Isolation By Distance (IBD) signals in the deep-water rose shrimp *Parapenaeus longirostris* (Lucas, 1846) (Decapoda, Panaeidae) in the Mediterranean Sea

S. Lo Brutto*, T. Maggio, M. Arculeo

Dept. STEBICEF, University of Palermo, Via Archirafi 18, 90123 Palermo, Italy

ABSTRACT

The identification of boundaries of genetic demes is one of the major goals for fishery management, and few Mediterranean commercial species have not been studied from a genetic point of view yet. The deep-water rose shrimp *Parapenaeus longirostris* (Lucas, 1846) is one of the most important components of commercial landings in Mediterranean, its fishery aspects have received much attention, regrettably without any concern for the genetic architecture of its populations. The population structure in the central and eastern Mediterranean Sea (captures from six Italian and two Greek landings) has been analysed on the basis of surveys carried out with mitochondrial and AFLP markers. Data revealed the presence of a gradual discrepancy along a west–east axis. This species, occurring mainly at a depth of between 100 and 400 m, is not strongly confined in isolated demes, but it demonstrates an 'Isolation By Distance' model, within the Mediterranean Sea, which includes geographical areas with a some degree of isolation. The role of hydrodynamic forces, such as currents, water fronts, is discussed; and a further evidence of the 'Levantine isolation' within Mediterranean basin is shown.

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1. Introduction

The deep-water rose shrimp, *Parapenaeus longirostris* (Lucas, 1846) is an epibenthic, natantian shrimp with thermophilic behaviour, a preferential range of 14–15 °C (Ungaro and Gramolini, 2006), a rapid growth rate and a wide geographic distribution from the Western Atlantic Ocean throughout the entire Mediterranean Sea (Sobrino et al., 2005). In the latter, the species inhabits sandy–muddy bottoms (Nouar and Maurin, 2001) and its bathymetric distribution ranges from between 50 m and 750 m although its maximum abundance has been observed between depths of 100 and 500 m (Holthius, 1980; Tom et al., 1988; Lembo et al., 1999; Abelló et al., 2002). *P. longirostris* shows a marked size-dependent distribution along depth, with small individuals being found at the edge of the continental shelf and larger specimens distributed at greater depths (Froglia, 1982; Ardizzone et al., 1990; Lembo et al., 1999; Abelló et al., 2002; Bilgin et al., 2009; Guijarro et al., 2009). Both sexes reach maturity in the first year of life (Froglia, 1982) and adults move during the spawning period to shallower waters where planktonic larvae have been found. After spawning, the post

larva reaches the sandy-muddy bottoms on the continental shelf and begins a bentho-pelagic cycle (Heldt, 1938; Tom et al., 1988; Dos Santos, 1998). *P. longirostris* does not have a single reproductive peak, as found in shrimps occurring at greater depths (like *Aristeus antennatus*), and maturing females can be found all year round in the Mediterranean waters (Bayhan et al., 2005; Dereli and Erdem, 2011; Nouar et al., 2011).

Whilst natantian and a feeder of zooplankton (Nouar et al., 2011), *P. longirostris* is a eurybathic species, having a tight relationship with the sea bottom (Colloca et al., 2004). Indeed, it can be considered as an active, carnivorous predator of bathypelagic, benthic and endobenthic preys (Kapiris, 2004), displaying a secondary activity of scavenging, which is commonly attributed to reptantian decapods, (Cartes, 1995).

P. longirostris is one of the most important fishery resources of the epibathyal grounds along Mediterranean coasts, and recent FAO statistics regarding landings have reported it as the fifth species among crustaceans. It is also the target species for the Italian, Spanish and French bottom trawl (FAO FishStat plus vers 2.3 http://www.fao.org/fishery/statistics/software/fishstat/en). The exploitation of this species takes place all year round, even if peak landings occur from March to July (Levi et al., 1995; Abdel Razek et al., 2006). Studies regarding the deep-water rose shrimp have primarily focused on its distribution, biology (Bombace, 1975; Lembo et al.,

2000; Mori et al., 2000; Abelló et al., 2002; Bayhan et al., 2005; Arculeo, 2009), and fishery aspects (Sbrana et al., 2003; Sobrino et al., 2005; Sbrana et al., 2006; Ligas et al., 2011; Aydın and Tosunoğlu, 2012). The population dynamics has been studied, in particular regarding the link between population abundance and environmental factors (Guijarro et al., 2009; Ligas et al., 2011). Unfortunately, there is a paucity of studies investigating the genetic population structure of *P. longirostris*, although such an integrative approach would be a helpful tool in understanding the effects of environmental changes on the species.

