Acta Oceanol. Sin., 2015, Vol. 34, No. 6, P. 49–58
DOI: 10.1007/s13131-015-0687-7
http://www.hyxb.org.cn
E-mail: hyxbe@263.net

Patterns of genetic and morphometric diversity in the marbled crab (*Pachygrapsus marmoratus*, Brachyura, Grapsidae) populations across the Tunisian coast

Temim Deli[1]*, Hiba Bahles[1], Khaled Said[1], Noureddine Chatti[1]

[1] Laboratory of Genetics, Biodiversity and Enhancement of Bioresources (LR11ES41), University of Monastir, Higher Institute of Biotechnology of Monastir, Monastir 5000, Tunisia

Received 16 April 2014; accepted 28 October 2014

Abstract

The present study reports on population structure analysis of the marbled crab *Pachygrapsus marmoratus* (Fabricius, 1787) from the Tunisian coast, an appropriate location to study biogeographical processes because of the presence of a well-known discontinuous biogeographic area (the Siculo-Tunisian Strait). Patterns of morphological and genetic variation of this highly dispersive and continuously distributed decapod species were assessed among its geographically close populations which cover almost the entire Tunisian coastline. A total of 386 specimens from nine sites were collected and examined for morphometric variability at 14 morphometric traits. The results of multivariate analyses of linear morphometric traits showed the existence of sexual dimorphism in this species by PERMANOVA (Permutational multivariate analysis of variance). In addition, both CDA (Canonical discriminant analysis) and NPMANOVA (Non parametric MANOVA test) analyses revealed statistically significant differences among the studied locations for both sexes. Overall, the outcome of CDA analysis showed that over 87% of individuals could be assigned correctly to three regional groups in both sexes (North, Center and South). Specifically, SIMPER (Similarity Percentages) analysis showed that carapace length, carapace width and merus length were major contributors to the morphometric separation between populations. The pattern of phenotypic variation suggested by morphometric analyses was found to be highly discordant with that suggested by the analysis of a mitochondrial marker (cytochrome oxidase I, COI). Indeed, the results inferred from restriction fragment analysis of the COI in 180 crabs, suggested high genetic homogeneity. Very low levels of haplotype diversity (h) were found in almost all the studied populations, associated with non significant genetic distances for nearly all population comparisons. Explanations to these morphometric and mtDNA patterns as well as the discrepancy between them are discussed.

Key words: *Pachygrapsus marmoratus*, Tunisian coast, multivariate analysis, morphological differentiation, COI, genetic homogeneity

Citation: Temim Deli, Hiba Bahles, Khaled Said, Noureddine Chatti. 2015. Patterns of genetic and morphometric diversity in the marbled crab (*Pachygrapsus marmoratus*, Brachyura, Grapsidae) populations across the Tunisian coast. Acta Oceanologica Sinica, 34(6): 49–58, doi: 10.1007/s13131-015-0687-7

1 Introduction

It has been shown that species with long larval stage and continuous distribution pattern, usually exhibit mild population differentiation as a result of high exchange of individuals among populations, as noted in the two intertidal starfish species: *Paterielle calcar* and *P. exigua* (Hunt, 1993) and the Indo-Pacific giant tiger prawn *Penaeus monodon* (Brooker et al., 2000). However, genetic analyses of highly dispersive decapods have revealed complex patterns of genetic differentiation at various geographic scales, and even over short distances of 40–225 km (Weber and Levy, 2000; Weber et al., 2000; Jørstad et al., 2004; Weetman et al., 2007). For example, mtDNA analysis of European lobster *Homarus gammarus* revealed significant structuring and distinct genetic clusters across its European distribution range (Triantafyllidis et al., 2005). Similarly, significant differentiation was revealed among samples of Norway lobster *Nephrops norvegicus* from the North Sea, Irish Sea, Portugal, and the Mediterranean Sea (Stamatis et al., 2004, for mtDNA RFLP; Stamatis et al., 2006, for allozymes).

The marbled crab *Pachygrapsus marmoratus* (Fabricius, 1787) is one of the most distributed and abundant grapsid species in intertidal environments (Cannicci et al., 1999). This species is considered as the most common grapsid crab in the intertidal belt of rocky shores throughout the Mediterranean Sea, Black Sea and northeastern Atlantic from Brittany to Morocco including the Canary Islands, the Azores and Madeira (Ingle, 1980; Cannicci et al., 1999; Flores and Paula, 2002) and can colonize the whole intertidal belt regardless of its width (Cannicci et al., 1999). This grapsid species is a very adaptable rocky shore dweller, capable of modifying its use of time and space according to the different characteristics of the shores it inhabits. Futhermore, it is a highly dispersive species with larval phase that can last for up to 30 d in plankton (Cuesta and Rodriguez, 2000). *P. marmoratus* has a semiterrestrial life-style and is found to be a select-

*Corresponding author, E-mail: temimdeli@yahoo.co.uk

ive omnivorous species that actively searches for food relying on the intertidal community throughout its post-larval stage. This highlights its significant role in shaping intertidal community structure by selectively choosing food items instead of randomly browsing on the most frequent ones (Cannicci et al., 2002). Like all decapod species with strong capability of spreading during larval stage, *P. marmoratus* larvae could theoretically recruit into other spatially isolated populations through long distance transportation by oceanic circulation (Johnson et al., 1984, 1986; Hobbs et al., 1992; Mense et al., 1995). However, oceanographic discontinuities, biogeographic boundaries, varying climatic regimes and diversity of habitats can limit their movements. In addition, physical factors (e.g., refuge availability) and biotic factors (e.g., presence / absence of competitors and predators) could be involved in shaping phenotypic and genotypic variation in this species. Previous studies of *P. marmoratus* population structure over microgeographic scales revealed the existence of mild to absent genetic differentiation. For example, while microsatellite investigations showed the existence of subpopulations structuring across the Italian (Fratini et al., 2008, 2013) and Portuguese coasts (Silva et al., 2009), a combined mitochondrial and morphometric examination of populations of this species along the Portuguese coast failed to show concordant patterns of variability, suggesting plastic response of this species to environmental conditions (Silva and Paula, 2008). Thereby, we address the question whether morphometric and genetic subunits could exist in other part of *P. marmoratus* distribution range, namely across the Tunisian coast. The Tunisian shores provide an excellent location to study biogeographical processes given the presence of a well documented genetic boundary between populations of several vertebrate and invertebrate species around the Siculo-Tunisian Strait (e.g., Quesada et al., 1995; Borsa et al., 1997; Bahri-Sfar et al., 2000; Nikula and Väinölä, 2003; Arnaud-Haond et al., 2007; Zitari-Chatti et al., 2008; Zitari-Chatti et al., 2009).

Morphological variation among populations of the same species can be used to identify and delineate morphotypes, and may also be useful in examining the stock structure within a morphotype (Cadrin, 1995; Duffy, 1996; Spotte, 1997). Similarly, mitochondrial DNA is highly variable in natural populations because of its elevated mutation rate, which can generate a signal about population history over short time frames and can be used as a marker to infer population divergences and genetic stock identification (Cassone and Boulding, 2006; Silva et al., 2010). Both mtDNA and morphometric markers would be helpful for optimizing population structure investigation in the marbled crab. Concordant results of the two approaches would highlight the involvement of genetic and environmental factors in shaping morphological variation. Whereas, discordant patterns would mainly invoke the impact of abiotic factors as major contributors.

