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Genetic variations of the Lessepsian rabbitfish populations from Red and Mediterranean Sea

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ABSTRACT

Rabbitfish (*Siganus rivulatus*) considers the most abundant of all species in Red Sea and played an important role in the local fisheries worldwide and migrated through Suez Canal to the Mediterranean Sea. The current study was carried out in Animal Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Sadat City, and The Faculty of Agriculture, Agricultural Botany Department, Saba Basha, Alexandria University, to study are study the morphological characters of *Siganus rivulatus* from the two different locations (Mediterranean Sea and Red Sea), and to detect the genomic variations between the two locations fish by using simple Sequence Repeats (SSR) markers. The results showed that there are no observed variations based on morphological characters and all the six SSR markers loci primers that tested for 38 *S. rivulatus* samples from the Red and Mediterranean Sea gave clear amplified fragments of DNA. The amplified fragments were obtained with the all studied samples. The number of alleles ranged from 1-2 alleles. 17 alleles were obtained with an average of 1.4 alleles per locus. The allele's size ranged from 100-250 bp. The data indicated some diversty between the two-population based on molecular data.

***Keywords*:** Rabbitfish, morphology, SSR, Red, Mediterranean Sea

Introduction

Population genetics can be defined as the science of how genetic variation is spread between species, populations and individuals. Its focused on how the evolutionary forces of mutation, selection, and migration affect the distribution of genetic variations (Hansen, 2003). Genetic diversity between populations could provide clues to the population's life histories and degree of evolutionary. Its states as alterations in alleles (quantity and quality), genes, chromosomes, and gene arrangements on the chromosomes that are existing within/among constituent populations (Williamson, 2001).

The genetic variations data has diverse application in research on evolution, conservation and management of natural resources and genetic improvement programmes (Tanya and Kumar, 2010). Ben Tuvia, (1966) reported that four species of *Siganus* genus (Rabbitfish) are lived in the Red Sea. The survey showed that two species of *Siganus* migrated through Suez Canal to the Mediterranean Sea. Rabbitfish (*Siganus rivulatus*) considers the most abundant of all species in Red and Mediterranean Sea and played an important role in the local fisheries worldwide. The Lessepsian rabbitfish, (family Siganidae) is an herbivorous fish. genus *Siganus*, feeding mainly on green algae; spawning season in both the eastern Mediterranean and the northern Red Sea from April to July.

The Red Sea and the western Indian Ocean, the natural range encompasses from. By Lessepsian migration through the Suez Canal, it colonized the Mediterranean Sea and first founded in the Mediterranean of Palestine coast in 1924. Since this time, it has spread northwards and westwards as far as Tunisia, Turkey and Malta (Insacco and Zava 2016). Many different members of the family Siganidae (Rabbitfishes) are widely spread throughout the tropical and subtropical waters in the Western Indo-Pacific region (Bariche, 2006). The rabbitfish (*Siganus rivulatus)*, locally known as "Sigan", is the most common among the five species of the genus *Siganus* that exist in fisheries (Hashem, 1983).

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Nowadays, many fish species that entered the Mediterranean Sea through Suez Canal, have established dense populations. To date, *via* the Suez Canal there about 100 Indo-Pacific fish species have been introduced. Golani, (1996) detected, after opening of the Suez Canal, many fish species from Red Sea recognized in the Mediterranean. *Siganus rivulatus* is one of the first Lessepsian migrants to the Mediterranean recorded by Steinitz, (1929). Philips and Akel (2003) reported that the first species of *Siganus rivulatus* was dominant in the Egyptian waters of Mediterranean, in Alexandria (Abu-Qir Bay).

Development of molecular markers has the ability to detect genetic studies of individuals, populations. Molecular markers, protein or DNA (mt-DNA or nuclear DNA such as microsatellites, SNP or RAPD) are now being used in fisheries and aquaculture (Tanya and Kumar, 2010). DNA molecular markers and Mitochondrial Cytochrome c oxidase gene can be used to study if they have some demographic bottleneck, if the colonization is a result of a few individuals, or whether the migrant's influx has been a steady process. Molecular markers such as PCR-based Markers have been demonstrated to be valuable methods to characterize and evaluate of genetic diversity between and within species and populations. PCR-based Markers offer another approach for species identification and have applied towards a wide range of fish, including closely related species. SSRs, is often used for barcoding to identify animal species. (Zong *et al*., 2013). the aims of this study are study the Morphological characters of *Siganus rivulatus* from the two different locations (Mediterranean Sea and Red Sea), and detect the genomic variations between the two locations fish by using simple Sequence Repeats (SSR) markers.

