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ORIGINAL ARTICLE

Phylogeography of the marbled crab *Pachygrapsus marmoratus* (Decapoda, Grapsidae) along part of the African Mediterranean coast reveals genetic homogeneity across the Siculo-Tunisian Strait versus heterogeneity across the Gibraltar Strait

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ABSTRACT
We investigate the influence of previously postulated biogeographic barriers in the Mediterranean Sea on the population genetic structure of a highly dispersive and continuously distributed coastal species. In particular, we examine nuclear and mitochondrial genetic variation in the marbled crab, *Pachygrapsus marmoratus*, across part of the African Mediterranean coast in order to assess the influence of the Siculo-Tunisian Strait on its population genetic structure. Four polymorphic microsatellite loci were genotyped for 110 individuals, collected from eight locations covering parts of the Algerian, Tunisian and Libyan coasts. In addition, mtDNA corresponding to the Cox1 gene was sequenced for 80 samples. The corresponding results show contrasting patterns of genetic differentiation. While mtDNA results revealed a homogeneous haplotype composition in our study area, microsatellite data depicted genetic differentiation among populations, but not associated with any geographic barrier. This pattern, already recorded for this species from different geographic regions, may hint at the involvement of a complex series of abiotic and biotic factors in determining genetic structure. Demographic history reconstruction, inferred from mtDNA data, supports demographic and spatial expansion for the North African metapopulation dating back to the Mid-Pleistocene and following an historical bottleneck. Comparison of these African mitochondrial sequences with new sequences from a Turkish population and previously published sequences revealed a weak but significant separation of Atlantic and Mediterranean populations across the Gibraltar Strait, which was not recorded in previous studies of this grapsid species.

Introduction

The Mediterranean Sea is characterized by a complex circulation system in which two main water bodies meet, namely the Modified Atlantic Water (MAW) and the Levantine Intermediate Water (LIW) (Astraldi et al. 1999). The hydrology is also shaped by the presence of physical barriers such as straits and channels (Béranger et al. 2004). The most important Mediterranean barriers are the Almeria-Oran Oceanographic Front, the Siculo-Tunisian Strait and the hydrographic isolation of the Aegean-Ionian and Adriatic Seas (Astraldi et al. 1999; Patarnello et al. 2007). These barriers are known to restrict gene flow in some species, leading to genetic differentiation among populations (Borsa et al. 1997; Patarnello et al. 2007).

The transition zone between the Eastern and Western Mediterranean Basins constitutes one of the best documented biogeographic transitions in the Mediterranean Sea (see Arnaud-Haond et al. 2007). The Siculo-Tunisian Strait, located between Cap Bon in Tunisia and Mazara Del Vallo in Sicily (Italy), is postulated to separate these two Mediterranean basins (Quignard 1978). On both sides of this barrier, water bodies circulate with different hydrological, physical and chemical characteristics (Béranger et al. 2004). It has been documented as a geographic break, causing population genetic differentiation in several marine species (e.g. Quesada et al. 1995; Borsa et al. 1997; Bahri-Sfar et al. 2000; Nikula & Vainola 2003; Arnaud-Haond et al. 2007; Mejri et al. 2009; Zitari-Chatti et al. 2009; Kaouèche et al. 2011; Ragionieri & Schubart

2013). Therefore, population genetic analyses across this potential barrier may contribute to the identification of the phylogeographic patterns and to depict historical events that might have shaped the genetic structure of marine species.

The African Mediterranean coast, especially the part encompassing the Tunisian and Libyan littoral, is considered as an appropriate area to study biogeographic processes because it harbours populations located west and east of the well-known biogeographic barrier of the Siculo-Tunisian Strait. Furthermore, it is hypothesized that episodes of oceanic fluctuation experienced within the central Mediterranean, specifically across the transition zone of the Siculo-Tunisian Strait, during the Pleistocene, may have affected both the genetic and demographic structure of populations for a broad variety of marine taxa (Bahri-Sfar et al. 2000; Stefanni & Thorley 2003; Zardoya et al. 2004; Mejri et al. 2009; Zitari-Chatti et al. 2009; Kaouèche et al. 2011).

The marbled crab *Pachygrapsus marmoratus* (Fabricius, 1787) is one of the most widely distributed and abundant decapod crustacean species in the Mediterranean and northeastern Atlantic rocky intertidal environment, and colonizes the whole intertidal belt regardless of its width (Cannici et al. 1999). Despite its high dispersal capacity, this grapsid species represents a good model to test for population subdivision, because of its local abundance and the accumulated knowledge of its development, ecology and genetics (Cannicci et al. 1999, 2002; Cuesta & Rodríguez 2000; Ingle & Clark 2006; Fratini et al. 2008, 2011, 2013; Silva & Paula 2008; Ferreira Silva et al. 2009; Silva et al. 2009a, 2009b). Factors expected to promote genetic homogeneity in this species include high fecundity (Flores & Paula 2002), a planktonic larval period that can last more than four weeks (Cuesta & Rodríguez 2000; Cuesta et al. 2011), and a continuous coastal distribution throughout the Mediterranean Sea, Black Sea and northeastern Atlantic coast from Brittany to Morocco, including the Canary Islands, the Azores and Madeira (Ingle 1980).

Previous studies on *P. marmoratus* revealed different patterns of mitochondrial and nuclear DNA variation and suggested relatively complex local genetic structure (Fratini et al. 2008, 2011, 2013; Silva et al. 2009b). Indeed, population genetic investigations at macro- to meso-geographic scales using mtDNA failed to reveal separation among populations across known phylogeographic barriers in the Mediterranean Sea, such as the Gibraltar Strait and the Siculo-Tunisian Strait (Fratini et al. 2011). However, population genetic studies, performed on local scales and investigating polymorphism at microsatellite loci, recorded

patterns of genetic differentiation among geographically close populations from the coast of Tuscany (Fratini et al. 2008, 2011) and the Tuscan archipelago (Fratini et al. 2013), with a genetic separation between the Ligurian and Tyrrhenian Seas populations. Another micro-geographic survey along the Portuguese coast, using the same nuclear markers, revealed the existence of genetic patchiness among populations of *P. marmoratus*, unrelated to any geographic gradient (Silva et al. 2009b). No genetic structure was found at a micro-geographic scale along the Tunisian coast under the likely influence of the Siculo-Tunisian Strait, but this study was based only on restricted fragment length polymorphisms (RFLPs) analyses of the mitochondrial cytochrome oxidase I (Cox1) gene (Deli et al. 2015a). The use of more variable markers, such as nuclear microsatellites, may allow for the detection of subtle population structure along the African Mediterranean coast, as these polymorphic markers provided high genetic resolution in *P. marmoratus* over small geographic scales (see Fratini et al. 2008, 2011, 2013; Silva et al. 2009b).

In light of these considerations, the main aim of the present study was to re-investigate the influence of the Siculo-Tunisian Strait on the genetic structure of populations of *P. marmoratus* along the African Mediterranean coast with molecular markers having higher resolution than those previously used by Deli et al. (2015a). For this purpose, a set of polymorphic microsatellite loci, specifically isolated and characterized for this grapsid species by Fratini et al. (2006), were genotyped in samples from eight locations covering parts of the Algerian, Tunisian and Libyan coasts.

Cox1 sequences were also obtained for all the North African samples and for one population from southern Turkey. Furthermore, these new sequences were combined with the existing dataset by Fratini et al. (2011) (including mainly Italian, but also Atlantic populations) to investigate an overall mitochondrial geographic structure within the Mediterranean Sea and in comparison to the adjacent Atlantic Ocean. This allowed a re-analysis of possible genetic separation across the Siculo-Tunisian Strait, but also a preliminary West-East comparison with the geographic extremes, Turkey and the Canary Islands.

Materials and methods

Sampling and DNA extraction

Specimens of *Pachygrapsus marmoratus* were sampled during field missions from intertidal or shallow subtidal locations along the African Mediterranean coast
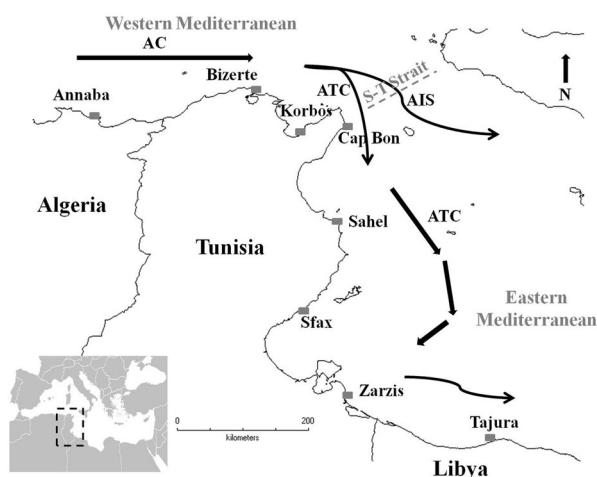
Figure 1. Sampling locations of *Pachygrapsus marmoratus* from the African Mediterranean coast, with sea surface currents of the studied region (according to Béranger et al. 2004). AC: Algerian Current; AIS: Atlantic Ionian Stream; ATC: Atlantic Tunisian Current.

