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Eco-taxonomic study for some tuna genotypes collected from Egyptian and Libyan coasts on the molecular level

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ABSTRACT

This study aimed at assessing the genotype variation among six tuna fish collected from certain Libyan and Egyptian coastal regions and the molecular characterization due to the prevailing ecological parameters using SRAP and ISSRs. In addition, the current work addressed the impact of some ecological factors in water for tuna biodiversity analysis. ISSR analysis is useful for the assessment of genetic diversity among the genotypes of bluefin tuna. It is possible to conclude that, the short the distance between any two locations, the more they increase the similarity between the two genotypes collected from these two locations. SRAP analysis is useful for assessing the genetic diversity among the genotypes of bluefin tuna. The SRAP results confirmed that when the distance between the locations increases, the tuna genotypes become distantly related and vice versa. The ISSR and SRAP combined data revealed that the highest similarity value among these six tuna genotypes (0.90) was recorded between ELkhoms and Serit genotypes (the two most closely related genotypes). On the other hand, the lowest value (0.60) was recorded between the Egyptian genotype and the Sebrata genotype and between the Egyptian genotype and ELkhoms genotype, with the same similarity value (the two most distantly related genotypes). It is possible to conclude that, the similarity value increased as much as distances between locations decreased and vice versa. The ecological impact of the four measured ecological factors revealed that the variation in these factors could act as barriers that decrease the tuna fish migration and thus decrease the chances of mating between the different genotypes among the different locations. This low chance of mating between the tuna fish genotypes could lead to the speciation proved by means of molecular investigation.

INTRODUCTION

Thunnus (*Perciformes, Scombridae* tunas) are highly migratory fishes that are mainly distributed in tropical and temperate oceans. **Abu-Almaaty *et al*. (2017)** determined the molecular genetic variations among three species of family Osphronemidae; namely, *Trichogaster trichopterus, Trichogaster leeri* and *Colisa lalia*by using ISSR-PCR. The previous researchers collected samples from ornamental fish farms in Egypt. The ISSR-

PCR analysis was carried out using ten primers; the results indicated that ISSR analysis are very useful in the determination of genetic molecular variations and genetic relationships among the different species which belong to the same family.

Labastida-Estrada *et al*. (2019) stated that the genetic analysis of lionfish collected from the Mexican coasts was addressed to detect their connectivity with other Caribbean localities (Belize, Cuba, Puerto Rico) and determine the role of ocean currents on population structure. They collected 213 lionfish samples from seven locations in four countries. To evaluate genetic structure, mitochondrial control region and nuclear inter-simple sequence repeat (ISSR) markers were used. They found that lionfish collected from the Mexican coasts showed a similar haplotype composition (H02 followed by H01 and H04) to other Caribbean locations, and the H03 rare haplotype was not found. Haplotype composition in the southwest Gulf of Mexico suggests a discontinuity between the southern and northern areas of the Gulf of Mexico. The southern area is clustered more strongly to the Caribbean region, and this is supported by the complexity of water circulation in the semi-enclosed region of the Gulf of Mexico. Mitochondrial genetic diversity parameters show small values; whereas, nuclear markers produce medium to high values. Only nuclear markers highlighted significant genetic differentiation between the southwest Gulf of Mexico and the Caribbean region, confirming a phylogeographic break between both regions. Separate analysis of the Caribbean locations indicates restricted larval exchange between the southern and northern regions of the Mesoamerican Barrier Reef System, potentially in response to regional oceanographic circulation.

Wuping *et al*. (2019) used sequence-related amplified polymorphism (SRAP) markers to assess the genetic diversity and genetic structure of 20 natural populations of *M. oblongifolius* collected from different eco-geographical regions of China. The results revealed a considerable genetic diversity and weak genetic differentiation. Data analyses supported a habitat-specific genetic clustering model for *M. oblongifolius*, indicating a local adaptive divergence for the studied populations.

Takeshi *et al*. (2020) reported that, marine invertebrates with pelagic larvae can migrate for long distances using ocean currents, suggesting reduced genetic diversification. Cluster analysis for *Pinctada fucata*, in the Indo- Pacific Ocean showed that the western Pacific population is distinct from that of the Indian Ocean, and that it is divided into northern (Japanese mainland) and southern (Nansei Islands, China, and Cambodia) populations. Genetic differentiation of *P. fucata* can be explained by geographic barriers in the Indian Ocean and a local lagoon, and by environmental gradients of sea surface temperature (SST) and oxygen concentration in the western Pacific. A genome scan showed evidence of adaptive evolution in genomic loci, possibly associated with changes in environmental factors, including SST and oxygen concentration.

Gaber *et al*. (2020) stated that the genetic relationship between two different subspecies of *T. chloris* occurs in the Arabian Peninsula: *T. c. abyssinicus* from the Red Sea coast

and *T. c. kalbaensis* from the Arabian Sea coast in the United Arab Emirates and Oman assessed using ISSR. The genetic profile or fingerprint for both subspecies was compared using ten primers of the highly polymorphic nuclear markers. They distinguished between the specimens of the two subspecies using ISSR markers. These results suggested that *T. c. abyssinicus* and *T. c. kalbaensis* are not identical, and thus they belong to different subspecies.

Syahida *et al*. (2020) studied the population genetic diversity and demographic history of the longtail tuna *Thunnus tonggol* collected from 11 localities around the Malaysian coastal waters using mitochondrial DNA. Low genetic differentiation between populations was found, possibly due to the past demographic history, seasonal migration in adults and the lack of geographical barriers. The gene trees revealed a single population with unsupported internal clades, which indicates an absence of structure among the studied populations.