Recently, García-Merchán et al. (2012) analyzed a partial region of the mitochondrial cytochrome oxidase subunit I (COI) of seven decapod species, among which four samples of *P. longirostris*, collected at both sides of the oceanographic discontinuity area separating Atlantic and Mediterranean. Their results did not score a genetic break within *P. longirostris*, highlighting that the effect of the fronts, placed in the Gibraltar Strait area, though barriers to gene flow for several species, cannot be generalized for all the decapods. García-Merchán et al. (2012) proposed that deeper is the decapod species lower is the genetic structure detected, being less affected by oceanographic processes occurring in the upper layers. However, they argued that the relatively low number of species studied recommends further studies and that the results could be due to the characteristics of the single molecular marker used.

The paper by García-Merchán et al. is a good example of how genetic approach can be a helpful tool in understanding the effects of environmental variables within a species.

As a matter of fact, studies regarding the genetic population analysis of other Mediterranean decapods (*A. antennatus* in Maggio et al. (2009a); Lo Brutto et al., 2012; *Melicertus* (*Penaeus*) *kerathurus* in Pellerito et al. (2009); *Nephrops norvegicus* in Stamatis et al. (2006)) describes patterns explained by three causes acting solely or in combination: (i) the effect of oceanographic features such as currents, water fronts, physical barriers; (ii) features relating to life history, such as larval dispersal or adult displacement ability; and (iii) the role of geographical distance on gene flow (Isolation By Distance, IBD). Generally, the genetic architecture depicted by Mediterranean decapods reveals species-specific patterns (García-Merchán et al., 2012), which make it difficult to design management actions suitable for commercial species.

The aim of this Paper is to assess the genetic population structure of *P. longirostris*, employing nuclear (Amplified Fragment Length Polimorphism, AFLP) and mitochondrial (Control Region sequences) markers to assess whether distinct stocks occur in the Mediterranean Sea. The comparison of these two markers enable us to make a more comprehensive investigation into genetic diversity; specifically, highly resolving DNA finger-printing techniques (such as AFLP markers and mtDNA control regions) have proven to be valuable in estimating genetic diversity in natural populations (Lo Brutto et al., 2012; García-Merchán et al., 2012).

2. Methods

2.1. Sampling

Samples of *P. longirostris* from the Mediterranean Sea were collected from the Tyrrhenian sub-basin (TYR1, TYR2 and TYR3), the Strait of Sicily (SS1 and SS2), Adriatic Sea (AD1) and from the Aegean Sea (AEG1 and AEG2) (Table 1 and Fig. 1 for details). Genomic DNA was extracted from 20 mg of ethanol-preserved pleopods accordingly to the protocol of the DNeasy Tissue Kit (Qiagen).

Table 1
Samples sites of *Parapenaeus longirostris*, analyzed with AFLP and mtDNA.

	Location of landings	Mediterranean sub-basin	Sample code	N AFLP	N mtDNA
1	Porto S. Stefano (Tuscany, Italy)	Tyrrhenian Sea	TYR1	40	14
2	Terrasini (north Sicily, Italy)		TYR2	22	17
3	Porticello (north Sicily, Italy)		TYR3	22	15
4	Scoglitti (south Sicily, Italy)	Strait of Sicily	SS1	30	17
5	Porto Palo (south Sicily, Italy)		SS2	30	14
6	Fano (The Marches, Italy)	Adriatic Sea	AD1	24	15
7	Crete (Greece)	Aegean Sea	AEG1	24	6
8	Kavala (Greece)		AEG2	48	9

N, sample size.

2.2. AFLP analysis

The AFLP analysis was performed in accordance with the description by Vos et al. (1995). Restriction digests were carried out in 40 μl reactions, using 200 ng of genomic DNA, 5 U *Taq* I, 5U *Eco*RI, 1 × buffer RL (50 mM TrisHAc, 50 mM MgCl$_2$, 250 mM KAc, 25 mM DTT, 25 ng/μl BSA) for 1 h at 65 °C and 1 h at 37 °C. Diluted ligation product was used as template for a pre-selective amplification. The successive selective PCR was performed by using the pre-selective PCR products. Twelve primer pairs were analysed and five selective primer combinations were chosen to generate all AFLP profiles: *Eco*RI-AAA + *Taq*I-AGG, *Eco*RI-AAA + *Taq*I-ATG, *Eco*RI-AAA + *Taq*I-ACA, *Eco*RI-ATT + *Taq*I-AGG and *Eco*RI-ATT + *Taq*I-ATG. Selective PCR products were separated on an ABI PRISM 310 automated sequencer (Applied Biosystem) with a GeneScan Rox 500 standard internal size. Electropherograms were subsequently analysed using Genescan 2.02 and Genotyper 2.5 (Applied Biosystems).

The percentage of polymorphic loci (5% level) and unbiased estimates of genetic diversity were computed using Aflp-Surv 1.0 software with a non-uniform prior distribution of allele frequencies (Vekemans et al., 2002). Allelic frequencies at AFLP loci were calculated from the observed frequencies of the fragments using the Bayesian approach, as proposed by Zhivotovsky (1999) for diploid species. Statistics relating to gene diversity were computed, using Aflp-Surv 1.0 software, strictly following the treatment proposed by Lynch and Milligan (1994); total gene diversity H_T (Nei, 1987) was calculated by pooling all individuals into a single metapopulation. H_E, *within-population* Nei's gene diversity (Nei, 1987), was also computed.

