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RESEARCH NOTE

Genetic variation in the small bivalve *Nuculana inaequisculpta* along a retreating glacier fjord, King George Island, Antarctica

Variación genética en el pequeño bivalvo *Nuculana inaequisculpta* a lo largo de un fiordo con retroceso glaciar, Isla Rey Jorge, Antártica

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Abstract. Climate change is strongly influencing regions of Antarctica but the consequences on microevolutionary processes have been little studied. Patterns of population genetic diversity were analysed in the Antarctic bivalve *Nuculana inaequisculpta* (Protobranchia: Nuculanidae) from a fjord with 70 years of documented climate-forced glacier retreat. Thirty-nine individuals from five sites at different distances from the glacier terminus were collected, and the COI gene was sequenced from each individual. No statistically significant genetic differentiation was found between sites nor a significant correlation between the proximity of glaciers and genetic diversity, suggesting a high dispersal capability and therefore, a planktonic larval stage for this species. Nevertheless, we encourage increasing the sample size and number of loci in future studies to confirm our findings.

Key words: COI, climate change, dispersal capability, Western Antarctic Peninsula

INTRODUCTION

West Antarctica is a hotspot for physical Climate Change, and ~90% of all glaciers along the West Antarctic Peninsula (WAP) are rapidly retreating (Cook *et al.* 2016). Marine species, especially those in the shallows, are subject to drastic environmental changes, including those which may negatively impact species survival (*e.g.*, increased ice scour) or favor colonization of new available habitats exposed by retreating glaciers. Several studies have documented ecological impacts of glacial retreat and melting on marine communities (*e.g.*, Moon *et al.* 2015, Sahade *et al.* 2015, Barnes *et al.* 2020, Bascur *et al.* 2020). However, it is less well known how such perturbations may drive microevolutionary responses of marine species at population genetic scales. There is a lack of studies examining this question in Antarctic ecosystems. Studies testing patterns of genetic diversity in relation to climate-change forced distributional shifts in other areas have found mixed responses. For instance, simulation

studies have found that the edge of a shifting range, under climate change, has low levels of neutral genetic diversity (Cobben *et al.* 2011, Arenas *et al.* 2012). In contrast, some empirical data, for example of *Larix decidua*, have shown high genetic diversity at the leading edge of an expanding population following a retreating glacier in the Swiss Alps (Pluess 2011).

The genetic population response of a species after perturbation is also related to aspects of its biology, such as its dispersal capability. For instance, a species with low dispersal capability might be expected to present a stronger pattern of genetic diversity (*e.g.*, decreasing genetic diversity along an expansion transect) than a species with high dispersal capability. This is because migration between demes is not fast enough to dilute the pattern (Klopfstein *et al.* 2006). In contrast, high dispersal capability would be likely to drive high gene flow between populations and therefore, rapid homogenization of any incipient genetic differentiation (Slatkin 1987).

In Antarctica, it has been argued that climatic conditions have filtered for life-history strategies that reduce or lack larval stages in benthic organisms, especially those which are planktotrophic (Pearse *et al.* 2009). However, planktonic larvae have been described for many Antarctic benthic species (*e.g.*, Stanwell-Smith *et al.* 1997, 1999), and their actual number may have been greatly underestimated due to the lack of detailed morphological and molecular studies on planktonic larvae (Thatje 2012). Studies around Antarctica have shown that developmental type influences patterns of genetic differentiation across barriers. For example, Poulin *et al.* (2014) found that species lacking planktonic larvae were more divergent across the Antarctic Circumpolar Current than species with planktonic larvae. Similarly, Muñoz-Ramírez *et al.* (2020) found that *Aequiyoldia eightsii*, a species with a lecithotrophic larvae, showed genetic patterns that were congruent with ocean current dispersal. Dispersal capability of species likely influences colonization rates and genetic patterns on ecological time scales. However, no studies have examined the genetic consequences at the temporal scale of current climate change in Antarctica.