Xiaopei *et al*. (2020) analyzed the genetic diversity among 33 *P. polyphylla* samples using SRAP, revealing the genetic relationships among their resources and providing a theoretical basis for genetic improvement and conservation. The results indicated that, SRAP markers were suitable for the genetic diversity analysis of *P. polyphylla*. Molecular markers such as SRAP have widely been used in genetic diversity research on germplasm resources, genetic relatedness and genetic conservation.

Ağdamar *et al*. (2020) stated that SRAP is an easy, reliable, dominant and iterative way for genetic variation of different species. SRAPs can also be used for assessing invasion genetics and discover variations in genetic structure of native and invasive freshwater fish. They suggested that the SRAPs could be applied to the subjects of invasion genetics.

Wannapimol *et al*. (2021) reported that ISSR is considered as new approach that can contribute to a better monitoring of stranded species. They stated that ISSR-HRM method is a new approach for marine mammal species identification. They developed ISSR markers and recorded a success rate of 100% in terms of discrimination of all marine mammals under investigation. Hence, ISSR-HRM analysis could serve as an effective alternative tool in the species identification process.

(Hassanien & Al-Rashada, 2021) stated that understanding fish genetic characterization plays a vital role in the conservation and utilization of fish genetic resources of grouper species. The authors assessed the genetic diversity and phylogenetic relationships in five grouper species, *Epinephelus* spp. from eastern Saudi Arabian coast using inter simple sequence repeat (ISSR). The results proved that the ISSR markers were highly informative and efficient in detecting genetic variability and relationships of the *Epinephelus* spp.

Ağdamar (2021) conducted (ISSR and SRAP) markers to characterize the genetic performance of gibel carp samples collected from eight locations in western Turkey. The results of ISSR and SRAP markers revealed a low level of gene flow between these populations and inter-population variation, while the rest was at an intra-population level.

These results indicated that this population of the gibel carp is the result of several colonization events originating from the different sources. The genetic relationships among the populations suggested that there were two independent major introduction events, one in the Marmara region and the other in southern Turkey.

Zhu *et al*. (2021) studied the genetic biodiversity and differentiation of three representative goldfish species in Beijing using SRAP. They found that a high polymorphism with a percentage of 80.47 was detected. Diversity indexes of Nei's genes were determined in short-tailed bubble-eye goldfish, redhead goldfish and black dragon-eye goldfish, with values of 0.225, 0.208 and 0.238, respectively. The genetic distance indicated that genetic divergence existed between the three groups. The two most distantly related groups were bubble-eye goldfish and redhead goldfish, while redhead goldfish and black dragon-eye goldfish were the most two closely related groups.

This study aimed at assessing the genotypes variation among some tuna fish collected from certain Libyan and Egyptian coastal regions and determining the molecular characterization due to prevailing ecological parameters. Moreover, to study the genetic relationships among these different tuna fish under investigation, the molecular level was addressed using SRAP and ISSR techniques. Additionally, this study was conducted to draw the consensus tree and calculate the similarity index among these six genotypes.

Tunas are significant economic species; however, they are recently facing population collapse **(Fromentin & Powers, 2005; MacKenzie *et al*. 2009)**. Regional tuna fishery organizations have taken management actions and set quotas in different countries to protect tuna resources for sustainable use.

The methodology is usually based on Polymerase Chain Reaction (PCR), which targets a specific genetic marker that is able to discriminate species. Some of the methodologies described to date focus on reducing protocol steps and avoiding DNA sequencing **(Rasmussen & Morrissey, 2008)**.Nevertheless, most studies require detailed knowledge on the DNA sequences of target species prior to setting-up the methodology, and in insecure cases, the final assignation should always be validated afterwards by DNA sequencing **(Rob, 2008)**.

Pecoraro *et al*. (2016) illustrated for the first time, the utility of 2b-RAD genotyping technique for investigating population genetic diversity in high gene-flow species. Running de novo pipeline in *Stacks*, a total of 6772 high-quality genome-wide SNPs were identified across the Atlantic, Indian and Pacific population samples representing all the major distribution areas. Preliminary analyses showed significant population structure among oceans. Discriminant Analysis of Principal Components endorsed the presence of genetically discrete yellowfin tuna populations among three oceanic pools. These results confirmed the efficiency of this genotyping technique in assessing genetic divergence in a marine fish with high dispersal potential.

Brophy *et al*. (2018) studied two stocks of bluefin tuna (*Thunnus thynnus*) collected from the North Atlantic; the western and eastern stocks spawn in the Gulf of Mexico and

the Mediterranean Sea, respectively. They reported that, the otolith shape could be used in combination with other population markers to improve the accuracy of mixing rate estimates for the Atlantic bluefin tuna.

MATERIALS AND METHODS

Tuna fish samples were collected from seven different locations of the Mediterranean Sea along the two countries (Egypt and Libya) extending from Dumyat in the East to Sebrata in the West, which is located between longitudinal 12.483824 west to 31.684428 east. The locations and their longitudinal and altitudinal lines are shown in Table (1). Molecular analysis was conducted in the laboratories of the Genetics Department, Faculty of Agricultural, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt.

Table 1. Locations of samples collection and their longitudinal and altitudinal lines

Location	altitudinal	longitudinal
Sebrata	**32.819337**	**12.483824**
Tripoli	**32.919314**	**13.174221**
ELkhoms	**32.658553**	**14.270410**
Misratah	**32.415534**	**15.107958**
Seret	**31.215221**	**16.558945**
Bin Ghazi	**32.130648**	**20.048561**
Dumyat	**31.506771**	**31.684428**

Molecular genetic analysis

The extracted DNA using DNeasy animal tissue Mini Kit (Biobasic com.) of the healthy fish tissues of each of the six different and afore-mentioned locations were selected and subjected to molecular investigation using two molecular approaches; namely, the inter simple sequence repeats (ISSR) and the sequence related amplified polymorphism (SRAP) analysis. The quality of the extracted DNA was tested using 1.2% agarose /TB buffer as described in **Sambrook *et al*. (1989)**. The extracted DNA was quantified using spectrophotometer on UV lengths of 260/280nm. **Inter simple sequence repeats (ISSR):** PCR amplification was performed using eleven primers with the following sequences Table (2). PCR was performed in 30-µl volume tubes according to **Zietkiewicz *et al.* (1994)**. PCR products were dissolved in agarose gel, and banding patterns were recorded as 1 for band presence and 0 for band absence.

