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Genetic divergence and phylogenetic relationship among three species of Mullet inferred from RAPD markers in Egypt

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ABSTRACT

The present study was carried out to determine the genetic divergence and phylogenetic relationship for three species of mullet (*Mugil cephalus*, *Liza ramada*, and *Valamugil seheli*) in Egypt using random amplified polymorphic DNA (RAPD) markers. two hundred and twenty-five specimens were randomly collected from Qarun, Manzala, and Burullus lakes and some commercial farms neighboring each of the three lakes. The results indicated that the highest genetic variation of 37.08% in *Liza ramada* from lake Manzala and the lowest difference genetic variation of 24.97% in *Mugil cephalus* from lake Manzala were observed. Dendrogram showed the highest genetic similarity between *Liza ramada* of lake Qarun and surrounding farms. It could be concluded that *Liza ramada* of lake Manzala is best to work for selection programs. There is a need for further genetic studies to give a more complete picture of mullets in Egypt.

INTRODUCTION

The total fish production in Egypt is 1.71 million tons annually where 1.4 million tons were produced through aquaculture, it represents more than 80% of the total fish production **(GAFRD, 2018)**. Tilapia and mullet are dominant species. They accounts for 85.1% of the total aquaculture production **(El-Sayed, 2015)**. In addition, Egypt is the world's top producer of cultured mullet. Most of the mullet farms are located in Delta region in Egypt, with three different species from *Mugilidae* family. Flathead mullet (*Mugil cephalus*), thinlip grey mullet (*Liza ramada*), and bluespot mullet (*Valamugil seheli*) constitute the most mullet aquaculture in Egypt **(Saleh, 2008)**. So far in Egypt, fish farms rely on the collection of mullet fry from fisheries and there are no artificial hatcheries to meet the demand for mullet fry **(Suloma and Ogata, 2006)**. According to

GAFRD (2018) during the period from 2006 to 2016 collection of mullet fry from the fisheries increased from 38.8 to 92.9 million, and mullet aquaculture decreased from 252.5 to 114.0 thousand tons, as a result, mullet fry decreased in fisheries and mullet production also declined. Despite aquaculture importance of mullet species, there is no information available on genetic background species **(Suresh *et al*., 2013)**. Genetic information is necessary to provide baseline data for identifying stock with superior traits for breeding programs and also to formulate management strategies for sustainable utilization of the species **(Jahromi and Othman, 2011)**. Many studies have been reported to determine genetic diversity and population structure of family *Mugilidae* based on molecular marker systems including allozyme in *Mugil cephalus* **(Rossi *et al*., 1998)**, mitochondrial DNA **(Huei-Mien *et al*., 2009; Jamandre *et al*., 2009; Shen *et al*., 2011 and Livia *et al*., 2011)**, microsatellites **(Miggiano *et al*., 2005; Xu *et al*., 2010; Mai *et al*., 2014 and Behrouz *et al*., 2018)** and RAPDs **(Kapila and Mishra, 2006 and Suresh *et al*., 2013)**. RAPD technique can be successfully used as a rapid and easy way for estimate genetic diversity in different fish species such as Nile tilapia **(Hassanien *et al*., 2004)** and green tiger prawn **(Hassanien and Al-Rashada, 2019)**. Therefore, the aim of this work was to assess the genetic divergence and phylogenetic relationship among three economically important species of *Mugilidae* (*Mugil cephalus*, *Liza ramada*, and *Valamugil seheli*) using RAPD markers. In addition, to provide genetic information about the level of genetic variability within farmed and wild Egyptian *Mugilidae* family.

MATERIALS AND METHODS

Fish samples

Two hundred and twenty five samples of three mullet species, 15 populations of family Mugilidae (*Mugil cephalus*, *Liza ramada*, and *Valamugil seheli*) were randomly collected from commercial landings and fish farms from three different lakes in Egypt: Qarun Lake (29°25`34" N , 30°34`49" E), Manzala Lake (31°15'36" N, 31°50'54" E), and Burullus Lake(31°28'35" N, 30°51'35" E). Sampling from fish mullet farms was carried out from farms adjacent to Qarun, Manzala and Burullus lakes (Figure 1). This study did not include *Valamugil seheli* from farms because they are not used for aquaculture in the regions of this study. The samples were identified based on morphological characters following **Saleh (2008)** and **Durand *et al.* (2012)** and were photographed using a digital camera (Figure 2).

