



HAL
open science

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

Claudio A. González-Wevar, Karin Gérard, Sebastián Rosenfeld, Thomas Saucède, Elie Poulin, Javier Naretto, Angie Díaz, Simon A. Morley, Paul Brickle

► To cite this version:

Claudio A. González-Wevar, Karin Gérard, Sebastián Rosenfeld, Thomas Saucède, Elie Poulin, et al.. Cryptic speciation in Southern Ocean *Aequiyoldia eightisii* (Jay, 1839): Mio-Pliocene trans-Drake Passage separation and diversification.. *Progress in Oceanography*, 2019, 174, pp.44-54. 10.1016/j.pocean.2018.09.004 . hal-02182480

HAL Id: hal-02182480

<https://hal.science/hal-02182480>

Submitted on 7 Feb 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1
2
3
4 Cryptic speciation in Southern Ocean *Aequiyoldia eightsii* Jay, 1839:
5 Mio-Pliocene trans-Drake separation and diversification
6
7
8

9 Claudio González-Wevar^{a,b,c}, Karin Gérard^{a,b*}, Sebastian Rosenfeld^a, Elie Poulin^c, Thomas
10 Saucède^d, Javier Naretto^e, Angie Díaz^e, Simon Morley^f, Paul Brickley^g
11
12

- 13
14 a) Departamento de Recursos Naturales, Universidad de Magallanes, Bulnes 01890, Punta Arenas,
15 Chile
16
17 b) Laboratorio de Ecología Molecular Antártica y Subantártica, Bulnes # 01890, Universidad de
18 Magallanes, casilla 113-D, Punta Arenas, Chile
19
20 c) Instituto de Ecología y Biodiversidad (IEB), Departamento de Ciencias Ecológicas, Facultad de
21 Ciencias, Universidad de Chile, Las Palmeras # 3425, Ñuñoa, Santiago, Chile
22
23 d) Biogéosciences, UMR CNRS 6282, Université de Bourgogne, 6, boulevard Gabriel, 21000,
24 Dijon, France
25
26 e) Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de
27 Concepción, Concepción, Chile
28
29 f) British Antarctic Survey (BAS), Natural Environment Research Council, Madingley Road, High
30 Cross, Cambridge CB3, 0ET, UK
31
32 g) South Atlantic Environmental Research Institute (SAERI), PO Box 609, Stanley Cottage,
33 Stanley, Falkland Islands
34
35

36 * Corresponding author: karin.gerard@umag.cl
37

38 Keywords: Biogeography; Southern Ocean; Antarctic-Magellan Connection; Kerguelen Islands, Antarctic
39 Peninsula, South America, morphometry; systematics; phylogeny
40
41

42 **Abstract**
43

44 The species of the genus *Aequiyoldia* Soot-Ryen, 1951, previously known as *Yoldia*, are common, soft-
45 substratum, saredtid bivalves. In the Southern Ocean, *Aequiyoldia eightsii* (Jay, 1839) was originally
46 described from the Antarctic Peninsula and has also been reported in southern South America, whereas *A.*
47 *woodwardi* (Hanley, 1960) was recorded for the Falkland/Malvinas Islands and Tierra del Fuego,
48 southern South America. *Aequiyoldia* has received little attention across its distribution in the Southern
49 Ocean, and although its taxonomy and systematics remain uncertain, all species of *Aequiyoldia* have been
50 grouped under the same broadly distributed unit: *A. eightsii*. However, molecular studies have
51 demonstrated a marked mtDNA genetic divergence (> 7%) between *A. eightsii* populations from South
52 America and Antarctic Peninsula. In order to further understand the diversity and biogeography of
53
54
55
56
57
58
59

60
61
62 *Aequiyoldia*, we analyzed *A. eightsii* populations from different provinces of the Southern Ocean
63 including South America (SA), the Falkland/Malvinas Islands (FI), Antarctic Peninsula (AP), and
64 Kerguelen Islands (KI). Individuals were characterized according to typical diagnostic morphological
65 measurements, and phylogenetic relationships were reconstructed based on mtDNA (Cytochrome c
66 Oxidase Subunit I). Patterns of genetic divergence of nucDNA intergenic transcribed spacers (ITS1,
67 ITS2) were also estimated. The statistical analysis of external diagnostic characteristics revealed two
68 morphotypes: (1) individuals with the morphology recorded for the nominal FI species, *A. woodwardi*,
69 and (2) individuals from SA, AP, and KI, with the morphology recorded for *A. eightsii*. However,
70 phylogenetic reconstructions based on mtDNA and nucDNA suggest the presence of at least five lineages
71 within *A. eightsii* including: one lineage in Kerguelen Island, two lineages in Antarctic Peninsula, one
72 lineage in South America, and the last one restricted to the Falkland/Malvinas Islands. Despite their very
73 similar morphologies, AP, SA, and KI populations of *Aequiyoldia* exhibited also marked levels of genetic
74 divergence. Such results are evidence that the Antarctic Polar Front represents an historical biogeographic
75 barrier for this group and that after the separation of these lineages, they followed independent
76 evolutionary pathways in different provinces of the Southern Ocean. Estimates of divergence time
77 suggest that KI separated from the other lineages close to the middle Miocene. Following this, the
78 separation between the AP and SA lineages occurred at the end of the Miocene around 7.5 Ma. Finally,
79 *Aequiyoldia* diversified during the Pliocene in Antarctic Peninsula (~4.5 Ma) and South America (~3.0
80 Ma). Individuals from FI exhibited morphological differences, and 4% of divergence from South
81 American individuals, suggesting that *A. woodwardi* could be revalidated. Similarly, the marked
82 molecular divergence between the KI and the rest of the recorded lineages also support the validity of *A.*
83 *kerquelenensis* (Thiele, 1931).
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118

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% to 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 much 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; 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 *Astrotoma agassizii* (Hunter and Halanych 2008), the pycnogonid *Collossedeis megalonix* (Krabbe et al., 2010), the bivalve *Yoldia* (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). Conversely, in 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

178
179
180 sub-Antarctic provinces (Griffiths et al., 2009; Pierrat et al., 2013; Griffiths and Waller, 2016). During the
181 last decade, the use of molecular tools for the taxonomic identification of organisms with conserved
182 morphology greatly increased the number of taxa in the Southern Ocean through the recognition of
183 cryptic species in several invertebrates groups such as crustaceans (Held and Wägele, 2005; Raupach and
184 Wägele, 2006; Leese et al., 2008; Krabbe et al., 2010; Arango et al., 2011; Baird et al., 2011; Dietz et al.,
185 2015), mollusks (Strugnell et al., 2008, 2012; Wilson et al., 2009; González-Wevar et al., 2010; Allcock
186 et al., 2011), echinoderms (Wilson et al., 2007; Heimeier et al., 2010; Janosik et al., 2011; Hemery et al.,
187 2012; Gubili et al., 2016), nematodes (Lee et al., 2017), and even macroalgae (Billard et al., 2015). In this
188 context, the broadly distributed Southern Ocean bivalve *Yoldia eightsii* shows a similar pattern with the
189 presence of different lineages at both sides of the Drake Passage (González-Wevar et al., 2012; Poulin et
190 al., 2014).
191

192 The genus *Yoldia* includes ~ 20 species of saltwater bivalves broadly distributed around the globe. Until
193 2013, the higher-latitude species, *Yoldia eightsii*, was recognized as broadly distributed in different
194 provinces of the Southern Ocean including South America, Antarctic Peninsula, and the Kerguelen
195 archipelago. Nevertheless, multi-locus molecular reconstructions identified *Yoldia* as paraphyletic with
196 the Antarctic *Y. eightsii* completely separated from the rest of the species (Sharma et al., 2013). Following
197 this, higher latitude *Y. eightsii* has been included in the genus *Aequiyoldia* Soot-Ryen, 1951. This genus
198 of sareptid bivalves is currently distributed in Antarctic and sub-Antarctic soft-substratum ecosystems and
199 includes only two nominal species: *A. defossata*, a Pliocene fossil from East Antarctica (Quilty et al.,
200 2016) and the broadly distributed *A. eightsii* (Jay, 1839). Several synonymous species have been grouped
201 within *A. eightsii*, including *Yoldia kerguelensis* (Thiele, 1931) and *Y. subaequilateralis* (E.A. Smith,
202 1875) from the Kerguelen archipelago, and *Yoldia woodwardi* (Hanley, 1860) from the
203 Falkland/Malvinas Islands.
204

205 The absence of clear diagnostic characters and the highly conserved morphology of protobranch bivalves
206 impose major taxonomic uncertainties within this group (Bouchet and Huber, 2015; Sartori, 2016a, b). In
207 fact, Antarctic Peninsula and South American populations of *A. eightsii* are morphologically identical,
208 although Antarctic individuals are larger than those recorded off South America (Villarroel and Stuardo,
209 1998). At the same time, morphological characteristics of the Kerguelen Islands species, *A. kerguelensis*
210 and *A. subaequilateralis*, completely overlap those recorded for *A. eightsii* from Antarctic Peninsula and
211 South America (Rabbarts & Whyborn 1979). Following this, *A. eightsii* is currently recognized in
212 different Southern Ocean provinces including the Falkland/Malvinas Islands, Kerguelen Islands,
213 Antarctic Peninsula, and South Shetland Islands (Linse, 2014). However, recent molecular analyses using
214 the mitochondrial locus cytochrome c oxidase subunit I (COI) recognized more than 7% divergence
215 between South American and Antarctic Peninsula populations of *A. eightsii*, which is far beyond the
216 intraspecific level (Gonzalez-Wevar et al., 2012; Poulin et al., 2014).
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236

237
238
239 In this study, we performed phylogenetic reconstructions, divergence-time estimations and morphological
240 comparisons of *Aequiyoldia* populations from different provinces of the Southern Ocean. We extended
241 previous molecular and morphological analyses by including *Aequiyoldia* populations from the Kerguelen
242 Islands and the Falkland/Malvinas Islands. We also included more populations from South America and
243 from Antarctic Peninsula. Through this, we obtained a clear picture about the evolutionary relationships
244 within *Aequiyoldia*, the taxonomic status of different populations across the distribution of the group, the
245 number of evolutionary units, and their respective divergences based on mitochondrial (COI) gene
246 sequences. We also compared *A. eightsii* populations using nucDNA sequences (ITS1, ITS2) to
247 corroborate the biogeographic patterns recorded with the mitochondrial markers. Finally, shell
248 morphometry was conducted by applying the criteria described by Rabarts and Whybrow (1979).
249
250
251
252
253
254

255 **2. Material and Methods**

257 *2.1 Sample collection*

258
259 *Aequiyoldia eightsii* specimens were collected by SCUBA diving off the Kerguelen (KI) and Falkland
260 Islands (FI), South America (SA) (Porvenir, San Juan River, Skyring Sound), and Antarctic Peninsula
261 (AP) (Admiralty Bay, Fildes Bay, Deception Island, Rothera Station, Marguerite Bay, Adelaide Island)
262 (Fig. 1 and 2, Table 1). All specimens were stored in 96° ethanol for future molecular analyses. For
263 comparative purposes, we also included one individual of *Portlandia isonota* collected off the Kerguelen
264 Islands.
265
266
267
268

269 *2.2 DNA preparation and sequence edition*

270
271 Genomic DNA was extracted from mantle and branchial tissues using the E.Z.N.A. Mollusc DNA Kit
272 (Omega bio-tek). A 621-bp fragment of the cytochrome c oxidase subunit I (COI) was amplified using
273 universal primers (Folmer et al., 1994). The PCR program consisted of 5 min at 95°C followed by 40
274 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
275 of up to 1190 bp containing the internal transcribed spacers 1 and 2 (ITS1 and ITS2) were amplified using
276 universal primers (Oliverio and Mariottini, 2001). The PCR program was 5 min at 95°C, then 40 cycles at
277 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
278 products were purified using QIAquick Gel Extraction Kit (QIAGEN) and sequenced in both direction
279 with an Automatic Sequencer ABI3730 x 1 at Macrogen Inc. (Seoul, South Korea). Chromatograms were
280 inspected visually, and multiple alignments were obtained using MUSCLE (Edgar, 2004) implemented in
281 CodonCode Aligner v7.1 (CodonCode Corporation). Base composition of nucleotide sequences was
282 analysed with MEGA 6 (Tamura et al., 2013) and codon usage was determined using the effective
283 number of codon value (ENC; Wright, 1990) with DnaSP 5.0 (Librado and Rozas, 2009). New
284 *Aequiyoldia* sequences will be submitted to GenBank under accession numbers: XXXXXX – XXXXXX.
285
286
287
288
289
290
291
292
293
294
295

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 (Darriba *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). This methodology allowed the reconstructions of phylogenies based on intra- and interspecific molecular data sets.

