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Phylogeography of the European spiny lobster (*Palinurus elephas*): Influence of current oceanographical features and historical processes

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ABSTRACT

The European spiny lobster (*Palinurus elephas*) is a suitable model organism to study the effects of past history and current oceanographic processes on the genetic diversity and population structure of marine species with a long-lived larval phase. A portion of the COI gene was sequenced in 227 individuals from 11 localities, covering most of the present distribution of the species. Divergence was found between Atlantic and Mediterranean regions, which could be explained by restricted gene flow between populations. Moreover, a principal component analysis detected differences within basins. The existence of genetic differentiation between Brittany and Ireland–Scotland populations could be accounted for by the large effect of the Gulf Stream, while mesoscale processes suffered by the incoming Atlantic waters could be responsible of genetic differentiation within the Mediterranean. Furthermore, historical processes could be responsible for a reduction on the overall genetic variability of *P. elephas*. The haplotypic distribution found in *P. elephas*, with the presence of one abundant haplotype and a large number of closely related haplotypes, is typical of species experiencing reduction in variability and subsequent expansions. Climatic fluctuations related to glacial cycles could explain the present level of variability and nucleotide diversity found. Interestingly, these glacial events do not seem to have the same impact in other species of the same genus. Our results indicate that recent glacial events could have had a lower impact on *Palinurus mauritanicus,* a congeneric species that presents an overlapping distribution area but is found in cooler waters than *P. elephas*.

1. Introduction

Genetic variability and population genetic structure of a species are shaped by both past and present ecological processes, such as recent paleoecological history (e.g., glaciations) and current pressures (e.g., over-exploitation, habitat degradation, introduction of invasive species). Taking into account the influence of present gene flow on the genetic structuring of the species is crucial in order to protect those populations with higher genetic diversity and greater ability to effectively be able to export individuals to other areas (Palumbi, 2004). Furthermore, recent paleoecological history should also be taken into account in order to protect regions containing higher diversity and distinct lineages (Fraser and Bernatchez, 2001). Neutral mtDNA markers can provide information about past events, while giving a picture of the overall gene flow between populations (Grant and Waples, 2000).

Population connectivity is mainly determined by the potential dispersal of the species, in a way that species with a restricted dispersive ability tend to present more genetically structured populations (Palumbi, 2003). Planktonic larval duration (PLD) is a key factor in shaping patterns of dispersal and degree of connectivity between populations of marine species (Shanks et al., 2003). Thus, species with a longer PLD should present extensive gene flow (panmixia) and, therefore, low or no population structuring. However, even though larvae can potentially disperse over long distances, population structuring can be found at lower levels than expected under the theoretical limit of larval dispersal (Taylor and Hellberg, 2003; Rocha et al., 2005), given that larval behavior coupled with oceanographic structures at different scales may be conducive to larval retention (Naylor, 2006).

Achelata lobsters (spiny, slipper and coral lobsters) are decapod crustaceans characterized by the presence of the phyllosoma, a larval phase specially adapted for long time dispersal. Dispersive ability of phyllosoma larvae is among the highest found in crustaceans, with an estimated duration of up to 24 months in some species of the genus *Jasus* (Booth, 1994). Accordingly, no evidence of subdivision was found among *Jasus edwardsii* populations from Southern

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Australia or New Zealand (Ovenden et al., 1992), among *Panulirus argus* populations from the Caribbean Sea (Silberman et al., 1994), or *Palinurus gilchristi* populations from South Africa (Tolley et al., 2005). On the contrary, RFLPs analysis of mtDNA showed a genetic subdivision in *Jasus verreauxi* populations from Southern Australia and New Zealand, probably due to a fall in larval survivorship while crossing the Tasman Sea (Brasher et al., 1992). A clear differentiation was also found between *P. argus* populations from the Caribbean Sea and Brasil (Diniz et al., 2005), related to an oceanic barrier formed by the drop in salinity caused by the Amazon and Orinoco rivers. More recently, Gopal et al. (2006) found shallow genetic partitioning for *Palinurus delagoae* along the South African coast, caused by the retention of some of the larvae in slow-moving anticyclonic eddies.

The European spiny lobster (*Palinurus elephas*) is present in the Mediterranean Sea and along the eastern Atlantic coasts from Morocco (28°N) to Norway (60°N) (Holthuis, 1991). Mean annual catches of *P. elephas* have decreased alarmingly during recent decades along its entire distribution area due to stock over-exploitation (Goñi and Latrouite, 2005), which makes *P. elephas* a primary target for conservation plans. Estimates of the duration of larval development from hatching to first juvenile stage are in the range of 5–6 months in the Mediterranean (Marin, 1985) and one year in the Atlantic (Mercer, 1973). Consequently, low or no population structuring is expected to be present in *P. elephas*, since this species has a large dispersal potential due to the long duration of its planktonic larval phase. However, juveniles of the congeneric species *Palinurus gilchristi* migrate along distances of up to 700 km, which could counteract the dispersal effect of the larval phase (Groeneveld and Branch, 2002).

Moreover, oceanic fronts and mesoscale gyres could also reduce the potential range of dispersal of a species by retaining even long-lived larvae such as phyllosoma larvae (Naylor, 2005). One of the most well-known oceanic fronts in the Northern Hemisphere is that found between the Atlantic Ocean and the Mediterranean Sea, the Almeria–Oran Front, formed by the encounter of the incoming Atlantic surface water with the modified higher density Mediterranean water (Tintoré et al., 1995). Most studies on population genetics of marine species with an Atlantic–Mediterranean distribution have focused on the genetic differentiation of populations found at both sides of the Strait of Gibraltar. Many of them have revealed some degree of genetic differentiation between populations from both marine areas, with some exceptions (Patarnello et al., 2007). Nevertheless, the interaction between Atlantic and Mediterranean waters is known to spread on a much wider scale since Mediterranean waters are known to move north as deep waters to southern Britain, whereas surface Atlantic waters travel all along the North-African Mediterranean coast (Millot, 2005). Consequently most of the picture could be lost when the focus is set on a small part of the distribution of the species of interest.

