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Cryptic speciation in Southern Ocean *Aequiyoldia eightsii* (Jay, 1839): Mio-Pliocene *trans*-Drake Passage separation and diversification

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ABSTRACT

The species of the genus *Aequiyoldia* Soot-Ryen, 1951, previously known as *Yoldia*, are common, soft-substratum, saredid bivalves. In the Southern Ocean, *Aequiyoldia eightsii* (Jay, 1839) was originally described from the Antarctic Peninsula and has also been reported in southern South America. The species *A. woodwardi* (Hanley, 1960) was reported for the Falkland/Malvinas Islands and Tierra del Fuego, but this taxon has been recently synonymised within the broadly distributed *A. eightsii*. *Aequiyoldia* has received little attention across its distribution in the Southern Ocean, and although its taxonomy and systematics remain uncertain, all the species have been grouped under a single and broadly distributed unit: *A. eightsii*. Nevertheless, preliminary mtDNA comparisons demonstrated a marked genetic divergence (> 7%) between *A. eightsii* populations from South America and Antarctic Peninsula. In order to further understand the diversity and biogeography of *Aequiyoldia*, we analyzed *A. eightsii* populations from different provinces of the Southern Ocean including South America (SA), the Falkland/Malvinas Islands (FI), the Antarctic Peninsula (AP), and Kerguelen Islands (KI). Individuals were characterized according to typical diagnostic morphological measurements and phylogenetic relationships were reconstructed based on mtDNA (cytochrome *c* oxidase subunit I). Patterns of genetic divergence of nucDNA intergenic transcribed spacers (ITS1, ITS2) were also estimated. The statistical analysis of external diagnostic characteristics revealed two morphotypes: (1) individuals with the morphology recorded for the nominal FI species, *A. woodwardi*, and (2) individuals from SA, AP, and KI, with the morphology recorded for *A. eightsii*. However, phylogenetic reconstructions based on mtDNA and nucDNA suggest the presence of at least five lineages within *A. eightsii* including: one lineage in Kerguelen Island, two lineages in the Antarctic Peninsula, one lineage in South America, and the last one restricted to the Falkland/Malvinas Islands. Such results are evidence that the Antarctic Polar Front represents an historical biogeographic barrier for this group and that after the separation of these lineages, they followed independent evolutionary pathways in different provinces of the Southern Ocean. Estimates of divergence time suggest that KI separated from other *Aequiyoldia* lineages close to the middle Miocene. Following this, the separation between the AP and SA lineages occurred at the end of the Miocene around 7.5 Ma. Finally, *Aequiyoldia* diversified during the Pliocene in Antarctic Peninsula (~4.5 Ma) and South America (~3.0 Ma). Individuals from FI exhibited morphological differences, and 4% of divergence from South American individuals, suggesting that *A. woodwardi* could be revalidated. Similarly, the marked molecular divergence between the KI and the rest of the recorded lineages also support the validity of *A. kerguelensis* (Thiele, 1931).

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1. Introduction

Southern-Ocean marine fauna has been traditionally associated with low diversity, mainly because of extreme environmental conditions and several groups including teleost fish, gastropods, bivalves, and some crustacean groups are scarcely represented in this region (Chown et al., 2015). Nevertheless, more than 8000 marine species are currently known and most of them have high levels of endemism (50–97%) (De Broyer and Koubbi, 2014). In fact, the expected number of Antarctic species ranges between 11,000 and 17,000 species. Accordingly, this continent seems to have intermediate species richness compared to other tropical, temperate or Arctic environments (Gutt et al., 2004). Therefore, the Antarctic marine biota is likely to be more diverse than previously thought, and its distribution, composition, and abundance is the outcome of major tectonic, oceanographic, and climatic changes operating in the region since the Mesozoic (Crame, 1999; Zachos et al., 2001; Clarke et al., 2005; Linse et al., 2006; Aronson et al., 2007; Griffiths et al., 2009; Poulin et al., 2014).

The opening of major gateways in the region, the isolation of the Antarctic continent and the establishment of the Antarctic Circumpolar Current (ACC) are profoundly coupled to the origin and diversification of several Southern Ocean marine benthic groups (Clarke and Crame, 1989; Clarke et al., 1992, 2005; Aronson et al., 2007; Griffiths and Waller, 2016). The ACC is the major current system on the planet and is delimited by two main fronts: (1) the sub-Antarctic Front (SAF) and (2) the Antarctic Polar Front (APF) that have major influence over the distribution of the marine benthic biota (Dell, 1972; Crame, 1999; Rintoul et al., 2001; Barnes et al., 2006; Aronson et al., 2007; Knox, 2007; Griffiths et al., 2009; Pierrat et al., 2013; Saucède et al., 2014; Griffiths and Waller 2016; González-Wevar et al., 2017). The APF might limit latitudinal movement of species enough to act as an effective barrier to connectivity between Antarctic and sub-Antarctic provinces. In fact, molecular analyses in several co-distributed Antarctic and South American invertebrates including the nemertean *Parborlasia corrugatus* (Thornhill et al., 2008), the brittle star *Astrofoma agassizii* (Hunter and Halanych 2008), the pycnogonid *Collossedeis megalonix* (Krabbe et al., 2010), the bivalve *Aequiyoldia* (González-Wevar et al., 2012; Poulin et al., 2014), and the red algae *Gigartina skottsbergii* (Billard et al., 2015) have revealed differences in evolutionary history with marked molecular divergence, showing that they have been isolated for several millions of years (Clarke et al., 2005; Barnes et al., 2006; Aronson et al., 2007; Wilson et al., 2009; González-Wevar et al., 2010, 2017; Moon et al., 2017). Integrative biogeographic studies in the Southern Ocean have demonstrated that the origin and diversification of several marine groups occurred long after the physical separation of the continental landmasses (Near et al., 2012; Dueñas et al., 2016; González-Wevar et al., 2017). Occasional dispersal has occurred across the APF on long (evolutionary) timescales, but little ongoing gene flow occurs across this front (Moon et al., 2017). On the one hand, for deep-sea organisms the ACC doesn't seem to represent an effective barrier and several groups of Southern-Ocean cephalopods colonized lower latitudes through the deep oceans (Strugnell et al., 2008). On the other hand, for shallow benthic marine invertebrates there has been almost no evidence of biological movement into the Antarctic from lower latitude since the Last Glacial Maximum, except via anthropogenic dispersal (Barnes et al., 2006; Fraser et al., 2018). Nevertheless, new records of floating kelps at sea indicate that several marine organisms may drift across the APF (Fraser et al., 2016). Moreover, recent genomic-based analyses revealed that rafting keystone kelps (*Durvillaea antarctica*) travelled for more than 20,000 km across oceanographic barriers to reach the Antarctic Peninsula from mid-latitude sub-Antarctic source populations (South Georgia and Kerguelen Islands) (Fraser et al., 2018). This new evidence highlights that Antarctica is not biologically isolated as previously proposed, that rafting is a frequent phenomenon across the ACC, and that this process could constitute a feasible transport mechanism for invasive kelp-dwelling organisms across Antarctic and

sub-Antarctic provinces. Moreover, the establishment of non-native species between Antarctic and sub-Antarctic provinces is thus presumably prevented by environmental differences and physiology rather than by transport (Fraser et al., 2018). Across the sub-Antarctic, the SAF can transport organisms between geographically distant provinces. This is especially true for species with high dispersive potential (Gérard et al., 2008; Díaz et al., 2011; Mortimer et al., 2011), kelps (Waters, 2007; Fraser et al., 2009; Macaya and Zuccarello, 2010), kelp-associated organisms (Fraser et al., 2011; Nikula et al., 2010; Cumming et al., 2014; González-Wevar et al., 2018; for a review see Moon et al., 2017), and even non-buoyant macroalgae (Fraser et al., 2013).

Recent biogeographic analyses based on taxonomic lists recognized a highly endemic Southern-Ocean biota, the presence of a single Antarctic province, and a clear separation between Antarctica and other sub-Antarctic provinces (Griffiths et al., 2009; Pierrat et al., 2013; Griffiths and Waller, 2016). During the last decade, the use of molecular tools for the taxonomic identification of organisms with conserved morphology greatly increased the number of taxa in the Southern Ocean through the recognition of cryptic species in several invertebrate groups such as crustaceans (Held and Wägele, 2005; Raupach and Wägele, 2006; Leese et al., 2008; Krabbe et al., 2010; Arango et al., 2011; Baird et al., 2011; Dietz et al., 2015), mollusks (Strugnell et al., 2008, 2012; Wilson et al., 2009; González-Wevar et al., 2010; Allcock et al., 2011), echinoderms (Wilson et al., 2007; Heimeier et al., 2010; Janosik et al., 2011; Hemery et al., 2012; Gubili et al., 2016), nematodes (Lee et al., 2017), and even macroalgae (Billard et al., 2015). In this context, the broadly distributed Southern Ocean bivalve *Aequiyoldia eightsii* shows a similar pattern with the presence of different lineages at both sides of the Drake Passage (González-Wevar et al., 2012; Poulin et al., 2014).

The genus *Yoldia* includes ~ 20 species of marine bivalves that are broadly distributed around the globe. Until 2013, the higher-latitude species, *Aequiyoldia eightsii*, was included within *Yoldia* and exhibited a broad distribution across different Southern-Ocean provinces such as South America, the Antarctic Peninsula, and the Kerguelen Islands. Nevertheless, multi-locus molecular reconstructions identified *Yoldia* as paraphyletic with the Antarctic *A. eightsii* completely separated from the rest of the species (Sharma et al., 2013). Following this, higher latitude *A. eightsii* has been included in the genus *Aequiyoldia* Soot-Ryen, 1951. This genus of saretid bivalves includes shallow-burrowing deposit feeders that are currently distributed in Antarctic and sub-Antarctic soft-substratum ecosystems. *Aequiyoldia* includes two nominal species: *A. defossata*, a Pliocene fossil from East Antarctica (Quilty et al., 2016) and the broadly distributed *A. eightsii* (Jay, 1839). Several synonymous species have been grouped within *A. eightsii*, including *Yoldia kerguelensis* (Thiele, 1931) and *Y. subaequilateralis* (E.A. Smith, 1875) from the Kerguelen Islands, and *Yoldia woodwardi* (Hanley, 1860) from the Falkland/Malvinas Islands.