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. defossatum* (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

355
356
357 sample size values were estimated for each parameter to ensure adequate mixing of the MCMC (ESSs >
358 1000).
359

360 361 362 *2.5 Morphological analysis*

363 Shell morphology of *Aequiyoldia eightsii* populations from different provinces of the Southern Ocean
364 (Fig. 2) was characterized following the criteria of Rabarts and Whybrow (1979). For the pallial sinus
365 shape, callipers were used to determine three parameters for each individual shell: H = Height, L =
366 Length, and W = Width. Discriminating scores (D) were obtained through the following formula $D=6H-$
367 $(3L+2I)$. The matrix of D was then analyzed through non-metric, multidimensional scaling (NMDS) using
368 Euclidean distances.
369
370
371
372

373 374 **3. Results**

375 376 *3.1 DNA polymorphism*

377 Phylogenetic reconstructions including populations of *Aequiyoldia eightsii* revealed clearly discriminated
378 evolutionary lineages associated to different provinces of the Southern Ocean (Fig. 3-4, Table 2). For
379 instance, *A. eightsii* populations from KI differed markedly (> 19%) from those collected in other the
380 Southern Ocean provinces (AP, SA, FI). In fact, more than 27 amino acid substitutions separated KI from
381 the rest of the analyzed populations (AP, SA, FI). No indels or stop codons were identified within *A.*
382 *eightsii* populations from AP, SA, or FI. A total of eight amino-acid substitutions were recorded among
383 individuals from these areas (see below). Mitochondrial DNA alignments (621 bp) included 79 variable
384 positions (12.72%) of which 73 (92.4%) were parsimoniously informative. Nuclear ITSs (1190 bp) were
385 more conserved with only 28 variable positions (2.35%), 19 of which (67.85%) were parsimoniously
386 informative. Mitochondrial and nuclear sequences were not saturated at any position and no evidence for
387 codon bias was detected for COI (ENC = 35.89).
388
389
390
391
392
393

394 395 *3.2 Phylogenetic relationships*

396 Phylogenetic reconstructions from mitochondrial data were consistent with previous analyses in
397 protobranch bivalves (Sharma et al. 2013) that discriminated major taxonomic protobranch groupings
398 with high bootstrap and posterior probabilities. For instance, the single individual of *Portlandia isonota*
399 from KI fell within the diversity of *Yoldiella* from different regions (Fig. 3). At the same time, these
400 analyses highlighted the presence of several evolutionary lineages within the nominal species, *A. eightsii*
401 (Fig. 3). The monophyly of *Aequiyoldia eightsii* was highly supported by the different markers and
402 reconstruction methods. Nevertheless, the high divergence level (> 19%) and phylogenetic position of *A.*
403 *eightsii* individuals from KI (Fig. 3) allowed us to discriminate them as a different species. However, the
404 exact systematic position of KI individuals remains uncertain. Although KI specimens may represent a
405 basal branch of *Aequiyoldia*, considering the high level of genetic divergence, we tend to think that they
406
407
408
409
410
411
412
413

414
415
416 constitute a different *Aequiyoldia* species endemic to KI. We recognized the presence of at least two
417 evolutionary lineages in AP that were sympatric at two sites (i.e., Rothera Station and Admiralty Bay)
418 (Fig. 3). We also recognized the presence of a different lineage in SA and another endemic to the FI (Fig.
419 3). Genetic divergence between Antarctic and South American *A. eightsii* populations ranged between
420 6.3% and 7.5% (Table 2), whereas these levels were lower between SA and FI populations (4%) and
421 among the different AP units (~3%).
422
423
424

425 426 427 3.3 Divergence time estimates

428 Divergence time estimates based on mtDNA sequences suggested that the *A. eightsii* populations from KI
429 separated from the remaining lineages during the middle Miocene c. 16.5 Ma (22.5–12 Ma) (Fig. 4).
430 Following this, the separation between Antarctic and South American lineages occurred at the end of the
431 Miocene, c. 8.5 Ma (13–6.5 Ma) (Fig. 4). Finally, the diversification of *Aequiyoldia* in AP and SA
432 occurred during the Pliocene, beginning with the separation between the AP lineages ~ 4.5 Ma (6.5–3.5
433 Ma), followed by the separation between SA and FI ~ 3.0 Ma (5.5–2.5 Ma) (Fig. 4).
434
435
436
437

438 439 3.4 Morphological analysis

440 The nMDS analysis separated individuals of *A. eightsii* into two main groups (Fig. 5). The first included
441 all individuals assigned to the broadly distributed nominal species *A. eightsii* collected at AP, SA, and KI,
442 which exhibited a u-shaped pallial sinus. The second group included individuals from FI that represented
443 a different morphological entity that exhibited a v-shaped pallial sinus.
444
445
446
447

448 4. Discussion

449 4.1 Cryptic speciation in the Southern Ocean

451 Recent molecular studies in broadly distributed Southern-Ocean organisms have demonstrated the
452 presence of several cryptic species complexes. Examples include crustaceans of the genus *Eusirus* (Baird
453 et al., 2011) and *Serolis* (Leese et al., 2008), the nudibranch *Doris kerguelenensis* (Wilson et al., 2009),
454 octopuses of the genus *Pareledone* (Allcock et al., 2011), pycnogonids of the genus *Nymphon* (Arango et
455 al., 2011), *Pallenopsis* (Weis et al., 2014; Harder et al., 2016), *Colossendeis* (Dietz et al., 2015), the
456 brittle star *Astrotoma* (Heimeier et al., 2010) the ophiurid *Promachochrinus* (Hemery et al., 2012), the
457 macroalgae genus *Gigartina* (Billard et al., 2015), and *Durvillaea* (Fraser et al., 2009). Given the high
458 number of cryptic species complexes discovered in the Southern Ocean, the region's biodiversity has been
459 severely underestimated. As recorded in *Aequiyoldia*, cryptic species complexes consist of closely related
460 species with incomplete morphological differentiation as opposed to distantly related species that have
461 acquired a misleading degree of morphological similarity through other evolutionary processes (i.e.,
462 convergent evolution) (Koubbi et al., 2014). Besides increasing diversity patterns and species counts, the
463 discovery of cryptic species often results in a shift from a small number of broadly distributed,
464
465
466
467
468
469
470
471
472

473
474
475 morphologically variable species to a series of less variable species with smaller, often allopatric
476 distributions. Notable exceptions of newly delimited species with larger distributions in the Southern
477 Ocean do exist (Fraser et al., 2009; Nikula et al., 2010; Cumming et al., 2014; González-Wevar et al.,
478 2018). New molecular advances have started to change our understanding of circumpolar and
479 cosmopolitan species, indicating the need for further, more detailed taxonomic and integrative
480 biogeographic work on potential cryptic species (Grant et al., 2010). Biogeographic patterns may change
481 with increased knowledge of diversity both at the genetic and species levels and how much these patterns
482 are likely to change is still unclear. Nevertheless, it seems probable that the overall pattern of rich
483 Antarctic fauna distinct from that of its neighbours will hold true and even strengthen (Koubbi et al.,
484 2014). Species numbers in the Southern Ocean are likely to increase with further sampling, taxonomic
485 work, and molecular analyses, helping provide a better understanding of global diversity, distribution, and
486 evolutionary history (Griffiths et al., 2011).

494 495 *4.2 Cryptic speciation in Aequiyoldia*

496 The highly conserved morphology and absence of genetic data led researchers to synonymize several
497 nominal species of *Aequiyoldia* (i.e., *A. kerguelensis*, *A. subaequilateralis*, *A. woodwardi*) into a single,
498 nominal, broadly distributed species: *A. eightsii*. Accordingly, *A. eightsii* has exhibited an extended
499 distribution across the sub-Antarctic from South America to the Kerguelen Island as well as a circum-
500 Antarctic distribution (Linse, 2014). Herein, the monophyly of *Aequiyoldia* in the Southern Ocean was
501 supported by mtDNA sequences and different reconstruction methods. Mitochondrial and nuclear
502 diversity within *Aequiyoldia* across its distribution (AP, SA, FI, and KI) as well as its morphological
503 characterization uncovered the presence of at least five cryptic species currently distributed in AP, SA, FI
504 and KI. We found a clear distinction between sub-Antarctic and Antarctic lineages of *A. eightsii*,
505 corroborating previous molecular studies in the group (González-Wevar et al., 2012, 2017; Poulin et al.,
506 2014). In Falkland/Malvinas Islands, we recorded the presence of an endemic lineage clearly separated
507 from its closest continental South American relative. Finally, along the Antarctic Peninsula, we recorded
508 at least two sympatric evolutionary units in Marguerite Bay (West Antarctic Peninsula) and Admiralty
509 Bay (South Shetlands).

