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Research article

Genetic diversity and population structure of the banded Murex, *Hexaplex trunculus* (Linnaeus, 1758) across the Siculo-Tunisian Strait

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ABSTRACT
Population structure of *Hexaplex trunculus* sampled along four Tunisian coasts was studied. We have scored the genetic variation of the mitochondrial Cytochrome c oxidase subunit I gene (*COI*) among a total of 45 specimens. Our analyses revealed 14 different haplotypes and two groups of haplotypes, separated by the Siculo-Tunisian Strait. Haplotype diversity showed an increasing north to south gradient which may be explained by the hydrographical patterns in the study area. The overall estimate of genetic divergence (F_{ST}) revealed significant genetic differentiation between all population pairs. Finally, populations seem to be deviated from mutation/drift equilibrium suggesting a recent demographic expansion or natural selection. These results may have management implications since *H. trunculus* is a harvested species with a high commercial value.

Key word: *Hexaplex trunculus*, Genetic diversity, *COI*, Tunisian coasts.

1. INTRODUCTION

The marine environment is considered to be highly dispersive (Hauser and Carvalho, 2008). However, populations can be genetically structured across a broad range of geographic scales. The patterns of population genetic structure in the marine environment reflect events of different ages ranging from allopatric divergence linked to historical climate shifts, geographical or oceanographic barriers, to present day oceanographic conditions that limit genetic exchanges among populations, in interaction with species life history traits and behavior (Selkoe et al., 2008). During the Pleistocene glacial episodes, sea level and climate changes have caused physical barriers to gene flow in the Mediterranean Sea and shaped the distribution of genetic diversity in this region. The common physical barriers in the Mediterranean Sea were the Gibraltar Strait (Quesada et al., 1995), the Almería-Oran front (Patarnello et al., 2007) and the Siculo–Tunisian Strait (Zittari-Chatti et al., 2009; Fadhlaoui-Zid et al., 2012). The banded murex, *H. trunculus*, is a common species of the family Muricidae and is distributed in the Mediterranean Sea and adjacent coast of the Atlantic Ocean (Houart, 2001). This species occurs in intertidal and infralittoral zones between 1 m and 100 m depth (Chiavarini et al., 2003). It inhabits both hard and soft substrates, from rocky shores to sandy–muddy. *H. trunculus* is a gonochoric species with collective spawns which produces intracapsular development larvae and therefore no planktonic larval stage. It is also highly restricted in mobility at the adult stage which potentially limits its colonization abilities (Vasconcelos et al., 2004). This species is regularly or occasionally fished for human consumption in several countries in the Mediterranean Sea (Houart, 2001). *H. trunculus* is considered as an ideal candidate for studding physical and evolutionary factors inducing population genetic structure in the Mediterranean Sea.
In this work, a partial sequence of mtDNA gene (*COI*) was used to study the genetic structure of the banded murex collected from Tunisian coasts. This genetic marker exhibits levels of sequence variation suitable for population analysis of numerous marine invertebrates (Calvo et al., 2009; Penant et al., 2013, Deli et al., 2015).

2. MATERIAL AND METHODS

2.1. Sample collection

A total of 45 specimens of *H. trunculus* collected from 4 Tunisian sites across the Siculo–Tunisian Strait (Fig. 1) were used in this study.

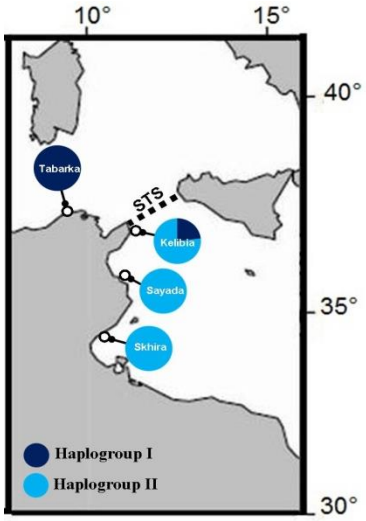

Fig. 1. Map showing the sampling sites of *H. trunculus*. Pie charts show the distribution of the major haplogroups. STS: Siculo-Tunisian Strait.

2.2. DNA extraction, amplification and sequencing

Total genomic DNA was extracted with Chelex resin (BioRad) solution at 10% (Walsh et al., 1991) and preserved at −20 °C until processing. Polymerase chain reaction (*PCR*) was used to amplify was used to amplify 710 bp of the mitochondrial Cytochrome Oxydase I gene (*COI*), using the primers LCO1490 and HCO2198 from Folmer et al. (1994). PCR was conducted using the following program: initial denaturation 3 min at 94°C; 35 cycles, denaturation 1 min at 94°C, primer annealing for 1 min at 42°C, extension for 1 min at 72°C, and final elongation with 5 min at 72°C. PCR reactions were carried out in 25 µL reaction volumes, with 1X buffer, 2mM $MgCl_2$, 0.2mM dNTP, 2µM of each primer, 1U Taq polymerase (Promega) and 2 µL of DNA. PCR products were sequenced on ABI automated sequencers in LGC Genomics Firm using the LCO1490 primer.