In the present work we have analyzed *P. elephas* individuals from a total of 11 localities, covering most of the present distribution of the species. A portion of the COI gene was used as genetic marker to study the variability and genetic differentiation found in populations. This sampling scheme will allow us to assess the genetic population structure between and within Atlantic and Mediterranean basins. Furthermore, we also aim to analyse whether the present genetic variability and population structure of *P. elephas* is influenced by current and/or historical factors. In order to infer the influence of historical processes, we used for

Table 1
Haplotype frequency distribution for each locality

Haplotype	WSCO	WIRE	SWEN	BRIT	BISC	SPOR	WMED	NWME	SARD	TUNI	SICI	Total
I	11	14	12	14	10	16	16	19	11	7	13	143
II							3	3				6
III	2		2	1	2	4	4	1	3	4	4	27
IV							1					1
V							1					1
VI						2	1	1	1	1		6
VII									1			1
VIII				1					1			2
IX									1			1
X									1			1
XI	1									3	1	5
XII											1	1
XIII										1		1
XIV	2		1		1					1		5
XV										1		1
XVI			1							1		2
XVII										1		1
XVIII					2	1						3
XIX	1	1				1						3
XX				1								1
XXI				1								1
XXII				1								1
XXIII				1								1
XXIV				1								1
XXV			1									1
XXVI			1									1
XXVII		1										1
XXVIII		1										1
XXIX	1	1										2
XXX		1										1
XXXI	2											2
XXXII	1											1
XXXIII	1											1
N	22	19	18	21	15	24	26	24	19	20	19	227

(*N*) number of specimens per locality. Haplotypes in bold present non-synonymous changes in comparison to haplotype I.
Localities: Western Scotland (WSCO); Western Ireland (WIRE); South Western England (SWEN); Brittany (BRIT); Bay of Biscay (BISC); South Portugal (SPOR); Western Mediterranean (WMED); North Western Mediterranean (NWME); Sardinia (SARD); Tunisia (TUNI); Sicily (SICI).

comparison the congeneric species *Palinurus mauritanicus*, since it is the only species of the genus with an overlapping distribution area. The pink spiny lobster *P. mauritanicus* occurs in the Northeast Atlantic from Senegal to western Ireland and in the western Mediterranean as far as Sicily, but not in the Adriatic (Holthuis, 1991). *P. mauritanicus* is generally found in deeper waters (180–600 m) than *P. elephas* (5–160 m), with greatest densities occurring between 200 and 400 m (Holthuis, 1991). This species inhabits the edge of the continental shelf, especially the canyons, and prefers muddy and coralligenous substrates near rocky outcrops (Goñi and Latrouite, 2005). Given that both species have similar biogeographic distributions and, therefore, could have been influenced by similar paleoecological events, we would expect past events affecting large areas in the Northern Hemisphere (e.g., glaciations) to have left a genetic signature in both species of *Palinurus*.

2. Materials and methods

Tissue samples of *P. elephas* individuals (n = 227) were obtained from several localities covering most of the present distribution of the species (Table 1 and Fig. 1) (GenBank Accession Nos. EU573030–EU573062). The sampled area included 11 different localities (Fig. 1): Western Scotland (WSCO): Oban; Western Ireland (WIRE): Galway; South Western England (SWEN): Newlyn; Brittany (BRIT): Le Conquet; Bay of Biscay (BISC): Gijón; South Portugal (SPOR): Sagres; Western Mediterranean (WMED): Cullera; North Western Mediterranean (NWME): Cap de Creus; Sardinia (SARD): Sassari; Tunisia (TUNI): Tunis; Sicily (SICI): Isola delle Femmine. A total of 20 individuals of *P. mauritanicus* from both North Western Mediterranean (Catalonia (2), Spain) and Atlantic waters (Tanger (18), Morocco) were sampled for interspecific comparisons and divergence time estimation (Accession Nos. EU573063–EU573075). The *Panulirus argus* COI sequence retrieved from GenBank (Accession No. AF339452.1) was used as an outgroup to assess for evolutionary rate constancy.

One pleopod from each individual was preserved in 100% ethanol and total genomic DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen Inc.). Amplification of part of the COI gene was achieved using the universal primers LCO1490 and HCO2198 (Folmer et al., 1994). Amplification was carried out with 30 ng of genomic DNA in a reaction containing 1 U of *Taq* polymerase (Amersham), 1× buffer (Amersham), 0.2 μM of each primer and 0.12 mM dNTPs. The PCR thermal profile used was 94 °C for 4 min for initial denaturation, followed by 35 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 4 min. Amplified PCR products were purified with QIA-Quick PCR Purification Kit (Qiagen Inc.) prior to direct sequencing of the product. The sequences were obtained using the Big-Dye Ready-Reaction kit v3.1 (Applied Biosystems) on an ABI Prism 3770 automated sequencer from the Scientific and Technical Services of the University of Barcelona.

2.1. Intraspecific variability

A 499 bp region was aligned using ClustalX with the default alignment parameters as implemented in BioEdit v7.0.1 (Hall, 1999) and checked manually for misalignments. Nucleotide diversity (π) and haplotype diversity (h) and its variance and standard deviation were calculated for each population using the software DnaSP v.4.10 (Rozas et al., 2003). In order to compare haplotype diversity values, the statistics and asymptotic confidence intervals derived by Salicrú et al. (1993) were used for both overall diversity comparison and pairwise comparisons between populations.

