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FURTHER INVESTIGATIONS ON POPULATIONS OF THE DEEP-WATER BLUE AND RED SHRIMP *ARISTEUS ANTENNATUS* (RISSO, 1816) (DECAPODA, DENDROBRANCHIATA), AS INFERRED FROM AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) AND MTDNA ANALYSES

BY

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ABSTRACT

The aim of this study was to integrate existing mitochondrial DNA data relating to the deep-sea blue and red shrimp *Aristeus antennatus* (Risso, 1816) with data obtained by Amplified Fragment Length Polymorphism (AFLP). A total of 145 AFLP polymorphic loci were scored in 236 specimens collected from one Atlantic and seven Mediterranean sample sites. AMOVA results revealed that the overall genetic variation among-populations was lower (11.81%) than within-populations (88.19%). The genetic variation between the Atlantic and Mediterranean samples was found to be not significant ($\Phi_{CT} = -0.007$; N.S.), indicating that the transition area between the Atlantic Ocean and the Mediterranean Sea does not act as a barrier to gene flow. Bayesian analysis also demonstrated the absence of genetic differentiation between the Atlantic and Mediterranean populations and within the Mediterranean basin. The results are in agreement with those previously published using mitochondrial markers. Some considerations on the life history traits of the species are discussed.

Key words. — AFLP, mtDNA, *Aristeus antennatus*, Atlanto-Mediterranean region

RIASSUNTO

Lo scopo del lavoro è stato quello di integrare dati precedenti ottenuti dall'analisi del DNA mitocondriale nel gambero viola *Aristeus antennatus* (Risso, 1816) con dati ottenuti dall'analisi di un marcatore nucleare (Amplified Fragment Length Polymorphism, AFLP). In totale sono stati identificati 145 loci polimorfici AFLP in 236 esemplari raccolti da sette località del Mediterraneo e da un sito dell'Oceano Atlantico. I risultati dell'analisi AMOVA hanno rivelato che la variazione genetica tra le popolazioni è inferiore (11,81%) alla variazione intra-popolazionale (88,19%). Il

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livello di variazione genetica tra l'Atlantico e i campioni mediterranei è risultato non significativo ($\Phi_{CT} = -0{,}007$; N.S.), mostrando l'assenza di "punti di rottura" associati all'area di transizione tra l'Oceano Atlantico e il Mar Mediterraneo. L'analisi bayesiana ha ulteriormente supportato l'assenza di differenziazione genetica tra l'Atlantico e le popolazioni del Mediterraneo, e tra le popolazioni mediterranee. Questi risultati sono in accordo con quelli precedentemente pubblicati e ricavati dall'analisi di marcatori mitocondriali. Vengono discusse le caratteristiche biologiche della specie e il pattern di omogeneità intraspecifica.

Parole chiave. — AFLP, mtDNA, *Aristeus antennatus*, Area atlanto-mediterranea

INTRODUCTION

The interactions between oceanographic conditions and biological characteristics of a species, for instance the dispersal capability, make the use of different molecular markers essential for understanding the causes of the inter-population structure of marine taxa. The more slowly evolving and conservative markers typically indicate older processes that have affected the genetic structure of the species; in contrast, rapidly evolving and highly polymorphic markers can help to hypothesize more recent mechanisms, processes or events. Thus, markers evolving at different rates provide information relating to different time/space scales; and this explains why, in detecting effective genetic intra-species architecture, markers with different properties are used, irrespective of the species studied. This has been widely demonstrated by the large number of articles that involve species of commercial interest: e.g., *Merluccius merluccius* L., 1758 (cf. Lo Brutto et al., 2004); *Solea vulgaris* Quensel, 1806 (cf. Garoia et al., 2007); *Salmo trutta* L., 1758 (cf. Lo Brutto et al., 2010; Apostolidis et al., 2011).