518 519 *4.3 A highly divergent Aequiyoldia lineage in Kerguelen Islands*

520 The *A. eightsii* population from KI was the most divergent and showed a basal position in the
521 phylogenies. All COI haplotypes from KI clustered together in a monophyletic group separated from the
522 remaining lineages from AP, SA, and FI by more than 19% divergence (Table 2). Such level of
523 divergence could not be corroborated by nuclear data, since ITSs sequences from KI did not align
524 properly with those from AP, FI, and SA. However, it may also reflect a long divergence history between
525 KI lineage from the South American and Antarctic ones. Hence, the amount of molecular divergence
526
527
528
529
530
531

532
533
534 accumulated in KI was extreme, despite morphological similarities among the KI, AP, and SA
535 populations. Because of its geographic isolation, Kerguelen Archipelago is indeed characterized by a high
536 level of endemism in a variety of terrestrial and marine species (Briggs, 1966; McDowall, 1968; Poulin
537 and Féral, 1995; Hennion and Walton, 1997; Brandt et al., 1999; Chapuis et al., 2000; Emerson, 2002;
538 Ledoux et al., 2012; González-Wevar et al., 2017). Consequently, this sereptid bivalve endemic to KI
539 should clearly be considered as a different *Aequiyoldia* species. Accordingly, and considering that an
540 *Aequiyoldia* species has been previously described in Kerguelen, our study supports the re-validation of
541 *A. kerguelensis* (Thiele, 1931).
542
543
544
545
546

547 4.4 Trans-Drake speciation: new *Aequiyoldia* species in South America

549 Phylogenetic reconstructions and molecular divergence within *Aequiyoldia* support previous Antarctic
550 and South American population-based comparisons in the group (González-Wevar et al., 2012; Poulin et
551 al., 2014) by recognizing the presence of different evolutionary lineages on both sides of the Drake
552 Passage. Such conclusions are supported by the mtDNA (6.5% - 7.5%) and nucDNA (1.2%) molecular
553 divergence recorded between Antarctic Peninsula and South American *Aequiyoldia* populations (Suppl.
554 Fig. 1). Consequently, we suggest that South American *A. eightsii* deserves specific status and that
555 integrative taxonomic revisions are required within *Aequiyoldia*.
556
557
558
559
560

561 4.5 Re-validating *Aequiyoldia woodwardi* (Hanley, 1860)

563 The morphological revision of *Aequiyoldia* shells confirmed the current presence of a broadly distributed
564 morphotype in different provinces including AP, SA, and KI (Fig. 4). In a revision of the group,
565 Villarroel and Stuardo (1998) concluded that the external anatomy of *A. eightsii* individuals from South
566 America and Antarctic Peninsula is very similar but they differ in their respective sizes, being the
567 Antarctic populations bigger than the South American ones. However, molecular data do not support the
568 presence of a single genetic unit across the Southern Ocean. Rather, the data highlighted the presence of
569 hidden cryptic diversity with at least five evolutionary lineages. Together with this broadly distributed
570 morphotype, we also recorded another one currently restricted to FI. Mitochondrial (COI) and nuclear
571 (ITSs) sequences of *Aequiyoldia* individuals from FI revealed the presence of a monophyletic group in
572 these islands separated from its sister South-American lineage by 4% and 0.5% of divergence,
573 respectively (Suppl. Fig. 1). Such molecular results corroborated previous morphological revision in the
574 group (Rabarts and Whybrow, 1979) that recorded the presence of a different species off these islands:
575 *Yoldia woodwardi* Hanley, 1860. Consequently, based on morphological and molecular evidence, we
576 propose a revalidation of the specific status conferred to the *Aequiyoldia* from FI and propose *Aequiyoldia*
577 *woodwardi* be used for this species. These results support recent population-based studies of several
578 marine, near-shore, benthic invertebrates including isopods of the genus *Serolis* (Leese et al., 2008), the
579 patellogastropods *Nacella magellanica* (González-Wevar et al., 2012) and *Nacella mytilina* (González-
580
581
582
583
584
585
586
587
588
589
590

591
592
593 Wevar et al., 2016), and pulmonates of the genus *Siphonaria* (González-Wevar et al., 2018); all those
594 studies recognized FI and SA populations as different genetic and sometimes as evolutionary units. As
595 stated by Leese et al. (2008), whereas the biota of FI shared most of their faunal inventory with SA,
596 molecular studies have demonstrated that shallow benthic species may, in fact, be strongly differentiated
597 or even reproductively isolated. The genetic differentiation between SA and FI populations of
598 *Aequiyoldia* may be a consequence of their distinct glaciological histories during the coldest glacial
600 periods. The southern tip of South America was almost completely covered by ice during the last Glacial
601 Maximum (LGM), and shallow marine habitats were largely absent. In contrast, little evidence of ice
602 advances during the LGM existed in FI apart from small cirques and short glacially eroded valleys
603 (Clapperton and Sugden, 1976; Clapperton, 1994; Hodgson et al., 2014), and no study shows evidence of
604 LGM glaciers extending offshore (Wilson et al., 2002, 2008).

609 610 4.6 Divergent lineages in *Aequiyoldia* off the West Antarctic Peninsula

611
612 Phylogenetic reconstructions and levels of mtDNA divergence, as well as divergence time estimates
613 indicated the presence of at least two different *Aequiyoldia* lineages off the Antarctic Peninsula. These
614 lineages are separated by 5.78% of mtDNA divergence and are currently found in sympatry in Marguerite
615 Bay and off the South Shetland Islands. These divergent Antarctic *Aequiyoldia* lineages could either be
616 the consequence of sympatric cryptic species or persistent ancestral polymorphism with incomplete
617 lineage sorting. To discriminate between these hypotheses, additional nuclear genetic data are required.
618
619
620
621

622 623 4.7 Biogeography in *Aequiyoldia* spp.

624 Molecular data suggest that the tmrca of *Aequiyoldia* spp., separating KI from the rest of the analyzed
625 provinces of the Southern Ocean (AP, SA and FI), occurred c. 16.5 Ma (22.5–12 Ma), close to the middle
626 Miocene transition (MMCT). This period represents an epoch of major oceanographic and climatic
627 changes in the Southern Ocean that were probably associated with the intensification of the ACC and the
628 re-establishment of a continental Antarctic Ice Sheet (Zachos et al., 2001; DeConto and Pollard, 2003;
629 Mackensen, 2004; Verducci et al., 2009). Major oceanic circulation changes during this period were
630 probably associated with the full development of a deep ACC, a process that was only achieved during
631 the late Miocene around 12 Ma (Dalziel et al., 2013). As recorded for other Southern-Ocean invertebrates,
632 fluctuations in latitudinal positioning and strengthening of the ACC during this period seemed to be
633 important drivers in the separation of lineages over geographically distant Southern Ocean provinces
634 (González-Wevar et al., 2017). Alternatively, the establishment of a strong and deep ACC may have
635 generated an effective oceanographic barrier that can be invoked to explain the diversification of
636 *Aequiyoldia* in different areas of the Southern Ocean. Following this, the separation between Antarctic
637 and South American lineages of *Aequiyoldia* occurred at the end of the Miocene c. 8.5 Ma (13–6.5 Ma).
638
639
640
641
642
643
644
645
646
647
648
649

650
651
652 In this context, late Miocene phases of Southern Ocean diversifications have been identified in different
653 groups of marine invertebrates including some octopus clades (Strugnell et al., 2008), octocorals (Dueñas
654 et al., 2016), patellogastropods (González-Wevar et al., 2017), muricid gastropods (Barco et al., 2012),
655 and fishes (Near et al., 2012). For instance, the limpet genus *Nacella* shows a particular radiation at the
656 end of the Miocene 8.5–7.0 Ma (González-Wevar et al., 2017), and the muricid gastropod of the
657 subfamily Pagodulinae diversified in the Southern Ocean between the late Miocene and Pliocene (Barco
658 et al., 2012). Following this, during the Pliocene, *Aequiyoldia* diversified both off South America and
659 across the Antarctic Peninsula. In both Antarctic and South America, this diversification started around ~
660 4.5 Ma (6.5–3.5 Ma) and ~ 3.0 Ma (5.5–2.5 Ma), respectively. Diversification processes during the
661 Pliocene have been reported for other Southern-Ocean marine benthic invertebrates including the
662 Antarctic amphipods of the genus *Epimeria* (Verheye et al., 2017), deep-sea octopuses (Strugnell et al.,
663 2008), and deep-sea octocorals (Dueñas et al., 2016). Molecular population-based studies have provided
664 strong evidence from a wide range of marine and terrestrial taxa that fragmentation of populations into
665 refugia during glacial maxima and subsequent expansions during interglacial periods may represent an
666 important mechanism enhancing cryptic speciation processes (Allcock and Strugnell, 2012; Fraser et al.,
667 2012; Poulin et al., 2014). Hence, the presence of isolated refugia during glacial maxima may have
668 enhanced such process and the re-colonization of newly de-glaciated habitats could have favoured the
669 recent radiation recorded in *Aequiyoldia* off South America and the Antarctic Peninsula.
670
671
672
673
674
675
676
677
678

681 **ACKNOWLEDGEMENT**

682
683 This study was funded by the following projects and institutions: An Initiation Fondecyt Project
684 (11140087) and the Program FONDAP, Project N° 15150003 to CAGW. Regular Fondecyt projects
685 (1116358) to KG and (1151336) EP. We greatly appreciate support from the projects P05-002 ICM and
686 PFB 023 (Instituto de Ecología y Biodiversidad IEB) to EP, SR, JN, and CAG-W; CD GAIA Antártica,
687 Universidad de Magallanes, to CAG-W and KG; CONICYT PIA ACT172065 to EP, AD, KG and CAG-
688 W. All specimens were collected in accordance with Chilean legislation (Technical Memorandum P.INV
689 N° 429/2015 SUBPESCA). Permits to undertake field studies and collect specimens in Falkland Islands
690 were issued by the Environmental Planning Department, the Falkland Islands Government. Field access
691 facilities at Kerguelen Archipelago were supported 415 by IPEV program 1044 Proteker to E.P, C.A.G-
692 W, and T.S. We recognize the SCAR AntEco and EBA programs, CAML, and SAERI for encouraging
693 and supporting Antarctic and Subantarctic evolutionary biology studies. This manuscript contributes to
694 the SCAR 'State of Antarctic Ecosystem' (AntEco) program.
695
696
697
698
699
700
701
702
703
704
705
706
707
708

709
710
711 **References**
712

- 713 Allcock, A., Barratt, I., Eleaume, M., Linse, K., Norman, M., Smith, P., Steinke, D., Stevens, D.W.,
714 Strugnell, J.M., 2011. Cryptic speciation and the circumpolarity debate: A case study on endemic
715 Southern Ocean octopuses using the COI barcode of life. *Deep-Sea Res. II* 58, 242–249.
716
717 Allcock, A., Strugnell, J., 2012. Southern Ocean diversity: new paradigms from molecular ecology.
718 *Trends Ecol. Evol.* 27, 520–528.
719
720 Arango, C., Soler-Membrives, A., Miller, K., 2011. Genetic differentiation in the circum-Antarctic sea
721 spider *Nymphon australe* (Pycnogonida; Nymphonidae). *Deep-Sea Res. II* 58, 212–219.
722
723 Aronson, R., Thatje, S., Clarke, A., Peck, L., Blake, D., Wilga, C., Seibel, B., 2007. Climate change and
724 invasibility of the Antarctic benthos. *Annu. Rev. Ecol. Evol. S.* 38, 129–154.
725
726 Baird, H.P., Miller, K., Stark, J.S., 2011. Evidence of hidden biodiversity, ongoing speciation and diverse
727 patterns of genetic structure in giant Antarctic amphipods. *Mol. Ecol.* 20, 3439–3454.
728
729 Barco, A., Schiaparelli, S., Houart, R., Oliverio, M., 2012. Cenozoic evolution of Muricidae (Mollusca,
730 Neogastropoda) in the Southern Ocean, with the description of a new subfamily. *Zool. Scripta* 41,
731 596–616.
732
733 Barnes, D., Hodgson, D., Ryan, P., Allen, C., Clarke, A., 2006. Incursion and excursion of Antarctic
734 biota: past, present and future. *Global Ecol. Biogeogr.* 15, 121–142.
735
736 Beu, A.G., 2009. Before the ice: Biogeography of Antarctic Paleogene molluscan faunas. *Palaeogeogr.*
737 *Palaeoclim. Palaeoecol.* 284, 191–226.
738
739 Billard, E., Reyes, J., Mansilla, A., Faugeron, S., Guillemin, M.-L., 2015. Deep genetic divergence
740 between austral populations of the red alga *Gigartina skottsbergii* reveals a cryptic species endemic
741 to the Antarctic continent. *Polar Biol.* 38, 2021–2034.
742
743 Bouchet, P., Huber, M., 2015. *Aequiyoldia*. In MolluscaBase. Accessed through WoRMS: World Register
744 of Marine Species. <http://www.marinespecies.org/aphia.php>.
745
746 Brandt, A., Linse, K., Mühlenhardt-Siegel, U., 1999. Biogeography of Crustacea and Mollusca of the
747 Subantarctic and Antarctic regions. *Sci. Mar.* 63, 383–389.
748
749 Briggs, J.C., 1966. Oceanic islands, endemism, and marine paleotemperatures. *Syst. Biol.* 15, 153–163.
750
751 Chapuis, J-L., Hennion, F., Le Roux, V., Le Cuizat, J., 2000. Growth and reproduction of the endemic
752 cruciferous species *Pringlea antiscorbutica* in Kerguelen Islands. *Polar Biol.* 23, 196–204.
753
754 Chown, S.L., Clarke, A., Fraser, C.I., Cary, S., Moon, K.L., McGeoch, M.A., 2015. The changing form of
755 Antarctic biodiversity. *Nature* 522, 431–438.
756
757 Clapperton, C.M., Sugden, D.E., 1976. The maximum extent of glaciers in part of west Falkland. *J.*
758 *Glaciol.* 17, 73–77.
759
760 Clapperton, C., 1994. The quaternary glaciation of Chile: a review. *Rev. Chil. Hist. Nat.* 67, 369–383.
761
762
763
764
765
766
767