2.2. Population genetic differentiation

Genetic differentiation was estimated by measuring GammaST (γST) (Nei, 1982) and Snn (Hudson, 2000). The significance of the Snn estimates of genetic differentiation was tested with a permutation (randomization) test using DnaSP. In order to test for isola-

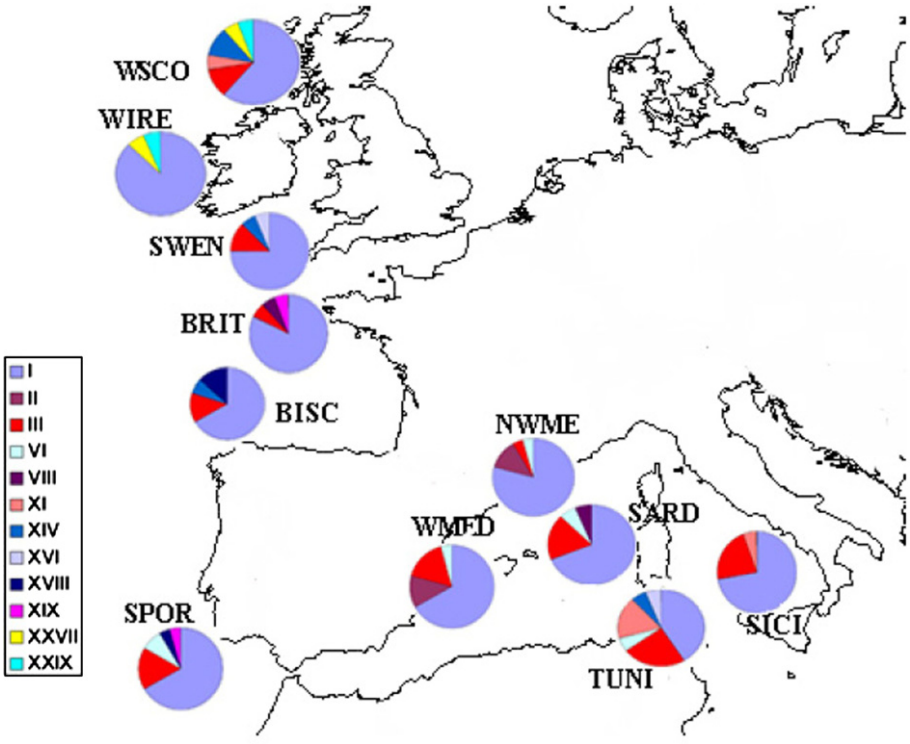

Fig. 1. Frequency of haplotypes shared among *Palinurus elephas* localities. Acronyms as described in Table 1.

tion by distance (IBD), a comparison of pairwise genetic distances between populations (GammaST/(1 − GammaST)) and geographical distances (log km) was carried out by using the Mantel test implemented in GENEPOP v3.4 (Raymond and Rousset, 1995). To visualize the relationship between populations, haplotype frequency data excluding private alleles was transformed to $2\sqrt{p_{ij}}$ (Balanya et al., 2006), and a principal components analysis (PCA) was carried out using Gingko Multivariate Analysis System (De Caceres et al., 2003). The distribution of genetic variance at different geographical levels was estimated by an analysis of molecular variance (AMOVA) using Arlequin v.3.01 (Excoffier et al., 2005).

A haplotype network was constructed using the program TCS 1.2.1 (Clement et al., 2000), which implements the statistical parsimony algorithm described by Templeton et al. (1992). A nested clade analysis (NCA) was performed in order to differentiate between population history events and population structure (Templeton, 1998). We used the empirical predictions derived from coalescent theory (Posada and Crandall, 2001) to solve network ambiguities. Nested clade contingency tests were carried out in GeoDis v2.5 (Posada et al., 2000). The inference key used is available at http://darwin.uvigo.es/software/geodis.html. Nested clade analyses can discriminate between phylogeographical associations due to recurrent but restricted gene flow vs. historical events operating at the population level (e.g., range expansion events), thereby yielding greater insight into both the evolutionary history and population structure of the species.

2.3. Neutrality tests and demographic inferences

Tajima's *D* (Tajima, 1989) and Fu's Fs (Fu, 1997) were used to test for deviation from mutation-drift equilibrium for all areas combined. The McDonald and Kreitman (1991) test was carried out using *P. mauritanicus* as outgroup to determine whether deviations are caused by selection acting upon this gene. Both *r* statistics (Harpending, 1994) and R^2 statistics (Ramos-Onsins and Rozas, 2002) were calculated and the mismatch distribution was plotted considering the number of pairwise differences in order to investigate the possibility of demographic changes. These statistics and their confidence intervals were obtained by coalescence simulations as implemented in DnaSP.

A relative ratio test implemented in MEGA v3.1 (Kumar et al., 2004) was performed in order to test for evolutionary rate constancy along *Palinurus* lineages. A likelihood ratio test for trees obtained both enforcing and not enforcing a molecular clock was also carried out to test for the molecular clock assumption. The substitution rate used for dating the speciation event and the haplotypes coalescent time within species (μ_s = 0.9–1.1% divergence/My) was estimated for the COI region in different decapods related to the formation of the Isthm of Panama (reviewed in Ketmaier et al., 2003). Since substitution rate represents a lower boundary for the mutation rate of a particular lineage, we followed a conservative approach after Emerson (2007). Thus, an intraspecific mutation rate 3–10 times faster than the interspecies substitution rate was used for dating haplotype coalescent time in *P. elephas* and *P. mauritanicus*. Harpending's population expansion model $T = \tau/2u$ (Harpending, 1994) with an intraspecific mutation rate 3–10 times faster than the interspecies substitution rate (Emerson, 2007) was used for obtaining population expansion estimates in *P. elephas*.

