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Asexual reproduction and heterozygote selection in an Antarctic demosponge (*Stylocordyla chupachus,* Suberitida)

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Abstract

Antarctic bottoms harbor stable, benthic communities, subjected to low temperatures. Environmental stability may promote the asexual (clonal) reproduction of sponges to maintain adapted genotypes to those particular conditions. *Stylocordyla chupachups* forms patchy populations across the Antarctic continental shelf. Individuals are mostly similar in size without distinct cohorts, which indicates fast growth of the new recruits. Settlement of incubated (clonal?) functional sponges may accelerate sponge growth and success at early colonization phases. To analyze the weight of clonal reproduction in the species, a genetic study was performed on three close populations using eight polymorphic microsatellite loci that were designed from massive sequencing. The three study populations showed a relatively low genetic diversity and low loci polymorphism (from 2 to 6 alleles). The estimators of genetic structure, the Analysis of the Molecular Variance (AMOVA), and the presence of private alleles indicated low but significant structure between the populations. A relatively high rate of asexual reproduction (ca. 25% of the individuals) was detected. The program MLGsim found five identical multilocus genotypes (MLGs) with an asexual origin. An excess of heterozygotes (in five out of the eight loci genotyped) was found, which suggests a positive selection mechanism for heterozygotes. The relatively high rates of asexual reproduction may be the result of adaptation to the environmental stability, while heterozygote selection would help maintain some genetic diversity in the populations. *S. chupachups* has been reported to be one of the first sponge species recolonizing bare areas resulting from iceberg scouring, which indicates a high species fitness and adaptation to Antarctic bottoms. Two out of the three study populations showed bottleneck, which may indicate a recent founder effect and supports the pioneer nature of this species.

Keywords Antarctic demosponges · Microsatellites · Population genetics · Clonal reproduction · Heterozygote selection

Introduction

Sponges are one of the most important taxa in terms of diversity and abundance in marine benthic ecosystems (Gili and Coma 1998). They are even more abundant in the Antarctic region, where sponges dominate many benthic communities between 100 and 200 m of depth, and account for ca. 75% of the benthic biomass (Belyaev and Ushakov 1975).

Recent studies (Fraser et al. 2018) have shown that dispersal of shallow organisms may be higher than traditionally thought in some Antarctic areas due to kelp rafting caused by storms. However, it is also true that in general the Antarctica had remained isolated from the rest of continents since it was separated from Gondwana ca. 40 millions of years ago, due to the formation of the circumpolar current (Dayton 1990; Thomson 1991). Moreover, although global warming is weakening Antarctic barriers (Bromwich et al. 2013; Duffy et al. 2017; Griffiths et al. 2017), the Antarctic continental shelf presents extreme low temperatures. These features may have contributed to the biogeographic isolation of many Antarctic invertebrates such as sponges, promoting a high degree of species endemism in the area (Sarà et al. 1992; McClintock 2005; Ríos 2006), which has been calculated to range from 43 to 68%, depending on the sponge Class (Downey et al. 2012).

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However, morphological convergence between species as a result of adaptation to similar environmental conditions, such as low temperature and stability, has caused misidentification of some species in the Antarctic, which were ascribed to other foreign species. Among them, *Stylocordyla borealis* from the Norwegian coasts was recorded from several Antarctic localities, but later identified as a new Antarctic species named *Stylocordyla chupachups* (Uriz et al. 2011). Some constant, cryptic, differences allowed differentiating both species. *S. borealis* surface is smooth and has a flat apical zone surrounded by a spicule fringe, while *S. chupachups* is hispid and almost spherical without any spicule fringe (Uriz et al. 2011).

Patchy distributions are common for Antarctic sponges (Barthel and Gutt 1992; Gutt and Koltun 1995; Gatti 2002). Some species form dense populations in some areas. while they are absent from many others, despite apparently similar environmental conditions (e.g., *Rossella racovitzae*, *Cinachyra barbata*, *Antarctotetilla leptoderma,* and *Stylocordyla chupachups*).

