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Restricted geographic distribution and low genetic diversity of the brooding sea urchin *Abatus agassizii* (Spatangoidea: Schizasteridae) in the South Shetland Islands: A bridgehead population before the spread to the northern Antarctic Peninsula?

Distribución geográfica restringida y baja diversidad genética en el erizo incubante *Abatus agassizii* (Spatangoidea: Schizasteridae) en las Islas Shetland del Sur: ¿Una población de avanzada antes de su expansión hacia el norte de la Península antártica?

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ABSTRACT

The glacial cycles of the Pleistocene have promoted the principal climatic changes of the Southern Ocean, and motivated scientific interest regarding the strategies developed by marine benthic invertebrates to tolerate and overcome the extension and contraction of the ice sheet on the Antarctic continental platform. A recent study of the bathymetric zonation and distribution of macro-invertebrates in a shallow subtidal area of Fildes Bay (King George Island, South Shetlands Islands, Antarctica) highlighted the presence of a large aggregation of the brooding sea urchin *Abatus agassizii*, whose geographic distribution is known only for localities south of the Antarctic convergence (Antarctic Peninsula and South Shetland and South Georgia Islands in the Scotia Arc). Its presence is atypical, given that these shallow populations should have been erased from the vicinity of the Antarctic Peninsula by the advances and retreats of the ice sheet, and the absence of a larval stage associated with brooding should limit re-colonization from northern Subantarctic areas. The aim of the study was to evaluate whether *A. agassizii* may have survived the glaciations in its narrow bathymetric range in the South Shetland Islands, or whether this population corresponds to a newcomer that re-colonized the area despite its low dispersal capacities. For this, we combined multidisciplinary approaches based on the geographical distribution of *A. agassizii*, its genetic diversity and its phylogenetic relationships with other species of the genus. In spite of an intensive sampling effort, the low occurrence of *A. agassizii* indicated that its distribution is very scarce along the Shetlands Islands and the Antarctic Peninsula, and seems to be restricted to protected and ice-free areas of Fildes Bay in King George Island. Moreover, this population presented very low genetic diversity associated with the signal of a recent demographic expansion. Finally, the reconstruction of the phylogenetic relationships among species of *Abatus* using mitochondrial COI sequences established the affinity of the Antarctic *A. agassizii* with Subantarctic species. Based on these results we consider that the presence of this species in the Shetland Islands more likely corresponds to a recent re-colonization from Antarctic Islands located further north.

Key words: Antarctic benthic fauna, COI phylogenetic relationships, King George Island, Southern Ocean, survivor population.

RESUMEN

Los periodos glaciales del Pleistoceno han promovido los principales cambios climáticos del océano Austral. Han generado el interés científico sobre las estrategias desarrolladas por la fauna bentónica para tolerar y superar la extensión y contracción de la capa de hielo sobre la plataforma continental. Un estudio reciente acerca de la zonación batimétrica y distribución de macro-invertebrados en una zona submareal somera de la Bahía Fildes (Isla Rey Jorge, islas Shetlands del Sur), resalta la presencia de una importante agregación del erizo incubante *Abatus agassizii*, cuya distribución geográfica es conocida solo para localidades al sur de la convergencia antártica (Península Antártica, islas Shetland del Sur e Isla Georgias del Sur en el Arco de Escocia). Sin embargo, su presencia es atípica dado a que, primero, sus poblaciones someras debieron haber sido extintas desde la vecindad de la Península Antártica por los avances y retrocesos de la capa de hielo y segundo, la ausencia de estadios larvales asociado a la estrategia de incubación como modo de desarrollo debería limitar una recolonización desde áreas subantárticas más al norte. El objetivo de este estudio fue evaluar si *A. agassizii* puede haber sobrevivido a las glaciaciones en su estrecho rango batimétrico en las islas Shetland del Sur o ha recolonizado recientemente el área, a pesar de su baja capacidad de dispersión. A pesar de un intensivo esfuerzo de muestreo, la baja ocurrencia de *A. agassizii* indica que su distribución es muy escasa a lo largo de las islas Shetland del Sur y la Península Antártica y parece estar limitada a densas poblaciones someras en áreas protegidas y libres del hielo en la Bahía Fildes en la Isla Rey Jorge. Además, estas poblaciones presentan una muy baja diversidad genética asociada a una señal de una expansión demográfica reciente. Finalmente, la reconstrucción de las relaciones filogenéticas entre las especies de *Abatus*, con secuencias mitocondriales del gen COI, establecieron la afinidad de la especie antártica *A. agassizii* con especies subantárticas. De acuerdo a los resultados obtenidos, consideramos que la presencia de esta especie en las islas Shetlands del Sur probablemente corresponde a una reciente recolonización desde las islas antárticas situadas más al norte.

Palabras clave: fauna bentónica antártica, Isla Rey Jorge, océano Austral, población sobreviviente, reconstrucción filogenética.

INTRODUCTION

The marine benthic fauna of the Antarctic continental shelf is considered to be the most isolated on the planet, due to the extreme environmental conditions and the surrounding barriers such as geographic distances, oceanic currents and deep waters (Lawver et al. 1992, Crame 1999, Clarke et al. 2005, Pfuhl & McCave 2005, Tripati et al. 2005). The current Antarctic biodiversity results from biogeographic and evolutionary processes driven by tectonic and climatic events since the Mesozoic (Clarke & Crame 1989, Crame 1999, Zachos et al. 2001, Brandt & Andreoli 2005, Pfuhl & McCave 2005, Scher & Martin 2006, Brandt et al. 2007, Rogers 2007, Aronson et al. 2007).

