Characterization of the molecular genetic variation in wild and farmed Nile tilapia *Oreochromis niloticus* in Ghana for conservation and aquaculture development

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ABSTRACT (ACADEMIC)

The Nile tilapia *Oreochromis niloticus* is native to Africa and middle East, and is an important source of nutrition for many in sub-Saharan Africa. Understanding the genetic diversity within and differentiation among wild populations can help identify *O. niloticus* populations that are imperiled and require directed management, especially because of increasing threats to the species' long-term persistence in the wild, including habitat destruction, overfishing, climate change, and hybridization with farmed populations. Knowledge of the genetic variation among wild populations also can contribute to foundation and selection of genetically diverse populations for aquaculture. I assessed the genetic variation among tilapia populations using fin-clips collected between December 2014 and July 2017 from 14 farmed sources, mostly originating from cage farms on the Volta Lake, and 13 wild sources from nine river basins in Ghana. I also conducted a laboratory growth experiment in Ghana with two wild populations to evaluate the tolerance of different genotypes to high temperatures, to inform their development for aquaculture in West Africa. I found that pure *O. niloticus* populations persist in the wild but some have been extensively introgressed with the closely related species, *O. aureus*, which has not previously been documented in Ghana. Additionally, some wild populations appear to have recently declined significantly in numbers, likely due to overfishing and habitat modification, the latter primarily as a result of illegal alluvial mining ongoing in Ghana. Analysis of the farmed populations revealed that at least two farms were growing the unapproved genetically improved farmed tilapia (GIFT) and related strains, and that escaped individuals are admixed into some wild populations. The results of my laboratory experiment showed that *O. niloticus* populations occurring in northern Ghana already may be adapted to warmer temperatures and could be developed and used purposefully in aquaculture, taking advantage of their adaptation. To protect remnant pure *O. niloticus* populations in the wild, timely conservation decisions should be made

and implemented. Protecting wild *O. niloticus* populations also would ensure that pure germplasms are available to develop aquaculture stocks from native populations.

ABSTRACT (PUBLIC)

The Nile tilapia *Oreochromis niloticus* is an important food source for many people in Africa. However, many wild populations may be at risk of population decline and extinction because of increasing human activities such as overfishing and farming of non-native strains. Understanding the genetic differences among wild populations and comparing them with farmed strains can inform protection of wild populations and also help develop aquaculture strains using native populations as genetic resources. I assessed the genetic differences among tilapia populations using fin-clips I collected between December 2014 and July 2017 from 14 farmed sources, mostly originating from cage farms on the Volta Lake, and 13 wild sources from nine river basins in Ghana. I also conducted a laboratory study with two wild populations to test their tolerance to high water temperature. My research showed that pure *O. niloticus* populations still occur in Ghanaian rivers, but some have reproduced widely with a similar species, *O. aureus*, which is not known to occur in Ghanaian rivers. I also found that some wild populations may have reduced population sizes because of overfishing or because their environments have been impacted by illegal mining occurring in almost all Ghanaian rivers. My results indicated that at least two farms were growing the genetically improved farmed tilapia (GIFT) and related varieties, some of which have escaped the farms and mixed with wild populations. The results of my laboratory experiment showed that *O. niloticus* populations occurring in northern Ghana may be adapted to warmer water temperatures and could be selectively bred and used in aquaculture. The information generated from my research should help in making timely conservation decisions, which should help protect the remnant pure *O. niloticus* populations in the wild and contribute to developing aquaculture responsibly.

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Chapter 1: General Introduction

The Nile tilapia (*Oreochromis niloticus*) is one of the most cultured fish worldwide (FAO 2018). In Ghana and in many parts of Africa, *O. niloticus* also contributes significantly to total freshwater capture fisheries. The success of *O. niloticus* as a global aquaculture species can be attributed, in part, to many years of extensive research (Watanabe et al. 1985; Binh et al. 1996; Garduño-Lugo et al. 2003; Ridha 2010; Mugo-Bundi et al. 2015). However, despite the wealth of information available on the species, we are still limited in our understanding of the genetics of natural populations of *O. niloticus*. Agnèse et al. (1997) presented one of the first studies that utilized allozymes and mitochondrial DNA to cluster natural populations of *O. niloticus* in Africa into three groups: 1) West African populations, 2) Ethiopian Rift Valley populations, and 3) Nile drainage and Kenyan Rift Valley populations. Later studies using novel molecular genetics tools and a suite of analytic methods have shown that fine-scale analysis within individual drainage basins is required to identify conservation units within the species (Hassanien and Gilbey 2005; Hallerman and Hilsdorf 2014).

Knowledge of the genetic variability within the species is important not only for monitoring the diversity within natural populations in order to conserve the species' genetic resources, but is also vital for advancing aquaculture, through the selection of appropriate resource stocks. This knowledge is particularly relevant to Ghana given a widespread concern that the Akosombo strain (the eighth to tenth generation of a local selective breeding program; Dewedar 2013) may not be a fast-growing strain. Additionally, anecdotal reports suggested that some farmers in Ghana were already growing the Genetically Improved Farmed Tilapia (GIFT) strain (Dey et al. 2000) or crossing the Akosombo strain with wild strains (Ansah et al. 2014). The absence of clear guidelines for obtaining fingerlings for aquaculture hampers aquaculture development in Ghana and may pose a significant threat to natural diversity in wild populations of *O. niloticus*, since fish escapes from farms are common (Safo 2007; Fisheries Commission 2012; Attipoe et al. 2013; Hatchery International 2014).

Climate change also poses a threat to natural populations of *O. niloticus*. Among the impacts of climate change on aquatic systems in Africa and other parts of the world are the increase in temperature and an associated decrease in dissolved oxygen levels (Ficke et al. 2007). Many tilapia species occurring in already-warm conditions in Africa may be threatened by an increase in regional temperatures (Case 2006). The climate in the north of Ghana and the northern sections of the Volta River basin is hot and drought-prone, compared to a relatively cooler south. The annual mean temperature for the north is 29^{o}C, while that for the south is 26^{o}C (Ghana Meteorological Agency 2016). As expected, the average water temperatures in rivers vary along the latitudinal gradient of Ghana (Frimpong et al. 2016). The Intergovernmental Panel on Climate Change projects a mean global temperature increase of 1.4 – 5.8^{o}C at the close of the 21st century, if greenhouse gas emissions remain unabated (IPCC 2001). Ghana may experience as much as a 2^{o}C increase in annual temperature over the next century (Hulme et al. 2001). At this projected rate of temperature increase, it is reasonable to expect that southern Ghana will experience temperature conditions that approximate the current temperature conditions in the north. Warmer temperatures in the south could present stressful environmental conditions to the outdoor culture systems predominantly used in Ghana since fish farming is concentrated in southern Ghana. However, an increase in water temperature also presents an opportunity for aquaculture development if *O. niloticus* populations occurring in the northern latitudes are already adapted to high temperature conditions compared to the Akosombo strain of *O. niloticus,* which is widely farmed in Ghana (Frimpong et al. 2016). Thus, proactive interventions will be necessary to ensure that the *O. niloticus* genetic resources are not compromised, will be appropriately used in resilient aquaculture systems, and that the species persists in the face of climate change.

Population and quantitative genetics studies with direct aquaculture applications, which recognize the potential negative effects of climate change, could help conserve the natural variability in *O. niloticus* populations in Ghana and promote the aquaculture industry. Frimpong et al. (2016) recently used a population genetics approach combined with experimental studies to provide fresh insights into the relatively understudied subject of tilapia population genetics within their native range. The goal of their study was to identify *O. niloticus* populations with potential adaptation to predicted future stressful climatic conditions for southern Ghana. The authors conducted water quality sampling in three rivers (Afram, Oti, and White Volta) within the Volta River basin, collected fish samples for population genetic analysis, and investigated phenotypic variation for tolerance of high temperature, low dissolved oxygen, and high salinity in *O. niloticus* from the three rivers. The authors found considerable molecular genetic differentiation among the three wild populations of *O. niloticus* studied, and their results suggested that at least one northern population of *O. niloticus* may be adapted to the high temperature and low dissolved oxygen (DO) levels predicted for southern Ghana (Frimpong et al. 2016).

Building upon these results, I conducted this research to characterize the genetic variability of *O. niloticus* within different drainage basins in Ghana and assessed potential genetic impacts of aquaculture on wild populations of *O. niloticus* to provide the basis for conserving the local adaptation of natural populations. I also sought to identify populations with desirable traits, such as tolerance to high water temperature, that can be incorporated into future selective breeding programs.

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Chapter 2: High genetic variation among wild and farmed Nile tilapia in Ghana evident in mitochondrial DNA

Abstract

In many African countries where the Nile tilapia *Oreochromis niloticus* is the main aquaculture species, native strains are widely considered inferior to the Genetically Improved Farmed Tilapia (GIFT) strain. The need for improved strains has led to a wide call to introduce the GIFT strain into Africa. However, the infrastructure for preventing the possible escape of farmed populations into wild populations and ecosystems is generally lacking, and there are limited baseline studies to guide conservation planning for wild populations. The present study was conducted in order to: 1) characterize the genetic background of Nile tilapia *O. niloticus* populations from different river basins in Ghana to provide baseline information on the species' genetic differentiation to guide management and conservation decisions, and 2) assess selected stocks of *O. niloticus* currently being farmed in Ghana. A phylogenetic analysis of mitochondrial *D-loop* region, and *ND1* gene, and *COI* gene DNA sequences from 184 tilapia samples collected from 14 farmed sources, mostly from cage farms on the Volta Lake; and 13 wild sources from nine river basins in Ghana, revealed three genetically distinct groups (with 100% bootstrap support) of *Oreochromis* spp. in Ghana: 1) pure *O. niloticus* populations in all rivers sampled with private haplotypes in the Black Volta River and the Afram River, 2) established *O. niloticus* populations introgressed with *O. aureus* mitochondrial DNA in almost all rivers, introgressed populations that have not been previously described, and 3) the GIFT (genetically improved farmed tilapia) strain of *O. niloticus* on some farms, introgressed with *O. mossambicus*, some of which have escaped into surrounding rivers. The results underscore the importance of complementing morphological traits with genetic data to identify populations and species of conservation concern. In order to protect pure populations of *O. niloticus*, it is important to prevent all upstream movement of farmed tilapia, especially since wild populations are already threatened by natural hybridization with *O. aureus.*

Introduction

The Nile tilapia (*Oreochromis niloticus*) is one of the most widely introduced species outside its native range. Originally from Africa and the Middle East, the Nile tilapia has been introduced to nearly all tropical and sub-tropical regions, primarily for aquaculture purposes (De Silva et al.

2004; Eknath and Hulata 2009). Many genetically improved tilapia strains have been developed for aquaculture production including GIFT, GET-EXCEL, BEST, GMT, Chitralada, YY-male, COLD, and Florida red (Ordoñez et al. 2017). The Genetically Improved Farmed Tilapia (GIFT) strain of *O. niloticus* was founded using parental stocks from eight countries, including four African countries (Egypt, Ghana, Kenya, and Senegal), and developed by 15 generations of selective breeding. The subsequent distribution of the GIFT strain for commercial culture in Asia revolutionized tilapia aquaculture in Asia and contributed to increased global tilapia production (Eknath et al. 1993; Eknath et al. 1998; Dey 2000; Eknath et al. 2007; Ponzoni et al. 2011). However, due to the risk of contamination of locally adapted native genetic stocks, the WorldFish Center and other development partners responsible for the GIFT strain adopted a policy that did not allow the dissemination of GIFT to African countries where the original parental stocks were collected (Gupta and Acosta 2004).

In Ghana, like in many other sub-Saharan African countries where *O. niloticus* is the main aquaculture species, locally available native strains are widely considered inferior to the GIFT and related strains with respect to growth performance. Recognition of the need for improved strains of tilapia in Africa, and to ensure that Africa benefits from the GIFT project without the associated ecological and genetic risks of introducing the GIFT strain resulted in the development of the Ghanaian Akosombo and the Egyptian Abbassa strains using the GIFT selective breeding methodology (Gupta and Acosta 2004; Attipoe et al. 2013; Dewedar 2013; Mireku et al. 2017). In recent years, however, many commercial farmers in Ghana have expressed discontentment with the growth and survival rates of the Akosombo strain. As part of the development and validation of the Akosombo strain, the GIFT was imported by the Ghanaian government’s Aquaculture Research Development Center (ARDEC) in 2012 for experimentation alongside the Akosombo strain (Ansah et al. 2014).

Ansah et al. (2014) analyzed the economic benefits and the long-term ecological risks of introducing the GIFT strain to Africa and suggested that practical biosecurity measures be implemented prior to any future GIFT introductions. However, the development and implementation of biosecurity measures would prove effective only if countries properly defined their conservation goals through a careful evaluation of the differentiation of populations requiring protection from genetic introgression in specific geographic regions. In Ghana, there are unconfirmed reports of farmers growing GIFT even though the strain has not been officially approved for commercial farming. Hence, it is urgent to assess *O. niloticus* genetic resources in Ghana to inform conservation of any genetically distinct populations remaining.

A systematic assessment of the genetic differentiation of *O. niloticus* populations in Ghana would be important not only for developing conservation goals and protecting locally adapted wild populations from introgression with introduced improved strains, but also could help improve aquaculture locally. Regardless of whether the government plans to continue improving the Akosombo strain or develop a new improved strain, an understanding of the genetic differentiation among wild *O. niloticus* populations would benefit future development of regionally appropriate stocks for aquaculture. In the Ghanaian context, research focusing on the genetic variation among *O. niloticus* populations using both mitochondrial and nuclear DNA markers have been limited to the Volta system (Agnèse et al. 1997; Falk et al. 2003; Falk and Abban 2004; Attipoe et al. 2013; Mireku et al. 2017), and have not explored the possibility that samples morphologically identified as *O. niloticus* could be introgressed or different species.

Hybridization and gene flow occur easily in tilapiine cichlids, many of which have undergone a recent evolutionary radiation and show evidence of incomplete speciation (Wohlfarth and Hulata 1981; Trewavas 1983; Agnèse et al. 1998; Nagl et al. 2001; Cnaani et al. 2003; Romana-Eguia et al. 2004; D’Amato et al. 2007; Ndiwa et al. 2014). Both natural and anthropogenic factors have contributed to gene flow between the three main tilapia species cultured globally; Nile tilapia *Oreochromis niloticus*, Blue tilapia *Oreochromis aureus*, and Mozambique tilapia *Oreochromis mossambicus*. The natural range of *O. aureus* largely overlaps that of *O. niloticus*, which is widely distributed across Africa and Middle East (Trewavas 1983; Rognon and Guyomard 2003). In contrast, the distributions of both *O. niloticus* and *O. aureus* do not naturally overlap with that of *O. mossambicus*, which is restricted to southern African in basins including the Lower Zambesi, the Brak River, the Boesmans River, and the Limpopo system (de Moor and Bruton 1988; Nagl et al. 2001; D’Amato et al. 2007).

O. niloticus is native to the Senegal, Gambia, Niger, Volta, Benoue, and Chad basins within West and Central Africa (Rognon and Guyomard 2003; Teugels and Thys van den Audenaerde 2003). *O. niloticus* also occur in the Nile basins including many East African rivers and lakes (Trewavas 1983; Rognon and Guyomard 2003), and in coastal rivers of Israel (Trewavas and Teugels 1991). Within Africa, sympatric populations of *O. aureus* and *O. niloticus* occur in the Senegal River, middle Niger River, and the upper tributaries of the Benoue and Chad basins (Rognon and Guyomard 2003). However, in the Middle East, *O. aureus* is restricted to the Nile delta, the Jordan basin, and some rivers in Israel (Rognon and Guyomard 2003). There are apparently no records of *O. aureus* in the Volta basin (Rognon and Guyomard 2003), nor in East Africa.

Natural hybridization in sympatric populations of *O. niloticus* and *O. aureus* has been documented in West Africa in the Senegal River (Rognon and Guyomard 2003); and the two species appear genetically more closely related than West African and East African *O. niloticus* populations (Rognon and Guyomard 2003). Natural hybridization also has been documented between *O. niloticus* and *O. mossambicus* in southern Africa (D'Amato et al. 2007), where the former was introduced through aquaculture operations. For aquaculture purposes, many different production strains have been produced by hybridizing *O. niloticus*, *O. aureus*, and *O. mossambicus*, including the red tilapias widely used in Asia, which were produced by hybridizing male *O. niloticus* and female *O. mossambicus* (Romana-Eguia et al. 2004).

Given how easily tilapias hybridize, it is important to combine both morphological and genetic data to identify genetically pure *O. niloticus* populations in order to inform their conservation. Therefore, this study was conducted in order to: 1) characterize the genetic background of Nile tilapia *O. niloticus* from different river basins in Ghana to provide baseline information of the species' genetic resources in order to contribute to management and conservation, and 2) assess strains of *O. niloticus* currently being farmed in Ghana using samples from selected farms and the government-approved Akosombo strain.

Methods

Fish sampling and DNA extraction

The main morphological trait used to distinguish *O. niloticus* from sympatric tilapia species is the presence of dark vertical bands on caudal fins (Eccles 1992; Teugels and Thys van den Audenaerde 2003). Other distinguishing traits include the lack of enlargement of jaws in mature males (Froese and Pauly 2018), and a non-tessellated genital papilla in breeding males (Trewavas 1983). In this study, fish were morphologically identified as *O. niloticus* based on the presence dark vertical bands on caudal fins. Fin-clips from nearly 700 fish were collected between December 2014 and July 2017. The samples included wild individuals from 13 sites, which represented eight major rivers and one coastal lagoon in Ghana: Afram River, Oti River, White Volta River, Black Volta River, Pra River, Tano River, Ankobra River, Lower Volta River and Juen Lagoon (Figure 1).

Farmed *O. niloticus* samples were obtained from 14 sources including selected farms and commercial hatcheries, as well as wholesale distribution points and local markets (Table 1). Samples of the Akosombo strain of *O. niloticus* were obtained from the Aquaculture Research and Development Centre (ARDEC) and the Pilot Aquaculture Centre (PAC) as reference samples for evaluating the farmed populations. All fin-clips were stored in paper envelopes, dried, and transported to Virginia Tech for laboratory analysis. Tilapia from two U.S. grocery stores, which had originated from China and Ecuador (Table 2), were purchased in October 2017 as reference populations for the GIFT strains of *O. niloticus* reportedly grown illegally in Ghana. Fish samples from the neighboring country, Cote d'Ivoire, were obtained as West African reference samples (Table 2).

Total genomic DNA was extracted using the DNeasy extraction kit (Qiagen) according to the manufacturer's instructions. The DNA was quantified using a BioDrop spectrophotometer and concentrations were standardized for use in the polymerase chain reaction (PCR). For this study, 184 samples were selectively sequenced and analyzed. The study samples included 93 wild samples, 53 samples from seven farms and hatcheries, and 38 market samples originating from cage farms on the Volta Lake.

Polymerase chain reaction and DNA sequencing

Three mitochondrial DNA markers - the displacement loop (*D-loop*) region, the first subunit of the NADH dehydrogenase (*ND1*) gene, and the cytochrome c oxidase subunit 1 (*CO1*) gene - were selected and amplified by polymerase chain reaction (PCR). The *D-loop* was chosen because it is the most variable region in the mitochondrial genome and known to be a "hot spot" for mutation. The *ND1* gene was chosen as it is a relatively more conserved region compared to the *D-loop* region. *CO1* was chosen because it is used widely as a barcoding gene marker to help identify different putative species. Primers (forward 5'-ACCCCTAGCTCCCAAAGCTA-3' and reverse 5'- CCTGAAGTAGGACCAGATG-3') previously designed for *O. niloticus* (Nyingi et al. 2009) were used for the *D-loop.* New primers were developed for *ND1* with the Primer 3 program in GenBank using *O. niloticus ND1* sequences available in GenBank (He et al. 2011). Of the ten candidate primer pairs generated by Primer 3, three pairs (*ND1Pr1*, ND1Pr5, and

ND1Pr10) were evaluated. A multiplex of *ND1*Pr5 (forward 5'- TGCAACTACGAAAAGGCCCA-3' and reverse 5'- TAAGTGCTAGGGTGAGGGCT-3') and *ND1*Pr10 (forward 5'- TAATCCTTCCCGCCTGACCT-3' and reverse 5'- GCCTGTTAGTGCGATTGGGA-3') amplified nearly 80% of the entire *ND1* gene, which included the region amplified by the primer *ND1*Pr1. Thus, only *ND1Pr5* (forward and reverse) and *ND1Pr10* (forward and reverse) were utilized for this study. Universal fish primers (forward F2 and VF2; reverse R2 and FR1d; Ivanova et al. 2007) were used for *CO1* gene amplification.

