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Genetic and morphological divergence in the purple sea urchin *Paracentrotus lividus* (Echinodermata, Echinoidea) across the African Mediterranean coast

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Abstract

The present investigation focuses on population structure analysis of the purple sea urchin *Paracentrotus lividus* across the African Mediterranean coast, with the main aim of assessing the influence of the Siculo-Tunisian Strait on gene flow disruption in this highly dispersive echinoid species. For this purpose, patterns of morphological and genetic variation were assessed among its populations from the western and eastern Mediterranean coasts. A total of 302 specimens from seven Tunisian sites were collected and examined for morphometric variability at twelve morphometric traits. Concordant results, inferred from CDA (canonical discriminant analyses), pairwise NPMANOVA (non parametric multivariate analysis of variance) comparisons and MDS (multidimensional scaling) plot, unveiled significant inter-population differences in the measured traits among the studied populations. Furthermore, the combined use of the one way ANOSIM (analysis of similarities) and the Discriminant/Hotelling analysis allowed unravelling two morphologically differentiated groups assigned to both western and eastern Mediterranean basins. The SIMPER (similarity percentages) routine analysis showed that total dry weight, test diameter and spine length were major contributors to the morphometric separation between locations and between groups. Pattern of phenotypic divergence discerned in *P. lividus* across the Siculo-Tunisian Strait is interestingly in congruence with that inferred from the genetic investigation of the purple sea urchin populations from the same region based on the analysis of the mtDNA COI (cytochrome oxidase I) gene in 314 specimens from nineteen locations covering a wider geographic transect, streching westward to the Algerian coast and eastward to the Libyan littoral. The specific haplotypic composition characterizing each Mediterranean basin, as inferred from the minimum spanning network, confirmed the geographic partioning of genetic variation, as revealed by F-statistics and AMOVA (analysis of molecular variance) analyses, yielding significant genetic differentiation between eastern and western Mediterranean populations. The newly detected phylogeographic patterns, observed for the first time in *P. lividus* throughout the explored distribution range, suggest the involvement of different biotic and abiotic processes in shaping such variation, and provide evidence that a large and geographically exhaustive dataset is necessary to unveil phylogeographic structure within widespread marine species, previously cathegorized as panmictic in part of their distribution range.

Key words: *Paracentrotus lividus*, Siculo-Tunisian Strait, morphological differentiation, COI, genetic divergence

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1 Introduction

Barriers to gene flow in the marine environment are known to generate marked levels of population differentiation in marine invertebrate species (Hellberg, 1996; Lessios et al., 2001; Hedgecock et al., 2007). Gene flow between populations, promoted by migration and dispersion of both larvae and adults, does not only preclude local adaptation (Barton and Hewitt, 1985) but also introduces new polymorphisms in the populations on which selection can potentially act. Hence, dispersal distances may not only influence geographical range and genetic structure of the species, but also play an important role in population differentiation and speciation processes with profound consequences for the species's phylogeography (Solé-Cava and Thorpe, 1991). It has been demonstrated that potential long-distance larval movements, characterizing most marine invertebrate species, may be hindered by the existence of physical and historical factors acting on population's connectivity and promoting the occurrence of spatial patterns of genetic diversity and therefore the existence of relatively strong population structure (Launey et al., 2002).

Marine areas separated by physical barriers such as straits are of greater importance in phylogeographic and population genetics investigations. Indeed, these specific zones, dominated by sudden changes on depth and strong current regimes, have been repeatedly subjected to disconnections and reconnections during the Quaternary glaciation events (Maggs et al., 2008). One of the best documented biogeographical transitions in the marine environment is the one between the eastern and western Mediterranean basins (Arnaud-Haond et al., 2007; Mejri et al., 2009; Zitari-Chatti et al., 2009; Deli et al., 2016). 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, wa-

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ter bodies circulate with different hydrological, physical, and chemical characteristics (Béranger et al., 2004). Shaped by both present and past physical oceanographic properties, this region has been affected by dramatic geological events and climatic fluctuations, with the occurrence of numerous episodes of sea level regressions (Thiede, 1978) and notable different hydrographic features of both Mediterranean basins (Pinardi and Masetti, 2000). During the last glacial maximum (LGM, between 26 500–20 000 before present; Clark et al., 2009), oscillations of sea level led to periods of reduced connectivity between the eastern and western basins, which stabilized at about 11 000 years before present (Collina-Girard, 2001). Contemporary factors proposed to account for the maintenance of a biogeographical barrier in the area include the water circulation pattern, characterized mainly by a constant and unidirectional east-south-east flow of a marine surface current arriving from Gibraltar (known as the Algerian Current) and bifurcating offshore of the northeastern tip of the Tunisian coast. This contrasts with the rest of the Eastern Mediterranean Basin, which is characterized by a very weak circulation (Pinardi and Masetti, 2000). Bunch of studies analysed variable mitochondrial or/and nuclear markers in populations of several vertebrate and invertebrate species around the Siculo-Tunisian Strait and revealed the existence of a genetic boundary in this region, which is probably the signature of past isolation events (e.g., Quesada et al., 1995; Borsa et al., 1997; Bahri-Sfar et al., 2000; Nikula and Väinölä, 2003; Mejri et al., 2009; Zitari-Chatti et al., 2009; Deli et al., 2015b). Therefore, population genetic analyses across this potential barrier may contribute to better identify the phylogeographic patterns and depict historical events that might have shaped the genetic structure of widely-distributed marine invertebrate species such as benthic sea urchins characterized by high dispersal life - history with high fecundity and external fertilization (Lawrence and Agatsuma, 2001).

The purple sea urchin *Paracentrotus lividus* (Lamarck, 1816) represents a good model to test for population subdivision and genetic structuring across marine biogeographic boundaries. This species is widely distributed in shallow marine coastal rocky habitats of the Mediterranean Sea, the northeastern Atlantic Ocean, from Scotland and Ireland to Morocco, and found also in the Azores, as well as the Canary Islands, Madeira, and Cape Verde (Boudouresque and Verlaque, 2001a) where it occurs in the sublittoral down to 20 m (Turon et al., 1995). The common sea urchin is relevant both ecologically, as it is a keystone herbivorous due to its ability to transform macroalgal-dominated communities into barren areas (Boudouresque and Verlaque, 2001a) and commercially, as it is harvested for human consumption (Guidetti et al., 2004). Furthermore, it exhibits long planktonic larval stage owing to a planktotrophic larva called pluteus which can survive in the plankton for 20–40 days (Pedrotti, 1993; Lozano et al., 1995).

Although numerous investigations have focused on the biology and the ecology of this commercially harvested sea urchin (e.g., Pedrotti, 1993; Lozano et al., 1995; Sala and Zabala, 1996; Fernandez and Boudouresque, 1997; Boudouresque and Verlaque, 2001a, b; Guidetti and Dulčić, 2007; Sellem and Guillou, 2007), phylogeography and population genetics studies are relatively scarce and recent. The first examination, using allozyme markers, found no significant genetic divergence between samples from Western Greece and Northern Sicily which were characterized by different mean body size (Arculeo et al., 1998). Analysis of other nuclear markers (RAPD, random amplification of polymorphic DNA) showed that relatively small and medium-sized populations of *P. lividus* from the Amvrakikos Gulf were genetically differentiated from normal sized populations originating from the Ionian and Tyrrhenian Seas (Rizzo et al., 2009). While these investigations were of limited relevance for phylogeographical inference owing to the examined geographic scale and the characteristics of the markers used, mitochondrial-based phylogeographic studies (at a macro-geographic scale) have evidenced clear discontinuities between the Mediterranean and the Atlantic (Duran et al., 2004; Calderón et al., 2008; Maltagliati et al., 2010) and between the Mediterranean and the Adriatic (Maltagliati et al., 2010). Spatial genetic structure has been also observed within the Western Mediterranean Basin (along the Spanish coast) by means of the mitochondrial cytochrome oxidase subunit I (COI) marker (Calderón et al., 2012). However, such a structure was not observed in all years and was not consistent among all populations. Moreover, this genetic differentiation disappeared when cohorts were pooled. At the local scale, analysis of the nuclear rDNA ITS2 spacer and the two mitochondrial genes 16S and COI in geographically close populations from the Gulf of Naples (Italy) did not show any signal of genetic structure in the surveyed region (Iuri et al., 2007). Population genetic investigations, based on temporal comparisons, revealed significant differentiation among cohorts using the bindin gene (Calderón and Turon, 2010). Such pattern of population structure appeared occasionally with the mtDNA COI gene (Calderón et al., 2012), and was not observed with nuclear microsatellite loci (Calderón et al., 2009). Recently, Penant et al. (2013) revisited population genetic structure of the purple sea urchin, reanalyzing the data from the three published phylogeographic studies (Duran et al., 2004; Calderón et al., 2008; Maltagliati et al., 2010) and combining these with new mitochondrial (COI) and nuclear (calpain exon-primed intron crossing, EPIC) datasets. Their multi-loci phylogeographic investigation showed within-basin and within-region differentiation with each genetic marker, providing, for this species, the first report of a significant and consistent genetic structure within regions in which no stable or identified oceanographic barriers had ever been reported.

