This study uses mitochondrial DNA sequence data of the mitochondrial ribosomal subunit (16S) and cytochrome c oxidase subunit 1 (CO1), and the nuclear Internal Transcribed Spacer (ITS) region. ITS data sets were analysed with all possible combinations of ITS-1 and ITS-2 to assess various sequence length and individual comparisons. Metazoan specific CO1 primers were designed by Geller *et al* (2013), universal primers were used to amplify ITS and hydrozoan primers were used for 16S (summarised in Table 1). Each 12.5 µl Polymerase Chain Reaction (PCR) reaction contained Promega GoTaq® Green Master Mix, 0.5µl of a 10 µM solution of forward and reverse primer pairs, < 250 ng of DNA template, and nuclease-free water. The thermal cycling profile

varied by gene region. For ITS this consisted of an initial denaturation at 95ºC for 2 minutes, then 35 cycles of a three step program 95ºC for 30 sec, 40-60ºC for 45 sec (annealing temperatures varied Table 1.) and 72ºC for one minute, with a final extension at 72ºC. This protocol was modified for CO1 by running 40 cycles of the same three step program. The 16S protocol was taken from Lindner *et al* (2008). A positive control of *E. fissurata* was used to identify the validity of PCR reactions. Sufficient master mix was retained for each PCR trial to be used as a negative control which contained all listed reagents, but no template DNA in order to control for contamination and the amplification of non-target DNA. Successful PCRs were purified using the Qiagen MinElute purification kit designed to produce high end concentrations of DNA. To elute the DNA, 30 µl of milliQ was used in place of a buffer. To increase the overall DNA yield prior to sequencing, two PCR reactions were purified for each sample. The two purified PCRs were then combined into one microcentrifuge sample to make a total concentration 3-6 ng/uL which was prepared for sequencing. Samples were sequenced in both directions at the Australian Genome Research Facility (AGRF) in Brisbane using the ABI platform.

Table 2. Details of PCR primers and annealing temperatures used to amplify three DNA regions from the seven species of deep-sea stylasterid coral. The resulting, or expected amplicon size (in base pairs) is indicated.

DNA Region & Primers	Direction	Primer Sequence	Annealing Temp (°C)	Fragment Size (bp)	Reference
16S					
SHA	5'-3'	TCGACTGTTTACCAAAAACATAGC	35-60	~600	Cunningham & Buss, 1993
SHB	3'-5'	ACGGAATGAACTCAAATCATGTAAG	35-61	~600	
ITS					
ITS-5	5'-3'	GGAAGTAAAAGTCGTAACAAGG	56	~600	White *et al.*, 1990
ITS-4	3'-5'	TCCTCCGCTTATTGATATGC	56	~600	
CO1					
jgHCO2198-1	5'-3'	TABACYTCBGGRTGBCCRAARAAYCA	50-60	~200	Geller *et al.*, 2013
jgLCO1490-1	3'-5'	TBTCBACBAAYCAYAARGAYATTGG	50-61	~200	

Data Analysis

Sequences for each species were edited and checked for errors using the software program Molecular Evolutionary Genetic Analysis (MEGA) 5.0 (Tamura *et al*, 2011). Contiguous sequences were generated for each specimen using forward (5'–3') and reverse (3'–5') primer sequences (Table 4.). Occasionally it was not possible to obtain sequence in both directions. In these cases, sequence data were only used if the sequences were clean and reliable. All sequences generated in this study will be lodged with GenBank (###). Basic Local Alignment Search Tool (BLAST; NCBI; www.ncbi.nlm.nih.gov) searches were performed on each sequence to confirm that 1) each sequence was in fact a stylasterid coral, and 2) the appropriate DNA region had been amplified. Additionally, multiple alignments across all samples were used to identify outliers within the data set; these outliers may have been the result of contamination and were removed prior to analysis. Two Sub-Antarctic samples from Lindner *et al*., (2008) were used as a check (*C. verrucosa* EU645274 and EU645273) and compare alignment against 16S sequences within the final data set confirming accurate identifications for these species. If sequences contained only a small fraction of double peaks then ambiguity codes were assigned based on fasta DNA codes (www.boekhoff.info/Data/FASTA DNA Codes), which prevented distortion of genetic signal. To further combat misalignment associated with ITS hypervariablity the genetic alignment program G blocks (http://www.phylogeny.fr) was used to investigate all possible ITS alignments. The most parsimonious data sets that optimised both sequence length and species comparison were used in the final analysis (Table 3 and 4).

Population Level Analysis

To assess intra-specific genetic diversity, the frequency of haplotypes for each of the three DNA regions (ITS, CO1 and 16S) was summarised for each morphological species using GenAlEx v6.501 (Peakall & Smouse, 2006). Haplotype networks were generated to resolve the geographic distribution of shared and unshared ITS, CO1 and 16S haplotypes, where applicable and informative to do so (e.g., where there were > 3). These data were used to obtain a graphical representation of haplotype relationships using the program Network v4.5.1.6 (http://www.fluxus-technology.com) using the median-joining algorithm (Bandelt *et al*., 1999), a 95% plausible connection limit, and with gaps treated as missing data.

Analysis of Molecular Variance (AMOVA), calculated using Arlequin v3.01 (Excoffier *et al*, 2005) was used to determine the level of population subdivision and test for

departures from panmixia among populations. Significance of the F-statistics were assessed with permutation tests using 10,000 iterations, at the 95% level of significance. Data sets were compiled to compare connectivity between seas (large scale connectivity), and within a sea or fiord system (local scale connectivity) for each species where possible (Table 2.). Large-scale, qualitative, geographic comparison was possible for all seven coral species. However, low sample sizes limited statistical analysis and AMOVA was not possible for all data sets. AMOVA was only applied to populations with ≥ 3 individuals (Table 2). Shared and unique haplotypic diversity was assessed where sample sizes were too small for statistical comparison, or where AMOVA was not necessary due to a lack of shared haplotypes between regions

To establish the amount of genetic exchange between seas, gene flow among populations was estimated as $N_em = (1/F_{ST} - 1)/4$ (effective number of migrants per generation). This model assumes that 1) all loci are selectively neutral; 2) genetic drift and gene flow are in equilibrium; and 3) there is random mating. Populations are maximally divergent when $N_em = 0$ and $F_{ST} = 1$ (Freeland, 2005). Gene flow estimates based on F_{ST} are limited by the underlying assumption of the island model (Wright 1978), which assumes that all populations exchange migrants. To account for this bias, a rarefaction curve (the most commonly used method for population estimates) was considered, but was deemed inappropriate due to an inability to measure sampling effort over approximately eighteen research voyages and through multiple international collaborations to collect in the deep-sea. Instead, a summary of Antarctic connectivity research is presented in the discussion for comparison to show that the sample sizes included here are within the range possible for such studies (Appendix Table 3).

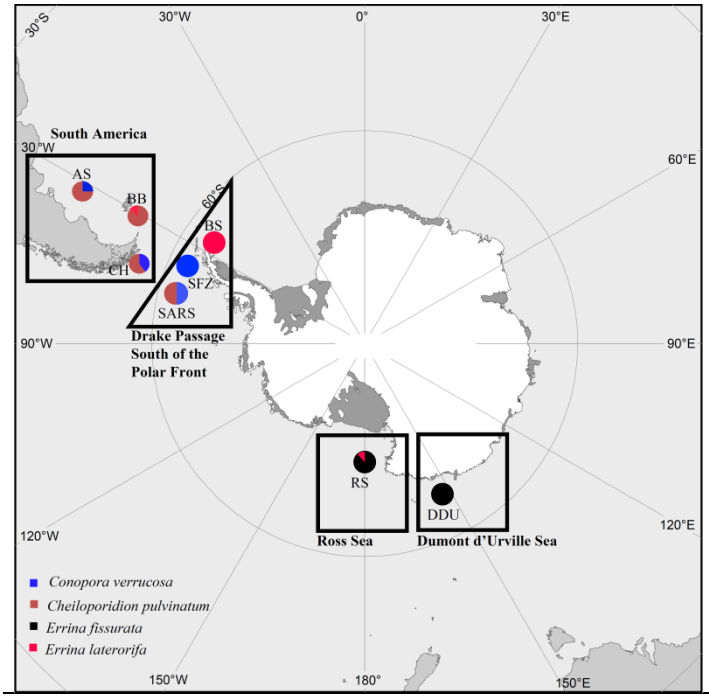


Table 3. Summary of samples used to explore genetic similarities among different seas, and across the Polar Front (a potential oceanographic barrier to dispersal). Geographic abrivieviations from left to right include: Arigentinian Shelf (AF), Burdwood Bank (BB), Cape Horn (CH), Bransfield Strait (BS), Shackleton Fracture Zone (SFZ), Sars Seamount (SARS), Ross Sea (RS), Dumont d'Urville Sea (DDU). Sample listing is included in the appendix table 3. Map edited from the AAD data centre. * Multiple data-sets based on ITS-1, 18S, ITS-2 alignments were compared.

Species	DNA region	South America	South of the PF	Ross Sea	Dumont d'Urville Sea
Conopora verrucosa	16S	4	7	-	-
Cheiloporidion pulvinatum	16S	12	2	-	-
Errina fissurata	ITS	-	-	5-19*	2-12*
Errina laterorifa	16S	3	2	1	-

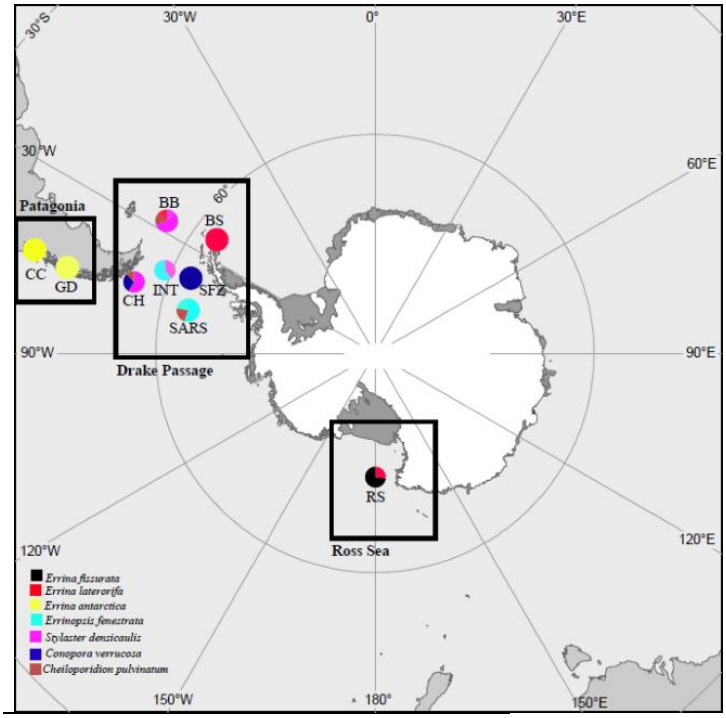


Table 4. Summary of samples used to assess genetic differences among sites within seas. AMOVA was used to statistically compare among sites where n>=3 for each species. Geographic abrivieviations from left to right include: Canal Copihue (CC), Grupo Dacres (GD), Burdwood Bank (BB), Cape Horn (CH), Interim Seamount (INT), Bransfield Strait (BS), Shackleton Fracture Zone (SFZ), Sars Seamount (SARS), Ross Sea (RS). Sample listing is included in the appendix table 3.1 See figure 1b and 1c for sample station/site locations. Map edited from the AAD data centre.

Species	DNA region			
		Geographic location – Patagonia		
		Canal Copihue	Grupo Dacres	
Errina antarctica	ITS	9	7	
		Geographic location – Drake Passage		
		Sars Seamount	Interim Seamount	
Errinopsisfenestrata	16S	3	3	
Errinopsis fenestrata	CO1	2	4	
		Geographic location – Drake Passage		
		Interim Seamount	Cape Horn	Burdwood Bank
Stylaster densicaulis	16S	2	5	5
Stylaster densicaulis	CO1	2	3	4
		Geographic location – Drake Passage		
		Sars Seamount	Cape Horn	Burdwood Bank
Cheiloporidion pulvinatum	16S	2	2	4
Cheiloporidion pulvinatum	CO1	-	2	4
		Geographic location – Drake Passage		
		Cape Horn	Shackleton Fracture Zone	
Conopora verrucosa	16S	3	7	

Conopora verrucosa		CO1				4			7	
						Geographic location – Drake Passage				
						Bransfield Strait			Burdwood Bank	
Errina laterorifa		16S				2			3	

Species	DNA region									
		Geographic location – Ross Sea stations								
		154	157	82	78	116	112	150	156	277
Errina laterorifa	ITS	3	5							
Errina fissurata	ITS	3	4	2	6	2	1	1	1	1
		Geographic location – Ross Sea stations								
Errina fissurata	CO1	23	24	27	28	41				
		3	2	3	2	1				

Results

Unfortunately, genetic amplification success was hindered by a number of potential variables (e.g., preservation type and method), and sequence quality was variable by gene region with a final genetic data set from just 151 specimens of a total of > 700 samples. To try to improve the quality of sequencing in poorly preserved or degraded samples alternative extraction, PCR protocols, primers, final elution and sequencing methods were tested, but these proved no more successful than the original methods used. Sequencing issues associated with the nuclear ITS region revealed multiple copies, and it was only possible to sequence portions (18S, ITS-1, 5.8S, ITS-2, 28S) rather than the complete 670 base pair alignment of the ITS region. The final ITS data sets were only comparable for *Errina* species (n = 57), and ITS-2 had the highest variability. In general 16S sequenced most effectively across species (n = 57). CO1 sequenced reliably for a small subset of samples, but proved to be a more difficult region to amplify overall (n = 40). CO1 was generally more variable than 16S; the proportion of variable sites ranged from 3.56 to 25.40% in CO1, and 0 to 10.1% in 16S (Tables 5 - 11). Similarly, nucleotide diversity was higher in CO1 compared to 16S, and polymorphism estimates ranged from 0.012 to 0.11 in CO1 and 0 to 0.05 in 16S (Table 5 - 11). Concatenated data sets were only available for a few individuals, and to capture the maximum comparative variation it was more appropriate to compile final data sets based on individual DNA regions, and to assess each geographic region independently (Tables 3 & 4). Small and varying sample sizes for each species and the associated uncertainty when examining genetic relationships should be noted, and consequently the capacity for comprehensive connectivity assessments were limited. Therefore, this results section addresses four aspects of connectivity in the Antarctic region:

1) A brief assessment of regional scale (> 6000 km) connectivity estimates based on only one species, *Errina laterorifa* and one gene region 16S.
2) Connectivity estimates across the Polar Front between South America and Antarctica (~ 1000 km) based on five species and mitochondrial 16S and CO1 DNA, as well as connectivity among seamount sites north of the Polar Front (South American) and among seamount sites south of the Polar Front (300 - 900 km) (Antarctic).

3) Connectivity in *Errina antarctica* in the Chilean Patagonian fiords based on the rapidly evolving ITS nuclear DNA sequences (~ 200 km) to provide fine scale connectivity estimates across a local scale.

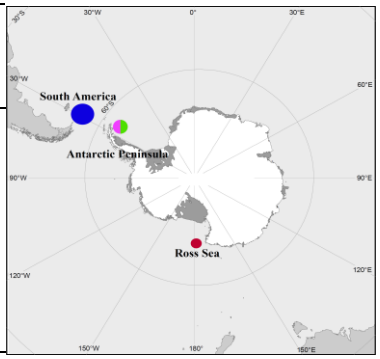
4) Connectivity across East Antarctica including large scale connectivity between the Ross and Dumont d'Urville Sea (~ 2000 km), and local scale connectivity among seamounts within the Ross Sea (~ 10 – 70 km) based on two species within the genus *Errina* and using ITS sequence data.

1) Regional Scale Connectivity Estimates

There were genetic differences between *Errina laterorifa* from the Ross Sea, Antarctic Peninsula and South America, locations that are separated by a maximum of ~ 6000 km. The six available 16S sequences revealed four unique haplotypes, and none were shared between regions. The haplotype network contains a median vector between regional haplotype associations, signifying a hypothetical ancestral sequence, suggesting a substantial level of separation (Bandelt *et al*., 1999) (Fig 2). However, although this provides some evidence of genetic separation, this result is based on a single individual from the Ross Sea and more sampling would be needed to properly understand if there are any links or shared haplotypes between regions.

Table 5. The 16S haplotype distribution and abundance of *Errina laterorifa* populations in three regions: South America, the Antarctic Peninsula and Ross Sea. Percent variation is the proportion of variable sites in DNA sequences; π signifies nucleotide diversity, used as a measure of DNA polymorphism in sequences for each species. Table includes information on the base pair number (bp), sample size and number of haplotypes per site (n). Antarctic map depicts the distribution and abundance of haplotypes for each species, pie charts sized according to relative sample size; all haplotypes are unique to their designated region. Maps edited from the AAD data centre.

	Haplotype	bp	n	South America	Antarctic Peninsula	Ross Sea
E.laterorifa		485				
16S	h1		3	3		
n = 6	h2		1		1	
(10.10% variation)	h3		1		1	
π 0.05	h4		1			1



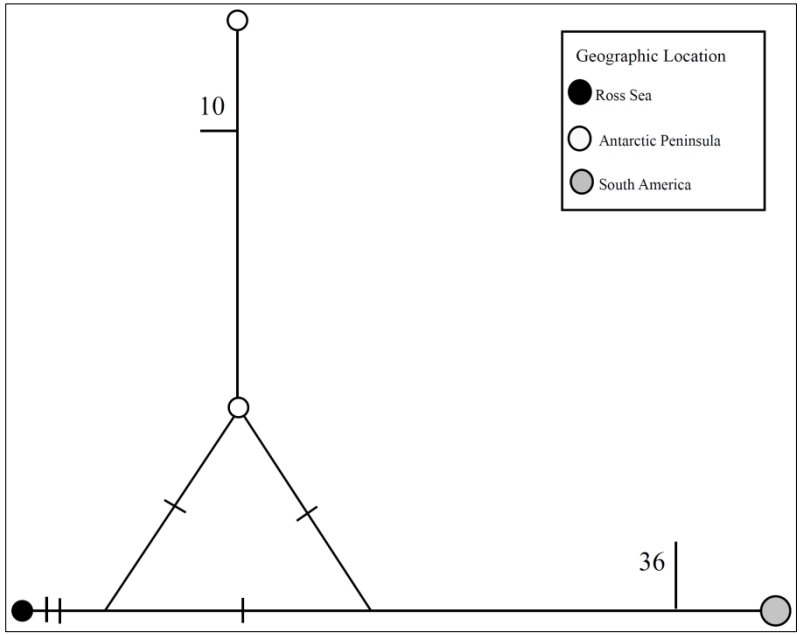


Figure 2. Haplotype network based on 6 16S DNA sequences from *Errina laterorifa* (total alignment length = 485 bp). Each node at a branch joining point represents a parallel mutation between a median vector (a triangle or square configuration in the network), each node on a branch represents a base pair mutation (represented numerically where there are >5bp mutations). Haplotypes are sized according to abundance.

2) Genetic Differences among Regions in the Sub-Antarctic and South America

2.1 Dispersal across the Polar Front

Genetic comparisons among stylasterid populations in the Drake Passage and South America from six locations (Fig 1b) across spatial scales ranging from > 300 - 900 km, show evidence of genetic subdivision across the Polar Front (PF). This is based on mitochondrial sequence data from four species, including: *Stylaster densicaulis*, *Errina laterorifa*, *Conopora verrucosa*, and *Cheiloporidion pulvinatum*. It is evident that the 16S gene region is more conserved in stylasterids, than CO1. In all sequence comparisons, the proportion of variable sites and estimated sequence polymorphism is higher in CO1 than 16S (Tables 6 – 11; Figs 4 - 7). In some instances, this resulted in evidence of genetic structure in CO1 at spatial scales of > 300 - 500 km, whilst no genetic structure was evdent in the 16S data. For example in *S. densicaulis* populations, there is a clear pattern of genetic sub-division across the PF between South America (Burdwood Bank and Cape Horn), and Interim Seamont south of the PF, based on a lack of shared CO1 haplotypes. However, most 16S haplotypes are shared across these locations (Table 10, Fig 7).

At larger spatial scales (~ 900 km), 16S proved sufficiently variable to differentiate the two most distant sampling sites of Bransfield Strait and Burdwood Bank based on a lack of shared haplotypes in *E. laterorifa* sequence comparisons (Table 6, Fig 3). However, in this instance, CO1 was not available for comparison, and inadequate sampling from the Bransfield Strait limits the ability to make meaningful conclusions.

At spatial scales > 500 km, genetic subdivision was evident for two species, *C. pulvinatum* and *C. verrucosa* based on an absence of shared haplotypes in pooled data from South America and populations south of the PF (Table 7). The haplotype networks for *C. verrucosa* and *C. pulvination* re-affirm genetic differences (Fig 4). Individual location information (un-pooled data) further illustrates genetic structure across the PF in these species, based on the absence of shared haplotypes between Cape Horn, north of the PF, and the Shackleton Fracture Zone, south of the PF (~ 500 km). In this instance, both CO1 and 16S were sufficiently variable to identify genetic difference in *C. verrucosa* and *C. pulvinatum* populations (Table 8 & 9). Overall, the finding that all species had unique haplotypes on either side of the PF strongly suggests the absence of connectivity across this oceanic feature (Table 6 - 11).

2.2 Dispersal among Sites on Either Side of the Polar Front

Genetic comparisons among stylasterid populations north of the Polar Front provide evidence of limited connectivity. Cape Horn and Burdwood Bank (both north of the Polar Front) are separated by ~ 300 km and there were no shared CO1 haplotypes in either *S. densicaulis* or *C. pulvinatum* sequence comparisons (Tables 9 & 10). However, where this result is well supported in CO1 sequence data for both species, genetic differences were apparent in 16S data only for *C.pulvinatum* (Table 9), but not *S. densicaulis* (Table 10). AMOVA between Cape Horn and Burdwood Bank indicated no genetic differentiation between sites north of the PF for *S. densicaulis* populations (16S F_{ST} = 0.1, P = 0), with an N_em of 3.33 (Table 10). Comparisons based on 16S in *C. pulvinatum* included only a single individual at Cape Horn (Table 9), therefore smaller sample sizes in *C. pulvinatum* populations limited statistical comparison (Table 9, Fig 6). Nevertheless, genetic differences are apparent among sites north of the PF for both species.

