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ORIGINAL PAPER

Genetic homogeneity and circum-Antarctic distribution of two benthic shrimp species of the Southern Ocean, *Chorismus antarcticus* and *Nematocarcinus lanceopes*

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Abstract During the last years, molecular studies revealed significant population differentiation and cryptic species within various benthic and pelagic marine Antarctic taxa. This is unexpected due to the lack of obvious barriers to gene flow and strong current systems. Using mitochondrial (COI, 16S rDNA) and nuclear (28S rDNA: D2) gene fragments, we tested whether two circum-Antarctic benthic shrimps with planktotrophic larvae, *Chorismus antarcticus* and *Nematocarcinus lanceopes,* show patterns of regional differentiation. For both species, the 16S and the 28S fragment were invariant. However, for COI we found 24 different haplotypes for *Chorismus antarcticus* and 54 for *Nematocarcinus lanceopes*. No significant differentiation was observed among populations or regions. Furthermore, we found signatures of a population expansion in the late Pleistocene hinting at an impact of large-scale glaciations in particular on the shallow-water shrimp *Chorismus antarcticus*, supporting a (re)colonization and demographic expansion of this shrimp species in response to climate oscillation.

Introduction

Biogeographic studies on marine Antarctic species have been conducted for over 150 years and represent some of the most fascinating examples in ecology and evolutionary

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biology. As consequence of the unique tectonic history, the long isolation, and recurrent large-scale glaciations of Antarctica, the Antarctic marine biota are unique in terms of their physiology, ecology, biogeography, and phylogeny (e.g. Clarke and Johnston 2003; Aronson et al. 2007). However, many aspects are still little understood in detail. Prominent examples are differences in species richness in various taxa, which have undergone a significant radiation in continental shelf waters of Antarctica, e.g. notothenioid fishes, ascidians, crinoids, molluscs, pycnogonids, amphipods, and isopods (Brandt 2000; Eastman and McCune 2000; Held 2000; Clarke and Johnston 2003; Raupach and Wägele 2006; Wilson et al. 2007; Near and Cheng 2008; Krabbe et al. 2010; Wilson et al. 2009).

In contrast to these taxa, the number of known decapod shrimp (Caridea) species on the Antarctic continental shelf is less than ten (Macpherson 1988; Gorny et al. 1992; Klages et al. 1995; Gorny 1999). Although low in species number, the Caridea represent an important and most abundant element of the Antarctic shelf and deep-sea benthos (Arntz and Gorny 1991; Gutt et al. 1991; Thatje and Arntz 2004). For example, the shallow-water species *Chorismus antarcticus* Pfeffer 1887 (Hippolytidae) represents the most abundant shelf inhabiting Antarctic shrimp, distributed around the Antarctic continent (Pfeffer 1887; Kirkwood 1984; Arntz and Gorny 1991; Gutt et al. 1991). The species exhibits a well-defined bathymetric distribution, with abundance values confirming a preference for depths ≤400 m with up to four specimens per m^2 (Gutt et al. 1991). In contrast to this species, the deep-sea shrimp *Nematocarcinus lanceopes* Bate 1888 (Nematocarcinidae) is known from the deeper waters in the Indian Ocean sector of East Antarctica, the Weddell Sea, the Antarctic Peninsula, the Magellan region, the deep waters west of Chile, and off South Africa (Cape and Agulhas basin; Bate 1888; Barnard 1950; Kirkwood 1984; Ledoyer 1989; Guzmán and Quiroga 2005). Large numbers (up to 9 specimens per m^2) were recorded between 550 and 1,200 m depth in the Weddell Sea (Arntz and Gorny 1991; Gutt et al. 1991), and it has been found as deep as 4,000 m (Thatje et al. 2005a), indicating a broad bathymetric distribution range from the Antarctic continental slope to the Southern Ocean abyssal plains.

As marine Antarctic species with pelagic larvae (e.g. Caridea) or any kind of drifting stage are often regarded to have a circum-Antarctic distribution (e.g. Knox 1994; Clarke and Johnston 2003; Vermeeren et al. 2004), for both studied species a circumpolar distribution can be expected. This seems particularly likely as no obvious barriers divide the Antarctic continental shelf nowadays, and actually strong current systems, such as the strong Antarctic Circum Polar Current, facilitate the transport of pelagic distribution stages. However, several recent molecular studies demonstrated that species distributions are often much more restricted, and population differentiation is significantly higher than expected according to the physical characteristics of the habitat. For example, various molecular studies of sequence variation of Antarctic Teleostei (Patarnello et al. 2003; Kuhn and Gaffney 2006; Kuhn and Gaffney 2008), Euphausiacea (Zane et al. 1998; Zane and Patarnello 2000; Jarman and Nicol 2002; Jarman et al. 2002) or Cephalopoda (Allcock et al. 1997; Sands et al. 2003) revealed significant genetic differences in geographically separated populations. Hence, in the case of species with pelagic life stages it cannot be assumed a priori that they are broadly distributed and lack genetic differentiation, either due to a lower mobility than expected or characteristic behaviour such as swarming, affecting the spatio-temporal distribution.

In the present study, we assessed and evaluated the genetic structure of the decapod shrimps *Chorismus antarcticus* and *Nematocarcinus lanceopes* from various locations around Antarctica in order to test whether disruptive or unifying ecological and evolutionary forces affect both species' gene pools. Furthermore, we assessed whether populations of the shallow-water species *Chorismus antarcticus* compared to the deep-sea species *Nematocarcinus lanceopes* show reduced genetic diversity and stronger signatures of recent expansions following a past population bottleneck or founding event. This can be expected as a feasible consequence of recurring disturbances of the inhabited shelf areas by advancing grounded glaciers during the ice ages in the late Cenozoic, in particular during the Last Glacial period (Thatje et al. 2005b; Janko et al. 2007). We utilized two fragments of fast evolving mitochondrial genes, the cytochrome c oxidase subunit I (COI) gene and the large ribosomal subunit 16S rRNA gene, as well as the D2 expansion fragment of the nuclear 28S rRNA gene, a promising nuclear marker for molecular taxonomy and hybrid detection (Monteiro et al. 2000, Ninet et al. 2003; Schmidt et al. 2006; Sonnenberg et al. 2007), for all analysed specimens.