(Figure 1). From each crab, muscle tissue was removed from a pereiopod and stored in absolute ethanol at −30°C until needed. A total of 80 African crabs were used for mtDNA sequencing and 110 for microsatellite analyses (Table I). Total genomic DNA was extracted from pereiopod muscle tissue, using the Wizard® genomic DNA purification kit (Promega).

Microsatellite loci amplification and allele sizing

A total of 110 individuals of *Pachygrapsus marmoratus* were screened for polymorphisms at four variable microsatellite loci (pm-101, pm-99, pm-183 and pm-187), specifically isolated and described for the species (Fratini et al. 2006). DNA amplification was carried out in 10.2 µl reactions with the following components: ddH$_2$O, dNTPs (0.12 mM each), PCR buffer (1×), MgCl$_2$ (1.23 mM), forward and reverse primers (0.4 µM each), Taq polymerase (0.5 U; MBI Fermentas, St Leon-Rot, Germany) and diluted DNA. PCR thermal cycling conditions were adapted from Fratini et al. (2006), with the following profile: 35 cycles with 30 s for denaturation at 94°C, 45 s for annealing at 57–65°C and 180 s for extension at 72°C, preceded by 5 min of initial denaturation at 94°C and followed by 30 min of final extension at 72°C. For detection of polymorphisms, the forward primer for each locus was 5′-labelled, and labelled amplicons from the four loci were loaded in three different, partly multiplexed sets (pm-101 FAM + pm-99 NED; pm-183 NED; and pm-187 VIC). For each set, 1 µl of each diluted PCR product was loaded with 25 µl of formamide and 0.5 µl of TAMRA 500 size standard in a final volume of 26.5 µl for successive dimensional analysis. Sizing was performed with reference to the internal size standard TAMRA 500 in an ABI Prism 310 Genetic Analyzer (Applied Biosystems) at the

Table I. Sampling information for the marbled crab (*Pachygrapsus marmoratus*), including collecting sites, countries/regions, geographic coordinates and the number of specimens examined for mtDNA and microsatellite analyses. Note: coordinates from Fratini et al. (2011) corrected.

			Number of examined specimens	
Collection site	Country/region	Geographic coordinates	mtDNA	Microsatellite loci
Newly analysed data				
Annaba	Algeria	36°54′N 07°45′E	10	10
Bizerte	Tunisia	37°16′N 09°52′E	8	20
Korbos	Tunisia	36°49′N 10°34′E	9	10
Cap Bon	Tunisia	36°51′N 11°05′E	12	20
Sahel	Tunisia	36°10′N 10°49′E	11	20
Sfax	Tunisia	34°44′N 10°45′E	9	10
Zarzis	Tunisia	33°30′N 11°06′E	11	10
Tajura	Libya	32°52′N 13°20′E	10	10
Beldibi	Turkey	36°52′N 28°15′E	18	–
Previously investigated data by Fratini et al. (2011)				
Calafuria	Italy	43°17′N 10°12′E	15	–
Rocchette	Italy	42°46′N 10°47′E	14	–
Porto Ercole	Italy	42°23′N 11°12′E	15	–
Giglio Island	Italy	42°21′N 10°55′E	11	–
Montecristo	Italy	42°20′N 10°19′E	15	–
Gaeta	Italy	41°11′N 13°35′E	15	–
Fusaro Lagoon	Italy	40°48′N 14°02′E	15	–
Crotone	Italy	39°04′N 17°08′E	16	–
Messina	Italy	38°12′N 15°34′E	16	–
St Florent	Corsica	42°41′N 09°16′E	15	–
Tarragona	Spain	41°07′N 01°16′E	20	–
Valencia	Spain	39°26′N 00°19′W	20	–
Cala Iris	Morocco	35°09′N 04°21′W	15	–
Sesimbra	Portugal	38°24′N 09°04′W	18	–
Matorral	Fuerteventura	28°02′N 14°19′W	18	–

University of Regensburg, using GENOTYPER 3.5 and GENESCAN 3.5 (Applied Biosystems).

Mitochondrial DNA amplification and sequencing

The mitochondrial cytochrome oxidase subunit I (Cox1) gene, consisting of approximately 1500 basepairs (bp), was amplified using the primers COL6a and COH16, specifically designed for brachyuran crab species (see Schubart 2009). The PCR reaction mix contained: ddH$_2$O, dNTPs (0.12 mM each), PCR buffer (1×), MgCl$_2$ (4 mM), both primers (0.24 µM each), Taq polymerase (0.5 U; MBI Fermentas), and diluted genomic DNA, adding up to a final master mix volume of 25 µl. PCR amplifications were carried out with an initial denaturation phase of 4 min at 94°C, followed by 40 cycles, each composed of 45 s of denaturation at 95°C, 60 s of annealing at 52°C and 90 s of extension at 72°C. These cycles were followed by 8 min of final extension at 72°C. PCR products were loaded on 1.5% agarose gel with GelRed staining and visualized under UV light. Only strong products were outsourced for sequencing with primer COL6a to LGC Genomics (Berlin). The sequences obtained were visually corrected with Chromas Lite 2.1.1 (Technelysium Pty Ltd 2012), aligned with BIOEDIT (Hall 1999) and trimmed to a 739 bp fragment for subsequent analyses.

Statistical analysis of microsatellite data

Nuclear microsatellite loci were investigated in North African populations of *Pachygrapsus marmoratus* from the Algerian, Tunisian and Libyan coasts. Deviations from Hardy–Weinberg equilibrium (HWE) in the marbled crab populations were tested using the Markov chain method with 5000 iterations as implemented in GENEPOP 4.2 (Raymond & Rousset 1995). Genetic variability (Ar: allelic richness, Ho: observed heterozygosity and He: expected heterozygosity) at the typed microsatellite loci, as well as Weir & Cockerham's (1984) estimation of F_{IT}, F_{ST} and F_{IS}, were calculated using FSTAT 9.2.3.2 (Goudet 1995). Genetic diversity measures in each population (Ho: observed heterozygosity, He: expected heterozygosity and F_{IS}: inbreeding coefficient) were estimated with GENEPOP using the Markov chain parameter with 10,000 dememorizations, 20 batches and 5000 iterations per batch. Since all populations showed highly significant heterozygote deficiencies, the dataset was checked for genotyping errors (i.e. null alleles) by means of Equation 2 from Brookfield (1996), as implemented in MICRO-CHECKER 2.2.3 (van Oosterhout et al. 2004). We then

used the software FREENA to compute a genotype dataset corrected for null alleles, following the INA method described in Chapuis & Estoup (2007).

Genetic differentiation was estimated by means of the exact test of population differentiation (Raymond & Rousset 1995), as implemented in GENEPOP. This test verifies the existence of differences in allele frequencies at each locus and for each population. Single locus P values were calculated using a Markov chain with 10,000 dememorizations, 100 batches and 5000 iterations per batch, combined over loci, using the Fisher exact test (Fisher 1935). The existence of genetic differentiation was also assessed by one-level AMOVA (Excoffier et al. 1992), using ARLEQUIN 3.1. Correlations between genetic (F_{ST} values) and geographic distances were assessed by the Mantel Test (Mantel 1967), as implemented in ARLEQUIN, with 10,000 random permutations.

An additional two-level AMOVA was performed, grouping populations according to a specific biogeographic hypothesis: African western Mediterranean versus African eastern Mediterranean. The exact test (G) of genetic differentiation, implemented in GENEPOP, was applied to the two groups of populations already tested by the structured AMOVA in order to assess both genic and genotypic differentiation.

The Bayesian approaches implemented in the two software packages, STRUCTURE 2.3.4 (Pritchard et al. 2000) and BAPS 3.2 (Corander et al. 2003), were also used to identify clusters of genetically similar populations based on the nuclear microsatellite data. BAPS generally tends to recover more clusters than STRUCTURE, and it has been suggested that both approaches should be used, particularly where levels of differentiation between populations may be low (Latch et al. 2006). For STRUCTURE analysis, the admixture model was processed using correlated allele frequencies. The probability of the number of populations (K) for the pooled data was estimated by fixing prior values of K (K was set from one to eight in our study) and comparing the Ln P (D) (the log-probability of the data) and the log-likelihood of the data. Three replicates for each K were independently performed, giving reproducible results. The analysis was run for 500,000 generations with a burn-in length of 20,000 iterations. For the BAPS analysis, the number of genetically diverged groups (K) varied from $K = 1$ to $K = 8$ in both clustering of individuals and clustering of groups of individuals. In the admixture analysis based on mixture clustering, 200 iterations were used for the number of times that individuals in the data were analysed using different simulated allele frequencies for the estimation of reference individuals, as suggested by Corander et al. (2005).

