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Do Habitat Features Modify Remarkable Genetic Homogeneity of *Oreochromis niloticus* Linnaeus, 1758 (Actinopterygii: Perciformes) Native to Burullus Lake, Rivulet and River Nile?

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Abstract: The Cichlidae family, including the Nile tilapia (*Oreochromis niloticus*), is a key model for studying evolutionary genetic homogeneity. This study examined remarkable genetic homogeneity among tilapia populations in different Egyptian habitats; Burullus Bay, Shakhlouba watershed, a rivulet, and the River Nile using the ITS DNA region. Results showed 100% genetic similarity across populations, suggesting either frequent migration or a robust genetic structure enabling adaptation to diverse environments. However, future habitat fragmentation due to water shrinkage and salt intrusion may disrupt this genetic uniformity over time.

Comparative BLAST analysis revealed high genetic similarity (93.77–100.00%) between Egyptian and Chinese *O. niloticus* isolates. For instance, an Egyptian isolate (On309) matched Chinese isolates at 99.87% (three samples) and 99.74% (six samples). Similarly, isolates from Burullus Bay (On80), the Damietta Nile branch (On77), and Shakhlouba watershed (On7785) showed 99.78% identity with multiple Chinese samples. The slightly lower pairwise identity (93.77%) for seven Chinese isolates may reflect habitat-specific influences. Notably, Shakhlouba's polluted conditions—receiving wastewater from residential, agricultural, and religious sources—could contribute to minor genetic homogeneity.

Overall, the study highlights the Nile tilapia's genetic stability across varied habitats but warns that environmental changes may alter population structures. The high cross-regional genetic similarity suggests either recent common ancestry or strong adaptive capacity, though localized ecological factors may introduce subtle variations. These findings underscore the importance of monitoring habitat changes to predict long-term genetic impacts on this ecologically and economically vital species.

keywords: Nile Tilapia, Genetic Homogeneity, Shakhlouba watershed, Rivulet, River Nile.

Introduction

Wide, streams are typically referred to as rivers, however smaller, less substantial, and more sporadic or intermittent streams are referred to as rivulet, brook, creek, tributary, rill, run, feeder, freshet, minor stream and streamlet. A river embodies the spirit of a location, encompassing its history and cultural heritage, while also supporting vital systems including agriculture, aquaculture, transportation, industry, recreation, and numerous others. A river embodies the spirit of a location, encompassing its history and cultural heritage, while also supporting vital systems including agriculture, aquaculture, transportation, industry, recreation, and many others [1].

More than 75 species of tilapias exist worldwide, with approximately nine species utilized in global aquaculture. A moderate rise in worldwide Nile tilapia (*Oreochromis niloticus*) production has occurred, representing 8.3% of global aquaculture finfish output in 2018 [2]. As a result, it markedly boosts global tilapia output from freshwater aquaculture. Numerous tilapia species exist in the wild in Nigeria, with some being farmed, yet *Oreochromis niloticus* seems to be particularly

favored by fish farmers [3], likely because of its economic advantages in semi-flow-through culture systems [4]. Genetic analysis of this species in Nigerian waters is crucial for identifying differences and commonalities among populations. This will also be beneficial in aquaculture and managing fisheries stocks. Molecular genetic markers serve as powerful instruments for assessing the genetic distinctiveness of individuals, populations, or species [5 – 7].

Cichlids (Teleostei: Perciformes: Cichlidae) are recognized as one of the most diverse families of fish. Currently, they encompass over 1600 valid species taxa [8], with estimates suggesting that the total may reach up to 3000 species. These species are found across the Neotropics, Africa, the Middle East, Madagascar, as well as in Southern India and Sri Lanka [9]. The remarkable morphological, behavioral, and ecological diversity of cichlids has intrigued biologists, particularly since the vast diversity observed in the East African cichlid radiation endemic to Lakes Tanganyika, Malawi, and the Lake Victoria region became evident [10]. In recent decades, cichlids have emerged as a key model system in evolutionary biology, particularly in the study of speciation [11]. The Nile Tilapia, *Oreochromis niloticus* Linnaeus, 1758, holds significant global importance for aquaculture [12]. Molecular phylogenetics has shown that the origin of the East African cichlid radiation is situated within a paraphyletic group that includes, among other tilapiine genera, members of the genus *Tilapia* Smith, 1840 [13].

The null hypothesis of the present study postulates that habitat features have no impact on remarkable genetic homogeneity of tilapia *Oreochromis niloticus* Linnaeus, 1758, irrespective of the variation of physicochemical and biotic aspects in diverse habitats. In view of this hypothesis, it is likely that the populations of *Oreochromis niloticus* existing in Burullus Lake hold comparable genetic physiognomies to their corresponding populations in the River Nile. This null hypothesis was verified in the current investigation by performing a comparative exploration of the genetic morphemes of tilapia (phylogenetic tree and genomic structure). The genomic structure pertains to the layout and configuration of an organism's DNA, encompassing genes and various functional sequences. It includes the physical and chemical traits of DNA, along with the spatial organization of chromosomes and chromatin inside the nucleus.

2. Materials and methods

The studied ecosystems are located in the Nile Delta, north Egypt (Fig. 1). Three differing–water quality habitats were explored, namely Burullus Bay (coordinates: 31.57907533856282, 30.976486990850756), Shakhlouba watershed (coordinates: 31.40296082479885, 30.75881721856754) and a rivulet, namely, Drain-7 (coordinates: 31.40392892446864, 30.77444568047246), in addition to Damietta Branch of the River Nile (coordinates: 31°03′00″N 31°23′00″E) as the original habitat of *Oreochromis niloticus*.