An identification of outlier loci was carried out in accordance with the approaches of Beaumont and Nichols (1996) and Beaumont and Balding (2004), as implemented by the Dfdist software (http://www.rubic.rdg.ac.uk), following the procedure modified by Caballero et al. (2008). The procedure of identifying outlier loci is based on the assumption that loci under selection exhibit higher or lower F_{ST} values than the majority of neutral loci. F_{ST} is an estimate of genetic differentiation among subpopulations related to gene flow. Dfdist initially calculates empirical F_{ST} values for each locus and, from the empirical distribution, the trimmed mean F_{ST} is determined by removing the highest and lowest 30% observed in the empirical dataset. The software performs a coalescent simulation (50,000 realizations) to generate data sets with a mean F_{ST}, which equals the trimmed mean F_{ST}, to obtain significant values which are higher or lower than the quantile limits (5%, 50% and 95%). Four loci with higher or lower F_{ST} values were considered under directional and balancing selection respectively, and, as outliers, excluded from the subsequent analysis.

Population differentiation was assessed by a hierarchical Analysis of MOlecular VARiance (AMOVA), using Arlequin 3.0 (Excoffier et al., 2005), clustering the samples in different ways according to
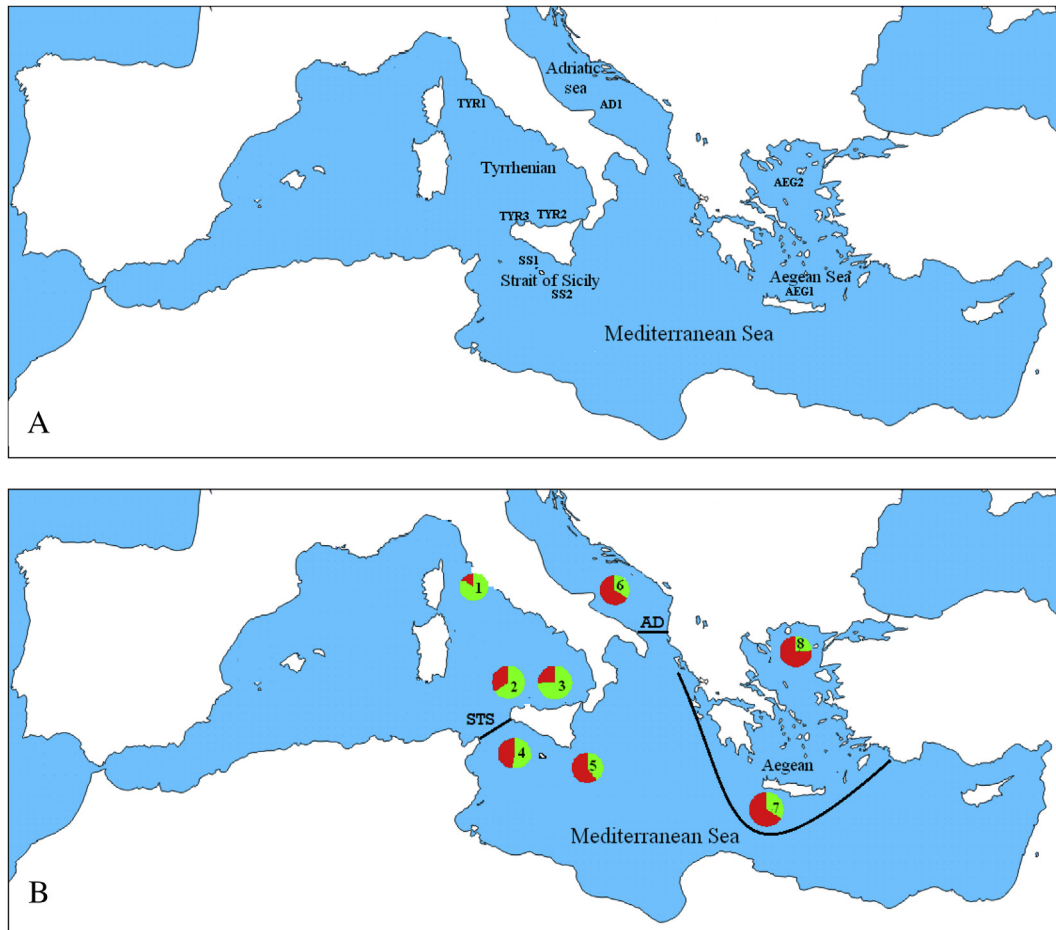
Fig. 1. A: location of *Parapenaeus longirostris* samples analysed with AFLP and mitochondrial control region (see Table 1 for details). B: bi-coloured pies representing the distribution of the two AFLP clusters resulting from the SᴛRUCTURE performance ($K = 2$) for the eight samples; and the main oceanographic fronts and boundaries (STS, Siculo Tunisian Strait; AD, Adriatic barrier; Aegean or 'Levantine isolation') affecting genetic pattern of the deep-water rose shrimp.

