ISSN 2347-6893

# Mitochondrial DNA variability of Octopus vulgaris (Mollusca, Cephalopoda) in the Tunisian Waters

Basma NAFKHA[1], Soufia EZZEDDINE[1]& Karima FADHLAOUI-ZID[1]
[1] Institut National des Sciences et Technologies de la Mer (INSTM)
2025, Rue du 2 mars 1934, Carthage Salammbô, Tunisia
basmanafkha@yahoo.fr
Soufia.ezzeddine@instm.rnrt.tn
karimafadhlaoui@yahoo.fr

## ABSTRACT

This study provides data on the genetic structuring of *Octopus vulgaris* in the Tunisian waters. A total of 121 specimens were collected in four locations (one in the western Mediterranean and three in the Central Mediterranean). A portion of the cytochrome b gene (485pb) of the mitochondrial DNA was analysed. The most relevant result was the high genetic structuring among populations in the study area (ΦST = 0.06851, p <0.0001). Median-joining network analysis did not separate any haplotype group, and haplotype distribution did not mirror the geographic origin of the samples. Populations pairwise ΦST and MDS analyses clearly showed that the populations from the coast are differentiated from the deep sea populations. For purposes of stock management, these two groups should be considered as separate stocks.

## Indexing terms/Keywords

*Octopus vulgaris*; Tunisia; Mitochondrial DNA; Cytochrome b gene; Genetic structure.

## Academic Discipline And Sub-Disciplines

Biology

## SUBJECT CLASSIFICATION

Genetics

## TYPE (METHOD/APPROACH)

Molecular Genetic Analysis of populations

Council for Innovative Research
Peer Review Research Publishing System
Journal: JOURNAL OF ADVANCES IN BIOLOGY
Vol 5, No.1
editor@cirjab.com
www.cirjab.com, www.cirworld.com

ISSN 2347-6893

## 1.INTRODUCTION

The common octopus (*Octopus vulgaris*) is the most important and valuable commercial fishery species along the Tunisian coasts especially the south East coasts including the Gulf of Gabes. Indeed, the biotic and abiotic factors of Gulf of Gabes distinguish it from the rest of Tunisian fisheries [1]. Due to the important contribution to regional fishery landings as well as its ecological importance, *Octopus vulgaris* is the most studied cephalopod in the Mediterranean (*e.g.* [2, 3, 4, 5, 6, 7-8]). However, the intense fishing activity of *Octopus vulgaris* which is carried out by trawling and by various small-scale gears, such as traps, pots, fykenets, and setnets [9,10], required knowledge in order to better manage the exploited stock level. The first results of octopus population assessment from the whole southern Tunisian area showed a fully exploited stock despite the fishing regulations in 1994 by the Tunisian legislation [2]. These results remained approximate, since there is little information available on genetic variation between populations of this specie. Knowledge of genetic diversity and population structure is a prerequisite for the successful management of germplasm conservation programmes. In fact, these analyzes of genetic population structure can provide clear and basic information on the geographical limits of stocks and the existence or otherwise of gene flows between populations [11]. Molecular markers have proved to be an exceptional indicator of genetic variation within and between populations of many fishery animals, including invertebrates [12].

Thus, in order to ensure a better management and hence sustainable fisheries of this marine resource, we used a genetic approach to verify whether the local Tunisian stock of common octopus belongs to one or several genetic stocks. This latter has been applied successfully to the stock discrimination in fisheries [13-14]. Early genetic studies have mainly focused on phylogenetic analyses [15,16,17,18,19,20-21].

Concerning the genetic structure and variability of *octopus vulgaris* stocks, different molecular markers have been analysed: allozymes [9], microsatellite [22-10] and mitochondrial DNA [23]. All these studies point to significant genetic structuring in the *O. vulgaris* in the study areas (Mediterranean and Atlantic ocean). The allozymes and the microsatellites markers used by Maltagliati et al. [9] and Casu et al. [22], were consistent in showing that within the Mediterranean, O. vulgaris is not constituted by a single panmictic unit. These studies found that this species presents a genetic break between the Western and Eastern Mediterranean basins. Cabranes et al. [10] have identified significant population structuring among five samples from the Atlantic sites and one from the Mediterranean Sea by using five microsatellite loci. Recently Fadhlaoui-Zid et al. [23] showed a significant break between eastern and western Mediterranean basins which corroborating the findings by Maltagliati et al. [9] and Casu et al. [22].
In the present study, we tested the hypothesis of the genetic structuring of *O. vulgaris* along the Tunisian coasts by analysing the mitochondrial cytochrome b gene (Cyt b). The goals of this study were : (1) to improve the knowledge of this species, (2) to contribute to a better understanding of its genetic population structure and (3) to provide suggestions for the management of this important biological resource.

