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ORIGINAL PAPER

Low-mitochondrial diversity and lack of structure in the velvet swimming crab Necora puber along the Galician coast

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Abstract The velvet swimming crab *Necora puber* is a common species along the European Atlantic coasts. Due to its increasing commercial importance, many studies have been carried out to characterize its growth and reproduction, but no genetic assessment has ever been attempted at the population level. Here, we describe the genetic diversity and population structure of *N. puber* in northwestern Spain (Galicia), including additional samples from France, Portugal, and southern Spain. To do so, we analysed two mitochondrial fragments of the COI and 16S genes in 217 individuals collected from ten localities. Our results unveil low-genetic diversity and weak population structure along the studied range. A range expansion after the last glacial maximum, followed by ongoing gene flow, seems to be the most likely explanation for the observed genetic pattern.

Introduction

The velvet swimming crab *Necora puber* (Linnaeus, 1767) is a common portunid in western Europe. It is found in the northeast Atlantic from Norway to Morocco; and in the western Mediterranean along Spanish, French, and Adriatic coasts. It lives on rocky shores from shallow subtidal to 70 m (Hearn 2004), where it is one of the dominant epibenthic predators (Freire and González-Gurriarán 1995). This crab is of particular importance in terms of commercial fisheries, and several studies have focussed on basic biological aspects such as growth and reproduction patterns throughout its range. These traits depend on water temperature, and therefore show a latitudinal gradient: toward the south of the distribution, velvet crabs grow faster, reach higher sizes, attain sexual maturity earlier, and show higher fecundity (Hearn 2002). Accordingly, minimum landing sizes and closed seasons have been established in each geographic region, in order to regulate its exploitation. As with other crustaceans, the life cycle is complex. Fertilization is internal, it occurs in the female's oviduct just before spawning. At the southernmost localities analysed (northwestern Spain, Galicia) females spawn twice a year, in January and March, with a mean clutch size of 200,000 eggs. These are incubated under the female's abdomen and hatching takes place a month and a half later. Larvae spend around 2 months in the water column, zoeas develop offshore, and megalopae return onshore to settle and metamorphose. Juveniles appear in intertidal areas in early autumn, where they remain for 1 year until they suffer the pubertal moult and reach sexual maturity. Adults are active swimmers and probably perform reproductive migrations to deeper habitats, but females move again to shallow and softer substrates to spawn, beginning a new cycle. Adults can live up to 4 years, although second and third year classes are the most frequent (González-Gurriarán 1985b; Lee et al. 2005).

The main fishing area for *N. puber* is the United Kingdom (UK), especially Scotland, where it has become the second most exploited crab after *Cancer pagurus* (Lee et al. 2006). The catches have been growing since 1984, when this fishery began to develop to supply Spanish markets. In Spain, the velvet crab is a highly relished seafood, and the local stocks suffered a decline in the 1980s due to overexploitation (González-Gurriarán 1985a). Nowadays, 90% of

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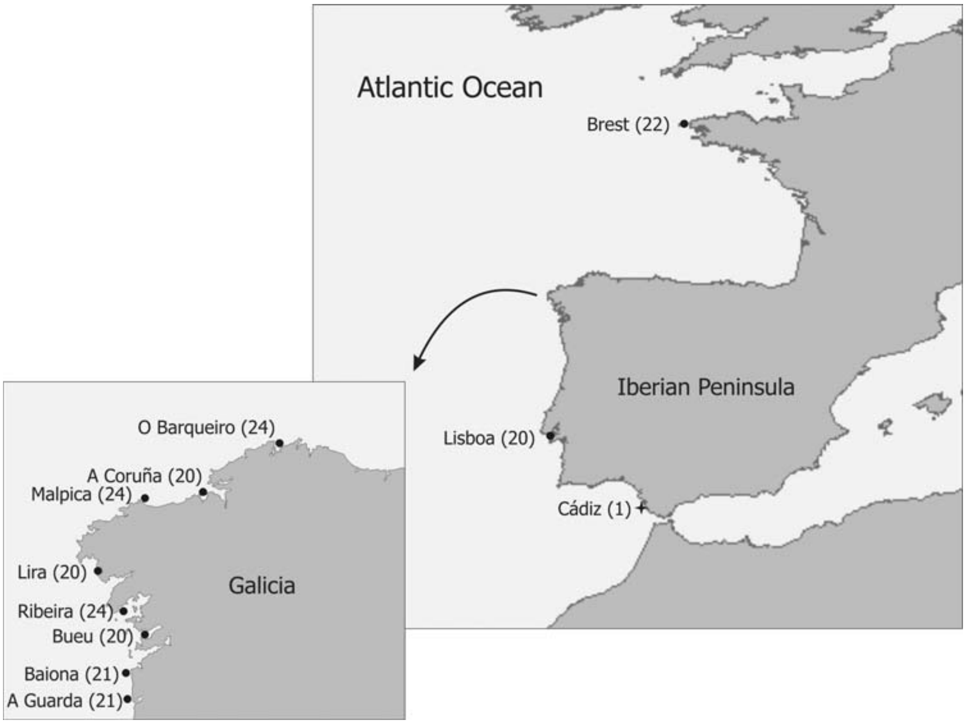