The Pleistocene glacial cycles were marked in Antarctica by the extension of the ice coverage over the continental shelf, and up to 100 km on the continental slope (Grobe & Mackensen 1992). Certainly, the glacial periods of the Cenozoic have attracted a lot a scientific attention regarding the strategies developed by the benthic fauna to tolerate these huge climatic changes in the Southern Ocean and the extension and contraction of the ice sheet on the Antarctic continental platform (Kennet 1977, Brey et al. 1996, Zachos et al. 2001, Convey et al. 2008). Three main hypotheses have been proposed to explain the survival during glacial maxima of benthic species that inhabit the Antarctic continental shelf. First, many benthic Antarctic taxa have extended bathymetric ranges compared to their relatives on other continental shelves. This allows movement in and out of deeper areas and could be an evolutionary adaptation (Brey et al. 1996), thus maintaining large population size and genetic diversity during glacial periods, as observed in the nudibranch *Doris kerguelensis* (Wilson et al. 2009) and in the pycnogonid *Nymphon australe* (Mahon et al. 2008, Arango et al. 2011). Second, according to the expansion-contraction model of Quaternary biogeography (Provan & Bennet 2008), it is possible that taxa with narrow bathymetric ranges, whose ecosystems were entirely disturbed during ice extension, could have endured the glacial maxima in the northern extremes of their distribution such as the Antarctic and Subantarctic islands of the Scotia Arc and other Subantarctic areas (González-Wevar et al. 2011, Hoffmann et al. 2011), where the shallow ecosystems may have been less impacted (Convey et al. 2008). According to this, current populations may represent recent re-colonization processes. The third hypothesis is the in situ persistence in Antarctic glacial refugia as occurred in terrestrial invertebrates including acarians, colembola and copepods (Convey & Stevens 2007, Rogers 2007, Convey et al. 2008) and green algae (De Wever et al. 2009).

The sea urchin genus *Abatus* (Spatangoidea: Schizasteridae) includes 11 brooding species distributed in different provinces of the Southern Ocean (David et al. 2005). Two species are distributed in Subantarctic Provinces, *A. cordatus* (Verrill 1876) endemic to the Kerguelen Archipelago in the Indian Ocean, and *A. cavernosus* (Philippi 1845) in Patagonia, southern South America. The rest of the species (nine), *A. agassizii* (Mortensen 1910), *A. beatriceae* (Larrain 1986), *A. bidens* (Mortensen 1910), *A. curvidens* (Mortensen 1936), *A. elongatus* (Koehler 1908), *A. ingens* (Koehler 1926), *A. nimrodi* (Koehler 1911), *A. philippii* (Lovén 1871) and *A. shackletoni* (Koehler 1911) are distributed in the Antarctic continent (David et al. 2005). These species are deposit feeders that inhabit muddy to sandy ecosystems (Arnaud 1974, Poulin & Féral 1995), and are found mainly in the upper part of the continental shelf (David et al. 2005).

In a recent study of the bathymetric zonation and distribution of macro-invertebrates in a shallow subtidal zone of Fildes Bay (King George Island, South Shetlands Islands; Fig. 1), Palma et al. (2007) discovered a large aggregation of the brooding sea urchin *Abatus agassizii* between 3 and 8 meters depth. Such shallow distributions with high density have been described for the Subantarctic species *A. cordatus* (Poulin & Féral 1995, Ledoux et al. 2012) and *A. cavernosus* (Gil, pers. comm.), in sheltered areas of the intertidal and infralitoral zones. In contrast, Antarctic representatives including *A. nimrodi*, *A. ingens* and *A. shackletoni* are usually found at lower densities and below 20-25 meters depth (Chenuil et al. 2004, E Poulin & J-P Féral, unpublished data). Another remarkable characteristic of *A. agassizii* is the low number of occurrences reported, particularly around the Antarctic continent, where the species was found only once, in the eastern Weddell Sea, during the Polarstern Expedition Antarktis IX (OBIS). By contrast, this species has been reported recurrently from shallow subtidal areas from the South Georgia and South Shetland Islands.

Based on the above information, and in the context of the hypotheses previously advanced about the strategies developed by Antarctic species to overcome the glacial maxima, we provide here new information about geographic and bathymetric distribution, genetic diversity and phylogenetic relationships of *A. agassizii*. First we further explore the extent of the distribution of *A. agassizii* along the South Shetlands Islands and the Antarctic Peninsula, compiling presence and absence records accumulated during several field trips and diving surveys between 2004 and 2011. Second, given that past demographic changes may leave a strong signal in the present genetic diversity,