their geographic location. This analysis permitted us to verify the partitioning of genetic variation *among-populations* on the whole, without the design of groups of populations, and a upper hierarchical level of groups of populations. Within and among the groups generated the following partition of diversity: $\Phi_{ST,}$ (the average *within-populations* diversity within groups), Φ_{SC} (the average *among-populations* diversity within groups) and Φ_{CT} (*among-groups*). Population relationships were also investigated by building a UPGMA (Unweighted Pair Group Method with Arithmetic mean) tree with MEGA 4 (Tamura et al., 2007), based on Nei's genetic distance, as calculated in Aғʟᴘ-Sᴜʀᴠ, and topological confidence was evaluated with 1000 bootstrap replicates.

The values for pairwise Reynolds genetic distances, as calculated in Aғʟᴘ-Sᴜʀᴠ, were plotted against geographical distances, under the null hypothesis of a one-dimensional stepping-stone model. The strength and significance of this relationship were tested by Mantel's permutation test (Mantel, 1967), on a Aʀʟᴇǫᴜɪɴ platform. Geographic distances between sampling sites were calculated as the shortest water-way along the 200 m bathymetric line.

Population differentiation was also inferred using a Bayesian approach, as implemented by Sᴛʀᴜᴄᴛᴜʀᴇ (Pritchard et al., 2000). This software was used to examine the most likely number of distinct genetic clusters (K), assigning individuals to populations and to identify migrants and admixed individuals. Ten replicates for every value of K were performed, with K ranging from 1 to 8 (burn-in length 100,000). The true number of genetic clusters, K, is

commonly identified as that with the highest posterior probability ($P(X|K)$), but since an increase in the probability values could lead to an overestimation of the number of genetic clusters, the procedure suggested by Evanno et al. (2005) was used to search for the lowest number of K based on the second order rate of variation of $\ln P\,(D)$. Delta (K) is the mean of the second order rate of change of the $\ln P\,(D)$ values of a given K divided by the $\ln P\,(D)$ standard deviation.

2.3. mtDNA analysis

The putative mitochondrial non-coding control region was amplified by using Ile-3 and 12S-2 primers (Chu et al., 2003). PCR was carried out in a Perkin Elmer Cetus Thermal cycler in a 25 μl solution containing 1 ng genomic DNA, 0.2 μM each dNTPs, 0.1 μM each primer, buffer 10 × containing 10 mM Tris−HCl (pH 9), 50 mM KCl, 1.5 mM MgCl$_2$ and 2.5 units of Perkin Elmer *Taq* polymerase. The thermal cycling profile began at 94 °C for 3 min as a hot start, followed by 5 cycles of 94 °C (60 s), 48 °C (45 s) and 72 °C (60 s), 35 subsequent cycles of 94 °C (60 s), 52 °C (45 s) and 72 °C (60 s), with a final step of 10 min at 72 °C for the termination of PCR. PCR products were separated on a 2% agarose gel which had been stained with ethidium bromide. Thereafter, they were purified using a Qiaquick PCR purification Kit and sequenced on an ABI Prism 310 automated sequencer (Applied Biosystems). The sequences were aligned using Cʟᴜsᴛᴀʟ X (Thompson et al., 1997) on a

default setting and thereafter the sequence alignment was visually rechecked. The data were analysed for estimating intra-population diversity, including nucleotide (π) and haplotype (h) diversity. Statistical data analyses were conducted using DnaSP 4.10 (Rozas et al., 2003), MEGA 4 and Arlequin 3.0 softwares.

The overall genetic differentiation among samples was computed by AMOVA as described above. Pairwise Φ_{ST} values were calculated to test genetic differentiation between each sample by using Arlequin 3.0, and the sequential Bonferroni test (Rice, 1989) was used to test significance. The pairwise values of $F_{ST}/(1 - F_{ST})$ were then plotted against geographical distances (one-dimensional stepping-stone model) to verify the Isolation By Distance (IBD) model (Slatkin, 1993). The null hypothesis of no correlation between genetic differentiation and geographic distances was tested by applying the Mantel permutation test. A UPGMA dendrogram illustrating the pattern of differentiation among samples was constructed, using Reynolds Distance by applying the method implemented in the MEGA software.

3. Results

3.1. AFLP results

The three primer combinations yielded a total of 143 polymorphic loci in the 240 analysed specimens from 8 populations. The proportion of polymorphic loci across the populations ranged from 45% to 95%. Total gene diversity, H_T was 0.291 and the within-population Nei's gene diversity, H_E, ranged from 0.175 (AD1) to 0.325 (TYR1).