Materials and methods

Sampling and DNA extraction

All analysed decapod specimens were collected in the Southern Ocean (Fig. 1) during various expeditions in the years 1996 to 2008. Decapod shrimps were caught using a variety of trawling gear, for example epibenthic sledges, bottom trawls, Rauschert dredges, or Agassiz trawls in depths ranging from 166 to 2,134 m. A detailed sampling record and expedition references are provided in the electronic supplementary Table S1. After collection, samples

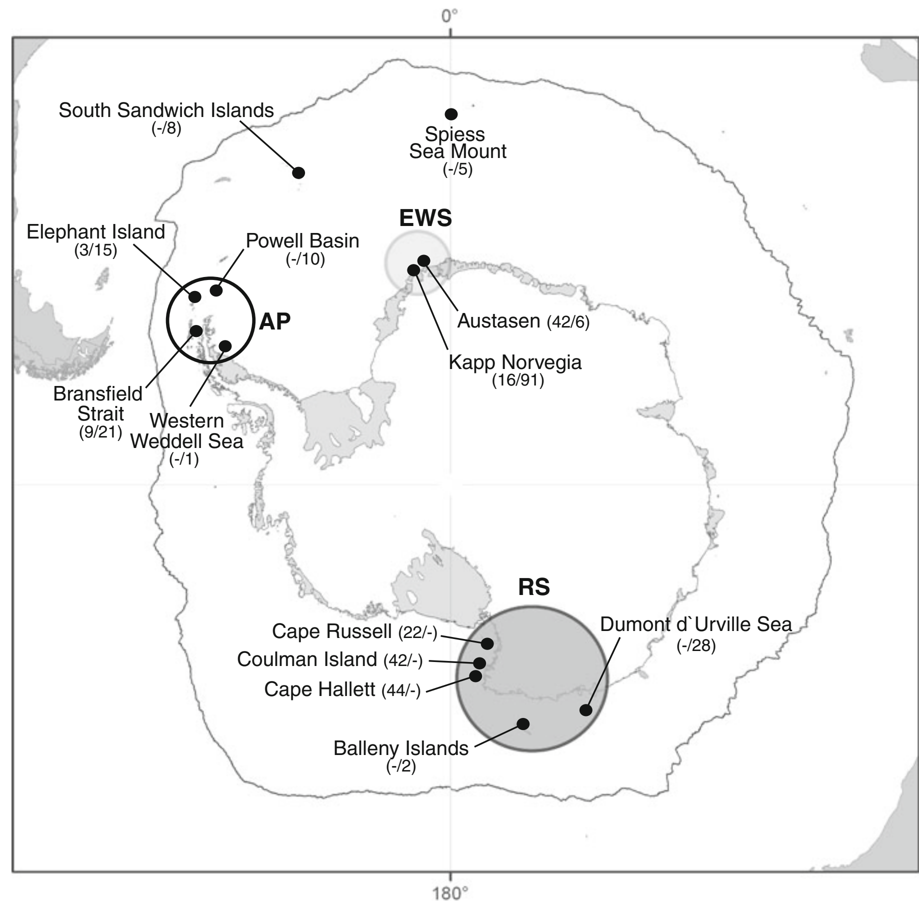

Fig. 1 Map of the Southern Ocean showing sample areas. *Numbers in parentheses* refer to the number of sampled specimens of *Chorismus antarcticus* (*left*) and *Nematocarcinus lanceopes* (*right*). The *black line* indicates the Antarctic Polar Front. *Circles* indicate three geographic groupings of populations (*AP* Antarctic Peninsula: transparent, *EWS* Eastern Weddell Sea: *light grey*; *RS* Ross Sea: *grey*) for the analysis of molecular variance (AMOVA). For *Nematocarcinus lanceopes*, Spiess Seamount and South Sandwich Islands have not been pooled in a geographic group because of their distinct location

were stored for at least 24 h at 0° C. Total genomic DNA was extracted from pleon muscle tissue of 178 specimens of *Chorismus antarcticus* and 187 individuals of *Nematocarcinus lanceopes*, using a commercial extraction kit (QIAmp© Blood and Tissue Kit, Qiagen GmbH) and following the tissue extraction protocol.

DNA amplification and sequencing

Amplification reactions for all gene fragments were carried out on a Progene Thermocycler (Techne Ltd.) or Thermal Cycler GeneAmp© PCR System 2700/2720 (Applied Biosystems) in 20 µl volumes, containing each 4 µl Q-Solution, 2 µl 10× Qiagen PCR buffer, 2 µl dNTPs (2 mmol/µl), 0.1 µl of each primer (both 50 pmol/µl), 1 µl of DNA template, and 0.2 µl Qiagen Taq (5 U/µl) and filled up to 20 µl with sterile H_2O. The PCR temperature profile for the mitochondrial COI fragment (~650 bp) using the primers LCO1490 and HCO2198 (Folmer et al. 1994) consisted of an initial denaturation at 94°C (5 min), followed by 38 cycles with 94°C (denaturation, 45 s), 44°C (annealing, 45 s), 72°C (extension, 80 s), and a final extension at 72°C (7 min). The amplification reactions and the temperature profile for the 16S rRNA gene were according to previous studies (Raupach and Wägele 2006). Approximately 200 bp of the 28S rDNA: D2 region was amplified with the newly designed forward primer CD2F (5′-GGACCCGTC TTGAAACAC-3′) and reverse primer CD2R (5′-GCATA GTTCACCATCTTTC-3′), using a PCR protocol of 94°C for 5 min (initial denaturation), 38 cycles with 94°C denaturation for 45 s, 52°C annealing for 45 s, and 72°C extension for 80 s, followed by a final 72°C extension for 7 min.

Negative and positive controls were included in every PCR setup. Three microlitres of amplified product (~0.7 kb) were controlled by electrophoresis on a 1% TAE agarose gel with ethidium bromide using DNA size standards, and the remaining PCR product was purified with a commercial kit (QIAquick© PCR Purification Kit, Qiagen GmbH). Purified PCR products were outsourced for sequencing to a contract sequencing facility (Macrogen, Seoul, Korea) on an ABI3730 XL automatic DNA sequencer, using the same primer set as for PCR. Both sequencing reads were assembled with the program Seqman™ II (DNASTAR, Inc.), while the identity of all new sequences was confirmed with BLAST searches (Altschul et al. 1990). All new sequences were deposited in GenBank (see electronic supplementary Table S2 for accession numbers).