- 768
769
770 Clarke, A., Crame, J., 1989. The origin of the Southern Ocean marine fauna. *J. Geol. Soc. London* 47,
771 253–268.
772
773 Clarke, A., Crame, J., Stromberg, J., Barker, P., 1992. The Southern Ocean benthic fauna and climate
774 change: A historical perspective [and discussion]. *Philos. S. T. Soc. B.* 338, 299–309.
775
776 Clarke, A., Barnes, D., Hodgson, D., 2005. How isolated is Antarctica? *Trends Ecol. Evol* 20, 1–3.
777
778 Crame, J., 1999. An evolutionary perspective on marine faunal connection between southernmost South
779 America and Antarctica. *Sci. Mar.* 63, 1–14.
780
781 Cumming, R.A., Nikula, R., Spencer, H., Waters, J.M., 2014. Transoceanic genetic similarities of kelp-
782 associated sea slug populations: long-distance dispersal via rafting? *J. Biogeogr.* 41, 2357–2370.
783
784 Dalziel, I., Lawver, L., Pearce, J., Barker, P., Hastie, A., Barfod, D., Schenke, H-W., Davis, M.B., 2013.
785 A potential barrier to deep Antarctic circumpolar flow until the late Miocene? *Geology* 41, 947–
786 950.
787
788 Darriba, D., Taboada, G., Doallo, R., and Posada, D., 2012. jModelTest 2: more models, new heuristics
789 and parallel computing. *Nat. Methods* 9, 772–772.
790
791 De Broyer, C., Koubbi, P., 2014. The biogeography of the Southern Ocean. In De Broyer C., Koubbi, P.,
792 Griffiths, H.J., Raymond, B., Udekem d’Acoz, C., Van de Putte A.P., Danis, B., Grant, S., Gutt, J.,
793 Held, C., Hosie, G., Heutmann, F., Post, A., Ropert-Coudert, Y. (Eds.), *Biogeographic Atlas of the*
794 *Southern Ocean*. Scientific Committee on Antarctic Research, Cambridge. pp. 2–9.
795
796 DeConto, R., Pollard, D., 2003. Rapid Cenozoic glaciation of Antarctica induced by declining
797 atmospheric CO₂. *Nature* 421, 245–249.
798
799 Dell, R., 1972. Antarctic Benthos. *Adv. Mar. Biol.* 10, 1–216.
800
801 Díaz, A., Féral, J-P., David, B., Saucède, T., Poulin, E., 2011. Evolutionary pathways among shallow and
802 deep-sea echinoids of the genus *Sterechinus* in the Southern Ocean. *Deep-Sea Res. II* 58, 205–211.
803
804 Dietz, L., Arango, C., Dömel, J. S., Halanych, K., Harder, A. M., Held, C., Mahon, A.R., Mayer, C.,
805 Melzer, R.R., Rouse, G.W., Weis, A., Wilson, N.G., Leese, F., 2015. Regional differentiation and
806 extensive hybridization between mitochondrial clades of the Southern Ocean giant sea spider
807 *Colossendeis megalonyx*. *R. Soc. Open Sci.* 2, 140424.
808
809 Drummond, A., Rambaut, A., 2007. BEAST: Bayesian Evolutionary Analysis by Sampling Trees. *BMC*
810 *Evol. Biol.* 7, 214.
811
812 Drummond, A., Suchard, M., Xie, D., Rambaut, A., 2012. Bayesian Phylogenetics with BEAUti and the
813 BEAST 1.7. *Mol. Biol. Evol.* 29, 1969–1973.
814
815 Dueñas, L.F., Tracey, D.M., Crawford, A., Wilke, T., Alderslade, P., Sánchez, J.A., 2016. The Antarctic
816 Circumpolar Current as a diversification trigger for deep-sea octocorals. *BMC Evol. Biol.* 16, 2.
817
818 Edgar, R., 2004. MUSCLE: a multiple sequence alignment method with reduced time and space
819 complexity. *BMC Bioinformatics* 5, 113.
820
821
822
823
824
825
826

- 827
828
829 Emerson, B.C., 2002. Evolution on oceanic islands: molecular phylogenetic approaches to understanding
830 pattern and process. *Mol. Ecol.* 11, 951–966.
831
832 Felsenstein, J., 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. *J. Mol.*
833 *Evol.* 17, 368–376.
834
835 Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of
836 mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar.*
837 *Biol. Biotech.* 3, 294–299.
838
839 Fraser, C., Nikula, R., Spencer, H., Waters, J., 2009. Kelp genes reveal effects of subantarctic sea during
840 the Last Glacial Maximum. *Proc. Natl. Acad. Sci. USA* 106, 3249–3253.
841
842 Fraser, C., Nikula, R., Waters, J., 2011. Oceanic rafting by a coastal community. *Proc. Roy. Soc. B-Biol.*
843 *Sci.* 278, 649–655.
844
845 Fraser, C., Nikula, R., Ruzzante, D., Waters, J., 2012. Poleward bound: biological impacts of Southern
846 Hemisphere glaciation. *Trends Ecol. Evol.* 27, 462–471.
847
848 Fraser, C., Zuccarello, G., Spencer, H., Salvatore, L., Garcia, G., Waters, J., 2013. Genetic affinities
849 between trans-oceanic populations of non-buoyant macroalgae in the high latitudes of the Southern
850 Hemisphere. *PLoS ONE* 8, e69138.
851
852 Gérard, K., Bierne, N., Borsa, P., Chenuil, A., Féral, J.-P., 2008. Pleistocene separation of mitochondrial
853 lineages of *Mytilus* spp. mussels from Northern and Southern Hemispheres and strong genetic
854 differentiation among southern populations, *Mol. Phylogenet. Evol.* 49, 84–91.
855
856 González-Wevar, C.A., Nakano, T., Cañete, J., Poulin, E., 2010. Molecular phylogeny and historical
857 biogeography of *Nacella* (Patellogastropoda: Nacellidae) in the Southern Ocean. *Mol. Phylogenet.*
858 *Evol.* 56, 115–124.
859
860 González-Wevar, C.A., Díaz, A., Gérard, K., Cañete, J., Poulin, E., 2012. Divergence time estimations
861 and contrasting patterns of genetic diversity between Antarctic and southern South America benthic
862 invertebrates. *Rev. Chil. Hist. Nat.* 85, 445–456.
863
864 González-Wevar, C.A., Rosenfeld, S., Segovia, N.I., Hüne, M., Gérard, K., Ojeda, J., Mansilla, A.,
865 Brickle, P., Díaz, A., Poulin, E., 2016. Genetics, gene flow, and glaciation: the case of the South
866 American limpet *Nacella mytilina*. *PLoS ONE* 11, e0161963.
867
868 González-Wevar, C.A., Hüne, M., Segovia, N.I., Nakano, T., Spencer, H.G., Chown, S.L., Saucède, T.,
869 Johnstone, G., Mansilla, A., Poulin, E., 2017. Following the Antarctic Circumpolar Current:
870 patterns and processes in the biogeography of the limpet *Nacella* (Mollusca: Patellogastropoda)
871 across the Southern Ocean. *J. Biogeogr.* 44, 861–874.
872
873 González-Wevar, C.A., Segovia, N.I., Rosenfeld, S., Ojeda, J., Saucède, T., Brickle, P., Morley, S.,
874 Spencer, H.G., Poulin, E., 2018. Unexpected absence of island endemics: long-distance dispersal in
875
876
877
878
879
880
881
882
883
884
885

- 886
887
888 higher latitude sub-Antarctic *Siphonaria* (Gastropoda: Euthyneura) species. J. Biogeogr. DOI:
889 10.1111/jbi.13174
890
- 891 Grant, R., Griffiths, H., Steinke, D., Wadley, V., Linse, K., 2010. Antarctic DNA barcoding; a drop in the
892 ocean? Polar Biol. 34, 775–780.
893
- 894 Griffiths, H., Barnes, D., Linse, K., 2009. Towards a generalized biogeography of the Southern Ocean
895 benthos. J. Biogeogr. 36, 162–177.
896
- 897 Griffiths, H., Arango, C., Munilla, T., McInnes, S., 2011. Biodiversity and biogeography of Southern
898 Ocean pycnogonids. Ecography 34, 616–627.
899
- 900 Griffiths, H., Waller, C.L., 2016. The first comprehensive description of the biodiversity and
901 biogeography of Antarctic and Sub-Antarctic intertidal communities. J. Biogeogr. 43, 1143–1155.
902
- 903 Gubili, C., Ross, E., Billett, D. S., Yool, A., Tsairidis, C., Ruhl, H. A., et al., 2016. Species diversity in
904 the cryptic abyssal holothurian *Psychropotes longicauda* (Echinodermata). Deep Sea Research Part
905 II: Topical Studies in Oceanography 137, 288–296.
906
- 907 Gutt, J., Sirenko, B., Smirnov, I., Arntz, W., 2004. How many macrozoobenthic species might inhabit the
908 Antarctic shelf? Ant. Sci. 16, 11–16.
909
- 910 Harder, A.M., Halanych, K.M., Mahon, A.R., 2016. Diversity and distribution within the sea spider genus
911 Pallenopsis (Chelicerata: Pycnogonida) in the Westerns Antarctic as revealed by mitochondrial
912 DNA. Polar Biol. 39, 677–688.
913
- 914 Held, C., Wägele, J-W., 2005. Cryptic speciation in the giant Antarctic isopod *Glyptonotus antarcticus*
915 (Isopoda: Valvifera: Chaetiliidae). Sci. Mar. 69, 175–181.
916
- 917 Heimeier, D., Lavery, S., Sewell, M., 2010. Molecular Species identification of *Astrotooma agassizii* from
918 planktonic embryos: further evidence for a cryptic species complex. J. Hered. 101, 775–779.
919
- 920 Hemery, L., Eleaume, M., Roussel, V., Ameziane, N., Gallut, C., Steinke, D., Cruaud, C., Couloux, A.,
921 Wilson, N.G., 2012. Comprehensive sampling reveals circumpolarity and sympatry in seven
922 mitochondrial lineages of the Southern Ocean crinoid species *Promachocrinus kerguelensis*
923 (Echinodermata). Mol. Ecol. 21, 2502–2518.
924
- 925 Hennion, F., Walton, D.W.H., 1997. Ecology and seed morphology of endemic species from Kerguelen
926 phytogeographic zone. Polar Biol. 18, 229–235.
927
- 928 Hodgson, D.A., Graham, A.G.C., Roberts, S.J., Bentley, M.J., Cofaigh, C.Ó., Verleyen, E., Vyverman,
929 W., Jomeli, V., Favier, V., Brunstein, D., Verfaillie, D., Colhoun, E.A., Saunders, K.M., Selkirk,
930 P.M., Mackintosh, A., Hedding, D.W., Nel, W., Hall, K., McGlone, M.S., Van der Putten, N.,
931 Dickens, W.A., Smith, J.A., 2014. Terrestrial and submarine evidence for the extent and timing of
932 the Last Glacial Maximum and the onset of deglaciation on the maritime-Antarctic and sub-
933 Antarctic islands. Quat. Sci. Rev. 100, 137–158.
934
935
936
937
938
939
940
941
942
943
944