Over the past two decades, many researchers have studied the biology, ecology and the exploitation levels of one of the most important of the Mediterranean fishery resources, the deep-water blue and red shrimp *Aristeus antennatus* (Risso, 1816) (cf. Demestre & Lleonard, 1993; Cartes, 1994; Arculeo et al., 1995; Ragonese & Bianchini, 1996; Kapiris & Thessalou-Legaki, 2001, 2006; Cau et al., 2002; Arculeo et al., 2011). *Aristeus antennatus* is distributed throughout the Mediterranean Sea, with the exception of the Adriatic Sea (Holthuis, 1980), and along the eastern Atlantic coast to the Cape Verde Islands (Ribeiro-Cascalho & Arrobas, 1982), and in the Indian Ocean (Crosnier, 1978). It has a wide bathymetric distribution, occurring at depths between 200 and 3300 m (Sardà et al., 2004) and is usually caught by trawlers on muddy bottoms at depths between 400 and 800 m. The presence of younger fractions of the population living at depths inaccessible to trawlers, called virgin grounds, reduces the effect of overexploitation of this species (Sardà et al., 1994; Sardà & Cartes, 1997; Papaconstantinou & Kapiris, 2001). In fact, *A. antennatus* has been intensively trawled in the deep sea for

more than 70 years as target species of a mono-specific fishery; nonetheless the Mediterranean populations of the blue and red shrimp have not collapsed (Caddy, 1999; Roberts, 2002; Morato et al., 2006; Company et al., 2008; Maiorano et al., 2010).

Recently, some authors demonstrated that the population dynamics of *A. antennatus* are strictly linked to the environmental conditions of its deep-sea habitat, in terms of climatic events, hydrographic factors and trophic resources (Cartes, 1994; Company et al., 2008; Guijarro et al., 2008; Maynou, 2008; Lo Brutto et al., 2011), thereby suggesting that this species merits attention.

Studies regarding stock delimitations were initially performed through morphometric and allozyme analyses by Sardà et al. (1998). Morphometric parameters revealed that populations from different parts of the Mediterranean and the adjacent Atlantic area were significantly different; conversely, allelic frequencies indicated low levels of differentiation among the same samples. More recently, various authors have analysed the genetic variation of mitochondrial (mt) DNA and demonstrated no strong population differentiation, neither along a depth gradient in the western Mediterranean (Sardà et al., 2010; Cannas et al., 2012) nor along the Atlantic Ocean–western Mediterranean–eastern Mediterranean axis (Maggio et al., 2009; Roldán et al., 2009; Fernández et al., 2011). All studies concur with the absence of a divergent genetic partition in the Mediterranean, attributed to the species' biology, particularly to its high dispersion capability, and to the effects of prevailing marine currents (Maggio et al., 2009; Roldán et al., 2009; Sardà et al., 2010; Fernández et al., 2011).

The aim of this study was to improve the data relating to the genetic variation of *A. antennatus* in a sampling area ranging from the Mediterranean to the adjacent waters of the Atlantic, using two differently evolving molecular markers (nuclear AFLP and mitochondrial control region), bearing in mind that AFLP, together with other markers, can provide more robust and comprehensive estimates of the genetic population structure as already demonstrated for other species (Lu et al., 2000; Weetman et al., 2007).

MATERIAL AND METHODS

The specimens of *Aristeus antennatus* from the Mediterranean Sea, analysed here with AFLP, originating from the Algero-Provençal, the Tyrrhenian Sea and the Strait of Sicily (see table I), were captured and analysed by means of mtDNA sequencing by Maggio et al. (2009). Furthermore, a sample from the Atlantic Ocean was collected close to Faro in southern Portuguese waters (fig. 1 and table I).

The Atlantic sample was analysed here for the first time by direct sequencing of a 369-bp mtDNA control region fragment and included in the mitochondrial

TABLE I

AFLP and mtDNA analysis: site locations, codes and number of the analysed specimens of *Aristeus antennatus* (Risso, 1816)

Geographical region	Sub-basin	Site location	Code	Sample size	
				AFLP	mtDNA
Atlantic Ocean		Faro (Portugal)	ATL	46	46
Mediterranean Sea	Algero-Provençal	Cataluña (north-western Spain)	CA	30	14
		Sanremo (north-western Italy)	SR	30	28
		Santa Margherita Ligure (north-western Italy)	SM	15	26
		Sant'Antioco (south-western Sardinia Island)	SW-SA	30	22
	Tyrrhenian	Siniscola (north-eastern Sardinia Island)	NE-SA	27	8
		Terrasini (northern Sicily Island)	TE	30	29
	Strait of Sicily	Strait of Sicily (southern Sicily Island)	SS	28	19

Mediterranean Sea specimens previously sequenced and analysed by using mtDNA in Maggio et al. (2009).

dataset previously published by Maggio et al. (2009). Analysis of mitochondrial diversity was done with DNASP (ver. 5) software (Librado & Rozas, 2009).