There was limited evidence of genetic subdivision across distances of ~ 300 km between two populations at Sars and Interim seamounts, south of the PF in the Drake Passage for *Errinopsis fenestrata*. There were no variable sites and no evidence of polymorphism in 16S data (all individuals had identical haplotypes; Table 11). Variation in CO1 was low at 3.56% and nucleotide polymorphism was 0.012, with shared haplotypes at Interim and Sars seamounts, and two unique haplotypes at Interim Seamount (Table 11). Low replication made AMOVA between sites and a haplotype network impracticable. A greater sampling for this species is needed to confirm gene flow between populations; nonetheless data suggests connectivity may exist between *E. fenestrata* populations south of the PF within the confluence of the ACC system (Fig 1). The presence of shared 16S and CO1 haplotypes in *C. pulvinatum* at Sars seamount (Table 9), adds further evidence to suggest that factors controlling connectivity either side of the PF, may differ at the scales tested in stylasterid coral populations.

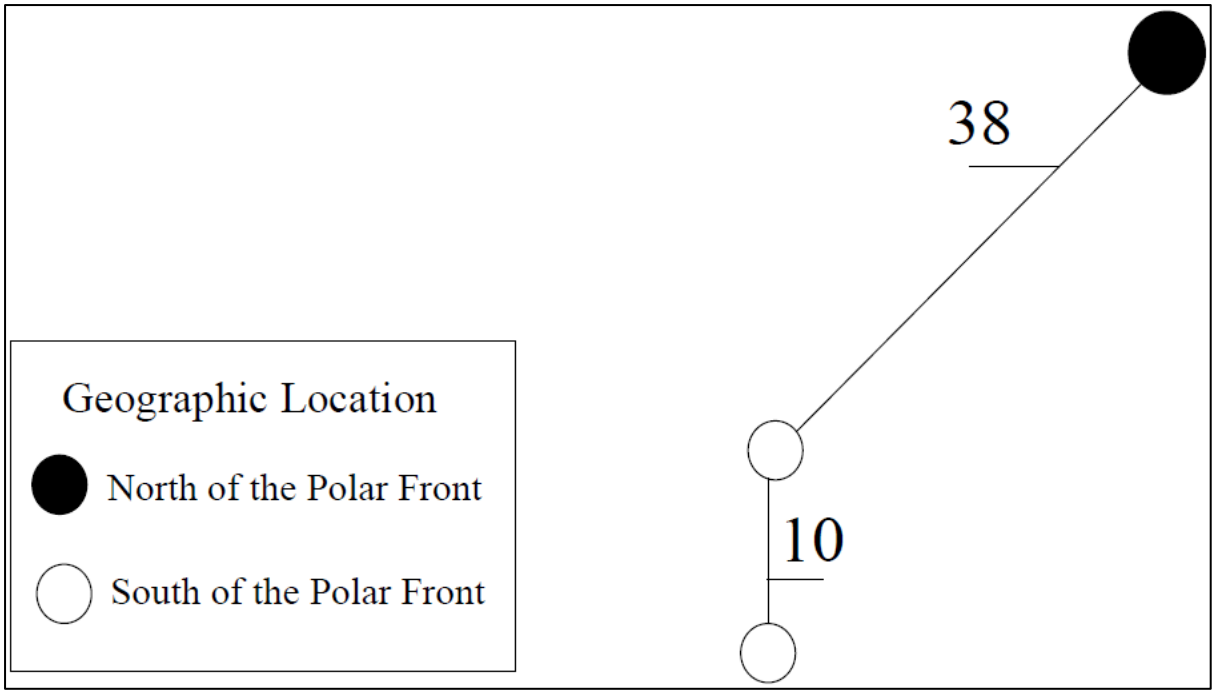
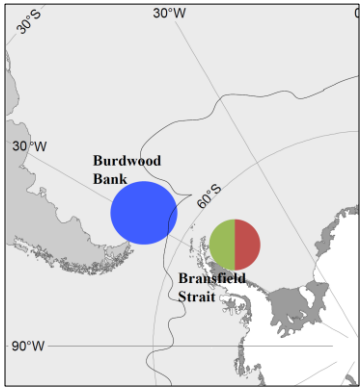


Figure 3. Haplotype network based on 5 x 16S DNA sequences from *Errina laterorifa* (total alignment length 16S = 485 bp). Each node at a branch joining point represents a parallel mutation between a median vector (a triangle in the network), each node on a branch represents a base pair mutation (represented numerically where there are >5bp mutations). Haplotypes are sized according to abundance.

Table 6. The distribution and abundance of 16S haplotypes of *Errina laterorifa* in the Drake Passage. Percent variation is the proportion of variable sites in DNA sequences; π signifies nucleotide diversity, used as a measure of DNA polymorphism in sequences for each species. Geographic locations are abbreviated as follows: Burdwood Bank (BB) and Bransfield Strait (BS). Table includes information on the base pair number (bp), sample size and number of haplotypes per site (n). The Polar Front is drawn to show coral populations in relation to the Polar Front. Pie charts are sized according to relative abundance. Maps edited from the AAD data centre.

Errina laterorifa	**Haplotype**	**Bp**	**n**	**BB**	**BS**
		485			
16S n = 5	h1		3	3	
(9.90% variation)	h2		1		1
π = 0.05	h3		1		1



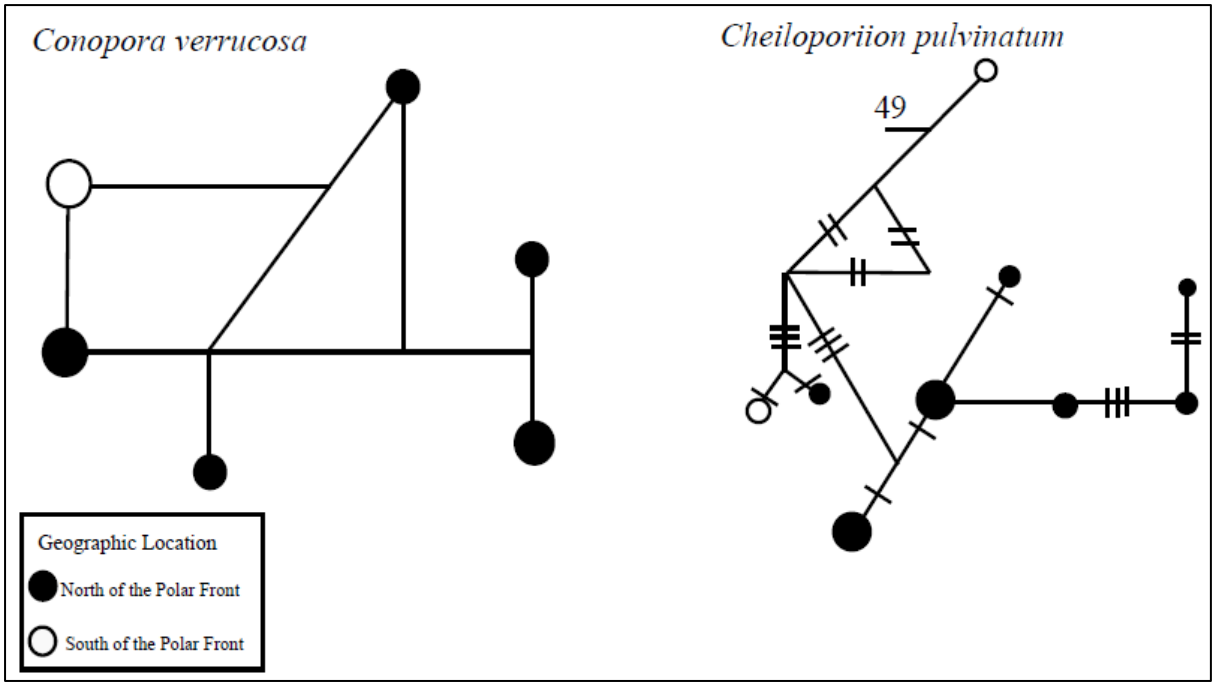
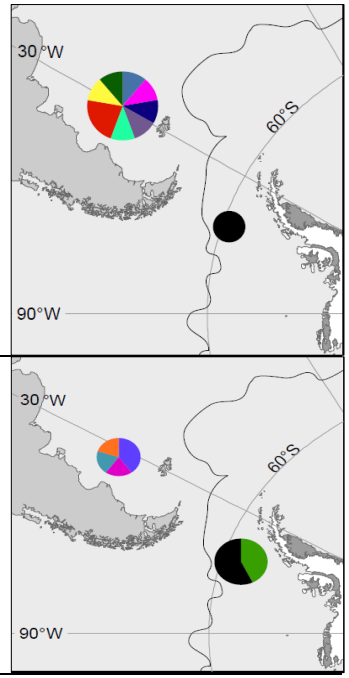


Figure 4. *Conopora verrucosa* and *Cheiloporidion pulvinatum* haplotype networks based on 16S (n = 11/12) DNA sequence data (total alignment length 16S = 544/607). Each node at a branch joining point represents a parallel mutation between a median vector (a triangle or square configuration in the network), each node on a branch represents a base pair mutation (represented numerically where there are >5bp mutations). Haplotypes are sized according to abundance.

Table 7. The 16S haplotype distribution and abundance of *Cheiloporidion pulvinatum* and *Conopora verrucosa* populations in the Drake Passage and South West Atlantic region of South America. Percent variation is the proportion of variable sites in DNA sequences; π signifies nucleotide diversity, used as a measure of DNA polymorphism in sequences for each species. Table includes information on the base pair number (bp), sample size and number of haplotypes per site (n). Antarctic maps depict the distribution and abundance of haplotypes for each species, pie charts sized according to relative sample size. The estimated boundary of the Polar Front is designated by a line. Maps edited from the AAD data centre.

	Haplotype	bp	N	South America	South of PF	
C. pulvinatum		607				
16S n = 13	h1		1	1		
(9.88% variation)	h2		1	1		
π 0.02	h3		1	1		
	h4		1	1		
	h5		1	1		
	h6		2	2		
	h7		2		2	
	h8		1	1		
	h9		1	1		
C. verrucosa		544				
16S n = 12	h1		2	2		
(6.98% variation)	h2		1	1		
π = 0.02	h3		3		3	
	h4		4		4	
	h5		1	1		
	h6		1	1		



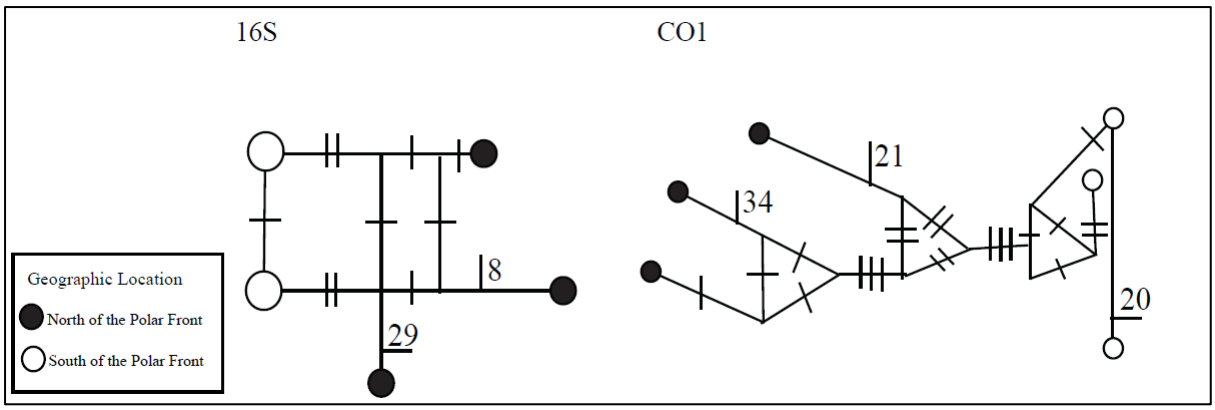
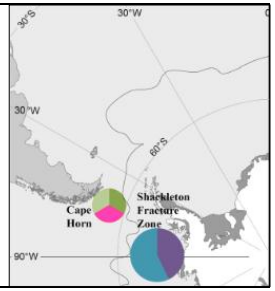
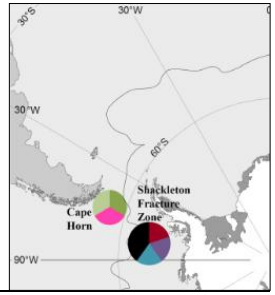


Figure 5. Haplotype network based on 10 16S and 8 CO1 DNA sequences from *Conopora verrucosa* (total alignment length 16S = 544 bp CO1 = 306 bp). Each node at a branch joining point represents a parallel mutation between a median vector (a triangle or square configuration in the network), each node on a branch represents a base pair mutation (represented numerically where there are >5bp mutations). Haplotypes are sized according to abundance.

Table 8. The distribution and abundance of CO1 and 16S haplotypes of *Conopora verrucosa* in the Drake Passage. Percent variation is the proportion of variable sites in DNA sequences; π signifies nucleotide diversity, used as a measure of DNA polymorphism in sequences for each species. Geographic locations are abbreviated as follows: Cape Horn (CH) and the Shackleton Fracture Zone (SFZ). Table includes information on the base pair number (bp), sample size and number of haplotypes per site (n). The Polar Front is included on maps of the Drake Passage to show coral populations either South of the Polar Front or North of the Polar Front in the South American region. Pie charts are sized according to relative abundance. The Polar Front is drawn to show coral populations in relation to the Polar Front. Pie charts are sized according to relative abundance. Maps edited from the AAD data centre.

	Haplotype	bp	n	CH	SFZ	
Conopora verrucosa		544				
16S n = 10	h1		1	1		
(6.80% variation)	h2		1	1		
π = 0.015)	h3		3		3	
	h4		4		4	
	h5		1	1		
Conopora verrucosa		306				
CO1 n = 8						
(22.55% variation)	h1		1	1		
π = 0.069	h2		1		1	
	h3		1		1	
	h4		1		1	
	h5		2		2	
	h6		1	1		
	h7		1	1		

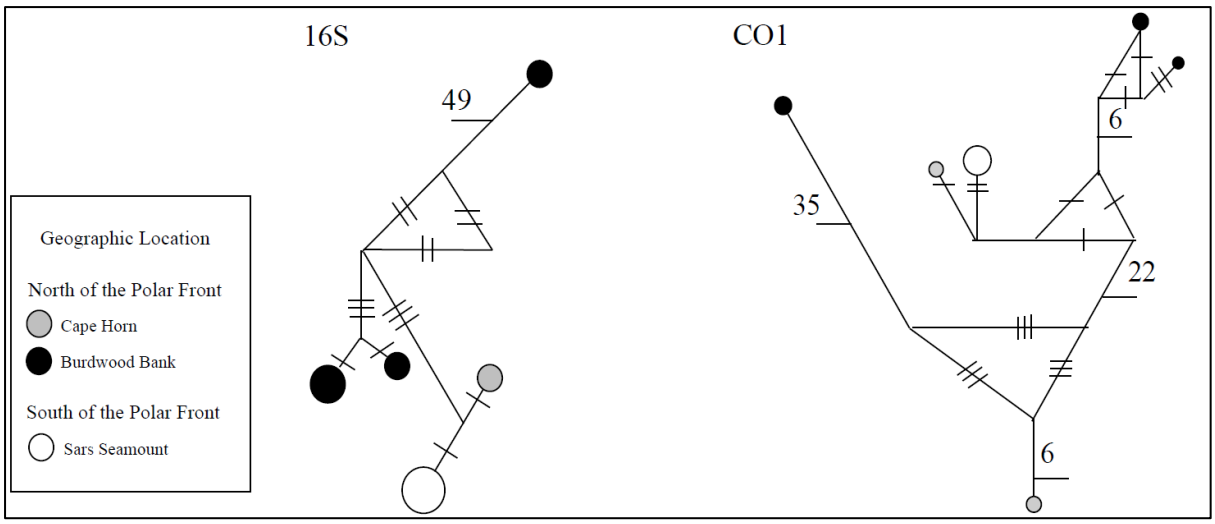
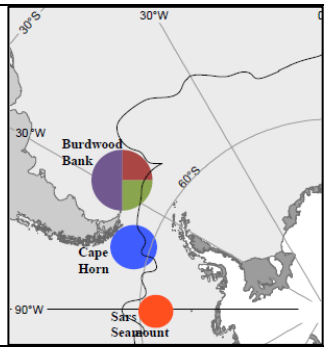


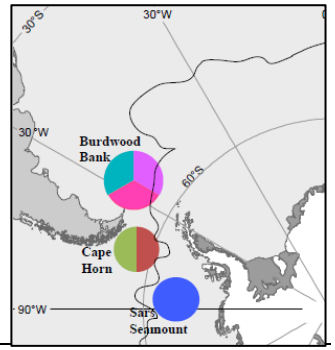
Figure 6. Haplotype network based on 7 16S and CO1 DNA sequences from *Cheiloporidion pulvinatum* (total alignment length 16S = 599 bp CO1 = 424 bp). Each node at a branch joining point represents a parallel mutation between a median vector (a triangle configuration in the network), each node on a branch represents a base pair mutation (represented numerically where there are >5bp mutations). Haplotypes are sized according to abundance.

Table 9. The distribution and abundance of CO1 and 16S haplotypes of *Cheiloporidion pulvinatum* in the Drake Passage. Percent variation is the proportion of variable sites in DNA sequences; π signifies nucleotide diversity, used as a measure of DNA polymorphism in sequences for each species. Geographic locations are abbreviated as follows: Burdwood Bank (BB), Sars Seamount (SARS) and Cape Horn (CH). Table includes information on the base pair number (bp), sample size and number of haplotypes per site (n). The Polar Front is drawn to show coral populations in relation to the Polar Front. Pie charts are sized according to relative abundance. Maps edited from the AAD data centre.

Cheiloporidion pulvinatum	**Haplotype**	**Bp**	**n**	**BB**	**SARS**	**CH**
16S n = 7		599				
(9.06% variation)	h1		1	1		
π = 0.03	h2		1	1		
	h3		2	2		
	h4		2		2	
	h5		1			1



Cheiloporidion pulvinatum						
		424				
CO1 n = 7	h1		1			1
(17.45% variation)	h2		1	1		
π = 0.07	h3		1	1		
	h4		1	1		
	h5		2		2	
	h6		1			1



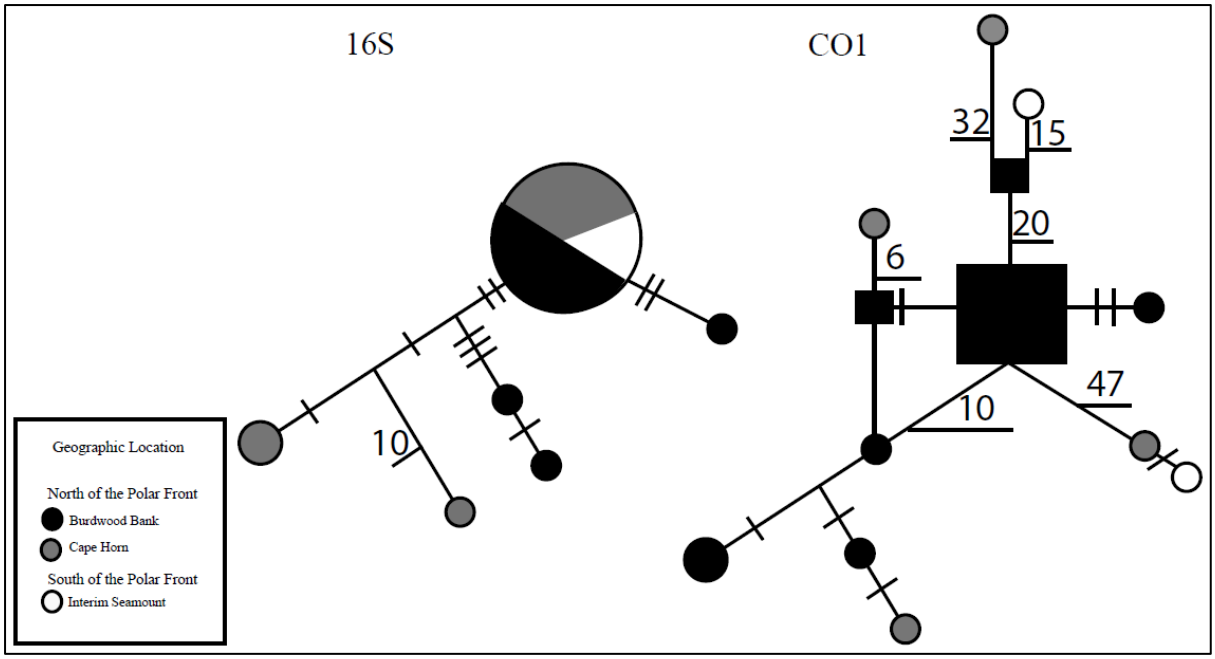
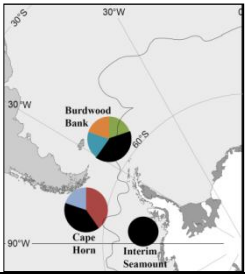


Figure 7. Haplotype network based on 12 16S and 9 CO1 DNA sequences from *Stylaster densicaulis* (total alignment length 16S = 600 bp CO1 = 308 bp). Each node at a branch joining point represents a parallel mutation between a median vector (a triangle configuration in the network), each node on a branch represents a base pair mutation (represented numerically where there are >5bp mutations). Haplotypes are sized according to abundance.

Table 10. Distribution and abundance of CO1 and 16S haplotypes of *Stylaster densicaulis* in the Drake Passage. Percent variation is the proportion of variable sites in DNA sequences; π signifies nucleotide diversity, used as a measure of DNA polymorphism in sequences for each species. Geographic locations are abbreviated as follows: Burdwood Bank (BB), Interim Seamount (INT) and Cape Horn (CH). Table includes information on the base pair number (bp), sample size and number of haplotypes per site (n) F_{ST}, P and N_em based on AMOVA. The Polar Front is drawn to show coral populations in relation to the Polar Front. Pie charts are sized according to relative abundance. Maps edited from the AAD data centre.

	Haplotype	bp	n	BB	INT	CH	F_{ST}	P	Nem
Stylaster densicaulis		600					0.1	<0.001	3.33
16S n = 12	h1		2			2			
(2.83% variation)	h2		1	1					
π = 0.008	h3		6	2	2	3			
	h4		1	1					
	h5		1	1					
	h6		1			1			
Stylaster densicaulis		308							
	h1		1		1				
CO1 n = 9	h2		1			1			
(25.40% variation)	h3		1		1				
π = 0.11	h4		1	1					
	h5		1	1					
	h6		1	1					
	h7		1			1			
	h8		1			1			
	h9		1	1					



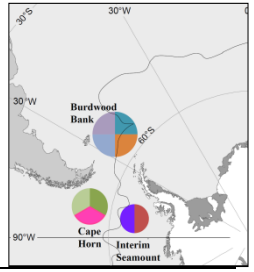
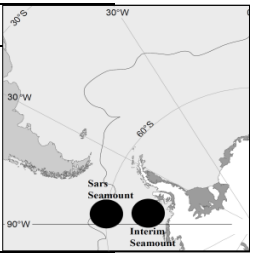
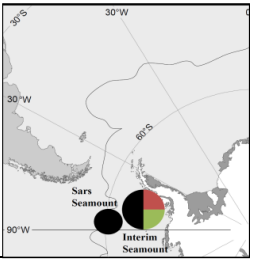


Table 11. The distribution and abundance of CO1 and 16S haplotypes of *Errinopsis fenestrata* in the Drake Passage. Percent variation is the proportion of variable sites in DNA sequences; π signifies nucleotide diversity, used as a measure of DNA polymorphism in sequences for each species. Geographic locations are abbreviated as follows: Sars Seamount (SARS) and Interim Seamount (INT). Table includes information on the base pair number (bp), sample size and number of haplotypes per site (n). The Polar Front is drawn to show coral populations in relation to the Polar Front. Pie charts are sized according to relative abundance. Maps edited from the AAD data centre.

