1 Is reproductive strategy a key factor in understanding the evolutionary history

2 of Southern Ocean Asteroidea (Echinodermata)?

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Running title: Phylogeography of Southern Ocean sea stars

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Data accessibility

All data and meta-data used in this study are publicly available on BOLD Systems (www.boldsystems.org) under the Dataset code DS-ASTROULB as a compilation of the following projects: SEAST; EAR; DSPEC; CHEC; SOA; SWEMA; TNBA; TCTNB; ODTNB; AIIIS; SORK; NZEC and NZECA.

Abstract

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51 Aim To investigate asteroid genetic structure in the Southern Ocean and test whether evolutionary and diversity 52 patterns differ due to reproductive strategy (brooding versus broadcasting). We hypothesise that brooding species 53 will show higher genetic diversity and greater species richness with a more pronounced genetic structuring in 54 space, compared to broadcasting species. Revealing these contrasting patterns should contribute to improving 55 our knowledge of asteroid evolutionary history. 56

57 Location Southern Ocean, south of 45 °S.

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4 6 7 8 $9 \\ 101 \\ 12 \\ 134 \\ 156 \\ 178 \\ 190 \\ 212 \\ 234 \\ 256 \\ 27$ 28 29 $\begin{array}{c} 30\\ 31\\ 32\\ 33\\ 34\\ 35\\ 36\\ 37\\ 38\\ 39\\ 40\\ 42\\ 43\\ \end{array}$ 44 45 46 47 48 49 59 **Taxon** Echinodermata, Asteroidea 60

61 Methods Over 1,400 mtDNA cytochrome C oxidase subunit I (COI) sequences were analysed using several
 62 species delineation methods, phylogenetic reconstructions, and molecular clock calibrations, in order to examine
 63 the role of reproductive strategy in the observed evolutionary and phylogeographic patterns.

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65 Results Genetic diversity was not found to be significantly different between brooders and broadcasters. 66 Similarly, species richness was no greater in brooding genera. Broadcasters are less structured spatially than 67 brooders, revealing their contrasting evolutionary histories, Phylogeographic patterns of broadcasters indicate 68 potential scenarios of deep-sea colonisation routes, bipolarity or cosmopolitan species, sub-Antarctic emergence, 69 and faunal exchanges between the Antarctic and New Zealand across the Polar Front. Phylogeographic patterns 70 of brooders could support the hypothesis of an established past trans-Antarctic seaway between the Ross and 71 Weddell seas during the Plio-Pleistocene. Our results also propose, for the first time, that the Weddell Sea is 72 composed of a mixed asteroid fauna originating from both the East and West Antarctic.

Main conclusions Reproductive strategy does not affect genetic diversity nor species richness of Southern
 Ocean asteroids. Nevertheless, reproductive strategy has played a key role in the spatial patterns of asteroid
 diversification in the Southern Ocean. Present spatial patterns of genetic diversity are contrasting between
 brooders and broadcasters; illuminating the evolutionary history of this key Southern Ocean benthic fauna.

79 Keywords

80 Antarctica, Asteroidea, bipolarity, brooding, Echinodermata, invertebrate, thermohaline expressway, trans-

81 Antarctic seaway, emergence

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83 Introduction

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85 The diversity of marine life in the Southern Ocean (SO) has long been underestimated and many taxa could be 86 overlooked or misidentified due to the reliance on systematics based solely on morphological characters (Gutt et 87 al., 2004; Clarke, 2008). The growing awareness of these issues and the lack of representative sampling (Griffiths 88 et al., 2014), have prompted Antarctic marine biologists to undertake a series of comprehensive census surveys 89 covering a wide range of taxa in the last two decades. International efforts such as the International Polar Year 90 (IPY 2007-2008) and the Census of Antarctic Marine Life (CAML 2005-2010) have been the launching pads for a 91 better assessment of SO biodiversity and its underlying ecological processes. Recent studies that have applied 92 molecular techniques to these SO specimens, exploring diversity, systematics, and phylogeography, have 93 significantly increased our understanding of Antarctic benthic ecosystems (e.g. Sands et al., 2015). These 94 international SO sampling expeditions have achieved several major objectives, such as the creation of a baseline 95 census of biodiversity (De Broyer & Danis, 2011; Griffiths et al., 2011), proposing how evolution has been 96 influenced by the regional geological, climatic, and oceanographic histories (e.g. Fraser et al., 2009, González-97 Wevar et al., 2018), and disentangling phylogeographic patterns at lower taxonomic levels to better understand 98 relationships among populations and species on a case by case strategy (e.g. Brasier et al., 2017; Dömel et al., 99 2017). This extensive work led to the discovery and the description of many new species (e.g. Janosik & 100 Halanych, 2010; d'Udekem d'Acoz & Verheye, 2017). More importantly, these studies show the frequent 101 discordance existing between traditional (morphology based) and molecular (DNA based) methods for assessing 102 species diversity (e.g. Dömel et al., 2017; Janosik et al., 2011). Cryptic speciation is a documented source of 103 species diversity underestimation, but it is not the only one. A recent study on the most studied sea star species in 104 the SO, Odontaster validus, Peck et al. (2018) showed that polymorphism in some morphological characters 105 could lead to misidentification of this frequently encountered species. Incorrect taxonomic assignments due to the 106 lack of clear identification keys (Allcock & Griffiths 2014), descriptions of nominal species based on distribution 107 only (Diaz et al. 2011, Saucède et al., 2015), descriptions based on juvenile specimens (Roberts et al. 2014) or 108 on deteriorated specimens due to inappropriate conservation practices, are all common limitations in meaningful 109 biodiversity assessments (Meyer, 2016).

