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# Mitochondrial DNA sequence variations in populations of *Sardina pilchardus* (Walbaum 1792) along the Tunisian coasts

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## Abstract

Sardine is a fish species of great economic importance to Tunisia. Knowledge of genetic diversity and population distribution is essential for efficient management and sustainability of any regional fisheries. This study is aimed to assess the genetic structure of the sardine and to specify the stocks of the European sardine (*Sardina pilchardus*). In all, 83 specimens were collected from three locations along the Tunisian coast and analyzed using mitochondrial DNA sequences. The results of sequence analysis determined the existence of variations in 40 single nucleotide sites within the 307 bp fragment of the cytb gene examined and defined twenty different haplotypes. Genetic diversity, estimated by haplotypic diversity, was high in all samples. Tunisian *S.pilchardus* samples show some level of genetic structuring. First, genetic differentiation between localities ($\Phi_{ST}$ estimates) was significant for all comparisons. Second, the analysis of molecular variance AMOVA indicates a high level of genetic variation ($\Phi_{ST}$ = 0.093; P<0.001). The structural patterns identified can be explained largely in relation to the regional oceanographic features. In conclusion, this study provided initial genetic data in making inferences of the genetic structure of *S. pilchardus* along the Tunisian coasts.

**Keywords:** *Sardina pilchardus*; Mediterranean Sea; Tunisian coasts; Population genetics; Genetic structure; Mitochondrial DNA.

## Introduction

Population genetic structure is determined by the level of connectivity or exchange between individuals and dispersal potential of the individuals (Nathan, 2001). It can be difficult to apply the concept of structural subdivisions in marine ecosystems, which is lack of obvious barriers (Waples, 1998). In fact, marine populations often consist of localized sub-populations that are relatively independent and have distinct ecological and genetic properties (Gaffney, 2000). In marine species, it is generally assumed that a high capacity for dispersal in the early life history stage results in reduced intra-specific differentiation over smaller spatial scales (Palumbi, 1995). In general, marine pelagic species have a high capacity for dispersal and as such, is expected to display less genetic structure. Nevertheless, gene-flow in marine species can be constrained by dispersal barriers, such as narrow water passages between land masses, sharp salinity gradients or different types of currents e.g., circular currents (eddies) or downward currents. The Mediterranean Sea is an area where there is evidence of breakpoints in gene flow which has been demonstrated widely in the Almeria-Oran Front (AOF) and the Siculo-Tunisian Strait (STS) (Bahri-Sfar *et al*., 2000; Patarnello *et al*., 2007). The latter one is a boundary area which divides the Mediterranean Sea into two basins (eastern and western) with different hydrological features. For some marine organisms, the Siculo-Tunisian Strait is considered as gene-flow barrier, regardless of their dispersal ability (Borsa *et al*., 1997; Patarnello *et al*., 2007), leading to genetic differentiation among populations. There have been numerous methods used to study the movement patterns and population structure of marine species, including genetic markers. Studies of fish population structure and genetic diversity are of interest in terms of management and sustainability of fish stocks.

One of the marine species not well studied in terms of genetic structure in Tunisian costal water, is the sardine, *Sardina pilchardus*, a small pelagic clupeoid fish, distributed in the Mediterranean Sea, the Sea of Marmara, the Black Sea and the coasts of the eastern North Atlantic Ocean (from the North Sea to Senegal) (Parrish *et al*., 1989). In Tunisia, the sardine is widespread, inhabiting bays and open sea areas from Tabarka to the Gulf of Gabes. In Tunisia commercial exploitation of small pelagic group, which sardine dominates, has been significant since the early 2000s and with the improvements in fishing technology, the fishing may be carried out further offshore. Consequently, sardine catches have increased

substantially (from 10000 tons in 1997 to 27000 tons in 2012; National Statistics).

As a direct consequence of its economic importance in Tunisia, the species has been studied mainly in terms of its biology, growth and morphometry (Khemiri & Gaamour, 2005; Khemiri, 2006). However, up to now, nothing is known about the genetic population structure of *Sardina pilchardus* species in Tunisian waters.

