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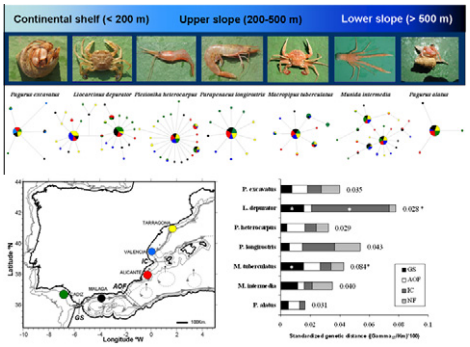
Graphical abstract

Phylogeographic patterns of decapod crustaceans at the Atlantic–Mediterranean transition **pp xxx–xxx**

V.H. García-Merchán *, A. Robainas-Barcia, P. Abelló, E. Macpherson, F. Palero, M. García-Rodríguez, L. Gil de Sola, M. Pascual



Highlights

▶ Genetic diversity of different species within families is related to depth. ▶ Shallow-water species present higher genetic diversity and structure levels. ▶ Oceanographic discontinuities have a different impact in different decapods. ▶ Phylogeographic patterns are affected by historical and contemporary processes.

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Phylogeographic patterns of decapod crustaceans at the Atlantic–Mediterranean transition

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ABSTRACT

Comparative multispecies studies allow contrasting the effect of past and present oceanographic processes on phylogeographic patterns. In the present study, a fragment of the COI gene was analyzed in seven decapod crustacean species from five families and with different bathymetric distributions. A total of 769 individuals were sampled along the Atlantic–Mediterranean transition area in order to test the effect of three putative barriers to gene flow: Strait of Gibraltar, Almeria–Oran Front and Ibiza Channel. A significant effect of the Strait of Gibraltar was found in the crabs *Liocarcinus depurator* and *Macropipus tuberculatus*. The Ibiza Channel had a significant effect for *L. depurator*. However, the Almeria–Oran front was not found to have a significant effect on any of the studied species. Higher levels of population structure were found in shallow-water species, although the number of species sampled should be increased to obtain a conclusive pattern. The haplotypes within the different species coalesced at times that could be related with past climatic events occurring before, during and after the last glacial maximum. Given the large diversity of phylogeographic patterns obtained within decapods, it is concluded that both historical and contemporary processes (marine current patterns, bathymetry and life-history traits) shape the phylogeographic patterns of these crustaceans.

1. Introduction

Decapod crustaceans are speciose and abundant, with more than 500 recognized species in the NE Atlantic and Mediterranean Sea (d'Udekem d'Acoz, 1999). They play an important role in most marine ecosystems, occupying a variety of trophic niches (Cartes et al., 2010). Many decapod species are of high commercial value and studies on their population biology and ecology have increased during the last decades (e.g. Company et al., 2008; Guijarro et al., 2009). Despite growing interest in this group, genetic structure, variability, and phylogeography of decapod species remain still poorly known (Palero et al., 2008; Sotelo et al., 2009; Kelly and Palumbi, 2010). Defining the genetic diversity and population structure of these species is necessary to better understand the influence of past and present climatic and oceanographic processes on the structure of their populations.

The use of molecular tools to study marine species has shown that both genetic variability and population structure are shaped by processes occurring at different time scales (Palumbi, 2004). Contemporary processes, such as permanent or semi-permanent oceanographic discontinuities, are among the main factors defining the population genetic structure of marine organisms (Ayre et al., 2009; Galarza et al., 2009a). Likewise, the distribution of genetic diversity levels has also been related to past events shaping the evolution and present distribution of species (e.g. Pleistocene glaciations: Hewitt, 2000; Maggs et al., 2008). In this context, mtDNA genes have been the main markers of choice, given that they provide information about past events while providing an overall picture of gene flow among populations (Avise, 2000; Reece et al., 2010) although nuclear markers have also proved to be powerful indicators of past and present events (Kenchington et al., 2009).

The Mediterranean Sea is a semi-enclosed marine basin surrounded by large continental masses and connected with the Atlantic Ocean through the Strait of Gibraltar. The patterns of water circulation in the Western Mediterranean, characterized by

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the inflow of surface Atlantic water and outflow of deeper Mediterranean water (Millot, 2005), were already established during the Pleistocene (Cacho et al., 1999). The circulation pattern and topography along the southern and eastern coasts of the Iberian Peninsula originate three main oceanographic discontinuities (Fig. 1): (1) around the Strait of Gibraltar, (2) the Almeria–Oran Front, and (3) the Ibiza Channel. The discontinuity around the Strait of Gibraltar is caused by Atlantic water fluxing into the Mediterranean through epipelagic layers (maximum depth around 100 m) and Mediterranean water exiting the basin through deep water layers (Gómez et al., 2000). Before the entry of the Atlantic waters throughout the Gibraltar Strait a branch of these waters recirculates near the Strait, in front of Cape Trafalgar, towards the northwest along the coast of Cadiz. This area is also influenced by the intense tidal-current regime of the Strait of Gibraltar and the strong topographic interaction between the swift along-shore tidal flow and a submerged ridge running perpendicular to the shoreline (García-Lafuente and Ruiz, 2007). These processes originate persistently a patch of cold water that can also affect the connectivity between populations at both sides of the Gibraltar Strait (Galarza et al., 2009b). The Almeria–Oran Front (AOF) is a semi-permanent dynamic oceanographic front connecting the main jet of incoming Atlantic water and the Mediterranean Sea (Tintoré et al., 1988). Depending on winter conditions, the AOF may decrease its strength or even disappear (Tintoré et al., 1988). Finally, the current flowing southwest along the continental slope of the northeastern Iberian Peninsula often turns around the Ibiza Channel (IC) towards the Balearic Islands (García-Lafuente et al., 1995; Salat, 1996) generating a disruptive effect on the circulation and the enclosing of Mediterranean water in the northwestern basin (Pinot et al., 2002).

