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Review and synthesis

Detecting glacial refugia in the Southern Ocean

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Throughout the Quaternary, the continental-based Antarctic ice sheets expanded and contracted repeatedly. Evidence suggests that during glacial maxima, grounded ice eliminated most benthic (bottom-dwelling) fauna across the Antarctic continental shelf. However, paleontological and molecular evidence indicates most extant Antarctica benthic taxa have persisted in situ throughout the Quaternary. Where and how the Antarctic benthic fauna survived throughout repeated glacial maxima remain mostly hypothesised. If understood, this would provide valuable insights into the ecology and evolution of Southern Ocean biota over geological timescales. Here we synthesised and appraised recent studies and presented an approach to demonstrate how genetic data can be effective in identifying where and how Antarctic benthic fauna survived glacial periods. We first examined the geological and ecological evidence for how glacial periods influenced past species demography in order to provide testable frameworks for future studies. We outlined past ice-free areas from Antarctic ice sheet reconstructions that could serve as glacial refugia and discussed how benthic fauna with pelagic or non-pelagic dispersal strategies moved into and out of glacial refugia. We also reviewed current molecular studies and collated proposed locations of Southern Ocean glacial refugia on the continental shelf around Antarctica, in the deep sea, and around sub-Antarctic islands. Interestingly, the proposed glacial refugia based on molecular data generally do not correspond to the ice-free areas identified by Antarctic ice sheet reconstructions. The potential biases in sampling and in the choice of molecular markers in current literature are discussed, along with the future directions for employing testable frameworks and genomic methods in Southern Ocean molecular studies. Continued data syntheses will elucidate greater understanding of where and how Southern Ocean benthic fauna persisted throughout glacial periods and provide insights into their resilience against climate changes in the future.

Keywords: Antarctic, benthic, genetic, glacial cycles, invertebrates, refugia

Introduction

Throughout the Quaternary ice ages, extensive climatic oscillations caused shifts in global species distributions in a cyclical rhythm (Hewitt 1996, 2004, Maggs et al. 2008,

Provan and Bennett 2008). During periods of glaciation, ice sheets expanded over high and mid latitude areas. In the Northern Hemisphere, biogeographic evidence suggests many Arctic and temperate taxa, from both terrestrial and marine environments, migrated to warmer lower latitude areas during glacial cycles, where climatic conditions were similar to that in their previous distributional range (Maggs et al. 2008, Provan and Bennett 2008). In the Southern Hemisphere, the continental-based East, West and Antarctic Peninsula Ice Sheets (collectively forming the Antarctic Ice Sheet (AIS)) also expanded and contracted repeatedly (Ingólfsson 2004). The seafloor of the Antarctic continental shelf was eroded repeatedly by the advance and retreat of grounded ice sheets (i.e. ice sheets resting on the seabed) (Pollard and DeConto 2009). Across the Antarctic continental shelf, these grounded ice advances are hypothesised to have catastrophically eliminated most seafloor habitat and communities (Clarke and Crame 1992, Thatje et al. 2005).

For most benthic fauna that lived on the Southern Ocean continental shelf, migration to lower latitudes during the Quaternary seems improbable (Thatje et al. 2005). The Southern Ocean contains the Antarctic Circumpolar Current (ACC) system with associated fronts and meridional overturning circulations that penetrate into the deep sea (Rintoul et al. 2001). These are hypothesised to have isolated most Southern Ocean fauna from other oceans since the onset of ACC around ~34 million yr ago (Clarke et al. 2004). However, faunal migration to lower latitudes is not impossible (Clarke et al. 2004), and molecular evidence has shown an evolutionary radiation of Southern Ocean octopods into adjacent deep sea basins (Strugnell et al. 2008). But fundamentally, the majority of paleontological and molecular evidence suggests that many extant Antarctic benthic taxa appeared to have evolved, diversified and persisted in situ since the early to middle Eocene (Crame 2018). Therefore, it has been argued that Antarctic benthic fauna likely survived glacial periods on the continental shelf (Convey et al. 2009, 2018), but the exact locations of glacial refugia for marine fauna remain mostly unknown. The benthic fauna makes up ~88% of extant Southern Ocean marine species, and are well-represented by phyla such as Arthropoda, Bryozoa, Cnidaria, Echinodermata, Mollusca, Nematoda and Porifera (De Broyer et al. 2011). These species would have to be able to settle, mature and reproduce in a stable environment in order to persist throughout these periods. Similarly, evidence from the Antarctic terrestrial realm also suggests terrestrial biota persisted in situ over a multimillion year timescale (Convey et al. 2008). Ice free areas sustained by high geothermal activities are suggested to have served as local glacial refugia for some Antarctic terrestrial taxa (Fraser et al. 2014). However, these geothermal refugia appear restricted to the terrestrial realm, as marine geothermal vents tend to support small-scale endemic communities (Rogers et al. 2012, Roterman et al. 2016). If glacial advances were extensive and severe during past glacial maxima, where and how did the Antarctic continental shelf benthic fauna survive?

Understanding how Southern Ocean benthic fauna persisted over multimillion year timescales, despite repeated environmental extremes, could give insights into their evolutionary histories, resilience and vulnerabilities over time. This has been challenging to examine, as biological samples from this remote ecosystem are limited, and for many years they were rarely preserved in a way that could be used for molecular sequencing. However, 'DNA-friendly' samples have become increasingly available following voyages to the Southern Ocean, such as those supporting the Census of Antarctic Marine Life (CAML; 2000–2010) and the International Polar Year (IPY; 2008–2009). Sequencing the 'barcoding gene' (a portion of the mitochondrial cytochrome c oxidase subunit I; COI) from Southern Ocean biota was championed by the Marine Barcode of Life (BOLD) project and CAML (Grant and Linse 2009). These, and similar, co-ordinated sampling and sequencing initiatives have facilitated the description of emerging genetic patterns and evolutionary histories in Southern Ocean species.

This review aims to examine the geological and ecological evidence of how glacial periods influenced past species demography in order to 1) provide testable frameworks to locate glacial refugia for Southern Ocean benthic taxa, 2) examine the molecular signatures of glacial refugia in published studies and 3) highlight future directions for studies that seek to use a genetic approach to detect glacial refugia. To construct testable frameworks, we first identify the potential locations of glacial refugia through past AIS reconstructions. Then, we discuss how benthic fauna with different dispersal strategies could have moved into and out of glacial refugia. In particular, we examine the long-standing hypotheses of whether survival in glacial refugia positively selected non-pelagic development in the Southern Ocean. We also explore how dispersal into and out of glacial refugia might have impacted species demography in the Southern Ocean. The molecular evidence for glacial refugia from extant Southern Ocean taxa is synthesised. We also suggest future directions for critically examining signatures of glacial refugia for Southern Ocean benthic taxa with testable frameworks and genomic methods.

Potential glacial refugia inferred from past Antarctic Ice Sheet reconstructions

Considering all past glacial maxima, the configuration and behaviour of the AIS during the most recent Last Glacial Maximum (LGM; ~20 ka BP) is the most well-understood (Bentley et al. 2014). Since the AIS expanded across almost the entire Antarctic continental shelf during the LGM, glacial refugia for marine benthic fauna on the shelf are often suggested to be highly limited throughout the Quaternary (Thatje et al. 2005). Nonetheless, various locations of glacial refugia for benthic marine fauna have been proposed within and around the Southern Ocean and adjacent regions. These hypothesised glacial refugia include unglaciated areas on the

Antarctic continental shelf and deeper areas near the continental shelf margin, as well as the continental slope, deep sea and adjacent sub-Antarctic islands (Kott 1969, Brey et al. 1996, Crame 1997, Thatje et al. 2005, Convey et al. 2009). The overall locations of glacial refugia were largely proposed based on the understanding of possible ice-free areas on the Antarctic continental shelf during the LGM. However, over repeated glacial–interglacial cycles, refugia on the Southern Ocean continental shelf would establish in ice-free areas during AIS expansions and subsequently dissolve during AIS retreats, when previously glaciated areas become available for colonisation. Throughout the Quaternary, the formation of ice-free refugia with the same size at the same locations on the shelf at each glacial maximum would require glacial periods to exhibit constant, repeated fluctuations. It is known that the total ice volume of the AIS and the magnitude of glacial extent across the continental shelf varied between glacial maxima throughout the different phases of the Quaternary (Pollard and DeConto 2009). So at each glacial maximum, while the size of glacial refugia free from grounded ice was unlikely to be constant, whether glacial refugia were located in the same area on the shelf requires further examination of past AIS morphology in high resolution.