Until recently, few genetic studies have been performed on sardine species using different molecular markers such as allozymes (Spanakis *et al*., 1989; Chlaida *et al*. 2006., Laurent *et al*., 2007; Chlaida *et al*., 2009), mitochondrial DNA (Tinti *et al*., 2002; Atarhouch *et al*., 2006 ; Imsiridou *et al*., 2019) and microsatellites (Gonzalez & Zardoya, 2007). The results of these studies, though not completely congruent, suggest a very weak genetic structure.

The goal of the present study is to reveal genetic population structure of *Sardina pilchardus* species along the Tunisian coast, using mitochondrial DNA (mtDNA) marker. The fast evolution rate of mtDNA coupled with maternal inheritance have made mtDNA an extremely useful genetic system for studying gene flow, population structure and phylogeny (Saccone *et al*., 1999; Hebert *et al.*, 2004; Avise, 2006).

In addition, the data from present study will be compared with previously published, geographically targeted reference samples from different areas of the Mediterranean Sea (Table 1) in an attempt to compare the levels of genetic variability of *S. pilchardus* and and to define the species' genetic structure.

## Materials and Methods

### *Sampling*

A total of 83 individuals belonging to three different geographic locations were collected during scientific fishing surveys (OASIS 12) (Fig. 1, Table 2). Morphological identification of the specimens was performed according to fish identification keys of the World Register of Marine Species, WoRMS (https://readtiger.com/wkp/en/World_Register_of_Marine_Species#Contents) and the Integrated Taxonomic Information System (Whitehead, 1985). After morphological examination, muscle tissue samples were dissected from each specimen and stored at -20°C or preserved in absolute ethanol until molecular processing.

DNA Extraction, Amplification, and Sequencing

Total genomic DNA was extracted using a QIAGEN DNeasy© tissue kit following the manufacturer's recommendations. A partial 307 base pair (bp) of the cytb gene was amplified as follows. The PCRamplificationwascarriedout in a25 µl volume reactions and included 1 x PCR 1 buffer (Promega), 2.5 mM 2MgCl2, 1 mg/ml BSA, 0.5 µM forward and reverse primers, 0.2 mM dNTPs, 1 U of Taq DNA polymerase (Go Taq© DNA polymerase, Promega), and 25-50 ng of DNA. The thermal cycling conditions consisted of 2 min at 95øC followed by 35

**Table 1.** Reference populations.

| Sample | Abbreviation | N | References |
|---|---|---|---|
| Tunisiancoasts | | | |
| Gulf of Tunis | GT | 30 | Presen tstudy |
| Eastern area | EA | 31 | Presen tstudy |
| Gulf of Gabes | GG | 22 | Presen tstudy |
| AdriaticSea | | | |
| Gulf of Venice | GVE | 29 | Tinti *et al.,* 2002 |
| Otok Pag, Croatiancoasts | OPCr | 19 | Tinti *et al.*, 2002 |
| Rimini, Italiancoasts | RNIt | 20 | Tinti *et al.*, 2002 |
| Adriatic Middle 1 | AdM1 | 25 | Tinti *et al.,* 2002 |
| DugiOtok, Croatiancoasts | DOCr | 22 | Tinti *et al.,* 2002 |
| Adriatic Middle 2 | AdM2 | 24 | Tinti *et al.,* 2002 |
| Adriatic Middle 3 | AdM3 | 21 | Tinti *et al.,* 2002 |
| Pescara, Italiancoasts | PEIt | 28 | Tinti *et al.*, 2002 |
| Lesina, Italiancoasts | LEIt | 28 | Tinti *et al.,* 2002 |
| Barletta, Italiancoasts | BAIt | 22 | Tinti *et al.*, 2002 |
| IonianSea | | | Tinti *et al.,* 2002 |
| Siracausa | SRIon | 19 | Tinti *et al.*, 2002 |
| Spanish | | | |
| Barcelona | BAR | 5 | Grant *et al.,* 1998 |

N: number of individuals.