Fig. 1 Map of sampled localities. Sample size is indicated within *parentheses*. Additionally, a single individual from Cádiz was also sequenced but was not included in the analyses

the product commercialized in Spain comes from the UK and only a 10% belongs to local extractions. Despite this reduction, there is still an important artisanal Spanish fishery operating on velvet crabs, mainly in the northwestern region (Galicia), and that is very relevant for the local economy.

Indeed, efforts should be made to preserve this resource through a more effective management, starting with a detailed characterization of the unit to be managed. Stock assessment requires a multidisciplinary approach that considers both environmental and species' biological characteristics (Ward 2000). A common definition of stock is that proposed by Ihssen et al. (1981): "an intraspecific group of randomly mating individuals with temporal and spatial integrity". In the marine realm, this integrity should be related to the availability of suitable habitats (e.g., temperature, salinity, substrate, food) and to the oceanographic circulation patterns connecting them (e.g., direction and strength of currents and eddies). These factors should determine population structure in conjunction with the species' ecology (ethology, life cycle, dispersal capacity) (Quinteiro et al. 2007). In fact, independent studies should be carried out on each species at each particular region in order to develop effective management strategies (Thorpe et al. 2000; Ward 2000).

Genetic analyses are very useful describing population structure and dynamics, and can help to understand when/whether populations conform to a random mating unit (Thorpe et al. 2000; Ward 2000). So far, only a few interspecific genetic studies (allozymes) included *N. puber* (Mantovani et al. 1992; Passamonti et al. 1997), and the population structure of this crab remains mostly unknown. To complement the extensive ecological data compiled across the northeastern Atlantic (revised in Hearn 2002), we present the first intraspecific phylogeographic study of the velvet swimming crab. Specifically, we analysed the variation at two mitochondrial DNA fragments of *N. puber* along the northwestern Spanish coast in order to describe the genetic diversity and structure of these populations.

Materials and methods

Sampling

We analysed a total of 217 individuals, mostly sampled at eight Galician localities (northwestern Spain) (Fig. 1). Also, we obtained two samples from France and Portugal, plus one individual from Cádiz (Andalucía, southern Spain). Samples were collected between 2005 and 2008 by local fishermen and sent alive to the lab, where muscle tissue from walking legs was conserved in pure ethanol. DNA extraction was performed with the Genomic DNA from tissue kit (Macherey-Nagel) following manufacturer's instructions.

Mitochondrial DNA sequencing

We amplified two mitochondrial fragments: 709 bp of COI gene using LCO1490 and HCO2198 primers (Folmer et al.

