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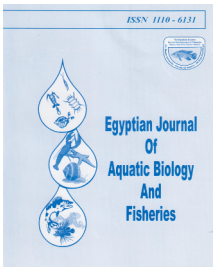


# Molecular Identification of Grey Mullet species in the Mediterranean Sea of Egypt

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## ABSTRACT

Mullet fish provide a valuable role in aquaculture to meet the rising demands for fish production in Egypt. The accurate estimate of species limits is a key factor in improving mullet fish for the application in evolution, conservation and management of the genetic programs. Four species of mullet fish, "*Mugil cephalus*, *Liza ramada*, *Liza aurata*, and *Liza saline*" were investigated by RAPD analysis to assess the genetic structure. Mullet fish were collected from three locations in the Mediterranean Sea (Al Maadyeah – Al Manzala – Bardawil). RAPD assay was performed using 10 decamers random primers; all the ten primers were succeeded in producing distinct bands.The obtained results revealed that the number of reproducible bands analyzed for ten primers for Al Maadyeah fishes was 181 bands in all four species, but in Bardawil and Manzala were 224 and 229 respectively. In each interspecies Mullet variation, the polymorphic amplified bands were 78.12% from it (20.98% *M. cephalus* 5.35% *L. aurata*, and 16.07 % *Liza ramada*unique bands) in Al Manzala, and 73.79% polymorphic from it (20.96% *M. cephalus*, 4.36% *L. aurata* and 12.2% *L. ramada* unique bands) in Bardawil and 66.85% polymorphic bands for Al Maadyeah from it (11.6% *M. cephalus* 8.2% *L. aurata*, 1.1% for *L. ramada* and *L. saline*unique Bands). Nei's genetic distance between (0.503 – 0.719) in Al Manzala, (0.263 – 0.514) in Al Maadyeah and (0.271 – 0.537) in Bardawil. The RAPD technique can be introduced as an easy tool in the population genetics of marine fishes, providing supplementary information in their genetic stock structure analysis.

## INTRODUCTION

Family Mugilidae including Grey mullets, a family of Teleostean fishes, are distributed worldwide populate marine and freshwater environments in all modest and equatorial regions (Polyakova N, *et al.* 2013) and they have great importance in aquaculture and fishery trade of many countries as well as a protein source. Several studies clarified the taxonomic status of Mugilidae based on traditional analysis of morphological traits, and the obtained results were in some cases controversial.

The ultimate contemporary classification showed that the Mugilidae containing 72 species belonging to seventeen genera (Nelson *et al.* 2006), the systematic status of some genera and species of this family is still uncertain (Semina *et al.,* 2007).

The most commonly distributing species of mullets are *Liza ramada* and *Mugil cephalus* (Lai *et al.*, 2011). Earlier studies reported that Egyptian water includes five species of Grey mullets (El-Sayed, 1991). The total catch percentage from the Egyptian natural fisheries in 2014 was 23%, the quantity and value of mullet family production from the Egyptian natural fisheries were 32853 MT (3131 Mediterranean Sea/ 185 Red Sea/ 28958 lakes/ 579 River Nile) (GAFRD. 2016).

Because e of its economic importance, considerable research has been devoted to the study of its biology, however, knowledge of its genetic population structure is lacking, although this could provide important information for effective fishery management (Carvalho & Hauser, 1994). The relative usefulness of different methods in the assessment of intraspecific genetic variation depends largely upon the species and geographical scale to which they are applied.

The identification and maintenance of differentiated stocks are very important for fishery activities because of their direct relation to the total productivity and sustainable use of resources (Carvalho and Hauser, 1994). It is particularly relevant in the marine environment, where physical barriers seem to be less effective (Palumbi, 1994), suggesting a tendency towards genetic homogenization (Bonhomme *et al.,* 2002).

Generally, many species of mullets group have a marked uniformity in their external morphology, which complicates their identification and this leads to taxonomical problems (Papasotiropoulos *et al.,* 2001). In fact, this consider as a limiting factor for addressing the questions concerning phylogenic relationships (particularly at intraspecies level) of mullets (Papasotiropoulos *et al.,* 2007; Liu *et al.,* 2010), moreover the identification of the fry to be used for stocking purposes, especially *M. cephalus* and others, is of practical interest and often causes problems for farmers who do not want to stock the ponds with other species. Information on the molecular structure of fish species is useful for optimizing identification of stocks, stock enhancement, breeding programs, management for sustainable yield and preservation of genetic diversity (Tassanakajon, *et al.* 1998).

Molecular techniques based on DNA sequence polymorphism are now used in population genetics studies, systematic and molecular taxonomy to get an answer to systematic related problems. Molecular techniques played an important role to understand the basis of polymorphism of a species, species diagnostics and population differentiation (Avise, 1996). Within the last decade, scientific progression has increasingly supported the use of molecular techniques in determining population diversity. Many molecular techniques are now available, which allow ecologists and evolutionary biologists to determine the genetic architecture of a wide variety of closely related individuals. The discovery of the PCR had a major impact on the research of eukaryotic genomes and contributed to the development and application of various DNA markers (Schlötterer, 2004)

RAPDs are particularly useful to study the genetic structure of populations because they reveal polymorphisms in non-coding regions of the genome. Random primers produce RAPDs that have been used extensively as molecular markers (Shikano and Taniguchi, 2002). The RAPD markers method has been reported to be an effective tool to differentiate geographically and genetically isolated population and has been used to verify the existence of a population of species that have arisen either through genetic selection under different environmental conditions or as a result of genetic drift (Fuchs *et al.*, 1998). During the last few years, RAPD-PCR has been shown a wide range of applications in fisheries and poultry (Salem *et al.*, 2005).