Genotypic structure analysis was also assessed by ordinating genotypes in multidimensional space by principal component analysis (PCA) of gene frequency data, using the program PCA-GEN 1.2.1 (Goudet 1999). Individuals were plotted by scores along the two principal axes (PC-I and PC-II), explaining the cumulative percentage of the total genetic diversity, testing for the existence of population groupings. Genotypic assignment was also assessed by GENECLASS 2.0 (Piry et al. 2004). The most probable origin of each individual was calculated by comparing the likelihood of the multi-locus genotype of a given individual in a set of pre-determined populations. The Bayesian method, proposed by Rannala & Mountain (1997), was chosen, together with a threshold of 0.05. GENECLASS also allowed for the detection of the number of migrants. The frequency-based method, described by Paetkau et al. (1995), was used and a threshold of 0.01 was set. For both tests, the rejection probability was obtained by simulating 10,000 individuals from allelic frequencies based on the simulation algorithm of Paetkau et al. (2004).

In order to test for evidence of a recent population bottleneck in African Mediterranean populations of *P. marmoratus*, we applied the BOTTLENECK test (Cornuet & Luikart 1996), using the heterozygosity excess method of Luikart et al. (1998), as implemented in BOTTLENECK 1.2.02 (Piry et al. 1999). This program generates the heterozygosity of each locus expected under mutation-drift equilibrium, given the observed number of alleles (Heq) under the three mutation models: the infinite allele model (IAM), the two phase mutation model (TPM) and the stepwise mutation model (SMM), using 10,000 simulations. The obtained Heq values are then averaged across loci and compared with the observed level of heterozygosity (Ho). The SMM and TPM are most appropriate for microsatellite data (Luikart & Cornuet 1998), with the TPM providing a more realistic picture of mutational events in microsatellite loci (Di Rienzo et al. 1994; Piry et al. 1999). For the TPM, 70 multi-step mutations were set, with a variance among multi-steps of 12%, as recommended for microsatellites (Piry et al. 1999), and statistical significance based on 1000 replications was obtained. In a population at mutation-drift equilibrium (i.e. the effective size of which has remained constant in the past), there is approximately an equal probability that a locus shows a heterozygosity excess or a heterozygosity deficit. We then recorded the number of loci for which Ho was greater than Heq and determined whether the overall set of deviations was statistically significant using the sign rank and Wilcoxon tests (Luikart et al. 1998).

Statistical analysis of mtDNA data

Data analysis procedure

Genetic analyses were carried out using two alignments: (1) a long alignment of 739 base pairs (bp), with sequences from North African populations, and (2) a shorter alignment of 596 bp for comparison and population genetic investigation of *Pachygrapsus marmoratus* across a wider geographic scale. The latter included the European Atlantic and western Mediterranean dataset previously investigated by Fratini et al. (2011) and the newly examined North African and Turkish sequences (Table I).

Genetic diversity and population structure

The nucleotide composition was assessed using MEGA 5.2 (Tamura et al. 2011). Measurements of DNA polymorphism, including the number of haplotypes, haplotype diversity (h; Nei 1987) and nucleotide diversity (π; Tajima 1983; Nei 1987), were calculated for each population using DNASP 5.10 (Librado & Rozas 2009). Intraspecific evolutionary relationships among the Cox1 haplotypes of *Pachygrapsus marmoratus*, for both alignment datasets, were estimated using the software TCS 1.21 (Clement et al. 2000).

We measured levels of population subdivision using both unordered (G_{ST}) and ordered alleles (N_{ST}) in order to assess the relationship between phylogeny and the geographic distribution of haplotypes. These analyses were carried out using a distance matrix (number of polymorphic sites that differed between haplotypes) that was based on the larger Cox1 data for *Pachygrapsus marmoratus*. Both population differentiation parameters (G_{ST} and N_{ST}) were estimated following the methods described by Pons & Petit (1995, 1996), using the program PERMUT & CPSRR 2.0 (Pons & Petit 1996). G_{ST} is based solely on haplotype frequencies, whereas N_{ST} also takes into account the genetic relationship among haplotypes. An N_{ST} higher than the G_{ST} usually indicates that closely related haplotypes are more often found in the same area than less closely related haplotypes (Pons & Petit 1996; Petit et al. 2005). These two parameters were compared statistically using the U-test (Pons & Petit 1996).

Evidence of population genetic differentiation within each data alignment was assessed by one-level AMOVAs (Excoffier et al. 1992), as implemented in ARLEQUIN 3.1 (Excoffier et al. 2005), based on nucleotide diversity and haplotype frequency. The extent of genetic differentiation between populations was estimated using the fixation indices: Φ_{ST} (based on a Tajima-Nei model, suggested for unequal

nucleotide frequencies, as observed in our dataset; Tajima & Nei 1984) and F_{ST} (computed using haplotypic frequencies). Significance levels of pairwise genetic distances, estimated among populations, were assessed by a randomization procedure with 10,000 permutations. Analyses of molecular variance (two-level AMOVA) were also used to examine population genetic structure of *P. marmoratus* along the West–East geographic gradient, testing for the separation across (1) the Gibraltar Strait (Atlantic vs. Mediterranean) and (2) across both the Gibraltar and Siculo-Tunisian Straits (Atlantic vs. Western Mediterranean vs. Eastern Mediterranean). A test of isolation by distance, for the whole dataset, was assessed by the Mantel test (Mantel 1967), as implemented in the program AIS 1.0 (Alleles in Space; Miller 2005). The statistical significance of the test was assessed assuming 10,000 random permutations.

Demographic history

Signatures of population demographic changes were investigated in African Mediterranean *Pachygrapsus marmoratus* using three neutrality tests: Tajima's *D* (Tajima 1989), Fu's *F*s (Fu, 1997), and Ramos-Onsins & Rozas's R_2 (Ramos-Onsins & Rozas 2002). Tajima's *D* and Fu's *F*s statistics were used to determine whether data departed from neutrality due to factors such as a population bottleneck or sudden expansion. The examination of deviation from neutrality by both *D* and *F*s indices was based on 1000 coalescent simulations, as implemented in ARLEQUIN. Significant negative *D* and *F*s values can be interpreted as signatures of population expansion. The R_2 statistic of Ramos-Onsins & Rozas (2002), which has more statistical power when population sizes are small, was calculated using a coalescent simulation algorithm implemented in DNASP, with 1000 simulations. Demographic changes in *P. marmoratus* were also examined by calculating the Harpending's raggedness index *rg* (Harpending 1994) of the observed mismatch distribution for each of the populations, according to the population expansion model implemented in ARLEQUIN. The significance of this measure, which quantifies the smoothness of the observed mismatch distribution, was tested using parametric bootstrapping (10,000 replicates). Tajima's *D*, Fu's *F*s, Ramos-Onsins & Rozas's R_2 and Harpending's raggedness index *rg* were calculated for each population and the whole dataset.

To provide other estimates of population size changes, we also examined site mismatch distributions for the whole dataset. Contrasting plots of observed and theoretical distributions of site differences yield insight into past population demographics. The expected mismatch distributions under a sudden expansion model were computed in ARLEQUIN. The sum of squared deviations (SSD) between observed and expected distributions was used as a measure of fit, and the probability of obtaining a simulated SSD greater than or equal to the expected was computed by randomization. If this probability was > 0.05, the expansion model was accepted. Graphical representation was made by means of the growth-decline model implemented in DNASP. It was investigated whether *P. marmoratus* underwent a range expansion by the spatial expansion model (Excoffier 2004) in ARLEQUIN. The mismatch distribution analysis also provides an estimation for the expansion parameters Tau (τ), Theta 0 (θ_0) and Theta 1 (θ_1), under a demographic or spatial expansion hypothesis. The value of τ can be used to calculate the time (*t*) at which the demographic or spatial expansion began, using the formula $t = \tau/2u$ (Rogers & Harpending 1992), with *u* defined as the mutation rate per site per year (Li 1977). A conventional mutation rate for the Cox1 gene in brachyuran Crustacea (1.66% per million years; Schubart et al. 1998) was used in our analysis.