Materials and Methods

The current study was carried out in Animal Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Sadat City, Minufiya, Egypt and The Faculty of Agriculture, Agricultural Botany Department, Saba Basha, Alexandria University, Alexandria, Egypt. The experimental fish which was used in the present study was rabbitfish, *Siganus rivulatus* from the family Siganidae. A total of 38 fish samples (37-48 gm weight) were collected from the Red and Mediterranean Sea in Egypt (19 fish samples from each Sea, 10 males and 9 females).

The samples were collected in plastic sachet, immediately transported to the laboratory in ice pack container and stored at -20^{o}C until used. The means of different body measurements for the collected samples are illustrated as flow: total length (cm), total width (cm), head length (cm), head width (cm), percentage of width to length, percentage of head length to total length.

Genomic DNA was extracted from the muscles tissues of *S. rivulatus* by using genomic DNA extraction kit (G-Spin) TM for cell/ tissue extraction (iNtRON Biotechnology, Inc). The kit was designed for rapid isolation of genomic DNA from different samples. DNA extraction from fish muscles tissues was completed according to the manufacturer's protocol. The obtained DNA for all samples was stored at -20^{o}C until used. The quality of the extracted DNA was detected by using 1% agarose gel electrophoresis as follow: 1 gm of agarose was dissolved in 100 ml of TBE buffer (100 mM tris, 90 mM boric acid, and 1 mM EDTA, PH 6.8) and cooked in a microwave oven for 2.0 mins. The cooked Agarose medium was cooled down to about 60°C. For DNA staining, the Ethidium bromide was added before pouring the gel in the submarine of the electrophoresis unit, to give a final concentration of 0.5 µg/ml of Eth Br. The gel was left to solidify at the room temperature, the TBE buffer was added to fill the electrode chamber, and loading buffer (1 ml glycerol, 10 mM Na_2EDTA, 50 mg SDS, 100 mg bromophenol blue, 13 mg xylene cyanol and water up to50 ml) was added to 10µl of DNA and loaded in the gel.

The electrophoresis run was performed at 80 V in DNA Bio-Rad electrophoresis unit for 60 mins. The Obtained DNA bands were visualized using UV-trans-illuminator and photographed by digital camera with orang filter. For the determination of the DNA concentration, one microliter of the extracted DNA was used in spectrophotometry (NanoDrop™ 2000 Spectrophotometer, thermo Scientific). The concentrations of extracted DNA samples ranged from 30 to 50 ng/ µl. PCR amplification was conducted by using Applied Biosystems® ProFlex™ PCR System.

The PCR amplification conditions were optimized for the all reactions by using extracted DNA in a total volume reaction of 20µl containing 10.0 µl of Master Mix. 0.4µM of each Primer (forward

and reverse), 100 ng of DNA template and de-ionized water up to 20µl. In the present study, 6 SSR markers loci were screened from 38 *S. rivulatus* individuals to detect the differences between the samples from the Red and Mediterranean Seas (18 samples from each Sea, 9 males and 9 females).

The PCR primers that were used in the present study were reported by Ravago-Gotanco and Candice, 2010. The primers were used in PCR amplification with modification in PCR conditions (the temperatures and the times for PCR steps). 100bp DNA ladder plus (iNtRON Biotechnology, Inc. Korea) was used to detect the obtained PCR products. The electrophoresis run was achieved at 75 V in DNA electrophoresis unit (Bio-Rad) for 90 mins. UV-trans-illuminator was used to visualize the obtained DNA bands and the gel was photographed by digital camera with orang filter.

Results and Discussion

Morphological measurements

In this study, the results of morphological measurements of *S. rivulatus* studied samples indicated that the fish has a total body length of 17.5-19.5 cm and have a depth of 6.0-6.5 cm for Red and Mediterranean Sea samples, respectively, as shown in Table 1. The total body length was about 2.9 times the depth. The percent of depth to length for the two seas samples was 34%. For the head measurements, the head length was 4.0 cm and the width were 4.5 cm in Red sea samples whereas the head length in Mediterranean Sea samples was more than that of Red sea (4.5 cm). The percent of head depth to head length ranged from 100-113%. It was 113% for Rea Sea and 100% for Mediterranean Sea samples. For the all studied samples, there were 13 spines in the dorsal fin and 7 spines in the anal fin.

Table 1: The means of different body measurements of the collected samples of *S. rivulatus* from Red and Mediterranean Sea.