## 2. MATERIALS AND METHODS

### 2.1 Sampling and DNA extraction

A total of 121 common octopus specimens of different sizes, were collected by trawling and coastal fishing at four different localities of Tunisian water : North (NOR), Deep water of Kerkennah Islands (DWK), Coastal Kerkennah Islands (COK), and Zarzis (ZAR) (Table 1 ; Figure 1).

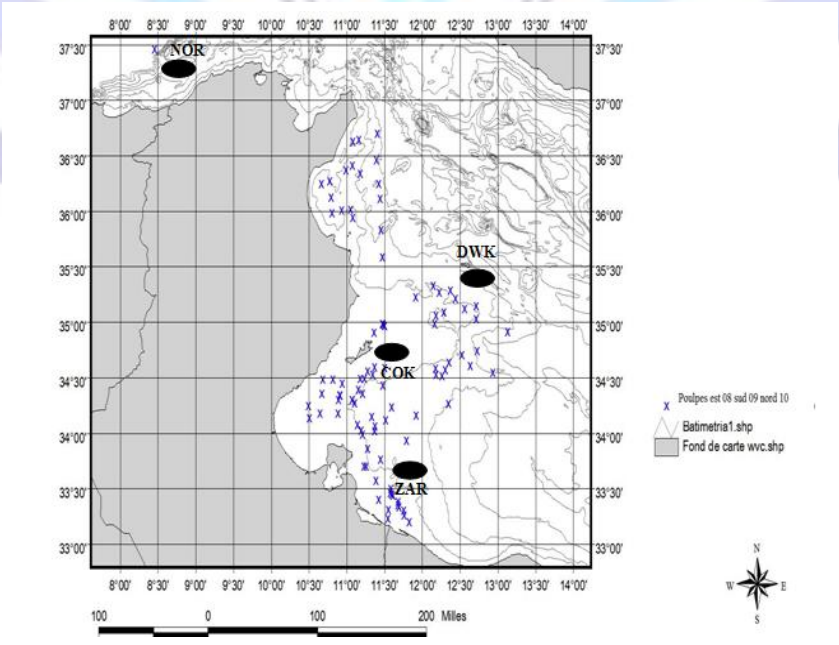


**Figure 1. Location of sampling sites. Details of sampling localities are provided in Table 1**

ISSN 2347-6893

From each animal, a small piece of muscular tissue from the tip of the arm or from the mantle was excised using a sterilised blad bistouri and subsequently preserved in absolute ethanol.

**Table 1. Samples of *Octopus Vulgaris* in this study**

| Collecting location | Code | Date | Coordinates (Lat, Lon) |
|---|---|---|---|
| North | NOR | 04/2010 | 42°23'N, 08°56'W |
| Deep-water of Kerkennah Islands | DWK | 05/2009 | 35°00'N, 12°20'E |
| Coastal Kerkennah Islands | COK | 05/2009 | 34°36'N, 11°17'E |
| Zarzis | ZAR | 05/2008 | 33°21'N, 11°37' |

Genomic DNA was extracted from 25 mg of muscle tissues using the QIAGEN DNeasy Tissue kit following the manufacturer's recommendations. The quality and the purity of DNA extracted were determined using a nanodrop spectrophotometer.

## 2.2 Mitochondrial DNA analysis

A segment of 485 bp of the mitochondrial DNA (mtDNA) cytochrome b (ctyb) gene was amplified using a sense primer (F 5´-TAT GTA CTA CCA TGA GGA CAA ATA TC-3′) and anti-sense primer (R 5´-ATT ACA CCT CCT AAT TTA TTA GGA AT-3′) [24]. Each mixture contained 5–10 ng DNA template, 5 µL 10X reaction buffer, 20 pmol forward and reverse primer, 10 mM dNTPs, 75 mM MgCl2, 5 units of Taq polymerase enzyme and 0.5 µg BSA; sterile distilled water was added to 50 µL. Thermal-cycle profiles consisted of an initial denaturation at 94°C for 5 min followed by 35 cycles of 94°C for 1 min50° C for 1,5 min and 72°C for 1 min), with a post-cycle extension at 72°C for 5 min.

