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Phylogenetics and Molecular Divergence of Tilapia Fish (Oreochromis Species) Using Mitochondrial D-Loop and Cytochrome b Regions

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Abstract

Understanding the level of genetic diversity in any population is an important requisite towards strategizing measures for conservation and improvement of stocks. This study focused on the assessment of phylogenetics and molecular divergence of tilapia fish species obtained from two populations (Domita in South-South and Odeda in South-West, Nigeria) using the displacement loop (D-loop) and cytochrome b region of the mitochondrial deoxyribonucleic acid (mtDNA). A total of 28 samples (15 from South-South and 13 from South-West) were used for the genetic analysis. DNA was extracted from the tissue of all the samples using Quik-gDNA™ miniPrep kit. The D-loop containing the hypervariable region was sequenced for all samples from the two populations, while cytochrome b (Cyt b) region of mtDNA was only sequenced for samples from South-South population. Chromatograms of the sequences were viewed and edited using Bioedit software. Multiple sequence alignment was carried out using molecular evolutionary genetic analysis (MEGA) software before subsequent genetic analyses. Phylogenetic analysis grouped the samples into two clusters based on population. Also, when the two mitochondrial regions were pooled together, they clustered into two major groups based on mitochondrial regions. Analysis of molecular variance (AMOVA) revealed 37.32% variation within population and 62.68% variation among population with a significant fixation index of 0.627 ($p < 0.05$). The genetic distance inferred between D-loop regions of South-South and South-West populations was 0.243. Maternal lineage analysis revealed that the origin of tilapia fish

from both populations could be traced to *Oreochromis spirilus* and *Oreochromis leucostictus* based on mitochondrial D-loop region. The findings of this study revealed molecular divergence among the tilapia populations and may serve as pivot information for the genetic improvement of this important species.

1. Introduction

The Nile tilapia (*Oreochromis niloticus*) is the most commonly farmed tilapia species in Nigeria with good aquaculture qualities such as ability to withstand poor water quality and wide range of feed that support its cultivation. Reference [1] reported that tilapia has fast growth rate that can be further improved in a more favourable environment. According to [2], Nile tilapia has special features such as cycloid scales, grey/black body bars, pointed mouth part that ease it identification. As a maritime nation, integrating tilapia farming into agricultural schemes will be a necessary step towards addressing the problem of protein malnutrition, which is currently a challenge faced by many impoverished Nigerians. Comparing the nutritional values of tilapia to other animal protein sources, [3] documented protein content of 20.1 g, 18.6 g and 21.2 g and cholesterol content of 50 mg, 68 mg and 64 mg per 100 g of tilapia, beef and chicken respectively. Although catfish is the most farmed fish in Nigeria, tilapia is a good candidate that could compete with catfish farming and consumption if given adequate research attention. It had been earlier reported that the total fat of 6 g and 18 g; 59 g and 42 g protein content; 130 mg and 145 mg cholesterol per 80 ounce are available in tilapia and catfish, respectively [4]. With the growing rate of insecurity in the country, the number of internally displaced people is consequentially increased daily. These people are faced with malnutrition, especially protein malnutrition in their various camps across the country leading to high mortality rate. Although government has made effort to meet the nutrient requirement of these unfortunate Nigerians, this effort is not just sufficient and as such, sourcing protein from tilapia fish to meet this need may be advantageous considering that it is cheaper, readily accessible, with high protein content and other nutritionally essential elements needed for body growth. The challenge in achieving this objective is that Nigeria is far behind in fish production [5] with an estimated N125 billion spent annually on fish importation that has left the country as the highest importers of fish in developing countries [6]. Also, there is a serious concern over the possible genetic erosion of tilapia fish perceived from the continuous exploitation and indiscriminate fishing from the wild by local fishermen to meet market demand.

Overcoming the present doom demands intensified efforts and interest in fish research and production especially tilapia fish which has received very little or

no research interest over the past years. This will pave new way for possible integration of tilapia fish farming into agricultural programmes. To achieve this, it is necessary to assess the different ecosystems where tilapia fish species are available as well as identifying reliable methods for their proper management, conservation and genetic improvement. Genetic variation and phylogenetic studies which assess the variation and relationships existing within and between populations have recently become an integral part in agricultural programmes as a tool for selection of breeding stock and characterization of species for possible conservation [7]. A good insight into the pattern of variation as well as the relationship in a population is a requisite for producing improved breeds. These relationships could be studied via morphological data from a population. Although morphometric method is the simplest and the most direct way used for fish identification and characterization [8] [9], it may not be very reliable for phylogeny considering that it is more subjected to environmental influences [10] [11] [12]. To fill this gap, different marker techniques have been developed and used for fish phylogenetics. The use of biochemical methods such as isozymes and protein banding patterns has been promising [13] [14]. Protein banding methods are often less informative and are used as preliminary phylogenetic investigative tool [15]. The advent of genetic markers such as RAPD, RFLP, and microsatellite in conjunction with statistical tools has revolutionized population studies geared towards exploring the phylogenetic relationships within and between populations. Importantly, DNA sequence data have proven to be the most direct and reliable method of assessing the pattern of variation and relationships among species.

Sequence data may be obtained from mtDNA or nuclear DNA. However, mtDNA found within the cytoplasm of a cell remains a good choice over nuclear DNA for phylogenetic studies due to the fast rate of mutation in the region [16] [17] and specificity as it is inherited only through maternal cell line [18]. These features of mtDNA are used by evolutionary biologists to establish relationships and to trace maternal lineage of species using array of sequence data. The displacement loop (D-loop) is the most variable region of mtDNA [19] and previous research reported that high variation in the region is useful in characterizing tilapia species [20] [21] [22]. Similarly, cytochrome b (Cytb) region of mtDNA has been used to establish the phylogenetic relationships within and between populations [17] [23] [24] [25] [26] [27]. In the present study, it was our aim to assess the phylogenetics of *Oreochromis niloticus*, a common species of tilapia fish in Nigeria using mitochondrial D-loop and Cytb.