3. Results

3.1. Intraspecific variability

A total of 33 haplotypes were detected in the 227 individuals of *P. elephas* sequenced. Overall, there were 30 variable sites (6%), of which only 5 yielded non-synonymous changes (Table 1). The most abundant haplotype (Hap1) was found in 63% of the individuals sequenced. A second haplotype (Hap3) was relatively common, occurring in 12% of all lobsters sequenced. A total of 12 haplotypes were shared among localities (Fig. 1). The majority of haplotypes (63.4%) were private from population, all but one being singletons. The mean number of haplotypes per population was 6.2 and ranged between 4 and 9. The presence of an extremely abundant haplotype and many low-frequency, closely related haplotypes is reflected both in the low nucleotide diversity (π = 0.0016) and fairly high haplotype diversity (h = 0.588) of the overall sample as well as in each locality (Table 2). Haplotype diversity values were significantly different among populations according to the χ^2 test developed by Salicrú et al. (1993) (χ^2 = 22.49, $P < 0.05$). Pairwise comparisons between localities showed that Western Scotland and Tunisia haplotype diversity values were significantly higher than those found in most populations (see Appendix). Haplotype diversity (h = 0.905) and nucleotide diversity (π = 0.0045) values were higher in *P. mauritanicus*.

Table 2
Genetic variability in *Palinurus elephas*

Sampled locality	*N*	N_H	$h \pm$ sd	$\pi \pm$ sd
WSCO	22	9	0.7489±0.0940	0.0023±0.0005
WIRE	19	6	0.4678±0.1400	0.0013±0.0005
SWEN	18	6	0.5621±0.1340	0.0013±0.0004
BRIT	21	8	0.5667±0.1290	0.0015±0.0005
BISC	15	4	0.5524±0.1370	0.0013±0.0004
SPOR	24	5	0.5399±0.1090	0.0012±0.0003
WMED	26	6	0.6031±0.0990	0.0017±0.0004
NWME	24	4	0.3696±0.1170	0.0008±0.0003
SARD	19	7	0.6608±0.1140	0.0018±0.0004
TUNI	20	9	0.8421±0.0610	0.0030±0.0005
SICI	19	4	0.5088±0.1170	0.0015±0.0005
Total	227	33	0.5880±0.0380	0.0016±0.0001

Note: Number of specimens (*N*), number of observed haplotypes (N_H), haplotype diversity (h), nucleotide diversity (π) and standard deviation (sd). Locality acronyms as in Table 1.

3.2. Genetic differentiation

Overall genetic differentiation among localities was significant according to the Snn statistic (Snn: 0.109, $P < 0.05$). Significant pairwise comparisons involved mainly Irish and Tunisian populations, while comparatively high Snn values involving other populations were not found significant (Table 3). This could be consequence of the most frequent haplotype, as well as the presence of many unique haplotypes, affecting the permutation test result. Nevertheless, global genetic differentiation was still significant when the Irish population (Snn: 0.119, $P < 0.05$), the Tunisian population (Snn: 0.115, $P < 0.05$) or both localities were excluded (Snn: 0.127, $P < 0.05$). When the pairwise genetic distances (γ_{ST}) were correlated to the geographic distances using the Mantel test, a shallow although significant correlation was detected (R^2 = 0.1436; P = 0.027), which indicates a pattern of isolation by distance (Fig. 2). However, when analyzed separately, this pattern disappeared both in Atlantic (R^2 = 0.048; P = 0.228) and Mediterranean (R^2 = 4E −05; P = 0.669) populations.

An analysis of molecular variance was carried out to evaluate the differentiation between the two putative regions (Atlantic and Mediterranean). Most of the variation (98%) was explained by the variability within samples (Table 4). This result is in agreement with the fairly high level of haplotype diversity in almost every population. Nonetheless, differences between regions were always significant, although *P*-values changed when SPOR—the population from South Portugal, which occupies an intermediate

Table 3
Pairwise genetic distances between *Palinurus elephas* localities

	WSCO	WIRE	SWEN	BRIT	BISC	SPOR	WMED	NWME	SARD	TUNI	SICI
WSCO		0.033	0.025	0.035	0.029	0.033	0.029	0.030	0.038	0.026	0.033
WIRE	0.531		0.033	0.022	0.045	0.040	0.047	0.036	0.050	0.070	0.068
SWEN	0.501	0.516		0.023	0.022	0.020	0.025	0.034	0.024	0.036	0.028
BRIT	0.555	0.474	0.478		0.032	0.024	0.032	0.026	0.029	0.056	0.047
BISC	0.530	0.564*	0.471	0.521		0.017	0.028	0.047	0.028	0.037	0.030
SPOR	0.547	0.533	0.510	0.510	0.513		0.017	0.036	0.012	0.041	0.021
WMED	0.563	0.577*	0.525	0.526	0.568	0.498		0.058	0.017	0.026	0.013
NWME	0.565*	0.527	0.523	0.490	0.575	0.521	0.475		0.045	0.052	0.056
SARD	0.523	0.545	0.476	0.461	0.505	0.472	0.495	0.522		0.034	0.016
TUNI	0.519	0.623*	0.487	0.563*	0.525	0.532	0.549	0.598*	0.496		0.021
SICI	0.523	0.555*	0.469	0.510	0.502	0.545	0.504	0.546	0.472	0.479	

GammaST: upper diagonal; Snn: lower diagonal.
Asterisks indicate significant Snn differentiation assessed by a permutation test ($P < 0.05$). Locality acronyms as in Table 1.