Stylocordyla chupachups behaves as a pioneer species in the colonization of disturbed areas by iceberg scouring (Gutt 2000). Its populations are mostly formed by individuals of the same size (i.e., no cohorts were clearly differentiated in the multiple videos recorded during the Polastern campaign). Moreover, ca. 1 mm in size internal bodies, which have been proposed to have a clonal origin (Bergquist 1972), were found in many specimens sampled, which permitted us hypothesizing a high rate of asexual reproduction in this species. To assess the extent of clonal reproduction in this species and the genetic traits of its populations, we performed a population genetics' study using microsatellite markers.

Microsatellites are among the most variable types of DNA sequences in the genome (Weber 1990). As each microsatellite contains many mutation sites, they have been considered suitablemarkers for studies of population genetics (Csilléry 2009), in particular where only a small number of samples are available (Haasl and Payseur 2011) as often occurs in sponges (Duran et al. 2004a; Blanquer et al. 2009; Blanquer and Uriz 2010; Dailianis et al. 2011; Guardiola et al. 2012; Pérez-Portela et al. 2015).

In the present study, we developed a set of microsatellite markers by sequencing from a part of the *Stylocordyla chupachups* genome, following the protocol in Guardiola et al. (2016), analyzed their suitability for a population genetics' study, and used these markers for assessing the extent of clonal reproduction in this sponge.

Materials and methods

Sampling

The samples of *Stylocordyla chupachups* were collected during the Polarstern ANT-XXVII/3 expedition (from February to April 2011) in the Antarctic region (− 70°50′33.0″S, − 10°35′21.6″W, Fig. 1). Three populations: Ant1 (18 individuals), Ant2 (20 individuals), and Ant3 (20 individuals) 58 individuals in total, were collected from an area with a diameter of ca. 1.8 km and depth between 238 and 268 meters, during three separate trawling operations (Agassiz trawl). Fragments of the external zone of the specimens, deprived of the incubated bodies (Sarà et al. 2002), were fixed in ethanol on board immediately after collection, placed in hermetic sealed plastic bowls, transported in a freezer to the CEAB (Centre d'Estudis Avançats de Blanes, Spain) at − 20 ºC, and stored there at − 20°C until DNA extraction.

The number of internal bodies was calculated for each individual analyzed by cutting the sponge manually in several pieces and counting the number of bodies found, which were larger enough to be detected with naked eye.

DNA extraction, pyrosequencing, and microsatellite selection

The DNA of *Stylocordyla chupachups* was extracted for pyrosequencing with QIAmp DNA stool kit (Qiagen). DNA concentration and quality were assessed using a Qubit fluorometer (Invitrogen) and a 2100 Bioanalyzer (Agilent Technologies), respectively. Sequencing (1/2 run) was performed in a 454 GS-FLX sequencer (Roche) at the Scientific and Technological Centre of the University of Barcelona (CCiTUB). Sequences were analyzed with the open-access program QDD v0.2.1 (Meglécz et al. 2010), which is a useful tool for the discovery and selection of microsatellites, and for primer design. The QDD package integrates the software programs BLAST, ClustalW (Larkin et al. 2007), and Primer 3-1.1.4 (Rozen and Skaletsky 2000), and works in three steps: sequence cleaning and microsatellite recognition, detection of sequence similarity, and primer design. Among, the best 100 microsatellites, we selected ten taking into account product size (120–285 bp), suitable flanking region, self-complementarity, guanine–cytosine (GC) content (50–60%), and GC clamp regions: Stylo_A, Stylo_D, Stylo_H, Stylo_K, Stylo_G, Stylo_M, Stylo_N, Stylo_R, Stylo_S, and Stylo_T.