Table 2. The 12 used ISSR primers names and sequences

ID	Sequence	ID	Sequence
807	5’ AGA GAG AGA GAG AGA GT 3`	HB-10	5` GAG AGA GAG AGA CC 3`
98A	5` CA CA CA CA CA CA AC 3`	HB-11	5` GTG TGT GTG TGT CC 3`
49B	5` CAC ACA CAC ACA GG 3`	HB-12	5`CAC CAC CAC GC 3`
HB-1	5` CAA CAA CAA CAA CAA 3`	HB-13	5´ GAG GAG GAG GC 3`
HB-4	5` GAC AGA CAG ACA GACA 3`	HB-15	5´ GTG GTG GTG GC 3`
HB-9	5`GTG TGT GTG TGT GC 3`	HB-10	5` GAG AGA GAG AGA CC 3`

Sequence Related Amplified Polymorphism (SRAP): PCR amplification was performed using seven primer pairs with the following sequences, as shown in Table (3). PCR was performed in 30-µl volume tubes according to the study of **Yang and Quiros (1993)**. PCR products were dissolved in agarose gel, and banding patterns were recorded as 1 for the band presence and 0 for the band absence.

Table 3. List of names and sequences of the 7 used SRAP primer pairs

NO.	Primer	Forward Primers (5′→ 3′)	Primer	Reverse Primers (5′→ 3′)
1	Me-2	TGAGTCCAAACCGGAGC	Em-5	GACTGCGTACGAATTAAC
2	Me-4	TGAGTCCAAACCGGACC	Em-4	GACTGCGTACGAATTTGA
3	Me-5	TGAGTCCAAACCGGAAG	Em-3	GACTGCGTACGAATTGAC
4	Me-6	TGAGTCCAAACCGGACA	Em-6	GACTGCGTACGAATTGCA
5	Me-7	TGAGTCCAAACCGGACG	Em-7	GACTGCGTACGAATTCAA
6	Me-9	TGAGTCCAAACCGGAGG	Em-9	GACTGCGTACGAATTCGA
7	Me-10	TGAGTCCAAACCGCAAA	Em-10	GACTGCGTACGAATTCGA

The similarity matrices were done using Gel works ID advanced software UVP-England program. The relationships among genotypes as revealed by dendrograms were done using SPSS windows (Version 10) program. DICE computer package was used to calculate the pairwise difference matrix and plot the phenogram among genotypes **(Yang & Quiros, 1993)**.

Ecological study

Some environmental parameters were measured using the portable multi-meter instrument (Hach hq40d). The measured ecological parameters in water were pH, salinity, dissolved oxygen and temperature.

RESULTS AND DISCUSSION

Two techniques, ISSRs and SRAP, were applied to assess the genetic diversity among six tuna fish genotypes at the molecular level. Sixteen primers of the two used techniques were selected for the molecular studies of genetic diversity assessment among the six blue fin tuna fish genotypes from six different locations in the Mediterranean Sea. Nine ISSR primers and seven SRAP primers gave prominent and reproducible banding patterns.

Biodiversity assessment using ISSRs technique

The nine primers successfully amplified DNA fragments for the six genotypes. A total number of 45 fragments were observed across the six investigated genotypes. The used primers produced number of bands that ranged from two fragments with primer HB-12 to nine fragments with primer HB-11.

Among the 45 amplified fragments across the 6 blue fin tuna genotypes collected from the six locations using the 9 ISSR primers, 22 common bands (monomorphic bands) and 23 bands were polymorphic with a total ratio of polymorphism (51.11%). The total

numbers of amplified, monomorphic and polymorphic fragments generated by each primer are shown in Table (4) and Fig. (1). The lowest number of ISSR amplified fragments was detected with primer HB-12 where it generated two bands only. While, the highest numbers of ISSR amplified fragments were detected with primer HB-11, where it emphasized nine bands, followed by primer HB-4 which produced total number of eight bands, two of these fragments were unique in HB-4 and five bands in primer HB-11. Primer HB-12 had no polymorphism, while primer HB-15 produced the highest ratio of polymorphism (75 %). Primers HB-1 gave one negative unique fragment which could be used as negative genotype marker. These results confirmed that ISSRs-PCR analysis are useful for the characterization of genotypes and the assessment of genetic diversity among the genotypes of blue fin tuna collected from the different locations under investigation; this finding coincides with that of **Pecoraro *et al*. (2016)** whothere reported the efficiency of molecular genotyping technique in assessing genetic divergence in a marine fish with high dispersal potential. Furthermore, this result concurs with that of **Abu-Almaaty *et al*. (2017)** who postulated that ISSR analysis is very useful in the determination of genetic molecular variations and genetic relationships among the species which belong to same family.