Nuculana inaequisculpta (Lamy, 1906) (Fig. 1D) is a small bivalve species that belongs to the Protobranchia subclass, a taxonomic group that contains the most ancient bivalves in the world (Yonge 1939, Villarroel & Stuardo 1998). It is an abundant infaunal species, patchy distributed in the South Shetland Islands and along the

Antarctic Peninsula (Cattaneo-Vietti *et al.* 2000, Lovell & Trego 2003, Gordillo *et al.* 2017). This species lives in benthic, muddy marine habitats and feeds mainly on organic deposits in sediments (Zardus 2002). It occurs as *ca.*, 800 m, and range in size from 2.5-16.0 mm in shell length (Cattaneo-Vietti *et al.* 2000, Gordillo *et al.* 2017). In general, protobranchia species have two separate sexes, with a similar (1:1) sex ratio, high fecundity, and lecithotrophic larval development with a pericalymma larva (Zardus 2002). Recent bioenergetic analyses have found that *Nuculana inaequisculpta* are sensitive to food availability and exhibit lower fitness in recently colonized areas, perhaps due to lower food supply and higher perturbations near the glacier fronts (Bascur *et al.* 2020).

In this study, sequences of the Cytochrome C Oxidase Subunit I (COI) gene obtained from *Nuculana inaequisculpta* were analysed to investigate patterns of genetic diversity along a fjord with a retreating glacier. A strong pattern of decreasing genetic diversity towards the glacier front could be considered indicative of low dispersal capability as expected under a direct development life-history strategy or benthic larvae, and would suggest impacts of current climate change on population diversity. In contrast, a lack of correlation would indicate high dispersal capability, as expected for planktonic larval stages, and highlight the importance of high dispersion as a mechanism of resilience against climate-change induced perturbations.

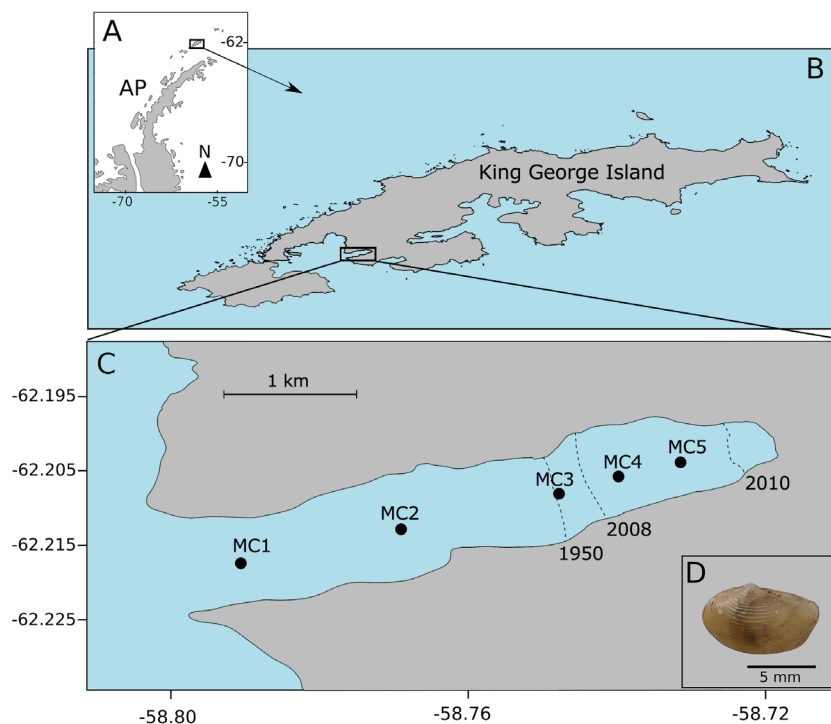


Figure 1. Sample sites for *Nuculana inaequisculpta* at Marian Cove, King George Island, South Shetland Islands, Antarctica. A) Antarctic Peninsula. B) King George Island. C) Marian Cove and sampling sites. D) *Nuculana inaequisculpta* specimen. AP: Antarctic Peninsula. Dashed lines across the fjord represent the location of the glacier front in different years / Sitios de muestreo para *Nuculana inaequisculpta* en Marian Cove, Isla Rey Jorge, Islas Shetland del Sur, Antártida. A) Península Antártica. B) Isla Rey Jorge. C) Marian Cove con los sitios de muestreo. D) Espécimen de *Nuculana inaequisculpta*. AP: Península Antártica. Líneas segmentadas a lo largo del fiordo indican la posición del frente glaciar en diferentes años