2. Materials and Methods

2.1. Location and Sample Collection

A total of 28 samples of *O. niloticus* were collected from two populations for this study. The samples were identified in the Fishery Unit of the Institute of Oceanography, University of Calabar. Fifteen samples were obtained from Domita
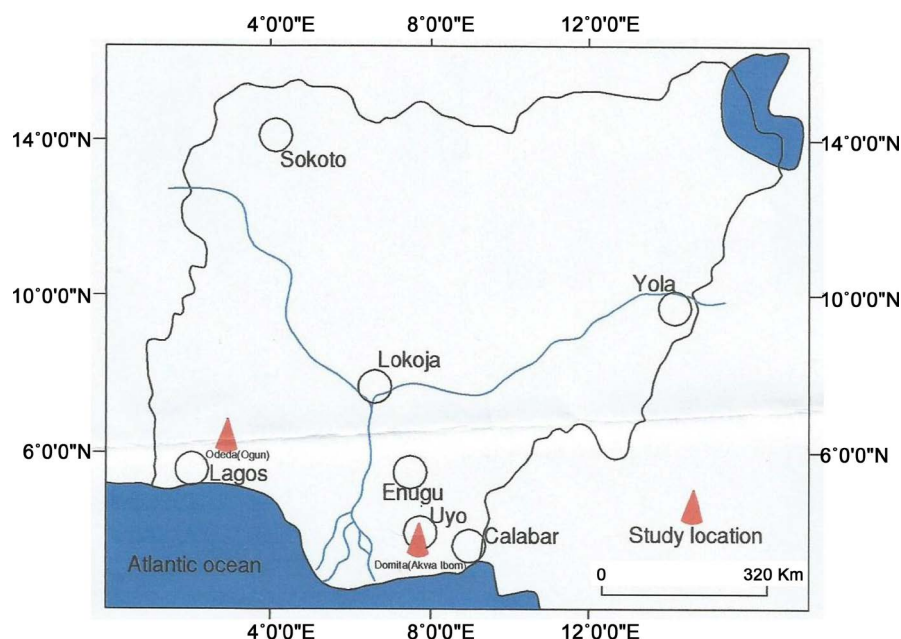
Figure 1. Map of Nigeria showing sampling locations.

Farm in Akwa Ibom State, a South-South region of Nigeria located at approximately 5˚1'4"N, 7˚59'52"E. Thirteen samples were obtained from Odeda Farm in Ogun State, a South-West region of Nigeria located at 7˚13'00"N, 3˚31'00"E (**Figure 1**).

2.2. DNA Isolation

DNA was extracted from the tissue samples of all the fish using Quick-gDNA™ MiniPrep kit (Zymo Research, USA). Beta-mecaptoethanol was added to the lysis buffer to a final solution of 500 μl per 100 ml according to the manufacturer's instruction. Approximately 25 mg of each fish tissue was homogenized in 500 μl of genomic lysis buffer in Ependorff tube. The lysate was centrifuged at 10000 × g under room temperature for five minutes using a Centurion Scientific microcentrifuge (Model: C2015, USA). The supernatant was carefully transferred to a Zymo-Spin™ column in a collection tube and centrifuged at 10,000 × g for one minute. Both the flow through and the collection tube were then discarded before transferring the Zymo-Spin™ column to a new collection tube. 200μl of DNA pre-wash buffer was added to the spin column and centrifuged at 10,000 × g for one minute. 500 μl of g-DNA wash buffer was added to the spin column and further centrifuged at 10000 × g for one minute. The spin column was transferred to a clean microcentrifuge tube and ≥50 μl of DNA elution buffer was added to the spin column. This was incubated for two to five minutes at room temperature and afterward centrifuged at top speed (14,000 × g) for 30 seconds to elute the DNA. The eluted DNA was stored at −20˚C for further analysis.

2.3. PCR Amplification

PCR amplification was carried out using the primers Fish-comum-D-loop For-

ward (5'-GGATTYTAACCCYTRCCCC-3') and Czilli Reverse (3'-AGTAAAG TCAGGACCAAGCC-5') for D-loop region as previously used by [22]. Czilli Forward (5'-GGATTTTAACCCTTACCCC-3') and Czilli Reverse (3'-AGTAAAGTCAGGACCAAGCC-5') were designed and used for cytochrome b region of the mtDNA. PCR for each mtDNA region was carried out using 25 μl reaction volume containing 1 μl genomic DNA, 2 mM MgCl₂, 200 μM of dNTP, 2.5 μl of 10 × PCR buffer, 1 μM of each primer and two units of STABVIDA proprietary Taq polymerase. This was performed using the GeneAmp˚ PCR system (9700 thermal cycler, USA) with the cycling condition of initial denaturation step at 95˚C for five minutes, followed by 25cycles of denaturation at 94˚C for 40 seconds, annealing at 54˚C for 45 seconds, extension at 72˚C for one minute and final extension at 72˚C for seven minutes. PCR products were purified using exofast protocol according to the manufacturer.

2.4. Sequencing of D-Loop and Cytochrome b Regions of mtDNA

D-loop and cytochrome b region of the mtDNA were sequenced for all tissue-DNA extracts using the primers (5'-GGATTYTAACCCYTRCCCC-3') and Czilli Reverse (3'-AGTAAAGTCAGGACCAAGCC-5') for D-loop region and Czilli Forward (5'-GGATTTTAACCCTTACCCC-3') and Czilli Reverse (3'-AGTAAAGTCAGGACCAAGCC-5') for cytochrome b region. Sequencing reaction was performed with AB13730 × L sequencer using 20 μl reaction comprising approximately 20 ng of purified PCR product as template DNA, 8 μl of Big Dye Terminator Reaction Mix (dNTPs, ddNTPs, buffer, enzyme and MgCl₂), 8 μl of deionized water, 2 μl of primer programmed as 25 cycles at 96˚C for 10 seconds, 50˚C for five seconds and 60˚C for four minutes [28]. The length of the sequenced fragments ranged from 950 to 1366 bp for D-loop and 1241 to 1401 bp for cytochrome b.