The PCR products were purified with a QIAquick PCR purification Kit (Qiagen), following manufacturer's instructions and were sequenced with an ABI Prism 3130 genetic analyser (Applied biosystems) using the forward and reverse primers noted above.

## 2.3 Data analysis

The mtDNA forward and reverse sequences were aligned using the programme CLUSTAL W [25], implemented in the BioEdit 7.0.5.2 software package [26] and then verified by eye. The genetic variation was assessed estimating the number of polymorphic sites (S), the number of haplotypes (K), haplotype diversity (h), nucleotide diversity (π), and the mean number of pairwise differences (MPD) using the software package DnaSP 5.10 [27] and Arlequin 3.5 [28].

Genetic relationships among haplotypes were investigated by a median-joining network [29] using the software package Network 4.5.0.1 (www.fluxus-engineering.com).

Genetic differentiation among populations was quantified by computing pairwise Φst estimates calculated using conventional F-statistics based on mtDNA haplotype frequencies and the pairwise difference distance method. Significance of pairwise population comparisons was assessed by 1,000 permutations. All analyses were conducted using Arlequin 3.5 [28].

An analysis of molecular variance (AMOVA; [30]) was performed in order to assess the geographical patterns of differentiation. First, the AMOVA was used to investigate genetic differentiation across all populations. Second, a hierarchical AMOVA was used to partition genetic variation 1) between regions (Western Mediterranean /Central Mediterranean) and (Coastal Central Mediterranean/Oceanic Central Mediterranean, 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 [28].

In order to establish the genetic relationships between Tunisian *Octopus vulgaris* samples, the Φst genetic distance matrix between populations was used to plot multidimensional scaling (MDS) [31] using the STATISTICA 8.0 package.

# 3. RESULTS

## 3.1 Sequence diversity

The analysis of mitochondrial DNA cytochrome b of common octopus *Octopus vulgaris* produced 121 sequences of 485 pb each, which characterise by 16 parsimony informative sites. A total of 31 distinct haplotypes were recovered and their sequences were submitted to GenBank (accession number JX 512814 to JX 512844). The haplotype diversity of samples (Table 2) was generally moderate. The highest value was recorded for the sample of deep water kerkennah islands (h= 0.736), while the lowest value was observed in the zarzis site (h=0.66). However, the samples from Zarzis (Za) show the

most important values of nucleotide diversity (π =0, 00339 ± 0, 00572) and mean number of pairwise nucleotide differences (MPD= 1.64 ± 0.99), indicating that this later contains the most divergent haplotypes.

**Table 2. Sample sizes and genetic diversity estimates obtained for the mitochondrial cyt b of the four *Octopus vulagaris* Tunisian *samples* (N: number of samples; K: number of haplotypes; h: haplotype diversity; p: nucleotide diversity; MPD: Mean number of pairwise differences)**

| Locality | N | K | h | π | MPD |
|---|---|---|---|---|---|
| North | 22 | 8 | 0,697± 0,102 | 0,00277± 0,00452 | 1.341991 ± 0.864742 |
| Deep-water of Kerkennah Islands | 42 | 13 | 0,736± 0,069 | 0,00329± 0,00479 | 1.595819 ± 0.966587 |
| Coastal Kerkennah Islands | 27 | 8 | 0,698± 0,070 | 0,00226± 0,00374 | 1.094017 ± 0.740611 |
| Zarzis | 30 | 8 | 0,660± 0,088 | 0,00339± 0,00572 | 1.645977 ± 0.997507 |
| Total | 121 | 31 | 0,713± 0,044 | 0,00316± 0,00883 | 1.530301 ± 0.841246 |

Generally, the Cytochrome b sequences of *Octopus vulgaris* examined were characterised by high haplotype diversity and relatively low nucleotide diversity, which may correspond to a fast growth of the population and an accumulation of mutations from a population with low individual effectif [32, 33].