Hence, the objective of the present study is to evaluate patterns of morphological and genetic variation of the highly dispersive decapod species, *P. marmoratus* across a well-known discontinuous biogeographic area along its North-African distribution range. To this end, several locations covering almost the entire Tunisian coastline were sampled and compared by means of multivariate analyses of linear morphometric traits and restriction fragment analysis of the mitochondrial gene cytochrome oxidase I (COI), a method has been shown to be variable enough for population studies in marine crabs (Fratini and Vanini, 2002; Roman and Palumbi, 2004; Darling et al., 2008). The results would be beneficial to future study of the evolutionary history of marine decapod fauna in the region and provide indirect information in different aspects of biology and ecology of the species that can be used for its management and conservation.

2 Materials and methods

2.1 *Sample collection*

Only adult marbled crabs were collected from nine sites in the littoral fringe off the Tunisian coast (Fig. 1). All samples were collected during the summer of 2008. For the morphometric comparisons, a total of 192 males and 194 females were measured. Nearly all sampled shore crabs exhibit none regenerated or absent claws and pereiopods (walking legs) except three males and one female which were excluded from the analysis. Only specimens with full morphometric characters were used in the statistical analysis, i.e., a total of 189 males (Tabarka: 23, Bizerte: 20, Korbos: 12, Kelibia: 21, Benikhiar: 21, Monastir: 27, Chebba: 16, Sfax: 24 and Zarzis: 25) and 193 females (Tabarka: 17, Bizerte: 22, Korbos: 38, Kelibia: 20, Benikhiar: 20, Monastir: 18, Chebba: 24, Sfax: 21 and Zarzis: 13). For the genetic analysis, a total of 180 specimens, 20 crabs per sampling site, were taken alive to the laboratory and one pereiopod from each individual was desiccated and preserved in 100% ethanol.

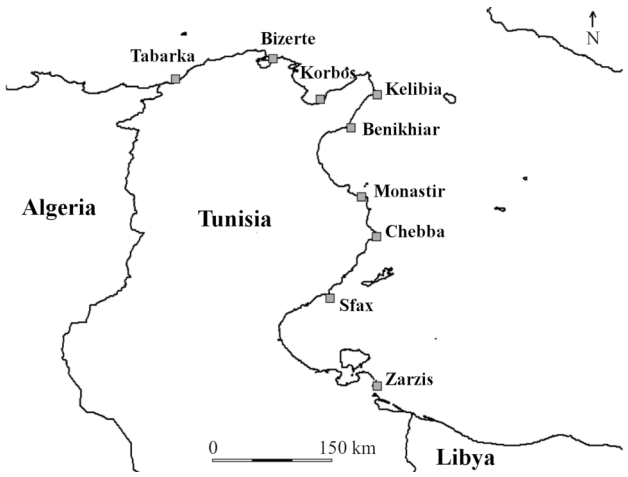

Fig. 1. Sampling locations of the marbled crab *P. marmoratus* along the Tunisian coast.

2.2 *Morphometric measurements*

The morphological measurements used for the morphometric analyses were: carapace width at the third spine (CW-3rd spine), carapace width at the first spine (CW-1st spine), carapace length (CL), carapace height (CH), right chela depth (RCHD), left chela depth (LCHD), right chela width (RCHW), left chela width (LCHW), merus length (ML), merus width (MW), propodus length (PL), propodus width (PW), dactylus length (DL) and largest abdominal width (LABW). These characters were measured using vernier calipers to the nearest 0.01 mm. The length, width, and height of the carapace were measured at the widest, longest, and deepest points respectively. Similarly, chela depth and width were measured at the deepest and widest points. Lengths and widths of merus and propodus as well as dactylus lengths were recorded for the second, third, fourth and fifth pairs of limbs respectively. For these latter traits, measurements were made on both sides and then averaged.

2.3 *PCR–RFLP screening*

Genomic DNA was extracted from the muscle tissue of the pereiopods using the Wizard® genomic DNA purification kit (promega). Twenty individuals per sampling site were examined. Genetic polymorphism was screened by PCR-RFLP. About 710 bp fragment of the mitochondrial gene cytochrome oxidase I (COI) was amplified using the universal primers: LCO1490 and HCO2198 (Folmer et al., 1994). PCR was set up in a 50 μL mix composed of PCR buffer, 200 μmol/L of each dNTP, 0.16 μmol/L of each primer, 1 U of Taq, approximately 0.2 μg of DNA and bidistilled sterile water. Amplification conditions were as follows: one preliminary denaturation at 95°C for 5 min, followed by 35 cycles of 95°C denaturation for 30 s, annealing at 40°C for 50 s and 72°C extensions for 60 s. A final extension of 8 min at 72°C was performed. Obtained PCR products were then digested by five restrictions enzymes: *Mbo*I (GATC), *Nla*III (CATG), *Taq*I (TCGA), *Msp*I (CCGG) and *Mae*I (CTAG). These enzymes were determined following the establishment of the restriction map of *P. marmoratus* using RestrictionMapper V.3. This latter was defined based on COI sequences from two individuals of the population of Monastir. Restriction enzyme digestions were carried out in 20 μL mixtures containing 2 μL enzyme buffer, 2.5 U restriction enzyme, 8 μL PCR product and bidistilled sterile water. Digestions were done at 37°C for 4 h, except for digests with *Taq*I, which were performed at 65°C for 2 h. Resulting fragments were separated electrophoretically in a 3% agarose gel, with ethidium bromide staining, and visualized under UV light. Fragment lengths were determined using a low range DNA ladder (50–1 000 bp) as size marker.