	Haplotype	bp	N	SARS	INT	
Errinopsis fenestrata		608				
16S n= 6	h1		6	3	3	
(0% variation)						
π = 0						
Errinopsis fenestrata		487				
CO1 n=6	h1		1		1	
(3.56% variation)	h2		1		1	
π = 0.012	h3		4	2	2	





3) Connectivity across the Chilean Fiords of Patagonia

Errina antarctica populations from the two Chilean fiords in Patagonia separated by ~200km (Fig 1c), were genetically similar. The proportion of variable sites within ITS-2 data sequences was low compared to mitochondrial data sets for other species (Tables 6 – 11), at 2.37%, and nucleotide diversity was 0.003 (Table 12). There were three ITS-2 haplotypes in the final data alignment, and two of these were common to both fiords (Table 12, Fig 9). Statistical comparisons indicated no genetic difference between fiords (F_{ST} = 0, p = 0.6).

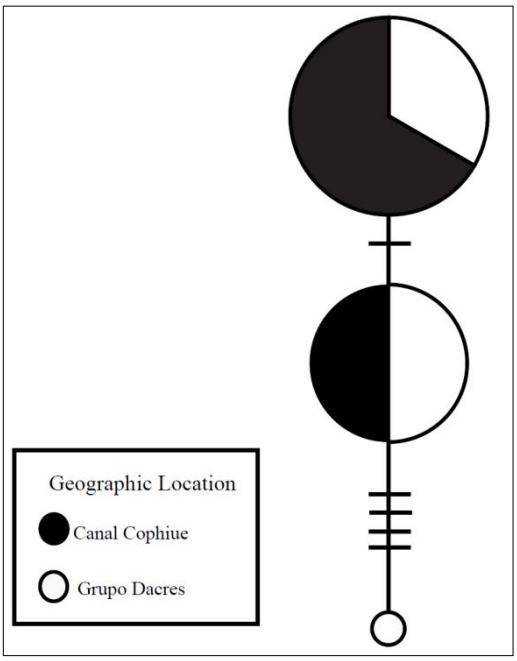
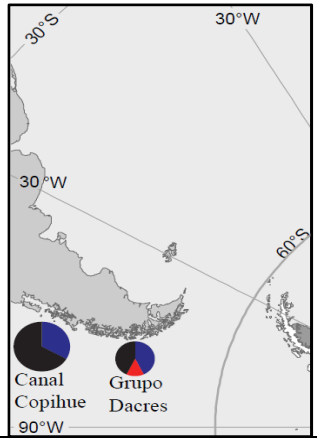


Figure 8. Haplotype network based on 16 ITS-2 DNA sequences from *Errina antarctica* (total alignment length = 169 bp). Each node on a branch represents a base pair mutation (represented numerically where there are >5bp mutations). Haplotypes are sized according to abundance.

Table 12. Results from AMOVA testing for genetic differences between *Errina antarctica* populations and the distribution and abundance of ITS-2 *Errina antarctica* haplotypes in the Patagonian fiords of Chile. Percent variation is the proportion of variable sites in DNA sequences; π signifies nucleotide diversity, used as a measure of DNA polymorphism in sequences for each species. Fiord locations are abbreviated as follows: Canal Copihue (CC), Grupo Dacres (GD). Table includes information on base pair number (bp), sample size and number of haplotypes per site (n) F_{ST}, P and N_em. Map depicts the distribution and abundance of haplotypes, black and blue represent shared haplotypes. The red haplotype is from a single specimen at Grupo Dacres. Pie charts are sized according to relative abundance. Map edited from the AAD data centre.

	Haplotype	bp	n	CC	GD	F_{ST}	P	N_em
Errina antarctica		169				0	0.6	0
ITS-2 n = 16	h1		6	3	3			
(2.37% variation)	h2		1		1			
π = 0.003	h3		9	6	3			



4. Genetic Differences in the Ross Sea and Dumont d'Urville Sea, East Antarctica

4.1 Large Scale Connectivity between the Ross and Dumont d'Urville Sea (~ 2000 km)

There was no significant genetic differentiation between *Errina fissurata* populations on shelf regions in the Ross Sea and Dumont d'Urville Sea, two protected field-like aggregations, separated by > 2000 km based on AMOVA (ITS-1 F_{ST} = 0.03 P = 0.165,and ITS-2 F_{ST} = 0.002, P = 0.27) (Table 13). The most variable region was the ITS-2, which showed the proportion of variable sites at 24%, compared with 4.3% in ITS-1 for a similar sequence length. Nucleotide diversity was low, 0.02 in ITS-2 and 0.002 in ITS-1. The effective gene flow across generations (N_em) between the Ross Sea and Dumont d'Urville Sea was estimated at seven based on ITS-1 data, and126 individuals in ITS-2, suggesting sufficient gene flow to prevent genetic divergence (Table 13). ITS-2 and ITS-1 sequence data revealed one common haplotype that was abundant in both regions but also the presence of unique haplotypes in each location. Five unique haplotypes are only 1 – 2 base pair mutations from the main shared/basal haplotype and there is evidence of a star phylogeny in ITS-1, but due to a higher number of base pair mutations this pattern is less obvious in ITS-2. In both networks, a number of sequences radiate from the common haplotype, and all other haplotypes were either rare or unique (Fig 9).

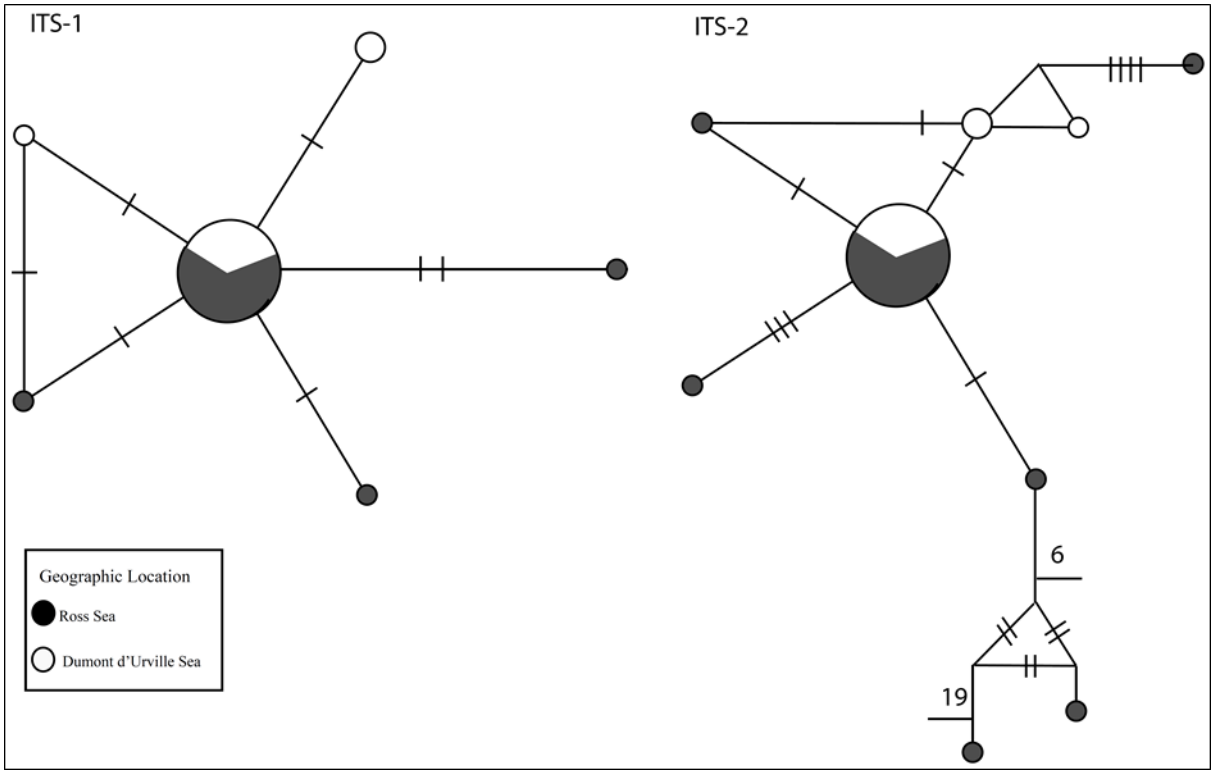
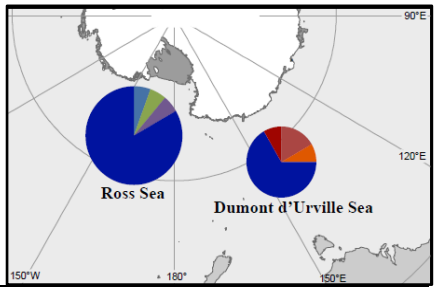
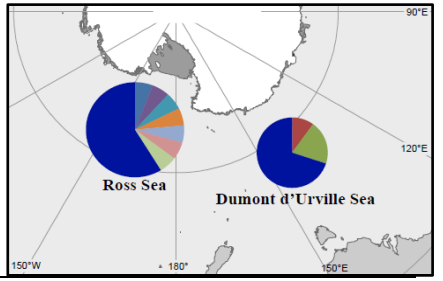


Figure 9. Haplotype network based on 30 ITS-1, and 27 ITS-2 DNA sequences from *Errina fissurata* (total alignment length = 114 bp). Each node represents a base pair mutation. Haplotypes are sized according to abundance.

Table 13. Results from AMOVA testing for genetic differences between *Errina fissurata* populations in the Ross Sea and Dumont d'Urville Sea in East Antarctica. Data sets designate the portions of the ITS region aligned (ITS-1, ITS-2), total sequence length in base pairs (bp), sample size and number of haplotypes per site (n) F_{ST}, P and N_em, π represents nucleotide diversity used as a measure of DNA polymorphism compared to percentage of variation within sequences. Antarctic maps depict the distribution and abundance of haplotypes for each species, the most prevalent and shared haplotype is designated in dark blue, all other haplotypes are unique. Pie charts are sized according to relative sample size. Maps edited from the AAD data centre.

	Haplotype	Bp	n	Dumont D'Urville	Ross Sea	F_{ST}	P	N_em
Errina fissurata		114				0.03	0.165	7.47
ITS-1	h1		1		1			
n = 30	h2		2	2				
(4.38% variation)	h3		1		1			
π 0.002	h4		1		1			
	h5		1	1				
	h6		23	8	15			
	h7		1	1				
Errina fissurata		130				0.002	0.27	126.013
ITS-2	h1		1		1			
n = 27	h2		1	1				
(24.62% variation)	h3		2	2				
π 0.02	h4		1		1			
	h5		1		1			
	h6		1		1			
	h7		1		1			
	h8		1		1			
	h9		1		1			
	h10		17	7	10			





4.2 Local Scale Connectivity within the Ross Sea (~ 10 – 70km)

Comparison of *E. fissurata* specimens and *E. laterorifa* specimens from 13 stations in the Ross Sea separated by ~ 10 – 73 km (Fig 1b) revealed genetic similarity at smaller spatial scales. There were only low levels of variation among ITS sequences of *E. laterorifa* specimens (0.28%), and nucleotide polymorphism was 0. In contrast, the ITS-2 region in *E. fissurata* was more variable, with a proportion of variable sites within sequences at 21.97%, and nucleotide polymorphism at 0.03. CO1 variation in *E. fissurata* was intermediate in comparison with 1.13% as the proportion variable sites and 0.005 nucleotide polymorphism. These estimates are much lower than those seen across laRger geographic divides (> 300 km Tables 6 - 11).

There was no evidence of genetic differentiation on a local scale in *E. laterorifa* populations in the Ross Sea. Statistical comparison between *E. laterorifa* samples from stations 154 and 157 suggest gene flow occurs at the scale of ~ 25 km in this species (F_{ST} = 0.15, P = 0.45), with an estimate of effective gene flow (N_em) of 4.75 migrants across generations (Table 14, Fig 1b). Two ITS haplotypes occurred across multiple stations (h2 and h3). The corals from the sites not included in the AMOVA (116 and 277) had two haplotypes; one that was unique to a single station (h1 at site 116), the colony from Station 277 had an ITS haplotype that was common across three stations, and was the dominant haplotype, found in six of the ten individuals (Table 14, Fig 11).

In *E. fissurata*, AMOVA indicated statistically significant genetic differentiation between stations 154, 157 and 78 (F_{ST} = 0.18, P = 0.02), with an N_em of 1.14 migrants across generations. This comparison was based on more variable ITS-2 data, and a higher sample size than *E. laterorifa* estimates (Table 14). The ITS-2 *E. fissurata* network showed that most specimens (from four of the five stations) shared a single common haplotype. The most divergent haplotypes were represented by singletons, and were found only at station 24, 27 and 28 (Fig 11).

Estimates of genetic differentiation in *E. fissurata* from stations 23 & 27 based on CO1 sequence data were not significantly different from panmixis (F_{ST} = 0.15, P = 0.65, N_em = 1.42) (Table 15). These two stations are geographically adjacent, and separated by only ~ 10 km) (Fig 1b).

Table 14. Results from AMOVA testing between *Errina* populations and the distribution and abundance of ITS *Errina laterorifa* and *Errina fissurata* haplotypes from stations in the Ross Sea. Percent variation is the proportion of variable sites in DNA sequences; π signifies nucleotide diversity, used as a measure of DNA polymorphism in sequences for each species. Table includes information on base pair number (bp), sample size and number of haplotypes per site (n) F_{ST}, P and N_em. Refer to Ross Sea station map (Fig 1c) (see Appendix Table 3.1 for station listing).

	Haplotype	**bp**	**n**	**154**	**157**	**82**	**78**	**116**	**277**	**150**	**156**	**F_{ST}**	**P**	N_em
Errina laterorifa		356										0.15	0.45	4.75
	h1		1					1						
ITS n = 10	h2		6	1	4				1					
(0.28% variation)	h3		3	2	1									
π = 0														
Erinna fissurata		130										0.18	0.02	1.14
	h1		1	1										
ITS-2 n = 17	h2		1		1									
(21.97% variation)	h3		1			1								
π = 0.03	h4		1			1								
	h5		1		1									
	h6		1					1						
	h7		10	2			6			1	1			
	h8		1		1									

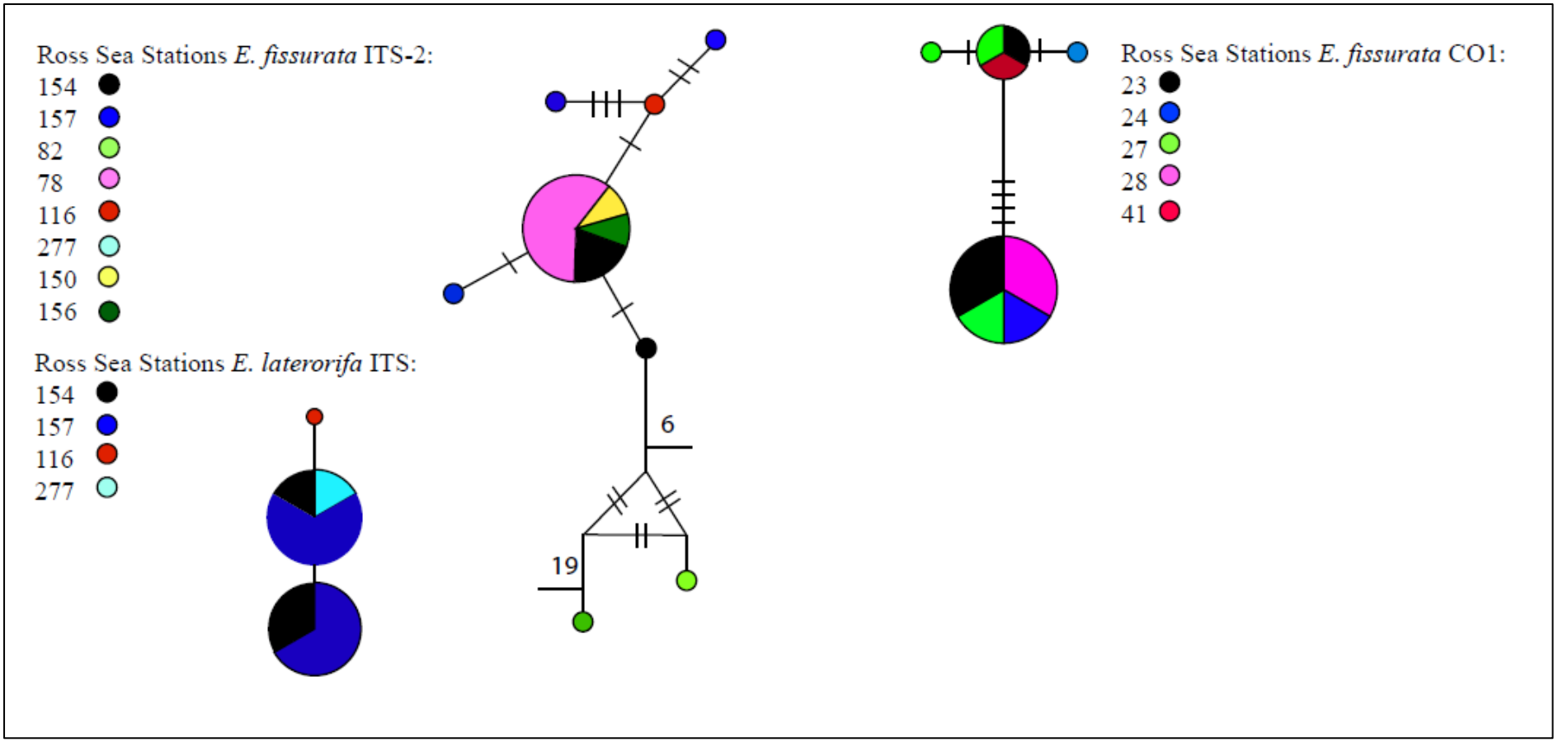


Figure 11. Haplotype network based on ITS-2 (n = 17) and CO1 (n = 11) DNA sequence data from *E. fissurata* populations, and E. laterorifa ITS (n = 10) within the Ross Sea (total alignment length = 130/531/356 bp). Each node represents a base pair mutation. Haplotypes are sized according to abundance.

Table 15. Results from AMOVA testing for genetic differences and the distribution and abundance of CO1 *Errina fissurata* haplotypes from stations in Ross Sea. Percent variation is the proportion of variable sites in DNA sequences; π signifies nucleotide diversity, used as a measure of DNA polymorphism in sequences for each species. Table includes information on base pair number (bp), sample size and number of haplotypes per site (n) F_{ST}, P and N_em. Refer to Ross Sea station map (Fig 1c) (see Appendix Table 2. for station listing).

				Station							
		bp	**n**	**23**	**24**	**27**	**28**	**41**	**F_{ST}**	**P**	**N_em**
		531							0.15	0.65	1.42
E.fissurata	h1		1		1						
CO1 n = 11	h2		6	2	1	1	2				
(1.13% variation)	h3		1			1					
π = 0.005	h4		3	1		1		1			

Discussion

The results of this study reveal both genetic sub-division and gene flow between stylasterid coral populations. Final interpretations vary by species, molecular marker, study region and spatial scale, suggesting that a multitude of factors may influence connectivity estimates. Conclusions of this study are generally restricted by low sample sizes (Table 3 & 4). For example, regionally the Ross Sea and Drake Passage are separated by more than ~ 6000 km, and there is a large genetic differentiation between populations of *Errina laterorifa* (Table 5, fig 2). This finding is unsurprising given the distance between populations, and oceanic and geographic barriers to dispersal. However, this result is based on only one sample from the Ross Sea, preventing solid conclusions. These limitations are common in Antarctic research, and most connectivity studies are similarly affected by low sample size (and associated low replication across sites). This restricts our ability to measure species richness, and gage if sampling intensity accurately represents a community (Arango *et al*., 2011). To emphasize this point a summary of Antarctic connectivity research to date is included in the appendix (Appendix Table 3.2).

Genetic Differences among Regions in the Sub-Antarctic and South America

Dispersal across the Polar Front

Gene flow estimates among populations from the Sub-Antarctic and South America show evidence of isolation across the Polar Front and a general pattern of genetic sub-division in stylasterid population assemblages, based on a lack of shared haplotypes (Tables 6 – 11; Figs 4 - 7). This conclusion holds true across six locations (Fig 1b), and spatial scales ranging from ~ 300 ≥ 1000 km in all species available for study, based on CO1 data (Tables 6 – 11; Figs 4 - 7). These estimates corroborate other invertebrate studies on connectivity in the Antarctic peninsula region (e.g., Wilson *et al*, 2007; Leese *et al*, 2010; Baird *et al*, 2011; 2012), and for the most part connectivity estimates conform to a pattern of isolation within self-recruiting populations, common of the Antarctic benthos (Thatje, 2012). However, incongruence in connectivity estimates was observed in some instances, and differential estimates of gene-flow in mitochondrial DNA sequence data revealed 16S as a more conservative gene region, compared to CO1 in all species (Tables 6 – 11; Figs 4 - 7). This pattern is common in coral and hydroid genomes (Hellberg, 2006; Cartwright *et al*., 2008), and stylasterids are no exception based on the data contained herein.

The genetic differentiation observed between 16S haplotypes of *E. laterorifa* in the Bransfield Strait (a geographically isolated sample site) and Burdwood Bank across ~ 900 km (a pattern not seen across shorter distances in other species based on 16S), supports an isolation by distance model of dispersal across the Drake Passage (see Slatkin, 1993) (Table 6) and suggests that in 16S, more time and geographic distance maybe needed to produce genetic changes compared to CO1. Very few migrants are needed to genetically connect populations (Hellberg, 1995), and for this reason it seems plausible to explain links (across multiple generations) based on 16S data as representing historical connectivity or retention of ancestral links. This scenario was detailed by Wilson *et al*., (2009) to explain their finding of higher genetic differentiation in CO1 than 16S in the Antarctic nudibranch *Doris kerguelensis*. Correspondingly, invertebrate connectivity studies by Thornhill *et al*., (2008) and Arango *et al*., (2011) also found CO1 to be more variable and informative than 16S. All three of these studies have sampling locations which overlap regionally (e.g., Antarctic Peninsula and Burdwood Bank), substantiating a conclusion of historic connections between invertebrate ecosystems in South America and the Sub-Antarctic.