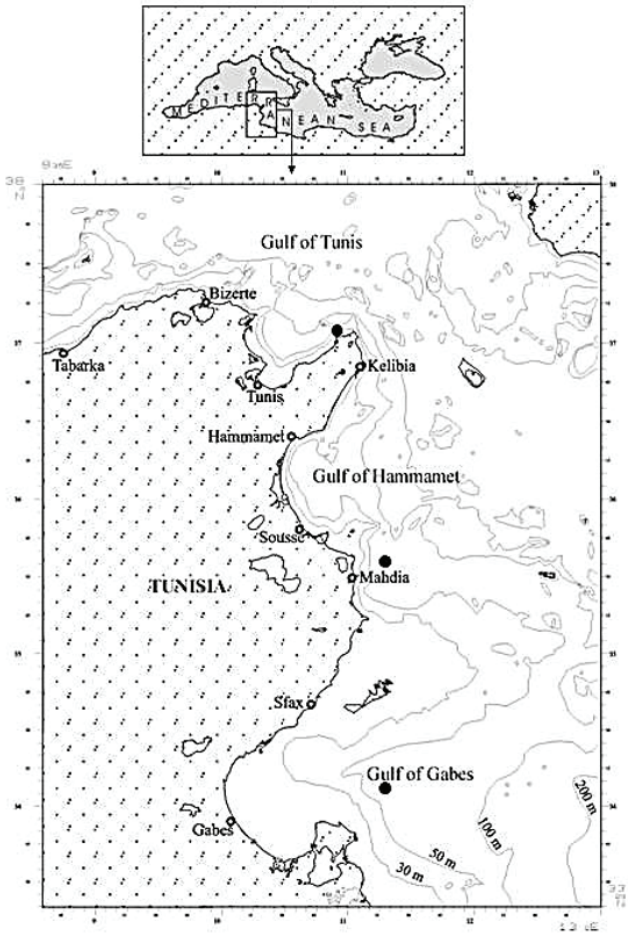


***Fig. 1:*** *Sardina pilchardus* sampling locations in the coastal zones of Tunisia. Solid circles represent the sampling sites.

cycles of 30 s at 95°C, 30 s at 50°C and 1 min at 72°C and a final 10 min extension at 72°C. Primers L14841 and H15149 (Kocher *et al*., 989) were used in the PCR and sequencing reactions. PCR products were purified using QIAquick PCR Purification Kit (Qiagen) following the manufacturer's instructions. Both strands of a sequence were aligned with CLUSTAL W algorithm (Thompson *et al*., 1994) as implemented in the software BioEdit v. 7.0.5.3 (Hall, 1999) and confirmed by eye.

### *Statistical analysis*

Estimates of genetic variation were obtained in the form of haplotype diversity h (Nei, 1987), nucleotide diversity π (Nei, 1987), and mean number of nucleotide differences among all haplotypes in a putative population with the software ARLEQUIN 3.5 (Excoffier & Lischer. 2010).

Pairwise genetic distances between samples were estimated with the ARLEQUIN 3.5 package. The significance of pairwise population comparisons were tested with 10, 000 permutations and P-values were adjusted with the sequential Bonferroni correction (Rice, 1989). The Neighbour-Joining Tree was employed to illustrate pairwise values between populations using MEGA 5 (Tamura *et al*., 2011) based on Kimura two parameter distances (Kimura, 1980).

An analysis of molecular variance AMOVA (Excoffier *et al*., 1992) was performed in order to assess the geographical patterns of differentiation. First, the AMOVA was used to look at differentiation across all populations. Second, a hierarchical AMOVA was used to partition genetic variation 1) between regions (Tunisian coasts/ Ionian Sea/ Adriatic Sea/ Western Mediterranean Sea), 2) among sites within regions, and 3) within populations. 10,000 permutations were run to test for statistically significant fixation indices in ARLEQUIN 3.5 (Excoffier & Lischer, 2010).

Median networks for cytb haplotypes detected in our dataset and in Adriatic, Ionian and Western Mediterranean Sea (Barcelona) were generated with the median joining algorithm (Bandelt *et al*., 1999) using the NETWORK 4.5.1.6 program (http://www.fluxus-engineering.com).

## Results

Out of total of 349 bp of Cytochrome b mitochondrial geneamplified, 307 bp fragment were analyzed to determine genetic variation. A total of 20 distinct haplotypes were identified among 83 analyzed specimens (Table 3). Sequences of the haplotypes were deposited in GenBank (accession numbers JQ237098- JQ237114; JQ585750-JQ585753; J Q621900).