Body part	Sample source	Total length	The depth	Head length	Head depth	% of depth to length	% of head length to total length	% of head deapth to head length
Mean (cm)	Red	17.5	6.0	4.0	4.5	34 %	22	113
	Mediterranean	19.0	6.5	4.5	4.5	34%	23	100

Extracted DNA quality quantity

The quality of the extracted DNA was detected by using 1% agarose gel electrophoresis for Red and Mediterranean Sea samples. The results indicated that there is no fragmentation was observed in extracted DNA. The quantity of extracted DNA samples was determined by using Nano drop Spectrophotometer and the concentration ranged from 30 - 50 ng/µl. The extracted DNA was directly used in PCR amplification.

SSR markers detection

All the six SSR markers loci primers that tested for 38 *S. rivulatus* samples from the Red and Mediterranean Sea gave clear amplified fragments of DNA. The amplified fragments were obtained with the all studied samples. As presented in Table 2, the number of alleles ranged from 1-2 alleles. 17 alleles were obtained with an average of 1.4 alleles per locus.

The allele's size ranged from 100-250 bp. As presented in Table 2 and Figures 1 to 5, the data showed that 4 from the 6 tested loci were homozygous in different studied samples of the two location. The amplified fragments sizes of these loci were 250, 165, 190 and 180 bp for Sfus-6, 9, 21 and 22 respectively.

There are no differences between different studied samples with primers (P6, 9, 21 and 22) that gave one allele for each locus. In addition, the males and females from the Red and Mediterranean Sea were homozygous for the same allele in the previous studied loci (Sfus-6, 9, 21 and 22). On the other hand, Sfus-5 and 8 loci were homozygous in some individuals and heterozygous in the others or homozygous for alternative alleles and (Figures 1 - 5).

Data indicate that Red Sea samples 1, 2, 3, 4, 7, 8, 9 and 10 are heterozygous for the Sfus-5 locus and 5, 6 and 11 to 19 were homozygous. Samples 5 and 6 are homozygous for alleles (males) and 11 to 19 were homozygous for the alternative alleles (females). The same results were observed in Mediterranean Sea samples 1, 2, 5, 6, 7, 8, 17, and 18 that are heterozygous whereas samples 3, 4, 9-16, and 19 are homozygous but for different alleles. Sfu**s-**8 locus of *S. rivulatus* from Red Sea exhibit different homozygous alleles in males and females without any specific profile for each. In Mediterranean Sea samples, the locus exhibit the same alleles as in Red Sea but some samples (females, 15 and 16) was homozygous for the alternative allele of others.

In conclusion, the results of SSR markers in 6 loci that were studied showed that, there are no differences between different studied samples and different sexes from Red and Mediterranean Sea in the loci, Sfus-6, 9, 21 and 22. The all samples were homozygous for the same alleles. Whereas, the results of some loci (Sfus-5 and 8) that were heterozygous in some samples and homozygous in the others, indicated that there are differences between the Red and Mediterranean Sea.

In addition, the males and females from the two Seas were homozygous for the same allele in the previous studied loci. Sex determination is controlled by several genes on different chromosomes and by environmental factors (Lee *et al*. 2004 and Zhu *et al*. 2016) detected only 99-bp allele in the female samples, of Tilapia nilotica while 141, 149 and 157-bp alleles were present in both male and female samples. The same results were obtained from Sfus-5 locus in the two Seas samples.

These results support the studies of Zhu *et al*. (2016). They found five sex-associated markers on LG1, LG3, LG19 and LG23. Two unlinked microsatellite markers, one located on LG3 and other on LG1 were found to be linked to phenotypic sex in a single family of blue tilapia (Lee *et al*., 2004). Zhu *et al*. (2016) reported that SSR markers can be used in fish breeding programs as a differentiated method between males and females.

Table 2: Simple sequence repeats Markers (SSR) primers sequences that were used with *S. rivulatus* individuals from the Red and Mediterranean Seas, their loci, repeated motif and number of alleles and their sizes.

