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Genetic Differentiation among Populations of the Green Crab *Carcinus aestuarii* (Nardo, 1847) (Brachyura, Carcinidae) from the Eastern and Western Mediterranean Coast of Tunisia

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Abstract: This study focuses on the population genetic structure of the green crab *Carcinus aestuarii* (Nardo, 1847) from five locations covering almost the entire Tunisian coastline. Its main objective was to seek for genetic subdivision across the Siculo-Tunisian region, characterised by a well-documented genetic boundary. Genetic polymorphism was screened in 88 specimens using PCR-RFLP of the mitochondrial marker COI. The results of the present investigation showed a specific haplotype distribution in two sets of populations from two different regions: the western (Tabarka and Bizerte populations) and eastern (Monastir, Sfax and Djerba populations) Mediterranean regions. The general trend of partitioning of genetic variation among populations was suggested by 1-level AMOVA (F_{ST} = 0.535, $P < 0.001$). It was confirmed by population structure analysis using 2-level AMOVA which showed strong genetic differentiation for both eastern and western Mediterranean localities in Tunisia. More than 62% of the variation was among groups (F_{CT} = 0.629, $P < 0.001$). No significant correlation was found between genetic and geographic distances, suggesting lack of isolation by distance. Possible drivers of this pattern of genetic differentiation were discussed.

Keywords: phylogeographic break, population genetics, larval dispersal, COI, PCR-RFLP

Introduction

It has been known that gene flow in marine invertebrates is generally expected to be correlated with larval dispersal potential (Shanks *et al.* 2003, Dibacco *et al.* 2006, Kelly, Palumbi 2010), which may, in turn, strongly influence the geographical range and genetic structuring of populations (Hedgecock 1986, Kelly, Palumbi 2010). Increased time that larvae spent in plankton is usually linked with less differentiation among populations (Scheltema 1971, Avise 1994). However, high dispersal potential may not always translate into high levels of gene flow (Knowlton, Keller 1986), reflecting the potential action of local retention mechanisms, such as temperature gradients (Newman 1979, Wares *et al.* 2001), oceanographic circulation patterns (Ketchum 1954, Hedgecock 1986, Mcconaugha 1992), and larval behaviour (Burton, Feldman 1982, Raimondi, Keough 1990). The Mediterranean green crab *Carcinus aestuarii* (Nardo, 1847) is a common inhabitant of estuaries and lagoons of the Mediterranean and Black Seas (Mori *et al.* 1990, Behrens Yamada, Hauck 2001). It can be found from a wide range of habitats including rocky intertidal, unvegetated intertidal, subtidal mud and sand, saltmarshes and seagrasses (Ray 2005). It is a voracious omnivore and aggressive competitor, which potentially impacts fisheries. Furthermore,

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the Mediterranean green crab has a wide tolerance for salinity, temperature, oxygen, and habitat type (Mori *et al.* 1990, Mistri *et al.* 2001). The female can spawn up to 185,000 eggs at a time (Cohen, Carlton 1995), and dispersal occurs at all life history stages (Cohen *et al.* 1995). Its planktonic larval stage can last for up to 50 days (Crothers 1967, Rainbow *et al.* 1999).

In the last few centuries, specimens of *C. aestuarii* have been accidentally introduced into several regions outside their native range as a result of maritime commerce and ballast transport, for instance to the Canaries Islands (Almaca 1962) and Tokyo Bay, Japan (Furota *et al.* 1999). Recently, Darling *et al.* (2008) provided evidence of *C. aestuarii* invasion to South Africa, suggested previously by Geller *et al.* (1997) and Carlton, Cohen (2003). Having such life history characteristics, this species is expected to exhibit lack of population divergence and weakly structured or seemingly unstructured populations (Hilbish 1996, Waples 1998). Nevertheless, population genetics studies on *C. aestuarii* from native and invaded sites (Darling *et al.* 2008, Marino *et al.* 2011, Ragionieri, Schubart 2013) have revealed extensive genetic variability and population differentiation linked mainly to oceanographic discontinuities that characterise these areas. Marino *et al.* (2011) and Ragionieri, Schubart (2013) found significant genetic differentiation among populations from the European western and eastern Mediterranean coast, and suggested the potential action of the Siculo-Tunisian Strait as a barrier to gene flow in *C. aestuarii*. This area, considered as one of the most important transition zones in the Mediterranean Sea, has been described as a genetic boundary between populations 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, Zitari-Chatti *et al.* 2008, 2009). In order to verify and correctly localise the phylogeographic break suggested by Marino *et al.* (2011) and Ragionieri, Schubart (2013), we sampled for the first time geographically close populations of *C. aestuarii* around the Siculo-Tunisian strait and along the Tunisian coast (*i.e.* the southern Mediterranean coast).. A recent study of several populations in the same region has shown, by means of multivariate analyses of linear morphometric traits, marked morphological differentiation (Deli *et al.* 2014). Thereby, we addressed the question whether this morphological differentiation was owing simply to phenotypic plasticity or it could be produced through genetic differences. Could this species, with high larval dispersal potential, exhibit genetic differentiation over a microgeographic spectrum?