The magnitude of historical demographic events for the African Mediterranean population of *P. marmoratus* was investigated using Bayesian Skyline Plots (BSP; Drummond et al. 2005). In comparison with simple parametric and older coalescent demographic methods, the smoother estimates and sensitivity of this method, together with a credibility interval, provide a realistic population size function and enable retrieval of more details than just summary statistics. Analyses were run in BEAST 1.7.5 (Drummond et al. 2012) using a GTR model and a strict molecular clock. The Markov chain Monte Carlo simulations were run with 30,000,000 iterations, while genealogies and model parameters were sampled every 1000 iterations. The first 3,000,000 iterations were discarded as burn-in, whereas the remaining results were combined in LogCombiner (Drummond et al. 2012) and summarized as BSPs after analysing their convergence in TRACER 1.5 (Rambaut & Drummond 2007).

Results

Micro-geographic scale

Microsatellites

Overall, a total of 85 alleles were detected at the four genotyped microsatellite loci. All loci were polymorphic, with the number of alleles per locus ranging from 17 (locus pm-183) to 25 (locus pm-101). The mean

Table II. The exact test for Hardy–Weinberg equilibrium in the marbled crab (*Pachygrapsus marmoratus*) and genetic diversity measures in each population, based on the analysis of microsatellite loci.

Population	Size	P	Ho	He	F_{IS}
Annaba	10	0.269 ns	0.800	0.818	0.022
Bizerte	20	0.056 ns	0.750	0.811	0.075
Korbos	10	0.567 ns	0.775	0.748	−0.035
Cap Bon	20	0.168 ns	0.700	0.712	0.017
Sahel	20	0.438 ns	0.775	0.794	0.024
Sfax	10	0.593 ns	0.825	0.798	−0.033
Zarzis	10	0.467 ns	0.800	0.781	−0.023
Tajura	10	0.081 ns	0.800	0.841	0.049

P: the P-value of departure from the Hardy–Weinberg equilibrium for each population; ns: no significant departure from Hardy–Weinberg equilibrium; Ho: observed heterozygosity; He: expected heterozygosity; F_{IS}: inbreeding coefficient; Corrected genotype dataset for null alleles was computed following the INA method (Chapuis & Estoup 2007).

number of alleles per locus was 21. Based on the original dataset, all populations strongly deviated from Hardy–Weinberg equilibrium (HWE), with an excess of homozygotes, at all examined loci. The software MICRO-CHECKER suggested the presence of null alleles for these loci. Therefore, we corrected the original matrix for null alleles. Re-analysis of the revised dataset revealed no significant departure from HWE for each population (Table II), as well as for the whole sample ($P = 0.089$). The mean observed and expected heterozygosities across loci were 0.778 ± 0.128 and 0.788 ± 0.123, respectively. The mean allelic richness across loci was 9.170 ± 1.969. Weir & Cockerham's (1984) estimation of F-statistics showed that the highest values of F_{IT} (0.084 ± 0.048) and F_{ST} (0.040 ± 0.016) corresponded to locus pm-183, whereas pm-101 exhibits the highest value of F_{IS} (0.067 ± 0.024). Overall, F_{IT} values were higher than those recorded for F_{IS} within the examined loci. The observed and expected heterozygosities and the F_{IS} estimates within the eight studied populations are shown in Table II. The mean observed and expected heterozygosities across populations were 0.778 ± 0.028 and 0.787 ± 0.030, respectively. Heterozygosity deficit, as measured by Wright's F_{IS} (Table II), showed low levels in most populations when averaged across loci, ranging from −0.035 (population at Korbos) to 0.075 (population at Bizerte). The average value of F_{IS} across populations was 0.012 ± 0.031, indicating a low heterozygote deficiency.

Overall, our results showed highly significant genetic differentiation among populations. Based on the outcome of the Fisher exact test, we rejected the hypothesis of genetic homogeneity in the distribution of allele frequencies for the entire microsatellite dataset ($\chi^2 = \infty$, df = 8, $P < 0.001$). The one-level AMOVA also confirmed the hypothesis of a partitioning of genetic variation among populations ($F_{ST} = 0.014$, df = 219, $P < 0.001$). No relationship was found between genetic and geographic distances ($r =$

−0.095, $P = 0.704$) by means of the Mantel test, excluding the hypothesis of isolation by distance to explain population separation. Population genetic structure was examined by means of the two-level AMOVA, grouping populations according to their geographic origin: African western Mediterranean (Annaba, Bizerte, Korbos and Cap Bon) versus African eastern Mediterranean (Sahel, Sfax, Zarzis and Tajura). Our results showed a lack of genetic subdivision across the Siculo-Tunisian Strait ($F_{CT} = -0.001$, df = 219, $P = 0.659$). Conversely, the exact test of genetic differentiation (G) showed weak but significant genic differentiation ($\chi^2 = 16.973$, df = 8, $P = 0.030$) between these two groups of populations. Nevertheless, comparison at the genotypic level did not lead to the same result ($\chi^2 = 15.084$, df = 8, $P = 0.057$).

A lack of population genetic structure was revealed based on genotypic assignment. The Bayesian clustering program STRUCTURE recorded a consistent decrease in the $LnP(D)$ (the log-probability of data) from K = 1 ($LnP(D) = -2057.1$) to K = 8 ($LnP(D) = -2158.2$), supporting a single population. Although BAPS analysis yielded three clusters, specimens of the examined populations were shown to mix and it was not possible to achieve a strong clustering pattern. However, cluster 3 seemed to differentiate the two populations of Korbos and Tajura, based on the average admixture coefficients for the specimens according to their clusters (Figure 2). This result was consistent with that given by the statistical approach of PCA-GEN software. The result of the PCA on the genotypes, ordered in the space defined by the two main axes (PC-I and PC-II, explaining 51.04% of the total genetic diversity), did not show significant groupings, even though populations like Bizerte and Tajura were shown to be separated from the others by means of axis I and II, respectively (Figure 3). Furthermore, only 16.4% of individuals were assigned by GENECLASS (with a quality index of 17.21%) to the populations from which they were sampled. GENECLASS also
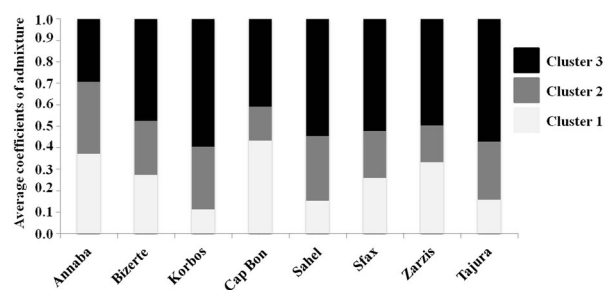

Figure 2. BAPS analysis depicting average coefficients of admixture for *Pachygrapsus marmoratus* specimens belonging to eight populations according to their clusters.
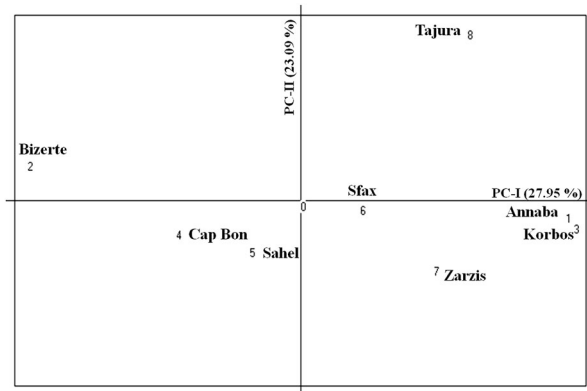
Figure 3. Scores of microsatellite genotypes of *Pachygrapsus marmoratus* populations, plotted on the first two axes (PC-I vs. PC-II) of a principal component analysis (PCA) performed using PCA-GEN version 1.2.1.

revealed that eight individuals ($P < 0.01$) were potential first generation migrants.

The hypothesis of a possible bottleneck in the North African metapopulation of *Pachygrapsus marmoratus* was tested. The results of bottleneck analysis using the sign rank test and Wilcoxon test for each of three mutation models are depicted in Table III. The probability values from the Wilcoxon test for the IAM, TPM and SMM models indicated the acceptance of mutation-drift equilibrium under all the models. Indeed, the Wilcoxon test revealed no significant patterns of heterozygosity excess ($P = 0.968$). Accordingly, these results excluded the hypothesis of a recent genetic bottleneck.