1994); and 674 bp of 16S gene using 16L29 and 16HLeu (Schubart 2009). PCR reactions were carried out in a final volume of 20 µl, containing 1 µl of DNA extraction, 2 µl of 10× PCR buffer [160 mM $(NH_4)_2SO_4$, 670 mM Tris–HCl pH 8.8, 0.1% Tween 20], 1 µl of 50 mM $MgCl_2$, 1 µl of 0.1% BSA (Amersham Life Science), 1 µl of 10 mM dNTP Mix (Applied Biosystems), 0.5 µl of each primer (20 µM), 0.2 µl BIOTAQ polymerase (5 U/µl, Bioline) and 13 µl of sterile bidistilled water. PCR profiles were as follows: 5 min at 95°C, 35 cycles of 20 s at 95°C, 20 s at 42°C, 20 s at 72°C, and 7 min at 72°C for COI; and 5 min at 95°C, 35 cycles of 20 s at 95°C, 20 s at 55°C, 30 s at 72°C, and 7 min at 72°C for 16S. For verification, PCR products were run in 2% agarose gels stained with ethidium bromide. Both fragments were sequenced for all individuals using the forward primer, and some samples were also sequenced using the reverse primers to check for consistency. PCR products were purified with the PCR clean-up and gel extraction kit (Macherey-Nagel). Sequences were performed with BigDye v1.1 chemistry (Applied Biosystems), precipitated with ethanol and run in an ABI PRISM 310 (Applied Biosystems). Electropherograms were visualized with BioEdit (Hall 1999). Sequences were aligned with ClustalW (Thompson et al. 1994) and revised by eye.

Data analysis

For the most part, the COI and 16S datasets were treated independently, but for some analyses they were combined. Substitution models were selected under the Akaike information criterion (AIC, Akaike 1974) using PAUP*b4.10 (Swofford 2003) and Modeltest v3.6 (Posada and Crandall 1998).

A number of genetic diversity indexes were calculated for each locality with Arlequin v3.01 (Excoffier et al. 2005): number of haplotypes (h), number of segregating sites (S), haplotype diversity (hd), nucleotide diversity (π), and the population mutation parameter from the number of segregating sites (θ_S) (Watterson 1975).

Demographic changes within populations were tested with Fu's F_S (Fu 1997), and R_2 (Ramos-Onsins and Rozas 2002) statistics using DnaSP v4.50 (Rozas et al. 2003). The 95% confidence intervals were obtained by coalescent simulation (1,000 replicates) conditioned on the nucleotide diversity (θ_π). We estimated the current effective population size (N_e) through the expression $N_e = \theta_\pi/\mu$, where μ is the substitution rate per site per generation, assuming that mtDNA in this species shows only maternal inheritance and that the sex ratio is 1:1. We considered a mean substitution rate of 1 and 0.7% per lineage per million years for COI (Ketmaier et al. 2003) and 16S (Schubart et al. 2000), respectively; and a mean generation time of 2 years.

Pairwise F_{ST} values were estimated with Arlequin v3.01, taking into account the haplotype frequencies and their nucleotide distances under the best-fit model of nucleotide substitution. The null distribution of the F_{ST}'s was obtained by bootstrapping (1,000 replicates), and the corresponding P-values were corrected for multiple tests with the modified false discovery rate (FDR) procedure described in Narum (2006). Geographical distances between populations were calculated with ArcGIS (ESRI), following the coastline. To detect any association between the genetic and the geographical distances, Mantel tests (Mantel 1967) were performed with 10,000 permutations using PopTools (available at http://www.cse.csiro.au/poptools/).

Population structure was also investigated using SAMOVA v1.0 (Dupanloup et al. 2002). This approach combines both genetic and geographic information in an annealing procedure that clusters adjacent populations in a way that maximizes the proportion of genetic variance due to differences between groups. Each run consisted of 1,000 annealing replicates and the number of groups tested ranged from 2 to 5.

Phylogenetic networks representing haplotype relationships were constructed with TCS v1.21 for each fragment (Clement et al. 2000). In order to separate population structure and history, the nested clade phylogeographic analysis (NCPA) (Templeton et al. 1995) was also implemented. Before building the nesting design, ambiguities in the network were resolved as described in Pfenninger and Posada (2002). NCPA statistics and their statistical significance were calculated with the program Geodis v2.5 (Posada et al. 2000) with 10,000 replicates. A revised version (15 December 2008) of the inference key (Templeton 2004), available at http://darwin.uvigo.es/software/geodis.html, was used to infer the processes likely responsible for the observed NCPA statistics.