Based on current ice sheet models, the amplitude of Antarctic ice volume changes over glacial–interglacial periods only became highly profound in the past one million years (Pollard and DeConto 2009, de Boer et al. 2014, McKay et al. 2016). Similar fluctuations in Antarctic atmospheric conditions between glacial and interglacial periods were also detected in the last one million years (Jouzel et al. 2007, Elderfield et al. 2012). The AIS configuration at glacial maxima throughout the past one million years may have been similar to the LGM configuration. While it is also generally regarded that the LGM grounded ice was extensive and eroded most of the Southern Ocean continental shelf, reconstruction of the LGM AIS configuration indicates that the grounded ice did not always extend to some parts of the continental shelf break. Although these appear limited to areas along the continental shelf break, areas free from grounded ice present on the outer shelf during the LGM have been detected in the Eastern Weddell Sea, Bellingshausen Sea along Western Antarctic Peninsula, Eastern Ross Sea, between Adélie Land and Bruce Rise in East Antarctica, and Astrid Ridge and Gunnerus Ridge near Dronning Maud Land (Golledge et al. 2012, Bentley et al. 2014) (Fig. 1). Even though the ice-free seabed was likely covered by floating ice shelves on the surface (Denton and Hughes 2002), modern observations of Southern Ocean benthic communities surviving beneath present-day ice shelves suggest faunal survival in these areas during glacial periods was possible (Dayton and Oliver 1977, Bruchhausen et al. 1979, Riddle et al. 2007, Gutt et al. 2011).

Past AIS dynamics also offer valuable insights into whether these suggested ice-free areas were habitable. For example, modelled sediment fluxes during the LGM suggest ice fee areas in the Eastern Ross Sea, Eastern Weddell Sea and Bellingshausen Sea received high cumulative sediment fluxes ($> 1000\,\mathrm{m^3\,yr^{-1}}$ per metre width) through ice streams (Golledge et al. 2013). If glacial refugia did exist in these regions, benthic fauna would likely to have only been able to survive near the shelf break away from the direct sediment flow and/or retreated into deeper areas following the seafloor bathymetry. Ephemeral ice-free areas that provided additional refugia on the shelf are also known to have existed during the LGM, including polynyas, which are open marine habitats within grounded ice driven by katabatic winds (Smith et al. 2010). In addition, the timing and extent of ice sheet advance and retreat were likely to be asynchronous between different regions (Anderson et al. 2002, Hillenbrand et al. 2014, Mackintosh et al. 2014). These variable and dynamic ice-free areas on the continental shelf may have provided refugia for populations of benthic species enabling their survival throughout glacial cycles (Thatje et al. 2005, Allcock and Strugnell 2012).

From the current geological evidence, it is becoming clear that ice-free areas did exist on the Southern Ocean continental shelf even during the extreme phase of glacial period in the Pleistocene (i.e. the LGM). Nonetheless, the spatial coverage of research effort varies across the continent and estimates of the past AIS extent over time remain limited and at coarse resolution (Brook and Buizert 2018). While the potential glacial refugia inferred from past Antarctic Ice Sheet reconstructions identified here provide hypotheses of refugial locations and scenarios, the knowledge identified from biological data can also be transferred vice versa.

Pathways to glacial refugia through larval dispersal

For benthic fauna to effectively retreat into glacial refugia, life history characteristics such as larval dispersal likely played an important role (Poulin et al. 2002, Thatje et al. 2005, Allcock and Strugnell 2012). Feeding (planktotrophic) swimming larvae are normally associated with dispersal capacity over a relatively wide geographical range (Paulay and Meyer 2006). Conversely, non-feeding (lecithotrophic) swimming larvae and non-pelagic development (i.e. benthic, direct developing juveniles) are generally believed to be associated with reduced and limited dispersal ability, respectively (Paulay and Meyer 2006). In the Southern Ocean, it is also believed that glacial refugia on the continental shelf were small, ephemeral in nature with limited primary productivity received (Poulin et al. 2002, Thatje et al. 2005, Convey et al. 2009, Pearse et al. 2009). Therefore, it has been hypothesised that direct development experienced strong positive selection (Poulin et al. 2002, Pearse et al. 2009). This was likely a beneficial strategy for reproducing in low food conditions and may have been driven by allopatric speciation in isolated refugia (Poulin et al. 2002, Pearse et al. 2009)

However, genetic evidence to date indicates survival in Southern Ocean glacial refugia does not seem to be specific to a single mode of dispersal, instead it can be associated with both pelagic and non-pelagic development (discussed within Allcock and Strugnell 2012). Molecular signatures
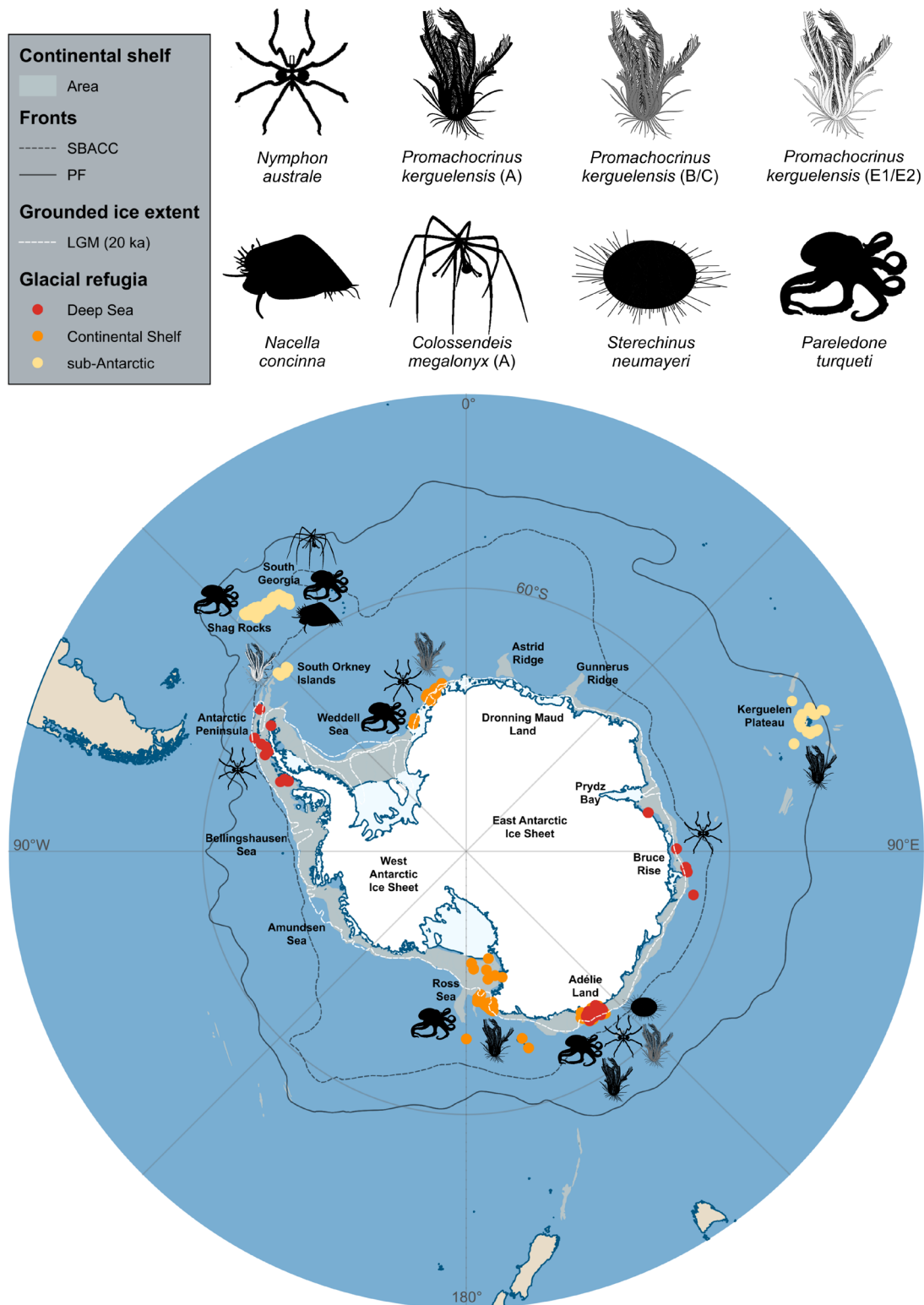
Figure 1. Map of Antarctica and proposed areas of Southern Ocean glacial refugia. Areas of glacial refugia were inferred from the sample locations that were associated with molecular signatures of glacial refugia, with each dot representing a sample location. Each organism on the map reflects the number of times an area was proposed as a refugium for that taxon. Data of grounded ice extent during Last Glacial Maximum (LGM) at 20 ka was extracted from model reconstruction in Bentley et al. (2014). Ice-free areas present on the outer continental shelf during the LGM are observed in the Eastern Weddell Sea, Bellingshausen Sea along Western Antarctic Peninsula, Eastern Ross Sea, between Adélie Land and Bruce Rise in East Antarctica, and Astrid Ridge and Gunnerus Ridge near Dronning Maud Land. SBACC, southern boundary of Antarctic Circumpolar Current; PF, Polar Front.