Most population genetic studies in this area have focused on coastal or shallow water species, which generally have epipelagic larvae that can be strongly influenced by surface oceanographic fronts and eddies. In fact, the AOF is known to affect the population structure of some species with an Atlantic–Mediterranean distribution (Patarnello et al., 2007; Galarza et al., 2009a). However, not so much is known about the effect of GS or IC, given that very few studies have considered the possible effect of each front independently. A restrictive effect of the GS has been described in a few fishes (Galarza et al., 2009b; Sala-Bozano et al., 2009; Fruciano et al., 2011) and crustaceans (Papetti et al., 2005; Fernández et al., 2011). The IC has also shown a restrictive effect in the comber fish (Schunter et al., 2011) and in the red gorgonian (Mokhtar-Jamaï et al., 2011). Furthermore, most oceanographic processes are seasonal (Salat, 1996) and could affect gene flow between populations differentially, depending on the reproductive season of the species or the dispersal capacity of the larvae. Species having a long larval phase are generally more panmictic than those with short planktonic life (Planes and Fauvelot, 2002; Selkoe and Toonen, 2011), although some studies have questioned this relationship (e.g. Galarza et al., 2009a). Therefore, a comparative study using multiple species with different dispersal capabilities, bathymetric distributions and reproducing at different seasons is needed in order to define the relevance of these oceanographic discontinuities in shaping the genetic structure.

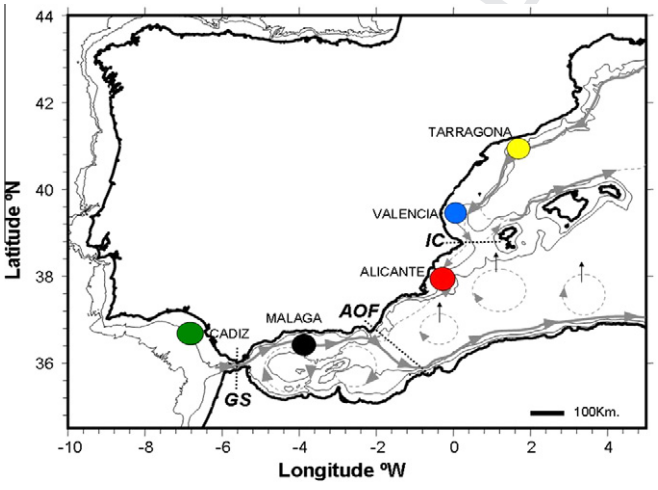

Fig. 1. Map showing the sampling localities and major oceanographic discontinuities found in the Western Mediterranean Sea and Gulf of Cadiz. Sampling localities are indicated by colored circles. The thick gray lines with arrows indicate the main direction of marine currents, and the thin lines correspond to 200 m isobaths. Dotted lines: oceanographic fronts (GS: Gibraltar Strait, AOF: Almeria–Oran Front, IC: Ibiza Channel). Solid gray lines: permanent currents. Dashed gray lines: semi-permanent gyres and currents.

The present study aims at investigating the potential effect of oceanographic discontinuities in the genetic structure of seven decapod crustacean species. A partial region of the cytochrome oxidase subunit I (COI) was analyzed in samples collected at both sides of every oceanographic barrier along the south-eastern Iberian Peninsula. The seven species, characteristic of muddy bottoms of the continental shelf and slope, have been selected according to their bathymetric distribution to evaluate whether the effect of oceanographic barriers varies with depth. We also analyzed whether the population structure is influenced by species life-history traits putatively involved in population connectivity (e.g. number of larval stages, main reproductive period). Finally, the analysis of the genetic variability in each species was used to trace historical processes in the Mediterranean Sea influencing the species phylogeography.

2. Materials and methods

2.1. Study area and sample collection

The study area encompassed the continental shelf and slope along the southern and eastern Iberian Peninsula (Fig. 1). Samples were obtained from the MEDITS_ES (Bertrand et al., 2002) and ARSA (López de la Rosa, 1997; Silva et al., 2011) fishery research surveys. The MEDITS survey, which targets the main demersal fisheries around the European Union and adjacent Mediterranean countries, is based on a common sampling protocol (Bertrand et al., 2002). The Spanish surveys were performed on board R/V 'Cornide de Saavedra'. Samples were based on a sample design randomly stratified by geographical sector and five depth strata (<50 m, 50–100, 100–200, 200–500 and 500–800 m). Each haul was performed along a fixed isobath during day-time hours. The bottom trawl gear used had a codend stretched mesh size of 20 mm which allows the capture of epibenthic and benthopelagic fish and crustaceans.

The sampling design allowed delimitation, for the present study, of several sub-areas, according to their geographic location in relation with putative oceanographic structures which might influence species connectivity: (1) Cadiz, located west of the Strait of Gibraltar, in Atlantic waters; (2) Malaga, between the Strait of Gibraltar and the Almeria–Oran Front; (3) Alicante, between the Almeria–Oran Front and the Ibiza Channel; (4) Valencia, and (5) Tarragona both located north of the Ibiza Channel. Each sampling sub-area encompassed several hauls taken within a *ca.* 50 km coastal sector. This sampling scheme, with areas evenly spaced, encompassing a broad geographic zone and with samples located at either sides of putative barriers to genetic dispersal, has been shown to be adequate in recent genetic studies carried out in the area (e.g. Calderón et al., 2008; Galarza et al., 2009a,b; Reuschel et al., 2010; Mokhtar-Jamaï et al., 2011; Schunter et al., 2011).

In order to analyze the effect of these oceanographic discontinuities on genetic population differentiation, the species were

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Table 1
Sampling locations, number of individuals sampled and diversity indices for the seven decapod species analyzed.