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111 Processes that have and continue to drive complex diversity patterns in the SO are far from being fully

112 understood, but the role of certain drivers has been demonstrated in a number of molecular studies. Processes

can be extrinsic (e.g. paleogeographic, climatic, oceanographic) or intrinsic (life history traits) (Allcock & Strugnell,
 2012; Thatje, 2012), and their combined effects cumulative, making identification of explanatory processes a

- 114 2012; Thatje, 2012), and their combined effects cumulative, making identification of explanatory processes a difficult endeavour. Life bioteny traits such as reproductive strategies have been proven to share the genetic.
- 115 difficult endeavour. Life history traits such as reproductive strategies have been proven to shape the genetic

- 116 structure of species in contrasting ways (Modica et al., 2017). Most marine benthic organisms show low to zero
- 117 mobility during the adult stage. The dispersal capacity of larvae is thus expected to condition population genetic
- 118 structure (Bowen et al., 2006; Purcell et al., 2006). Larval development (e.g. planktotrophic, lecithotrophic), 119
- parental care (brooders versus broadcasters) and pelagic larval duration (from days to several months; Selkoe & 120 Toonen, 2011), are intrinsic factors that can combine with extrinsic factors, such as oceanographic currents or
- 121 geological history, to determine the population genetic structure (e.g. Hoffman et al., 2011, Jossart et al., 2017).
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123 As a consequence of their high diversity and differentiated functional roles, benthic invertebrates have been the 124 subject of many studies on genetic diversity and connectivity (see Riesgo et al., 2015 for a review). Sea stars 125 (Asteroidea, Echinodermata) represent a diversified, abundant and common ecological group in SO benthic 126 habitats (Jossart et al., 2015). To date, around 300 species have been recorded from the SO (Moreau et al., 127 2015; Moreau et al., 2018) but it is likely that many more remain to be described. Recent studies based on 128 species morphological identification tried to disentangle distribution patterns and biogeographic relationships in 129 sea stars at the scale of the entire SO (Moles et al., 2015; Moreau et al., 2017). These studies described the 130 spatial structure of asteroid assemblages and stressed the major influence of life history traits, among which 131 reproductive strategy appeared to have the most notable effect (Moreau et al., 2017).

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133 Few molecular studies have been performed on SO asteroids and they are nearly exclusively focused on the 134 common Odontaster genus (Janosik & Hallanych, 2010; Janosik et al., 2011). The phylogeny and evolutionary 135 history of the Asteroidea in the SO has, however, been discussed in several studies (Mah & Foltz, 2011a, 2011b, 136 2014; Mah et al., 2015). In their comprehensive studies on the molecular phylogeny of the superorders 137 Valvatacea and Forcipulatacea, Mah & Foltz (2011a, b) describe some diversification processes in several 138 Antarctic and sub-Antarctic families (e.g. Odontasteridae, Antarctic Asteriidae) but they did not analyse the lower

- 139 taxonomic levels (i.e., genera and species).
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141 Brooders and broadcasters have contrasting dispersal capabilities (low range dispersal in brooders vs high range 142 in broadcasters) and brooding taxa are usually hypothesised to display 1) higher genetic diversity, 2) greater 143 species richness and 3) more genetic differentiation through space than broadcasters (e.g. Modica et al., 2017; 144 Purcell et al., 2006). In the present study, we tested these assumptions by investigating the genetic diversity and 145 the phylogeography of five widely distributed asteroid genera across the SO. We selected genera with contrasting 146 reproductive strategies in two distinct clades: the three brooding genera Diplasterias, Lysasterias, and Notasterias 147 in the monophyletic family Asteriidae (Foltz et al., 2007; Mah & Foltz, 2011b), and the two broadcasting genera 148 Psilaster and Bathybiaster in a monophyletic group of the family Astropectinidae (Mah & Foltz 2011a, Bosch & 149 Pearse, 1990).