Results

Sequence ambiguities

We identified double peaks in the electropherograms of COI (in the same seven positions in 13 individuals) and 16S (in the same two positions in 18 individuals). Noticeably, 12 of these individuals presented double peaks in both fragments; and nine of these 12 individuals were found in the same locality, Ribeira (Tables 1, 2). DNA from these individuals was extracted de novo and sequenced in both directions, but the ambiguities remained. The nine ambiguous positions were practically invariable in the remaining individuals, and two of them were nonsynonymous for COI. We performed all the population analyses twice, for the full dataset with the ambiguities coded according to the IUPAC, and for a smaller dataset in which all individuals presenting ambiguities were removed. The results of both

Table 1 Absolute frequency of COI haplotypes across localities

COI haplotypes	Brest	O Barqueiro	A Coruña	Malpica	Lira	Ribeira	Bueu	Baiona	A Guarda	Lisboa	Cádiz	Total
h1	18	18	16	15	14	11	13	18	17	15		155
h2	3	1	2	2	1	1	2			1		13
h3	1											1
h4		1										1
h5		1										1
h6		1										1
h7		1										1
h8		1										1
h9			1	1	1				1			4
h10			1									1
h11				1								1
h12				1	2		1		1	1		6
h13				1		2		2	1	1		7
h14				1						1		2
h15					1							1
h16					1		1					2
h17						1						1
h18[a]						1						1
h19							1					1
h20							1					1
h21							1					1
h22								1				1
h23										1		1
h24[b]				2		8			1		1	12
Total	22	24	20	24	20	24	20	21	21	20	1	217

[a] h18 differs from h1 by seven ambiguities and one substitution

[b] h24 differs from h1 by seven ambiguities

analyses were largely concordant. Hereafter we will refer to the full dataset unless noticed.

Genetic variation

The final COI and 16S alignments consisted of 658 and 609 bp, respectively. The best-fit models of nucleotide substitution were HKY (Hasegawa et al. 1985) for COI and TrN (Tamura and Nei 1993) for 16S. A total of 24 COI haplotypes (GenBank accession numbers xxx–xxx) were defined by 27 variable sites (22 haplotypes and 20 variable sites when excluding individuals with ambiguities), with five nonsynonymous changes (three excluding the two ambiguous positions mentioned above). For 16S, 17 haplotypes (GenBank accession numbers xxx–xxx) and 15 variable sites were identified (14 haplotypes and 13 variable sites after excluding ambiguous individuals). COI haplotype 1 (frequency = 0.71), and 16S haplotypes 2 and 3 (frequency = 0.62 and 0.21, respectively) were uniformly distributed along the geographical range (Tables 1, 2). For both fragments, most haplotypes were singletons, and all haplotypes were closely related, with a mean difference of one and a maximum of four (Fig. 2), resulting in moderate levels of haplotype diversity and low levels of nucleotide variability for both loci across all localities (hd = 0.3–0.7, $\pi \leq 0.001$) (Table 3).

Demographics

The estimated N_e ranged from 21,000 to 52,400 for COI, and from 39,500 to 117,300 for 16S (Table 3). According to the F_S statistics, most of the populations did not fit a model of constant size following the COI data, while for 16S the constant size model was only rejected in one population (Table 4). The R_2 test was more conservative in general. For the combined dataset the constant size model was rejected for most populations. It was also rejected when all samples were pooled (except for R_2 when ambiguities were included).

Table 2 Absolute frequency of 16S haplotypes across localities

16S haplotypes	Brest	O Barqueiro	A Coruña	Malpica	Lira	Ribeira	Bueu	Baiona	A Guarda	Lisboa	Cádiz	Total
h1	6											6
h2	10	17	15	17	13	12	16	11	13	11		135
h3	4	3	3	2	6	2	4	7	7	7		45
h4	1											1
h5	1											1
h6		1								1		2
h7		1										1
h8		1										1
h9		1										1
h10			1						1			2
h11				1								1
h12				1								1
h13						1						1
h14[a]						1						1
h15										1		1
h16[b]			1	2	1	7		1			1	13
h17[b]				1		1		2				4
Total	22	24	20	24	20	24	20	21	21	20	1	217