2.5. Statistical Analysis

Bioedit software version 7.2.5 [29] was used to view and edit the sequences. MEGA 6.06 was used for multiple sequence alignment of all the samples [30] excluding all the gaps. Analysis of molecular variance (AMOVA) was determined using Arlequin 3.5.1.3 software [31]. Arlequin was also used to compute the fixation index among the populations which was computed as the average of the variance among the populations by the total variance observed in the population. The genetic distance and phylogenetic analysis of samples from the populations was performed using MEGA 6.06 [30]. To classify the species into maternal lineage, D-loop sequences of other tilapia species were retrieved from GenBank with the following accession numbers: KX757836.1, KX767829.1, EU163710.1, FJ664225.1, EU308500.2, EU430997.2, AF296483.1, FJ664233.1, AF296455.1, AF296460.1 and FJ664215.1 while cyt reference with the following accession numbers: AB018989.1, X81565.1, AF375617.1, HM049957.1, EF059808.1, KX347644.1, JQ755415.1, EF679275.1, KX347652.1, EF679291.1, KX347643.1

and AF370632.1 were used. These were all aligned with the query sequences obtained from tilapia in the studied populations to construct a maximum likelihood tree using MEGA 6.06 with 1000 bootstrap replications [30].

3. Results

3.1. Phylogenetic Relationship Analysis

The Neighbor-Joining phylogenetic relationship of tilapia fish from the two populations based on mitochondrial D-loop is presented in **Figure 2**. The numbers at the node represent the percentage bootstrap values for branches with
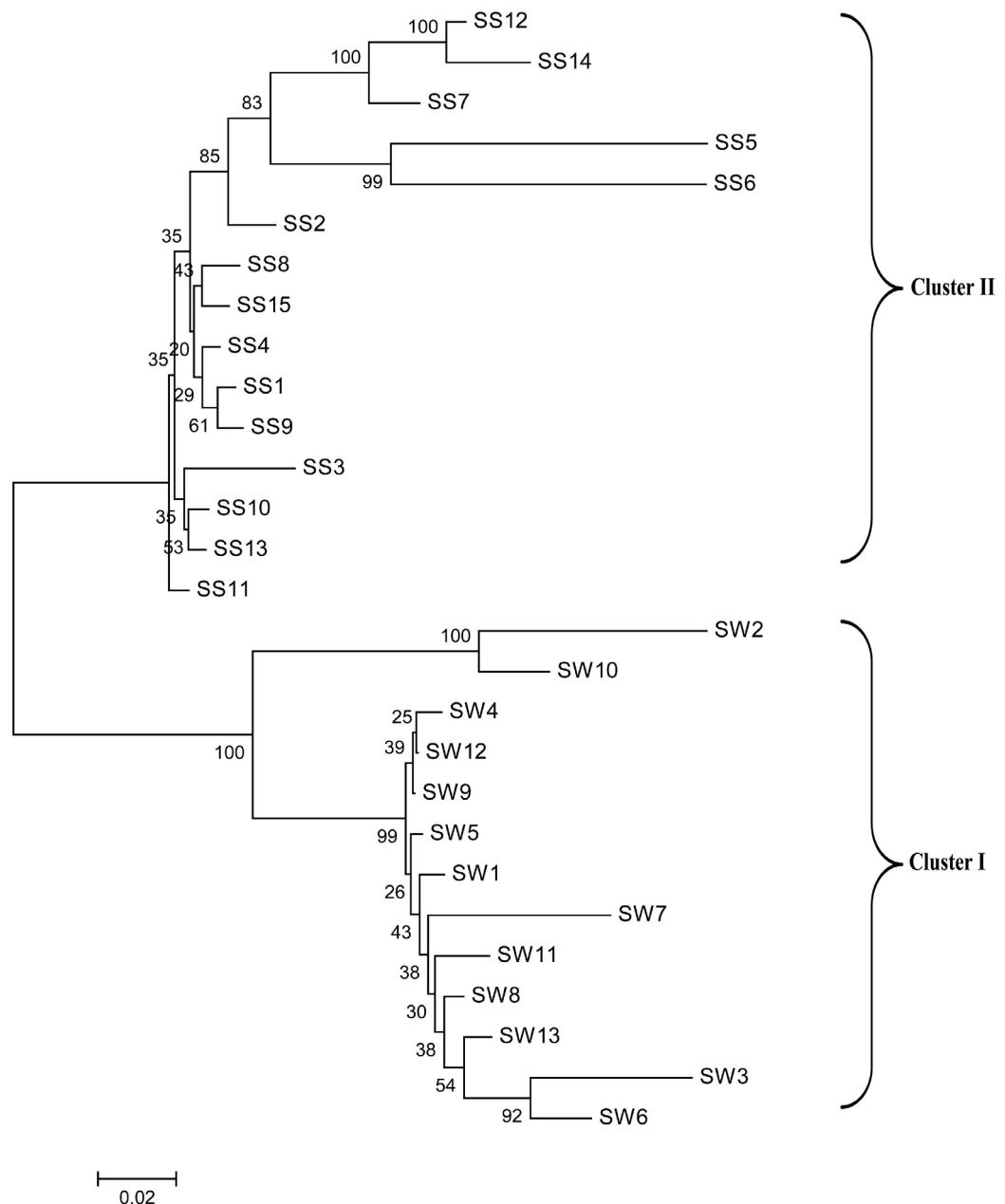

Figure 2. Neighbor-Joining phylogenetic tree showing the relationship between tilapia fish from two populations based on mtDNA D-loop region (SS = South-South; SW = South-West).
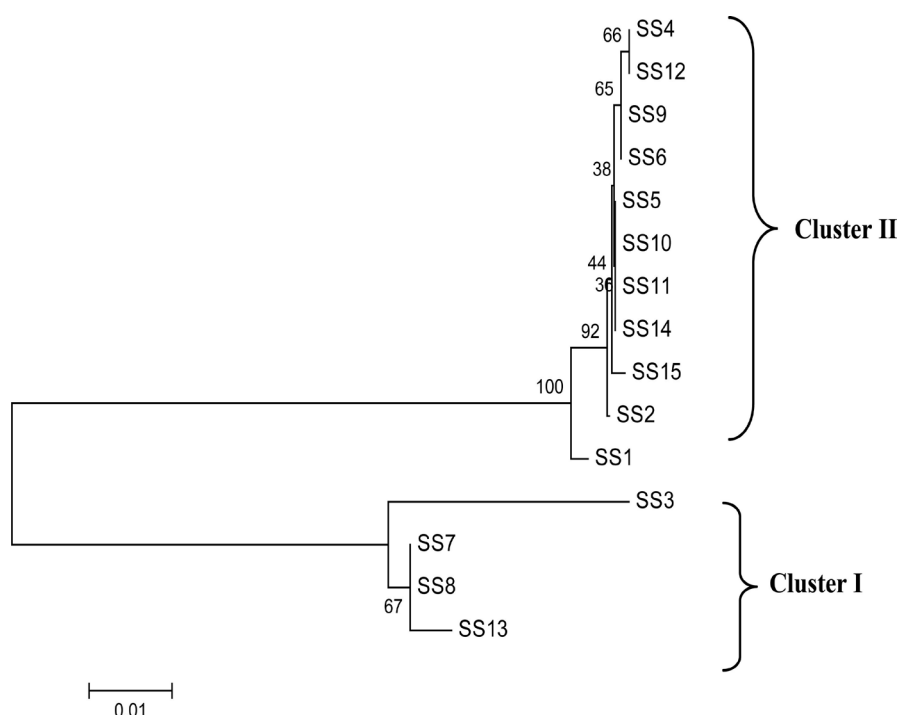
Figure 3. Neighbor-Joining phylogenetic tree showing the relationship between tilapia fish from South-South population based on mtDNA Cyt b (SS = South-South).