Haplotype diversity (h) within populations was high, ranging from 0.798 in Gulf of Tunis (GT) to 0.870 in Gulf of Gabes (GG). Nucleotide diversity (π) was low, ranging from 0.00484 in Gulf of Tunis to 0.01692 in Gulf of Gabes (Table 2).

The estimated genealogical relationship among the 20 haplotypes is shown in the median-joining network (Fig. 2). Only three haplotypes occurred in more than one location; all others were restricted to a single population. The most common haplotype in the network (Hap TM; Fig.2) comprised 34% of the total sample and had the highest frequency of occurrence in the three populations and it is the only haplotype shared among the three populations. Haplotype TN was shared between the Gulf of Tunis and the Eastern populations whereas haplotype TR was shared between two Gulf populations (GT and GG). The Median joining network including haplotypes from Western Mediterranean (Barcelona; Grant *et al*., 1998) and Adriatic and Ionian Sea (Tinti *et al*., 2002) has a star-like configuration with a very common central haplotype that was spread across all locations except for the area of Barcelona (Suppl. Fig.1). Many additional haplotypes were directly connected to the central one by one to three mutations. Four haplotypes were shared between Tunisian coast and Adriatic samples, whereas only one haplotype was shared between Ionian and Adriatic. Additionally, haplotypes identified in Barcelona region were unique to their geographical location.

An analysis of the molecular variance (AMOVA) was performed with the sample data set described in this

**Table 2.** Measures of haplotypes and nucleotide diversity within populations of *Sardina Pilchardus* analyzed by location.

| Samples | Acronym | Geographic location | Depth | N | K | h | π | MPD |
|---|---|---|---|---|---|---|---|---|
| Gulf of Tunis | GT | 37° 05N, 10° 50E | 67 | 30 | 7 | 0.798 ± 0.055 | 0.00484 ± 0.003 | 1.485 ± 0.922 |
| Eastern area | EA | 35° 37N, 11° 18E | 77 | 31 | 8 | 0.860 ± 0.034 | 0.00790 ± 0.004 | 2.425 ± 1.351 |
| Gulf of Gabes | GG | 34° 08N, 11° 17E | 58 | 22 | 9 | 0.870 ± 0.050 | 0.01692 ± 0.009 | 5.194 ± 2.613 |
| All data set | ALL | | | 83 | 20 | 0.865 ± 0.030 | 0.009645±0.005 | 2.960 ± 1.5651 |

N: Number of samples, K: Number of haplotypes, h: haplotype Diversity, π: nucleotide diversity, MPD: Mean number of pairwise differences.

**Table 3.** Segregating sites (40 bp) in the 307-bp segment of the cytochrome b (cytb) gene defining 20 different haplotypes and their distributions across 3 populations of *Sardina pilchardus*.

| Haplotype | Nucleotide positions | Locality[a] | | | |
|---|---|---|---|---|---|
| | [0000000000000011111111111111222222222333] | GG | GT | EA | |
| | [3566778888889123445556688891234557899000] | | | | |
| | [2569680123596517780470312406058496125012] | | | | |
| | | | | | All |
| Hap TM | GCAATAATCGAGCTCCGTTCATATTTCACGAGCTATTTCT | 7 | 12 | 9 | 28 |
| Hap TI | . . . . . G . . . . . | 1 | - | - | 1 |
| Hap TJ | . . . . . T . . . . . C . . . . . C | 2 | - | - | 2 |
| Hap TK | . . . . G . . . . . C G T . . . . . G . . . . . C . . . . | 2 | - | - | 2 |
| Hap TL | . . . . . T . C . . . . T . C . . . G . C A . A C . . . . . | 3 | - | - | 3 |
| Hap TO | . . . . . C . . . . . G . . . . T . . . . C . . | 1 | - | - | 1 |
| Hap TR | . . . . . T . | 2 | 3 | - | 5 |
| Hap TP | . . C . . . . . T C . . C . . C . . . . . A . . . . . | 1 | - | - | 1 |
| Hap TQ | . . . . . T . . . . C . . . . . | 3 | - | - | 3 |
| Hap TA | . . . . . T . . . . . | - | 4 | - | 4 |
| Hap TB | . . . . . A . . . . . G . . . . . | - | 2 | - | 2 |
| Hap TN | . . . . . C . . . . . | - | 5 | 3 | 8 |
| Hap TC | . T . C . . . . G . . . . . | - | 2 | - | 2 |
| Hap TS | . . . . . G . . . . . | - | 2 | - | 2 |
| Hap TD | A . . . . . | - | - | 1 | 1 |
| Hap TE | . . . . . C . . . . . A . . . . . | - | - | 5 | 5 |
| Hap TF | . . . . . A . . . . . | - | - | 4 | 4 |
| Hap TG | . . . . . C C . . . . . C . . . . . C . . . . . C . . . | - | - | 4 | 4 |
| Hap TH | . . . . . C . . . . . C . . . . . | - | - | 2 | 2 |
| Hap TT | . . . . . T . . . . . | - | - | 3 | 3 |
| Total | | 22 | 30 | 31 | 83 |