The absence of clear diagnostic characters and the highly conserved morphology of protobranch bivalves impose major taxonomic uncertainties within this group (Bouchet and Huber, 2015; Sartori, 2016a, 2017b). In fact, Antarctic Peninsula and South American populations of *A. eightsii* are morphologically identical, although Antarctic individuals are larger than those recorded off South America (Villarroel and Stuardo, 1998). At the same time, morphological characteristics of the Kerguelen Islands species, *A. kerguelensis* and *A. subaequilateralis*, completely overlap those recorded for *A. eightsii* from Antarctic Peninsula and South America (Rabarts and Whybrow, 1979). Following this, *A. eightsii* is currently recognized in different Southern-Ocean provinces including the Falkland/Malvinas Islands, Kerguelen Islands, Antarctic Peninsula, and South Shetland Islands (Linse, 2014). However, recent molecular analyses using the mitochondrial locus cytochrome *c* oxidase subunit I (COI) recognized more than 7% divergence between South American and Antarctic Peninsula populations of *A. eightsii*, which is far beyond the intraspecific level (González-Wevar et al., 2012; Poulin et al., 2014).

In this study, we performed phylogenetic reconstructions, divergence-time estimations and morphological comparisons of *Aequiyoldia* populations from different provinces of the Southern Ocean. We extended previous molecular and morphological analyses by including *Aequiyoldia* populations from the Kerguelen Islands and the Falkland/Malvinas Islands. We also included more populations from South America and from Antarctic Peninsula. Through this, we obtained a clear picture about the evolutionary relationships within *Aequiyoldia*, the taxonomic status of different populations across the distribution of the group, the number of evolutionary units, and their respective divergences based on mitochondrial (COI) gene sequences. We also compared *A. eightsii* populations using nucDNA sequences (ITS1, ITS2) to corroborate the biogeographic patterns recorded with the mitochondrial markers. Finally, shell morphometry was conducted by applying the criteria described by Rabarts and Whybrow (1979).

2. Material and methods

2.1. Sample collection

Aequiyoldia eightsii specimens were collected by SCUBA divers at

Port-aux-Français, Kerguelen Islands (KI) and at Hookers Point, Falkland/Malvinas Islands (FI). South American samples of *Aequiyoldia* were collected across the Strait of Magellan at: (1) Porvenir, (2) San Juan River, and (3) Skyring Sound. Finally, Antarctic samples were collected at (1) Admiralty Bay, King George Island, South Shetland Islands, (2) Fildes Bay, King George Island, South Shetland Islands, (3) Deception Island, and (4) Marguerite Bay, Adelaide Island (Figs. 1 and 2, Table 1). All specimens were stored in 96% ethanol for future molecular analyses. For comparative purposes, we also included one individual of *Portlandia isonota* collected off the Kerguelen Islands.

2.2. DNA preparation and sequence edition

Genomic DNA was extracted from mantle and branchial tissues using the E.Z.N.A. Mollusc DNA Kit (Omega bio-tek). A 621-bp fragment of the cytochrome *c* oxidase subunit I (COI) was amplified using universal primers (Folmer et al., 1994). The PCR program consisted of 5 min at 95 °C followed by 40 cycles at 95 °C for 40 s, 45 °C for 40 s, 72 °C for 1 min, and a final extension of 7 min at 72 °C. Fragments of up to 1190 bp containing the internal transcribed spacers 1 and 2 (ITS1 and ITS2) were amplified using universal primers (Oliverio and

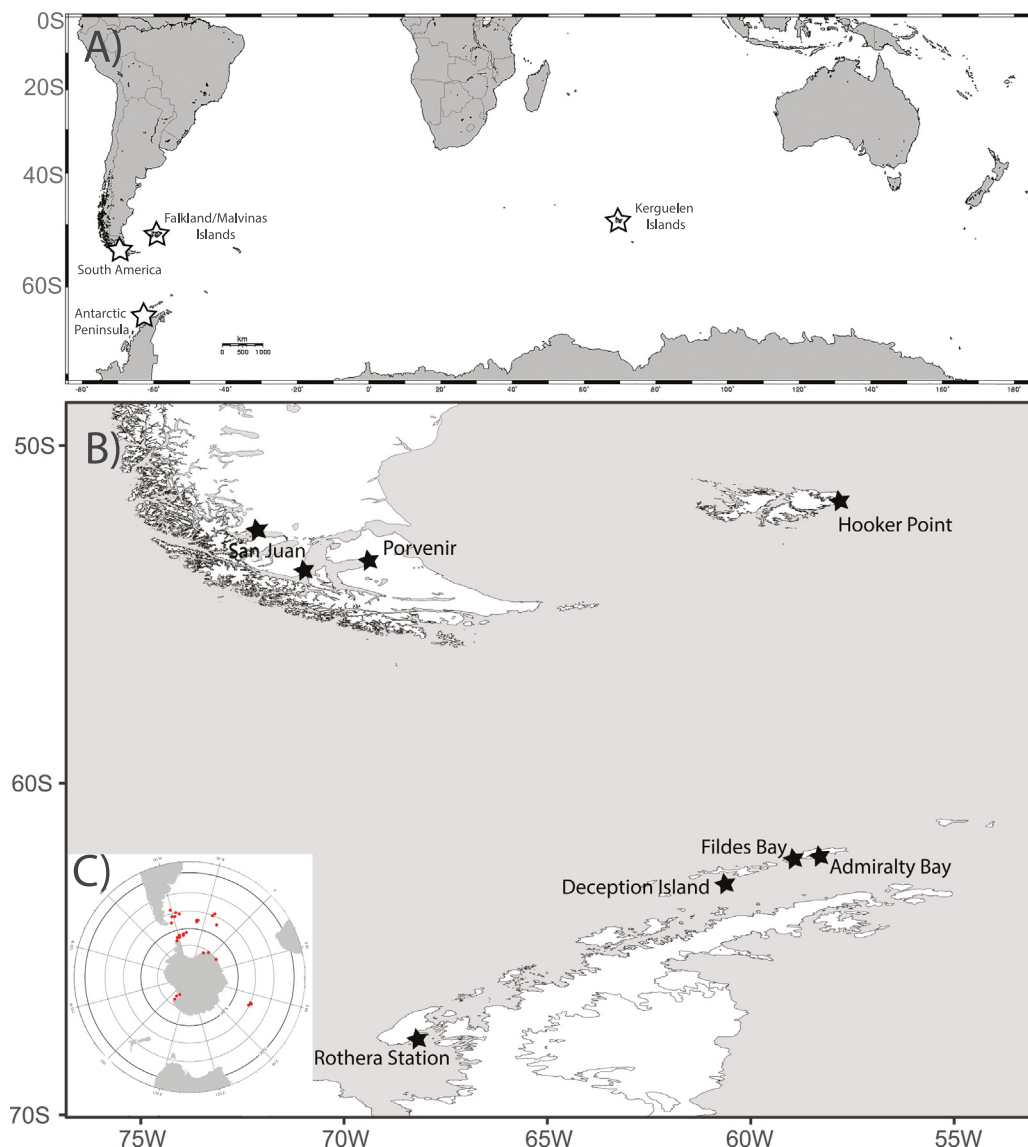


Fig. 1. (A) Main Southern Ocean provinces included in the study. (B) Sampling sites of *Aequiyoldia eightsii* off South America (SA), Falkland/Malvinas Islands (FI), and Antarctic Peninsula (AP). (C) Distribution of *Aequiyoldia eightsii* in the Southern Ocean (Linse, 2014).

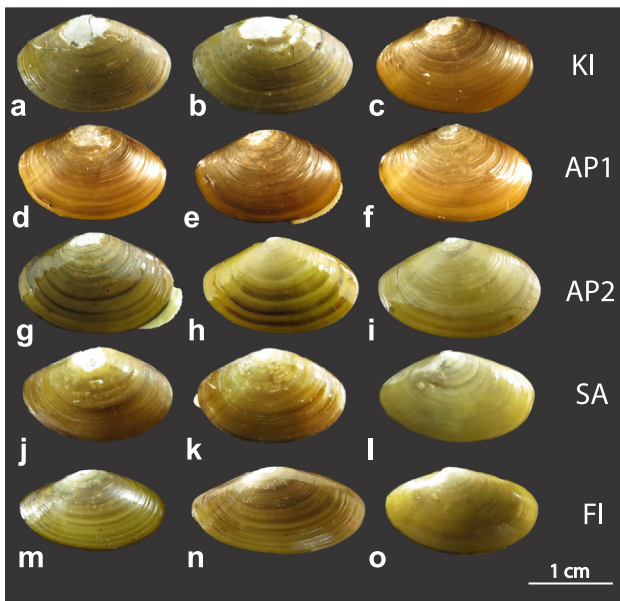


Fig. 2. *Aequiyoldia eightstii* individuals from: (a–c) Kerguelen Islands (KI = L1), (d–f) Rothera Station (AP1 = L2), (g–i) Admiralty Bay (AP2 = L3), (j–l) South America (SA = L4), and (m–o), and the Falkland/Malvinas Islands (FI = L5).