of deep sea and continental shelf refugia have been suggested for species with pelagic development including the shrimp *Nematocarcinus lanceopes* (Raupach et al. 2010), the sea urchin *Sterechinus neumayeri* (Díaz et al. 2018) and two cryptic species within the crinoid *Promachocrinus kerguelensis* (Hemery et al. 2012), as well as those with direct development including the sea spider *Nymphon australe* (Soler-Membrives et al. 2017), and the octopod *Pareledone turqueti* (Strugnell et al. 2012, 2017). Despite the apparent evolutionary success of a direct developing strategy to survive glacial cycles, pelagic larvae are found in Antarctic waters from a wide range of phyla, including Arthropoda, Bryozoa, Cnidaria, Echinodermata, Mollusca, Nematoda and Porifera (Shreeve and Peck 1995, Stanwell-Smith et al. 1999, Sewell 2005, Freire et al. 2006, Bowden et al. 2009, Sewell and Jury 2011, Ameneiro et al. 2012, Gallego et al. 2015). The molecular evidence of glacial survival and modern observations of pelagic and non-pelagic development in extant benthic fauna reflect that both strategies clearly contributed unique ways of assisting fauna to persist through glacial periods.

Pelagic larval survival throughout glacial cycles

Of the pelagic larval development strategies in the Southern Ocean, lecithotrophy is hypothesised to be favoured during the food impoverished glacial maxima and therefore a trait that could be more prevalent than planktotrophy (Pearse et al. 2009). In recent surveys, planktotrophy and lecithotrophy were detected simultaneously all year round along the Western Antarctic Peninsula (Shreeve and Peck 1995, Freire et al. 2006, Bowden et al. 2009). In an effort to examine whether Thorson's rule (i.e. high latitudes do not favour planktotrophy in marine invertebrates, Mileikovsky 1971) applies to the Western Ross Sea, Gallego et al. (2015) performed plankton surveys to measure the larval diversity characterised by planktotrophy and lecithotrophy over multiple summer seasons. This study revealed that at least 70% of larval diversity sampled (Molecular Operational Taxonomical Units; mOTUs) during summer seasons were comprised of planktotrophic mOTUs (Gallego et al. 2015). Similarly, planktotrophic larvae were also observed in high abundance along the Western Antarctic Peninsula during summer, coinciding with the timing of the annual phytoplankton bloom (Bowden et al. 2009). In the Southern Ocean, links between pelagic larval development and evolutionary selection have been studied in detail in sea snails. While most non-Antarctic Capulidae snails are planktotrophic, Antarctic lineages are mostly lecithotrophic (Fassio et al. 2015), thus reflecting the links between selection and developmental strategy in this region. However, based on current evidence, selection for lecithotrophy does not seem to be a general trend as Antarctic Velutinidae snails appear to exhibit planktotrophy similar to other non-Antarctic lineages, although with a reduced pelagic larval duration (PLD) (Fassio et al. 2019). The persistence of planktotrophy through time can be explained by

species selection, despite frequent transitions to lecithotrophy (Krug et al. 2015). The current evidence indicating the year-round presence of planktotrophic and lecithotrophic larvae, as well as a reduction in PLD in some groups, highlights that species with pelagic larvae likely possess a range of strategies that have enabled persistence throughout glacial cycles in the Southern Ocean.

Instead of only favouring direct developers over species with pelagic larvae over glacial cycles, selection likely acted differently on pelagic and non-pelagic larval development. The survival of direct developers could have been facilitated by isolated refugia and low primary production in the water column (Poulin et al. 2002, Pearse et al. 2009). The year-round presence of lecithotrophic larvae along the Western Antarctic Peninsula (Shreeve and Peck 1995, Freire et al. 2006, Bowden et al. 2009), and up to 30% of larval diversity (mOTUs) were found to be lecithotrophic during summer seasons in the Ross Sea (Gallego et al. 2015) when the annual summer primary production exclusively promotes planktotrophy, also highlight lecithotrophy can be a successful strategy in the Southern Ocean. Lecithotrophy and reduced PLD in planktotrophy could have also aided pelagic larvae to survive glacial cycles by reducing the reliance on long periods of feeding in the water column. Faunal recolonisation from the shelf break or non-shelf refugia to the inner continental shelf may seem impossible if direct development with limited dispersal ability was exclusively favoured during glacial cycles. Modern observations on benthic fauna assemblages in the Weddell Sea also indicate that after local iceberg disturbance destroyed the previous benthic community, four out of five recolonising species displayed pelagic larval development (Teixidó et al. 2004). After local volcanic eruptions at Deception Island, South Shetland Islands, species with planktotrophic larvae also recolonised and dominated the newly established benthic community in the caldera (Barnes et al. 2008). However, a decade after volcanic eruptions at Southern Thule, South Sandwich Islands, species with apparent limited dispersal ability (brooding and lecithotrophic larvae) were found to dominate the local benthic community (Kaiser et al. 2008). Notably, although highly relevant to signatures of glacial survival, limited studies have examined post glaciation recolonisation pathways and ecological succession in relation to larval dispersal strategies throughout glacial periods.

Investigating past changes in the demographic history of extant species can shed light onto how species survived repeated glacial–interglacial cycles (Clarke et al. 2004), and how glacial periods also selected for both pelagic and non-pelagic development (Thatje 2012). However, it is also important to note that dispersal ability does not follow a linear, uniform pattern in nature. The relationship between PLD and dispersal range is also not strongly correlated in the marine realm (Weersing and Toonen 2009, Selkoe and Toonen 2011). For most Southern Ocean benthic species, their larval and reproductive ecology remains largely unexplored, and thus most hypotheses regarding past demographic history can only be constructed based on the simplified difference

between pelagic and non-pelagic development (Pearse et al. 1991, Barnes and Conlan 2007).