The Median-joining network of 31 haplotypes revealed a star-shaped phylogeny with many haplotypes originating from the most abundant haplotype (Figure 2). Most haplotypes were separated by a single mutation, with no clear assortment of haplotypes based on geographic locality. Three haplotypes occurred in more than one sampling site (H_1,H_14 and H_3). The dominant haplotype 1 (H_1) accounted for 52% of all O*. vulgaris* specimens and appears in all putative populations. The second most common haplotype (H_14) was found in 14 sequences representing three geographic regions (NOR, COK, ZAR). The next shared haplotype (H_3) was observed in a smaller proportion (6%) in only North and Deep-Water Kerkennah islands samples. Six haplotypes (H9, H10, H11, H23, H24 and H28) occur in more than one individual. The remaining haplotypes (Twenty two) were represented by only one individual. These unique haplotype variants were present in all sampled localities. In fact, the unique haplotypes (H15, H16, H17, H18, H19 and H20) were observed only in the coastal sample of Kerkennah islands, whereas (H27, H29, H30 and H31) were only found in the North sample. The haplotypes (H21, H22 ,H25 and H26) were detected only in the Zarzis sample. Finally, the deep-water sample of Kerkennah Islands showed the following haplotypes (H2, H4, H5, H6, H7,H8, H12 and H13).

ISSN 2347-6893

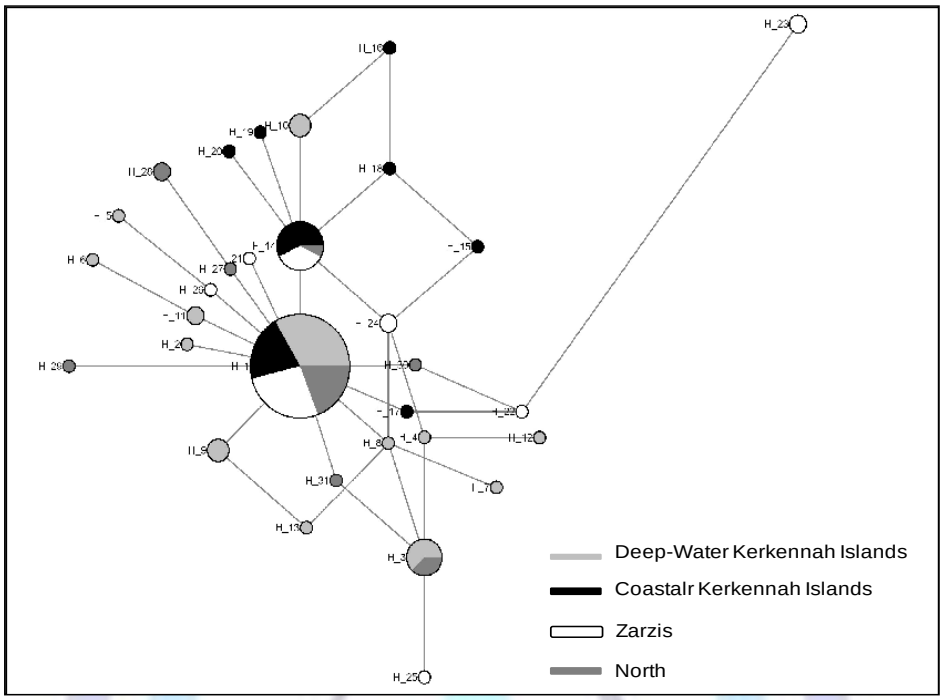


**Figure 2. Median-joining network of haplotypes for the 4 Tunisian localities of *Octopus vulgaris*. Size of circles is proportional to the frequency of each haplotype**

## 3.2 Genetic structure

The overall estimate of ΦST among all *octopus vulagaris* populations showed a significant value (ΦST = 0.06851, p <0.0001) indicating a relevant population differentiation. The exact test of population differentiation corroborated this result (p<0.001). In order to highlight possible population subdivision among Tunisian Octopus vulgaris populations, an analysis of molecular variance (AMOVA) was conducted first between two groups according to geographical criteria (Western Mediterranean versus Centrel Mediterranean).

Results revealed no genetic differentiation was observed between these two biogeographic units corresponding to the western Mediterranean basin in one hand and the central Mediterranean basin in the other hand ($\Phi_{CT}$=-0.007, p = 0. 74 ) , most of the molecular variation (7.12%) was found to lie among populations within groups (Table 3). The results show that the Siculo-Tunisian Strait does not play the role of barrier to gene flow and can not explain the differentiation among Tunisian O*. vulgaris* populations.

When focussing on Central Mediterranean region, the AMOVA conducted between coastal and deep Sea areas indicated a non significant $\Phi_{CT}$ value ($\Phi_{CT}$ = 0.064, P= 0.335), suggesting a lack of genetic differentiation between the two groups (Table 3).