- 945
946
947 Huelsenbeck, J., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees.
948 Bioinformatics 17, 754–755.
- 950 Hunter, R., Halanych, K., 2008. Evaluating connectivity in the brooding brittle star *Astrotoma agassizii*
951 across the Drake Passage in the Southern Ocean. J. Hered. 99, 137–148.
- 953 Janosik, A., Mahon, A., Halanych, K., 2011. Evolutionary history of Southern Ocean *Odontaster* sea star
954 species (Odontasteridae; Asteroidea). Polar Biol. 34, 575–586.
- 956 Knox, G. (2007). Biology of the Southern Ocean (2nd ed). CRC Press Taylor and Francis Group.
- 958 Koubbi, P., De Broyer, C., Griffiths, H.J., Raymond, B., d’Udekem d’Acoz, C., Van de Putte, A.P.,
959 Danis, B., David, B., Grant, S., Gutt, J., Held, C., Hosie, G., Huettmann, F., Post, A., Ropert-
960 Coudert, Y., Stoddart, M., Swadling, K.M. and Wadley, V. (2014) Conclusions: present and future
961 of Southern Ocean biogeography. In De Broyer C., Koubbi, P., Griffiths, H.J., Raymond, B.,
962 Udekem d’Acoz, C., Van de Putte A.P., Danis, B., Grant, S., Gutt, J., Held, C., Hosie, G.,
963 Heutmann, F., Post, A., Ropert-Coudert, Y. (Eds.), Biogeographic Atlas of the Southern Ocean.
964 Scientific Committee on Antarctic Research, Cambridge. pp. 470–475.
- 968 Krabbe, K., Leese, F., Mayer, C., Tollrian, R., Held, C., 2010. Cryptic mitochondrial lineages in the
969 widespread pycnogonid *Colossendeis megalonyx* Hoek, 1881 from Antarctic and Subantarctic
970 waters. Polar Biol. 33, 281–292.
- 973 Ledoux, J.B., Tarnakowska, K., Gérard, K., Lhuhier, E., Jacquemin, B., Weydmann, A., Féral, J-P.,
974 Chenuil, A., 2012. Fine-scale spatial genetic structure in the brooding sea urchin *Abatus cordatus*
975 suggests vulnerability of the Southern Ocean marine invertebrates facing global change. Polar Biol.
976 35, 611–623.
- 979 Lee, M. R., Canales-Aguirre, C. B., Nuñez, D., Perez, K., Hernandez, C. E., Brante, A., 2017 The
980 identification of sympatric cryptic free-living nematode species in the Antarctic intertidal. PLoS
981 ONE 12(10), e0186140. doi.org/10.1371/journal.pone.0186140
- 983 Leese, F., Kop, A., Wägele, J-W., Held, C., 2008. Cryptic speciation in a benthic isopod from Patagonian
984 and Falkland Island waters and the impact of glaciations on its population structure. Front. Zool. 5,
985 1–15.
- 988 Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism
989 data. Bioinformatics 25, 1451–1452.
- 991 Linse, K., Griffiths, H., Barnes, D., Clarke, A., 2006. Biodiversity and biogeography of Antarctic and
992 sub-Antarctic mollusca. Deep-Sea Res. II 53, 985–1008.
- 994 Linse K., 2014. Bivalves. In De Broyer C., Koubbi, P., Griffiths, H.J., Raymond, B., Udekem d’Acoz, C.,
995 Van de Putte A.P., Danis, B., Grant, S., Gutt, J., Held, C., Hosie, G., Heutmann, F., Post, A.,
996 Ropert-Coudert, Y. (Eds.), Biogeographic Atlas of the Southern Ocean. Scientific Committee on
997 Antarctic Research, Cambridge. pp. 126–128.
- 1000
1001
1002
1003

- 1004
1005
1006 Macaya, E., Zuccarello, G., 2010. DNA barcoding and genetic divergence in the giant kelp *Macrocystis*
1007 (Laminariales). *J. Phycol.* 46, 736–742.
1008
1009 Mackensen, A., 2004. Changing Southern Ocean palaeocirculation and effects on global climate. *Ant.*
1010 *Sci.* 16, 369–386.
1011
1012 McDowall, R.M., 1968. Oceanic islands and endemism. *Syst. Zool.* 17, 346–350.
1013
1014 Moon, K.L., Chown, S.L., Fraser, C.I., 2017. Reconsidering connectivity in the sub-Antarctic. *Biol. Rev.*
1015 92, 2164–2181.
1016
1017 Mortimer, E., Jansen van Vuuren, B., Lee, J.E., Marshall, D.J., Ryan, P., Chown, S.L., 2011. Mite
1018 dispersal among the Southern Ocean Islands and Antarctica before the last glacial maximum. *Proc.*
1019 *Biol. Sci.* 278, 1247–1255.
1020
1021 Near, T., Dornburg, A., Kuhn, K., Eastman, J., Pennington, J., Patarnello, T., Zane, L., Fernández, D.A.,
1022 Jones, C.D., 2012. Ancient climate change, antifreeze, and the evolutionary diversification of
1023 Antarctic fishes. *Proc. Natl. Acad. Sci. USA* 109, 3434–3439.
1024
1025 Nikula, R., Fraser, C., Spencer, H., Waters, J., 2010. Circumpolar dispersal by rafting in two subantarctic
1026 kelp-dwelling crustaceans. *Mar. Ecol-Prog. Ser.* 405, 221–230.
1027
1028 Oliverio, M., Mariottini, P., 2001. Contrasting morphological and molecular variation in *Coralliophila*
1029 *meyendorffii* (Muricidae, Coralliophilinae). *J. Moll. Stud.* 67, 243–246.
1030
1031 Pierrat, B., Saucède, T., Brayard, A., David, B., 2013. Comparative biogeography of echinoids, bivalves
1032 and gastropods from the Southern Ocean. *J. Biogeogr.* 40, 1374–1385.
1033
1034 Poulin, E., Féral, J-P., 1995. Pattern of spatial distribution of brood-protecting schizasterid echinoid,
1035 *Abatus cordatus*, endemic to the Kerguelen Islands. *Mar. Ecol-Prog. Ser.* 118, 179–186.
1036
1037 Poulin, E., Gonzalez-Wevar, C.A., Díaz, A., Gérard, K., Hüne, M., 2014. Divergence between Antarctic
1038 and South American marine invertebrates: What molecular biology tells us about Scotia Arc
1039 geodynamics and the intensification of the Antarctic Circumpolar Current. *Global Planet. Change*
1040 123, 392–399.
1041
1042 Quilty, P.G., Darragh, T.A., Gallagher, S.J., Hardin, L.A., 2016. Pliocene Mollusca (Bivalvia,
1043 Gastropoda) from the Sørsdal Formation, Marine Plain, Vestfold Hills, East Antarctica: taxonomy
1044 and implications for Antarctic Pliocene palaeoenvironments, *Alcheringa* 40, 556–582.
1045
1046 Rabarts, I., Whybrow, S., 1979. A revision of the Antarctic and Subantarctic members of the genus
1047 *Yoldia* Moller, 1842 (Bivalvia: Nuculanidae). *J. Natural Hist.* 13, 161–183.
1048
1049 Raupach, M., Wägele, J-W., 2006. Distinguishing cryptic species in Antarctic Asellota (Crustacea:
1050 Isopoda) - a preliminary study of mitochondrial DNA in *Acanthaspidia drygalskii*. *Antarct. Sci.* 18,
1051 191–198.
1052
1053 Rintoul, S., Hughes, C., Olbers, D., 2001. The Antarctic Circumpolar Current System. In Siedler, G.,
1054 Church, J., Gould, J. (Eds.), *Ocean Circulation and Climate*, Oxford Academic Press. pp. 1–32.
1055
1056
1057
1058
1059
1060
1061
1062

- 1063
1064
1065 Salzburger, W., Ewing, G.B., Von Haeseler, A., 2011. The performance of phylogenetic algorithms in
1066 estimating haplotype genealogies with migration. *Mol. Ecol.* 20: 1952–1963.
1067
1068 Sartori, A. F., 2016a. *Aequiyoldia* Soot-Ryen, 1951. In: MolluscaBase (2017). Accessed through: World
1069 Register of Marine Species at <http://www.marinespecies.org/aphia.php?p=taxdetails&id=868254>
1070 on 2017-12-16.
1071
1072 Sartori, A.F., 2016b. *Aequiyoldia eightsii* Jay, 1839. In: MolluscaBase (2017). Accessed through: World
1073 Register of Marine Species at <http://www.marinespecies.org/aphia.php?p=taxdetails&id=868255>
1074 on 2017-12-01.
1075
1076
1077 Saucède, T., Pierrat, B., Danis, B. David, B., 2014. Biogeographic processes in the Southern Ocean. In De
1078 Broyer C., Koubbi, P., Griffiths, H.J., Raymond, B., Udekem d’Acoz, C., Van de Putte A.P., Danis,
1079 B., Grant, S., Gutt, J., Held, C., Hosie, G., Heutmann, F., Post, A., Ropert-Coudert, Y. (Eds.),
1080 Biogeographic Atlas of the Southern Ocean. Scientific Committee on Antarctic Research,
1081 Cambridge. pp. 456–463.
1082
1083
1084 Sharma, P.P., Zardus, J.D., Boyle, E.E., González, V.L., Jennings, R.M., McIntyre, E., Wheeler, W.C.,
1085 Etter, R.J., Giribet, G., 2013. Into the deep: A phylogenetic approach to the bivalve subclass
1086 Protobranchia, *Mol. Phylogenet. Evol.* 69, 188–204.
1087
1088
1089 Strugnell, J., Rogers, A., Prodöhl, P., Collins, M., Allcock, A., 2008. The thermohaline expressway: the
1090 Southern Ocean as a centre of origin for deep-sea octopuses. *Cladistics* 24, 853–860.
1091
1092 Strugnell, J., Watts, P., Smith, P., Allcock, A., 2012. Persistent genetic signatures of historic climatic
1093 events in an Antarctic octopus. *Mol. Ecol.* 21, 2775–2787.
1094
1095
1096 Tamura, K., Strecher, G., Peterson, D., Filipski, A., and Kumar, S., 2013. MEGA6: Molecular
1097 Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.* 30, 2725–2729.
1098
1099 Thornhill, D., Mahon, A., Norenburg, J., Halanych, K., 2008. Open-ocean barriers to dispersal: a test case
1100 with the Antarctic Polar Front and the ribbon worm *Parborlasia corrugatus* (Nemertea: Lineidae).
1101 *Mol. Ecol.* 17, 5104–5117.
1102
1103 Verducci, M., Foresi, L., Scott, G., Sprovieri, M., Lirer, F., Pelosi, N., 2009. The Middle Miocene
1104 climatic transition in the Southern Ocean: Evidence of paleoclimatic and hydrographic changes at
1105 Kerguelen plateau from planktonic foraminifers and stable isotopes. *Palaeogeogr. Palaeoclim.*
1106 *Palaeoecol.* 280, 371–386.
1107
1108
1109 Verheye, M.L., Backeljau, T., d’Udekem d’Acoz, C., 2017. Locked in the icehouse: Evolution of an
1110 endemic Epimeria (Amphipoda, Crustacea) species flock on the Antarctic shelf. *Mol. Phylogenet.*
1111 *Evol.* 114, 14–33.
1112
1113
1114 Villarroel, M., Stuardo, J., 1998. Protobranchia (Mollusca: Bivalvia) chilenos recientes y algunos fósiles.
1115 *Malacologia* 40, 113–229.
1116
1117
1118
1119
1120
1121