The AFLP analysis was performed in accordance with the description given by Vos et al. (1995). Restriction digests were carried out in 40-μl reactions, using 200 ng of genomic DNA, 5 U *Taq*I, 5 U *Eco*RI and 1× RL buffer (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. After digestion, 9 μl of ligation solution, including 5 pmol *Eco*RI adaptor and 50 pmol *Taq*I adaptor, 1 U T4 ligase, 1× RL buffer RL and 10 mM ATP was added to the restricted DNA solution followed by incubation at 37°C. Pre-selective amplification was performed in 50 μl of reaction sample containing 5 μl of diluted ligation mixture, 75 ng/μl of pre-amplification primers with a single selective base, 10 mM dNTPs, 1× PCR buffer, 25 mM $MgCl_2$ and 0.2 U *Taq* DNA polymerase. Temperature cycles followed this protocol: initial denaturation at 95°C for 15 s, then 20 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 60 s, followed by a final extension of 10 min at 72°C. The selective amplification reaction was performed in 10 μl containing 1 μl of pre-selective product diluted 20-fold in distilled water, 10 μM of selective primers with three selective bases, 10 mM dNTPs, 1× PCR buffer, 25 mM $MgCl_2$ and 0.2 U *Taq* DNA polymerase. Selective amplification was performed with 15 s denaturation at 95°C, then 11 cycles of 30 s at 94°C, 30 s at 65°C and 1 min at 72°C, with a 0.5°C decrease in the annealing temperature each cycle, followed by 23 cycles of 30 s at 94°C, 30 s at 56°C and 1 min at 72°C, with a final extension of 10 min at 60°C.