Table 4. Total bands, (monomorphic and polymorphic bands), negative markers and polymorphism percentage of the 6 tuna genotypes based on nine ISSR primers

Primer Name	Total Bands	Monomorphic bands	Polymorphic bands	Negative markers	Polymorphism %
98A	4	2	2	-	50.00%
807	5	2	3	-	60.00%
HB-1	5	2	3	1	60.00%
HB-4	8	2	6	-	75.00%
HB-9	3	2	1	-	33.33%
HB-10	5	4	1	-	20.00%
HB-11	9	5	4	-	44.44%
HB-12	2	2	-	-	00.00%
HB-15	4	1	3	-	75.00%
Total	**45**	**22**	**23**	**1**	**Mean 51.11%**

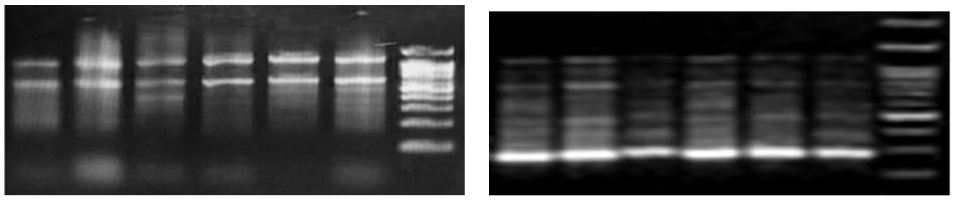

Fig. 1. Banding patterns using ISSR primerHB4 and HB11 for the six tuna fish genotypes collected from (1- Egypt), (2- Sebrata), (3- ELkhoms), (4- Serit), (5- Benghazi), (6- Darnah) and (M- Molecular size Marker)

Genetic similarity indicies and cluster analysis for 6 tuna genotypes using ISSR

The ISSR data were used to estimate the genetic similarity indices among the six tuna genotypes which collected from different locations Table (5). The highest similarity value 0.990 was recorded between the two genotypes which collected from El khoms and Serit, followed by the similarity between Serit and Benghazi with value of 0.940. On the other

hand, the lowest similarity values were recorded between the two genotypes which collected from Egypt and Sebrata with value of 0.710 followed by the two genotypes which collected from (Egypt and Serit) and (Egypt and El khoms) with similarity value of 0.720. The high similarity value between the two genotypes which collected from El khoms and serit revealed a kind of closely genetic relationship among these two genotypes. Also, the high similarity value between the two genotypes which collected from Serit and Ben ghazi revealed a kind of closely genetic relationship among these two genotypes based on ISSR data analysis.

Table (5): Dice similarity coefficient of the 6 tuna genotype which collected from the six different locations under investigation based on ISSR data analysis

	Egypt	Sebratah	El khomos	Seret	Benghazi	Darnah
Egypt	1.000					
Sebratah	0.710	1.000				
El khomos	0.720	0.920	1.000			
Seret	0.720	0.900	0.990	1.000		
Benghazi	0.760	0.930	0.930	0.940	1.000	
Darnah	0.920	0.740	0.780	0.790	0.810	1.000

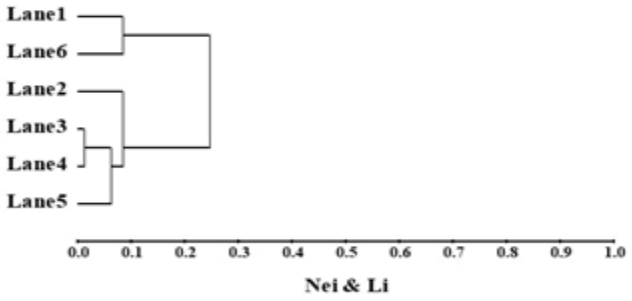

Figure (2): Consensus tree showed the genetic distance among the 6 tuna genotypes which collected from the six different locations under investigation based on ISSR data analysis where (1- Egypt), (2- Sebrata), (3- ELkhoms), (4- Serit), (5- Benghazi) and (6- Darnah)

The consensus tree based on ISSR data is shown in Fig. (2). The consensus tree was divided into two main clusters. The first cluster contain Egypt genotype and Darnah genotype, while the second cluster was divided into two sub-clusters; the first sub-cluster included sebrata genotype, while the second sub-cluster was divided into two sub-sub clusters, the first sub-sub cluster included sebrata while the second sub-sub cluster included El khoms and serit in a branch while Benghazi lays in the second branch of the second sub-sub cluster.

The relationship as detected from the tree indicated that genotype El khoms and serit genotypes were the two most closely related genotypes; moreover it's possible to conclude that (Egypt and Sebrata) genotypes were the two most distantly related genotypes as detected from the consensus tree based on the data analysis of the nine ISSR primers which used in this investigation.

Based on the ISSRs data analysis, it is possible to conclude that, as short as the distance between any two locations under investigation, the similarity between the two genotypes which collected from these two locations increased. This conclusion is in agreement with the results which obtained by **Alexander *et al*. (2018)** who demonstrated that both life- history characteristics and habitat play a role in shaping patterns of genetic diversity in fishes and should be considered when prioritizing species for conservation Furthermore, fishes biodiversity increase need to be contended with the effects of global climate change, which is driving ocean acidification and increases in aquatic temperatures and is expected to impose regional changes to salinity, dissolved oxygen availability, and circulation patterns in aquatic environments **(Crozier and Hutchings, 2014; O'Reilly *et al*., 2015).** Thus, disentangling species that are likely to adapt to future environmental changes from those that will require intervention remains a fundamental challenge for successful conservation and management of fishes.