Tilapia (*Oreochromis niloticus*) muscle piece is fixed in 96% ethyl alcohol and proceed for DNA extraction and amplification with PCR using specific primers (18S RNA, 28S RNA, ITS1 and ITS2 according to Mendoza-Palmero *et al*. [14]. The coding regions are used to determine the phylogenetic relationship between phyla while the non-coding regions are used to study the relationships among closely related genera congeneric species [15]. The ITS regions (non-coding) are used as molecular markers for taxonomy and phylogenetic analyses.

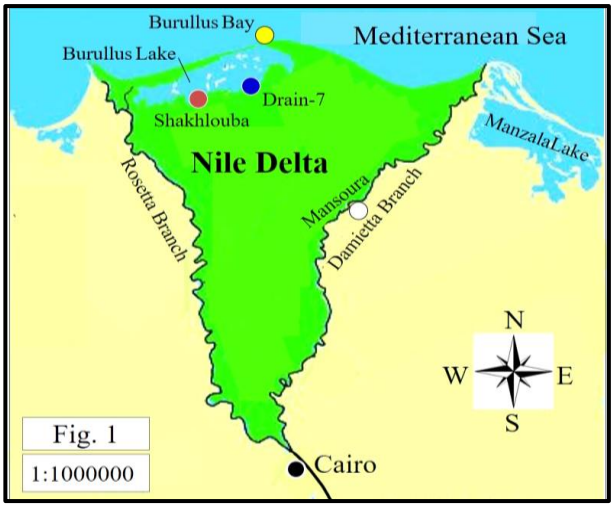

Figure 1. Hand drawn map of the Nile Delta, Egypt showing: Burullus Bay (yellow solid circle), Shakhlouba watershed (red solid circle), Drain-7 (blue solid circle) and Damietta Branch of the River Nile in the vicinity of Mansoura (white solid circle). Note that the bright green color in the map reflects the fertility of this

magic landscape (Nile Delta), however yellow color reflects the eastern and western desert around Nile Delta. Note the directions according to the compass.

DNA Extraction Procedure: Begin by carefully excising 100 mg of muscle tissue (dorsolateral aspect of tilapia) using a sterile scalpel and forceps. Next, homogenize the muscle in 600 µl of extraction buffer and transfer the resulting paste into a 1.5 ml Microcentrifuge Tube (MCT). Genomic DNA was extracted via the salting out technique following the procedure of Lopera-Barrero *et al*. [16]. Muscles were stored in Eppendorf microtubes containing 90% absolute ethanol and kept in a freezer at -20°C. Lysis buffer (550 µL) with 7 µL of 200 µg mL^{-1} proteinase K was incorporated. The samples were placed in a water bath at 50°C for 12 hours. Sodium chloride (660 µL of 5 M) was introduced and centrifuged at 12,000 rpm for 10 minutes. The supernatant was placed into a new tube and 700 µL of cold absolute ethanol was added to precipitate the DNA. The samples were stored at -20°C for 2 hours. The DNA sample was spun in a centrifuge and rinsed with 700 µL of 70% v/v ethanol. The sample was re-dissolved in 80 µL of TE buffer (10 mM Tris pH 8.0 and 1 mM EDTA). Ribonuclease (30 µg mLG1) was introduced and allowed to incubate at 42EC for 4 hours. This was kept in the freezer at -20°C prior to PCR. The DNA's concentration and purity were assessed by measuring absorbance at 260 and 280 nm using a Biorad Nano Spectrophotometer. The PCR reactions were carried out in a Biorad I-cycler following this program. Denaturation occurs initially at 95°C for 5 minutes, followed by 35 cycles consisting of 30 seconds at an annealing temperature (45-60°C) and 1 minute at 72°C, concluding with a final elongation phase of 10 minutes at 72°C. The enhanced DNA fragments were subjected to electrophoresis with 1.5% (w/v) agarose gel and stained using Ethidium bromide. The amplified products were observed on a UV transilluminator and captured in photographs. The SSR primers chosen were obtained from the database at the National Center for Biotechnology Information [17].

Heat maps are commonly used to visualize grouped data. Basically, a heat map consists of a grid made up of rectangles or squares, where each cell corresponds to an entry in the data table, representing both a row and a data set. Each rectangle or square is assigned a color based on the value contained in that particular cell of the table [18]. A heatmap (also known as a heat map) illustrates values for a primary variable of interest across two axis variables arranged as a grid of colored squares. The axis variables are categorized into ranges similar to a bar chart or histogram, with the color of each cell reflecting the value of the main variable pertinent to that specific cell range. Typically, a sequential color gradient is used where lighter shades represent lower values and darker shades denote higher values, or the other way around. Since color alone does not have an intrinsic link to value, a key is crucial for viewers to understand the values represented in a heatmap [18].

3. Results

3.1. Habitat features in explored ecosystems

3.1.1. Heat Map of Selected Physicochemical Factors

As shown in Figure 2, summer showed the highest thermal regime at the three explored habitats. However, water temperatures exhibited obviously lower levels throughout the study period. The lowest water temperature was obtained at Drain-7 during winter.

	Burullus Bay	Shakhlouba watershed	Drain-7
Winter	20	18.3	17.7
Spring	23.6	18.4	18.2
Summer	29.4	30	31
Autumn	19.9	21.5	21.3

Figure 2. A heat map (colour scale: Red-Yellow-Green) showing seasonal fluctuations in water temperature at Burullus Lake, Shakhlouba watershed and Drain-7. Note that dark red colour reflects maximum levels, while dark green colour reflects minimum levels. Note also that yellow colur is intermediate and can blend with either the red or green colour to display a definite gradient.