The 22-µl PCR amplification reaction consisted of 50-100 ng of genomic DNA, 5 U/µl *Taq* DNA polymerase, 5x PCR buffer, 25mM $MgCl_2$, 2.5 mM dNTP mix, 5 µM bovine serum albumin, and 5 µM of primers. The *CO1* amplification reaction used 10 µM of primers. The following thermal cycling conditions were used for *D-loop* and *CO1*: 94^{o}C for 3 min; 35 cycles of 94^{o}C for 40 sec, 52^{o}C for 30 sec, and 72^{o}C for 1 min; a final extension of 72^{o}C for 5 min; and a 4^{o}C hold. Thermal cycling conditions for *ND1* were similar to those for the *D-loop* except that the annealing temperature was 56^{o}C and the final extension time was 10 min. PCR products were visualized with agarose gel electrophoresis to confirm amplicon sizes prior to sequencing with an ABI3730 automated DNA sequencer at the Virginia Tech Biocomplexity Institute (Blacksburg, VA).

Data analysis

DNA sequences and polymorphism – The raw DNA sequences were assembled using the program Geneious® 11.1.2 (Biomatters Ltd., Auckland, New Zealand). The consensus sequences obtained then were aligned and edited with the program ClustalW (Larkin et al. 2007) embedded in GeneStudio ™ v2.2. (http://genestudio.com/). Variable sites and parsimony informative sites were determined using MEGA7 (Kumar et al. 2016). The numbers of haplotype sequences were determined separately for each mtDNA marker with the program DnaSP 6.11.01 (Rozas et al. 2017). Using the Basic Local Alignment Search Tool (BLAST) feature in GenBank, haplotype sequences for both DNA markers were queried, and highly homologous sequences obtained from the BLAST searches were retrieved and used as reference sequences for the phylogenetic analysis.

All sequences were realigned and the best substitution model of sequence evolution was determined using the program MrModeltest 2.3 (Nylander 2008) implemented within PAUP 4.0

(Swafford 2002). The best substitution model selected using the Akaike Information Criterion was the General Time Reversible model with gamma-shaped distribution (GTR+G) for *D-loop*, the Hasegawa, Kishino, and Yano model with gamma-shaped distribution (HKY+G) for *ND1*, and the GTR with a proportion of invariable sites with gamma-shaped distribution (GTR+I+G) for *CO1*. Phylogenetic analysis based on Bayesian inference was conducted with the program MrBayes 3.2.6 (Ronquist et al. 2012) using the parameters specified from MrModeltest. The resulting phylogenetic trees were visualized with the program FigTree 1.4.2 (Rambaut 2014). Evolutionary divergence between haplotypes was estimated using pairwise percentage sequence divergence (*p*-distance) implemented in the program MEGA7 (Kumar et al. 2016).

Phylogenetic analysis – Phylogenetic analysis was conducted using both phylogenetic trees (*D-loop* and *ND1*) and haplotype networks (*D-loop* and *CO1).* The phylogenetic tree for *D-loop* was constructed using the Ghanaian *O. niloticus* haplotype sequences, and six groups of reference samples (Table 2). The reference samples were: 1) U.S. grocery store tilapia originating from China and Ecuador , 2) GenBank sequences of the GIFT strain of *O. niloticus* and Egyptian, Philippino, and American strains of *O. niloticus* which were studied in China, 3) GenBank sequences of *Oreochromis aureus* and *O. mossambicus*, as well as *O. niloticus* x *O. aureus* and *O. niloticus* x *O. mossambicus* hybrids, 4) West African reference samples represented by *O. niloticus* samples obtained from Cote d’Ivoire and sequenced as part of the present study, and a GenBank sequence of *O. niloticus* sampled from Senegal, and 5) GenBank sequences of select subspecies of *O. niloticus* sampled from Kenya, representing East African samples. All GenBank accession numbers are presented in Table 2. The phylogenetic tree for *ND1* was constructed with Ghanaian haplotypes and only reference groups 1 to 3 described above. *Coptodon zillii* was chosen as the outgroup for both mtDNA markers. The *Coptodon zillii D-loop* sequence was obtained from an individual collected from the Volta River in Ghana during the field sampling. The *Coptodon zillii ND1* sequence with accession number KM658974 was obtained from GenBank.

Results

D-loop haplotypes

A partial sequence of 391 bp covering the hypervariable region (280 bp) and the first part of the central conserved region (111 bp) of the mitochondrial *D-loop* region was analyzed. There were

99 variable sites and 83 parsimony-informative sites. Thirty-eight haplotypes were identified among the 184 individuals sequenced. The haplotype data showed 77 variable sites (Table 3), and 68 parsimony-informative sites. Of the 38 haplotypes identified, 15 haplotypes were shared among populations, while 23 were private (Table 4). Two haplotypes (haplotypes 2 and 3) were exhibited among more than 50% (*N* = 95) of all the individuals, with every geographic site exhibiting at least one of those two haplotypes (Table 4). Additionally, haplotypes 2 and 3 were highly frequent in samples from the Volta system (White Volta River, Black Volta River, Oti River, and Lower Volta River), and were exhibited by eight of the 10 ARDEC individuals. Nine of the ten Ankobra River individuals exhibited haplotype 2. The other haplotype observed in the Ankobra River sample was haplotype 33, which was shared with individuals from ARDEC and the Afram River (Table 4).

Four of the shared haplotypes were noteworthy. Haplotypes 1 and 15 were observed only in samples collected from wild populations. Haplotype 1 was shared by the White Volta River, Black Volta River, Tano River, and the Juen Lagoon, while haplotype 15 was shared by the Oti River and Black Volta River populations. Haplotype 9 was shared by the Tano River and the Black Volta at Talewona, and haplotype 11 was restricted to the Tano River population (Table 4). Nine of the 23 private haplotypes were observed in five collections from the wild; Afram River (haplotypes 36 and 37, *N* = 6), Black Volta River (haplotypes 29, 30, 31, and 32; *N* = 16), Pra River (haplotype 12; *N* = 9), Lower Volta River (haplotype 5, *N* = 11), and Juen Lagoon (haplotype 14, *N* =5). Additionally, haplotype 38, which was private to the Adjei Farm at Elubo, may be characteristic of the Tano River. The remaining 13 private haplotypes mostly were observed in collections from aquaculture facilities on the Volta Lake, and dominated by samples from Fujian Farm (Table 4).

D-loop phylogenetic relationships

The results of the phylogenetic analysis of mitochondrial *D-loop* sequences showed four genetically distinct clusters with 100% bootstrap support (Figure 2), which also were distinct from that of the outgroup species, *C. zillii*. The first cluster, the *O. niloticus* cluster, contained 17 haplotypes, which clustered with one sample from Cote d'Ivoire (Onilo_CD), and the GenBank sequence Kpa11 originally sampled from the Volta basin in Ghana by Falk et al. (2003) as part of a genetic study of black-chinned tilapia, *Sarotherodon melanotheron*. The *O. niloticus* cluster contained haplotypes 2 and 3 (the haplotypes observed in the majority of both wild and farmed individuals), as well as the two “wild” haplotypes, 1 and 15 (Figure 2). Haplotypes 3, 10, 15, 31, and 32 clustered with GenBank-derived sequences of *O. niloticus* x *O. aureus* hybrids (Figure 2).

The second cluster, the inferred *O. aureus* cluster, contained 11 haplotypes from individuals from the Black Volta, White Volta, and Pra rivers, and nearly all the farm and market samples. The *O. aureus* cluster also contained the Senegal sample, one sample from the U.S. grocery-store tilapia reference group (Onilo_WA1), and all three *O. aureus* sequences from GenBank (Figure 2). Two of the haplotypes in the *O. aureus* cluster were private haplotypes from wild sources, haplotype 12 from the Pra River and haplotype 30 from the Black Volta River. To assess the observation of samples morphologically identified as *O. niloticus* appearing distinctly as *O. aureus*, the haplotype network constructed based on the method of Templeton, Crandall, and Sing (TCS; Templeton et al. 1992); using TCS network (Clement et al. 2002) with haplotypes from both the *O. niloticus* cluster and the *O. aureus* cluster. The results supported the monophyletic grouping of *O. niloticus* and *O. aureus* previously observed from the *Dloop* analysis, and showed genetic distinctiveness, with about 141 mutation steps between the two clusters (Figure 3).

The third cluster, the *O. niloticus* x *O. mossambicus* cluster, contained a GenBank sequence of a *O. niloticus* x *O. mossambicus* hybrid, six haplotypes including four private haplotypes from three sites – Fujian Farm (haplotypes 22 and 24), the Lower Volta River (haplotype 5), and Juen Lagoon (haplotype 19); haplotype 5 shared by the Fujian Farm, Lower Volta River, and Akosombo tilapia farm; and haplotype 33 shared by ARDEC, Ankobra River, and Afram River. The *O. niloticus* x *O. mossambicus* cluster also contained all five East African reference sequences; seven of the nine U.S. grocery tilapia reference samples and the three GIFT-related strains (Egyptian strain, Filipino strain and the American strain); and two samples from Noe, Cote d’Ivoire. Haplotype 19 observed in the Juen Lagoon sample was identical to that in the Onilo_Egypt strain and Onilo_eduardianus from East Africa. The third cluster may be best described as an inferred *O. niloticus* x *O. mossambicus* cluster because of the common practice of hybridizing male *O. niloticus* and female *O. mossambicus* to produce red tilapias (Romana-Eguia et al. 2004). This may also explain why the individuals in this cluster appear morphologically as *O. niloticus* (for example, the East African reference samples) but contain *O. mossambicus* mtDNA.

The fourth cluster, the *O. mossambicus* cluster, consisted of all five GenBank sequences for *O. mossambicus*; four haplotypes (private and shared) from the Fujian Farm and the Akosombo tilapia farm; one of the U.S. grocery store tilapia samples (KB2); and one of the Noe samples from Cote d'Ivoire. The GenBank *O. niloticus*_GIFT strain sequence and another *O. niloticus* sequence also grouped with the *O. mossambicus* cluster.

Pairwise nucleotide *p*-distances provided further support for the clustering observed. The genetic distances were considerably larger between clusters than within clusters (Table 5). For instance, the genetic distances (i.e., dissimilarity) between the *O. niloticus* cluster and the *O. aureus* cluster, *O. niloticus* x *O. mossambicus* cluster, and the *O. mossambicus* cluster were about 38%, 57% and 66-71%, respectively, compared to the largest within-group distance of 1.6% for the *O. niloticus* cluster (Table 5). However, divergence based on fixed nucleotide differences at the variable sites showed that individuals in the *O. niloticus* cluster were genetically more similar to individuals in the inferred *O. aureus* cluster; and genetically distinct from individuals in the inferred *O. mossambicus* clusters (Table 3). The variable sites of the *D-loop* haplotypes in comparison to all reference samples are provided in Table S1.

ND1 haplotypes

Sixty individuals, including the nine U.S. grocery-store reference samples, were sequenced at the *ND1* gene, which represented a subset of the samples analyzed at the *D-loop* region. Sixteen sequences (mostly farmed, market and reference samples) were excluded in the analysis because they were either too short or too divergent. A total of 44 sequences with length 675 bp were analyzed. There were 72 variable sites, 65 parsimony information sites, and 19 haplotypes. The number of variable sites were the same for both the full dataset and the haplotype dataset. Table 6 provides haplotype and variable sites information. The 19 haplotypes observed included seven shared haplotypes and 12 private haplotypes, and the pattern of individual groupings into haplotypes closely matched the groupings for the *D-loop* sequences. The relatively high percentage of *ND1* private haplotypes was expected due to the small sample sizes for the sites included in the analysis.

ND1 phylogenetic relationships

The phylogenetic relationships observed for the mitochondrial *ND1* haplotypes were congruent with the *D-loop* results, except that the clustering was not as distinct for *O. niloticus* and *O.*

aureus as was observed for the *D-loop* tree. Two main clusters were observed. Cluster one contained *O. niloticus*, *O. aureus* and hybrids of the two species as evidenced by individuals that grouped with the respective clusters in the *D-loop* tree and with the GenBank reference sequences (Figure 4). Cluster two included two sub-clusters. The first sub-cluster, the inferred *O. niloticus* x *O. mossambicus* cluster, contained the U.S. grocery store tilapia reference samples, the GIFT-related strains, and two *ND1* haplotypes from the Lower Volta River and Fujian Farm, all of which had clustered similarly in the *D-loop* tree (Figure 4). The second sub-cluster, the *O. mossambicus* cluster, contained the GIFT strain and two GenBank sequences from *O. mossambicus*. Table 7 provides the haplotype frequency distribution across sampling sites.

The pairwise genetic distances calculated for *ND1* haplotypes showed that the *O. niloticus*/*O. aureus* cluster and the *O. niloticus* x *O. mossambicus* cluster were distinct. Haplotypes in the *O. niloticus*/*O. aureus* cluster were separated from the *O. niloticus* x *O. mossambicus* cluster by a distance of 9.2-10.6%, while the largest intraspecific distance between haplotypes in the *O. niloticus*/*O. aureus cluster* and in the *O. niloticus* x *O. mossambicus* were 1.7% and 0.4%, respectively (Table 8). Genetic divergence based on the fixed nucleotide differences at the *ND1* sequence variable sites was congruent with the findings from *D-loop* sequence analysis and showed the observed genetic similarity between individuals in the *O. niloticus* / inferred *O. aureus* cluster; and the dissimilarity between the *O. niloticus* cluster and the inferred *O. mossambicus* cluster (Table 6).

CO1 relationships

I verified the presence of GIFT and related strains in Ghana by sequencing the *CO1* gene of two farmed samples (FFARM28 and FFARM29) previously identified as GIFT haplotypes from the *D-loop* and *ND1* analyses. The *COI* sequences were compared with *CO1* sequences from GIFT samples from GenBank (accession numbers KU565825 and KU565864) and related strains (e.g. EXCEL, BEST, PNTO4) collected in the Philippines (Ordoñez et al. 2017). This verification step was necessary because no information on the GenBank Onilo_GIFT (used for the *D-loop* and *ND1* gene phylogenetic analysis) linked the sequence to the GIFT project, and no other appropriate GIFT sequences were found on GenBank for the *D-loop* and *ND1* analysis. I also sequenced one farmed sample identified as a native haplotype (PAC1) as a reference sample. The relationships among the samples were visualized using a haplotype network and were congruent with the results obtained for both *D-loop* and *NDI* analyses. The results showed that the Fujian Farm samples were distinct from the native stock. The Philippines GIFT strain sequences clustered with FFARM29, other improved strains, and the Egyptian and GIFT-related strains used in the *D-loop* and *ND1* analyses (Figure 5). Additionally, FFARM28 clustered with GenBank O. *mossambicus* sequences, similar to findings from the *D-loop* and *ND1* analyses (Figure 5).

Discussion

Native populations of O. niloticus in Ghana

The results of the phylogenetic reconstruction presented here suggest the presence of distinct natural populations of *O. niloticus* in Ghana (*Dloop*_cluster 1). These distinct populations appear to have originated in the Volta River basin, with ancestral populations from mainly the Black Volta, White Volta, and the Oti rivers (e.g., haplotypes 1, 2, 3, 29, and 31). Populations in the Afram River and the Lower Volta River were later established from these source populations (Figure 3). The Tano River also appears to be a source population for *O. niloticus* in Ghana, judging from the number of haplotypes shared with the Black Volta, White Volta, and Tano rivers; and the fact that those rivers are not currently connected (e.g., haplotype 9 is shared by the Tano River and the Black Volta River). The fact that one sample of *O. niloticus* from Cote D’Ivoire (Onilo_CD) also clustered with the Ghanaian haplotypes may be an indication that *O. niloticus* from the Tano River shared ancestry with that from Cote D’Ivoire. Additionally, it appears that *O. niloticus* independently colonized the Pra and the Ankobra rivers through the Tano River, although the mechanism of colonization should be explored since all three rivers share a geographically restricted tilapia species, *Tilapia pra* (Dunz and Schliewen 2000). The results of the study suggested that the Akosombo strain (ARDEC) is widely used by farmers across the country, even though the use of wild strain was detected at farms in Elubo, Western Region. This inference was supported by the haplotype distribution among sites and the number of haplotypes that the ARDEC strain shared both with the source drainages, as well as with the farm and market samples.

New records of O. aureus in Ghana?

The clustering of Ghanaian *O. niloticus* haplotypes with GenBank sequences of *O. aureus* with 100% bootstrap support and the presence of sympatric populations of *O. niloticus* x *O. aureus* hybrids suggest that *O. aureus* occurred in Ghana but had not yet been documented. These are two possible explanations for this observation: 1) *O. aureus* populations occurred in Ghana, but have not yet been identified because the species' geographic distribution previously was thought to not extend to Ghana (Rognon and Guyomard 2003), and 2) samples morphologically identified as *O. niloticus* carried mtDNA nearly identical to *O. aureus*. Given that the samples collected phenotypically appeared as *O. niloticus*, the occurrence of introgressive hybridization could account for why they are genetically similar to *O. aureus*. Introgressive hybridization is the result of hybridization and repeated backcrossing, leading to the mtDNA of one species completely replacing the mtDNA of the other (Rognon and Guyomard (2003).

Hybridization between *O. aureus* and *O. niloticus* is known (Agnèse et al. 1997; Rognon and Guyomard 2003; Bakhoum et al. 2009), and introgressive hybridization has been reported for the two species in West African rivers. For instance, Rognon and Guyomard (2003) found that introgressed *O. niloticus* individuals from Senegal showed mtDNA identical to *O. aureus*, but were distinct based on morphological and nuclear DNA analysis. These results also may explain why the GenBank *O. niloticus* sequence from Senegal (accession number EF016715) grouped with the *O. aureus* cluster in this study. This finding is congruent with the high genetic differentiation observed by Nyingi et al. (2009) when the same Senegal-derived mtDNA sequence was analyzed together with *O. niloticus* sequences collected in Kenya. D'Amato et al. (2007) also reported introgressive hybridization between *O. niloticus* and *O. mossambicus,* where morphologically identified *O. mossambicus* contained *O. niloticus* mtDNA.

The occurrence of private haplotypes of "*O. aureus*" in the Black Volta River and the White Volta River further supports the possible presence of *O. aureus* populations in Ghana. In Africa, *O. aureus* naturally occurs in the Lower Nile (Egypt), Chad Basin (Chad), Benue (middle and upper Niger River; Nigeria, Niger, Cameroon, and Mali) and Senegal River (Senegal; Froese and Pauly 2018). Thus, it is likely that *O. aureus* naturally colonized the Volta River basins from the upper Niger River (Mali – Burkina Faso – Ghana). Further studies involving a detailed survey of rivers within the Volta River basin should help assess the presence of *O. aureus* in Ghana.

Another important factor to note is that *O. aureus* and *O. niloticus* show 88.2% similarity at the non-coding *D-loop* region and 91.6% at mitochondrial coding genes (He et al. 2011). The high nucleotide similarities between the species especially at coding genes could explain why the *D-loop* provided higher resolution for observing the genetic distinctiveness between the two species compared to *ND1* and other coding genes (Ordoñez et al. 2017). However, although a high bootstrap support (100%) was obtained for the *D-loop* tree, the relatively small nucleotide differences observed in the *O. niloticus* and inferred *O. aureus D-loop* DNA sequences was congruent with the *ND1* results, and suggests that the two "species" may be two different lineages within one species.