[a]Locality information: GG, Gulf of Gabes, GT, Gulf of Tunis, EA, Eastern area.

study and those from Adriatic Sea, Ionian Sea and Western Mediterranean Sea (Table 1) to test for differentiation between geographical populations within species.

When all samples were considered as a single group, most of the molecular variance was present within samples (90.69%) and 9.31% of genetic heterogeneity was apportioned among samples. The Φ-statistics value associated to the remaining part of variance was significant ($\Phi_{ST}$ = 0.093, P < 0.001) (Table 4) indicating genetic differentiation among samples. Genetic differentiation was also detected when considering only Tunisian collections ($\Phi_{ST}$ = 0.073, P < 0.001). AMOVA applied to sequences pooled by geographical regions (Tunisian Coast vs Ionian Sea vs Adriatic Sea vs Western Mediterranean Sea) revealed significant genetic differentiation among all populations studied (7.20 % variation among regions, $\Phi_{CT}$ = 0.072, p < 0.001). When focusing on the Eastern Mediterranean region (Tunisian Coast vs Ionian Sea and Adriatic Sea) significant population genetic structure was detected (8.70 % variation among regions, $\Phi_{CT}$ = 0.087, p < 0.05).

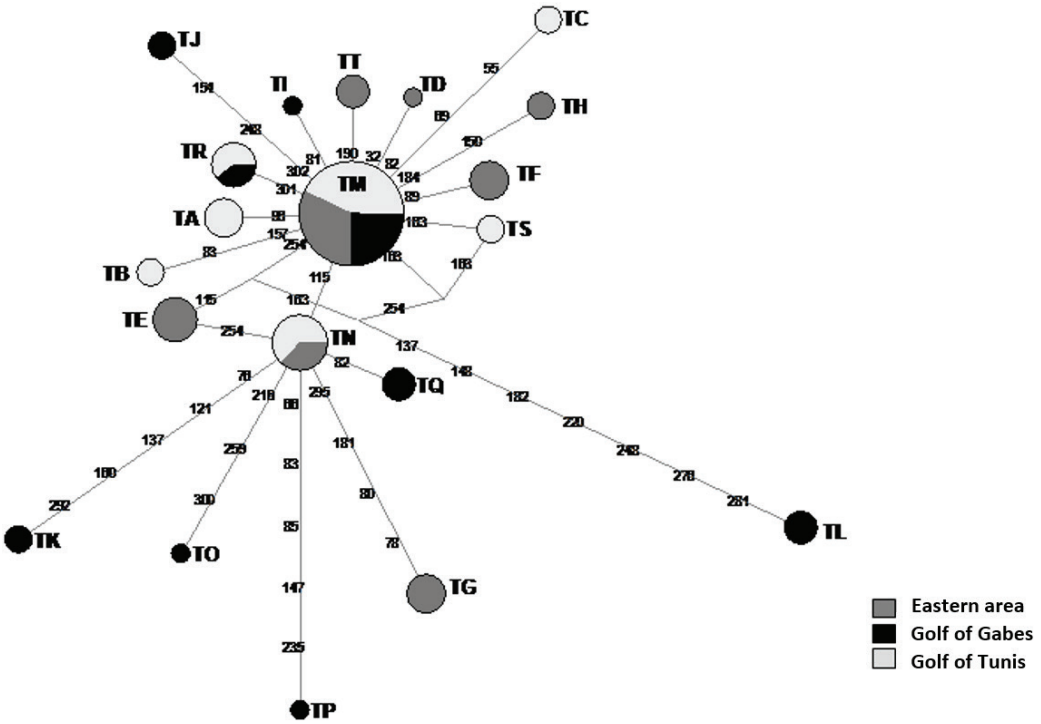


***Fig. 2:*** Mitochondrial DNA haplotype network. Each pie chart represents a haplotype partitioned into frequency of occurrence in the three samples for *Sardina pilchardus.* The size of each haplotype corresponds to the frequency of the haplotype within the entire sample.

