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Study of genetic variation in populations of *Coptodon zilliii, Oreochromis aureus, and Oreochromis niloticus* in two areas South of Iraq, in Khaur Abdallah and Shatt Al-Arab Mitochondrial Cytochrome b Gene Analysis

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Abstract

This study has a specific goal in mind: Genetic Variation Study Within Populations of *Coptodon zilliii, Oreochromis aureus, and Oreochromis niloticus,* and analysed the partial Cytb gene sequence of mtDNA to determine genetic diversity among these species in the Khaur Abdallah and Shatt Al-Arab in the south of Iraq, where the mitochondrial cytochrome b gene was used. DNA was isolated from 40 specimens of the three species collected from two different sites and examined. The gene size for *Coptodon zilliii,* Oreochromis aureus, and *Oreochromis niloticus* was (439, 326 & 473 bp long (amplified Cytb gene)), respectively. The study found that two haplotypes in Khaur Abdallah and one in the Shatt-Al-Arab river in *Coptodon zilliii* populations, while was found one haplotype in Khaur Abdallah and one in the Shatt-Al-Arab in both *Oreochromis aureus*, and *Oreochromis niloticus* populations by using Software DnaSP v5.1. In each of the species *Oreochromis aureus* and *Oreochromis niloticus*, the research identified two types of unique haplotypes, one in Khaur Abdallah and the other in Shatt-Al-Arab. However, three haplotype patterns for *Coptodon zilliii* populations were discovered: one in the Shatt al-Arab River and two in Khaur Abdallah. Low nucleotide (π) and haplotype diversity (Hd) values were found in both *Oreochromis aureus* and *Oreochromis niloticus* populations in the Shatt Al-Arab river and Khaur Abdallah, indicating low genetic variation among populations for both species in the two regions. However, there was no diversity within the *Oreochromis aureus* population in both Shatt Al-Arab and Khaur Abdallah. The population of *Oreochromis niloticus* has the same indication. Three haplotypes for *Coptodon zilliii* indicate to genetic variance across populations in the Shatt Al-Arab river and Khaur Abdallah regions, although no variety within the *Coptodon zilliii* species was found in the Shatt Al-Arab river. In the Khaur Abdallah district, there was little genetic diversity among them.

Keywords: allozymes, mtDNA, PCR, population genetics, genetic diversity.

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I. INTRODUCTION

The occurrence of distinct alleles, or alternative forms, in a collection of genes is referred to as genetic variety in a community. Individuals in a population with genetic variety have different alleles, which implies they have different genetic composition. Polymorphic genetics refers to local genetics in which numerous alleles are present. An alternate variation or form of a gene or chromosomal location is known as Allen (usually a group of genes). There are at least two or three different versions of the gene. Alleles with differences in the genetic coding can sometimes create distinct acetates (skin or eye color). Many variances, on the other hand, result in little or no discernible change. Human traits like as eye color and blood type, for example, are polymorphic. One of the most broad facets of the idea of phenotypic variation is genetic variation.

Differences in the traits of individuals in a population are referred to as phenotypic variation. The development of proved phenotypes leaving more descendants is of important to biologists because of what natural selection is: various forms of phenotype may have varying fitness, and selection leads to the emergence of proven phenotypes leaving more descendants.

Gene variety is a process in which independent genetic changes (mutations) develop throughout time when two or more sets of ancestors have established populations that have been isolated for a length of time. In certain situations, subpopulations dwell in biologically separate marine settings. This might indicate genetic variance from the rest of the population, especially in very large populations. Genetic variations across populations, on the other hand, might result in mutations (which do not impact external shape) or raise the relevance of morphological or even physiological changes. It will also be accompanied by genetic variety, which will always be associated with reproductive isolation, either by definition of these novel adaptations or by genetic drift, which is the major process underlying that belonging (Redon et al., 2006). Furthermore, genetic heterogeneity among individuals in a population can be characterized at many levels. Variations in the apparent pattern of quantitative characteristics can be used to define genetic variation (constantly changing traits that are coded by many genes such as dog length or separate traits, traits that fall into separate categories and are coded by a gene or number). Several genes, such as white, red, or pink, are found in the petals of various flowers (Pavlopoulos et al., 2013).