		Pagurus excavatus	*Liocarcinus depurator*	*Plesionika heterocarpus*	*Parapenaeus longirostris*	*Macropipus tuberculatus*	*Munida intermedia*	*Pagurus alatus*
(bp)		540	573	548	561	571	566	512
Accession #		JN564868-JN564873	JN564801-JN564829	JN564874-JN564895	JN564896-JN564906	JN564854-JN564863	JN564830-JN564853	JN564864-JN564867
Cadiz	*N* (*H*)	21 (3)	22 (10)	26 (9)	22 (5)	25 (5)	22 (8)	28 (2)
	h	0.185 ± 0.150	0.710 ± 0.106	0.622 ± 0.107	0.338 ± 0.128	0.653 ± 0.088	0.775 ± 0.068	0.071 ± 0.084
	π	0.0004 ± 0.0003	0.0029 ± 0.0008	0.0015 ± 0.0003	0.0007 ± 0.0002	0.0016 ± 0.0003	0.0033 ± 0.0005	0.0001 ± 0.0001
Malaga	*N* (*H*)	23 (3)	24 (9)	19 (6)	21 (2)	22 (4)	25 (9)	24 (2)
	h	0.245 ± 0.120	0.746 ± 0.046	0.596 ± 0.122	0.095 ± 0.084	0.333 ± 0.124	0.817 ± 0.055	0.083 ± 0.078
	π	0.0006 ± 0.0004	0.0040 ± 0.0004	0.0012 ± 0.0003	0.0002 ± 0.0001	0.0006 ± 0.0002	0.0031 ± 0.0005	0.0002 ± 0.0001
Alicante	*N* (*H*)	24 (2)	20 (6)	25 (7)	24 (1)	25 (5)	20 (9)	21 (2)
	h	0.083 ± 0.070	0.789 ± 0.057	0.537 ± 0.115	0	0.363 ± 0.147	0.747 ± 0.098	0.095 ± 0.081
	π	0.0002 ± 0.0001	0.0038 ± 0.0003	0.0011 ± 0.0003	0	0.0007 ± 0.0003	0.0033 ± 0.0007	0.0002 ± 0.0001
Valencia	*N* (*H*)	23 (4)	22 (6)	23 (5)	13 (3)	23 (5)	24 (6)	4 (1)
	h	0.249 ± 0.102	0.411 ± 0.131	0.391 ± 0.125	0.294 ± 0.135	0.324 ± 0.124	0.739 ± 0.070	0
	π	0.0006 ± 0.0003	0.0021 ± 0.0007	0.0007 ± 0.0002	0.0005 ± 0.0002	0.0006 ± 0.0002	0.0026 ± 0.0004	0
Tarragona	*N* (*H*)	23 (1)	27 (9)	25 (7) 0.590 ±	21 (4)	20 (4)	22 (9)	16 (1)
	h	0	0.604 ± 0.108	0.112	0.2714 ± 0.138	0.363 ± 0.131	0.762 ± 0.080	0
	π	0	0.0029 ± 0.0006	0.0014 ± 0.0003	0.0005 ± 0.0003	0.0006 ± 0.0002	0.0034 ± 0.0006	0
Total	*N* (*H*)	114 (6)	115 (29)	118 (22)	101 (11)	115 (10)	113 (24)	93 (4)
	h	0.152 ± 0.048	0.752 ± 0.027	0.542 ± 0.055	0.189 ± 0.054	0.420 ± 0.059	0.765 ± 0.034	0.063 ± 0.038
	π	0.0004 ± 0.0001	0.0039 ± 0.0001	0.0012 ± 0.0001	0.0003 ± 0.0001	0.0009 ± 0.0001	0.0031 ± 0.0002	0.0002±0.0001

bp: sequence length in base pairs, *N*: Number of samples, *H*: number of haplotypes, *h*: haplotype diversity, π: nucleotide diversity. Standard errors were computed from 1000 bootstrap replicates.

chosen by being present throughout the study area, belonging to different zoological groups within the Decapoda and encompassing different bathymetric distributions. The seven species are representative components of the soft bottom communities of the Western Mediterranean (Abelló et al., 1988, 2002). Two species occur on the continental shelf (<200 m): the swimming crab *Liocarcinus depurator* (Portunidae) and the hermit crab *Pagurus excavatus* (Paguridae), four species on the upper slope (200–500 m): the squat lobster *Munida intermedia* (Munididae), the crab *Macropipus tuberculatus* (Portunidae), the peneid shrimp *Parapenaeus longirostris* (Penaeidae), and the caridean shrimp *Plesionika heterocarpus* (Pandalidae), and one in the lower slope (>500 m): the hermit crab *Pagurus alatus* (Paguridae). Sample sizes per location and species are given in Table 1. The mean number of sampled individuals per population was 23 ± 1, with the exception of *P. alatus*, which could only be sampled in a lower number (19 ± 9) due to its very low frequency of occurrence and density in the Valencia sector (Abelló et al., 2002). The mean depth of occurrence, northernmost latitude, number of larval stages and main reproductive period of each species were the main life-history traits considered in the present study (summarised in Table 2). Given that there are no direct estimates for potential larval dispersal capabilities of the studied species, the number of larval stages has been used as a proxy (González-Gordillo et al., 2001). Whenever the number of larval stages was unknown for a given species we used as a proxy the value from other species of the same genus or family, since this is a rather conservative character in phylogenetically close species (Anger, 2001). The latitudinal range was used to define species as being tropical (species reproducing in summer and distributed principally between 23°S and 23°N) or mostly temperate (reproducing in winter and outside that range).

2.2. DNA extraction, amplification and sequencing

Muscle tissue from each individual was preserved in 100% ethanol and total genomic DNA extraction was performed with Chelex 10% following Estoup et al. (1996). The cytochrome oxidase I (COI) gene was amplified using the universal primers LCO1490 and HCO2198 (Folmer et al., 1994). The sequence lengths (bp) for each species are given in Table 1. PCR reactions were carried out in a 13 μl volume reaction with approximately 40 ng of genomic DNA containing 1 U of Taq polymerase (Amersham), 1× buffer (Amersham), 0.2 μM of each primer and 0.12 mM of dNTPs. The reaction profile was 94 °C for 4 min for initial denaturation, followed by 36 cycles at 94 °C for 1 min, 54 °C for 1 min, 72 °C for 1 min and a final extension at 72 °C for 7 min. A small volume (2 μl) from each PCR product was purified using the Exo-SAP method with 0.34 μl of exonuclease I (ThermoScientific) and 0.66 μl of shrimp alkaline phosphatase (Promega), incubated at 37 °C for 15 min and at 80 °C for 15 min. Cycle-sequencing was carried out using the Big Dye terminator sequencing kit v3.1 (Applied Biosystems) following the manufacturer's instructions. The sequences were obtained with an ABI PRISM®3770 automated sequencer (Applied Biosystems) from the Scientific and Technical Services of the University of Barcelona.