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151 Materials and methods 152

Comprehensive sampling & DNA sequence compilation

153 154 Studied specimens were sampled during several expeditions in the SO (Fig. 1; Appendix S1 in Supporting 155 Information), all were preserved in 96% ethanol or frozen, and identified at species or genus level (either by 156 Christopher Mah or Camille Moreau). Genomic DNA was extracted using either a salting-out protocol (modified 157 from Sunnucks & Hales, 1996, with larger volumes and incubation at 70°C for 10 minutes to inhibit protein activity 158 after digestion) or QIAGEN DNeasy extraction kits. A fragment (612 nucleotides) of the mitochondrial gene 159 cytochrome c oxidase subunit I (COI) was then amplified using the specific forward primer LCOech1aF1 for the 160 class Asteroidea and the universal HCO2198 Reverse primer (Folmer et al., 1994). COI sequences amplified 161 using the same primers were also obtained through The Barcode of Life Data System (BOLD, Ratnasingham & 162 Hebert, 2007) in both public and private datasets, accounting for 460 of the 1,416 sequences analysed in this 163 study (Appendix S1). As the Astropectinidae genera are also recorded outside the SO (under different nominal 164 species), all available sequences for these from outside the SO were included within this study (Appendix S1). 165 Reverse and forward sequences were edited and assembled using CodonCode Aligner v6.0.2 and translated 166 using the echinoderm mitochondrial genetic code to ensure the absence of a stop codon. Sequences were 167 aligned using the MUSCLE alignment process (Edgar, 2004). Base compositional heterogeneity was examined 168 using match-paired tests for symmetry (Ababneh et al., 2006) in SeqVis v1.5 (Ho et al., 2006). 169

Phylogenetic reconstruction

170 171 Due to relatively high genetic distances, phylogenetic relationships were reconstructed independently within the 172 Asteriidae and the Astropectinidae. Coscinasterias muricata and Thrissacanthias penicillatus were used as

173 outgroups respectively, following previous phylogenetic studies (Mah & Foltz, 2011a, 2011b). Maximum 174 Likelihood (ML) and Bayesian Analyses (BA) were used to construct the trees using only unique haplotypes. ML 175 reconstructions were generated using a codon partitioned model and the GTR+G substitution model in RAxML v 176 8.1.2 (Stamatakis, 2014) through the RAxMLGUI interface (Silvestro & Michalak, 2012). To assess branch 177 support, 10 runs were realised with 1,000 thorough bootstraps each. The PartitionFinder v2 software (Lanfear et 178 al., 2016) was used for the BA reconstructions on the CIPRES Science Gateway (Miller et al., 2010) to select for 179 best-fit partitioning schemes and models of evolution. An XML file was created with BEAUti v1.8.4 (Drummond et 180 al., 2012) using a partition for each codon position as specified by PartitionFinder v2, a strict clock model with a 181 lognormal prior for "clock.rate" such that the median reflected a universal divergence time of echinoderm COI of 182 3.1 to 3.5% per Myr (McCartney et al., 2000), a Markov chain Monte Carlo run of 20 x 10⁶ generations sampling 183 every 1000 trees and a Yule process speciation prior. The XML file was used through the software BEAST v1.8.4 184 on the CIPRES Science Gateway (Miller et al., 2010) to reconstruct time-calibrated trees. Tracer v1.6 allowed us 185 to ensure an appropriate effective sampling size (ESS > 200) as recommended by the software documentation. 186 TreeAnnotator v1.8.4 calculated a consensus tree which was visualised using FigTree v1.4.3

187 (http://tree.bio.ed.ac.uk/software/figtree/).

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- Species delineation

190 Several single-locus methods of species delineation were used to delineate and explore diversity among the 191 studied genera. Two of these methods, the Generalized Mixed Yule Coalescent (GMYC - Pons et al., 2006; 192 Fujisawa & Barraclough, 2013) and the multi-rate Poisson Tree Processes (mPTP - Kapli et al., 2017) are tree-193 based methods requiring an ultrametric tree for the former and a maximum likelihood tree for the latter. Both 194 single (sGMYC) and multiple-threshold (mGMYC) models were investigated using the R package SPLITS (Ezard 195 et al. 2009) and the ultrametric tree was obtained using BEAST during the phylogenetic reconstruction. The online 196 webservice (available at http://mptp.h-its.org) was used for mPTP with the ML tree constructed using RAxML. We 197 also performed a distance-based analysis using the Automatic Barcode Gap Discovery (ABGD - Puillandre et al., 198 2012) on the online server (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) with default settings for the 199 prior range (0.001, 0.1), K80 corrected distances and a value of 1.5 for the relative gap width (X). We also used 200 the newly developed ASAP method (http://wwwabi.snv.jussieu.fr/public/asap/; Puillandre et al., in prep.) with 201 default settings and K80 corrected distances. For subsequent analyses we have selected the method giving the 202 smallest number of delineated entities as recommended to avoid false interpretation (Carstens et al., 2013). All 203 molecular diversity indexes were calculated using the DNAsp v6 software (Rozas et al., 2017) for each previously 204 delineated species. Differences in haplotype and nucleotide diversity between brooders and broadcasters were 205 tested using a Wilcoxon-Mann-Whitney test (R Core Team, 2018). Only calculations for sample sizes ≥25 206 individuals, were discussed as suggested by Goodall-Copestake et al. (2012). Intra and inter-specific genetic 207 distances within each genus were calculated using MEGA v7.0.18 (Kumar et al., 2016) and the Kimura 2-208 parameter model. Haplotype networks were generated using a TCS network method (Clement et al., 2002) 209 inferred in the software PopART (http://popart.otago.ac.nz). The genus Psilaster being recovered as polyphyletic, 210 we focus on the SO nominal species Psilaster charcoti. 211