Genetic and environmental variation can both cause phenotypic variance. Only genetic variations, however, may be handed on to future generations. Furthermore, only a small portion of the genetic component fluctuates, i.e., the extra genetic variation(Persson et al., 1998).

Heredity is the amount of offspring who resemble their parents as a result of phenotypic variation.

In the 1960s, there was a lot of debate over how much genetic variety was already present in the population, and the general consensus was that polymorphic sites were rather uncommon.

Following that, scientists were able to investigate patterns of protein variation in populations and discover genetic variation thanks to the invention of gel electrophoresis technology.

A remarkable amount of genetic variety has been uncovered by biologists.

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In most vertebrate species, for example, about 30% of genes are known to be polymorphism (Feuk et al., 2006). Genetic variety in humans is about the same as it is in other animal species, according to research undertaken in the 1970s. Human studies have also demonstrated that the supposed human species are not biologically distinct biological groupings. It has been discovered that there is far more genetic variety within races. Since then, the lack of genetic variety has been seen as unusual. The lack of genetic variety in population groupings implies a recent bottleneck in the group's history, when the population number shrank dramatically.

As a result of the population bottleneck, all present community members are descended from a small number of individuals and so have little genetic variety. When new mutations emerge in these populations, it is predicted that genetic variety would accumulate over time (Futuyma, 1998).

The finding of enormous quantities of genetic variety in practically all populations raised a new question: how is genetic variation preserved? Natural selection, after all, eliminates genetic variety in many circumstances by removing genotypes that are less advantageous. The level of genetic variety in a society is influenced by a number of variables. Mutation is one of these, and it is the ultimate cause of all variants. Mutations, on the other hand, are rare, occurring in per 100,000 to 1,000,000 genomic locations every generation (GOULD, 1996). Most of the forms that arise in regular groupings can't be explained at this rate. However, the mutation might explain some of the extremely unusual traits that have been observed in humans and other species, including albinism.

Selective neutrality is the second factor that affects genetic variation in natural populations. Selective neutrality refers to circumstances in which two gene variants have little fitness differences. Natural selection may be defeated by the random force of genetic drift because minor changes in fitness result in only weak natural selection. Alleles that are selectively neutral are those whose frequency is determined by genetic drift rather than natural selection. Allele frequencies fluctuate over time in neutrality, randomly rising or dropping (Maunder, 1992). Random changes in the relative frequency of various alleles over time may eradicate some of them from the species. The genetic forms, on the other hand, are long-term, and new neutral alleles may appear on a regular basis as a result of mutation. Various types of natural selection aim to retain genetic diversity rather than eradicate it. Budget selecting, frequency-based selection, and evolving natural selection patterns with time and velocity are examples.

When a location has a heterogeneous characteristic, the heterozygous genotype (one with two distinct alleles) is more suited than either of the two homozygous species (one with the same allele) (Futuyma, 1998). All of the alleles included in the group will be kept under the heterozygote feature.

The growth and function of the brain, as well as the protection of neurological illnesses, are dependent on hereditary and genetic rules. Currently, there are no evident biological pathways underpinning neuropsychiatric illnesses or successful treatment options in the sector. Because most of these disorders are not monogenic and are likely to have a large environmental effect, epigenetics adds a whole new dimension to therapeutic interventions (Roth et al., 2011);(Feil and Fraga, 2012). Environmental variables including as diet, chemical pollutants, unpleasant early-life events, temperature variations, and exercise have a big impact on the genome, but how they effect brain development isn't fully known.

More crucially, the effects of the environment on epigenetics are not confined to postpartum development, but may also affect uterine development. It has recently been proposed that early life emphasises this because

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cellular genetic "priming" may produce long-term genetic alterations. When a specific environmental exposure happens and changes the epigenetic state of a gene, that gene is now in a "moderate response" and will respond quicker if the same environmental exposure occurs repeatedly (Vineis et al., 2017). The notion of epigenetic memory in response to environmental cues can be used to identify people who are predisposed to neuropsychiatric disorders. Rivers have influenced the development of human civilisation all across the planet. Rivers and floodplains have long been a part of human interaction. The study of rivers as a science began far later than it should have. The study of genetic diversity among populations and its link to the features of various river environments was the primary focus of biologists.