[a] h14 only differs from h2 by two ambiguities and one substitution

[b] h16 differs from h2 by two ambiguities as well as h17 from h3

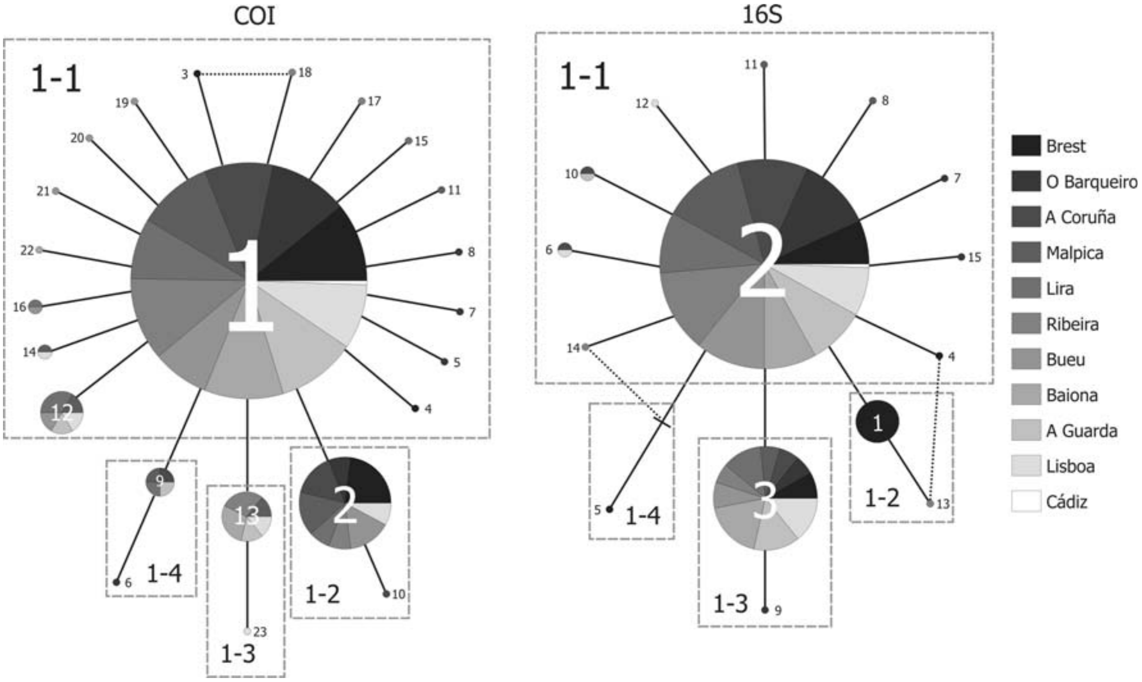

Fig. 2 Phylogenetic network and nested design for the COI and 16S haplotypes. *Dashed lines* connecting haplotypes represent loops solved before constructing the cladogram. A *cross-bar* on the *right* diagram represents a missing or unsampled haplotype. *Circle sizes* represent haplotype frequency, but are not completely proportional in order to maintain readability

Population differentiation

Differentiation between populations was in general very low, especially for COI (Table 5); and it was significantly correlated between the two markers ($r = 0.43$, P-value = 0.004; and $r = 0.30$, P-value = 0.048 when ambiguities were excluded). There were no significant F_{ST} values, with the exception of the comparison between Brest and Baiona

Table 3 Genetic variability and effective population sizes across localities for COI and 16S datasets

Locality	n	COI dataset						16S dataset					
		S	h	hd	π	θ_S	N_e	S	h	hd	π	θ_S	N_e
Brest	22	2	3	0.3247	0.0005	0.0008	25,650	5	5	0.7143	0.0016	0.0023	117,286
O Barqueiro	24	7	7	0.4457	0.0009	0.0028	44,350	5	6	0.4964	0.0010	0.0022	73,071
A Coruña	20	3	4	0.3632	0.0007	0.0013	35,600	2	4	0.4316	0.0006	0.0009	43,214
Malpica	24	6	8	0.6123	0.0009	0.0024	43,800	3	6	0.5000	0.0006	0.0013	46,286
Lira	20	5	6	0.5158	0.0009	0.0021	44,800	1	3	0.5105	0.0007	0.0005	51,857
Ribeira	24	4	6	0.6957	0.0006	0.0016	31,100	4	6	0.6812	0.0008	0.0018	56,071
Bueu	20	6	7	0.5842	0.0010	0.0026	52,400	1	2	0.3368	0.0006	0.0005	39,500
Baiona	21	2	3	0.2667	0.0004	0.0008	21,000	1	4	0.6333	0.0008	0.0005	60,286
A Guarda	21	3	5	0.3524	0.0004	0.0013	21,700	2	3	0.5286	0.0009	0.0009	65,929
Lisboa	20	5	6	0.4474	0.0009	0.0021	44,800	3	4	0.6000	0.0011	0.0014	79,643
TOTAL	217	20	24	0.4827	0.0007	0.0051	35,850	13	17	0.5675	0.0009	0.0036	64,429