2.4 *Data and statistical analysis*

Sexual dimorphism in morphological characters was tested prior to data analysis in order to decide whether to consider the sex of specimens as an independent factor in the analysis. Multidimensional analyses based on permutation tests were performed to compare sex differences within and between the studied locations using the software PERMANOVA V.1.6 (Anderson, 2005). These were tested with the following design: the factor sex was analyzed as crossed, fixed factor with two levels (female and male), while the factor location was analyzed as crossed, fixed factor with nine levels (Tabarka, Bizerte, Korbos, Kelibia, Benikhiar, Monastir, Chebba, Sfax and Zarzis). No adjustment of values was used and size-uncontroled values were entered into the analysis. Choice of this method was based on the fact that it allows testing of complex experimental designs using multivariate data and interaction terms (Anderson and Ter Braak, 2003). The analysis was based on Bray-Curtis dissimilarities on fourth-root transformed data (Clarke, 1993). Patterns of morphometric relationships can be influenced by the effect of allometric growth and size in species of undetermined age. Regressions, performed within the nine studied locations for the different measurement of body dimensions versus the carapace width (CW-3rd spine), used as independent variable and considered as adjusted trait values (Reist, 1985; Debuse et al., 2001), showed a positive and consistent allometry of each measured trait among populations separately for females and males. Therefore, the effect of maximum carapace width (X) variation on each measured trait (Y) within each location was removed by using the allometric equation $Y=aX^b$. All measured traits were standardized using the equation: $Y_i=Y_i\,(X_{\mathrm{m}}/X_i)^b$ where Y_i is the standardized measurement from the measured trait Y_i of the ith specimen, X_{m} is the mean value of maximum carapace width for the examined location, X_i is the measured maximum carapace width of the ith specimen and b is the standardizing parameter obtained from the allometric equation (Anastasiadou and Leonardos, 2008). Standardized values were then plotted against carapace width and arc-sinus/tangent-transformed to achieve normality, before being processed with multivariate analyses (CW-3rd spine was not considered since it was used to adjust all the remaining parameters). To see whether natural variability of the parameters measured is different between locations, we used the non parametric test of significant difference between two or more groups (Non-Parametric MANOVA), implemented in the software PAST V.2.17 (Hammer et al., 2001), based on the Bray-Curtis distance (Anderson, 2001). Pairwise NPMANOVAs between all pairs of groups were provided as a post-hoc test. The significance was computed by permutation of group membership, with 9 999 replicates. Variables, responsible for the eventual separation of the locations were identified using the Similarity Percentages (SIMPER) routine in PAST V.2.17. In addition, the discriminant function analyses were carried out on both sexes separately using the arc-sin/tan-transformed variables. Canonical discriminant analysis (CDA) finds linear combinations of variables (roots), that maximise differences among a priori defined groups, with the hit ratio (percentage correctly classified) providing a goodness of fit measure. A canonical analysis, performed on the arc-sin/tan-transformed variables for the nine studied populations (with each population considered as a separate group), showed a clear distinction between the locations of North Tunisia (Tabarka, Bizerte, Korbos, Kelibia and Benikhiar) and those of the south (Chebba, Sfax and Zarzis) with a noticeable separation of the central location of Monastir from them. These preliminary insights resulted in reanalyzing the data by considering three groups (Group1 = Tabarka, Bizerte, Korbos, Kelibia and Benikhiar; Group 2 = Monastir; Group 3 = Chebba, Sfax and Zarzis). Canonical discriminant analysis was used, thereafter, to find out how well discriminant functions allow classification of individuals to groups. Mahalanobis distances, based on the correlation between variables, were determined to test for significant difference among the three defined groups for both sexes of *P. marmoratus*. In all CDA analyses, all variables were entered simultaneously, with the relative contributions of each variable assessed on the basis of the structure correlations (discriminant loadings), rather than the discriminant coefficients, as the former are considered more valid in interpreting the discriminating power of the independent variables. The following specifications were used for all CDA runs: Backward stepwise, Tolerance=0.01, F to enter=1.0, F to remove=0.0, and a priori probabilities were estimated to be proportional to group sizes. For all canonical discriminant analyses, the Statistica for Windows program V.4.3 (StatSoft, Inc, 1993) was used.

For genetic analysis, restriction patterns, generated by each enzyme, were identified and then combined to define composite mtDNA haplotype patterns. Restriction site data were analyzed with the computer package ARLEQUIN V.3.01 (Excoffier et al., 2005). Within-population diversity was estimated by haplotype diversity h (Nei, 1987) and the extent of genetic differentiation between populations, was estimated using the fixation index F_{ST} (Wright, 1950). The significance of pairwise F_{ST} estimates among all populations was assessed by randomization procedure with 10.000 permutations. B-Y FDR correction (Narum, 2006) was

then applied to yield the exact level of significance (critical value=0.011 98 with 36 hypothesis tests and alpha=0.05).

3 Results

3.1 *Linear-based morphometric analyses*

PERMANOVA analysis revealed significant difference between both sexes of *P. marmoratus* for the analyzed traits (F_{sex}=12.581, *P* (permutation)=0.000 1). Furthermore, a significant interaction between both factors, sex and location ($F_{sex\times location}$=13.289, *P* (permutation)=0.000 1), was noted as significant differences have been shown to exist between both sexes at all locations (Table 1). This required that female and male crabs should be considered independently for further morphometric

Table 1. Summary of results of permutational multivariate analysis of variance (PERMANOVA) comparing sexual dimorphism in *P. marmoratus* at nine locations

Source	df	SS	MS	*F*	*P* (Perm)	*P* (MC)			
Sex	1	39.812 9	39.812 9	12.581 5	***	***			
Location	8	159.537 1	19.942 1	6.302 0	***	***			
Sex×Location	8	336.431 8	42.054 0	13.289 7	***	***			
Residual	162	512.631 6	3.164 4						
Total	179	1 048.413 3							
Comparison	Tabarka	Bizerte	Korbos	Kelibia	Benikhiar	Monastir	Chebba	Sfax	Zarzis
Male vs. female	*	*	***	***	***	***	***	*	*

Notes: df represents degree of freedom, SS sum of squares, MS mean square, *F* morphometric distance inferred from Bray-Curtis dissimilarities, Perm permutation procedure, andMC Monte-Carlo asymptotic procedure. Number of permutations used is 9 999. * Significant difference at *P*<0.05 and *** significant difference at *P*<0.001.

3.1.1 *Inter-population morphometric variation*

CDA analyses were highly significant for all sampled locations. Eight roots were defined, but only six were significant in female and male-analysis (Table 2). The first two functions provided the best overall discrimination between populations. Marbled crab individuals were correctly assigned to the defined locations in 88.60% of the cases for females, and in 88.35% for males (Table 3). In addition, NPMANOVA analyses showed relatively significant differentiation among locations for both sexes with a clear distinction of the population of Monastir (Table 4). SIMPER analyses showed that CW-1st Sp, CL, LABW and ML contributed the most to the separation between female populations, while only CW-1st Sp, CL and ML were major contributors to the separation between male populations with a cumulative contribution of 59.95% in female crabs and 61.49% in male crabs. The distribution of the individuals in the canonical discriminant space formed by Root 1 and Root 2 (both roots explained 80% of the total discrimination in both sexes), showed overlapping between northern locations (Tabarka, Bizerte, Korbos, Kelibia and Benikhiar) and their southern counterparts (Chebba, Sfax and Zarzis) with the existence of a remarkable signal of separation between them. However, no overlapping between the population of Monastir and the remaining ones was shown, indicating the most morphological distinction. Specimens from this location were separated from the others mainly by the means of Root 1 in both sexes.

3.1.2 *Inter-group morphometric variation*

Discriminant canonical analysis yielded highly significant discrimination between the three defined groups in external morphometric characters for *P. marmoratus* females (Wilk's lambda=0.122, *P*<0.001) and males (Wilk's lambda=0.060, *P*<0.001) (Table 2). This morphological differentiation among groups was confirmed by the highly significant inter-group Mahalanobis distances for females ($D^2_{G1\text{-}G2}$=39.324, *P*<0.001; $D^2_{G1\text{-}G3}$=7.083, *P*<0.001; $D^2_{G2\text{-}G3}$=25.766, *P*<0.001) as well as for males ($D^2_{G1\text{-}G2}$=46.857, *P*<0.001; $D^2_{G1\text{-}G3}$=7.468, *P*<0.001; $D^2_{G2\text{-}G3}$=45.973, *P*<0.001). Marbled crab females were correctly assigned to the defined groups in 94.30% of the cases, while males were corrcetly classified in 94.17% of the cases (Table 3). The individuals plotted in the discriminant canonical space, formed by Root 1 and Root 2, clustered according to the predefined grouping (three groups) (Fig. 2). Despite some overlap, the three groups were well separated on both roots for both sexes. Hence, the discriminant analysis by grouping populations into three groups, suggests the presence of three metapopulations of *P. marmoratus* that could be geographically delimited on the basis of morphology: the first, localized in the Northern Tunisian coast, consists of the locations of Tabarka, Bizerte, Korbos, Kelibia and Benikhiar; the second includes the central location of Monastir; the third is situated in the South, and consists of the locations of Chebba, Sfax and Zarzis. Consequently, the defined three groups could be assigned to two biogeographically different regions: Group 1 representing the Western Mediterranean and Groups 2 and 3 belonging to the Eastern Mediterranean.