Rognon and Guyomard (2003) discussed two hypotheses, which could explain the low mtDNA sequence divergence recorded between *O. niloticus* and *O. aureus*, and affect the assessment of whether the two warrant separate designation as valid species. The two hypotheses are: 1) the conservation of ancestral polymorphism, or 2) differential introgressive hybridization (Avise 1994; Rognon and Guyomard 2003). The arguments supporting the first hypothesis suggest that the speciation of *O. aureus* occurred relatively recently from the West African *O. niloticus* group. However, the coalescence times estimates of 200,000 and 300,000 years between the two species, based on previously reported substitution rates of 3.6% my^{-1} (Donaldson and Wilson 1999); and 5.6% my^{-1} (Nagl et al. 2001) respectively do not agree with protein data (Rognon and Guyomard 2003), fossil records, morphology or biogeographical history (Trewavas 1983). On the contrary, available fossil records and protein data suggest that *O. niloticus* and *O. aureus* diverged about 3.3 ± 1.5 million years ago during the Upper Pliocene (Trewavas 1983; Rognon and Guyomard 2003). Biogeographical information suggests that the *O. aureus* ancestral populations occupied the Jordan basin and the Nile delta and encountered *O. niloticus* ancestors from the Nile basins prior to their independent colonization of West African river basins around Pleistocene times (Trewavas 1983; Agnèse et al. 1997; Lowe-McConnell 1998).

Given this divergence time, the second hypothesis supports introgressive hybridization between *O. niloticus* and *O. aureus,* and would best explain the low divergence between the two sympatric West African populations compared to the significantly high divergence between West

African and East African *O. niloticus* populations (Rognon and Guyomard 2003). These authors suggested that long-term unidirectional introgression from *O. aureus* into *O. niloticus* is likely the case in West Africa because *O. aureus is* outnumbered by *O. niloticus* in the region (Trewavas 1983; Lowe-McConnell 2000). Hence, hybrids are likely the products of mating between male *O. niloticus* and female *O. aureus* because unidirectional introgression most frequently involves females from the less-common species (Avise 1994; Wirtz 1999). Also, given that crossings between male *O. niloticus* and female *O. aureus* tend to produce relatively more female offspring (Wohlfarth and Hulata 1983), unidirectional introgressive hybridization may become recurrent, producing more and more females appearing phenotypically as *O. niloticus* with *O. aureus* mtDNA.

The above discussions clearly support two assertions: 1) *O. niloticus* and *O. aureus* are two valid species, which have naturally hybridized extensively within West African river basins where they exist in sympatry; and 2) the likelihood of introgressed *O. niloticus* populations occurring within the Volta River basin in Ghana is very plausible, which would require further studies to ascertain their possible distribution. The fact that *O. niloticus* x *O. aureus* hybrids were detected on all farms samples suggests that the parental populations that ARDEC used to develop the Akosombo strain included such hybrids, which went undetected. Thus, through the nationwide dissemination of the Akosombo strain, these hybrids have become widely spread across the country.

The presence of GIFT and related improved strains in Ghana

The clustering of Ghanaian haplotypes of *O. niloticus* with *O. mossambicus* x *O. niloticus* hybrids, U.S. grocery-store tilapia from China and Ecuador (*D-loop* cluster 3, *ND1* cluster 2), *O. mossambicus* sequences, and GenBank sequence Onilo_GIFT (*D-loop* cluster 4) suggests a recent introduction of GIFT or the related improved strains into Ghana. The clustering of Onilo_GIFT with *O. mossambicus* further indicates that the original GIFT included founding *O. niloticus* populations which were introgressed with *O. mossambicus* prior to their inclusion in the GIFT project. The most likely *O. mossambicus* hybrid population used for developing the GIFT may be *O. niloticus vulcani* from Kenya. Eknath et al. (1991) reported that *O. n. vulcani* was genetically distant from the seven other GIFT founding populations examined, including populations from Ghana, Egypt, and Senegal. Such genetic distinction between East African and

West African *O. niloticus* has been observed consistently (Agnèse et al. 1997; Rognon and Guyomard 2003; Nyingi et al. 2009), and in the present study, it was expected that all West African haplotypes would be distinctly separated from the East African haplotypes.

As expected, the East African *O. niloticus* sequences were genetically distinct from the pure and hybrid *O. niloticus* populations discussed above. However, the East African *O. niloticus* sequences also clustered with some Ghanaian haplotypes distinct from the other Ghanaian haplotypes, *O. niloticus* x *O. mossambicus* hybrids, and the U.S. grocery-store tilapia samples. This observation supports the hypothesis that there was widespread hybridization between East African *O. niloticus* populations and *O. mossambicus*. The results from the *CO1* analysis provided evidence of the ongoing farming of the GIFT and other improved strains in Ghana and supported the findings that the GIFT strains are hybrids between *O. niloticus* and *O. mossambicus*.

The distribution of haplotypes showed that the GIFT and related strains detected among Ghanaian *O. niloticus* haplotypes were introduced into the country through aquaculture and were predominantly represented by samples from two farms, Fujian Farm and Akosombo Tilapia Farm. The finding that one of the two haplotypes observed from the Lower Volta River was shared with the Fujian Farm and the Akosombo Tilapia farm further suggests that those individuals were escapees or descendants of escapees from the two farms. The signal of *O. mossambicus* in the ARDEC samples suggests experimental cross-contamination of the GIFT strain with the Akosombo strain during performance trials with the GIFT strain. The three other sites where *O. mossambicus* hybrids were detected were the Juen Lagoon, Ankobra River, and Afram River. It is plausible that the hybrids were introduced to these basins through farm escapees because of past or present fish farming activities in or near these basins. For instance, local fishermen operating on the Juen Lagoon reported a significant increase in the catches of *O. niloticus* in the landings from the lagoon in recent years. They attributed the increased catches of *O. niloticus* to fish escapes into the Tano River when a cage aquaculture facility on the river was destroyed by unidentified persons. This essentially represents a stocking event with farmed strains. Considering the close proximity of Noe, Cote d’Ivoire with the Tano River, and the fact that the Noe samples clustered with the GIFT and related strains in this study, it is highly likely that the Tano River contains the GIFT strains.

Conclusion and implications

This study was conducted to characterize the genetic background of Nile tilapia *O. niloticus* from different river basins and selected farms in Ghana to provide baseline information of the species' genetic resources, in order to inform management and conservation. The results of the phylogenetic analysis revealed: 1) that pure *O. niloticus* populations occurred in all rivers sampled, with private haplotypes in the Black Volta River and the Afram River, 2) that established *O. niloticus* populations likely introgressed with *O. aureus* mitochondrial DNA occur in all Ghanaian rivers except the Ankobra, although this introgression has not been described previously, and 3) the GIFT and other genetically improved tilapia strains are already present on some farms, and such stocks have escaped into surrounding rivers.

The results of the present study underscore the importance of complementing morphological trait data with genetic data to identify species and differentiated populations of conservation concern. The finding that the GIFT strain has already escaped from farms into the wild is particularly worrisome considering the interconnectedness among rivers in the Volta basin, which has resulted in the observed distribution of *O. niloticus* in Ghana. Having established from the present study that there are likely thriving populations of *O. niloticus* x *O. aureus* hybrids in the Volta Basin, other rivers in Ghana and on fish farms, the introduction of GIFT strains could mean that "pure" populations of *O. niloticus* in Ghana would be difficult or impossible to find in the foreseeable future.

Within the Volta basin, it appears that the escaped GIFT populations are restricted to the Lower Volta River and the Afram River. This inference suggests that upstream populations in the Black Volta River, White Volta River and the Oti River are not currently impacted by the GIFT strain. The Pra River also appears unimpacted with the GIFT strain. However, more samples would have to be screened to test this interpretation and to guide the development of conservation plans to prevent these wild populations from potentially hybridizing with the improved strains. Upstream movement of all farmed strains of tilapia in the Volta basin should be avoided.

Furthermore, the possibility that the Akosombo strain disseminated by ARDEC is contaminated with the GIFT strain should be investigated as a matter of urgency because of the potential to spread Akosombo strain and GIFT-strain hybrids to other farms. In the meantime, the two farms currently containing the improved *O. niloticus* strains should be required to practice strict confinement measures to prevent further escapes or intentional spread to other farms. The broader implication of the findings presented here is the possibility of the spread of GIFT strains to neighboring countries through the Volta River basin and shared basins such as the Tano. More samples should be collected from the Ankobra River, the Juen Lagoon, and the Tano River to assess the presence of the introduced strains. In light of the results of this study, it is imperative that baseline studies be conducted for all neighboring countries to provide data for the conservation of pure populations of *O. niloticus* in those countries.

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Figures and Tables

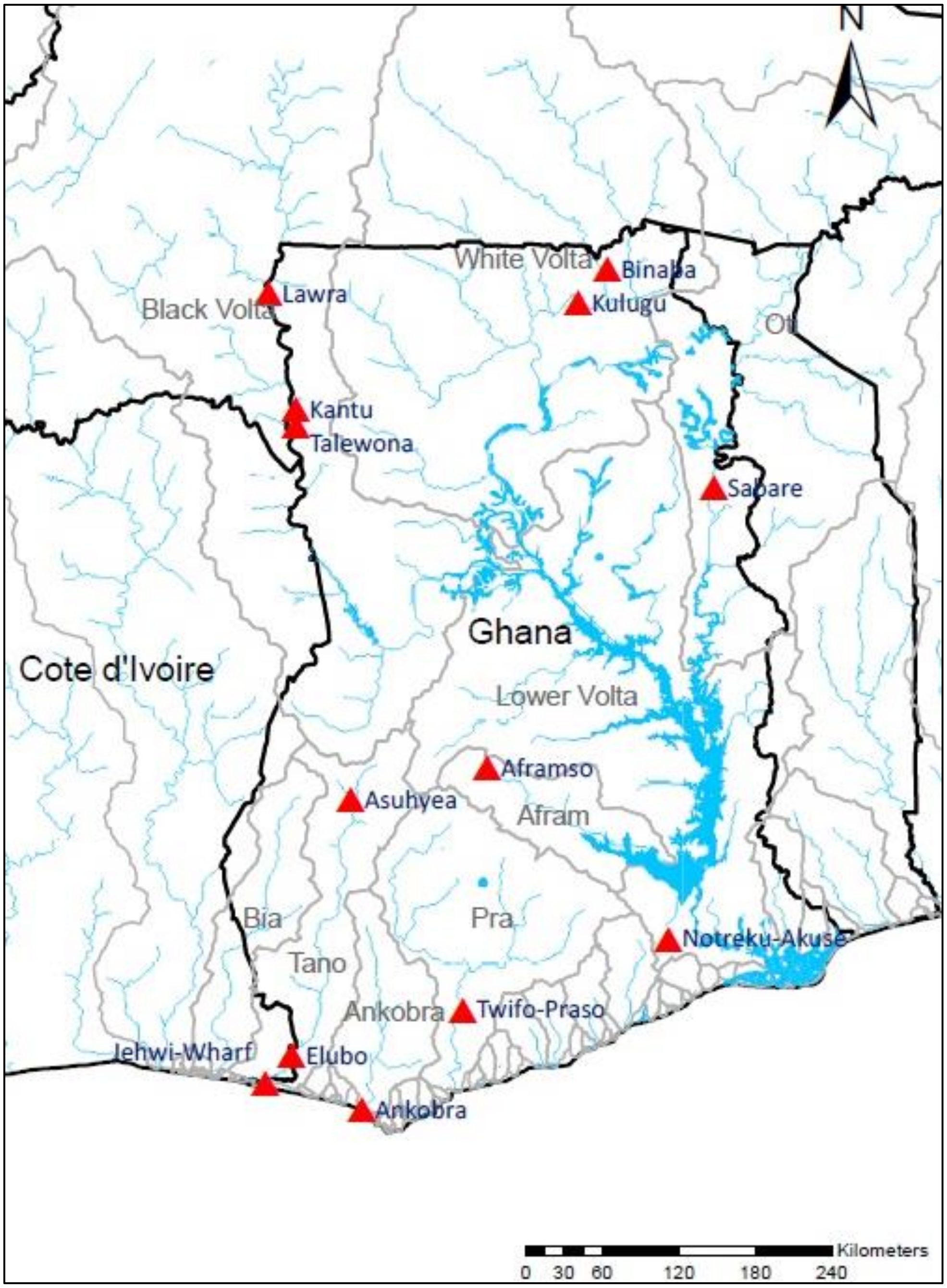

Figure 1. Sampling locations for wild *Oreochromis* spp. collected in eight rivers and one coastal lagoon in Ghana from December 2014 through July 2017. Sites are indicated with red triangles.

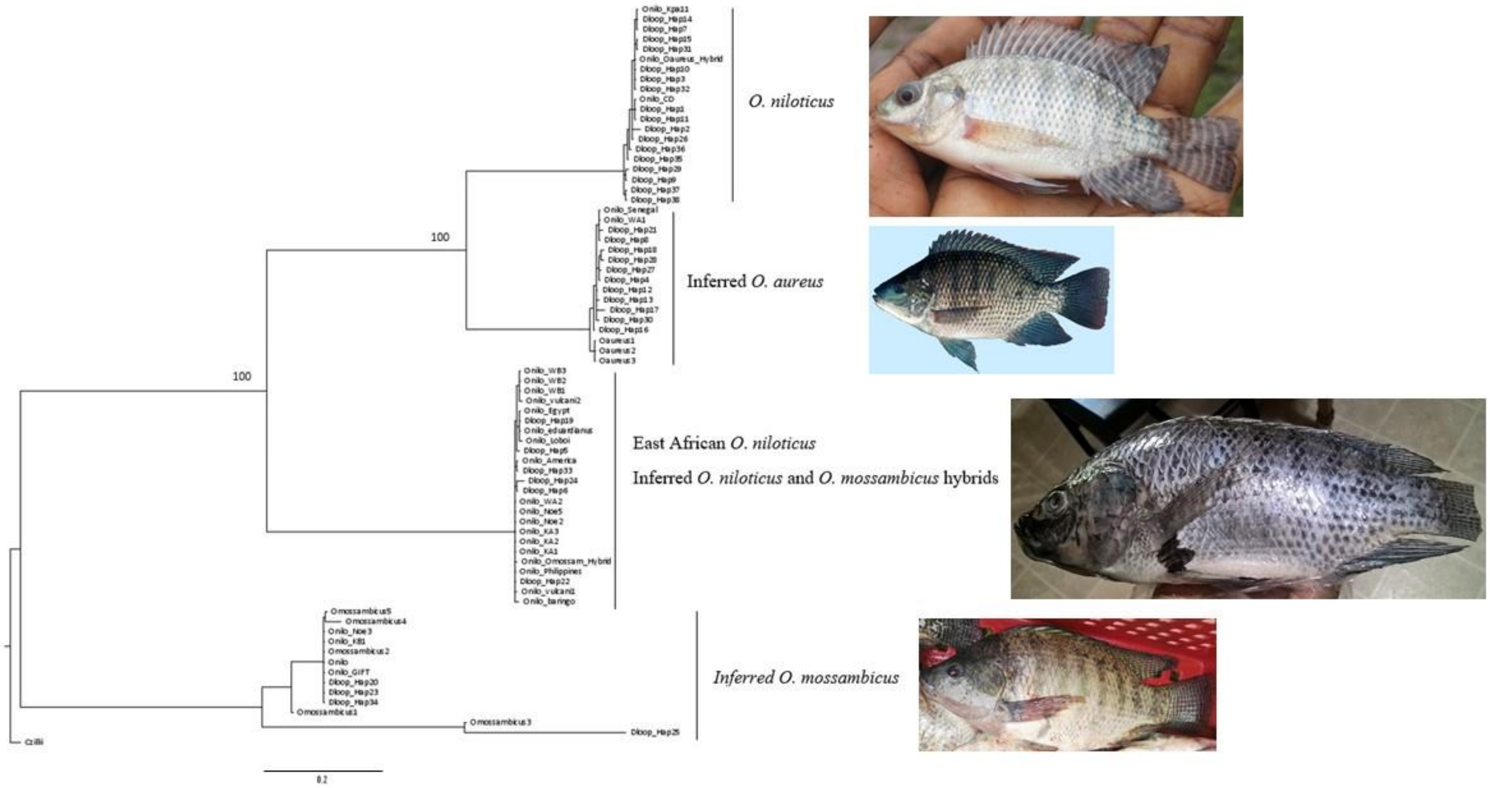

Figure 2. Phylogenetic tree constructed from 391-bp *D-loop* sequences for *Oreochromis* spp. using Bayesian analysis. The analysis was performed with 2 million Markov chain Monte Carlo generations with four chains, a sample frequency of every 100 generations, and a burn-in of 500,000 generations. The analysis was performed in two runs. A total of 30,002 trees were sampled. The average standard deviation of split frequency was 0.0073 with a -Ln likelihood of -3687.36. Bootstrap support values are indicated to the left of each node. Photo credits: *O. aureus* (Froese and Pauly 2018); all other photos are from this study.

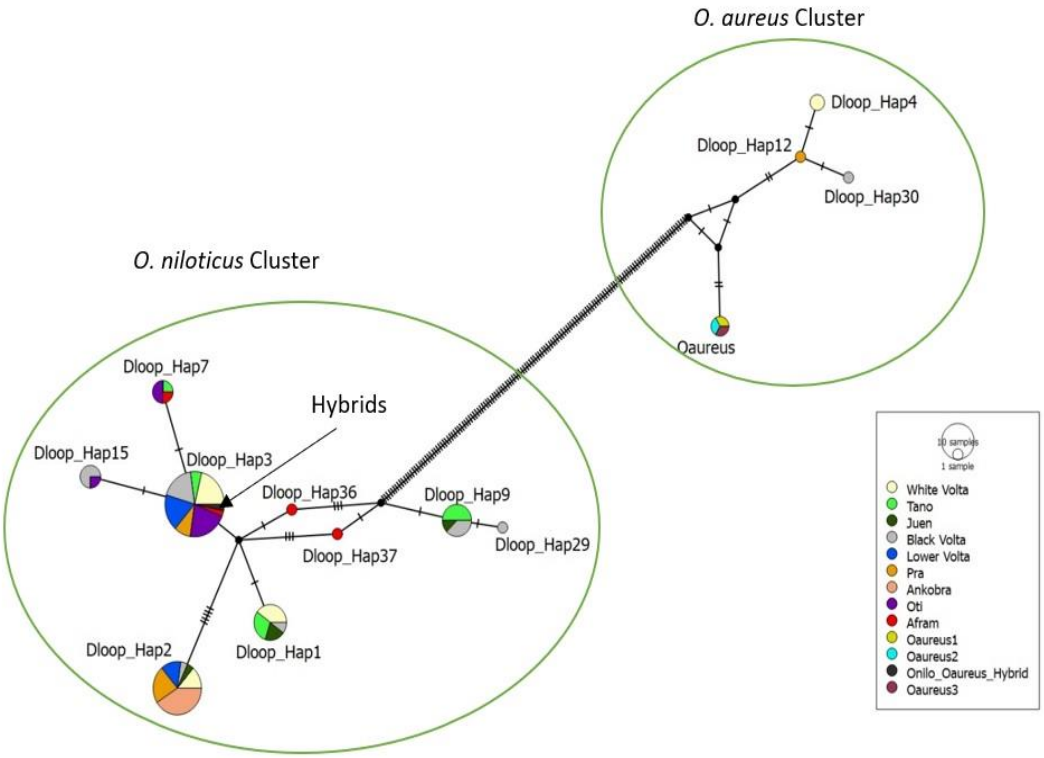

Figure 3. TCS network of 12 native *Oreochromis* spp. haplotypes from nine drainages in Ghana inferred from 391 bp of sequence from the mitochondrial *D-loop* region. *O. aureus* sequences are included as outgroups. Inferred mutations are indicated by hash marks.