Sequence alignment, haplotype genealogy, and population structure analysis

All sequences of each marker were aligned using Muscle version 3.6 (Edgar 2004) with default settings, generating three individual data sets. Alignments were tested for nucleotide bias using a chi-square test of base composition homogeneity across taxa implemented in PAUP*4.0b10 (Swofford 2002). All aligned COI sequences were translated to amino acid sequences to check for nuclear mitochondrial pseudogenes (numts) using BioEdit 7.0.9.0 (Hall 1999).

For the COI data sets, a statistical parsimony network was constructed with TCS 1.21 (Clement et al. 2000), using default settings (Fig. 2). Population structure analyses for both species were performed using Arlequin 3.11 (Schneider et al. 2000). Haplotype diversity (h) and nucleotide diversity (π; Nei and Miller 1990) were calculated for each population (Table 1). Pairwise F_{ST} (fixation index among populations) were calculated using both haplotype frequencies with and without genetic distances. To estimate differentiation among regions, an analysis of molecular variance (AMOVA) was performed when no geographical differentiation of population within regions was observed (Excoffier et al. 1992; Table 2). To this end, sampled areas were grouped into three geographic regions ("Antarctic Peninsula" vs. "Eastern Weddell Sea" vs. "Ross Sea") for both species (see Fig. 1). For *Nematocarcinus lanceopes*, specimens sampled from the South Sandwich Islands and Spiess Seamount (close to Bouvet Island) were not pooled to any of the other regions but remained as a fourth and fifth separate population for the AMOVA due to their distinct and remote geographic location. Covariance components were used to calculate fixation indices (F) among groups of populations (F_{CT}), among populations within groups (F_{SC}), or among populations (F_{ST}). Statistical significances of differences among the geographic regions were assessed by comparing the observed distribution with randomly permuted distributions (10,000 permutations), in which individuals were randomly reallocated to each population.

Population history

In order to test whether recent demographic processes have influenced the genetic diversity, we performed four tests to assess whether populations are in mutation-drift equilibrium. First, coalescent modelling of changes in effective population size were analysed using the program Fluctuate vers. 1.4 (Kuhner et al. 1998), which coestimates the parameters θ (the effective population size scaled by the mutation rate, i.e. $N_e\mu$) and the exponential rate of population growth or decline relative to the neutral mutation rate "g" for both species. Hence, positive values indicate growth, while negative values give evidence for a population decline. The initial value of θ was estimated using the approach of Watterson (1975). We tested several settings and used 1,000 short Markov chains with 200 generations, and two long Markov chains with 400,000 generations. Sampling increment was 20 for short and long chains. The initial Transition/Transversion ratio was set to 5.

In a second approach, we calculated mismatch distributions of pairwise differences between the specimens using Arlequin 3.11 (Schneider et al. 2000). For each species, the shape of the distribution was compared to a distribution of a simulated data set under a spatial expansion model (Ray et al. 2003; Excoffier 2004) and a sudden population expansion (Rogers and Harpending 1992). The fit between observed and estimated distribution under a sudden expansion model was subjected to two different goodness-of-fit tests (standardized squared differences (SSD) and raggedness index tests; Harpending 1994). The goodness-of-fit tests provide information on how well the simulated model of population expansion fits the observed mismatch distribution, while the parameters Tau (τ), Theta 0, and Theta 1 were inferred directly from the model of demographic expansion in Arlequin 3.11. Furthermore, departures from mutation-drift equilibrium were assessed by calculating Tajima's D (Tajima 1989) and Fu's F's (Fu 1997). Both neutrality tests provide information on possible population expansions or indicate recent selective sweeps. Significance for these two neutrality tests was obtained by simulating 10,000 samples in accordance with the assumptions of selective neutrality and population equilibrium using a coalescent approach.

To approximately date the timing of population expansions (T) for the COI gene according to the formula $T = \tau/2u$ following the approach of Rogers (1995) with τ being calculated from the mismatch distribution and u, i.e. for the substitution rate for the 657 bp fragment per year with a bias correction implemented by Excoffier and Schneider (1999), we used several molecular clock estimates. Since no molecular clock for the Antarctic shrimps exists, we used estimates from other decapods that range from 1.4 to 2.6% sequence divergence per MY (Knowlton et al. 1993; Knowlton and Weigt 1998; Schubart et al. 1998). As consequence, we used an average divergence rate of 2% per MY and calculated the lower (more recent) and upper (more ancient) confidence intervals using the divergence rates of 2.6 and 1.4% per MY and the 5 and 95% percentile values for τ, respectively.

Results

Our COI data analyses for 178 individuals of *Chorismus antarcticus* from 7 populations revealed 24 different