Restriction fragment length polymorphism (PCR-RFLP) of the mitochondrial marker COI was applied to analyse population genetic structure of the green crab *C. aestuarii* from the eastern and western Mediterranean coast of Tunisia. The COI gene has been shown to be variable enough for population studies in marine crabs (Fratini, Vannini 2002, Roman, Palumbi 2004, Darling *et al.* 2008).

Material and Methods

Sample Collection and DNA Extraction

During field-trips in Tabarka (20), Bizerte (20), Monastir (16), Sfax (14) and Djerba (18) (Fig. 1), 88 adult specimens of *C. aestuarii* were collected from five sampling sites, covering almost the entire coastline of Tunisia. Specimens of the Mediterranean green crab can be distinguished mainly by a flat and protruded frontal area beyond the eyes, straight and parallel male pleopods and width to length ratio of the carapace under 1.27 (Behrens Yamada, Hauck 2001). Crabs were taken alive to the laboratory and genomic DNA was extracted from the muscle tissue of the walking legs using Wizard® genomic DNA purification kit (promega).

PCR-RFLP Screening

Genetic polymorphism was screened using PCR-RFLP. About 700 bp fragment of the mitochondrial gene cytochrome oxidase I (COI) was amplified using the degenerate universal primers: COI F and COI R (Darling *et al.* 2008). PCR was set up in a 50 µl mix composed of PCR buffer, 200 µM of each dNTP, 0.16 µM of each primer, 1U of Taq, almost 0.2 µg of DNA and bidistilled sterile water. The amplification included one preliminary denaturation at 95°C for 5 minutes, followed by 35 cycles of 95°C denaturation for 30 seconds, annealing at 40°C for 50 seconds and 72°C extensions for 60 seconds. A final extension of 8 minutes at 72°C was performed. The PCR products were then digested with seven restrictions enzymes: *Hin*fI (GANTC), *Mae*I (CTAG), *Mbo*I (GATC), *Msp*I (CCGG), *Nla*III (CATG), *Rsa*I (GTAC) and *Taq*I (TCGA). These enzymes were determined following the establishment of restriction map of *C. aestuarii* using RestrictionMapper version 3. This map was defined based on COI sequences obtained from two individuals belonging to the populations of Tabarka and Djerba. Restriction enzyme digestions were carried out in 20 µl mixtures containing 2 µl of enzyme buffer, 2.5 U of restriction enzyme, 8µl of PCR product and bidistilled sterile water. Digestions were done at 37°C for 4 hours, except for digests with *Taq*I, which were performed at

65°C for 2 hours. At the end of the reactions, 10 µl of the resulting fragments were separated on a 3% agarose gel, with ethidium bromide staining, electrophoresed for 2 hours at 90 V, and visualized under UV light. Fragment lengths were determined using a low range DNA ladder (50-1000 bp) as size marker.

Data and statistical analyses

Only four enzymes (*Hin*fI, *Mbo*I, *Msp*I and *Nla*III) out of the seven used in this study, yielded clear and reliable digestion patterns. Consequently, restriction patterns, generated by each of these enzymes, were identified and then combined to define mtDNA haplotype patterns. Restriction site data were analysed with the computer package ARLEQUIN V.3.01 (Excoffier *et al.* 2005). Within-population diversity was estimated by haplotype diversity *h* (NEI 1987). The existence of genetic differentiation was assessed using 1-level AMOVA (Excoffier *et al.* 1992), based on haplotype frequencies. The extent of genetic differentiation between populations was estimated using the fixation index F_{ST} (Wright 1951). The significance of pair-wise F_{ST} estimates among all populations was assessed by randomisation procedure with 10,000 permutations. Bonferroni correction (Rice 1989) was then applied to yield the exact level of significance (critical value = 0.005 with 10 hypothesis tests and alpha = 0.05). Test of isolation by distance, assessed by the Mantel test, was carried out based on correlations between genetic (F_{ST} values) and geographic distances, as implemented in ARLEQUIN, with 10,000 random permutations. Based on the result of 1-level AMOVA and the outcome of significant pair-wise population comparisons, we defined groups for a structured AMOVA to examine population genetic structure, within samples of *C. aestuarii*. Significance levels of fixation indices (F_{CT}, F_{SC} and F_{ST}) were assessed by randomisation procedure with 10,000 permutations. A minimum spanning network between the found haplotypes was computed in ARLEQUIN and designed to infer phylogenetic relationships between haplotypes. The number of mutational steps connecting the haplotypes was inferred, based on the minimum spanning tree computation from the matrix of pair-wise distances calculated between all pairs of haplotypes using a modification of the algorithm described in Rohlf (1973).

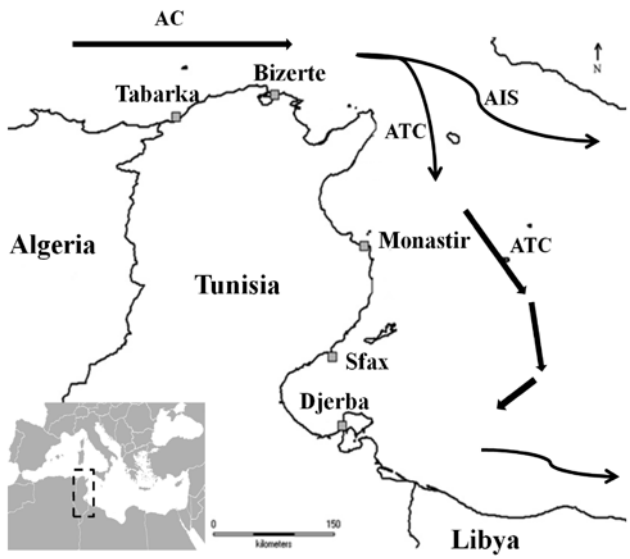

Fig. 1. Sampling locations of C. aestuarii along the Tunisian coast with sea surface currents of the studied region (sea surface currents pattern is in agreement with Beranger *et al.* (2004)). AC: Algerian Current; AIS: Atlantic Ionian Stream; ATC: Atlantic Tunisian Current. Source of base map: DIVA-GIS 7.5.0.