2.3. Diversity estimates and genetic differentiation

Sequences were visually inspected, aligned and trimmed with BioEdit v7.0.1 (Hall, 1999). Nucleotide diversity (π), haplotype diversity (*h*) and their standard deviations were calculated for each area and species using DnaSP v5 (Librado and Rozas, 2009). Haplotype networks were constructed for each species using the Median Joining network algorithm (Bandelt et al., 1999) as implemented in Network v4.5.1.6 (Fluxus Technology). The resulting networks illustrate the relationship among haplotype sequences and allow examining the geographic partitioning of the data. Haplotype sequences were deposited in GenBank under accession numbers (JN564801-JN564906) (Table 1).

Pairwise genetic differentiation among sampling sites was estimated measuring $Gamma_{ST}$ values and its significance was obtained using the Snn statistic (Hudson, 2000) as implemented in DnaSP. Pairwise $Gamma_{ST}$ values were standardized by dividing each pairwise value by its corresponding geographic distance. In this way, a genetic distance per km of geographic distance was obtained and used to evaluate the relative effect of each front on each species.

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ANOVA tests were carried out considering genetic diversity and $Gamma_{ST}$ values as dependent variables and life history traits as factors. Depth was initially classified in three levels: continental shelf (<200 m), upper (200–500 m) and lower (>500 m) slope. Northernmost latitude was classified in two levels: high (≥65°N) and low (≤50°N). Number of larval stages was grouped in two levels: low (≤6) and high (≥11). Main reproductive period in the study area was summarized in two levels: winter and summer. ANOVA tests were also used to evaluate the effect of depth within the families Paguridae (*P. excavatus* and *Pagurus alatus*) and Portunidae (*L. depurator* and *M. tuberculatus*). Before carrying out the ANOVA analyses, dependent variables were tested for normality using the Shapiro–Wilk test. Haplotype diversity followed a normal distribution. Nucleotide diversity did not fit a normal distribution after transformation and was not used. $Gamma_{ST}$ values were Ln-transformed and fit normality. ANOVA tests were performed with STATISTICA v8.0. The homogeneity of variances was evaluated with both the Figner–Killeen test and the Bartlett test as implemented in R (R Development Core Team, 2008). None of the test gave significant results and thus variances could be considered homogeneous.

In order to test for patterns of isolation by distance, comparisons between pairwise genetic and geographical distances were carried out through a Mantel test using the GenAlEx package v6.4 (Peakall and Smouse, 2006). The geographical distances were measured along the 200 m isobath using the software Karto v5.2 (Cadiou, 1994).

2.4. Neutrality tests, demographic inferences and coalescence time

To test for patterns that deviate from neutrality Fu's *Fs* (1997) was computed using DnaSP v5 (Librado and Rozas, 2009). The McDonald and Kreitman (MK) test (McDonald and Kreitman, 1991), that compares the ratio of polymorphism to divergence at non-synonymous and synonymous sites, was carried out to detect selection acting directly on the COI gene. Outgroup selection was based on sequence similarity assessed through blast searches in GenBank. *Liocarcinus maculatus* (FJ174949) was used as outgroup for *L. depurator*, *Neosarmatium fourmanoiri* (FN392165) for *P. heterocarpus*, *Alpheus cristulifrons* (FJ013896) for *P. longirostris*, *P. alatus* for *P. excavatus* and vice versa, *L. depurator* for *M. tuberculatus*, and *Munida delicata* (EU418001) for *M. intermedia*. Time elapsed since population expansion was inferred from pairwise nucleotide site differences (Mismatch distribution) for each species assuming the "sudden expansion" model and the equation: $t = \tau/2\mu k$, where τ (Tau) is the date estimate measured in units of mutational time, k is the sequence length and μ is the mutation rate per nucleotide (Rogers and Harpending, 1992). Following Rogers (1995), we assumed theta final (theta after the population growth) to be infinite in order to estimate theta initial and τ from the data. The substitution rate (μ_S) per nucleotide for the COI region was estimated from sister decapod species separated by the Isthmus of Panama (μ_S = 0.9–1.1% divergence/My) as reviewed in Ketmaier et al. (2003). Since substitution rate (μ_S) represents a lower boundary for the mutation rate within species, we followed a conservative approach after Emerson (2007). Thus, an intraspecific mutation rate (μ_1) three times faster than the substitution rate (Howell et al., 2003) was also used for dating haplotype coalescence time in all species.

3. Results

3.1. Genetic variability

A total of 769 samples were analyzed in seven decapod crustaceans, with final fragment sizes ranging from 512 bp in *P. alatus* to 573 bp in *L. depurator* (Table 1). Genetic diversity levels varied across species, with total number of haplotypes ranging between 4 and 29 (Table 1; see Appendix A for details), haplotype diversity (h) from 0.063 to 0.765, and nucleotide diversity (π) ranging from 0.0002 to 0.0039 (Table 1). When comparing haplotype diversity levels between species, three groups were observed when considering non-overlapping 95% confidence intervals (1) a high diversity group: *L. depurator* and *M. intermedia*; (2) an intermediate diversity group: *P. heterocarpus* and *M. tuberculatus*; (3) and a low diversity group: *P. excavatus*, *P. longirostris* and *P. alatus*) (Fig. 2 and Table 1).