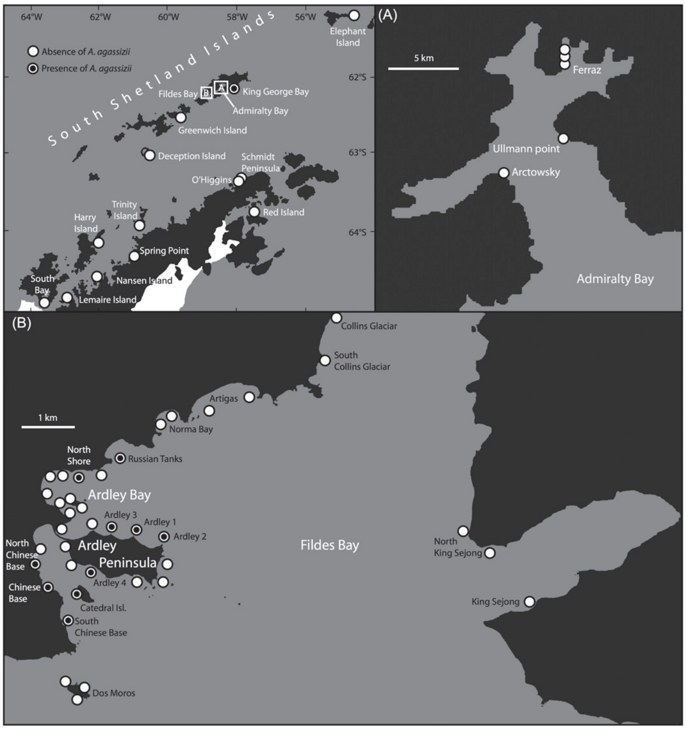

Fig. 1: Distribution of sites where the presence of *Abatus agassizii* was investigated by scuba diving. Top left corner: Antarctic Peninsula and surrounding Islands. Top right and bottom frames are zooms of King George Island and the South Shetlands. A: Admiralty Bay. B: Fildes Bay.

Distribución de los sitios donde se investigó la presencia de *Abatus agassizii* mediante buceo. Cuadro izquierdo superior: Península Antártica e islas cercanas. Cuadros derecho superior y inferior son zoom de la Isla Rey Jorge, Shetland del Sur; A: Bahía Almirantazgo. B: Bahía Fildes.

we evaluated the genetic diversity of the mitochondrial Cytochrome c Oxidase subunit I (COI) gene in *A. agassizii* in order to detect signals of recent population expansion. Third, we reconstructed phylogenetic relationships between Antarctic and Subantarctic *Abatus* species using the COI sequences. Finally, based on our results and other information available on distribution and life-history traits of *Abatus* species, we discuss whether a population of *A. agassizii* could have survived the glaciations in the South Shetland Islands within its narrow bathymetric range, or whether it may represent a newcomer that re-colonized the area despite its low dispersal capacities.

METHODS

Sampling effort in the Antarctic Peninsula and surrounding islands

We compiled presence-absence records collected during eight successive field trips at 57 geo-referenced locations between the latitudes 61-65°S (Table 1; Fig. 1), including the Shetland Islands, Palmer Archipelago, West Antarctic Peninsula and Red Island in the northeastern extremity of the Antarctic Peninsula. Exploration using scuba diving was made at different scales; first at hundreds of kilometers to evaluate the overall distribution of the species along the northern Antarctic Peninsula and surrounding islands, and second at the scale of large bays (Admiralty and Fildes Bays). Surveyed areas were chosen based on typical environmental characteristics described for *Abatus* species (Poulin & Féral 1995, Palma et al. 2007); sheltered areas with medium to fine sand substrate and moderate to slight slope. Scuba diving samplings between 0 – 15 m depth consisted of gently removing by hand the upper layer of sediment to detect directly the presence of *Abatus* individuals.

Samples and mtDNA sequencing

27 specimens of *A. agassizii* were collected in the subtidal zone of the Ardley Peninsula (Ardley 1), while *A. cordatus* (13 individuals) and *A. cavernosus* (27 individuals) were obtained from the infra-littoral of Kerguelen Island (48°56'S, 69°02'E) and Argentinean Patagonia (47°45'S, 65°52'W), respectively. Specimens were fixed in 96° ethanol and DNA was prepared from the gonads or spines using the salting-out method (Aljanabi & Martínez, 1997). We amplified by polymerase chain reaction (PCR) a fragment of 799 base pairs (bp) of the COI gene using the primers described by Stockley et al. (2005). However, it was necessary to design internal specific primers for *A. agassizii* (ABAin-F: TTG CCA TTT TCT CAC TCC ACC T; ABAin-R: AAG AAT CAG AAT AGA TGY TGA AAT A). PCRs were performed in a Thermo PxE 0.5 thermocycler using TaqDNA polymerase (Invitrogen, Recombinant). Each reaction tube (25 µL) contained: 1 µL of extracted DNA (approx. 10 ng), 2.5 µL 10X PCR buffer, 2 µL 1.5 mM $MgCl_2$, 2.5 µL 10 mM of each dNTP, 10 pmol of each primer, 0.2 U Taq polymerase. After 4 min of initial denaturation at 94° C, amplifications were performed using 35 cycles of 94° C for 50 s, 56.4° C for 45 s, 72° C for 1 min and a final extension of 10 min at 72° C. PCR products were purified with Qiagen Qiaquick columns and sequenced by Macrogen (South Korea) in an ABI 3100 automatic sequencer (Applied Biosystems). The sequences were aligned with the programs Proseq v. 2.91 and ClustalX 1.8 (Thompson et al. 1997, Filatov 2002).