- 1122
1123
1124 Waters, J., 2007. Driven by the West Wind Drift? A synthesis of southern temperate marine
1125 biogeography, with new directions for dispersalism. *J. Biogeogr.* 35, 417–427.
1126
1127 Weis, A., Meyer, R., Dietz, L., Dömel, J.S., Leese, F., Melzer, R.R., 2014. *Pallenopsis patagonica* (Hoek,
1128 1881) – a species complex revealed by morphology and DNA barcoding, with description of a new
1129 species *Pallenopsis* Wilson, 1881. *Zool J. Linn. Soc.* 170, 110–131.
1130
1131 Wilson, N., Hunter, R., Lockhart, S., Halanych, K., 2007. Multiple lineages and absence of panmixia in
1132 the “circumpolar” crinoid *Promachocrinus kerguelensis* from the Atlantic sector of Antarctica.
1133 *Mar. Biol.* 152, 895–904.
1134
1135 Wilson, N., Schrödl, M., Halanych, K., 2009. Ocean barriers and glaciation: evidence for explosive
1136 radiation of mitochondrial lineages in the Antarctic sea slug *Doris kerguelensis* (Mollusca,
1137 Nudibranchia). *Mol. Ecol.* 18, 965–984.
1138
1139 Wilson, P., Clark, R., Birnie, J., Moore, D.M., 2002. Late Pleistocene and Holocene landscape evolution
1140 and environmental change in the Lake Sullivan area, Falkland Islands, South Atlantic. *Quat. Sci.*
1141 *Rev.* 21, 1821–1840.
1142
1143 Wilson, P., Bentley, M.J., Schnabel, C., Clark, R., Xu, S., 2008. Stone run (block stream) formation in the
1144 Falkland Islands over several cold stages, deduced from cosmogenic isotope (^{10}Be and ^{26}Al) surface
1145 exposure dating. *J. Quat. Sci.* 23, 461–473.
1146
1147 Wright, F., 1990. The effective number of codons' used in a gene. *Gene* 87, 23–29.
1148
1149 Zachos, J., Pagani, M., Sloan, L., Thomas, E., Billups, K., 2001. Trends, rhythms, and aberrations in
1150 global climate 65 Ma to present. *Science* 292, 686–693.
1151
1152
1153
1154
1155
1156
1157
1158
1159
1160
1161
1162
1163
1164
1165
1166
1167
1168
1169
1170
1171
1172
1173
1174
1175
1176
1177
1178
1179
1180

1181
1182
1183 **Figure Captions**
1184

1185 **Figure 1.** A) Main Southern Ocean provinces included in the study. B) Sampling sites of *Aequiyoldia*
1186 *eightsii* off South America (SA), Falkland/Malvinas Islands (FI), and Antarctic Peninsula (AP). C)
1187 Distribution of *Aequiyoldia eightsii* in the Southern Ocean (Linse et al., 2014).
1188

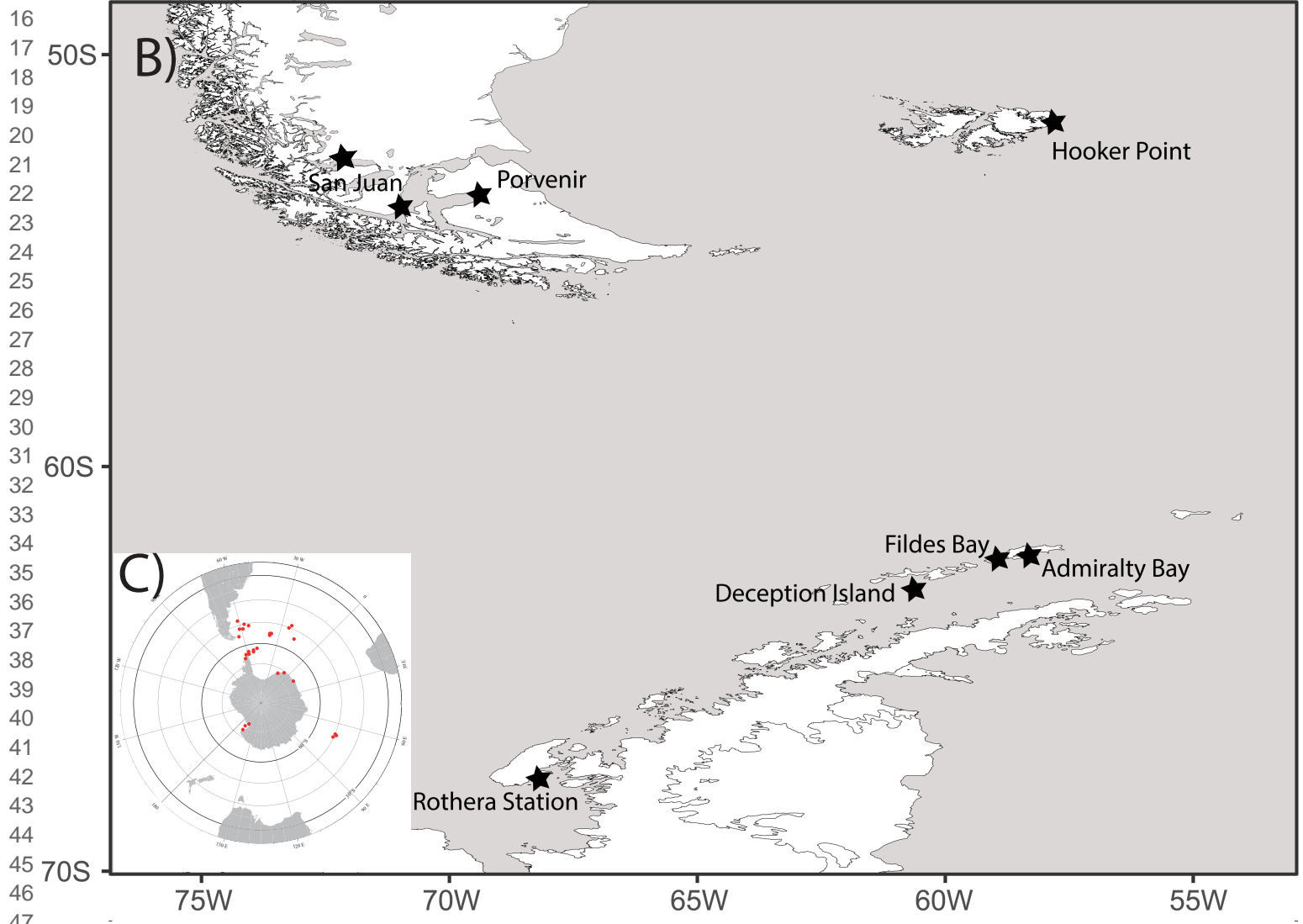
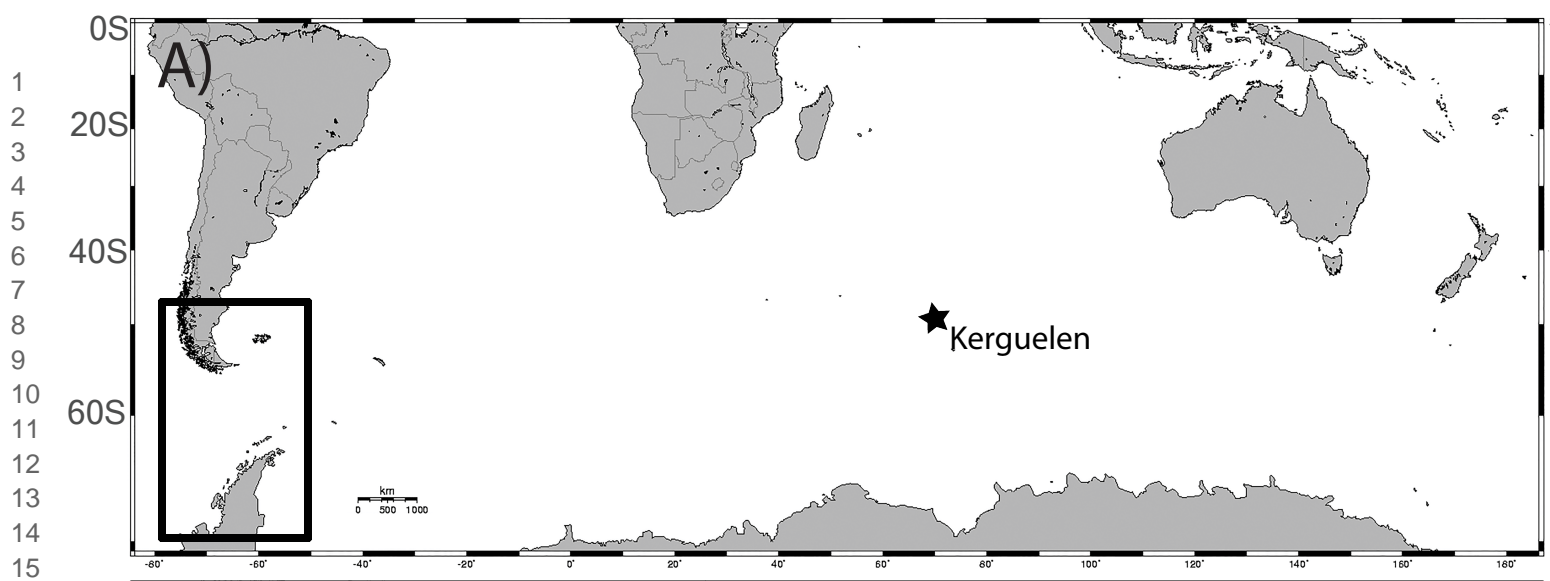
1189
1190 **Figure 2.** *Aequiyoldia eightsii* individuals from: a-c) Kerguelen Islands (KI = L1), d-f) Rothera Station
1191 (AP = L2), g-i) Admiralty Bay (AP = L3), j-l) South America (SA = L4), m-o), and the
1192 Falkland/Malvinas Islands (FI = L5).
1193