As shown in Figure 3, the highest electrical conductivity at Burullus Lake during autumn. An obviously high electrical conductivity level was obtained at the same habitat during spring, however the lowest level was reached during winter. Comparatively lower electrical conductivity levels could be recognized in Shakhlouba during spring and summer, as well

as in Drain-7 during winter, summer and autumn.

	Burullus Bay	Shakhlouba watershed	Drain-7
Winter	2.94	4.5	1.53
Spring	5.75	1.57	2.53
Summer	3.75	1.94	1.92
Autumn	7.5	3.71	1.63

Figure 3. A heat map (colour scale: Red-Yellow-Green) showing seasonal fluctuations in water electrical conductivity at Burullus Lake, Shakhlouba watershed and Drain-7.

Figure 4 illustrates seasonal fluctuations in total dissolved solids at Burullus Lake, Shakhlouba watershed and Drain-7. This physicochemical parameter attains a similar seasonal pattern to that displayed by electrical conductivity. The highest total dissolved solids at Burullus Lake during autumn. An obviously high total dissolved solids level was obtained at the same habitat during spring, however the lowest level was reached during winter. Comparatively lower total dissolved solids levels could be recognized in Shakhlouba during spring and summer, as well as in Drain-7 during winter, summer and autumn.

	Burullus Bay	Shakhlouba watershed	Drain-7
Winter	1882	2880	979.2
Spring	3680	1004.8	1619.2
Summer	2400	1241.6	1228.8
Autumn	4800	2374.4	1043.2

Figure 4. A heat map (colour scale: Red-Yellow-Green) showing seasonal fluctuations in water total dissolved solids at Burullus Lake, Shakhlouba watershed and Drain-7.

Figure 5 illustrates seasonal fluctuations in amounts of dissolved oxygen at Burullus Lake, Shakhlouba watershed and Drain-7. Water was highly oxygenated during spring in Drain-7 and Burullus Lake, and during autumn at Shakhlouba watershed. Water showed relative oxygen depletion during winter in Shakhlouba watershed as well as during summer in Drain-7.

	Burullus Bay	Shakhlouba watershed	Drain-7
Winter	7	4.8	6.5
Spring	7.7	6.6	8.5
Summer	6	5.3	4.4
Autumn	6.9	8.5	7.1

Figure 5. A heat map (colour scale: Red-Yellow-Green) showing seasonal fluctuations in water dissolved oxygen at Burullus Lake, Shakhlouba watershed and Drain-7.

3.1.2. Distinctive abiotic elements of water

The electrical conductivity in water from Burullus Bay is noticeably higher than corresponding levels in Shakhlouba watershed and Drain-7 (4.99±2.05 dS/m, 3.17±1.07 dS/m and 1.67±0.16 dS/m, respectively). Similarly, the amount of total dissolved solids is remarkably higher than corresponding levels in Shakhlouba watershed and Drain-7 (3190.50±1312.40 mg/L, 1875.2±831.16 mg/L and 1217.6±266.54 mg/L, respectively). Amounts of bicarbonates in water of Burullus Bay were obviously higher than those of Shakhlouba watershed and Drain-7 (376.38±213.66 mg/L, 270.84±116.49 mg/L and 126.42±133.93 mg/L, respectively). Similar trends were documented for amounts of sulphates (631.92±458.36 mg/L, 524.95±203.87 mg/L and 256.62±131.58 mg/L, respectively) and chlorides (2182.46±719.69 mg/L, 1229.84±692.38 mg/L and 670.67±136.80 mg/L, respectively). The thermal regime was homogenous at Burullus Bay, Shakhlouba watershed and Drain-7 (23.23±4.46°C, 22.05±5.50°C and 22.05± 6.18°C, respectively). The pH is located within fairly alkaline scale at Burullus Bay, Shakhlouba watershed and Drain-7 (8.69±0.56, 8.62±0.22 and 8.54±0.45, respectively). The water at Burullus Bay, Shakhlouba watershed and Drain-7 were fairly oxygen-rich (6.90±0.70 mg/L, 6.30±1.65 mg/L and 6.63±1.70 mg/L, respectively). The water body of Burullus Bay (308.6±72.61 cm) is evidently deeper than water bodies at Shakhlouba watershed (135.20±17.53 cm) and Drain-7 (78.03±17.22 cm). The water body at Drain-7 showed higher turbidity levels (14.5±5.26 cm) than water bodies at Burullus Bay (15.50±4.20 cm) and Shakhlouba watershed (36.25±11.09 cm).

3.2. Genetic map of *Oreochromis niloticus*

Ten studied isolates from *Oreochromis niloticus* isolate species collected from each of the four locations (Drain 7 connecting to south of Burullus Lake, Burullus Bay (north Burullus Lake) at Kafr El-Sheikh Governorate, Shakhlouba watershed (south Burullus Lake) at Kafr El-Sheikh Governorate and Damietta branch of the River Nile at Dakahlia Governorate (Egypt) were compared to relatives and identified on molecular basis

using ITS region. From Blast analysis, studied isolates displayed 100% - 93.77% similarity with the formerly identified isolates of *Oreochromis niloticus* on the Genbank were illustrated in Tables (1-4). Figures (6-9) represent the constructed phylogenetic tree of the studied two isolates and their relatives which comes in line with the previous morphological identification. The GenBank accession numbers and sequences of the studied isolates were illustrated in Table (5).

Table (1): Comparison of the similarity percentage of Oreochromis niloticus isolate On309 collected from Drain-7 connecting to south of Burullus Lake (Egypt) with the other Oreochromis niloticus isolates previously registered in NCBI.