Despite the existence of such considerable mass of genetic data on *P. lividus*, the phylogeography of this echinoid species in the Mediterranean basin is still poorly investigated. Even less is known about particular areas, such as the African Mediterranean coast. Moreover, none of the genetic investigation, carried so far on this species throughout its Mediterranean distribution range, has revealed patterns of genetic differentiation between western and eastern Mediterranean basins. Accordingly, genetic investigation across this geographic spectrum is highly recommended to unveil patterns of genetic connectivity among populations and unravel eventual divergent patterns across the Siculo-Tunisian Strait. The North-African coast, especially the part encompassing the Tunisian littoral, is considered as an appropriate area to study biogeographical processes because it harbors populations located west and east of the well-known biogeographic barrier of the Siculo-Tunisian Strait. Recent population genetic studies on other macro-invertebrates, with similar life-hitory traits as *P. lividus*, revealed patterns of genetic (i.e., for the shrimp *Penaeus kerathurus*, Zitari-Chatti et al., 2009; and the green crab *Carcinus aestuarii*, Deli et al., 2015b) and morphological (i.e., for the two coastal crab species: *C. aestuarii*, Deli et al., 2014 and *Pachygrapsus marmoratus*, Deli et al., 2015a; and the black sea urchin *Arbacia lixula*, Deli et al.[①]) diver-

[①] Deli T, Ben Monhamed A, Ben Attia M H, et al. High genetic connectivity among morphologically differentiated populations of the black sea urchin *Arbacia lixula* (Echinoidae: Arbacioida) from the Eastern and Western Mediterranean coasts of Tunisia. Marine Biodiversity (in press)

gence among populations from the African western and eastern Mediterranean coasts. These patterns were extended in certain cases to sharp discontinuity as has been revealed for the green crab *C. aestuarii* (Deli et al., 2016). For this latter, both morphological as well as genetic markers, including the mitochondrial COI gene and nuclear microsatellite loci, revealed concordant patterns of divergence across the Siculo-Tunisian Strait and suggested Pleistocene vicariant event in this highly dispersive species (Deli et al., 2016).

Although the prolonged larval stage of *P. lividus* is expected to facilitate high levels of gene flow as marine currents could drive circulation of plutei between distant localities, it is possible that complex oceanographic features across the African Mediterranean coast may disrupt gene flow and promote population subdivision. Hence, we predict that the Algerian current would move larvae (main moving stage) of *P. lividus* along the African western Mediterranean coast and homogenize populations there but could not reach those on African eastern coast. In the opposite side, water current would probably take *P. lividus* larvae away to farther eastern Mediterranean locations and may lead, therefore, to reduced connectivity among both Mediterranean basins.

In light of these considerations, the main aim of the present study was to assess patterns of population differentiation in this highly dispersive sea urchin species across the Siculo-Tunisian Strait and delineate possible morphometric and genetic subunits. To achieve this target, several locations covering almost the entire Tunisian coastline were sampled and compared by means of multivariate analyses of morphometric traits. Specimens from nineteen locations covering a wider geographic transect, including the Algerian, Tunisian and Libyan coasts, were then analyzed genetically by means of restriction fragment length polymorphism (PCR-RFLP) of the mitochondrial COI gene. The use of both morphometric and mtDNA markers would be helpful for optimizing population structure investigation in the purple sea urchin. The results would have important implications not only for ecology and evolutionary history of the species across the surveyed region but also for conservation and management of biodiversity.

2 Material and methods

2.1 *Sample collection*

Specimens of *P. lividus* were collected from nineteen locations along the African Mediterranean coast, including the Algerian, Tunisian and Libyan littorals, and the Italian site of Palermo (Fig. 1). Sea urchins were captured by snorkelling in shallow rocky habitats. A total of 302 adult specimens (diameter >4 cm) from seven sampling sites covering almost the entire Tunisian coastline (Tabarka: 46, Menzel Abderrahmen: 40, Sidi Rais: 42, Kelibia: 48, Monastir: 44, Chebba: 42, Djerba: 40) were collected and measured for the morphometric comparisons. While a total of 314 specimens, from the entire sampled region covering the nineteen examined sites, were considered for the genetic analyses (Table 1). One gonad from each individual was desiccated and stored in absolute ethanol at –20°C until processed.

2.2 *Morphometric measurements*

Nine linear measurements (Test Diameter (TD), Test Height (TH), Aristotle's Lantern Diameter (LD), Lantern Height (LH), Periproct Diameter (PPD), Peristome Diameter (PSD), Ambulacral Zone Width (AZW), Auricle Height (AH), Spine Length (SL)) and three weight measurements (Total Wet Weight (TWW), Total Dry Weight (TDW), Lantern Dry Weight (LDW)) were used for the morphometric analyses. Linear characters were measured using vernier calipers to the nearest 0.01 mm. Weight measurements of each specimen were recorded to the nearest 0.01 g by means of an electronic balance.

2.3 *DNA extraction and PCR-RFLP screening*

Total genomic DNA was extracted from a tiny portion of gonad, using the Wizard® genomic DNA purification kit (Promega). A fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using specific primers defined from the

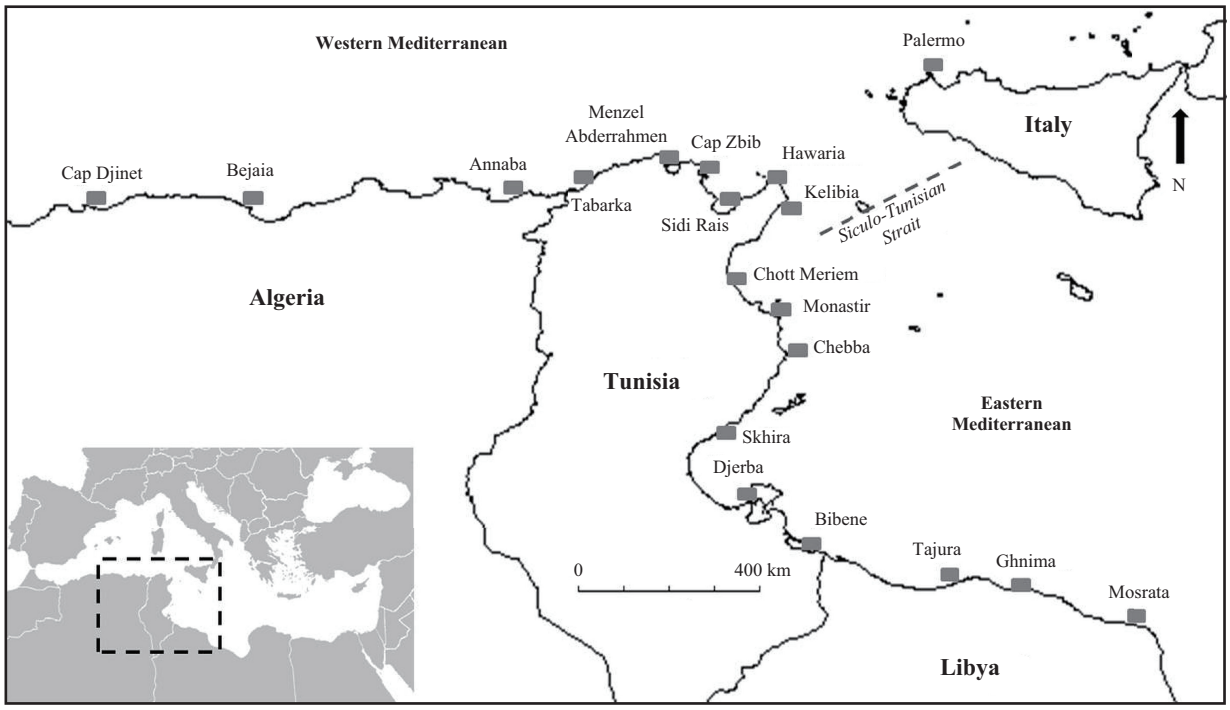

Fig. 1. Sampling locations of *P. lividus* along the African Mediterranean coast. Source of base map: DIVA-GIS 7.5.0.