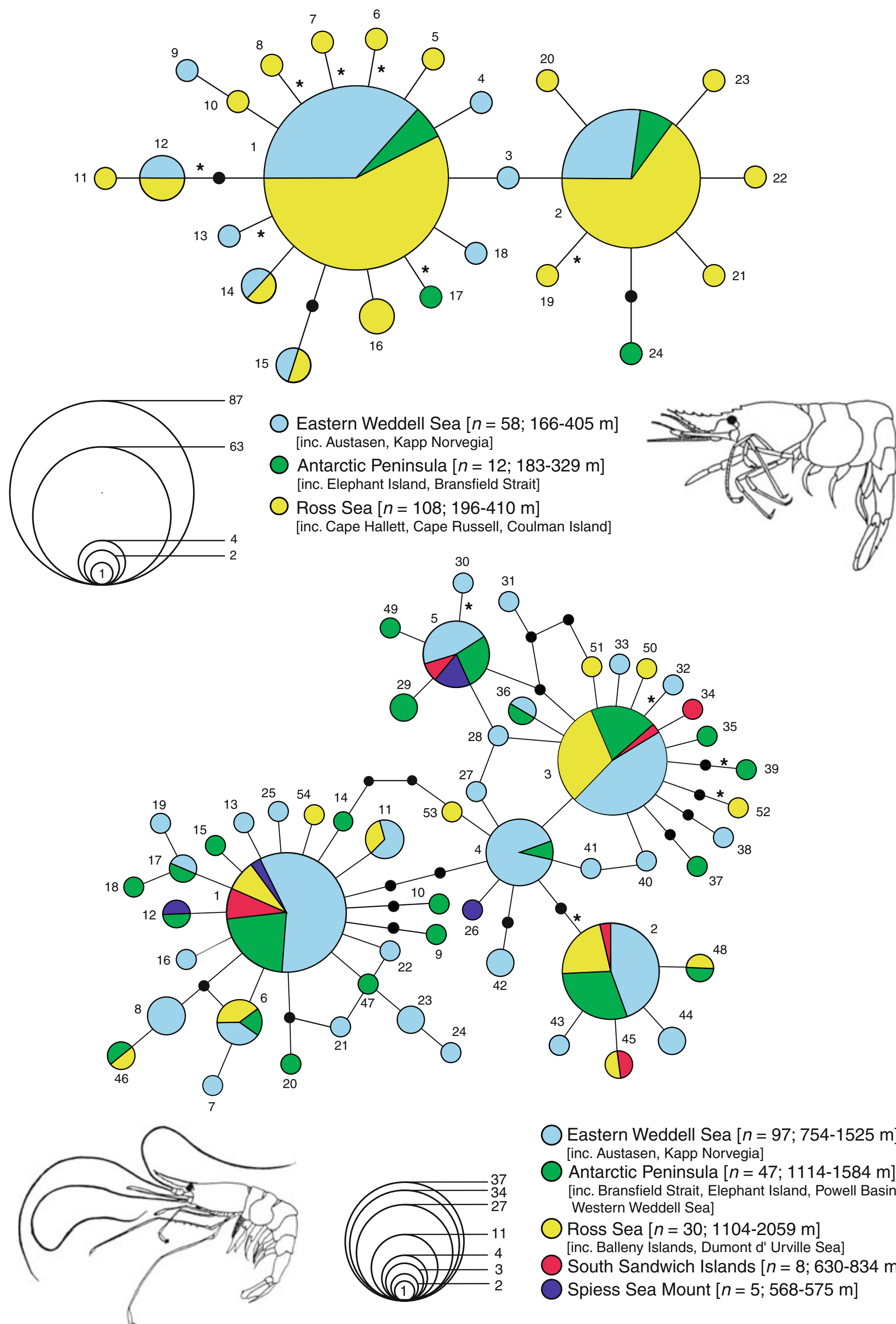

Fig. 2 Statistical parsimony network showing the mutational relationships among the analysed mitochondrial COI haplotypes of the shallow-water shrimp *Chorismus antarcticus* (*above*) and the deep-sea species *Nematocarcinus lanceopes* (*below*). *Each line* in the network represents a single mutational change, *small black dots* indicate missing haplotypes. The *colour* of the full circles of the network corresponds to the presented colour code of the sampled region (as in the AMOVA). The number of analysed specimens (n) and the depth range are listed [*in brackets*]. *Numbers at the full circles* of the networks indicate the haplotype code number (see Table 3 and S1). The diameter of the circles is proportional to the number of haplotypes sampled (see *Open circles with numbers*). *Asterisks* indicate amino acid replacement substitutions (colour figure online)

Table 1 Sampled regions, depth range, number of analysed specimens (*n*), and identified COI haplotypes of the studied populations of *Chorismus antarcticus* and *Nematocarcinus lanceopes*

Species	Sample area [Code]	Depth range [m]	*n*	Number of COI haplotypes	*h*	π
Chorismus antarcticus	Austasen [A]	166–405	42	8	0.626 ± 0.055	0.001916 ± 0.00137
	Bransfield Strait [BS]	291–329	9	3	0.6389 ± 0.1258	0.002368 ± 0.001764
	Cape Hallett [CH]	196	44	8	0.6385 ± 0.0447	0.002037 ± 0.001431
	Coulman Island [CI]	410	42	8	0.6028 ± 0.0637	0.001913 ± 0.001368
	Cape Russell [CR]	330	22	6	0.71 ± 0.0705	0.002524 ± 0.001721
	Elephant Island [EI]	183–277	3	2	0.6667 ± 0.3143	0.003044 ± 0.00287
	Kapp Norvegia [KN]	191–228	16	5	0.65 ± 0.1083	0.002169 ± 0.001564
	Total		178	24	0.6394 ± 0.0247	0.002092 ± 0.001434
Nematocarcinus lanceopes	Austasen [A]	1,488–1,525	6	5	0.9333 ± 0.1217	0.005277 ± 0.003609
	Balleny Islands [BI]	1,395	2	2	1 ± 0.5	0.00761 ± 0.008337
	Bransfield Strait [BS]	2,108–2,124	21	13	0.919 ± 0.0418	0.006451 ± 0.00372
	Dumont d'Urville Sea [DS]	1,104–2,059	28	12	0.9074 ± 0.0308	0.005182 ± 0.003043
	Elephant Island [EI]	1,144	15	11	0.9048 ± 0.0719	0.006059 ± 0.003599
	Kapp Norvegia [KN]	754–1,055	91	28	0.8979 ± 0.0175	0.005441 ± 0.00309
	Powell Basin [PB]	1,181–1,584	10	6	0.8929 ± 0.1113	0.006393 ± 0.00392
	Spiess Sea Mount [SM]	568–575	5	4	0.9 ± 0.161	0.006697 ± 0.004641
	South Sandwich Islands [SSI]	630–834	8	6	0.8929 ± 0.1113	0.00598 ± 0.003893
	Western Weddell Sea [WWS]	1,114–1,115	1	1	1	0
	Total		187	54	0.9025 ± 0.0119	0.005679 ± 0.003184

Statistical parameters indicate haplotypic diversity *h* (± SD) and nucleotide diversity π (± SD) observed for the COI fragment of the analysed populations

haplotypes with a length of 657 bp with no significant differences in base composition (Table 1). For *Nematocarcinus lanceopes*, a 657-bp fragment was obtained for 187 individuals from 10 different sampling locations. For this deep-sea shrimp species, 54 different haplotypes were found (Table 1). However, there were no intraspecific variations within the analysed mitochondrial 16S rDNA and the nuclear 28S rDNA: D2 sequences (*P*-distances = 0) for all specimens of both shrimp species. As consequence, population structure analyses were performed only for the COI data sets.