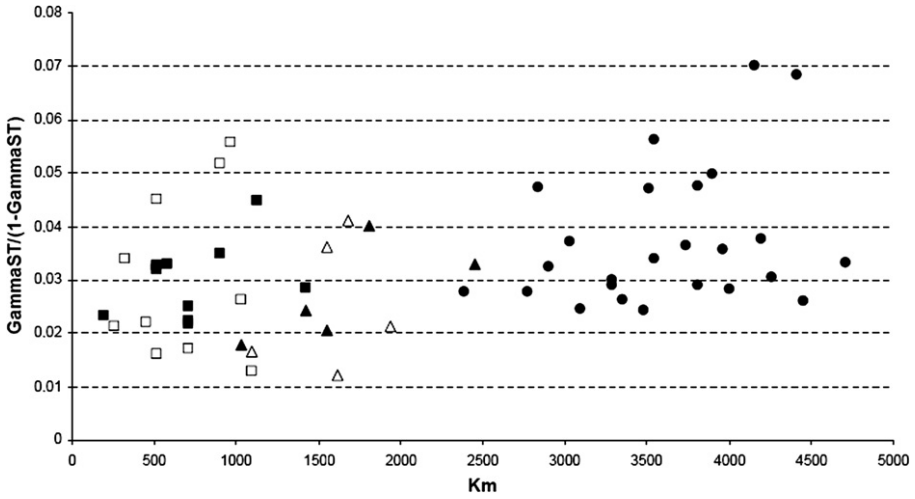

Fig. 2. Correlation in *Palinurus elephas* populations between genetic and geographic distances. Symbols indicate comparison between (□) Mediterranean populations, (■) Atlantic populations, (●) Mediterranean and Atlantic populations, (▲) South Portugal and Atlantic populations, and (Δ) South Portugal and Mediterranean populations.

Table 4
Hierarchical analysis of molecular variance for *Palinurus elephas*

	Source of variation	Sum of Squares	% Total variance	*P*
Atlantic + SPOR	Among regions	1.074	1.29	0.050
	Among localities/ within regions	4.259	0.89	0.068
	Within localities	86.178	97.82	0.034
Excluding SPOR	Among regions	1.173	1.63	0.037
	Among localities/ within regions	3.857	0.87	0.148
	Within localities	78.783	97.50	0.031
Mediterranean + SPOR	Among regions	1.179	1.50	0.026
	Among localities/ within regions	4.308	0.76	0.049
	Within localities	89.109	97.74	0.036

position between all other samples—was included in the Atlantic region or in the Mediterranean region. The highest percentage of variance explained by differences between the Atlantic and Mediterranean regions (1.63% of total variance) was found when SPOR was excluded from the analysis ($P = 0.036$). Moreover, differences between localities within regions were only significant when SPOR was included in the Mediterranean region.

To further investigate genetic differentiation among populations a principal component analysis was carried out using haplotype frequency data of shared alleles. The first principal component (%Var = 27.64) was significantly correlated to haplotype diversity in each population ($R^2 = 0.624$; $P < 0.01$). Thus, in order to reveal the relationships between populations without considering haplotype diversity, we plotted the values of second (%Var = 25.99) and third (%Var = 12.16) principal components (Fig. 3). Mediterranean and Atlantic localities showed a different behavior with respect to these two components so that four groups could be identified. The two northernmost Atlantic localities (Western Scotland and Western Ireland) were clearly differentiated from the rest because they presented negative PC2 values. Negative PC3 values were found in three Atlantic localities (South Western England, Brittany and Bay of Biscay). The two western Mediterranean localities exhibited the largest PC3 values. Finally, the other four localities (South Portugal, Tunisia, Sicily and Sardinia) showed intermediate PC3 values.

The haplotype network constructed by statistical parsimony showed a star-like phylogeny in which most of the unique haplotypes were closely related to the common central haplotype (Fig. 4). This haplotype network is typical of species which have suffered a bottleneck and a subsequent expansion. Three loops were found in the haplotype network (dashed lines in Fig. 4) and the frequency and shared location criteria were followed to solve the ambiguities, since all the connections were transitional changes. The first loop was broken by connection (A), since haplotypes IX and XV were found in different localities and this breakage allowed them to remain connected to the most frequent haplotype found in the same location. The second and the third loops were

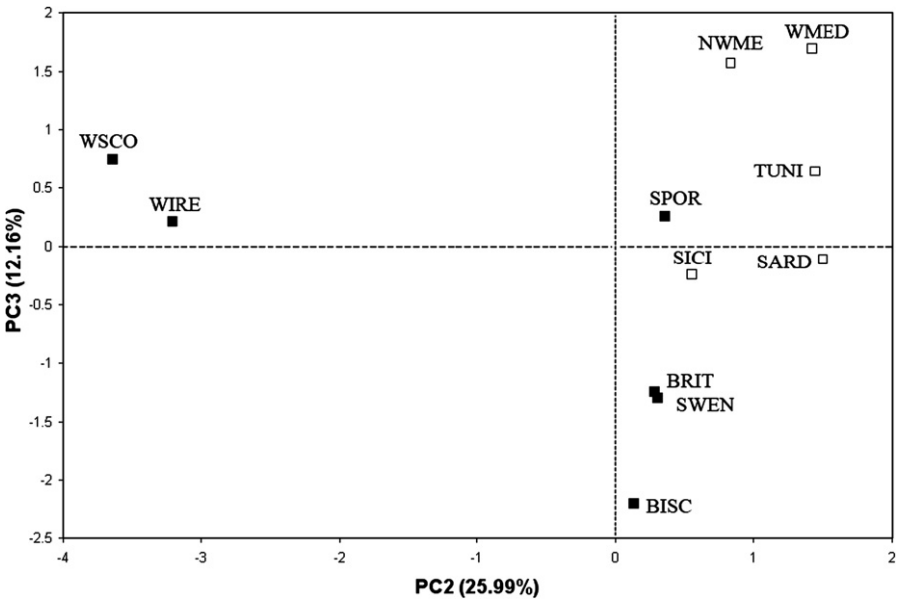

Fig. 3. Principal components plot for *Palinurus elephas* populations. PC1 is strongly correlated to haplotype diversity thus PC2 and PC3 are only plotted. Acronyms as described in Table 1.