Locus	Primer sequence (5'—3')	Repeated motif	No. of alleles	Size (bp)
Sfus-5	F: GGTAAGGGGCCAGCAAAT R: CCATTTCAGGTTTGCATGTG	$(GT)_{16}$	2	100-180
Sfus-6	F: ACAATCCAGGATGCAAGTCC R: CGAATTGCCACATGCAATAA	$(AC)_{10}$	1	250
Sfus-8	F: TCAAAAAGAAGAGCAAGGAGAA R: AAGAGAGGATGGGTTTGTGG	$(AGAA)_{17}$	2	160-200
Sfus-9	F: CAATGTGTCACAGATGGTAACAA R: TGGCCTGGTGCTTTTCTACT	$(AG)_{8}$	1	165
Sfus-21	F: CCCAGCTTTTGTTTTATTCA R: TGCAAGCTTTTAGAAGACTGTAT	$(CT)_{27}$	1	190
Sfus-22	F: GAGCACAACAGGCATTTGAA R: CTGGGATCAGAGGGTGAAAG	$(AC)_{19}$	1	180

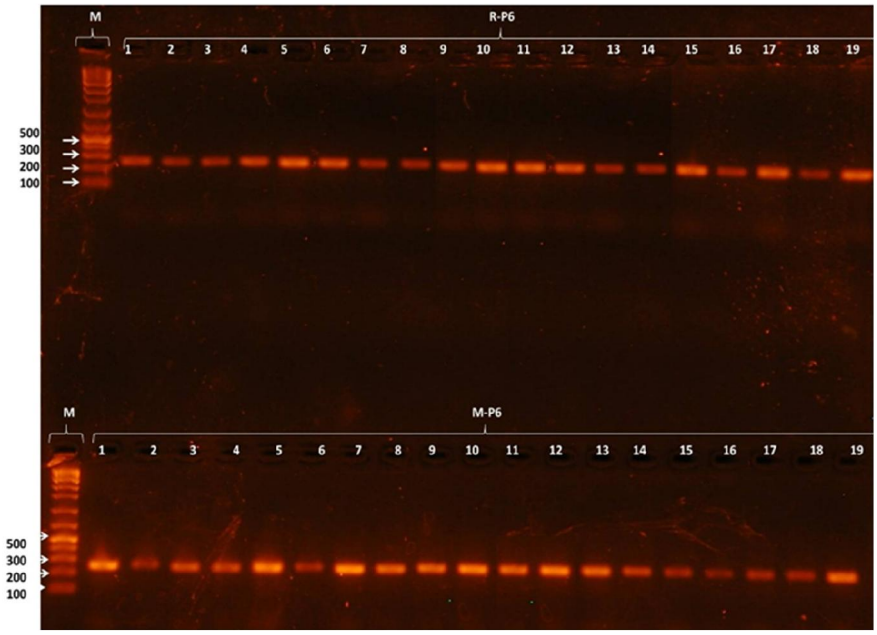

Fig. 1: Agarose gel electrophoresis for amplified Sfus-6 loci of *S. rivulatus* from Red and Mediterranean Seas samples. Lines 1-10 are males and 11-19 are females. M: Molecular marker, R: Red Sea samples, M: Mediterranean Sea samples.

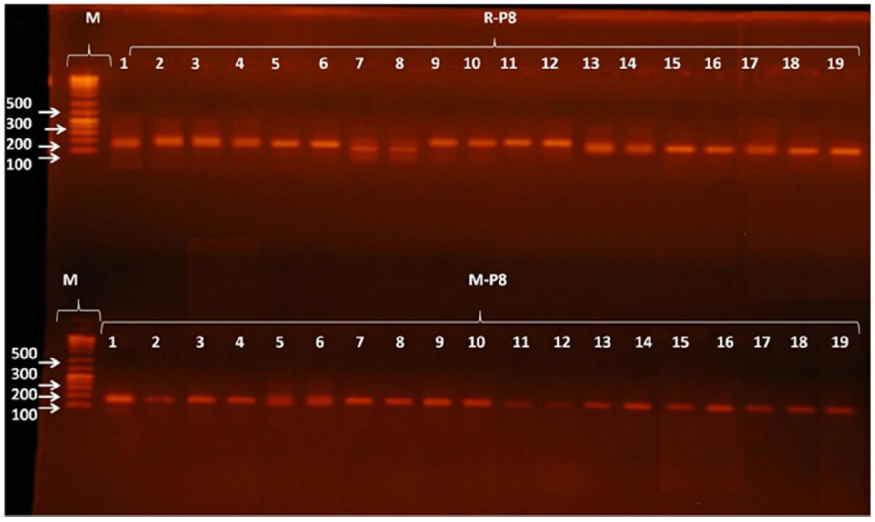

Fig. 2: Agarose gel electrophoresis for amplified Sfus-8 locus of *S. rivulatus* from Red and Mediterranean Seas samples. Lines 1-10 are males and 11-19 are females. M: Molecular marker, R: Red Sea samples, M: Mediterranean Sea samples.