Variability of the COI gene fragment in *Chorismus antarcticus*

Of 25 polymorphic sites, four (16%) were at the first, three (12%) at the second, and 18 (72%) were at the third codon position. In total, seven amino acid replacement substitutions (first position: 2, second position: 3, and third position: 2) were detected. Uncorrected pairwise genetic distances (*p*-distances) among haplotypes of *Chorismus antarcticus* ranged from 0 to 0.0106. Two haplotypes (1 and 2) were present at high frequencies in most analysed populations, representing 48.3 and 35.9% of the entire data set (Fig. 2). Almost all other haplotypes were derived from these haplotypes, while haplotype 1 represented the putative ancestral haplotype, as most other haplotypes derived from this particular haplotype. Of the remaining 22 haplotypes, four were shared among populations, while the other 18 haplotypes were only scored in one specimen (singletons; Table 3). Overall haplotype diversity *h* was moderate (0.6394 ± 0.0247), while nucleotide diversity π values were low (0.0021 ± 0.0014; Table 1). Most haplotypes differed from each other by only one or two mutations, and there was no evidence for any significant differentiation among populations. Therefore, populations from the three major geographic regions were pooled and analysed in an AMOVA. Results of the AMOVA provided also no evidence for genetic differentiation with >98% of the variation being distributed within populations, supporting the hypothesis of panmixia of the analysed specimens ($F_{CT} = 0$, $F_{SC} = 0.015$, $F_{ST} = 0$; Table 2).

Population history of *Chorismus antarcticus* based on COI

Sequences of all specimens were pooled as a single group prior to analyses as we found no evidence for population structure. Estimates for the growth rate g were high and

Table 2 Results of AMOVA testing significance of population structure of *Chorismus antarcticus* and *Nematocarcinus lanceopes* based on the COI data sets, with fixation indices (*F*) among groups of populations (F_{CT}), among populations within groups (F_{SC}), or among populations (F_{ST}), and the statistical significance (*P*), based on conventional *F*-statistics

Species	Grouping	Source of variation	*df*	Sum of squares	Variance component	Percent of total	F_{CT}	F_{SC}	F_{ST}	*P*
Chorismus antarcticus	"Antarctic Peninsula" [BS, EI] vs. "Eastern Weddell Sea" [A, KN] vs. "Ross Sea" [CR,CI,CH]	Between regions	2	0.38	−0.0051	−1.50	−0.016			0.645
		Among stations within regions	4	1.74	0.0048	1.50		0.015		0.287
		Within stations	171	54.47	0.3185	100			−0.001	0.351
		Total	177	56.59	0.3182	100				
Nematocarcinus lanceopes	"Antarctic Peninsula" [BS, EI, PB, WWS] vs. "Eastern Weddell Sea" [A, KN] vs. "Ross Sea" [BI, DS] vs. South Sandwich Islands [SSI] vs. Spiess Seamount [SM]	Between regions	4	2.163	0.00161	0.36	0.0036			0.203
		Among stations within regions	12	5.744	0.00358	0.79		0.008		0.223
		Within stations	170	75.922	0.4466	98.85			0.011	0.145
		Total	186	83.829	0.45178	100				

positive, with the most likely value for $g = 8{,}212$ (SD = 187.9) and $\theta = 0.0538$ (SD = 0.0025), indicating population growth. Replicate runs with alternate random seeds and also different chain settings produced comparable results, ensuring convergence on the correct parameter estimates. Values for g varied in a range of about 3,500–9,000 depending on the number of chains used. Values of both Tajima's *D* and Fu's *F*'s were negative and significant (Table 4). These negative values indicated an excess of low frequency polymorphisms which is contrary to expected values under a neutral model of sequence evolution.

In line with these findings, distributions of the pairwise differences between sequence pairs showed a bimodal distribution as a result of the two very dominant haplotypes H1 and H2 separated by only two mutation steps (Fig. 2). Both models of expansion cannot be rejected on a 5% confidence level, and also neutrality tests showed significant deviations from mutation-drift equilibrium values (Table 4, Fig. 3). Molecular clock estimates dated the onset of the expansion to 85 KY BP (CI: 0–270 KY BP) for *Chorismus antarcticus*.

For COI, most replacement substitutions were located at the tips of the network (Fig. 2), indicating an expanding population. Furthermore, the low COI diversity values and the lack of any variation for the mitochondrial 16S rDNA and nuclear 28S rDNA D2 expansion segment indicated that genetic variability was low and not as expected from old viable populations at mutation-drift equilibrium. This genetic population characteristic can be interpreted as a current expansion after a severe past bottleneck but also as a result of a selective sweep.

Variability of the COI gene fragment in *Nematocarcinus lanceopes*

In total, 43 polymorphic sites were detected: four (9.3%) were first positions, one (2.3%) second position, and 38 (88.4%) third positions, defining 54 distinct haplotypes with no significant differences in base composition (Table 1). Five replacement substitutions (first position: 2, second position: 1, and third position: 2) were detected; all are located at the tips of the network. Pairwise nucleotide differences between sequences ranged from 0 to 0.0152. Three dominating haplotypes, haplotype 1 (19.8% of the entire data set), haplotype 2 (14.4%), and haplotype 3 (18.2%), were detected, with haplotype 1 representing the ancestral haplotype (Fig. 2). Of the remaining 51 haplotypes, 11 were shared among different populations (Table 3). The majority of haplotypes (36 = 66.7%) were singletons (Table 3). Haplotypic diversity *h* in total was high (0.9025 ± 0.0119), while nucleotide diversity π values were low (0.0057 ± 0.0032; Table 1). Exact test of differentiation among populations revealed significant differences among populations from Balleny Islands and Spiess Seamount for *F*-statistics ($P = 0.0256$). Since only two specimens from the Balleny Islands and only five shrimps

Table 3 Distribution of COI haplotypes of *Chorismus antarcticus* and *Nematocarcinus lanceopes* among sampling localities