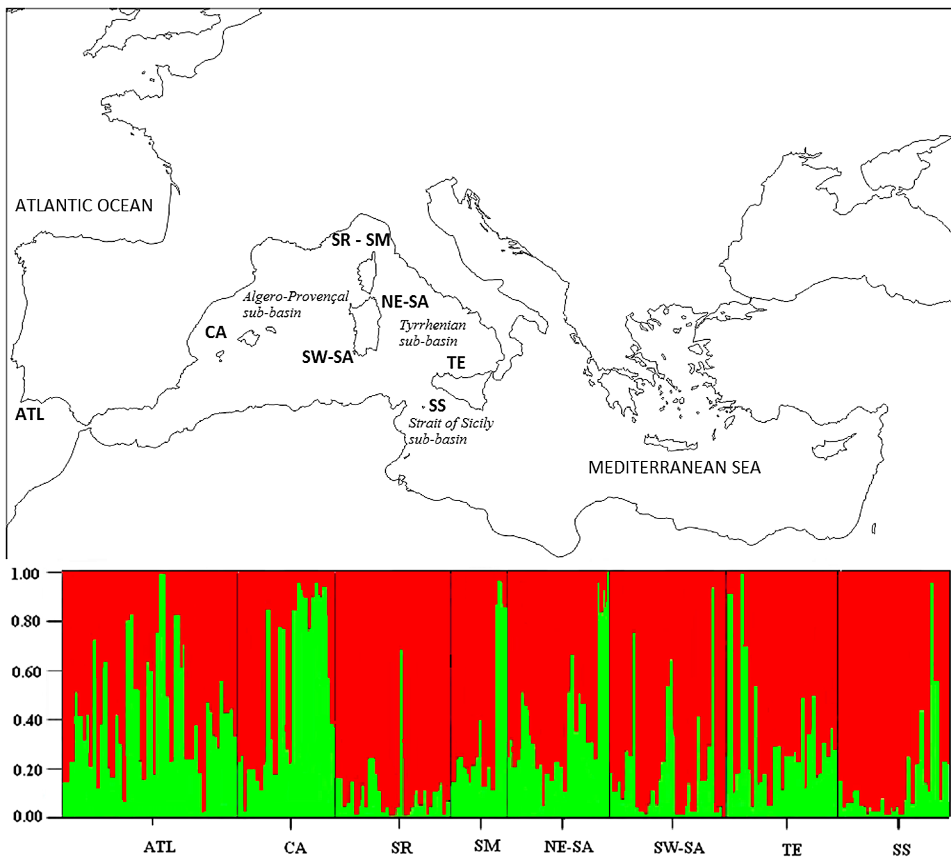

Fig. 1. Top, sampling sites of *Aristeus antennatus* (Risso, 1816) in the Atlanto-Mediterranean region: 1, Faro, Portugal (ATL); 2, Cataluña (CA), north-western Spain; 3, Sanremo (SR); and, 4, Santa Margherita Ligure (SM) in north-western Italy; 5 and 6, Siniscola (NE-SA) and Sant'Antioco (SW-SA) in the north-eastern and south-western Sardinia, respectively; 7 and 8, Terrasini (TE) and Strait of Sicily (SS), northern and southern Sicily, respectively. Bottom, summary plot of q estimates (proportion of membership) for $K = 2$ from the AFLP analysis. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/15685403.

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 Biosystems) with a GeneScan Rox 500 internal size standard. Electropherograms were subsequently analysed using Genescan 2.02 and Genotyper 2.5 (Applied Biosystem).

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 & Milligan (1994). Total gene diversity H_T (Nei, 1987) was calculated by pooling all individuals in a population; considering each population, H_E, Nei's gene diversity within-population, was also computed; total variance of H_E was subdivided into the variance due to sampling of individuals, *Var*I, and the variance due to sampling of loci *Var*L. Finally, H_S was calculated as the average within-population Nei gene diversity and its variance subdivided into *Var*I (due to sampling of individuals), *Var*L (due to sampling of loci) and *Var*P (due to sampling of populations).

Identification of outlier loci was carried out in accordance with the approaches of Beaumont & Nichols (1996) and Beaumont & Balding (2004), as implemented in the DFDIST software (http://www.rubic.rdg.ac.uk) following the procedure modified by Caballero et al. (2008). The procedure to identify outlier loci is based on the assumption that loci under selection exhibit higher or lower F_{ST} values than the majority of neutral markers. 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 that are higher or lower than the quantile limits (5, 50 and 95%). Loci with higher or lower F_{ST} values were considered under directional and balancing selection, respectively, and were, 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 their geographic location. This analysis allowed to verify the partitioning of genetic variation among populations on the whole and within and among the groups generated, the last value reporting the correspondent fixation indices: Φ_{ST} (the average within-populations within a group), Φ_{SC} (the average among-populations within a group) and Φ_{CT} (among groups).

Population differentiation was also inferred using the Bayesian approach, as implemented in the Structure software (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 done, with K ranging from 1 to 8 (burning length 100 000). The true number of genetic clusters, K, is commonly identified

as that with the highest posterior probability ($P(X|K)$), but as 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 $\ln P(D)$.

Subsequently, the Doh assignment test calculator (available online at http://www2.biology.ualberta.ca/jbrzusto/Doh.php) was used to test whether individuals could be assigned to the samples from which they had been sampled. This software identifies genotypes of individuals from several populations and determines the population of origin for each individual, by using an assignment index, which is associated to the highest probability (Paetkau et al., 1995).

RESULTS

A total of 145 AFLP polymorphic loci were scored in 236 specimens of *Aristeus antennatus*. Within-population Nei gene diversity, H_E, ranged from 0.288 to 0.392 (table II). Total gene diversity, H_T, was 0.361; the average gene diversity, H_S, was 0.340; and the percentage contributions of *Var*I, *Var*L and *Var*P to the variance of H_S were 1.50, 6.95 and 91.55%, respectively, thereby demonstrating that the greater part of gene diversity was attributed to variation within-populations.