Table 1

Sampling sites of *Aequiyoldia eightstii*: Geographical coordinates, n: number of analyzed individuals per locus.

| Sites | Lat. | Long. | n (COI) | n (ITS) |
|--|---------|---------|---------|---------|
| Port-aux-Français, Kerguelen Islands | 49°21'S | 70°13'W | 11 | 9 |
| Hookers Point, Falkland/Malvinas Islands | 51°42'S | 57°46'W | 20 | 7 |
| San Juan River, Strait of Magellan | 53°38'S | 70°56'W | 20 | 9 |
| Porvenir, Tierra del Fuego | 53°18'S | 70°22'W | 5 | – |
| Skyring Sound, Strait of Magellan | 52°34'S | 70°56'W | – | 3 |
| Admiralty Bay, South Shetland Islands | 62°09'S | 58°27'W | 6 | – |
| Fildes Bay, South Shetland Islands | 62°12'S | 58°57'W | – | – |
| Deception Island, South Shetland Islands | 62°56'S | 60°36'W | 20 | 8 |
| Rothera Station, Antarctic Peninsula | 67°34'S | 68°07'W | 20 | – |

Mariottini, 2001). The PCR program was 5 min at 95 °C, then 40 cycles at 95 °C for 1 min, 54 °C for 1 min, 72 °C for 1 min, and a final extension of 7 min at 72 °C. Amplified PCR products were purified using QIAquick Gel Extraction Kit (QIAGEN) and sequenced in both direction with an Automatic Sequencer ABI3730 XL at Macrogen Inc. (Seoul, South Korea). Chromatograms were inspected visually, and multiple alignments were obtained using MUSCLE (Edgar, 2004) implemented in CodonCode Aligner v7.1 (CodonCode Corporation). Base composition of nucleotide sequences was analysed with MEGA 6 (Tamura et al., 2013) and codon usage was determined using the effective number of codon value (ENC; Wright, 1990) with DnaSP 5.0 (Librado and Rozas, 2009).

2.3. Phylogenetic reconstructions

Mitochondrial reconstructions included the different haplotypes of *Aequiyoldia* recorded in SA, AP, FI, and KI. For comparative purposes, we included other protobranch genera (*Yoldiella*, *Yoldia*, *Tindaria*, *Neilonella*) in the reconstructions following Sharma et al., (2013). Phylogenetic relationships were estimated using maximum parsimony (MP), maximum likelihood (ML), and Bayesian analyses (BA). MP analyses were performed using MEGA v.6.0 (Tamura et al., 2013) and the following assumptions: characters were treated as equally weighted using a heuristic search and tree bisection reconnection (TBR) with the branch swapping option. Non-parametric bootstrap (BS) values were

used to infer nodal support for both the MP and ML with 1000 pseudo-replicates (Felsenstein, 1981). Nucleotide substitution models for ML and BA analyses were selected using the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC), with jmodeltest v.2.0 (Darrriba et al., 2012), respectively. The GTR+I+G (COI) substitution model was used for ML and BA analyses using the programs MEGA and MrBayes 3.1.2. (Huelsenbeck and Ronquist, 2001). Bayesian inference posterior probabilities were estimated using the Metropolis coupled Markov chain Monte Carlo algorithm (MCMC), running four chains for 50×10^6 generations with trees sampled every 1000 generations. Stationarity was inferred when the average standard deviation of split frequencies was less than 0.01 (Huelsenbeck and Ronquist, 2001). The initial 10% of the trees were discarded (burn-in), and posterior probabilities were estimated as the fraction of trees showing a particular node. Posterior probability density was summarized as a Maximum Clade Credibility Tree (MCCT) using TreeAnnotator v.1.6.1 (<http://beast.bio.ed.ac.uk/TreeAnnotator>) and visualized with FigTree v.1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree>). For comparative purposes, we also reconstructed mtDNA and nucDNA genealogical relationships within sub-Antarctic and Antarctic *Aequiyoldia* populations using maximum-likelihood networks in Hapview (Salzburger et al., 2011).

2.4. Divergence time estimations

Relaxed molecular clock analyses were used for mtDNA sequences with an uncorrelated lognormal (ucln) model of molecular evolutionary rate heterogeneity and the GTR+I+G substitution model implemented in BEAST v.1.6.2 (Drummond and Rambaut, 2007; Drummond et al., 2012). An age prior with a normal distribution was applied (mean, 44; SD 4.4) to the time of the oldest Antarctic *Aequiyoldia* fossil from La Meseta Formation during the Middle Eocene (Beu, 2009). At the same time, we included another prior within *Aequiyoldia* from Antarctica: *A. defossata* (mean, 5, SD, 0.5), a Pliocene fossil from Vestfold Hills, East Antarctica (Quilty et al., 2016). A birth-death speciation prior was used for branching rates in the phylogeny; four chains were run twice for 50×10^6 generations, and trees were sampled every 1000 generations. The convergence of model parameters was estimated by plotting marginal posterior probabilities versus the generation state in TRACER 1.5 (<http://beast.bio.ed.ac.uk/Tracer>). Effective sample size values were estimated for each parameter to ensure adequate mixing of the MCMC (ESSs > 1000).

2.5. Morphological analysis

Shell morphology of *Aequiyoldia eightstii* populations from different provinces of the Southern Ocean (Fig. 2) was characterized following the criteria of Rabarts and Whybrow (1979). Callipers were used to measure three dimensions for each individual shell: H = Height, L = Length, and W = Width. Discriminating scores (D) were obtained through the following formula $D = 6H - (3L + 2W)$. The matrix of D was then analyzed through non-metric, multidimensional scaling (NMDS) using Euclidean distances.

3. Results

3.1. DNA polymorphism

Phylogenetic reconstructions including populations of *Aequiyoldia eightstii* revealed clearly discriminated evolutionary lineages associated with different provinces of the Southern Ocean (Figs. 3 and 4, Table 2). For instance, *A. eightstii* populations from KI differed markedly (> 19%) from those collected in other the Southern Ocean provinces (AP, SA, FI). In fact, more than 27 amino acid substitutions separated KI from the rest of the analyzed populations (AP, SA, FI). No indels or stop codons were identified within *A. eightstii* populations from AP, SA, or FI.

A total of eight amino-acid substitutions were recorded among individuals from these areas (see below). Mitochondrial DNA alignments (621 bp) included 79 variable positions (12.72%) of which 73 (92.4%) were parsimoniously informative. Nuclear ITSs (1190 bp) were more conserved with only 28 variable positions (2.35%), 19 of which (67.85%) were parsimoniously informative. Mitochondrial and nuclear sequences were not saturated at any position and no evidence for codon bias was detected for COI (ENC = 35.89).

3.2. Phylogenetic relationships

Phylogenetic reconstructions from mitochondrial data were consistent with previous analyses in protobranch bivalves (Sharma et al., 2013) that discriminated major taxonomic protobranch groupings with high bootstrap and posterior probabilities. For instance, the single individual of *Portlandia isonota* from KI fell within the diversity of available *Yoldiella* sequences from the Northern Hemisphere (Fig. 3). At the

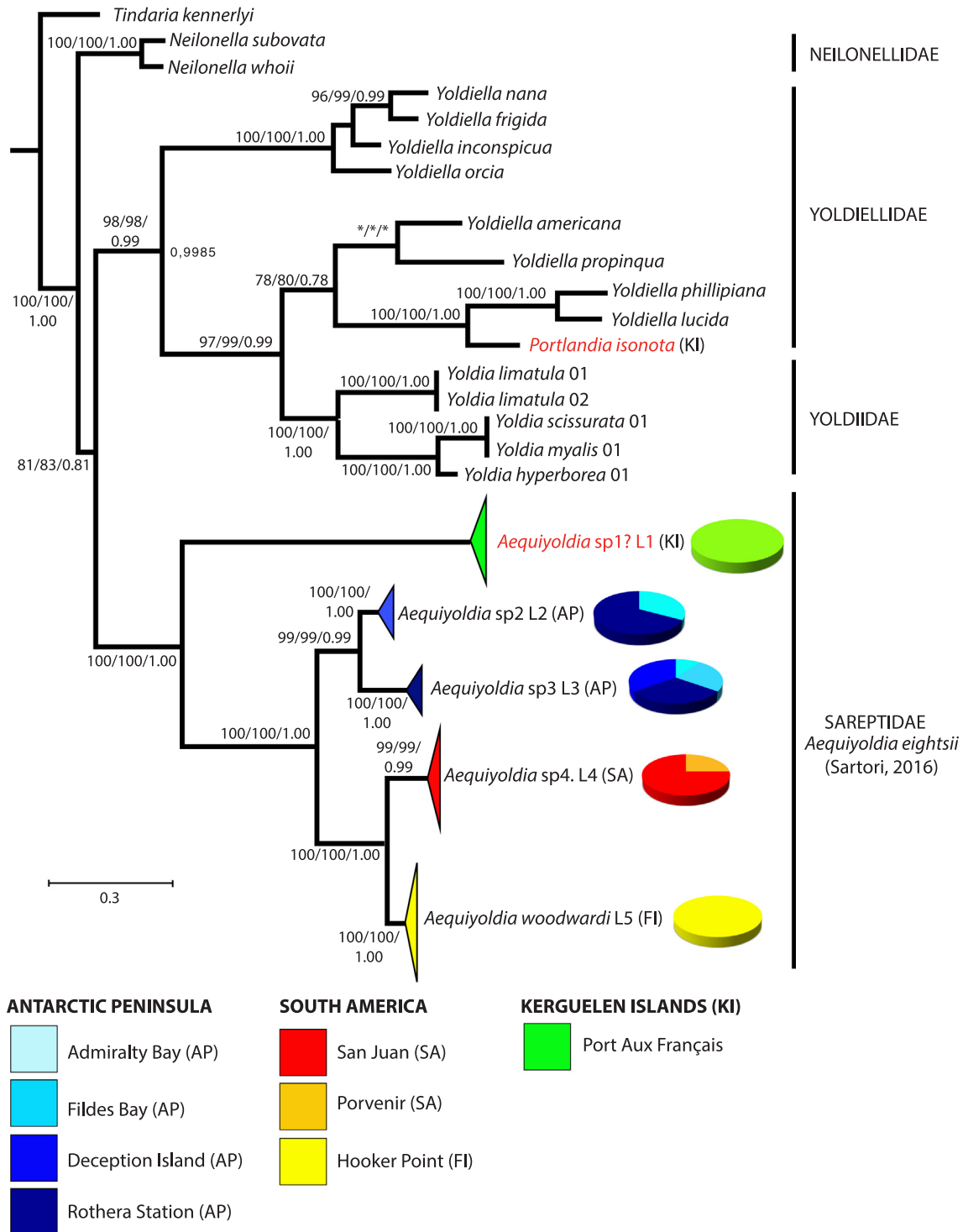


Fig. 3. Bayesian maximum clade credibility tree of *Aequiyoldia* relationships based on mtDNA (COI) sequences with special emphasis on Antarctic Peninsula and South American populations. Bootstrap support values (MP and ML) and BPP are shown above the nodes (in that order). Map and colors indicate major sampled areas. Round charts indicate the frequency of the recorded *Aequiyoldia* lineages across the sampling sites.