Table 1. Genetic diversity within the examined populations of the purple sea urchin *P. lividus*

Population/**Region**	Sample size	Number of haplotypes	Number of polymorphic sites	Haplotype diversity (h)	Nucleotide diversity (π (10^2))
Cap Djinet	14	5	5	0.703 3±0.100 8	0.011 1
Bejaia	16	3	2	0.241 7±0.135 3	0.002 4
Annaba	16	5	4	0.608 3±0.130 2	0.009 5
Tabarka	18	6	6	0.686 3±0.111 3	0.018 2
Menzel Abderrahmen	16	6	5	0.741 7±0.083 6	0.013 3
Cap Zbib	10	3	3	0.511 1±0.164 3	0.010 7
Sidi Rais	18	7	6	0.784 3±0.084 9	0.014 9
Hawaria	11	4	5	0.490 9±0.175 4	0.008 9
Kelibia	23	7	6	0.770 8±0.070 8	0.017 7
Palermo	15	6	7	0.800 0±0.071 1	0.019 4
Western Mediterranean	**157**	**18**	**14**	**0.633 8±0.003 1**	**0.012 6±0.000 003**
Chott Meriem	10	2	2	0.200 0±0.154 1	0.003 7
Monastir	22	7	8	0.684 0±0.097 2	0.013 8
Chebba	20	5	5	0.652 6±0.092 7	0.013 7
Skhira	17	3	4	0.227 9±0.129 5	0.004 4
Djerba	21	4	4	0.533 3±0.111 4	0.011 9
Bibene	9	3	4	0.722 2±0.096 7	0.018 2
Tajura	20	5	6	0.678 9±0.079 8	0.017 2
Ghnima	19	2	1	0.491 2±0.068 3	0.004 5
Mosrata	19	5	6	0.731 0±0.080 5	0.014 8
Eastern Mediterranean	**157**	**10**	**11**	**0.546 8±0.004 7**	**0.011 4±0.000 004**
Total	314	23	16	0.592 6±0.001 9	0.012 0±0.000 001

complete genome sequence of *P. lividus* mitochondrion (GenBank accession number J04815). These primers, designed with PRIMER 3.0 (Rozen and Skaletsky, 2000), allowed for the amplification of 712 base pairs of the echinoderm COI gene, corresponding to positions (5′–3′): 6 066–6 777 of the mitochondrial genome (Cantatore et al., 1989). The PCR reaction mix was set up in a 50 μL total-reaction volume with 1.25 units of AmpliTaq DNA Polymerase, 200 μmol/L of dNTPs and 0.16 μmol/L of each primer. PCR amplifications were carried out with an initial denaturation phase of 2 min at 95°C, followed by 30 cycles, each composed of 30 s of denaturation at 95°C, 30 s of annealing at 59°C and completed with an extension time of 60 s at 72°C. These cycles were followed by 8 min of final extension at 72°C. PCR products were loaded and visualized on 1% agarose gel. The amplified fragments were subsequently screened for polymorphism using six restriction endonucleases: *Hin*fI (GANTC), *Msp*I (CCGG), *Nci*I (CCSGG), *Rsa*I (GTAC), *Sac*I (GAGCTC) and *Taq*I (TCGA). These enzymes were determined following the establishement of restriction map of *P. lividus* COI gene using RestrictionMapper V.3. Aliquots (6–9 μL) of each PCR product were digested overnight with 5 U of the restriction enzyme in a final volume of 20 μL at 37°C. Only digests with *Taq*I were performed at 65°C for 1–2 h. Variant RFLP patterns were visualized under UV light on 3% agarose gels or 10% polyacrylamide gels. Fragment lengths were determined using a low range DNA ladder (50–1 000 bp) as size marker. The obtained sizes were specified later with the sequence data.

2.4 *Statistical analyses*

2.4.1 *Morphometric data*

Patterns of morphometric relationships can be influenced by the effect of sex as well as allometric growth and size in species of undetermined age. Test of sexual dimorphism in the examined morphological characters of *P. lividus* revealed no significant difference between both sexes. Accordingly, female and male purple sea urchins were combined for subsequent morphometric analyses. Regressions performed within the seven studied locations for the eleven measurement of body dimensions (TDW, LDW, TD, TH, LD, LH, PPD, PSD, AZW, AH, SL) versus the total wet weight (TWW), used as independent variable and considered as adjusted trait values (Fernandez and Boudouresque, 1997; Middleton et al., 1998), showed a positive and consistent allometry of each measured trait among populations (TDW: R=0.948, P<0.001; LDW: R=0.876, P<0.001; TD: R=0.884, P<0.001; TH: R=0.865, P<0.001; LD: R=0.844, P<0.001; LH: R=0.826, P<0.001; PPD: R=0.560, P<0.001; PSD: R=0.774, P<0.001; AZW: R=0.907, P<0.001; AH: R=0.682, P<0.001; SL: R=0.208, P<0.001). Therefore, the effect of total wet weight (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: $Y_i=Y_i(X_m/X_i)^b$ where Y_i is the standardized measurement from the measured trait Y_i of the ith specimen, X_m is the mean value of total wet weight for the examined location, X_i is the measured total wet weight 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 total wet weight and arc-sinus-transformed to achieve normality, before being processed with multivariate analyses (TWW was not considered since it was used to adjust all the remaining parameters).

Canonical discriminant analysis (CDA), implemented in Statistica for Windows program V.4.3 (StatSoft, Inc., 1993), was used to find out how well discriminant functions allow classification of individuals to groups. The analysis was performed on the arc-sin-transformed variables for the seven studied populations (with each population considered as a separate group). 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. The follow-

ing specifications were used for all CDA runs: Backward stepwise, Tolerance equals 0.01, F to enter equals 1.0, F to remove equals 0.0, and a priori probabilities were estimated to be proportional to group sizes. In order to find out whether natural variability of the measured parameters 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 9999 replicates. A non-metric Multidimensional Scaling (MDS), implemented in PAST V.2.17, was then applied to the Bray-Curtis distances between every pair of population, applying the Sammon (1969) non-linear mapping method for the iterative stress minimization (Johnson and Wichern, 1998; Venables and Ripley, 2002). Using a set of similarities, this technique finds a representation of the samples in few dimensions so that the inter-samples proximities in the low-dimensional space match the original similarities. Variables, responsible for the eventual morphometric separation among locations, were identified using the Similarity Percentages (SIMPER) routine in PAST V.2.17. We tested the correlation between the squared Mahalanobis distances (D^2) noted among the studied populations and the geographical distances separating them in order to find out whether morphological separation between populations is linked to isolation by distance phenomenon.

Based on the preliminary insights inferred from CDA and MDS analyses, yielding a signal of separation between western Mediterranean populations (Tabarka, Menzel Abderrahmen, Sidi Rais, Kelibia) and their eastern Mediterranean counterparts (Monastir, Chebba, Djerba), we assessed pattern of morphometric dissimilarities among both groups of populations using the non-parametric test of significant difference between two or more groups (the one way Analysis of Similarities, ANOSIM), implemented in PAST V.2.17, based on the Bray-Curtis distance (Clarke, 1993; Anderson, 2001). The distances are converted to ranks. In a rough analogy with ANOVA, the test statistic R is based on comparing distances between groups with distances within groups. Large positive R (up to 1) signifies dissimilarity between groups. The one-tailed significance is computed by permutation of group membership, with 9 999 replicates. The Discriminant/Hotelling analysis, implemented in PAST V.2.17, was also applied on the two sets of multivariate data to assess morphological differentiation among them. Given two sets of multivariate data, an axis is constructed which maximizes the difference between the sets (Davis, 1986). The two sets are then plotted along this axis using a histogram. Equality of the means of the two groups is tested by a multivariate analogue to the t test, called *Hotelling's T-squared*, and a P value for this test is given to confirm or reject the hypothesis that the two groups are morphologically distinct. The SIMPER routine analysis was used to account for traits contributing to differences among both regional groups.

2.4.2 *Mitochondrial DNA data*

The presence or absence of restriction sites were inferred for each enzyme from completely additive fragment patterns, and composite haplotypes were assigned to each individual. The composite haplotype data and the restriction site matrix were used for subsequent genetic analyses. The REAP software package (Restriction Enzyme Analysis Package; McElroy et al., 1992) was used to derive estimates of haplotype diversity h (Nei, 1987) and nucleotide diversity π (Nei and Tajima, 1981) within populations and regions. Whereas between-population diversity was estimated as net nucleotide sequence divergence d (Nei and Tajima, 1981).

Intraspecific evolutionary relationships among the recorded COI haplotypes of *P. lividus* were analyzed in a parsimony network estimated with MINSPNET program implemented in ARLEQUIN V.3.01 (Excoffier et al., 2005). This method provides a 95% plausible set for all haplotype type linkages within an unrooted tree. Heterogeneity of haplotype frequencies distribution among samples was tested by means of χ^2-test through a Monte Carlo simulation approach with 1 000 replicates, as described by Roff and Bentzen (1989), using the MONTE program from the REAP package.

Evidence of population genetic differentiation within the examined *P. lividus* dataset was assessed by one-level AMOVA (Excoffier et al., 1992), as implemented in ARLEQUIN V.3.01, based on haplotype frequency. The extent of genetic differentiation between populations was estimated using the fixation index F_{ST} (Wright, 1950). Significance levels of pairwise F_{ST} estimates among all populations were assessed by a randomization procedure with 10 000 permutations. Bonferroni correction (Rice, 1989) was then applied to yield the exact level of significance. Test of isolation by distance was carried out based on assessment of correlations between genetic (F_{ST} values) and geographic distances, using the Mantel test as implemented in ARLEQUIN V.3.01, with 10 000 random permutations. Based on the outcome of significant pairwise population comparisons and evolutionary relationships among the COI haplotypes, inferred from the generated minimum spanning network, we used structured analysis of molecular variance (two-level AMOVA) to examine population genetic structure of *P. lividus* along West-East geographic gradient testing for the separation across the Siculo-Tunisian Strait (Western Mediterranean vs. Eastern Mediterranean). Significance levels of fixation indices (F_{CT}, F_{SC} and F_{ST}) were assessed by randomization procedure with 10 000 permutations. Population genetic structure of the purple sea urchin was further explored by analyzing the genetic relationships among the western and eastern Mediterranean populations. For this purpose, the pairwise matrix of nucleotide sequence divergence d, generated with the REAP software, was used to construct a neighbor-joining tree using NEIGHBOR and DRAWTREE programs from the PHYLIP package (Felsenstein, 1989).