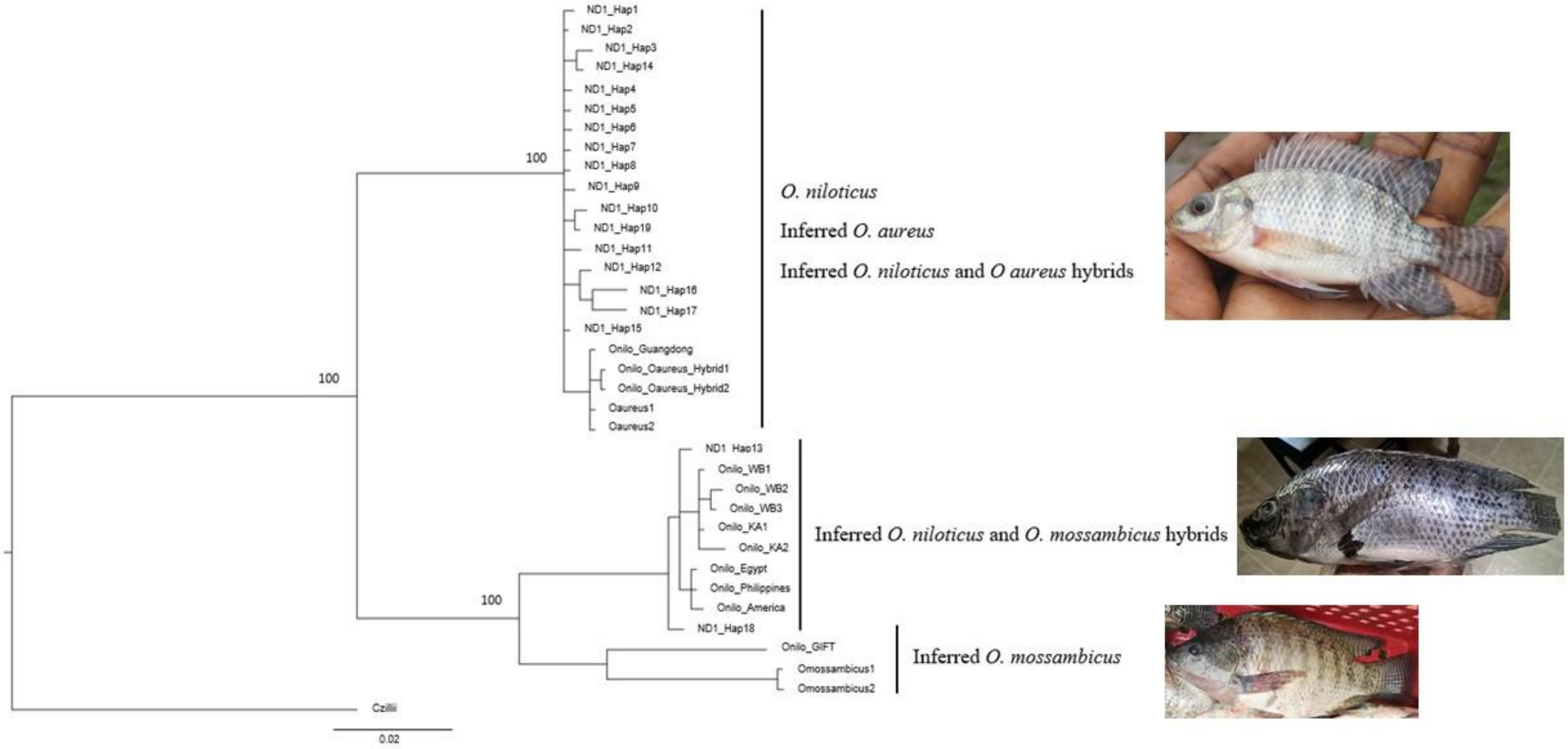

Figure 4. Phylogenetic tree constructed with 675-bp *ND1* gene sequences for *Oreochromis* spp. using Bayesian analysis. The analysis was performed with 1 million Markov chain Monte Carlo generations with four chains, a sample frequency of every 100 generations, and a burn-in of 250,000 generations. The analysis was performed in two runs. A total of 15,000 trees were sampled. The average standard deviation of split frequency was 0.0071 with a -Ln likelihood of -2113.78. Bootstrap support values are indicated to the left of each node. All photos are from this study.

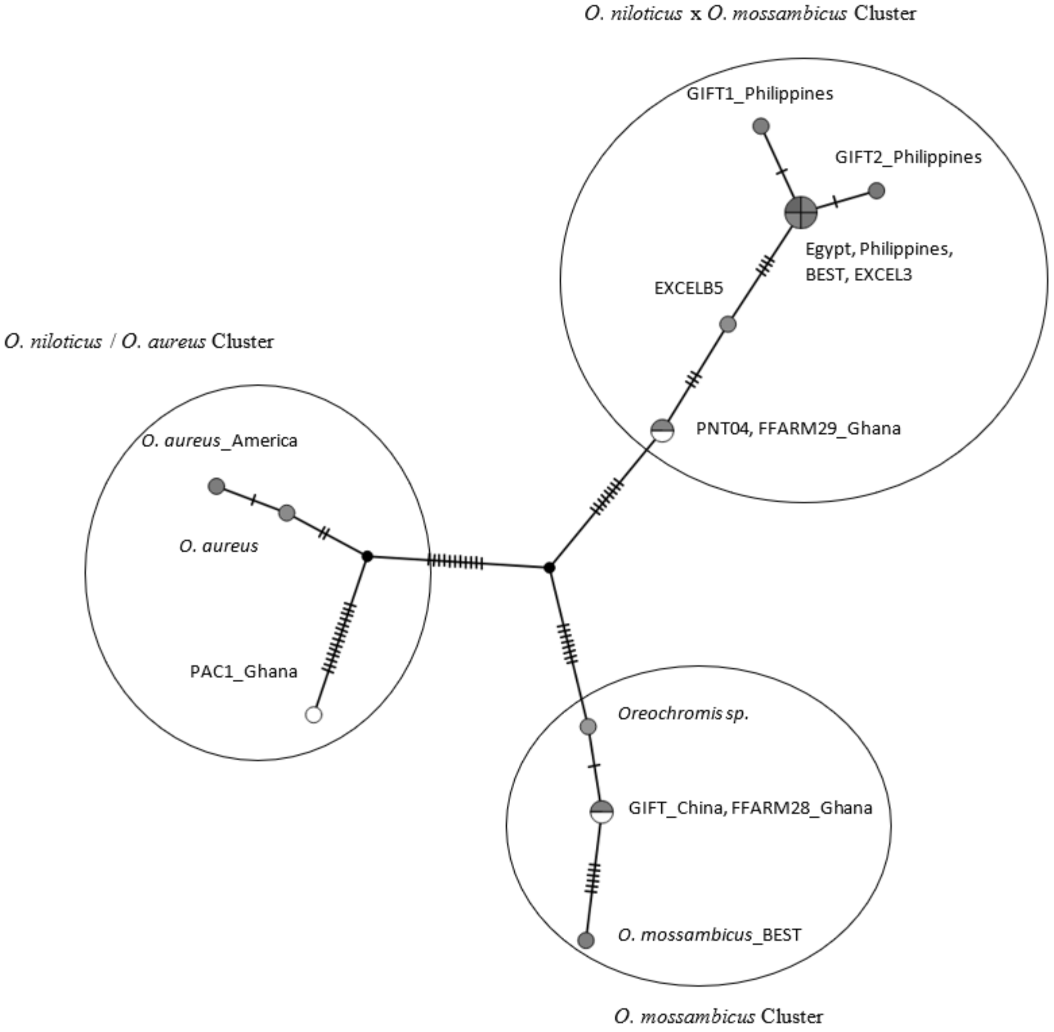

Figure 5. TCS network of three farmed tilapia haplotypes from Ghana (white shading), and the GIFT and related strains of *O. niloticus* (gray shading) observed among 315 bp of the mitochondrial *CO1* gene sequence. PAC1_Ghana (native *O*. *niloticus*), *O. aureus* and *O. mossambicus* sequences are included as reference samples to show the close relations between the native *O*. *niloticus* and *O*. *aureus* populations; and the dissimilarity between *O*. *niloticus* and *O*. *mossambicus*, and the genetically improved strains.

Table 1. Sample and site information for *Oreochromis* spp. collected in Ghana from December 2014 through July 2017.

Sample Code	Site Name	Sampling Location	Latitude	Longitude	Source	*N*
AFRAM	Afram River	Aframso	7.32070	-1.38333	Wild	6
ANKOB	Ankobra River	Ankobra	4.90340	-2.26845	Wild	10
BVKAN	Black Volta	Kantu	9.84236	-2.73142	Wild	7
BVLAW	Black Volta	Lawra**	10.65846	-2.92331	Wild	3
BVTAL	Black Volta	Talewona	9.72958	-2.73142	Wild	6
JUEN	Juen Lagoon	Jehwi-Wharf	5.08845	-2.94539	Wild	5
TANASU	Tano	Asuhyea	7.08884	-2.34260	Wild	5
TANELU	Tano	Elubo	5.29009	-2.75927	Wild	5
WVBIN	White Volta	Binaba	10.83529	-0.53601	Wild	10
WVKUL	White Volta	Kulugu	10.59808	-0.74306	Wild	6
LVOLTA	lower Volta River	Notreku-Akuse	6.11059	-0.10695	Wild	11
OTI	Oti River	Sabare	9.31770	-0.25450	Wild	10
PRA	Pra Pra	Twifo-Praso	5.61004	-1.55516	Wild	9
FFARM	Fujian Farm	Asutuare	6.09807	-0.20962	Farm	9
LFARM	Lee Farm	Akosombo	6.30079	-0.03457	Farm	10
PAC	Pilot Aquaculture Centr	Kona-Odumasi	6.88538	-1.50333	Farm	9
ARDEC	ARDEC*	Akosombo	6.28229	-0.05571	Farm	10
NABFARM	Nabrunye Farm	Nabrunye**	10.64546	-2.88305	Farm	5
ADFARM	Adjei Farm	Elubo	5.28910	-2.75556	Farm	5
NIFARM	Nii Farm	Elubo	5.33118	-2.76083	Farm	5
VCATCH	Volta Catch	Asokwa-Kumasi**	6.66667	-1.60429	Market	8
AKTIL	Akosombo Tilapia	Ashaiman**	5.69251	-0.01278	Market	5
VALUE	Value Concern	Kumasi**	6.68832	-1.61571	Market	6
TILAD	Tilapia Ladies	Kumasi**	6.68832	-1.61571	Market	5
HASSIN	Half-Assini Pub	Half-Assini**	5.04884	-2.87573	Market	4
ECOLD	Elubo Cold Store	Elubo**	5.29075	-2.77833	Market	5
SUBZ	Sub Zero	Kumasi**	6.67330	-1.64088	Market	5

*ARDEC = Aquaculture Research and Development Center

**Approximate locations

Table 2. *Oreochromis* spp. reference samples used in the *D-loop* and *ND1* phylogenetic analysis.

Sample ID	Sampling Location	Country of Origin	GenBank Accession	Mitochondrial DNA Marker	Reference
Oaureus1	Taiwan	Taiwan	EU430994	*D-loop*	GenBank
Oaureus2 / Oaureus1	China	China	GU370125	*D-loop* / *ND1*	He et. 2011
Oaureus3 / Oaureus2	China	China	GU477629	*D-loop* / *ND1*	GenBank
Onilo_Oaureus_Hybrid /Hybrid1	China	China	KM434157	*D-loop* / *ND1*	GenBank
Onilo_Oaureus_Hybrid2	China	China	NC_025669	*ND1*	GenBank
Omossamicus1	Mozambique	Mozambique	AF296466	*D-loop*	Nagl et al. 2001
Omossamicus2	Taiwan	Taiwan	EU430997	*D-loop*	GenBank
Omossamicus3	Mozambique	Mozambique	AF328843	*D-loop*	Nagl et al. 2001
Omossamicus4	Boesmans River	South Africa	AY833436	*D-loop*	D'Amato et al. 2007
Omossamicus5	Sucoma	Malawi	AY833448	*D-loop*	D'Amato et al. 2007
Omossamicus1	Taiwan	Taiwan	AY597335	*ND1*	GenBank
Omossamicus2	Taiwan	Taiwan	NC007231	*ND1*	GenBank
Onilo_Omossam_Hybrid	Olifants River	South Africa	AY833481	*D-loop*	D'Amato et al. 2007
Onilo	China	China	GU238433	*D-loop*	GenBank
Onilo_Guangdong	China	China	GU477627	*ND1*	GenBank
Onilo_GIFT	China	China	GU477624	*D-loop* / *ND1*	GenBank
Onilo_Egypt	China	China	GU477625	*D-loop* / *ND1*	GenBank
Onilo_Philippines	China	China	GU477626	*D-loop* / *ND1*	GenBank
Onilo_America	China	China	GU477628	*D-loop* / *ND1*	GenBank
Onilo_Kpa11	Ghana	Ghana	AF485083	*D-loop*	Falk et al. 2003
Onilo_Senegal	Senegal	Senegal	EF016715	*D-loop*	Nyingi et al. 2009
Onilo_barigo	Kenya	Kenya	AJ237397	*D-loop*	Nyingi et al. 2009
Onilo_eduardianus	Kenya	Kenya	EF016672	*D-loop*	Nyingi et al. 2009
Onilo_Loboi	Kenya	Kenya	FJ440579	*D-loop*	Nyingi et al. 2009
Onilo_vulcani1	Kenya	Kenya	EF016688	*D-loop*	Nyingi et al. 2009
Onilo_vulcani2	Kenya	Kenya	AF296468	*D-loop*	The present study
Onilo_CD	Noe River	Cote d'Ivore	-	*D-loop*	The present study
Onilo_Noe2	Ghana	Cote d'Ivore	-	*D-loop*	The present study
Onilo_Noe3	Ghana	Cote d'Ivore	-	*D-loop*	The present study
Onilo_Noe5	Ghana	Cote d'Ivore	-	*D-loop*	The present study
Onilo_KA1	USA (Kroger)	China	-	*D-loop* / *ND1*	The present study
Onilo_KA2	USA (Kroger)	China	-	*D-loop* / *ND1*	The present study
Onilo_KA3	USA (Kroger)	China	-	*D-loop*	The present study
Onilo_KB1	USA (Kroger)	Ecuador	-	*D-loop*	The present study
Onilo_WA1	USA (Walmart)	China	-	*D-loop*	The present study
Onilo_WA2	USA (Walmart)	China	-	*D-loop*	The present study
Onilo_WB1	USA (Walmart)	China	-	*D-loop* / *ND1*	The present study
Onilo_WB2	USA (Walmart)	China	-	*D-loop* / *ND1*	The present study
Onilo_WB3	USA (Walmart)	China	-	*D-loop* / *ND1*	The present study

Table 3. Variable nucleotide sites for haplotypes at the mitochondrial *D-loop* region for Ghanaian *Oreochromis* spp. Alignment gaps are indicated by “-”. Nucleotides identical to those in the first sequence are indicated with a dot.

			Base Pairs																																																																										
																				1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3
			1	1	1	2	3	5	5	5	6	6	8	8	8	9	9	9	9	0	0	0	0	1	1	1	1	2	2	2	3	3	3	4	5	5	5	5	6	6	7	7	7	7	7	8	8	8	8	8	9	9	9	0	0	0	2	2	3	4	5	5	6	6	6	7	7	7	7	7	9	1	1	1	4	5	5	7	7
Cluster	Haplotype	N	5	6	8	5	0	5	6	9	1	6	6	7	9	1	3	8	9	0	1	6	7	2	3	4	5	1	8	9	1	8	9	1	0	1	2	5	3	5	1	2	4	5	7	2	5	6	7	9	0	3	4	0	6	9	7	9	9	7	6	9	3	5	8	0	1	4	5	8	0	4	7	8	8	4	6	1	6
C1- *O. niloticus*	Dloop_Hap1	7	T	G	A	G	G	C	T	A	C	A	T	T	A	T	C	T	T	A	A	A	G	C	C	C	C	A	A	C	T	T	C	T	C	A	T	A	A	C	A	A	C	T	C	T	-	T	T	G	A	A	A	G	C	T	C	T	C	A	T	A	T	G	T	A	T	T	C	A	G	T	T	A	A	C	T	A	C
	Dloop_Hap2	39	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.
	Dloop_Hap3	56	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Dloop_Hap7	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Dloop_Hap9	9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	-	A	.	T	.	.	G	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
	Dloop_Hap10	5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Dloop_Hap11	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Dloop_Hap14	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Dloop_Hap15	3	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Dloop_Hap26	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Dloop_Hap29	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	G	.	.	.	.	-	A	.	T	.	.	G	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
	Dloop_Hap31	1	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Dloop_Hap32	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Dloop_Hap35	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
	Dloop_Hap36	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Dloop_Hap37	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	-	A	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
	Dloop_Hap38	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	-	A	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
C2 - Inferred *O. aureus*	Dloop_Hap4	11	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.
	Dloop_Hap8	2	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.
	Dloop_Hap12	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.
	Dloop_Hap13	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	G	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.
	Dloop_Hap16	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.
	Dloop_Hap17	1	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	C	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	T	.	.	.	.	.	.	C	.	.	.	A	.	C	.	.	.	.	.	.
	Dloop_Hap18	1	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.
	Dloop_Hap21	1	.	.	.	.	.	T	C	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.
	Dloop_Hap27	1	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	G	.	.
	Dloop_Hap28	2	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	G	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.
	Dloop_Hap30	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.
C3 - *O. niloticus* x *O . mossambicus*	Dloop_Hap5	1	C	A	G	A	A	T	.	T	.	.	.	A	.	C	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	C	T	.	A	G	A	.	.	A	.	.	T	A	T	.	T	A	A	T	.	T	.	.	T	.	T	C	T	.	C	.	C	T	A	T	.	.	A	T	.	C	A	T	.	T	.	T	T
	Dloop_Hap6	3	C	A	G	A	.	T	.	T	.	.	.	A	.	C	.	A	-	.	.	.	C	T	T	T	T	.	T	T	.	C	T	.	A	G	A	.	.	A	.	.	T	A	T	.	C	G	A	T	.	T	.	.	T	.	T	C	T	.	C	.	C	T	A	T	.	.	A	T	.	C	A	T	.	T	.	T	T
	Dloop_Hap19	1	C	A	G	A	A	T	.	T	.	.	.	A	.	C	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	C	T	.	A	G	A	.	.	A	.	.	T	A	T	C	C	A	A	T	.	T	.	.	T	.	T	C	T	.	C	.	C	T	A	T	.	.	A	T	.	C	A	T	.	T	.	T	T
	Dloop_Hap22	1	C	A	G	A	A	T	.	T	.	.	.	A	.	C	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	C	T	.	A	G	A	.	.	A	.	.	T	A	T	.	C	G	A	T	.	T	.	.	T	.	T	C	T	.	C	.	C	T	A	T	.	.	A	T	.	C	A	T	.	T	.	T	T
	Dloop_Hap24	1	C	A	G	A	.	T	.	T	.	G	.	A	.	C	.	A	-	.	.	.	C	G	T	T	T	T	T	T	.	C	G	.	A	G	A	.	.	A	.	.	T	A	T	.	C	G	A	T	.	T	.	.	T	.	T	C	T	.	C	.	C	T	A	T	.	.	A	T	.	C	A	G	.	T	.	T	T
	Dloop_Hap33	4	C	A	G	A	A	T	.	T	.	.	.	A	.	C	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	C	.	.	A	G	A	.	.	A	.	.	T	A	T	.	C	G	A	T	.	T	.	.	T	.	T	C	T	.	C	.	C	T	A	T	.	.	A	T	.	C	A	T	.	T	.	T	T
C4 - *O. mossambicus*	Dloop_Hap20	1	C	A	G	A	.	T	.	.	.	.	.	A	T	.	T	A	-	.	G	C	C	.	T	.	.	.	C	.	A	.	T	C	G	.	A	.	G	A	G	.	.	.	T	A	C	A	.	T	C	.	.	A	.	.	T	C	T	.	C	C	C	T	A	T	.	C	G	T	.	.	C	.	G	.	.	T	T
	Dloop_Hap23	2	C	A	G	A	.	T	.	.	.	.	.	A	T	.	T	A	-	.	G	C	C	.	T	.	.	.	C	.	A	.	T	C	G	.	A	.	G	A	G	.	.	.	T	A	C	A	.	T	C	.	.	A	.	.	T	C	T	.	C	C	C	T	A	T	.	C	G	T	.	.	C	.	G	.	.	T	T
	Dloop_Hap25	1	C	A	G	A	.	T	.	.	.	.	.	A	T	.	T	A	-	.	G	C	C	.	T	.	.	.	C	.	A	.	T	C	G	.	A	.	G	A	G	.	.	.	T	A	C	A	.	T	C	.	.	A	.	.	T	C	T	.	C	C	C	T	A	T	.	C	G	T	.	.	C	.	G	.	.	T	T
	Dloop_Hap34	1	C	A	G	A	.	T	.	.	.	.	.	A	T	.	T	A	-	.	G	C	C	.	T	.	.	.	C	.	A	.	T	C	G	.	A	.	G	A	G	.	.	.	T	A	C	A	.	T	C	.	.	A	.	.	T	C	T	.	C	C	C	T	A	T	.	C	G	T	.	.	C	.	G	.	.	T	T

Table 4. Frequency distribution of *D-loop* haplotypes for all geographic sites, and the corresponding species clusters inferred for Ghanaian *Oreochromis* spp.