Analysis of genetic diversity

The genetic diversity of the *A. agassizii* populations from Antarctica was evaluated from COI fragments of 799 bp. We estimated the number of haplotypes (K), haplotype diversity (H), the number of polymorphic sites (S) and the mean number of pairwise differences (Π) of each sequence set obtained for the three species using the program DNAsp v5 (Librado & Rozas 2009). The genealogical relationships among haplotypes were built using the Median Joining algorithm (Bandelt et al. 1999), implemented in the program Network v.4.1.5 (Röhl 2002). To determine past demographic changes in A. agassizii, Tajima's D and Fu's F_S tests were performed to evaluate the assumption of selective neutrality of mtDNA sequences and population mutation-drift equilibrium. Significant negative values for these tests are due to an excess of rare polymorphisms in a population, indicating either recent demographic expansion or positive selection. To evaluate the existence of a signal that this population has undergone a sudden population growth we constructed the mismatch distribution of the COI sequences, comparing it to the Poisson expectation for varying population sizes under a sudden expansion model (Slatkin & Hudson 1991, Rogers & Harpending 1992). For this, we estimated the raggedness statistic r (Harpending 1994) and evaluated its significance by computer simulations using the coalescent. This statistic quantifies the smoothness of the observed distribution of pairwise differences. Past population dynamics over time for *A. agassizii* were estimated using the Bayesian Skyline model in BEAST (Drummond et al. 2005; 2012). The appropriate model of nucleotide substitution was GTR+G, determined using ModelTest ver. 3.06 (Posada & Crandall 2001). Two independent runs of 2 x 10^8 iterations were performed; the model parameters were sampled every 1000 iterations. We used the mutation rate estimated by Lessios (2008) for sea urchins COI (3.5 % Ma-1). Demographic plots were visualized using Tracer v1.0.1 (Drummond & Rambaut 2007).

Phylogenetic relationships

The phylogenetic relationships among eight of the eleven existing *Abatus* species were reconstructed using mtDNA COI sequences: *A. agassizii* from Fildes Bay (King George Island), *A. cordatus* from Kerguelen Island, *A. cavernosus* from Argentinean Patagonia, *A. elongatus* from the Weddell Sea (Antarctic Peninsula), *A. bidens, A. shackletoni, A. ingens* and *A. nimrodi* from Adélie Land, East Antarctic. We rooted the tree with three outgroups: two belonging to the Order Spatangoidea: *Paleopneustes cristatus* (AJ639908) and *Spatangus raschi* (AJ639911.1), following the phylogeny reconstruction proposed by Smith et al. (2006), and one outgroup belonging to the Family Schizasteridae, *Brachisternaster chesheri* (JX000215). The size of the common fragment after alignment of all sequences was 690bp. Sequences were deposited in GenBank (JX000212-JX000219). Phylogenetic trees were constructed by Bayesian analysis using the program MrBayes v.3.1.2 (Huelsenbeck & Ronquist 2003), with the substitution model GTR+G as indicated by the Modeltest program (Nylander 2004). This method estimates the posterior probability of each taxon being a monophyletic unit. All the Markov Chain Monte Carlo analyses (MCMC) were performed twice, using independent runs with random trees for a total of 7 x 10^6 generations. Trees were sampled every 500 generations and the first 1000 trees were discarded as burn-in. The remaining trees were used to construct a consensus phylogram with the majority rule and posterior probability for the nodes. In addition we performed a Maximum Likelihood analysis using RaxML-VI v.7.0.4. (Stamatakis 2006), implemented in the Phylobench Phylogenetic Software Benchmarking Suite. Branch support was assessed with 100 non-parametric bootstrap replicates under the GAMMA model, and bootstrap values were plotted on the ML tree with the best likelihood.

RESULTS

Occurrence and absence of A. agassizii

No specimen of *A. agassizii* was found either along the west coast of the Antarctic Peninsula or on the east side (Fig. 1). Searches were also unsuccessful in the South Shetlands, except for King George Island. At this latter location, *A. agassizii* was absent from Admiralty Bay (Fig. 1A); it was found mainly in Fildes Bay, but also in King George Bay (only 1 specimen found after an extensive search, but not processed in this study) (Table 1). In Fildes Bay, *A. agassizii* was found in the southwestern part, outside the area affected by the glacier. Ten local populations were identified in the proximity of the Ardley Peninsula, Fildes Bay, King George Island (Fig. 1B). In several opportunities another irregular sea urchin, *Trypilus abatoides* (Schizasteridae) was found in syntopy with *A. agassizii*, but its occurrence never exceeded 5 % of the total abundance.