212 Results

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In total, 1,416 sequences of 612bp were included in the analyses (Appendix S1, Table 1, Fig. 1), varying from 224
 for *Lysasterias* to 318 for *Notasterias*. No stop codons nor significant evidence for base heterogeneity were
 observed.

- Species delineation

219 Species delineation applied to all genera show contrasting results dependent upon the method applied (Fig. 2, 3, 220 4; Table 2). Overall, the mGMYC method delineated the highest number of entities (79), while mPTP was the 221 most conservative (19). Following a conservative approach, we based our work on the following delineated 222 entities: two for Bathybiaster, three for Diplasterias and Notasterias and four for P. charcoti and Lysasterias. 223 These results are in line with haplotype networks and phylogenetic groupings (Fig. 2,3, 4; Appendix 2). Among 224 the brooding entities, only one matches with an identified morphological species: Diplasterias meridionalis. In 225 broadcasters, one delineated entity of the genus Bathybiaster comprises specimens from the Arctic identified as 226 Bathybiaster vexillifer and Psilaster andromeda. 227

Genetic diversity of the delineated entities ranged from 0.00160 (*P. charcoti* - clade 3) to 0.01547 (*Diplasterias*sp2) for nucleotide diversity and from 0.394 (*P. charcoti* - clade 3) to 0.924 (*P. charcoti* - clade 1) for haplotype
diversity (Table 1). The number of segregating sites varied from 11 (*D. meridionalis*) to 50 (*P. charcoti* - clade 1).
Mean intraspecific distances varied from 0.16% (*P. charcoti* - clade 3) to 1.6% for *Diplasterias* sp2 (Table 1) while

inter-specific distances ranged from 2% between clades 2 and 3 (P. charcoti) to 6.8% between Lysasterias sp1
 and Lysasterias sp4 (Appendix 2).

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Haplotype and nucleotide diversity are not significantly different between brooders and broadcasters (Wilcoxon Mann-Whitney tests; p-values: 0.1709 and 0.9433, respectively).

- Phylogenies and divergence time estimates

In total, 578 sequences in broadcasters and 838 in brooders were used for ML and BA reconstructions. Both
methods give congruent results for broadcasters with similar taxonomic groupings and high node supports (Fig.
Node support was higher using the BA method for brooders. Branching patterns were however identical in all
reconstructions.

243 The monophyly of the genus Psilaster is not supported by our analysis but SO Psilaster representatives are 244 monophyletic (i.e. the nominal species P. charcoti). The genus Bathybiaster is monophyletic and includes 245 specimens from the SO and from the Northern Hemisphere (Fig. 2). Psilaster specimens collected outside the SO 246 (Psilaster acuminatus from New Zealand and Australia, Psilaster andromeda from Sweden and Psilaster 247 pectinatus from the Arctic Ocean) are retrieved as monophyletic in a sister clade to Bathybiaster. The monophyly 248 of each brooding genus is supported but one sub-clade only matches with the morphological taxonomy: the 249 species D. meridionalis. All other sub-clades are composed of specimens belonging to distinct morphospecies 250 questioning current taxonomy at the species level.

Divergence time estimates suggest numerous divergence events both in brooders and broadcasters over the last 5 Myr and particularly, over the last 2.5 Myr (Fig. 2). Results also indicate that members of the Asteriidae under study diverged from its Pan-tropical outgroup around 21 Myr ago (Appendix 3). Main divergence events (Fig. 2, Appendix 3) in *P. charcoti* and *Bathybiaster* occurred around 1.6 \pm 0.8 Myr ago (Node C and D in Fig. 2). In brooders, divergence time estimates (Fig. 2, Appendix 3) between East Antarctic sub-clades and those of the Antarctic Peninsula all fall within the same time range of 2.1 \pm 1.2 Ma (Nodes III, V and VI in Fig. 2). This is also in line with the time range computed for broadcasters.