Thus, considering the diversity of mullet species in the Mediterranean coast of Egypt and the difficulty in differentiating them based on morphological characteristics, the main goals of the present study were to employ the RAPD method to study molecular differences and to evaluate the levels of genetic variation within and among Mugiliadae family taken from three locations of the Egyptian Mediterranean.

## MATERIALS AND METHODS

**Sample collection:**

Total of thirty-six samples of Mullet species was collected from the three different locationsof the Egyptian Mediterranean Sea (Bardawil, Al Manzala, and Al Maadeyah) from October 2016 to February 2017 and three individuals were sampled from each species associated to each location. The species in this study are *Mugil cephalus*, *Liza aurata*, *Liza ramada* and *Liza saliens*. The samples then transferred to the genetics laboratory at the National Institute of Oceanography and fisheries. Tissue samples were dissected from the body muscle and preserved in 95% ethanol.

**DNA Extraction:**

For the isolation of total genomic DNA, a modified protocol was followed using Joseph Sambrook and David W. Russell 2001 Manual. Nano-drop was used to check the quality as well as the quantity of isolated DNA. Optical densities of the DNA samples were measured at 260 nm and 280nm.

**RAPD-PCR amplification:**

In the present study, ten primers were used to initiate PCR amplification. Primers were randomly selected on the basis of GC content and the annealing temperature as showed in (Table 1) according to Mishra 2008 and Rajakumaran, 2014, and applied them for examination on the gel.

Table 1: RAPD-PCR primers sequences and GC% contents.

| Primers | *Sequence* | *GC %* |
|---|---|---|
| RAPD 1 | AATCGGGCTG | 60% |
| RAPD 2 | GAAACGGGTG | 60% |
| RAPD 3 | CAATGCCCGT | 60% |
| RAPD 4 | GTATTGCCCT | 50% |
| RAPD 5 | TGCCTCGTGC | 70% |
| RAPD 6 | GCGCCTGGAG | 80% |
| RAPD 7 | AACGGGCAGG | 70% |
| RAPD 8 | GGCTGCGGTA | 70% |
| RAPD 9 | GCGGAGGTCC | 80% |
| RAPD 10 | CGACGCCCTG | 80% |

The PCR products were performed into PCR tubes containing a 10 µl of the PCR mixture according to Williams *et. al.* 1990. (5 µl of 2x PCR Master Mix Solution (i - Taq™), 0.8 µl primer, 1 µl Template DNA and 3.2 µl $H_2O$ (D.W)). The amplification Thermo-cycler condition according to Williams *et. al.* (1990) was programmed by using gradient PCR (Applied Biosystems life technologies™) initial denaturation of 5 min at 94°C, followed by 30 cycles of denaturation for 30 s at 94°C, 30 s annealing at 42-50°C, 2 min extension at 72°C, and one 10 min cycle at 72°C for final extension. Amplified products were separated on 2.5% agarose gel stained with ethidium bromide, run in 1 X TAE buffer at a constant 80 V (Sambrook and Russell 2001). The gels were imaged using a Biometra gel documentation system (UK).

**Data analysis:**

Bands observed in each lane were compared with all the other lanes of the same gel and reproducible bands were scored as present (1) or absent (0). Fragment sizes were estimated based on the 1Kp DNA Ladder (Intronbio - Korea). Data were analyzed using the GenAlEx 6.5 (Rod and Peter, 2012) and used PAST318 to construct dendrograms based on unbiased Nei's genetic distances (Nei, 1972).

## RESULTS

In the present study, four mullet species (*M. cephalus, L. aurata, L. ramada and L. saline*) were identified using a molecular technique (RAPD) to detect genetic variability of these economic fish. The profile of amplified DNA primarily depends on nucleotide sequence homology between the template DNA and oligonucleotide primer at the end of each amplified product. Nucleotide variation between different sets of template DNAs will result in the presence or absence of bands because of changes in the priming sites. All the ten primers examined produced different RAPD fragment patterns. The number of amplified bands detected varied, depending on the primers, species, and phenotypes. All amplification products were found to be reproducible when reactions were repeated using the same reaction conditions.

With respect to the four species of mullet, RAPD electrophoresis profiles are represented in gel figures from Figs. 1& 3, It is clear from figures that DNA polymorphism can be seen from to viewpoint; first the presence or absence of one or more RAPD bands which possess RAPD patterns, second change in the intensity of bands which having the same size (Table 2).