Differential mutation rates in stylasterid mitochondrial DNA regions was exemplified in *Stylaster densicaulis* where 16S haplotypes are shared either side of the PF, between Cape Horn, Burdwood Bank and Interim Seamount, whilst CO1 haplotypes were unique at all three locations (Table 10). Interim Seamount, south of the PF, represents a relatively short dispersal route across the PF into South America of ~ 300 km, and sea floor bathymetry connects this region to Cape Horn (Waller *et al*., 2011) (Fig 1b), therefore benthic connections may enable dispersal between locations. Based on the life history characteristics of stylasterid corals (Stratford, 2002; Miller *et al*., 2004), it is unlikely that current-mediated dispersal is common. However, over evolutionary time scales the survival of a small number of recruits across the PF could contribute to the prevalence of stylasterids in the Drake Passage. Assessment of gene flow in other species provides evidence to suggest that genetic sub-division across the PF increases with geographic distance (> 300 km). For example, *Conopora verrucosa* populations at Cape Horn and the Shacklton Fracture Zone (SFZ) separated by ~ 500 km, further south than Interim Seamount, were genetically unique at both DNA regions (Table 8). *Cheiloporidion pulvinatum* 16S and CO1 haplotypes are also unique at sample locations, including Cape Horn and Sars Seamount, substantiating isolation south of the PF at spatial scales > 400 km. However, in *C. pulvinatum* the estimated dispersal distance of > 400 km is not dissimilar to that of *S. densicaulis* from Cape Horn to Interim

seamount, suggesting that factors other than distance may contribute to gene-flow estimates across the PF.

The finding of genetic subdivision at Sars Seamount in *C. pulvinatum* (Table 9, Fig 6), and at SFZ in *C. verrucosa* (Table 8, Fig 5), but not in *S. densicaulis* at Interim Seamount (Table 9, Fig 6 based on 16S) may signify site-specific isolation across the PF (Fig 1b). A recent study by Margolin *et al*., (2014) provides evidence to support this conclusion at Sars Seamount. Their study, based on carbon dated scleractian coral age estimates, showed a population shift from south to north, including a transition zone at Sars Seamount during the Last Glacial Maximum (LGM), ~ 23 - 19 thousand years ago. Livermore *et al*., (2004) describe the SFZ is a structurally unique oceanic transverse ridge, formed by uplift 8 million years ago. The ACC is more restricted at the SFZ forming a 'pinch point', where several enduring and semi-enclosed eddies exist within the ACC and Antarctic Bottom Water (ABW) is restrained. Therefore, restricted dispersal and more isolated faunal assemblages are possible at both the SFZ and Sars Seamount. Additionally, these two examples of climactic change on evolutionary time scales provide evidence to support an overall conclusion of historic gene-flow, and isolation among self-recruiting populations either side of the PF.

Cold-water coral habitats are extremely dynamic and the opportunity to diversify and colonise deep benthic environments may have been frequent through evolutionary time (e.g., Lindner *et al*., 2008), but comparatively low over ecological (recent) time scales (e.g., Le Goff-Vitry *et al*., 2004). The cosmopolitan distribution of *S. densicaulis* and *C. verrucosa* in the Sub-Antarctic reflects a biogeographic distribution pattern of dispersal and colonisation throughout the Drake Passage and Scotia Arc over time (Bax & Cairns, 2014). Similarly, the large aggregation of *S. densicaulis* and *C. verrucosa* at Cape Horn (Waller & Robinson, 2011 pers. identification), and aggregations of *C. pulvinatum* in the South West Atlantic (Bax *et al*., unpublished data), signal the capacity of these species to predominate in select habitats. Therefore, the historical connections present in 16S data may be related to evolutionary change effecting the dispersal of many Antarctic animals (e.g., opening of the Drake Passage, ACC formation, LGM Clarke *et al*., 2005), including corals (Margolin *et al*., 2014). The higher variation displayed in CO1 may represent more recent (and therefore more ecologically informative) isolation among self-recruiting populations across the PF, at the scales tested.

Dispersal among Sites on Either Side of the Polar Front

The presence of both CO1 and 16S shared haplotypes in *Errinopsis fenestrata* (Table 11), substantiate a conclusion of panmixia south of the Polar Front between Interim and Sars seamounts. More population comparisons are needed to determine if this pattern is congruent with other stylasterid populations documented south of the PF. However, despite limited comparative data, genetic connectivity is in alignment with other studies (Gutt *et al*., 2013), and the ACC system is thought to facilitate the transport of larvae around the Antarctic continent in a number of invertebrate populations (see Thatje *et al*., 2012 for a summary). Therefore, in *E. fenestrata,* this biogeographic pattern may reflect geneflow linked to the ACC and a heightened ability to disperse through current-aided means, compared to species outside of the ACC at similar spatial scales (~ 300 km Table 9 & 10). However, this does not preclude that *E. fenestrata* is a successful primary coloniser in the Antarctic benthos, with a circum-Antarctic dispersal capacity. In fact, *E. fenestrata* is listed as a rare and endemic species with a limited geographic range (Bax & Cairns, 2014). Therefore, the exertion to which ACC forcing determines stylasterid population connectivity remains to be tested, and linkages may be historic rather than ecological.

Comparisons north of the Polar Front, away from the main confluence of the ACC, show a pattern of genetic sub-division in *S. densicaulis* and *C. pulvinatum* populations between the South American sampling locations of Burdwood Bank and Cape Horn (Table 9 & 10). This finding is consistent with the pattern of isolation seen in other stylasterid species (Tables 6 – 8; Figs 4 - 7), and it seems fair to assume that differences based on the more variable CO1 reflect isolation in *S. densicaulis* populations, despite 16S AMOVA suggesting no genetic differentiation between populations (16S F_{ST} = 0.1, P = < 0.001) (Table 10). Furthermore, the presence of shared 16S and CO1 haplotypes in *C. pulvinatum* at Sars Seamount (Table 9) north of the PF, is similar to the pattern of shared haplotypes at both loci in *E. fenestrata* (Table 11). Therefore, geneflow either side of the PF may differ at the spatial scale of ~ 300 km.

Fine Scale Connectivity in Chilean Patagonian Fiords (~ 200 km)

Antarctic sampling is extremely challenging; sample sizes and experimental design are often less than ideal as a result (Appendix Table 3.2). The shallow coral fields of *Errina antarctica* in the Patagonian fiords provide the unique opportunity to gain insight into the dispersal capacity of corals in more inaccessible ecosystems such as sea-mount and submarine ridges in the Southern Ocean, and AMOVA revealed no genetic difference

between two Chilean fiords (Table 12). Geographically the Chilean fiords comprise a complicated maze of channels, with a steep and narrow topography across an archipelago formed during the submersion of a glacial valley encompassing multiple openings towards the sea (Marden & Clapperton, 1995). Therefore, this environment is highly dynamic and factors controlling larval dispersal are likely complicated. Dispersal may occur through deep channels into the surrounding ocean (see Fillinger & Richter, 2013). However dispersal within the fiord system would be more complex. *Errina antarctica* populations in Canal Copihue and Grupo Dacres are separated by ~ 200 km (by the most direct dispersal route), and a number of dispersal barriers exist between these two regions, including at least five island groupings, and fiords which diverge in multiple directions (Fig 1c). Therefore, demographic connectivity between the two populations tested appears unlikely, as dispersal would involve the capacity of a larva to negotiate a complex maze of dead ends, both strong and weak currents and selectively settle in suitable habitats. Furthermore, studies of the congeneric *E. novazelandiae* in the New Zealand fiords (Miller *et al*., 2004) indicated dispersal across such distances within a fiord system was unlikely.

This is the first study to use ITS DNA sequence data to assess population structure in stylasterid corals. A lack of comparative sequences and the complications associated with sequencing the gene (e.g., multi-copy and hyper-variability) lead to smaller than anticipated sample sizes, and the elimination of the majority of sequences. The only other study on coral connectivity in the fiord region of Patagonia was Miller *et al.*, (2011) on the scleractinian coral *Desmophyllum dianthus*. Miller *et al*., (2011) studied sites separated by ~ 900 km and also found no genetic differentiation between fiords based on 16S, ITS or MtC data. Therefore, either gene flow exists between fiords, or ITS may not be sufficiently variable to pick up fine scale genetic structure in *E. antarctica*.

The usefulness of ITS DNA sequence data may be taxon and region specific. For example, Flot *et al*., (2013) studied *Lophelia pertusa* corals collected 7500 km apart which shared identical nuclear ITS-2 and near-identical mitochondrial genomes. However, Miller *et al* (2010) studied ITS sequence data from antipatharian corals and found genetic structure across ~500 – 1000 km. Alternate gene regions and primer combinations were trialled over the course of this study including variations of the ITS-2, ITS-3, SRP-54 and D-loop (Bax, 2009). However, amplification success and sequence quality was low, and these regions were not useful for further analysis. It is possible that more variable or more appropriate markers (ie. Microsatellites or SNPs) may better clarify connectivity in Patagonian stylasterids.

Given the urgent need for conservation managers to have estimates of contemporary gene flow (Almany *et al*., 2009), this area of research should be assigned the highest priority. Therefore, despite results suggesting gene flow between *E. antarctica* populations a precautionary approach to conservation management is advised until more ecologically informative genetic markers are available for study. This recommendation is especially so when we consider the mounting evidence on the fragility of the fiord ecosystem (Fillinger & Richter, 2013; Häussermann & Försterra, 2007), the recent observation of devastated *E. antarcitca* fields (Häussermann & Försterra, 2014; Fig 12), and the lack of gene flow in similar species found in fiord environments (Miller *et al*., 2004).

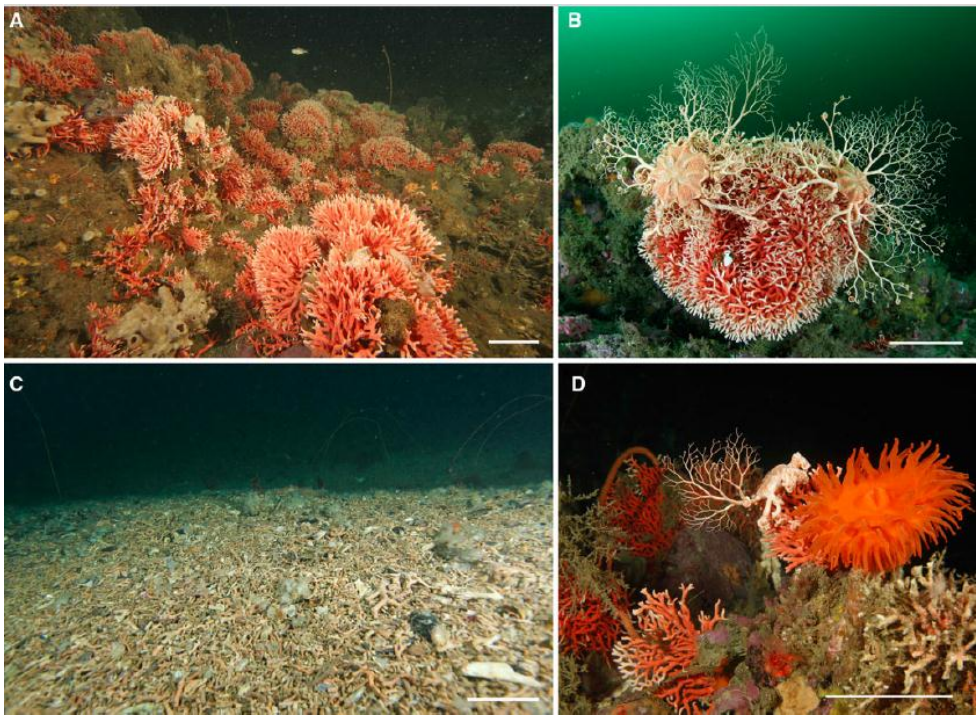


Figure 12. Reef-like aggregations of *Errina antarctica* were discovered by Häussermann and Försterra (2007) in aggregations of ±80% coverage across a 10,000m² area at Canal Copihue (A, B, D). This site was re-visited during a 2012 expedition and coral coverage was already depleted (pers. obs.), the entire population has since been reduced to coral rubble (Fig C from Häussermann & Försterra, 2014), and the exact cause is unknown.

Large Scale Connectivity between the Ross and Dumont d'Urville Sea (~2000 km)

Geographically the Ross Sea and Dumont d'Urville Sea are separated by approximately 2100 km and large scale AMOVA analysis of ITS data suggests no genetic subdivision among *Errina fissurata* populations (Table 13). Despite this finding of genetic similarity, there were unique haplotypes in both seas. These haplotypes may be common by descent, but to effectively assess connectivity estimates it is important to consider other

factors that may have contributed to this result. The *E. fissurata* ITS-1 haplotype network illustrates what is described as a star phylogeny (Martins & Housworth, 2002) (Fig 9), and in ITS-2 a similar pattern illustrating a branch multi-furcation from a common haplotype, and few base pair mutations in unique haplotypes (Fig 9). This pattern is often representative of a recent population expansion event from a founder population (e.g., Hellberg, 2006 as an example in corals).

Errina fissurata is described as a circum-Antarctic species (Cairns, 1983; Bax & Cairns, 2014), and may have gradually spread around the Antarctic continent through evolutionary time. Therefore, ITS sequence data may not reflect ongoing gene-flow between geographically distant *E. fissurata* populations, and may instead represent adaptive radiation from a basal lineage (or founder population). In this fashion, a small percentage of larvae may be retained within a geographic locality due to factors such as oceanographic conditions, nutrient supply etc. ., eventually resulting in unique haplotype diversity through mutation, and overtime - specific local adaptations and reproductive isolation (e.g., Arango *et al*., 2011). This hypothesis may explain the comparatively high abundance of *Errina* species in the Dumont d'Urville Sea (Post *et al*., 2010), and regional separation of circum-Antarctic stylasterid genera and species in general (Cairns, 1983; Bax & Cairns, 2014). During Antarctic voyages not every trawl survey collects benthic fauna, often across great distances of sampled area; this suggests that benthic diversity in the Antarctic deep-sea is sparsely distributed, which could lead to isolation among self–recruiting populations at the scale tested, and their subsequent radiation is likely dependent upon suitable habitat, a limited resource in the Antarctic deep-sea.

Local Scale Connectivity within the Ross Sea (~ 10 – 70 km)

Connectivity in the Ross Sea was investigated on a local scale (across ~ 10 – 73 km), and similarly to large scale analysis in East Antarctic stylasterid populations, there was a lack of significant genetic differentiation. This result is based on both ITS and CO1 sequence comparisons (Table 14, 15 & Fig 11) in *E. fissurata* and *E. laterorifa* populations. The apparent lack of differentiation between Ross Sea stations suggests that populations are not self-recruiting and that, at the local scale, there is gene flow between neighboring populations. These results maybe reflective of the current systems that operate between the patchy distributions of suitable habitat (e.g., Adelie Bottom Water, Post *et al*., 2010). However, despite apparent panmixia, unique haplotypes were present and the N_em values between stations in the Ross Sea was comparatively much lower than the estimated number

of migrants between the Ross Sea and the Dumont d'Urville Sea (N_em=1.14 and 4.15 *vs.* 7 and 126) (Table 14 *vs.* 15).

Estimates of gene flow from shallow water coral studies show similar discrepancies in N_em estimates across varying spatial scales. For example, a study of connectivity between the Great Barrier Reef and Lord Howe Island (considered a geographically isolated location) found estimates of N_em ranged from 0.6 to 6.0 for five brooding corals. The coral *Stylophora pistillata* had a greater level of genetic connectivity between geographic distances of 500 – 1200 km (N_em = 1.4) compared to local scale connectivity (< 5 km, N_em = 0.6). Ayre and Hughes (2000) concluded that populations with low N_em (0.6 – 3.3) were effectively subdivided, regardless of scale, and that the majority of recruitment was local. Based on this conclusion, the Ross Sea *Errina* spp. populations are more distinct at local spatial scales (< 100 km) than at large spatial scales (> 2000 km). However, variability estimates in CO1 are much lower than those seen across lager geographic divides (e.g., > 300 km Tables 6 - 11), and there is limited evidence of genetic subdivision at larger geographic scales in East Antarctica (> 2000 km) (Table 12). Therefore, variability in ITS and CO1 sequence data may not accurately represent fine-scale connectivity within Ross Sea *Errina* spp. Ayre and Hughes (2000) also speculated that long distance dispersal may be more important over evolutionary time scales, and it is difficult to determine, without comparative genetic data, if the results here are informing ecological or historical connections. Taken together results suggest historical connections are more likely across data sets.

Conclusion

This study found significant population structure across the Polar Front based on CO1 and, and in most cases for 16S sequence data at scales ranging from ~ 300 – 1000 km, in all species available for connectivity estimates in the Sub-Antarctic and South America. In contrast, 16S and CO1 data suggest panmixia in Antarctic *E. fenestrata* populations south of the Polar Front across ~ 300 km within the ACC confluence. Whilst, ITS data suggests that gene-flow is present between the Ross Sea and Dumont d'Urville in East Antarctica, and within the Patagonian fiords in Chile. Therefore, large (~ 1000s of km), and local scale (~ 100s of km) water masses may enable connectivity between stylasterid populations in East Antarctica, Patagonia and on the Antarctic Peninsula within the ACC system, but not in the

Sub-Antarctic and South America, due to the presence of the PF and isolating eddies and gyres at seamount and shelf locations (see Livermore *et al*., 2004). However, in regard to conservation management, these findings are potentially misleading, as the DNA markers used here may insufficiently differentiate ecological from evolutionary connectivity and sample sizes were limited. To conclude that gene-flow estimates are representative of present day dispersal may be detrimental to conservation goals, as it appears to be the consensus within this data set, that genetic connections are historical in origin.

For specific management outcomes, it is important to consider that a far greater number of migrants are needed to make demographic changes such as replenishing a devastated population (as in ecological connectivity Botsford 2003) than the number of migrants that would aid in the genetic maintenance of a population (as in evolutionary connectivity Burridge *et al*., 2012). Results suggest that ITS, 16S and CO1 gene regions may inform connectivity across different temporal scales. ITS estimates of connectivity may help us understand adaptive radiation in the Ross and Dumont d'Urville seas, and the Patagonian fiords. 16S shared haplotypes may explain historical connections and speciation south of and across the Polar Front, potentially linked to time frames such as the LGM (as in Margolin *et al*., 2014), whilst the higher variability in CO1, and a lack of shared haplotypes in most species maybe more ecologically informative for stylasterid corals at spatial scales >300 km (e.g Sub-Antarctic species), but not at local scales < 100 km (e.g Ross Sea *E. fissurata*).

This is the first study to provide genetic data for Antarctic stylasterids, therefore more comparative sequences are needed to substantiate a baseline for their genetic diversity. More variable markers, and preferably larger sample sizes, are needed before spatial structure can be explicitly resolved. It is recommended that future studies focus on the field-forming *Errina* species, for which only ITS data were available for most connectivity assessments. These populations are of particular conservation concern as VMEs throughout the Antarctic and Patagonian benthos - providing habitat for a diverse fauna. Distinguishing ecological from evolutionary connectivity, and recognising the regional and species level differences in stylasterid populations will be key to their continued preservation under current CCAMLR conservation management regimes (Table 2), and future conservation initiatives in South America and Patagonia. In the interim, a precautionary approach is advocated for stylasterid coral population conservation and management - especially when considering the extreme fragility of the deep-sea environment to climate change and economic interest in Southern Ocean fisheries.

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Final Discussion and Synthesis to Address the Management Questions and Conservation Implications of this thesis

Stylasterids were used as model organisms to understand the diversity, structure and function of cold water coral ecosystems within the framework of Antarctic Vulnerable Marine Ecosystems (VMEs). Research was focused on biogeographic patterns, species identification tools, evolutionary responses to climate change and an assessment of connectivity in order to best inform conservation strategies, and contribute to the protection of Antarctic coral communities from the multiple threats to their survival. These threats include: deep-sea trawl and long-line fisheries which permanently remove coral fauna and damage benthic habitat (Clark & Rowden, 2009), and climate change and ocean acidification which threaten to inhibit essential biological processes, lower diversity, and cause successional shifts in benthic communities (Ingels *et al*., 2012). This thesis presents several key findings that will inform conservation of these poorly understood and threatened coral ecosystems:

- Field-like aggregations of stylasterids are patchily distributed throughout the Antarctic, Sub-Antarctic and Patagonia. The biogeographic distribution of these coral aggregations and the relatively localised distribution of common species is considered to reflect insulated and isolated self-recruiting populations (Chapter 2, Bax & Cairns, 2014).
- Phylogenetic mtDNA sequence data was sufficiently variable to resolve genetic and morphological relationships in stylasterid corals, and 16S and CO1 sequences may work for future DNA barcoding studies. This has important practical implications regarding the documentation of new species in the Antarctic deep-sea (Chapter 3).
- Phylogenetic data combined with the fossil record and skeletal mineralogy show stylasterids may have radiated within the Drake Passage following de-acidification of the world's oceans linked to the Eocene/Oligocene boundary (~ 34 MYA). This finding may help us predict how stylasterid populations will respond to future ocean acidification, and decreasing saturation states in the Sub-Antarctic (Chapter 4).
- Population structure and limited gene-flow is evident across the Polar Front between the Antarctic Peninsula and South America, and among populations north of the Polar Front that are separated by ~ 300 km. In contrast, evidence of connectivity was apparent among populations south of the Polar Front at similar spatial scales,

suggesting connectivity mediated by the Antarctic Circumpolar Current (ACC). However, all genetic links were likely ancestral and there is little evidence to suggest stylasterid coral populations are connected by ongoing gene-flow (Chapter 5).

- VME listed *Errina fissurata* and *Errina laterorifa* populations in East Antarctica and *Errina antarctica* populations within Patagonia show no evidence of intra-specific genetic differences at spatial scales of ~ 10 - 2000 km based on ITS sequence data. However, evidence of genetic links may not represent ongoing gene-flow, linked to the resolution of the markers used. Haplotype networks indicate an evolutionary pattern of adaptive radiation, whereby species gradually disperse from a natal population into available habitat, and over time become regionally abundant in specialised localities (Chapter 5).

Taken together, the results from this study reveal a diverse, yet isolated Antarctic and Sub-Antarctic stylasterid fauna that has been affected by past climatic changes, and highlights the vulnerability of present day populations. These findings are of considerable scientific and conservation value, improving our knowledge of key habitat-forming species that form VMEs in the remote and understudied Antarctic deep-sea, and provide invaluable data to underpin the management and conservation of ecosystems in the vast Southern Ocean, Sub-Antarctic and Patagonia.