DNA extraction and quantification

Caudal fin clips were cut from representative family *Mugilidae* specimens. The tissues were immediately placed in separate 2 ml polypropylene tubes containing 95% ethanol for storage until conducting RAPD-PCR analysis. DNA was isolated from fin tissue using a standard Phenol/Chloroform extraction method **(Sambrook *et al.*, 1989)**. The DNA samples were checked for quantity and quality by 1% agarose gel electrophoresis and spectrophotometer respectively. The DNA samples were diluted to 20 ng/µl then suspended in TE buffer (50 mM Tris-HCL; 10mM EDTA) and then stored at 4°C until used.

Fig. 1. Map of Egypt showing the three sampling sites.

Fig. 2. Illustration the three mullet species which used in the present study, a. *Mugil cephalus*, b. *Liza ramada*, and c. *Valamugil seheli*

RAPD-PCR analysis

Out of the 100 RAPD primers (Five kits: kit OPA, kit OPB, kit OPC, kit OPD and kit OPH) Operon Technologies, Almeda were tested, only 14 primers giving sharp, polymorphic, reproducible bands were selected to estimate of genetic diversity and phylogenetic analysis between wild and farmed mullet populations (Table 1). Using Thermocycler GeneAmp® PCR System 9700. PCR reactions were conducted in 25µl,

containing PCR master mix 12 µl, double distilled water10 µl, random primer 1 µl, and 2 µl template DNA (40ng). RAPD-PCR conditions consist of an initial denaturation for 5 min at 94° C, then 40 cycles: 30 s at 94°C, 30 s at 44°C, 30 s at 72°C and final extension for 7 min at 72°C. RAPD-PCR products were electrophoresed on 1.5% agarose gel in 1 X TBE buffer system. The gels were stained by ethidium bromide. Then gels were imaged by a Kodak Science 120ds Imaging System. GeneRuler 100bp DNA ladder was used to estimate the size of the RAPD bands.

Data analysis

In this study, RAPD bands were scored as present "1" or absent "0", carried out the data to a binary matrix. For each RAPD markers, total number of bands, level of polymorphism, genetic variability, and genetic diversity indices **(Nei, 1978)** were calculated by using POPGENE Version 1.32 Software **(Yeh *et al.*, 1999)** and GenAlEx Version 6.5 Software **(Peakall and Smouse, 2012)** programs. Genetic distances and phylogenetic tree in all mullet populations were analyzed using MEGA software version 5 **(Tamura *et al.*, 2011)**.

RESULTS AND DISCUSSION

1. Genetic diversity

The genetic diversity of populations is an important way for successful management of these species and provides a useful vision into breeding programs development in fish species. Despite the economic importance of mullet, there is little information available on the phylogenetic relationships and genetic variation among and within mullet species in Egypt. Therefore, the aim of the present work was to explore the genetic differentiation among and within *M. cephalus*, *L. ramada*, and *V. seheli* populations collected from three geographical sites (Qarun Lake, Manzala Lake, and Burullus Lake) using RAPD markers data. According to DNA molecular markers, random amplified polymorphic DNA (RAPD) is the dominant marker, quickest, cheapest, and can determine high levels of DNA polymorphisms system **(Bartish *et al.*, 2000; Hassanien *et al.*, 2004 and Hassanien and Al-Rashada, 2019)**. Of fourteen 10-mer primers examined for 15 mullet populations (225 specimens), generated a total of 191 reproducible and analysable RAPD bands, with an average of 13.64 bands per primer. All these primers gave polymorphic RAPD bands patterns among the three studied mullet species, generating a total of 115 bands (60.21%) (Table 1). The molecular size of DNA fragments ranged from 300 (OPA-11) to 1500 bp (OPD-07). The number of polymorphic bands ranged between 6 bands (OPA-05) to 11 bands (OPB-3). Figure 3 shows representative profiles obtained with the RAPD marker (OPA-11). The percentage of polymorphism in wild *Mugil cephalus* populations from Qarun, Burullus, and Manzala lakes were high, (75.10%, % 62.31 and 57.36%, respectively). **Suresh *et al.* (2013)** concluded that RAPD data will be supportive for *M. cephalus* when developing a superior strain for aquaculture.

Mugil cephalus populations are widely distributed in different parts of the world. Grey mullet is a complex species because of great differing spawning seasons, nursery areas, occupied habitats, and migratory behavior **(Rossi *et al.*, 1996 and Whitfield *et al.*, 2012)**.