Molecular assessment for biodiversity using SRAP technique

In this study, seven SRAP primers were used to develop molecular markers to assess the genetic diversity among the 6 tuna genotypes as shown in Table (6). The resulted amplified fragments are shown in Figures (3) and their densitometric analyses are illustrated in Tables (6). All the seven used primers successfully amplified DNA fragments. A total number of 59 fragments were obtained across the six investigated genotypes. Primers produced number of bands ranged from three fragments (ME9xEM9) to fourteen fragments (ME2xEM5) across the genotypes under investigation with total number of produced bands of 59, out of these 15 monomorphic bands and 44 polymorphic bands were obtained. Eight negative markers were produced out of the polymorphic ones with a total ratio of polymorphism (74.57%). The lowest number of SRAP amplified fragments was detected with primer pair ME9xEM9 where it generated three bands only. While the highest numbers of SRAP amplified fragments were detected with primer pair EM2xME5, where it showed 14 bands followed by primer pair ME5xEM3 which produced total number of 13 bands, five fragments were considered as unique bands in EM4xME4 and three bands in primer ME2xEM5. Primer pair ME7xEM7 produced one unique band also primer pair ME9xEM9 produced two unique bands. These results confirmed that SRAP-PCR analysis is useful for characterization genotypes and assessment of genetic diversity among the six genotypes of blue fin tuna. These results was agreed with the previous studies of **Pecoraro *et al*. (2016),** as they showed the efficiency of molecular genotyping technique in assessing genetic divergence in a marine fish with high dispersal potential. Also these results were in agreement with those results which obtained by **Ji *et al*. (2014)** where they studied the genetic diversity of wild and natural populations of (*Megalobrama amblycephala*) fish using SRAP markers and assessed the genetic diversity among three natural populations.

They reported that, SRAP marker technique is a simple and efficient method to quantify genetic diversity within and among fish populations.

Table (6): Total bands, (Monomorphic and Polymorphic bands), negative markers and polymorphism percentage of the 6 tuna genotypes based on seven SRAP primers

Primer name	Total bands	Monomorphic bands	Polymorphic bands	Negative markers	Polymorphism %
EM2xME5	14	3	11	1	78.57%
ME5xEM3	13	0	13	4	100.00%
ME4xEM4	7	5	2	0	28.57%
ME6xEM6	5	1	4	0	80.00%
ME7xEM7	9	1	8	3	88.88%
ME9xEM9	3	2	1	0	33.33%
ME10xEM10	8	3	5	0	62.50%
summation	59	15	44	8	**Mean 67.41%**

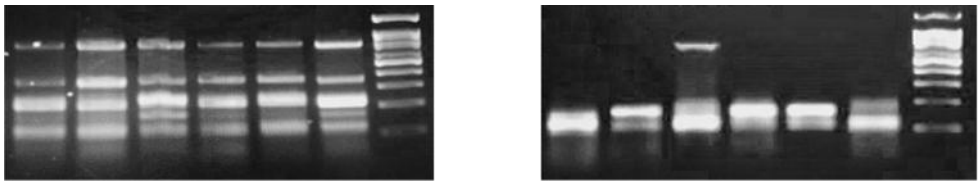

Figure (3): banding patterns using SRAP primer pairs ME4XEM4 and ME6XEM6 for six different genotypes collected from (1- Egypt), (2- Sebrata), (3- ELkhoms), (4- Serit), (5- Benghazi), (6- Darnah) and (molecular size marker).

Genetic similarity and cluster analysis based on SRAP data

The SRAP data were used to estimate the genetic similarity indices among the six tuna genotypes (Table 7). The highest similarity value among the six tuna genotypes was (0.870) that recorded between Darnah and Benghazi genotypes, Sebrata and ELkhoms (0.850) than then (0.820) between Serit and ELkhoms. The lowest similarity value (0.480) was observed between the Egyptian genotype and Sebrata, followed by the similarity value of (0.490) between Egyptian genotype and ELkhoms genotype, also the same value was resulted between Egyptian genotype and Serit genotype. These results revealed that SRAP-PCR analysis are useful to detect genetic variability and relationships between the tuna genotypes that agreed with **Che *et al.* (2009) and AL-Somain *et al.* (2017)** who reported that SRAP-PCR analysis is a reliable and powerful tool for assessing genetic polymorphisms and genotypes relationships.

Table (7): Similarity indices for the six tuna genotypes which collected from six different locations under investigation based seven used SRAP primer pairs data analysis

	Egypt	**Sebratah**	**El khomos**	**Seret**	**Benghazi**	**Darnah**
Egypt	**1.000**					
Sebratah	**0.480**	**1.000**				
El khomos	**0.490**	**0.850**	**1.000**			
Serit	**0.49**	**0.810**	**0.820**	**1.000**		
Benghazi	**0.570**	**0.740**	**0.780**	**0.790**	**1.000**	
Darnah	**0.610**	**0.760**	**0.770**	**0.760**	**0.870**	**1.000**

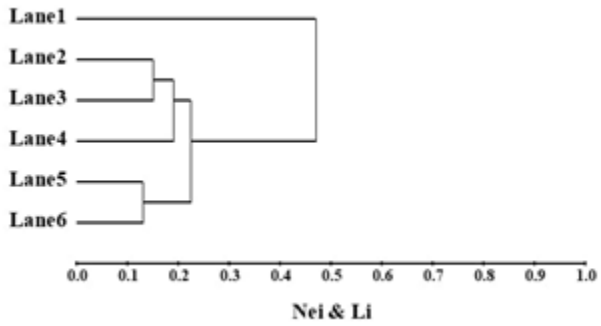

Figure (4): Consensus tree showed the genetic distance among the 6 tuna genotypes which collected from the six different locations under investigation based on ISSR data analysis where (1- Egypt), (2- Sebrata), (3- ELkhoms), (4- Serit), (5- Benghazi) and (6- Darnah)