3.2 *Population genetics analyses*

Restriction profiles corresponding to each enzyme were analyzed and allowed to define a composed haplotype for each individual. We distinguished ten different haplotypes, of which seven were unique. Haplotype 1 was major and found in 169 specimens and in the whole studied populations. Whereas Haplotype 2 and Haplotype 3 were present each in two individuals of the populations of Korbos and Sfax respectively. The population of Zarzis harbors the highest number of haplotypes (8, with seven haplotypes were specific to this region) (Table 5). Haplotype diversity was low in almost all the studied populations, and ranged from 0 in the populations of Tabarka, Bizerte, Kelibia, Benikhiar, Monastir, and Chebba to 0.589±0.129 in the population of Zarzis (Table 6). Genetic analysis of *P. marmoratus* populations via the mitochondrial marker COI revealed the existence of high genetic homogeneity. Indeed, nearly all F_{ST} values among populations were not significant (*P*>0.05) except those noted between the population of Zarzis and the populations of Tabarka, Bizerte, Kelibia, Benikhiar, Monastir and Chebba respectively after B-Y FDR correction (Table 7).

Table 2. The examination of eigenvalues and the hypothesis test for those eigenvalues by Chi-square approach in discriminant analysis of *P. marmoratus* populations and groups

Removed roots	Sex	Eigenvalue	Canonical correlation	Wilks'lambda	Chi-square	df	*P*
Populations							
0	female	7.833	0.941	0.003	996.778	224	***
	male	7.382	0.938	0.002	1050.892	224	***
1	female	2.716	0.854	0.028	618.807	189	***
	male	2.253	0.832	0.017	690.520	189	***
2	female	1.178	0.735	0.104	391.049	156	***
	male	1.557	0.780	0.055	490.565	156	***
3	female	0.827	0.672	0.228	255.966	125	***
	male	0.924	0.693	0.141	331.388	125	***
4	female	0.426	0.546	0.417	151.370	96	***
	male	0.771	0.659	0.272	220.404	96	***
5	female	0.303	0.482	0.596	89.785	69	*
	male	0.493	0.574	0.482	123.469	69	***
6	female	0.141	0.351	0.776	43.804	44	ns
	male	0.254	0.450	0.720	55.523	44	ns
7	female	0.127	0.336	0.886	20.895	21	ns
	male	0.106	0.310	0.903	17.152	21	ns
Groups							
0	female	3.368	0.878	0.122	370.499	56	***
	male	5.483	0.919	0.060	482.491	56	***
1	female	0.867	0.681	0.535	110.278	27	***
	male	1.528	0.777	0.395	160.047	27	***

Notes: * Significant difference at $P<0.05$, *** significant difference at $P<0.001$, and ns means $P>0.05$.

Table 3. Percentage of correct assignment by sex of *P. marmoratus* populations and groups based on the morphometric classification function of all the variables

Population	Sex	Correct assignment/%	Group	Sex	Correct assignment/%
Tabarka	female	88.23	Group 1	female	96.58
	male	91.30		male	94.84
Bizerte	female	95.45			
	male	75.00			
Korbos	female	100			
	male	100			
Kelibia	female	65.00			
	male	80.95			
Benikhiar	female	75.00			
	male	76.19			
Monastir	female	100	Group 2	female	100
	male	100		male	100
Chebba	female	95.83	Group 3	female	87.93
	male	93.75		male	90.76
Sfax	female	85.71			
	male	95.83			
Zarzis	female	76.92			
	male	84.00			
Total	female	88.60	Total	female	94.30
	male	88.35		male	94.17

4 Discussion

This study is the first report on population structure of *P. marmoratus* along part of the African Mediterranean coast. Previous investigations were focused on European Atlantic and Mediterranean populations (Silva and Paula, 2008; Ferreira Silva et al., 2009; Silva et al., 2009; Fratini et al., 2008; Fratini et al., 2011; Fratini et al., 2013). Our results demonstrated clear discordance between levels of phenotypic and preliminary genetic variability among populations over the geographical range surveyed, and the extensive morphological differences between populations. Based on the traits examined, at least three morphometric "units" could be distinguished, which is consistent with the findings of previous studies using similar sampling scheme on other shore crabs (Bentley et al., 2002; Brian, 2005, Lye et al., 2005; Brian et al., 2006; Duarte et al., 2008; Silva and Paula, 2008; Hopkins and Thurman, 2010). Recently, similar morphometric study of the green crab *Carcinus aestuarii* populations from the same locations along the Tunisian coast (Deli et al., 2014) showed the

same pattern of variability as that found in *P. marmoratus*. These findings provide evidence that despite their immense capacity of dispersal, shore crabs may exhibit phenotypic variability even within restricted geographical areas. Moreover, this may hint to the possible impact of the studied environment on yielding biogeographic units in marine crabs across a given sampling scale (Brian et al., 2006; Silva and Paula, 2008; Deli et al., 2014). Pairwise NPMANOVA comparisons showed that both female and male shore crabs exhibiting significant inter-population differences in the measured traits. It is generally assumed that intraspecific polymorphism arises from divergent selection pressures between alternative environments (Schluter, 2000). Population-level phenotypic divergence can result from either genetic differentiation or phenotypic plasticity; both processes can represent adaptive responses to natural and sexual selection that may vary across a species' range (Orr and Smith, 1998; Schluter, 2000; Bell et al., 2004). Hence, variations in the morphology of the characteristics analyzed could be associated with the differential ability of shore crabs to compete or reproduce in the environment at each site. The SIMPER routine analysis showed that morphometric differences among the sampling sites of *P. marmoratus* were driven mainly by carapace dimensions for both sexes. This could be related to agonistic interactions and reproductive behaviour as noted for predatory portunid crabs (Lee and Seed, 1992). Otherwise, it may suggest the effect of population density, feeding efficiency and food availability as diet has been shown to be a major cause of phenotypic differences in other shore crab species (Freire et al., 1996). The morphometric population structure of *P. marmoratus*, noted in the present study, could be also a response to differing environmental conditions such as exposure to wave action and linked-prey availability. It is known that *P. marmoratus* has a flexible omnivorous diet (Cannicci et al., 2002; Cannicci et al., 2007). Furthermore, this species is an opportunistic forager so the diet composition should reflect prey availability, and the abundance of intertidal prey species is known to vary along the wave exposure gradient (Burrows et al., 2008). Ferreira Silva et al. (2009) showed that phenotypic variation in *P. marmoratus* can result from differing food availability on sheltered and exposed shores. We can also hypothesize that the different morpho-groups, noted in our study, could be the result of a regional adaptation to specific abiotic features such as temperaure and salinity. It is known that the Eastern Mediterranean basin is warmer and more saline than the Western part and its average water temperature ranges 16–29°C with average salinity of 39 while the Western basin displays lower temperatures (12–23°C) and salinity (36) (Serena, 2005). Shirley et al. (1987) showed that water temperature during incubation had a considerable effect on zoeal morphology in the Dungeness crab *Cancer magister*. It is possible that intraspecific differences due to latitude or habitat depend on how steep the thermal gradient is, leading to local adaptation of thermal tolerance. Specifically, the striking separation of the specimens of Monastir from the others deserves a detailed investigation as the same pattern was observed recently in *C. aestuarii* (Deli et al., 2014). Being unable at this stage to disentangle precisely the driving mechanisms that led to such pattern of morphometric variability, additional work is needed to identify the biotic and abiotic factors that vary among locations and evaluate their respective roles in promoting inter-population morphological divergence.