Mitochondrial DNA

Genetic analyses of the African Mediterranean populations of *Pachygrapsus marmoratus* were carried out using the longer alignment of 739 bp, generated for the mtDNA cytochrome oxidase I (Cox1) gene from 80 individuals. In all, 31 nucleotide sites were variable, of which 13 were parsimony-informative and 18 were autapomorphic (singletons). Among these sequences, 32 haplotypes were identified (Figure 4a). The nucleotide composition of the analysed fragment showed an A-T bias (C = 20.29%; T = 36.80%; A = 25.37%; G = 17.54%), as previously mentioned for invertebrate mitochondrial DNA (Simon et al. 1994). Overall, genetic diversity analysis of the mitochondrial dataset showed high haplotypic diversity ($h = 0.812 \pm 0.044$) and low nucleotide diversity ($\pi = 0.0029 \pm 0.000$) (Table IV). The statistical parsimony procedure yielded one network with several connections (Figure 4a). The network had a star-like shape in which the proposed ancestral haplotype (haplotype 19, found in the population of Korbos) occupied a central position (Figure 4a). Most of the haplotypes differed by few mutations. The most common haplotype (haplotype 1), found in 34 specimens from all populations, was separated from the ancestral haplotype by one mutational step. The two most divergent haplotypes (28 and 29) were from the population of Tajura, being separated from the ancestral haplotype by at least five mutational steps. The population of Korbos was characterized by the highest genetic variability with the existence of nine haplotypes, of which seven were site-specific.

The one-level AMOVA showed a lack of population genetic structure among African Mediterranean populations of *P. marmoratus* ($\Phi_{ST} = 0.014$, df = 79, $P = 0.215$, based on Tajima–Nei distances; $F_{ST} = -0.006$, df = 79, $P = 0.593$, based on haplotype frequency). All pairwise comparisons, estimated from both genetic divergence and haplotype frequencies, were not significant (data not shown), except the one between the population of Korbos and the population of Sahel ($\Phi_{ST} = 0.149$, $P < 0.05$).

Table III. Genetic bottleneck analysis within the North African metapopulation of *Pachygrapsus marmoratus* based on three microsatellite evolutionary models.

Locus	Under the IAM (Infinite allele model)				Under the TPM (Two phase model)				Under the SMM (Stepwise mutation model)			
	Heq	S.D.	DH/sd	Prob	*Heq*	S.D.	DH/sd	Prob	*Heq*	S.D.	DH/sd	Prob
pm101	0.916	0.024	−2.736	0.016	0.941	0.012	−7.496	0.001	0.948	0.012	−8.138	0.003
pm99	0.904	0.029	2.014	0.000	0.935	0.013	2.172	0.000	0.943	0.012	1.619	0.000
pm183	0.860	0.046	−2.787	0.019	0.903	0.022	−7.803	0.000	0.919	0.014	−13.28	0.000
pm187	0.885	0.039	−8.177	0.000	0.922	0.015	−23.596	0.000	0.933	0.015	−23.55	0.000
Sign rank test (number of loci	Expected	2.42			Expected	2.33			Expected	2.37		
with heterozygosity excess)	Observed	1			Observed	1			Observed	1		
	Probability	0.172			Probability	0.199			Probability	0.189		
Wilcoxon test (probability)		0.968				0.968				0.968		

Heq: expected heterozygosity at the mutation-drift equilibrium, generated from the number of alleles at a locus and the sample size; S.D.: the standard deviation (SD) of the mutation-drift equilibrium distribution of the heterozygosity; DH/sd: the standardized differences for each locus ((Ho-He)/SD); Prob: the distribution obtained through simulation enables also the computation of a *P*-value for the observed heterozygosity. Test of whether observed heterozygosity deviates from that expected under mutation-drift equilibrium, using the three mutation models, was assessed by the probability of Heterozygosity excess (*P*) yielded by the Wilcoxon test.
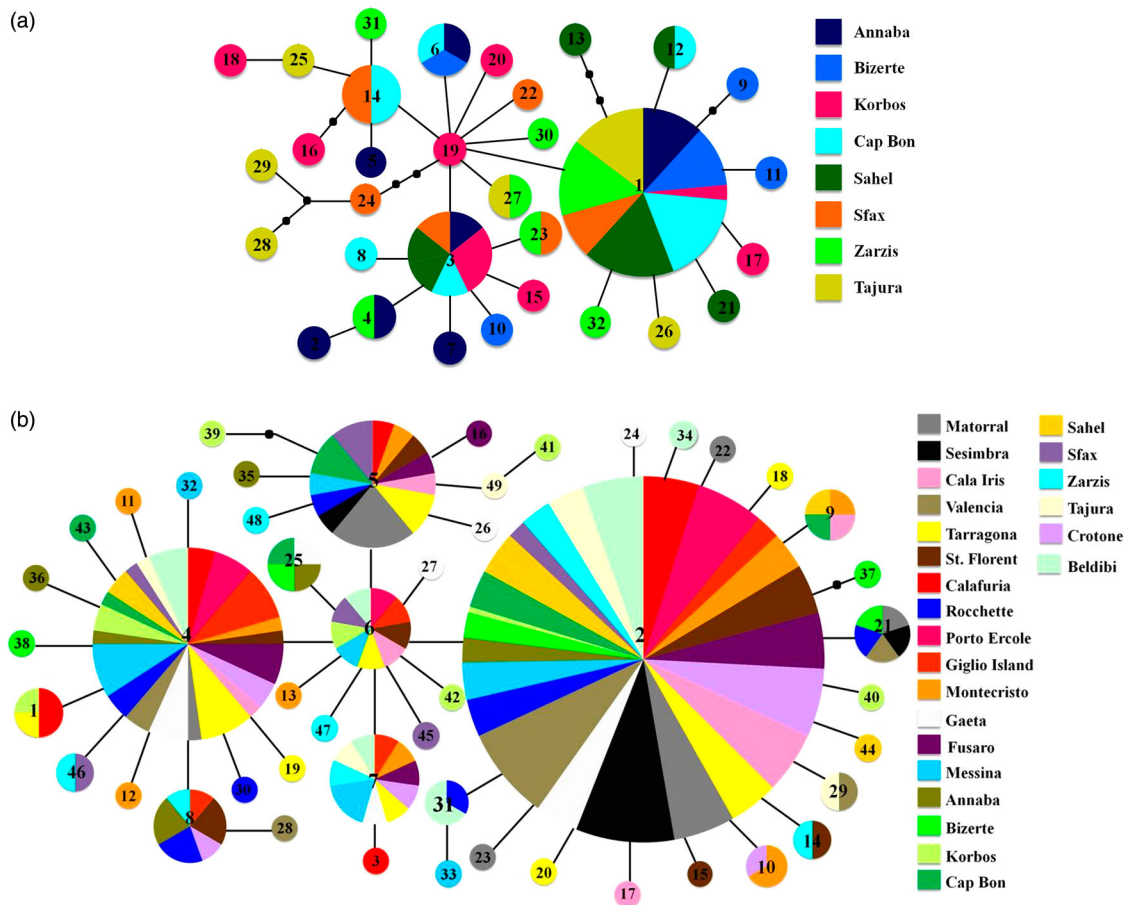
Figure 4. TCS haplotype network of *Pachygrapsus marmoratus*, based on the alignment of 739 bp (a) and 596 bp (b) of the mito-chondrial gene Cox1, showing the relationships among the recorded haplotypes across different investigated regions. Haplotypes 19 (a) and 6 (b) correspond to the ancestral haplotype. Each line between two points represents one mutational step. Circle sizes depict proportions of haplotypes; the smallest corresponds to 1 and the largest to 34 (a) and 182 (b) individuals. Notation: Each network includes its own haplotype organization driven from the corresponding alignment.

Population demographic history was recon-structed using three neutrality tests, mismatch distri-bution analysis and BSP analysis. The applied neutrality tests revealed significant deviations from mutation-drift equilibrium for the populations of Annaba, Bizerte, Korbos, Cap Bon, Zarzis and Tajura, showing mainly significant results for F_S and R_2

tests (Table IV) and suggesting recent population expansion events. Moreover, the negative and signifi-cant values of Tajima's D and Fu's F_S tests, as well as the significant value of Ramos-Onsins & Rozas $R2$, together with the small and non-significant value of Harpending's raggedness index rg, revealed signifi-cant deviations from neutrality for the whole

Table IV. Genetic diversity and historical demographic results for *Pachygrapsus marmoratus* populations from the African Mediterranean coast, based on the analysis of the mtDNA Cox1 gene.