- Phylogeographic patterns

260 All brooders display clear geographical patterns, with a distinction between the East Antarctic and the Antarctic 261 Peninsula (Fig. 2, 3; Appendix 2). In Diplasterias, D. meridionalis displays a specific distribution as it is shared 262 between South Georgia and the Kerguelen Plateau. Diplasterias sp1, includes specimens from the Antarctic 263 Peninsula along with five specimens from the Magellanic region and five from the Weddell Sea. The last sub-264 clade (Diplasterias sp2) is mainly composed of specimens from the East Antarctic among which seven specimens 265 from the Weddell Sea, 14 from the South Sandwich Islands, four from Bouvet Island and one from the South 266 Orkney Islands. For Notasterias, (Table 2, Fig. 2,3; Appendix 2) one sub-clade (Notasterias sp1) mainly contains 267 specimens from the Antarctic Peninsula and the Weddell Sea in addition with one sample from the Amundsen 268 Sea, seven from South Georgia and five from East Antarctica. Specimens belonging to the second sub-clade 269 (Notasterias sp2) are mainly from East Antarctica (66 specimens) along with two from the Amundsen Sea, four 270 from the Antarctic Peninsula and five from the Weddell Sea. The remaining sub-clade (Notasterias sp3) only 271 contains two deep-sea specimens from the Scotia Arc (3,800m) and the Weddell Sea (2.100m). Within the genus 272 Lysasterias, (Table 2, Fig. 2,3; Appendix 2) the first entity (Lysasterias sp1) nearly exclusively contains specimens 273 from East Antarctica with one from the Weddell Sea. The second entity (Lysasterias sp2) mainly contains 274 specimens from the Antarctic Peninsula together with one specimen from the South Sandwich Islands and nine 275 from the Weddell Sea. The third entity is mainly composed of specimens from the Antarctic Peninsula together 276 with nine specimens from South Georgia, three from Bouvet Island and two from the South Sandwich Islands. 277 Finally, the last entity (Lysasterias sp4) is composed of nine specimens and is endemic to the Weddell Sea. 278 Broadcasters show contrasting biogeographic patterns. P. charcoti displays a complex haplotype network with 279 sub-clade 4 endemic to Adélie Land and the three others circumpolar in distribution with two of them containing 280 specimens collected off New Zealand (Appendix 2). Sub-clades of the genus Bathybiaster are mainly 281 differentiated according to depth: Bathybiaster sp1 gathers specimens from the Antarctic shelf and the shallows of 282 the Scotia Arc, along with a deep specimen from the South Sandwich Islands (Fig. 4; Appendix 2). Bathybiaster 283 sp2 is composed of representatives from the Antarctic slope, deep troughs, and canyons along with all specimens 284 from the Kerguelen plateau and slope (Fig. 4; Appendix 2).

- 285 286 Discussion
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- Species richness, genetic diversity and taxonomic implications

290 We found that genetic diversity did not significantly differ between brooders and broadcasters. This may reflect 291 the presence of species complexes, related to recurrent disturbances. Recent glacial and interglacial cycles could 292 have promoted high rates of differentiation in SO species (O'Hara et al., 2019), regardless of their dispersal 293 capabilities. Discordances between the different delineation methods were detected in all groups. This had 294 already been highlighted in recent studies (Kekkonen et al., 2015; Blair & Bryson, 2017) and great care should be 295 taken when interpreting the results, as further investigations are needed. At best, these methods should be 296 considered as a first step towards subsequent and more integrative taxonomic works (Kekkonen et al., 2015) and 297 therefore, a conservative approach is recommended (Carstens et al., 2013). Genetic diversity among the 298 delineated species falls into the range of intra-specific values obtained in other taxa for COI mtDNA (Goodall-299 Copestake et al 2012), supporting this approach. Similarly, the measured inter-specific (2-6.8%) distances are 300 within the range obtained in previous studies for sea stars (Janosik et al., 2011; Foltz et al., 2013). DNA barcoding 301 has proven to be an efficient method to differentiate echinoderm species (Ward et al., 2008) with ~98% of the 191 302 studied species being distinguished based on their COI barcodes. Rapid diversification can however make intra-303 and inter-species genetic distances difficult to interpret using species delineation methods. The clear discrepancy 304 between current taxonomy and our results emphasises the urgent need for a complete reassessment of SO 305 asteroid taxonomy based on multiple genes and an integrative taxonomic approach at both species and genus 306 levels.

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Dispersal capacity and biogeographic patterns

309 Several mechanisms have been proposed to account for the unusually high proportion of brooding species in the 310 SO and the evolutionary success of Antarctic brooders is now widely recognised within the community of Antarctic 311 biologists (Poulin et al., 2002). While adaptation to polar environmental conditions is not considered a determining 312 mechanism (Poulin et al., 2002; Pearse et al., 2009), reproductive strategy has been hypothesised as the main 313 factor structuring the evolution and the diversity of SO benthic life (e.g. Raupach et al., 2010; Thatje, 2012). 314 Whether they are based on morphology (e.g. Moreau et al., 2017) or genetic markers (e.g. Hoffman et al., 2011), 315 most studies agree that biogeographic structures are more pronounced in brooders than in broadcasters due to 316 contrasting dispersal capacities.