While morphometric analysis data pointed to the existence of

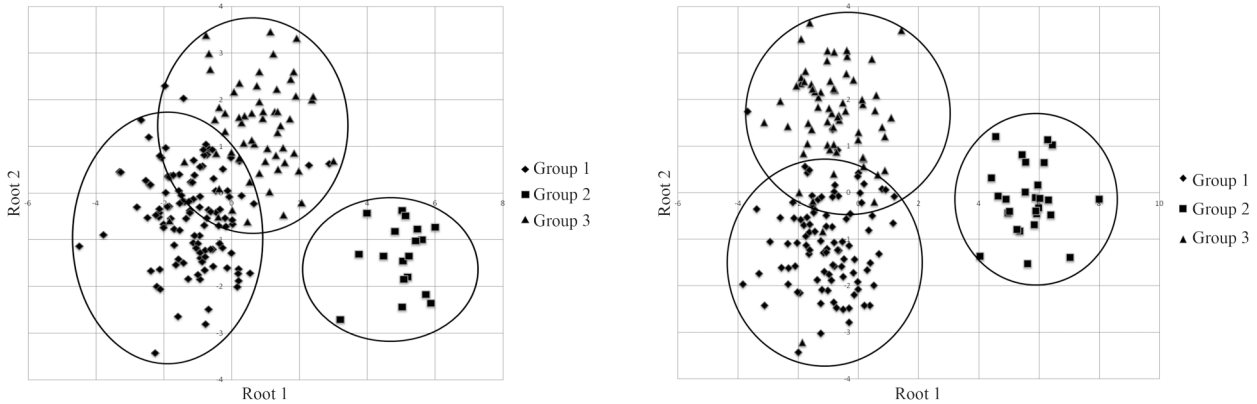

Fig. 2. Results of the canonical discriminant analyses for the morphometric data of female (a) and male (b) *P. marmoratus*. Plot of the first two canonical variates. Dependent categories: three groups.

Table 4. Pairwise NPMANOVAs between all pairs of the marbled crab *P. marmoratus* populations, based on Bray-Curtis distances

	Tabarka	Bizerte	Korbos	Kelibia	Benikhiar	Monastir	Chebba	Sfax	Zarzis
Tabarka	–	ns	ns	ns	ns	***	*	ns	ns
Bizerte	**	–	ns	ns	ns	***	**	ns	ns
Korbos	*	ns	–	ns	ns	***	**	ns	ns
Kelibia	ns	ns	ns	–	ns	***	ns	ns	ns
Benikhiar	ns	*	*	ns	–	***	ns	ns	ns
Monastir	***	***	***	***	***	–	***	***	***
Chebba	ns	ns	ns	ns	ns	***	–	**	*
Sfax	ns	ns	ns	ns	ns	***	ns	–	ns
Zarzis	ns	ns	ns	ns	ns	***	ns	ns	–

Notes: Level of significance, inferred from pairwise male (below the diagonal) and female (above the diagonal) comparisons, is computed by permutation of group membership with 9 999 replicates: * significant difference at $P<0.05$, ** significant difference at $P<0.01$, *** significant difference at $P<0.001$, and ns $P>0.05$.

three differentiated groups, mitochondrial DNA data recovered only a single evolutionary lineage. Restriction fragment analysis of the mitochondrial marker COI, carried on the same populations, revealed low mitochondrial diversity and lack of structure in the marbled crab *P. marmoratus* along the Tunisian coast. Estimated haplotype frequencies and *F* statistics showed a high level of genetic homogeneity. This finding contrasts with those previously noted in other decapod species such as the caramote prawn *Penaeus kerathurus* (Zitari-Chatti et al., 2009) and the green crab *C. aestuarii* [①] across the investigated region. Using the same marker for genetic investigation, populations of the latter species were shown to be structured across the Siculo-Tunisian region. This suggests that lack of mtDNA-based genetic structure, noted in *P. marmoratus*, could be linked to incomplete lineage sorting because of a slow mtDNA mutation rate in this particular taxonomic group. Although the COI gene has been shown to be variable enough in different decapod species (Fratini and Vannini, 2002; Roman and Palumbi, 2004; Darling et al., 2008), it failed to reveal genetic structuring in others like the blue crab *Callinectes bellicosus* (Pfeiler et al., 2005). Otherwise, the low genetic diversity could represent a possible signature of historical events that happened in the entire region (i.e., possible bottleneck during the glaciations periods in the Mediterranean). It is very unlikely that present day selection could shape similarly the genetic diversity in environmentally different localities, i.e., Tabarka in the western Mediterranean and Chebba in the eastern Mediterranean. Lack of COI divergence patterns was recorded previously in *P. marmoratus* from the European western Mediterranean and eastern Atlantic coasts (Fratini et al., 2011). The authors argued that the distribution of genetic variation of the species could be the residual effect of a recent evolutionary history. The mtDNA is strictly a useful marker in the description of population genetic structure owing to its high level of polymorphism and evolutionary rate ten times faster than nuclear genomes. In our case, female-biased dispersal may contribute to the pattern of genetic variation observed using the maternally inherited mtDNA marker (Awadalla et al., 1999). Nevertheless, mtDNA-derived dispersal estimates, in the marine environment, most likely represent the overall dispersal rather than exclusively for female because sex-biased dispersal is highly unlikely for planktonic larvae (Barber et al., 2006).