In all cases, haplotype networks showed one or two widely distributed haplotypes and several derived haplotypes found in one population only (Fig. 3). Most of those private haplotypes were singletons (present in one individual only) and separated from the common haplotypes by one or two mutational steps. *L. depurator* had a particularly structured haplotype network, with two abundant haplotypes showing opposite geographic frequency clines. Ldep02 was present in all Mediterranean areas but not in Cadiz, and Ldep03 was predominantly present in Cadiz, Malaga and Alicante (i.e. the Atlantic area and Mediterranean areas under strong Atlantic influence) (Appendix A). No haplotype frequency clines were observed in any of the other six species.

The ANOVA test was only significant for haplotype diversity with depth (F = 6.50, P = 0.004). Furthermore, Fig. 2 suggests that within a family, haplotype diversity is higher in the shallower species than in the deeper (e.g. *L. depurator* vs. *M. tuberculatus* and *P. excavatus* vs. *P. alatus*). However when evaluating the effect of depth within families, a significant relationship between h and depth was observed only for portunid crabs (F = 7.12, P = 0.03).

3.2. Neutrality tests, demographic inferences and coalescent time

In agreement with the star-like shape of most species haplotype networks, Fu's *Fs* test yielded negative and significant values in all species, which is indicative of deviations of neutral expectation that can be due to recent expansions or selection (Table 3). When the test was independently computed for each significantly differentiated unit of *M. tuberculatus* (see below) no significant values were obtained for Cadiz (*Fs* = −0.925, P > 0.05) but significant for the grouping of the remaining populations (*Fs* = −8.746, P < 0.01). For *L. depurator* Fu's *Fs* values were also independently estimated for the three genetically differentiated units (see below) and significant values were obtained for Cadiz (*Fs* = −5.087, P < 0.05) and the populations north of the IC (*Fs* = −7.049, P < 0.05) and not significant for the group constituted by the two populations separated by the AOF (*Fs* = −3.589, P > 0.05). The MK test was only significant in *P. excavatus* and *M. tuberculatus* due to the larger frequency of non-synonymous changes when comparing polymorphism within species (Table 3, Appendix B). Pseudogene amplification can be ruled out in these species since the sequences we obtained were good and no double peaks were observed.

When haplotype coalescent times within each species were estimated from Tau using the substitution rate (μ_S), an older coalescence time of approximately 100–138 kya was found for *L. depurator* and *M. intermedia*, an intermediate coalescent time of 44–68 kya for *P. heterocarpus* and *M. tuberculatus*, and a younger coalescent time of 6–20 kya for *P. longirostris* and *P. alatus* (Table 3). For *P. excavatus* it was not possible to estimate its haplotype coalescence time given that the observed variance was larger than the mean haplotype diversity (Rogers, 1995). When we used an intraspecific mutation rate (μ_1) three times faster than the substitution rate, the estimates were placed before the Last Glacial Maximum (LGM), with 34–46 kya for *L. depurator* and *M. intermedia*, during the LGM (15–23 kya) for *P. heterocarpus* and *M. tuberculatus* and more recently (2–7 kya) for *P. longirostris* and *P. alatus* (see Table 3).

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Table 2
Main distribution and life history traits of the seven species of decapod crustaceans analyzed. Species are ordered according to mean depth of occurrence.

Sea habitat	Species	Family	Mean depth of occurrence	Latitudinal range	Main reproductive period	Number of larval stages
Continental shelf	*Pagurus excavatus*	Paguridae	92	10 N–44 N	Winter	5
	Liocarcinus depurator	Portunidae	159	20 N–68 N	Winter	6
	Plesionika heterocarpus	Pandalidae	220	17S–45 N	Summer	11
Upper slope	*Parapenaeus longirostris*	Penaeidae	250	17S–44 N	Summer	15
	Macropipus tuberculatus	Portunidae	277	27 N–65 N	Winter	6
	Munida intermedia	Munididae	379	15 N–50 N	Winter	5
Lower slope	*Pagurus alatus*	Paguridae	574	20 N–65 N	Winter	5

Note: Mean depth of occurrence from Abelló et al. (2002). Latitudinal range and mean reproductive period from d'Udekem d'Acoz (1999) and references therein. Number of larval stages from González-Gordillo et al. (2001).

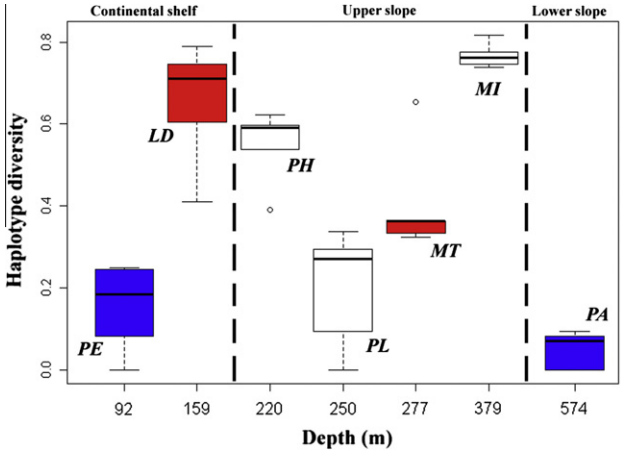

Fig. 2. Boxplot for the haplotype diversity values in the seven species. Those pairs of species belonging to the same family are highlighted in color (blue: Paguridae; red: Portunidae). (PE: *Pagurus excavatus*, LD: *Liocarcinus depurator*, PH: *Plesionika heterocarpus*, PL: *Parapenaeus longirostris*, MT: *Macropipus tuberculatus*, MI: *Munida intermedia* and PA: *Pagurus alatus*). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Genetic differentiation and oceanographic processes

Global genetic differentiation within species was only significant for *L. depurator* ($Gamma_{ST}$ = 0.228, $P < 0.001$) and *M. tuberculatus* ($Gamma_{ST}$ = 0.084, $P < 0.05$) (Fig. 4). Pairwise comparisons between Cadiz and Malaga populations showed that the Gibraltar Strait had a significant effect in these two species (Fig. 4 and Appendix C). This front had no significant effect for *M. intermedia*, despite the $Gamma_{ST}$ value between populations at both sides of the front was highest (Fig. 4). Almeria–Oran front did not cause significant genetic differentiation between populations located at both sides for none of the seven studied species. However, for *P. heterocarpus* the populations separated by this front presented the largest $Gamma_{ST}$ value. Finally, Ibiza Channel showed a significant effect only on *L. depurator*. The correlation between geographic and $Gamma_{ST}$ genetic distances assessed by the Mantel test revealed isolation by distance patterns for *L. depurator* (r = 0.779, $P < 0.05$) and *M. tuberculatus* (r = 0.695, $P < 0.05$) and yielded a marginally significant value for *P. excavatus* (r = 0.513, P = 0.054). No significant correlations between genetic and geographic distances were obtained for the other species.