Taxa	Country	The most similar sequences in Gene Bank database	
		GenBank Accession Number	Identity
Oreochromis niloticus isolate On309	Egypt (Drain-7 south Burullus Lake)	PP091930	100 %
Oreochromis niloticus isolate 58	China	MF460357	99.87 %
Oreochromis niloticus isolate 55	China	MF460354	99.87 %
Oreochromis niloticus isolate 15	China	MF460314	99.87 %
Oreochromis niloticus isolate 19	China	MF460318	99.74%
Oreochromis niloticus isolate 40	China	MF460339	99.74%
Oreochromis niloticus isolate 20	China	MF460319	99.74%
Oreochromis niloticus isolate 5	China	MF460304	99.74%
Oreochromis niloticus isolate 28	China	MF460327	99.74%
Oreochromis niloticus isolate 43	China	MF460342	99.74%

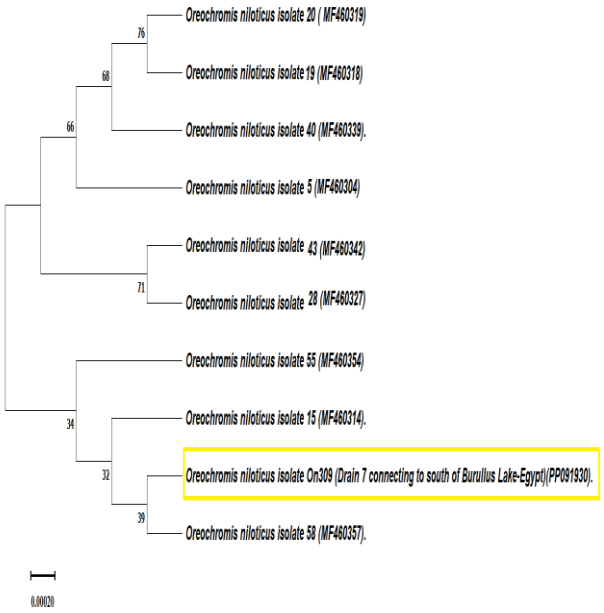

Figure 6. Phylogenetic tree for *Oreochromis niloticus* isolate On309 collected from Drain 7 connecting to south of Burullus Lake) from Egypt inferred from ITS sequences obtained from Gene Bank (highlighted in yellow color). Bootstrap tests were performed with 1000 replications.

Table (2): Comparison of the similarity percentage of *Oreochromis niloticus* (isolate On80) collected from Burullus Bay connecting Burullus Lake to Mediterranean Sea at Kafr El-Sheikh Governorate (Egypt) with the other Oreochromis niloticus isolates previously registered in NCBI.

Taxa	Country	The most similar sequences in Gene Bank database	
		GenBank Accession Number	Identity
Oreochromis niloticus isolate On80	Egypt (Burullus Bay Kafr El-Sheikh Governorate)	PP085551	100 %
Oreochromis niloticus isolate 53	China	MF460352	99.78 %
Oreochromis niloticus isolate 19	China	MF460318	99.78 %
Oreochromis niloticus isolate 40	China	MF460339	99.78 %
Oreochromis niloticus isolate 20	China	MF460319	99.78 %
Oreochromis niloticus isolate 5	China	MF460304	99.78 %
Oreochromis niloticus isolate 43	China	MF460342	99.78 %
Oreochromis niloticus isolate 37	China	MF460336	99.78 %
Oreochromis niloticus isolate 31	China	MF460330	99.78 %

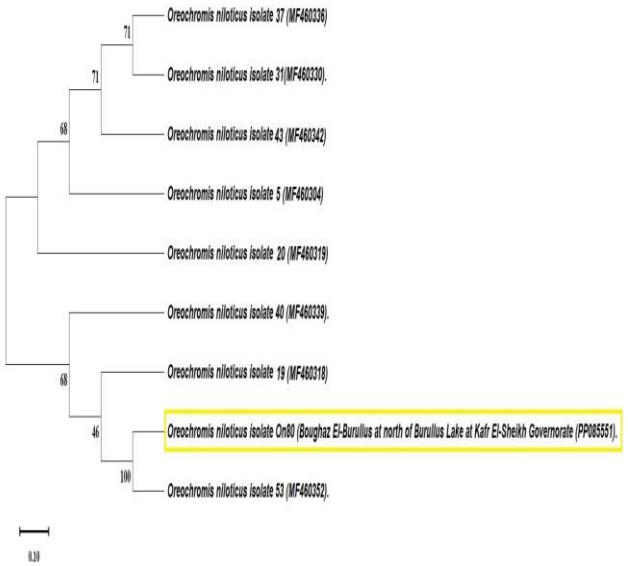

Figure 7. Phylogenetic tree for *Oreochromis niloticus* (isolate On80) collected from Burullus Bay, north Burullus Lake at Kafr El-Sheikh Governorate (Egypt) inferred from ITS sequences obtained from Gene Bank (highlighted in yellow color). Bootstrap tests were performed with 1000 replications.

3.2.1. *Oreochromis niloticus* isolate On309 collected from Drain-7 connecting to south of Burullus Lake (Egypt)

The sequence length, AT and GC content for studied taxa are recorded as in Table (6). The sequence length was 760 bp. AT% content also was 34.6% while GC% content was 65.4 %. The BLAST search showed a pairwise identity (PI) of 99.87% for three *Oreochromis niloticus*

isolates (15, 55 and 58) and identity (PI) of 99.74 % for six *Oreochromis niloticus* isolates (5, 19, 20, 28, 40 and 43) from China (Table 1).

Table (3): Comparison of the similarity percentage of *Oreochromis niloticus* On7785 collected from Shakhlouba watershed, south Manzala Lake at Kafr El-Sheikh Governorate (Egypt) with other *Oreochromis niloticus* isolates previously registered in NCBI.