MATERIALS AND METHODS

SAMPLING SITES AND MOLECULAR DATA COLLECTION

Thirty-nine individuals of *N. inaequisculpta* were collected from five sites (6-8 individuals per site) at Marian Cove, King George Island, Antarctica, a fjord with a significant glacier retreat documented since 1950 (Park *et al.* 1998, Cook *et al.* 2016, Barnes *et al.* 2020) (Fig. 1, Table 1). Genomic DNA was extracted with the Qiagen DNeasy Blood & Tissue kit, following the manufacturer's protocol. A 688 bp fragment of the cytochrome c oxidase subunit I (COI) gene was amplified using universal primers from Folmer *et al.* (1994) with final concentrations for PCR components per 25 μ L reaction as follows: 25 ng template DNA, 0.25 μ M of each primer, 0.625 units of GoTaq DNA polymerase (Promega, Madison, WI, USA), 0.1 mM of each dNTP, 2.5 μ L of 10 reaction buffer and 2.5 mM $MgCl_2$. Amplification parameters were as follows: 95 $^{\circ}C$ for 2 min followed by 35 cycles of 95 $^{\circ}C$ for 30 s, 48 $^{\circ}C$ for 30 s, 72 $^{\circ}C$ for 90 s, and 72 $^{\circ}C$ for 7 min. Purification and sequencing were conducted at MACROGEN Inc. (South Korea). Chromatograms were edited in CodonCode Aligner 8.0.2 (Dedham, MA, USA). Sequences were imported to BioEdit (Version 7.0.5.2) (Hall *et al.* 1999), aligned using the Clustal W algorithm, available within BioEdit, and checked by eye. All sequences were deposited in GenBank (accession numbers MT510924-MT510962).

GENETIC DIFFERENTIATION AND DIVERSITY

To estimate levels of population differentiation, F_{st} values between sites were calculated using the R-packages *ADEGENET* (Jombart 2008), and *HIERFSTAT* (Goudet 2005). Genetic diversity indexes, haplotype richness (S), haplotype diversity (Hd) and nucleotide diversity (π) for each site and for the entire fjord were estimated using *PEGAS* (Paradis 2010). To visually display relationships between haplotypes, a haplotype network was estimated using the median-joining network algorithm of Bandelt *et al.* (1999) implemented in the program POPART (Leigh & Bryant 2015).

Based on results of low genetic diversity for the closest site to the glacier terminus, a test was conducted to estimate the probability of obtaining the lowest π value for that site under a model of panmixia and chance. For this, a panmictic population was assumed and samples were randomly sampled to build a frequency distribution of 1,000 simulated π values, selecting six samples at each iteration to calculate π . Subsequently, the empirical π value was compared to the frequency distribution of random π values. Support for a significantly low value is given if the empirical π value is lower than the 95% of the simulated π values.

Table 1. Sampling details, genetic diversity and genetic differentiation (F_{st}) between sampling sites based on COI sequences for the Antarctic bivalve *Nuculana inaequisculpta*. F_{st} values were not statistically significant based on a 999-permutation test / Detalles de muestreo, diversidad genética y diferenciación genética (F_{st}) entre sitios de muestreo basado en secuencias del gen COI para el bivalvo antártico *Nuculana inaequisculpta*. Los valores de F_{st} no fueron significativos basado en un test de 999 permutaciones

Sites	Latitude	Longitude	N	Hd	π	
MC1	-62.2176	-58.7292	8	0.6429	0.0032	
MC2	-62.21273	-58.7689	8	1.0000	0.0036	
MC3	-62.20815	-58.7479	8	0.9286	0.0040	
MC4	-62.20588	-58.7399	9	0.9722	0.0053	
MC5	-62.20373	-58.7314	6	0.8000	0.0021	
F_{st} between populations	MC1	MC2	MC3	MC4	MC5	
	MC1	0.0000	0.0819	0.0279	0.0425	0.0848
	MC2	0.0819	0.0000	0.0630	0.0388	0.1057
	MC3	0.0279	0.0630	0.0000	0.0279	0.0583
	MC4	0.0425	0.0388	0.0279	0.0000	0.0758
	MC5	0.0848	0.1057	0.0583	0.0758	0.0000