Locating Southern Ocean glacial refugia through demographic history

Ice sheet expansions occurring during severe glaciations in the last one million years would have been catastrophic to the Southern Ocean seafloor communities. As a result, significant changes in demography would occur according to species responses to environmental changes (Thatje et al. 2008). Individualistic species response to climate change may also lead to different strategies of survival in glacial refugia which in turn can reflect different patterns of past species demography (Hofreiter and Stewart 2009, Stewart et al. 2010). Such past demographic history would be encoded in the genomes of those extant species that have persisted through glacial cycles (Hewitt 2000). Therefore, understanding the key historical processes related to persistence, migration and extinction during glacial cycles can offer testable frameworks to deduce the locations of glacial refugia in the Southern Ocean (Avise 1989, Davis et al. 2005). For studies that detect signatures of glacial refugia in Northern Hemisphere terrestrial and marine taxa, signals of past changes in population size can indicate population persistence, and signals of shifts in species distribution can reflect migration to southernly warmer areas during glacial cycles. However, these patterns cannot be directly applied to Antarctic benthic fauna because of the isolated nature of the Southern Ocean. For example, glacial cycles have been proposed as drivers of allopatric speciation in the Southern Ocean because of repeated glacial advances, retreats and refugium isolation (the Antarctic diversity pump hypothesis) (Clarke and Crame 1989, 1992, Crame 1997). In contrast to the Northern Hemisphere where glacial cycles were suggested to have reduced genetic diversity in terrestrial and marine taxa, repeated AIS expansions and retreats have been proposed to increase genetic diversity in the Southern Ocean (Wilson et al. 2009). Nonetheless, concepts developed from Northern Hemisphere refugia research could be adapted as frameworks within the Southern Ocean context. As extant benthic fauna in the Southern Ocean could have persisted on the continental shelf or migrated to deep sea and/or sub-Antarctic refugia during glacial cycles, if true, these hypothesised survival scenarios would also be reflected by different patterns in changes in population size, and population connectivity and isolation.

Population size

As ice-free areas on the continental shelf (such as areas along the shelf break and open marine habitats within grounded ice) could have enabled the in situ survival of benthic fauna, populations may have experienced demographic bottlenecks within these glacial refugia instead of complete eradication (Allcock and Strugnell 2012). Evidence of founder effects may also be observed in individuals reaching new refugia in

deeper waters and sub-Antarctic islands for refugia, where an establishing population is based on a subset of newly colonising individuals from a source population. Given that ice sheet reconstructions suggest glacial advances did not extend beyond the continental shelf break throughout glacial cycles (Bentley et al. 2014), a supposed stable continental slope and the deep sea would have provided ice-free habitat (but see Thatje et al. 2005). During glacial cycles, subsets of benthic shelf populations could have migrated to deeper waters for refuge following seafloor bathymetry and subsequently expanded in population size.

Individuals of species that found refuge around sub-Antarctic islands could have dispersed via the ACC (Matschiner et al. 2009). Although larval state is typically thought of as the key dispersive stage, adults may disperse as well. Adult dispersal could be achieved via rafting and attachment to other organisms (Helmuth et al. 1994, Leese et al. 2010, Nikula et al. 2010) or to drift pieces of anchor ice (Dayton et al. 1969, Teixidó et al. 2004, Thatje 2012). Because many of the sub-Antarctic islands were also heavily glaciated with limited ice free areas at the LGM (Hodgson et al. 2014), newly colonising individuals were likely to experience limited expansion in population size in comparison to populations that found refuge in deep sea areas.

Connectivity and isolation

If deep sea and sub-Antarctic refugia were used by species, then we would expect to see some residual signals of connectivity between the shelf and these places (Clarke 2008). However, given the Antarctic continental shelf is geographically isolated from sub-Antarctic islands via deep water channels, but is relatively connected to the deep sea along the seafloor bathymetry, different patterns of connectivity may be expected to be derived from surviving in deep sea versus sub-Antarctic island refugia. For example, repeated glacial cycles would encourage populations that were able to seek deep sea refugia to persist and diversify on the continental slope and in the deep sea throughout glacial periods (Kaiser et al. 2011, Allcock and Strugnell 2012). Recolonisation of the continental shelf from deep sea refugia would also be reflected by stepwise connectivity between the deep sea and continental shelf along seafloor bathymetry. Thus, an isolation by distance and depth pattern may be expected between deep sea and continental shelf populations in extant taxa.

It could be challenging for populations that persisted in sub-Antarctic refugia to recolonise the continental shelf as individuals would need to penetrate across different frontal boundaries within the ACC in order to reach the continent. Nonetheless, recolonisation from sub-Antarctic refugia is not impossible and has been suggested for the benthic shrimp *Chorismus antarcticus* (Raupach et al. 2010) and limpet *Nacella concinna* (González-Wevar et al. 2013). In both species, a founder effect on the continental shelf was observed, indicating connectivity between continental shelf and sub-Antarctic islands can be achieved via a few colonist individuals.

For populations that had survived in glacial refugia on the continental shelf, those with non-pelagic development and lecithotrophic larvae would slowly expand to nearby glacial refugia or even remain isolated among refugia owing to a lower dispersal rate (Pearse et al. 1991). Pelagic development with planktotrophic larvae might enable populations to be distributed broadly and swiftly over habitable areas across long geographic distances (Pearse et al. 1991), leading to connectivity between refugia (Allcock and Strugnell 2012). High levels of admixture may also be seen in populations with pelagic development, as populations that were previously separated between different refugia could face secondary contact during deglaciations. Admixture (in the form of high genetic diversity) driven by secondary contact during post-glacial recolonisation has notably been observed in European trees and shrubs with high seed dispersal abilities (Petit et al. 2003) and in the Antarctic springtail *Desoria klovstadi* in Victoria Land, Antarctica (Stevens et al. 2007).

It should be noted that the degree to which dispersal strategy structures connectivity patterns appears to vary between Southern Ocean taxa, and connectivity between populations can also be influenced by seascape dynamics and ecological responses. Genetic evidence has demonstrated refugial populations of species with pelagic planktotrophic larvae (Hemery et al. 2012) and also direct development (Dietz et al. 2015) have encountered secondary contact along recolonisation routes in the Southern Ocean, despite the supposed contrasting difference in dispersal abilities. Other factors, including heterogeneous seascapes and oceanic currents, are also known to be dispersal barriers and influence population connectivity in the Southern Ocean (Thornhill et al. 2008, Wilson et al. 2009, Dambach et al. 2016, Strugnell et al. 2017, Moore et al. 2018). Ecological responses to glaciations may also be significant in structuring population connectivity and isolation during glacial cycles. Molecular evidence has highlighted contrasting genetic structures between cryptic species of the Antarctic benthic amphipod genus *Eusirus*; and was proposed as a result of different species-specific survival strategies during glacial cycles (Baird et al. 2011). Similarly contrasting patterns of connectivity were detected in closely related species of Southern Ocean crinoid with pelagic development (Hemery et al. 2012) and in octopus with direct developing young (Strugnell et al. 2017). Disparate patterns of population connectivity and isolation between closely related species have also been detected in other non-Antarctic marine species, and these contrasting patterns have been attributed to differences in dispersal barriers, larval duration and ecological responses (Marko 2004, Hickerson and Cunningham 2005, Crandall et al. 2008).

What is the current molecular evidence of glacial refugia in the Southern Ocean?

Genetic data from present day taxa can reveal past species demography and evidence of glacial refugia (Box 1). Therefore, we systematically reviewed published genetic studies of Southern Ocean species to evaluate current molecular signatures of glacial survival. A keyword search of 'Antarctic' AND 'genetic' was conducted on Web of Science on 26 September 2019. Out of the 893 journal articles that were gathered through the Web of Science search, 73 analysed the genetic patterns of Southern Ocean continental shelf benthic fauna. These articles were published between 1991 and September 2019. Of these 73 articles, only six studies used molecular data to propose locations of where Southern Ocean benthic fauna had survived glacial cycles using molecular data (Box 1) (Table 1). Other studies that did not explicitly point to locations were also discussed in the context of how preliminary evidence can be better incorporated into investigations of the locations of glacial refugia.