**Table 3 . Analysis of Molecular Variance (AMOVA)**

| Source of variation | df | | Φ- Statistic | P value |
|---|---|---|---|---|
| Group 1 (NOR)/ | 1 | Among Groups | Fct = - 0.00739 | 0.74 |
| Group 2 (COK, DWK,ZAR) | 2 | Among population | Fsc = 0.0712 | <0.0001 |
| | 117 | Within population | Fst = 0.06441 | <0.00001 |
| Group 1 (DWK)/ | 1 | Among Groups | Fct = 0.06429 | 0.335 |
| Group 2 (COK , ZAR) | 1 | Among population | Fsc = 0.022 | 0.1 |
| | 96 | Within population | Fst = 0.0848 | <0.0001 |

Significant pairwise $\Phi_{ST}$ values were related to the comparison involving Deep water samples (NOR and DWK) and the two coastal samples (ZAR and COK) (Table 4). All the other pairwise comparisons were not significant (Table 4).

**Table 4. Matrix of pairwise population Φst values**

| | DWK | COK | ZAR | NOR |
|---|---|---|---|---|
| DWK | 0.00000 | | | |
| COK | 0.12019*** | 0.00000 | | |
| ZAR | 0.05034** | 0.02559NS | 0.00000 | |
| NOR | 0.01563NS | 0.16277*** | 0.05536* | 0.00000 |

NS: not significant; *P < 0.05; **P < 0.01; ***P < 0.001

On the basis of these genetic distances, a multidimensional scaling plot (MDS) was performed. The obtained MDS shows that the samples are scattered in the plot and did not reveal clear geographical patterns at regional scale (Figure 3).

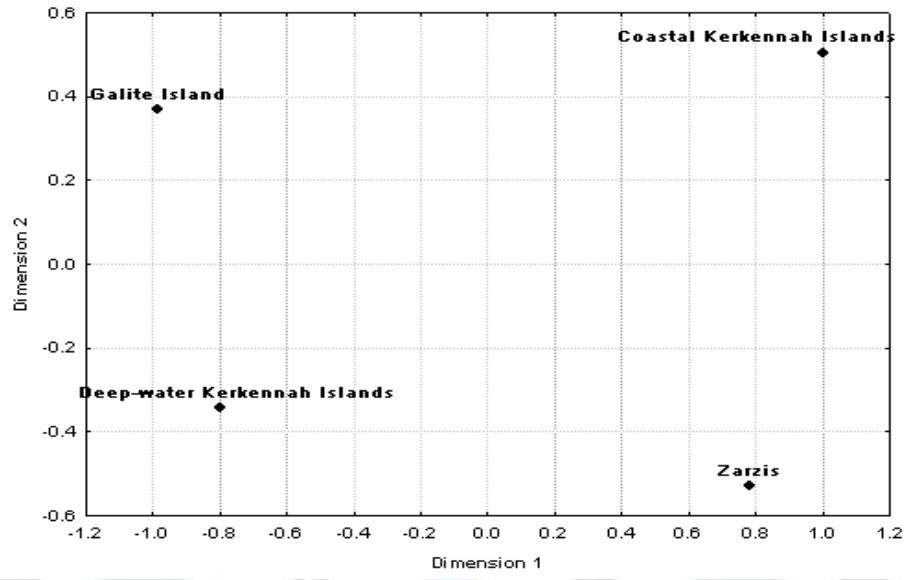


**Figure 3. *Octopus vulgaris* multidimensional scaling Plot obtained from nonmetric of pairwise Fst among Tunisian sampling sites.**

## 4. DISCUSSION AND CONCLUSION

The present investigation provided some insights into the genetic structure of *O. vulgaris* in the Tunisian waters. Results suggest a significant genetic structuring in the study area (the overall genetic divergence Φst = 0.06851, p <0.0001). This pattern of genetic structure displayed by *O. vulgaris* seems to be common for species of the genus octopus. A previous allozyme analysis of *O. vulgaris* showed significant genetic divergence among the nine locations from the Mediterranean Sea [9]. Another study using a single microsatellite locus found high levels of genetic differentiation between nine samples of O. vulgaris across the Mediterranean Sea and one Atlantic locality [22]. In a second microsatellite study Murphy and collaborators analyzed *O. vulgaris* populations from the northwestern coast of Africa revealed highly significant genetic structuring among specimens from Mauritania and the Western Sahara [13].