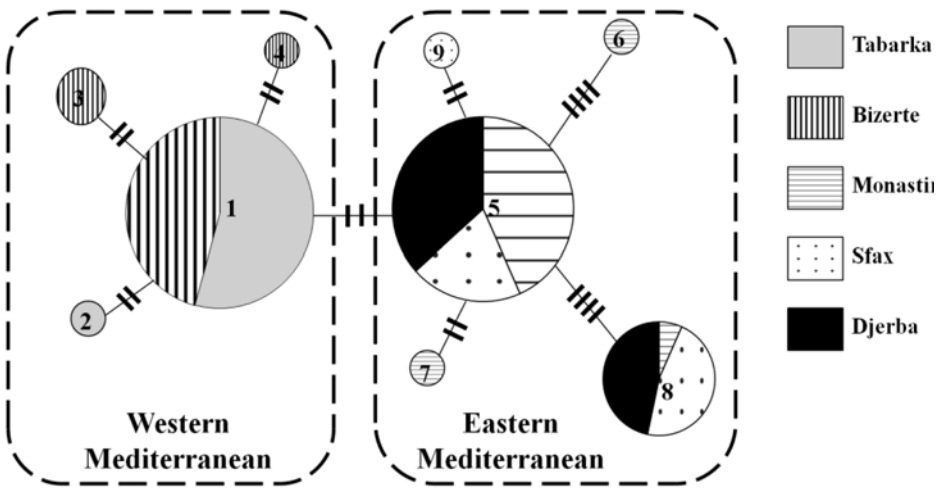

Fig. 2. Minimum spanning network for the nine COI haplotypes of C. aestuarii. Estimated number of mutations, connecting the found haplotypes, is presented in dashes. Circle sizes depict proportions of haplotypes; the smallest corresponds to 1 and the largest to 35

Results

Assignment of the restriction patterns of the four enzymes to the 88 individuals, analysed in the studied populations, yielded a total of nine haplotypes (Table 1). Among these, five haplotypes were unique. Four haplotypes (1, 2, 3 and 4) were found in the populations of Tabarka and Bizerte, whereas the remaining five (5, 6, 7, 8 and 9) were present in the populations of Monastir, Sfax and Djerba. The minimum spanning network showed clustering of all haplotypes according to their geographic origins. Two star-like clades could be defined. In each clade, less frequent haplotypes were connected to the major central haplotype and were separated from it by few mutational steps (Fig. 2). Haplotype diversity was low in almost all the studied populations (0.364 ± 0.099) and ranged from 0.100 ± 0.088 in the population of Tabarka to 0.604 ± 0.075 in the population of Sfax (Table 2). Six out of ten comparisons between *C. aestuarii* populations showed high and significant pair-wise F_{ST} values (Table 3). These were between populations belonging to both the western (Tabarka and Bizerte) and eastern (Monastir, Sfax and Djerba) Mediterranean regions. The 1-level AMOVA analysis also confirmed the general trend of partitioning of genetic variation among populations *(*F_{ST} = 0.535, df = 87, $P < 0.001$). The Mantel test revealed non-significant correlation between genetic *(*F_{ST} values) and geographic distances (r = 0.756, $P = 0.067$). Based on the geographical distribution of the analysed specimens, the outcome of the haplotype network clustering and the significant pair-wise population comparisons, we tested population genetic structure of Tunisian *C. aestuarii* under the biogeographic hypothesis: western Mediterranean (Tabarka and Bizerte) versus eastern Mediterranean (Monastir, Sfax and Djerba) regions. The global 2-level AMOVA results showed that more than 62% of the variation was among groups (F_{CT} = 0.629, $P < 0.001$; Table 4).We included these five populations, previously investigated in our study on *C. aestuarii* (Deli *et al.* 2014), and reanalysed their morphometric data in order to assess morphological differentiation between the two groups of populations. Therefore, we applied the Discriminant/Hotelling analysis, as implemented in PAST V.2.17, on the two sets of multivariate data. The *Hotelling's T-squared test* showed a highly significant difference among both groups of populations belonging to the eastern and western Mediterranean regions for females (*Hotelling's* T^2 = 286.16, $F = 26.522$, $P < 0.001$; Fig. 3a), as well as for males (*Hotelling's* $T^2 = 147.45$, $F = 13.692$, $P < 0.001$; Fig. 3b).