Taxa	Country	The most similar sequences in Gene Bank database	
		GenBank Accession Number	Identity
Oreochromis niloticus isolate On7785	Egypt (Shakhlouba, south Manzala Lake at Kafr El-Sheikh Governorate)	PP085499	100 %
Oreochromis niloticus isolate 19	China	MF460318	100 %
Oreochromis niloticus isolate 40	China	MF460339	93.77 %
Oreochromis niloticus isolate 20	China	MF460319	93.77 %
Oreochromis niloticus isolate 58	China	MF460357	93.77 %
Oreochromis niloticus isolate 5	China	MF460304	93.77 %
Oreochromis niloticus isolate 43	China	MF460342	93.77 %
Oreochromis niloticus isolate 37	China	MF460336	93.77 %

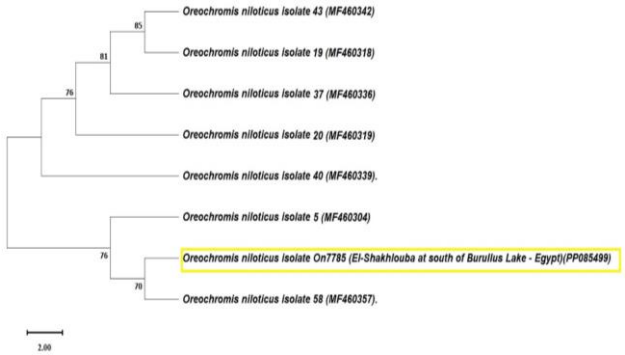

Figure 8. Phylogenetic tree for *Oreochromis niloticus* isolate On7785 collected from Shakhlouba watershed, south Manzala Lake at Kafr El-Sheikh Governorate (Egypt) inferred from ITS sequences obtained from Gene Bank (highlighted in yellow color). Bootstrap tests were performed with 1000 replications.

Table (4): Comparison of the similarity percentage of *Oreochromis niloticus* (isolate On77) collected from Damietta branch of the River Nile at Dakahlia Governorate (Egypt) with other *Oreochromis niloticus* isolates previously registered in NCBI.

Taxa	Country	The most similar sequences in Gene Bank database	
		GenBank Accession Number	Identity
Oreochromis niloticus isolate On77	Egypt (Damietta branch of the River Nile at Dakahlia Governorate)	PP085498	100 %
Oreochromis niloticus isolate 19	China	MF460318	99.87 %
Oreochromis niloticus isolate 40	China	MF460339	99.87 %
Oreochromis niloticus isolate 20	China	MF460319	99.87 %
Oreochromis niloticus isolate 5	China	MF460304	99.87 %
Oreochromis niloticus isolate 28	China	MF460327	99.87 %
Oreochromis niloticus isolate 43	China	MF460342	99.87 %
Oreochromis niloticus isolate 37	China	MF460336	99.87 %
Oreochromis niloticus isolate 25	China	MF460324	99.87 %

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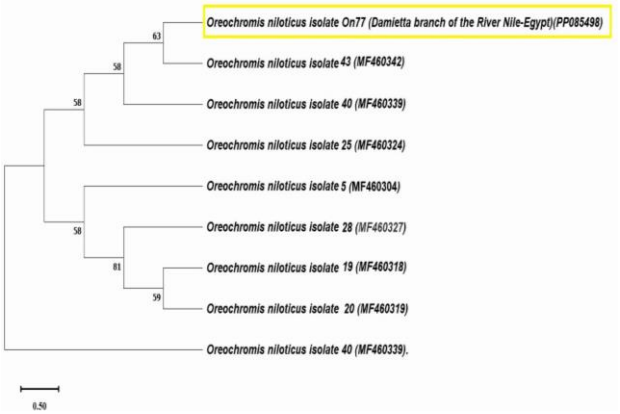

Figure 9. Phylogenetic tree for *Oreochromis niloticus* (isolate On77) collected from Damietta branch of the River Nile at Dakahlia Governorate (Egypt) inferred from ITS sequences obtained from Gene Bank (highlighted in yellow color). Bootstrap tests were performed with 1000 replications

Table (5): Accession numbers and sequences of the studied isolates from *Oreochromis niloticus* and their relatives.