In Western Asia, the Tigris and Euphrates, together with their tributaries, comprise an important river system. They originate in eastern Turkey and flow through Syria, Iraq, and into the Arabian Gulf. The Tigris–Euphrates nature reserve comprises Iraq, Turkey, Syria, Iran, Saudi Arabia, Kuwait, and Jordan. The rivers flow from their headwaters and upper courses in the mountains of eastern Anatolia through valleys and gorges to the uplands of Syria and northern Iraq, and finally to the alluvial plain of central Iraq. The rivers travel south-east through the middle plain, merging at Al-Qurnah to create the Shatt al-Arab, which empties into the Arabian Gulf(Abdal-Satter et al., 2020).Predation may also benefit from frequency-dependent selection: if predators form a search image for more popular prey varieties, concentrating on capturing those, less common phenotypes may have a chance to survive in the end; modifying selection patterns over time or space can help preserve genetic variation in a population. Various alleles or genotypes may exhibit superior fitness at various periods if selection processes change throughout time. Both alleles could persist in a population as a result of the overall impact. A grasshopper population with two colour morphs, a brown morph and a green morph, is subjected to shifting selection pressures throughout time. The better-camouflaged brown grasshoppers enjoy more protection from predators early in the year, when the environment is browner. Nonetheless, as the season progresses, the environment becomes greener, and the green grasshoppers get more fit. Another explanation is that selection patterns vary from one location to the next due to habitat & environment differences (Hay et al., 1995).

Multiple alleles can be maintained in a species due to the abundance of various genotypes in various environments coupled with gene flow between environments; one instance is the allele for resistance to copper toxicity in grass species. Copper-tolerant alleles are widespread in locations where the soil is affected by copper mining (Ridley and Baker, 1993).

In uncontaminated areas, however, where they are less suited than conventional alleles, they are not predicted. The goal of this study is to use the cytochrome b gene to discover genetic diversity among and among three invasive Tilapia species in two separate locations south of Iraq. Gametes, on the other hand, can spread over broad distances due to wind contamination of species of plants.

The Aim of the Study

The study's goal is to "use mitochondrial gene to investigate genetic diversity across and within three invading Tilapia populations in two distinct locales in southern Iraq."

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II. METHODS AND MATERIAL

The Euphrates and Tigris meet near the village of Al-Qurnah in the county of Basra in southern Iraq, and the Shatt al-Arab is around 200 kilometres (120 miles) long.

The river's southern end defines the boundary between Iraq and Iran, and it pours into the Arabian Gulf at its mouth. Its breadth varies between 232 metres (761 feet) in Basra and 800 metres (2,600 feet) in its mouth. The Khawr Abd Allah is now an estuary, although it was previously the site of the Shatt al-discharge Arab's into the Arabian Gulf. It is located in southern Iraq and northern Kuwait; the Iraqi-Kuwaiti boundary separates the lower half of the estuary, although the inlet becomes entirely Iraqi near to the port of Umm Qasr. East of the Khawr Abd Allah, the Shatt al-Arab is now the location where the rivers pour out. It changes its name to Khawr az-Zubayr as it moves northwestward into Iraq, passing through Umm Qasr. It then connects to the northwest through canal and into the Tigris and Euphrates proper. It forms the northeast and north coasts of Jazirat Bubiyan and Jazirat Warbah, respectively, see figure 1 (Bury, 2003).

Sample Collection

Forty specimens of *Coptodon zillii* , *Oreochromis niloticus* , and *Oreochromis aureus*, were collected from January 2020 to September 2020, from two regions (Khaur Abdullah &Shatt Al-Arab) fifty: fifty from each region, as shown at (Table 1).

by Gill nets additional to electroshocking, and fin clip tissue samples having approximate size of (2 x 2 mm) were collected from caudal fin / left side of pectoral or pelvic fin without disturbing their morphology. Specimens were transferred to biotechnology Lab. in Marine Science Centre. The Size of gene was 439 bp for *Cuptodon zillii* , 326 bp for *Oreochromis aureus* & 473 bp for *Oreochromis niloticus* species.