3 Results

3.1 *Morphometric analyses*

Patterns of morphometric relationships between the seven studied populations of *P. lividus* as well as among the two investigated biogeographic regions of the Mediterranean were assessed by the examination of twelve measurement of body dimensions (total wet weight, total dry weight, lantern dry weight, test diameter, test height, lantern diameter, lantern height, periproct diameter, peristome diameter, ambulacral zone width, auricle height, spine length). The outcome of regressions analyses showed a positive and consistent allometry of each measured trait versus the total wet weight among populations. Hence, the total wet weight was used as independent variable and considered as adjusted trait values. It was not considered in subsequent morphometric analyses. Examination of the analyzed morphological characters of *P. lividus* revealed no significant difference between both sexes. Accordingly, female and male purple sea urchins were combined for subsequent morphomet-

ric analyses involving assessement of inter-population and inter-group variations.

3.1.1 *Inter-population morphometric variation*

CDA analyses were highly significant for all sampled locations. Five out of the six defined roots were highly significant in the analysis (Table 2). The first two functions provided the best overall discrimination between populations. Specimens of *P. lividus* were correctly assigned to the defined locations in 79.13% of the cases. Furthermore, pairwise NPMANOVA comparisons showed significant differentiation among locations (F=41.56; P<0.001). Nineteen among the twenty one comparisons between *P. lividus* populations showed significant pairwise Bray-Curtis values (Table 3). Results of MDS plot are well concordant with this finding, showing a clear morphometric separation among locations with remarkable distinction of the population of Sidi Rais (Fig. 2). MDS plot also yielded a signal of separation between western Mediterranean populations (Tabarka, Menzel Abderrahmen, Sidi Rais, Kelibia) and their eastern Mediterranean counterparts (Monastir, Chebba, Djerba) by means of Dimension 2. SIMPER analyses showed that TDW, TD and SL were major contributors to the separation between populations with a cumulative contribution of 55.97% (Table 4). The distribution of the individuals in the canonical discriminant space, formed by Root 1 and Root 2 (both roots explained more than 80% of the total discrimination), proved the results of NPMANOVA and MDS analyses and showed the existence of a remarkable signal of separation between western and eastern Mediterranean locations, despite some overlap between them. Both groups of populations were separated mainly by the means of Root 2. The correlation between the squared Mahalanobis distances (D^2) noted among the studied populations (Table 3) and the geographical distances separating them was significant (R=0.458, P=0.036), supporting the hypothesis of isolation by distance (IBD) to explain population separation.

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

Removed roots	Eigenvalue	Canonical correlation	Wilks'lambda	Chi-square	Degree of freedom	P
0	2.714	0.854	0.044	910.652	66	***
1	1.249	0.745	0.164	527.490	50	***
2	0.532	0.589	0.369	290.746	36	***
3	0.446	0.555	0.566	166.074	24	***
4	0.177	0.387	0.818	58.383	14	***
5	0.037	0.190	0.963	10.771	6	ns

Note: *** significant difference at P<0.001 and ns means non significant difference (P>0.05).

Table 3. Level of significance, inferred from Pairwise NPMANOVAs (non-parametric multivariate analysis of variance) between all pairs of the purple sea urchin *P. lividus* populations, based on Bray-Curtis distances (below the diagonal) and squared Mahalanobis distances (D^2) recorded among locations (above the diagonal)

	Western Mediterranean				Eastern Mediterranean		
	Tabarka	Menzel Abderrahmen	Sidi Rais	Kelibia	Monastir	Chebba	Djerba
Tabarka	–	13.237	4.813	4.903	6.329	7.663	24.115
Menzel Abderrahmen	***	–	21.497	15.913	8.092	19.079	12.013
Sidi Rais	***	***	–	4.410	10.564	7.440	26.154
Kelibia	ns	***	***	–	4.277	8.341	20.035
Monastir	*	***	***	**	–	8.795	16.855
Chebba	*	***	***	***	***	–	15.464
Djerba	***	ns	***	***	***	***	–

Note: * significant difference at P<0.05, ** significant difference at P<0.01, *** significant difference at P<0.001, and ns P>0.05.

3.1.2 *Inter-group morphometric variation*

In order to confirm the pattern of morphological divergence already inferred from MDS and CDA analyses, we assessed morphological differentiation among both groups of populations belonging to the western and eastern Mediterranean basins: (Tabarka, Menzel Abderrahmen, Sidi Rais, Kelibia) vs. (Monastir, Chebba, Djerba) by means of the one way Analysis of Similarities (ANOSIM) and the Discriminant/Hotelling analysis, applied on the two sets of multivariate data. The one way ANOSIM plot exhibited marked phenotypic differences (Fig. 3a) and yielded highly significant morphological dissimilarity (R=0.041; P<0.001) among both western and eastern Mediterranean populations. These results were confirmed by the outcome of the Discriminant/Hotelling analysis showing a clear separation among both groups of populations despite the existence of some overlap between them (Fig. 3b). Statistical analysis of the obtained trend also revealed highly significant difference (Hotelling's T^2=215.250, F=18.916, P<0.001) and allowed for the acceptance of the hypothesis that the two examined groups are morphologically distinct, hinting to the presence of two metapopulations of *P. lividus* that could be geographically delimited on the basis of morphology. SIMPER analysis of traits contribution for differences accounted among purple sea urchin groups yielded similar result to that found for locations showing that total dry weight (TDW), test diameter (TD) and spine length (SL) contributed the most to the separation between groups, with a cumulative contribution of 55.48% (Table 4).

3.2 *Population genetics analyses*

3.2.1 *Genetic diversity*

Genetic analyses of *P. lividus* examined populations were carried out using PCR-RFLP of a 712 base pairs fragment of the mtDNA COI gene. A total of 19 restriction sites were inferred rep-

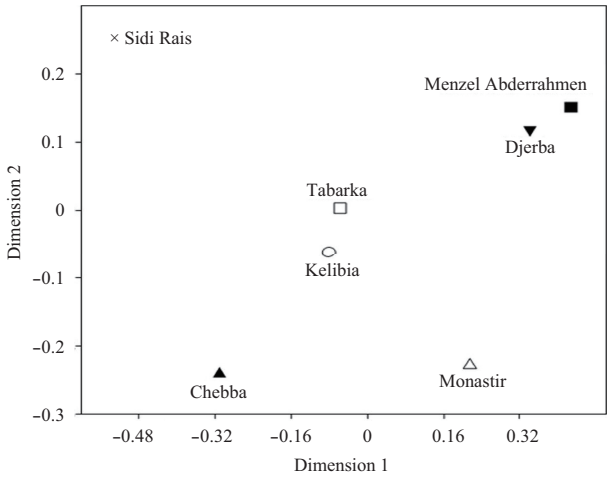

Fig. 2. Non-metric Multidimensional Scaling (MDS) based on Bray-Curtis distance, among populations of *P. lividus* plotted in two dimensions.

resenting an average of 85 bases surveyed (12% of the total fragment). Of these, 16 sites were variable. Assignment of the restriction patterns of the six polymorphic restriction enzymes, to the 314 individuals analyzed in the studied populations, yielded a total of 23 composite haplotypes (Fig. 4, Table 1). Overall, genetic diversity analysis of the mitochondrial dataset showed considerably high haplotypic diversity (h=0.592 6±0.001 9) and low nucleotide diversity (π (10^2)=0.012 0±0.000 001). Levels of these measures were the lowest in the population of Chott Meriem and the highest in the population of Palermo (Table 1). At the regional scale, the Western Mediterranean populations exhibited higher haplotype (h=0.633 8±0.003 1) and nucleotide (π (10^2)= 0.012 6±0.000 003) diversities than those recorded for the Eastern Mediterranean (h=0.546 8±0.004 7; π (10^2)=0.011 4±0.000 004). The minimum spanning network exhibited a double star-like shape, in that all haplotypes were very closely related to the two common Haplotypes 1 and 2 which were distributed across the sampled range (Fig. 4). Haplotype 2 was the most common (recorded in 122 specimens) and was found with higher frequency in the eastern Mediterranean samples, while the next most common haplotype (Haplotype 1, present in 95 individuals) was more frequent in the samples from the western Mediterranean region. Haplotpe 3 was found at the junction of the branches connecting the two main haplotypes (1 and 2) and allowed for their separation by two mutational steps. Alternatively, a set of six