317 Most of the results obtained in the present study are in line with this expected biogeographic pattern, with 318 the prevalence of clear spatial structures in brooders. This is in agreement with our previous work describing the 319 SO asteroid biogeographic patterns and demonstrating the importance of life history traits to understanding the 320 structure of spatial distributions (Moreau et al. 2017). Our results support the differentiation of species between 321 the Antarctic Peninsula and the East Antarctic. The past collapse of the West Antarctic Ice Sheet (Bamber et al., 322 2009; Pollard & DeConto, 2009) and the putative existence of a subsequent trans-Antarctic seaway separating 323 the West and the East Antarctic have been proposed as possible mechanisms leading to such patterns (Barnes & 324 Hillenbrand, 2010; Pierrat et al., 2013; Linse et al., 2006). The role of the Weddell Sea Gyre (Linse et al., 2006), 325 environmental dissimilarities, and contrasting glacial histories have also been proposed as possible explanations 326 (Anderson et al., 2002). Interestingly, in brooding genera, haplotypes of specimens from the Weddell Sea are 327 present in both the East Antarctic and the Antarctic Peninsula clades. Recent faunal exchanges between the two 328 regions could explain the occurrence in the Weddell Sea of a mixed fauna of East Antarctic and Antarctic 329 Peninsula origin as the Weddell Sea is located in between these two regions.

330 Divergence dates suggest that a vicariance event occurred ~2 Myr ago, while the last collapse of the 331 West Antarctic ice sheet is hypothesised to have occurred more recently, in the last 1.1 Myr (Pollard & DeConto, 332 2009; Scherer et al., 2008). This last dating is in line with previous work on SO asteroids (Janosik et al., 2011). 333 Contrasting divergence dating between phylogeographic studies is not uncommon and absolute ages should be 334 taken with caution when using a molecular clock (Thomas et al., 2006), given the uncertainties in molecular 335 divergence rates, especially when a single locus is used and when fossil calibrations are not available. However, 336 similar divergence dates were obtained for all brooding genera, which strongly suggests the effect of a common 337 and significant event. Unfortunately, only a few abyssal samples were available for study (only two for the entity 338 Notasterias sp3). They are strongly divergent from all other Notasterias specimens, suggesting the absence of 339 population mixing and independent evolution of populations of Notasterias on both the continental shelf and in the 340 deep-sea. 341

In contrast to the marked genetic structures described above, exceptions have been in certain brooders due to the effect of unusual dispersal vectors reported (Cumming et al., 2014; Diaz et al., 2011). This is also the case in this study, as the brooding species *D. meridionalis* shares genetic units between South Georgia and the Kerguelen Islands, which suggests an ongoing connectivity between the two distant regions. This pattern could be explained by kelp rafting via the Antarctic Circumpolar Current (ACC), which has previously been found in other SO brooding echinoderms (O'Hara, 1998) and taxa such as in isopods and molluscs (Leese et al., 2010, González-Wevar et al., 2018). Kelp rafting was suggested as a viable dispersal vector for shallow-water species
living in macro-algal beds (O'Hara, 1998), which is the case of *D. meridionalis*.

351 Genetic structures in broadcasters under study are quite different from the patterns observed in 352 brooders. The studied broadcasters show circumpolar structures that suggest the prevalence of gene flow across 353 the SO promoted by higher larval dispersal capacities. Geographic patterns in P. charcoti, with several 354 circumpolar entities, suggest the existence of past refugia during past glacial maxima and subsequent dispersal 355 centres (Hemery et al., 2012). Unfortunately, little is known about asteroid larval biology in the SO, apart from a 356 handful of well-studied species (Pearse et al., 1991; Aguera et al., 2015; Peck et al., 2013; Souster et al., 2018). 357 Major differences exist between the genetic structure of Psilaster and Bathybiaster. (1) clades of P. charcoti are 358 found to have a greatly fragmented pattern, potentially as a result of lower dispersal capacity than representatives 359 of B. loripes (found at continental shelf depths) but this could also reflect a longer evolutionary history as these 360 clades are older (Fig. 2), (2) some specimens of P. charcoti occur on both sides of the PF and (3) Bathybiaster 361 sp2 shows a potential bipolar distribution (sensu Darling et al., 2000) and likely corresponds to the nominal 362 species B. vexillifer. The occurrence of shared haplotypes between the Northern and Southern Hemispheres in 363 Bathybiaster sp2 suggests the existence of recent gene flow between the two hemispheres. Morphological 364 similarities between North American and South African specimens had already been indicated in previous work 365 on the echinoderm fauna of South Africa (Clark, 1923). This could be the result of a deep-sea dispersal route, a 366 scenario coined the "thermohaline expressway" by Strugnell et al. (2008) but a wide, cosmopolitan distribution of 367 the species is probably the most reasonable hypothesis. Unfortunately, no tropical deep-sea samples were 368 available for study. Bathybiaster sp2 is recorded on the Kerguelen Plateau around the Kerguelen Islands at 369 shallow depths, giving credit to a possible sub-Antarctic emergence scenario and colonisation of the Kerguelen 370 Plateau from the deep sea (Diaz et al., 2011; Aronson et al., 2007).