1000 bootstrap replications. Two major clusters were revealed, which were based on populations. Cluster I consisted of all the samples from South-West D-loop with assigned sequence accession number: MF385002 sub-clustered into two groups. Similarly, cluster II consisted of all the samples from South-South D-loop with assigned sequence accession number: MF385001. SS11 was separated into a different lineage from other samples within the South-South population. For mtDNA Cyt b with sequence accession number: MF384326, two major clusters were also revealed where SS3, SS7, SS8 and SS13 shared the same lineage (Figure 3). The comparative clustering of D-loop and cyt b separated the samples into two major clusters based on mitochondrial DNA regions. Samples from South-South and South-West D-loop were grouped together under the same major cluster while samples sequenced for cyt b had a different lineage as shown in Figure 4.

3.2. Analysis of Molecular Variance (AMOVA)

From Table 1, AMOVA revealed that molecular genetic variation among populations was higher (62.68%) than within population variation (37.32%) inferred from the D-loop of the two populations. Additionally, fixation index was 0.627, which made the variation significant ($p < 0.05$).

3.3. Genetic Distance

The genetic distance between tilapia fish from South-South and South-West population was 0.243. From Table 2, the highest genetic distance within mito-
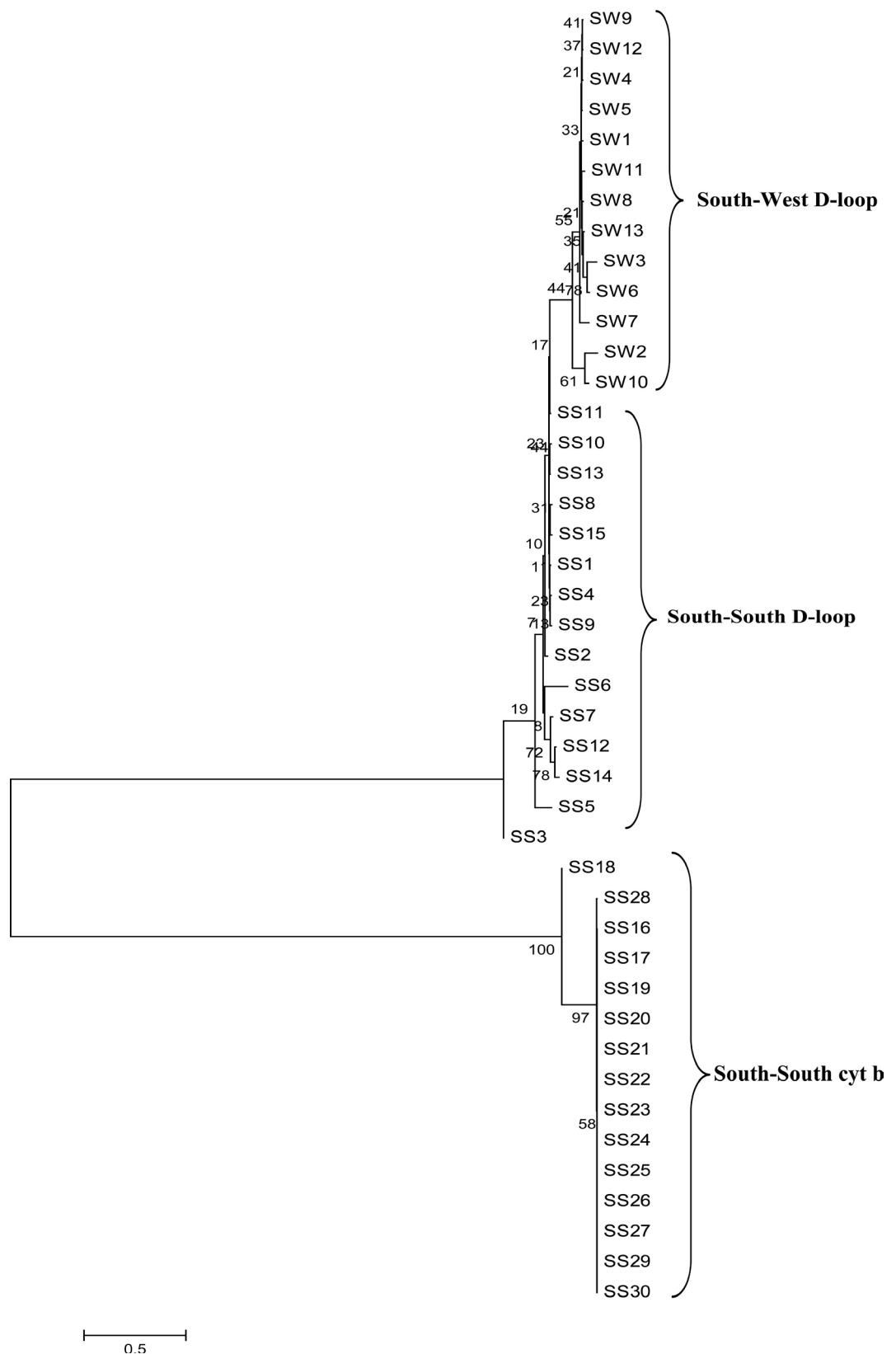
Figure 4. Neighbor-Joining phylogenetic tree showing the relationship between tilapia fish based on two mtDNA regions [SS1-15 = South-South D-loop; SS16-SS30 = South-South cyt b; SW1-13 = South-West D-loop].

Table 1. AMOVA showing genetic variation within and between tilapia in South-South and South-West Nigeria.