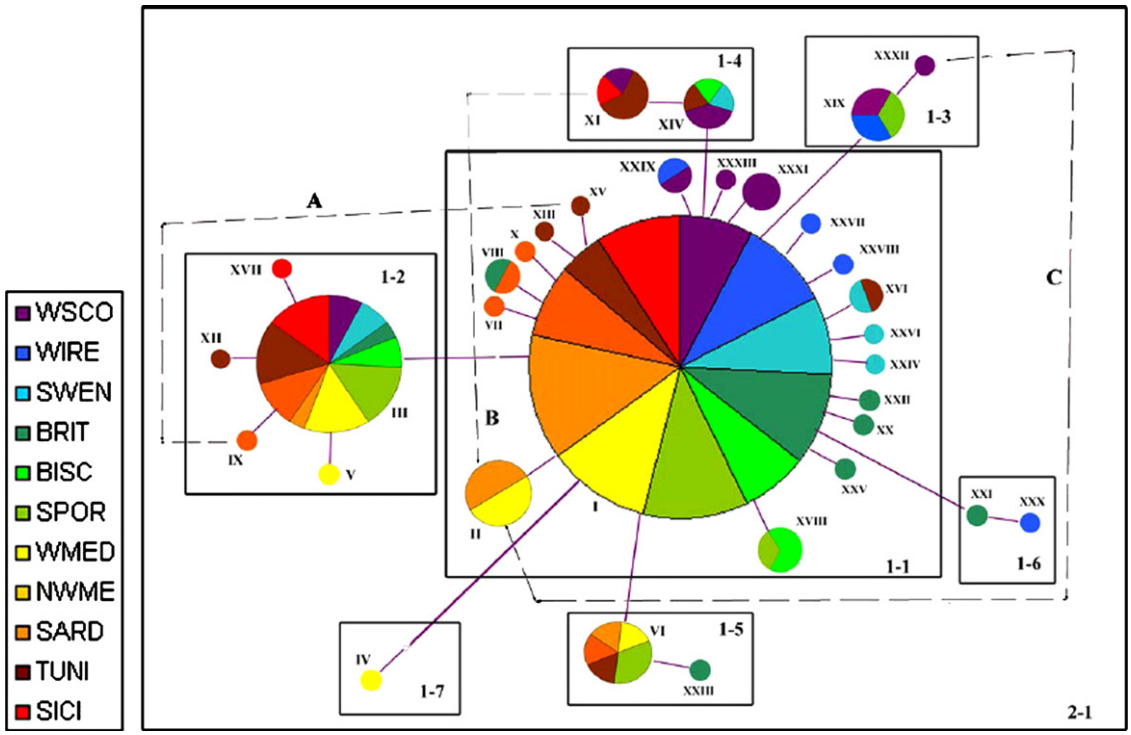

Fig. 4. Statistical parsimony haplotype network for *Palinurus elephas*. Dashed lines in the network indicate connections broken in order to solve network ambiguities (see text for details). See Table 1 for abbreviations.

opened through connections B and C, respectively, also following the frequency and shared population criteria. Permutation analysis gave significant results for clade 1-1 and clade 2-1 (Appendix). For both clades Templeton's inference key revealed the existence of restricted gene flow/dispersal but with some long distance dispersal.

3.3. Neutrality tests and demographic inferences

Both Tajima's *D* test (Tajima's *D*: −2.34, $P < 0.01$), and Fu's Fs test (Fu's Fs: −4.81, $P < 0.001$) indicated a significant deviation from neutrality. This deviation could be explained by either selection or demographic factors and can be related to the presence of a large number of unique haplotypes. Using *P. mauritanicus* as outgroup, the McDonald and Kreitman test showed non-significant differences in the proportion of synonymous and non-synonymous changes within and between species (G: 1.04; $P = 0.308$). Even though the value of the raggedness statistic was not significant ($r = 0.078$; $P = 0.097$), the R^2, which is a more powerful test for detecting population growth (Ramos-Onsins and Rozas, 2002), was significant ($R^2 = 0.014$; $P = 0.015$) and suggested the occur-

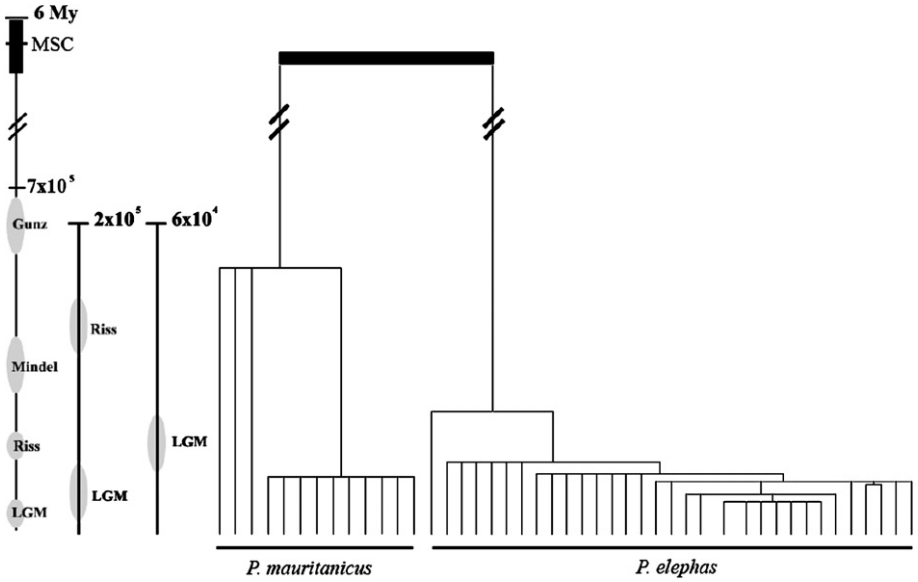

Fig. 5. Haplotype cladogram in the *Palinurus* species of the Northern Hemisphere: *P. elephas* and *P. mauritanicus*. Different dates for haplotype coalescence time are obtained when using the substitution rate for decapod crustaceans (Ketmaier et al., 2003), and a more conservative approach using a 3–10 times faster mutation rate (Emerson, 2007).

rence of a population expansion. The same results were obtained when Atlantic and Mediterranean localities were analyzed separately.