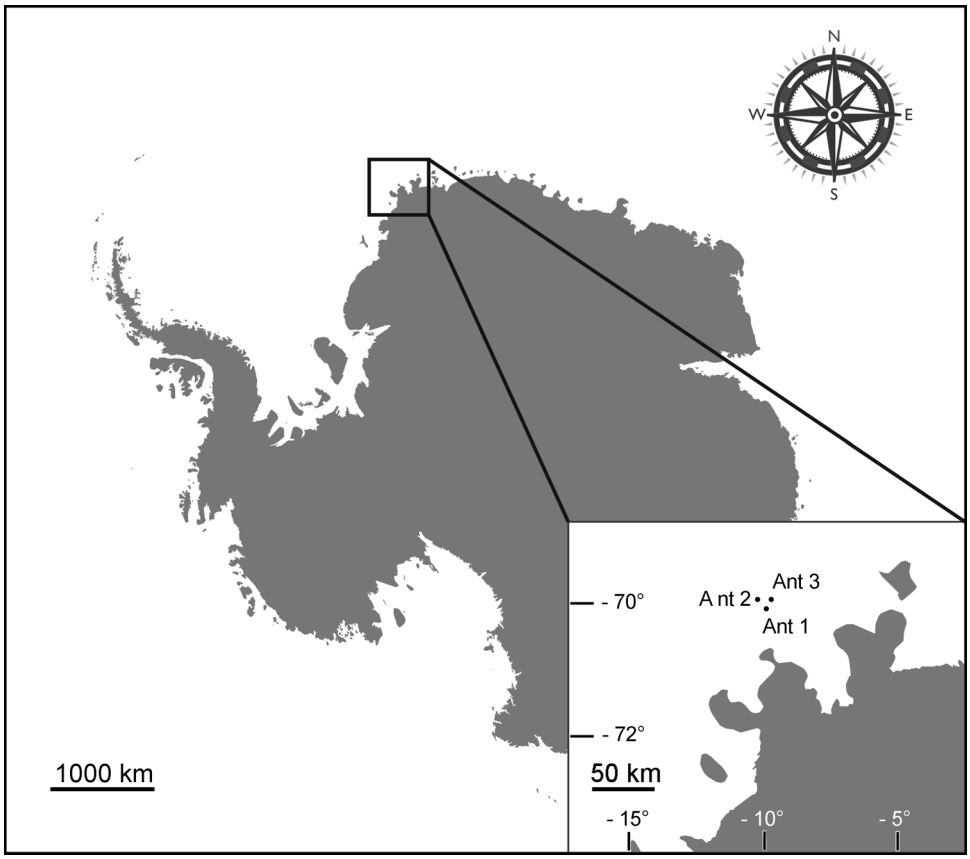

Fig. 1. Sampling sites near Newmayer, Antarctica. The inset image indicates the sampling locations of the three study populations: Ant1, Ant2, and Ant3.

Amplification and microsatellite genotyping

From the ten selected loci, Stylo_R and Stylo_T failed to amplify. Thus, all the analyses were performed with the remaining eight microsatellites (Stylo_A, Stylo_D, Stylo_H, Stylo_K, Stylo_G, Stylo_M, Stylo_N, and Stylo_S) (Table 1). The forward primers of each locus were labeled with fluorescent dyes (NED, PET, VIC, 6-FAM; Applied Biosystems) for screening and were then amplified in a final volume of 25 µL (10–30 ng of DNA), which contained 17.15 µL H20, 2.5 µL 10X buffer (BIO LINE), 2 mmol/L µL MgCl (BIO LINE), 1.2 µL DMSO (dimethyl sulfoxide), 1 µL BSA, 0.25 mmol/L of dNTP mix (Sigma-Aldrich), 0.25 µmol/L of each primer, 1 U Taq (BIO LINE) 1 µL of DNA template.

Amplification was performed in Bio Applied and Bio-Rad PCRs with the following parameters: 1 min denaturation at 94 °C, followed by 40 cycles of 30 s at 94 °C, 40 s at a locus-specific annealing temperature (temperature varies from 52 to 63 °C), 50 s at 72 °C, followed by an extension cycle of 3 min at 72 °C. The resulting PCR products were then visualized on 1.3% agarose gel stained with GelRed (Biotium) and then genotyped in an ABI Prism 3700 automated sequencer (Applied Biosystems) at the CCiTUB. The length of the PCR products was estimated relative to the internal size standard GeneScan 500LIZ and determined using GeneMapper and Peak Scanner software. The raw data generated were reviewed using AutoBin v0.0.9 (Excel macro written in Microsoft Visual Basic, F. Salin unpubl.), in order to automatically detect relevant gaps in allele size. Moreover, three independent readers checked the AutoBin results to ensure a lack of scoring errors.