Population	N	Na	h	π (%)	D	F_S	R_2	rg
Annaba	10	7	0.86 ± 0.10	0.32 ± 0.03	−0.61	**−2.67**	**0.12**	0.12
Bizerte	8	5	0.78 ± 0.15	0.26 ± 0.02	−1.35	−1.23	**0.12**	0.06
Korbos	9	8	0.97 ± 0.06	0.37 ± 0.03	−0.74	**−4.62**	**0.11**	0.06
Cap Bon	12	6	0.75 ± 0.12	0.22 ± 0.02	−0.67	−1.87	**0.11**	0.22
Sahel	11	5	0.70 ± 0.13	0.21 ± 0.02	−1.40	−0.97	0.13	0.03
Sfax	9	6	0.88 ± 0.09	0.32 ± 0.03	−0.85	−1.71	0.14	**0.32**
Zarzis	11	7	0.81 ± 0.11	0.29 ± 0.02	−1.26	**−2.65**	**0.08**	0.17
Tajura	10	6	0.77 ± 0.13	0.37 ± 0.03	−1.00	−1.04	**0.10**	0.02
Total	80	32	0.81 ± 0.04	0.29 ± 0.00	**−2.03**	**−27.12**	**0.03**	0.05

Values reported for each population and for the total dataset are: sample size (*N*), number of haplotypes (*Na*), haplotype (*h*) and nucleotide (*π*) diversity, Tajima's F_S test (*D*), Fu's F_S test (F_S), Ramos-Onsins and Rozas's R_2 test (R_2), and mismatch distribution raggedness index (*rg*). Nucleotide diversity (*π*) was calculated in per cent. Significant values are in bold.
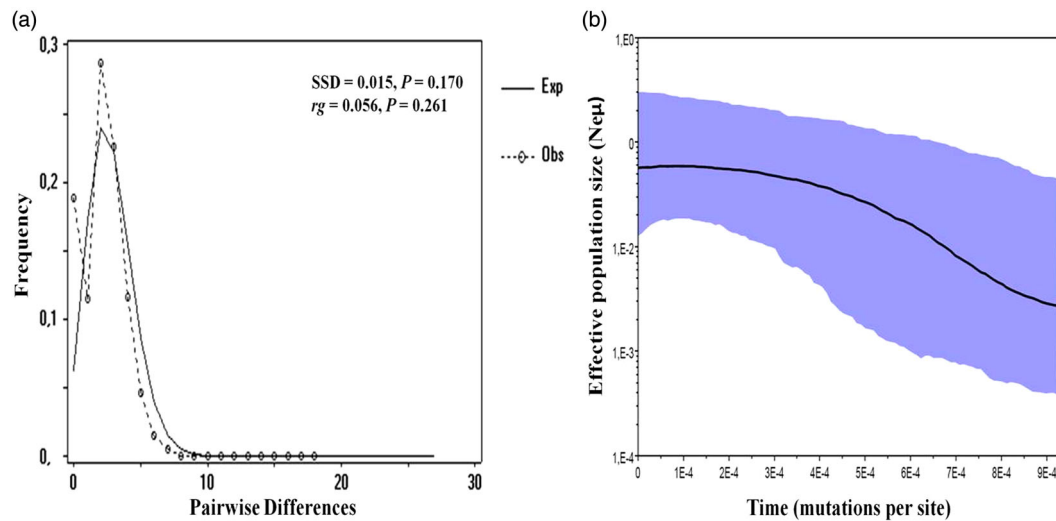
Figure 5. Mismatch distribution (a) and Bayesian skyline plot (BSP, implemented in BEAST) (b) for the North African populations of *Pachygrapsus marmoratus*. (a) Observed frequencies (dotted line) were compared with the expected frequencies (continuous line), estimated by the growth-decline model implemented in DNASP. The demographic expansion parameters used, τ and $\theta_{initial}$, were calculated in ARLEQUIN ($\tau = 2.781$ and $\theta_{initial} = 0.000$); θ_{final} value was fixed at 1000 to simulate the infinite. The two demographic indices SSD and rg, provided for the observed mismatch distributions, were calculated under the assumption of a demographic expansion model, implemented in ARLEQUIN. (b) BSP showing changes in effective population size (expressed as effective population size [Ne] multiplied per generation time [μ]) over time (measured in mutations per site). The thick solid line depicts the median estimate, and the margins of the surrounding area represent the highest 95% posterior density intervals.

dataset (Table IV and Figure 5a), consistent with a scenario of a sudden demographic expansion. The unimodal mismatch distribution obtained for the North African metapopulation of *P. marmoratus* (Figure 5a) also suggested a recent demographic expansion. Statistical analysis of the mismatch distribution supported both models of demographic (SSD = 0.015; P = 0.170) and spatial expansion (SSD = 0.009; P = 0.545, associated with a strictly positive migration rate: M = 7.810 [2.575–99999, for a 95% confidence interval]). Under the assumption of a sudden demographic expansion, the value of τ for the whole sample was 2.781 (95% confidence interval: 1.111–4.494). Thus, assuming a Cox1 mutation rate of 1.66% per million years (Schubart et al. 1998), the most recent population expansion began at around 0.837 million years ago. Under the spatial expansion hypothesis, the value of τ decreased to 2.619 (95% confidence interval: 0.850–4.097), corresponding to an expansion time of 0.788 million years ago.

Historical demography of the North African population of *P. marmoratus* was also inferred from the coalescent approach in the BSP analysis. The pattern of effective population size change over time was shown to be a remarkably progressive increase (Figure 5b) and invoked the possible scenario of population demographic recovery following an historical bottleneck (Hoffman et al. 2011).

Macro-geographic scale

The inclusion of previously published data allowed for the analysis of 336 Cox1 sequences (Table I) with an alignment length of 596 bp. A total of 41 variable sites (17 informative) defined 49 haplotypes. Among these, 16 haplotypes were newly detected in this study. Nucleotide composition of the analysed fragment was found to be unbalanced (C = 20.97%; T = 35.91%; A = 25.08%; G = 18.04%). Overall haplotype diversity (h) and nucleotide diversity (π), calculated for the investigated geographic range, were 0.684 ± 0.020 and 0.0024 ± 0.000, respectively. The haplotype network had a star-like shape, centred on two main haplotypes (2 and 4; Figure 4b). Haplotype 6, found at the junction of the branches connecting the main haplotypes, occupied a central position and was therefore proposed to be the ancestral haplotype (Figure 4b). Haplotype 2, found in 182 individuals, was present in all populations. In contrast, haplotype 4, found in 44 specimens, was not recorded from the Portuguese location of Sesimbra or the Tunisian populations of Bizerte and Zarzis. Calculations of N_{ST} (0.015) and G_{ST} (0.012) revealed that the N_{ST} value was not significantly higher than the G_{ST} value (U-test = 0.70, P = 0.05), inferring the lack of relationship between phylogeny and the geographic distribution of haplotypes.

The one-level AMOVA showed weak but significant genetic differentiation based on nucleotide divergence ($\Phi_{ST} = 0.022$, df = 335, P = 0.031). The general trend of

partitioning genetic variation among populations was not confirmed based on haplotype frequency ($F_{ST} = 0.016$, df = 335, $P = 0.058$). Pairwise comparisons of genetic differentiation, estimated from genetic divergence and haplotype frequency, are presented in Table V. Population genetic structure was investigated grouping populations under different biogeographic hypotheses (Table VI). Analysis of molecular variance (two-level AMOVA) showed significant population structure along the West-East geographic gradient, depicting a weak but significant difference between the Atlantic and Mediterranean based on nucleotide divergence ($\Phi_{CT} = 0.049$; $P < 0.05$). Although a significant result was also noted when considering three groups (Atlantic vs. Western Mediterranean vs. Eastern Mediterranean), partitioning the genetic variance among Atlantic and Mediterranean Basins yielded the highest Φ_{CT} and explained most of the population genetic structure of *Pachygrapsus marmoratus*. The Mantel test revealed no significant correlation between genetic and geographic distances ($r = -0.069$, $P = 0.741$), suggesting a lack of isolation by distance.

Discussion

The present investigation mainly focuses on the population genetic structure of the intertidal marbled crab *Pachygrapsus marmoratus* across the Siculo-Tunisian Strait, investigating mtDNA and nuclear variation among eight North African populations. Analyses of mtDNA and microsatellite loci showed contrasting patterns of population differentiation, but both markers revealed no significant genetic structure across the Siculo-Tunisian Strait.

Overall, our mtDNA results showed a relatively homogeneous mtDNA haplotype composition among populations of the African Mediterranean coast belonging to distinct basins. The geographic distribution of haplotypes also showed that the most common haplotype (haplotype 1, Figure 4a) was present in all populations in high frequencies. This suggests the existence of high gene flow connecting populations separated by hundreds of kilometres (such as the Algerian population of Annaba and the Libyan population of Tajura) and maintaining the observed genetic homogeneity. This finding is in contrast with the genetic structure previously recorded with the same marker in other decapod species, such as the caramote prawn *Penaeus kerathurus* Forskål, 1775 (see Zitari-Chatti et al. 2009) and the green crab *Carcinus aestuarii* Nardo, 1847 (see Deli et al. 2015b) across the investigated region. This suggests that the lack of mtDNA-based genetic structure in *Pachygrapsus*

marmoratus could be linked to incomplete lineage sorting, owing to a slow mtDNA mutation rate in this species. Although the Cox1 gene has been shown to be variable enough in different decapod species (Fratini & Vannini 2002; Roman & Palumbi 2004; Darling et al. 2008; Silva et al. 2010; Laurenzano et al. 2014), it also failed to reveal genetic structuring in other brachyuran species, like the blue crab *Callinectes bellicosus* Stimpson, 1859 (see Pfeiler et al. 2005) or the fiddler crab *Uca (Leptuca) uruguayensis* Nobili, 1901 (see Laurenzano et al. 2013). The absence of genetic structure could also indicate the possible signature of historical mixing events that took place in the investigated region (i.e. through a possible bottleneck during the glaciation periods in the Mediterranean).