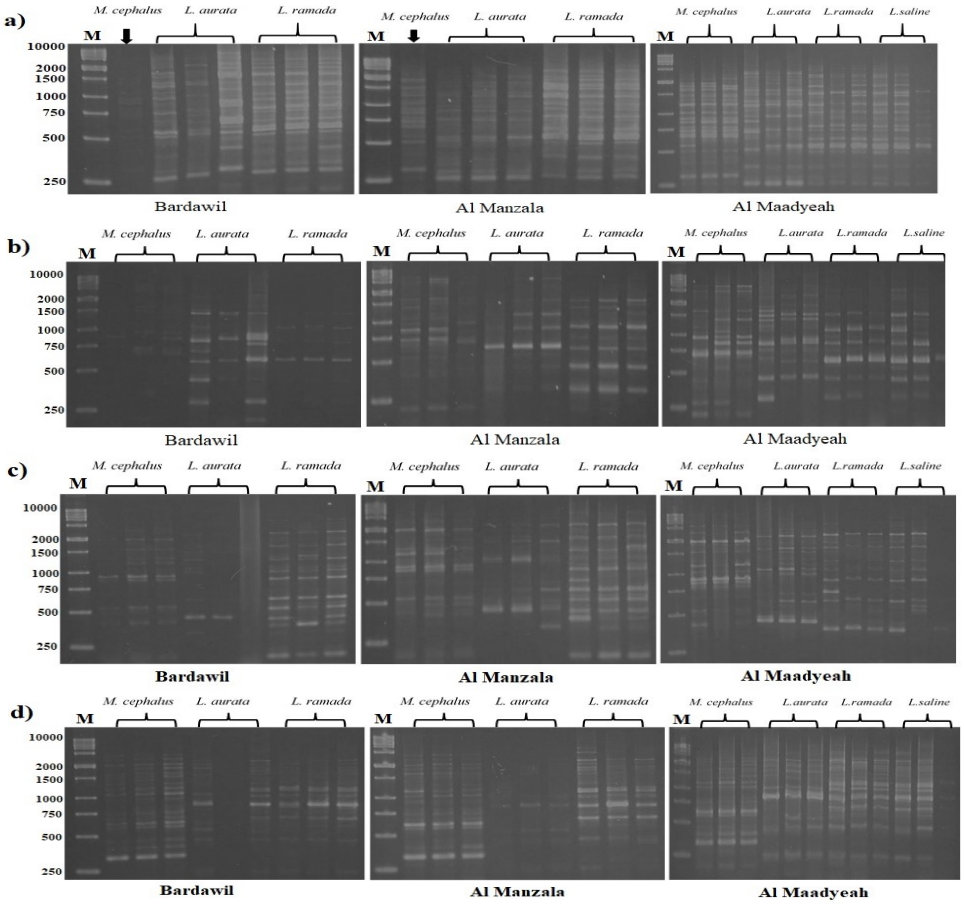


Fig. 1 :Electrophoretic bands pattern of RAPD PCR for species generated by; a) primer 1, b) primer 2, c) primer 3 and d) primer 4 in Bardawil, Al Manzala and Al Maadyeah.

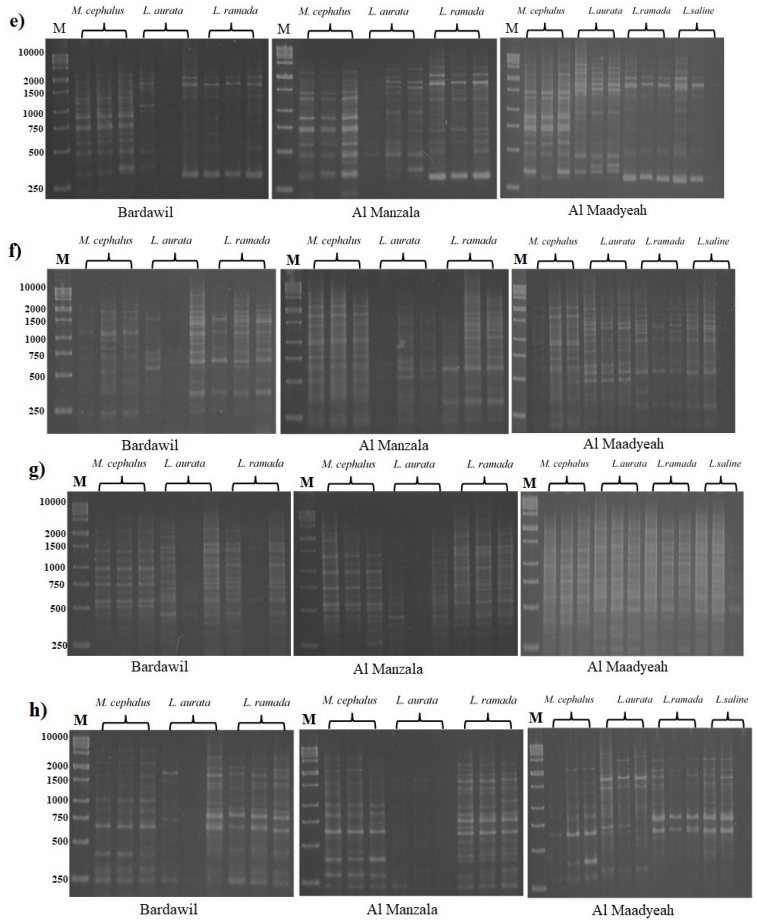


Fig. 2: Electrophoretic bands pattern of RAPD PCR for species generated by; e) primer 5, f) primer 6, g) primer 7 and h) primer 8 in Bardawil, Al Manzala and Al Maadyeah.