Data analysis

To evaluate the extent of clonal reproduction, we recorded the presence of identical multilocus genotypes (MLGs) in our populations. To assess whether individuals sharing MLGs could have resulted from sexual or asexual reproduction we used the program MLGsim (10,000 simulations; Stenberg et al. 2003). The p values of all analyses involving multiple comparisons were corrected by the False Discovery Rate (FDR) (Benjamini and Yekutieli 2001).

The number of alleles per locus, the expected (He) and observed (Ho) heterozygosity, genotypic frequency, the number of private alleles, linkage disequilibrium, departure from Hardy–Weinberg equilibrium, heterozygote deficit or excess, allele frequencies, and the inbreedingcoefficient (F_{is}) (Weir and Cockerham 1984) for each locus individually, as well as for all loci combined, were calculated using

Table 1 Identical multilocus genotypes (MLGs) identified in *Stylocordyla chupachups* populations

	Genotypes								Population/individuals	MLGsim
	Stylo_A	Stylo_D	Stylo_H	Stylo_K	Stylo_G	Stylo_M	Stylo_N	Stylo_S		
MLG1	122/127	239/264	160/166	278/302	149/164	117/120	260/263	255/255	ANT 1 IND. 13	***
									ANT 1 IND. 18	***
MLG2	127/127	239/264	160/166	278/302	164/164	117/120	260/263	255/255	ANT 1 IND. 16	*
									ANT 2 IND. 11	*
MLG3	127/127	239/264	160/166	285/302	157/157	117/120	260/263	255/255	ANT 2 IND. 10	**
									ANT 2 IND. 18	**
MLG4	127/127	239/264	160/166	285/302	157/164	117/120	260/263	255/255	ANT 2 IND. 7	***
									ANT 2 IND. 8	***
									ANT 2 IND. 9	***
									ANT 2 IND. 12	***
									ANT 2 IND. 13	***
									ANT 2 IND. 14	***
MLG5	127/127	244/264	160/166	285/302	164/164	117/120	260/263	255/255	ANT 2 IND. 2	*
									ANT 2 IND. 3	*
MLG6	127/127	239/264	160/166	285/302	164/164	117/120	260/263	255/255	ANT 1 IND. 12	ns
									ANT 2 IND. 5	ns
MLG7	127/127	244/264	160/166	000/000	157/164	117/120	260/263	255/255	ANT 3 IND. 8	ns
									ANT 3 IND. 13	ns

Seven MLGs were found in the three populations

Significant p values (i.e., $p<0.05$) in the MLGsim analysis allowed rejecting the null hypothesis of a sexual origin for all the MLGs except MLG6 and MLG7 (significant values: $*p<0.05$, $**p<0.01$, $***p<0.001$)

GENEPOP, web version 4.0 (Raymond and Rousset 1995; Rousset 2008), after leaving only one genet when several ramets of a MLG were found. The inbreeding coefficient was also calculated using F-STAT version 2.9.3 (Goudet 1995, 2001) to confirm the results obtained by GENEPOP. The presence of null alleles was assessed using MicroChecker v.2.2.3 (Van Oosterhout et al. 2004).

The genetic differentiation of the three populations, once leaving only genets, was assessed by two estimators: the differentiation index D, which is independent of within-population heterozygosity (Jost 2008) and the F_{st} statistic, which measures allele fixation and depends on within-population heterozygosity (Weir and Cockerham 1984). Both estimators (F_{st} and D) range from 0 to 1 (0 = no differentiation; 1 = absolute differentiation). The Pair-wise F_{st} was assessed with ARLEQUIN (Excoffier et al. 2005), while D values were calculated with DEMEtics (Gerlach et al. 2010). The molecular variances among populations, within populations, and within individuals, were analyzed by *AMOVA*, using ARLEQUIN (Excoffier et al. 2005). Whether populations deviate or not from mutation/drift equilibrium was analyzed by Bottleneck versus 1.2.02 (Cornuet and Luikart, 1996; Piry et al. 1999). The Wilcoxon test was chosen because it has been reported to be the most powerful and robust for populations with few polymorphic loci (Piry et al. 1999).