RAPD results suggested close relationship between *Mugil cephalus* Qarun farms and *Mugil cephalus* Qarun wild populations and separated *Mugil cephalus* Qarun wild population as an out-group of *Mugil cephalus* Manzala and Burullus wild populations (Table 2).

Table 1. RAPD primers used in genetic diversity analysis of *Mugilidae* populations.

Primer	Sequence	Total loci*	Polymorphic loci	Polymorphism (%)
OPA-05	AGGGGTCTTG	13	6	46.15
OPA-08	GTGACGTAGG	16	7	43.75
OPA-09	GGGTAACGCC	12	8	66.67
OPA-11	CAATCGCCGT	11	7	63.64
OPB-03	CATCCCCCTG	15	11	73.40
OPB-07	GGTGACGCAG	17	9	52.94
OPB-11	GTAGACCCGT	10	7	70.00
OPB-17	AGGGAACGAG	14	9	64.28
OPB-18	CCACAGCAGT	16	10	62.50
OPC-10	TGTCTGGGTG	12	7	58.40
OPD-02	GGACCCAACC	17	10	58.82
OPD-07	TTGGCACGGG	14	8	57.14
OPH-05	AGTCGTCCCC	9	7	77.80
OPH-08	GAAACACCCC	15	9	60
Total		191	115	60.21
Mean per primer		13.64	8.21	

*. Numbers of total loci produced through PCR amplification and of polymorphic for all samples.

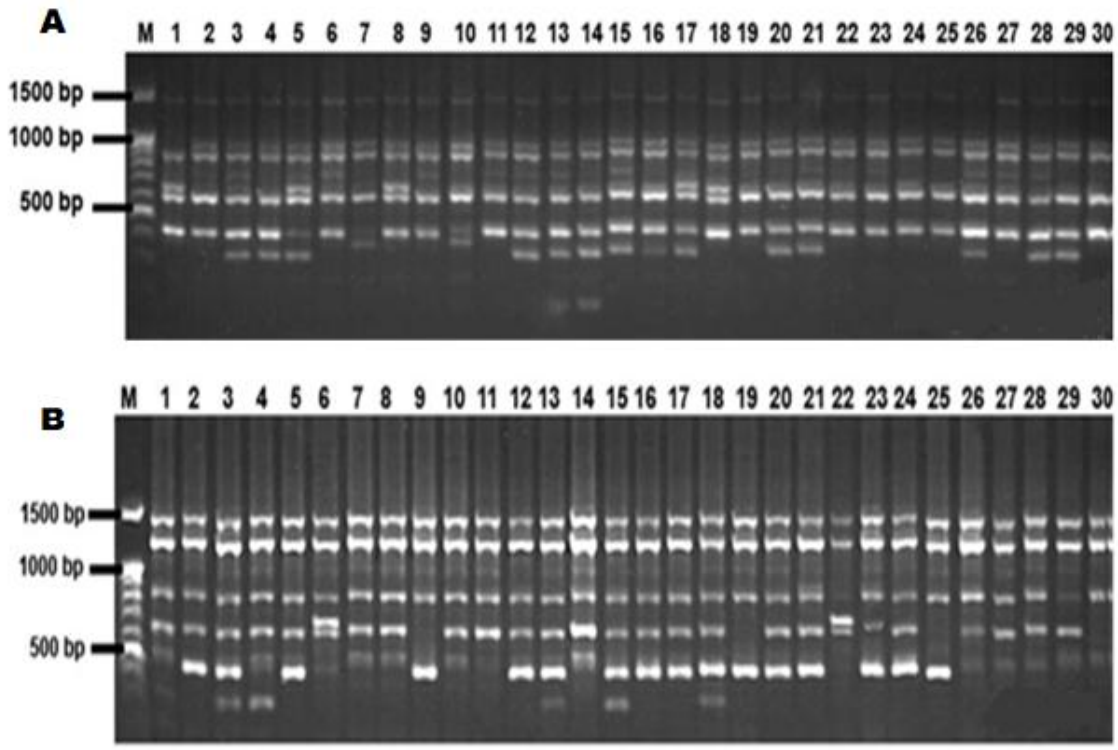

Fig. 3. RAPD gel profile with fragment generated by primer OPA-11. (M): marker 100 bp DNA Ladder, A (1-15) wild Qarun *M. cephalus* and (16-30) farmed Qarun *M. cephalus*. B (1-15) wild Qarun *L. ramada* and (16-30) and farmed Qarun *L. ramada.*