No	Isolates	Sequences	Genbank accession numbers
1	*Oreochromis niloticus isolate On309*	CTACCGATTGGATGGTTTAGTGAGGTCCTCGGATCGGCCCCGCCGGGGTCGGTCACGGCCCTGGCGGAGCGCCGAGAAGACGATCAAACTTGA CTATCTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACTGGCTACACCGAGCGGCCCGCCTGCGGCG CACCCGGTGTTCTCCCTCTTTGCCGCCGAGGGTCTCCCGCCACCGTCACCGGTGCGGGTCTCCCGAGGTTGTCGGCTCGCGCGTCCCCCACCGGA GCTCGAGCCTTAGTCTGGGCCTGGTCACCGGCCGGACGACCCGACGGCCCCGCCCCGCCTCTACGCCAAAGCGAGCCCGCTGCCCCGACCGGCT CCTCCGAGGCCGACGGAGGAGAGTCGGACTCGCGCTCGTTGAGAGCGGGCACCGGGGTCCCGTCCGGCAACCACCGGTCCCGGCCCCCA CGAGAACCTCAACCGAAAGCGCGGGCTGGCGGTTTCGCCCTGACCGCTGCCCGCGCGCCTCCGGGTACCCAACTCTCCTCCCTCCTGCGGAGG AGCACGGGGGGTTCAATGTCTCCTTCCCCTGCCCCGGCGGAGGAAGGAGCGCCCGGGGGTTTTTTCCTTCCTTTAAACCCCTTATCCCGTCTA CGAATGTGGCAACCCACGGTAAAACAAAAAACAATACGACTCTTAGCGGTGGATCACTCGGCTCGTGCGTCGATGAAGAACGCAGCTAGCTG CGAGAACTAA	PP091930
2	*Oreochromis niloticus isolate On80*	CTACCGATTGGATGGTTTAGTGAGGTCCTCGGATCGGCCCCGCCGGGGTCGGTCACGGCCCTGGCGGAGCGCCGAGAAGACGATCAAACTTGA CTATCTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACTGGCTACACCGAGCGGCCCGCCTGCGGCG GCACCCGGTGTTCTCCCTCTTTGCGCCGAGGGTCTCCCGCCACCGTCACCGGTGCGGGTCTCCCGAGGTTGTCGGCTCGCGCGTCCCCCACCGG AGCTCGAGCCTTAGTCTGGGCCTGGTCACCGGCCGGACGACCCGACGGCCCCGCCCCGCCTCTACGCCAAAGCGAGCCCGCTGCCCCGACCGGC CTCCTCCGAGGCCGACGGAGGAGAGTCGGGACTGCGGCTCGTTGGAGCGGGCGCCGGGGTCCCGTCCGGCAACCACCGGTCCCGGCCCCCG CACGAGAACCTCAACCGAAAGCGCGGGCTGGCGGTTTCGCCCTGACCGCTGCCCGCGCGCCTCCGGGTACCCAACTCTCCTCCCTCCTGCGGAG GGAGCACGGGGGGTTCAATGTCTCCTTCCCCTGCCCCGGCGGAGGAAGGAGCGCCCGGGGGTTTTTTCCTTCCTTTAAACCCCTTATCCCGTC TACGAATGTGGCAACCCACGGTAAAACAAAAAACAATACGACTCTTAGCGGTGGATCACTCGGCTCGTGCGTCGATGAAGAACGCAGCTAGC TGCGAGAACTAA	PP085551
3	*Oreochromis niloticus isolate On77*	TACCGATTGGATGGTTTAGTGAGGTCCTCGGATCGGCCCCGCCGGGGTCGGTCACGGCCCTGGCGGAGCGCCGAGAAGACGATCAAACTTGAC TATCTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACTGGCTACACCGAGCGGCCCGCCTGCGGCGC ACCCGGTGTTCTCCCTCTTTGCCGCCGAGGGTCTCCCGCCACCGTCACCGGTGCGGGTCTCCCGAGGTTGTCGGCTCGCGCGTCCCCCACCGGA GCTCGAGCCTTAGTCTGGGCCTGGTCACCGGCCGGACGACCCGACGGCCCCGCCCCGCCTCTACGCCAAAGCGAGCCCGCTGCCCCGACCGGCT CCTCCGAGGGCCGACGGAGGAGAGTCGGGACTGCGGCTCGTTGGAG GCGGGCGCCGGGGTCCCGTCCGGCAACCACCGGTCCCGGCCCGCCACGAGAACCTCAACCGAAAGCGCGGGCTGGCGGTTTCGCCCTGACCG CTGCCCGCGCGGCCTCCGGGTACCCAACTCTCCTCCCTCCTGCGGAGGGAGCACGGGGGGTTCAATGTCTCCTTCCCCTGCCCCGGCGGAGGAA GGAGCGCCCGGGGGTTTTTCCCTTCCTTTAAACCCCTTATCCCGTCTACGAATGTGGCAACCCACGGTAAAACAAAAAACAATACGACTCTT AGCGGTGGATCACTCGGCTCGTGCGTCGATGAAGAACGCAGCTAGCTGCGAGAACTAA	PP085498
4	*Oreochromis niloticus isolate On7785*	CTACCGATTGGATGGTTTAGTGAGGTCCTCGGATCGGCCCCGCCGGGGTCGGTCACGGCCCTGGCGGAGCGCCGAGAAGACGATCAAACTTGA CTATCTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACTGGCTACACCGAGCGGCCCGCCTGCGGCG CACCCGGTGTTCTCCCTCTTTGCCGCCGAGGGTCTCCCGCCACCGTCACCGGTGCGGGTCTCCCGAGGTTGTCGGCTCGCGCGTCCCCCACCGGA GCTCGAGCCTTAGTCTGGGCCTGGTCACCGGCCGGACGACCCGACGGCCCCGCCCCGCCTCTACGCCAAAGCGAGCCCGCTGCCCCGACCGGCT CTCCGAGGGCCGACGGAGGAGAGTCGGGACTGCGGCTCGTTGGA GGCGGGCGCCGGGGTCCCGTCCGGCAACCACCGGTCCCGGCCCGCCACGAGAACTACCGGTCCAGGCCCACCATGAGAACCTCAACCGAAAG GGCGGGCTGGTGGTTTCTCCCTGACCGCTGCCCCCGCGCCTCCGGGTACCCAATTCTCCTCCCTCGTGGGGAGGGAGCACGGGGGGCTTCAATG TTTCCTCTCCCCTGCCCCGGCGGAGGAAGCAGCGCCCGGGGTTTTTTCCTTAACCCCCTTGTCCTGTCTACGAATGGGGCAACCAACGGTAAAA ACAAAAAACAATACGACTCTTAGCAGTGGATCACTCGGCTCGTGCGTCAATGAAGAACGCAGCTAGCTGCGAGAACTAA	PP085499

3.2.2. *Oreochromis niloticus* isolate On80 collected from Burullus Bay connecting Burullus Lake to Mediterranean Sea at Kafr El-Sheikh Governorate (Egypt)

The sequence length, AT and GC content for studied taxa are recorded as in Table (6). The sequence length was 761 bp. AT% content also was 34.3% while GC% content was 65.7%. The BLAST search showed a pairwise identity (PI) of 99.78% for eight *Oreochromis niloticus* isolates (5, 19, 20, 31, 37, 40, 43 and 53) from China (Table 2).