The presence of most common haplotype (Haplotype 1) in all

Table 5. Distribution pattern of the found haplotypes in the studied populations of the marbled crab *P. marmoratus*

Haplotype	Population								
	Tabarka	Bizerte	Korbos	Kelibia	Benikhiar	Monastir	Chebba	Sfax	Zarzis
1	20	20	18	20	20	20	20	18	13
2	0	0	2	0	0	0	0	0	0
3	0	0	0	0	0	0	0	2	0
4	0	0	0	0	0	0	0	0	1
5	0	0	0	0	0	0	0	0	1
6	0	0	0	0	0	0	0	0	1
7	0	0	0	0	0	0	0	0	1
8	0	0	0	0	0	0	0	0	1
9	0	0	0	0	0	0	0	0	1
10	0	0	0	0	0	0	0	0	1
Total number of specimens	20	20	20	20	20	20	20	20	20

Table 6. Haplotype diversity within the marbled crab *P. marmoratus* populations

Population	*N*	*N*h	*h*
Tabarka	20	1	0.000±0.000
Bizerte	20	1	0.000±0.000
Korbos	20	2	0.189±0.108
Kelibia	20	1	0.000±0.000
Benikhiar	20	1	0.000±0.000
Monastir	20	1	0.000±0.000
Chebba	20	1	0.000±0.000
Sfax	20	2	0.189±0.108
Zarzis	20	8	0.589±0.129
Total	180	10	0.107±0.187

Notes: *N* represents number of samples per population, *N*h number of haplotype, and *h* haplotype diversity. Each value is the mean±standard deviation.

populations with high frequency suggests that gene flow could be operating in this area, maintaining the genetic homogeneity observed. *P. marmoratus* juvenile and adult movements alone were not able to homogenize populations. Instead, planktonic larvae seem to have a high dispersive capacity. This characteristic, shared with many other marine invertebrates, could enhance

[①] Deli T, Said K, Chatti N. 2015. Genetic differentiation among populations of the green crab *Carcinus aestuarii* (Brachyura, Carcinidae) from the Eastern and Western Mediterranean coasts of Tunisia. Acta Zoologica Bulgarica, 67 (in press)

gene flow and maintain connectivity between populations. It is admitted that migration of few individuals per generation is sufficient to prevent genetic differentiation (Slatkin and Barton, 1989). *P. marmoratus* larvae can drift in open waters for almost 30 d (Cuesta and Rodriguez, 2000), a period long enough to permit homogenization of geographically distant populations. Theoretically drifting *P. marmoratus* larvae can reach very distant locations on the Tunisian littoral, an area characterized by a unidirectional surface current called "the Algerian current" originating from the Atlantic, moving eastward along the North-African coast and flowing into the eastern basin. This marine current and the continuous Tunisian coast could have enhanced genetic homogeneity of the populations studied according to the linear stepping stone model. Indeed, this model is common in continuously-distributed species like *P. marmoratus* where homogenization of distant populations is maintained by a gene flow that let exchange of individuals between adjacent or nearby populations (Kimura and Weiss, 1964; Slatkin and Maddison, 1990). Despite the lack of geographically associated haplotypes and genetic structure among populations, our analyses suggested genetic differentiation between the southernmost population of Zarzis in the Eastern Mediterranean coast of Tunisia and the remaining populations except Korbos and Sfax. Surprisingly, the population of Zarzis showed the highest level of genetic diversity and harbored high number of private haplotypes. This suggests that Zarzis could actually represent a continuity of a more eastern genetically different unit or probably reflects a signature of a historic distribution of the genetic polymorphism in the region. Extending the analyses to a more complete sampling would shed more light on this issue.

Table 7. Pairwise F_{ST} values among populations of the marbled crab *P. marmoratus*, based on haplotype frequencies

	Tabarka	Bizerte	Korbos	Kelibia	Benikhiar	Monastir	Chebba	Sfax	Zarzis
Tabarka	–								
Bizerte	0.000	–							
Korbos	0.052	0.052	–						
Kelibia	0.000	0.000	0.052	–					
Benikhiar	0.000	0.000	0.052	0.000	–				
Monastir	0.000	0.000	0.052	0.000	0.000	–			
Chebba	0.000	0.000	0.052	0.000	0.000	0.000	–		
Sfax	0.052	0.052	0.002	0.052	0.052	0.052	0.052	–	
Zarzis	**0.157**	**0.157**	0.061	**0.157**	**0.157**	**0.157**	**0.157**	0.061	–

Notes: Bold values indicate significant difference, obtained after B-Y FDR correction (critical value=0.011 98 with 36 hypothesis tests and alpha=0.05).

The discordance between morphometric and genetic levels of variability has been shown previously in other shore crab species like *Carcinus meanas* along the British coasts, where low levels of allozyme diversity contrasted with extensive morphometric variability (Brian et al., 2006). Silva and Paula (2008) showed the same pattern when combining both levels of mitochondrial and morphometric variability in population analysis of the Portuguese *P. marmoratus*. As far as the outcome of the present investigation analyses could infer, the pattern of discrepancy between morphological and mtDNA COI data in Tunisian *P. marmoratus* might refer to the involvement of abiotic factors as possible contributors to the noted morphometric separation. Indeed, Brian et al. (2006) hinted that patterns of morphological variability in the Atlantic green crab *C. maenas* are largely determined by local environmental conditions and the species may exhibit phenotypic plasticity in UK populations. In addition, Schubart et al. (2001) suggested that *Brachynotus gemmellari* and *B. sexdentatus* possibly represent different ecophenotypes of a single species at different depths. Similarly, molecular and morphometric comparisons in *Cyrtograpsus affinis* and *C. altimanus* revealed no genetic structure but two different morphs that were always associated with subtidal versus intertidal habitats (Spivak and Schubart, 2003).

Based on the current data, no conclusion can be made on the driving mechanisms that might have shaped morphometric and mitochondrial DNA variation patterns in Tunisian *P. marmoratus*. Further ecological and genetic investigations are needed. Particularly, investigation of highly variable markers such as the nuclear microsatellites, which allowed high genetic resolution in this intertidal crab over small geographic scales (Fratini et al., 2008; Fratini et al., 2011; Fratini et al., 2013; Silva et al., 2009), is required. These nuclear markers would be more suitable for the investigation of contemporary gene flow. DNA sequence data from the mtDNA control region (CR) would be desirable as it is also characterized by high levels of variability and susceptibility to genetic drift (Avise, 2000). The development of adequate PCR primers for the survey of mtDNA CR sequence variation in *P. marmoratus* will specifically facilitate the population-level research. In addition, analysis of populations covering a wider distribution range would help getting a more complete picture about patterns of intra and inter population phenotypic and genetic variability of this species and enrich our knowledge about its evolutionary history.

Acknowledgements

The authors want to thank Christoph D Schubart and Silke Reuschel, from the University of Regensburg (Germany), for their valuable and interesting advices on the appropriate choice of morhometric traits used in this study. The authors also would like to thank three anonymous reviewers for their helpful and constructive comments that greatly contributed to improving the manuscript quality.

References

Anastasiadou C, Leonardos I D. 2008. Morphological variation among populations of *Atyaephyra desmarestii* (Millet, 1831) (Decapoda: Caridea: Atyidae) from freshwater habitats of northwestern Greece. Journal of Crustacean Biology, 28(2): 240–247

Anderson M J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology, 26(1): 32–46

Anderson M J. 2005. Permanova-Permutational multivariate analysis of variance [dissertation]. Auckland: Department of Statistics University of Auckland

Anderson M J, Ter Braak C J F. 2003. Permutation tests for multifactorial analysis of variance. Journal of Statistical Computation and Simulation, 73(2): 85–113

Arnaud-Haond S, Migiaccio M, Diaz-Almela E, et al. 2007. Vicariance patterns in the Mediterranean Sea: east-west cleavage and low dispersal in the endemic seagrass *Posidonia oceanica*. Journal of Biogeography, 34 (6): 963–976

Avise J C. 2000. Phylogeography: the History and Formation of Species. Cambridge, Massachusetts: Harvard University Press, 464

Bahri-Sfar L, Lemaire C, Hassine O K B, et al. 2000. Fragmentation of sea bass populations in the western and eastern Mediterranean as revealed by microsatellite polymorphism. The Royal Society Publishing: Proceedings B, 267(1446): 929–935