The consensus tree based on SRAP data is shown in Figure (4). The consensus tree was divided into two main clusters. The first cluster contains the genotype which collected from Egypt (Egyptian genotype). While the second cluster was divided into two sub-clusters, the first sub-cluster included the genotype which collected from Darnah and the genotype which collected from Benghazi (the east part of Libya), meanwhile the second sub-cluster included Serit, El khoms and Sebrata (the west part of Libya). The relationship as detected from the tree indicated that the genotypes which collected from the east part of Libya are close to each other while they are varied from those which collected from west part of Libya. Moreover the Egyptian genotype is completely varied from the Libyan genotypes. These results reflected the fact that as long as the distance between the locations increased, the tuna genotypes became distantly related and vice versa. The two most distantly related genotypes as detected from the consensus tree were the Egyptian genotype and the genotype which collected from Sebrata. These results were in agreement with the findings of **Nebuchadnezzar and Aris (2018)** where they reported that the pairwise comparison test (Fst) showed a few genetic differentiations between yellowfin tuna populations. The value (Fst) of the yellowfin tuna population shows a strong gene flow between populations. The haplotype distribution exhibits a relationship between haplotypes in both yellowfin tuna, thus failing to show clade between different geographic locations. Unsustainable use can harm the population through genetic quality. Several approaches should be taken to support the life cycle of yellowfin tuna.

Brophy *et al*. (2018) studied two stocks of the blue-fin tuna (*Thunnus thynnus*) collected from the north Atlantic; the western and eastern stocks spawn in the Gulf of Mexico and the Mediterranean Sea respectively. Otolith shape descriptors were used to characterize western and eastern stocks of Atlantic blue-fin tuna and to estimate stock composition in catches of unknown origin, Otolith shape varied with length and between locations and years, the two stocks were distinguished with an accuracy of 83%. Bayesian stock mixture analysis indicated that samples from the east Atlantic and Mediterranean were

predominantly of eastern origin, the proportion assigned to the eastern stock showed slight spatial variation; yet, overlapping 95%.

Similarity and cluster analysis based on the combined data

The combined data of ISSR and SRAP were used to estimate the genetic similarity indices among the six tuna genotypes under investigation (Table 8). The highest similarity value among these six tuna genotypes (0.90) was recorded between El Khoms and Serit genotypes (the two most closely related genotypes) followed by (0.88) between El Khoms and Sebratah. On the other hand the lowest value (0.60) was recorded between (Egyptian genotype and Sebratah genotype) and (Egyptian genotype and El Khoms genotype) with he same similarity value (the two most distantly related genotypes), followed by (0.61) between Egyptian genotype and Seret genotype. From this dendrogram it possible to concluded that similarity value increased as much as distances between the location decreased, vice versa (i.e.) there are a negative relation between the similarity and the distance between these genotypes under investigation.

Table (8): Dice similarity coefficient of the six tuna genotypes based on ISSR and SRAP data

	Egypt	Sebratah	El khomos	Seret	Benghazi	Darnah
Egypt	**1.000**					
Sebratah	**0.600**	**1.000**				
El khomos	**0.600**	**0.880**	**1.000**			
Seret	**0.610**	**0.850**	**0.900**	**1.000**		
Benghazi	**0.670**	**0.830**	**0.860**	**0.870**	**1.000**	
Darnah	**0.770**	**0.750**	**0.810**	**0.780**	**0.840**	**1.000**

Whereas: 1= Egypt genotype, 2 Sebratah, 3 El Khoms, 4 Serit, 5 Benghazi, 6 Darnah genotypes

The consensus tree based on the combined data of ISSRs and SRAP is shown in Figure (5). It was divided into two main clusters. The first cluster contain Egyptian genotype, while the second cluster was divided into two sub-clusters; the first sub-cluster included Benghazi and Darnah genotypes, while the second sub-cluster included Serit, El khoms and Sebrata. The relationship as detected from the tree indicated that genotype El khoms and Serit were the two most closely related genotypes (90%), moreover it's possible to conclude that genotypes Egyptian genotype and Sebrata genotype were the two most distantly related genotypes as detected from the consensus tree.

In comparison to SRAP primers, ISSRs generated less scorable and polymorphic bands per primer Table (9). SRAP marker polymorphism is higher than ISSR marker, SRAP as well as ISSR markers are useful to study tuna genetic diversity and understand the relationship of those indigenous genotypes that would direct the selection, protection and use of tuna genotypes to improve tuna fish productivity.

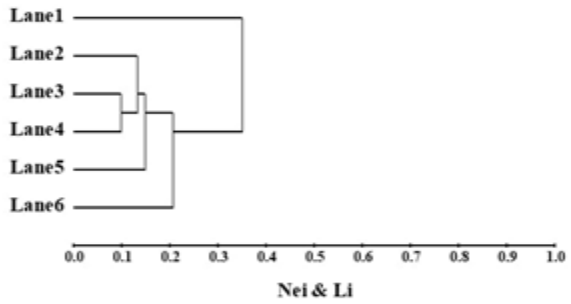

Fig. (5): Consensus tree showed the genetic distance among the 6 different Tuna genotypes based on the combined data of ISSR and SRAP where (1- Egypt), (2- Sebrata), (3- ELkhoms), (4- Serit), (5- Benghazi) and (6- Darnah).

Table (9): Total bands, (Monomorphic and Polymorphic bands), unique bands and polymorphism percentage generated with 11 ISSR primers and 7 SRAP primer combinations for 6 tuna genotypes

Primer Name	Total bands	Monomorphic bands	Polymorphic bands	Unique bands	Polymorphism %
ISSR	46	24	22	3	47.820%
SRAP	59	15	44	16	74.576%
Combined	105	39	66	19	62.857%

The pairwise comparison test shows a few genetic differentiations between blue-fin tuna populations. The distribution shows a relationship between different geographic locations. They stated that several approaches should be taken to support the life cycle of blue-fin tuna. The overall result shows that there has not been any change of genetic structure of blue-fin tuna **(Nakamura *et al*., 2013).**

Julia *et al*. (2017) reported the recent developments in the field of genomics have provided new and powerful insights into population structure and dynamics that are essential for the conservation of biological diversity. They use whole-genome sequencing in concert with a draft genome assembly to decipher the global population structure of the yellowfin tuna, and to investigate its demographic history.