The hierarchical AMOVA was conducted on the neutral dataset excluding the detected outlier and clustering the samples in different ways in order to define the distribution of genetic variation. In the first clustering (i.e. *all together*) the highest proportion of genetic variation was found *within-population* and a significant value of Φ_{ST} was reported (Table 2). When the samples were clustered into four groups (analysis 2$_{AFLP}$), according to their location in the Mediterranean sub basins (TYR + SS + AD + AEG), the greater portion of genetic variation was always *within-populations* (79.53%) and the *among-groups* fixation index proved to be significantly different from zero ($\Phi_{CT} = 0.118$; $p < 0.001$). Subsequently, the exclusion of the AEG samples (analysis 3$_{AFLP}$) resulted in the reduction of the *among-groups* fixation index ($\Phi_{CT} = -0.001$; N.S.); the same result was obtained in the Analysis 4$_{AFLP}$ in which the TYR samples were excluded ($\Phi_{CT} = -0.001$; N.S.). The results of Analysis 3$_{AFLP}$ and 4$_{AFLP}$ indicated that the greatest contribution to the differences among the four Mediterranean sub basins depended on the Aegean (AEG) and Tyrrhenian (TYR) areas, which represented the most divergent groups.

The UPGMA tree revealed the deep separation of the Aegean samples (AEG) from the remaining populations (Fig. 2). The Mantel's test, applied to the relationship between genetic vs. geographical distances, indicated a significant relationship ($p = 0.007$, $r = 0.88$) and thus supporting an Isolation By Distance model (Fig. 3). The strongest differentiation occurred between the most distant samples (TYR1-2 and AEG1-2) and the remaining samples fitted a clinal distribution of the gene pool.

The Bayesian analysis implemented in Structure yielded the highest value for posterior probability for $K = 8$, indicating the presence of a substructure among *P. longirostris* samples. The ΔK vs K curve indicated most support for $K = 2$. In accordance with this model, the specimens from TYR were strongly assigned to the first cluster, and the samples from SS and AD were scattered more or less randomly to the two clusters, with the AEG samples being primarily assigned to the second cluster (Fig. 4).

Table 2
AMOVA conducted on samples of *Parapenaeus longirostris* with AFLP (analyses 1$_{AFLP}$, 2$_{AFLP}$, 3$_{AFLP}$ and 4$_{AFLP}$) and mtDNA (analyses 1$_{mtDNA}$, 2$_{mtDNA}$, 3$_{mtDNA}$ and 4$_{mtDNA}$) data, clustering in four different ways. Analysis 1: all together; Analysis 2: grouping Tyrrhenian Sea, Strait of Sicily, Adriatic Sea, Aegean Sea, (TYR + SS + AD + AEG); Analysis 3: grouping TYR + SS + AD, excluding Aegean samples; Analysis 4 three groups Strait of Sicily (SS), Adriatic sea (AD), Aegean sea (AEG) excluding Tyrrhenian sea; * indicates $p < 0.05$ and **$p < 0.001$.

|	Sample clustering	Variation source	Degrees of freedom	Variation (%)	Fixation indices
1$_{AFLP}$	All together	Among-populations	7	18.5	Φ_{ST} 0.185**
		Within-populations	125	81.5	
2$_{AFLP}$	Four groups:	Among-groups	3	11.78	Φ_{CT} 0.118**
	TYR + SS + AD + AEG	Among-populations within groups	4	8.69	Φ_{SC} 0.098**
		Within-populations	125	79.53	Φ_{ST} 0.204**
3$_{AFLP}$	Three groups:	Among-groups	2	−0.08	Φ_{CT} −0.001
	TYR + SS + AD	Among-populations within groups	3	14.02	Φ_{SC} 0.140*
		Within-populations	67	86.06	Φ_{ST} 0.139*
4$_{AFLP}$	Three groups:	Among-groups	2	7.25	Φ_{CT} 0.090
	SS + AD + AEG	Among-populations within groups	2	20.76	Φ_{SC} 0.207*
		Within-populations	57	71.99	Φ_{ST} 0.280*
1$_{mtDNA}$	All together	Among-populations	7	5.10	Φ_{ST} 0.051**
		Within-populations	99	94.90	
2$_{mtDNA}$	Four groups:	Among-groups	3	4.12	Φ_{CT} 0.046*
	TYR + SS + AD + AEG	Among-populations within groups	4	1.76	Φ_{SC} 0.022*
		Within-populations	100	94.12	Φ_{ST} 0.058**
3$_{mtDNA}$	Three groups:	Among-groups	2	−0.51	Φ_{CT} −0.005
	TYR + SS + AD	Among-populations within groups	3	0.99	Φ_{SC} 0.009
		Within-populations	86	99.52	Φ_{ST} 0.005
4$_{mtDNA}$	Three groups:	Among-groups	2	0.42	Φ_{CT} 0.004
	SS + AD + AEG	Among-populations within groups	2	0.95	Φ_{SC} 0.009
		Within-populations	57	98.63	Φ_{ST} 0.013*
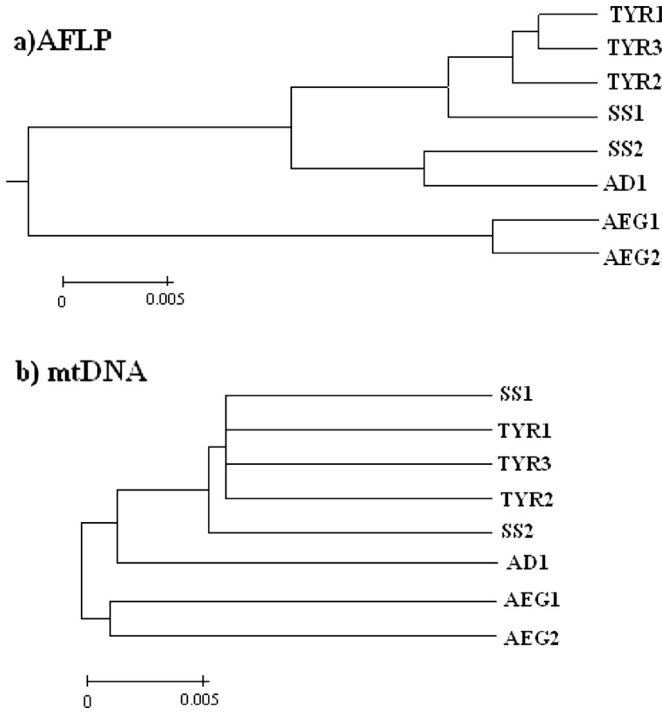
Fig. 2. UPGMA dendrograms based on Nei's genetic distance for AFLP (a) and on Reynolds distance for mtDNA (b). The scale bars represent the genetic distance.