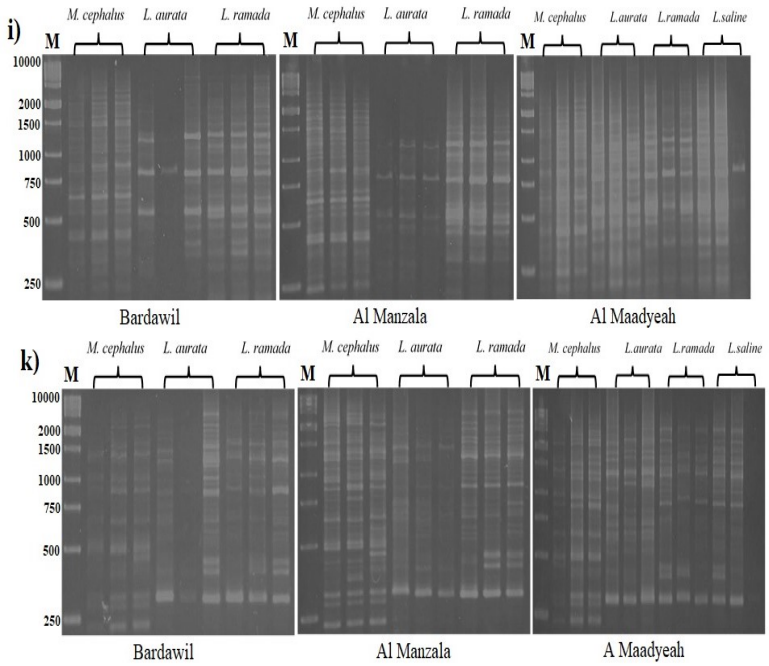


Fig. 3 :Electrophoretic bands pattern of RAPD PCR for species generated by; i) primer 9, k) primer 10, in Bardawil, Al Manzala and Al Maadyeah.

Table 2: Densitometric analysis and calibrated quantity (ng) of DNA RAPD bands generated by 10 primers for the M. cephalus, L. aurata, L. ramada and L. saline in Bardawil, Al Manzala and Al Maadyeah.

| ***Primer*** | ***Species*** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ***M. cephalus*** | | | ***L. aurata*** | | | ***L. ramada*** | | | ***L. saline*** |
| | **Br** | **Mz** | **Ma** | **Br** | **Mz** | **Ma** | **Br** | **Mz** | **Ma** | **Ma** |
| ***RAPD 1*** | - | - | 300 | 700-800-1500 | 250 | 250 | 500 | 500-1400-1500 | 500 | 500 |
| ***RAPD 2*** | - | - | 700 | 600-800 | 700 | 300-450 | - | 300 | 600 | 600 |
| ***RAPD3*** | - | - | 900 | - | 450 | 450 | - | - | 400 | 400 |
| ***RAPD4*** | 300 | 300 | 300-600 | - | - | 800 | - | 800 | 800-1200 | 800-1200 |
| ***RAPD5*** | 750 | 750 | 350-750-800 | - | - | 1500-1600 | - | 300 | 300-1600 | 300-1600 |
| ***RAPD6*** | 1200 | 1000 | 900 | 1400-350 | - | - | 350 | 350 | - | 500 |
| ***RAPD7*** | - | - | - | 1400-1500 | - | 450-1400 | - | 1400 | 1300 | 600-1000-1300 |
| ***RAPD8*** | - | 650 | 450 | 650-700 | - | 1500 | 750 | 750 | 700-800 | 700-800 |
| ***RAPD9*** | - | 600 | 450 | 500-800-1300 | 800-1300 | 800 | 500-800 | - | 500-800 | 400-500-800 |
| ***RAPD 10*** | - | - | 500 | 300-1400 | 350 | 300 | 300 | 350 | 360 | 360 |

Number of reproducible and well-resolved bands analyzed per primer in all populations ranged from 181 to 229, the genetic variability of the fishes as analyzed from (Tbles 3,4 & 5), can be interpreted in the following points: Out of the 224 bands analyzed for all the ten random primers in Al Manzala, 175 were polymorphic, and (47 *M. cephalus* – 12 *L. aurata* – 36 *L. ramada*) were unique band (Table 3), out of 229 bands analyzed in Bardawil 169 were polymorphic, and (48 *M. cephalus* – 10 *L. aurata* – 28 *L. ramada*) were unique bands (Table 4). While in AlMaadyead zoone the tolal number of the band were 181; the polymorphic band was 121 and (21 *M. cephalus*, 15 *L. auarta*, 2 *L. ramada* and 2 *L. saline*) were unique bands (Table 5).