Barber P H, Erdmann M V, Palumbi S R. 2006. Comparative phylogeography of three codistributed stomatopods: origins and timing of regional lineage diversification in the coral triangle. Evolution, 60(9): 1825–1839

Bell M A, Aguirre W E, Buck N J. 2004. Twelve years of contemporary armor evolution in a threespine stickleback population. Evolution, 58(4): 814–824

Bentley M G, Clare A S, Scott C L, et al. 2002. A1.10–Multi-level analyses of endocrine disruption in shore crabs from estuarine and coastal environments. Comparative Biochemistry and Physiology Part A, 132: S1–S12

Borsa P, Blanquer A, Berrebi P. 1997. Genetic structure of the flounders *Platichthys flesus* and *P. stellatus* at different geographic scales. Marine Biology, 129(2): 233–246

Brian J V. 2005. Inter-population variability in the reproductive morphology of the shore crab (*Carcinus maenas*): evidence of endocrine disruption in a marine crustacean? Marine Pollution Bulletin, 50(4): 410–416

Brian J V, Fernandes T, Ladle R J, et al. 2006. Patterns of morphological and genetic variability in UK populations of the shore crab *Carcinus maenas* Linnaeus, 1758 (Crustacea: Decapoda: Brachyura). Journal of Experimental Marine Biology and Ecology, 329(1): 47–54

Brooker A L, Benzie J A H, Blair D, et al. 2000. Population structure of the giant tiger prawn *Penaeus monodon* in Australian waters, determined using microsatellite markers. Marine Biology, 136(1): 149–157

Burrows M T, Harvey R, Robb L. 2008. Wave exposure indices from digital coastlines and the prediction of rocky shore community structure. Marine Ecology Progress Series, 353: 1–12

Cadrin S X. 1995. Discrimination of American lobster (*Homarus americanus*) stocks off southern New England on the basis of secondary sex character allometry. Canadian Journal of Fisheries and Aquatic Sciences, 52(12): 2712–2723

Cannicci S, Gomei M, Boddi B, et al. 2002. Feeding habits and natural diet of the intertidal crab *Pachygrapsus marmoratus*: opportunistic browser or selective feeder? Estuarine, Coastal and Shelf Science, 54(6): 983–1001

Cannicci S, Gomei M, Dahdouh-Guebas F, et al. 2007. Influence of seasonal food abundance and quality on the feeding habits of an opportunistic feeder, the intertidal crab *Pachygrapsus marmoratus*. Marine Biology, 151(4): 1331–1342

Cannicci S, Paula J, Vannini M. 1999. Activity pattern and spatial strategy in *Pachygrapsus marmoratus* (Decapoda: Grapsidae) from Mediterranean and Atlantic shores. Marine Biology, 133(3): 429–435

Cassone B J, Boulding E G. 2006. Genetic structure and phylogeography of the lined shore crab, *Pachygrapsus crassipes*, along the northeastern and western Pacific coasts. Marine Biology, 149(2): 213–226

Clarke K R. 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology, 18(1): 117–143

Cuesta J A, Rodriguez A. 2000. Zoeal stages of the intertidal crab *Pachygrapsus marmoratus* (Fabricius, 1787) (Brachyura, Grapsidae) reared in the laboratory. Hydrobiologia, 436(1–3): 119–130

Darling J A, Bagley M J, Roman J, et al. 2008. Genetic patterns across multiple introductions of the globally invasive crab genus (*Carcinus*). Molecular Ecology, 17(23): 4992–5007

Debuse V J, Addison J T, Reynolds J D. 2001. Morphometric variability in UK populations of the European lobster. Journal of the Marine Biological Association of the United Kingdom, 81(3): 469–474

Deli T, Said K, Chatti N. 2014. Morphological differentiation among geographically close populations of the green crab *Carcinus aestuarii* Nardo, 1847 (Brachyura, Carcinidae) from the Tunisian coast. Crustaceana, 87(3): 257–283

Duarte C M, Dennison W C, Orth R J W, et al. 2008. The charisma of coastal ecosystems: addressing the imbalance. Estuaries and Coasts, 31(2): 233–238

Duffy J E. 1996. Species boundaries, specialization, and the radiation of sponge-dwelling alpheid shrimp. Biological Journal of the Linnean Society, 58(3): 307–324

Excoffier L, Laval G, Schneider S. 2005. Arlequin ver.3.0: an integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online, 1: 47–50

Ferreira Silva A C, Brazão S, Hawkins S J, et al. 2009. Abundance, population structure and claw morphology of the semi-terrestrial crab *Pachygrapsus marmoratus* (Fabricius, 1787) on shores of differing wave exposure. Marine Biology, 156(12): 2591–2599

Flores A A V, Paula J. 2002. Population dynamics of the shore crab *Pachygrapsus marmoratus* (Brachyura: Grapsidae) in the central Portuguese coast. Journal of the Marine Biological Association of the United Kingdom, 82(2): 229–241

Folmer O, Black M, Hoeh W, et al. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3(5): 294–299

Fratini S, Ragionieri L, Cutuli G, et al. 2013. Pattern of genetic isolation in the crab *Pachygrapsus marmoratus* within the Tuscan Archipelago (Mediterranean Sea). Marine Ecology Progress Series, 478: 173–183

Fratini S, Schubart C D, Ragionieri L. 2011. Population genetics in the rocky shore crab *Pachygrapsus marmoratus* from the western Mediterranean and eastern Atlantic: complementary results from mtDNA and microsatellites at different geographic scales. In: Held C, Koenemann S, Schubart C D, eds. Crustacean Issues 19: Phylogeography and Population Genetics in Crustacea. Boca Raton, Florida: Taylor & Francis/CRC Press, 191–213

Fratini S, Vannini M. 2002. Genetic differentiation in the mud crab *Scylla serrata* (Decapoda: Portunidae) within the Indian Ocean. Journal of Experimental Marine Biology and Ecology, 272(1): 103–116

Fratini S, Zane L, Ragionieri L, et al. 2008. Relationship between heavy metal accumulation and genetic variability decrease in the intertidal crab *Pachygrapsus marmoratus* (Decapoda; Grapsidae). Estuarine, Coastal and Shelf Science, 79(4): 679–686

Freire J, Sampedro M P, González-Gurriarán E. 1996. Influence of morphometry and biomechanics on diet selection in three portunid crabs. Marine Ecology Progress Series, 137: 111–121

Hammer Ø, Harper D A T, Ryan P D. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica, 4(1): 9

Hobbs R C, Botsford L W, Thomas A. 1992. Influence of hydrographic conditions and wind forcing on the distribution and abundance of Dungeness crab, *Cancer magister*, larvae. Canadian Journal of Fisheries and Aquatic Sciences, 49(7): 1379–1388

Hopkins M J, Thurman C L. 2010. The geographic structure of morphological variation in eight species of fiddler crabs (Ocypodidae: genus *Uca*) from the eastern United States and Mexico. Biological Journal of the Linnean Society, 100(1): 248–270