			Site Code																										
Cluster	Haplotype	N	AFRAM (6)	ANKOB (10)	BVKAN (7)	BVLAW (3)	BVTAL (6)	JUEN (5)	TANASU (5)	TANELU (5)	WVBIN (10)	WVKUL (6)	LVOLTA (11)	OTI (10)	PRA (9)	FFARM (9)	LFARM (10)	PAC (9)	ARDEC (10)	NABFARM (5)	ADFARM (5)	NIFARM (5)	VCATCH (8)	AKTIL (5)	VALUE (6)	TILAD (5)	HASSIN (4)	ECOLD (5)	SUBZ (5)
C1- *O. niloticus*	Dloop_Hap1	7					1	1	1		2	2																	
	Dloop_Hap2	39		9	1			1			1	2	3		5			3	5	2	1	3			1	1	1		
	Dloop_Hap3	56	1		2	1	1			1	5	2	6	7	3	1	3	3	3	1			6		2	2	1	1	4
	Dloop_Hap7	8								1				1			3								2		1		
	Dloop_Hap9	9				1	2	1	2	2																1			
	Dloop_Hap10	5								1							1		1	1							1		
	Dloop_Hap11	4						1	2													1							
	Dloop_Hap14	3	1											1				1											
	Dloop_Hap15	3			2									1															
	Dloop_Hap26	1														1													
	Dloop_Hap29	1					1																						
	Dloop_Hap31	1				1																							
	Dloop_Hap32	2			2																								
	Dloop_Hap35	1																						1					
	Dloop_Hap36	1	1																										
	Dloop_Hap37	1	1																										
	Dloop_Hap38	2																			2								
C2 - Inferred *O. aureus*	Dloop_Hap4	11									2						1	1			2		2			1		1	1
	Dloop_Hap8	2																						1	1				
	Dloop_Hap12	1													1														
	Dloop_Hap13	1																1											
	Dloop_Hap16	2																		1		1							
	Dloop_Hap17	1															1												
	Dloop_Hap18	1															1												
	Dloop_Hap21	1														1													
	Dloop_Hap27	1																										1	
	Dloop_Hap28	2																										2	
	Dloop_Hap30	1					1																						
C3 - *O. niloticus* x *O* . *mossambicus*	Dloop_Hap5	1											1																
	Dloop_Hap6	3											1			1								1					
	Dloop_Hap19	1						1																					
	Dloop_Hap22	1														1													
	Dloop_Hap24	1														1													
	Dloop_Hap33	4	2	1															1										
C4 - *O. mossambicus*	Dloop_Hap20	1														1													
	Dloop_Hap23	2														1								1					
	Dloop_Hap25	1														1													
	Dloop_Hap34	1																						1					

Table 5. Pairwise nucleotide uncorrected *p*-distances between haplotypes[1] at the mitochondrial *D-loop* region for Ghanaian *Oreochromis* spp. Representative samples for the haplotypes are in parentheses.

	Hap1	Hap2	Hap3	Hap4	Hap5	Hap6	Hap8	Hap10	Hap19	Hap20	Hap22	Hap23	Hap24	Hap25	Hap33	Hap34
Hap1 (WVBIN)	0.001															
Hap2 (WVBIN)	0.015	0.002														
Hap3 (WVBIN)	0.005	0.015	0.000													
Hap4 (WVBIN)	0.386	0.381	0.386	0.001												
Hap5 (LVOLTA)	0.569	0.569	0.566	0.559	N = 1											
Hap6 (LVOLTA)	0.569	0.569	0.566	0.559	0.010	0.000										
Hap8 (VALUE)	0.386	0.381	0.386	0.005	0.554	0.554	0.000									
Hap10 (TANELU)	0.005	0.015	0.000	0.386	0.566	0.566	0.386	0.000								
Hap19 (JUEN)	0.569	0.569	0.566	0.559	0.005	0.010	0.554	0.566	N = 1							
Hap20 (FFARM)	0.706	0.706	0.706	0.701	0.672	0.675	0.698	0.706	0.675	N = 1						
Hap22 (FFARM)	0.571	0.571	0.569	0.561	0.005	0.005	0.556	0.569	0.005	0.675	N = 1					
Hap23 (FFARM)	0.706	0.706	0.706	0.701	0.672	0.675	0.698	0.706	0.675	0.000	0.675	0.000				
Hap24 (FFARM)	0.574	0.574	0.571	0.561	0.023	0.013	0.556	0.571	0.023	0.675	0.018	0.675	N = 1			
Hap25 (FFARM)	0.658	0.661	0.664	0.677	0.651	0.656	0.677	0.664	0.651	0.611	0.653	0.611	0.658	N = 1		
Hap33 (ARDEC)	0.574	0.574	0.571	0.564	0.008	0.008	0.559	0.571	0.008	0.672	0.003	0.672	0.018	0.653	0.000	
Hap34 (AKTIL)	0.706	0.706	0.706	0.701	0.672	0.675	0.698	0.706	0.675	0.000	0.675	0.000	0.675	0.611	0.672	N = 1

[1] The *O. niloticus* cluster is represented by haplotypes 1, 2, 3, and 10; the *O. aureus* cluster by haplotypes 4 and 8; and the *O. niloticus* x *O. mossambicus* cluster haplotypes 5, 6, 19, 22, 24, and 33. *O. mossambicus* is represented by haplotypes 20, 23, 25, and 34. WVBIN = White Volta River-Binaba, LVOLTA = Lower Volta River, VALUE = Value Concern, TANELU = Tano River-Elubo, JUEN = Juen Lagoon, FFARM = Fujian Farm, ARDEC = Aquaculture Research and Development Centre, AKTIL = Akosombo Tilapia Farm. Positions containing gaps and missing data were eliminated. Interspecific distances were not calculated for haplotypes with only one sample and are indicated with *N* = 1.

Table 6. Variable nucleotide sites for haplotypes at the mitochondrial *ND1* gene for Ghanaian *Oreochromis* spp. Alignment gaps are indicated by “-”. Nucleotides identical to those in the first sequence are indicated with a dot.

			Base Pairs																																																																					
Cluster	Haplotype	N	4	8	10	11	16	61	67	82	88	91	97	109	118	145	169	178	184	187	220	222	224	225	226	227	228	229	232	241	253	265	268	277	280	287	289	292	301	302	313	314	317	325	358	367	376	388	394	424	433	445	448	457	469	509	520	529	536	548	580	589	592	595	610	634	637	640	643	646	656	664	674	675
C1- *O. niloticus / O. aureus / Hybrids*	ND1_Hap1	2	T	A	C	C	A	C	C	C	C	C	C	C	T	A	T	C	C	C	A	G	T	T	A	G	G	G	C	A	T	C	C	A	A	G	C	C	C	A	C	T	C	G	C	A	C	C	G	C	C	C	C	C	G	T	C	C	T	G	T	C	C	C	C	C	T	A	C	A	T	C	A	A
	ND1_Hap2	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	ND1_Hap3	1	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	C	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	ND1_Hap4	5	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	ND1_Hap5	3	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	ND1_Hap6	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	ND1_Hap7	1	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	ND1_Hap8	1	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	ND1_Hap9	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	ND1_Hap10	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	ND1_Hap11	1	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	ND1_Hap12	1	.	C	A	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
	ND1_Hap14	1	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	ND1_Hap15	5	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	ND1_Hap16	1	.	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	C	C	.	.	C	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.
	ND1_Hap17	1	.	.	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.
	ND1_Hap19	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C2 - *O. niloticus* x *O. mossambicus*	ND1_Hap13	1	C	.	.	.	G	T	T	.	T	T	T	T	C	G	C	T	T	T	C	.	C	.	.	.	.	A	A	T	C	T	T	G	C	A	T	T	T	G	.	C	.	A	T	T	T	T	A	T	T	T	T	T	A	C	T	T	A	A	C	T	T	T	A	T	C	G	T	G	C	.	T	G
	ND1_Hap18	1	C	.	.	.	G	T	T	.	T	T	T	T	C	G	C	T	T	T	C	.	C	.	.	.	.	A	A	T	C	T	T	G	C	A	T	T	T	G	.	C	.	A	T	T	T	T	A	T	T	T	T	T	A	C	T	T	A	A	C	T	T	T	A	T	C	G	T	G	.	.	G	.

Table 7. Frequency distribution of *ND1* haplotypes for all geographic sites, and the corresponding species and inferred clusters for Ghanaian *Oreochromis* spp.

			Site Code													
Cluster	Haplotype	N	AFRAM (3)	ANKOB (3)	BVKAN (2)	BVLAW (2)	BVTAL (2)	TANASU (2)	TANELU (3)	WVBIN (5)	WVKUL (2)	LVOLTA (4)	OTI (4)	PRA (3)	FFARM (2)	PAC (2)
C1- *O. niloticus / O. aureus / Hybrids*	ND1_Hap1	2	1							1						
	ND1_Hap2	4	1				1						1	1		
	ND1_Hap3	1	1													
	ND1_Hap4	5		2	1							1		1		
	ND1_Hap5	3		1		1					1					
	ND1_Hap6	6			1			1	2			1	1			
	ND1_Hap7	1						1								
	ND1_Hap8	1								1						
	ND1_Hap9	2								1	1					
	ND1_Hap10	1								1						
	ND1_Hap11	1								1						
	ND1_Hap14	1				1										
	ND1_Hap15	5					1		1			1	1	1		
	ND1_Hap19	1											1			
	ND1_Hap12	1													1	
	ND1_Hap16	1													1	
	ND1_Hap17	1													1	
C2 - *O. niloticus* x *O. mossambicus*	ND1_Hap13	1													1	
	ND1_Hap18	1										1				

Table 8. Pairwise nucleotide distances between haplotypes at the mitochondrial *ND1* gene for Ghanaian *Oreochromis* spp.

	Hap1	Hap2	Hap3	Hap4	Hap5	Hap6	Hap7	Hap8	Hap9	Hap10	Hap11	Hap12	Hap13	Hap14	Hap15	Hap16	Hap17	Hap18	Hap19
Hap1	0.000																		
Hap2	0.001	0.000																	
Hap3	0.009	0.010	N = 1																
Hap4	0.004	0.003	0.010	0.000															
Hap5	0.004	0.003	0.007	0.003	0.000														
Hap6	0.003	0.001	0.012	0.001	0.004	0.000													
Hap7	0.006	0.004	0.009	0.001	0.001	0.003	N =1												
Hap8	0.003	0.004	0.006	0.004	0.001	0.006	0.003	N = 1											
Hap9	0.004	0.003	0.010	0.003	0.003	0.001	0.001	0.004	0.000										
Hap10	0.003	0.004	0.009	0.007	0.004	0.006	0.006	0.003	0.004	N = 1									
Hap11	0.006	0.007	0.006	0.004	0.004	0.006	0.003	0.003	0.004	0.006	N = 1								
Hap12	0.007	0.009	0.011	0.009	0.006	0.011	0.008	0.004	0.009	0.008	0.008	N = 1							
Hap13	0.098	0.100	0.105	0.100	0.100	0.098	0.098	0.098	0.096	0.098	0.098	0.100	N = 1						
Hap14	0.004	0.006	0.004	0.006	0.003	0.007	0.004	0.001	0.006	0.004	0.004	0.006	0.100	N = 1					
Hap15	0.003	0.001	0.009	0.001	0.001	0.003	0.003	0.003	0.004	0.006	0.006	0.007	0.101	0.004	0.000				
Hap16	0.013	0.015	0.010	0.012	0.012	0.013	0.010	0.010	0.012	0.013	0.007	0.009	0.099	0.012	0.013	N = 1			
Hap17	0.014	0.015	0.008	0.015	0.012	0.017	0.014	0.011	0.015	0.014	0.014	0.009	0.106	0.009	0.014	0.012	N = 1		
Hap18	0.094	0.096	0.101	0.096	0.096	0.094	0.094	0.094	0.092	0.094	0.094	0.098	0.004	0.095	0.097	0.097	0.104	N = 1	
Hap19	0.001	0.003	0.007	0.006	0.003	0.004	0.004	0.001	0.003	0.001	0.004	0.006	0.096	0.003	0.004	0.012	0.012	0.092	N = 1

The variation among sites was modeled with the best substitution model of evolution, Hasegawa-Kishino-Yano (HKY). Positions containing gaps and missing data were eliminated. Intraspecific genetic distances are indicated in the diagonal. Interspecific distances were not calculated for haplotypes with only one sample and are indicated with *N* = 1.

Table S1. Variable nucleotide sites for *Oreochromis* spp. haplotypes at the mitochondrial *D-loop* region and the reference sequences used for the phylogenetic analysis. Alignment gaps are indicated by "-". Nucleotides identical to those in the first sequence are indicated with a dot.

		Base Pairs																																																																																			
																					1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3
		1	1	1	2	2	3	5	5	5	6	6	8	8	8	9	9	9	9	9	0	0	0	0	1	1	1	1	2	2	2	3	3	3	3	4	5	5	5	5	6	6	6	7	7	7	7	7	7	8	8	8	8	8	8	9	9	9	0	0	0	2	2	2	3	4	5	5	6	6	6	7	7	7	7	7	7	9	1	1	1	4	5	5	7	7
Haplotype	N	5	6	8	5	8	0	5	6	9	1	6	6	7	9	1	3	4	8	9	0	1	6	7	2	3	4	5	1	8	9	1	5	8	9	1	0	1	2	5	3	5	8	1	2	4	5	6	7	1	2	5	6	7	9	0	3	4	0	6	9	1	7	9	9	7	6	9	3	5	8	0	1	3	4	5	8	0	4	7	8	8	4	6	1	6
Dloop Hap1	7	T	G	A	G	A	G	C	T	A	C	A	T	T	A	T	C	T	T	T	A	A	A	G	C	C	C	C	A	A	C	T	C	T	C	T	C	A	T	A	A	C	C	A	A	C	T	T	C	A	T	-	T	T	G	A	A	A	G	C	T	C	C	T	C	A	T	A	T	G	T	A	T	C	T	C	A	G	T	T	A	A	C	T	A	C
Dloop Hap2	39	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.
Dloop Hap3	56	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Dloop Hap4	11	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.
Dloop Hap5	1	C	A	G	A	.	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	.	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	.	T	A	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	T	T
Dloop Hap6	3	C	A	G	A	.	.	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	T	.	.	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	.	C	G	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	T	T
Dloop Hap7	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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Dloop Hap9	9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	-	.	.	G	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
Dloop Hap10	5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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Dloop Hap12	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.
Dloop Hap13	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	G	.	T	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.
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Dloop Hap18	1	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.
Dloop Hap19	1	C	A	G	A	.	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	.	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	C	C	A	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	T	T
Dloop Hap20	1	C	A	G	A	.	.	T	.	.	.	.	.	A	T	.	T	.	A	-	.	G	C	C	.	T	.	.	.	C	.	A	.	.	T	C	G	.	A	.	G	-	A	G	.	.	.	.	T	.	A	C	A	.	T	C	.	.	A	.	.	.	T	C	T	.	C	C	C	T	A	T	.	.	C	G	T	.	.	C	.	G	.	.	T	T
Dloop Hap21	1	.	.	.	.	.	.	T	C	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.
Dloop Hap22	1	C	A	G	A	.	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	.	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	.	C	G	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	T	T
Dloop Hap23	2	C	A	G	A	.	.	T	.	.	.	.	.	A	T	.	T	.	A	-	.	G	C	C	.	T	.	.	.	C	.	A	.	.	T	C	G	.	A	.	G	-	A	G	.	.	.	.	T	.	A	C	A	.	T	C	.	.	A	.	.	.	T	C	T	.	C	C	C	T	A	T	.	.	C	G	T	.	.	C	.	G	.	.	T	T
Dloop Hap24	1	C	A	G	A	.	.	T	.	T	.	G	.	A	.	C	.	.	A	-	.	.	.	C	G	T	T	T	T	T	T	.	.	C	G	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	.	C	G	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	G	.	T	.	T	T
Dloop Hap25	1	C	A	G	A	.	.	T	.	.	.	.	.	A	T	.	T	.	A	-	.	G	C	C	.	T	.	.	.	C	.	A	.	.	T	C	G	.	A	.	G	-	A	G	.	.	.	.	T	.	A	C	A	.	T	C	.	.	A	.	.	.	T	C	T	.	C	C	C	T	A	T	.	.	C	G	T	.	.	C	.	G	.	.	T	T
Dloop Hap26	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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Dloop Hap33	4	C	A	G	A	.	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	.	C	.	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	.	C	G	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	T	T
Dloop Hap34	1	C	A	G	A	.	.	T	.	.	.	.	.	A	T	.	T	.	A	-	.	G	C	C	.	T	.	.	.	C	.	A	.	.	T	C	G	.	A	.	G	-	A	G	.	.	.	.	T	.	A	C	A	.	T	C	.	.	A	.	.	.	T	C	T	.	C	C	C	T	A	T	.	.	C	G	T	.	.	C	.	G	.	.	T	T
Dloop Hap35	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.
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Omossambicus1	1	C	A	G	A	.	.	T	.	.	.	.	.	A	T	.	T	.	A	-	.	G	C	C	.	T	.	.	.	C	.	A	.	.	T	C	G	.	A	.	G	-	A	G	.	.	.	.	T	.	A	C	A	.	T	C	.	.	A	.	.	.	T	C	T	.	C	C	C	T	A	T	.	.	C	G	T	.	.	C	.	G	.	.	T	T
Omossambicus2	1	C	A	G	A	.	.	T	.	.	.	.	.	A	T	.	T	.	A	-	.	G	C	C	.	T	.	.	.	C	.	A	.	.	T	C	G	.	A	.	G	-	A	G	.	.	.	.	T	.	A	C	A	.	T	C	.	.	A	.	.	.	T	C	T	.	C	C	C	T	A	T	.	.	C	G	T	.	.	C	.	G	.	.	T	T
Omossambicus3	1	C	A	G	A	.	.	T	.	.	.	.	.	A	C	.	T	.	A	-	.	.	C	C	.	T	.	.	.	C	.	A	.	.	T	C	A	.	A	.	G	-	A	.	.	T	C	C	T	.	A	C	A	.	T	C	.	.	A	.	.	T	T	C	T	.	C	C	C	T	A	T	.	T	C	G	T	.	.	.	.	.	T	.	T	.
Omossambicus4	1	C	A	G	A	.	.	T	.	.	.	.	.	A	T	.	T	C	A	-	.	.	C	C	.	T	.	.	.	C	.	A	.	.	T	C	G	.	A	.	G	-	G	.	.	T	C	.	T	.	A	C	A	.	T	C	.	.	A	.	.	.	T	C	T	.	C	C	C	T	A	T	.	.	C	A	T	.	.	.	.	.	T	.	T	.
Omossambicus5	1	C	A	G	A	.	.	T	.	.	.	.	.	A	T	.	T	.	A	-	.	G	C	C	.	T	.	.	.	C	.	A	.	.	T	C	G	.	A	.	G	-	A	G	.	.	.	.	T	.	A	C	A	.	T	C	.	.	A	.	.	.	T	C	T	.	C	C	C	T	A	T	.	.	C	G	T	.	.	.	.	G	.	.	T	T
Onilo	1	C	A	G	A	.	.	T	.	.	.	.	.	A	T	.	T	.	A	-	.	G	C	C	.	T	.	.	.	C	.	A	.	.	T	C	G	.	A	.	G	-	A	G	.	.	.	.	T	.	A	C	A	.	T	C	.	.	A	.	.	.	T	C	T	.	C	C	C	T	A	T	.	.	C	G	T	.	.	C	.	G	.	.	T	T
Onilo America	1	C	A	G	A	.	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	.	C	.	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	.	C	G	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	T	T
Onilo baringo	1	C	A	G	A	.	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	T	.	.	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	.	C	G	A	T	.	T	.	.	.	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	G	T	.	T	.	-	-
Onilo CD	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Onilo eduardianus	1	C	A	G	A	.	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	.	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	C	C	A	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	-	-
Onilo Egypt	1	C	A	G	A	.	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	.	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	C	C	A	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	T	T
Onilo GIFT	1	C	A	G	A	.	.	T	.	.	.	.	.	A	T	.	T	.	A	-	.	G	C	C	.	T	.	.	.	C	.	A	.	.	T	C	G	.	A	.	G	-	A	G	.	.	.	.	T	.	A	C	A	.	T	C	.	.	A	.	.	.	T	C	T	.	C	C	C	T	A	T	.	.	C	G	T	.	.	C	.	G	.	.	T	T

Table S1. Continued.