6.1 Stylasterid Biogeography

Chapter two reviewed the biogeography of stylasterid corals in Antarctica and the Sub-Antarctic and concluded that Stylasterids are characterised by localised species aggregations throughout their geographic range, and suggests the biogeographic distribution of stylasterid corals is representative of population isolation and endemicity (Bax & Cairns, 2014).

The following geographic regions are proposed for conservation consideration:

Stylasterid coral fields

1) Isolated areas of particularly high stylasterid occurrence including the coral-fields of the Dumont d'Urville Sea (Post *et al*., 2010) and Ross Sea (Bax & Cairns, 2014), the Larsen B ice shelf in the Weddell Sea (Fillinger, 2013), Cape Horn (Waller & Robinson, 2011) and Burdwood Bank (Bax & Cairns, 2014). These areas are recommended for priority protection, in order to maintain benthic biodiversity, as these field-like aggregations support a diverse

array of associated fauna (see Post *et al*., 2010; Waller *et al*., 2011; Kaiser *et al*., 2013), and may provide important ecosystem services such as substrate for attachment, refuge for juveniles, aggregation sites for spawning and feeding, and potentially factor in the trajectory of deep-water current systems and house economically important fish populations and bio-medical compounds (see NOAA, 2010).

Endemic faunal assemblages along the Macquarie Ridge

2) The Sub-Antarctic Region south of New Zealand, including Macquarie Ridge, is shown to have a potentially endemic fauna (11 species), and may act as a transition region between New Zealand and the Antarctic, enabling colonisation either into or out of Antarctic waters. Such transition zones are of high conservation value, providing corridors to dispersal (Lenihan & Oliver, 1995; Linse *et al*., 2008; Jörger *et al*., 2014) and a buffer against climate change (Brandt & Gutt, 2011).

Stylasterid biodiversity hot-spots at South Georgia and Shag Rocks

3) South Georgia and Shag Rocks, in the Scotia Arc have the highest documented stylasterid diversity (16, of the 33 known Antarctic species), and it would be beneficial to include these regions within the already established adjacent South Orkney Islands Marine Protected Area (Brooks, 2013), to build on the MPA network scheme advocated under regulated fisheries management regimes in the Sub-Antarctic (Rochette *et al*., 2014).

Marine Protected Areas (MPAs) have been established world-wide to protect marine biodiversity and, especially where deep-sea fisheries are concerned, they are considered the best conservation model available (Morato *et al*., 2010; Clark *et al*., 2012; Grant *et al*., 2012). However, despite this consensus among marine scientists and conservation managers, there are only two regional MPAs designated to protect deep-sea VMEs in the world: 1) The Charlie Gibbs MPA in the North East Atlantic (O'Leary *et al*., 2012), and 2) The South Orkney Islands MPA in the Sub-Antarctic (Brooks, 2013). There are a number of factors limiting MPA designation in the deep-sea such as: a lack of consensus regarding political and economic factors (Fabra & Gascón, 2008), incomplete practical descriptions of species assemblages, communities, and habitats (Griffiths *et al*., 2010), and biological and ecological functions are unknown for most deep-sea taxa (Rogers, 2004), this is especially true of the Antarctic benthos (Brandt *et al*., 2007; Clarke, 2008).

In the Southern Ocean, the biogeographic atlas publication (De Broyer *et al*., 2014) provides a classification system for Antarctic taxa and habitats, through the synthesis of all

available spatial and biological estimates, and compilation of the Census of Antarctic Marine Life (CAML) research collections. This information combined with the ecosystem management approach, advocated under the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) governing Antarctic fisheries, provides a political and economic pathway for conservation discussion and implementation (Teschke *et al*., 2014). However, despite decadal advances in Antarctic benthic research (including habitat mapping and global datasets, see Kaiser *et al*., 2013), biological and geographic spatial alignment is still lacking for many deep-sea invertebrates (De Broyer *et al*., 2014). Sampling is predominantly shallow (rarely below 500 m) (Chapter 5, Appendix table 3.2), and Peninsula Antarctica, being the most accessible region is also the most heavily studied (Clarke & Johnston, 2003; Linse *et al*., 2006; Gutt *et al*., 2011). When making conservation management decisions, it is important to note that studies on the Peninsula, and shallow continental shelf, may not fully capture the complexity of deeper, more isolated habitats (e.g., stylasterid coral fields in East Antarctica (Post *et al*., 2010) (*Thesis statement image page 6*). Therefore, although informative, the occurrence data now available for Southern Ocean stylasterids (Bax & Cairns, 2014), in and of itself tells us very little about how benthic populations interact across spatial and temporal scales. While, molecular data offers to build on distribution data and delineate ecologically and evolutionarily informative patterns (Chapters 3 - 5).

6.2 Stylasterid Taxonomic and Phylogenetic Relationships

Chapter three addressed the genetic relationships among morphologically delineated Antarctic stylasterid species, and found that morphological and genetic relationships are congruent in Antarctic stylasteridae, based on mtDNA (16S and CO1). It appears that the 16S gene region is also suitably variable for consideration as a candidate for DNA barcoding (a taxonomic method that uses a single short genetic DNA marker, usually CO1, to identify species (Hebert & Gregory, 2005). Few hydrozoan studies use CO1 sequence data, due to amplification difficulties (Govindarajan *et al*., 2005: Peña Cantero *et al*., 2009). The primers developed by Geller *et al*., (2013), amplified successfully in this study (Chapter 3). However, only for a limited subset of samples (CO1 n = 47), and only for ~289bp of a 648bp region (Hebert & Gregory, 2005). Therefore, the effectiveness of CO1 as a universal barcoding gene, may not apply to stylasterids, and 16S, although less variable than CO1, successfully differentiates between genera and species, and amplifies more easily (16S n = 72) across a similar base pair length of 293bp out of a ~600bp total sequence length (Cunningham &

Buss, 1993). Comparitive studies on hydrozoans substantiate the utility of 16S to identify genera (Govindarajan *et al*., 2005; Peña Cantero *et al*., 2009). Therefore, 16S is recommended, above CO1, for future barcoding studies on stylasterids.

This findng has practical implications regarding the identification of species, for cataloguing of marine life, and the identification of specimens that lack morphological integrity. Ideally, now that the technology and reference material is available, the incorporation of molecular taxonomy as a complementary tool to traditional taxonomy can be prioritised in future studies on stylasterid corals. However, in studies where time or expertise for traditional morphological taxonomy is limited, DNA barcoding offers an efficient method for species identification. DNA sequence data generated in this project will also enable the incorporation of additional stylasterid sequences in large data sets assessing biodiversity, such as the international Barcode of Life (BOLD) initiative, aimed at compiling a phylogenetic 'tree of life' (Hajibabaei *et al*., 2007).

While species genetic identification was in alignment with morphology for the Antarctic and sub-Antarctic species studied here, phylogenetic clade arrangement is counterintuitive to traditional taxonomic assessments of phylogeny (outlined in Cairns, 1983; Cairns, 2011). The only other genetic phylogeny of stylasterids available for comparison is that of Lindner *et al*., (2008), and their phylogenetic arrangement also supports a reassignment of synapomophies in stylasterids. This conclusion is based on the basal placement of genera containing cyclosystems (e.g., *Conopora*), the derived placement of species with gastrostyles (e.g., *Stellapora*), and the concurrent divergence of genera with both gastrosyles and cyclosystems (e.g., *Stylaster* and *Stellapora*) in both phylogenies (Chapter 3, Lindner *et al.*, 2008). This indicates that the MRCA to stylasteridae was a cyclosystemate species, and the fossil record supports this conclusion (Jell *et al*., 2011; Lindner *et al*, 2008). Therefore, it is recommended based on these genetic data sets, that the cyclosystem be attributed as a basal character state, and the gastrostyle be attributed as a more derived character in future phylogenetic and morphological assessments.

6.3 Stylasterid Evolution in the Drake Passage

Mitochondrial phylogenetic data combined with the fossil record (~ 65 – 50 MYA) revealed that stylasterids radiated within the Drake Passage following the Eocene/Oligocene boundary ~34 MYA (Chapter 4). This time period is linked to a rapid de-acidification of the

world's oceans (Merico *et al*., 2008), and a shift from calcite to aragonite sea conditions (Stanley & Hardie, 1998). Therefore, stylasterid speciation may be linked to changes in ocean chemistry affecting carbonate mineralogy. In context, this may inform future research on Antarctic corals and ocean acidification.

Most stylasterids have an aragonitic skeletal composition, and this is likely to be the ancestral character state in these corals. However, the two most derived genera, *Errinopsis* and *Cheiloporidion*, have calcite skeletons which likely evolved during the Eocene (< 34 MYA) when ocean chemistry favoured calcite. Some modern populations of *Errinopsis* and *Cheiloporidion* contain aragonite, suggesting a return to the ancestral aragonite mineralogy in response to oceanic conditions (the favourable calcification state of modern oceans). This result suggests at least some capacity for stylasterids to adapt to changing ocean chemistry, and further research in this area may help understand their ability to buffer impending ocean acidification, as the chemical state of the Southern Ocean shifts rapidly from aragonite to calcite sea conditions (IPCC, 2013).

6.4 Estimates of Genetic Isolation among Stylasterid Populations

Intra-specific genetic relationships among geographically isolated populations of stylasterids in the Drake Passage demonstrate limited to no dispersal across the Polar Front between Antarctica and South America across spatial scales > 500 km (Chapter 5). However, where this finding of genetic structure is evident in CO1 estimates at all spatial scales > 300 km and in all species studied, genetic structure is not apparent within some 16S comparisons. This discordance in connectivity estimates is attributed to the conserved nature of the 16S gene region in corals (Miller *et al*., 2010), and invertebrates generally (Wilson *et al*., 2009). Geneflow estimates from 16S may be explained by heightened historic geneflow linked to the ACC south of the Polar Front (e.g., *E. fenestrata*), or evolutionary linkages which are no longer present such as dispersal prior to the Last Glacial Maximum (LGM) (~ 23 - 19 thousand years ago, e.g., Margolin *et al*., 2014). Therefore, apparent genetic similarity between stylasterid populations based on 16S may be due to the retention of ancestral haplotypes (Wilson *et al*., 2009; Thornhill *et al*., 2008; Arango *et al*., 2011). At the same time, CO1 haplotypes confirm a present day pattern of isolation among populations at the spatial scales tested.

The conclusion of ancestral genetic linkages in stylasterid populations is further substantiated in ITS data from *Errina* species in East Antarctica. Despite estimates of gene flow that were not significantly different from panmixia, haplotype networks illustrated a phylogenetic configuration representing population expansion from a common ancestor (basal haplotype) and subsequent local adaptation and divergence (as in Arango *et al*., 2011) (Chapter 5). The localised abundance of *Errina* species in the Dumont d'Urville Sea (Post *et al*., 2010) and the disjunct species distributions common of circum-Antarctic stylasterid populations (Cairns, 1983; Bax & Cairns, 2014) fit with this scenario. Furthermore, during benthic sampling expeditions, faunal assemblages were patchily distributed (Bax & Cairns, 2014; Waller *et al*., 2011). This could reflect isolation and a subsequent pattern of radiation into different habitats.

The interpretation of genetic data to infer radiation from founder populations is supported within the limited stylasterid literature available for comparison. Miller *et al*., (2004) provide evidence of low genetic diversity in New Zealand fiord *Errina* spp., and relate their findings to a recent colonisation of the fiord system, ~ 18,000 years ago (Pickrill *et al*., 1992; Smith, 2001). In this manner, *Errina* fiord populations represent satellite populations from deeper waters, and Cairns (1991) outline a similar hypothesis describing New Zealand as the centre of diversity for the *Errina* genus, and subsequent radiation into surrounding waters. Based on this hypothesis, it is also likely that the shallow water *Errina antarctica* coral populations characteristic of the Patagonian fiord system (Häussermann & Försterra, 2007), are similarly derived from deep water relatives that colonised the fiords upon glacial retreat (Fillinger, 2013). This is substantiated by the presence of *E. antarctica* in deeper waters in the Sub-Antarctic (Cairns, 1983; Bax & Cairns, 2014). Furthermore, the Lindner *et al*., (2008) study which describes stylasterid colonisation from deep-sea ancestors into shallower habitats on at least three consecutive occasions over the last ~ 65 MYA, solidifies this scenario as the most likely evolutionary trajectory for *Errina* corals, if not all stylasterid coral populations. To what extent these evolutionary connections are maintained remains to be tested. However, in the context of conservation value, stylasterid corals rank as a high priority (Post *et al*., 2010; CCAMLR, 2008 - 2012), therefore in regards to effective management - evidence of limited to no gene-flow between modern stylasterid populations (Chapter 5), dictates that the maintenance of historic linkages between deep and shallow waters is likely minimal.

6.5 Future Research Priority - Conservation and Molecular Biology

The level of genetic exchange between stylasterid coral populations within (local scale connectivity) and between (large scale connectivity) regions based on 16S and ITS revealed only historic links, whilst the variability of CO1 proved sufficient to determine genetic structure in modern Drake Passage populations across spatial scales > 300 km (Chapter 5). However, due to limited DNA amplification success, fine scale estimates of gene flow using CO1 were not possible, and at spatial scales of 10 – 73 km gene flow estimates were not significantly different from panmixia (F_{ST} = 0.15, P = 0.65). To best inform conservation strategies it is recommended that future studies focus on fine scale connectivity estimates and incorporate microsatellite or Next Generation Sequencing (NGS) techniques to identify informative loci for population comparison. More variable markers have been used to differentiate genetic structure across smaller spatial scales in Antarctic invertebrates (< 10s of km e.g., Baird *et al*., 2012), and deep-sea corals (> 35 km e.g., Dahl *et al*., 2012). Therefore, the technology is available, and not only does such research directly link to conservation goals, it will also benefit the broader research community and fits well within the goals of the BOLD project (http://www.barcodeoflife.org/): to increase our available knowledge on a unique, ecologically important and rarely studied groups of corals.

The fact that high mitochondrial genetic variability was identified here (Chapter 3 & 5), compared to other deep-sea corals (Miller *et al*., 2010), provides a good basis for additional research. Single-Nucleotide Polymorphisms (SNPs) analysis using techniques such as restriction-site-associated DNA (RAD) could complement existing CO1 and 16S markers and increase resolution in connectivity studies on stylasterids. However, NGS relies upon high quality DNA (Shendure & Ji, 2008). The low sequencing yield found here (Chapters 3 - 5) and the variability in specimen quality and preservation in Antarctic collections may limit the potential for such techniques at this stage. Furthermore, large sample sizes are hindered by the expense and inaccessibility of the Antarctic deep-sea (Chapter 5, Appendix Table 3.2). This will severely limit statistical rigor, and spatial replication in microsatellite and SNP studies.

Microsatellite population genetic studies rely upon sample replication with, ideally, >25 - 30 individuals per site to accurately estimate allele frequencies (Hale *et al*., 2012). Only two Antarctic studies, Baird *et al*., (2012) and Leese *et al*., (2010), have been able to acquire high

enough replication across sites to conduct statically informative microsatellite studies on Antarctic invertebrates to date. Both these studies were on abundant crustaceans, which are easily identifiable as a single animal, in waters from 10 m in Baird *et al*., (2012), and down to 200 m in Leese *et al*., (2010) (Appendix Table 3.2). It is unlikely that studies on Antarctic stylasterid corals can replicate these types of sampling protocols, due to: 1) a predominantly deep habitat (> 500 m), 2) a skewed population demography (dissimilar species compositions across neighbouring sample sites) (Bax & Cairns, 2014) and 3) the only collection methods available are destructive and non-selective practices, such as trawling and fisheries by-catch (Parker & Bowden, 2009). These methods break coral skeletons and often make it impossible to determine a single individual of the same species from the multiple branch fragments in collections (pers. obs.). Therefore, without substantial research investment, population genetic studies using SNPs and microsatellite data would be best focused on the fiord populations of *Errina* spp., corals which are accessible by SCUBA, in New Zealand (Miller *et al*., 2004), and Chile (Häussermann & Försterra, 2007). These *Errina* spp., populations share affinities with deep-sea populations in Antarctica (Cairns, 1983), and allow for selective replication across fiord sites at ~10 – 30 m (Häussermann & Försterra, 2007, Chapter 5) (Fig 1).

6.6 Links between Habitat, Ecology and Field-like Aggregations of *Errina* spp., and their Implications for Stylasterid Conservation

The biogeographic assessment of stylasterid coral fields defined two regions with the highest stylasterid aggregations (Bax & Cairns, 2014) the East Antarctic VME in the Dumont d'Urville sea (Post *et al*., 2010); and 2) the Chilean fiord populations of *E. antarctica* (Häussermann & Försterra, 2007; Fillinger, 2013). When these two ecosystems are compared, similarity in habitat preference is apparent (Fig 1). The Ross and Dumont d'Urville Sea regions of high *Errina* spp. field-like aggregation are associated with bottom currents in regions of strong upwelling (Post *et al.*, 2010; Barnes pers. com.). In Patagonia, the highest abundance of *Errina antarctica* was found along vertical walls, in strong nutrient rich currents where there is ample rocky substrate for larval attachment (pers. obs., Fillinger, 2013). For comparison, *Errina novaezelandiae* and *Errina dendyi* are abundant constituents on steep rock walls throughout Fiordland in New Zealand and in areas of increased nutrient rich current (Miller, pers. com.; Wing & Jack, 2014), and low sedimentation (Grange, pers. com.). This suggests that suitable habitat and nutrient availability are essential to stylasterid

colonisation success in the Southern Ocean, Patagonia and New Zealand. These are the only Southern Hemisphere *Errina* spp. aggregations where any information is available, and rocky substrate for attachment is a key limiting feature in all three locations. This is in agreement with Cairns' (1992; 2011) reviews of stylasterid distributions world-wide. Therefore, until this hypothesis of habitat preference can be more rigorously tested, conservation resources would be best focused on the preservation of habitat which meets these criteria.

To provide a framework to inform conservation, Miller *et al*., (2004) conducted the only ecological study available for comparison in a regionally accessible environment. Their study on the New Zealand *Errina* spp. populations found evidence of vulnerable life history characteristics such as slow growth, longevity and a pattern of natal recruitment within genetically isolated populations, and evidence of inbreeding within a 0.93 sq. km marine reserve. These characteristics make *Errina* spp. corals particularly susceptible to disturbance, stress and colony damage. Once a population is depleted it will be slow to recover due to limited external recruitment, low genetic diversity leading to decreased fitness, and an inability to re-establish population density through local reproduction. If the same pattern holds true in an Antarctic context, as it likely does, *Errina* spp. are especially vulnerable to the synergistic effects of disturbance from increasing ice-berg scour (Post *et al*., 2010), climate change (Barnes & Souster, 2011), stress from chemical changes in their environment linked to ocean acidification (Guinotte *et al*., 2006; Chapter 4), and colony damage in the face of destructive fishing practices such as long lining and trawling (Parker *et al*., 2009).

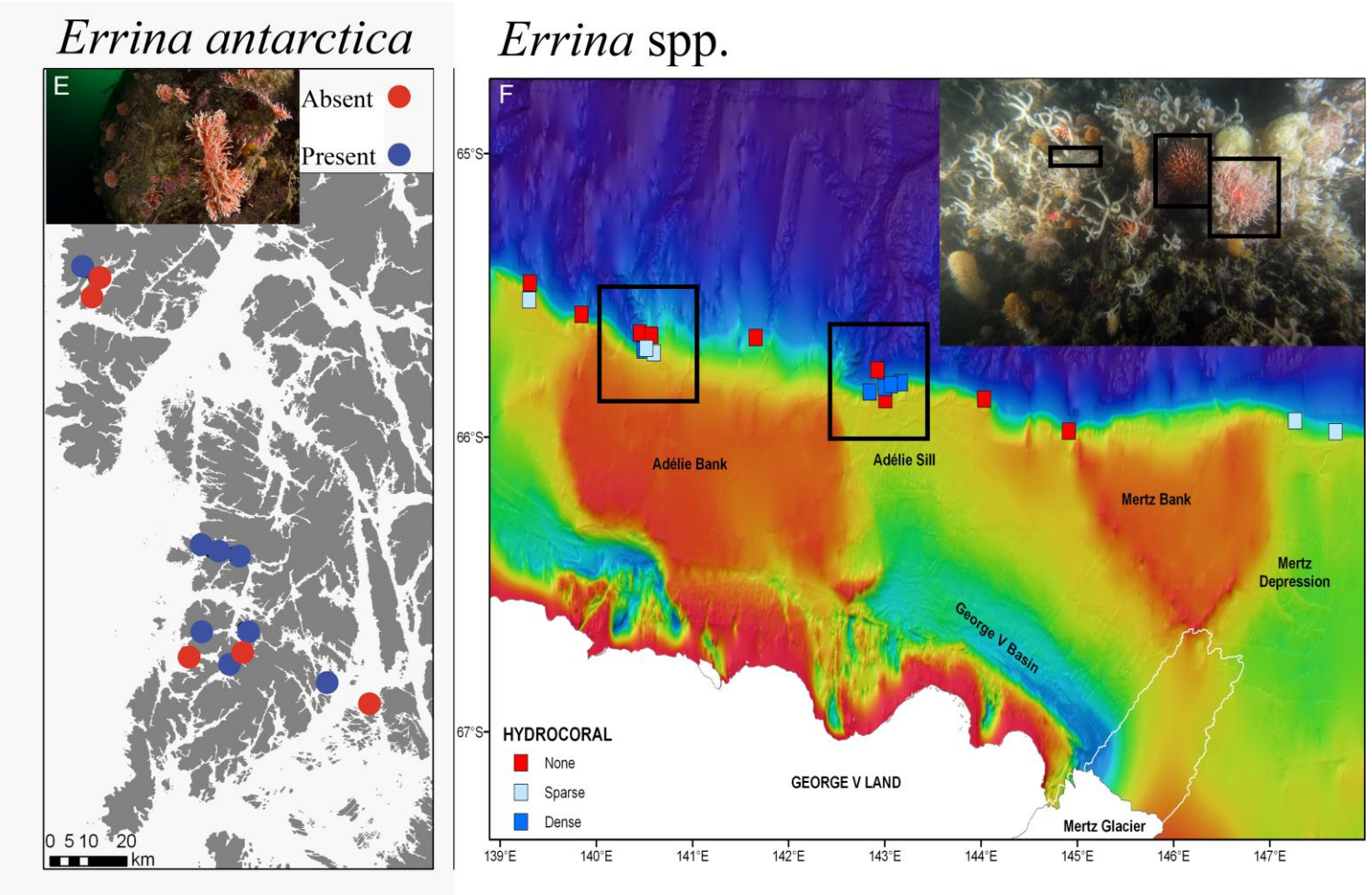

Figure 1. Distribution and presence (blue) or absence (red) of *Errina* spp. coral fields in the Patagonian fiords (left), and the Dumont d'Urville Sea (right). *Errina antarctica* is found in field-like abundance in patchily distributed populations in the Chilean fiord region of Patagonia (Figure edited from Fillinger 2013). *Errina gracilis/Inferiolabiata labiata*, *Errina fissurata*, *Errina laterorifa* (pictured in boxes from left to right) are found in field-like abundance in the Dumont d'Urville Sea, East Antarctica (Bax & Cairns, 2014). This region is listed as a VME, under CCAMLR (CCAMLR, 2009a). Image AAD ©.