SOV	Df	SS	Variance components	%Variance	F_{ST}
Among populations	1	613.704	42.255	62.68	
Within populations	26	654.046	25.156	37.32	0.627 (p < 0.05)
Total	27	1267.750	67.410		

SOV (source of variation); df (degree of freedom); SS (sum of squares); F_{ST} (fixation index).

Table 2. Pairwise genetic distance of tilapia fish from South-South Nigeria based on mitochondrial D-loop region.

	SS1	SS2	SS3	SS4	SS5	SS6	SS7	SS8	SS9	SS10	SS11	SS12	SS13	SS14	SS15
SS1	0														
SS2	0.036	0													
SS3	0.047	0.053	0												
SS4	0.014	0.025	0.044	0											
SS5	0.152	0.147	0.155	0.145	0										
SS6	0.154	0.139	0.160	0.147	0.160	0									
SS7	0.075	0.057	0.076	0.066	0.160	0.154	0								
SS8	0.019	0.033	0.050	0.019	0.145	0.147	0.066	0							
SS9	0.011	0.030	0.050	0.014	0.154	0.152	0.069	0.027	0						
SS10	0.019	0.039	0.033	0.019	0.152	0.150	0.072	0.022	0.022	0					
SS11	0.014	0.033	0.036	0.016	0.149	0.147	0.075	0.025	0.019	0.016	0				
SS12	0.090	0.060	0.091	0.078	0.172	0.154	0.039	0.081	0.081	0.090	0.090	0			
SS13	0.023	0.037	0.034	0.020	0.149	0.144	0.068	0.023	0.023	0.009	0.015	0.089	0		
SS14	0.110	0.080	0.099	0.098	0.174	0.162	0.052	0.101	0.101	0.110	0.107	0.026	0.102	0	
SS15	0.022	0.030	0.047	0.014	0.150	0.144	0.060	0.016	0.019	0.022	0.022	0.078	0.018	0.092	0

SS (South-South).

chondrial D-loop of tilapia from South-South population was between SS5 and SS14 (0.174), while the lowest distance was between SS10 and SS13 (0.009). The highest genetic distance within South-West population was 0.133 recorded between SW2 and SW6 and between SW3 and SW10, while the lowest distance was between SW9 and SW12 (0.002), Table 3. Similarly, Table 4 showed that the highest genetic distance within mitochondrial Cyt b of South-South population was 0.149 (SS3 and SS12) while the lowest genetic distance was 0.000 (SS7 and SS8, SS6 and SS9, SS5 and SS10, SS5 and SS11, SS10 and SS11, SS4 and SS12, SS5 and SS14, SS10 and SS14, SS11 and SS14).

3.4. Maternal Lineage

Unweighted Pair Group Method with Arithmetic Means (UPGMA) tree constructed using the query sequence of tilapia fish from South-South and South-West populations with 10 reference sequences obtained from GenBank (KX757836.1, KX767829.1, EU163710.1, FJ664225.1, EU308500.2, EU430997.2, AF296483.1,

Table 3. Pairwise genetic distance of tilapia fish from South-West Nigeria based on mitochondrial D-loop region.

	SW1	SW2	SW3	SW4	SW5	SW6	SW7	SW8	SW9	SW10	SW11	SW12	SW13
SW1	0												
SW2	0.116	0											
SW3	0.052	0.123	0										
SW4	0.010	0.117	0.055	0									
SW5	0.007	0.117	0.053	0.006	0								
SW6	0.033	0.133	0.039	0.034	0.033	0							
SW7	0.039	0.097	0.060	0.042	0.040	0.065	0						
SW8	0.009	0.119	0.045	0.012	0.009	0.025	0.042	0					
SW9	0.009	0.116	0.056	0.005	0.004	0.037	0.043	0.013	0				
SW10	0.088	0.052	0.133	0.081	0.083	0.111	0.123	0.093	0.078	0			
SW11	0.016	0.123	0.058	0.015	0.012	0.038	0.043	0.014	0.016	0.092	0		
SW12	0.009	0.118	0.056	0.005	0.005	0.037	0.045	0.011	0.002	0.080	0.016	0	
SW13	0.014	0.120	0.045	0.011	0.014	0.026	0.042	0.014	0.016	0.090	0.017	0.016	0

SW (South-West).

Table 4. Pairwise genetic distance of tilapia fish from South-South Nigeria based on mitochondrial Cyt b region.

	SS1	SS2	SS3	SS4	SS5	SS6	SS7	SS8	SS9	SS10	SS11	SS12	SS13	SS14	SS15
SS1	0														
SS2	0.007	0													
SS3	0.144	0.146	0												
SS4	0.008	0.003	0.149	0											
SS5	0.008	0.001	0.148	0.002	0										
SS6	0.007	0.002	0.148	0.001	0.001	0									
SS7	0.118	0.119	0.032	0.123	0.120	0.122	0								
SS8	0.118	0.119	0.032	0.123	0.120	0.122	0.000	0							
SS9	0.007	0.002	0.148	0.001	0.001	0.000	0.122	0.122	0						
SS10	0.008	0.001	0.148	0.002	0.000	0.001	0.120	0.120	0.001	0					
SS11	0.008	0.001	0.148	0.002	0.000	0.001	0.120	0.120	0.001	0.000	0				
SS12	0.008	0.003	0.149	0.000	0.002	0.001	0.123	0.123	0.001	0.002	0.002	0			
SS13	0.122	0.126	0.035	0.127	0.127	0.126	0.005	0.005	0.126	0.127	0.127	0.127	0		
SS14	0.008	0.001	0.148	0.002	0.000	0.001	0.120	0.120	0.001	0.000	0.000	0.002	0.127	0	
SS15	0.010	0.003	0.145	0.004	0.002	0.003	0.120	0.120	0.003	0.002	0.002	0.004	0.125	0.002	0

SS (South-South).