Hunt A. 1993. Effects of contrasting patterns of larval dispersal on the genetic connectedness of local populations of two intertidal starfish, *Pateriella calcar* and *P. exigua*. Marine Ecology Pro-

gress Series, 92: 179–186
Ingle R W. 1980. British Crabs. British Museum (Natural History). London: Oxford University Press, 222
Johnson D F, Botsford L W, Methot R D Jr, et al. 1986. Wind stress and cycles in Dungeness crab (*Cancer magister*) catch off California, Oregon, and Washington. Canadian Journal of Fisheries and Aquatic Sciences, 43(4): 838–845
Johnson D R, Hester B S, McConaugha J R. 1984. Studies of a wind mechanism influencing the recruitment of blue crabs in the Middle Atlantic Bight. Continental Shelf Research, 3(4): 425–437
Jørstad K E, Prodöhl P A, Agnalt A-L, et al. 2004. Sub-arctic populations of European lobster, *Homarus gammarus*, in northern Norway. Environmental Biology of Fishes, 69(1–4): 223–231
Kimura M, Weiss G H. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics, 49: 561–576
Lee S Y, Seed R. 1992. Ecological implications of cheliped size in crabs: some data from *Carcinus maenas* and *Liocarcinus holsatus*. Marine Ecology Progress Series, 84: 151–160
Lye C M, Bentley M G, Clare A S, et al. 2005. Endocrine disruption in the shore crab *Carcinus maenas*—a biomarker for benthic marine invertebrates? Marine Ecology Progress Series, 288: 221–232
Mense D J, Posey M H, West T, et al. 1995. Settlement of brachyuran postlarvae along the North Carolina coast. Bulletin of Marine Science, 57(3): 793–806
Narum S R. 2006. Beyond Bonferroni: less conservative analyses for conservation genetics. Conservation Genetics, 7(5): 783–787
Nei M. 1987. Molecular Evolutionary Genetics. New York: Columbia University Press, 512
Nikula R, Väinölä R. 2003. Phylogeography of *Cerastoderma glaucum* (Bivalvia: Cardiidae) across Europe: a major break in the Eastern Mediterranean. Marine Biology, 143(2): 339–350
Orr M R, Smith T B. 1998. Ecology and speciation. Trends Ecol Evol, 13(12): 502–506
Pfeiler E, Hurtado L A, Knowles L L, et al. 2005. Population genetics of the swimming crab *Callinectes bellicosus* (Brachyura: Portunidae) from the eastern Pacific Ocean. Marine Biology, 146(3): 559–569
Quesada H, Beynon C M, Skibinski D O F. 1995. A mitochondrial DNA discontinuity in the mussel *Mytilus Galloprovincialis* Lmk: pleistocene vicariance biogeography and secondary intergradation. Molecular Biology and Evolution, 12(3): 521–524
Reist J D. 1985. An empirical evaluation of several univariate methods that adjust for size variation in mor-phometric data. Canadian Journal of Zoology, 63(6): 1429–1439
Roman J, Palumbi S R. 2004. A global invader at home: population structure of the green crab, *Carcinus maenas*, in Europe. Molecular Ecology, 13(10): 2891–2898
Schluter D. 2000. Ecological character displacement in adaptive radiation. Amer Nat, 156(S4): S4–S16
Schubart C D, Cuesta J A, Rodriguez A. 2001. Molecular phylogeny of the crab genus *Brachynotus* (Brachyura: Varunidae) based on the 16S rRNA gene. Hydrobiologia, 449(1–3): 41–46
Serena F. 2005. Field identification guide to the sharks and rays of the Mediterranean and Black Sea. FAO Species Identification Guide for Fishery Purposes. Rome: FAO, 97
Shirley S M, Shirley T C, Rice S D. 1987. Latitudinal variation in the Dungeness crab, *Cancer magister*: zoeal morphology explained by incubation temperature. Marine Biology, 95(3): 371–376
Silva I C, Mesquita N, Paula J. 2010. Genetic and morphological differentiation of the mangrove crab Perisesarma guttatum (Brachyura: Sesarmidae) along an East African latitudinal gradient. Biological Journal of the Linnean Society, 99(1): 28–46
Silva I C, Mesquita N, Schubart C D, et al. 2009. Genetic patchiness of the shore crab *Pachygrapsus marmoratus* along the Portuguese coast. Journal of Experimental Marine Biology and Ecology, 378(1–2): 50–57
Silva I C, Paula J. 2008. Is there a better chela to use for geometric morphometric differentiation in brachyuran crabs? A case study using *Pachygrapsus marmoratus* and *Carcinus maenas*. Journal of the Marine Biological Association of the United Kingdom, 88(5): 941–953
Slatkin M, Barton N H. 1989. A comparison of three indirect methods for estimating average levels of gene flow. International Journal of Organic Evolution, 43(7): 1349–1368
Slatkin M, Maddison W P. 1990. Detecting isolation by distance using phylogenies of genes. Genetics, 126(1): 249–260
Spivak E D, Schubart C D. 2003. Species status in question: a morphometric and molecular comparison of *Cyrtograpsus affinis* and *C. altimanus* (Decapoda, Brachyura, Varunidae). Journal of Crustacean Biology, 23(1): 212–222
Spotte S. 1997. Sexual and regional variation in the morphology of the spotted anemone shrimp *Periclimenes yucatanicus* (Decapoda: Caridea: Palaemonidae). Journal of Crustacean Biology, 17(3): 389–397
Stamatis C, Triantafyllidis A, Moutou K A, et al. 2004. Mitochondrial DNA variation in Northeast Atlantic and Mediterranean populations of Norway lobster *Nephrops norvegicus*. Molecular Ecology, 13(6): 1377–1390
Stamatis C, Triantafyllidis A, Moutou K A, et al. 2006. Allozymic variation in Northeast Atlantic and Mediterranean populations of Norway lobster *Nephrops norvegicus*. ICES Journal of Marine Science, 63(5): 875–882
StatSoft, Inc. 1993. STATISTICA (data analysis software system: for the windows operating system reference for statistical procedures), version 4.5. www.statsoft.com
Triantafyllidis A, Apostolidis A P, Katsares V, et al. 2005. Mitochondrial DNA variation in the European lobster (*Homarus gammarus*) throughout the range. Marine Biology, 146(2): 223–235
Weber L I, Hartnoll R G, Thorpe J P. 2000. Genetic divergence and larval dispersal in two spider crabs (Crustacea: Decapoda). Hydrobiologia, 420(1): 211–219
Weber L I, Levy J A. 2000. Genetic population structure of the swimming crab *Callinectes danae* (Crustacea: Decapoda) in southern Brazil. Hydrobiologia, 420(1): 203–210
Weetman D, Ruggiero A, Mariani S, et al. 2007. Hierarchical population genetic structure in the commercially exploited shrimp *Crangon crangon* identified by AFLP analysis. Marine Biology, 151(2): 565–575
Wright S. 1950. The genetical structure of populations. Nature, 166 (4215): 247–249
Zitari-Chatti R, Chatti N, Elouaer A, et al. 2008. Genetic variation and population structure of the caramote prawn *Penaeus kerathurus* (Forskäl) from the eastern and western Mediterranean coasts in Tunisia. Aquaculture Research, 39(1): 70–76
Zitari-Chatti R, Chatti N, Fulgione D, et al. 2009. Mitochondrial DNA variation in the caramote prawn *Penaeus* (*Melicertus*) *kerathurus* across a transition zone in the Mediterranean Sea. Genetica, 136(3): 439–447