N: Number of individuals. Hd : Haplotype diversity. π : Nucleotide diversity

RESULTS AND DISCUSSION

A total of eighteen haplotypes were present among the thirty-nine individuals of *N. inaequisculpta* at Marian Cove. The average number of nucleotide differences between samples was 0.402. Genetic structure between populations was low. Genetic differentiation was greater between sites MC2 and MC5 ($F_{st} = 0.1057$), whilst the lowest differentiation values were between MC3 versus MC1 and MC3 versus MC4 (both $F_{st} = 0.0279$). However, none of the F_{st} values between sites were statistically significant at the 0.05 level. A similar lack of structure was observed in the haplotype network (Fig. 2A). A central, high-frequency haplotype was present at all sites at relatively high frequency. Most of the remaining haplotypes were derived from that, with low frequency and separated by only few mutational steps. This star-like topology is typical of populations that have undergone a recent bottleneck and subsequent population expansion (Slatkin & Hudson 1991). Living space on the Antarctic shelf has undergone cyclic expansions and contractions due to the advance and retreat of the Antarctic Ice Shield during glacial cycles (Augustin *et al.* 2004). These major historical events can explain the signal of demographic expansion, which could have initiated after the Last Glacial Maximum. However, this could also be explained, at least in part, due to recent colonization of the fjord. Data are not available to distinguish these hypotheses, but larger geographic sampling, including populations from the entire

distribution of the species could help resolve this question in a future study.

Haplotype diversity (Hd) across sites varied from 0.643 (in MC1) to 1 (in MC2) (Fig. 2B). There was no clear tendency for Hd to increase or decrease with glacier proximity, and the lowest values were found at the opposites sites of the transect ($Hd_{MC1} = 0.643$; $Hd_{MC5} = 0.8$). Nucleotide diversity tended to increase with proximity to the glacier from sites MC1 ($\pi_{MC1} = 0.0032$) to MC4 ($\pi_{MC4} = 0.0053$), but it strongly decreased at site MC5, with MC5 presenting the lowest value among all sites ($\pi_{MC5} = 0.0021$) (Fig. 2C). No significant correlation was found between geographic proximity to the glacier terminus and genetic diversity for both Hd ($r = -0.307$; P -value = 0.6157) and π ($r = 0.06$; P -value = 0.9216), suggesting high dispersal capability for *N. inaequisculpta*, and a planktonic larval stage. However, the lowest π value found at MC5 (the closest site to the glacier terminus) suggests that strong perturbations (*e.g.*, ice scouring, glacier meltwater discharge, high sedimentation) near the glacier terminus may impact genetic diversity. To test if this low value of genetic diversity could imply a deterministic force (*e.g.*, impact of perturbations on genetic diversity), the probability of having the lowest π value at MC5 was estimated under population panmixia and chance. The probability of obtaining a π value equal or lower than the observed value ($\pi = 0.00201$) only by chance was $P = 0.1897$, and therefore, not significant. This indicates that