Discussion

The results of the present study showed a non-random distribution of the genetic variation of *C. aestuarii* across the surveyed geographic range. AMOVA analyses and F-statistics suggested strong genetic structure among the studied populations and allowed the characterisation of two genetically distinct groups from the eastern and western Mediterranean coasts of Tunisia. Patterns of genetic differentiation have already been studied in other decapod species, with quite similar life history traits as *C. aestuarii*, across a microgeographic spectrum in other regions, such as the swimming crab *Callinectes danae* in Southern Brazil (Weber, Levy 2000), the two spider crab species *Inachus dorsettensis* and *Hyas coarctatus* from two areas off the coast of the Isle of Man (Weber *et al.* 2000), the European lobster *Homarus gammarus* in Northern Norway (Jørstad *et al.* 2004), the shrimp *Crangon crangon* along the British coast and the Baltic Sea (Weetman *et al.* 2007), and the Italian *C. aestuarii* from the Venice Lagoon (Marino *et al.* 2010). Our results and comparisons do not allow agreeing with the general concept that gene flow in continuous marine systems is generally expected to homogenise populations over large distances (Scheltema 1975, Hedgecock 1986). Several biotic and abiotic factors may lead to genetic differentiation between geographically close populations (Kyle, Boulding 2000, Selkoe *et al.* 2008, 2010, Schunter *et al.* 2011). Nevertheless, their role and respective contributions still need more empirical clarifications and support. For instance, we can hypothesise that the genetic differentiation among *C. aestuarii* populations could be the result of a regional adaptation to specific abiotic conditions such as temperature and salinity. It is known that the Eastern Mediterranean Basin is warmer and more saline: its average water temperature ranges between 16 and 29°C with average salinity of 39 ppt while the western basin displays lower temperatures (12-23°C) and salinity (36 ppt) Serena 2005). In addition, we could speculate that biotic factors linked to reproduction and recruitment events might have triggered genetic differentiation among the studied green crab populations. Marino *et al.* (2010) argued that selective forces acting during the recruitment should have produced the genetic differentiation among local samples of *C. aestuarii* within the Venice Lagoon. Phylogenetic relationships between haplotypes exhibited by the minimum spanning network confirmed the geographic partitioning of genetic variation and showed strict clustering of all haplotypes depending on geography. Within this phylogeographical pattern,