Although there are only six molecular studies unequivocally proposing locations of glacial refugia in Southern Ocean benthic fauna, together they suggested multiple refugial locations on the continental shelf around Antarctica, in the deep sea, and also around sub-Antarctic islands (Fig. 1). Signatures of putative Southern Ocean glacial refugia have so far been inferred from the pycnogonids *Nymphon australe* (Soler-Membrives et al. 2017) and *Colossendeis megalonyx* (clade A) (Dietz et al. 2015), crinoids *Promachocrinus kerguelensis* (clade A, B/C and E1/E2) (Hemery et al. 2012), the echinoid *Sterechinus neumayeri* (Díaz et al. 2018), the gastropod *Nacella concinna* (González-Wevar et al. 2013) and the octopod *Pareledone turqueti* (Strugnell et al. 2012). These studies mainly utilised COI data to infer past species demography in species with either pelagic or non-pelagic development. The sampling distribution of these studies was either restricted to the Scotia Sea and Antarctic Peninsula or broadly-distributed taxa. The locations of glacial refugia were primarily inferred from where animals with a high number of private alleles were collected, in the form of a high proportion of private haplotypes or via high haplotypic diversity (Table 1). Past population size changes and population connectivity and isolation were also commonly discussed in the context of how populations were affected by glacial cycles. Even though different dispersal strategies can structure different patterns of species demography, these patterns are challenging to detect from current molecular evidence due to the limited number of studies outlined here (n = 6).

Evidence of shelf refugia

Although the proposed locations of refugia included the continental shelf around Antarctica (Fig. 1), the data suggest that some continental shelf species with sub-Antarctic populations also persisted around sub-Antarctic islands, presumably throughout glacial cycles (Table 1). The proposed locations of shelf refugia include Shag Rocks, South Georgia and South Orkney Islands in the Scotia Sea, and Eastern Weddell Sea, Western Ross Sea, Adélie Land, as well as the Kerguelen Plateau off East Antarctica. Some locations have been suggested as glacial refugia for multiple species, and these locations include South Georgia, Eastern Weddell Sea, Eastern Ross Sea and Adélie Land in East Antarctica. With

Box 1. How to identify demographic history associated with glacial refugia?

Phylogeography (the analysis of spatially distributed genetic data, Avise et al. 1987) has rapidly expanded since its inception, and has become one of the key disciplines in understanding the demographic and evolutionary history of species (Avise 2009). In turn, a phylogeographic approach has been a popular tool in investigating refugia and ecological processes during glacial–interglacial cycles globally (Provan and Bennett 2008, Gavin et al. 2014). Phylogeography connects the field of population genetics and systematics and is built upon coalescent theory (Avise et al. 1987, Avise 2009). Coalescent theory is tightly coupled with the theories of population genetics, which assumes 1) neutral evolution, 2) that all lineages can be traced back to a single common ancestor and 3) that an idealised population should persist with random mating and neutral mutations in DNA (Rosenberg and Nordborg 2002). Any deviations from these assumptions may signify that population structure was influenced by variations in population size and unequal gene flow between populations in the past (Hey and Machado 2003, Grant 2015). Several recent reviews have examined the theoretical complexities in investigating phylogeography and highlighted different methods that can offer independent lines of evidence to infer how past climate changes influenced demographic histories (Crisci et al. 2012, Cutter 2013, Bank et al. 2014, Lowe et al. 2017). Although haplotype networks linking to alternative survival scenarios during glacial cycles and population connectivity have been widely discussed for marine taxa (Avise et al. 1987, Maggs et al. 2008, Allcock and Strugnell 2012), other commonly reported population genetic metrics might also be useful in pinpointing possible refugial locations and scenarios.

In global animal phylogeographic studies, COI is traditionally the most commonly used genetic marker due to its low intraspecific variability but relatively high interspecifically variability (Avise et al. 1987). In the Southern Ocean phylogeographic studies, COI has frequently been sequenced due to the discounted sequencing campaigns through CAML and BOLD, as well as its widespread utility in delineating species. However, COI does not represent the multiloci genealogical evolution of the studied species. Instead, mitochondrial DNA (mtDNA) analyses only investigate the evolution of a mostly non-recombining, maternally inherited mitochondrial genome. Factors such as introgression (Ballard 2000), hybridisation (Dowling et al. 2008), selection against assumed neutrality (Ballard and Rand 2005) and mutation rate variations within mtDNA (Galtier et al. 2009) can violate the assumptions of coalescent theory in population genetics. Nonetheless, it is important to note that single locus studies can still offer highly transferable knowledge to establish hypotheses for future research, therefore data derived from COI data is still highly relevant in the genomic era (see Bowen et al. 2014 for detailed discussion).

Genetic variation

Genetic variation between populations (both nucleotide and haplotypic diversity) is widely used to distinguish between refugial and recolonised populations. Individuals isolated within glacial refugia are likely to experience long-term isolation and harbour more endemic diversity (private alleles). Recolonising individuals are assumed to diverge from a subset of individuals dispersed from glacial refugia, leading to lower levels of genetic diversity compared to those that persisted in refugia over multiple glacial cycles (Petit et al. 2003, Maggs et al. 2008, Provan and Bennett 2008). Areas where colonisers from previously isolated refugia come into secondary contacts are suggested to exhibit the highest genetic diversity (Petit et al. 2003).

Neutrality tests

Population genetic data can be analysed using neutrality tests such as Tajima's D (Tajima 1989) and Fu's F (Fu and Li 1993) to test for significant deviations from neutral evolution, which can reflect past changes in population size and therefore demographic histories of refugial and recolonised populations. For example, positive and negative values derived from neutrality tests can represent historical population size reduction (loss of rare alleles) and population expansion (excess of rare alleles) respectively (Fay and Wu 1999). However, if a bottleneck was extremely severe then neutrality tests may produce negative values; an outcome expected for population expansion because most alleles were lost to a strong bottleneck effect (Fay and Wu 1999). Therefore, a range of methods and measurements need to be incorporated in the analyses to counteract potential method-specific errors.

Past changes in population size

Population size changes can also be investigated using the nucleotide differences between paired individuals visualised in the form of frequency distribution (pairwise/mismatch distribution) (Slatkin and Hudson 1991, Rogers and Harpending 1992). This pairwise difference is theorised to exhibit a unimodal distribution under the model of recent population expansion, with indication of equilibrium (i.e. constant population size) is expected to exhibit bimodal or multimodal distributions (Rogers and Harpending 1992). In addition, a 'ragged' bimodal or multimodal distribution may represent deep divergences between populations (Grant 2015). Skyline-plots are another popular demographic tool for reconstructing past population sizes through time based on estimated patterns of coalescence (Ho and Shapiro 2011). They can reconstruct magnitude and timing of population bottleneck and subsequent expansion simultaneously.

Hypothesised refugia scenarios testing

Recent theoretical advances have also made it possible to combine genomic data derived from neutral loci with range dynamics models to reconstruct past demographic scenarios (Nunziata et al. 2017, Pujolar et al. 2017, Roman et al. 2018). By visualising genomic data in the form of allele frequency distribution across polymorphic sites (i.e. size frequency spectrum, SFS), the observed shape of the SFS can be used to test for deviations from neutral evolution (Rosen et al. 2018). The SFS can be computed and species demographic history can be inferred through SFS-based interface models, which can investigate complex scenarios involving divergence and historical migrations, as well as being able to distinguish which scenario scores the highest likelihood between contrasting hypothesised scenarios (Gutenkunst et al. 2009, Excoffier et al. 2013).

Table 1. Summary of molecular signatures of cytochrome c oxidase subunit I (COI) linked to glacial refugia in the Southern Ocean. * Significantly different from zero.