A further microsatellite study [10] compared populations from the eastern Atlantic and the Mediterranean, identified significant genetic structure following the isolation by distance model. Similarly, Moreira and collaborators identified 4 subpopulations of *O. vulgaris* off southern Brazil, with a tendency for greater genetic differentiation between geographically more distant populations [34]. More recently, analysis of the mitochondrial cytochrome oxydase subunit (COIII) gene identified significant genetic structure among seven Mediterranean samples [23]. Even populations of *O. maorum*, a species commonly distributed throughout southern Australian and New Zealand temperate and sub-Antarctic waters, evaluated by five polymorphic microsatellite loci, revealed significant genetic divergence among the five sites sampled [35] .

The results of the current study also indicated that the two coastal samples belonging to the south-eastern coast of Tunisia (Gulf of Gabes) (Zarzis and coastal Kerkennah) were genetically distinct from the remaining oceanic populations. This feature was clearly seen in ΦST pairwise estimates (Table 4) and MDS based on genetic distances (Figure 3). This outcome corroborated results obtained by Fadhlaoui-Zid et al[23] .

A similar pattern was observed by Fassatoui et al. [36] for the sparid fish Pagellus erythrinus, as they detect a genetic differentiation between Zarzis population and all other populations from northwestern Mediterranean and Eastern Mediterranean off the Tunisian coast. Such genetic differentiation was also reported for other marine species in the Mediterranean Sea [37,38-39] .This differentiation was interpreted as the reduction in gene flow related to the transition zone at the Siculo-Tunisian strait between the Eastern and Western Mediterranean basins. The sampling scheme of the present study didn't allow us to verify this hypothesis. Interestingly, in our study, the transition zone for this species is further south, as was the case for Pagellus erythrinus studied by Fassatoui et al.[36]. A likely explanation lies in the oceanographic properties of the Gulf of Gabes, which is characterised by a large continental shelf and is dominated by sandy and muddy demersal habitats. Unlike the northern coast of Tunisia, which is under the influence of Atlantic currents, the Gulf of Gabes has hydrodynamic characteristics more typical of the Central Mediterranean [40]. 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. Given the fact that the octopus vulgaris specie is subject to intense fishing activities carried out in the Gulf of Gabes (Ezzeddine-Najai S. personal communication) and the particularly environmental conditions of this area low genetic diversity can indeed be expected. This degree of genetic structuring among samples of common octopus in the study area between coastal and deep water areas of Tunisia, provide a new perspective on the management of the local common octopus, requiring separate management. These measures are important in order to ensure better management for a sustainable exploitation of common octopus fisheries.

In conclusion, this study provided genetic data in making inference of the genetic structure of *O. vulgaris* in the Central Mediterranean Sea. However, these inferences are preliminary. Further future studies with more sampling from Western and Eastern Mediterranean could provide more information on genetic structure and differentiation. In addition more efficient molecular markers (i.e., highly variable marker-microsatellite) are needed to provide a clear understanding of the genetic distribution and population dynamics of the *O. vulgaris* populations in the Mediterranean Sea.

## ACKNOWLEDGMENTS

We are grateful to Dr. Chammam Bachra for her assistance. This work was supported by the Tunisian Ministry of High Education and Scientific Research and the FAO Project ''Assessment and monitoring of fisheries resources and the ecosystems in the Straits of Sicily'' (MedSudMed), funded by the Italian Ministry of Agriculture, Food and Forestry Policies, MiPAAF.

## REFERENCES

[1] Najai,S. 1981. La pêche côtière des Céphalopodes en Tunisie. Rapp. Comm. Int. Mer Médit. 21 (10): 779-781.

[2] Ezzeddine, S. 1992. Biologie et pêche du poulpe *Octopus vulgaris* (Cephalopoda, Octopoda) du golfe de Gabès. Bull. Inst. Natl. Sci. Tech. Mer, Salammbô, 19: 5-19.

[3] Sanchez, P. and Obarti , R. 1993. The biology and fishery of *Octopus vulgaris* caught with clay pots on the Spanish Mediterranean coast. In: Recent Advances in Cephalopod Fisheries Biology, Okutani, T., O'Dor, R.K. & Kubodera, T. (Eds.), Tokai University Press, Tokyo, 477-487 pp.