		Base Pairs																																																																																				
Haplotype	N	15	16	18	25	28	30	55	56	59	61	66	86	87	89	91	93	94	98	99	100	101	106	107	112	113	114	115	121	128	129	131	135	138	139	141	150	151	152	155	163	165	168	171	172	174	175	176	177	181	182	185	186	187	189	190	193	194	200	206	209	221	227	229	239	247	256	259	263	265	268	270	271	273	274	275	278	290	304	317	318	348	354	356	371	376
Onilo KA1	1	C	A	G	A	.	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	.	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	.	C	G	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	T	T
Onilo KA2	1	C	A	G	A	.	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	.	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	.	C	G	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	T	T
Onilo KA3	1	C	A	G	A	.	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	.	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	.	C	G	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	T	T
Onilo KB1	1	C	A	G	A	.	.	T	.	.	.	.	.	A	T	.	T	.	A	-	.	G	C	C	.	T	.	.	.	C	.	A	.	.	T	C	G	.	A	.	G	-	A	G	.	.	.	.	T	.	A	C	A	.	T	C	.	.	A	.	.	.	T	C	T	.	C	C	C	T	A	T	.	.	C	G	T	.	.	C	.	G	.	.	T	T
Onilo Kpa11	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Onilo Loboi	1	C	A	G	A	.	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	.	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	C	C	A	A	T	.	T	.	.	T	.	.	T	C	T	.	C	G	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	-	-
Onilo Noe2	1	C	A	G	A	.	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	.	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	.	C	G	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	T	T
Onilo Noe3	1	C	A	G	A	.	.	T	.	.	.	.	.	A	T	.	T	.	A	-	.	G	C	C	.	T	.	.	.	C	.	A	.	.	T	C	G	.	A	.	G	-	A	G	.	.	.	.	T	.	A	C	A	.	T	C	.	.	A	.	.	.	T	C	T	.	C	C	C	T	A	T	.	.	C	G	T	.	.	C	.	G	.	.	T	T
Onilo Noe5	1	C	A	G	A	.	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	.	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	.	C	G	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	T	T
Onilo Oaureus Hybrid	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Onilo Omossam Hybrid	1	C	A	G	A	.	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	T	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	.	C	G	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	T	T
Onilo Philippines	1	C	A	G	A	.	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	.	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	.	C	G	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	T	T
Onilo Senegal	1	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	-	-
Onilo vulcani1	1	C	A	G	A	.	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	.	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	.	C	G	A	T	.	T	.	.	T	.	.	.	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	-	-
Onilo vulcani2	1	C	A	G	A	G	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	.	T	.	T	.	.	.	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	.	C	A	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	-	-
Onilo WA1	1	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.
Onilo WA2	1	C	A	G	A	.	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	.	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	.	C	G	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	T	T
Onilo WB1	1	C	A	G	A	G	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	.	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	.	C	A	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	T	T
Onilo WB2	1	C	A	G	A	G	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	.	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	.	C	A	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	T	T
Onilo WB3	1	C	A	G	A	G	A	T	.	T	.	.	.	A	.	C	.	.	A	-	.	.	.	C	T	T	T	T	.	T	.	.	.	C	T	.	A	G	A	.	.	-	A	.	.	T	A	.	T	.	.	C	A	A	T	.	T	.	.	T	.	.	T	C	T	.	C	.	C	T	A	T	.	.	.	A	T	.	C	A	T	.	T	.	T	T

Chapter 3: Recent genetic bottlenecks within wild tilapia populations in Ghana and the risk of admixture with non-native farmed strains

Abstract

Despite the global importance of the Nile tilapia *Oreochromis niloticus*, especially to aquaculture, knowledge of genetic variability within native populations is still limited. Many native populations of *O. niloticus* face multiple threats, including habitat destruction and hybridization with closely related species. The purpose of this study was to: 1) assess genetic variation in *O. niloticus* within and among drainage basins in Ghana using nuclear microsatellite DNA markers, and 2) assess the genetic impact of aquaculture on wild populations focusing on selected farms on the Volta Lake in Ghana. I used eight nuclear microsatellite DNA markers to screen 452 wild and farmed tilapia collected from nine river basins and five aquaculture facilities. I found moderate to high genetic diversity within and differentiation among wild populations, with strong signals of recent demographic bottlenecks in several populations. I also found significant genetic structure among the populations, suggesting the presence of up to ten management units (MUs) within the 11 wild populations studied. In particular, the Black Volta and the Tano-Asuhyea populations were the most genetically distinct and geographically isolated, and may be most at risk of loss of genetic diversity over time. Therefore, at the minimum, the Black Volta and Tano-Asuhyea populations should be prioritized for conservation and designated as MUs to sustain them over the long-term. Genetic variability among farmed populations revealed that populations from two farms were distinct from the native Akosombo strain and suggested that those farms cultured non-native tilapia strains. Observation of admixed individuals in the Lower Volta population confirmed earlier findings that some non-native farmed tilapias have escaped into the wild and bred with local populations. Potentially admixed individuals also were found in the three other farmed populations, including the Akosombo strain. Timely management decisions to reduce the further spread of non-native farmed tilapia populations should include the suspension of the reservoir stocking program planned for northern Ghana because *O. niloticus* populations in the Black Volta, White Volta, and the Oti are potentially unaffected by admixture with non-native strains.

Introduction

The Nile tilapia (*Oreochromis niloticus*) is the second most cultured species worldwide, with 2016 production of 7.9 million metric tons (FAO 2018). Given the importance of *O. niloticus* to aquaculture and commercial fisheries, particularly in Africa, it is surprising that our knowledge of genetic diversity within the species is still rather limited. Most research has focused on characterizing aquaculture performance differences among geographically defined strains (Eknath et al. 1993, Eknath et al. 1998, Falk and Abban 2004), with comparatively little attention to population genetic differentiation in the wild.

While several early studies attempted to cover all major drainage basins in Africa where *O. niloticus* occurs (Rognon et al. 1996, Agnèse et al. 1997, Bezault et al. 2011), relatively little effort has focused on characterizing genetic differentiation at finer scales. Further, while past studies have shown regional genetic differences among populations, sampling effort across drainages often has been unequal. The outcome is disparate sample sizes for analysis among basins, complicating generalization across basins. Thus, when populations from one region are considered collectively as, for example, as “West African” populations, inferences could be misleading, especially in cases when entire drainages are not sampled (Rognon et al. 1996). Regional clustering of populations also tends to have limited utility when the focus is to identify populations that may be demographically isolated from other populations (as management units, or MUs; Moritz 1994), or populations that are genetically distinct, have unique adaptations, and require conservation management (evolutionarily significant units, or ESUs; Waples 1991).

With the availability of microsatellite DNA markers for tilapias (Lee and Kocher 1996, Carleton et al. 2002, Streelman and Kocher 2002), we are able to quantify genetic differentiation among *O. niloticus* populations, building upon earlier studies that relied on allozymes or mitochondrial DNA (Rognon et al. 1996; Agnèse et al. 1997; Vreven et al. 1998; Rognon and Guyomard 2003) That is, our understanding of differentiation among *O. niloticus* populations can be advanced by conducting drainage-scale, local and regional studies, which incorporate multiple, highly variable molecular markers (D’Amato et al. 2007; Ndiwa et al. 2014).

Frimpong et al. (2016) showed the importance of fine-scale population genetic studies within individual drainage basins as opposed to non-systematic sampling among multiple large rivers. The authors screened five microsatellite loci among collections of *O. niloticus* from three major tributaries of the Volta River system within Ghana (the Oti, White Volta, and Afram rivers). Their analysis revealed that the three wild populations showed moderate levels of genetic differentiation (Afram and White Volta, $F_{ST} = 0.049$; Afram and Oti, $F_{ST} = 0.027$; and White Volta and Oti, $F_{ST} = 0.054$), suggesting that widening the sampling effort to include multiple sites within different rivers would reveal patterns of significant genetic variation within and among populations. In Ghana, the largest populations of *O. niloticus* occur within the Volta system, which drains about 70% of the country. However, *O. niloticus* also occurs in almost all Ghanaian rivers, including also the Pra, Densu, Ankobra and Bia systems (Ansah 2010, Dunz and Schliewen 2010, Kuranchie-Mensah et al. 2013). No population genetics studies of *O. niloticus* outside of the Volta system in Ghana have been conducted.

Given the considerable historical connectivity apparent within the Volta River basin (McCartney et al. 2012), and the moderate genetic differentiation observed among *O. niloticus* populations from the Oti, White Volta, and Afram rivers, we expect genetic differentiation among populations among different river basins in Ghana. Such differentiation may be the result of both non-selective and adaptive population genetic processes. Many rivers in West Africa are stressed from increased anthropogenic activities, such as overfishing, alluvial mining, water abstraction, and deforestation within the catchment and riparian zones, which pose significant threats to aquatic life. Without timely intervention, uncharacterized populations of *O. niloticus* could become extirpated, posing loss of valuable genetic resources.

Understanding the genetic variability within and among populations is vital for promoting long-term persistence of the species, especially because of growing threats to their persistence in the wild. Rognon and Guyomard (2003) reported widespread genetic introgression by *O. aureus* into several West African *O. niloticus* populations. Similar results were reported for wild tilapia samples collected in Ghana (Chapter 2). However, pure populations of *O. niloticus* persist in Ghanaian rivers. Therefore, it is important that we assess population genetic data to inform management and conservation planning. Analysis of mitochondrial DNA sequence data showed high genetic diversity among *O. niloticus* populations across nine drainage basins and also revealed the presence of non-native tilapia strains on some aquaculture operations, from which some stocks have escaped into the wild and interbred with native populations (Chapter 2). However, the extent of mixing of farmed and wild populations is unknown, and it is vital to obtain baseline information on the genetic diversity among natural populations of *O. niloticus* in aquaculture receiving streams for purposes of ongoing monitoring of aquaculture impacts on aquatic ecosystems in Ghana.

To complement the results from the phylogenetic analysis, the purposes of this study were to: 1) assess population genetic variation in *O. niloticus* within and among drainage basins in Ghana using nuclear microsatellite DNA, and 2) assess the extent of genetic impact of aquaculture on wild populations using selected farms operating on the Volta Lake in Ghana.

Methods

Populations sampled

A total of 452 individual fish - 312 wild and 140 farmed fish - were analyzed in this study. The wild populations were sampled from eight major rivers and one coastal lagoon in Ghana: Afram River, Oti River, White Volta River (two sites), Black Volta River (three sites), Pra River, Tano River (two sites), Ankobra River, Lower Volta River, and Juen Lagoon (Table 1). Due to small sample sizes in two Black Volta sites (Talewona and Lawra), all Black Volta individuals were combined and analyzed as one population. To assess the genetic impact of aquaculture on native populations, samples were collected from five major aquaculture facilities operating on the Volta Lake, and also samples from the Aquaculture Research and Development Centre (ARDEC), the government hatchery responsible for the development and dissemination of the native Ghanaian Akosombo strain of *O. niloticus*.

Polymerase chain reaction and genotyping

I genotyped individual fish from all sites using polymerase chain reaction (PCR) with ten microsatellite DNA markers designed for *O. niloticus*; *UNH123, UNH130, UNH178, UNH180, UNH203, UNH858, UNH898, UNH925*, *UNH934,* and *UNH991* (Carleton et al. 2002; Streelman and Kocher 2002). These markers have shown high allelic variation in previous studies (Streelman and Kocher 2002, Cnaani et al. 2003, Frimpong et al. 2016), and were screened for polymorphism in our populations prior to including them in the study.

The 11-µl PCR amplification reactions consisted of 50-100 ng of genomic DNA, 5 U/µl *Taq* DNA polymerase, 5x PCR buffer, 25mM $MgCl_2$, 2.5 mM dNTP mix, 5 µM bovine serum albumin, and 5 µM of primers. The following thermal cycling conditions were used: 94^{o}C for 3 min; 35 cycles of 94^{o}C for 40 sec, 54-58^{o}C depending on marker for 30 sec, and 72^{o}C for 1 min;

a final extension of 72°C for 5 min; and a 4°C hold. Technical information for the respective microsatellite markers is presented in Table 2. PCR products were visualized using agarose gel electrophoresis to confirm amplification and amplicon sizes prior to genotyping with an ABI3730 automated DNA sequencer at the Virginia Tech Biocomplexity Institute (Blacksburg, VA) and Cornell University (Ithaca, NY). Amplification products were visualized with GeneMarker version 2.6.4 and scored by eye.

Data analysis

I used the program MICROCHECKER (Van Oosterhout et al. 2004) to check for genotyping errors in the data set resulting from null alleles, false peaks, and short-allele dominance using a Monte Carlo simulation of differences in the expected allele size. I screened populations for linkage disequilibrium (LD) between all loci pairs (Sokal and Rohlf 1994) and for Hardy-Weinberg equilibrium (HWE) deviations at each locus implemented in the program ARLEQUIN, version 3.1 (Excoffier et al. 2005). I determined the HWE significance using the Fisher's exact test with a Markov chain of 1,000,000 steps and 100,000 dememorization steps (Guo and Thompson 1992) and LD pairwise tests' significance using the likelihood-ratio test with 10,000 permutations (Slatkin and Excoffier 1996). I then used the sequential Bonferroni correction, an approach used to account for possible Type 1 errors associated with multiple pairwise evaluations (Rice 1989).

I quantified genetic variation across the ten microsatellite loci for both the wild and farmed tilapia populations using number of alleles per locus, observed and expected heterozygosities, allele frequencies at each locus, and number of private alleles. I used the Garza-Williamson Index (*M*-Ratio, the ratio of allelic richness to allelic range, the latter being the difference between the largest allele and the smallest allele per locus, Garza and Williamson 2001) to assess random genetic drift, and F_{IS} inbreeding coefficients to assess inbreeding within populations. I also estimated effective population size (N_e) using the program NeEstimator version 2.1 (Do et al. 2014), and bottleneck using the program BOTTLENECK (Cornuet and Luikart 1996).

I quantified population differentiation and structure using multiple methods. I used the program ARLEQUIN to calculate the F_{ST} (Wright 1978) metric of differentiation and perform analysis of molecular variance (AMOVA). I also calculated locus-by-locus genic differentiation using the Fisher's exact *G* test executed in Genepop on the web version 4.2 (available at: http://genepop.curtin.edu.au/genepop_op3.html; Raymond and Rousset 1995). I also calculated locus-by-locus F_{ST} estimates in ARLEQUIN. I further estimated $G_{ST'}$, a standardized measure of differentiation developed by Hendrick (2005); and Jost' *D* (Jost 2008) an actual measure of differentiation, i.e., metrics which often estimate differentiation more accurately than F_{ST}. Both $G_{ST'}$ and *D* were calculated using the program POPTREE2 (Takezaki et al. 2010). I then compared the locus-by-locus F_{ST} estimates to the Fisher's exact *G* test, $G_{ST'}$ and *D* estimates.

I assessed population structure among wild populations and the genetic impact of farmed populations on wild populations using the program STRUCTURE version 2.3.4 (Pritchard et al. 2000). I used both the admixture and no-admixture models to assess underlying patterns in the structure of the wild populations, but I used only admixture models for assessing the extent of mixing of farmed populations with wild populations. I executed the models with 100,000 burn-in and 500,000 MCMC replicates with the ancestry correlated option using default settings. I set the number of clusters *K* sequentially from 1 to 11 populations for the wild populations. I selected the Black Volta and Lower Volta populations as the upstream- and downstream-most reference populations for assessing introgression from the five populations from farms currently operating on the Volta Lake. Consequently, I set $K = 1$ to 7 for analyses using each farmed population. The most likely *K* value was selected using the Evanno et al. (2005) and Pritchard and Wen (2003) methods implemented online in STRUCTURE HARVESTER (Earl and vonHoldt 2012). I constructed an unrooted phylogenetic tree using the nuclear microsatellite DNA data to further visualize the relationships among populations using the program POPTREE2 (Takezaki et al. 2010). I also tested for the likelihood of isolation by distance (IBD), that is, whether genetic dissimilarity among populations increased with increasing geographic distances among sites. I performed a Mantel test to assess IBD using Genepop on the web (Raymond and Rousset 1995) and R© statistical software version 3.5.1 (R Core Team 2018). The general equation for a Mantel test statistic is:

$$Zm = \sum_{i=1}^{n} \sum_{j=1}^{n} gij \text{ x } dij$$

where g_{ij} and d_{ij} are the genetic and geographic distances, respectively, between populations *i* and *j*, given *n* populations (Mantel 1967). For genetic distances, I used pairwise F_{ST} values transformed as $F_{ST}(1-F_{ST})$; and for the geographic distances, I used both the raw distances between sites (km) and the log (ln)-transformed distances.

Results

Genetic diversity in wild populations

All ten loci screened were polymorphic. However, results from the MICROCHECKER analysis showed evidence of null alleles at locus *UNH925* for seven of the 11 collections from the wild and consistently showed departure from HWE across all sites. Locus *UNH130* showed evidence for segregation of null alleles in some collections from the wild and consistently showed departure from HWE. Thus, I excluded data from loci *UNH925* and *UNH130* from subsequent analysis. Table 3 provides the summary statistics calculated to show the variation across the eight loci included in the study. Table S1 (*Supplemental Material*) provides the allele frequencies for each of the eight microsatellite loci across sites.

After Bonferroni correction ($\alpha = 0.05/28$ pairs of comparison; adjusted $\alpha = 0.0018$), significant linkage disequilibrium was observed at one pair of loci for the Tano (Elubo) and four pairs of loci for the Black Volta populations. Significant departures from HWE ($\alpha = 0.05$) were observed in the Afram population at *UNH180*; White Volta-Binaba at *UNH123*, *UNH858*, *UNH898*, and *UNH991*; White Volta (Kulugu) at *UNH123*, *UNH178*, and *UNH898*; Oti at *UNH123* and *UNH180*; Ankobra at *UNH178*; Tano (Asuhyea) at *UNH178*, Juen at *UNH991*; Black Volta at all loci except *UNH991*; and Lower Volta at *UNH123*, *UNH180*, and *UNH934*. Because such departures were distributed among the respective populations, data for all loci was retained in the analysis.