Species	Larval dispersal mode	Marker used in the study	Area sampled	Sampled depth range (m)	Proposed area of glacial refugia		Nucleotide diversity	Haplotype diversity	Fu's Fs	Pairwise difference distribution	Proportion of private haplotype	References
Nymphon australe	Non-pelagic	COI	South Sandwich Islands, West Antarctic Peninsula, East Weddell Sea, West Ross Sea, Davis Station, Bruce Rise, Adélie Land	25–1261	West Antarctic Peninsula	Deep sea	0.00538	0.7940	−17.764*	Unimodal	89.7	Soler-Membrives et al. (2017)
					East Antarctica (Davis Station, Bruce Rise and Adélie Land)	Deep sea	0.00496	0.8570	−49.372*	Unimodal	78.0	
					East Weddell Sea	Continental shelf	0.00133	0.4290	−3.961*	Unimodal	71.4	
Colossendeis megalonyx (A)	Non-pelagic	COI	South Georgia, South Sandwich Islands, South Orkney Island, Elephant Island, South Shetland Islands	81–2285	South Georgia	Sub-Antarctic island		High				Dietz et al. (2015)
Promachocrinus kerguelensis (A)	Pelagic	COI, Cytb, 16S, 28S, ITS	South Georgia, South Shetland Islands, South Sandwich Islands, Bouvet Island, East Weddell Sea, West Ross Sea, Adélie Land, Kerguelen Plateau	106–541	Adélie Land	Continental shelf	0.00430	0.6855	−1.397	Unimodal	66.7	Hemery et al. (2012)
					West Ross Sea	Continental shelf	0.00480	0.6621	−3.256	Bimodal	60.0	
					Kerguelen Plateau	Sub-Antarctic island	0.00120	0.4300	−4.900*	Unimodal	85.7	
Promachocrinus kerguelensis (B/C)	Pelagic		South Orkney Island, South Shetland Island, South Sandwich Islands, West Antarctic Peninsula, East Weddell Sea, West Ross Sea, Davis Station, Adélie Land	65–1157	Adélie Land	Continental shelf	0.00400	0.7423	−2.056	Bimodal	64.3	
					East Weddell Sea	Continental shelf	0.00590	0.8072	−3.258	Bimodal	68.8	
Promachocrinus kerguelensis (E1/E2)	Pelagic		South Georgia, South Orkney Island, South Shetland Islands, West Ross Sea, Adélie Land	147–525	South Orkney Islands	Sub-Antarctic island	0.00490	0.7423	−2.056	Bimodal	64.3	
Sterechinus neumayeri	Pelagic	COI, Microsatellite	West Antarctic Peninsula, East Antarctic Peninsula, East Weddell Sea, Adélie Land	Shallow water	Adélie Land	Continental shelf	0.00115	0.4760	−2.679*	Unimodal	80.0	Díaz et al. (2018)
Nacella concinna	Pelagic	COI	South Georgia, South Orkney Island, South Shetland Islands, West Antarctic Peninsula, East Antarctic Peninsula	Intertidal	South Georgia	Sub-Antarctic island	0.00353	0.8800	−3.440*	Unimodal	72.7	González-Wevar et al. (2013)
Pareledone turqueti	Non-pelagic	COI, Microsatellite	Shag Rocks, South Georgia, South Orkney Island, South Sandwich Islands, Elephant Island, King George Island, Livingstone Island, West Antarctic Peninsula, East Antarctic Peninsula, East Weddell Sea, Prydz Bay, West Ross Sea, Adélie Land	95–1044	Adélie Land	Continental shelf	0.00530	0.7420	1.060	Bimodal	50.0	Strugnell et al. (2012)
					West Ross Sea	Continental shelf	0.00340	0.6270	−2.220	Multimodal	58.3	
					East Weddell Sea	Continental shelf	0.00360	0.6670	0.590	Multimodal	80.0	
					Shag Rocks	Sub-Antarctic island	0.00120	0.6130	−4.060*	Unimodal	50.0	
					South Georgia	Sub-Antarctic island	0.00050 (NW/0.00120 (SE)	0.3070 (NW/0.5830 (SE)	−3.810*	Unimodal		

the exception of outer shelf in the Eastern Ross Sea, these proposed glacial refugia do not correspond with the LGM ice-free areas inferred from past AIS reconstructions. This may reflect sampling effort to some extent, as these studies lacked samples collected from most of the proposed ice-free areas from past AIS reconstructions (i.e. outer shelf of Bellingshausen Sea, between Adélie Land and Bruce Rise in East Antarctica, and Astrid Ridge and Gunnerus Ridge near Dronning Maud Land). Future studies should target the same studied species at these locations to be able to test this hypothesis directly.

Inferred changes in demographic history in relation to survival in glacial refugia have often been linked to the LGM via molecular dating (González-Wevar et al. 2013, Soler-Membrives et al. 2017, Díaz et al. 2018), or simply hypothesised (Hemery et al. 2012, Díaz et al. 2018). Population bottlenecks during the LGM, followed by expansions during subsequent deglaciation, were suggested in species with either pelagic or non-pelagic development, and were supported by significantly negative neutrality tests and unimodal pairwise mismatch distributions (Table 1). These populations include *P. kerguelensis* (A) from the Kerguelen Plateau (Hemery et al. 2012), *S. neumayeri* from off Adélie Land (Díaz et al. 2018) and *N. australe* in the Weddell Sea (Soler-Membrives et al. 2017). However, other populations of *P. kerguelensis* (A) in the Western Ross Sea, as well as populations in the two other cryptic species of *P. kerguelensis* (B/C; E1/E2), also appeared to have experienced relatively stable population size in some areas on the continental shelf, most probably throughout the LGM, as indicated by non-significant negative neutrality tests and bimodal mismatch distributions (Table 1). In the cryptic species B/C, stable populations persisted in Adélie Land and East Weddell Sea; and in E1/E2, a stable population was detected off the South Orkney Islands.

More interestingly, past changes in the demographic history of the direct developing *P. turqueti* were dated back to the mid-Pleistocene preceding the LGM, with various populations also experiencing contrasting demographic histories. One *P. turqueti* population in the Weddell Sea was detected to have experienced a relatively stable population size throughout glacial maxima on the continental shelf (Table 1). Bayesian Skyline Plot analysis of *P. turqueti* populations from off the Adélie Land and in the Ross Sea indicated in situ survival on the continental shelf was possibly not associated with a population bottleneck, but instead, an overall population expansion was detected in these two locations (Strugnell et al. 2012).

Evidence of recolonisation of the continental shelf

Molecular evidence of recolonisation of the Southern Ocean continental shelf is limited, with only two studies so far proposing faunal recolonisation of the shelf from sub-Antarctic island and deep sea refugia (González-Wevar et al. 2013, Soler-Membrives et al. 2017). Recolonisation of the continental shelf from sub-Antarctic island refugia was detected in the population history of the limpet *Nacella concinna* after the

LGM (González-Wevar et al. 2013). This was demonstrated through significant genetic differentiation between Antarctic continental shelf and sub-Antarctic South Georgia populations, combined with an older demographic history and a high proportion of private haplotypes in the South Georgia population (González-Wevar et al. 2013). Demographic model analysis (approximate Bayesian computation) was also employed to confirm the shelf recolonisation from sub-Antarctic island population as a highly likely scenario (González-Wevar et al. 2013).

Signatures of shelf recolonisation from putative deep sea refugia were suggested from the populations of *Nymphon australe* sampled mostly from the continental shelf adjacent to the Antarctic Peninsula and areas near Davis Station, Bruce Rise and Adélie Land in east Antarctica. These areas were proposed as deep sea refugia, and the sampled populations exhibited a high proportion of private haplotypes, high genetic diversity and signature of past population expansion without signs of a bottleneck (Soler-Membrives et al. 2017). The evidence of deep sea refugia was mainly supported by signatures of population expansion, as well as high genetic variation hypothesised as a result of repeated colonisations from shelf to deep sea (and vice versa). Although AIS reconstructions indicate the LGM grounded ice extended near the continental shelf break, limited ice-free areas were also observed on the continental shelf edge near Bruce Rice and Adélie Land (Fig. 1). In addition, minimal samples were available (n = 8) of *N. australe* from the deeper waters (> 1000 m) to be included in the dataset. Since relatively high genetic variation and population expansion without prior bottlenecks have also been found to be associated with continental shelf refugia (Table 1), the molecular signatures differentiating deep sea and continental shelf refugia remain largely unexplored.