Then this tissue samples were preserved in100% ethanol in 1.5ml micro tubes with appropriate code as tagged on specimens. The fish specimens were preserved in 70% ethanol. The voucher specimens were collected in Marine Science Centre /Basra University. As well as, the genomic DNA was extracted from ethanol – preserved fin clips using C-TAB method (Innis *et* al., 2012) / DNeasy Blood & Tissue Kit according to the manufacturer's instructions.

DNA Quantification

Nano-drop (260/280 ratio) was used to quantify the DNA extracted.

Samples were found to be contaminated with RNA or phenolic chemicals, and samples were processed further for PCR reactions. Primers for the cytb gene were constructed using Primer-BLAST(Ye et al., 2012) from the NCBI database (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) based on sequences available in GenBank (http://www.ncbi.nlm.nih.gov/tools/ (Table 2). Initial PCR amplifications were performed to identify the optimum annealing temperature and to assess the influence of template DNA, dNTPs, Mg++, and Taq DNA

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polymerase concentrations, largely following the procedure outlined by Williams et al. (1990) with minor annealing temperature adjustments (Williams et al., 1990).

The cytochrome b gene was amplified in 96-well plates using specified primers for PCR reactions utilizing the Kappa bio systems kit. 9.6 µl 10% trehalose, 7 µl H_2O, 2.5 µl 10X PCR buffer 'B', 0.8 µl MgCl2, 2 µl 2.5mM DNTP, 1 µl 10mM forward primer, 1 µl 10mM reverse primer, and 0.1 µl taq polymerase made up the reaction master mix (5 units).

An initial step of 3 minutes at 95°C was followed by 35 cycles of 30 seconds at 94°C denaturation, 30 seconds at (51-48) °C annealing range for both species, and 30 seconds at 72°C extension, with a final extension at 72°C for 10 minutes. On a 2 percent agarose gel, amplicons were detected as Shawn at figure 2. PR omega's Wizard® SV Gel and a PCR Clean-Up System were used to purify the Amplicons (Promega Madison, WI). The Big Dye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.) was used in the sequencing procedures, and the purified PCR product was sequenced on an ABI 3130 Genetic analyzer capillary sequencer.

The barcode product was paired-end sequenced to facilitate full-length barcode sequence by eliminating signal degradation issues that sometimes arise near the conclusion of a read.

Sequence Analysis

Sequence polymorphisms were deduced from sequence comparisons in Alignment Explorer (Molecular Evolutionary Genetics Analysis software). Haplotypes and haplotype frequencies were determined within each (cytb) gene individual using DnaSPv5.10 (Khlestkina et al., 2004) and Arlequin v3.1 (Hackauf and Wehling, 2002), respectively.

Data Analysis

To discover the level of genetic variation, sequences were edited using the UPGMA method in MEGA7 (Kumar et al., 2016) and aligned with Clustal W 1.6, which was installed in the same software, molecular diversity indices [number of haplotypes, haplotype diversity (Hd), and nucleotide diversities (π)].(Schneider et al., 2000). AMOVA (analysis of molecular variance) is a technique for detecting genetic differences (Excoffier et al., 1992).

III. RESULTS

The present study reveals low genetic variation among Oreochromis aureus, and Oreochromis niloticus populations in Shatt Al-Arab and Khour Abdallah regions, where the individuals cytb genes were dominated by (2) haplotypes, whereas, no diversity within each population in each station, where the haplotypes within the individuals cytb genes was only one haplotype, according to an analysis of molecular variance of cytb gene. Number of individuals whose have H1 haplotype were 16 in Shatt Al-Arab, while there were 14 individuals which have H2 in Khour Abdallah successful of total 20 *Oreochromis aureus* individuals.

For Oreochromis niloticus 18 individuals have H1 haplotype in Shatt Al-Arab and 12 have H2 haplotype in Khour Abdallah successful of total 20 individuals from each region, as shown at Table 4.

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There was genetic heterogeneity among the Shatt Al-Arab and Khour Abdallah populations of *Coptodon zillii* with three haplotypes dominating the individuals' cytb genes.

However, within the *Coptodon zilliii* Shatt Al-Arab species, there was no diversity, but within the *C. zilliii* Khour Abdallah group, there was no variety. Eight individuals with haplotype H1 were successful, whereas 11 individuals with H2 were successful out of a total of 20 individuals from Khour Abdallah, and 17 individuals with H3 were successful out of a total of 20 individuals, see Table 4.