Durand *et al.* (2013) reported that genetic divergence of *Mugil cephalus* populations in the Mediterranean and Black Seas was very low (0.6%) indicating the same strain in this area. Primers which generated polymorphic amplification, produced high percentage of polymorphism in wild *Liza ramada* Qarun, Manzala and Burullus lakes, (73.10%, 60.51% and 55.84% respectively). There were close relationship between *Liza ramada* Qarun wild and *Liza ramada* Qarun farms populations which separated *Liza ramada* Qarun wild population as an out-group of *Liza ramada* Manzala and Burullus wild populations (Table 2). The primers that generated polymorphic amplification, produced high percentage of polymorphism in *Valamugil seheli* populations from Manzala, Burullus and Qarun lakes, (70.11 %, 69.35%, and 66.80%, respectively). There were close relationship between *Valamugil seheli* Burullus wild and *Valamugil seheli* Qarun wild populations which separated *Valamugil seheli* Manzala wild population as an out-group of *Valamugil seheli* Burullus and Qarun wild populations. The heterozygosity (He) ranged between 0.249 (wild *Mugil cephalus* from Manzala lake) to 0.361 (wild *V. seheli* from Burullus lake). For overall species, observed alleles, effective number of alleles (Ne), Shannon index (I) and the heterozygosity (He) were 1.71, 1.51, 0.237 and 0.322 respectively (Table 2). Molecular markers and their statistical analysis play an important role to describe genetic structure. DNA molecular marker systems, dominant or co-dominant (mtDNA or nuclear DNA such as RAPD, ISSR, AFLP, SSR, or SNP) are now being used in fisheries conservation and aquaculture **(Chauhan and Rajiv, 2010)**.

2. Genetic variation

Genetic variation within population indicated highest genetic variation within *Mugil cephalus* Qarun population 32.99% and lowest genetic variation within *Mugil cephalus* Manzalla population 24.97% and *Mugil cephalus* Burullus population had intermediate value 29.93%, respectively. *Liza ramada* Manzala population had indicated highest genetic variation 37.08%, lowest genetic variation within *Liza ramada* Burullus population 30.09% and *Liza ramada* Qarun population had intermediate value 34.25%, respectively. *Valamugil seheli* Burullus population had highest genetic variation 36.18%, lowest genetic variation was observed within *Valamugil seheli* Qarun population 31.56%, while, *Valamugil seheli* Manzala population had intermediate value 35.33%, respectively. Assuming that Qarun lake populations originated from the same founder stock in Manzalla and Burullus lakes, the genetic drift and natural selection forces may have accounted for the reduction of genetic diversity within Qarun lake populations. The same pattern of geographic differentiation was found between Manzalla and Burullus lakes. **Sun *et al.*, (2012)** studied the genetic variability of *Mugil cephalus* in china sea using mtDNA marker. They reported that Bohai and east China populations had lower genetic variation (0.0725) than that of the southern population (South China) (from 0.4530 to 0.6827), concluding two discriminated genetic populations in Chinese waters.

Table 2. Average genetic diversity indices in different *Mugilidae* populations generated by 14 RAPD loci.

Site	Species	Poly (%)	Na	Ne	He	I
Qarun Lake	*M. cephalus* wild	75.10	1.73	1.41	0.329	0.290
	M. cephalus farmed	74.23	1.76	1.59	0.326	0.211
Manzala Lake	*M. cephalus* wild	57.36	1.57	1.29	0.249	0.197
	M. cephalus farmed	60.45	1.75	1.54	0.308	0.203
Burullus Lake	*M. cephalus* wild	62.31	1.68	1.32	0.299	0.205
	M. cephalus farmed	64.38	1.8	1.55	0.312	0.243
Qarun Lake	*L. ramada* wild	73.10	1.83	1.61	0.322	0.265
	L. ramada farmed	72.90	1.85	1.60	0.344	0.293
Manzala Lake	*L. ramada* wild	60.51	1.75	1.68	0.350	0.271
	L. ramada farmed	62.25	1.85	1.57	0.328	0.178
Burullus Lake	*L. ramada* wild	55.84	1.69	1.50	0.300	0.254
	L. ramada farmed	58.36	1.75	1.62	0.338	0.253
Qarun Lake	*V. seheli*	66.80	1.55	1.46	0.315	0.222
Manzala Lake	*V. seheli*	70.11	1.60	1.44	0.353	0.201
Burullus Lake	*V. seheli*	69.35	1.60	1.47	0.361	0.274
	Overall	**65.53**	**1.71**	**1.51**	**0.322**	**0.237**