Timing of glacial refugia in the Southern Ocean

Genetic signals of glacial survival derived from syntheses on Northern Hemisphere taxa often suggest the locations of refugia were unchanged throughout glacial cycles, leading to refugial populations to exhibit long-term isolation, strong genetic differentiation and older genealogical splits from other lineages (Provan and Bennett 2008, Bálint et al. 2011). However, given the locations and size of Southern Ocean glacial refugia were likely to be variable throughout different phases of the glacial periods, Southern Ocean taxa may not have experienced long-term isolation in a single area. From the reported genetic signatures characterising Southern Ocean glacial refugia, divergence between individuals that were sampled in putative refugia and recolonised areas can sometimes be apparent, but not definitive. For example, microsatellite data highlighted very low or no population structure between putatively refugial (Adélie Land) and non-refugial (Antarctic Peninsula and Weddell Sea) populations in *S. neumayeri* (Díaz et al. 2018). In contrast, approximate Bayesian computation analysis supported the scenario of continental shelf populations diverging from sub-Antarctic island refugial population in *N. concinna* after the LGM

(González-Wevar et al. 2013). The divergence of refugial and non-refugial populations in *P. turqueti* was dated back to 1.25 million yr ago at the start of the Mid-Pleistocene Transition (Strugnell et al. 2012). Although not dated, various levels of divergence were also found between cryptic species of *P. kerguelensis* (Hemery et al. 2012). In contrast to Northern Hemisphere taxa, the current genetic evidence suggests that refugial populations in the Southern Ocean do not necessarily exhibit long-term isolation, highlighting possible evidence of glacial refugia locations being different throughout glacial cycles.

However, current molecular data also show different divergence times between refugial and non-refugial populations in different Southern Ocean species. Different population divergence times between species could be due to taxon-specific mutation rates, but it is also likely to be the product of population-level survival and extinction events being different between species over many glacial maxima in the Quaternary (Avise 2009, Hemery et al. 2012). Avise (2009) speculated that even though some species might have persisted in an area acting as glacial refugium, populations of a particular species (or more) could undergo extinction in the same area. During the following interglacial period, this area could be recolonised again by the species that had previously been wiped out but persisted in other refugia (Avise 2009). Therefore, the temporal depth of lineage split could represent the last time a population faced extinction and recolonised in an area, with a shallow split representing a more recent extinction (Avise 2009). From the reported demographic histories in the Southern Ocean, it is clear that the effects of recent glacial cycles, particularly population bottlenecks and expansions during and post LGM, have been superimposed on existing genetic patterns, thus masking the molecular signatures of older glacial events (as discussed in Maggs et al. 2008, Grant 2015). Phylogenetic reconstruction can also be influenced by the loci of choice, number of individuals sequenced, and population substructure (Kim et al. 2015). However, if estimated correctly, the chronology of lineage splitting appears to be able to give clues as to how multiple glacial cycles may have influenced diversification processes differently between species, and more importantly the periodicity of glacial refugia. For example, the divergence of continental shelf populations from sub-Antarctic island populations in *N. concinna* after the LGM suggests South Georgia served as an LGM glacial refugia. The mid-Pleistocene divergence and subsequent long-term persistence of *P. turqueti* populations suggests glacial refugia for this species occurred at Shag Rocks, South Georgia, East Weddell Sea, West Ross Sea and Adélie Land since the mid-Pleistocene.

The complexity of population genetic metrics

The demographic histories of extant Southern Ocean benthic fauna appear to be characterised by different combinations of population bottlenecks, expansions and stable population sizes throughout different phases of glacial cycles. These are highlighted by some species reported in Table 1, which do not show significantly negative neutrality tests and/or non-unimodal mismatch distributions. Although this could reflect different survival strategies of pelagic and non-pelagic development, the reported population genetic metrics may be confounded by complex evolutionary histories and sequencing biases.

While it is hypothesised that population persistence in a glacial refugium results in endemic diversity (Schmitt 2007, Provan and Bennett 2008), the highest genetic diversity is thought to be found in areas where colonisers from previously isolated refugia come into secondary contact along recolonisation routes (Petit et al. 2003). When comparing reported molecular population genetic metrics, proposed Southern Ocean refugial locations are not solely characterised by a similar threshold of genetic diversity. Instead, a range of intraspecific genetic diversity from low to high were found in suspected refugial populations irrespective of pelagic or non-pelagic development (Table 1). The wide range of genetic diversity associated with glacial refugia could be due to limited representative samples within species distributional range, small sample size, and/or over pooling of samples, thus resulting incorrect estimations of genetic metrics (Goodall-Copestake et al. 2012, Bertl et al. 2018). The known widespread occurrence of grounded ice during the LGM may have induced severe population bottlenecks and therefore could even result in low genetic diversity in areas of glacial refugia (Amos and Harwood 1998). An alternative and more persuasive explanation is that the genetic patterns of Southern Ocean benthic fauna observed today are built upon eroded signals gathered over many glacial maxima, and superimposed by the LGM and modern genetic patterns, as highlighted above. Past population and demographic histories are encoded in the genomes of extant taxa (Hewitt 2000), but studies employing single locus and microsatellite loci (all studies in Table 1) may lack power to investigate Southern Ocean benthic species with complex evolutionary histories.

The haplotype networks associated with the proposed locations of glacial refugia (Table 1) generally correspond to the patterns expected for species' dispersal strategy and the kind of glacial refugium as outlined in Allcock and Strugnell (2012). Additional factors likely also play a role in shaping patterns of haplotype networks. For example, shared haplotypes between multiple regions were often detected and suggested as a result of present-day connectivity via long distance dispersal and oceanic currents (Hemery et al. 2012, Strugnell et al. 2012, Soler-Membrives et al. 2017, Díaz et al. 2018), as well as ancient seaways from past West Antarctic Ice Sheet collapses (Strugnell et al. 2012). While signatures of glacial survival are encoded in the genome of Southern Ocean benthic taxa, other evolutionary forces are also embedded within it. These evolutionary signals range from high diversification rates as a result of rebound from extinctions associated with the formation of the Antarctic environment during the mid-Cenozoic (Dietz et al. 2019, O'Hara et al. 2019), to the evolutionary drivers associated with the local environments (e.g. water

depths) (Linse et al. 2007, Strugnell et al. 2017). A better understanding of where and how the Antarctic continental shelf benthic fauna survived glacial cycles can be achieved by resolving current sampling and sequencing biases, exploiting genomic data using appropriate hypothesis driven methods.

Sampling bias and data syntheses

Although the studies examined here have suggested locations of glacial refugia across the continental shelf, deep sea and sub-Antarctic islands for benthic fauna, the emerging patterns of glacial refugia may be partially a result of sampling bias. Benthic sampling in the Southern Ocean is often conducted in areas where access is relatively easy and close to supply routes to national research stations (Griffiths et al. 2014). Almost all locations suggested as glacial refugia (with the exception of Adélie Land) have been identified as benthic sampling hotspots in the Southern Ocean (Griffiths et al. 2014). Areas on the inner and outer continental shelf along the Bellingshausen Sea, Amundsen Sea, West Weddell Sea and East Antarctica are rarely sampled and therefore often excluded in the analysis of glacial refugia. Specimens from the continental slope and deep sea are also scarce, and balanced sampling designs are rarely applied. Given that molecular evidence of glacial refugium has only been suggested from eight species with inherent sampling bias, the emerging patterns of glacial refugia reported here invites continued data syntheses, sample collection from poorly sampled locations, and with respect to glacial refugia predicted from past AIS reconstructions.

The studies identified here that explicitly propose where Antarctic benthic fauna survived glacial cycles do not represent the majority of Southern Ocean molecular studies. In fact, more than 500 Southern Ocean species have been sequenced for molecular analyses (Riesgo et al. 2015) and many genetic studies have discussed some preliminary evidence of glacial refugia for benthic taxa. However, the data analysis and/or interpretation of preliminary evidence were constrained by various factors, leaving the refugia hypotheses unanswered. Some studies suggested that evidence of glacial survival is reflected by overall genetic patterns but did not propose explicit locations of glacial refugia (Raupach et al. 2010, Dömel et al. 2015, Sromek et al. 2015, Leiva et al. 2018, Miller et al. 2018), while others had limited sample coverage (Janko et al. 2007, Baird et al. 2012, Díaz et al. 2012, Wiernes et al. 2013) and were confounded by discoveries of multiple cryptic species in datasets (Wilson et al. 2009, Allcock et al. 2011, Baird et al. 2011, Wiernes et al. 2013, Harder et al. 2016, González-Wevar et al. 2019).