Mean observed and expected heterozygosities were moderate to high across sites, and ranged between 0.60 and 0.82, and 0.58 and 0.79 respectively (Table 3). The Black Volta, Ankobra, and Tano-Asuhyea populations had the lowest, while the Afram and Tano-Elubo populations showed the highest heterozygosities. Similarly, allelic richness was moderate to high across sites, and ranged from 5.88 – 9.75; the Tano-Asuhyea population showed the lowest number of alleles per locus, while Black Volta, Afram, and Lower Volta populations showed the highest mean number of alleles across the eight loci (≥ 9.0). Private alleles were observed at all loci for all sites except White Volta-Binaba and Tano-Asuhyea; numbers of private alleles ranged from 1 (Ankobra) to 8 (Black Volta). The highest numbers of private alleles were observed at loci *UNH123* (9) and *UNH180* (Table S1).

Effective population size estimates ranged from 21 to infinity using both the linkage disequilibrium and heterozygote excess methods. The linkage disequilibrium method was more conservative and showed that seven populations - Aframso, White Volta-Binaba, Oti, Tano-Elubo, Juen, and Black Volta, and Lower Volta rivers - had N_e estimates ranging between 21.4 and 178.2 (Table 4). Both methods estimated *Ne* for Tano-Elubo to be below 30. The heterozygote excess method estimated *Ne* for Tano-Asuhyea at approximately 54 even though the upper confidence interval suggested the *Ne* for the population could be significantly larger (Table 4).

The results of the bottleneck tests showed that the stepwise mutation model (SMM) was the best in capturing the demographics within the populations. Three out of the four tests under the SMM showed several bottlenecked populations. The sign test suggested that White Volta-Kulugu, Oti, Pra, Ankobra, and Black Volta were bottlenecked (Table 5). The significant differences test suggested that all populations were bottlenecked while the Wilcoxon two-tailed test suggested that all but the White Volta-Binaba and Tano-Asuhyea populations were bottlenecked (Table 5). Mean *M*-ratios ranged between 0.37 and 0.49 for all populations across the eight loci, while F_{IS} ranged from -0.04 to 0.16. These low ratios supported the inference that genetic bottlenecks had occurred.

Genetic differentiation among wild populations

Pairwise F_{ST} estimates revealed moderate to high genetic differentiation among sites (Table 6), which was statistically significant ($p = 0.000$). In general, Tano-Asuhyea was differentiated from all but the Tano-Elubo and Juen populations. The lowest differentiation was observed between the Oti and Afram populations ($F_{ST} = 0.02$), while the highest differentiation was observed between Tano-Asuhyea and Oti ($F_{ST} = 0.18$). The AMOVA results also supported moderate to high differentiation among populations, with over 8% of the variance explained by differences among populations (Table 7). Genic differentiation (Fisher's exact *G* test) and the locus-by-locus F_{ST} estimates all showed highly significant statistical differences ($p < 0.000$) across all loci for all sites combined (Table 8). Similarly, both Hendrick's $G_{ST'}$ and Jost's *D* supported high genetic differentiation among populations (Table 8).

STRUCTURE analysis showed significant differentiation among the populations at the 11 sites. The admixture model showed highest support for grouping populations into four clusters ($K = 4$, results not shown), with several apparent migrants and admixed individuals at several sites. Results from use of the no-admixture model using location information, while generally comparable to those of the admixture model, were better supported in terms of higher Ln$P(D)$ values. The best-supported model for genetic structure using the Evanno et al. (2005) method was $K = 5$, suggesting five clusters within and among individuals from the 11 sites (Figure 2). However, the Ln$P(D)$ values suggested that the most probable number of clusters was $K = 10$ (Figure 3, Table S2). STRUCTURE plots for both $K = 5$ and $K = 10$ are presented for comparison (Figure 4).

Considering $K = 5$ (Figure 4; top plot), the first cluster (shown in red) contained individuals from the two White Volta sites (Q, the level of individual membership in the inferred cluster, ranged between 0.92 and 0.99) and the Oti River ($Q = 0.11$). The second cluster (shown in green) contained individuals mainly from Afram River ($Q = 0.98$) and Oti River ($Q = 0.88$). The third cluster (shown in blue) was almost exclusive to the Black Volta River ($Q = 0.98$). The fourth cluster (shown in yellow) contained individuals from Pra River ($Q = 0.97$), Ankobra River ($Q = 0.\ 98$), Juen ($Q = 0.20$), and Lower Volta ($Q = 0.91$). Nearly 10% of individuals from the Lower Volta shared genotypes with individuals in the White Volta/Oti and Afram/Oti cluster, while almost no individuals shared genotypes with individuals from the Black Volta ($Q = 0.0001$). The fifth cluster (shown in pink) contained individuals from Tano-Asuhyea River ($Q = 0.98$), Tano-Elubo ($Q = 0.97$), and Juen ($Q = 0.76$). There was no signal of admixture or introgression of genes from the Black Volta population into individuals in the fifth cluster ($Q = 0.0000$), even though very weak signals were detected in the reverse direction (Q ranged between 0.0004 and 0.0079).

Considering $K = 10$ (Figure 4; bottom plot), each population was clustered distinctly, with the exception of the two White Volta sites, the Tano River basin sites, and the Black Volta. The two White Volta sites clustered together, with Q values similar to those observed when $K = 5$. The three Tano River basin sites grouped into two clusters; Tano-Asuhyea in one cluster, and Tano-Elubo and Juen in another cluster. Interestingly, individuals in the Black Volta population grouped into two clusters. The first Black Volta cluster was comprised of 30 individuals originally sampled from Kantu; and the second cluster was comprised of nine individuals sampled from Lawra and Talewona.

The structure of the phylogenetic tree constructed with the nuclear microsatellite DNA data was consistent with the clustering observed at $K = 5$ in the STRUCTURE results (Figure 5). The Mantel test showed a significant positive relationship between genetic distances and geographic distances; and provided modest support for isolation-by-distance (IBD) (adjusted $r^2 = 0.08$, and $p = 0.023$). However, the genetic differentiation across space showed a weak relationship between genetic distances and geographic distances, even after log-transforming the geographic distances (adjusted $r^2 = 0.10$, and $p = 0.012$) (Figures 6 and 7).

Genetic variation in farmed populations and admixture with native populations

Observed and expected heterozygosities were moderate in the Volta Catch and ARDEC populations, but high in Lee's Farm, Akosombo Tilapia Farm, and Fujian Farm samples. Observed heterozygosities ranged between 0.61 ±0.20 and 0.80 ±0.15, while expected heterozygosities ranged between 0.64 ±2.34 and 0.80 ±0.08. Similarly, mean numbers of alleles per locus were moderate in the Volta Catch and ARDEC populations (6.63 ± 2.34 and 6.88 ± 2.47 respectively), and higher in Lee's Farm (8.00 ± 2.40); Akosombo Tilapia Farm (8.25 ± 2.99); and Fujian Farm (9.38 ± 2.34) samples.

Private alleles were observed in all but the Akosombo population. The highest number of private alleles was observed in the Fujian Farm stock ($N = 7$), followed by Akosombo Tilapia Farm ($N = 4$). The Lee's Farm and Volta Catch samples had three and two private alleles, respectively. The Fujian Farm and Akosombo Tilapia Farm stocks shared eight infrequent alleles. The two farmed populations also shared a number of infrequent alleles exclusively with either the ARDEC population or with Lee's Farm population. The Lower Volta River population also shared infrequent alleles with the Fujian and Akosombo Tilapia farm stocks. Significant departure from HWE was evident in the Akosombo strain at loci *UNH858* and *UNH898*; Lee's Farm at *UNH180* and *UNH858*; Volta Catch at *UNH180* and *UNH898*; and Fujian Farm at *UNH180* and *UNH858*. The Akosombo Tilapia Farm population showed no departures from HWE. Significant departures from linkage equilibrium were observed only in the Fujian Farm (one pair of loci) and Lee's Farm (seven pairs of loci) samples after Bonferroni correction.

Pairwise F_{ST} estimates revealed low to moderate genetic differentiation among the farmed populations, in general, and somewhat higher than that among wild populations (Table 9). In general, the Fujian Farm and Akosombo Tilapia Farm stocks were similar to one another, but differentiated from all the other farmed populations. The least differentiation was observed between Fujian Farm and Akosombo Tilapia Farm (F_{ST} = - 0.000), while the greatest was between Fujian Farm and Volta Catch (F_{ST} = 0.21). Similarly, the AMOVA results also showed high differentiation among populations, with 11% of the variance explained by differences among populations (Table 10). Similarly, the Fisher's exact *G* test and the locus-by-locus F_{ST} also showed highly significant differentiation ($p < 0.000$) across all loci for all sites combined.

STRUCTURE analysis using the admixture model and the most probable number of *K* selected using the Evanno et al. (2005) method revealed greatest support for $K = 2$ clusters within and among the farmed and nearby wild populations analyzed, with high associated probabilities of assignment (*Q* ranged between 0.95 and 0.99; Figure 8). In general, individuals from Fujian Farm and Akosombo Tilapia Farm grouped into one cluster (shown in green), while all other farmed populations and the reference wild populations grouped into another cluster (shown in red). On the other hand, the Ln*P*(*D*) values revealed the greatest support for $K = 6$ clusters (Figure 8; bottom plot). However, two distinct groups were evident within the six clusters. The first group comprised individuals in the ARDEC, Volta Catch, Lee's Farm, Lower Volta River, and Black Volta River populations while the second group comprised individuals from Fujian and Akosombo Tilapia farms. These outcomes suggested two populations, a native Nile tilapia group and a non-native Nile tilapia group. Two individuals from the Lower Volta River (LV02 and LV03) ($N = 33$) showed evidence of high levels of admixture and clustered with the non-native tilapia group from Fujian Farm ($Q = 0.86$ and 0.88). In contrast, none of the Black Volta individuals ($N = 39$) showed evidence of admixture ($Q > 0.98$). The non-native tilapia group showed no admixture with the native populations. However, the Lee's Farm stock contained several individuals apparently admixed with the non-native tilapia populations (*Q* ranged between 0.11 and 0.74). Two individuals, one each in the Akosombo and Volta Catch populations, appeared admixed with the non-native populations ($Q = 0.13$).

Discussion

Genetic diversity within wild O. niloticus *populations: evidence of recent bottlenecks*

The genetic diversity observed among wild populations of Nile tilapia in this study was high compared to that found in similar studies conducted in Ghana within the Volta River basin (Mireku et al. 2017). However, the finding that significant departures from Hardy-Weinberg Equilibrium (HWE) occurred at several loci for all populations except Pra River and Ankobra and significant linkage disequilibrium (LD) observed especially in the Black Volta River suggests the occurrence of recent bottlenecks within the populations. Further, the results of bottleneck tests conducted suggested that even the Ankobra and Pra populations are bottlenecked, and the mean *M*-ratio estimates in this study were below the 0.7 threshold value used to assess population bottlenecks within populations (Garza and Williamson 2001). It is plausible that there were natural historical bottlenecks and that the resulting demographic effect on populations was exacerbated with recent anthropogenic impacts. Effective population size estimates also suggested declining population numbers at several sites, which is alarming for a highly fecund species like *O. niloticus*. Inbreeding among relatives is also likely due to the low to moderate and positive values of the inbreeding index (F_{IS} ranged between 0.04 to 0.16). Deviations from HWE may be due to multiple causes, including genetic drift, inbreeding, or introgression with *O. aureus* (Chapter 2). The inference of segregation of null alleles at three loci (*UNH178, UNH203,* and *UNH898*) in the Black Volta population also could have contributed to the departures from HWE and linkage equilibrium observed. Nonetheless, the Black Volta population requires further assessment and monitoring in order to understand the genetic dynamics within the population. This is especially pertinent since many private alleles were observed in the Black Volta population, as well as private mitochondrial DNA (mtDNA) haplotypes; and they are likely the main ancestral population of *O. niloticus* in Ghana (Chapter 2).

The recent genetic bottlenecks inferred within the study populations may be the consequence of anthropogenic impacts upon the river systems in question. Many tilapia populations have been subject to heavy exploitation. Further, within the last decade, drastic habitat alteration has occurred in almost all freshwater habitats in Ghana, especially due to illegal alluvial gold mining. Turbidity levels of many rivers in Ghana are significantly above recommended limits for aquatic life, and cichlids in particular do not thrive under such environmental conditions. Dankwa et al. (2005) found that in heavily mined areas (turbidity levels between 100 and 1110 NTU), cichlids were completely absent, as were their plankton prey. Physical and environmental bottlenecks can affect reproduction and long-term persistence of *O. niloticus* in Ghana. High turbidity could affect the selection of breeding mates, which also could result in inbreeding. Management effort should therefore focus on removing these bottlenecks to ensure the long-term persistence of *O. niloticus*.

Genetic differentiation among wild populations: barriers to dispersal and a unique dispersal mechanism

The metrics used to assess genetic differentiation and structure among populations showed moderate to high genetic differentiation as a function of geographic location. The pairwise F_{ST} estimates showed that the Afram and Oti populations were the least differentiated. This finding was supported by the clustering of Afram River and Oti River populations into the same group by the STRUCTURE analysis and in the phylogenetic population tree. Even though moderately differentiated, some level of gene flow was apparent between *O. niloticus* populations in the Afram and Oti rivers across a distance of about 254 km (158 mi) between the two populations.

The clustering of the Oti and the White Volta populations was not as surprising because the distance between the Oti site and the farthest of the two White Volta sites (Binaba) is relatively short, 172 km (106 mi). The distance between the Afram site and the Lower Volta site is about 195 km (121 mi). The finding that some level of genetic exchange among Nile tilapia occurred between the Lower Volta and all the rivers within the Volta basin (except the Black Volta) suggests that isolation-by-distance is not as important in mediating gene flow within the Volta basin as the presence of physical barriers such as the 53-year-old Akosombo Dam. The presence of the Akosombo dam and other irrigation reservoirs also could explain why the Black Volta River populations are highly differentiated from all other populations with the Volta basin. The results of the Mantel test further supported the notion that isolation-by-distance alone could not explain the genetic structuring observed across space for the populations. For instance, significant genetic structuring was observed between the Black Volta sites even though two of the sites (Kantu and Talewona) are only 13 km (about 8 mi) apart.

On the other hand, both isolation-by-distance and physical barriers could be operating together to limit gene flow between the Black Volta and all sites outside the Volta basin.

However, I observed that the Black Volta was more differentiated from the Tano-Asuhyea (F_{ST} = 0.143) than from the Tano-Elubo (F_{ST} = 0.083) population, which suggests some genetic exchange between the latter two population pairs. This result corroborates findings that the Black Volta population shared mitochondrial *COI* haplotypes with the Tano-Elubo population (Chapter 2), and suggests *O. niloticus* movement, likely through human intervention, between Ghana and Cote d'Ivoire at the shared border along the Black Volta River.

The pairwise F_{ST} estimates also revealed that the Tano-Asuhyea population was very distinct from all populations outside the Tano basin and moderately differentiated from the Tano-Elubo and Juen Lagoon populations. The apparent genetic differentiation from other populations, relatively low allelic richness, and the lack of private alleles within the Tano-Asuhyea population suggests loss of diversity within the population due to genetic drift. This assertion is supported by the low mean *M*-ratio estimated for the Tano-Asuhyea population (0.37) and significant bottleneck test results (Table 5). Occurring in the headwaters of the Tano River, the Tano-Asuhyea population is relatively isolated and may be exchanging relatively few migrants with the Tano-Elubo population.

STRUCTURE analysis and the population phylogenetic tree also showed clustering of individuals from the Tano basin (Tano-Asuhyea, Tano-Elubo, and Juen Lagoon) and individuals from the Pra, Ankobra and the Lower Volta rivers. Given that the Pra, Ankobra, Tano, and Lower Volta rivers all run parallel to one another and drain into the Atlantic Ocean, oceanic dispersal appears a plausible mechanism for the genetic exchange apparent among *O. niloticus* populations. It appears that these populations are migrating from one freshwater environment through brackish water into another freshwater environment. Oceanic dispersal is possible because tilapias, in general, are euryhaline (Philippart and Ruwet 1982; Stickney 1986), and the salinity tolerance in *O. niloticus* has been documented (Watanabe et al. 1985; Likongwe et al. 1996; Kamal and Mair 2005). Genetic data have been useful in detecting oceanic dispersal for species presumed to be restricted to freshwater environments, such as river sharks (Li et al. 2015). Oceanic dispersal also may explain the colonization of *O. niloticus* in the Ankobra and Pra rivers (from the Tano River, Chapter 2), and further explain why *Tilapia pra* is restricted to the Ankobra, Tano, and Pra rivers (Dunz and Schliewen 2000).

Taken together, the genetic differentiation observed within and among the wild populations of Nile tilapia provide support for developing effective management and conservation plans for preserving these populations. The STRUCTURE results suggested that of the eleven populations studied, there may be as many as ten populations requiring separate management, which should be designated as management units (MUs). Additionally, the genetic distinctiveness of the Tano-Asuhyea and Black Volta populations and their isolation from other populations suggested the possibility that these populations may represent evolutionary significant units (ESUs) within *O. niloticus* populations in Ghana. Further studies focusing upon elucidation of any adaptive differences among candidate ESUs should help establish this possibility.

Genetic variation in farmed populations and admixture with native populations

The genetic diversities within and among the farmed populations were higher than expected. Overall, the diversity observed in the ARDEC and Volta Catch populations were similar to those of the wild populations, but significantly lower than the diversity observed in the Lee's, Akosombo Tilapia, and Fujian farm stocks. The high genetic diversity observed in individuals from Lee's Farm compared to the ARDEC and Volta Catch populations showed an apparent introduction of new alleles into the Lee's Farm populations' gene pool. However, the likelihood for both hybrid breakdown and outbreeding depression potentially could offset any potential benefits to Lee's Farm admixed population. Both the genetic diversity within and differentiation among the farmed populations clearly showed that two distinct tilapia strains were farmed in Ghana; a native strain and a non-native farmed strain (likely the genetically improved farmed tilapia, GIFT, and related strains*;* Chapter 2). Due to the escape of the non-native tilapia strains into at least the Lower Volta River, potentially negative genetic impacts due to introgression with farmed stocks are posed to wild populations in the long-term.

Results of the present study confirm that non-native tilapia strains are farmed predominantly by two farms, the Fujian and Akosombo Tilapia farms. The fact that the two farms with the non-native tilapia shared some alleles with the other farmed populations and the observation that admixed individuals occurred in these populations suggests possible cross-breeding between native *O. niloticus* populations and the non-native farmed tilapia populations. Admixture was observed in one individual each in the ARDEC and Volta Catch populations and could be due to misclassification errors. However, the presence of admixed individuals within the ARDEC population could be explained by possible crossing of the Akosombo strain of *O.*

niloticus with the GIFT strain during recent experimentation with the two strains (Chapter 2, Ansah et al. 2014). Several admixed individuals were found in Lee's Farm, and the nature of the admixture suggests cross-breeding between non-native farmed tilapia with native *O. niloticus* either deliberately or accidently.

The STRUCTURE results also showed that two individuals in the Lower Volta River population were admixed. One of the two admixed individuals (LV03) also shared a mitochondrial DNA haplotype with Fujian and Akosombo Tilapia farms (Chapter 2), supporting the inference that the non-native farmed tilapia females have escaped into the wild and interbred. The other admixed Lower Volta individual (LV02) was not among the ten individuals selectively screened at the mtDNA *D-loop* region (Chapter 2), suggesting the presence of multiple escapes of the non-native fish into the Lower Volta River. - The detection of admixed individuals both in farmed and Lower Volta populations requires that proactive measures be taken in a timely fashion to prevent further and routine escapes of the non-native farmed tilapia into the wild.