Results

From 2 to 7 spherical bodies ca. 1mm in diameter, purportedly with an asexual origin, were found in the samples used for the population genetics' study. Among the microsatellites selected, two loci (Stylo_D and Stylo_H) were perfect and six loci (Stylo_A, Stylo_G, Stylo_K, Stylo_S, Stylo_M and Stylo_N) were compound or imperfect. Null alleles were found for locus Stylo_S, exclusively. Analyses were repeated removing locus Stylo_S, and analogous results were obtained.

All loci showed low polymorphism, with a mean number of alleles per locus ranging from 2 to 6. The numbers of alleles per locus are given in Online Resource (EM_1.pdf). No loci showed linkage disequilibrium after False Discovery Rate correction (FDR) for multiple comparisons.

Seven identical multilocus genotypes, accounting for 18 individuals, were found in the three populations analyzed. The MLGsim test indicated that five (from MLG1 to MLG5, Table 1) of them were produced by asexual reproduction, as their p values, which reflect the likelihood that they have been produced sexually, were significant ($p<0.001$ in two cases, $p<0.05$ in other two cases. and <0.01 in one case), and then the null hypothesis on their sexual origin has to be rejected. However, two identical multilocus genotypes (MLG6 and MLG7) could be the result of sexual

reproduction as no significant values were retrieved by the MLGsim analyses. Most identical MLGs consisted of two ramets except MLG4, which consisted of six ramets.

From these groups of identical MLG, four (MLG1, MLG3, MLG4, and MLG5) were found in the same population, while MLG2 was found in two separate populations (Table 1).

The observed heterozygosity (Ho) was higher than that expected one in six out of the eight loci analyzed in the three populations. The exact test for Hardy–Weinberg equilibrium indicated significant deviations from equilibrium with mean F_{is} values negative in the three populations, when all loci were considered together (Table 2). The heterozygote loci represented 71.43% of all loci in the clonal genotypes and 66.97% in the sexually produced genotypes. Two private alleles were found in population ANT1, one in ANT2, and four in ANT3 (Table 2). The mean frequency of private alleles for the eight loci, in the three populations was 0.08.

Genetic differentiation was low among the three target populations as indicated by the estimators F_{st} and D. The values of these estimators were low but significant between populations Ant1 and Ant2 (F_{st} 0.048, $p < 0.001$ and D 0.032, $p < 0.05$), between Ant1 and Ant3 (F_{st} 0.029 $p < 0.05$ and D 0.018 n.s.), and between Ant2 and Ant3 (F_{st} 0.028 $p < 0.05$ and D 0.018 $p < 0.05$). The hierarchical AMOVA confirmed the low but significant genetic differentiation among populations ($F_{st} = 0.063$, $p < 0.001$).

Bottleneck analyses indicated that population ANT2 deviated significantly (Wilcoxon test, $p < 0.05$) from mutation/drift equilibrium under the three mutation models (IAM, TPM, SMM) and population ANT1 did it just under IAM and TPM models. However, ANT3 did not deviate from the mutation/drift equilibrium (Wilcoxon test, $p > 0{,}05$).

Table 2 Genetic information on the eight microsatellite loci for each population of *Stylocordyla chupachups*