The table displays the number of segregating sites (S), number of haplotypes (h), haplotype diversity (*hd*), nucleotide diversity (π), genetic diversity (θ_S) and effective population size (N_e)

Table 4 F_S and R_2 tests for COI, 16S and combined datasets

Locality	COI dataset		16S dataset		Combined dataset	
	F_S	R_2	F_S	R_2	F_S	R_2
Brest	−0.8698	0.1210	−0.9925	0.1168	−2.2983	0.1076
O Barqueiro	**−5.3476**	**0.0769**	**−3.5017**	0.0800	**−10.3026**	**0.0495**
A Coruña	−1.7129	0.1096	−0.7746	0.1280	**−2.8227**	**0.0893**
Malpica	**−5.4028**	**0.0690**	−1.9364	0.0997	**−7.9714**	**0.0549**
Lira	**−4.0149**	**0.0822**	1.1690	0.2211	**−3.4294**	**0.0849**
Ribeira	**−3.3438**	0.0850	−1.4990	0.1134	**−5.0593**	**0.0694**
Bueu	**−5.0483**	**0.0739**	0.7208	0.1684	**−6.5741**	**0.0724**
Baiona	−1.2590	0.1157	1.4737	0.2571	−0.4937	0.1359
A Guarda	**−2.8198**	0.1166	0.0449	0.1580	**−2.6974**	0.0914
Lisboa	**−4.0149**	**0.0822**	−0.8819	0.1280	**−3.9662**	**0.0812**
Total	**−32.7911**	0.0151	**−14.6014**	0.0233	**−57.2025**	**0.0153**

Significant values (P-value <0.05) after the FDR correction are indicated in bold

Table 5 Pairwise F_{ST} values between populations based on COI (upper diagonal) and 16S (lower diagonal)

	Brest	O Barqueiro	A Coruña	Malpica	Lira	Ribeira	Bueu	Baiona	A Guarda	Lisboa
Brest		0.0025	0	0	0.0113	0	0	0.0544	0.0335	0.0113
O Barqueiro	0.0614		0	0	0	0.0016	0	0.0084	0	0
A Coruña	0.0639	0		0	0.0022	0.0172	0	0.0531	0.0295	0.0114
Malpica	0.0695	0	0		0	0	0	0	0	0
Lira	0.0721	0	0.0115	0.0347		0.0123	0	0.0235	0	0
Ribeira	0.0183	0	0	0	0.0313		0.0048	0	0	0
Bueu	0.0651	0	0	0	0	0		0.0214	0.0022	0
Baiona	0.1157	0.0807	0.1167	0.1436	0	0.1325	0.0684		0	0
A Guarda	0.0809	0.0174	0.0226	0.0561	0	0.0518	0	0		0
Lisboa	0.0799	0.0177	0.0390	0.0628	0	0.0583	0.0040	0	0	

There were not significant values after the FDR correction

in the case of the combined dataset. Moreover, the Mantel tests did not reveal any significant association between the F_{ST}'s and the coastline distances between populations. However, this test was marginally significant for the combined dataset (P-value = 0.029), and when we excluded the individuals with ambiguities from the 16S dataset (P-value = 0.025). Remarkably, in both cases significance disappeared after removing Brest, the northernmost locality.

The SAMOVA results also reflected a lack of population structure. Independently of the number of clusters tested, more than 99% of the COI variation and more than 89% of the 16S variation were found within localities. In the different partitions, the southern samples tended to cluster together (Baiona, A Guarda and Lisboa) as well as the northern one (Brest) tended to form another differentiated cluster.