371 Interpretation of the present results is limited by the use of a single mtDNA locus. The observed patterns 372 could be due to mechanisms such as adaptive introgression, demographic disparities or sex-biased asymmetries 373 arising from the sole use of mtDNA (Toews & Brelsford, 2012). Furthermore, if data derived from COI analyses 374 reflect a long-term effect of contrasting dispersal capacities, other fast evolving nuclear markers such as 375 microsatellites or SNPs (RAD-seq data) will be needed in the future to investigate these recent and ongoing 376 processes. Nevertheless, the causal relationship between genetic patterns and dispersal capacities of asteroids, 377 can be linked to past climatic and geological events and give some clues to the upstream drivers of species 378 evolution. 379

- The role of past climate change

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381 The SO has been partially isolated for nearly 40 million years since the first opening of the Drake Passage, which 382 led to the onset of the ACC and subsequent cooling of the ocean when the ACC intensified. However, isolation of 383 the SO was reduced several times during periods of climate warming and the decreasing ACC intensity 384 (Lagabrielle et al., 2009; Dalziel et al., 2013). In a recent review of key stages in the evolution of the Antarctic 385 marine fauna, Crame (2018) emphasizes the influence of past climate on the current distribution of modern fauna. 386 At the scale of the Cenozoic (c. 65 Myr), he points out that the succession of several major extinction and 387 radiation events are linked to important drops in sea water temperature. The fossil record of SO sea stars is very 388 poor but it suggests that the extinction of an older asteroid fauna was followed by the diversification of the 389 Antarctic Asteriidae and of other modern Antarctic forcipulataceans (Mah & Foltz, 2011b). The origin of the 390 Antarctic Asteriidae has been dated to 21 Ma in this study, suggesting a diversification of the family starting in the 391 Miocene after the initial opening of the Drake passage (c. 34 Ma) and before the full establishment of the ACC (c. 392 14 Ma) (Lawver & Gahagan, 2003). The global phylogeny of Astropectinidae, reconstructed by Mah & Foltz 393 (2011a), does not show any regional pattern, which makes any speculation as to the origin of Bathybiaster and 394 Psilaster in the SO problematic. Our results, however, suggest a recent evolutionary history of broadcasting 395 genera in the SO (c. 5 Myr). At a more recent time scale, all the studied groups show a high diversification rate 396 over the last ~2.5 Myr, suggesting that recent climate events could account for these observed patterns. Naish et 397 al. (2009) estimated that at least 38 distinct glacial cycles occurred over the last 5 Myr. These glacial cycles are 398 believed to have been major drivers of species differentiation as hypothesised by the "Antarctic diversity pump" 399 hypothesis (Clarke & Crame 1989, 1992) and even promoting evolutionary radiations of the benthic fauna (Wilson 400 et al., 2009; Allcock, 2005; Raupach et al., 2007). These major environmental changes could have also resulted 401 in cryptic speciation in brooding species and broadcasting species with limited larval dispersal capabilities, by the 402 maintenance of genetic differentiation at local scales (Thatje, 2012). Finally, the ACC hypothesis predicts the 403 "existence of many species in clades of varied divergence times, at a wide range of depths, but with highest 404 diversity downstream of the Drake Passage, in the Scotia Arc and Weddell Sea" (sic. Pearse et al., 2009). This 405 hypothesis could explain some of the diversification patterns we have observed in asteroids. Nevertheless, the

406 apparent correlation with past geological and climatic events should be taken with great care due to the lack of 407 fossil records and uncertainties in calibration of the molecular clock also known to be taxon and climate regime 408 dependent (Gillooly et al., 2005).

Conclusion & future prospects:

410 411 The observed genetic patterns of SO sea stars highlight the relevance of considering life history traits to 412 understand spatial patterns of genetic diversity. Our results suggest that reproductive strategy could have 413 conditioned the spatial structuring of genetic diversity, but with no apparent effect on the level of genetic diversity. 414 Molecular results also indicate that an in-depth taxonomic revision of the group is needed based on an integrative 415 taxonomy approach (combining genetic and morphological data). The increasing development of genetic surveys 416 in biodiversity conservation and management plans (Goodall-Copestake et al., 2012) stresses the need for robust 417 estimates of species diversity. Species complexes are frequent in the SO (e.g. Hemery et al., 2012) and 418 estimates of species diversity based on morphology can lead to under- or alternatively, overestimating 'true' 419 biological diversity. In that context, multi-locus approaches are essential to detect evolutionary processes within 420 species. Finally, the apparent bipolar or cosmopolitan distribution of the species B. vexillifer should be further 421 investigated using additional specimens and multiple molecular markers. This will bring new insights on a 422 potential deep-sea route connecting the two polar oceans and the relevance of biogeographic scenarios such as 423 the thermohaline expressway (Strugnell et al., 2008) and the sub-Antarctic emergence (Diaz et al., 2011). The 424 presence of B. loripes on the continental shelf could be the result of a past colonisation from the deep sea 425 (Strugnell et al., 2011), which potentially challenges the established paradigm of an isolated SO benthic diversity 426 (Clarke et al., 2004).