The analysis conducted with DFDIST software identified two different loci out of the 145, polymorphic at the 95% confidence level. When the detected outliers were excluded, the neutral dataset was used to conduct a hierarchical AMOVA. This analysis was performed in different ways: clustering the samples "all together" (analysis 1_{AFLP}, table III) and clustering samples into groups coherent

TABLE II

AFLP analysis: genetic diversity data of *Aristeus antennatus* (Risso, 1816) based on the Lynch & Milligan (1994) method

Site location	PLP (0.05)	H_E	*Var*I (%)	*Var*L (%)
ATL	93.4	0.321	12.7	87.3
CA	97.5	0.327	45.8	54.2
SR	97.5	0.392	18.2	81.8
SM	94.2	0.322	24.4	75.6
SW-SA	100.0	0.378	22.0	78.0
NE-SA	92.6	0.305	15.3	84.7
TE	81.0	0.288	8.3	91.7
SS	97.5	0.386	18.4	81.6

PLP, proportion of polymorphic loci within-populations at levels of 5%; H_E, gene diversity within-population; *Var*I, the percentage of variance attributed to individuals; *Var*L, percentage of variance attributed to loci.

TABLE III

AFLP and mtDNA analysis: AMOVA conducted on samples of *Aristeus antennatus* (Risso, 1816) on the basis of the AFLP markers clustered in four different ways (1_{AFLP}, 2_{AFLP}, 3_{AFLP} and 4_{AFLP}) and on the basis of the mtDNA marker with samples clustered in three different ways (1_{mt}, 2_{mt} and 3_{mt}); the grouping for the mtDNA analysis of the three Mediterranean sub-basins Algero-Provençal + Tyrrhenian + Strait of Sicily is taken from Maggio et al. (2009)

Cluster	Grouping	Source of variation	df	Percentage of variation	Fixation index
1_{AFLP}	All together'	Among populations	7	11.81	Φ_{ST} 0.118**
		Within populations	224	88.19	
2_{AFLP}	Atlantic + Mediterranean'	Among groups	1	0	Φ_{CT} −0.077
		Among populations within groups	7	15	Φ_{SC} 0.120**
		Within groups	253	85	Φ_{ST} 0.114**
3_{AFLP}	Atlantic + Algero-Provençal + Tyrrhenian + Strait of Sicily'	Among groups	3	−0.04	Φ_{CT} −0.004
		Among populations within groups	4	11.84	Φ_{SC} 0.118**
		Within groups	224	88.20	Φ_{ST} 0.119**
4_{AFLP}	Algero-Provençal + Tyrrhenian + Strait of Sicily'	Among groups	2	4	Φ_{CT} 0.040
		Among populations within groups	5	12	Φ_{SC} 0.124**
		Within groups	208	84	Φ_{ST} 0.162**
1_{mt}	All together	Among populations	7	3.60	Φ_{ST} 0.036
		Within populations	183	96.40	
2_{mt}	Atlantic + Mediterranean	Among groups	1	−0.89	Φ_{CT} −0.009
		Among populations within groups	7	1.36	Φ_{SC} 0.013*
		Within groups	183	99.54	Φ_{ST} 0.040*
3_{mt}	Atlantic + Algero-Provençal + Tyrrhenian + Strait of Sicily	Among groups	3	−0.020	Φ_{CT} −0.002
		Among populations within groups	5	1.20	Φ_{SC} 0.012*
		Within groups	183	98.80	Φ_{ST} 0.012*

df, degree of freedom; $^{*}p < 0.05$; $^{**}p < 0.001$.

with their geographical location in order to understand where the observed genetic variation lay. The first grouping was 'Atlantic + Mediterranean' (analysis 2_{AFLP}, table III). Subsequently, the samples were pooled into four groups: the three Mediterranean sub-basins and the Atlantic ocean, 'Atlantic + Algero-Provençal + Tyrrhenian + Strait of Sicily' (analysis 3_{AFLP}, table III). Finally, the Mediterranean samples were grouped into the three sub-basins 'Algero-Provençal + Tyrrhenian + Strait of Sicily', excluding the Atlantic sample (analysis 4_{AFLP}, table III). AMOVA results revealed that the overall genetic variation among-populations which were grouped 'all together' in analysis 1_{AFLP} was lower (11.81%) than within-populations (88.19%) though the fixation index proved to be significant ($\Phi_{ST} = 0.118$; $p < 0.001$). The genetic variation between the Atlantic and Mediterranean samples in analysis 2_{AFLP} was found to be not significant ($\Phi_{CT} = -0.007$; N.S.), indicating that genetic variation is not associated with the transition between the Atlantic Ocean and the Mediterranean Sea but rather to the variation found among populations within groups (15% of genetic variation) and within populations (85%).