Through an analysis of molecular variance of cytb gene for *C. zilliii* species, analyses of nucleotide and haplotype diversity were were done independently for each population for *Coptodon zilliii* species using the software DnaSP v5.10. Low of the genetic variation was found between individuals within Khour Abdallah population, where the individuals cytb genes were dominated by (2) haplotypes, and no diversity in the Shatt Al-Arab population, with just one haplotype dominating their cytb genes.

Nucleotide and haplotype diversity of cytb genes were range values $\pi = (0.161 \pm 0.084)$ and $Hd = (1.00 \pm 0.024)$, respectively among the populations in both regions. While, Nucleotide and haplotype diversity of cytb genes were range values $\pi = (0.048 \pm 0.014)$ and $Hd = (0.89 \pm 0.003)$, respectively among *Oreochromis aureus* populations in both stations. $\pi = (0.050 \pm 0.016)$ and $Hd = (0.90 \pm 0.003)$ cytb genes were range values for *Oreochromis niloticus* also in both regions Shatt Al-Arab and Khour Abdallah, see table 3

UPGMA Dendrogram based on cytb gene sequences shows that three different clusters of *Coptodon zilliii* among Shatt Al-Arab & Khour Abdallah populations were formed, and two different clusters *Oreochromis niloticus* were formed (Figure 4), also two different clusters of *Oreochromis aureus* among Shatt Al-Arab and Khour Abdallah populations were formed, see Figure 3.

The main findings of this study are as follows:

- Study reveals genetic variation within and among three populations in two locations using cytb gene.
- *Oreochromis niloticus* and *Oreochromis aureus* populations had not have genetic variation within their individuals, whereas there was low diversity among Shatt Al-Arab and Khour Abdallah species.
- Genetic diversity of *Coptodon zilliii* among Shatt Al-Arab & Khour Abdallah populations observed in means of haplotype diversity & nucleotide diversity, despite the low diversity within Khour Abdallah population and no diversity within Shat Al-Arab species.

IV. DISCUSSION

The genetic variation of *Oreochromis aureus*, and *Oreochromis niloticus* populations was not observed within species from Shatt Al-Arab and Khour Abdallah populations, where Data was inferred from mtDNA cytochrome b gene sequences analysis. The nucleotide and haplotype diversity values revealed low genetic variation among *O. aureus*, and *O. niloticus* populations in Shatt Al- Arab river and Khour Abdallah southern of Iraq where two haplotypes for *O. aureus sp.* and two haplotypes for *O. niloticus sp.* were

revealed in spite of the expectation that the species are invading species will affect the genetic diversity, as it came from different environments compared with local species.

These fish are native to Africa and the Middle East, where Tilapias were imported to Iraq in the late 1990s for aquaculture rather for natural freshwater stocking.

Tilapia eventually made its way to Iraq's rivers and lakes, where it formed a viable population.

In addition to the success of tilapia culture expansion increase of culture activities, several problems have arisen, among these: the development of new strains and hybrids, mono-sex male culture, formulated diets, a variety of semi-intensive and intensive culture systems.

For *Coptodon zilliii* populations where pi (π) and Hd values revealed genetic variation among Shatt Al-Arab and Khour Abdallah populations where two haplotypes for Khour Abdallah group and one haplotype for Shatt Al-Arab species, but haplotype sharing and low nucleotide mutation within Khour Abdallah population was apparent from the data. no genetic variation in Shatt Al -Arab population. Overall, this study provided relevant genetic information for *Coptodon zilliii*, *Oreochromis aureus*, and *Oreochromis niloticus* populations planning management, conservation and ranching guidelines for Fishes south of Iraq.

V. CONCLUSION

The different environments that those tilapia species came from had no effect on genetic diversity as expected, where Shatt Al-Arab is formed by the confluence of the Euphrates and Tigris rivers in southern Iraq, where the Euphrates river flows by/through Turkey, Syria, and Iraq into the Arabian Gulf through Shatt Al-Arab.