The ANOVA tests comparing $Gamma_{ST}$ values in relation to the northernmost latitude showed that species reaching higher latitudes have significantly greater population genetic structuring (F = 8.45, P = 0.005). Furthermore, a positive significant relationship was observed between $Gamma_{ST}$ and depth (F = 7.37, $P < 0.001$). The higher genetic differentiation in shallow water species was also observed when evaluating the effect of depth within both families (F = 6.62, P = 0.02, for *Portunidae* and F = 6.60, P = 0.02, for *Paguridae*).

4. Discussion

In the present study, we have analyzed the effects of the three main oceanographic discontinuities occurring in the Western Mediterranean on the phylogeography and genetic structure of seven crustacean species using mitochondrial genes which integrate information of present and past processes (Avise, 2000). We used haplotype networks and coalescence times to enquire about historical events that could be related to glaciations during the Pleistocene. Our results showed that shallow water species present higher genetic differentiation than deep water species as also shown by Etter et al. (2005). Furthermore, species living at lower latitudes were less likely to present population genetic structure. Other life history traits such as the number of larval stages (as a proxy of planktonic larval duration) and main reproductive period did not influence the genetic diversity or structure patterns, as observed by Galarza et al. (2009a). However, the relatively low number of species considered in the present study recommends that further studies would strengthen the validity of these relationships. In the evaluation of oceanographic discontinuities, only the Strait of Gibraltar (for the crabs *L. depurator* and *M. tuberculatus*) and the Ibiza Channel (for *L. depurator*) seemed to act as barriers to gene flow. Surprisingly, the Almeria–Oran front, previously defined as a barrier in numerous marine organisms (e.g. Patarnello et al., 2007; Galarza et al., 2009a), showed no effect on the genetic structure on any of the studied species. This result could be due to sampling limitations or could be related to the characteristics of the molecular marker used (e.g. low diversity found in *Parapenaeus* and the pagurid crabs).

4.1. Genetic variability, population history and haplotype coalescence time

The signature of historical demographic or selection processes can be inferred from the observed genetic variability levels in natural populations. Three groups of species were identified based on mean haplotype diversity values (Fig. 2): high diversity in *L. depurator* and *M. intermedia*, intermediate levels in *P. heterocarpus* and *M. tuberculatus* and low diversity in *P. excavatus*, *P. alatus* and *P. longirostris*. The high and intermediate diversity values are similar to those reported for other crustacean species of the Atlantic-Mediterranean area such as *Carcinus maenas* (Roman and Palumbi, 2004), *Palinurus elephas* (Palero et al., 2008), or *Aristeus antennatus* (Roldan et al., 2009). Low diversity values are characteristic of populations having experienced strong bottlenecks due to founder effects (Roman, 2006), although they could also result from low lineage-specific mutation rates or natural selection. For the two studied hermit crabs (*P. excavatus* and *P. alatus*), low lineage

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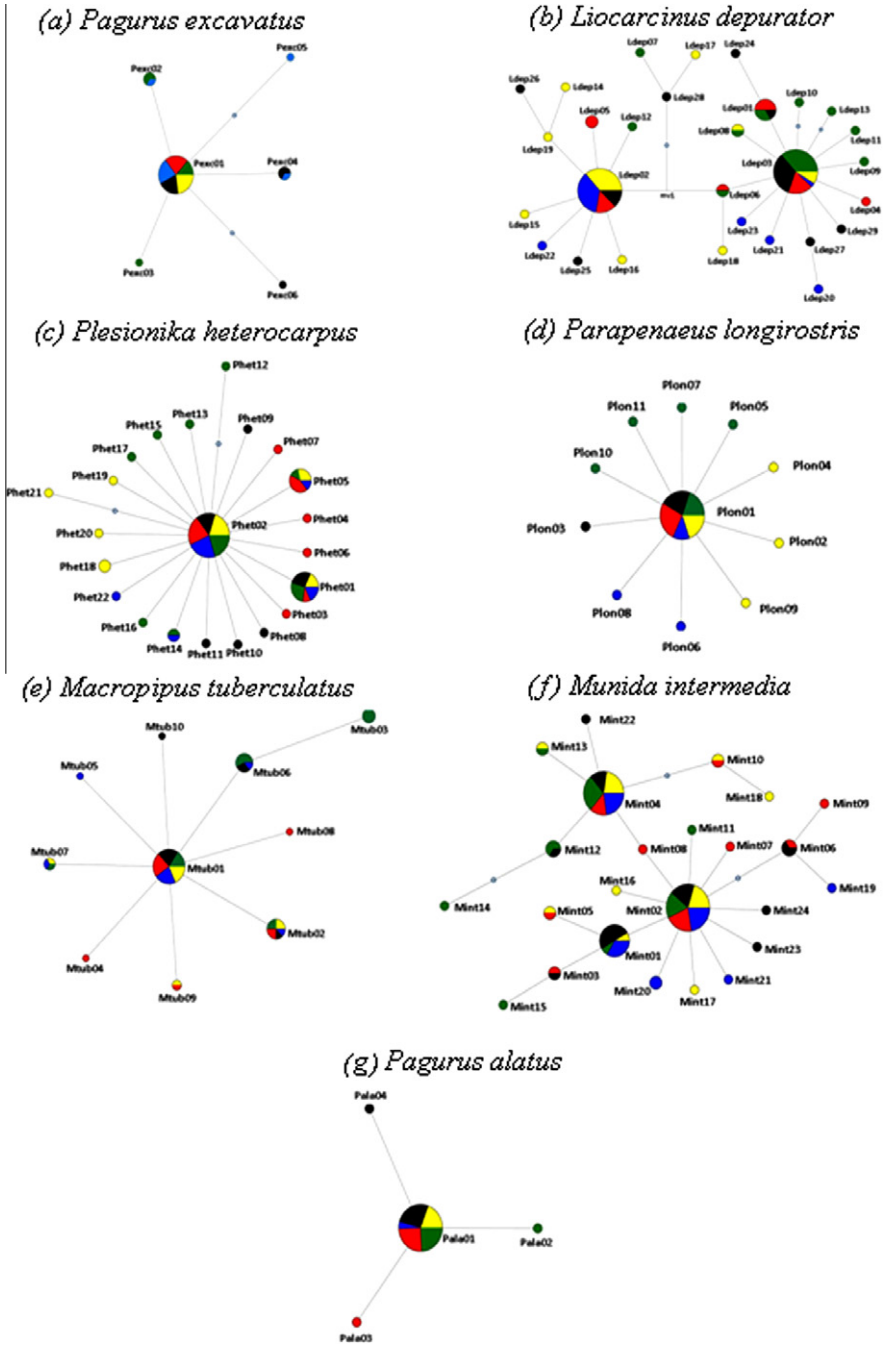