The molecular clock assumption fits well the species group under study, since the likelihood ratio test for the tree topologies obtained with and without enforcing a molecular clock assumption showed no significant differences ($\chi^2 = 1.88$, $P > 0.05$). The relative ratio test using *Panulirus argus* as outgroup showed that the substitution rate has remained constant for the *Palinurus* species analyzed ($P > 0.05$). Consequently, an approximate dating of the speciation processes was carried out, since no significant difference in branch-length was found between the *Palinurus* species and the outgroup. Using the substitution rate for the COI gene established in several decapod crustaceans ($\mu = 0.9$–1.1% divergence/My; Ketmaier et al., 2003), we dated the divergence of *Palinurus mauritanicus* from *P. elephas* around 5.52–6.75 My ago. If the same substitution rate is used for dating the time to the most recent common ancestor within each species, we find a coalescence time of approximately 266,000–355,000 years ago for the *P. elephas* haplotypes and approximately 477,000–583,000 years ago for the *P. mauritanicus* haplotypes (Fig. 5). However, the intraspecific mutation rate could be considered 3–10 times faster than the interspecies substitution rate (Emerson, 2007). Using this approach the coalescence time was approximately 26,000–118,000 years ago for the *P. elephas* haplotypes and 48,000–194,000 years ago for the *P. mauritanicus* haplotypes (Fig. 5). The mismatch distribution indicated that the observed differences in *P. elephas* followed the distribution of expected differences under an expanding population model ($\tau = 0.807$). Using the intraspecific mutation rate the timing of the population expansion for *P. elephas* would be within a range of 8000–48,000 years ago. Mismatch distribution in *P. mauritanicus* did not follow an expanding population model.

4. Discussion

4.1. Genetic differentiation among populations

Even though the presence of one abundant haplotype all over the distribution area of the species indicated a high degree of genetic homogeneity among populations, the analysis of the shared haplotype frequency data revealed the existence of geographic population structuring. The significant genetic differentiation found between the Atlantic and Mediterranean regions could be explained by restricted gene flow among populations, in agreement with the nested clade analysis results. While the Mantel test including all the sampled localities showed a pattern of isolation by distance (IBD), the analysis of Mediterranean and Atlantic localities separately failed to detect it, indicating that this pattern might be spurious and result from restricted gene flow through the Gibraltar Strait. These results point out to a possible reduction in the dispersal capability of the phyllosoma as compared to expected dispersal levels given its long planktonic duration (Mercer, 1973; Marin, 1985). This reduction could be related to the dynamics of mesoscale oceanic processes or to the possible phylopatry of juveniles, which might have a behavioral compensation effect on the overall dispersal (Naylor, 2005, 2006).

Interestingly, the principal components analysis suggests the existence of four groups of samples in *P. elephas*. The two northernmost Atlantic localities form the most differentiated group. The existence of genetic differentiation between Brittany and Ireland–Scotland samples has also been found in the fish *Pomatoschistus microps* and the brown alga *Fucus serratus* (Gysels et al., 2004; Hoarau et al., 2007) and could be accounted for by the large effect of the Gulf Stream hitting the European subcontinent at approximately the south of Ireland (Bower et al., 2002; Jakobsen et al, 2003). The Gulf Stream splits into a northern current and a southwestern current towards Britain and continental Europe south of Britain in a way that its influence could reduce gene flow between these areas and therefore promote genetic differentiation. Accordingly, a second group was formed by the Atlantic populations found in the western Channel and the Bay of Biscay area, which are influenced by the southern branch of the Gulf Stream split. The south Portugal sample grouped with those Mediterranean populations (Sardinia, Tunisia and Sicily) that receive a stronger influence of Atlantic waters (Millot, 2005). The surface incoming Atlantic water enters the Mediterranean through the Gibraltar Strait and, after crossing the Alboran Sea while forming two large mesoscale anticyclonic gyres, heads towards the northern shores of Africa, forming the Almeria–Oran front, and then eastwards towards the Sicily Channel which separates the western from the eastern Mediterranean basins. Some eddies often detach northwards towards Sardinia and the Balearic Islands (Millot, 2005).

The two most differentiated *P. elephas* populations sampled within the Mediterranean are those located in the northwestern basin, which are precisely the areas under the farthest influence from the incoming Atlantic water and constitute the most differentiated Mediterranean waters (Salat, 1996). In this area, the occurrence of the Northern and Balearic Currents forms a mesoscale cyclonic gyre, which may favor larval retention. Our results indicate that even though population structuring in a marine species with Atlantic–Mediterranean distribution may be influenced by the Almeria–Oran Front (Bargelloni et al., 2005), mesoscale processes suffered by the incoming Atlantic waters could be responsible for further genetic differentiation within the Mediterranean.