Haplotype code	Austasen	Balleny Islands	Bransfield Strait	Cape Hallett	Coulman Island	Cape Russell	Dumont d' Urville Sea	Elephant Island	Kapp Norvegia	Powell Basin	Spiess Sea Mount	South Sandwich Islands	Western Weddell Sea	Total number
Chorismus antarcticus														
1	22		5	19	24	7			9					86
2	14		3	19	12	10		2	4					64
3	1													1
4									1					1
5					1									1
6				1										1
7					1									1
8					1									1
9	1													1
10				1										1
11					1									1
12	1					2			1					4
13	1													1
14	1					1								2
15				1					1					2
16				1	1									2
17								1						1
18	1													1
19					1									1
20						1								1
21				1										1
22				1										1
23						1								1
24			1											1
Nematocarcinus lanceopes														
1	1		5				3	1	20	3	1	3		37
2	2		4				6	1	10	3		1		27
3		1	2				9	5	16			1		34
4								1	10					11
5			1					1	5	1	2	1		11
6			1				2		2					5
7									1					1
8									3					3
9			1											1
10			1											1
11		1							2					3
12								1			1			2
13									1					1
14			1											1
15			1											1
16									1					1
17									1	1				2
18			1											1
19									1					1
20													1	1
21									1					1
22									1					1

Table 3 Distribution of COI haplotypes of *Chorismus antarcticus* and *Nematocarcinus lanceopes* among sampling localities

Haplotype code	Austasen	Balleny Islands	Bransfield Strait	Cape Hallett	Coulman Island	Cape Russell	Dumont d' Urville Sea	Elephant Island	Kapp Norvegia	Powell Basin	Spiess Sea Mount	South Sandwich Islands	Western Weddell Sea	Total number
23									2					2
24									1					1
25									1					1
26											1			1
27	1													1
28	1													1
29			1					1						2
30									1					1
31									1					1
32									1					1
33									1					1
34												1		1
35			1											1
36	1									1				2
37										1				1
38									1					1
39			1											1
40									1					1
41									1					1
42									2					2
43									1					1
44									2					2
45							1					1		2
46							1	1						2
47								1						1
48							1	1						2
49								1						1
50							1							1
51							1							1
52							1							1
53							1							1
54							1							1

from Spiess Seamount were sampled, this result can be neglected. For AMOVA, populations from the three major geographic regions were pooled (Fig. 1, Table 2); populations from the South Sandwich Islands and from Spiess Seamount were not pooled but kept as a separate region due to their distinct geographic.

The analysis of molecular variance revealed no significant partitioning of genetic variation within *Nematocarcinus lanceopes* when stations were grouped by geographic region ($F_{CT} = 0.0036$, $F_{SC} = 0.008$, $F_{ST} = 0.011$; Table 2), giving evidence of a homogeneous population. Furthermore, our data revealed no differences within all analysed mitochondrial 16S rDNA and nuclear 28S rDNA: D2 sequences (*P*-distances = 0).

Population history of *Nematocarcinus lanceopes* based on COI

Sequences of all specimens were pooled as a single group. Estimates for the growth rate g were high and positive, with the most likely value for $g = 3.783$ (SD = 134.1) and $\theta = 0.2386$ (SD = 0.0215), indicating strong and significant population growth. The estimate of the growth rate g was lower and θ higher in *Nematocarcinus lanceopes* compared to *Chorismus antarcticus*. As for the *Chorismus antarcticus* data set, replicate runs with alternate random seeds and different chain settings produced comparable results, ensuring convergence on the correct parameter estimates. Values for g varied in a range of about 1.500–4.000 depending on

Table 4 Genetic diversity and historical demography parameters inferred for the complete COI data sets of *Chorismus antarcticus* and *Nematocarcinus lanceopes*

	Chorismus antarcticus (shallow water)	*Nematocarcinus lanceopes* (deep sea)
Diversity indices		
Haplotype diversity	0.639 (SD ± 0.025)	0.901 (SD ± 0.012)
Nucleotide diversity	0.002 (SD ± 0.001)	0.0057 (SD ± 0.003)
Neutrality tests		
Tajima's *D*	−1.943**	−1.469*
Fu's *Fs*	−19.244**	−25.808**
Mismatch analysis		
$\text{Tau}_{\text{sudd. exp.}}$	2.271 (CI 0–4.943)	4.871 (CI 1.678–7.932)
$\text{Theta 0}_{\text{sudd exp}}$	0 (CI 0–0.257)	0.007 (CI 0–2.051)
$\text{Theta 1}_{\text{sudd. exp}}$	2.59 (CI 1.131–inf)	11.895 (CI 6.868–inf)
$\text{Tau}_{\text{dem. exp}}$	2.271 (CI 0.303–4.326)	4.871 (CI 2.252–7.267)
$\text{Theta 0}_{\text{dem. exp}}$	0 (CI 0–0.028)	0 (CI 0–1.371)
$\text{Theta 1}_{\text{dem. exp}}$	2.59 (CI 1.723–inf)	11.895 (CI 8.223–inf)
SSD	0.061 NS	0.003 NS
Raggedness index	0.222 NS	0.010 NS

For the mismatch analysis, estimates simulated under a sudden range expansion (sudd. exp.) and a demographic expansion (dem. exp.) are listed. SD indicates the standard deviation and CI indicates the 95% confidence interval ranges. Asterisks (*/**) indicate a significance level of <0.05/0.01, respectively, *NS* non-significant

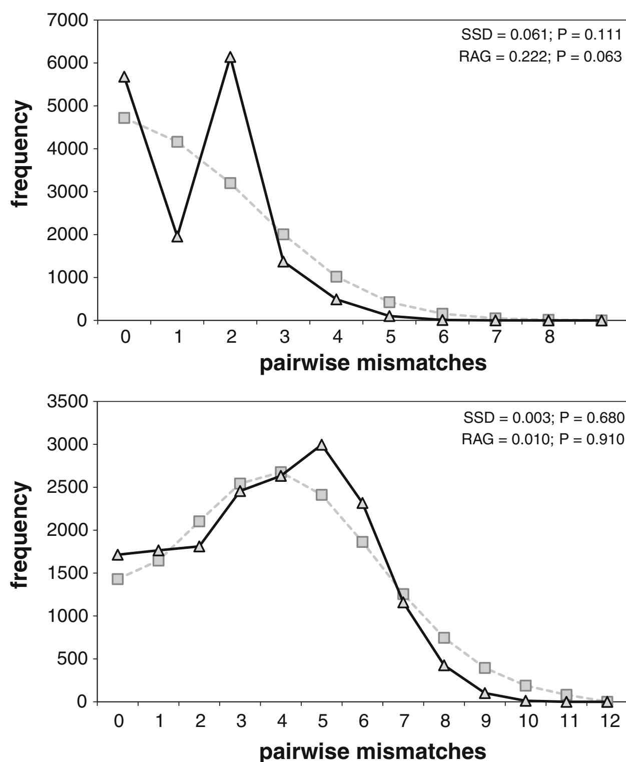

Fig. 3 Observed (*black line*) and simulated (*grey line*) mismatch distributions of pairwise haplotype differences of COI sequences between the specimens of *Chorismus antarcticus* (*above*) and *Nematocarcinus lanceopes* (*below*). For details see text