3.2. mtDNA results

The 307 bp fragment revealed 77 different haplotypes among 107 analyzed specimens, of which only 8 were shared among different individuals and the remaining 69 were unique. The shared
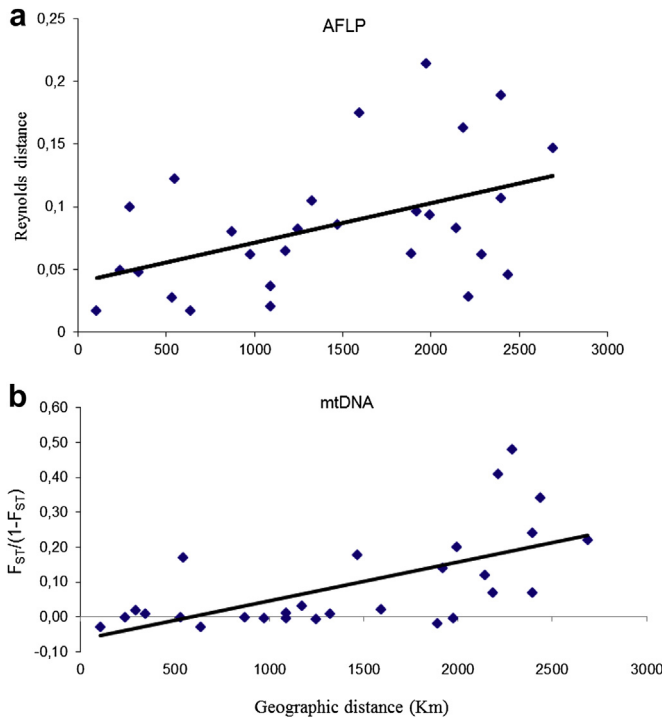

Fig. 3. Relationship between genetic vs. geographical distances showing an Isolation By Distance (IBD) pattern for AFLP (a) and mtDNA (b) data. Genetic differentiation was computed as Reynolds distance for AFLP and as $F_{ST}/(1 - F_{ST})$ for mtDNA and plotted against geographic distance in Km. Lines represent the regression lines over all pairwise comparisons.
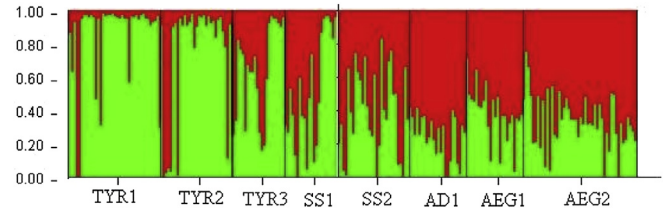

Fig. 4. Bayesian assignment analysis for $K = 2$. Each bar represents an individual of *Parapenaeus longirostris* and its associated probability of belonging to one of the two genetic clusters detected (green and red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

haplotypes frequency ranged from 0.018 to 0.159; the 77 haplotypes differed from one another by 1–51 mutations. The most frequent haplotype was common to all the samples excluding from AEG. Haplotype diversity (h) within populations was high, ranging from 1 (in TYR1, AD1, AEG1 and AEG2) to 0.867 (TYR2). Nucleotide diversity (π) was low, ranging from 0.022 (TYR3) to 0.054 (AD1) (Table 3).