4.2. Intraspecific variability, demographic changes and divergence

Historical processes may influence the resolution of genetic markers to study population differentiation, since reductions on population size or selective sweeps would be responsible of a reduction on the overall genetic variability. A fairly high haplotype diversity was observed in *P. elephas* samples, with the presence of one abundant haplotype and a large number of closely related haplotypes. These results are consistent with those found in other studies of palinurid lobsters using mtDNA control region (Diniz et al., 2005; Tolley et al., 2005; Gopal et al., 2006). However, it should be pointed out that one previous study with both *P. elephas* and *P. mauritanicus* failed to detect variability in the COI gene (Cannas et al., 2006), while a more recent paper found intraspecific variability in both species (Groeneveld et al., 2007), in agreement with our results. Therefore, we can conclude that there is an overall tendency for this group of crustaceans to present high levels of haplotype diversity with most haplotypes being closely related. A genetic bottleneck caused by either selection or demography would reduce the effective population size and cause the present day haplotypes coalesce at a recent time. Since the COI region seems to have evolved neutrally in both *P. elephas* and *P. mauritanicus*, this pattern could indicate that demographic fluctuations have been responsible for shaping their genetic variation and causing the current shallow intraspecific phylogenies (Harpending, 1994). However, given that mtDNA acts as a single locus, selection acting elsewhere on the mtDNA molecule would exactly mimic a demographic bottleneck; thus, nuclear genes should be used to completely discard the hypothesis of selection (Bazin et al., 2006). Nonetheless, the distribution of the number of pairwise differences and the shallow pattern of mtDNA found in the present work seems a general trend present in species belonging to diverse taxonomic groups (Domingues et al., 2005; Jolly et al., 2006). Such a pattern can be related to environmental changes that have dramatic effects over a wide area such as glacial periods, which can cause sea level declines of more than 100 m (Lambeck et al., 2002). The impact of glacial periods on demographic fluctuations has been remarked in different species (Bargelloni et al., 2005; Domingues et al., 2005). If the mutation rate is assumed to be equal to the substitution rate, the date of coalescence for *P. elephas* haplotypes—approximately 266,000–355,000 years ago—indicates that this pattern of genetic variation could be related to the climatic changes of the Mindel glacial period (230–300 ky ago) (Fig 5). Accordingly, the dates of mtDNA coalescence obtained for *P. mauritanicus* would be approximately 477,000–583,000 years ago, indicating the possible influence of the Günz glaciation (620–680 ky ago).

However, there has been recently much debate about intraspecific mutation rates being much higher than interspecific substitution rates (Ho et al., 2005). Taking these observations into account and using a mutation rate 3–10 times faster than the substitution rate (Emerson, 2007), the coalescence time for *P. elephas* haplotypes was much more recent and could be related to the last glacial maximum (LGM) (Fig 5). Nevertheless, using this faster rate, *P. mauritanicus* haplotypes would still coalesce at an older age than *P. elephas* haplotypes, indicating a smaller effect of the last glacial event compared to *P. elephas*. Previous studies had emphasized the role of glaciations in causing a larger depletion in levels of genetic variation among high intertidal species, where exposure times to cold stress were longer than for species living lower on the shore (Marko, 2004). Since *P. mauritanicus* is generally found in deeper waters than *P. elephas* (Holthuis, 1991), sea level or temperature changes could have affected *P. mauritanicus* populations to a lesser extent. A cold temperature refuge can be found in deeper waters during warm periods (Graham et al., 2007), whereas a warm refuge in cold periods would be only found by heading latitudinally south, and remaining in shallow waters. These processes would cause population bottlenecks that would imply a decrease in genetic diversity as found in *P. elephas* and a smaller effect in colder water species, as in *P. mauritanicus*. Additionally, glaciations could have a differential impact on population effective sizes reducing or shifting differentially the overall distribution areas of the two species. In this way, a larger distribution area during glaciations could have allowed *P. mauritanicus* to retain larger populations as compared to *P. elephas* and thus promote the maintenance of more ancestral polymorphisms.

The time of divergence between *Palinurus elephas* and *P. mauritanicus* lineages (5.52–6.75 My) is much older than the coalescence time of haplotypes for any of the two species. This time of speciation could be related to the Messinian Salinity Crisis (MSC), which caused the desiccation of the Mediterranean and the resulting change in ocean currents between 5.96 and 5.33 million years ago (Duggen et al., 2003). There is an increasing body of evidence that indicates that the MSC could be related to speciation processes in marine taxa (e.g., Huyse et al., 2004; Carreras-Carbonell et al., 2005). The oceanographic processes and changes in oceanic currents that occurred after the MSC could have caused an allopatric speciation event in *Palinurus* since phyllosoma larvae could have been retained in separated oceanic systems (Pollock, 1990). Nevertheless, it is worth noting that a recent analysis of phylogenetic relationships within the genus *Palinurus*, including both COI and 16S rDNA sequences, points out to a much older divergence time between the *Palinurus elephas* and *P. mauritanicus* lineages (Groeneveld et al., 2007). These authors suggest that both species were separated during the Miocene (11.2–23 My), when the African plate moved north closing the Tethys Seaway in the east. Consequently, they obtained a very slow substitution rate. These discrepancies, therefore, recommend a further revision of the divergence time among palinurid species.

In conclusion, the present study shows that present oceanographic processes and paleoecological history (e.g., glaciations) have a role in shaping the genetic variability and population structure of the European spiny lobster *Palinurus elephas*. The variability and distribution of the number of pairwise differences in *P. elephas* haplotypes points to either demographic changes or a strong selective sweep related to glacial periods. Despite a reduction on the overall genetic variability and its long planktonic larval duration, restricted gene flow was found between *P. elephas* populations, causing genetic differentiation between Atlantic and Mediterranean regions and between different areas within each basin. The European spiny lobster example indicates that mesoscale circulation patterns can be responsible of genetic differentiation within marine regions, highlights the influence of historical processes in shaping genetic variation and points to the necessity of covering most of the distribution area of marine species when trying to define the existence of genetic differentiation and possible restrictions to dispersal.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2008.04.022.

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