the number of chains used. Values of both Tajima's *D* and Fu's *F*'s were negative and significant; only *F*'s was highly significant (Table 4). Distributions of pairwise differences between sequence pairs showed a unimodal distribution that shifted further to the right when compared to *Chorismus antarcticus*, hinting at a population expansion that may date back even further in the past. The goodness-of-fit tests cannot reject the model of rapid population expansion (Fig. 3, Table 4). Molecular clock estimates dated the onset of the expansion to have started ~185 KY BP (CI: 20–540 KY). Similar to *Chorismus antarcticus*, mitochondrial and nuclear patterns strongly deviate from what would be expected from populations at mutation-drift equilibrium.

Discussion

All analysed data sets showed no evidence for cryptic species complexes within *Chorismus antarcticus* and *Nematocarcinus lanceopes*. The uncorrected pairwise nucleotide distances were low for both species (*Chorismus antarcticus*: 0–0.0106; *Nematocarcinus lanceopes*: 0–0.0152), representing typical intraspecific diversity values of crustaceans (e.g. Lefébure et al. 2006; Bucklin et al. 2007; Costa et al. 2007; Radulovici et al. 2009). Furthermore, there was no evidence for a geographical substructure of genetic variability or any considerable differentiation among any population pair. Although we cannot present an all-encompassing sampling scheme due to limited availability of Antarctic marine specimens, our molecular data provide convincing evidence for a homogeneous genetic structure of the analysed populations, i.e. the absence of significant geographical partitioning of haplotype frequencies. Hence, our results strongly support a circum-Antarctic distribution of *Chorismus antarcticus* and *Nematocarcinus lanceopes*.

In most cases, the assumption of circum-Antarctic species distribution is primarily based on morphological evidence only. However, restricted gene flow or even cryptic speciation within species with pelagic distribution stages has been successfully demonstrated for a variety of taxa, e.g. Antarctic Cephalopoda (Brierley et al. 1993; Allcock et al. 1997; Sands et al. 2003), Teleostei (Patarnello et al. 2003; Shaw et al. 2004; Kuhn and Gaffney 2006; 2008), and Euphausiacea (Patarnello et al. 1996; Zane et al. 1998; Jarman and Nicol 2002; Jarman et al. 2002). On the other hand, some molecular studies showed a lack of genetic differentiation within broadly distributed species with pelagic larval stages that inhabit the Southern Ocean. For example, a study analysing mitochondrial and intron DNA markers of the Antarctic toothfish *Dissostichus mawsoni* revealed low genetic variation (Smith and Gaffney 2005). Two fragments of mitochondrial DNA and additional microsatellites were used to investigate the stock structure of the Patagonian toothfish *Dissostichus eleginoides*, providing little evidence for population sub-structuring (Appleyard et al. 2002; 2004). Allozymes provided evidence for negligible polymorphisms in the icefish *Champsocephalus gunnari* (Duhamel et al. 1995), while RFLP analysis of whole mtDNA molecules showed moderate nucleotide diversity but little evidence of population subdivision (Williams et al. 1994). Another study using the partial mitochondrial control region (D-loop) revealed a weak population structure for the abundant Antarctic silverfish *Pleuragramma antarcticum* (Zane et al. 2006). For invertebrates, evidence for a homogeneous genetic structure of populations sampled south of the Antarctic Polar Front was also shown for the abundant nemertean ribbon worm *Parborlasia corrugatus* using two mitochondrial gene fragments (Thornhill et al. 2008) and the pycnogonid *Colossendeis megalonyx* from the Atlantic sector of the Southern Ocean (Krabbe et al. 2010). Finally, a remarkable proof of genetic homogeneity and strong evidence for recent post-glacial recolonization of Southern Ocean habitats has been demonstrated for the widespread Southern Bull Kelp *Durvillaea antarctica* (Fraser et al. 2009). In summary, these examples across a broad variety of different taxa show that no universal or predictable trend can be seen easily and empirical assessment of the genetic structure is crucial.

Within our study on the genetic structure of two abundant decapod shrimps using mitochondrial and nuclear DNA sequence data, we show that species with low genetic variation (for *Nematocarcinus lanceopes* only for 16S and 28S rDNA) and pelagic larvae or drifting distribution stages occur in Antarctic waters (Thatje et al. 2005b). Patterns of genetic diversity of both species strongly deviate from the expectations of large and stable populations and show signatures that may result from recent spatial and/or demographic expansion. Haplotype diversity h and nucleotide diversity π was higher for the analysed *Nematocarcinus lanceopes* specimens ($n = 54$, $h = 0.9025 \pm 0.0119$, $\pi = 0.0057 \pm 0.0032$) than for *Chorismus antarcticus* ($n = 24$, $h = 0.6394 \pm 0.0247$, $\pi = 0.0021 \pm 0.0014$; Table 1). In general, genetic variation within a species should be positively correlated with the effective population size. Thus, the observed low mtDNA variation within *Chorismus antarcticus* and the star-like haplotype network give evidence for a smaller, non-equilibrium population when compared to *Nematocarcinus lanceopes* and in agreement with a population under expansion following a past bottleneck that eliminated polymorphisms (Nei et al. 1975). This is supported by results of Tajima's *D* and the coalescent modelling of population growth rates using Fluctuate, which provide lower estimates for Theta but higher values for the growth rates in *Chorismus antarcticus* when compared to *Nematocarcinus lanceopes*. However, absolute values of the growth rates cannot be compared as the algorithm is slightly biased upwards (Kuhner et al. 1998). The results of the mismatch distribution statistics provide somewhat stronger evidence for a spatial expansion than for a pure demographic expansion according to our analyses (not shown). Nevertheless, this should be regarded as first evidence only as a solid statistical framework for these tests is still not available (see Arlequin manual).