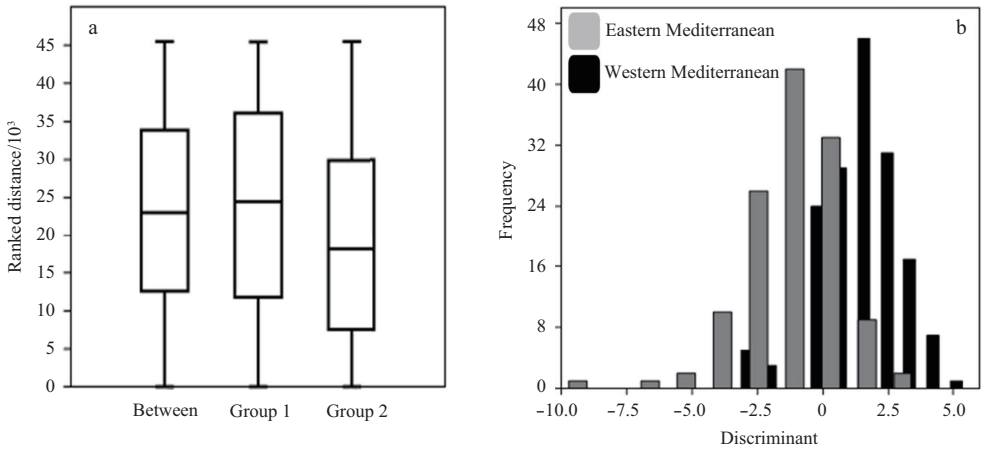

Fig. 3. Results of the one way ANOSIM (Analysis of Similarities) plot (a) depicting inter-group morphometric variation (Group 1: Western Mediterranean, Group 2: Eastern Mediterranean) and Discriminant/Hotelling analysis (b) applied on the two sets of multivariate data, for the purple sea urchin *P. lividus*. Western Mediterranean: Tabarka, Menzel Abderrahmen, Sidi Rais, Kelibia. Eastern Mediterranean: Monastir, Chebba, Djerba.

Table 4. SIMPER analysis of traits contribution for differences accounted among *P. lividus* populations (Tabarka, Menzel Abderrahmen, Sidi Rais, Kelibia, Monastir, Chebba, Djerba) and groups (Western Mediterranean, Eastern Mediterranean)

Traits	Populations			Traits	Groups		
	Average dissimilarity	Contribution/ %	Cumulative/ %		Average dissimilarity	Contribution/ %	Cumulative/ %
Total dry weight	3.772	26.03	26.03	Total dry weight	3.612	25.78	25.78
Test diameter	2.886	19.91	45.93	Test diameter	2.783	19.87	45.65
Spine length	1.454	10.03	55.97	Spine length	1.377	9.83	55.48
Test height	1.335	9.20	65.17	Test height	1.327	9.46	64.95
Peristome diameter	1.204	8.30	73.48	Peristome diameter	1.149	8.20	73.15
Ambulacral zone width	1.045	7.20	80.69	Ambulacral zone width	1.008	7.19	80.34
Lantern height	0.979	6.75	87.45	Lantern height	0.969	6.92	87.27
Lantern diameter	0.891	6.14	93.59	Lantern diameter	0.871	6.22	93.49
Auricle height	0.360	2.48	96.08	Auricle height	0.355	2.53	96.02
Lantern dry weight	0.308	2.12	98.20	Lantern dry weight	0.299	2.13	98.16
Periproct diameter	0.260	1.79	100	Periproct diameter	0.257	1.84	100

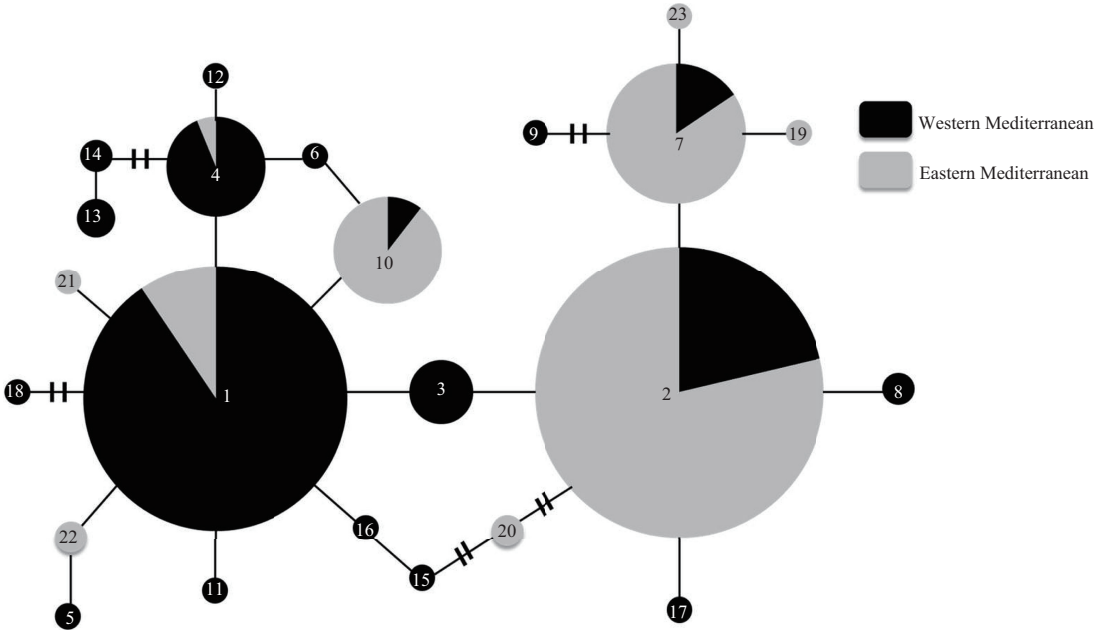

Fig. 4. Minimum spanning network for the recorded twenty three COI (cytochrome oxidase subunit I) gene haplotypes of *P. lividus*. Estimated number of mutations (more than one mutational step) is presented in dashes. Circle sizes depict proportions of haplotypes; the smallest corresponds to 1 and the largest to 122.

mutational steps could also connect both haplotypes via the intermediate connection of Haplotypes 15, 16 and 20 (Fig. 4). As far as could be inferred from the minimum spanning network, the genetic distinctiveness of the two haplogroups centred on the two common haplotypes (1 and 2) supports the existence of a phylogeographic structure within the analyzed samples of *P. lividus*. Indeed, the relatively less frequent haplotypes radiating the common Haplotype 1 characterized mainly the western Mediterranean basin (except Haplotypes 10, 21 and 22). While those connected to the major central Haplotype 2 were commonly found in the eastern Mediterranean specimens (excluding Haplotypes 8, 9 and 17).

3.2.2 *Genetic structure*

Overall, our results showed highly significant genetic differentiation among populations. We rejected the hypothesis of genetic homogeneity in the distribution of haplotype frequencies among samples of *P. lividus* (χ^2=589.82, P<0.001). This trend was also supported by the outcome of the one-level AMOVA, based on haplotype frequency, confirming the hypothesis of a partitioning of genetic variation among populations (F_{ST}=0.190, df=313, P<0.001). Pairwise comparisons of genetic differentiation, estimated from haplotype frequencies, showed that the majority of significant values were those between the Eastern and Western Mediterranean populations (Table 5). A significant relationship was found between genetic and geographic distances (r=0.437, P<0.001) by means of a Mantel test, allowing for the acceptance of isolation by distance hypothesis to explain population separation. Furthermore, populations assigned to both Mediterranean regions exhibited homogeneous haplotype frequencies distributions (Western Mediterranean: χ^2=57.27, P=0.648; Eastern Mediterranean: χ^2=85.72, P=0.119). Based on these insights, and those already inferred from the minimum spanning nework analysis, we examined population genetic structure, within the analyzed data, by means of two-level AMOVA, grouping specimens according to their geographic origin and testing for partitioning of the genetic variance under the biogeographic hypothesis: Western Mediterranean vs. Eastern Mediterranean. Our results showed significant genetic subdivision across the Siculo-Tunisian Strait (F_{CT}=0.272, P<0.001; Table 6). Neighbor-Joining phylogenetic analysis assessing the genetic relationships among the studied populations, based on the pairwise matrix of nucleotide divergence d (Table 5), also showed well clustering of all populations according to their geographic origins with a clear separation between the western and eastern Mediterranean regions (Fig. 5), supporting therefore the structured AMOVA results based on haplotype frequencies.

4 Discussion

This study is the first to focus on population structure of the purple sea urchin *P. lividus* across the African Mediterranean coast. Our results revealed concordant patterns of genetic and morphometric divergence across the Siculo-Tunisian Strait. They also unravelled for the first time significant pattern of genetic differentiation among the western and eastern Mediterranean purple sea urchins, based on the COI mtDNA gene analysis, which was not recorded in previous investigations on this echinoid species (Calderón et al., 2008; Maltagliati et al., 2010; Penant et al., 2013).