1194
1195 **Figure 3.** Bayesian maximum clade credibility tree of *Aequiyoldia* relationships based on mtDNA (COI)
1196 sequences with special emphasis on Antarctic Peninsula and South American populations. Bootstrap
1197 support values (MP and ML) and BPP are shown above the nodes (in that order). Map and colors indicate
1198 major sampled areas. Round charts indicate the frequency of the recorded *Aequiyoldia* lineages across the
1199 sampling sites.
1200

1201 **Figure 4.** Bayesian maximum clade credibility tree based on mtDNA (COI) showing divergence time
1202 estimates within A) protobranch bivalves and B) *Aequiyoldia*. Posterior probabilities are shown above the
1203 clades. Grey boxed regions depict 95% Bayesian credible intervals (BCIs) for relative divergence times.
1204

1205
1206 **Figure 5.** Non-metric, Multidimensional Scaling (NMDS) ordination of *A. eightsii* samples from the
1207 Kerguelen Islands (KI), Falkland/Malvinas Islands (FI), South America, and the Antarctic Peninsula
1208 using shell morphological data following Rabarts and Whybrow (1979).
1209

1210
1211 **Supplementary Figure 1.** A) mtDNA (COI) maximum parsimony networks including *Aequiyoldia*
1212 individuals collected at different localities in the Southern Ocean. B) nucDNA (ITS1 and ITS2) maximum
1213 parsimony network including *Aequiyoldia* individuals from different localities in the Southern Ocean. A
1214 coloured circle represents each haplotype and indicate the main area where it was collected. The size of
1215 the circle is proportional to its frequency in the whole sampling effort.
1216
1217
1218
1219
1220
1221
1222
1223
1224
1225
1226
1227
1228
1229
1230
1231
1232
1233
1234
1235
1236
1237
1238
1239



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56



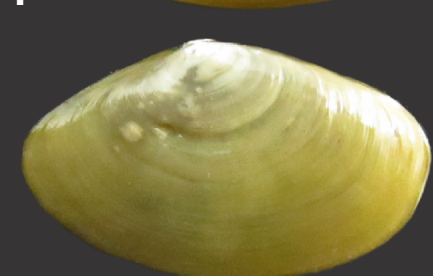
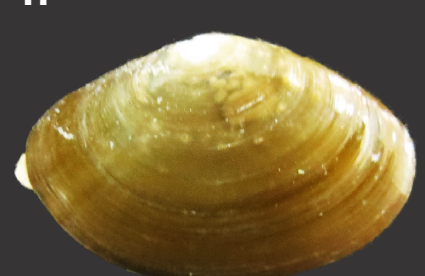
KI



AP1



AP2

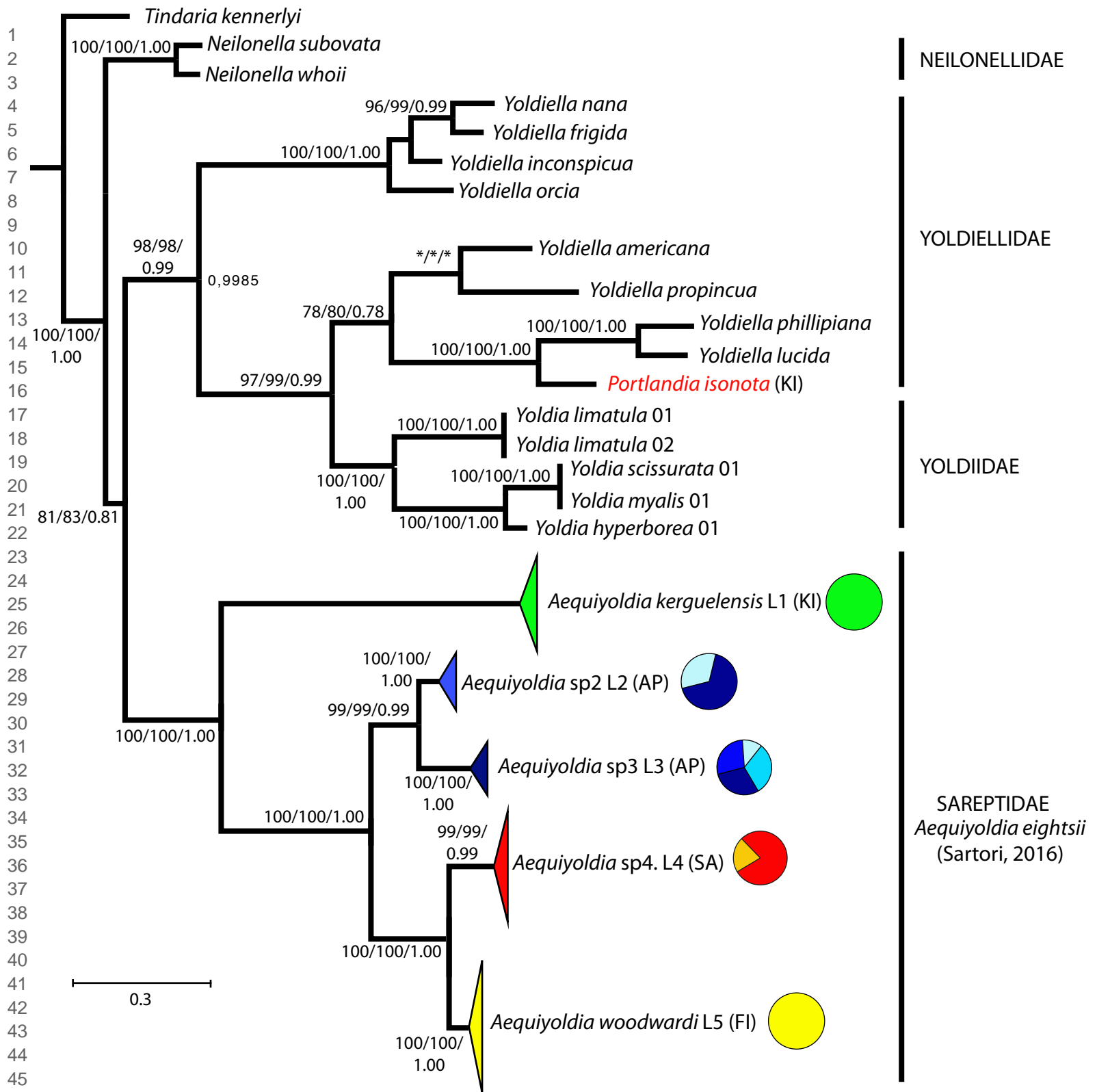


SA



FI

1 cm



ANTARCTIC PENINSULA

SOUTH AMERICA

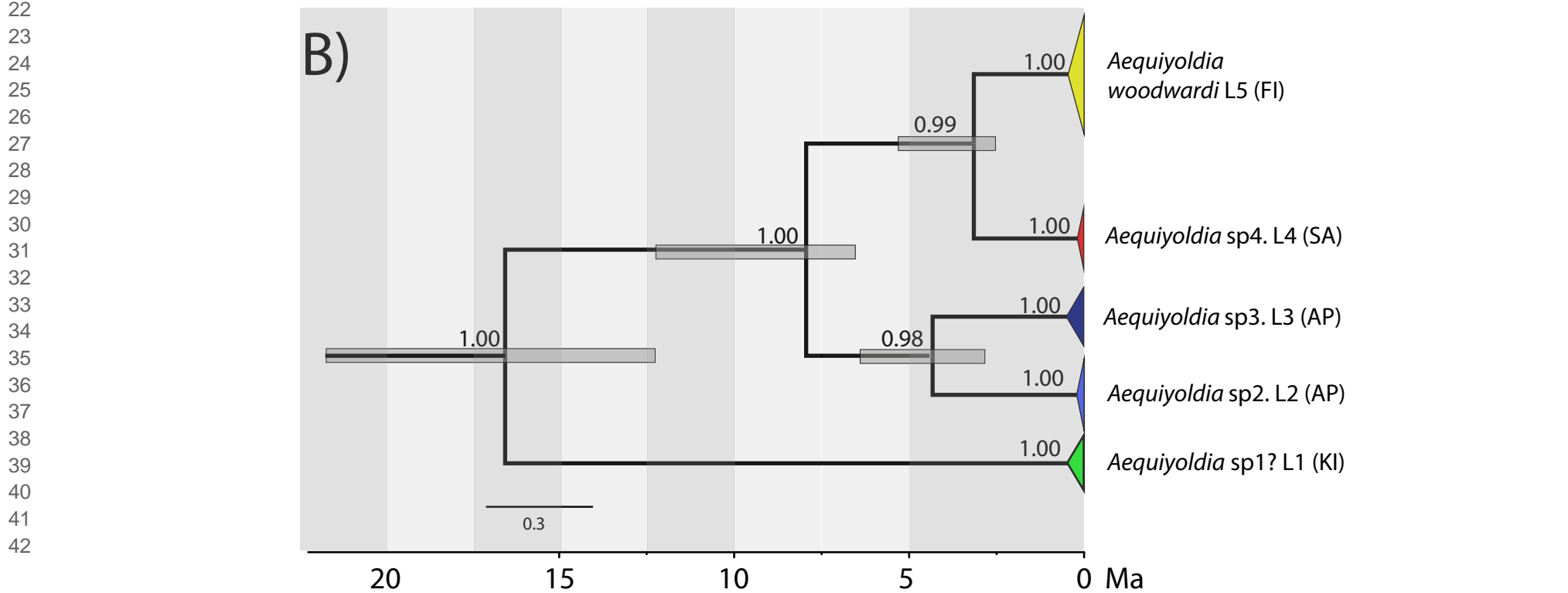
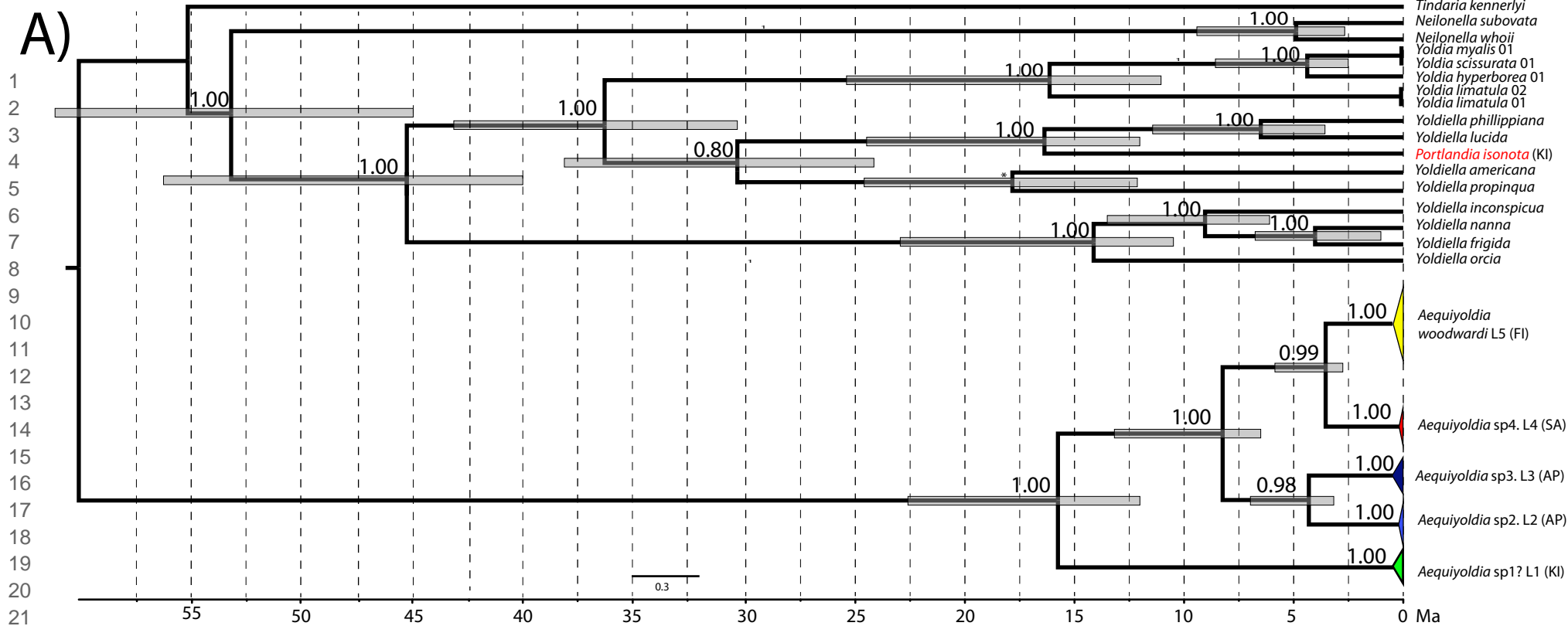
KERGUELEN ISLAND (KI)