Poly (%) =percentage of polymorphism, Na=observed alleles, Ne=effective alleles, He=heterozygosity, I=Shannon index

3. Dendrogram analysis and genetic distance

Dendrogram linked the three species of mullet family *Mugilidae* in three selected sites farms and wild populations. Wild *Mugil cephalus* Manzala lake and wild *Mugil cephalus* Burullus populations (genetic distance was 0.0286) were separated from wild *Mugil cephalus* Qarun population as an out-group. RAPD results suggested close relationship between Manzalla and Burullus populations (Table 3 and Figure 4). Wild *Liza ramada* Manzala lake and wild *Liza ramada* Burullus lake populations (genetic distance was 0.0493) were separated from wild *Liza ramada* Qarun lake population as an out-group. RAPD results suggested close relationship between *Liza ramada* populations from Manzalla and Burullus lakes. *Valamugil seheli* Burullus lake and *Valamugil seheli* Qarun populations (genetic distance was 0.045) were separated from *Valamugil seheli* Manzala wild population as an out-group. RAPD results suggested close relationship between Burullus and Qarun populations.

Phylogenetic tree obtained from the analysis of RAPD loci screened in the 15 mullet species populations showed a clustring of populations according to species between *Liza ramada* Qarun wild and farms population and clustring of populations according to species between *Mugil cephalus* Qarun wild and farms population. A close correspondence can be seen between the clustering of populations distinguished (Figure 4). Inter-populations comparisons among Qarun, Manzalla and Burullus populations, *Liza ramada* Qarun wild and farms population and *Mugil cephalus* Qarun wild and farms population showed the most similarity RAPD profiles while, *Mugil cephalus* wild populations from Manzalla and Burullus were more closely related to each other and also *Liza ramada* wild populations from Manzalla and Burullus were more closely related to each other. The lower genetic distance between populations of Manzalla and Burullus lakes than between those of Qarun lake populations could be demonstrated by the impact of man changes factors, salinity, and morphology on lake ecology **(Hassanien *et al.*, 2004 and Hassanien and Gilbey 2005)**. **Thorpe and Sol-Cave (1994)** concluded that genetic distance values for conspecific populations averaged 0.05 (from 0.002 to 0.07), for congeneric species averaged 0.30 (from 0.03 to 1.61), and for confamilial genera ranged from 0.58 to 1.21. The genetic distance values obtained in the present study fall within the range of conspecific, and congeneric species. **Liu *et al.*, (2010)** studied the phylogenetic relationships of mullet revealed by mtDNA marker. They found that *Mugil cephalus* is the most genetically diverse species among the mullet species. **Siccha-Ramirez *et al.*, (2014)** reported that genetic distance among 12 populations of mullet species (Mugil liza) collected from the Atlantic South Caribbean and South America were very high among species (from 3.0 to 20.1%) and very low within species (from 0.03 to 0.4%) and that all specimens analyzed of this species belong to a single population. Finally, in natural populations, it is difficult to isolate the effect of genetic, and non-genetic factors and these factors can influence these types of traits in fish **(Feral, 2002)**.

Table 3. Genetic distance matrix among 15 mullet populations based on RAPD data.

	MQW	MQF	MMW	MMF	MBW	MBF	LQW	LQF	LMW	LMF	LBW	LBF	VQW	VMW	VBW
MQW	0														
MQF	0.054	0													
MMW	0.138	0.174	0												
MMF	0.076	0.084	0.073	0											
MBW	0.130	0.139	0.028	0.104	0										
MBF	0.142	0.120	0.091	0.111	0.055	0									
LQW	0.058	0.075	0.072	0.034	0.056	0.054	0								
LQF	0.097	0.097	0.090	0.055	0.065	0.038	0.014	0							
LMW	0.115	0.091	0.143	0.082	0.102	0.073	0.054	0.040	0						
LMF	0.114	0.108	0.119	0.054	0.092	0.079	0.019	0.017	0.060	0					
LBW	0.136	0.137	0.218	0.137	0.153	0.119	0.076	0.065	0.049	0.084	0				
LBF	0.170	0.197	0.163	0.182	0.120	0.138	0.147	0.139	0.100	0.154	0.185	0			
VQW	0.119	0.187	0.219	0.161	0.192	0.201	0.151	0.152	0.124	0.153	0.188	0.069	0		
VMW	0.129	0.149	0.187	0.155	0.158	0.141	0.140	0.124	0.090	0.148	0.146	0.047	0.056	0	
VBW	0.090	0.106	0.200	0.114	0.161	0.184	0.112	0.118	0.076	0.110	0.142	0.074	0.045	0.053	0