The phylogenetic analysis for *Oreochromis niloticus* (isolate On80) collected from Burullus Bay using the ITS gene is presented in Figure (7). The phylogenetic tree showed two main

clades. The first clade includes *Oreochromis niloticus* isolate On80 (Egypt) and *Oreochromis niloticus* isolates (19, 40 and 53) with bootstrap support (68% BS). The studied *Oreochromis niloticus* (isolate On80) was related nearby to *Oreochromis niloticus* (isolate 53) with bootstrap support (100% BS). The second clade includes the other isolates from *Oreochromis niloticus* from China with bootstrap support (68% BS).

3.2.3. *Oreochromis niloticus* isolate On7785 collected from Shakhlouba watershed, south Burullus Lake at Kafr El-Sheikh Governorate (Egypt)

The sequence length, AT and GC content for studied taxa are recorded as in Table (6). The sequence length was 781 bp. AT% content also was 35.1% while GC% content was 64.9%. The BLAST search showed a pairwise identity (PI) of 100% for one *Oreochromis niloticus* isolate (19) and 93.77 % for six *Oreochromis niloticus* isolates (5, 20, 37, 40, 43 and 58) from China (Table 3).

The phylogenetic analysis for *Oreochromis niloticus* (isolate On7785) collected from Shakhlouba watershed, south Burullus Lake using the ITS gene is presented in Figure (8). The phylogenetic tree showed two main clades. The first clade includes five isolates from *Oreochromis niloticus* (19, 20, 37, 40 and 43) from china with bootstrap support (76% BS). The second clade includes *Oreochromis niloticus* isolates (isolate On7785) from Egypt related to *Oreochromis niloticus* isolates (5 and 58) with bootstrap support (76% BS).

3.2.34. *Oreochromis niloticus* isolate On77 collected from Damietta branch of the River Nile at Dakahlia Governorate (Egypt)

The sequence length, AT and GC content for studied taxa are recorded as in Table (6). The sequence length was 759 bp. AT% content also was 34.4% while GC% content was 65.6%. The BLAST search showed a pairwise identity (PI) of 99.78% for eight *Oreochromis niloticus* isolates (5, 19, 20, 25, 28, 37, 40 and 43) from China (Table 4).

The phylogenetic analysis for *Oreochromis niloticus* (isolate On77) collected from Damietta branch using the ITS gene is presented in Figure (9). The phylogenetic tree showed two main clades. The first clade includes *Oreochromis niloticus* isolate 40 (China) and the second clade includes the rest of isolates. The second clade divided into two sub-clades; the first sub-clade includes *Oreochromis niloticus* (isolate On77) related to *Oreochromis niloticus* isolates (25, 40 and 43) from China with bootstrap support (58% BS). The second sub-clade incudes four isolates from *Oreochromis niloticus* (5, 19, 20 and 28) with bootstrap support (58% BS).

Table (6). Data generated from DNA region (ITS) for *Oreochromis niloticus* isolates. GC content (the percentage of guanine- cytosine content) is percentage of nitrogenous bases in a DNA or RNA molecule that are either guanine (G) or cytosine (C). AT content (percentage of adenine- thymine nucleotides in a DNA sequence).

Nuclear region	Isolates	A	T	G	C	AT%	GC%	GenBank Accession No	Pairwise Identity (PI)	Nucleotide (bp)
ITS	*Oreochromis niloticus* isolate On309	132	131	234	263	34.6%	65.4%	PP091930	100%	760
	Oreochromis niloticus isolate On80	132	129	234	266	34.3%	65.7 %	PP085551	100 %	761
	Oreochromis niloticus isolate On77	132	129	235	263	34.4%	65.6 %	PP085498	100 %	759
	Oreochromis niloticus isolate On7785	139	135	239	268	35.1%	64.9%	PP085499	100%	781

4. Discussion

Members of the family Cichlidae represent one of the most diverse groups of vertebrates and exemplify key concepts in evolutionary biology. They are especially important for investigating the mechanisms that drive organismal diversification during evolutionary radiations [19 – 24]. Tilapia species are known to display a noticeable order of genetic constancy simplified by firm structure of chromosomes and adaptability to abroad spectrum environments, ranging from freshwater to salt water habitats. Numerous studies have focused on cichlid genomes to tackle essential questions in evolutionary biology. These inquiries include the identification of genomic signatures and potential factors that affect evolutionary radiations, the analysis of genetic variation regarding its quantity and distribution-both within individual genomes and across different species-and the consequences for the evolutionary divergence of populations into distinct species. Additionally, researchers seek

to comprehend the processes through which genetic variation is generated and sustained, particularly highlighting the significance of gene flow. Consequently, a substantial and rapidly growing repository of genetic and genomic data on cichlids has been assembled; to date, 116 studies have contributed to a cumulative total of 51.5 trillion base pairs available in the National Center for Biotechnology Information (NCBI) Sequence Read Archive [25]. However, compiling a thorough overview of genomic diversity in cichlids is hindered by methodological discrepancies across various studies, which encompass differing genetic and sequencing techniques, distinct evolutionary hypotheses, and focused investigations into specific cichlid evolutionary radiations.

Genetic variation within any species serves as a resource that is manifested at two distinct hierarchical levels. The first level is explicit and encompasses the genetic differences observed among individuals within a population. The second level of genetic variation pertains to the differences that exist among various populations [26 – 28].