6.7 Future Research Priority - Climate Change and Ecosystem Preservation

Climate change research is imperative in our current era of dramatic environmental degradation (IPCC, 2013). In deep benthic ecosystems this research centres upon the response of corals to ocean acidification (Turley *et al*., 2007; Thresher *et al*., 2011; Fillinger & Richter, 2013). The pattern of bi-minerality of field-forming genera of *Errina*, *Errinopsis* and *Cheiloporidion* is intriguing, and worthy of further investigation (Chapter 4). This would require the incorporation of habitat-specific data sets linking chemical regimes to calcite and aragonite production, a task beyond the scope of this thesis. Future studies investigating adaptations in *Errina-Errinopsis-Cheiloporidion* would benefit from the incorporation of other deep-sea coral groups to aid in conservation efforts and gauge responses to decreasing saturation states. In particular, the gorgonian corals for which a substantial literature is now available (McFadden *et al*., 2010; Thresher *et al*., 2010; Watling *et al*., 2011) and the colonial scleractinian corals, such as *Solenosmilia* and *Madrepora* (Miller *et al*., 2010; Williams *et al*., 2010). In combination with stylasterid corals, these coral taxa form a dominant component of VMEs throughout the Antarctic and Sub-Antarctic (CCAMLR, 2009c), and have been shown to have differential carbonate mineralogy (respectively as follows: calcite/aragonite Thresher *et al*., 2011; aragonite Margolin *et al*., 2014; calcite/aragonite Cairns & Macintyre, 1992). Therefore, they may be differentially affected by ocean acidification (see Thresher *et al*., 2011).

6.8 Marine Protected Areas (MPAs) in Antarctica

The Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR), the entity managing Antarctic VMEs, recently considered a proposal to establish the world's largest MPA, prohibiting fishing within a 1.25 million sq. km reserve in the Ross Sea and a 1.0 million sq. km reserve in East Antarctica (Fig 2), including the Dumont d'Urville *Errina* spp. VME (Fig 1). Unfortunately, this proposal was rejected due to a lack of consensus among delegate nations (Teschke *et al*., 2014). The proposal will hopefully be reconsidered again in 2015 (AAD, 2014), and all available evidence indicates the proposed MPA is vital to the protection of the Dumont d'Urville VME (Chapters 1 - 5).

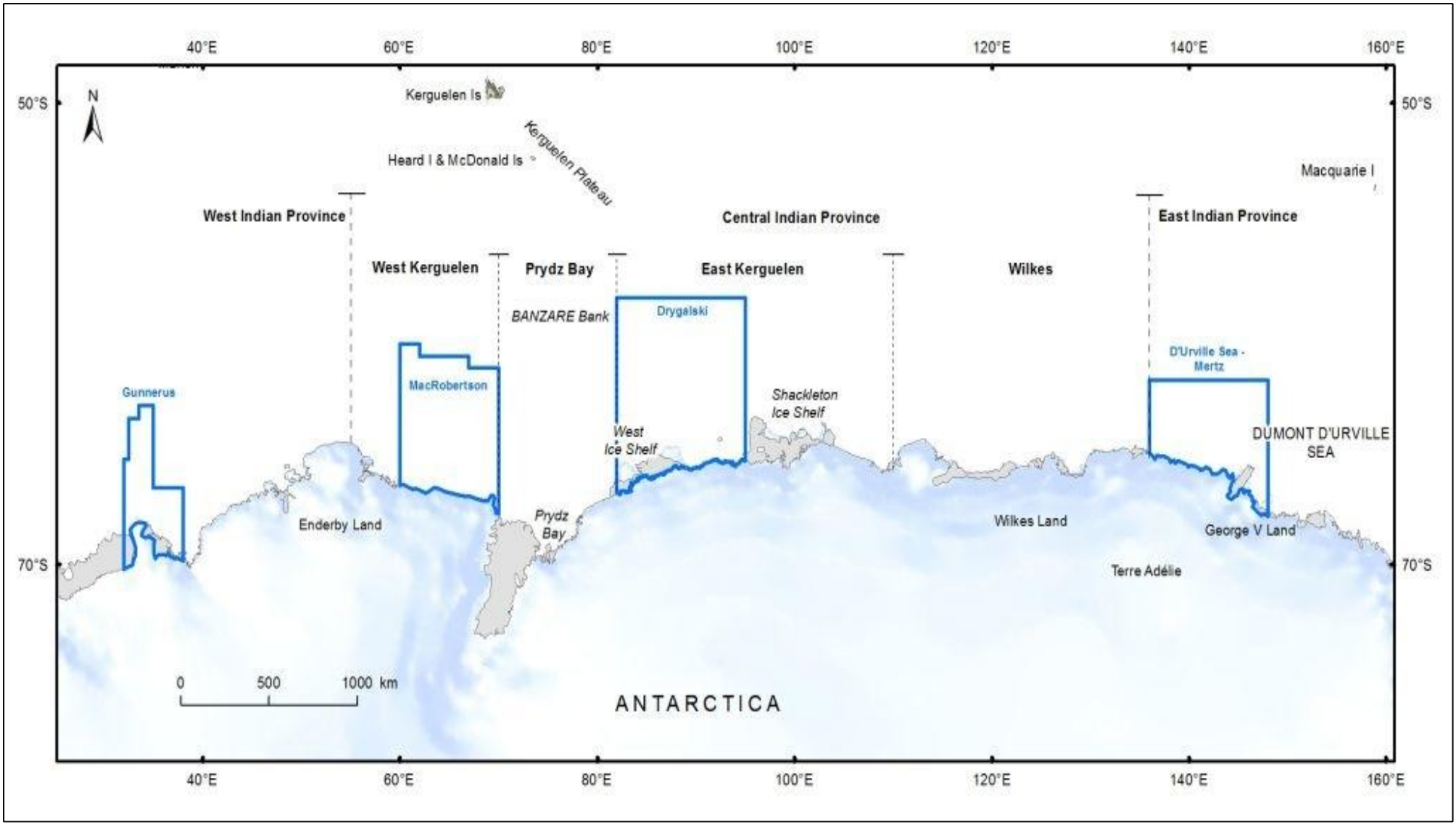

Figure 2. Map of the proposed CCAMLR MPA in East Antarctica. Image Justin Chambers AAD ©.

The results presented in this thesis provide support, and highlight the need for, the proposed East Antarctic MPA which would encompass continued priority listing of *Errina* spp. corals as VME taxa, and the elimination of destructive fisheries in habitat where stylasterids are prevalent. The vulnerability and need for conservation in Antarctic stylasterids is substantiated by data on the vulnerably of stylasterids generally (Stratford, 2002; Miller *et al*., 2004; Häussermann & Försterra, 2007; 2014), a lack of ongoing gene-flow between populations in Antarctica (Chapter 5), a skewed and isolated population demography (Chapter 2), and a high susceptibility to climate change (Chapter 4). Whilst most of the inferences in this thesis have focused on *Errina* spp., due to the comparative availability of information and samples within this genus, and the very little information beyond morphological data that is available for any other Antarctic stylasterid genera (Cairns, 1983). It is highly likely that all stylasterid corals are vulnerable based on shared life history characteristics (Chapter 1 - 6), hence it is imperative to conservation goals that the VME taxon listing is maintained for all stylasterid corals (CCAMLR, 2009c), to provide a frame work through which these ecosystems are given conservation significance and protection from anthropogenic threats.

Conclusion

This study has provided some evidence that skeletal minerology in stylasterids is linked with changes in ocean chemistry, and in this context, stylasterids may be able to adapt to a changing ocean. However, phylogenetic data (Chapter 4) combined with gene-flow estimates (Chapter 5) infer that a species' ability to adapt or disperse to new benthic habitats is limited. Stylasterids are not rapid and successful primary colonisers, the opposite – they are sessile with fragile skeletons (Cairns, 2011). Genetic sub-division among populations suggests that stylasterids are isolated and largely self-recruiting (Chapter 5). The same pattern is evident in more accessible fiord corals (Miller *et al*., 2004). The study by Häussermann & Försterra (2007) which documented fields of isolated *E. antarctica* (Fig 1) and their subsequent complete eradication within one fiord in a 6 - 7 year time frame (Häussermann & Försterra, 2014), vividly illustrates that even if adaptation to climate change is possible (Chapter 4), it may not occur rapidly enough to combat the multiple threats to coral populations in modern oceans (Chapter 1). Stylasterid life history characteristics (e.g., long lived, slow growing, late to reproduce Miller *et al*., 2004; Stratford, 2002) dictate that

localised impacts such as destructive fisheries, pose a direct and immediate threat to population persistence. Therefore, habitat protection is vital to the continued study, and long term survival of Antarctic, Sub-Antarctic and Patagonian coral ecosystems.

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Appendix

Table 1. Sample information for chapter 3: identification number, morphological species identification, gene region (CO1,16S, ITS), geographic location, latitude, longitude, depth and voyage recorded for all study specimens.

ID	Species	Gene region	Location	Geographic region	Latitude	Longitude	Depth (m)	Voyage	Notes
1	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.09	176.18	760	NIWA Mfish	
13	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.05	176.39	707	NIWA Mfish	
19	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.01	176.45	917	NIWA Mfish	
7	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.01	176.45	917	NIWA Mfish	
21	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.09	176.18	760	NIWA Mfish	
15	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.05	176.26	1054	NIWA Mfish	
8	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-74.41	177.04	958	NIWA Mfish	
2	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.05	176.39	707	NIWA Mfish	
10	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.05	176.39	707	NIWA Mfish	
4	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.05	176.26	1054	NIWA Mfish	
3	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.09	176.18	760	NIWA Mfish	
1C	*Cheiloporidion pulvinatum*	CO1	Burdwood Bank	South America	-54.71	-62.19	660	NBP-11-03	
15C	*Cheiloporidion pulvinatum*	CO1	Burdwood Bank	South America	-54.73	-62.22	804	NBP-08-05	
12C	*Cheiloporidion pulvinatum*	CO1	Cape Horn	South America	-57.32	-66.85	938	NBP-11-03	
19B	*Conopora verrucosa*	CO1	Cape Horn	Drake Passage	-57.21	-66.98	904	NBP-11-03	
20B	*Conopora verrucosa*	CO1	Cape Horn	Drake Passage	-57.17	-67.11	1059	NBP-11-03	
3B	*Conopora verrucosa*	CO1	Shackleton Fracture Zone	Drake Passage	-60.22	-57.69	909	NBP-11-03	
9B	*Conopora verrucosa*	CO1	Shackleton Fracture Zone	Drake Passage	-60.18	-57.85	1038	NBP-11-03	
11A	*Cheiloporidion pulvinatum*	CO1	Sars Seamount	Drake Passage	-59.72	-68.76	817	NBP-11-03	
10A	*Cheiloporidion pulvinatum*	CO1	Sars Seamount	Drake Passage	-59.72	-68.76	817	NBP-11-03	
6A	*Errinopsis fenestrata*	CO1	Interim Seamount	Drake Passage	-60.57	-65.98	950	NBP-11-03	
8A	*Errinopsis fenestrata*	CO1	Sars Seamount	Drake Passage	-59.72	-68.76	817	NBP-11-03	
14A	*Errinopsis fenestrata*	CO1	Interim Seamount	Drake Passage	-60.58	-65.99	884	NBP-08-05	
7A	*Errinopsis fenestrata*	CO1	Interim Seamount	Drake Passage	-60.56	-65.97	793	NBP-11-03	

ID	Species	Gene region	Location	Geographic region	Latitude	Longitude	Depth (m)	Voyage	Notes
19A	*Errinopsis fenestrata*	CO1	Interim Seamount	Drake Passage	-60.56	-65.97	896	NBP-08-05	
9A	*Errinopsis fenestrata*	CO1	Sars Seamount	Drake Passage	-59.72	-68.76	817	NBP-11-03	
13C	*Errinopsis reticulum*	CO1	Burdwood Bank	South America	-54.73	-62.22	804	NBP-08-05	*cf C. pulvinatum*
18A	*Errinopsis reticulum*	CO1	Burdwood Bank	South America	-54.73	-62.22	804	NBP-08-05	
16A	*Errinopsis reticulum*	CO1	Burdwood Bank	South America	-54.52	-62.23	331	NBP-11-03	
1A	*Errinopsis reticulum*	CO1	Burdwood Bank	South America	-54.46	-62.21	319	NBP-11-03	
4A	*Errinopsis reticulum*	CO1	Burdwood Bank	South America	-54.52	-62.23	331	NBP-11-03	
17B	*Stylaster densicaulis*	CO1	Cape Horn	South America	-57.21	-66.98	904	NBP-11-03	
5B	*Stylaster densicaulis*	CO1	Burdwood Bank	South America	-54.84	-62.13	1930	NBP-11-03	
2B	*Stylaster densicaulis*	CO1	Burdwood Bank	South America	-54.81	-62.17	1538	NBP-11-03	
1B	*Stylaster densicaulis*	CO1	Burdwood Bank	South America	-54.71	-62.19	660	NBP-11-03	
22B	*Stylaster densicaulis*	CO1	Cape Horn	South America	-57.36	-66.69	1420	NBP-11-03	
27B	*Stylaster densicaulis*	CO1	Burdwood Bank	South America	-54.52	-62.23	331	NBP-11-03	
17C	*Stellapora echinata*	CO1	Burdwood Bank	South America	-54.73	-62.22	804	NBP-08-05	
18C	*Stellapora echinata*	CO1	Burdwood Bank	South America	-54.73	-62.22	804	NBP-08-05	
12B	*Conopora verrucosa*	CO1	Shackleton Fracture Zone	Drake Passage	-60.28	-57.53	1597	NBP-11-03	
11B	*Conopora verrucosa*	CO1	Shackleton Fracture Zone	Drake Passage	-60.26	-57.60	1083	NBP-11-03	
13B	*Conopora verrucosa*	CO1	Site AA	Antarctic Peninsula	-63.08	-61.64	642	NBP-11-03	
6B	*Conopora verrucosa*	CO1	Shackleton Fracture Zone	Drake Passage	-60.14	-57.99	1213	NBP-11-03	
16B	*Conopora verrucosa*	CO1	Cape Horn	South America	-57.19	-67.01	1257	NBP-11-03	
4C	*Sporadopora dichotoma*	16S	Cape Horn	South America	-57.19	-67.01	1257	NBP-11-03	
5CA	*Sporadopora dichotoma*	CO1	Cape Horn	South America	-54.78	-62.23	904	NBP-11-03	
9C	*Stellapora echinata*	16S	Cape Horn	South America	-57.31	-66.86	740	NBP-11-03	
23C	*Stellapora echinata*	16S	Interim Seamount	South America	-60.56	-65.97	896	NBP-08-05	
11A	*Cheiloporidion pulvinatum*	16S	Sars Seamount	Drake Passage	-59.72	-68.76	817	NBP-11-03	
10A	*Cheiloporidion pulvinatum*	16S	Sars Seamount	Drake Passage	-59.72	-68.76	817	NBP-11-03	
5A	*Cheiloporidion pulvinatum*	16S	Burdwood Bank	South America	-54.81	-62.17	1538	NBP-11-03	
217	*Cheiloporidion pulvinatum*	16S	South West Atlantic	South America	-47.19	-59.76	934	PATA1008	

ID	Species	Gene region	Location	Geographic region	Latitude	Longitude	Depth (m)	Voyage	Notes
226	*Cheiloporidion pulvinatum*	16S	South West Atlantic	South America	-43.07	-58.74	1529	PATA0209	
212	*Cheiloporidion pulvinatum*	16S	South West Atlantic	South America	-42.01	-57.57	485	PATA0210	
15C	*Cheiloporidion pulvinatum*	16S	Burdwood Bank	South America	-54.73	-62.22	804	NBP-08-05	
1C	*Cheiloporidion pulvinatum*	16S	Burdwood Bank	South America	-54.71	-62.19	660	NBP-11-03	
209	*Cheiloporidion pulvinatum*	16S	South West Atlantic	South America	-42.07	-57.44	1048	PATA0210	
208	*Cheiloporidion pulvinatum*	16S	South West Atlantic	South America	-42.12	-57.50	1090	PATA0210	
12C	*Cheiloporidion pulvinatum*	16S	Cape Horn	Drake Passage	-57.32	-66.85	938	NBP-11-03	
18A	*Errinopsis reticulum*	16S	Burdwood Bank	South America	-54.44	-62.24	306	NBP-08-05	
16A	*Errinopsis reticulum*	16S	Burdwood Bank	South America	-54.52	-62.23	331	NBP-11-03	
1A	*Errinopsis reticulum*	16S	Burdwood Bank	South America	-54.46	-62.21	319	NBP-11-03	
4A	*Errinopsis reticulum*	16S	Burdwood Bank	South America	-54.52	-62.23	331	NBP-11-03	
15A	*Errinopsis fenestrata*	16S	Sars Seamount	Drake Passage	-59.72	-68.73	914	NBP-08-05	
19A	*Errinopsis fenestrata*	16S	Interim Seamount	Drake Passage	-60.56	-65.97	896	NBP-08-05	
7A	*Errinopsis fenestrata*	16S	Interim Seamount	Drake Passage	-60.56	-65.97	793	NBP-11-03	
8A	*Errinopsis fenestrata*	16S	Sars Seamount	Drake Passage	-59.72	-68.76	817	NBP-11-03	
9A	*Errinopsis fenestrata*	16S	Sars Seamount	Drake Passage	-59.72	-68.76	817	NBP-11-03	
6A	*Errinopsis fenestrata*	16S	Interim Seamount	Drake Passage	-60.57	-65.98	950	NBP-11-03	
37B	*Errina laterorifa*	16S	Burdwood Bank	South America	-54.41	-60.54	151	NBP-11-05	
38B	*Errina laterorifa*	16S	Burdwood Bank	South America	-54.41	-60.54	151	NBP-11-05	
40B	*Errina laterorifa*	16S	Burdwood Bank	South America	-54.41	-60.54	151	NBP-11-05	
120	*Errina laterorifa*	16S	Bransfield Strait	Antarctic Peninsula	-63.26	-59.90	296	NBP-11-05	
4	*Errina gracilis*	16S	Herdman Bank	Scotia Arc	-59.90	-32.44	521	NBP-11-05	
5	*Errina gracilis*	16S	Herdman Bank	Scotia Arc	-59.90	-32.44	521	NBP-11-05	
6	*Errina gracilis*	16S	Herdman Bank	Scotia Arc	-59.90	-32.44	521	NBP-11-05	
122	*Errina antactica*	16S	Burdwood Bank	South America	-54.68	-60.93	163	NBP-11-05	
222B	*Errina antarctica*	16S	South West Atlantic	South America	-41.59	-57.58	435	PATA0210	
16B	*Conopora verrucosa*	16S	Cape Horn	South America	-57.19	-67.01	1257	NBP-11-03	
18B	*Stylaster densicaulis*	16S	Cape Horn	South America	-54.73	-62.22	804	NBP-08-05	

ID	Species	Gene region	Location	Geographic region	Latitude	Longitude	Depth (m)	Voyage	Notes
27B	*Stylaster densicaulis*	16S	Burdwood Bank	South America	-54.52	-62.23	331	NBP-11-03	
237B	*Stylaster densicaulis*	16S	South West Atlantic	South America	-44.32	-59.37	1478	PAT0209	
1B	*Stylaster densicaulis*	16S	Burdwood Bank	South America	-54.71	-62.19	660	NBP-11-03	
17B	*Stylaster densicaulis*	16S	Cape Horn	South America	-57.21	-66.98	904	NBP-11-03	
32B	*Stylaster densicaulis*	16S	Interim Seamount	Drake Passage	-60.56	-65.96	1008	NBP-11-03	
23B	*Stylaster densicaulis*	16S	Cape Horn	South America	-57.18	-66.51	740	NBP-11-03	
2B	*Stylaster densicaulis*	16S	Burdwood Bank	South America	-54.81	-62.17	1538	NBP-11-03	
5B	*Stylaster densicaulis*	16S	Burdwood Bank	South America	-54.84	-62.13	1930	NBP-11-03	
30B	*Stylaster densicaulis*	16S	Interim Seamount	Drake Passage	-60.56	-65.96	1008	NBP-11-03	
22B	*Stylaster densicaulis*	16S	Cape Horn	Drake Passage	-57.36	-66.69	1420	NBP-11-03	
25B	*Stylaster densicaulis*	16S	Cape Horn	Drake Passage	-57.28	-67.24	1870	NBP-11-03	
2	*Inferiolabiata labiata*	16S	Discovery Bank	Scotia Arc	-60.12	-34.97	452	NBP-11-05	
7	*Inferiolabiata labiata*	16S	South Orkney Islands	Scotia Arc	-60.55	-45.37	125	NBP-11-05	
16	*Inferiolabiata labiata*	16S	Discovery Bank	Scotia Arc	-60.12	-34.97	452	NBP-11-05	
7B	*Inferiolabiata labiata*	16S	South Orkney Islands	Scotia Arc	-60.55	-45.37	125	NBP-11-05	
60B	*Inferiolabiata labiata*	16S	Discovery Bank	Scotia Arc	-65.87	-89.29	403.6	BR09	
10B	*Conopora verrucosa*	16S	Shackleton Fracture Zone	Drake Passage	-60.15	-57.93	1230	NBP-11-03	
16B	*Conopora verrucosa*	16S	Cape Horn	South America	-57.19	-67.01	1257	NBP-11-03	
13B	*Conopora verrucosa*	16S	Site AA	Antarctic Peninsula	-63.08	-61.64	642	NBP-11-03	
3B	*Conopora verrucosa*	16S	Shackleton Fracture Zone	Drake Passage	-60.22	-57.69	909	NBP-11-03	
9B	*Conopora verrucosa*	16S	Shackleton Fracture Zone	Drake Passage	-60.18	-57.85	1038	NBP-11-03	
6B	*Conopora verrucosa*	16S	Shackleton Fracture Zone	Drake Passage	-60.28	-57.53	1597	NBP-11-03	
11B	*Conopora verrucosa*	16S	Shackleton Fracture Zone	Drake Passage	-44.87	-59.64	1248	PAT1108	
12B	*Conopora verrucosa*	16S	Shackleton Fracture Zone	Drake Passage	-60.26	-57.60	1083	NBP-11-03	
7B	*Conopora verrucosa*	16S	Shackleton Fracture Zone	Drake Passage	-44.87	-59.64	1248	PAT1108	
19B	*Conopora verrucosa*	16S	Cape Horn	South America	-57.21	-66.98	904	NBP-11-03	
20B	*Conopora verrucosa*	16S	Cape Horn	South America	-57.17	-67.11	1059	NBP-11-03	
235	*Conopora verrucosa*	16S	South West Atlantic	South America	-54.71	-62.19	660	PATA	