[4] Belcari, P. and Sartor, P. 1999. Octopus vulgaris Cuvier, 1797, in: G. Relini, J. Bertrand, A. Zamboni (Eds.), Synthesis of the knowledge on bottom fishery resources in central Mediterranean (Italy and Corsica). Biol. Mar. Medit, 6 (suppl. 1), 757–766.

[5] Fiorentino, F., Ben Meriem, S., Bahri, T., Camilleri ,M., Dimech, M., Ezzeddine-Naja, S., Massa, F., Jarboui, O., and Zgozi S. 2008. Synthesis of information on some target species in the MedSudMed Project area (Central Mediterranean), MedSudMed Tech Doc, 15–67.

[6] Ben Youssef, S. 2006. Contribution à l'étude discriminatoire du poulpe commun Octopus vulgaris (Cuvier, 1797) du golfe de Gabès. Msc Thesis , ISBM, Tunisie. 118 pp.

[7] Nouira, T. 2006. Ecobiologie du poulpe commun Octopus vulgaris Cuvier, 1797 (Cephalopoda, Octopoda) de la région Est de Tunisie. Msc Thesis, ISBM, Tunisie. 105 pp.

[8] Garofalo, G., Ceriola, L., Gristina, M., Fiorentino, F., and Pace, R. 2010. Nurseries, spawning grounds and recruitment of *Octopus vulgaris* in the Strait of Sicily, central Mediterranean Sea, ICES J. Mar. Sci,67 ,1363–1371.

[9] Maltagliati, F., Belcari, P., Casu, D., Casu ,M., Sartor, P., Vargiu, G., and Castelli ,A. 2002. Allozyme genetic variability and gene flow in *Octopus vulgaris* (Cephalopoda, Octopodidae) from the Mediterranean Sea. Bull. Mar. Sci, 71: 473–486.

[10] Cabranes, C.,Fernandez-Rueda, P., and Martinez, J.L. 2008. Genetic structure of *Octopus vulgaris* around the Iberian Peninsula and Canary Islands as indicated by microsatellite DNA variation. ICES J. Mar. Sci., 65: 12–16.

[11] Begg, G.A., Friedland K.D., and Pearce, J.B. 1999**.** Stock identification and its role in assessment and fisheries management: an overview. Fish. Res, 43: 1–8.

[12] An, H.S., Lee, J.W., and Hong, S.W .2013. Population genetic structure of the Korean Pacific abalone Haliotis diversicolor supertexta inferred from microsatellite marker analysis.Biochem Syst Ecol ,48:76–94.

[13] Murphy, J.M., Balguerías, L.N ., and Boyle, P.R. 2002. Microsatellite DNA markers discriminate between two *Octopus vulgaris* (Cephalopoda: Octopoda) fisheries along the northwest African coast. Bull. Mar. Sci., 72 (1): 545–553.

[14] Fitzcharles, E.M. 2012. Rapid discrimination between four Antarctic fish species, genus *Macrourus*,using HRM analysis. Fisheries Research, 127. 166-170.

15] Oosthuizen, A., Jiwaji, M., and Shaw, P. 2004. Genetic analysis of the *Octopus vulgaris* population on the coast of South Africa. S. Afr. J. Sci, 100: 603–607.

[16] Teske, P. R., Oosthuizen ,A., Papadopoulos, I., and Barker, N. P. 2007. Phylogeographic structure of *Octopus vulgaris* in South Africa revisited: identification of a second lineage near Durban harbour. *Marine Biology*, 151: 2119–2122.

[17] De Los Angeles Barriga Sosa, I., Beckenbach, K., Hartwick, E.B., and Smith,M.J. 1995. The molecular phylogeny of five eastern North Pacific octopus species. Molec. Phylogenet. Evol. 4, 163–174.

[18] Söller, R., Warnke, K., Saint-Paul, U., and Blohm, D. 2000. Sequence divergence of mitochondrial DNA indicates cryptic biodiversity in *Octopus vulgaris* and supports the taxonomic distinctiveness of *Octopus mimus* (Cephalopoda: Octopodidae). Mar. Biol., 136: 29–35.

[19] Sosa, I.D.B., Beckenbach, K., Hartwick ,B ., and Smith, M.J. 1995. The Molecular Phylogeny of Five Eastern North Pacific Octopus Species. Original Research Article. Molecular Phylogenetics and Evolution, 4 (2): 163-174 .