Phylogeographic analyses

The haplotype networks for COI and 16S had a similar star-like shape (Fig. 2). The most frequent haplotypes were located in the centre of the network surrounded by several low-frequency haplotypes. The NCPA nested design consisted of four 1-step clades (Fig. 2). At the total cladogram level, significantly large nested distances were detected in clade 1–2 for both COI and 16S, suggesting a northward past range expansion (especially for 16S, clade 1–2 was more represented in the north).

Discussion

The observation of ambiguous positions in the sequences of some individuals, mainly linked between the two markers and mainly found in one locality (Ribeira), is an issue that deserves a further and detailed study. The fact that the ambiguities remained after repeating the extractions and performing forward and reverse sequencing suggests that contamination and technical artefacts are not very plausible explanations. Double peaks in mitochondrial sequences could also be due to heteroplasmy. However, this hypothesis does not seem very compatible with the ambiguities concentrating in one population and the predominant allele being always the same (the “higher” peaks always corresponded to the same nucleotides). Another source of ambiguities could be gene duplication within the mitochondrial genome or a nuclear copy of a mitochondrial fragment (a pseudogene or *numt*). In both cases, it would imply a large region of the mtDNA being affected, as the COI and 16S seem to be very distant genes according to the proposed ancestral gene order for the insect–crustacean clade (Crease 1999). Although several gene rearrangements have been described for other crustaceans (Hickerson and Cunningham 2000; Yamauchi et al. 2003; Miller et al. 2005; Segawa and Aotsuka 2005; Sun et al. 2005), they never seem to come close to each other. However, pseudogenes have been found in a wide range of organisms (Bensasson et al. 2001) including crustaceans (Williams and Knowlton 2001; Williams et al. 2002), and they could reach 4 kb in length as in the rodent *Microtus rossiaemeridionalis* (Triant and DeWoody 2008). Nonetheless, the putative nuclear copy in our samples does not disrupt the coding frame. Moreover, most changes are synonymous, which is not expected of a pseudogene (due to relaxed selection in the nuclear environment) unless it is very recent. Indeed, we do not seem to identify the cause of the ambiguities with confidence, but more in-depth procedures could be used to decipher the different scenarios (see Luttikhuizen et al. 2008 and references therein). Nevertheless, it is very important to stress that the conclusions derived from this study are not affected by these ambiguities.

The analysis of the COI and 16S fragments revealed very low levels of genetic diversity and a weak differentiation among populations. The observed nucleotide diversities ($\pi \leq 0.001$) are smaller than those found for other crustaceans in the area under study: for the green crab *Carcinus maenas* [$\pi = 0.003$–0.004 (Roman and Palumbi 2004)], the spider crab *Maja brachydactyla* [$\pi = 0.003$–0.005 (Sotelo et al. 2008)], the spiny lobster *Palinurus elephas* [$\pi = 0.001$–0.002 (Palero et al. 2008)] or the stalked barnacle *Pollicipes pollicipes* [$\pi = 0.004$ (Quinteiro et al. 2007)].

Our results clearly point out to a recent demographic expansion. Fu’s F_S test showed significant negative values for the COI and the combined datasets, but not for the 16S dataset. However, in the latter case, the lower polymorphism level, in terms of segregating sites and number of haplotypes, could have reduced its statistical power (Ramos-Onsins and Rozas 2002; Ramírez-Soriano et al. 2008). The F_S statistic was significant at more localities than the R_2 test. This was expected, as F_S has proven more powerful under demographic expansions on nonrecombining genomic regions (Ramírez-Soriano et al. 2008). The presence of a major haplotype equally distributed across all localities and an appreciable number of closely related singletons also fit a recent demographic expansion from an ancestral population with limited N_e (Grant and Bowen 1998), a common pattern described for several marine species and related to Pleistocene glaciations. In the northeast Atlantic, for example, it has been the case for the deep-sea fish *Helicolenus dactylopterus* (Aboim et al. 2005), the sea urchin *Paracentrotus lividus* (Calderón et al. 2008) and the crustaceans mentioned above. In agreement with this observation, the NCPA and the current haplotype distribution suggest that the demographic expansion has been accompanied by an increment in geographical range. Although there is a huge controversy about the validity of the NCPA (e.g., Garrick et al. 2008; Knowles 2008; Petit 2008a, b; Templeton 2008), in this case the conclusions derived from it are largely concordant with those derived from other tests.