2.3. Phylogenetic analyses

Sequences were aligned using *ClustalW* (Thompson et al., 1994) implemented in Bioedit (Hall, 1999) and deposited in the GenBank database (Accession numbers: KJ135252-KJ135266).

Haplotype network was constructed using *NETWORK* with the median joining algorithm (Bandelt et al., 1999). Nucleotide and haplotype diversities (π, *Hd*) were calculated using the DNAsp software (Librado and Rozas, 2009). Differentiation between populations was estimated with the θ estimator of F_{ST} using *ARLEQUIN* (Excoffier and Lischer, 2010) and the null hypothesis of no differentiation was tested by performing 10 000 permutations among individuals between populations. The historical population expansion events within *H. trunculus* populations were investigated by an analysis of the mismatch distribution. Since these tests were found to be conservative, we also computed Tajima's *D* test and Fu's *Fs* neutrality tests implemented in *DNAsp*.

3. RESULTS

A total of 45 *H. trunculus COI* sequences of 658were obtained. Among them, 14 different haplotypes were identified; 10 nucleotide sites were variable and 6 were parsimony informative. Overall, nucleotide diversity was π = 0.0018±0.0002 and haplotype diversity was *Hd*=0.831±0.001. Skhira and Kelibia populations presented nearly similar and height values of haplotype diversity, while Tabarka population has the lowest *Hd*. The nucleotide diversity was height in Skhira population and very low in Tabarka(Table 1).

Table 1. Estimates of gene diversity and neutrality tests of the four *H. trunculus* populations. (* P<0.05)

	N	h	π	*Hd*	*Fs*	*D*
Tabarka	16	3	0.0008	0.342	0.699	1.055
Kelibia	9	4	0.0061	0.833	-1.077*	-1.692*
Sayada	10	5	0.0013	0.667	-2.260*	-1.744*
Skhira	10	6	0.0106	0.844	-2.780*	-0.781*
Total	45	14	0.0018	0.831	-7.915*	-0.834*

The *H. trunculus* haplotypes were distributed into two main haplogroups, as evidenced by the haplotype network. The first haplogroup (I) occurred in Tabarka and Kelibia and the second one (II) occurred in Sayada, Skhira and Kelibia (Fig. 2). Haplotypes from Kelibia were shared between the two main haplogroups (Fig. 1). The overall estimate of genetic divergence using F_{ST} statistics revealed significant genetic differentiation (P<0.05) between all population pairs. Comparison between Skhira-Kelibia and Skhira-Sayada showed a significant low level of genetic differentiation. The other F_{ST} estimates showed a highly significant differentiation between pairs of population, indicating relatively high inter-population divergence (Table 2).

Table 2. Pairwise F_{ST} based on *COI* sequences among the populations of *H. trunculus*

	Tabarka	Kelibia	Sayada	Skhira
Tabarka	0.000			
Kelibia	0.453	0.000		
Sayada	0.520	0.252	0.000	
Skhira	0.440	0.161	0.196	0.000

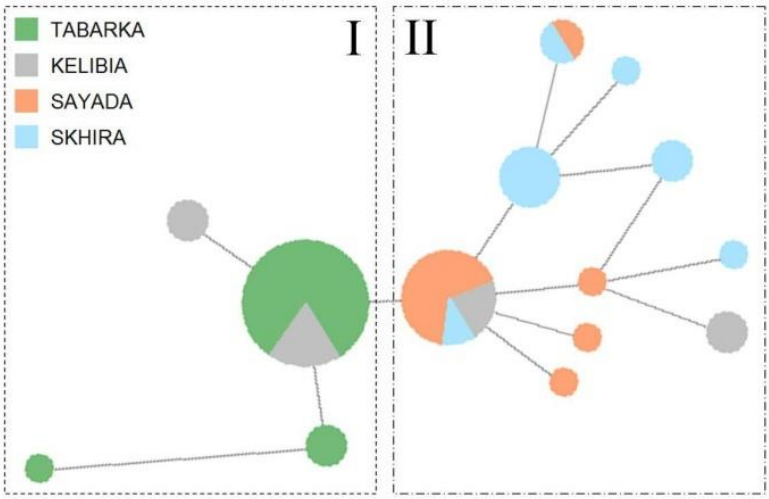

Fig. 2. Haplotype network for *COI*. Each circle represents an haplotype. The size of the circle is proportional to the number of individuals displaying the each haplotype. Colors indicate the origin of each haplotype as in the legend. Links are proportional to the number of mutations between 2 haplotypes