Locus	Subpopulations			
	Ant1	Ant2	Ant3	Mean Na Locus
Stylo_A				
Na	3	1	2	2
He	0.476	0	0.188	
Ho	0.647	0	0.211	
Fis	−0.333*	0	−0.09	
Stylo_D				
Na	3	3	4	3.3
He	0.678	0.565	0.594	
Ho	0.823	1	0.736	
Fis	−0.519***	−0.752***	−0.38	
Stylo_H				
Na	6	3	5	4.6
He	0.668	0.565	0.573	
Ho	1	1	1	
Fis	−0.474***	−0.752***	−0.731***	
Stylo_K				
Na	4	4	4	4
He	0.756	0.721	0.552	
Ho	0.471	0.615	0.368	
Fis	0.068	−0.081	−0.5	
Stylo_G				
Na	3	2	3	2.6
He	0.469	0.473	0.386	
Ho	0.353	0.307	0.368	
Fis	0.104	0.384	0.073	
Stylo_M				
Na	2	2	2	2
He	0.553	0.5	0.498	
Ho	0.941	1	0.947	
Fis	−1***	−1***	−0.9***	
Stylo_N				
Na	2	3	6	3.6
He	0.498	0.535	0.655	
Ho	0.941	1	0.789	
Fis	−0.882***	−0.857***	−0.179	
Stylo_S				
Na	2	2	2	2
He	0.484	0.311	0.361	
Ho	0.117	0.231	0.263	
Fis	0.77**	0.294	0.23	
Pa	2	1	4	
Mean Na\population	3.125	2.5	3.5	
Mean He	0.572	0.458	0.476	
Mean Ho	0.662	0.644	0.585	
Mean *Fis*	−0.295***	−0.441***	−0.343***	

Na number of alleles, *He* expected heterozygosity, *Ho* observed heterozygosity, *Fis* inbreeding coefficient,*Pa* number of private alleles per population

Only genets have been considered in the populations

Significant values: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Discussion

The microsatellites assayed were selected among the best 100 loci obtained from the sponge genome, taking into account the most suitable characteristics advised by the software developers (Primer 3-1.1.4, Rozen and Skaletsky 2000). Even so, they showed a very low polymorphism in the study populations, compared with microsatellites genotyped in sponge populations from other latitudes (e.g., Dailianis et al. 2011; González-Ramos et al. 2015). Although this low polymorphism should be confirmed in larger populations, we suggest that some mechanisms of DNA repair might be operating in sponges of Antarctic environments.

A relatively high rate of asexual reproduction (ca. 25% of the individuals) was detected in the study populations of *S. chupachups*. Similar percentages have only been recorded in some *Chondrilla* species living in stable environments

and are produced by fragmentation (Zilberberg et al. 2006). Since neither fragmentation nor external buds have been observed in *S. chupachups*, release of the internal bodies (Burton 1928; Bergquist 1972), which were frequent in many sampled individuals, might be responsible for the clones recorded. High rates of clonal reproduction may also occur in populations of other Antarctic sponge species, in particular of some hexactinellid that produce huge amounts of external buds (Teixidó et al. 2006). However, studies on population genetics' of these species are still absent.

Antarctic benthic invertebrates have been isolated from the rest of continents for several millions of years (Dayton 1990; Thompson 1991). Isolation and stability over evolutionary time scales enable us to foresee that some Antarctic invertebrates from ancient asexual lineages (Stoeckel et al. 2006) have perpetuated well-adapted genotypes to consistently low temperatures (Clarke 1988) and temporal trophic depletion (Clarke 1988; Gatti 2002).

Two clones were found in two populations located ca. 2 km apart. Rafting of internal bodies caused by the action of currents (Gili et al. 2001), storms (Fraser et al. 2018), iceberg scouring (Gerdes et al. 2003), and bottom trawling of research vessels, which operated in the area every two years (e.g., Polarstern campaigns), may be possible explanations for the presence of clones even relatively far from each other.

Asexual reproduction in invertebrates may prompt colonization of new substrates, as the resulting individuals may favorably compete for space, and decrease size-dependent mortality rates during recruitment with respect those developing from larvae (Hughes et al. 1992; Hall and Hughes, 1996). *Stylocordyla chupachups* has been reported to incubate internal, presumably clonal (Burton 1928; Bergquist 1972), functional individuals. These internal bodies have been proposed to have a parthenogenetic origin, as they are formed from purportedly nonfertilized, female gametes (Bergquist 1972) instead of from somatic cells, which is typical for asexual sponge gemmules (Fell 1993). Settlement of functional sponges, with radial skeleton, and choanocyte chambers already developed (Sarà et al. 2002) may accelerate individual growth and success at early colonization phases and might explain why *Stylocordyla chupachups* behaves as a pioneer species in recent bare areas resulting from iceberg scouring (Gutt 1996, 2000; Gerdes et al. 2003).