Genetic diversity

A low level of genetic diversity was observed in *A. agassizii*. Among 27 COI fragments of 799 bp, only four variable sites were found. One of the five haplotypes recovered was shared by 23 individuals (85.15 %), leading to extremely low values of haplotype diversity (H = 0.28) and mean number of pairwise nucleotide differences (Π = 0.296). Negative and significant values of Fu's F_S and Tajima's D indicated an excess of low frequency haplotypes (-3.024

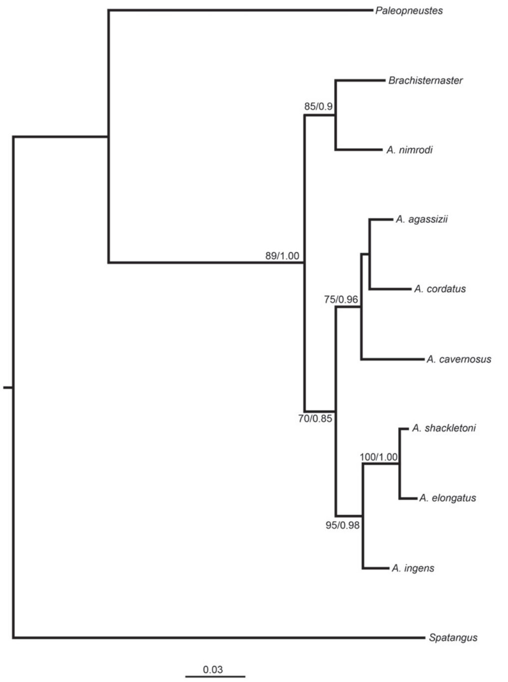

Fig. 2: Bayesian inference (BI) tree of the COI sequences obtained for the genus *Abatus*. The branch support values are from ML and BI analyses.

Árbol de Inferencia Bayesiana (IB) con las secuencias de COI obtenidas para el género *Abatus*. Los valores de soporte en cada nodo corresponden a ML y BI análisis.

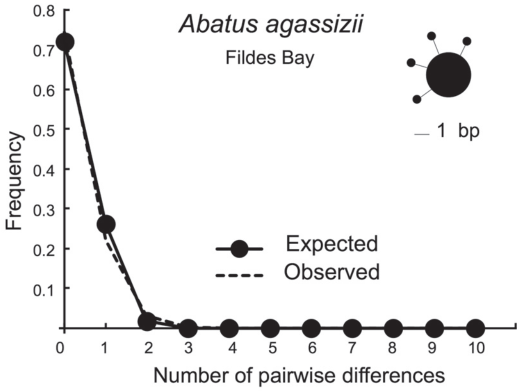

Fig. 3: Mismatch distribution and network of COI haplotypes of *A. agassizii*. Haplotypes are represented by circles whose size indicates their frequency. bp: Base pairs.

Distribución de *Mismatch* y red de haplotipos de COI para *A. agassizii*. Los haplotipos son representados por círculos cuyos tamaños dependen de la frecuencia. bp: pares de bases.

and -1.888, respectively), while the haplotype network exhibited a star-like structure (Fig. 3). The mismatch distribution showed a unimodal L-shaped pattern that fit the expected Poisson distribution under the sudden expansion model (r = 0.27, P = 0.51, Fig. 3), suggesting the existence of a recent demographic expansion or a selective sweep. Similarly, the Bayesian Skyline plot revealed a very recent expansion that started around 10000 yr. Since this time, population effective size increased from around 400 individuals up to 40000 at the present (Fig. 4).

Phylogenetic relationships among Abatus *species*

Bayesian and Maximum Likelihood methods produced similar phylogenetic hypotheses in terms of branching and the robustness of some nodes. The tree topology suggests that *Abatus* species do not represent a monophyletic clade. In fact, the analysis revealed that *Brachisternaster chesheri* is the sister species of *A. nimrodi*. However, the rest of the species did form a monophyletic group with two well-differentiated clades. The first one includes *A. agassizii* (Antarctica) and the Subantarctic species *A. cordatus* and *A. cavernosus*, with a bootstrap support of 75 % and posterior probability 0.96. The second clade includes the remaining Antarctic species with 95 % bootstrap support and 0.98 posterior probability (Fig. 2). Comparison of sequences among *A. agassizii*, *A. cordatus* and *A. cavernosus* revealed deep divergences among Antarctic, Patagonian and Kerguelenian species of *Abatus*. The average number of pairwise differences between *A. agassizii* (Antarctica) and *A. cavernosus* (Kerguelen) was 28.15, and between *A. agassizii* (Antarctica) and *A. cordatus* (Patagonia) the average was 22.15. Finally, the average number of differences between Subantarctic *A. cavernosus* (Kerguelen) and *A. cordatus* (Patagonia) was 30.

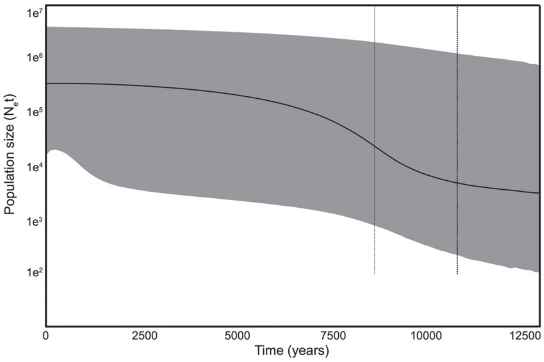

Fig. 4: Historical demographic trends of the effective population size (Ne) constructed using the Bayesian skyline plot approach based on Cytochrome oxidase subunit I (COI) haplotypes of *A. agassizii*. The x-axis is the time in units of years and the y-axis is equal to $N_e t$, the product of effective population size (Ne) and generation length in a log scale. The median estimate (black solid line) and 95 % highest probability density (HPD) limits (grey) are shown. The thick dashed line represents the time of the most recent ancestor (trcma) and the thin dashed line represents time for the expansion in the species.