FJ664233.1, AF296455.1, AF296460.1 and FJ664215.1) is presented in **Figure 5** and **Figure 6**. From the lineage analysis, tilapia fish from South-South population used in this study shared the same lineage with *Oreochromis aureus,* while tilapia from South-West population shared the same lineage with *Tilapia zillii*

from GenBank. Similar result was obtained when mtDNA cyt b reference sequences (AB018989.1, X81565.1, AF375617.1, HM049957.1, EF059808.1, KX347644.1, JQ755415.1, EF679275.1, KX347652.1, EF679291.1, KX347643.1 and AF370632.1) were compared with South-South population (Figure 7).
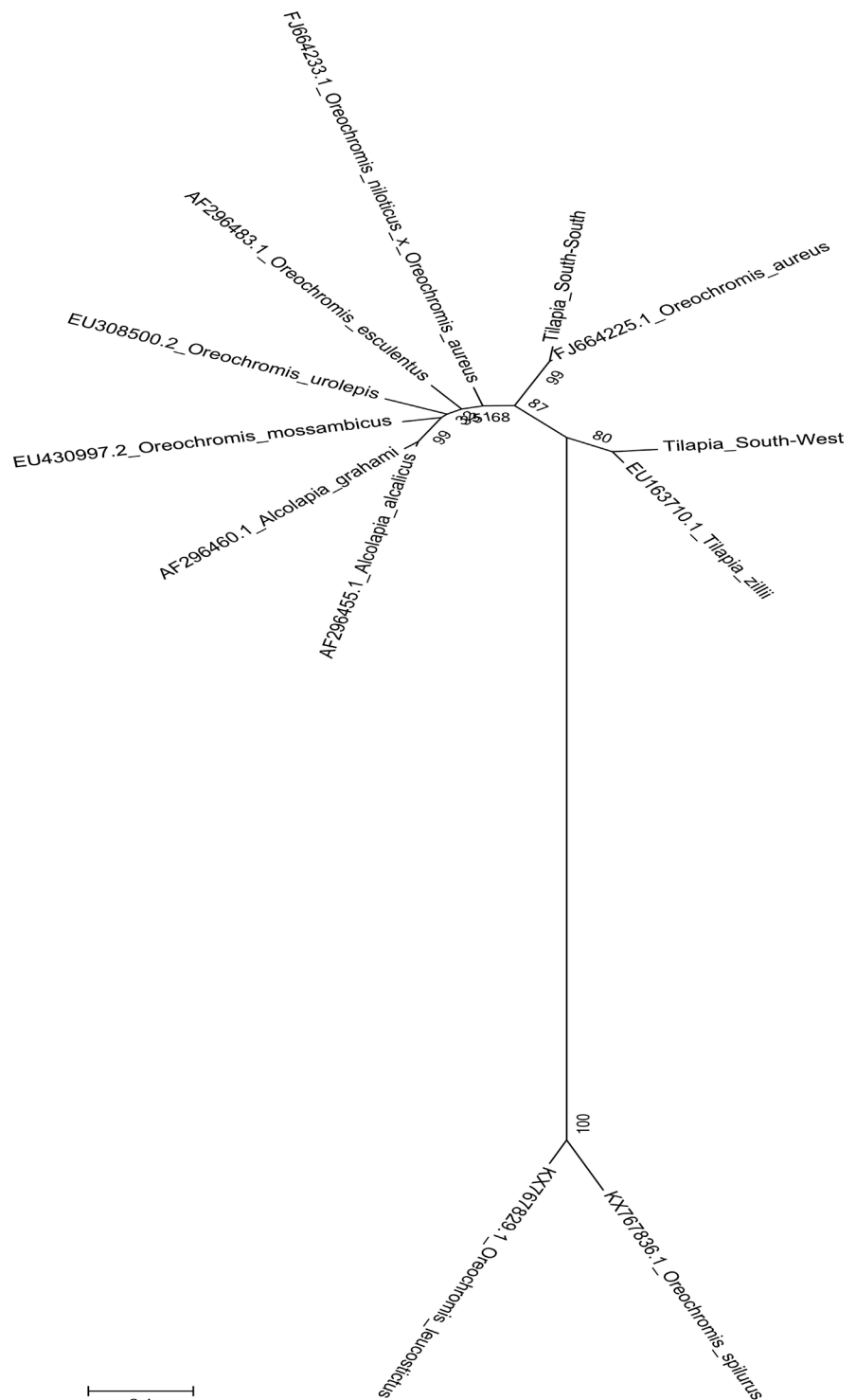