ID	Species	Gene region	Location	Geographic region	Latitude	Longitude	Depth (m)	Voyage	Notes
235	*Conopora verrucosa*	16S	South West Atlantic	South America	-44.87	-59.64	1248	PATA0209	
4	*Sporadopora dichotoma*	16S	Cape Horn	South America	-57.19	-67.01	1257	NBP-11-03	
8C	*Sporadopora dichotoma*	16S	Cape Horn	South America	-57.21	-66.98	904	NBP-11-03	
5CO	*Sporadopora dichotoma*	16S	Cape Horn	South America	-57.21	-66.98	904	NBP-11-03	
17C	*Stellapora echinata*	16S	Burdwood Bank	South America	-54.73	-62.22	804	NBP-08-05	
18C	*Stellapora echinata*	16S	Burdwood Bank	South America	-54.73	-62.22	804	NBP-08-05	
305	*Errina antarctica*	ITS-2	Canal Copihue	Chilean Patagonia	-50.20	-75. 22	15	Explorador II	
312	*Errina antarctica*	ITS-2	Canal Copihue	Chilean Patagonia	-50.20	-75.22	15	Explorador II	
314	*Errina antarctica*	ITS-2	Grupo Dacres	Chilean Patagonia	-51.36	-73.55	20	Explorador II	
316	*Errina antarctica*	ITS-2	Canal Copihue	Chilean Patagonia	-50.20	-75.22	15	Explorador II	
328	*Errina antarctica*	ITS	Isla Solar	Chilean Patagonia	-50.98	-74.95	25	Explorador II	
309	*Errina antarctica*	ITS-2	Canal Copihue	Chilean Patagonia	-50.20	-75.22	15	Explorador II	
310	*Errina antarctica*	ITS-2	Canal Copihue	Chilean Patagonia	-50.20	-75.22	15	Explorador II	
352	*Errina c.f gracilis n.sp*	ITS	Shackleton Glacier	East Antarctica	-64.27	97.08	1000.3	BR09	
39	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-54.69	-60.85	151	NBP-11-05	
104	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-71.98	172.17	737	TAN0402	
3	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.09	176.18	760	NIWA Mfish	
111	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.82	142.95	775	CEAMARC	
112	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.82	142.95	775	CEAMARC	
51	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-72.11	172.69	495	TAN0402	
113	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.82	142.95	775	CEAMARC	
43-B	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-71.99	172.20	312	TAN0402	
4	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.82	142.95	775	CEAMARC	
52	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-72.11	172.69	495	TAN0402	
53	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-72.11	172.60	495	TAN0402	
100	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-71.98	172.17	737	TAN0402	
48	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-72.06	172.90	526	TAN0402	
116	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.82	142.95	775	CEAMARC	

ID	Species	Gene region	Location	Geographic region	Latitude	Longitude	Depth (m)	Voyage	Notes
54	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-72.11	172.60	495	TAN0402	
117	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.82	142.95	775	CEAMARC	
58	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-71.99	172.20	675	TAN0402	
66	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-72.11	172.69	495	TAN0402	
69	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-71.97	171.96	480	TAN0402	
119	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.80	142.95	775	CEAMARC	
120	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.80	142.90	775	CEAMARC	
81	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-72.00	172.22	536	TAN0402	
73	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-72.00	172.22	536	TAN0402	
65	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-72.11	172.69	495	TAN0402	
129	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.80	142.90	775	CEAMARC	
2	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.82	142.95	775	CEAMARC	
5	*Errinopsis reticulum*	ITS	Burdwood Bank	East Antarctica	-54.81	-62.17	1538	NBP-11-03	*cf C. pulvinatum*
83	*Errina laterorifa*	ITS	Ross Sea	East Antarctica	-72.00	172.22	536	TAN0402	
85	*Errina laterorifa*	ITS	Ross Sea	East Antarctica	-72.00	172.22	536	TAN0402	
107	*Errina laterorifa*	ITS	Ross Sea	East Antarctica	-71.98	172.17	737	TAN0402	
106	*Errina laterorifa*	ITS	Ross Sea	East Antarctica	-71.98	172.17	737	TAN0402	
102	*Errina laterorifa*	ITS	Ross Sea	East Antarctica	-71.98	172.17	737	TAN0402	
86	*Errina laterorifa*	ITS	Ross Sea	East Antarctica	-65.40	160.88	103	TAN0402	
82-D	*Errina laterorifa*	ITS	Ross Sea	East Antarctica	-72.00	172.22	536	TAN0402	
7	*Errinopsis fenestrata*	ITS	Interim Seamount	Drake Passage	-60.56	-65.97	793	NBP-11-03	
6	*Errinopsis fenestrata*	ITS	Interim Seamount	Drake Passage	-60.57	-65.98	950	NBP-11-03	
16C	*Stylaster densicaulis*	16S	Burdwood Bank	South America	-57.36	-66.69	1420	NBP-11-03	
203	*Conopora verrucosa*	16S	South West Atlantic	South America	-44.87	-59.64	1248	PATA1108	

Table 2. Sample information for chapter 4: identification number, morphological species identification, gene region (CO1,16S), geographic location, latitude, longitude, depth and voyage recorded for all study specimens.

ID	Species	Location	Latitude	Longitude	Depth range (m)	Cruise
3	*Cheiloporidion pulvinatum*	Burdwood Bank	-54.90	-62.14	2215-2343	NBP0805
35	*Cheiloporidion pulvinatum*	Burdwood Bank	-54.72	-62.24	720-736	NBP1103
36	*Cheiloporidion pulvinatum*	Burdwood Bank	-54.72	-62.24	720-736	NBP1103
105	*Cheiloporidion pulvinatum*	Burdwood Bank	-54.47	-62.20	310-320	NBP1103
109	*Cheiloporidion pulvinatum*	Burdwood Bank	-54.51	-62.23	323-334	NBP1103
9	*Cheiloporidion pulvinatum*	Cape Horn	-54.73	-62.26	804-828	NBP0805
80	*Cheiloporidion pulvinatum*	Cape Horn	-57.16	-67.09	931-937	NBP1103
75	*Cheiloporidion pulvinatum*	Sars Seamount	-59.70	-69.01	550-650	NBP1103
84	*Conopora verricusa*	Cape Horn	-57.17	-67.11	975-1049	NBP1103
44	*Conopora verricusa*	Shackleton Fracture Zone	-60.18	-57.85	717-822	NBP1103
46	*Conopora verricusa*	Shackleton Fracture Zone	-60.18	-57.83	778-834	NBP1103
52	*Conopora verricusa*	Shackleton Fracture Zone	-60.18	-57.85	952-1045	NBP1103
103	*Errinopsis fenestrata*	Burdwood Bank	-54.90	-62.14	2215-2343	NBP0805
112	*Errinopsis fenestrata*	Burdwood Bank	-54.84	-62.13	1835-1922	NBP1103
120	*Errinopsis fenestrata*	Interim Seamount	-60.57	-65.98	1246-1326	NBP1103
121	*Errinopsis fenestrata*	Interim Seamount	-60.57	-65.98	1246-1326	NBP1103
123	*Errinopsis fenestrata*	Sars Seamount	-59.70	-69.01	550-650	NBP1103
124	*Errinopsis fenestrata*	Sars Seamount	-59.70	-69.01	550-650	NBP1103
125	*Errinopsis fenestrata*	Sars Seamount	-59.70	-69.01	550-650	NBP1103
126	*Errinopsis fenestrata*	Sars Seamount	-59.70	-69.01	550-650	NBP1103
127	*Errinopsis fenestrata*	Sars Seamount	-59.70	-69.01	550-650	NBP1103
128	*Errinopsis fenestrata*	Sars Seamount	-59.70	-69.01	550-650	NBP1103
10	*Errinopsis reticulum*	Burdwood Bank	-54.73	-62.26	804-828	NBP0805
11	*Errinopsis reticulum*	Burdwood Bank	-54.73	-62.26	804-828	NBP0805

ID	Species	Location	Latitude	Longitude	Depth range (m)	Cruise
102	*Errinopsis reticulum*	Burdwood Bank	-54.47	-62.19	312-314	NBP0805
106	*Errinopsis reticulum*	Burdwood Bank	-54.51	-62.23	323-334	NBP1103
108	*Errinopsis reticulum*	Burdwood Bank	-54.51	-62.23	323-334	NBP1103
4	*Sporadopora dichotoma*	Burdwood Bank	-54.90	-62.14	2215-2343	NBP0805
5	*Sporadopora dichotoma*	Burdwood Bank	-54.90	-62.14	2215-2343	NBP0805
17	*Sporadopora dichotoma*	Burdwood Bank	-54.51	-62.23	323-334	NBP1103
26	*Sporadopora dichotoma*	Burdwood Bank	-54.72	-62.24	720-736	NBP1103
27	*Sporadopora dichotoma*	Burdwood Bank	-54.72	-62.24	720-736	NBP1103
30	*Sporadopora dichotoma*	Burdwood Bank	-54.72	-62.24	720-736	NBP1103
33	*Sporadopora dichotoma*	Burdwood Bank	-54.72	-62.24	720-736	NBP1103
38	*Sporadopora dichotoma*	Burdwood Bank	-54.83	-62.10	1749-1918	NBP1103
77	*Sporadopora dichotoma*	Cape Horn	-57.16	-67.09	931-937	NBP1103
81	*Sporadopora dichotoma*	Cape Horn	-57.19	-67.01	1188-1257	NBP1103
89	*Sporadopora dichotoma*	Cape Horn	-57.28	-67.24	1869-1877	NBP1103
91	*Sporadopora dichotoma*	Cape Horn	-57.01	-67.57	447-689	NBP1103
79	*Stellopora echinata*	Cape Horn	-57.16	-67.09	931-937	NBP1103
85	*Stellopora echinata*	Cape Horn	-57.36	-66.68	1388-1494	NBP1103
92	*Stellopora echinata*	Cape Horn	-57.01	-67.57	447-689	NBP1103
12	*Stylaster densicaulis*	Burdwood Bank	-54.73	-62.26	804-828	NBP0805
13	*Stylaster densicaulis*	Burdwood Bank	-54.73	-62.26	804-828	NBP0805
37	*Stylaster densicaulis*	Burdwood Bank	-54.72	-62.24	720-736	NBP1103
82	*Stylaster densicaulis*	Cape Horn	-57.17	-67.11	975-1049	NBP1103
83	*Stylaster densicaulis*	Cape Horn	-57.17	-67.11	975-1049	NBP1103
90	*Stylaster densicaulis*	Cape Horn	-57.01	-67.57	447-689	NBP1103
59	*Stylaster densicaulis*	Interim Seamount	-60.56	-65.96	957-1007	NBP1103
60	*Stylaster densicaulis*	Interim Seamount	-60.56	-65.96	957-1007	NBP1103
40	*Stylaster densicaulis*	Shackleton Fracture Zone	-60.17	-57.55	868-888	NBP1103

ID	Species	Location	Latitude	Longitude	Depth range (m)	Cruise
42	*Stylaster densicaulis*	Shackleton Fracture Zone	-60.18	-57.85	717-822	NBP1103
43	*Stylaster densicaulis*	Shackleton Fracture Zone	-60.18	-57.85	717-822	NBP1103
47	*Stylaster densicaulis*	Shackleton Fracture Zone	-60.18	-57.83	778-834	NBP1103
50	*Stylaster densicaulis*	Shackleton Fracture Zone	-60.18	-57.83	790-856	NBP1103
53	*Stylaster densicaulis*	Shackleton Fracture Zone	-60.22	-57.69	979-1081	NBP1103
54	*Stylaster densicaulis*	Shackleton Fracture Zone	-60.26	-57.60	1223-1300	NBP1103

Table 3. Sample information for chapter 5: identification number, morphological species identification, gene region (CO1,16S, ITS), geographic location, latitude, longitude, depth and voyage recorded for all study specimens.

ID	Species	Gene region	Location	Geographic region	Latitude	Longitude	Depth (m)	Voyage
212	*Cheiloporidion pulvinatum*	16S	South West Atlantic	South America	-42.01	-57.57	485	PATA0210
152	*Cheiloporidion pulvinatum*	16S	South West Atlantic	South America	-46.24	-59.47	787	ATL10
217B	*Cheiloporidion pulvinatum*	16S	South West Atlantic	South America	-47.19	-59.76	934	PATA1008
209	*Cheiloporidion pulvinatum*	16S	South West Atlantic	South America	-42.07	-57.44	1048	PATA0210
208	*Cheiloporidion pulvinatum*	16S	South West Atlantic	South America	-42.12	-57.50	1090	PATA0210
226	*Cheiloporidion pulvinatum*	16S	South West Atlantic	South America	-43.07	-58.74	1529	PATA0209
5A	*Cheiloporidion pulvinatum*	16S	Burdwood Bank	South America	-54.81	-62.17	1538	NBP-11-03
1C	*Cheiloporidion pulvinatum*	16S	Burdwood Bank	South America	-54.71	-62.19	660	NBP-11-03
1C	*Cheiloporidion pulvinatum*	CO1	Burdwood Bank	South America	-54.71	-62.19	660	NBP-11-03
15C	*Cheiloporidion pulvinatum*	16S	Burdwood Bank	South America	-54.73	-62.22	804	NBP-08-05
13C	*Cheiloporidion pulvinatum*	16S	Burdwood Bank	South America	-54.73	-62.22	804	NBP-08-05
13C (1)	*Cheiloporidion pulvinatum*	CO1	Burdwood Bank	South America	-54.73	-62.22	804	NBP-08-05
13C (2)	*Cheiloporidion pulvinatum*	CO1	Burdwood Bank	South America	-54.73	-62.22	804	NBP-08-05
15C	*Cheiloporidion pulvinatum*	CO1	Burdwood Bank	South America	-54.73	-62.22	804	NBP-08-05
11A	*Cheiloporidion pulvinatum*	16S	Sars Seamount	Drake Passage	-59.72	-68.76	817	NBP-11-03
10A	*Cheiloporidion pulvinatum*	16S	Sars Seamount	Drake Passage	-59.72	-68.76	817	NBP-11-03
7C	*Cheiloporidion pulvinatum*	16S	Cape Horn	South America	-57.21	-66.98	904	NBP-11-03
7C	*Cheiloporidion pulvinatum*	CO1	Cape Horn	South America	-57.21	-66.98	904	NBP-11-03
12C	*Cheiloporidion pulvinatum*	16S	Cape Horn	South America	-57.32	-66.85	938	NBP-11-03
12C	*Cheiloporidion pulvinatum*	CO1	Cape Horn	South America	-57.32	-66.85	938	NBP-11-03
19B	*Conopora verrucosa*	CO1	Cape Horn	South America	-57.21	-66.98	904	NBP-11-03
3B	*Conopora verrucosa*	CO1	Shackleton Fracture Zone	Drake Passage	-60.22	-57.69	909	NBP-11-03
6B	*Conopora verrucosa*	16S	Shackleton Fracture Zone	Drake Passage	-60.14	-57.99	1213	NBP-11-03
7B	*Conopora verrucosa*	16S	Shackleton Fracture Zone	Drake Passage	-60.15	-57.93	1230	NBP-11-03
235	*Conopora verrucosa*	16S	South West Atlantic	South America	-44.87	-59.64	1248	PATA0209
203	*Conopora verrucosa*	16S	South West Atlantic	South America	-44.87	-59.64	1248	PATA1108

ID	Species	Gene region	Location	Geographic region	Latitude	Longitude	Depth (m)	Voyage
10B	*Conopora verrucosa*	16S	Shackleton Fracture Zone	Drake Passage	-60.12	-57.43	1425	NBP-11-03
3B	*Conopora verrucosa*	16S	Shackleton Fracture Zone	Drake Passage	-60.13	-57.41	909	NBP-11-03
9B	*Conopora verrucosa*	16S	Shackleton Fracture Zone	Drake Passage	-60.18	-57.85	1038	NBP-11-03
9B	*Conopora verrucosa*	CO1	Shackleton Fracture Zone	Drake Passage	-60.18	-57.85	1038	NBP-11-03
20B	*Conopora verrucosa*	16S	Cape Horn	South America	-57.17	-67.11	1059	NBP-11-03
20B	*Conopora verrucosa*	CO1	Cape Horn	South America	-57.17	-67.11	1059	NBP-11-03
11B	*Conopora verrucosa*	16S	Shackleton Fracture Zone	Drake Passage	-60.26	-57.60	1083	NBP-11-03
11B	*Conopora verrucosa*	CO1	Shackleton Fracture Zone	Drake Passage	-60.26	-57.60	1083	NBP-11-03
6B	*Conopora verrucosa*	CO1	Shackleton Fracture Zone	Drake Passage	-60.14	-57.99	1213	NBP-11-03
16B	*Conopora verrucosa*	16S	Cape Horn	South America	-57.19	-67.01	1257	NBP-11-03
16B	*Conopora verrucosa*	CO1	Cape Horn	South America	-57.19	-67.01	1257	NBP-11-03
12B	*Conopora verrucosa*	16S	Shackleton Fracture Zone	Drake Passage	-60.28	-57.53	1597	NBP-11-03
12B	*Conopora verrucosa*	CO1	Shackleton Fracture Zone	Drake Passage	-60.28	-57.53	1597	NBP-11-03
312	*Errina antarctica*	ITS-2	Canal Copihue	Chilean Patagonia	-50.20	-75. 22	15	Explorador II
307	*Errina antarctica*	ITS-2	Canal Copihue	Chilean Patagonia	-50.20	-75. 22	15	Explorador II
299	*Errina antarctica*	ITS-2	Canal Copihue	Chilean Patagonia	-50.20	-75. 22	15	Explorador II
303	*Errina antarctica*	ITS-2	Canal Copihue	Chilean Patagonia	-50.20	-75. 22	15	Explorador II
305	*Errina antarctica*	ITS-2	Canal Copihue	Chilean Patagonia	-50.20	-75. 22	15	Explorador II
309	*Errina antarctica*	ITS-2	Canal Copihue	Chilean Patagonia	-50.20	-75. 22	15	Explorador II
310	*Errina antarctica*	ITS-2	Canal Copihue	Chilean Patagonia	-50.20	-75. 22	15	Explorador II
316	*Errina antarctica*	ITS-2	Canal Copihue	Chilean Patagonia	-50.20	-75. 22	15	Explorador II
308	*Errina antarctica*	ITS-2	Canal Copihue	Chilean Patagonia	-50.20	-75. 22	15	Explorador II
320	*Errina antarctica*	ITS-2	Grupo Dacres	Chilean Patagonia	-51.36	-73. 55	20	Explorador II
322	*Errina antarctica*	ITS-2	Grupo Dacres	Chilean Patagonia	-51.36	-73. 55	20	Explorador II
319	*Errina antarctica*	ITS-2	Grupo Dacres	Chilean Patagonia	-51.36	-73. 55	20	Explorador II
318	*Errina antarctica*	ITS-2	Grupo Dacres	Chilean Patagonia	-51.36	-73. 55	20	Explorador II
315	*Errina antarctica*	ITS-2	Grupo Dacres	Chilean Patagonia	-51.36	-73. 55	20	Explorador II
314	*Errina antarctica*	ITS-2	Grupo Dacres	Chilean Patagonia	-51.36	-73. 55	20	Explorador II
326	*Errina antarctica*	ITS-2	Grupo Dacres	Chilean Patagonia	-51.36	-73. 55	20	Explorador II

ID	Species	Gene region	Location	Geographic region	Latitude	Longitude	Depth (m)	Voyage
14A	*Errinopsis fenestrata*	CO1	Interim Seamount	Drake Passage	-60.58	-65.99	884	NBP-08-05
15A	*Errinopsis fenestrata*	16S	Sars Seamount	Drake Passage	-59.72	-68.73	914	NBP-08-05
7A	*Errinopsis fenestrata*	16S	Interim Seamount	Drake Passage	-60.56	-65.97	793	NBP-11-03
7A	*Errinopsis fenestrata*	CO1	Interim Seamount	Drake Passage	-60.56	-65.97	793	NBP-11-03
9A	*Errinopsis fenestrata*	16S	Sars Seamount	Drake Passage	-59.72	-68.76	817	NBP-11-03
8A	*Errinopsis fenestrata*	16S	Sars Seamount	Drake Passage	-59.72	-68.76	817	NBP-11-03
9A	*Errinopsis fenestrata*	CO1	Sars Seamount	Drake Passage	-59.72	-68.76	817	NBP-11-03
8A	*Errinopsis fenestrata*	CO1	Sars Seamount	Drake Passage	-59.72	-68.76	817	NBP-11-03
19A	*Errinopsis fenestrata*	16S	Interim Seamount	Drake Passage	-60.56	-65.97	896	NBP-08-05
19A	*Errinopsis fenestrata*	CO1	Interim Seamount	Drake Passage	-60.56	-65.97	896	NBP-08-05
6A	*Errinopsis fenestrata*	16S	Interim Seamount	Drake Passage	-60.57	-65.98	950	NBP-11-03
6A	*Errinopsis fenestrata*	CO1	Interim Seamount	Drake Passage	-60.57	-65.98	950	NBP-11-03
97-C	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-71.29	170. 57	170	TAN0402
43-B	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-71.99	172. 20	312	TAN0402
69-C	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-71.97	171. 96	480	TAN0402
65-C	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-72.11	172. 69	495	TAN0402
66-B	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-72.11	172. 69	495	TAN0402
51-B	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-72.11	172. 69	495	TAN0402
52-C	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-72.11	172. 69	495	TAN0402
53-D	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-72.11	172. 6	495	TAN0402
54-E	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-72.11	172. 6	495	TAN0402
3	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-65.70	140. 59	500	CEAMARC
48-A	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-72.06	172. 90	526	TAN0402
49-B	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-72.06	172. 90	526	TAN0402
73-E	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-72.00	172. 22	536	TAN0402
81-E	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-72.00	172. 22	536	TAN0402
68	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-72.32	170. 42	536	TAN0402
58	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-71.99	172. 20	675	TAN0402
13	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.05	176.39	707	NIWA Mfish