MQW, *Mugil cephalus* Qarun wild, MQF, *Mugil cephalus* Qarun farm, MMW, *Mugil cephalus* Manzala wild, MMF, *Mugil cephalus* Manzala farm, MBW, *Mugil cephalus* Burullus wild, MBF, *Mugil cephalus* Burullus farm, LQW, *Liza ramada* Qarun wild, LQF, *Liza ramada* Qarun farm, LMW, *Liza ramada* Manzala wild, LMF, *Liza ramada* Manzala farm, LBW, *Liza ramada* Burullus wild, LBF, , *Liza ramada* Burullus farm, VQW, *Valamugil seheli* Qarun wild, VMW, *Valamugil seheli* Manzala wild, VBW, *Valamugil seheli* Burullus wild.

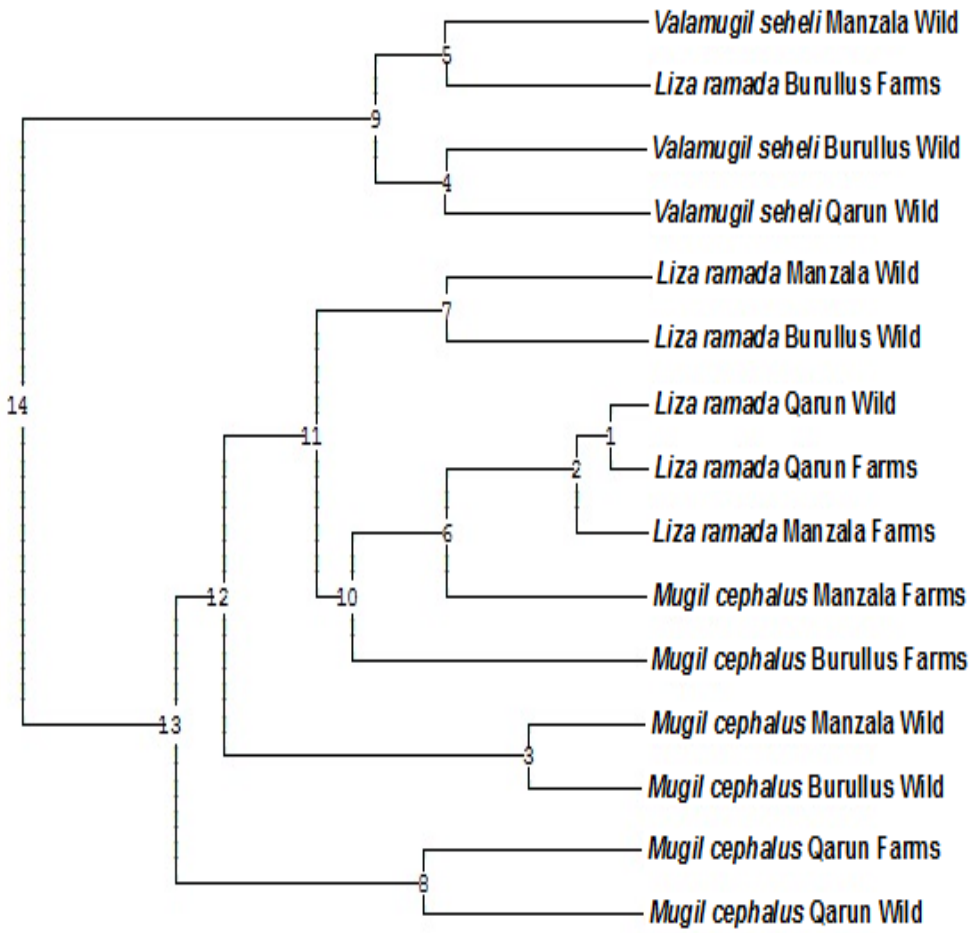

Fig. 4. UPGMA dendrogram of the 15 populations of mullet based on values of genetic distances calculated from RAPD data.

CONCLUSION

RAPD data revealed genetic differentiation among and within populations of *Mugil cephalus*, *Liza ramada* and *Valamugil seheli* populations in Egypt. There was a higher genetic variation within *Mugil cephalus*, *Liza ramada and Valamugil seheli* wild populations. This may have been caused by the forces of genetic drift and natural selection pressure. Further genetic studies on mullet populations in Egypt are needed with other DNA markers such as microsatellites marker (SSR) or mitochondria DNA markers (mtDNA). The results of these studies will give more complete picture of the genetic resources of mullet in Egypt.