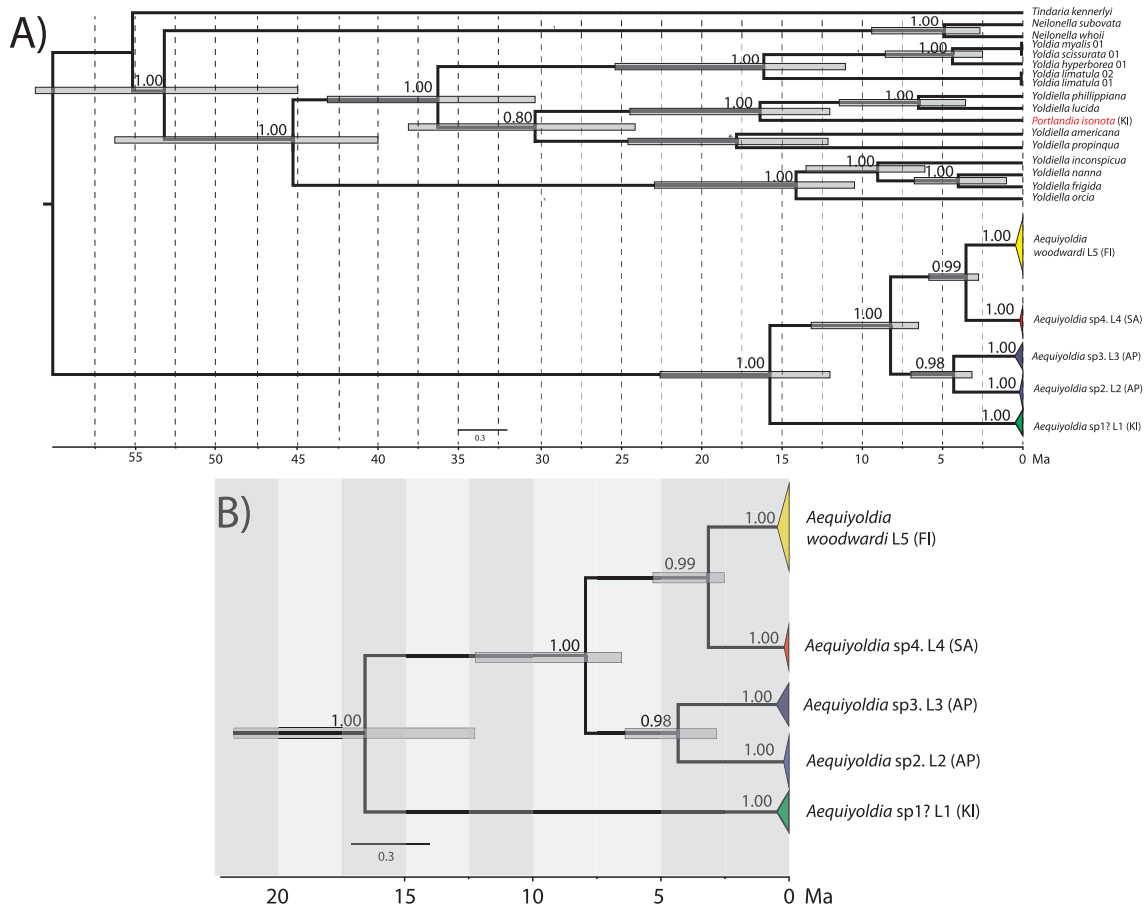


Fig. 4. Bayesian maximum clade credibility tree based on mtDNA (COI) showing divergence time estimates within (A) selected protobranch bivalves and (B) *Aequiyoldia*. Posterior probabilities are shown above the clades. Grey shaded regions depict 95% Bayesian credible intervals (BCIs) for relative divergence times.

Table 2

Uncorrected p-distances based on mtDNA COI sequences between the recorded lineages of *Aequiyoldia* from Kerguelen Islands (KI), the Antarctic Peninsula (AP), South America (SA), and the Falkland/Malvinas Islands (FI).

| | KI | AP1 | AP2 | SA | FI |
|-----|--------|-------|-------|-------|------|
| KI | **** | | | | |
| AP1 | 19.96% | **** | | | |
| AP2 | 20.61% | 5.79% | **** | | |
| SA | 20.45% | 6.60% | 8.53% | **** | |
| FI | 19.80% | 5.47% | 6.92% | 3.86% | **** |

same time, these analyses highlighted the presence of several evolutionary lineages within the nominal species, *A. eightsii* (Fig. 3). The monophyly of *Aequiyoldia eightsii* was highly supported by the different markers and reconstruction methods. Nevertheless, the high divergence level (> 19%) and phylogenetic position of *A. eightsii* individuals from KI (Fig. 3) allowed us to discriminate them as a different species. However, the exact systematic position of KI individuals remains uncertain. Although KI specimens may represent a basal branch of *Aequiyoldia*, considering the high level of genetic divergence, we tend to think that they constitute a different *Aequiyoldia* species endemic to KI. We recognized the presence of at least two evolutionary lineages in AP that were sympatric at two sites (i.e., Rothera Station and Admiralty Bay) (Fig. 3). We also recognized the presence of a different lineage in SA and another endemic to the FI (Fig. 3). Genetic divergence between Antarctic and South American *A. eightsii* populations ranged between 6.3% and 7.5% (Table 2), whereas these levels were lower between SA and FI populations (4%) and among the different AP units (~3%). In this context, levels of mtDNA (COI) genetic divergence recorded among

lineages of *Aequiyoldia* in the Southern Ocean (Table 2) are higher (between 20.61% and 3.86%) than those recorded in congeneric species ($n = 13,320$) of different taxonomic groups where most pairs of species (98%) showed greater than 2% sequence divergence (Hebert et al., 2003). Moreover, levels of genetic divergence recorded among *Aequiyoldia* lineages are similar and even higher than those recorded between clearly established *Yoldiella* species (Mikkelsen et al., 2017).

3.3. Divergence time estimates

Divergence time estimates based on mtDNA sequences suggested that the *A. eightsii* populations from KI separated from the remaining lineages during the middle Miocene c. 16.5 Ma (22.5–12 Ma) (Fig. 4). Following this, the separation between Antarctic and South American lineages occurred at the end of the Miocene, c. 8.5 Ma (13–6.5 Ma) (Fig. 4). Finally, the diversification of *Aequiyoldia* in AP and SA occurred during the Pliocene, beginning with the separation between the AP lineages ~ 4.5 Ma (6.5–3.5 Ma), followed by the separation between SA and FI ~ 3.0 Ma (5.5–2.5 Ma) (Fig. 4).

3.4. Morphological analysis

The nMDS analysis separated individuals of *A. eightsii* into two main groups (Fig. 5). The first included all individuals assigned to the broadly distributed nominal species *A. eightsii* collected at AP, SA, and KI, which exhibited a u-shaped pallial sinus. The second group included individuals from FI that represented a different morphological entity that exhibited a v-shaped pallial sinus.

4. Discussion

4.1. Cryptic speciation in the Southern Ocean

Recent molecular studies in broadly distributed Southern-Ocean organisms have demonstrated the presence of several cryptic species complexes. Examples include crustaceans of the genus *Eusirus* (Baird et al., 2011) and *Serolis* (Leese et al., 2008), the nudibranch *Doris kerguelensis* (Wilson et al., 2009), octopuses of the genus *Pareledone* (Allcock et al., 2011), pycnogonids of the genus *Nymphon* (Arango et al., 2011), *Pallenopsis* (Weis et al., 2014; Harder et al., 2016), *Colossendeis* (Dietz et al., 2015), the brittle star *Astrotooma* (Heimeier et al., 2010) the ophiurid *Promachochrinus* (Hemery et al., 2012), the macroalgae genus *Gigartina* (Billard et al., 2015), and *Durvillaea* (Fraser et al., 2009). Given the high number of cryptic species complexes discovered in the Southern Ocean, the region's biodiversity has been severely underestimated. As recorded in *Aequiyoldia*, cryptic species complexes consist of closely related species with incomplete morphological differentiation as opposed to distantly related species that have acquired a misleading degree of morphological similarity through other evolutionary processes (i.e., convergent evolution) (Koubbi et al., 2014). Besides increasing diversity patterns and species counts, the discovery of cryptic species often results in a shift from a small number of broadly distributed, morphologically variable species to a series of less variable species with smaller, often allopatric distributions. Notable exceptions of newly delimited species with larger distributions in the Southern Ocean do exist (Fraser et al., 2009; Nikula et al., 2010; Cumming et al., 2014; González-Wevar et al., 2018). New molecular advances have started to change our understanding of circumpolar and cosmopolitan species, indicating the need for further, more detailed

taxonomic and integrative biogeographic work on potential cryptic species (Grant et al., 2010). Biogeographic patterns may change with increased knowledge of diversity both at the genetic and species levels and how much these patterns are likely to change is still unclear. Species numbers in the Southern Ocean are likely to increase with further sampling, taxonomic work, and molecular analyses, helping provide a better understanding of global diversity, distribution, and evolutionary history (Griffiths et al., 2011).