Polymorphic bands were taken as RAPD markers, and they were combined in a simple matrix of binary data, with registers of presence or absence of bands. In each interspecies Mullet, population amplified DNA fragments counted for polymorphic bands as follows 78.12% Al Manzal, 73.79% Bardawil and 66.85% in Al Maadyeah. The Unique Band were as following: In Al Manzala 20.98% *M. cephalus* 5.35% *L. aurata*, and 16.07 % *Liza ramada*. For Bardawil 20.96% *M. cephalus*, 4.36% *L. aurata* and 12.2% *L.ramada*. Al Maadyeah 11.6% *M. cephalus* 8.2% *L. aurata*, 1.1% for *L. ramada* and *L. saline*.

Table 2 presents the values of amplified copies sequences differing in size (bp length). The highest amplified bands in *M. cephalus* were in Al Maadyeah ranged from (300-900), at all primers except primer number 7, while in *L. aurata* ranged from (300-1500) which were detected in Bardawil and Al Maadyeah except primer number 3,4,5 in Bardawil and number 1 in Al Maadyeah.

Table 3: Total number of bands, polymorphic bands and monomorphic bands obtained from a survey of 10 primers of a random sequence of Mullet species collected from Lake Manzala.

| Primers | No. of Band | Poly. | *M. cephalus* | | *L. auarta* | | *L. ramada* | |
|---|---|---|---|---|---|---|---|---|
| | | | Unq. band* | MW** | Unq. band* | MW** | Unq. band* | MW** |
| **RAPD 1** | 23 | 13 | 0 | - | 2 | 400-1550 | 5 | 400-3000 |
| **RAPD 2** | 15 | 13 | 6 | 100-2000 | 1 | 1300 | 2 | 300-1500 |
| **RAPD 3** | 18 | 16 | 3 | 100-2000 | 1 | 450 | 3 | 400-3000 |
| **RAPD 4** | 20 | 19 | 9 | 300-2500 | 1 | 500 | 2 | 450-700 |
| **RAPD 5** | 19 | 11 | 1 | 750 | 1 | 400 | 2 | 755-2000 |
| **RAPD 6** | 19 | 15 | 3 | 500-2000 | 1 | 500 | 4 | 400-1500 |
| **RAPD 7** | 26 | 19 | 6 | 500-3000 | 1 | 250 | 5 | 400-2000 |
| **RAPD 8** | 26 | 23 | 5 | 600-3000 | 2 | 400-600 | 8 | 500-2500 |
| **RAPD 9** | 29 | 25 | 10 | 250-3000 | 2 | 300-600 | 2 | 300-1400 |
| **RAPD 10** | 29 | 21 | 4 | 250-2500 | 0 | - | 3 | 480-4000 |
| **Total** | 224 | 175 | 47 | | 12 | | 36 | |
| *Unq. Band: Unique band, **Mw: Molecular Weight | | | | | | | | |

Table 4: Total number of bands, polymorphic bands and monomorphic bands obtained from a survey of 10 primers of a random sequence of Mullet species collected from Lake Bardawil.

| Primers | No. of Band | Poly. | *M. cephalus* | | *L. auarta* | | *L. ramada* | |
|---|---|---|---|---|---|---|---|---|
| | | | Unq. band* | MW** | Unq. band* | MW** | Unq. band* | MW** |
| **RAPD 1** | 25 | 16 | 4 | 700-1500 | 3 | 700-1600 | 2 | 300/400 |
| **RAPD 2** | 20 | 11 | 3 | 700-300 | 2 | 300/1400 | 0 | - |
| **RAPD 3** | 23 | 22 | 5 | 500-2000 | 0 | - | 5 | 150-3000 |
| **RAPD 4** | 26 | 19 | 10 | 300-3000 | 0 | - | 1 | 2100 |
| **RAPD 5** | 21 | 16 | 6 | 250-2500 | 1 | 2000 | 0 | - |
| **RAPD 6** | 21 | 16 | 2 | 450-755 | 1 | 760 | 7 | 400-2500 |
| **RAPD 7** | 23 | 15 | 5 | 400-3000 | 0 | - | 0 | - |
| **RAPD 8** | 19 | 13 | 1 | 3000 | 0 | - | 4 | 450-2000 |
| **RAPD 9** | 28 | 25 | 9 | 250-2100 | 0 | - | 6 | 470-4000 |
| **RAPD 10** | 23 | 16 | 3 | 250-500 | 3 | 600-1300 | 3 | 760-3000 |
| **Total** | 229 | 169 | 48 | | 10 | | 28 | |
| *Unq. Band: Unique band, **Mw: Molecular Weight | | | | | | | | |

Table 5:Total number of bands, polymorphic bands and monomorphic bands obtained from a survey of 10 primers of a random sequence of Mullet species collected from Lake Maadyeah.