Ecological study

To study the ecological impact on the tuna fish biodiversity four ecological factors were investigated. These four factors were water temperature (temp), water pH (pH), water salinity (salinity) and dissolved oxygen (DO). These four ecological factors were measured and their results were recorded in Table (10).

Table (10): Four measured ecological factors in the locations under investigation

Locations	Temp. ^{o}C	PH	Salinity X 1000	DO
Libya west	26.8	8.17	36.80	8.0
Libya Mid	29.0	8.06	36.50	7.3
Libya east	28.3	8.12	37.54	6.09
Egypt	24.0	8.66	37.86	8.30

The results in Table (10) show that water temperature ranged from 29 at Libya mid to 24 at Egyptian location. While water pH ranged from 8.66 at the Egyptian location to 8.06 at Libya mid. Moreover, water salinity ranged from 37.86 x 1000 ppm at the Egyptian location to 36.5 x 1000 ppm at Libya mid. The dissolved oxygen in sea water ranged from 8.3 at the Egyptian location to 6.09 at Libya east. These results indicated that, the four measured ecological elements were varied among the different locations under investigation with different proportion of each location. The results indicated that the four ecological were varied between the Egyptian location and the Libyan locations. The results emphasizes a wide range in water temperature, salinity and dissolved oxygen in water, while, water pH revealed a narrow range. As previously known tuna fish is an immigrant fish within or between marines and that increased the chance for mating between the genotypes in different locations in the marines. The variation in the ecological factors could acts as barriers witch decrease the tuna fish migration and thus decreased the chances of mating between the different genotypes among the different locations. This low chance of mating between the tuna fish genotypes could leads to the speciation which proved by means of molecular investigation. These findings are in agreement with what stated by **Prabhaker *et al*. (2020)** where they stated that in many aquatic species, alteration of habitats and human-induced barriers shape the population's genetic structure in rivers with longitudinal connectivity. **Dolph *et al*. (2021)** reported that genes and genomic regions having large effects on phenotypic differences between populations are known from numerous taxa, but fitness effect sizes have rarely been estimated. They mapped fitness over a generation in an F_2 intercross between a marine and a lake stickleback population introduced to a freshwater pond. A quantitative trait locus map of the number of surviving offspring per F_2 female detected a single, large-effect locus near *Ectodysplasin* (*Eda*), a gene having an ancient freshwater allele causing reduced bony armor and other changes. These findings are consistent with other studies suggesting strong selection on this gene (and/or linked genes) in fresh water. Selection on ancient genetic variants carried by colonizing ancestors is likely to increase the prevalence of large-effect fitness variants in adaptive evolution.

REFERENCES

Abu-Almaaty, A.; Hassan, M.; Bahgat, I. and Suleiman, M. (2017). Inter Simple Sequence Repeat (ISSR) and Cytogenetic Analysis of Three Fish Species of Family Osphronemidae; Egyptian Journal of Aquatic Biology and Fisheries, 21(2): 1-15.

Ağdamar, S. (2021). Sequence-Related Amplified Polymorphism (SRAP) Markers: A Feasible Tool for Studies in Invasion Genetics of Freshwater Fish; Proceedings, 68: impress.

Ağdamar, S.; Baysal, Ö.; Yıldız, A.; and Tarkan, A. S. (2020). Genetic differentiation of non-native populations of Gibel Carp, Carassius gibelio in Western Turkey by ISSR and SRAP markers; Zoology in the middle east, 66 (4): 55-68.

Alexander S.; Janna, M.; Willoughby, R. and Christie, M. R. (2018). Genetic diversity in fishes is influenced by habitat type and life-history variation; Ecology and Evolution. 18;1–10.

AL-somain B.H.A.; Migdadi, H.M.; Al-Faifi, S.A.; Alghamdi, S.S.; Muharram, A.A.; Mohammed, N.A. and Refay, Y.A. (2017). Assessment of genetic diversity of sesame accessions collected from different ecological regions using sequence-related amplified polymorphism markers; Biotech, 7(1), 82.

Brophy D.; Duncan, R.; and Arrizabalaga, H. (2018). Otolith shape analysis as a tool for stock separation of albacore tuna feeding in the Northeast Atlantic. *Fisheries Research*, 200, 68–74.

Che, Z.; Zhang, Y.; Sun, J.; Zhang, X.; Shang, X. and Wang, H. (2009). Genetic diversity analysis of black sesame (*Sesamum indicum* DC) core collection of China using SRAP markers; Acta Agronomica Sinica, 35(10), 1936-1941.

Crozier, L.G. and Hutchings, J.A. (2014). Plastic and evolutionary responses to climate change in fish. Evolutionary Applications, 7, 68–87.

Dolph, S.; Marchinko, K.B.; Arnegard, M.E.; Zhang, H.; Brady, S.D.; Jones, F. C.; Bell, M. A. and Kingsley, D.M. (2021). Fitness maps to a large-effect locus in introduced stickleback populations; Proc Natl Acad Sci, 118(3):e1914889118.

Fromentin, J. M. and Powers, J. E. (2005). Atlantic bluefin tuna: population dynamics, ecology, fisheries and management; Fish Fisheries, 6 (4): 281-306.