Figure 5. Maximum likelihood phylogenetic tree showing maternal lineage of tilapia fish from South-South and South-West Nigeria based on mitochondrial D-loop.
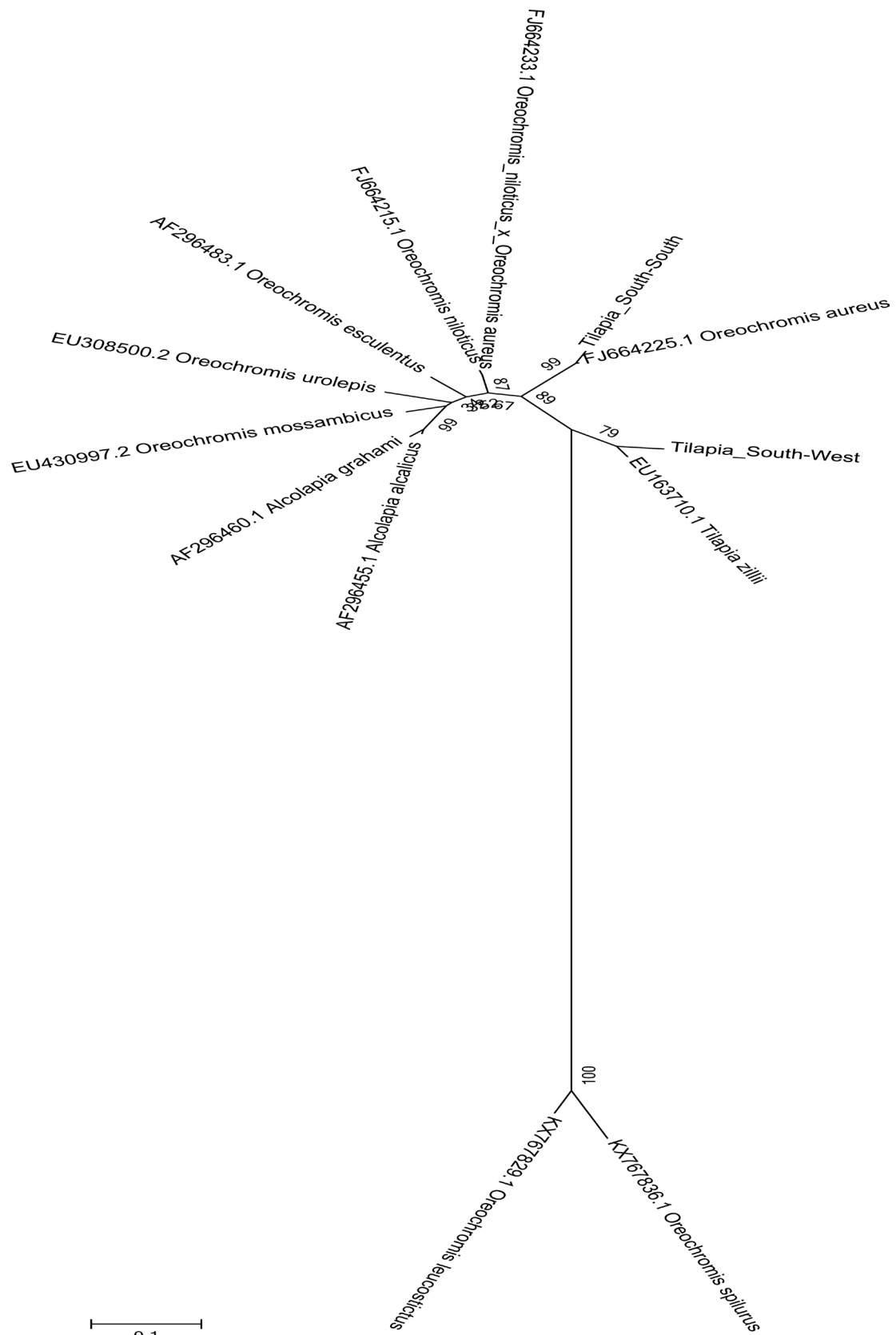
Figure 6. Maximum likelihood phylogenetic tree showing maternal lineage of tilapia fish from South-South and South-West Nigeria based on mitochondrial D-loop.
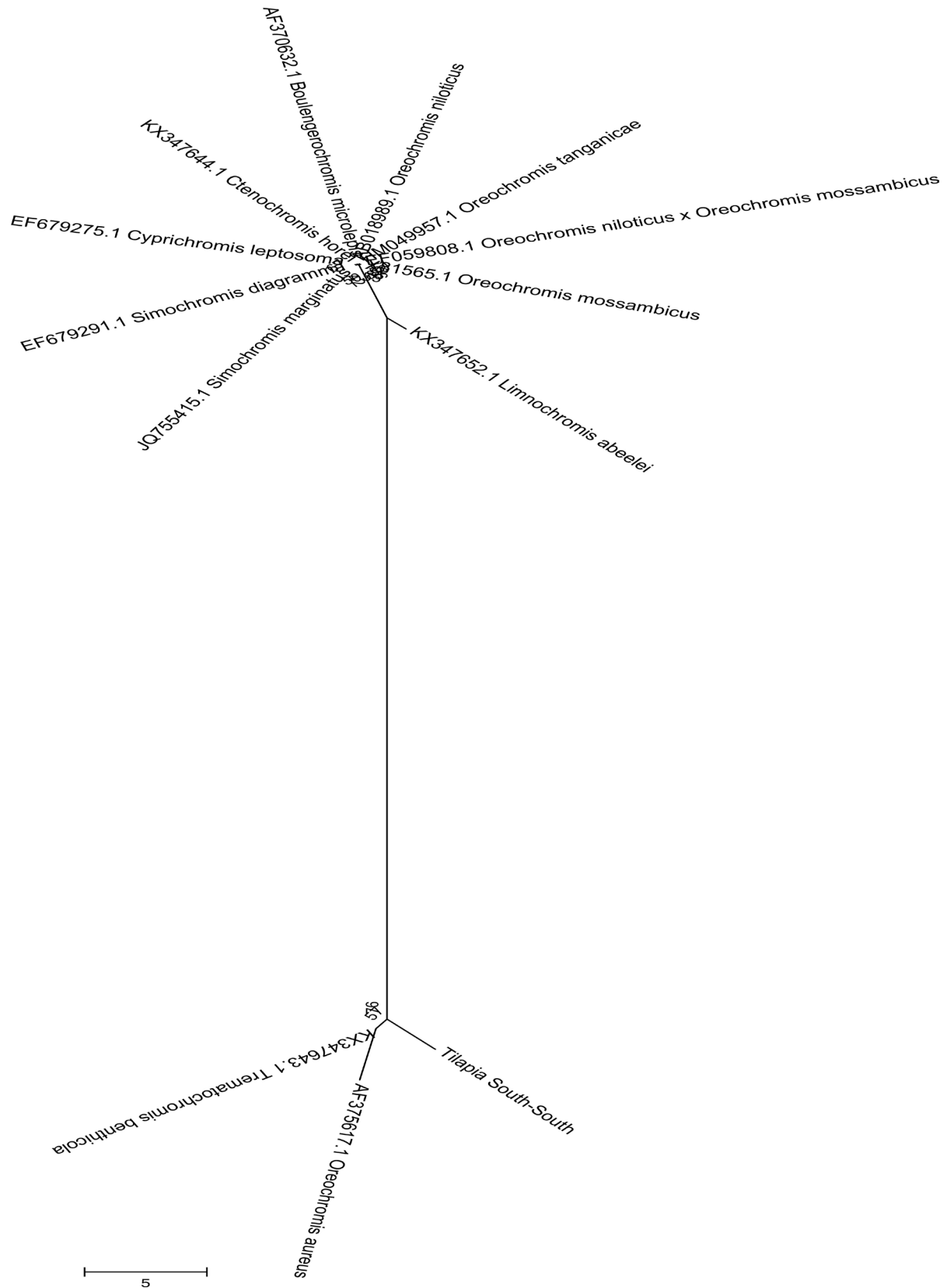
Figure 7. Maximum likelihood phylogenetic tree showing maternal lineage of tilapia fish from South-South Nigeria based on mitochondrial Cyt b.

4. Discussion

Understanding the genetic architecture of species in a population is very critical in developing conservation and management strategies for endangered species [32]. Tilapia fish is highly migratory species with high rate of introgression, which may alter their genetic originality. Thus, for management and improvement of this important species, it becomes imperative to assess their genetic blue-print and relationships.