| Primers | No. of Band | Poly. | *M. cephalus* | | *L. auarta* | | *L. ramada* | | *L. saline* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Unq. band* | MW** | Unq. band* | MW** | Unq. band* | MW** | Unq. band* | MW** |
| **RAPD 1** | 23 | 1 | 5 | 300-1500 | 1 | 2700 | 0 | - | 0 | - |
| **RAPD 2** | 18 | 14 | 2 | 300-4000 | 2 | 1400/ 2500 | 0 | - | 0 | - |
| **RAPD 3** | 24 | 23 | 3 | 600-3000 | 5 | 450-4000 | 1 | 300 | 0 | - |
| **RAPD 4** | 17 | 12 | 2 | 400/ 450 | 1 | 500 | 0 | - | 0 | - |
| **RAPD 5** | 18 | 13 | 2 | 600/ 2100 | 0 | - | 0 | - | 0 | - |
| **RAPD 6** | 9 | 6 | 0 | - | 0 | - | 0 | - | 0 | - |
| **RAPD 7** | 15 | 12 | 2 | 400/ 900 | 2 | 350/ 1600 | 0 | - | 0 | - |
| **RAPD 8** | 20 | 16 | 0 | - | 4 | 350-4000 | 0 | - | 0 | - |
| **RAPD 9** | 21 | 14 | 2 | 300/ 2000 | 0 | - | 0 | - | 0 | - |
| **RAPD 10** | 16 | 10 | 3 | 200-1300 | 0 | - | 1 | | 2 | 1000/ 2000 |
| **Total** | 181 | 121 | 21 | | 15 | | 2 | | 2 | |

*Unq. Band: Unique band, **Mw: Molecular Weight

In *L. ramada* it appeared in all primers in Al Manzala and Al Maadyeah except primer number 3 and 9 in Al Manzala and Number 6 in Al Maadyeah and ranged (350 – 1600). In *L. saline* Al Maadyeah it was found in all ten primers and ranged from (300-1600).

Estimates of Nei's unbiased genetic distances in Al Manzala were: 0.719 (*M. cephalus* / *L. aurata*), 0.514 (*M. cephalus* / *L. ramada*) and 0.503 (*L. aurata* / *L. ramada*). In Al Maadyeah there were 0.263 (*M. cephalus* / *L. auarata*), 0.325 (*M. cephalus* / *L. ramada*), 0.553 (*M. cephalus* / *L. saline*), 0.397 (*L. aurata* / *L. ramada*), 0.514 (*L. aurata* / *L. saline*), 0.446 (*L. ramada* / *L. saline*). And In Bardawil Nei's genetic distances 0.537 (*M. cephalus* / *L. aurata*), 0.628 (*M. cephalus* / *L. ramda*) and 0.271 (*L. aurata* / *L. ramada*).

A Paired group (UPGMA) based on Nei's genetic distance is shown in (Fie. 4 – Fig. 5 – Fig. 6). Two major clades were identified in Al Maadyeah Lake the first clade consisted of one sister group (*L. ramada and L. saline)*and one out-group (*L. aurata*), the second clade were *Mugil cephalus*. In Al Manzala and Bardawil Lake, there was two clade, the First clade consisted of one sister taxa (*L. aurata & L. ramada)* and the second clade was (*M. cephalus*)

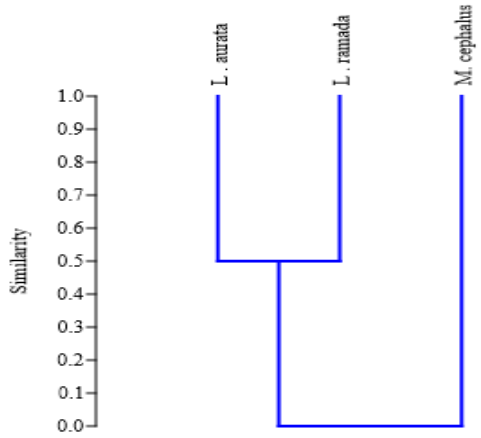


Fig. 3:Paired group (UPGMA) tree, based on Nei's genetic distance obtained from RAPD markers of three species of Mugilidea family individuals from Al *Manzala Lakes*

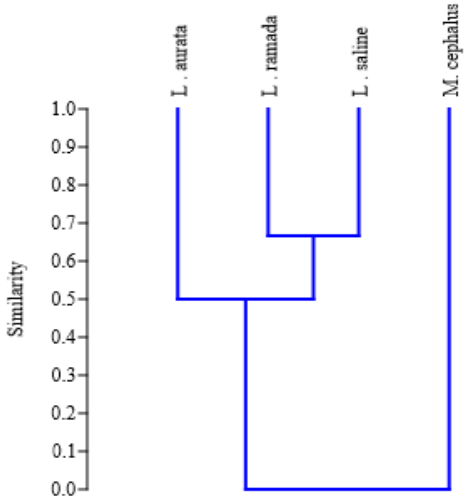


Fig. 3: Paired group (UPGMA) tree, based on Nei's genetic distance obtained from RAPD markers of four species of Mugilidea family individuals from Al Maadyeah Lakes.

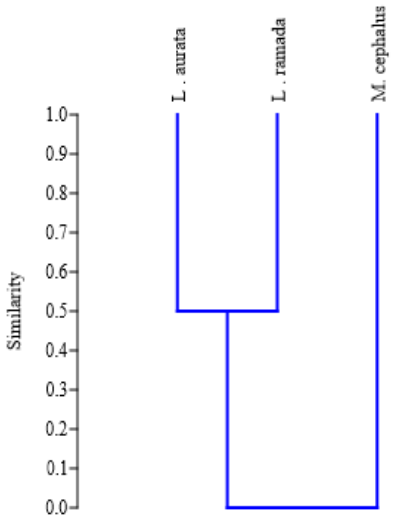


Figure 3: Paired group (UPGMA) tree, based on Nei's genetic distance obtained from RAPD markers of three species of Mugilidea family individuals from Bardawil Lakes.