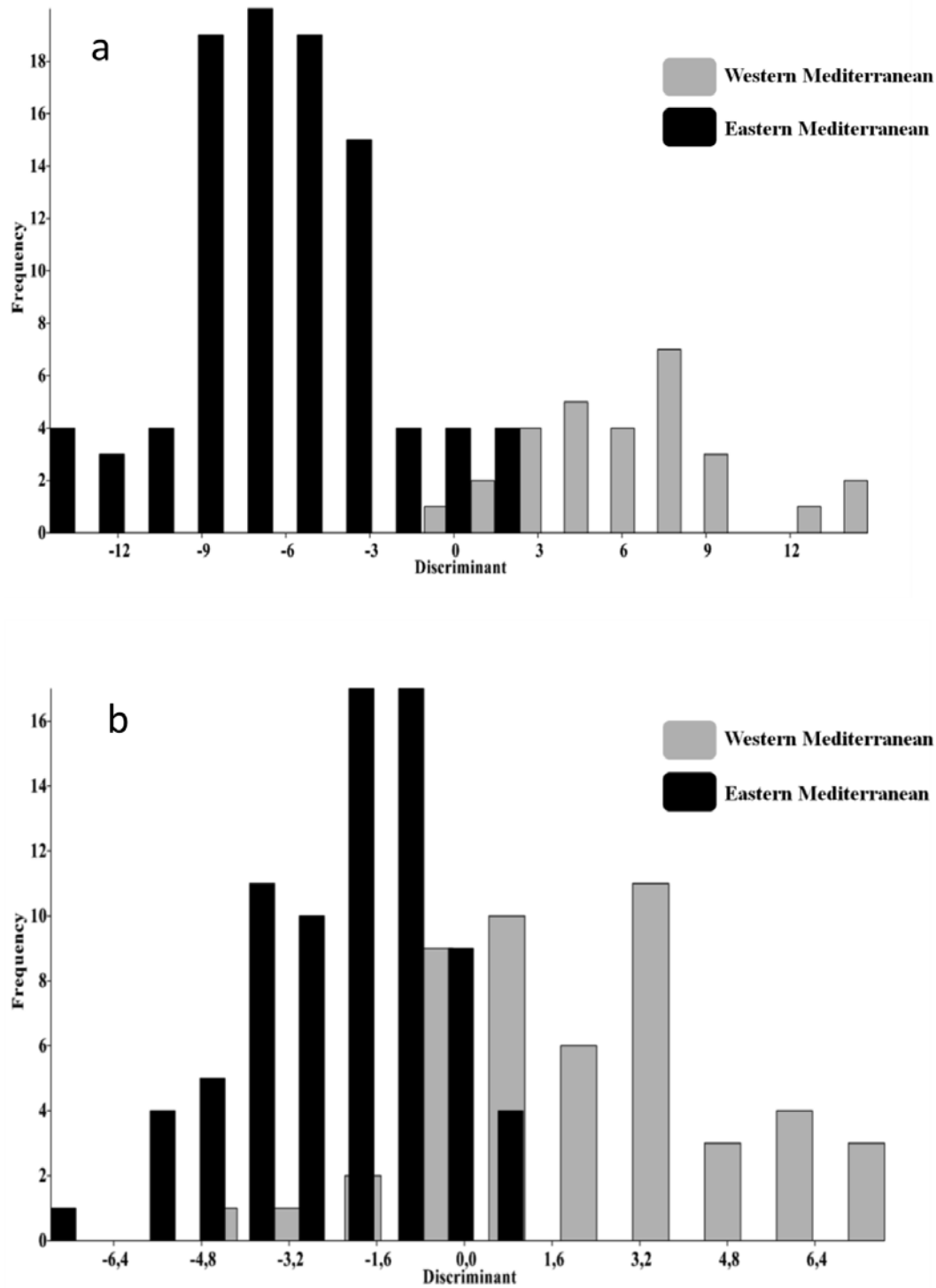

Fig. 3. Results of the Discriminant/Hotelling analysis applied on the two sets of multivariate data, for female (a) and male (b) C. aestuarii, corresponding to the Western Mediterranean (Tabarka and Bizerte) and Eastern Mediterranean (Monastir, Sfax and Djerba) (data adapted from DELI et al. 2014)

we distinguished two phylogroups. Although tightly connected, these groups were defined by exclusive haplotypes. This pattern, showing strong clustering of haplotypes to their geographic origin separated by few mutational steps, suggests that the eastern and western groups had not been sundered by long-term biogeographic barriers and their differentiation is probably maintained by restricted contemporary gene flow.

The contemporary water circulation along the Tunisian coast is characterised by a unidirectional surface current known as the Algerian current. It

Table 1. Distribution pattern of the found haplotypes in the studied populations of the green crab *C. aestuarii*

Haplotype	Population				
	Tabarka	**Bizerte**	**Monastir**	**Sfax**	**Djerba**
H1	19	16	0	0	0
H2	1	0	0	0	0
H3	0	3	0	0	0
H4	0	1	0	0	0
H5	0	0	13	6	11
H6	0	0	1	0	0
H7	0	0	1	0	0
H8	0	0	1	7	7
H9	0	0	0	1	0
Total	20	20	16	14	18

Table 2. Haplotype diversity within *C. aestuarii* populations. Legend: N- number of samples per population; Nh- number of haplotype; *h*- haplotype diversity (each value is the mean ± Standard deviation)

Population	**N**	**Nh**	***h***
Tabarka	20	2	0.100 ± 0.088
Bizerte	20	3	0.352 ± 0.122
Monastir	16	4	0.350 ± 0.147
Sfax	14	3	0.604 ± 0.075
Djerba	18	2	0.503 ± 0.063
Total	88	9	0.364 ± 0.099

Table 3. Pair-wise F_{ST} values among populations of *C. aestuarii*, based on haplotype frequencies (below the diagonal); *P* values, calculated from 10,000 permutations (above the diagonal). Bold values indicate significant difference, obtained after Bonferroni correction (critical value = 0.005 with 10 hypothesis tests and alpha = 0.05)

	Tabarka	**Bizerte**	**Monastir**	**Sfax**	**Djerba**
Tabarka	-	0.168	0.000	0.000	0.000
Bizerte	0.057	-	0.000	0.000	0.000
Monastir	**0.786**	**0.648**	-	0.012	0.051
Sfax	**0.681**	**0.535**	0.235	-	0.482
Djerba	**0.706**	**0.574**	0.107	-0.017	-

originates from the Atlantic, moves eastwards along the coast of North Africa and leaves the coastal zone opposite the north coast of Tunisia (around Kelibia) at the entering of the Eastern Mediterranean Basin (see main surface currents in Fig. 1). There is no surface current in the opposite direction: the Eastern Mediterranean Basin is characterised by very weak circulation (Pinardi, Masetti 2000). Hence, larvae, the main motile stage, drifting on water would move along the northern Tunisian coast and homogenise populations there (Bizerte and Tabarka) but could not reach those on the eastern-southern coast (Monastir, Sfax and Djerba). Water current would probably take *C. aestuarii* larvae away from Eastern Tunisian populations to farther eastern Mediterranean locations and may lead to reduced connectivity between both genetic groups. In future studies, analysing samples along an appropriate and wider West-East Mediterranean transect could help verify this hypothesis.

Even though the phylogeographical pattern suggesting two mitochondrial groups deserves to be verified by other mitochondrial and nuclear markers, it is interestingly notably congruent with previous morphometric investigation of *C. aestuarii* populations from the same region which showed strong morphometric differentiation between the eastern and western Mediterranean populations (Deli *et al.* 2014). The concordance of genetic and phenotypic characters in defining two distinct groups suggests geographic separation, triggered by divergent selection pressures between alternative environments. It may imply a possible signature of historical events that took place in the studied region and that might have shaped population genetic structure likely as for many marine fish species (Bahri-Sfar *et al.* 2000, Mejri *et al.* 2009, Kaouèche *et al.* 2011), molluscs (Gharbi *et al.* 2011) and shrimps (Zitari-Chatti *et al.* 2008, 2009). The striking similarity of biogeographic patterns reinforces the idea that these Mediterranean species were probably facing the same historical events and suggests strongly the existence of vicariance events due to Pleistocene glacial episodes characterised by strong fluctuations in climate (Thiede 1978). Analysing DNA sequence data in future studies will be more suitable for coalescence analyses when aiming to trace historical processes and to better estimate divergence levels and dates. The results of the present study support the West-East geographic separation (Marino *et al.* 2011, Ragionieri, Schubart 2013) of the European Mediterranean populations of *C. aestuarii*. It allowed to extend the investigated geographic spectrum of this species to the African Mediterranean coast and, therefore, widen the available genetic dataset. In addition, comparison of our findings to previous ones for other species from the same region proves the existence of similar marked divergence patterns between populations from the western and eastern Mediterranean coasts of Tunisia and therefore confirms the status of the Siculo-Tunisian Strait as a biogeographic boundary. While different patterns of water circulation and/or vicariance event might have promoted genetic differentiation among