ID	Species	Gene region	Location	Geographic region	Latitude	Longitude	Depth (m)	Voyage
2	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.05	176.39	707	NIWA Mfish
10	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.05	176.39	707	NIWA Mfish
91-A	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-71.98	172. 17	737	TAN0402
100-E	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-71. 98	172. 17	737	TAN0402
101-F	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-71.98	172. 17	737	TAN0402
104-I	*Errina fissurata*	ITS	Ross Sea	East Antarctica	-71.98	172. 17	737	TAN0402
1	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.09	176.18	760	NIWA Mfish
21	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.09	176.18	760	NIWA Mfish
3	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.09	176.18	760	NIWA Mfish
2	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.82	142. 95	775	CEAMARC
4	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.82	142. 95	775	CEAMARC
111-A	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.82	142. 95	775	CEAMARC
113-C	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.82	142. 95	775	CEAMARC
116-F	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.82	142. 95	775	CEAMARC
117-G	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.82	142. 95	775	CEAMARC
119-I	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.80	142. 95	775	CEAMARC
120-J	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.80	142. 9	775	CEAMARC
127-Q	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.80	142. 9	775	CEAMARC
128-R	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.80	142. 9	775	CEAMARC
129-S	*Errina fissurata*	ITS	Dumont d'Urville	East Antarctica	-68.80	142. 9	775	CEAMARC
19	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.01	176.45	917	NIWA Mfish
7	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.01	176.45	917	NIWA Mfish
8	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-74.41	177.04	958	NIWA Mfish
15	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.05	176.26	1054	NIWA Mfish
4	*Errina fissurata*	CO1	Ross Sea	East Antarctica	-75.05	176.26	1054	NIWA Mfish
86	*Errina laterorifa*	ITS	Ross Sea	East Antarctica	-65.40	160. 88	103	TAN0402
37B	*Errina laterorifa*	16S	Burdwood Bank	South America	-54.41	-60. 54	151	NBP-11-05
38B	*Errina laterorifa*	16S	Burdwood Bank	South America	-54.41	-60. 54	151	NBP-11-05
40B	*Errina laterorifa*	16S	Burdwood Bank	South America	-54.41	-60. 54	151	NBP-11-05

ID	Species	Gene region	Location	Geographic region	Latitude	Longitude	Depth (m)	Voyage
136	*Errina laterorifa*	16S	Bransfield Strait	Antarctic Peninsula	-63.26	-59.90	296	NBP-11-05
155	*Errina laterorifa*	16S	Bransfield Strait	Antarctic Peninsula	-63.26	-59.90	296	NBP-11-05
42-A	*Errina laterorifa*	ITS	Ross Sea	East Antarctica	-71.99	172. 20	312	TAN0402
260	*Errina laterorifa*	16S	Ross Sea	East Antarctica	-71.73	171. 75	451	TAN0402
82-D	*Errina laterorifa*	ITS	Ross Sea	East Antarctica	-72.00	172. 22	536	TAN0402
83-C	*Errina laterorifa*	ITS	Ross Sea	East Antarctica	-72.00	172. 22	536	TAN0402
85-A	*Errina laterorifa*	ITS	Ross Sea	East Antarctica	-72.00	172. 22	536	TAN0402
102-G	*Errina laterorifa*	ITS	Ross Sea	East Antarctica	-71.98	172. 17	737	TAN0402
106-K	*Errina laterorifa*	ITS	Ross Sea	East Antarctica	-71.98	172.17	737	TAN0402
107-L	*Errina laterorifa*	ITS	Ross Sea	East Antarctica	-71.98	172.17	737	TAN0402
101-F	*Errina laterorifa*	ITS	Ross Sea	East Antarctica	-71.98	172.17	737	TAN0402
103-H	*Errina laterorifa*	ITS	Ross Sea	East Antarctica	-71.98	172.17	737	TAN0402
23B	*Stylaster densicaulis*	16S	Cape Horn	South America	-57.18	-66.51	740	NBP-11-03
16C	*Stylaster densicaulis*	16S	Burdwood Bank	South America	-54.73	-62.22	804	NBP-08-05
18B	*Stylaster densicaulis*	16S	Cape Horn	South America	-57.21	-66.98	904	NBP-11-03
15B	*Stylaster densicaulis*	CO1	Cape Horn	South America	-57.09	-67.05	931	NBP-11-03
24B	*Stylaster densicaulis*	CO1	Cape Horn	South America	-57.32	-66.85	938	NBP-11-03
30B	*Stylaster densicaulis*	16S	Interim Seamount	Drake Passage	-60.56	-65.96	1008	NBP-11-03
32B	*Stylaster densicaulis*	16S	Interim Seamount	Drake Passage	-60.56	-65.96	1008	NBP-11-03
14B	*Stylaster densicaulis*	CO1	Interim Seamount	Drake Passage	-60.64	-66.04	1513	NBP-11-03
25B	*Stylaster densicaulis*	16S	Cape Horn	South America	-57.28	-67.24	1870	NBP-11-03
27B	*Stylaster densicaulis*	16S	Burdwood Bank	South America	-54.52	-62.23	331	NBP-11-03
27B	*Stylaster densicaulis*	CO1	Burdwood Bank	South America	-54.52	-62.23	331	NBP-11-03
1B	*Stylaster densicaulis*	16S	Burdwood Bank	South America	-54.71	-62.19	660	NBP-11-03
1B	*Stylaster densicaulis*	CO1	Burdwood Bank	South America	-54.71	-62.19	660	NBP-11-03
17B	*Stylaster densicaulis*	16S	Cape Horn	South America	-57.21	-66.98	904	NBP-11-03
17B	*Stylaster densicaulis*	CO1	Cape Horn	South America	-57.21	-66.98	904	NBP-11-03
22B	*Stylaster densicaulis*	16S	Cape Horn	South America	-57.36	-66.69	1420	NBP-11-03
22B	*Stylaster densicaulis*	CO1	Cape Horn	South America	-57.36	-66.69	1420	NBP-11-03

ID	Species	Gene region	Location	Geographic region	Latitude	Longitude	Depth (m)	Voyage
2B	*Stylaster densicaulis*	16S	Burdwood Bank	South America	-54.81	-62.17	1538	NBP-11-03
2B	*Stylaster densicaulis*	CO1	Burdwood Bank	South America	-54.81	-62.17	1538	NBP-11-03
5B	*Stylaster densicaulis*	16S	Burdwood Bank	South America	-54.84	-62.13	1930	NBP-11-03
5B	*Stylaster densicaulis*	CO1	Burdwood Bank	South America	-54.84	-62.13	1930	NBP-11-03

Table 3.1. Sample information for chapter 5: identification number, morphological species identification, gene region (ITS), geographic location, Station, latitude, longitude, depth and voyage recorded for all study specimens.

ID	Species	Gene region	Location	Latitude	Longitude	Depth (m)	Station	Voyage
1	*Errina fissurata*	CO1	Ross Sea	-75.09	176.18	760	23	NIWA Mfish
21	*Errina fissurata*	CO1	Ross Sea	-75.09	176.18	760	23	NIWA Mfish
3	*Errina fissurata*	CO1	Ross Sea	-75.09	176.18	760	23	NIWA Mfish
15	*Errina fissurata*	CO1	Ross Sea	-75.05	176.26	1054	24	NIWA Mfish
4	*Errina fissurata*	CO1	Ross Sea	-75.05	176.26	1054	24	NIWA Mfish
13	*Errina fissurata*	CO1	Ross Sea	-75.05	176.39	707	27	NIWA Mfish
2	*Errina fissurata*	CO1	Ross Sea	-75.05	176.39	707	27	NIWA Mfish
10	*Errina fissurata*	CO1	Ross Sea	-75.05	176.39	707	27	NIWA Mfish
19	*Errina fissurata*	CO1	Ross Sea	-75.01	176.45	917	28	NIWA Mfish
7	*Errina fissurata*	CO1	Ross Sea	-75.01	176.45	917	28	NIWA Mfish
8	*Errina fissurata*	CO1	Ross Sea	-74.41	177.04	958	41	NIWA Mfish
2	*Errina fissurata*	ITS	Dumont d'Urville	-68.82	142.95	775	65	CEAMARC
4	*Errina fissurata*	ITS	Dumont d'Urville	-68.82	142.95	775	65	CEAMARC
111-A	*Errina fissurata*	ITS	Dumont d'Urville	-68.82	142.95	775	65	CEAMARC
113-C	*Errina fissurata*	ITS	Dumont d'Urville	-68.82	142.95	775	65	CEAMARC
116-F	*Errina fissurata*	ITS	Dumont d'Urville	-68.82	142.95	775	65	CEAMARC
117-G	*Errina fissurata*	ITS	Dumont d'Urville	-68.82	142.95	775	65	CEAMARC
119-I	*Errina fissurata*	ITS	Dumont d'Urville	-68.82	142.95	775	65	CEAMARC
120-J	*Errina fissurata*	ITS	Dumont d'Urville	-68.82	142.90	775	65	CEAMARC
127-Q	*Errina fissurata*	ITS	Dumont d'Urville	-68.82	142.90	775	65	CEAMARC
128-R	*Errina fissurata*	ITS	Dumont d'Urville	-68.82	142.90	775	65	CEAMARC
129-S	*Errina fissurata*	ITS	Dumont d'Urville	-68.82	142.90	775	65	CEAMARC
65-C	*Errina fissurata*	ITS	Ross Sea	-72.11	172.69	495	78	TAN0402
66-B	*Errina fissurata*	ITS	Ross Sea	-72.11	172.69	495	78	TAN0402
51-B	*Errina fissurata*	ITS	Ross Sea	-72.11	172.69	495	78	TAN0402
52-C	*Errina fissurata*	ITS	Ross Sea	-72.11	172.69	495	78	TAN0402

ID	Species	Gene region	Location	Latitude	Longitude	Depth (m)	Station	Voyage
53-D	*Errina fissurata*	ITS	Ross Sea	-72.11	172.60	495	78	TAN0402
54-E	*Errina fissurata*	ITS	Ross Sea	-72.11	172.60	495	78	TAN0402
3	*Errina fissurata*	ITS	Dumont d'Urville	-65.70	140.59	500	79	CEAMARC
48-A	*Errina fissurata*	ITS	Ross Sea	-72.06	172.90	526	82	TAN0402
49-B	*Errina fissurata*	ITS	Ross Sea	-72.06	172.90	526	82	TAN0402
97-C	*Errina fissurata*	ITS	Ross Sea	-71.29	170.57	170	112	TAN0402
43-B	*Errina fissurata*	ITS	Ross Sea	-71.99	172.20	312	116	TAN0402
42-A	*Errina laterorifa*	ITS	Ross Sea	-71.99	172.20	312	116	TAN0402
69-C	*Errina fissurata*	ITS	Ross Sea	-71.97	171.96	480	150	TAN0402
68	*Errina fissurata*	ITS	Ross Sea	-72.32	170.42	536	154	TAN0402
73-E	*Errina fissurata*	ITS	Ross Sea	-72.00	172.22	536	154	TAN0402
81-E	*Errina fissurata*	ITS	Ross Sea	-72.00	172.22	536	154	TAN0402
82-D	*Errina laterorifa*	ITS	Ross Sea	-72.00	172.22	536	154	TAN0402
83-C	*Errina laterorifa*	ITS	Ross Sea	-72.00	172.22	536	154	TAN0402
85-A	*Errina laterorifa*	ITS	Ross Sea	-72.00	172.22	536	154	TAN0402
58	*Errina fissurata*	ITS	Ross Sea	-71.99	172.20	675	156	TAN0402
91-A	*Errina fissurata*	ITS	Ross Sea	-71.98	172.17	737	157	TAN0402
100-E	*Errina fissurata*	ITS	Ross Sea	-71.98	172.17	737	157	TAN0402
101-F	*Errina fissurata*	ITS	Ross Sea	-71.98	172.17	737	157	TAN0402
104-I	*Errina fissurata*	ITS	Ross Sea	-71.98	172.17	737	157	TAN0402
102-G	*Errina laterorifa*	ITS	Ross Sea	-71.98	172.17	737	157	TAN0402
106-K	*Errina laterorifa*	ITS	Ross Sea	-71.98	172.17	737	157	TAN0402
107-L	*Errina laterorifa*	ITS	Ross Sea	-71.98	172.17	737	157	TAN0402
101-F	*Errina laterorifa*	ITS	Ross Sea	-71.98	172.17	737	157	TAN0402
103-H	*Errina laterorifa*	ITS	Ross Sea	-71.98	172.17	737	157	TAN0402
86	*Errina laterorifa*	ITS	Ross Sea	-65.40	160.88	103	277	TAN0402

Table 3.2. Summary of connectivity research in Antarctic benthic invertebrates tested to date. The majority of genetic connectivity research in Antarctica has focused on a single species, and comparisons across multiple spatial scales are uncommon. This study has comparatively more species, includes multiple spatial scales (20 – 2000km). Most studies to date focus on a broad spatial scale (>1000km). Samples included herein are comparatively deeper, and across a broader depth range (~15-2000m) than the majority of studies to date (predominantly <500m). To illustrate this, studies with more than one species are highlighted in dark grey, low sample sizes (<150 samples) are highlighted in light grey, deeper depths (>500m) and studies incorporating a localised spatial scale (<200km) are in bold. (Edited from Thatje, 2012).

Species	Genetic pattern	Sample Size	Depth (m)	Study area	Spatial Scale (estimated km)	Reproductive mode	Genetic marker	Reference
Chorismus antarcticus (Decapoda)	Panmixia, reduced haplotype diversity, post-glacial expansion	2 - 44 samples per site (178 total)	**166 - 2,134**	Circum-Antarctic (shelf depth)	> 2,000	Planktotrophic larva	COI, 16S 18S, 28S	Raupach *et al*., 2010
Nematocarcinus lanceopes (Decapoda)	Panmixia, high haplotype diversity	2 - 44 samples per site (187 total)	**166 - 2,134**	Circum-Antarctic (deep-sea and deep continental slope)	> 2,000	Planktotophic larva	COI, 16S 18S, 28S	Raupach *et al*., 2010
Lissarca notorcadensis (Bivalvia)	Cryptic speciation	2 - 6 samples per site (58 total)	**231 - 622**	Sub-Antarctic, Weddell Sea, West Antarctic Peninsula, Ross Sea	> 1,000	Brooder	COI, 28S	Linse *et al*., 2007

Species	Genetic pattern	Sample Size	Depth (m)	Study area	Spatial Scale (estimated km)	Reproductive mode	Genetic marker	Reference
Margarella antarctica (Bivalvia)	non-restricted gene flow	43 - 48 samples per site (414 total)	10 - 28	West Antarctic Peninsula	**~ 2 - 15,00**	Brooder	AFLPs	Hoffman *et al.*, 2013
Nacella spp. (7 species) (Gastropoda)	Homogenous species, differentiation at population level	22 - 54 samples per site (208 total)	2 - 25	Patagonia	~ 200 - 1,000	Brooder	CO1	Gonzalez-Wevar *et al.*, 2011
Nacella concinna (Gastropoda)	Homogenous species, differentiation at population level	37 - 48 samples per site (405 total)	10 - 28	Scotia sea islands, West Antarctica Peninsula	**~ 2 - 1,500**	Planktotrophic larva	AFLPs	Hoffman *et al.*, 2011
Doris kerguelenensis (Nudibranchia)	Rapid post-glacial expansion; potentially cryptic species	1 - 25 samples per site (144 total)	24 - 520	Circum-Antarctic	**<50 - 6,200**	Direct developer	COI	Wilson *et al.*, 2009

Species	Genetic pattern	Sample Size	Depth (m)	Study area	Spatial Scale (estimated km)	Reproductive mode	Genetic marker	Reference
Promachocrinus kerguelensis (Crinoidea)	Some haplotype diversity found; intermediate, suggesting limited pelagic dispersal	3 - 16 samples per site (1,888 total)	**116 - 1,170**	West Antarctic Peninsula and Scotia sea	500 - >1,000	Short pelagic larva (?)	COI, CytB	Wilson *et al*., 2007
Promachocrinus kerguelensis (Crinoidea)	Circum-Antarctic; sympatric in seven mitochondrial lineages, restricted gene flow, East Antarctica	17 - 418 samples per site (1,307 total)	**200 - 1,000**	Circum-Antarctic	**~20 - 20,000**	Short pelagic larva (?)	COI, CytB, 16S, 28S, ITS	Hemery *et al*., 2012
Astrotoma agassizii (Ophiuoridea)	Homogenous population at intermediate scale (>500?km)	1 - 18 samples per site (118 total)	**96 - 854**	Drake Passage	**72 - >500**	Brooder, some dispersal potential shown	COII, 16S rRNA	Hunter & Halanych 2008
Astrotoma agassizii (Ophiuroidea)	Evidence for likely cryptic speciation in South America; homogenous populations in Ross Sea	3 samples per site (12 total)	50	Ross Sea	500 - 5,000	Possibly planktonic larva in Antarctica, brooding in South American lineages (?)	COI, 16S	Heimeier *et al*., 2010

Species	Genetic pattern	Sample Size	Depth (m)	Study area	Spatial Scale (estimated km)	Reproductive mode	Genetic marker	Reference
Ophionotus victoriae (Ophiuroidea)	Evidence for cryptic speciation; some genetically homogenous populations	5 - 127 per site (395 total)	**130 - 648**	West Antarctic Peninsula, Southern Ocean Islands	**20 - >1,500**	Planktotrophic larva (short duration?)	COI, 16S	Hunter & Halanych 2010
Odontaster species (Astyeroidea)	Multiple species found, cross ACC distribution in *O. meridionalis*, restricted distribution to either side of Polar Front in other Odontaster species.	1 - 12 per site (105 total)	**116 - 1,170**	West Antarctic Peninsula, sub-Antarctic/Atlantic, Ross Sea	**20 - >1,500**	Pelagic larva	COI, 16S	Janosik *et al.*, 2011
Abatus cordatus (Echinoidea)	Significant differentiation at the population level	41 - 136 per site (374 total)	<10	Kerguelen Islands (endemic)	**25**	Brooder	Microsats, EPIC markers	Ledoux *et al.*, 2012

Species	Genetic pattern	Sample Size	Depth (m)	Study area	Spatial Scale (estimated km)	Reproductive mode	Genetic marker	Reference
Parbolasia corrugatus (Nemertea)	Cryptic speciation (but low diversity; two forms)	20 - 336 per site (1,064 total)	<16	South Orkney Islands; sub-Antarctic and circum-Antarctic	**1 - 15**	Planktotrophic larva	COI	Rogers *et al*., 1998; Thornhill *et al*., 2008
Eusirus perdentatus, *Eusirus giganteus* (Amphipoda)	Highly restricted gene flow, possible cryptic speciation and/or speciation	1 - 18 per site (125 total)	**163 - 698**	Circum-Antarctic	**150 - 5,000**	Brooder	COI, CytB, ITS2	Baird *et al*., 2011
Durvillaea antarctica (*Limnoria stephenseni* and *Parawaldeckia kidderi* Amphipoda)	Single haplotype found	15 - 47 per site (151 total)	~ 1 (kelp collected at surface)	Circum-Antarctic through rafting on kelp	700 - 8,000	Brooder	COI	Nikula *et al*., 2010
Orchomenella franklini (Amphipoda)	High genetic differentiation	14 - 32 per site (718 total)	<10	East Antarctica	**<1 - 1,400**	Brooder	Microsats	Baird *et al*., 2012

Species	Genetic pattern	Sample Size	Depth (m)	Study area	Spatial Scale (estimated km)	Reproductive mode	Genetic marker	Reference
Acanthaspidia drygalskii (Isopoda)	Some evidence for cryptic speciation	2 - 9 per site (17 total)	**668 - 1119**	Circum-Antarctic, also deep-sea	>1,000	Brooder	16S	Raupach & Wägele 2006
Betamorpha fusiformis (Isopoda)	Cryptic speciation	1 - 15 per site (50 total)	**1030 - 4696**	Deep-sea - Weddell Sea up to South Africa	>1,000	Brooder	16S, 18S	Raupach *et al*., 2007
Septemserolis septemcarinata (Isopoda)	Significant genetic differentiation found, but one species still; result of recent expansion or rafting?	5 - 56 samples per site (95 total)	>200	Scotia sea to Bouvet	>1,000	Brooder	COI, Microsats.	Leese *et al*., 2010
Glyptonotus antarcticus (Isopoda)	Cryptic speciation	1 - 6 samples per site (56 total)	**231 - 622**	Circum-Antarctic	>500	Brooder	16S	Held & Wägele 2005

Species	Genetic pattern	Sample Size	Depth (m)	Study area	Spatial Scale (estimated km)	Reproductive mode	Genetic marker	Reference
Ceratoserolis trilobitoides (Isopoda)	Cryptic speciation	1 - 5 samples per site (28 total)	~20 -459	Circum-Antarctic	>500	Brooder	16S Microsat.	Held 2003, Leese &Held 2008
Macroscapha spp. (8 species) (Ostracoda)	Increased number of cryptic and morpho-species	1 - 78 samples per site (219 total)	**84 - 2893**	Circum-Antarctic (Weddell Sea, Ross Sea)	>500	Brooder	COI, ITS	Brandão *et al*., 2010
Nymphon australe (Pycnogonida)	Cryptic speciation	9 - 81 samples per site (131 total)		Circum-Antarctic	**10 - >1,000**	Brooder	COI, 16S	Arango *et al*., 2011
Colossendeis megalonyx (Pycnogonida)	Cryptic speciation	1 - 38 samples per site (96 total)	**75 - 648**	Antarctic and sub-Antarctic	>500	Brooder	COI	Krabbe *et al*., 2009

Species	Genetic pattern	Sample Size	Depth (m)	Study area	Spatial Scale (estimated km)	Reproductive mode	Genetic marker	Reference
Conopora verrucosa (Stylasteridae)	Significant differentiation at the population level	2 - 7 samples per site (20 total)	**660 - 1597**	South West Atlantic and Sub-Antarctic	<500 - 1,000	Brooder	CO1, 16S	This study
Chieloporidion pulvinatum (Stylasteridae)	Significant differentiation at the population level	2 - 6 samples per site (15 total)	**485 - 1529**	South West Atlantic and Sub-Antarctic	<500 - 1,000	Brooder	CO1, 16S	This study
Errina spp. (3 species) (Stylasteridae)	No significant differentiation at the population level, evidence of adaptive radiation from a basal ancestor	1 - 11 samples per site (61 total)	**15 - 1054**	Chilean Patagonia, Ross Sea and Dumont d'Urville Sea, East Antarctica	**<10 -2,000**	Brooder	CO1, ITS	This study
Errinopsis fenestrata (Stylasteridae)	Significant differentiation at the population level	1 - 3 samples per site (12 total)	**793 - 950**	Sub-Antarctic	**200 - 500**	Brooder	CO1, 16S	This study

Stylaster densicaulis (Stylasteridae)	Significant differentiation at the population level	2 - 5 samples per site (21 total)	**331 - 1930**	Sub-Antarctic	**200 - 500**	Brooder	CO1, 16S	This study