## DISCUSSION

Genetic approaches offer powerful tools for examining the current status of populations, for understanding the population changes for its conservation (Belfiore and Anderson, 2001). The RAPD technique is one of the most frequently used molecular methods for taxonomic and systematic analyses of various organisms (Garg *et al.,* 2009a, b). The RAPD PCR-based method is used for fish species

identification because it is a simple, specific and sensate method for genetic characterization (Asensio *et al.,* 2009). Moreover, just only one primer could obtain the different profiles for genomic analysis (Antunes *et al*., 2010).

However, it is important, to have an adequate knowledge on the genetic population structure of both the released stock and the wild population before carrying out Restocking and stock enhancement programs (Pereira *et al.,* 2010). So, the genetic markers should be conducted to provide the information needed for a sound management of economic aquatic resources in wild fish stocks and/or farms (Saad *et al.,* 2011)

The number of reproducible and well-resolved bands analyzed in all populations examined in this study using the ten primers resulted in 175 polymorphic in Al Manzala 169 polymorphic in Bardawil and 121 polymorphic bands in Al Maadyeah, Hilal and Ali (2016) mentioned that the DNA markers were polymorphic reflecting a rich allelic diversity in the applied fish species. So, the primers of these loci are recommended to detect the genetic polymorphism and inferring the genetic variations for the applied fish species.

The obtained unique bands in Al Manzala were (47 *M.cephalus*, 12 *L.aurata* and 36 *L. ramada*), the unique band in Bardawil were (48 *M.cephalus*, 11 *L.aurata* and 28 *L. ramada*), and in Al Maadyeah The obtained unique bands in Al Mazala were (47 *M. cephalus*, 12 *L. aurata* and 36 the unique band were (20 *M. cephalus*, 15 *L. aurata*, 2 *L. ramada*, and 2 *L. saline*). This result could be used as species-specific RAPD markers as mentioned by Rashid *et al.* (2009) who stated that the RAPD markers could be used in two different ways. In a first way, these markers will be used, as a species genetic signature. The second way, these markers are used as marker-assisted selection inbreeding (to develop local fish breeds) and restocking programs.On the other hand, Neekhra *et al.* (2014) indicated that coexist of monomorphic bands in all three fish species of the family: Cyprinidae (*Labeo rohita*, *Catla catla*, and *Cirrhina mrigala*) suggesting that they have a common ancestor and are genetically closer to each other.

The polymorphism in the intensity of bands indicates that these bands are highly amplified in comparison to the others. The increase in the number of highly amplified bands appeared in *M. cephalus* Al Maadyeah and *L. aurata* (Bardawil and Al Maadyeah), *L. ramada* (Al Manzala, Al Maadyeah) and *L. saline* in Al Maadyeah, this data proved a high degree of variability among the four species. Generally, the number and size of the bands generated strictly depend upon the nucleotide sequence of the primer used and the source of the template DNA; resulting in the genome-specific finger-prints of random DNA bands (Welsh *et al.,* 1991; Pereira *et al.,* 2010; Rashed *et al.,* 2008).

It is necessary to estimate intra and inter-population variations and phylogenetic relationships among fish genomes (Saad *et al.,* 2011) to help the breeder in designing suitable breeding programs for fish improving and /or conservation. In addition, this will be useful in detecting any genetic contamination in these fish genomes. According to Garg *et al.* (2009b) the intraspecific genetic similarity between the individuals of the population was due to geological variations or changes in the aquatic environment. In this context, we found that the three studied zones are varied greatly in their aquatic conditions whereas, Al Manzala Lake receives huge quantities of sewage, agricultural and industrial water, without any treatment. With some 650 million cubic meters of sewageThe highest salinity value of 66.24% was recorded with an average of 21.5%, Bardawil Lake is the least polluted of the northern lakesThe highest salinity value was 54.45% with a mean of 44.0% average annual

salinity of Al Maadyeah lake 2.86% The lake has about 2062 million cubic meters of agricultural drainage water /loaded with chemical fertilizers and agricultural pesticides as well as industrial drainage (EEAA. 2017).

Environmental factors may explain the clear differences in the total reproducible bands for the ten primers in Al Maadyeah (181 bands) than in Al Manzala and Bardawil (224, 229 respectively), which may stem out of point mutation in priming sites that cause misleading priming as a result to the environmental pollution,and may also consequently decrease the polymorphism among the four species in Al Maadyeah. From another side chemical pollution (fertilizers, pesticides and industrial drainage) has a deleterious effect on mullet genome as hall, and sewage pollution has lower effect on the allelic diversity of Mullet species than chemical pollution.