Table 4. Global 2-level AMOVA results as a weighted average over the 12 restriction sites, testing for partitioning the genetic variance under the biogeographic hypothesis: Western Mediterranean (Tabarka and Bizerte) vs. Eastern Mediterranean (Monastir, Sfax and Djerba). Statistical significance ***: *P* value < 0.001 from permutation test; Statistical significance **: *P* value < 0.01 from permutation test

Source of Variation	Sum of squares	Variance components	Percentage of variation	Fixation indices
Among groups	51.641	1.135	62.911	F_{CT} = 0.629***
Among populations within groups	6.082	0.083	4.623	F_{SC} = 0.124**
Within populations	48.618	0.585	32.464	F_{ST} = 0.675***
Total	106.341	1.804		

populations belonging to both Mediterranean basins, larval retention could also be essential for species with a long larval dispersal phase and with low adult migration in preventing high levels of gene flow between locations (Weber, Levy 2000).

In conclusion, regardless of the kind of the process susceptible to the genetic differentiation pattern found in this highly dispersive species, the information *per se* is of practical importance. Our results, along with those inferred from morphometric data, already obtained on *C. aestuarii* from the same surveyed geographic spectrum (Deli *et al.* 2014), yielded important knowledge regarding different aspects of the biology and ecology of this species that can be used for its management and conservation. Specifically, the genetic and morphometric heterogeneity among the populations across the investigated geographic area should be considered in order to develope appropriate population management programs. Our results could contribute in clarifying the adaptive capability of the studied invasive species to different environmental conditions, and in understanding the mechanisms of invading new locations. Future genetic studies of population connectivity should be extended to a greater geographic range of the species in order to generate more complete information and assist in getting an up-to-date overview of the whole distribution range.

References

Almaca C. 1962. Sur la distribution géographique du genre *Carcinus* Leach (Crust. Dec. Brach.). – *Revista da Faculdade de Ciências da Universidade de Lisboa*, **10:** 109-113**.**

Arnaud-Haond S., E. Diazalmela, S. Teixeira 2007. Vicariance patterns in the Mediterranean Sea: East-West cleavage and low dispersal in the endemic seagrass *Posidonia oceanica*. – *Journal of Biogeography*, **14:** 963-976**.**

Avise J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York. 511 p.

Bahri-Sfar L., C. Lemaire, O. K. B. Hassine, F. Bonhomme 2000. Fragmentation of sea bass populations in the western and eastern Mediterranean as revealed by microsatellite polymorphism. – *Proceedings of the Royal Society B: Biological Sciences*, **267:** 929-935**.**

Behrens Yamada S., L. Hauck 2001. Field identification of the European green crab species: *Carcinus maenas* and *Carcinus aestuarii*. – *Journal of Shellfish Research*, **20:** 905-912**.**

Béranger K., L. Mortiera, G. P. Gasparini, L. Gervasio, M. Astraldi, M. Crepon 2004. The dynamics of the Sicily Strait: a comprehensive study from observations and models. – *Deep-Sea Research II*, **51:** 411-440**.**

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

Burton R. S., M. Feldman 1982. Population genetics of coastal and estuarine invertebrates: does larval behaviour influence population structure? – In Kennedy VS (ed.): Estuarine comparisons. Academic, New York, 537-551.

Carlton J. T., A. N. Cohen 2003. Episodic global dispersal in shallow water marine organisms: the case history of the European shore crabs *Carcinus maenas* and *C. aestuarii*. – *Journal of Biogeography*, **30:** 1809-1820**.**

Cohen A. N., J. T. Carlton 1995. Nonindigenous aquatic species in a United States estuary: A case study of the biological invasions of the San Francisco Bay and Delta. –Report to the US Fish and Wildlife Service, Washington, DC, and Connecticut Sea Grant**.**

Cohen A. N., J. T. Carlton, M. C. Fountain 1995. Introduction, dispersal and potential impacts of the green crab *Carcinus maenas* in San Francisco Bay, California. – *Marine Biology*, **122:** 225-237**.**

Crothers J. H. 1967. The biology of the shore crab *Carcinus maenas* (L.): I. The background, growth and life history. – *Field Studies*, **2:** 407-434**.**

Darling J. A., J. M. Bagley, J. Roman, C. K. Tepolt, J. B. Geller 2008. Genetic patterns across multiple introductions of the globally invasive crab genus *Carcinus*. – *Molecular Ecology*, **17:** 4992-5007**.**

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

Dibacco C., L. A Levin, E. Sala 2006. Connectivity in marine ecosystems: the importance of larval and spore dispersal. – In: Crooks KR, Sanjayan M (eds.): Connectivity conservation. Conservation biology, Vol 14. Cambridge University Press, Cambridge, 184-213**.**

Excoffier L., G. Laval, S. Schneider 2005. Arlequin ver. 3.0:

An integrated software package for population genetics data analysis. – *Evolutionary Bioinformatics Online*, **1:** 47-50.