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REFERENCES

Bartish I. V.; Garkava L. P.; Rumpunen K. and Nybom H. (2000). Phylogenetic relationships and differentiation among and within populations of *Chaenomeles Lindl* (Rosaceae) estimated with RAPDs an isozymes. Theor. Appl. Genet.,101**:** 554-561

Behrouz, M.; Norouzi, M.; Samiei, M. H., and Heshmatzad, P. (2018). Microsatellite analysis of golden grey mullet *Chelon auratus (*Risso, 1810) in the Fereydoon-Kenar and Ramsar coasts (south caspian sea, Iran). Croatian Journal of Fisheries: Ribarstvo, 76: 35-40.

Chauhan, T. and Rajiv, K. (2010). Molecular markers and their applications in fisheries and aquaculture. Advances in Bioscience and Biotechnology,1:281-291.

Durand, J. D.; *Shen*, K. N.; Chen, W. J.; Jamandre, B. W.; Blel, H.; Diop, K.; Nirchio, M.; Garcia de León, F. J.; Whitfield, A. K.; Chang, C. W. and Borsa, P. (2012). Systematics of the grey mullets (Teleostei: Mugiliformes: Mugilidae): Molecular phylogenetic evidence challenges two centuries of morphology-based taxonomy. Molecular Phylogenetics and Evolution, 64:73-92.

Durand, J. D.; Blel, H.; Shen, K. N.; Koutrakis, E. T. and Guinand, B. (2013). Population genetic structure of *Mugil cephalus* in the Mediterranean and Black seas: a single mitochondrial clade and many nuclear barriers. Marine Ecology Progress Series, 474: 243-261.

El-Sayed, A. M. (2015). Regional review on status and trends in aquaculture development in the Near East and North Africa – 2015, by. FAO Fisheries and Aquaculture Circular No. 1135/6. Rome, Italy.

Feral, J. P. (2002). How useful are the genetic markers in attempts to understand and manage marine biodiversity? Journal of Experimental Marine Biology and Ecology, 268:121-145.

GAFRD (General Authority for Fishery Resources Development). (2018). Fish Statistics Yearbook 2016. Ministry of Agriculture and Land Reclamation, Egypt.

Hassanien, H. A., and Al-Rashada, Y. (2019). Estimation of genetic variation in green tiger prawn, Penaeus semisulcatus by using random amplified polymorphic DNA, inter simple sequence repeat and simple sequence repeat markers. Aquaculture Research, 50:1626-1633.

Hassanien, H. A.; Elnady, M.; Obeida, A. and Itriby, H. (2004). Genetic diversity of Nile tilapia populations revealed by randomly amplified polymorphic DNA (RAPD) Aquaculture Research, 35: 587-593.

Hassanien, H. A., and Gilbey, J. (2005). Genetic diversity and differentiation of Nile tilapia (*Oreochromis niloticus*) revealed by DNA microsatellites. Aquaculture Research, 36:1450-1457.

Huei-Mien; Wen-Wen Lin, and Hsiao-Wei Kao., (2009). Genetic diversity and differentiation of gray mullet (*Mugil cephalus*) in the coastal waters of Taiwan. Zoological science, 26: 421-428.

Jahromi, S. T. and Othman, A. S. (2011). Isolation and characterization of novel microsatellite loci in green tiger shrimp (*Penaeus Semisulcatus*). International Journal of Life Science and Pharma Research, 3:23-34

Jamandre, B. W.; Durand, J. D. and Tzeng, W. N. (2009). Phylogeography of the flathead mullet *Mugil cephalus* in the north- west Pacific as inferred from the mtDNA control region. Journal of fish biology, 75:393-407.

Liu, J. Y.; Brown, C. L. and Yong, T. B. (2010). Phylogenetic relationships of mullets (*Mugilidae*) in China Seas based on partial sequences of two mitochondrial genes. Biochemical Systematics and Ecology, 38:647-655.

Livi, S.; Sola, L. and Crosetti, D. (2011). Phylogeographic relationships among worldwide populations of the cosmopolitan marine species, the striped gray mullet (*Mugil cephalus*), investigated by partial cytochrome b gene sequences. Biochemical Systematics and Ecology, 39:121-131.