Our statistical analysis showed considerable genetic variation among the genotypes of Mullet species collected from different locations of Mediterranean.Our study values of pairwise comparisons of Nei's (1972) genetic distance (D) within populations, showed that EL-Maadyeah and Bardawil exhibited the lowest genetic distance. The highest Nei's unbiased genetic distance, estimated as 0.719 in Al Manzala between *Mugil cephalus* and *Liza aurata*, this result indicated that the genetic variability was not lost during the establishment of these two populations. Nei's genetic distances between species from the same genus have been found no lower than 0.162 and over 0.3 in most species, as estimated from enzyme studies (Thorpe and Solé-Cava, 1994).Our results revealed two separate clades (*L. ramada and L. saline L. aurata*). The second clade including *Mugil cephalus*. The results of this study are consistent with the findings reported by (Papasotiropoulos *et al*., 2001, 2002, 2007; Blel *et al.,* 2008; Semina *et al.,* 2007), they applied several techniques for genetic analysis among Mugilidae family and reported that *M. cephalus* is the most distinct species compared with *Liza* genus

The result of genetic similarity among Mullet species indicated highly expected success of interspecific hybridization between *Liza aurata*, *Liza saline*, and *Liza ramada* also the hybridization may be succeeded between *Mugil cephalus* and *Liza* group. The genetic similarity by RAPD marker can be used as a rapid method for predicting the specific hybridization between Mullet spp.

The RAPD assay has been used to construct phylogenetic trees for resolving taxonomic problems in many organisms (Barman *et al.,* 2003; Soliman *et al.,* 2003) population genetic differentiation may be due to ecological, geographical and evolutionary factors. The genetic diversity data has varied applications in research on evolution, conservation, and management of natural resources and genetic improvement programmers, etc. (Ferguson *et. al.,* 1995; Liu and Cordes, 2004). The present study showed that RAPD primers were informative in detecting species-specific DNA markers.

The present study provides us baseline information, not only for species identification but also valuable data for impact pollution on mullet genome. Therefore, it may serve as a reference point for future examination of genetic variations within the populations of fishes, which are commercially important and the possible use of DNA markers in future may create new avenues for fish molecular biological research.

## CONCLUSION

The genetic diversity data has varied applications in research on evolution, conservation, and management of natural resources and genetic improvement programmers, etc. RAPD analysis for genetic diversity study provides a basis to obtain genetic variation within and among populations. The present study showed that RAPD fingerprinting can generate unique, species-specific DNA profiles for the four Mullet species.Both unique and polymorphic RAPD markers identified in mullet can be used for species differentiation. Once the population structure is known, scientific management for optimal harvest and conservation of the fish fishery resource can be undertaken. Therefore, The present study will thus become extremely important when we think in terms of genetic selection and hybridization of commercially important mullet species for advanced aquaculture and may serve as a reference point for future genetic examinations and the possible use of much specific molecular biomarkers for more understanding the taxonomical relations of such family, which are widely distributed in various brackish water streams, lakes and fish farms of Egypt.

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## ARABIC SUMMARY

### التعرف الجزيئي على أنواع أسماك البوري في البحر المتوسط في مصر

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تلعب أسماك البوري دورا هاما في الاستزراع السمكى لتلبية الطلبات المتزايده على إنتاج الاسماك. يعد التحديد الدقيق للانواع عامل رئيسي في تحسين اسماك البوري وذلك لتطبيقه في تطوير وحفظ وإدارة البرامج الجينية.
تم دراسه أربعه أنواع من أسماك البوري *Mugil cephalus, Liza aurata, Liza ramada* and *Liza saline* بواسطه تحليل RAPD لتقيم البنيه الجينية. تم جمع أسماك البوري من ثلاث مناطق على البحر الأبيض المتوسط (المعدية – المنزلة – البردويل). وتم إستخدام ١٠ بادئات عشوائية في اختبار الـ RAPD، نجحت البادئات العشره في إنتاج حزم مميزة .

أظهرت النتائج التي تم الحصول عليها أن عدد الحزم القابلة للنسخ التي تم تحليلها لعشر بادئات في أسماك المعدية كانت ١٨١ حزمه للانواع الأربعه، ولكن في البردويل والمنزلة كانت ٢٢٤ و ٢٢٩ على التوالي. في كل الإختلافات بين أنواع أسماك البوري كانت الحزم متعددة الاشكال المتضخمه ٧٨.١٢% منها (٢٠.٩٨% *M. cephalus* ٣٥.٥% *L. aurata* و ١٦.٠٧% *L. ramada* ) حزم فريدة في منطقة المنزلة، و٧٣.٧٩% متعدد الاشكال منها (٢٠.٩٦% *M. cephalus*, 4.36% *L. aurata* و 12.2% *L. ramada*) حزم فريدة في منطقة البردويل، و ٦٦.٨٥% حزم متعددة الأشكال في منطقة المعدية منها (١١.٦% *M. cephalus*، 8.2% *L. aurata* ، ١.١% *L. ramada* و *L. saline*) حزم فريدة.

المسافة الجينية Nei's تتراوح بين (٠.٥٠٣-٠.٧١٩) في المنزلة، (٠.٢٦٣-٠.٥١٤) في المعدية، و(٠.٢٧١-٠.٥٣٧) في البرداويل. يمكن أستخدام تقنية RAPD كأداة سهلة في علم وراثة العشائر للأسماك البحرية مما يوفر معلومات تكميلية في التحليل الهيكلي للمخزون الجيني لهذه الاسماك.