Excoffier L., P. E. Smouse, J. M. Quattro 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. – *Genetics*, **131:** 479-491.

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

Furota T., S. Watanabe, T. Watanabe, S. Akiyama, K. Kinoshita 1999. Life history of the Mediterranean green crab, *Carcinus aestuarii* Nardo, in Tokyo Bay, Japan. – *Crustacean Research*, **28:** 5-15.

Geller J. B., E. D. Walton, E. D. Grosholz, G. M. Ruiz 1997. Cryptic invasions of the

crab *Carcinus* detected by molecular phylogeography. – *Molecular Ecology*, **6:** 901-906.

Gharbi A., R. Zitari-Chatti, A. Van Womhoudt, M. N. Dhraief, F. Denis, K. Said, N. Chatti 2011. Allozyme Variation and Population Genetic Structure in the Carpet Shell Clam *Ruditapes decussatus* Across the Siculo-Tunisian Strait. – *Biochemical Genetics*, **49:** 788-805.

Hedgecock D. 1986. Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? – *Bulletin of Marine Science*, **39:** 550-565.

Hilbish T. J. 1996. Population genetics of marine species: the interaction of natural selection and historically differentiated populations. – *Journal of Experimental Marine Biology and Ecology*, **200:** 67-83.

Jørstad K. E., P. A. Prodöhl, A. L. Agnalt, M. Hughes, A. P. Apostolidis, A. Triantafyllidis, E. Farestveit 2004. Subarcticpopulations of European lobster, *Homarus gammarus*, in northern Norway. – *Environmental Biology of Fishes*, **69:** 223-231.

Kaouèche M., L. Bahri-Sfar, M. González-Wangüemert, Á. Pérez-Ruzafa, O. K. Ben Hassine 2011. Allozyme and mtDNA variation of white seabream *Diplodus sargus* populations in a transition area between western and eastern Mediterranean basins (Siculo-Tunisian Strait). – *African Journal of Marine Science*, **33** (1)**:** 79-90.

Kelly R. P., S. R. Palumbi 2010. Genetic structure among 50 Species of the Northeastern Pacific rocky intertidal community. – *PLoS One*, **5**(1)**:** e8594.

Ketchum B. H. 1954. Relation between circulation and planktonic populations in estuaries. – *Ecology*, **35:** 191-200.

Knowlton K., B. D. Keller 1986. Larvae which fall short of their potential: highly localized recruitment in an alpheid shrimp with extended larval development. – *Bulletin of Marine Science*, **39:** 213-223.

Kyle C. J., E. G. Boulding 2000. Comparative population genetic structure of marine gastropods (*Littorina spp.*) with and without pelagic larval dispersal. – *Marine Biology*, **137:** 835-845.

Marino I. A. M., F. Barbisan, M. Gennari, F. Giomi, M. Beltramini, P. M. Bisol, L. Zane 2010. Genetic heterogeneity in populations of the Mediterranean shore crab, *Carcinus aestuarii* (Decapoda, Portunidae), from the Venice Lagoon. – *Estuarine, Coastal and Shelf Science*, **87:** 135-144.

Marino I. A. M., J. M. Pujolar, L. Zane 2011. Reconciling deep calibration and demographic history: Bayesian Inference of post glacial colonization patterns in *Carcinus aestuarii* (Nardo, 1847) and *C. maenas* (Linnaeus, 1758). – *PlosOne*, **6** (12)**:** e28567.

Mcconaugha J. R. 1992. Decapod larvae: dispersal, mortality, and ecology. A working hypothesis? – *American Zoologist*, **32:** 512-573.

Mejri R., S. L. Brutto, O. K. B. Hassine, M. Arculeo 2009. A study on *Pomatoschistus tortonesei* Miller 1968 (Perciformes, Gobiidae) reveals the Siculo-Tunisian Strait (STS) as a breakpoint to gene flow in the Mediterranean basin. – *Molecular Phylogenetics and Evolution*, **53:** 596-601.

Mistri M., R. Rossi, E. A. Fano 2001. Structure and secondary production of a soft bottom macrobenthic community in a brackish lagoon (Sacca di Goro, northeastern Italy). – *Estuarine, Coastal and Shelf Science*, **52:** 605-616.

Mori M., R. Mancon, G. Fanciulli 1990. Notes on the reproductive biology of *Carcinus aestuarii* Nardo (Crustacea, Decapoda) from the lagoon of San Teodoro (Island of Sardinia, Italy). – *Rivista di Idrobiologia*, **29:** 763-774.

Nei M. 1987. Molecular Euolutionary Genetics. Columbia University Press, New York. 512 p.

Newman W. A. 1979. California transition zone: significance of short-range endemics. – In Gray J, Boucot AJ (eds.): Historical biogeography, plate tectonics, and the changing environment. Oregon State University Press, Corvallis, 399-416.

NIKULA R., R. VAINOLA 2003. Phylogeography of *Cerastoderma glaucum* (Bivalvia: Cardiidae) across Europe: a major break in the Eastern Mediterranean. – *Marine Biology*, **143:** 339-350.