Kapila, R. and Mishra, D. P. (2006). Randomly amplified polymorphic DNA (RAPD) fingerprinting of *Schizothorax richardsonii* (Gray). Indian Journal of Fisheries, 53:219-224.

Mai, A. C.; Mino, C. I.; Marins, L. F.; Monteiro-Neto, C.; Miranda, L.; Schwingel, P. R. and Vieira, J. P. (2014). Microsatellite variation and genetic structuring in Mugil liza (*Teleostei: Mugilidae*) populations from Argentina and Brazil. Estuarine, Coastal and Shelf Science, 149:80-86.

Miggiano, E.; Lyons, R. E.; Li, Y., Dierens, L. M.; Crosetti, D. and Sola, L. (2005). Isolation and characterization of microsatellite loci in the striped mullet, *Mugil cephalus.* Molecular Ecology Notes, 5:323-326.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89:583-590.

Peakall, R. and Smouse, R. P. P. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28: 2537-2539.

Rossi, A. R.; Crosetti, D.; Gornung, E. and Sola, L. (1996). Cytogenetic analyses of global populations of *Mugil cephalus* (striped mullet) by different staining techniques and fluorescent in situ hybridization. Heredity, 76:77-82.

Rossi, A. R.; Capula, M.; Crosetti, D.; Campton, D. E. and Sola, L. (1998). Genetic divergence and phylogenetic inferences in five species of *Mugilidae (Pisces: Perciformes)*. Marine Biology, 131:213-218.

Saleh, M. (2008). Capture-based aquaculture of mullets in Egypt. In *A. Lovatelli* and *P.F. Holthus* (eds). Capture-based aquaculture. Global overview. FAO Fisheries Technical Paper. No. 508. Rome, FAO. pp. 109-126.

Sambrook, J, F. and Maniatis, T. (1989). Molecular cloning, a laboratory manual, 2nd edition cold spring harbor laboratory press, Cold Spring Harbor, N.Y. pp. 528-532.

Shen, K-N.; Jamandre, B. W.; Hsu, C-C.; Tzeng, W-N. and Durand, J-D. (2011). Plio-Pleistocene sea level and temperature fluctuations in the northwestern Pacific promoted speciation in the globally-distributed flathead mullet *Mugil cephalus*. BMC, Evol. Biol., 11:83-95.

Siccha-Ramirez, R.; Menezes, N. A.; Nirchio, M.; Foresti, F. and Oliveira, C. (2014). Molecular identification of mullet species of the Atlantic south Caribbean and south America and the phylogeographic analysis of *Mugil liza* , Reviews in Fisheries Science and Aquaculture, 22:86-96.

Suloma, A. and Ogata, H. Y. (2006). Future of rice-fish culture, desert aquaculture and feed development in Africa: The case of Egypt as the leading country in Africa. JARQ, 40:351-360.

Sun, P.; Shi, Z.; Yin, F. and Peng, S. (2012). Genetic variation analysis of *Mugil cephalus* in China sea based on mitochondrial COI gene sequences. Biochem Genet., 50:180-191.

Suresh, E.; Tiwari, V. K.; Sekar, M.; Sankar, M. and Kathirvelpandian, A. (2013). Genetic structure of populations of *Mugil cephalus* using RAPD markers. African Journal of Biotechnology, 12:6262-6266.

Tamura, K.; Peterson, D.; Peterson, N.; Stecher, G.; Nei's M. and Kumar, S. (2011). Mol Biol Evol, 28:2731–2740.

Thorpe, J. P. and sol-Cava, A. M. (1994). The use of allozyme electrophoresis in vertebrate systematics. Zool. Scripta., 23:3-18.

Whitfield, A. K.; Panfili, J. and Durand J. D. (2012). A global review of the cosmopolitan flathead mullet *Mugil cephalus* Linnaeus 1758 (Teleostei: Mugilidae), with emphasis on the biology, genetics, ecology and fisheries aspects of this apparent species complex Rev. Fish Biol. Fisheries, 22: 641-681.

Xu, T. J.; Sun, D. Q.; Shi, G. and Wang, R. X. (2010). Development and characterization of polymorphic microsatellite markers in the gray mullet (*Mugil cephalus*). Genet. Mol. Res., 9:1791-1795.

Yeh, F. C.; Yang, R. C. and Boyle, T. (1999). POPGENE version 1.32 Microsoft windows-based freeware for population genetic analysis. University of Alberta and centre for international forestry research, Alberta, Canada, 28pp.