**Table 4.** Measures of geographical population differentiation in *Sardina pilchardus* based on an analysis of molecular Variance approach with cytochrome b data.

| Source of variation | Source of variation | df | Variance components | Variation (%) | Fixation indices | P |
|---|---|---|---|---|---|---|
| Unstructured | Among sampling localities | 13 | 0.44654 | 9.31 | ΦST =0.093 | <0.001 |
| | Within sampling localities | 376 | 4.34922 | 90.69 | | |
| Four regions TC vs IO vs AD vs WM | Among regions | 3 | 0.35915 | 7.20 | ΦCT = 0.072 | <0.001 |
| | Among sampling localities within regions | 11 | 0.32079 | 6.43 | ΦSC = 0.069 | <0.001 |
| | Within sampling localities | 380 | 4.30555 | 86.36 | ΦST = 0.136 | <0.001 |
| Two regions TCvs IO and AD | Among regions | 1 | 0.44417 | 8.70 | ΦCT =0.087 | <0.05 |
| | Among sampling localities within regions | 12 | 0.28687 | 5.65 | ΦSC = 0.061 | <0.01 |
| | Within sampling localities | 376 | 4.34922 | 85.65 | ΦST = 0.143 | <0.001 |

TC: Tunisian coasts ; IO: Ionian sea ; AD: Adriatic ; WM : Western Mediterranean (Bar).

Pairwise estimates of $\Phi_{ST}$ calculated between pairs of populations are presented in Table 4. All pairwise $\Phi_{ST}$ tests between pairs of Tunisian collections were significant. Among these comparisons, the largest $\Phi_{ST}$ values occurred between Gulf of Tunis and Eastern area($\Phi_{ST}$ = 0.07823, P < 0.05after Bonferroni adjustment) and the lowest value is between Gulf of Gabes and Eastern area (ΦST = 0.07249, P < 0.05) (Table 5).These results perfectly match with the previous differentiation of these populations in the northern and southern part of Tunisia, and with the differentiation among eastern and western populations, caused by the Siculo-Tunisian Strait geneflow barrier.

Significant differences also appeared between the Tunisian collections (Gulf of Gabes and Eastern area) and all the Adriatic samples, except respectively for OPCr and RNIt samples, where $\Phi_{ST}$ becomes non-significant after Bonferroni correction. In contrast, the sample from Gulf of Tunis was not significantly different after Bonferroni adjustment from Adriatic and Ionian samples, with the following exceptions: PEIt and LEIt (Table 4). Additionally, only Tunisian sample from Eastern area show significant value of $\Phi_{ST}$ ($\Phi_{ST}$ = 0.12, P < 0.05 after Bonferroni adjustment) with Ionian sample (Table 4). Pair-

**Table 5.** Pairwise comparisons of genetic structure measured with $\Phi_{ST}$ among samples of *Sardina pilchardus* from Tunisian coastal waters, Barcelona, Ionian and Adriatic Sea.