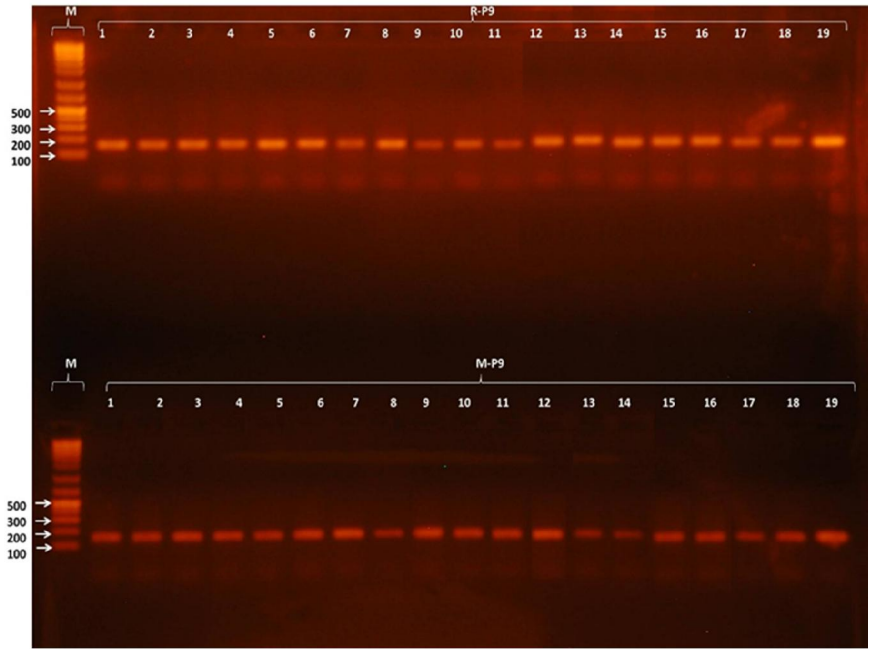

Fig. 3: Agarose gel electrophoresis for amplified Sfus-9 loci of *S. rivulatus* from Red and Mediterranean Seas samples. Lines 1-10 are males and 11-19 are females. M: Molecular marker, R: Red Sea samples, M: Mediterranean Sea samples.

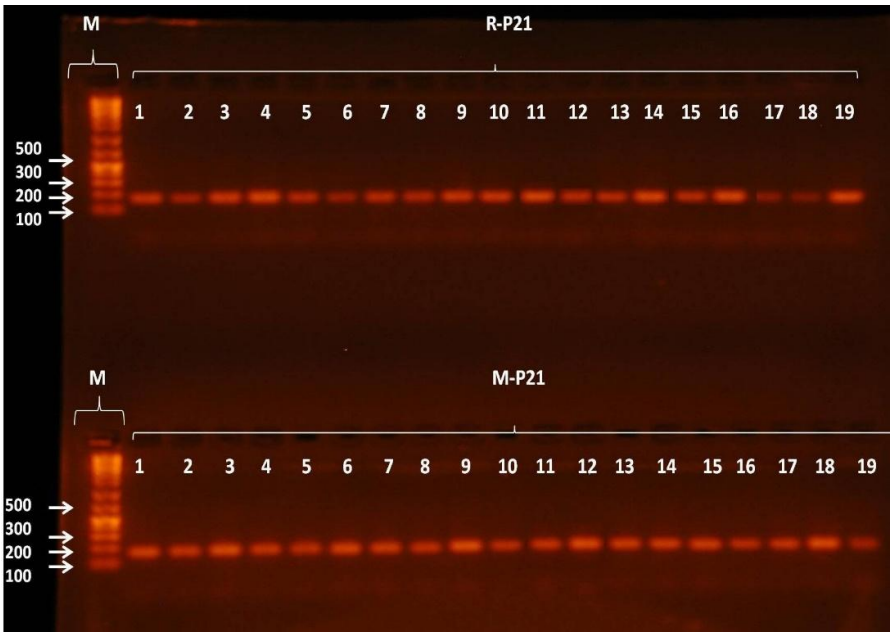

Fig. 4: Agarose gel electrophoresis for amplified Sfus-21 loci of *S. rivulatus* from Red and Mediterranean Seas samples. Lines 1-10 are males and 11-19 are females. M: Molecular marker, R: Red Sea samples, M: Mediterranean Sea samples.