We tried to estimate the time of the expansion from the mismatch distributions (Schneider and Excoffier 1999; Excoffier 2004), but the program used (Arlequin) constantly reported convergence failures. We only obtained τ (expansion parameter) values for half of the localities with the COI dataset. A rough mean estimate was 0.5, the same that for the pooled samples. Assuming a 1% substitution rate and a mean generation time of 2 years, and according to the expression $T = \tau/2\mu k$ (where T is the time since the expansion and k is the sequence length), the expansion was dated quite recently, around 19,000 years ago, after the last glacial maximum at the end of the Pleistocene (Depraz et al. 2008; Neuenschwander et al. 2008). A similar scenario has been proposed for the softshell clam *Mya arenaria* in the northwest Atlantic (Strasser and Barber 2009).

Population differentiation was null in Galicia and very low along the sampled range, although the power to detect it should be largely diminished by the low levels of genetic variation. Even in the face of a recent expansion, most variations are located within populations, suggesting that gene flow could be also operating in this area maintaining the genetic homogeneity observed. Postlarval stages of *N. puber* are active swimmers, but only reproductive migrations have been proposed. Instead, planktonic larvae seem to have a high dispersive capacity, characteristic of many marine invertebrates. Zoeas spend around 2 months offshore, going through five development stages. They remain in a 10-km-wide band along the coast, under the influence of oceanic and wind currents. In turn, megalopae are transported inshore, and arranged in patches parallel to the coast. There they are subject to down- and up-welling events and to the associated alongshore circulation (dos Santos et al. 2008). Indeed, larval behaviour promotes the connectivity of the local adult populations in accordance with hydrographic patterns. The Atlantic Iberian coast is under the influence of the Iberian Poleward Current (IPC). It flows mainly northward from the south of Portugal to Cape Finisterre, eastward along Cantabrian coast and northward from the Bay of Biscay. In addition, mesoscale features such as coastal topography, seasonal upwellings, gyres and eddies also govern water movements along this range (Quinteiro et al. 2007), facilitating homogenization. To the south, the Azores Current limits the IPC, as it enters the Gulf of Cádiz before moving southwards. The population structure described in the northeast Atlantic for the stalked barnacle *P. pollicipes* fits this scheme (Quinteiro et al. 2007), as well as the shallow differences reported for the spider crab *M. brachydactyla* (Sotelo et al. 2008). For *N. puber*, we observed a high genetic homogeneity from the French to the Portuguese coasts, as could be expected given its longer larval phases. A very slight differentiation was detected by the SAMOVA for Brest, the northernmost population, and to a less extent for the southern localities, Baiona, A Guarda and Lisboa, while some of the Mantel tests also pointed out to isolation by distance, although always dependent on the inclusion of the sample from Brest. Remarkably, the individual collected in Cádiz also carries the most common and widespread haplotype, found in all other localities, suggesting a large homogeneous region, although it is obvious that more extensive sampling is needed.

Despite the inferred historical population growth (since last glaciations) and the fact that N_e is in the order of several thousands, probably related to the high fecundity of this crab, these populations could have been suffering a demographic decline in recent years. Reduced abundance has been reported in the Galician coast since the 1980s as a consequence of overfishing (González-Gurriarán 1985a). Moreover, commercial catches have already diminished in Portugal and France (Hearn 2002), and signs of overexploitation are emerging in the UK as well (Lee et al. 2006). Although marine populations tend to fluctuate naturally due to a large variance in reproductive success, which largely depends on biotic and abiotic parameters (Grant and Bowen 1998), the impact of commercial captures cannot be ignored in terms of conservation of populations and sustainable development.

This study is the first population genetic description of *N. puber*. In Galicia, these crabs seem to conform to a single stock with low mitochondrial diversity. Indeed, every inference presented here is only based on mitochondrial data, so it will be convenient to contrast those using nuclear markers. In this sense, we have tried to isolate microsatellites using enriched libraries, and although the proportion of repetitive fragments was high, the optimization of variable loci was largely unsuccessful. Future studies could concentrate on a wider sampling and include other nuclear loci.

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