In addition to the success of tilapia culture expansion, breeders and fish farmers are importing fingerlings and small-scale fish of these species as economic fish species from other nations. In the southern Iraqi cities of Shatt Al-Arab River and Khour Abdallah, low genetic variation was identified among both (*Oreochromis aureus* and *Oreochromis niloticus*) populations, with no diversity within each.

Individuals of the *Coptodon zilliii* species were discovered to have genetic variation in Shatt Al-Arab and Khour Abdallah, but no genetic variation within Shatt Al-Arab indivdials and low genetic variation within Khour Abdallah individauls.

Our findings suggest that wild stocks of species in the south of Iraq require a variety of genetic management practises, such as stopping unintentional fish capture and establishing sanctuaries to protect small stocks, in order to preserve and conserve the current diverse gene pool and avoid genetic deterioration, which would have a negative impact on aquaculture productivity. There is also an essential conclusion that revealing the whole genetic variation before genetically contaminating is the ideal case.

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Table 1. List of specimens collected from two sampling stations

Species of samples	Shatt Al- Arab	Khour Abdallah
Oreochromis aureus	20	20
Oreochromis niloticus	20	20
Cuptodon zillii	20	20

Table 2: List of primers designed of Cytochrome b genes for three species of Tilapia

Species	Primer	Sequence(5'-3')	Sequence size(bp)
Oreochromis aureus	Left primer	CAGCTCCCTCAAACATCTCC	326
	Right primer	GCCCGATGTGGAGGTAAATA	
Oreochromis niloticus	Left primer	ACGGCTGACTCATTCGAAAC	473
	Right primer	GTCCTCATGGGAGGACGTAG	
Cuptodon zillii	Left primer	AGGAGAAGGCTGTTGCGATA	439
	Right primer	GCAAACGACGCACTAGTTGA	

Table 3. Genetic diversity parameters among three species of Tilapia Using mtCyt gene.

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Species Diversity indices	*Coptodon zillii*	*Oreochromis aureus*	*Oreochromis niloticus*
Number of Haplotypes	3	2	***2***
Haplotype diversities (Hd)	1.00 ± 0.024	0.89 ± 0.003	0.90 ± 0.003
Nucleotide diversities	0.161 ± 0.084	0.048 ± 0.014	0.050 ± 0.016

Table 4. Haplotype variation in mtCyt-b of the three species of tilapia fish from two regions south Iraq

Cytb								
Coptodon zillii (Shatt Al-Arab & Khour Abdallah)			***Oreochromis niloticus*** (Shatt Al-Arab & Khour Abdallah)			***Oreochromis aureus*** (Shatt Al-Arab & Khour Abdallah)		
Haplotypes	No. of samples	Frequency	Haplotypes	No. of samples	Frequency	Haplotypes	No. of Samples	Frequency
H1	8	(0.065)	HI	18	(0.298)	H1	16	(0.285)
H2	11	(0.131)	H2	12	(0.253)	H2	14	(0.273)
H3	17	(0.266)						