Mojekwu and Anumudu [29] found that *Oreochromis niloticus* dwelling aquatic ecosystems across six federations (geopolitical zones) of Nigeria exhibited varying degrees of genetic similarity. The intensity of this similarity may be attributed to the amplified levels of hybridization that take place in the wild among these species. Their findings advocated for the implementation of a more comprehensive genomic strategy, such as the exon gene-capture technique, to tackle the challenges posed by hybridization. The exon gene-capture technique is a method employed to enhance the specific coding regions (exons) of a genome, thereby facilitating efficient sequencing. This process entails hybridizing DNA libraries with probes that are complementary to the exons of interest, followed by the capture and sequencing of the enriched DNA [30].

In the present study, data generated from DNA (ITS region) for *Oreochromis niloticus* isolates revealed about 100% similarity among tilapia populations in Burullus Bay, Shakhlouba watershed, Drain-7 and River Nile. This may be attributed to regular migration of this cichlid fish among the four explored habitats or maintenance of the robust genetic map of this highly tolerant teleost that can invade diverse habitat types and reside therein. However, habitat fragmentation and geographical isolation over coming decades, due to water shrinkage and salt intrusion, may modify the gene map of different populations of the Nile tilapia. According to Mojekwu and Anumudu [29], the observed high levels of similarity in the gene map of *Oreochromis niloticus* may be attributed to significant proceedings such as flooding, which frequently occurs in Nigeria, or to escapes from limited living places such as fish farms. Previous research utilizing tilapia to evaluate genetic diversity among certain cichlids in Nigeria also revealed varying degrees of similarity [31, 32].

Even so, these differing levels of similarity might suggest that the species is hybridizing with other tilapia species. *Oreochromis niloticus*, similar to other cichlid species, has the capacity to hybridize with other closely related species, resulting in fertile offspring that sometimes exhibit the *O. niloticus* phenotype. Reports indicate that DNA fingerprints from mixed DNA samples are valuable for assessing relationships among closely related populations, owing to the significant genetic differentiation observed. This data reinforces the notion that the high similarity among tilapia correlates with an increased likelihood of hybridization, and based on the genetic distances between strains, we can infer that there are varying degrees of genetic variability among *O. niloticus* in the Nigerian water bodies examined. The genetic variability noted in this study aligns with earlier research on this species, which employed both mitochondrial DNA and nuclear markers [31 – 35].

The genetic variation among various tilapia species in Egypt's aquatic ecosystems has been examined by a limited number of researchers [36]. Tilapia fish have been examined at the genetic level to elucidate population genetic structure, genetic diversity, taxonomy, and species identification, which has been beneficial for fish farming and fisheries management [37, 38]. Soliman *et al*. [36] performed a comparative study on the genetic structure of the redbelly tilapia found in Lake

Nasser (freshwater environment), Lake Idku (brackish water environment), and Max Bay (marine environment). The authors utilized three mtDNA markers-COI, D-loop, and CYTB and revealed distinct genetic variation in the population structure of *T. zillii* across the three fragmented ecosystems. They linked the genetic diversity to physical obstacles like the Aswan High Dam at Lake Nasser, habitat segregation at Idku Lake, and the small population of *T. zillii* at Max Bay, which is occupied by fish fleeing from adjacent ponds and aquaculture sites. Additionally, the low genetic variability of the redbelly tilapiine fish in Max Bay was associated by Soliman *et al*. [36] with chemical barriers, as the bay displays higher salinity levels compared to other environments. In these habitat conditions, a restricted group of redbelly tilapia genotypes has adapted to thrive in salt-rich environments. At Lake Idku, the size of the habitat was related to the genetic diversity of the redbelly; there was a swift change in demographic features at Idku Lake as it has diminished by around 390 km² since 1800. Habitat-associated differences in genetic diversity were observed by Hassanien and Gilbey [39] in *Oreochromis niloticus*, by Szitenberg *et al*. [40] in redbelly tilapia residing in the Dead Sea System, and by Martinez *et al*. (2018) who collected estimates of genetic variation, taxonomic relationships, habitat data, conservation level, and life-history traits for a wide range of fish species. According to Martinez *et al*. [41], greater genetic diversity in marine fish is probably due to variations in the frequency, scale, and interplay of genetic drift and gene flow. Reduced genetic drift in marine species may help explain this phenomenon, particularly considering that marine fish populations are generally much larger than those of freshwater fish [42].

Soliman *et al*. [36] accomplished a comparative analysis on the population genetic structure of the redbelly tilapia inhabiting Lake Nasser (freshwater habitat), Lake Idku (brackish water habitat) and Max Bay (marine habitat). These authors employed three mtDNA markers, namely COI, D-loop, and CYTB), and demonstrated a clear genetic variation in the structure of the population of *T. zillii* from the three fragmented ecosystems. They correlated the genetic variation to physical barriers such as Aswan High Dam at Lake Nasser, habitat isolation at Idku Lake and limited population size of *T. zillii* at Max Bay that is inhabited by fish escaping from the nearby ponds and fish farming facilities. Moreover, low genetic diversity of the redbelly tilapiine fish at Max Bay was as correlated by Soliman *et al*. [36] to chemical barriers where the bay exhibits higher salinity level than other habitats. Under these habitat circumstances, a limited subset of the genotypes of the redbelly tilapia that has been adapted to survive salt-rich habitats. At Lake Idku, habitat size was correlated to the genetic diversity of the redbelly; at Idku Lake, there was a rapid change in the demographic aspects as the lake lost about 390 km² since 1800. Habitat-related variation in the genetic diversity was reported by Hassanien and Gilbey [39] in the Nile tilapia, *Oreochromis niloticus* and by Szitenberg *et al*. [40] in the redbelly tilapia inhabiting the Dead Sea System.

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