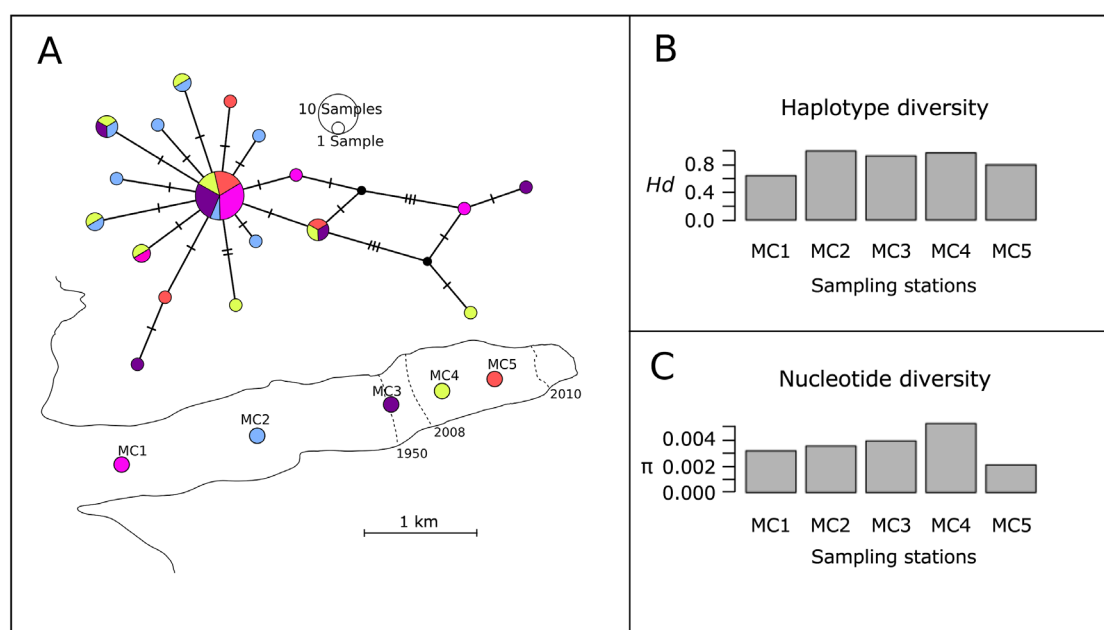


Figure 2. A) Haplotype network, B) Haplotype diversity (Hd), and C) Nucleotide diversity (π) for *Nuculana inaequisculpta* based on COI sequences. Black dots and dashes in the haplotype network represent nodes and mutational steps, respectively / A) Red de haplotipos, B) Diversidad haplotípica (Hd) y C) Diversidad nucleotídica (π) para *Nuculana inaequisculpta* basada en secuencias COI. Los círculos negros y las líneas cortas perpendiculares sobre las líneas que conectan haplotipos corresponden a nodos y pasos mutacionales, respectivamente

there is not enough evidence that the lowest π value at MC5 is due to deterministic forces (*e.g.*, recent colonization of the area or perturbations). However, evidence of low fitness in *Nuculana* specimens from near the glacier terminus has been recently provided by Bascur *et al.* (2020), who found, by conducting bioenergetic analyses, that individuals from MC5 had the lowest energy content in their tissues. This congruence between bioenergetic and genetic results strongly suggests an impact of glacier-derived perturbations on the species's performance.

The pattern of low genetic diversity towards MC1 (the farthest to the glacier terminus) is puzzling, and may suggest a more complex scenario including low competitive abilities. If *N. inaequisculpta* is a species that exploits its dispersal capability to reach recently emptied habitats, but is a generally weak competitor, populations that are present in longer-established communities (*e.g.*, those which are further away from the glacier terminus) may be at disadvantage, and therefore maintain lower population sizes. This could explain low genetic diversity at MC1 and the progressively higher diversity towards MC4 (although not significant). Indeed, an additional site (MC6) furthest from the glacier terminus (not shown on the map) was also sampled, but it yielded no individuals of *N. inaequisculpta*, so it was not included in this study. This MC6 site was rich and diverse in other species though (data not shown), suggesting that the community was old and long established. This seems to support the idea that *N. inaequisculpta* could be a weak competitor given its absence in this site. However, it is recognized that this is speculative, and as a tentative hypothesis it will require further study. Therefore, it is proposed that the comparative genetic study of multiple species with different life history strategies (*e.g.*, with and without planktonic larval stage) and competitive abilities will allow testing this hypothesis in future studies.

There are clear limitations to the inferential power of the marker used here (one single mitochondrial gene) and the low number of individuals used, so these results should be taken with caution. Nevertheless, the results provided in this study are a first step towards a more comprehensive understanding of the patterns and processes governing the genetic diversity of this bivalve species, which can be largely improved in future studies by increasing the sample size and the adoption of more powerful genomic markers such as RADSeq-derived single-nucleotide polymorphisms.

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