Tendencia demográfica histórica del tamaño poblacional efectivo (Ne) construido con la aproximación bayesiana Skyline plot basado en haplotipos del citocromo Oxidasa subunidad I (COI) de *A. agassizii*. El eje x es el tiempo en unidades de años y el eje y es igual a $N_e t$, el producto del tamaño poblacional efectivo (Ne) y la extensión de la generación en una escala logarítmica. Se muestran la estimación media (línea negra) y el 95 % de los límites más altos de densidad de probabilidad (HPD) (área gris). La línea discontinua gruesa representa el tiempo del ancestro común más reciente (tacmr) y la línea discontinua delgada representa el tiempo de la expansión en la especie.

DISCUSSION

The current distribution of a species is the result of a complex interplay between extrinsic factors such as isolation of landmasses, climatic conditions and niche availability, and intrinsic factors such as dispersal capacity and physiological tolerance (Leese et al. 2008). Although the presence of *A. agassizii* has been previously described in the South Shetlands Islands (OBIS 2007), the global occurrence data reported for *A. agassizii* are very scarce for a species accessible by Scuba diving. Our sampling effort allowed us to confirm that the presence of *A. agassizii* is sporadic along the South Shetland Islands and it is probably absent from the northern part of the Antarctic Peninsula. So far, *A. agassizii* populations are known to be common only in the eastern sector of Fildes Bay, in 10 sites located in Ardley Bay, around the Ardley Peninsula and the vicinity of the Great Wall Chinese Base.

In general, the presence of dense *A. agassizii* populations appears to be restricted by substrate type as well as wave exposure; it is found in patchy and dense aggregations at low

TABLE 1

Sites visited and sampled in the Antarctic Peninsula and surrounding islands: geographic area, details, GPS coordinates of the locations, presence/absence of *Abatus agassizii.*

Sitios visitados y muestreados en la península Antártica e islas cercanas: área geográfica, detalles, coordenadas GPS de las localidades, presencia/ausencia de *A. agassizii.*

Area	Location	Latitude S	Longitude W	Presence
South Shetlands	Elephant Isl.	61°07’07”	54°38’49”	no
South Shetlands, KG Isl.	King George Bay	62°08’18”	58°07’21”	yes
South Shetlands, KG Isl., Almirantazgo B.	Ullman Point	62°07’32”	58°23’47”	no
South Shetlands, KG Isl., Almirantazgo B.	Ferraz, Brasilian Base	62°05’00”	58°23’17”	no
South Shetlands, KG Isl., Almirantazgo B.	Ferraz, Brasilian Base	62°05’01”	58°23’12”	no
South Shetlands, KG Isl., Almirantazgo B.	Ferraz, Brasilian Base	62°05’07”	58°23’27”	no
South Shetlands, KG Isl., Almirantazgo B.	Arctowsky, Polish Base	62°09’19”	58°28’00”	no
South Shetlands, KG Isl., Fildes B.	Collins Glaciar	62°09’35,68”	58°50’23,60”	no
South Shetlands, KG Isl., Fildes B.	South Collins Glaciar	62°10’22,27”	58°51’20,28”	no
South Shetlands, KG Isl., Fildes B.	Artigas, Uruguayan Base	62°11’14,32”	58°53’55,43”	no
South Shetlands, KG Isl., Fildes B.	Artigas, Uruguayan Base	62°11’08,16”	58°52’59,27”	no
South Shetlands, KG Isl., Fildes B.	Norma Bay	62°11’35,70”	58°54’54,97”	no
South Shetlands, KG Isl., Fildes B.	Norma Bay	62°11’27,92”	58°54’45,94”	no
South Shetlands, KG Isl., Fildes B.	Russian Tanks Bay	62°11’48,66”	58°55’47,32”	yes
South Shetlands, KG Isl., Fildes B.	Ardley Bay	62°11’55,44”	58°56’18,77”	no
South Shetlands, KG Isl., Fildes B.	North Shore of Ardley Bay	62°11’59,17”	58°56’43,62”	yes
South Shetlands, KG Isl., Fildes B.	Ardley Bay	62°11’57,04”	58°57’14,63”	no
South Shetlands, KG Isl., Fildes B.	Ardley Bay	62°11’58,59”	58°57’25,63”	no
South Shetlands, KG Isl., Fildes B.	Ardley Bay	62°12’11,46”	58°57’25,01”	no
South Shetlands, KG Isl., Fildes B.	Ardley Bay	62°12’14,07”	58°57’13,84”	no
South Shetlands, KG Isl., Fildes B.	Ardley Bay	62°12’21,56”	58°56’59,26”	no
South Shetlands, KG Isl., Fildes B.	Becerra Isl.	62°12’16,92”	58°56’49,63”	no
South Shetlands, KG Isl., Fildes B.	Logger site, Becerra Isl.	62°12’12,17”	58°56’41,71”	no
South Shetlands, KG Isl., Fildes B.	North Arley Isthmus	62°12’28,95”	58°57’11,06”	no
South Shetlands, KG Isl., Fildes B.	North-East Penin. Ardley	62°12’29,67”	58°56’29,65”	no
South Shetlands, KG Isl., Fildes B.	Penin. Ardley 3	62°12’28,01”	58°56’03,08”	yes
South Shetlands, KG Isl., Fildes B.	Penin. Ardley 1	62°12’30,31”	58°55’34,78”	yes
South Shetlands, KG Isl., Fildes B.	Penin. Ardley 2	62°12’35,46”	58°54’53,14”	yes
South Shetlands, KG Isl., Fildes B.	South of Ardley Isthmus	62°12’40,28”	58°56’58,84”	no
South Shetlands, KG Isl., Fildes B.	South of Ardley Isthmus	62°12’52,10”	58°56’57,25”	no
South Shetlands, KG Isl., Fildes B.	Penin. Ardley 4	62°12’57,53”	58°56’30,62”	yes
South Shetlands, KG Isl., Fildes B.	South-East Penin. Ardley	62°13’03,27”	58°55’27,39”	no
South Shetlands, KG Isl., Fildes B.	South-East Penin. Ardley	62°13’03,44”	58°54’51,70”	no
South Shetlands, KG Isl., Fildes B.	South-East Penin. Ardley	62°12’53,79”	58°54’50,25”	no
South Shetlands, KG Isl., Fildes B.	North Great Wall, Chinese Base	62°12’34,60”	58°57’44,93”	no
South Shetlands, KG Isl., Fildes B.	North Great Wall, Chinese Base	62°12’50,58”	58°57’43,34”	yes