Fig. 3. Median–Joining haplotype networks of mtDNA COI sequences for each of the seven species, where (a and b) are continental shelf species, (c–f) are upper slope species and (g) is a lower slope species. Empty circles represent missing haplotypes. The haplotype pie sizes within each network are proportional to their frequency. Populations are color coded: Cadiz (green), Malaga (black), Alicante (red), Valencia (blue) and Tarragona (yellow).

specific mutation rate may be ruled out given that high nucleotide diversity values in COI gene have been found in other pagurid species (Kelly and Palumbi, 2010). Consequently, the low diversity values could be due to recent colonization of the studied area and/or selection. The low number of non-synonymous changes observed with the MK test (Appendix B) could be caused by purifying selection, as recently unveiled in other crustacean species (Palero et al., 2010). In particular, different selective pressures acting on mtDNA genes have been suggested to cause low genetic diversity estimates in species with shallow bathymetric distributions in contrast to species from the same group with a deeper distribution (Etter et al., 2005; Palero et al., 2010). On the contrary, the present study found higher genetic diversity levels in shallower water species compared to those with a deeper bathymetric distribution. However, this differentiation was only significant in portunid crabs and thus it could be species specific.

The significant Fu's *Fs* values and star-shaped haplotype networks (observed in all species included in the present work), are characteristic of species that have undergone a recent process of expansion or selection (Wares, 2010). Assuming Rogers and Harpending (1992) "sudden expansion" model allowed us to date haplotype coalescent times and therefore relate genetic diversity levels and historical processes. The time estimates found could be associated to abrupt climatic changes occurring during the late

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Table 3
Neutrality tests and coalescence times for seven decapod crustaceans distributed in the Western Mediterranean and adjacent Atlantic Ocean.

Species	Fu's *Fs*	MK test	Tau	Coalescence time (kya)	
				μ_S	μ_1
Pagurus excavatus	−6.088**	0.0024*	0.000	na	na
Liocarcinus depurator	−21.340***	1.0	1.426	113–138	38–46
Plesionika heterocarpus	−29.727***	1.0	0.672	56–68	19–23
Parapenaeus longirostris	−17.845***	1.0	0.202	16–20	5.3–6.5
Macropipus tuberculatus	−7.512***	0.0192*	0.549	44–53	15–18
Munida intermedia	−17.065***	1.0	1.269	102–125	34–42
Pagurus alatus	−5.562*	1.0	0.065	6.2–7.6	2.1–2.5

Coalescence times estimated from Tau using μ_S (substitution rate for the COI gene established in several Crustacea: 0.9–1.1% divergence/My; Ketmaier et al., 2003) and μ_1 (assuming the mutation rate is three times the substitution rate, according to Emerson (2007)). The symbol "na" indicates that haplotype coalescence could not be estimated (see main text for details).
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

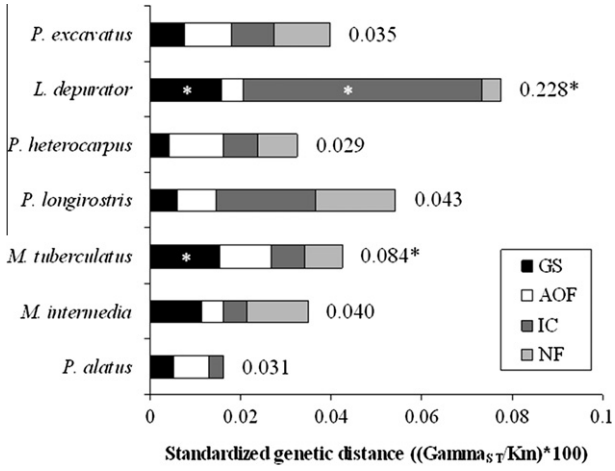

Fig. 4. Standardized pairwise Gamma$_{ST}$ values for the different decapod crustacean species across putative oceanographic discontinuities. The values in the right side of each species bar correspond to their global Gamma$_{ST}$. (GS: Gibraltar Strait (Cadiz vs. Malaga), AOF: Almeria–Oran Front (Malaga vs. Alicante), IC: Ibiza Channel (Alicante vs. Valencia), NF: No front (Valencia vs. Tarragona). *$P < 0.05$. Pairwise Gamma$_{ST}$ values for the seven species across all populations in the Atlantic–Mediterranean transition in Appendix C).