The BSP estimate of the effective population size fluctuations of the North African metapopulation showed a gradual increase over time, characteristic of a population in a phase of demographic recovery following an historical bottleneck (Hoffman et al. 2011). However, we should caution with the fact that the actual effective population size does not appear to be much greater than in the past. This might be due to the limited number of analysed specimens (less than 100), which can prevent the capture of the full extent of a population expansion (Grant 2015). The obtained BSP result is concordant with the ragged unimodal mismatch distribution and the star-like haplotype network. Furthermore, the estimated time of expansion under both demographic (0.837 million years ago) and spatial (0.788 million years ago) expansion models coincides well with Pleistocene glaciation periods, and seems to indicate that the North African metapopulation of *P. marmoratus* might have experienced rapid historical population growth from an ancestral population with a small effective population size in the Mid-Pleistocene. During this period, the Mediterranean Sea experienced strong climatic changes and sea level fluctuations, supposedly affecting the genetic population structure of many marine species (for a review see Hewitt 2000 and Patarnello et al. 2007).

Despite the lack of differentiated geographic groups within *P. marmoratus*, both one-level AMOVA and Fisher exact tests, based on microsatellite data, showed significant partitioning of genetic variation among North African populations. This result contrasts clearly with that inferred from mtDNA analyses of the same populations. However, the recorded population subdivision was not in accordance with geographic boundaries nor correlated with geographic distances, as revealed by the Mantel test. Accordingly, the genetic separation among populations could be attributed to the small effective size contributing to the next

Table V. Pairwise comparisons of genetic differentiation estimated from genetic divergence (Φ_{ST}, below the diagonal) and haplotype frequency (F_{ST}, above the diagonal), based on the mtDNA Cox1 gene. Values with $P < 0.05$ are reported in bold (significance levels calculated from 10,000 permutations).

	Atlantic		Western Mediterranean																Eastern Mediterranean					
	MAT	SSB	CAI	VAL	TRG	SFL	CAL	RCH	PER	GIG	MTC	GAE	FUS	MES	ANN	BIZ	KOR	CAP	SHL	SFX	ZRZ	TAJ	CRT	BLB
MAT	*	0.101	−0.01	0.037	0.00	−0.01	−0.01	−0.00	0.03	0.072	0.004	0.015	−0.01	0.029	0.027	−0.0	**0.131**	−0.04	0.002	−0.0	0.010	−0.00	0.017	0.013
SSB	0.051	*	0.031	0.003	**0.18**	**0.110**	0.086	**0.176**	0.05	**0.306**	**0.183**	**0.168**	0.097	**0.230**	**0.229**	**0.15**	**0.418**	**0.136**	0.085	**0.26**	**0.170**	0.088	0.040	**0.115**
CAI	−0.016	0.003	*	−0.02	0.01	−0.02	−0.03	0.011	−0.0	0.074	0.014	0.006	−0.03	0.041	0.033	−0.0	**0.164**	−0.02	−0.05	0.03	0.007	−0.04	−0.03	−0.01
VAL	0.028	0.011	−0.02	*	**0.07**	0.02	−0.00	0.059	−0.0	**0.133**	**0.078**	0.042	−0.00	**0.099**	0.090	0.03	**0.254**	0.038	−0.02	**0.13**	0.060	−0.02	−0.03	0.011
TRG	0.045	**0.195**	0.063	0.079	*	−0.00	−0.01	−0.02	0.04	−0.03	−0.01	−0.03	−0.01	−0.04	−0.01	−0.0	0.012	−0.02	−0.00	−0.0	−0.00	−0.01	0.028	−0.01
SFL	0.001	**0.101**	−0.00	−0.00	−0.00	*	−0.02	−0.04	0.00	0.015	−0.01	−0.01	−0.02	0.005	−0.03	−0.0	0.085	−0.03	−0.02	−0.0	−0.04	−0.03	−0.01	−0.01
CAL	0.017	**0.131**	0.011	0.015	−0.03	−0.03	*	−0.00	−0.0	0.034	0.005	−0.01	−0.04	0.013	0.012	−0.0	**0.107**	−0.02	−0.04	0.01	0.001	−0.04	−0.02	−0.02
RCH	0.063	**0.208**	0.076	0.061	−0.01	−0.00	−0.00	*	0.04	−0.02	−0.01	−0.02	−0.01	−0.01	−0.05	−0.0	0.034	−0.03	−0.01	−0.0	−0.02	−0.01	0.009	−0.02
PER	0.016	0.100	−0.02	−0.02	0.00	−0.03	−0.03	0.018	*	0.069	0.065	0.013	−0.03	0.056	0.073	0.04	**0.209**	0.027	−0.05	0.10	0.060	−0.02	−0.04	−0.01
GIG	**0.170**	**0.411**	**0.205**	**0.196**	−0.02	0.04	0.03	−0.02	0.11	*	0.010	−0.03	−0.00	−0.05	−0.01	0.04	−0.00	0.023	0.016	−0.0	0.022	0.015	0.058	−0.00
MTC	0.009	**0.116**	0.003	0.029	−0.02	−0.02	−0.03	−0.00	−0.0	0.020	*	−0.01	−0.00	−0.01	−0.01	−0.0	0.036	−0.03	0.000	−0.0	−0.02	−0.01	0.020	0.000
GAE	0.016	**0.158**	0.026	0.047	−0.03	−0.02	−0.03	−0.00	−0.0	0.000	−0.03	*	−0.03	−0.03	−0.02	−0.0	0.041	−0.02	−0.03	−0.0	−0.01	−0.03	−0.00	−0.03
FUS	−0.013	**0.137**	0.004	0.031	−0.03	−0.03	−0.04	−0.00	−0.0	0.029	−0.04	−0.04	*	−0.01	0.010	−0.0	**0.113**	−0.02	−0.05	0.01	0.000	−0.05	−0.04	−0.03
MES	**0.077**	**0.249**	0.098	0.110	−0.03	−0.00	−0.00	−0.02	0.03	−0.05	−0.01	−0.03	−0.02	*	−0.00	0.01	0.006	−0.00	0.005	−0.0	0.000	−0.00	0.040	−0.01
ANN	**0.109**	**0.315**	**0.143**	**0.142**	−0.01	0.00	0.02	−0.05	0.08	−0.06	0.010	−0.03	0.010	−0.03	*	−0.0	0.027	−0.02	0.008	−0.0	−0.03	−0.00	0.025	0.003
BIZ	−0.00	0.060	−0.01	−0.01	0.02	−0.02	−0.01	0.013	−0.0	0.098	−0.01	−0.01	−0.01	0.040	0.044	*	0.067	−0.04	−0.01	−0.0	−0.03	−0.03	0.008	−0.00
KOR	**0.149**	**0.378**	**0.207**	**0.244**	0.02	0.09	0.07	0.049	**0.16**	0.003	0.058	0.040	0.060	0.014	−0.01	0.08	*	0.070	**0.122**	−0.0	0.059	0.103	**0.183**	0.091
CAP	−0.02	**0.138**	−0.00	0.040	−0.02	−0.03	−0.02	−0.00	−0.0	0.039	−0.04	−0.04	−0.05	−0.01	−0.00	−0.0	0.039	*	−0.03	−0.0	−0.01	−0.03	0.004	−0.01
SHL	0.013	0.046	−0.04	−0.04	0.03	−0.02	−0.02	0.033	−0.0	0.143	−0.02	0.010	−0.00	0.064	0.095	−0.0	**0.171**	−0.00	*	0.03	0.002	−0.05	−0.04	−0.04
SFX	0.065	**0.347**	**0.141**	**0.183**	−0.03	0.03	0.03	0.00	0.10	−0.00	−0.00	−0.02	−0.01	−0.03	−0.03	0.06	−0.04	−0.02	**0.126**	*	−0.01	0.015	0.077	0.015
ZRZ	0.005	**0.146**	0.018	0.040	−0.00	−0.04	−0.03	−0.03	−0.0	−0.00	−0.02	−0.03	−0.04	−0.04	−0.03	−0.0	0.024	−0.04	0.003	−0.0	*	−0.04	0.007	−0.00
TAJ	−0.036	0.062	−0.03	−0.01	−0.00	−0.03	−0.03	0.015	−0.0	0.088	−0.04	−0.03	−0.05	0.009	0.049	−0.0	0.077	−0.04	−0.03	0.03	−0.04	*	−0.05	−0.04
CRT	0.016	0.067	−0.02	−0.03	0.01	−0.04	−0.03	0.007	−0.0	0.097	−0.02	−0.00	−0.02	0.030	0.068	−0.0	**0.162**	−0.00	−0.04	0.10	−0.01	−0.04	*	−0.02
BLB	0.019	0.075	−0.01	−0.02	0.01	−0.04	−0.02	−0.00	−0.0	0.091	−0.01	−0.00	−0.01	0.028	0.058	−0.0	**0.159**	−0.00	−0.03	0.09	−0.02	−0.03	−0.05	*