4.2. Cryptic speciation in *Aequiyoldia*

The highly conserved morphology and absence of genetic data led researchers to synonymize several nominal species of *Aequiyoldia* (i.e., *A. kerguelensis*, *A. subaequilateralis*, *A. woodwardi*) into a single, nominal, broadly distributed species: *A. eightsii*. Accordingly, *A. eightsii* has exhibited an extended distribution across the sub-Antarctic from South America to the Kerguelen Island as well as a circum-Antarctic distribution (Linse, 2014). Herein, the monophyly of *Aequiyoldia* in the Southern Ocean was supported by mtDNA sequences and different reconstruction methods. Mitochondrial and nuclear diversity within *Aequiyoldia* across its distribution (AP, SA, FI, and KI) as well as its morphological characterization uncovered the presence of at least five cryptic species currently distributed in AP, SA, FI and KI. We found a clear distinction between sub-Antarctic and Antarctic lineages of *A. eightsii*, corroborating previous molecular studies in the group (González-Wevar et al., 2012, 2017; Poulin et al., 2014). In Falkland/Malvinas Islands, we recorded the presence of an endemic lineage clearly separated from its closest continental South American relative. Finally, along the Antarctic Peninsula, we recorded at least two sympatric evolutionary units in Marguerite Bay (West Antarctic Peninsula)

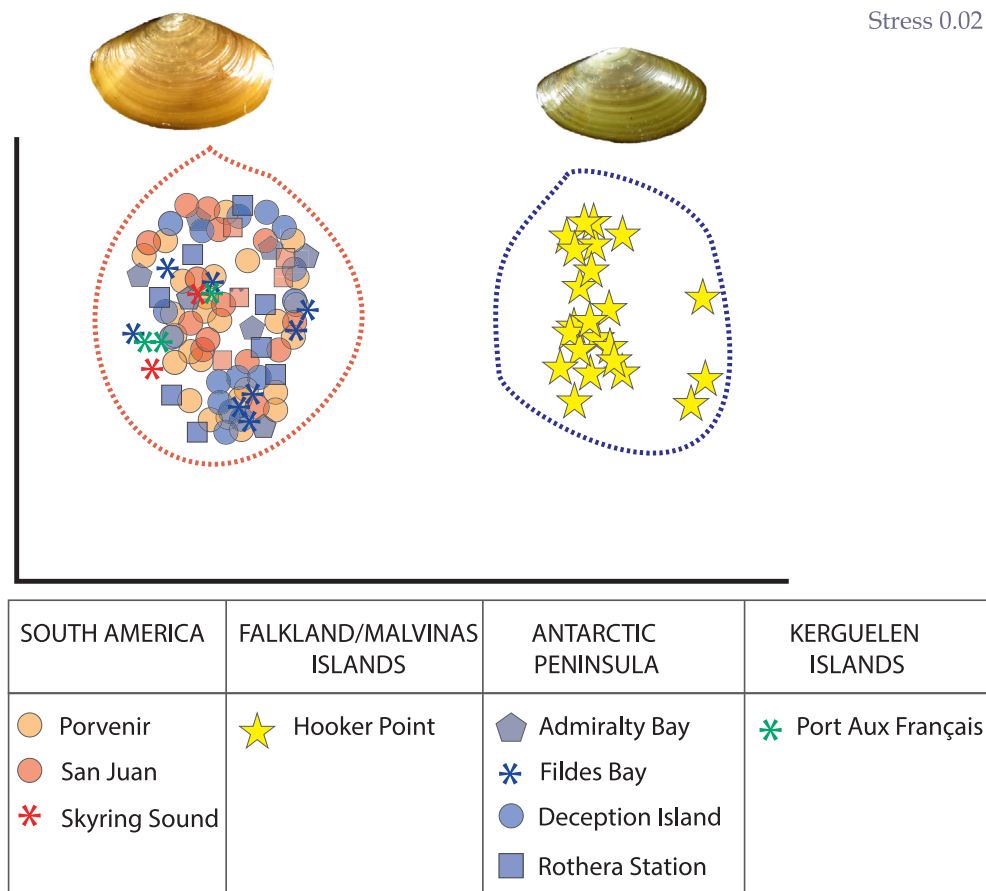


Fig. 5. Non-metric, Multidimensional Scaling (NMDS) ordination of *A. eightsii* samples from the Kerguelen Islands (KI), Falkland/Malvinas Islands (FI), South America, and the Antarctic Peninsula using shell morphological data following Rabarts and Whybrow (1979).

and Admiralty Bay (South Shetlands).

4.3. A highly divergent *Aequiyoldia* lineage in Kerguelen Islands

The *A. eightsii* population from KI was the most divergent and showed a basal position in the phylogenies. All COI haplotypes from KI clustered together in a monophyletic group separated from the remaining lineages from AP, SA, and FI by more than 19% divergence (Table 2). Such level of divergence could not be corroborated by nuclear data, since ITSs sequences from KI did not align properly with those from AP, FI, and SA. However, it may also reflect a long divergence history between KI lineage from the South American and Antarctic ones. Hence, the amount of molecular divergence accumulated in KI was extreme, despite morphological similarities among the KI, AP, and SA populations. Because of its geographic isolation, Kerguelen Islands is indeed characterized by a high level of endemism in a variety of terrestrial and marine species (Briggs, 1966; McDowall, 1968; Poulin and Féral, 1995; Hennion and Walton, 1997; Brandt et al., 1999; Chapuis et al., 2000; Emerson, 2002; Ledoux et al., 2012; González-Wevar et al., 2017). Consequently, this sereptid bivalve endemic to KI should clearly be considered as a different *Aequiyoldia* species. Accordingly, and considering that an *Aequiyoldia* species has been previously described in Kerguelen, our study supports the re-validation of *A. kerguelensis* (Thiele, 1931).

4.4. Trans-Drake Passage speciation: new *Aequiyoldia* species in South America

Phylogenetic reconstructions and molecular divergence within *Aequiyoldia* support previous Antarctic and South American population-based comparisons in the group (González-Wevar et al., 2012; Poulin et al., 2014) by recognizing the presence of different evolutionary lineages on both sides of the Drake Passage. Such conclusions are supported by the mtDNA (6.5–7.5%) and nucDNA (1.2%) molecular divergence recorded between Antarctic Peninsula and South American *Aequiyoldia* populations (Suppl. Fig. 1). Consequently, we suggest that South American *A. eightsii* deserves specific status and that integrative taxonomic revisions are required within *Aequiyoldia*.

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.pocan.2018.09.004>.

4.5. Re-validating *Aequiyoldia woodwardi* (Hanley, 1860)

The morphological revision of *Aequiyoldia* shells confirmed the current presence of a broadly distributed morphotype in different provinces including AP, SA, and KI (Fig. 4). In a revision of the group, Villarroel and Stuardo (1998) concluded that the external anatomy of *A. eightsii* individuals from South America and Antarctic Peninsula is very similar but they differ in their respective sizes, being the Antarctic populations bigger than the South American ones. However, molecular data do not support the presence of a single genetic unit across the Southern Ocean. Rather, the data highlighted the presence of hidden cryptic diversity with at least five evolutionary lineages. Together with this broadly distributed morphotype, we also recorded another one currently restricted to FI. Mitochondrial (COI) and nuclear (ITSs) sequences of *Aequiyoldia* individuals from FI revealed the presence of a monophyletic group in these islands separated from its sister South-American lineage by 4% and 0.5% of divergence, respectively (Suppl. Fig. 1). Such molecular results corroborated previous morphological revision in the group (Rabarts and Whybrow, 1979) that recorded the presence of a different species off these islands: *Yoldia woodwardi* Hanley, 1860. Consequently, based on morphological and molecular evidence, we propose a revalidation of the specific status conferred on the *Aequiyoldia* from FI and propose *Aequiyoldia woodwardi* be used for this species. These results support recent population-based studies of several marine, near-shore, benthic invertebrates including isopods of

the genus *Serolis* (Leese et al., 2008), the patellogastropods *Nacella magellanica* (González-Wevar et al., 2012) and *Nacella mytilina* (González-Wevar et al., 2016), and pulmonates of the genus *Siphonaria* (González-Wevar et al., 2018); all those studies recognized FI and SA populations as different genetic and sometimes as evolutionary units. As stated by Leese et al. (2008), whereas the biota of FI shared most of their faunal inventory with SA, molecular studies have demonstrated that shallow benthic species may, in fact, be strongly differentiated or even reproductively isolated. The genetic differentiation between SA and FI populations of *Aequiyoldia* may be a consequence of their distinct glaciological histories during the coldest glacial periods. The southern tip of South America was almost completely covered by ice during the last Glacial Maximum (LGM), and shallow marine habitats were largely absent. In contrast, little evidence of ice advances during the LGM existed in FI apart from small cirques and short glacially eroded valleys (Clapperton and Sugden, 1976; Clapperton, 1994; Hodgson et al., 2014), and no study shows evidence of LGM glaciers extending offshore (Wilson et al., 2002, 2008).

4.6. Divergent lineages in *Aequiyoldia* off the West Antarctic Peninsula

Phylogenetic reconstructions and levels of mtDNA divergence, as well as divergence time estimates indicated the presence of at least two different *Aequiyoldia* lineages off the Antarctic Peninsula. These lineages are separated by 5.78% of mtDNA divergence and are currently found in sympatry in Marguerite Bay and off the South Shetland Islands. These divergent Antarctic *Aequiyoldia* lineages could either be the consequence of sympatric cryptic species or persistent ancestral polymorphism with incomplete lineage sorting. To discriminate between these hypotheses, additional nuclear genetic data are required.

4.7. Biogeography in *Aequiyoldia* spp.

Molecular data suggest that the tmrca of *Aequiyoldia* spp., separating KI from the rest of the analyzed provinces of the Southern Ocean (AP, SA and FI), occurred c. 16.5 Ma (22.5–12 Ma), close to the middle Miocene transition (MMCT). This period represents an epoch of major oceanographic and climatic changes in the Southern Ocean that were probably associated with the intensification of the ACC and the re-establishment of a continental Antarctic Ice Sheet (Zachos et al., 2001; DeConto and Pollard, 2003; Mackensen, 2004; Verducci et al., 2009). Major oceanic circulation changes during this period were probably associated with the full development of a deep ACC, a process that was only achieved during the late Miocene around 12 Ma (Dalziel et al., 2013). As recorded for other Southern-Ocean invertebrates, fluctuations in latitudinal positioning and strengthening of the ACC during this period seemed to be important drivers in the separation of lineages over geographically distant Southern-Ocean provinces (González-Wevar et al., 2017). Alternatively, the establishment of a strong and deep ACC may have generated an effective oceanographic barrier that can be invoked to explain the diversification of *Aequiyoldia* in different areas of the Southern Ocean. Following this, the separation between Antarctic and South-American lineages of *Aequiyoldia* occurred at the end of the Miocene c. 8.5 Ma (13–6.5 Ma).