Gaber A.; Hassan, M. M.; Boland, C.; Alsuhaibany, A.; Babbington, J.; Pereira, J.; Budd, J. and Shobrak, M. (2020). Molecular identification of Todiramphus chloris subspecies on the Arabian Peninsula using three mitochondrial barcoding genes and ISSR markers; Saudi Journal of Biological Sciences,27(1): 480- 488.

Hassanien H. A. and Al-Rashada, Y. (2021). Assessment of genetic diversity and phylogenetic relationship among grouper species *Epinephelus* spp. from the Saudi waters of the Arabian Gulf; Saudi J Biol Sci.; (3):1779-1786.

Ji, W.; Zhang G.R.; Ran, W.; Gardner, J.P.; Wei, K.J.; Wang, W.M. and Zou, G.W. (2014). Genetic Diversity of and Differentiation among Five Populations of Blunt Snout Bream (*Megalobrama amblycephala*) Revealed by SRAP Markers: Implications for Conservation and Management; PLoS ONE; 9 (9)

Julia M.I.; Damerau,M.; and Hanel, R. (2017). Genomic Differentiation and Demographic Histories of Atlantic and Indo-Pacific Yellowfin Tuna (*Thunnus albacares*) Populations; Genome Biology and Evolution; 9 (4): 1084-1098.

Labastida-Estrada, E.; Salima Machkour-M'Rabet.; Laura Carrillo.; Hénaut, Y. and Castelblanco-Martínez, D. N. (2019). Genetic structure of Mexican lionfish populations in the southwest Gulf of Mexico and the Caribbean Sea; PLoS One 14 (10): 1379.

MacKenzie, B. R.; Mosegaard, H. and Rosenberg, A. A. (2009). Impending collapse

of bluefin tuna in the northeast Atlantic and Mediterranean; Conservation Letters. 2 (1):26-35.

Nebuchadnezzar Akbar and Muhammad Aris (2018). Genetic Population Structure of Yellowfin Tuna (*Thunnus albacares*) as Based Data of Fish Conservation in North Mallucas Sea; Omni-Akuatika, 14 (3): 75–85.

Nakamura Y.; Mori, K.; Saitoh,K.; Oshima, K.; Mekuchi, M.; Sugaya, T. and Inouye, K. (2013). Evolutionary changes of multiple visual pigment genes in the complete genome of Pacific bluefin tuna. *Proceedings of the National Academy of Sciences*, *110*(27), 11061-11066.

.

O'Reilly C.M.; Sharma, S.; Gray, D.K.; Hampton, S.E.; Read, J.S.; Rowley, R.J. and Zhang, G. (2015). Rapid and highly variable warming of Lake Surface waters around the globe. Geophysical Research Letters, 42, 10773–10781.

Pecoraro, C.; Babbucci, M.; Villamor, A.; Franch, R.; Papetti, C.; Leroy, B. and Murua, H. (2016). Methodological assessment of 2b-RAD genotyping technique for population structure inferences in yellowfin tuna (*Thunnus albacares*); Marine genomics, *25*: 43-48

Prabhaker Y.; Kumar, A.; Hussain, S. A. and Gupta, S. K. (2020). Evaluation of the effect of longitudinal connectivity in population genetic structure of endangered golden mahseer, Tor putitora (Cyprinidae), in Himalayan rivers: Implications for its conservation; PLoS One 15(6): e0234377.

Rasmussen, R. S. and Morrissey, M. T. (2008). DNA-Based Methods for the Identification of Commercial Fish and Seafood Species. Compr Rev Food. Sci Food Saf 7(3): 280–295.

Rob, O. (2008). Fisheries forensics: the use of DNA tools for improving compliance, traceability and enforcement in the fishing industry. Fish and Fisheries 9 (4): 462–472.

Sambrook, J.; Fritsch, E. R. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual (2nd ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Syahida N.K.; Nurul, T. A. M. Jaafar.; Piah, R. M.; Wahidah, M. Arshaad.; Nor, S. A. M.; Habib , M.; Abdel Ghaffar,Y.; Sung, Y.; Daniel, M. D. and Tan , M. P. (2020). Recent population expansion of longtail tuna *Thunnus tonggol* (Bleeker, 1851) inferred from the mitochondrial DNA markers; Peer J. 8: impress.

Takeshi T.; Masaoka, T.; Aoki, H.; Koyanagi, R.; Fujie, M. and Satoh, N. (2020). Divergent northern and southern populations and demographic history of the pearl oyster in the western Pacific revealed with genomic SNPs; Evol Appl.; 13(4): 837–853.

Wannapimol K.; Buddhachat, K.; Poommouang, A.; Chomdej, S.; Thitaram, C.; Kaewmong, P.; Kittiwattanawong, K. and Nganvongpanit, K. (2021).

Feasibility of melting fingerprint obtained from ISSR-HRM curves for marine mammal species identification; Peer J. 9: impress.

Wuping, Y.; Li, J.; Zheng, D.; Friedman, C. and Wang, H. (2019). Analysis of genetic population structure and diversity in Mallotus oblongifolius using ISSR and SRAP markers; Peer J.; 7: 7173.

Xiaopei Z.; Zou, G.; Zhao, J. and Linyi, H. (2020). Genetic relationships and diversity among populations of Paris polyphylla assessed using SCoT and SRAP markers; Physiology and Molecular Biology of Plants 26(1): 38-45.

Yang, X. and Quiros, C. (1993). Identification and classification of celery cultivars with RAPD markers. Theor Appl Genet 86:205-212

Zhu S. H.; Li, W.; Saisai, W. and Qu, J. (2021). SRAP Markers - Based Genetic Biodiversity and Differentiation of Three cultured Goldfish Strains Dongjie; IOP Conference Series: Earth and Environmental Science.

Ziekiewicz, E.; Rafalski, A. and Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchore PCR amplification. Genomics, 20: 176-183.