Morphometric analyses of the purple sea urchin *P. lividus* across the Tunisian coast showed extensive morphological variability among geographically close populations and allowed for the delineation of two morphologically differentiated groups assigned to the western and eastern Mediterranean basins. This finding is consistent with those of previous investigations using similar sampling scheme on *P. lividus* (Fernandez and Boudouresque, 1997; Arculeo et al., 1998; Rizzo et al., 2009) and on other echinoids such as *Strongylocentrotus* sp. (Hagen, 2008) and *Arbacia dufresnii* (Epherra et al., 2015). Recently, similar morphometric study of the black sea urchin *Arbacia lixula* populations from nearly the same locations along the Tunisian littoral① revealed the same pattern of variability as that found in *P. lividus*. Similar

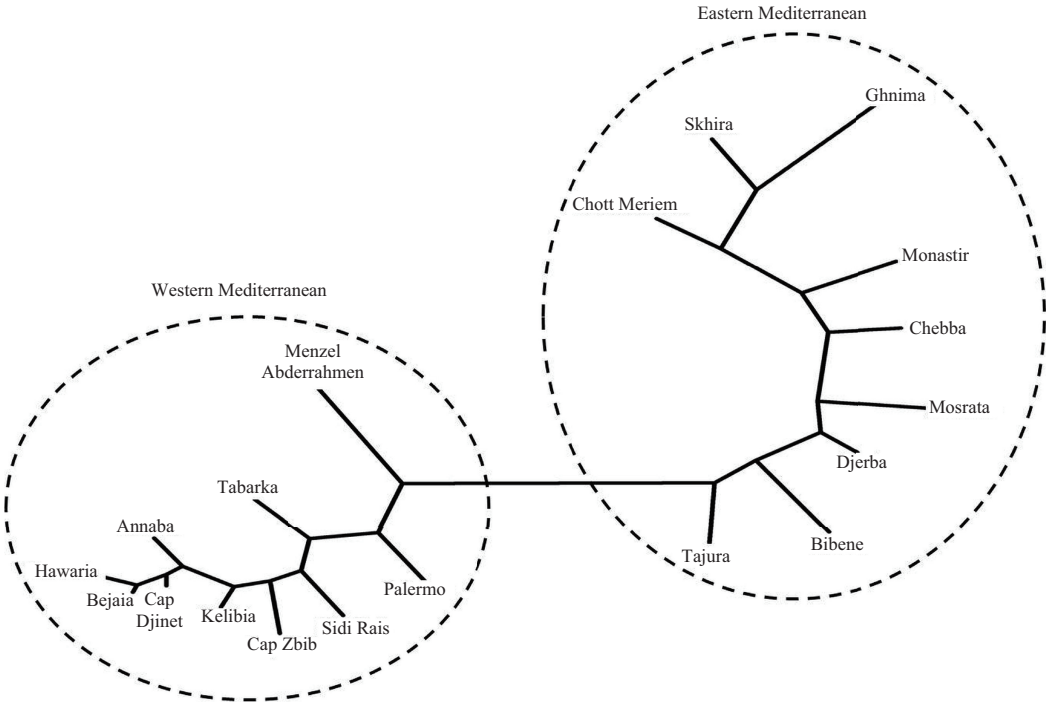

Fig. 5. Unrooted Neighbor-Joining tree assessing the genetic relationships among the examined *P. lividus* populations, based on the pairwise matrix of nucleotide sequence divergence *d* generated with the REAP (Restriction Enzyme Analysis Package) software.

trends of morphological differentiation have been also reported for other Tunisian macro-invertebrates species with similar life history-traits as *P. lividus*, i.e., the green crab *Carcinus aestuarii* (Deli et al., 2014) and the marbled crab *Pachygrapsus marmoratus* (Deli et al., 2015a), hinting at the impact of the studied environment on yielding similar biogeographic units in the investigated marine invertebrates across the Tunisian coast, and providing clear evidence that despite their high dispersal capacity (Pedrotti, 1993; Lozano et al., 1995), purple sea urchin specimens may exhibit phenotypic variability even within restricted geographical areas.

Significant inter-population differences in the measured traits among the studied populations of *P. lividus*, as revealed by CDA analyses, pairwise NPMANOVA comparisons and MDS plot, might have been driven from the impact of divergent selection pressures between alternative environments (Schluter, 2000). Hence, heterogenous environmental factors at different geographical locations across the Tunisian coast might have exerted strong selection pressures on the purple sea urchins in those environments, generating different phenotypic responses amongst them. Fernandez and Boudouresque (1997) showed that *P. lividus* specimens from two ecologically different sites may exhibit specific morphometric response to environmental conditions. This response seems to be mainly due to the quantity and quality of available food at each investigated site. The authors found that sea urchins, living in the sea grass beds, have a higher gonad, repletion and test index than specimens living in pebble zones where the food resources are low. Predation is another factor that could be involed in generating different morphological response among specimens of *P. lividus* inhabiting different ecological niches. This kind of selective pressure has been considered as an important process affecting the structure of sea urchin populations (McClanahan et al., 1994). Sala and Zabala (1996) found out that predation by fish appears to determine *P. lividus* abundance, mean size, size-frequency distribution and behaviour. During their experimental study, the authors noted that the decrease in *P. lividus* mean diameter was attributed mainly to fish predation. They argued that juvenile *P. lividus* that survive early mortality are able to escape efficiently from predation within shelters; a larger size, however, may reduce the ability to occupy small shelters leaving sea urchins more exposed to predation. Sala and Zabala (1996) also found out that during the predation experiment, fish feeding on medium-size urchins may cause morphological differences among purple sea urchins, observed between sites. This constatation has been recently confirmed by Guidetti and Dulčić (2007) showing that *P. lividus* medium-sized individuals are subject to a significantly higher predation than large size individuals which may lead in turn to the onset of morphometric differences among sea urchin populations.

The outcome of the SIMPER routine analysis revealed that total dry weight, test diameter and spine length were major contributors to the morphological differentiation recorded among the sampling sites of *P. lividus*. Plasticity in these characters could be related to food availability. Larger sea urchins would increase the strength of scraping (Ebert, 1980), therefore increasing the grazing potential, which is likely to be of adaptive significance in resource-limited environments (Black et al., 1984). The study by Fernandez and Boudouresque (1997) highlighted a positive relationship between the abundance of food and the thickness of the test (and thus its weight) in *P. lividus*. Greater test weight can also contribute to improving *P. lividus* stability when exposed to hydrodynamic forces (Lumingas, 1994; Fernandez and Boudouresque, 1997). In addition, a heavier test (greater mass) can increase the chances of the sea urchin's survival (Ebert, 1988). Fernandez and Boudouresque (1997) stated that increase in the thickness of the test could help specimens of the purple sea urchin *P. lividus* escape fish predation and thus increase their longevity. Variation in spine length may be linked to

Table 5. Pairwise comparisons of genetic differentiation estimated from haplotype frequency (F_{ST}, below the diagonal) and nucleotide divergence (d (10^2), above the diagonal)