In this context, late Miocene phases of Southern Ocean diversifications have been identified in different groups of marine invertebrates including some octopus clades (Strugnell et al., 2008), octocorals (Dueñas et al., 2016), patellogastropods (González-Wevar et al., 2017), muricid gastropods (Barco et al., 2012), and fishes (Near et al., 2012). For instance, the limpet genus *Nacella* shows a particular radiation at the end of the Miocene 8.5–7.0 Ma (González-Wevar et al., 2017), and the muricid gastropod of the subfamily Pagodulinae diversified in the Southern Ocean between the late Miocene and Pliocene (Barco et al., 2012). Following this, during the Pliocene, *Aequiyoldia* diversified both off South America and across the Antarctic Peninsula. In both Antarctic and South America, this diversification started around ~ 4.5 Ma

(6.5–3.5 Ma) and ~ 3.0 Ma (5.5–2.5 Ma), respectively. Diversification processes during the Pliocene have been reported for other Southern-Ocean marine benthic invertebrates including the Antarctic amphipods of the genus *Epimeria* (Verheye et al., 2017), deep-sea octopuses (Strugnell et al., 2008), and deep-sea octocorals (Dueñas et al., 2016). Molecular population-based studies have provided strong evidence from a wide range of marine and terrestrial taxa that fragmentation of populations into refugia during glacial maxima and subsequent expansions during interglacial periods may represent an important mechanism enhancing cryptic speciation processes in marine (Wilson et al., 2009; Arango et al., 2011; Baird et al., 2011; Allcock and Strugnell, 2012; Fraser et al., 2012; Poulin et al., 2014) and terrestrial (Stevens and Hogg, 2003, 2006; Stevens et al., 2007; McGeachran et al., 2010) ecosystems. Indeed, such processes are also probably associated to the strong patterns of regionalization and high levels of endemism recorded on terrestrial ecosystems (Chown & Convey, 2007; Convey & Stevens, 2007; Convey et al., 2008, 2009; Convey, 2010). As observed in terrestrial Antarctic ecosystems, the presence of isolated marine refugia during glacial maxima may have enhanced such process and the re-colonization of newly de-glaciated habitats could have favoured the recent radiation recorded in *Aequiyoldia* off South America and the Antarctic Peninsula.

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References

Allcock, A., Barratt, I., Eleaume, M., Linse, K., Norman, M., Smith, P., Steinke, D., Stevens, D.W., Strugnell, J.M., 2011. Cryptic speciation and the circumpolarity debate: A case study on endemic Southern Ocean octopuses using the COI barcode of life. *Deep-Sea Res. II* 58, 242–249.

Allcock, A., Strugnell, J., 2012. Southern Ocean diversity: new paradigms from molecular ecology. *Trends Ecol. Evol.* 27, 520–528.

Arango, C., Soler-Membrives, A., Miller, K., 2011. Genetic differentiation in the circum-Antarctic sea spider *Nymphon australe* (Pycnogonida; Nymphonidae). *Deep-Sea Res. II* 58, 212–219.

Aronson, R., Thatje, S., Clarke, A., Peck, L., Blake, D., Wilga, C., Seibel, B., 2007. Climate change and invasibility of the Antarctic benthos. *Annu. Rev. Ecol. Evol. S.* 38, 129–154.

Baird, H.P., Miller, K., Stark, J.S., 2011. Evidence of hidden biodiversity, ongoing speciation and diverse patterns of genetic structure in giant Antarctic amphipods. *Mol. Ecol.* 20, 3439–3454.

Barco, A., Schiaparelli, S., Houart, R., Oliverio, M., 2012. Cenozoic evolution of Muricidae (Mollusca, Neogastropoda) in the Southern Ocean, with the description of a new subfamily. *Zool. Scripta* 41, 596–616.

Barnes, D., Hodgson, D., Ryan, P., Allen, C., Clarke, A., 2006. Incurion and excursion of Antarctic biota: past, present and future. *Global Ecol. Biogeogr.* 15, 121–142.

Beu, A.G., 2009. Before the ice: Biogeography of Antarctic Paleogene molluscan faunas. *Palaeogeogr. Palaeoclim. Palaeoecol.* 284, 191–226.

Billard, E., Reyes, J., Mansilla, A., Faugeton, S., Guillemin, M.-L., 2015. Deep genetic divergence between austral populations of the red alga *Gigartina skottsbergii* reveals a cryptic species endemic to the Antarctic continent. *Polar Biol.* 38, 2021–2034.

Bouchet, P., Huber, M., 2015. *Aequiyoldia*. In: MolluscaBase. Accessed through WoRMS: World Register of Marine Species. <http://www.marinespecies.org/aphia.php>.

Brandt, A., Linse, K., Mühlenhardt-Siegel, U., 1999. Biogeography of Crustacea and Mollusca of the Subantarctic and Antarctic regions. *Sci. Mar.* 63, 383–389.

Briggs, J.C., 1966. Oceanic islands, endemism, and marine paleotemperatures. *Syst. Biol.* 15, 153–163.

Chapuis, J.-L., Hennion, F., Le Roux, V., Le Cuizat, J., 2000. Growth and reproduction of the endemic cruciferous species *Pringlea antiscorbutica* in Kerguelen Islands. *Polar Biol.* 23, 196–204.

Chown, S.L., Convey, P., 2007. Spatial and temporal variability across life's hierarchies in the terrestrial Antarctic. *Phil. T. R. Soc. B* 362, 2307–2331.

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

Clapperton, C.M., Sugden, D.E., 1976. The maximum extent of glaciers in part of west Falkland. *J. Glaciol.* 17, 73–77.

Clapperton, C., 1994. The quaternary glaciation of Chile: a review. *Rev. Chil. Hist. Nat.* 67, 369–383.

Clarke, A., Crame, J., 1989. The origin of the Southern Ocean marine fauna. *J. Geol. Soc. Lond.* 47, 253–268.

Clarke, A., Crame, J., Stromberg, J., Barker, P., 1992. The Southern Ocean benthic fauna and climate change: a historical perspective [and discussion]. *Philos. S. T. Soc. B.* 338, 299–309.

Clarke, A., Barnes, D., Hodgson, D., 2005. How isolated is Antarctica? *Trends Ecol. Evol.* 20, 1–3.

Convey, P., Stevens, M.I., 2007. Antarctic biodiversity. *Science* 317, 1877e1878.

Convey, P., Gibson, J.A.E., Hillenbrand, C.-D., Hodgson, D.A., Pugh, P.J.A., Smellie, J.L., Stevens, M.I., 2008. Antarctic terrestrial life – challenging the history of the frozen continent? *Biol. Rev.* 83, 103–117.

Convey, P., Stevens, M.I., Hodgson, D.A., Smellie, J.L., Hillenbrand, C.-D., Barnes, D., Clarke, A., Pugh, P.J.A., Linse, K., Cary, S.C., 2009. Exploring biological constraints on the glacial history of Antarctica. *Quaternary Sci. Rev.* 28 (27–28), 3035–3048.

Convey, P., 2010. Terrestrial biodiversity in Antarctica – Recent advances and future challenges. *Polar Sci.* 4, 135–147.

Crame, J., 1999. An evolutionary perspective on marine faunal connection between southernmost South America and Antarctica. *Sci. Mar.* 63, 1–14.

Cumming, R.A., Nikula, R., Spencer, H., Waters, J.M., 2014. Transoceanic genetic similarities of kelp-associated sea slug populations: long-distance dispersal via rafting? *J. Biogeogr.* 41, 2357–2370.

Dalziel, I., Lawver, L., Pearce, J., Barker, P., Hastie, A., Barfod, D., Schenke, H.-W., Davis, M.B., 2013. A potential barrier to deep Antarctic circumpolar flow until the late Miocene? *Geology* 41, 947–950.

Darriba, D., Taboada, G., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* 9, 772–772.

De Broyer, C., Koubbi, P., 2014. The biogeography of the Southern Ocean. In: In: De Broyer, C., Koubbi, P., Griffiths, H.J., Raymond, B., Udekem d'Acoz, C., Van de Putte, A.P., Danis, B., Grant, S., Gutt, J., Held, C., Hosie, G., Heutmann, F., Post, A., Ropert-Coudert, Y. (Eds.), *Biogeographic Atlas of the Southern Ocean Scientific Committee on Antarctic Research*, Cambridge, pp. 2–9.

DeConto, R., Pollard, D., 2003. Rapid Cenozoic glaciation of Antarctica induced by declining atmospheric CO₂. *Nature* 421, 245–249.

Dell, R., 1972. Antarctic benthos. *Adv. Mar. Biol.* 10, 1–216.

Díaz, A., Féral, J.-P., David, B., Saucedo, T., Poulin, E., 2011. Evolutionary pathways among shallow and deep-sea echinoids of the genus *Sterechinus* in the Southern Ocean. *Deep-Sea Res. II* 58, 205–211.

Dietz, L., Arango, C., Dömel, J.S., Halanaych, K., Harder, A.M., Held, C., Mahon, A.R., Mayer, C., Melzer, R.R., Rouse, G.W., Weis, A., Wilson, N.G., Leese, F., 2015. Regional differentiation and extensive hybridization between mitochondrial clades of the Southern Ocean giant sea spider *Colossendeis megalonyx*. *R. Soc. Open Sci.* 2, 140424.

Drummond, A., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* 7, 214.

Drummond, A., Suchard, M., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* 29, 1969–1973.

Dueñas, L.F., Tracey, D.M., Crawford, A., Wilke, T., Alderslade, P., Sánchez, J.A., 2016. The antarctic circumpolar current as a diversification trigger for deep-sea octocorals. *BMC Evol. Biol.* 16, 2.

Edgar, R., 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinf.* 5, 113.

Emerson, B.C., 2002. Evolution on oceanic islands: molecular phylogenetic approaches to understanding pattern and process. *Mol. Ecol.* 11, 951–966.

Felsenstein, J., 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* 17, 368–376.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotech.* 3, 294–299.

Fraser, C., Nikula, R., Spencer, H., Waters, J., 2009. Kelp genes reveal effects of sub-antarctic sea during the Last Glacial Maximum. *Proc. Natl. Acad. Sci. USA* 106, 3249–3253.