The importance of understanding the phylogenetic relationship existing within and between species in a population cannot be overemphasized as it has become a handy method used in tracing the origin and evolution of species, prediction of physiological, biochemical and structural features of sequences obtained from organism [33]. Phylogenetic tree can be constructed using different features and characters of species such as morphology, RAPD information or sequence data of the DNA. Important to note is the reliability of sequence data over other methods in establishing the relationship between closely related species [34]. In this study, the phylogenetic tree constructed from the sequence data (D-loon and cyt b) of samples from the two populations clearly separated the samples into two major clusters based on population. All samples from South-South population were grouped under the same major cluster while South-West samples clustered under a common group. This could suggest that the two populations are genetically distinct although they may share a common ancestor. The findings here corroborate the earlier report by [22] that populations of tilapia species in Nigeria showed no evidence of sub-structuring in their phylogeny but may be traced to a single ancestral lineage. Similarly, phylogenetic analysis by [35] and [36] also revealed population-based phylogenetic clustering of fish. Comparing the two mitochondrial DNA regions showed that the samples are genetically distinct as they were specifically grouped based on the two mitochondrial regions used for the study. Although D-loop was sequenced for two different populations, the samples from these populations shared the same major cluster. This might suggest that sequence evolution of mtDNA D-loop region is not environmentally influenced but the differences could be traced to other factors inherent in the sequence. Also, the fact that there was no intertwining of D-loop and cyt b samples on the phylogenetic tree could be an indication of the dissimilarity of these regions in predicting evolutionary trends [19]. Phylogeny of the two populations corroborates the findings of the genetic distance based on D-loop region. In South-South population, samples with the highest genetic distance (SS5 and SS14) were found in separate sub-clusters, while the lowest genetic distance (SS10 and SS13) were sub-clustered together with a bootstrap value of 83. In South-West population, the highest genetic distance (SW2 and SW6) were in different sub-clusters, while the lowest distance observed between SW9 and SW12 were in the same sub-clusters.

Analysis of molecular variance (AMOVA) using D-loop suggested low genetic variance within the population (37.32%) and high genetic variation among the

population (62.68%), which could be an indication of a strong population differentiation between the two populations. This agreed with the findings of [32], where low within population and high between population variations was reported among *Channa* fish. The AMOVA result on among population molecular variation (62.68%) is also a good explanation to the different clustering of tilapia populations observed, the mitochondrial DNA region notwithstanding. The molecular variation among populations may not have been unconnected with natural selection or genetic drifts, which are hinged on evolution via random mutation. Sewall Wright effect (allelic drift) changes the frequency of an existing allele in a population as a result of random sampling of organisms. This may however, cause gene variants to disappear completely and thereby reduce genetic variation [37]. This might implicitly account for the differences observed between tilapia fish population of South-South/South-West mtDNA D-loop region and mtDNA D-loop and cyt b of South-South. Fixation index is used to differentiate populations and has been employed by molecular biologists to examine the genetic variations in population and the contribution of such variations in differentiating populations [38]. The fixation index (F_{ST}) value was significant between the two populations used in this study (0.627) suggesting further that the two populations are genetically different. The result may be an indication that the two populations may have evolved in isolation after diverging from a common ancestor. This could be further explained by the result of the maternal lineage analysis using mitochondrial D-loop region where the origin of tilapia fish from the two populations was traced to *Oreochromis spirilus* and *Oreochromis leucostictus*. However, during evolution, tilapia from South-South population separated into a different lineage with *Oreochromis aureus* while tilapia from South-West population shared lineage with *Tilapia zillii*. Furthermore, our result on maternal lineage suggests that the mtDNA D-loop of tilapia fish sampled from South-South shared 99% similarity with *Oreochromis aureus*. It is important to mention that the most common tilapia fish species in Africa are *Oreochromis niloticus* and *Oreochromis aureus*. The difference between the mtDNA D-loop region of tilapia fish sampled from South-South and South-West may be traced to the fact that the tilapia fish species from South-South could be *Oreochromis aureus* rather than *Oreochromis niloticus*. To further confirm this claim, sequence of *O.niloticus* from database was also used to construct the maternal lineage. The *O. niloticus* from database was rather clustered with a hybrid between *O. niloticus* x *O. aureus*, while our query sequence from South-South D-loop was still at 99% similarity with *O. aureus*. Cyt b though sequenced only from one population also linked the South-South samples to *O. aureus*. The findings here are very critical to both fish farmers and breeders who in the quest of setting up fish farms may purchase stock without adequate knowledge of their genetic blue print/species involved. This lack of understanding may even be the major contributing factor to the poor performance of tilapia fish as documented in literature because the specific feed/environment required to attain the maxi-

mum performance of specific species of tilapia fish may not have been met. It therefore suggests that for better improvement of tilapia fish, there should be collaboration between geneticists, breeders and fish farmers.

The genetic distance between South-South and South-West populations based on D-loop was 0.243, which was higher than within population genetic distance. Genetic distance measures the genetic differences between species/populations. Populations with similar alleles are expected to have low genetic distance as an indication of their genetic relatedness [39]. Therefore, genetic distance between the populations may be further indication of the molecular divergence between tilapia from South-South and South-West Nigeria. The result on the genetic distance further support the speculation that tilapia fish sampled from South-South and South-West may not be the same species as the genetic distance is wide. Going by the report of [39], it does suggest that the two populations might not be genetically related especially using mtDNA D-loop reference. The genetic unrelatedness may also be from the fact that due to random mutation, alleles that were similar from the ancestral root had changed frequency [40]. This divergence is important for the survivability of tilapia fish in the advent of environmental hazard. Comparing the within group genetic distance of D-loop and Cyt b of South-South population, the highest distance from D-loop was observed between SS5 and SS14 (0.174), while SS3 and SS12 (0.149) was highest based on Cyt b. Although from the same population, D-loop and Cyt b seem to predict the within group genetic distance differently which could be an indication that the two regions of mtDNA may not estimate genetic relatedness in a similar pattern. As it is reported that the mutation rate of the different regions of mtDNA are different [19], the findings of the present study may not be unexpected.

5. Conclusion

The findings of this study revealed the existence of molecular divergence between tilapia fish from the two geographical regions of Nigeria. Also, the two mtDNA regions used in this study showed dissimilarity in estimating genetic relatedness.

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Conflict of Interest

We declare that there is no conflict of interest in the manuscript from any author.

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