Subsequently, when the samples were grouped into four groups ('Atlantic + Algero-Provençal + Tyrrhenian + Strait of Sicily'), the greater portion of the genetic variation was found within groups (88.2%), reporting significant fixation indices ($\Phi_{ST} = 0.119$; $p < 0.001$); no genetic differentiation was detected among groups ($\Phi_{CT} = -0.004$; N.S.). Finally, the analysis 4_{AFLP} revealed no differentiation among the three Mediterranean sub-basins as was evident by the non-significant fixation index ($\Phi_{CT} = 0.04$; N.S.) (table III). This is in accordance with the previous results obtained with the same samples from Maggio et al. (2009) using mtDNA. The three clustering methods proved that the high value of genetic variation is not due to differences at group level but at population and individual levels.

Confirming our results, the Bayesian analysis conducted with Structure software demonstrated the absence of genetic differentiation between the Atlantic and Mediterranean populations. The two modes in the graph of ΔK vs. K were observed for $K = 2$ (log $= -22\,879$) and $K = 5$ (log $= -20\,995$). When $K = 2$, the separation into two genetic clusters negated any geographical correspondence with the Atlantic-Mediterranean subdivision and revealed a high degree of membership to the first cluster for the Mediterranean samples (table IV and fig. 1). When $K = 5$, the average proportion of membership was low ($q < 0.70$) for all samples, indicating the presence of admixed individuals (table IV). The putative migrants identified by the Bayesian-based assignment method were distributed throughout all our samples and particularly the samples with numerous specimens considered as migrants were SM, SW-SA and TE.

TABLE IV

AFLP analysis: proportion of membership of each pre-defined population in the most probable genetic clusters of *Aristeus antennatus* (Risso, 1816) identified in the Bayesian analysis, as suggested by Evanno et al. (2005)

	$K = 2$		$K = 5$				
	1	2	1	2	3	4	5
ATL	0.592	0.408	0.355	0.055	0.073	0.448	0.069
CA	0.532	0.468	0.514	0.080	0.013	0.365	0.028
SR	0.900	0.100	0.056	0.482	0.062	0.334	0.067
SM	0.660	0.340	0.192	0.031	0.077	0.145	0.555
SW-SA	0.791	0.209	0.147	0.362	0.078	0.315	0.099
NE-SA	0.628	0.372	0.146	0.067	0.028	0.063	0.695
TE	0.698	0.302	0.167	0.267	0.474	0.050	0.042
SS	0.859	0.141	0.088	0.036	0.065	0.629	0.182

In the assignment analysis conducted with Doh software, 151 of the total of 236 specimens analysed were assigned to the sampling population, corresponding to 65% of correct assignment. Specifically, there was a high assignment success in ATL, NE-SA, TE, SS and CA (ranging from 73% to 89%) and a low assignment success, and consequently a high number of putative migrants, in SR, SM and SW-SA (table V).

The mitochondrial control region fragment was sequenced in the sample from the Atlantic and showed high haplotype diversity (0.934), and similar to the Mediterranean samples (Maggio et al., 2009); nucleotide diversity was low for both the Atlantic (0.019) and Mediterranean area (see Maggio et al., 2009). AMOVA results based on mtDNA were in accordance with the AFLP results, as reported in table III: the partitioning of genetic variation obtained by clustering the samples into two groups, the Atlantic and Mediterranean, revealed that the

TABLE V

AFLP analysis: results of the Doh assignment test scored in *Aristeus antennatus* (Risso, 1816)

	Population assigned							
	ATL	CA	SR	SM	NE-SA	SW-SA	TE	SS
ATL	41	1	2	0	0	1	0	1
CA	2	23	1	0	0	1	1	2
SR	0	0	15	1	0	3	1	10
SM	0	3	1	5	6	0	0	0
SW-SA	2	1	4	1	1	10	2	9
NE-SA	0	3	0	2	22	0	0	0
TE	0	2	3	0	0	3	22	0
SS	1	3	1	0	0	1	0	22

two geographical areas were not significantly different ($\Phi_{CT} = -0.009$; N.S.) and the greatest variation was among-populations within groups and within-populations (table III). Even clustering the samples into the four 'Atlantic + Algero-Provençal + Tyrrhenian + Strait of Sicily' groups did not account for significant differences among groups ($\Phi_{CT} = -0.0002$; N.S.).