[20] Warnke, K. 1999 Diversität des Artenkomplexes *Octopus cf. vulgaris* Cuvier, 1797 in beziehung zu seiner Verbreitung an der Ost- und Westküstes Lateinamerikas. Doctoral Thesis, Universität Bremen, Shaker Verlag, Aachen.256 pp.

[21] Guerra, A., Roura, A., Gonzalez, A. F., Pascual, S., Cherel, Y., and Perez-Losada, M. 2010. Morphological and genetic evidence that *Octopus vulgaris* (Cuvier, 1797) inhabits Amsterdam and Saint Paul Islands (southern Indian Ocean), ICES J. Mar. Sci, 67: 1401–1407.

[22] Casu, M. M., Maltagliati, F., Meloni, M., Casu ,D., Cossu, P., Binelli, G., Curini-Galletti, M ., and Castelli, A. 2002. Genetic structure of *Octopus vulgaris* (Mollusca, Cephalopoda) from the Mediterranean Sea as revealed by a microsatellite locus. Ital. J. Zool, 71: 473–486.

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

[24] Simon, C., Francisco, F., Andrew ,B., Berni, C., Hong, L ., and Paul, F. 1994. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved PCR primers. Ann.Entomol.Soc.Am,87 (6), 651-701.

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

[26] Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser., 41: 95–98.

[27] Librado, P . and Rozas, J. 2009. DnaSP v5: software for comprehensive analysis of DNA polymorphism data, Bioinformatics, 25, 1451–1452.

[28] Excoffier, L . and Lischer, H.E.L. 2010. Arlequin suite version 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Res*, 10: 564–567.

[29] Bandelt, H.J.,Forster P., and Rohl, A. 1999. Median-joining networks for inferring intraspecific phylogenies, Mol. Biol. Evol, 16: 37–48.

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

[31] Guiller, A., Bellido, A., and Madec, L. 1998. Genetic distances and ordination: the land snail Helix aspersa in North Africa as a test case. Syst. Biol,47: 208–227.

[32] Grant, W.S. and Bowen, B.W. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. The American Genetic Association,89: 415-426.

[33] Avise, J.C. 2000. Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge, MA. (447 pp).

[34] Moreira, A. A., Toma´ s, A. R. G., and Hilsdorf, A. W. S. 2011. Evidence for genetic differentiation of Octopus vulgaris (Mollusca, Cephalopoda) fishery populations from southern coast of Brazil as revealed by microsatellites. J. Exp. Mar. Biol. Ecol. 407:34–40.

[35] Doubleday, Z.A ., Semmens, J.M ., Smolenski, A.J and Shaw, PW .2009. Microsatellite DNA markers and morphometrics reveal a complex population structure in a merobenthic octopus species (Octopus maorum) in south-

east Australia and New Zealand. Marine Biology: international journal on life in oceans and coastal waters, 156 (6). pp. 1183-1192. ISSN 0025-3162.

[36] Fassatoui ,C., Mdelgi, E., and Romdhane, M.S. 2009. A preliminary investigation of allozyme genetic variation and population structure in common pandora (Pagellus erythrinus, Sparidae) from Tunisian and Libyan coasts. Ichthyological Research 56: 301-307.

[37] Borsa, P., Naciri, M., Bahri ,L., Chikhi, L., Garcia de Leon ,F.J., Kotoulas, G., and Bonhomme, F.1997. Zoogéographie infra-spécifique de la mer Méditerranée : analyse des données génétiques populationnelles sur seize espèces atlanto-méditerranéennes (Poissons et Invertébrés). Vie Milieu 47: 295-305.

[38] Bahri-Sfar ,L., Lemaire, C., Ben Hassine ,O.K., and Bonhomme, F. 2000. Fragmentation of sea bass populations in the western and eastern Mediterranean as revealed by microsatellite polymorphism. Proc. R. Soc. Lond., B. 267:929–935.

[39] Patarnello, T., Volckaert, F.A.M.J., and Castilho R. 2007. Pillars of hercules: is the Atlantic-Mediterranean transition a phylogeographical break? Mol. Ecol. 16: 4426–4444.

[40] Ben Othman, S. 1973. Le sud tunisien (Golfe de Gabès), hydrologie, sédimentologie, flore et faune. Thèse 3ème cycle, Faculté Sci. Tunis. 166p.