| | GVE | OPCr | RNIt | AdM1 | DOCr | AdM2 | AdM3 | PEIt | LEIt | BAIt | SRIon | GG | GT | EA | BAR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GVE | 0.00000 | | | | | | | | | | | | | | |
| OPCr | -0.01538 | 0.00000 | | | | | | | | | | | | | |
| RNIt | 0.01610 | -0.01179 | 0.00000 | | | | | | | | | | | | |
| AdM1 | 0.00969 | 0.01203 | -0.00601 | 0.00000 | | | | | | | | | | | |
| DOCr | -0.02206 | -0.04156 | 0.00245 | 0.00469 | 0.00000 | | | | | | | | | | |
| AdM2 | -0.01664 | -0.00575 | 0.02270 | -0.02081 | -0.01456 | 0.00000 | | | | | | | | | |
| AdM3 | 0.00023 | 0.03728 | 0.03511 | 0.02778 | 0.03870 | 0.02698 | 0.00000 | | | | | | | | |
| PEIt | 0.01635 | 0.05422 | 0.04797 | 0.00462 | 0.04656 | 0.00656 | 0.07074 | 0.00000 | | | | | | | |
| LEIt | 0.01585 | -0.03540 | -0.02498 | 0.01203 | -0.01654 | 0.01873 | 0.04438 | 0.05291 | 0.00000 | | | | | | |
| BAIt | 0.00608 | 0.03747 | 0.03228 | -0.00525 | 0.03175 | -0.00372 | 0.05323 | 0.00000 | 0.03922 | 0.00000 | | | | | |
| SRIon | -0.02916 | -0.03564 | 0.03529 | 0.03338 | -0.04094 | -0.01541 | 0.05326 | 0.08688 | 0.00455 | 0.06690 | 0.00000 | | | | |
| GG | **0.10594***** | 0.07580** | **0.08205*** | **0.11318**** | **0.08956**** | **0.10544**** | **0.09649**** | **0.12930***** | **0.10608***** | **0.10714***** | 0.08003** | 0.00000 | | | |
| GT | 0.04393* | 0.04085* | 0.02178 | 0.05108* | 0.04723* | 0.05439** | 0.03414 | **0.06750**** | **0.05243*** | 0.05309** | 0.04855* | **0.07497**** | 0.00000 | | |
| EA | **0.13273**** | **0.11810*** | 0.10248** | **0.13532**** | **0.12952*** | **0.13200**** | **0.12880*** | **0.15514***** | **0.14204**** | **0.13484*** | **0.12218*** | **0.07249*** | **0.07823*** | 0.00000 | |
| BAR | **0.92640***** | **0.89841***** | **0.90211***** | **0.94906***** | **0.92673***** | **0.94733***** | **0.94336***** | **0.98600***** | **0.88601***** | **0.98268***** | **0.93896**** | **0.44334***** | **0.72477***** | **0.62615***** | 0.00000 |

*** P <0.001 ; ** P <0.01 ; * P <0.05.

wise $\Phi_{ST}$ estimates showed that sample from Barcelona was significantly different from all samples.

The NJ relationships showed two clusters. The first cluster contained all Tunisian, Ionian and Adriatic collections and the second contained only the Barcelona sample (Fig. 3).

The first cluster was subsequently divided into three sub-groups. The Adriatic and Ionian collections formed one group; the Gulf of Tunis sample formed the second group and Eastern area and Gulf of Gabes samples formed the third group (Fig. 3). Hence, Tunisian collections from Gulf of Gabes and Eastern area appeared more genetically similar.

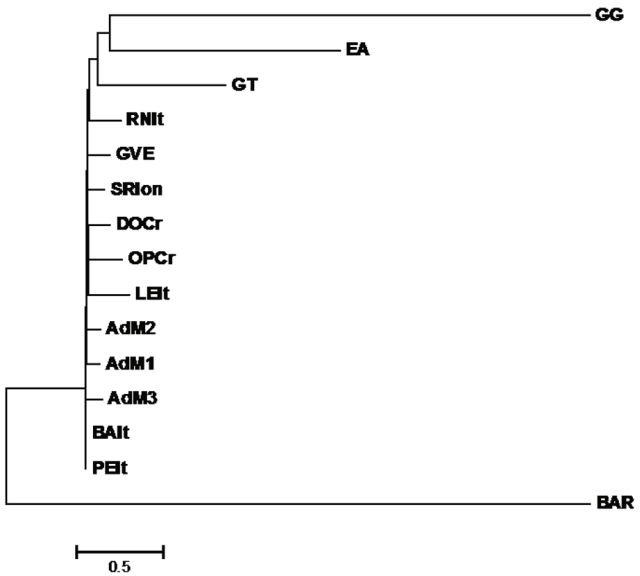


***Fig. 3:*** Neighbor-joining dendogram based on $\Phi_{ST}$ genetic distances among fifteen collections of *Sardina pilchardus.*