Area	Location	Latitude S	Longitude W	Presence
South Shetlands, KG Isl., Fildes B.	Great Wall, Chinese Base	62°13’07,64”	58°57’28,30”	yes
South Shetlands, KG Isl., Fildes B.	Catedral Isl.	62°13’09,05”	58°56’52,65”	yes
South Shetlands, KG Isl., Fildes B.	South Great Wall, Chinese Base	62°13’36,01”	58°56’54,35”	no
South Shetlands, KG Isl., Fildes B.	South Great Wall, Chinese Base	62°13’25,79”	58°57’01,04”	yes
South Shetlands, KG Isl., Fildes B.	Dos Moros Isl.	62°14’06,14”	58°57’03,49”	no
South Shetlands, KG Isl., Fildes B.	Dos Moros Isl.	62°14’12,98”	58°57’01,01”	no
South Shetlands, KG Isl., Fildes B.	Dos Moros Isl.	62°14’07,22”	58°56’39,01”	no
South Shetlands, KG Isl., Fildes B.	King Sejong, Korean Base	62°13’07,94”	58°46’10,73”	no
South Shetlands, KG Isl., Fildes B.	North King Sejong, Korean Base	62°12’42,75”	58°47’41,07”	no
South Shetlands, KG Isl., Fildes B.	North King Sejong, Korean Base	62°12’30,95”	58°48’11,45”	no
South Shetlands	Greenwich Isl.	62°28’38”	59°40’15”	no
South Shetlands	Deception Isl,	62°58’47”	60°33’29”	no
West Antarctic Peninsula	O’Higgins, Chilean Base	63°19’18,43”	57°54’01,76”	no
West Antarctic Peninsula	Schmidt Penin.	63°19’14”	57°53’52”	no
West Antarctic Peninsula	Trinity Isl.	63°54’12”	60°47’10”	no
West Antarctic Peninsula	Harry Point	64°07’42”	62°00’54”	no
West Antarctic Peninsula	Spring Point	64°17’13”	61°03’43”	no
West Antarctic Peninsula	Nansen Isl.	64°33’23”	61°59’33”	no
West Antarctic Peninsula	Lemaire Isl.	64°49’50”	62°54’41”	no
West Antarctic Peninsula	South Bay	64°52’25”	63°34’37”	no
East Antarctic Peninsula	Red Isl.	63°44’13”	57°52’51”	no

depth (1.5 to 8 meters), buried in a medium to fine sandy sediment. In this regard, Lopez & Levinton (1987) emphasized that the particle size composition of the substratum is usually a primary factor determining the habitat of deposit feeders, influencing their respiratory and locomotive functions. These sites were all swell-sheltered and far from iced shores such as the Collins ice cap that influences the northeastern side of the bay. For example, the northernmost site where *Abatus* was found, near the Russian Tanks Bay (Table 1), is more exposed to swell action. In this locality, only few specimens have been found in deeper zones, generally around 10 m depth, where swell action is less severe. This shallow distribution contrasts with those reported for other Antarctic *Abatus* species such as *A. nimrodi*, *A. ingens* and *A. shackletoni*, that are generally found bellow 25-30 m depth (Chenuil et al. 2004, Chiantore et al. 2006). In this respect, the bathymetric range of *A. agassizii* is very similar to those described for the Subantarctic species *A. cordatus* and *A. cavernosus*. In these species, some dense aggregations (up to 64 and 140 individuals m^{-2} in Argentinean Patagonia and Kerguelen Islands, respectively) are found in shallow water at the bottom of sheltered bays in sediments that range from medium to fine sand (Poulin & Féral 1995, Gil et al. 2009). Probably due to all of these characteristics, current habitats along the Antarctic Peninsula are unsuitable for the settlement of *A. agassizii* populations. In the context of the current rapid climatic changes in the Antarctic Peninsula and the acceleration of ice sheet retreat, it is likely that the most suitable sites for *A. agassizii* were made available in the very recent past. Alternatively, the absence of *A. agassizii* from the Antarctic Peninsula may suggest that it has not been able to reach those areas. The absence of a dispersal stage limits the effective colonization of distant newly available habitats. By contrast, at the scale of Fildes Bay *A. agassizii* seems able to extend its distribution to nearby areas even across unsuitable habitats, possibly by the way of greater bathymetric depth or the local dispersion of juveniles by bottom and tidal currents. The first alternative is not supported by our observations, since *A. agassizii* occurred only in very shallow waters, while the second is more plausible; indeed tidal currents and wave action, although weak in this part of the bay, could be strong enough to transport juveniles.