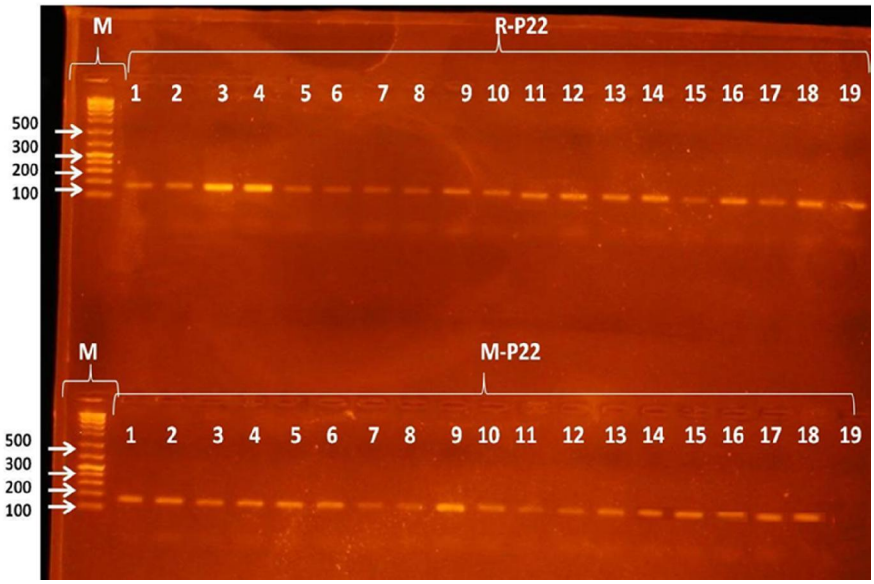

Fig. 5: Agarose gel electrophoresis for amplified Sfus-22 locus of *S. rivulatus* from Red and Mediterranean Seas samples. Lines 1-10 are males and 11-19 are females. M: Molecular marker, R: Red Sea samples, M: Mediterranean Sea.

References

Bariche, M., 2006. Diet of the Lessepsian fishes *Siganus rivulatus* and *S. luridus* (Siganidae) in the eastern Mediterranean: a bibliographic analysis. Cybium 30(1):41-49

Ben Tuvia, A., 1966. Red Sea fishes recently found in the Mediterranean Sea. Cope& No.2, 254-275.

Golani, D., 1996. The marine ichthyofauna of the eastern Levant - history, inventory and characterization. Isr. J. Zool., 42: 15-55.

Hansen, M.M., 2003asis on fishes. DSc Thesis, Faculty of Natural Sciences, University of Aarhus, 68 pp.

Hashem, M.T., 1983. Biological Studies on *S. rivulatus* (Forsskal) in The Red Sea. Journal of the Faculty of Marine Science, King Abdulaziz University: Jeddah, Saudi Arabia. 1983; 3:119-127.

Lee, B. Y., G. Hulata and T. D. Kocher, 2004. Two unlinked loci controlling the sex of blue tilapia (*Oreochromis aureus*). Heredity, 92, 543–549

Philips, A.E. and E.H. Akel, 2003. Investigation of beach seine catches of Abu-Qir Bay (Egypt). Bull. Nat. Inst. Oceanogr. and Fish AR E 1: 79-91.

Ravago-Gotanco, R., Candice Y. Lumibao, Ma. Josefa R. Pante, 2010. Isolation and characterization of thirteen microsatellite markers for the rabbitfish ,Siganus fuscescens, Conservation Genet Resour., 2:225–227

Steinitz, W., 1929. Beitrage zur Kenntnis der Küstenfauna Palästinas. Publ. Staz. Zool. Napoli, 8(3-4): 311-353.

Tanya, C. and R. Kumar, 2010. Molecular markers and their applications in fisheries and Aquaculture. Advances in Bioscience and Biotechnology, 2010, 1, 281-291. doi:10.4236/abb.2010.14037 published Online October 2010 (http://www. SciRP. org/ journal/abb/).

Williamson, J.H., 2001. Broodstock management for imperilled and other fishes. In G. A. Wedemeyer, (Ed.). Fish hatchery management, second edition. American Fisheries Society, Bethesda, Maryland: 397-482. Sunnucks, P. 2000. Efficient genetic markers of population biology. Trends in Ecology and Evolution, 15: 199- 203.

Zhu, H., Z. Liu, M. Lu, F. Gao, X. Ke, D. Ma, Z. Huang, J. Cao and M. Wang, 2016. Screening and identification of a microsatellite marker associated with sex in Wami tilapia, *Oreochromis urolepis hornorum.* J. Genet. 95, 283–289.