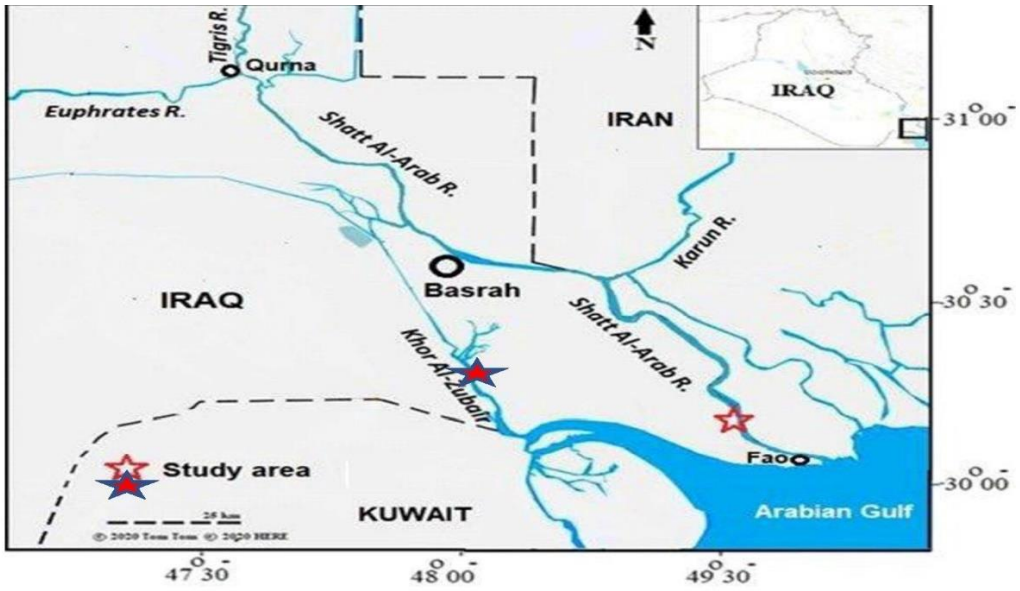

Fig.1 Refer to Area study 'Shatt Al-Arab river and Khour Abdallah', southern of Iraq (samples locations).

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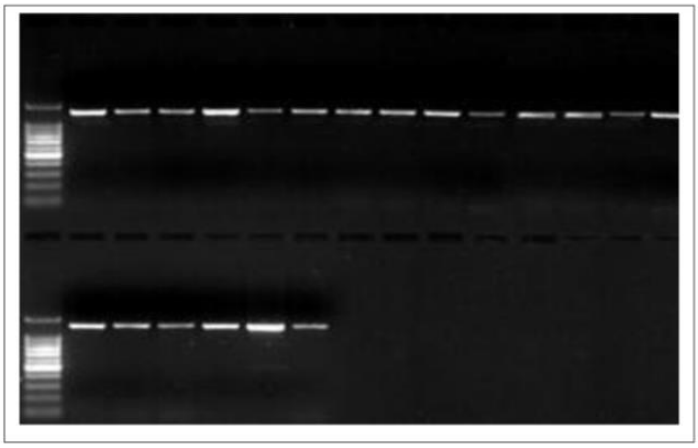

Fig.2 Agarose gel electrophoresis introducing PCR products of cytochrome b gene

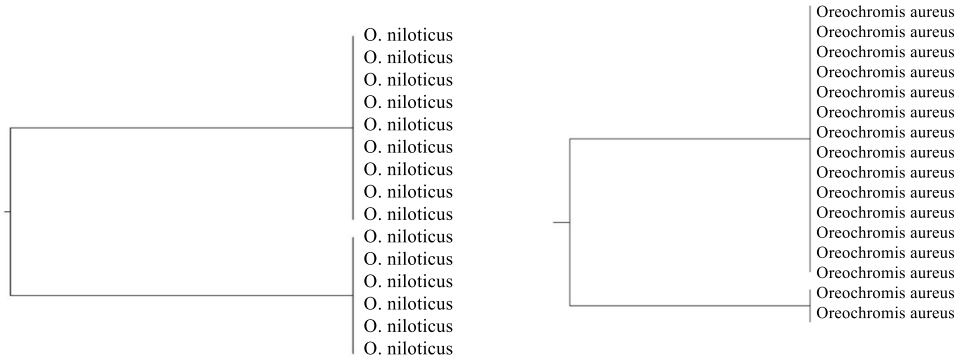

Fig. 3. UPGMA Dendrogram, based on the nucleotide divergence showing, left: the relationship between the Shatt Al-Arab and Kaur Abdallah populations for *Oreochromis niloticus.* Right: *Oreochromis aureus.*

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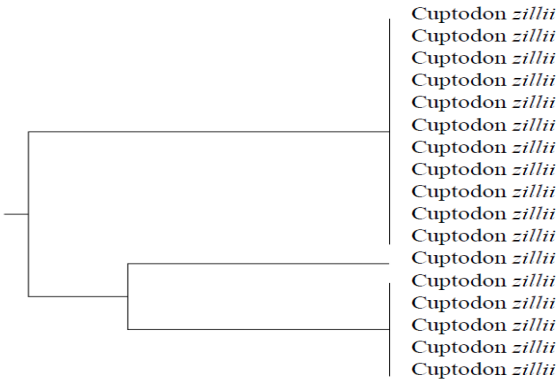

Fig. 4. UPGMA Dendrogram, based on the nucleotide divergence showing: The relationship between the Shatt Al-Arab and Kaur Abdallah populations for Coptodon zilliii species.