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683 Biosketch 684

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Tables

700 Table 1 Molecular diversity statistics for each delineated species. Statistics for taxonomic groups with n<25 are not represented. n : number of sequences. π : nucleotide diversity. H: haplotype diversity.

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Taxonomic group		n	Π	n of haploty pe	Н	Segregating sites	Mean intraspecific distance
Brooders	<i>Lysasterias</i> sp1	81	0.01000 ± 0.00062	16	0.831 ± 0.027	25	0.0103 ± 0.0069
-	Lysasterias sp2	78	0.01146 ± 0.00093	12	0.840 ± 0.019	26	0.0118 ± 0.0092
	<i>Lysasterias</i> sp3	56	0.00825 ± 0.00095	13	0.765 ± 0.055	21	0.0085 ± 0.0073
	Notasterias sp1	236	0.00561 ± 0.00032	23	0.733 ± 0.027	31	0.0057 ± 0.0044
	Notasterias sp2	80	0.01108 ± 0.00109	14	0.718 ± 0.037	31	0.0114 ± 0.0096
	Diplasterias sp1	105	0.01262 ± 0.00030	21	0.909 ± 0.013	32	0.0130 ± 0.0069
	Diplasterias sp2	116	0.01547 ± 0.00097	19	0.849 ± 0.021	36	0.0161 ± 0.0123
	Diplasterias meridionalis	75	0.00239 ± 0.00023	13	0.777 ± 0.031	11	0.0024 ± 0.0019
Broadcasters - -	Bathybiaster sp1	133	0.00347 ± 0.00042	25	0.790 ± 0.023	29	0.0035 ± 0.0039
	Bathybiaster sp2	110	0.00557 ± 0.00054	28	0.835 ± 0.026	34	0.0057 ± 0.0051
	Psilaster charcoti clade1	92	0.01457 ± 0.00069	29	0.924 ± 0.015	50	0.0151 ± 0.0085
	Psilaster charcoti clade2	55	0.00272 ± 0.00028	12	0.734 ± 0.052	12	0.0027 ± 0.0020
	Psilaster charcoti clade3	148	0.00160 ± 0.00026	19	0.394 ± 0.052	17	0.0016 ± 0.0025

Table 2 Number of species delineated by the different species delineation methods

Taxonomic group	ABGD	sGMYC	mGMYC	mPTP	ASAP	
Brooders	Lysasterias	4	17	17	4	4
	Diplasterias	3	14	15	4	3
	Notasterias	3	4	18	3	3
Broadcasters	Psilaster charcoti	9	9	17	4	9
	Bathybiaster	4	3	12	3	2
Total		27	47	79	19	30

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719 Figures

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721 722 723 724 Figure 1. Maps of the SO indicating sample location for each target group (red stars). Sampling locations are labelled: red - South Georgia; pink - South Sandwich Islands; yellow - Bouvet Island; dark blue - Kerguelen 725 726 Islands; light blue - East Antarctica; light green - Amundsen Sea; orange - Antarctic Peninsula; dark green -Burdwood Bank and purple - Weddell Sea. Projection: South Pole Stereographic.

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731 Figure 2. Bayesian chronograms of partitioned COI sequences derived from the brooding (left) and broadcasting

(right) groups of interest. The distribution of uncertainty of node placement is indicated around each of the main
nodes. Time scale is expressed in millions of years. Posterior probabilities and bootstrap values are provided
under main nodes. No value was indicated if bootstrap was less than 45%. Coloured patches indicate sampling
locations. Results for each species delineation method are reported as black bars representing the delineated
units.



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740 Figure 3. Brooders statistical parsimony network indicating genetic relationships with regards to sampling
741 locations. Delineated clades are represented and colour code follows the central map: red - South Georgia; pink –
742 South Sandwich Islands; yellow – Bouvet Island; dark blue – Kerguelen Islands; light blue – East Antarctica; light
743 green – Amundsen Sea; orange – Antarctic Peninsula; dark green – Burdwood Bank and purple – Weddell Sea.
744 Projection: South Pole Stereographic.



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Figure 4. Bathybiaster statistical parsimony network indicating genetic relationships with regards to A) sampling

geographic locations and B) sampling depths and Kerguelen Island (shallow and deep). Delineated clades are
 represented and colour code follows the appended legend.