	Western Mediterranean										Eastern Mediterranean								
	Cap Djinet	Bejaia	Annaba	Tabarka	Menzel Abderrahmen	Cap Zbib	Sidi Rais	Kelibia	Palermo	Hawaria	Chott Meriem	Monastir	Chebba	Skhira	Djerba	Bibene	Tajura	Ghnima	Mosrata
Cap Djinet	*	0.016 3	−0.040 9	0.081 7	0.169 0	0.030 5	0.064 5	0.017 6	0.214 9	−0.006 7	1.236 1	1.085 8	1.041 2	1.388 4	0.901 2	0.726 2	0.673 3	1.807 0	0.878 7
Bejaia	0.132 8	*	0.008 2	0.180 1	0.223 1	0.079 6	0.113 2	0.093 1	0.230 0	−0.024 5	1.383 3	1.220 9	1.158 3	1.542 5	1.010 2	0.806 8	0.769 2	1.965 8	0.978 3
Annaba	−0.026 7	0.037 2	*	0.037 4	0.071 0	−0.028 7	−0.004 7	−0.012 5	0.108 9	−0.028 2	1.031 6	0.886 3	0.856 1	1.165 4	0.699 6	0.522 2	0.496 8	1.562 5	0.684 9
Tabarka	−0.017 8	0.079 7	−0.036 0	*	−0.054 4	−0.064 5	−0.068 0	−0.039 9	−0.047 9	0.117 3	0.538 4	0.381 4	0.340 4	0.627 8	0.303 5	0.171 7	0.185 9	0.854 5	0.252 6
Menzel Abderrahmen	0.018 9	**0.193 2**	0.001 2	−0.007 7	*	−0.064 8	−0.057 9	0.019 4	−0.057 4	0.147 0	0.399 3	0.298 5	0.275 4	0.483 8	0.186 4	0.079 2	0.088 0	0.760 3	0.169 7
Cap Zbib	0.039 4	0.045 6	−0.044 0	−0.032 0	0.000 6	*	−0.078 9	−0.035 2	−0.040 3	0.014 5	0.600 1	0.499 0	0.466 3	0.714 3	0.362 9	0.248 6	0.231 6	1.051 8	0.344 8
Sidi Rais	−0.019 5	0.139 3	−0.018 8	−0.035 8	−0.041 6	0.002 2	*	−0.040 9	−0.042 2	0.055 0	0.613 8	0.468 3	0.441 5	0.711 1	0.347 1	0.199 9	0.211 7	0.993 2	0.314 9
Kelibia	−0.002 7	**0.148 9**	−0.009 1	−0.006 0	−0.034 8	0.005 3	−0.028 4	*	0.017 0	0.046 3	0.832 2	0.642 9	0.616 8	0.940 7	0.539 4	0.370 2	0.389 4	1.207 7	0.483 6
Palermo	0.071 1	**0.269 6**	0.061 9	0.045 7	−0.027 6	0.054 3	0.005 3	−0.010 6	*	0.145 9	0.448 2	0.296 1	0.273 6	0.531 0	0.229 8	0.108 7	0.139 9	0.747 7	0.162 2
Hawaria	0.007 5	−0.013 8	−0.053 7	−0.027 5	0.041 5	−0.059 7	0.012 5	0.026 3	0.104 0	*	1.212 7	1.073 5	1.018 3	1.366 5	0.869 3	0.688 0	0.654 5	1.786 0	0.845 0
Chott Meriem	**0.461 7**	**0.753 5**	**0.475 6**	**0.429 6**	0.264 4	**0.525 9**	**0.340 1**	**0.299 6**	0.216 6	**0.585 4**	*	0.010 8	0.020 4	−0.022 1	0.024 1	0.104 6	0.133 1	0.124 0	0.034 8
Monastir	**0.232 3**	**0.474 4**	**0.254 3**	**0.206 7**	0.078 6	**0.262 4**	**0.128 3**	**0.131 7**	0.060 6	**0.316 7**	0.076 1	*	−0.041 3	0.009 5	−0.009 1	−0.008 6	0.072 4	0.055 2	−0.049 7
Chebba	**0.277 3**	**0.518 0**	**0.298 4**	**0.242 5**	0.119 7	**0.307 4**	**0.164 0**	**0.170 0**	0.094 9	**0.361 9**	0.108 9	−0.028 3	*	0.027 6	0.003 9	0.007 3	0.071 7	0.070 8	−0.039 4
Skhira	**0.518 3**	**0.765 4**	**0.534 1**	**0.485 3**	**0.328 5**	**0.582 4**	**0.395 1**	**0.359 7**	**0.281 9**	**0.631 8**	−0.041 5	0.086 8	0.111 9	*	0.033 8	0.102 7	0.151 2	0.080 4	0.043 3
Djerba	**0.342 2**	**0.582 0**	**0.359 0**	**0.316 7**	**0.158 3**	**0.371 0**	**0.225 4**	**0.216 6**	0.132 1	**0.430 0**	0.043 5	−0.004 6	0.023 6	0.030 6	*	−0.085 1	−0.035 1	0.193 5	−0.043 9
Bibene	**0.264 8**	**0.564 7**	**0.303 3**	**0.242 0**	0.113 4	**0.326 3**	0.143 1	**0.170 0**	0.103 3	**0.374 5**	0.241 6	0.000 3	0.021 5	0.224 3	−0.004 3	*	−0.122 6	0.237 0	−0.081 8
Tajura	**0.265 1**	**0.503 6**	**0.288 1**	**0.246 3**	0.109 6	**0.298 3**	**0.156 6**	**0.165 5**	0.096 8	**0.349 0**	0.150 7	0.013 7	0.045 5	0.144 4	−0.014 0	−0.067 4	*	0.361 5	0.131 8
Ghnima	**0.382 7**	**0.626 0**	**0.405 8**	**0.338 9**	**0.213 7**	**0.428 3**	**0.254 7**	**0.263 2**	**0.189 1**	**0.479 0**	0.166 6	0.000 3	−0.020 7	0.141 7	0.057 8	0.062 9	0.095 5	*	−0.009 7
Mosrata	**0.191 0**	**0.423 1**	**0.202 8**	**0.152 4**	0.035 2	**0.203 7**	0.078 0	0.093 1	0.031 2	**0.259 7**	0.125 3	−0.037 5	−0.018 8	0.142 9	0.007 8	−0.027 2	−0.003 2	0.019 5	*

Note: Bold F_{ST} values indicate significant difference, obtained after Bonferroni correction.

Table 6. Analysis of molecular variance testing for genetic differentiation among the studied populations of *P. lividus* and partition of the genetic variance under the biogeographic hypothesis: Western Mediterranean vs. Eastern Mediterranean

Partition tested	Degree of freedom	Sum of squares	Variance components	Percentage of variation	Fixation indices (*P*)
Among examined locations					
Among populations	18	26.559	0.071	18.97	F_{ST}=**0.190** (*P*<0.001)
Within populations	295	89.619	0.304	81.03	
Among Western Mediterranean vs. Eastern Mediterranean					
Among groups	1	18.822	0.117	27.19	F_{CT}=**0.272** (*P*<0.001)
Among populations within groups	17	7.737	0.009	2.15	F_{SC}=**0.029** (*P*<0.05)
Within populations	295	89.619	0.304	70.66	F_{ST}=**0.293** (*P*<0.001)
Total	313	116.178	0.430		

Note: Significant values are in bold and calculated from 10.000 permutations.

the type of environment that purple sea urchins inhabit as well as to the availability of nutrients. Régis (1979) reported the existence of *P. lividus* with shorter spines living in *Posidonia oceanica* meadow than those inhabiting rocky substrate. Dance (1987) has linked this fact to the activity of *P. lividus* which would be less on *P. oceanica* meadow. Longer spines can also indicate the presence of a large amount of nutrients and would therefore be considered a morpho-functional adaptation to a more active and efficient management of this organic matter (Soualili, 2008).

The results inferred from both one way ANOSIM and Discriminant/Hotelling analysis revealed the existence of significant morphological differentiation among both Western and Eastern Mediterranean populations of *P. lividus*. This pattern of regional separation has been recently reported for the black sea urchin *A. lixula*① as well as for the green crab *C. aestuarii* (Deli et al., 2014) and the marbled crab *P. marmoratus* (Deli et al., 2015a) across the Tunisian coast. This finding supports the concept that environmental heterogeneity across biogeographic barriers yields similar patterns of morphometric variation amongst species inhabiting these areas (Hopkins and Thurman, 2010; Hampton et al., 2014). We may hypothesize, for instance, that the two morpho-groups of *P. lividus* could be the result of a regional adaptation to specific abiotic features such as temperature and salinity. It has been demonstrated that growth and size of echinoids may be altered significantly by temperature and salinity conditions (Forcucci and Lawrence, 1986; Wangensteen et al., 2013). Bressan et al. (1995) suggested that among the abiotic factors, temperature and salinity are considered as the most vital factors on the embryonic development of the purple sea urchin *P. lividus*. Somatic growth of *P. lividus* in the field appears to be related mainly to temperature, but also to other factors such as food quality and gonad development (Fernandez, 1996). Recently, Fernandez et al. (2006) found out that low salinity, turbidity and siltation could also greatly affect *P. lividus* stock. Hence, the notable difference in temperature and salinity among both Mediterranean basins (with the Eastern Mediterranean basin being warmer and more saline than the Western; Serena, 2005) might have promoted phenotypic divergence in Tunisian *P. lividus*. Parallely, habitats of different textures, characterizing the sampling sites, with muddy and sandy habitats in the Eastern locations versus rocky ones prevailing in the Westerns, could also yield different phenotypic response in delineated metapopulations of the purple sea urchin. Earlier experimental study carried out by Fernandez (1996), involving the transfer of sea urchin *P. lividus* specimens from the pebble zone to enclosures within the seagrass bed, showed rapid morphological modifications, resulting mainly in an increase in relative test and a decrease in lantern index. Alternatively, different recruitment strategies, characterizing both western and eastern Mediterranean populations of *P. lividus*, might also have induced differences in population morphometric structure of the purple sea urchin. Fernandez et al. (2006) showed the presence of high density of medium size *P. lividus* in a shallow Mediterranean brackish lagoon (Urbinu, Corsica), associated with a high recruitment episode. The authors suggested that high recruitment potential could be considered as adaptive strategy in *P. lividus* to maintain the population in this highly variable habitat. 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.

Pattern of phenotypic divergence discerned in *P. lividus* across the Siculo-Tunisian Strait is interestingly in congruence with that inferred from the genetic investigation. Analysis of the mtDNA COI gene in nineteen populations covering a wider geographic transect, streching westward to the Algerian coast and eastward to the Libyan littoral, yielded significant genetic differentiation between eastern and western Mediterranean populations, as revealed by the outcome of F-statistics and AMOVA analyses. Patterns of genetic structure in *P. lividus* have already been observed throughout its European Atlantic and Mediterranean distribution zone. Earlier investigations revealed significant patterns of genetic differentiation among the Atlantic and Mediterranean (Duran et al., 2004; Calderón et al., 2008; Maltagliati et al., 2010) and even between the Adriatic and the Mediterranean (Maltagliati et al., 2010). In particular, population genetic investigation of the purpule sea urchin, in other parts of the Mediterranean coast, i.e., along the Spanish coast (Western Mediterranean Basin), has also revealed marked spatial genetic structure by means of the mitochondrial cytochrome oxidase subunit I (COI) marker (Calderón et al., 2012) and significant differentiation among cohorts using the bindin gene (Calderón and Turon, 2010). However, such a structure was not observed in all years and was not consistent among all populations. Moreover, this genetic differentiation disappeared when cohorts were pooled and was not observed with nuclear microsatellite loci (Calderón et al., 2009). At the local micro-geographic scale of the Italian coast, analysis of the nuclear rDNA ITS2 spacer and the two mitochondrial genes 16S and COI in geographically close populations from the Gulf of Naples (Italy) did not show any signal of genetic structure in the surveyed region (Iuri et al., 2007). While these studies suggested shallowness of genetic structure resulting from the high dispersal potential of *P. lividus*, Penant et al. (2013) have recently questioned these constatations. Their multi-loci phylogeographic investigation, based on an enlargement of the previously examined data, retrieved newly significant within-basin and within-region differentiation with each examined genetic mark-

er. The study provided for this species the first report of a significant and consistent genetic structure within regions, i.e, a strong genetic differentiation between the easternmost Lebanese population and the rest of the populations. The very new picture revealed by our results, i.e., the detection of two well defined genetic entities characterizing both eastern and western Mediterranean basins, allowed for the confirmation of Penant et al. (2013) new vision regarding spatial structure of *P. lividus* and provides new and considerably important insights on phylogeography and population genetic structure of this species from poorly investigated southern Mediterranean coast.