For *Chorismus antarcticus*, the time since population expansion was estimated to be ~85 KY BP (CI: 0–270 KY BP), for *Nematocarcinus lanceopes* 185 KY BP (CI: 20–540 KY BP). Although molecular clock estimates should be regarded with caution when no strong calibration points exist, our data provide evidence that *Chorismus antarcticus* may have been experienced a more recent expansion compared to *Nematocarcinus lanceopes*. The averaged onset estimates calculated with a divergence rate of 2% nucleotide divergence for both species predate the onset of the last deglaciation of the Antarctic shelf. The lower confidence intervals, however, fall within this range. Evidence that values in the lower CI range, i.e. more recent estimates, may reflect better the true demographic scenario, stems from the lack of any variability at the 16S and 28S rRNA genes. Held (2001) demonstrated that mutation rates in the Antarctic could in principle be of similar value compared to non-Antarctic crustaceans. Therefore, the strong deviations of mutation-drift equilibrium conditions in our data support late Pleistocene population expansions following a bottleneck or a founding event.

For *Chorismus antarcticus*, the more recent estimates for the expansion event suggest a post-glacial expansion after the Last Glacial Maximum of survivors of these species around Antarctica. From the molecular clock data available, it cannot be excluded that the pattern of population expansion predates the Last Glacial Maximum (CI: 0–270

KY BP). The impact of this most recent large-scale glaciation makes this event a good explanation for the data observed. Modelling data suggest that grounded ice masses reached to the continental shelf edge around Antarctica and left no permanent glacial refuges at the Last Glacial Maximum (Huybrechts 2002). On the other hand, both glacial extent and retreat across the continental shelf may have been time transgressive leaving temporary shelters at different locations for benthic organisms (Thatje et al. 2005a). Also, recent geophysical surveys add support to the general small-scale availability of few small-scale shelf refugia (Anderson et al. 2002). Recent molecular genetic data for few benthic, direct-developing invertebrates that lack dispersive distribution stages support the concept of survival on the continental shelf during glacial maxima (Leese 2008; Wilson et al. 2009). Also, due to time transgressive ice extent and retreat patterns in glacial periods, temporarily geographical isolated refugia for benthic organisms may have existed on the continental shelf, allowing species to migrate from one shelter to another (see Thatje et al. 2005b; 2008). However, for shallow-water organisms with planktotrophic larval stages, such as *Chorismus antarcticus*, it may seem more plausible that they have survived the last ice age in shallow waters of sub-Antarctic islands or Patagonia (but see Thatje et al. 2008) and re-colonized the Antarctic continental shelf during interglacial periods. The strongly reduced or even absent molecular diversity and the much higher population growth rates inferred using the coalescent modelling approach of Fluctuate agree with such a re-colonization and spatial expansion scenario of the Antarctic shelf, possibly from one or very few refugia (Thatje et al. 2008).

In the case of the deep-sea shrimp *Nematocarcinus lanceopes* it is more likely that earlier events influenced the demography of these species, obviously independent from glacial periods (see Thatje et al. 2008). *Nematocarcinus lanceopes* basically shows a deep-sea distribution in the Southern Ocean down to about 4,000 m water depth (Thatje et al. 2005a), and only emerges on the Antarctic Continental Slope to about 550 m water depth (Gutt et al. 1991). Based on this distribution pattern, populations of this deep-sea shrimp should have been less affected by the advance of grounded ice sheets across the Antarctic Continental Shelf during glacial periods due to their potential to evade into the deep sea. Hence, they can have maintained a greater diversity when compared to shallow-water species, which is consistent with the findings presented here. These results coincide with other examples of moderate to high genetic variability, which have been observed within various other deep-sea decapod crustaceans (e.g. Shank et al. 1999; Weinberg et al. 2003) or fish (Hoarau and Borsa 2000; Kojima et al. 2001; Danielsdottir et al. 2008). However, as in *Chorismus antarcticus*, there is also evidence for non-equilibrium as indicated from the neutrality test, the mismatch distribution and the obvious lack of differentiation in the mitochondrial 16S and nuclear 28S rDNA marker. Although in comparison with *Chorismus antarcticus*, the unimodal distribution of pairwise mismatches in *Nematocarcinus lanceopes* is shifted to the right being indicative for a bottleneck that predates the Last Glacial Maximum. Thus, our results can provide evidence that in recent history, populations of *Nematocarcinus lanceopes* were less affected in their genetic diversity, which is consistent with a scenario of recent and recurrent glaciations of the continental shelf that should generally have affected benthic shallow-water shelf species far more than pelagic species (Janko et al. 2007) or primarily deep-sea distributed species. Nevertheless, the basic patterns of mutation-drift disequilibrium are prevailing and obvious. Although we cannot completely rule out the possibility of selective sweeps being responsible for the reduced 16S and 28S rDNA variability, the consistency of both markers and the COI data can explain recent colonization events and expansions of both species around Antarctica quite well.

Additional markers should be tested to understand historical processes and microevolutionary forces leading to the population structure of both analysed species in more detail. Molecular markers with a greater resolution on population level in particular independent SNP sets or microsatellites rather than single locus coding genes alone can reveal more insights into genetic variability and gene flow through migration and dispersal (Ballard and Whitlock 2004; Ballard and Rand 2005) and should be employed in particular for studying population genetics in an Antarctic context (Held and Leese 2007). Additional specimens from other regions of the Southern Ocean (e.g. Davis Sea or Amundsen Sea) are also needed to further verify a circum-Antarctic distribution.

In conclusion, our data provide important first insights into the population structure of two highly abundant species of Antarctic decapod shrimps that constitute significant faunal elements of the Antarctic benthos. The analysed mitochondrial and nuclear data sets reveal genetic homogeneity among populations with the dominance of a few haplotypes and a possible circum-Antarctic distribution of both analysed decapod shrimp species. We interpret the signatures of a population bottleneck dated to the late Pleistocene that in particular for shallow-water species such as *Chorismus antarcticus* the Last Glacial period with large-scale glaciations of shelf habitats may have had a severe impact and possibly have expelled populations temporarily from the High Antarctic Continental shelf. This effect is less prominent for the deep-sea species *Nematocarcinus lanceopes*, although the non-equilibrium in the genetic data is indicative also for an impact that may, however, predate the Last Glacial Maximum.

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