## Discussion

The current study provides the first examination of the population genetics of a sardine species in Tunisian coastal waters. The detection of 20 different haplotypes in only 83 individuals of three *S. pilchardus* samples underlines the usefulness of the cytochrome b gene as molecular markers for investigating the geographic structure of this species. High genetic diversity was obtained within each sample and for the overall sample (h= 0.865; Table 2). The AMOVA testing for overall population genetic structuring among Tunisian populations was highly significant ($\Phi_{ST}$ = 0.073, P<0.001). Moreover, we detected significant population genetic differentiation among sample pairs, which is consistent with the results of previous research of age and growth of the *Sardinapilchardus* caught in the northern and southern areas along the Tunisian coast (Gaamour & Khemir, 2010). However, a different growth pattern was found between the sardines from the north that have on average a statistically greater size at the same age than those from the south area. The authors concluded that there were two differentiated groups of sardines (Gaamour & Khemiri, 2010). This outcome is also consistent with the results of the NJ dendrogram; the Tunisian collections from Gulf of Gabes and Eastern area appeared more genetically similar. On the other hand, the population from the Gulf of Tunis forms a distinct group. Furthermore, this clustering agrees also with the genetic differentiation among eastern and western populations, caused by the Siculo-Tunisian Strait gene-flow barrier. Significant genetic divergence between western and eastern Mediterranean basins has been reported for many marine species (reviewed in Patarnello *et al.*, 2007) and interpreted as the restriction of gene flow related to the oceanographic transition zone at the Siculo-Tunisian Strait.

Our data on cytb sequence variation clearly indicate that population sub- structuring exists along the Tunisian coast.

This pattern of genetic structure displayed by *S. pilchardus* along the Tunisian coast seems to be largely due to the oceanographic conditions. In fact, the north area is under the influence of the Atlantic inflowing current (Brandhorst, 1977; Sammari & Gana, 1995). This current is characterized by a warm temperature and high salinity. Unlike the northern coast of Tunisia, the Gulf of Gabes has hydrodynamic characteristics more typical of the Central Mediterranean (Ben Othman, 1973). The many channels around the Kerkennah Islands, the largest tidal variations in the Mediterranean and the seasonal variability of cyclonic circulation from the Atlantic water which flows through the Strait of Sicily further define local oceanographic features.

Overall genetic structuring among samples was also detected when considering in addition data from previously published studies ($\Phi_{ST}$ = 0.093; P<0.001). With respect to the geographic regions, genetic differentiation was revealed between the Tunisian Coast vs Ionian and Adriatic Sea ($\Phi_{CT}$ = 0.087; P< 0.05).

The core of the network shows a star-like structure where the most frequent haplotype is made up of individuals from all regions, except for the Barcelona area probably due to the small sample size (five individuals) (Suppl. Fig. 1). This shared haplotype could represent a founder haplotype. Although results of this study have increased our understanding of the geographic genetic relationships among *S. pilchardus* populations, several pertinent questions remain unanswered such as the limited size of some sampled populations. However, investigations using other types of markers such as microsatellites should be conducted to provide a clear understanding of the genetic distribution and population dynamics and to estimate the effective sizes of *Sardina pilchardus* populations. In addition, sampling in areas between the studied sites should be conducted to further test migration and population structuring, as well as sampling throughout the range of this species to identify any discontinuities in gene flow.

## Conclusions

In this study, mitochondrial cytochrome b analysis revealed a high-level of genetic diversity in all Tunisian

*Sardinapilchardus* samples. AMOVA suggested significant population genetic structure between all regions considered and specifically in the eastern Mediterranean region (Tunisian Coast vs Ionian Sea and Adriatic) and would be suitable as genetic stocks for conservation programs.

## Conflict of interest

The authors confirm that there is no conflict of interest for the information presented in this manuscript.

## *Supplementary*

***Suppl. Fig. 1:*** Median-joining network of haplotypes detected for the cytb sequences at the sampling locations of the Tunisian coasts, Adriatic Sea, Ionian Sea and Western Mediterranean. The area of each circle is proportional to the number of individuals exhibiting that haplotype. Each line in the network represents one mutational step.


## Acknowledgements

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