Pinardi N., E. Masetti 2000. Variability of the large scale general circulation of the Mediterranean Sea from observations and modelling: a review. – *Paleogeography, Paleoclimatology, Paleoecology*, **158:** 153-174.

Quesada H., C. M. Beynon, D. O. F. Skibinski 1995. A mitochondrial DNA discontinuity in the mussel *Mytilus Galloprovincialis* Lmk: Pleistocene vicariance biogeography and secondary intergradations. – *Molecular Biology and Evolution*, **12:** 521-524.

Ragionieri L., C. D. Schubart 2013. Population genetics, gene flow, and biogeographical boundaries of *Carcinus aestuarii* (Crustacea: Brachyura: Carcinidae) along the European Mediterranean coast. – *Biological Journal of the Linnean Society*, **109:** 771-790.

Raimondi P. T., M. J. Keough 1990. Behavioural variability in marine larvae. – Australian *Journal of Ecology*, **4:** 427-437.

Rainbow P. S., C. Amiard-Triquet, J. C. Amiard, B. D. Smith, S. L. Best, Y. Nassiri, W. J. Langston 1999. Trace metal uptake rates in crustaceans (amphipods and crabs) from coastal sites in NW Europe differentially enriched with trace metals. – *Marine Ecology Progress Series*, **183:** 189-203.

Ray G. L. 2005. Invasive animal species in marine and estuarine environments: biology and ecology. – *Aquatic Nuisance Species Research Program, U.S. Army Corps of Engineers*. ERDC/EL TR-05-2. January 2005.

Rice W. R. 1989. Analysing tables of statistical tests. – *Evolution*, **43:** 223-225.

Rohlf F. J. 1973. Algorithm 76. Hierarchical clustering using the minimum spanning tree. –*The Computer Journal*, **16:** 93-95.

Roman J., S. R. Palumbi 2004. A global invader at home: population structure of the green crab, *Carcinus maenas*, in

Europe. – *Molecular Ecology*, **13:** 2891-2898.

Scheltema R. S. 1971. Larval dispersal as a means of genetic exchange between geographically separated populations of shallow-water benthic invertebrates. – *Biological Bulletin of the Marine Biological Laboratory, Woods Hole*, **140:** 284-322.

Scheltema R. S. 1975. Relationship of larval dispersal, gene flow and natural selection to geographic variation of benthic invertebrates in estuaries and along coastal regions. – *Estuarine Research*, **1:** 372-389.

Schunter C., J. Carreras-Carbonell, E. Macpherson, J. Tintore, E. Vidal-Vijande, A. Pascual 2011. Matching genetics with oceanography: directional gene flow in a Mediterranean fish species. – *Molecular Ecology*, **20:** 5167-5181.

Selkoe K. A., C. M. Henzler, S. D. Gaines 2008. Seascape genetics and the spatial ecology of marine populations. – *Fish and Fisheries*, **9:** 363-377.

Selkoe K. A., J. R. Watson, C. White, T. Ben Horin, M. Iacchei, S. Mitarai 2010. Taking the chaos out of genetic patchiness: seascape genetics reveals ecological and oceanographic drivers of genetic patterns in three temperate reef species. – *Molecular Ecology*, **19:** 3708-3726.

Serena F. 2005. Field identification guide to the sharks and rays of the Mediterranean and Black Sea. – *FAO Species identification Guide for Fishery Purposes*. Rome: FAO, 97 pages.

Shanks A. L., B. A. Grantham, M. H. Carr 2003. Propagule dispersal distance and the size and spacing of marine reserves. – *Ecological Applications*, **13:**159-169.

Thiede J. 1978. A glacial Mediterranean. – *Nature*, **276:** 680-683.

Waples R. S. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. – *Journal of Heredity*, **89:** 438-450.

Wares J. P., S. D. Gaines, C. W. Cunningham 2001. A comparative study of asymmetric migration events across a marine biogeographic boundary. – *Evolution*, **55:** 295-306.

Weber L., R. Hartnoll, J. Thorpe 2000. Genetic divergence and larval dispersal in two spider crabs (Crustacea: Decapoda). – *Hydrobiologia*, **420:** 211-219.

Weber L. I., J. A. Levy 2000. Genetic population structure of the swimming crab *Callinectes danae* (Crustacea: Decapoda) in southern Brasil. – *Hydrobiologia*, **420:** 203-210.

Weetman D., A. Ruggiero, S. Mariani, P. W. Shaw, A. R. Lawler, L. Hauser 2007. Hierarchical population genetic structure in the commercially exploited shrimp *Crangon crangon* identified by AFLP analysis. – *Marine Biology*, **151:** 565-575.

Wright S. 1951. The genetical structure of populations. – *Annals of Eugenics*, **15:** 323-353.

Zitari-chatti r., N. Chatti, a. Elouaer, k. Said 2008. Genetic variation and population structure of the caramote prawn *Penaeus kerathurus* (Forskal) from the eastern and western Mediterranean coasts in Tunisia. – *Aquaculture Research*, **39:** 70-76.

Zitari-Chatti R., N. Chatti, D. Fulgione, I. Gaiazza, G. Aprea, A. Elouaer, K. Said, T. Capriglione 2009. Mitochondrial DNA variation in the caramote prawn *Penaeus* (Melicertus) *kerathurus* across a transition zone in the Mediterranean Sea. – *Genetica*, **136:** 439-447.

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