MAT: Matorral; SSB: Sesimbra; CAI: Cala Iris; VAL: Valencia; TRG: Tarragona; SFL: St. Florent; CAL: Calafuria; RCH: Rocchette; PER: Porto Ercole; GIG: Giglio Island; MTC: Montecristo; GAE: Gaeta; FUS: Fusaro; MES: Messina; ANN: Annaba; BIZ: Bizerte; KOR: Korbos; CAP: Cap Bon; SHL: Sahel; SFX: Sfax; ZRZ: Zarzis; TAJ: Tajura; CRT: Crotone; BLB: Beldibi.

Table VI. Two-level AMOVA, assessing population genetic structure of *Pachygrapsus marmoratus* under different biogeographic hypotheses, based on the mtDNA Cox1 gene.

Grouping	Nucleotide divergence (Tajima and Nei distance)	Haplotype frequency
Across the Gibraltar Strait:		
Atlantic vs. Mediterranean	$\Phi_{SC} = 0.012$ *	$F_{SC} = 0.009$ ns
	$\Phi_{ST} = 0.060$ *	$F_{ST} = 0.042$ ns
	$\Phi_{CT} = 0.049$ *	$F_{CT} = 0.033$ ns
Across the Gibraltar and Siculo-Tunisian Straits:		
Atlantic vs. Western	$\Phi_{SC} = 0.012$ ns	$F_{SC} = 0.010$ ns
Mediterranean vs. Eastern	$\Phi_{ST} = 0.031$ *	$F_{ST} = 0.021$ ns
Mediterranean	$\Phi_{CT} = 0.018$ *	$F_{CT} = 0.010$ ns

Populations and grouping: Atlantic: Matorral and Sesimbra; **Mediterranean:** Cala Iris, Valencia, Tarragona, St. Florent, Calafuria, Rocchette, Porto Ercole, Giglio Island, Montecristo, Gaeta, Fusaro, Messina, Annaba, Bizerte, Korbos, Cap Bon, Sahel, Sfax, Zarzis, Tajura and Beldibi; **Western Mediterranean:** Cala Iris, Valencia, Tarragona, St. Florent, Calafuria, Rocchette, Porto Ercole, Giglio Island, Montecristo, Gaeta, Fusaro, Messina, Annaba, Bizerte, Korbos, Cap Bon; **Eastern Mediterranean:** Sahel, Sfax, Zarzis, Tajura, Crotone and Beldibi. *: $P < 0.05$; ns, non-significant difference ($P > 0.05$).

generation or a 'sweepstakes effect' (Hedgecock 1994). This view may be supported by the outcomes of the BAPS and PCA analyses of genotypes, showing remarkable genetic separation of certain populations, i.e. Bizerte, Korbos and Tajura. This pattern of separation, which does not seem to be associated with a clear geographic gradient, may rather follow a 'chaotic genetic patchiness' in the composition of recruits (Christie et al. 2010), as has been suggested previously for Portuguese (Silva et al. 2009b) and Italian (Fratini et al. 2011, 2013) populations of *P. marmoratus*.

This study provides evidence for the absence of a significant genetic structure across the Siculo-Tunisian Strait, as inferred from mtDNA and nuclear microsatellite loci analyses. The Siculo-Tunisian Strait is considered as a well-documented genetic boundary between African Mediterranean populations of many marine species of fishes (Bahri-Sfar et al. 2000; Mejri et al. 2009; Kaouèche et al. 2011), molluscs (Gharbi et al. 2011) and shrimps (Zitari-Chatti et al. 2009), but apparently does not seem to interrupt the connectivity among African populations of *P. marmoratus* from the Western and Eastern Mediterranean Basins. The lack of genetic structure could be explained by the passive dispersal of planktonic larvae, which could limit the formation of population substructure (Lessios et al. 2003; Waters & Roy 2004). *Pachygrapsus marmoratus* planktonic larvae seem to have a high dispersive capacity, characteristic of many marine invertebrates. Zoeae spend around one month offshore, going through at least six development stages (Cuesta & Rodríguez 2000). Movement of juveniles and adults of *P. marmoratus* is not expected to homogenize populations. Instead, larval dispersal during a

long planktonic phase could promote the connectivity of the local adult populations in accordance with hydrographic patterns. In theory, drifting *P. marmoratus* larvae can reach very distant locations along the North-African littoral. The latter is characterized by a unidirectional surface current, called the Algerian Current, originating from the Atlantic, moving eastwards along the North African coast and flowing into the Eastern Mediterranean Basin (Béranger et al. 2004, Figure 1). This marine current along the continuous African Mediterranean coast could have enhanced genetic homogeneity of the studied populations according to a linear stepping stone model. Indeed, this model is common in continuously distributed species, like *P. marmoratus*, in which homogenization of distant populations is maintained by gene flow through the exchange of individuals between adjacent or nearby populations (Kimura & Weiss 1964; Slatkin & Maddison 1990).

Analysis of a shorter fragment of the mitochondrial Cox1 marker across a broader geographic scale, covering almost the entire distribution range of this species, showed significant genetic differentiation among populations, as revealed by the one-level AMOVA. Based on this enlarged dataset, the results of the structured AMOVA analysis hint at the existence of significant genetic structure along a West-East geographic gradient, depicting weak but significant differentiation between Atlantic and Mediterranean populations. These results are the first to reflect such patterns of genetic structure for *P. marmoratus*. Previous mtDNA phylogeographic studies of the same species across different geographic scales failed to detect population differentiation and suggested the existence of overall genetic homogeneity (Fratini et al. 2011; Deli et al. 2015a). However, the genetic heterogeneity observed in this study is not due to isolation by distance. Indeed, the most significant pairwise differences were not between geographically extreme populations. The great diversity of habitats and, therefore, selective forces within both Mediterranean basins and the Atlantic could contribute to the onset of enclosures or dispatched isolates (genetic separation of specific populations that is not linked to any geographic gradient). Furthermore, the potentially random distribution of larvae according to oceanographic currents would be critical in the development of such isolates (Johnson & Black 2006). Accordingly, we can attribute the genetic differentiation among Atlantic and Mediterranean populations to the impact of various oceanographic circulation patterns in the area of the Gibraltar Strait, resulting in a barrier to gene flow (Quesada et al. 1995). The Almeria-Oran Oceanographic Front

constitutes an important barrier for many marine species with planktonic larval stages (Patarnello et al. 2007). Based solely on the analysis of mitochondrial DNA and in the absence of data from nuclear markers from this area, the exact barrier cannot be determined. It should be noted that although the mtDNA-derived estimates do represent female dispersal, it can be seen to be representative of overall dispersal, because sex-biased dispersal among planktonic larvae is not expected (Barber et al. 2006). It should be mentioned that the lack of a relationship between phylogeography and the geographic distribution of haplotypes, identified by the parsimony network and supported statistically by the program PERMUT, could be attributed to the fact that the Cox1 mitochondrial marker can retain the ancestral polymorphisms of *P. marmoratus* due to its low mutation rate.

As far as can be inferred from our results, mitochondrial and nuclear DNA patterns of population structure across part of the African Mediterranean coasts are in accordance with those previously reported for geographically close European populations (Fratini et al. 2008, 2013; Silva et al. 2009b). Mitochondrial DNA homogeneity vs. nuclear DNA patchiness, detected so far in different examined geographic regions (Fratini et al. 2008, 2011, 2013; Silva et al. 2009b; Deli et al. 2015a), probably result from the involvement of a complex series of abiotic and biotic factors in determining genetic structure, of which the origin and effect should be determined in future investigations. Furthermore, the observed weak mtDNA heterogeneity across the Gibraltar Strait cannot be considered a conclusive result, as more populations from the Atlantic and Mediterranean (including the Eastern Basin and Black Sea) need to be analysed in order to verify this pattern and to obtain a complete overview of phylogeographic structure of *P. marmoratus* across its distribution range. This would help to shed more light on inter-population and inter-regional differentiation and allow understanding of the evolutionary history of this species.

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