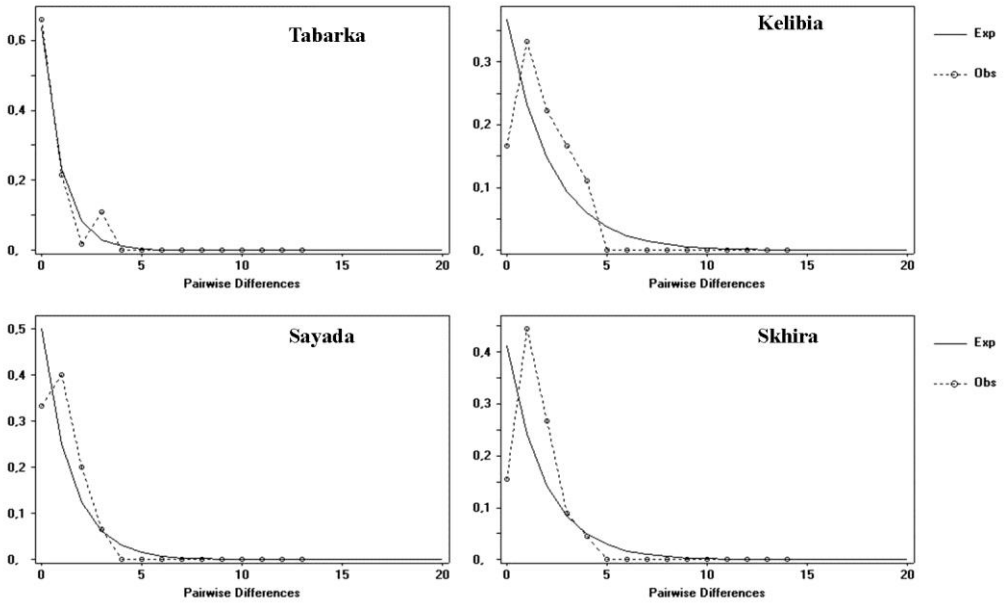

Fig. 3. Pairwise mismatch distribution for the studiedpopulation of *H. trunculus*.

Mismatch distributions were unimodal for all studied populations. Consequently, the shapes of these distributions suggested recent population expansions or selective sweeps (Fig.3). This hypothesis was supported by significant negative values of *Fs* and *D*, except for Tabarka (Table 1).

4. DISCUSSION

Using *COI* sequence data, a high haplotype and nucleotide diversity were detected for populations of Kelibia, Sayada and Skhira while population from Tabarka has the lowest variability. The haplotype diversity showed an increasing North to South gradient with the highest diversity value reported in the southernmost locality and the lowest in the northernmost one. This pattern seems to be associated to the Siculo-Tunisian Strait that separate two water bodies circulating with different hydrological, physical and chemical characteristics. Oueslati et al. (1993) indicate that the hydrodynamics was higher in the northern than in the southern Tunisian coasts, which could be probably the cause of this increasing gradient. In fact, currents could homogenize population in the northern part and perform same diversity in the southern part.

Many studies have brought to light variation of marine species in this transition area between western and eastern Mediterranean basins (Zitari-Chatti et al., 2009, 2008; Gharbi et al., 20011; Faddhlaoui-Zid et al., 2012; Deli et al., 2015). The F_{ST} values support this disjunction between western and eastern of Tunisia, this estimator was highly significant divergence between the eastern (Sayada and Skhira) and the western (Tabarka) coasts. Our results indicate that population from Kelibia contains a mixture of individuals from both haplogroups; this was probably due to its transition position in the Siculo-Tunisian Strait and indicates that gene flow was maintained between the eastern and the western of Tunisian coastlines. Additional samples and other molecular markers are needed to test if the two haplogroups constitute tow divergent lineages and if they interbreed in the Siculo-Tunisian strait where they are in sympatry.

Populations from Tabarka, Kelibia, Sayada and Skhira were not in mutation/drift equilibrium suggesting that demographic expansion affected these populations or selective sweeps affected the studied marker. In marine populations, such expansions after a period of small effective population size were often related to episodes of marine level oscillations occurred in Pleistocene (Patarnello et al., 2007). Deviation from mutation/drift equilibrium might be due not only to the demographic history of a species but also to natural selection (Bazin et al., 2006). Demographic and selective scenarios are not mutually exclusive, and are difficult to disentangle when using a single mtDNA marker (Haney et al., 2010). Population expansion of *H. trunculus* should therefore be confirmed using other markers from the nuclear genome which are inherited independently from the mitochondrial ones.