The hypothesis of panmixia among Mediterranean populations was rejected with the high statistical significance from the AMOVA analyses. Indeed, the *all together* and *four groups* analysis reported a significant value for the fixation indices (Analysis 1$_{mtDNA}$ $\Phi_{ST} = 0.051$; $p < 0.001$; Analysis 2$_{mtDNA}$ $\Phi_{CT} = 0.046$; $p < 0.001$). As reported in AFLP results, when the AEG and the TYR group were excluded from the analysis (Analysis 3$_{mtDNA}$ and Analysis 4$_{mtDNA}$, respectively; Table 2) no indication of an *among-groups* structure was found and the fixation index was not significantly different from zero. Pairwise Φ_{ST} values were also reported (Table 4) and, again the two Aegean populations (AEG1 and AEG2) differed significantly from all the others. A Mantel test revealed a significant correlation between $F_{ST}/(1 - F_{ST})$ and geographic distances ($r = 0.85$; $p = 0.005$), indicating an Isolation By Distance pattern among the populations of *P. longirostris* from central to eastern Mediterranean Sea, similarly in the mitochondrial dataset (Fig. 3). The UPGMA tree constructed with the Reynolds distance is shown in Fig. 2, which confirms a clear geographic partitioning of genetic distance with AEG and TYR samples as being the most divergent, though TYR forms a polytomy with the samples from SS.

4. Discussion

This study provides the first data regarding the genetic structure of the deep-water rose shrimp *P. longirostris* in the central and eastern Mediterranean Sea, using nuclear and mitochondrial markers.

AFLP and mitochondrial control region revealed concordant results. The markers rejected the null hypothesis of panmixia in favour of a heterogeneous distribution of the genotypes and haplotypes. Results of the genetic distances and assignment methods

Table 3
Genetic diversity of the mitochondrial control region of *Parapenaeus longirostris*.

Sample	Haplotypes	h	π
TYR1	14	1.000	0.034
TYR2	11	0.882	0.024
TYR3	11	0.933	0.022
SS1	14	0.971	0.046
SS2	11	0.956	0.027
AD1	15	1.000	0.054
AEG1	6	1.000	0.040
AEG2	9	1.000	0.039

h, haplotypic diversity; π, nucleotidic diversity.

Table 4
Pairwise Φ_{ST} values, based on the mitochondrial control region sequences of samples of Parapenaeus longirostris.

	TYR1	TYR2	TYR3	SS1	SS2	AD1	AEG1	AEG2
TYR1	–							
TYR2	−0.005							
TYR3	−0.001	−0.029						
SS1	0.011	−0.002	0.008					
SS2	−0.003	−0.018	−0.030	0.018				
AD1	−0.004	0.009	−0.007	0.020	−0.019			
AEG1	0.068*	0.168**	0.121*	0.068*	0.107*	0.032		
AEG2	0.184**	0.324**	0.290**	0.193**	0.252**	0.146**	0.144*	–

*$p < 0.05$ and **$p < 0.001$.

assessed a significant and gradual differentiation from the Tyrrhenian (TYR), Adriatic (AD), Strait of Sicily (SS), to the Aegean (AEG) area, describing an Isolation By Distance (IBD) model. This was evidenced by a positive correlation between the genetic and geographic distance (Fig. 3) along an ideal west–east axis (Fig. 1). The Bayesian analysis (Fig. 4), with the two hypothesized genetic clusters ($K = 2$), showed a clinal population structuring over a large range in which the nearest sampling areas were the most genetically similar, and the proportion of membership of the individuals to the two identified genetic clusters increased/decreased from west to east and vice versa.

The clinal, genetic variation of the deep-water rose shrimp shares similarities with other marine species in the Mediterranean Sea (De Matthaeis et al., 2000; Diaz-Almela et al., 2004; Vias et al., 2004; Pérez-Losada et al., 2007; Zulliger et al., 2009; Babbucci et al., 2010). In all these studies it is evident as the genetic differentiation is not sharp and does not show breakpoints due to well-defined physical barriers to gene flow but rather it arises as geographic distance increases.

As commonly observed in marine realm, such clinal variation is often detected on a wide geographical range, as the example of the European anchovy Engraulis encrasicolus, which exhibited an IBD pattern among the NE Atlantic samples from the Bay of Biscay to the Canary Islands, including the Alboran Sea (Zarraonaindia et al., 2012). Over long distances the role of hydrodynamic factors, such as currents, water fronts, eddies, is crucial.

Particularly in the Mediterranean sea, there are various potential physical barriers to individual displacements, mostly placed in the Siculo-Tunisian Strait (STS) (Fig. 1) (e.g. Mejri et al., 2009) and at the boundaries of the Adriatic sub-basin and the Aegean region (Fig. 1) (e.g. Maggio et al., 2009b; Mejri et al., 2011).