Fraser, C., Nikula, R., Waters, J., 2011. Oceanic rafting by a coastal community. *Proc. Roy. Soc. B-Biol. Sci.* 278, 649–655.

Fraser, C.I., Nikula, R., Ruzzante, D., Waters, J., 2012. Poleward bound: biological impacts of Southern Hemisphere glaciation. *Trends Ecol. Evol.* 27, 462–471.

Fraser, C.I., Zuccarello, G., Spencer, H., Salvatore, L., Garcia, G., Waters, J., 2013. Genetic affinities between trans-oceanic populations of non-buoyant macroalgae in the high latitudes of the Southern Hemisphere. *PLoS ONE* 8, e69138.

Fraser, C.I., Kay, G.M., du Pleiss, M., Ryan, P., 2016. Breaking down the barrier: dispersal across the Antarctic Polar Front. *Ecography* 39, 1–3.

Fraser, C.I., Morrison, A.K., Hogg, A.M., Macaya, E.C., van Sebille, E., Ryan, P.G.,

- Padovan, A., Jack, C., Valdivia, N., Waters, J.M., 2018. Antarctica's ecological isolation will be broken by storm-driven dispersal and warming. *Nat. Clim. Change* 8, 704–708.
- Gérard, K., Bierre, N., Borsa, P., Chenuil, A., Féral, J.-P., 2008. Pleistocene separation of mitochondrial lineages of *Mytilus* spp. mussels from Northern and Southern Hemispheres and strong genetic differentiation among southern populations. *Mol. Phylogenet. Evol.* 49, 84–91.
- González-Wevar, C.A., Nakano, T., Cañete, J., Poulin, E., 2010. Molecular phylogeny and historical biogeography of *Nacella* (Patellogastropoda: Nacellidae) in the Southern Ocean. *Mol. Phylogenet. Evol.* 56, 115–124.
- González-Wevar, C.A., Díaz, A., Gérard, K., Cañete, J., Poulin, E., 2012. Divergence time estimations and contrasting patterns of genetic diversity between Antarctic and southern South America benthic invertebrates. *Rev. Chil. Hist. Nat.* 85, 445–456.
- González-Wevar, C.A., Rosenfeld, S., Segovia, N.I., Hüne, M., Gérard, K., Ojeda, J., Mansilla, A., Brickle, P., Díaz, A., Poulin, E., 2016. Genetics, gene flow, and glaciation: the case of the South American limpet *Nacella mytilina*. *PLoS ONE* 11, e0161963.
- González-Wevar, C.A., Hüne, M., Segovia, N.I., Nakano, T., Spencer, H.G., Chown, S.L., Saucède, T., Johnston, G., Mansilla, A., Poulin, E., 2017. Following the Antarctic Circumpolar Current: patterns and processes in the biogeography of the limpet *Nacella* (Mollusca: Patellogastropoda) across the Southern Ocean. *J. Biogeogr.* 44, 861–874.
- González-Wevar, C.A., Segovia, N.I., Rosenfeld, S., Ojeda, J., Saucède, T., Brickle, P., Morley, S., Spencer, H.G., Poulin, E., 2018. Unexpected absence of island endemics: long-distance dispersal in higher latitude sub-Antarctic *Siphonaria* (Gastropoda: Euthyneura) species. *J. Biogeogr.* 45, 874–884.
- Grant, R., Griffiths, H., Steinke, D., Wadley, V., Linse, K., 2010. Antarctic DNA barcoding: a drop in the ocean? *Polar Biol.* 34, 775–780.
- Griffiths, H., Barnes, D., Linse, K., 2009. Towards a generalized biogeography of the Southern Ocean benthos. *J. Biogeogr.* 36, 162–177.
- Griffiths, H., Arango, C., Munilla, T., McInnes, S., 2011. Biodiversity and biogeography of Southern Ocean pycnogonids. *Ecography* 34, 616–627.
- Griffiths, H., Waller, C.L., 2016. The first comprehensive description of the biodiversity and biogeography of Antarctic and Sub-Antarctic intertidal communities. *J. Biogeogr.* 43, 1143–1155.
- Gubili, C., Ross, E., Billett, D.S., Yool, A., Tsairidis, C., Ruhl, H.A., et al., 2016. Species diversity in the cryptic abyssal holothurian *Psychropotes longicauda* (Echinodermata). *Deep Sea Res. Part II: Top. Stud. Oceanogr.* 137, 288–296.
- Gutt, J., Sirenko, B., Smirnov, I., Arntz, W., 2004. How many macrozoobenthic species might inhabit the Antarctic shelf? *Ant. Sci.* 16, 11–16.
- Harder, A.M., Halanych, K.M., Mahon, A.R., 2016. Diversity and distribution within the sea spider genus *Pallenopsis* (Chelicerata: Pycnogonida) in the Westerns Antarctic as revealed by mitochondrial DNA. *Polar Biol.* 39, 677–688.
- Hebbert, P.D.N., Ratsingham, S., deWaard, J.R., 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. Biol. Sci.* 270, S96–S99.
- Held, C., Wägele, J.-W., 2005. Cryptic speciation in the giant Antarctic isopod *Glyptonotus antarcticus* (Isopoda: Valvifera: Chaetiliidae). *Sci. Mar.* 69, 175–181.
- Heimeier, D., Lavery, S., Sewell, M., 2010. Molecular Species identification of *Astrotoma agassizii* from planktonic embryos: further evidence for a cryptic species complex. *J. Hered.* 101, 775–779.
- Hemery, L., Eleaume, M., Roussel, V., Ameziane, N., Gallut, C., Steinke, D., Cruaud, C., Couloux, A., Wilson, N.G., 2012. Comprehensive sampling reveals circumpolarity and sympatry in seven mitochondrial lineages of the Southern Ocean crinoid species *Promachocrinus kerguelensis* (Echinodermata). *Mol. Ecol.* 21, 2502–2518.
- Hennion, F., Walton, D.W.H., 1997. Ecology and seed morphology of endemic species from Kerguelen phytogeographic zone. *Polar Biol.* 18, 229–235.
- Hodgson, D.A., Graham, A.G.C., Roberts, S.J., Bentley, M.J., Cofaigh, C.Ó., Verleyen, E., Vyverman, W., Jomeli, V., Favier, V., Brunstein, D., Verfallie, D., Colhoun, E.A., Saunders, K.M., Selkirk, P.M., Mackintosh, A., Hedding, D.W., Nel, W., Hall, K., McGlone, M.S., Van der Putten, N., Dickens, W.A., Smith, J.A., 2014. Terrestrial and submarine evidence for the extent and timing of the Last Glacial Maximum and the onset of deglaciation on the maritime-Antarctic and sub-Antarctic islands. *Quat. Sci. Rev.* 100, 137–158.
- Huelsbeck, J., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754–755.
- Hunter, R., Halanych, K., 2008. Evaluating connectivity in the brooding brittle star *Astrotoma agassizii* across the Drake Passage in the Southern Ocean. *J. Hered.* 99, 137–148.
- Janosik, A., Mahon, A., Halanych, K., 2011. Evolutionary history of Southern Ocean *Odontaster* sea star species (Odontasteridae; Asteroidea). *Polar Biol.* 34, 575–586.
- Knox, G., 2007. *Biology of the Southern Ocean*, second ed. CRC Press Taylor and Francis Group.
- Koubbi, P., De Broyer, C., Griffiths, H.J., Raymond, B., d'Udekem d'Acoz, C., Van de Putte, A.P., Danis, B., David, B., Grant, S., Gutt, J., Held, C., Hosie, G., Huettmann, F., Post, A., Ropert-Coudert, Y., Stoddart, M., Swadling, K.M., Wadley, V., 2014. Conclusions: present and future of Southern Ocean biogeography. In: In: De Broyer, C., Koubbi, P., Griffiths, H.J., Raymond, B., Udekem d'Acoz, C., Van de Putte, A.P., Danis, B., Grant, S., Gutt, J., Held, C., Hosie, G., Heutmann, F., Post, A., Ropert-Coudert, Y. (Eds.), *Biogeographic Atlas of the Southern Ocean Scientific Committee on Antarctic Research*, Cambridge, pp. 470–475.
- Krabbe, K., Leese, F., Mayer, C., Tollrian, R., Held, C., 2010. Cryptic mitochondrial lineages in the widespread pycnogonid *Colossendeis megalonyx* Hoek, 1881 from Antarctic and Subantarctic waters. *Polar Biol.* 33, 281–292.
- Ledoux, J.B., Tarnakowska, K., Gérard, K., Lhuhier, E., Jacquemin, B., Weydmann, A., Féral, J.-P., Chenuil, A., 2012. Fine-scale spatial genetic structure in the brooding sea urchin *Abatus cordatus* suggests vulnerability of the Southern Ocean marine invertebrates facing global change. *Polar Biol.* 35, 611–623.
- Lee, M.R., Canales-Aguirre, C.B., Nuñez, D., Perez, K., Hernandez, C.E., Brante, A., 2017. The identification of sympatric cryptic free-living nematode species in the Antarctic intertidal. *PLoS ONE* 12 (10), e0186140. <https://doi.org/10.1371/journal.pone.0186140>.
- Leese, F., Kop, A., Wägele, J.-W., Held, C., 2008. Cryptic speciation in a benthic isopod from Patagonian and Falkland Island waters and the impact of glaciations on its population structure. *Front. Zool.* 5, 1–15.
- Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25, 1451–1452.
- Linse, K., Griffiths, H., Barnes, D., Clarke, A., 2006. Biodiversity and biogeography of Antarctic and sub-Antarctic mollusca. *Deep-Sea Res. II* 53, 985–1008.
- Linse, K., 2014. Bivalves. In: In: De Broyer, C., Koubbi, P., Griffiths, H.J., Raymond, B., Udekem d'Acoz, C., Van de Putte, A.P., Danis, B., Grant, S., Gutt, J., Held, C., Hosie, G., Heutmann, F., Post, A., Ropert-Coudert, Y. (Eds.), *Biogeographic Atlas of the Southern Ocean Scientific Committee on Antarctic Research*, Cambridge, pp. 126–128.
- Macaya, E., Zuccarello, G., 2010. DNA barcoding and genetic divergence in the giant kelp *Macrocystis* (Laminariales). *J. Phycol.* 46, 736–742.
- Mackensen, A., 2004. Changing Southern Ocean palaeocirculation and effects on global climate. *Ant. Sci.* 16, 369–386.
- McDowall, R.M., 1968. Oceanic islands and endemism. *Syst. Zool.* 17, 346–350.
- McGaughan, A., Stevens, M.I., Holland, B.R., 2010. Biogeography of circumpolar springtails. *Mol. Phylogenet. Evol.* 57, 48–58.
- Mikkelsen, N.T., Schander, C., Willassen, E., 2017. Local scale DNA barcoding of bivalves (Mollusca): a case study. *Zool. Scr.* 36, 455–463.
- Moon, K.L., Chown, S.L., Fraser, C.I., 2017. Reconsidering connectivity in the sub-Antarctic. *Biol. Rev.* 92, 2164–2181.
- Mortimer, E., Jansen van Vuuren, B., Lee, J.E., Marshall, D.J., Ryan, P., Chown, S.L., 2011. Mite dispersal among the Southern Ocean Islands and Antarctica before the last glacial maximum. *Proc. Biol. Sci.* 278, 1247–1255.
- Near, T., Dornburg, A., Kuhn, K., Eastman, J., Pennington, J., Patarnello, T., Zane, L., Fernández, D.A., Jones, C.D., 2012. Ancient climate change, antifreeze, and the evolutionary diversification of Antarctic fishes. *Proc. Natl. Acad. Sci. USA* 109, 3434–3439.
- Nikula, R., Fraser, C., Spencer, H., Waters, J., 2010. Circumpolar dispersal by rafting in two subantarctic kelp-dwelling crustaceans. *Mar. Ecol.-Prog. Ser.* 405, 221–230.
- Oliviero, M., Mariottini, P., 2001. Contrasting morphological and molecular variation in *Coralliophila meyendorffii* (Muricidae, Coralliophilinae). *J. Moll. Stud.* 67, 243–246.
- Pierrat, B., Saucède, T., Brayard, A., David, B., 2013. Comparative biogeography of echinoids, bivalves and gastropods from the Southern Ocean. *J. Biogeogr.* 40, 1374–1385.
- Poulin, E., Féral, J.-P., 1995. Pattern of spatial distribution of brood-protecting schizasterid echinoid, *Abatus cordatus*, endemic to the Kerguelen Islands. *Mar. Ecol.-Prog. Ser.* 118, 179–186.
- Poulin, E., Gonzalez-Wevar, C.A., Díaz, A., Gérard, K., Hüne, M., 2014. Divergence between Antarctic and South American marine invertebrates: What molecular biology tells us about Scotia Arc geodynamics and the intensification of the Antarctic Circumpolar Current. *Global Planet. Change* 123, 392–399.
- Quilty, P.G., Darragh, T.A., Gallagher, S.J., Hardin, L.A., 2016. Pliocene Mollusca (Bivalvia, Gastropoda) from the Sørsdal Formation, Marine Plain, Vestfold Hills, East Antarctica: taxonomy and implications for Antarctic Pliocene palaeoenvironments. *Alcheringa* 40, 556–582.
- Rabarts, I., Whybrow, S., 1979. A revision of the Antarctic and Subantarctic members of the genus *Yoldia* Moller, 1842 (Bivalvia: Nuculanidae). *J. Natural Hist.* 13, 161–183.
- Raupach, M., Wägele, J.-W., 2006. Distinguishing cryptic species in Antarctic *Asellota* (Crustacea: Isopoda) - a preliminary study of mitochondrial DNA in *Acanthaspida drygalskii*. *Antarct. Sci.* 18, 191–198.
- Rintoul, S., Hughes, C., Olbers, D., 2001. The Antarctic circumpolar current system. In: In: Siedler, G., Church, J., Gould, J. (Eds.), *Ocean Circulation and Climate Oxford Academic Press*, pp. 1–32.
- Salzburger, W., Ewing, G.B., Von Haeseler, A., 2011. The performance of phylogenetic algorithms in estimating haplotype genealogies with migration. *Mol. Ecol.* 20, 1952–1963.
- Sartori, A. F., 2016a. *Aequiyoldia Soot-Ryen, 1951*. In: *MolluscaBase* (2017). Accessed through: *World Register of Marine Species* at <http://www.marinespecies.org/aphia.php?p=taxdetails&id=868254> on 2017-12-16.
- Sartori, A.F., 2016b. *Aequiyoldia eightsi Jay, 1839*. In: *MolluscaBase* (2017). Accessed through: *World Register of Marine Species* at <http://www.marinespecies.org/aphia.php?p=taxdetails&id=868255> on 2017-12-01.
- Saucède, T., Pierrat, B., Danis, B., David, B., 2014. Biogeographic processes in the Southern Ocean. In: In: De Broyer, C., Koubbi, P., Griffiths, H.J., Raymond, B., Udekem d'Acoz, C., Van de Putte, A.P., Danis, B., Grant, S., Gutt, J., Held, C., Hosie, G., Heutmann, F., Post, A., Ropert-Coudert, Y. (Eds.), *Biogeographic Atlas of the Southern Ocean Scientific Committee on Antarctic Research*, Cambridge, pp. 456–463.
- Sharma, P.P., Zardus, J.D., Boyle, E.E., González, V.L., Jennings, R.M., McIntyre, E., Wheeler, W.C., Etter, R.J., Giribet, G., 2013. Into the deep: a phylogenetic approach to the bivalve subclass Protobranchia. *Mol. Phylogenet. Evol.* 69, 188–204.
- Stevens, M.I., Hogg, I.D., 2003. Long-term isolation and recent range expansion from glacial refugia revealed for the endemic springtail *Gomphiocephalus hodgsoni* from Victoria Land, Antarctica. *Mol. Ecol.* 12, 2357–2369.
- Stevens, M.I., Hogg, I.D., 2006. Contrasting levels of mitochondrial DNA variability between mites (Penthalodidae) and springtails (Hypogastruridae) from the Trans-Antarctic Mountains suggest long-term effects of glaciation and life history on substitution rates, and speciation processes. *Soil. Biol. Biochem.* 38, 3171–3180.
- Stevens, M.I., Frati, F., McGaughan, A., Spinsanti, G., Hogg, I.D., 2007. Phylogeographic structure suggests multiple glacial refugia in northern Victoria Land for the endemic