Regarding the genetic diversity pattern in the Fildes Bay population, our results clearly support the existence of a recent and rapid population growth. However, this pattern could reflect a demographic expansion associated with a founder effect following a re-colonization event as well as a re-expansion from a glacial refuge, and the results do not allow discrimination between these scenarios.

In addition to the evidence obtained on distribution and habitat characteristics, phylogenetic analysis clustered *A. agassizii* with the Subantarctic *Abatus* species, separating them from other Antarctic species. These genetic affinities are also supported by the subtle morphological differences that allow distinguishing these three species (presence of globiferous pedicellaria, number of teeth in pedicellaria valves) (David et al. 2005). In addition, Maturana (2011) showed the existence of an annual reproductive cycle in the *A. agassizii* populations of Fildes Bay similar to those described in Subantarctic *Abatus* populations from Argentina (*A. cavernosus*) and Kerguelen Island (*A. cordatus*), while Antarctic species such as *A. shackletoni* and *A. nimrodi* exhibited continuous reproduction (Pearse & McClintock 1990).

Considering life history trait similarities (habitat preference, bathymetry, and reproductive cycle) and phylogenetic affinity between *A. agassizii* and its Subantarctic relatives, we suggest that the presence of *A. agassizii* in Fildes Bay, King George Island may represent a recent (Holocene) colonization event from the northernmost. Islands of the Scotia Arc belonging to the Antarctic and Subantarctic Provinces such as South Orkney and South Georgia Islands, respectively (see Griffith et al 2008 for a review of biogeographic provinces in the Southern Ocean). At the same time, we challenge the possibility of re-colonization from Subantarctic regions such as Tierra de Fuego, considering the high levels of genetic divergence between *A. agassizii* and the Argentinean *A. cavernosus*. Such a level of divergence requires a long period of isolation, much longer than the time considered here.

However, re-colonization from northern Antarctic Islands to the South Shetlands is difficult to invoke, considering the low dispersal potential that the direct development of *A. agassizii* implies. However, exceptional long distance dispersal events through rafting have been described in some Southern Ocean brooders. For instance, colonization of juveniles by rafting in a brooding pelecypod, *Gaimardia trapesina*, has been described between Cape Horn, the Falkland Islands and the South Georgia Island in the Scotia Arc (1300-2000 km) (Helmuth et al. 1994). As shown in *G. trapesina*, it is possible that *A. agassizii* re-colonized King George Island through rafting from northern areas, although this is highly speculative and remains to be demonstrated. Alternatively, *A. agassizii* may have persisted in Fildes Bay through the existence of glacial refugia, and would represent a relict population that survived the entire glacial period in situ. However, such shallow costal marine refugia have not been identified, and as stated by Pugh & Convey (2008), the South Shetland Islands and the coastal terrain of the Antarctic Peninsula were probably covered by a deep and extensive ice cap during the LGM; it has been stated that present ecosystems are no older than 10000 to 6000 years (Fenton 1982, Björk et al. 1991). The South Georgia Islands remain a likely place where *A. agassizii* could have endured the coldest periods of the Pleistocene glacial cycles.

In conclusion, this study provides new evidence that supports the affinity between the Antarctic species *A. agassizii* and the Subantarctic congeneric species from the Magellanic and Kerguelenian Provinces. Even though *A. agassizii* is currently distributed in the Antarctic Province, it shares several genetic, reproductive and ecological characteristics with *A. cordatus* (Kerguelen Island) and *A. cavernosus* (Argentina). Although the in situ refugia hypothesis cannot be rejected, the probable absence of suitable habitats during glacial periods together with the extremely restricted geographic distribution of *A. agassizii* in the South Shetlands and Antarctic Peninsula, its very low level of population genetic diversity and its phylogenetic affinity with Subantarctic congeners, suggest a recent (re) colonization of the South Shetland Islands from the northernmost islands of the Antarctic Province. Under this scenario, we would expect to find more genetic diversity in these islands as an evidence of an older population. Also, we would expect to detect the dominant haplotype of the Fildes Bay population to support the hypothesis of a founder effect associated with the re-colonization process. Until we provide such information, we are aware that our hypothesis remains mainly speculative, even if supported by a large body of evidence. Further studies will include samples of *Abatus* populations from other islands such as South Georgia, South Sandwich and South Orkney to test our hypothesis. Including more Subantarctic populations from Kerguelen and Patagonia should also provide elements for evaluating the genetic diversity at the species level to infer the evolutionary and demographic history of *A. cordatus* and *A. cavernosus*. Finally, such studies will further enhance our understanding of the history of the Antarctic marine benthic species that inhabited the infralittoral and shallow subtidal zones during the coldest periods that have affected recurrently the Antarctic marine ecosystem during the Pleistocene.

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