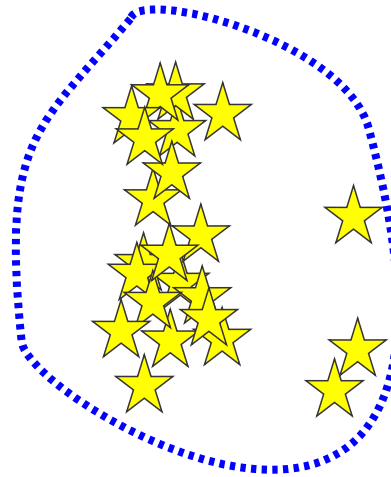
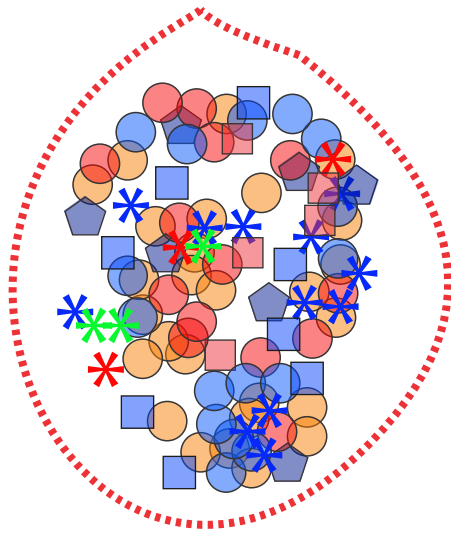
- 48 Admiralty Bay (AP)
- 49 Fildes Bay (AP)
- 50 Deception Island (AP)
- 51 Rothera Station (AP)

- 52 San Juan (SA)
- 53 Porvenir (SA)
- 54 Hooker Point (FI)

- 55 Port Aux Français

SAREPTIDAE
Aequiyoldia eightsii
(Sartori, 2016)





1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56

SOUTH AMERICA	FALKLAND/MALVINAS ISLANDS	ANTARCTIC PENINSULA	KERGUELEN ISLANDS
<p>○ Porvenir</p> <p>○ San Juan</p> <p>* Skyring Sound</p>	<p>★ Hooker Point</p>	<p>⬠ Admiralty Bay</p> <p>* Fildes Bay</p> <p>○ Deception Island</p> <p>■ Rothera Station</p>	<p>* Port Aux Français</p>

A) COI

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56

SOUTH AMERICA (SA)

- San Juan (Strait of Magellan)
- Porvenir (Tierra del Fuego)

FALKLAND/MALVINAS ISLANDS (FI)

- Hookers Point

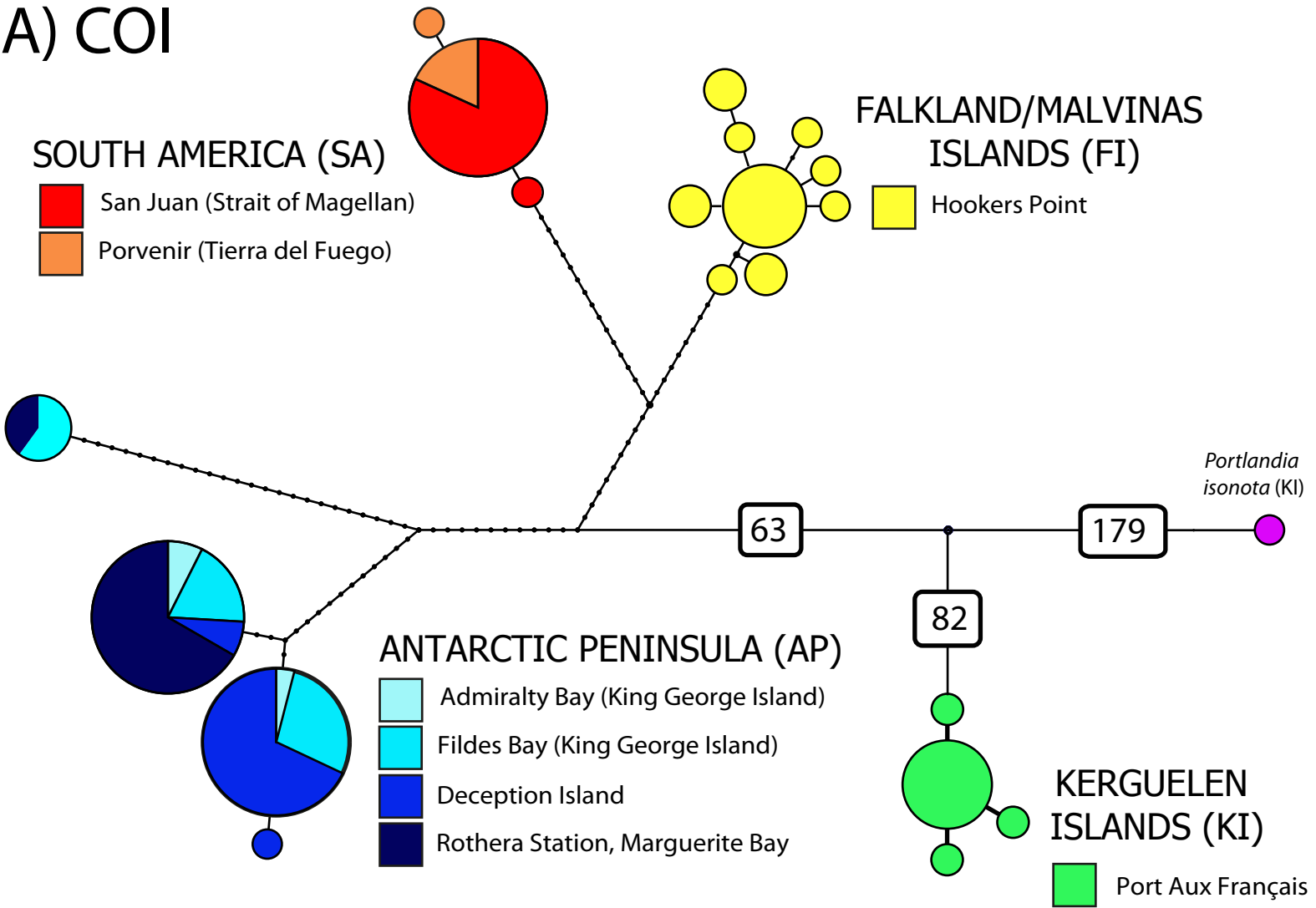
ANTARCTIC PENINSULA (AP)

- Admiralty Bay (King George Island)
- Fildes Bay (King George Island)
- Deception Island
- Rothera Station, Marguerite Bay

KERGUELEN ISLANDS (KI)

- Port Aux Français

*Portlandia
isonota* (KI)



B) ITS1 + ITS2

- Deception Island
- San Juan (Strait of Magellan)
- Porvenir (Tierra del Fuego)
- Hookers Point

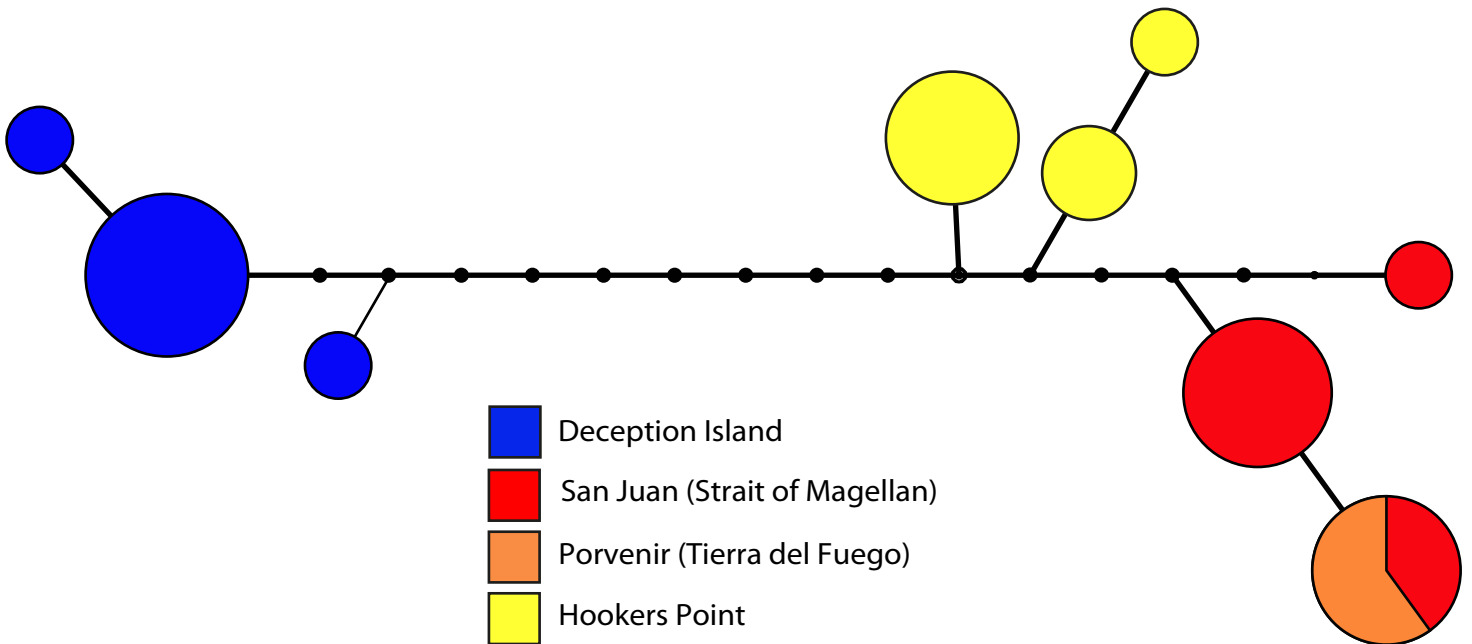


Table 1. Sampling sites of *Aequiyoldia eightsii*: Geographical coordinates, name of collector, n: number of analyzed individuals per locus.

Sites	Lat.	Long.	n (COI)	n (ITS)
Port-aux-Français, Kerguelen Islands	49°21'S	70°13'W	11	9
Hooker's 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	-

Table 2. Uncorrected p-distances based on mtDNA COI sequences between the recorded lineages of *Aequiyoldia* from Kerguelen Island (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%	****