Pleistocene and Holocene (Cacho et al., 2002; Frigola et al., 2007). During the last glacial maximum (30–20 kya) the sea level decreased up to 120 m (Lambeck and Chappell, 2001) although did not significantly change the oceanographic processes occurring in the area (Cacho et al., 1999). For both *L. depurator* and *M. intermedia* haplotypes, coalescence times may be related to an abrupt descent of sea temperatures in north Atlantic waters driving an intensive cooling of the Alboran Sea (westernmost portion of the Mediterranean Sea) at 38–40 kya (Cacho et al., 2002). For *P. heterocarpus* and *M. tuberculatus,* the haplotypes described within each species coalesced approximately at 20 kya coinciding with the Last Glacial Maximum (LGM). Sea level and sea surface temperatures are known to have increased in the studied area after the LGM (Cacho et al., 2002) so that higher temperatures could then have favoured the range expansion of species with a tropical distribution and summer reproduction such as *P. heterocarpus* and *P. longirostris* (Table 1). These species could postglacially colonize and further expand its distribution area towards the Mediterranean Sea as indicated in *Melicertus kerathurus*, which presents a similar distribution range (Pellerito et al., 2009). Finally, *P. alatus* presents the most recent haplotype coalescent time and could be related to a cold event detected in the North Atlantic 2.5 kya (Frigola et al., 2007). Despite this close agreement between coalescent times and past climatic events, it should be stressed that not only demographic but also other processes, such as selection linked to climatic events, may have influenced the observed COI diversity patterns.

4.2. Genetic differentiation and oceanographic discontinuities

The effect of the Strait of Gibraltar on genetic differentiation was only significant for the two portunid crabs, *L. depurator* and *M. tuberculatus*. Significant differences at both sides of the Strait of Gibraltar have been previously observed in a few crustacean and fish species (Papetti et al., 2005; Galarza et al., 2009b; Sala-Bozano et al., 2009; Fernández et al., 2011). The circulation pattern at the Strait of Gibraltar may affect species differentially according to the distribution of their larval phases along the water column. The Atlantic water flowing inwards could transport *L. depurator* epipelagic larvae (Abelló and Guerao, 1999) but prevent the outwards transport of larvae from the Mediterranean. This process is clearly observed in the distribution of the two most frequent *L. depurator* haplotypes, presenting opposite clinal patterns and with the most frequent Mediterranean haplotype being absent in the Atlantic area (see Appendix A). For *M. tuberculatus* the presence of an Atlantic private haplotype (Mtub03, Appendix A) seems to be the cause of the population differentiation between the two basins and suggests that Atlantic larvae have restricted movement towards the Mediterranean Sea and could be located in the deeper layers (Gómez et al., 2000). However, given that a single marker was used to assess genetic differentiation, the possibility of local adaptation cannot be ruled out in either *L. depurator* or *M. tuberculatus*. The fact that both species belong to the Portunidae and could be under similar selective pressures indicates that this point merits further consideration and that an independent set of nuclear neutral markers should be tested on these samples. As for the absence of genetic differentiation in the other species, it would seem to indicate that the depth distribution of their larval stages could encompass the whole water column (see Queiroga and Blanton, 2005; Dos Santos et al., 2008) and therefore facilitate genetic homogenization between populations. In any case, the lack of reliable data on larval behavior for these species recommends further studies to confirm the relationship between gene flow and water dynamics.

The Almeria–Oran Front (AOF) is a semi-permanent dynamic oceanographic structure (Tintoré et al., 1988) that has been described as the main barrier causing genetic discontinuities along the Atlantic–Mediterranean transition area (e.g. Patarnello et al., 2007; Galarza et al., 2009a; Reuschel et al., 2010). The AOF would affect larval dispersion mainly in those species having epipelagic stages while it would not affect so much those species whose

larvae are distributed throughout the water column. Despite the fact that our sampling strategy was specifically designed to include populations at both sides of the front, we did not detect its effect in any of the seven decapod studied species. This is in agreement with a recent phylogeography study on the red shrimp *A. antennatus* (Fernández et al., 2011). The absence of effect of this front in *L. depurator*, a species with coastal epipelagic larvae, could be related to the winter relaxation of the AOF (Tintoré et al., 1988) coinciding with the main planktonic larval development season of this species (Abelló, 1989).

Finally, the Ibiza Channel only showed a significant effect on the genetic structure in the case of *L. depurator* populations. The water masses transported by the northern current often block the circulation across the Ibiza Channel in the upper epipelagic layers, diverting large volumes of water to the northeastward Balearic Current (López-Jurado et al., 2008; Monserrat et al., 2008). The intensities of the oceanographic processes occurring in this area are stronger in winter (Pinot et al., 2002), coinciding with the main reproductive period of *L. depurator*, and can restrict the genetic connectivity between its populations at both sides of the Channel as observed in the red gorgonian and the comber fish (Mokhtar-Jamaï et al., 2011; Schunter et al., 2011). However, no significant association was found between genetic differentiation and main reproductive period for all species. Nevertheless, the significant isolation by distance patterns observed in *L. depurator* and *M. tuberculatus* suggest that their genetic population structure may not only be influenced by the oceanographic discontinuities and that active and passive dispersal, along with historical colonization and local adaptation processes, could be responsible for the observed patterns.

5. Conclusions

Overall, our results indicate that species living along the continental slope have a low genetic structure, being less affected by oceanographic processes occurring in the upper layers. The Almería–Oran Front, despite being considered as the main oceanographic discontinuity separating Atlantic and Mediterranean populations, showed no effect in the species analyzed in this study. This result indicates that the effect of this front cannot be generalized and that other discontinuities, such as the Gibraltar Strait, can reduce the gene flow between the two basins. The Ibiza Channel also appears as a significant barrier influencing connectivity between populations. Finally, the present study showed that both current and historical processes have to be considered together when analyzing genetic variability and population differentiation in marine species.

6. Uncited references

Garoia et al. (2004) and Guarniero et al. (2004).

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.11.009.

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