Inferring locations of glacial refugia through cryptic speciation patterns and processes

Information related to species glacial survival can be interpreted from genetic data through different population genetic analytical methods. Interestingly, glacial cycles have been proposed as drivers of allopatric speciation in the Southern

Ocean because of repeated glacial advances, retreats and refugia isolations (the Antarctic diversity pump hypothesis) (Clarke and Crame 1989, 1992, Crame 1997), which are relevant to the discoveries of multiple cryptic species in genetic datasets. As popular molecular methods that test for evidence of glacial refugia are based on population level processes, the discovery and inclusion of separate cryptic species could appear as an analytical dead end. Whether glacial cycles can drive allopatric speciation on the Southern Ocean continental shelf depends on the opportunities for refugia isolation and time since isolation within a grounded ice sheet. However, whether populations undergo speciation within isolated refugia is dependent upon reproductive isolation and genetic drift; both factors can be linked to dispersal strategies and small population size (e.g. a population bottleneck) (Palumbi 1994, April et al. 2013). The rate of speciation is also not necessarily determined by the duration of each glacial maximum per se, as speciation could occur during a short timeframe as a result of adaptation under local selective environmental pressure (Hendry et al. 2000). Therefore, understanding whether glacial survival could drive cryptic speciation processes could lead to the discovery of glacial refugia locations, and more importantly how Southern Ocean glacial cycles influenced speciation processes.

In the Southern Ocean, it has been proposed that allopatric glacial refugia could have driven the cryptic speciation within the direct developing pycnogonid *Pallenopsis patagonica* species complex (Dömel et al. 2019). Isolation by glacial refugia coupled with adaptation to local predation has also been suggested to drive cryptic speciation in the direct developing sea slug *Doris kerguelenensis* in the Scotia Sea and Antarctic Peninsula regions (Wilson et al. 2009, 2013). However, it is also clear there are Southern Ocean benthic species with populations distributed in allopatric refugia around the Southern Ocean and across the Scotia Sea and Antarctic Peninsula that did not speciate over glacial cycles, regardless of whether their larval type is benthic or pelagic (Table 1). This highlights the fact that glacial cycle survival can drive different levels of population divergence across taxa, and therefore a range of molecular signals and demographic scenarios should be expected in the analyses seeking to identify Southern Ocean glacial refugia. Increased efforts in systematic analyses, further population genetic data of Southern Ocean benthic taxa and investigations of the genetic processes driving cryptic speciation will undoubtedly unravel more mechanisms of glacial survival in the Southern Ocean.

Future directions of Southern Ocean molecular ecology

As discussed above, previous studies seeking to highlight possible signatures of glacial refugia have been restricted by sample availability, analyses using single locus, and limited examinations of potential refugium locations. Incorporation of benthic samples from rarely sampled regions in the Southern Ocean can be improved through international

initiates and cross-disciplinary collaborations. Investigating and comparing the signatures of glacial refugia in species with different dispersal strategies (direct developers, planktotrophic or lecithotrophic pelagic larvae) can offer insights into the evolutionary selection and survival of pelagic and non-pelagic development throughout glacial periods. The current collection of benthic samples and extracted genomic DNA can be re-sequenced and leveraged for more data through genomic analyses. Advances in genomic sequencing are now enabling the analysis of any regions of interest in the genome, ranging from whole genomes to the sequencing of particular regions such as the mitochondrial genome and/or exonic regions for understanding mutation rate variations and selection (Davey et al. 2011, Schraiber and Akey 2015).

One popular method in population genetics is to use neutral and unlinked loci with single nucleotide polymorphisms (SNPs), which are sequenced randomly across the genome to investigate past demographic processes based on neutral evolution (Schraiber and Akey 2015). The use of SNPs can untangle past complex demographic processes at population- and species-level compared to single locus and microsatellite analyses (Morin et al. 2004, Reitzel et al. 2013, Jeffries et al. 2016). SNP data can also be used to reconstruct past changes in population size and population connectivity and isolation over time based on hypothesis testing of simulated scenarios through demographic models, and can be executed based on different mathematical assumptions (e.g. fastsimcoal2, ∂a∂I and Moments) (Gutenkunst et al. 2009, Excoffier et al. 2013, Jouganous et al. 2017).

In Southern Ocean population genetic studies, SNPs have been employed to investigate the population divergence in the sponge *Dendrilla antarctica* (Leiva et al. 2019), the brittle stars *Ophionotus victoriae* (Galaska et al. 2017a) and *Astrotoma agassizii* (Galaska et al. 2017b), *N. australe* (Collins et al. 2018), and the speciation processes within the *P. patagonica* species complex (Dömel et al. 2019). The use of SNPs could also be useful for investigating past AIS configurations with respect to changes in past patterns of population connectivity and demography (Strugnell et al. 2018). More importantly, genomic data can also highlight other evolutionary drivers that may be associated with survival through glacial cycles in the Southern Ocean, including physical oceanographic forces and biological adaption to environments (Halanych and Mahon 2018). Future investigations employing SNPs will deepen our understanding of how different factors influence genetic variation and population structure, elucidate how different glacial refugial scenarios may apply across taxa, and will likely indicate pathways of recolonisation from refugia leading to present day distributions.

Aside from selectively analysing neutral and unlinked loci, model advances have also demonstrated that detailed past evolutionary histories can be inferred through whole genome-based approaches. Whole genome approaches that are built based on sequentially Markovian coalescent (SMC), e.g. pairwise SMC (PSMC) (Li and Durbin 2011), multiple SMC (MSMC) (Schiffels and Durbin 2014), SMC++

(Terhorst et al. 2017), minimal-assumption genomic inference of coalescence (MAGIC) (Weissman and Hallatschek 2017), can effectively estimate past population size and divergence from a single or multiple individuals due to the high data resolution offered by hundreds of thousands of independent loci. These SMC based approaches can investigate past population genetics and demographic processes at a much finer scale than SNPs methods, and can be effective in investigating complex evolutionary processes based on limited samples.

Detailed evolutionary histories can also be understood using approaches that dissect the genealogies of all loci within the genomes from a thousand or more samples, e.g. Relate (Speidel et al. 2019) and tsinfer (Kelleher et al. 2019). These approaches can be highly powerful in identifying mutation, natural selection and genetic drift down to a single gene and/or trait, and are more accurate in understanding modern and recent processes compared to SMC based methods (Harris 2019). Although the investigations of Southern Ocean evolutionary histories are likely to be restricted by sample constraints, advance in genome-based approaches highlights existing methods that can detect where and how benthic taxa persisted in the Southern Ocean over multimillion year timescales with high analytical power.

Concluding remarks

The questions of where and how Southern Ocean benthic fauna survived the Quaternary ice ages have intrigued Antarctic scientists for decades. It is important to recognise the size, and perhaps locations, of Southern Ocean glacial refugia were likely to be variable over time, and that contrasting dispersal mechanisms into and out of refugia could lead to different demographic signatures, even though these may challenge the reliability of accessing how species survived glacial periods using molecular data. The ice-free areas identified from past AIS reconstructions and the key process of how species survived through glacial periods outlined in this review (dispersal and patterns of past species demography) can offer testable frameworks to deduce where and how Southern Ocean benthic fauna survived the Quaternary, and consequently how glacial periods influenced evolutionary processes. These frameworks can be effectively exploited by genomic methods, which can untangle complex evolutionary histories with high analytical power. Even though only a handful of studies have proposed locations where Southern Ocean benthic fauna survived glacial cycles with potential sampling and molecular marker biases, together with other genetic studies in the Southern Ocean, they have provided essential knowledge that have progressed the critical understanding of where and how species survived glacial periods. Continued data syntheses and explorations using genomic and whole genome methods will undoubtedly reveal a greater understanding of where and how Southern Ocean benthic fauna persisted over multimillion year timescales,

providinginsightsintotheirresilienceagainstclimatechangesin the future.

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