The three study populations showed a relatively low genetic diversity. Bottleneck analyses indicate a founder effect in ANT 1 and ANT 2 populations, as they proved to deviate from mutation/drift equilibrium model, but not in ANT3. Moreover, they also show from moderate-to-low genetic structure, according to F_{st} and D values. The presence of some private alleles in all of them, however, indicates, poor gene flow among populations, despite their proximity, which seems to be a rule in marine sponges (Duran et al. 2004a, b, c; Nichols and Barnes 2005; Calderon et al. 2007; Blanquer et al. 2009; López-Legentil and Pawlik, 2009; Blanquer and Uriz, 2010; Dailianis et al. 2011), as a result of poor larval dispersal (Mariani et al. 2005).

Bottleneck analyses indicate a founder effect in ANT 1 and ANT 2 populations, which might represent examples of recent colonization events.

Inbreeding (i.e., positive F_{is} values) or homozygote excess is common among marine invertebrates (Addison and Hart, 2005) and particularly, in sponges (Duran et al. 2004a, b, c; Nichols and Barnes 2005; Calderon et al. 2007; Blanquer et al. 2009; López-Legentil and Pawlik 2009; Blanquer and Uriz 2010; Dailianis et al. 2011). Inbreeding has been attributed to several causes, such as restricted dispersal and population structure (Grosberg, 1987; Carlon 1999) genetic drift, bottleneck, and a decline in the effective population size (Pérez-Portela et al. 2015). Conversely, negative *F*IS values and heterozygote excess were found in the three study populations of *S. chupachups*.

Heterozygote excess has been poorly studied (Stoeckels et al. 2006), and the potential causes for the negative F_{is} values observed in *S. chupachups* populations can only be speculated upon. Negative assortative mating or active avoidance of self- and consanguineous mating (Storz et al. 2001) may favor heterozygosity, particularly in small-size populations, but this reproduction trait would affect the whole genome and thus cannot explain why the heterozygote excess only applied to five out of the eight loci genotyped in our study species. Overdominant selection of loci with heterozygote advantage (heterosis) might be more likely, since it may induce an excess of heterozygotes at neutral loci closely linked to those under selection (Strobeck 1979; Nei 1987; Coulson et al. 1998).

If the origin of *S. chupachups* clones is parthenogenetic, as has been proposed, but not proven (Bergquist, 1972), then it should be apomictic to avoid counteracting the purported heterozygosis selection. This type of parthenogenesis, which is far from rare in plants but also occurs in animals (Mittwoch 1978), is similar to mitosis (i.e., it occurs without meiosis), and it results in diploid individuals genetically identical to their mother (Bicknell and Koltunow 2004). Thus, in contrast to automixic parthenogenesis, which produces homozygotes (Engelstädter 2017), the subsequent clones can be heterozygotes if the sponge mother is. This type of reproduction, which might be related to both the particular environmental characteristics and the evolutionary history of the Antarctic organisms, has never been proposed for sponges and thus, merits additional histological and molecular studies to either confirm or discard the hypothesis.

To summarize, clonal reproduction, which represents a mechanism of genotype adaptation to stable environmental conditions, may contribute to the low genetic diversity found in the targeted populations of *S. chupachups*. At first sight,

a low genetic diversity resulting from a high rate of clonal reproduction would suggest vulnerability of sponge populations in the Antarctic ecosystems. However, compensatory genetic mechanisms, such an ancestral selection of loci with heterozygotic advantage (heterosis) might be acting, and preserving the minimal genetic diversity in *S. chupachups* populations to succeed in the Southern Ocean. Similar genetic strategies to those of the target species might be present in other Antarctic sponges with high rates of clonal reproduction, such as the hexactinellids of the genera *Rosella* (Carter) and *Anoxycalyx* (Topsent) (Teixidó et al. 2006), but studies on population genetics of those species are still missing.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interest.

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