DISCUSSION

Aristeus antennatus is a species with a wide geographical distribution and may, therefore, be composed of distinct genetic units as previous mitochondrial results had demonstrated on a macro-geographical scale (i.e., Mediterranean vs. Indian Ocean in Fernández et al., 2011). However, a low degree of differentiation in smaller spatial areas, such as those which cover the Mediterranean Sea, was found by using mtDNA (Maggio et al., 2009; Roldán et al., 2009).

In the present work, AFLP and the mitochondrial dataset showed congruent patterns of low genetic differentiation among the samples analysed, coupled with a high genetic variation within-populations clearly shown along the Mediterranean samples.

The low degree of genetic differentiation among Mediterranean populations of the blue and red shrimp may be a consequence of the mobility pattern of the species. Specifically, vertical and horizontal displacements of adults have been reported in relation to the spatio-temporal variation of the trophic resources in response to the action of chemical and physical factors in deep-sea habitats (Tudela et al., 2003; Cartes et al., 2008). Vertical mixing of different stocks has been recently demonstrated by analysing the genetic structure of *A. antennatus* along a depth gradient, proving that the gene flow is high and that the deeper living stocks are not isolated from those in the higher strata (Sardà et al., 2010; Cannas et al., 2012). Deep-water circulation and turbulent mixing phenomena, associated with the migration events of *A. antennatus*, seem to promote some degree of gene flow (Maggio et al., 2009; Roldán et al., 2009; Sardà et al., 2010; Fernández et al., 2011).

Besides, the present data provide evidence for a genetic similarity between the eastern North Atlantic Ocean and the Mediterranean basin, thus supporting the occurrence of gene flow between the two areas. With reference to the AMOVA, grouping the population according to their geographical location, 'Atlantic + Mediterranean' (table III), and to the Structure results obtained from AFLP (fig. 1) an absence of any genetic break between the Atlantic and Mediterranean is evident. Furthermore, the Bayesian analysis (table IV) and the Doh assignment test (table V) similarly supported the presence of migrants (population admixture) as

well among the various Mediterranean populations as between the Atlantic Ocean and the Mediterranean Sea.

The area of transition from the eastern Atlantic to the western Mediterranean involves inflowing Atlantic water, which forms two almost permanent anticyclonic gyres from Almeria in Spain to Oran in Morocco (the Almeria-Oran Front, AOF). This front has been considered a barrier to various species for displacements of larvae and adults (Patarnello et al., 2007). However, the inflow of enriched superficial water and a down-welling process determine high levels of nutrients and concentrations of small organisms, which have made this area a favourable feeding and recruitment ground, and a larval retention area for some other species (Caddy, 1993; Sanchez-Vidal et al., 2004).

Such characteristics could render the area suitable for the mixing of the pelagic and bentho-pelagic stages of *A. antennatus* and we may assume that the transition Atlantic-Mediterranean area cannot be considered a physical barrier to gene flow 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.

This study has provided a further assessment of the genetic population structure of *A. antennatus*, because nuclear results corroborated previous studies, and did not describe strong break-points in the populations of the species inhabiting the Mediterranean Sea.

As one of the most important deep-sea resources and considering the demonstrable and tight link between species and environmental features, *A. antennatus* should be further investigated as a model species in the light of the present-day climate warming. Climate changes strongly influence the hydrography of marine ecosystems and could, therefore, correlate with the change in population dynamics and population structure of *A. antennatus*, as suggested by several authors (Cartes, 1994; Company et al., 2008; Guijarro et al., 2008; Maynou, 2008; Lo Brutto et al., 2011). Additional samples from other Atlantic areas and more data regarding the influence of environmental features on the dynamics of populations are required to improve the documentation regarding the genetic differentiation of the species. An exhaustive understanding of population genetic structure will further be of fundamental importance in managing and conserving this species, taking into consideration the paucity of knowledge on the effects of increased deep-water trawling on benthic faunas as reported in Danovaro et al. (2010).

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