- Antarctic springtail *Desoria klovestadii*, (Collembola, Isotomidae). Zool. Scr. 36, 201–212.
- Strugnell, J., Rogers, A., Prodöhl, P., Collins, M., Allcock, A., 2008. The thermohaline expressway: the Southern Ocean as a centre of origin for deep-sea octopuses. Cladistics 24, 853–860.
- Strugnell, J., Watts, P., Smith, P., Allcock, A., 2012. Persistent genetic signatures of historic climatic events in an Antarctic octopus. Mol. Ecol. 21, 2775–2787.
- Tamura, K., Streecher, G., Peterson, D., Filipinski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.
- Thornhill, D., Mahon, A., Norenburg, J., Halanych, K., 2008. Open-ocean barriers to dispersal: a test case with the Antarctic Polar Front and the ribbon worm *Parborlasia corrugatus* (Nemertea: Lineidae). Mol. Ecol. 17, 5104–5117.
- Verducci, M., Foresi, L., Scott, G., Sprovieri, M., Lirer, F., Pelosi, N., 2009. The Middle Miocene climatic transition in the Southern Ocean: Evidence of paleoclimatic and hydrographic changes at Kerguelen plateau from planktonic foraminifers and stable isotopes. Palaeogeogr. Palaeoclim. Palaeoecol. 280, 371–386.
- Verheye, M.L., Backeljau, T., d’Udekem d’Acoz, C., 2017. Locked in the icehouse: Evolution of an endemic Epimeria (Amphipoda, Crustacea) species flock on the Antarctic shelf. Mol. Phylogenet. Evol. 114, 14–33.
- Villarroel, M., Stuardo, J., 1998. Protobranchia (Mollusca: Bivalvia) chilenos recientes y algunos fósiles. Malacologia 40, 113–229.
- Waters, J., 2007. Driven by the West Wind Drift? A synthesis of southern temperate marine biogeography, with new directions for dispersalism. J. Biogeogr. 35, 417–427.
- Weis, A., Meyer, R., Dietz, L., Dömel, J.S., Leese, F., Melzer, R.R., 2014. *Pallenopsis patagonica* (Hoek, 1881) – a species complex revealed by morphology and DNA barcoding, with description of a new species *Pallenopsis Wilson, 1881*. Zool. J. Linn. Soc. 170, 110–131.
- Wilson, N., Hunter, R., Lockhart, S., Halanych, K., 2007. Multiple lineages and absence of panmixia in the “circumpolar” crinoid *Promachocrinus kerguelensis* from the Atlantic sector of Antarctica. Mar. Biol. 152, 895–904.
- Wilson, N., Schrödl, M., Halanych, K., 2009. Ocean barriers and glaciation: evidence for explosive radiation of mitochondrial lineages in the Antarctic sea slug *Doris kerguelensis* (Mollusca, Nudibranchia). Mol. Ecol. 18, 965–984.
- Wilson, P., Clark, R., Birnie, J., Moore, D.M., 2002. Late Pleistocene and Holocene landscape evolution and environmental change in the Lake Sullivan area, Falkland Islands, South Atlantic. Quat. Sci. Rev. 21, 1821–1840.
- Wilson, P., Bentley, M.J., Schnabel, C., Clark, R., Xu, S., 2008. Stone run (block stream) formation in the Falkland Islands over several cold stages, deduced from cosmogenic isotope (^{10}Be and ^{26}Al) surface exposure dating. J. Quat. Sci. 23, 461–473.
- Wright, F., 1990. The effective number of codons used in a gene. Gene 87, 23–29.
- Zachos, J., Pagani, M., Sloan, L., Thomas, E., Billups, K., 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292, 686–693.