The finding of the present investigation is in line with the geographic separation of the western and eastern Mediterranean basins previously reported for other African Mediterranean macro-invertebrates with similar life-history traits as *P. lividus*, i.e., the caramote prawn *Penaeus kerathurus* (Zitari-Chatti et al., 2008, 2009) and the green crab *Carcinus aestuarii* (Deli et al., 2015b, 2016). The phylogeographical pattern we distinguished, based on the minimum spanning network, confirmed the geographic partitioning of genetic variation, as revealed by F-statistics and AMOVA analyses, and showed striking distribution differences among the two common Haplotypes 1 and 2. These latters present in different frequencies, across the Siculo-Tunisian Strait, whith Haplotype 1 commonly found in the Western Mediterranean and Haplotype 2 prevailing in the Eastern Mediterranean. The longitudinal clinal distribution of both common haplotypes probably suggest a sort of adaptation to the environment at both regions and imply the impact of divergent selective pressures across the Siculo-Tunisian Strait which might have modulated such structuring pattern. However, the question that could be raised here: does this divergent pattern imply local adaptation enhanced by the accumulation of phenotypic and genetic differences over time or it is just reflecting short-term impacts of selection and/or recruitment?

The concordance of genetic and phenotypic characters in defining two distinct groups supports the hypothesis that divergent selection pressures between alternative western and eastern Mediterranean environments might have possibly triggered both morphometric and genetic divergent patterns in Afrian Mediterranean populations of *P. lividus*, hence, implying local adaptation and reflecting a possible signature of historical events that took place in the studied region and that might have shaped population genetic structure of many marine species such as fishes (Bahri-Sfar et al., 2000; Mejri et al., 2009; Kaouèche et al., 2011), molluscs (Gharbi et al., 2011), shrimps (Zitari-Chatti et al., 2008, 2009) and crabs (Deli et al., 2014, 2015b). The striking similarity of biogeographic patterns reinforces the idea that these Mediterranean species were probably facing the same historical events and suggests strongly vicariance events due to Pleistocene glacial episodes characterized by strong climate fluctuations (Thiede, 1978). During this epoch, Temperature and sea level fluctuations, might have led to the fragmentation of marine species distribution range on either side of the Siculo-Tunisian Strait (Thiede, 1978; Pérès, 1985). Furthermore, during the last ice age (18 000 years before present), the Eastern Basin was about 10°C warmer than the Western Basin (Thiede, 1978) and this temperature gradient is preserved until present (Mojetta and Ghisotti, 1996). It is therefore quite possible that ancient separation between North-African populations of *P. lividus* from the two Mediterranean basins allowed for the accumulation of significant variations over time offering them the opportunity to diverge. Recently, similar patterns of morphometric and genetic differentiation were highlighted among the green crab *C. aestuarii* populations from the African western and eastern Mediterranean coasts (Deli et al., 2014, 2015b). Later on, detailed investigation by Deli et al. (2016) showed that this particular pattern of divergence was due to an historical separation dating back to the early Pleistocene.

Nevertheless, even if the outcome of phylogenetic analysis, based on the pairwise matrix of nucleotide divergence *d*, yielded a clear pattern of separation between the western and eastern Mediterranean regions, both haplogroups, centred on the two common Haplotypes 1 and 2, were shown to be separated by only few mutational steps. Hence, this finding may rather suggest recent differentiation between eastern and western Mediterranean groups, maintained by restricted contemporary gene flow. Previous phylogeographic investigations on this species, suggested recent and contemporary processes, i.e., oceanographic patterns, as the main causes of the found subtle genetic differentiation among *P. lividus* populations (Duran et al., 2004; Calderón et al., 2008; Maltagliati et al., 2010; Penant et al., 2013), where migration-drift dominates over mutation (Penant et al., 2013). In light of these considerations, we may propose that contemporary water circulation along the North-African littoral seems to be the likely process involved in shaping such patterns of genetic structure across the Siculo-Tunisian Strait. Accordingly, we may hypothesize that the unidirectional surface current called "the Algerian current", originating from the Atlantic (which moves eastward along the North-African coast and at the entering of the Eastern Mediterranean Basin leaves the coastal zone opposite the North coast of Tunisia around Kelibia; Béranger et al., 2004), would move purple sea urchin larvae (main moving stage) along the Algerian and Tunisian coasts and homogenize populations there but could not reach those on Eastern-Southern coast (eastern Tunisian and Libyan populations). As the Eastern Mediterranean, characterized by very weak circulation (Pinardi and Masetti, 2000), harbors no surface current in the opposite direction that could homogeneize gene pool of *P. lividus*, we might expect purple sea urchin larvae movement from eastern Tunisian and Libyan locations to farther Eastern Mediterranean locations, which may lead to reduced connectivity among both Mediterranean basins. With regards to earlier genetic investigations on European purple sea urchin, highlighting genetic homogeneity within the Mediterranean Sea, the particular complex circulation pattern across the African Mediterranean, differing from other patterns across the Northern Mediterranean coast, might provide convincing argument on why our study thrived in discerning gene flow disruption in North-African *P. lividus*, while earlier macro-geographic investigations by Calderón et al. (2008) and to a lesser extent Maltagliati et al. (2010) and Penant et al. (2013) failed to delineate such pattern of differentiation.

Alternatively, we might attribute genetic differentiation among both Mediterranean basins to different larval survival and recruitment strategies in *P. lividus* from both regions. Lopéz et al. (1998) showed that these parameters are highly dependent on spring planktonic blooms. The reduction of this bloom might decrease larval survival time, and therefore gene flow. This vision would be largely supported by the fact that most of the significant F_{ST} were observed between Western and Eastern Mediterranean populations. In this context, significant F_{ST} (based on haplotype frequency) might be observed after relatively few generations of genetic drift and would be expected to be more powerful than Φ_{ST} (based on nucleotide divergence between the observed haplotypes) to detect significant regional differentiation (Penant et al., 2013). However, the observed significant pat-

tern of isolation by distance (IBD) within the African Mediterranean coast may strongly suggest that gene flow is not only limited by fluctuating events, but also by distance and environmental factors, i.e., hydrological factors (affecting dispersal) or differential selection (affecting fitness). In addition to the previously discussed impact of oceanographic patterns on shaping contemporary genetic structure of African *P. lividus*, selection on mtDNA haplotypes would be another crucial factor that should be taken into account. The impact of selection on phenotypic and genetic structure has been already suggested in a study of stressed *P. lividus* populations from Greece (Rizzo et al., 2009). Recently, Penant et al. (2013) assumed that selection on the mitochondrial COI marker would be responsible of the differentiation observed in the Lebanese sample of *P. lividus*. The authors suggested the possibility that natural selection might have eliminated larvae containing the very common COI haplotypes from the Lebanese population, located in the region with the most oligotrophic waters and the highest mean seawater temperature. Based on these insights, and given the presence of different temperature gradients across the African western and eastern Mediterranean coasts with the eastern coast being warmer (Serena, 2005), we may favour the possible impact of different selective pressures, deriving from different levels of sea surface temperature, on gene pool of North-African *P. lividus*. Grant et al. (2006) have already reported a correlation between sea surface temperature and mitochondrial haplotypes for the North Pacific walleye pollock *Theragra chalcogramma*.

In conclusion, regardless the kind of the process susceptible of generating patterns of morphometric and genetic differentiation in *P. lividus*, the information *per se* is of practical importance for management and conservation of this exploited species. Altogether with previous insights inferred from the study of Penant et al. (2013), the newly detected phylogeographic patterns of the purple sea urchin *P. lividus* across part of the southern Mediterranean coast (African Mediterranean) prove that a large and geographically exhaustive dataset is necessary to unveil phylogeographic structure within widespread marine species, previously cathegorized as panmictic in part of their distribution range. Analysis of other variable nuclear markers in this highly dispersive species would allow confirming the obtained patterns of population structure and determining whether population differentiation is due to limited dispersal or local adaptation.

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