The IBD pattern describes at the same time a some homogeneity within the total meta-population, and heterogeneity among subgroups, detected at both side of important oceanographic transition zones. Such contradiction, homogeneity on the whole and heterogeneity spatially confined, is not uncommon, and several authors proved it (e.g. Zarraonaindia et al., 2012; Babbucci et al., 2010).

The Strait of Sicily represents a nursery and spawning area (Fortibuoni et al., 2010) and one of the most important fishing grounds for Mediterranean trawlers targeting P. longirostris (Levi et al., 1995; Abelló et al., 2002; Sobrino et al., 2005), and is characterised by a complex hydrodynamic system (Béranger et al., 2004). It is influenced principally by two main fluxes: the Atlantic Ionian Stream (AIS) from west to east, and the Levantine Intermediate Water (LIW) flowing in the opposite direction. Both are coupled with vortexes and fronts, such as the Adventure Bank Vortex (ABV), the Ionian Shelf-break Vortex (ISV), and the Ionian Slope Front (ISF) (Astraldi et al., 2002; Millot and Taupier-Letage, 2005).

According to Agostini and Bakun (2002), the Strait of Sicily shows the ocean triad conditions, which generate eggs and larvae retention. Enrichment processes, such as upwelling, mixing, cyclonic eddy formation, enhance food availability for predators through increasing the concentration of food particles, and contribute towards keeping individual members of a population in the appropriate place during the various parts of the life cycle.

The Atlantic Water (AW) flowing eastwards produces mesoscale eddies (Hamad et al., 2006) which further isolate the Aegean sub-basin. Thus, a turbulent regime which prevents a passive and plentiful displacement of individuals from the Tyrrhenian area through the Strait of Sicily towards the Adriatic and the Aegean, and vice versa, occurs.

In general, an IBD pattern can be characterized by different degrees of genetic discontinuities. The degree of genetic discontinuity can be different along the cline, so that a group of neighbouring populations can result much more differentiated than the rest of the group. In the light of such hypothesis, it cannot be excluded that the eastern P. longirostris populations are more differentiated from the others ('Levantine isolation'), though unsampled populations (ghost populations) may have obscured important information.

According to the present results, the deepest discrepancy in the genetic population structure has been observed between the Aegean samples and the others (shown in dendrograms of Fig. 2), highlighting a 'Levantin isolation' which characterizes the eastern Mediterranean basin. Hydrodynamic and ecological conditions of this Mediterranean region affect the distribution of genotypes. The Aegean sub-basin has been supposed to preserve genetically different populations within many species (e.g. Mejri et al., 2011; Borrero-Pérez et al., 2011), even in good swimmer fishes, for instance the sea bass Dicentrarchus labrax as reported by Castilho and Ciftci (2005).

In the case of P. longirostris, such hydrodynamic forces do not sufficiently act to prevent isolated demes, while long-distance dispersal is not durable enough to guarantee homogenization.

Other than the oceanographic barriers, the IBD pattern may reflect the dispersal of specific phenotypes/genotypes and it can be of biological significance. Indeed the genetic architecture has been demonstrated to be related to life history traits (Vias et al., 2004; Bradbury and Bentzen, 2007; García-Merchán et al., 2012).

The mortality of long-distance shrimp dispersers in concert with complex circulation can reduce the effective dispersal commonly associated with the pelagic larval period. However, the gene flow pattern cannot depend only on the passive larval dispersion but it can also be guaranteed by the active displacement of adults. The mobility of P. longirostris adults is considered to be high (Levi et al., 1995) although comprehensive studies are lacking. Adult migrations primarily depend on trophic resources; and, temperature values ($< 16\ °C$) are also considered a limiting factor regarding the distribution of the species (Tom et al., 1988; Ungaro and Gramolini, 2006).

The present dataset concurs to better design the genetic structure of P. longirostris; and integrates results by García-Merchán et al. (2012). They did not find heterogeneity among the P. longirostris samples at both sides of the oceanographic discontinuity area separating Atlantic and Mediterranean, most likely due to the restricted geographic sampling area. The characteristics of the Gibraltar Strait area, where fronts and eddies strongly influence the water circulation, could render the area suitable for the mixing of the pelagic and bentho-pelagic stadium of crustaceans. The transition Atlantic − Mediterranean area cannot be considered as a physical barrier to gene flow within some species, but rather an area in which the adults and the juveniles can mix horizontally and vertically along the water column in relation to the availability of trophic resources. Such proposition can be attributed to the ocean triad conditions likewise detected in the Alboran Sea by Agostini and Bakun (2002).

Many factors concur in depicting the various genetic patterns, locally and on a large spatial scale, and further studies needs characterizing the dispersal ability of the species. Of note is the importance of studying deep-water species in the Mediterranean Sea, where data linked to oceanographic features, trophic availability and ecological conditions could provide us with much more information relating to this scarcely known ecosystem, as also reported by several authors (Company et al., 2008; Nouar et al., 2011; García-Merchán et al., 2012).

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References

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