A better knowledge on population genetics of this species is necessary in order to management of the increasing commercial exploitation of this species. Additional sampling will be needed to confirm the above finding and to contribute in conservation plans for its protection. Moreover, Research on the patterns of marine population structure in this area, using nuclear genes would reinforce this finding.

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REFERENCES

Bandelt, H.J., P. Forster and A. Rohl. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution. 16: 37−48.

Bazin, E., S. Glemin and N. Galtier. 2006. Population size does not influence mitochondrial genetic diversity in animals. Science. 312: 570–572.

Calvo, M., J. Templado, M. Oliverio and A. Machordom. 2009. Hidden Mediterranean biodiversity: molecular evidence for a cryptic species complex within the reef building vermetid gastropod *Dendropoma petraeum* (Mollusca: Caenogastropoda). Biological Journal of the Linnean Society. 96: 898−912

Chiavarini, S., P. Massanisso, P. Nicolai, C. Nobili and R. Morabito. 2003. Butyltins concentration levels and imposex occurrence in snails from the Sicilian coasts (Italy). Chemosphere. 50: 311–319.

Deli, T., K. Said and N. Chatti. 2015. Genetic Differentiation among Populations of the Green Crab *Carcinus aestuarii* (Nardo, 1847) (Brachyura, Carcinidae) from the Eastern and Western Mediterranean Coast of Tunisia. Acta Zoologica Bulgarica. 67 (3): 327-335.

Excoffier, L., H.E.L. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resource. 10: 564−567

Fadhlaoui-Zid, K., L. Knittweis, D. Aurelle, C. Nafkha, S. Ezzedine, F. Fiorentino, H. Ghmati, L. Ceriola, O. Jarboui and F. Maltagliati.2012. Genetic structure of *Octopus vulgaris* (Cephalopoda, Octopodidae) in the central Mediterranean Sea inferred from the mitochondrial COIII gene. Comptes Rendus Biologie. 335: 625-636.

Folmer, O., M. Balck, W. Hoeh, R. Lutz and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochromec oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology. 3: 294–299.

Gharbi, A., R. Zitari-Chatti, A. Van Wormhoudt, M.N. Dhraief, F. Denis, K. Said and N. Chatti. 2011. Allozyme Variation and Population Genetic Structure in the Carpet Shell Clam *Ruditapes decussatus* Across the Siculo-Tunisian Strait. Biochemical Genetics. 49(12), 788-805.

Haney, S., L. Bardwell and Q. Nie. 2010. Ultrasensitive responses and specificity in cell signaling. BMC Syst Biol. 4: 119.

Hauser, L. and G.R. Carvalho. 2008. Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. Fish Fish. 9: 333−362

Houart, R. 2001. Areview of the recent Mediterranean and Northeastern Atlantic species of Muricidae. Rome, Edition Evolver.

Librado, P. and J. Rozas. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 25 : 1451.

Oueslati, A. 1993. Les côtes de la Tunisie, Géomorphologie Environnement et Aptitudes à l'aménagement. Centre National Pédagogique, Tunis.

Patarnello, T., F. Volckaert and R. Castilho. 2007. Pillars of Hercules: is the Atlantic-Mediterranean transition a phylogeographical break? Molecular Ecology. 16: 4426–4444

Penant, G., D. Aurelle, J. Feral and A. Chenuil. 2013. Planktonic larvae do not ensure gene flow in the edible sea urchin *Paracentrotus lividus*. Marine Ecology Progress Series. 480: 155–170.

Quesada. H., C.M. Beynon and D.O.F. Skibinski. 1995. A mitochondrial DNA discontinuity in the mussel *Mytilus galloprovincialis* Lmk: Pleistocene vicariance biogeography and secondary intergradation. Molecular Biology and Evolution. 12: 521–52.

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

Thompson, J.D., D.G. Higgins and T.J. Gibson.1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research. 22: 4673-4680.

Walsh, P.S., D.A. Metzger and R. Higushi. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques. 10:506-513

Zitari-Chatti, R., N. Chatti, A. Elouaer and K. Said. 2008. Genetic variation and population structure of the caramote prawn *Penaeus kerathurus* (Forskäl) from the eastern and western Mediterranean coasts in Tunisia. Aquaculture Research. 39(1): 70–76.

Zitari-Chatti, R., N. Chatti, D. Fulgione, I. Caiazza, G. Aprea, A. Elouaer, K. Said and 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.