The lack of evidence of admixed individuals in the Black Volta population support earlier findings from the mtDNA analysis that the escaped non-native farmed tilapia has been restricted to the Lower Volta and Afram rivers within the Volta basin (Chapter 2). The Lower Volta River is downstream of aquaculture operations on the Volta Lake, while there are aquaculture facilities in close proximity to the Afram River. On the other hand, the Black Volta is relatively isolated from aquaculture farms even though cage farming in irrigation reservoirs is increasing in the region. Given the possibility of ocean dispersal as a mechanism for migration of *O. niloticus* to otherwise isolated basins, restricting the dispersal of non-native tilapia populations would be very challenging if fish continue to escape from farms.

Conclusions and Implications

The objectives of this study were to assess genetic variation in *O. niloticus* in nine different drainage basins in Ghana using nuclear microsatellite DNA markers, and to assess the genetic impact of aquaculture on wild populations by characterizing the tilapia stocks at selected farms operating on the Volta Lake in Ghana. I found moderate to high genetic diversity within and differentiation among wild populations, and I also detected strong signals of recent demographic and genetic bottlenecks in several populations. I also found significant genetic structure among the populations, suggesting the presence of up to ten management units (MUs) within the 11 wild populations studied. In particular, the Black Volta and the Tano-Asuhyea populations were the most genetically distinct and isolated, and may be most at risk of genetic diversity loss over time. Therefore, in the minimum, both the Black Volta and Tano-Asuhyea populations should be designated as management units (MUs) and prioritized for conservation to sustain them in the long-term.

Farmed tilapia populations were more genetically diverse than expected. Individuals in two farmed populations, the Fujian and Akosombo Tilapia farm stocks, were genetically distinct from native *O. niloticus* populations and had high genetic diversity likely resulting from production of non-native tilapia strains. Admixed individuals observed in samples from the Lower Volta River population downstream of the aquaculture facilities confirmed earlier inferences that the non-native, farmed tilapia have escaped into the wild and interbred with native populations. The presence of several admixed individuals in the Lee's Farm stock suggests significant upstream migration of non-native tilapia escapees, assuming that Lee's Farm obtained no broodstock from Fujian and Akosombo Tilapia farms.

Given that the Black Volta, Oti, and White Volta rivers may yet be free of impacts from non-native farmed strains, it is important to restrict movement of farmed tilapia across the country. Since the Akosombo strain is potentially contaminated with the GIFT strain, timely management decisions to reduce the further spread of non-native farmed tilapia populations should include the suspension of the reservoir stocking program planned for northern Ghana because the proposed program would guarantee a first major movement of farmed tilapia strains to the Black Volta, White Volta, and the Oti river populations which appear to be unimpacted currently. The results from this study provide timely baseline information, which could be used for continuous monitoring of aquaculture receiving rivers and help to conserve native *O. niloticus* populations.

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Figures and Tables

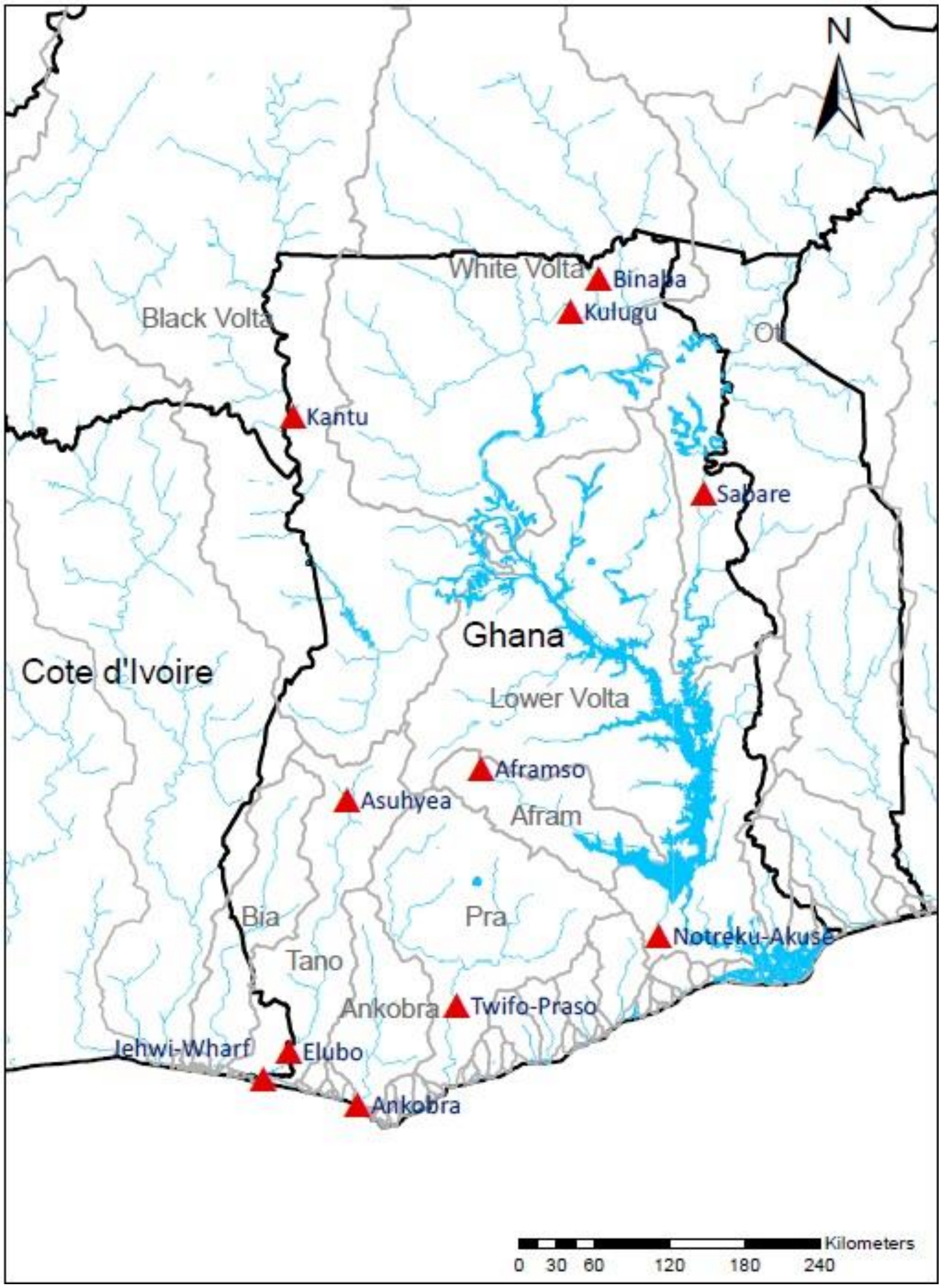

Figure 1. Sampling locations for wild *Oreochromis* spp. collected at 11 sites from eight rivers and one coastal lagoon in Ghana from December 2014 through July 2017. Sampling sites are indicated with red triangles.

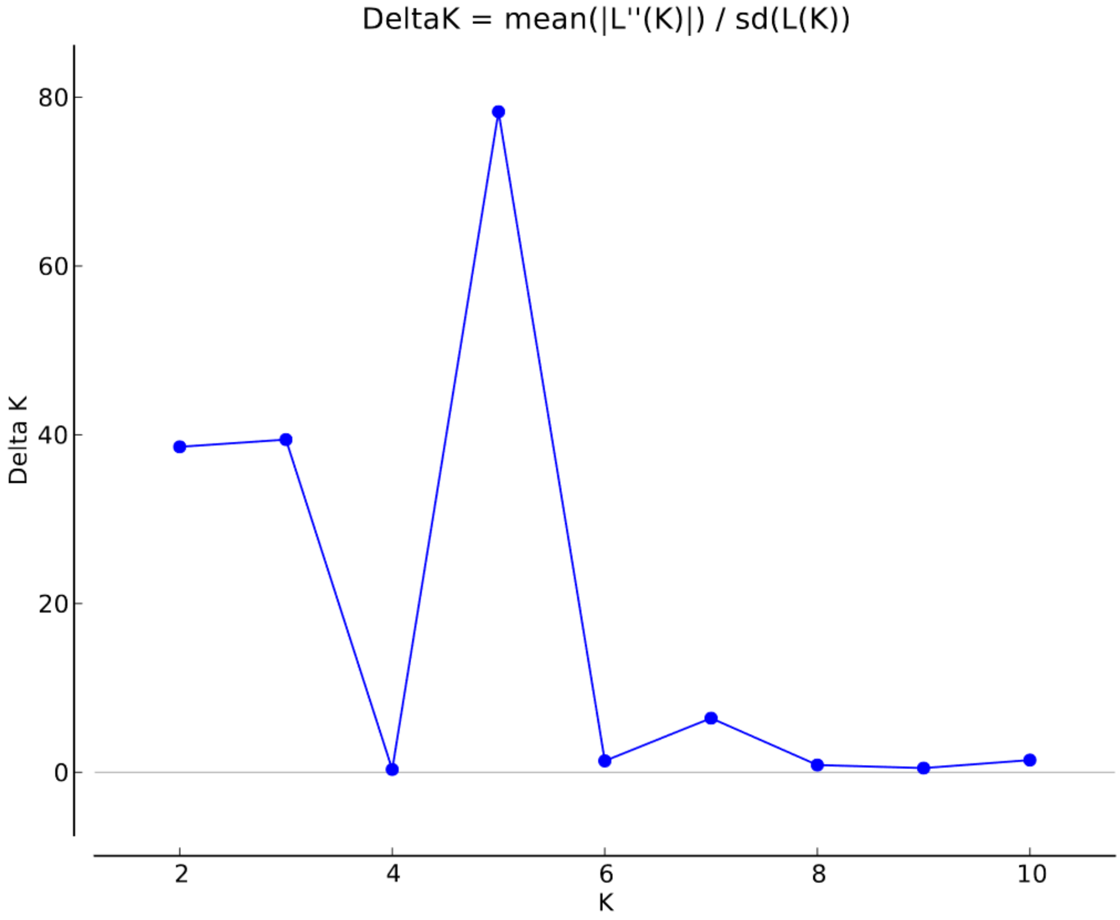

Figure 2. The Evanno et al. (2005) method supporting *K* = 5 as the most likely number of clusters for wild *O. niloticus* populations studied. All inputs were based on Structure analysis with MCMC = 500,000.

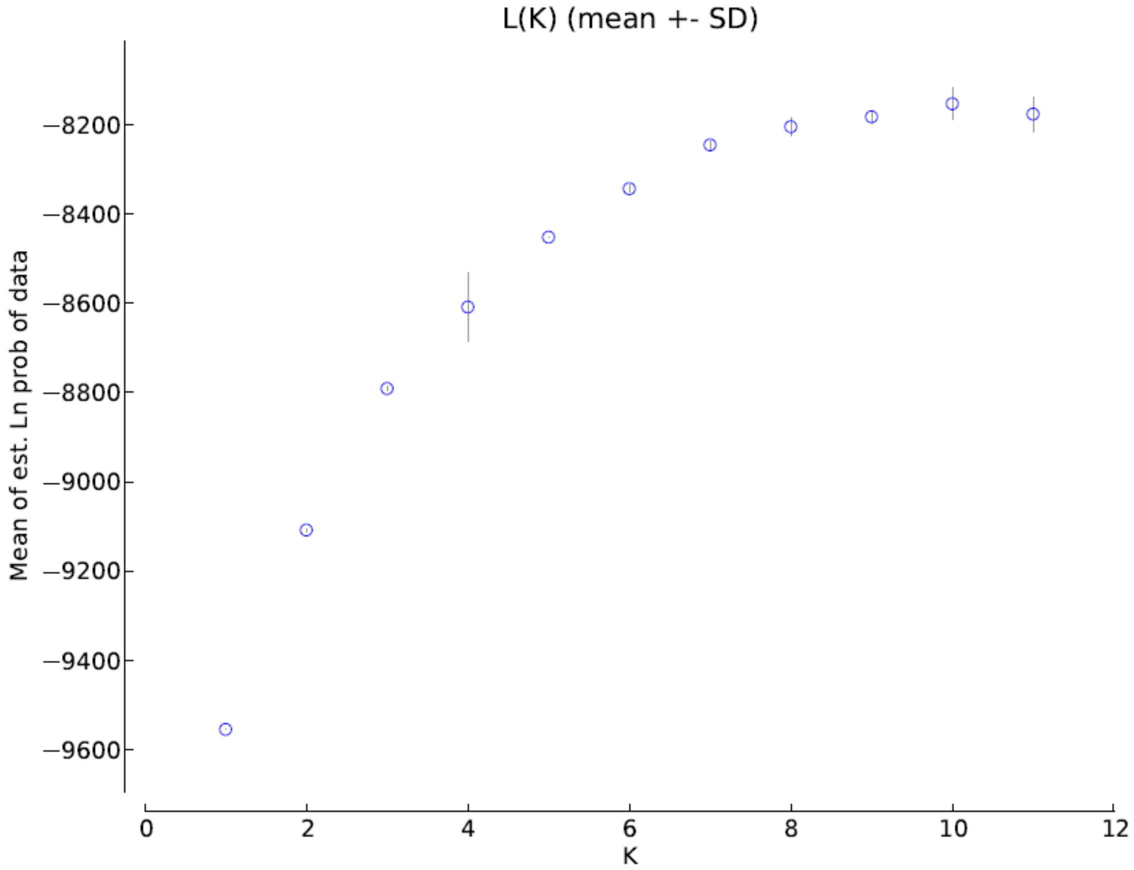

Figure 3. STRUCTURE results for mean likelihood values suggesting *K* = 10 clusters (MCMC = 500,000) among the *O. niloticus* populations studied.

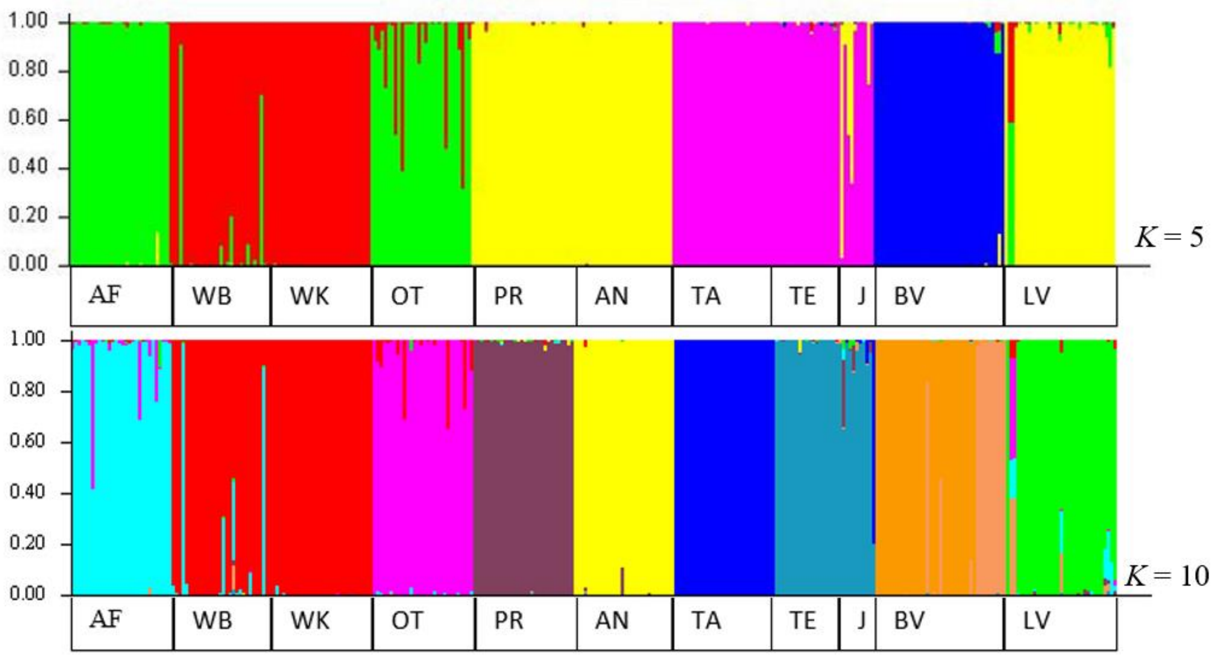

Figure 4. STRUCTURE results showing the proportion of each wild *O. niloticus* population's ancestry that was inferred to have come from each of *K* = 5 (top plot) and *K* = 10 (bottom plot) clusters (MCMC = 500,000). Populations are on the *x*-axis and *Q* values are on the *y*-axis. AF = Afram, WB = White Volta-Binaba, WK = White Volta- Kantu, OT = Oti, AN = Ankobra, TA = Tano-Asuhyea, TE = Tano-Elubo, J = Juen, BV = Black Volta, and LV = Lower Volta.

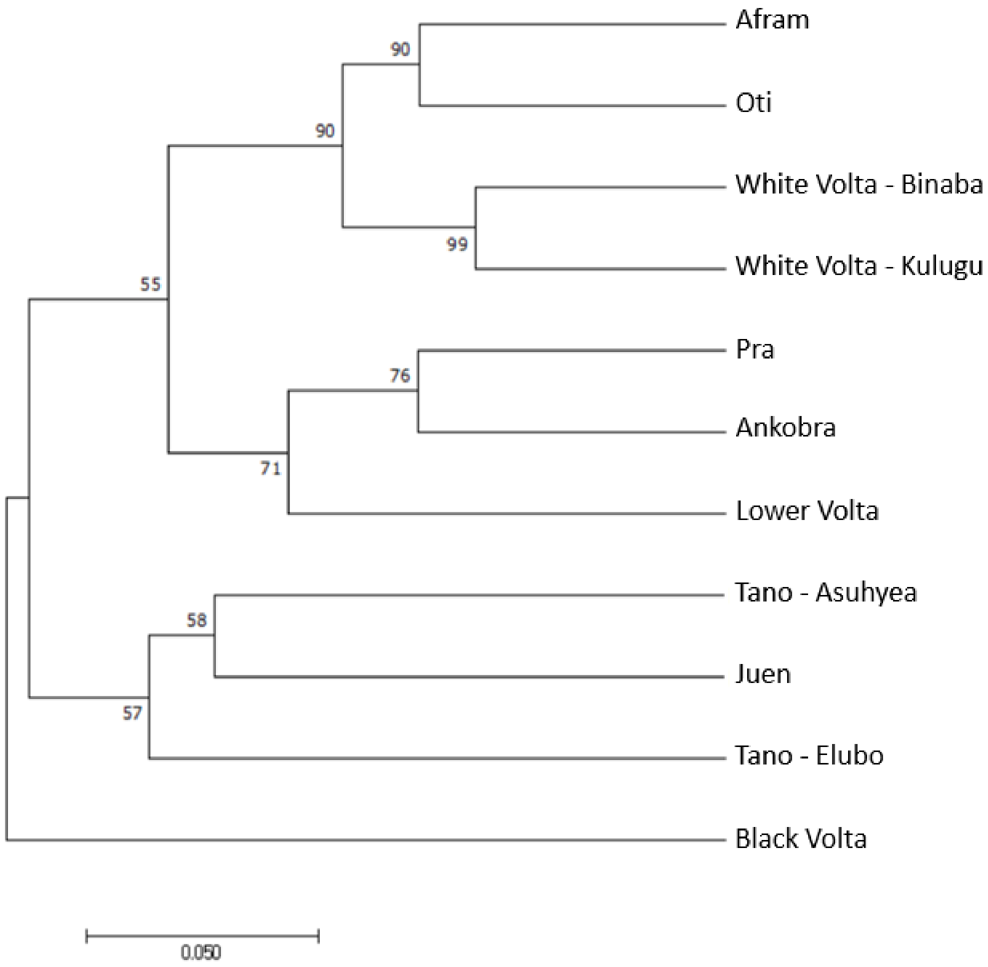

Figure 5. Phylogenetic tree constructed from microsatellite DNA data for *Oreochromis niloticus* collected from 11 sites in Ghana between December 2014 and July 2017. The tree was constructed using the unweighted pair-group method with arithmetic mean (UPGMA; Sneath and Sokal 1973) with distance measure D_A (Nei 1983; Takezaki and Nei 1996; 2008) corrected for population size. Bootstrap replication was 100,000.

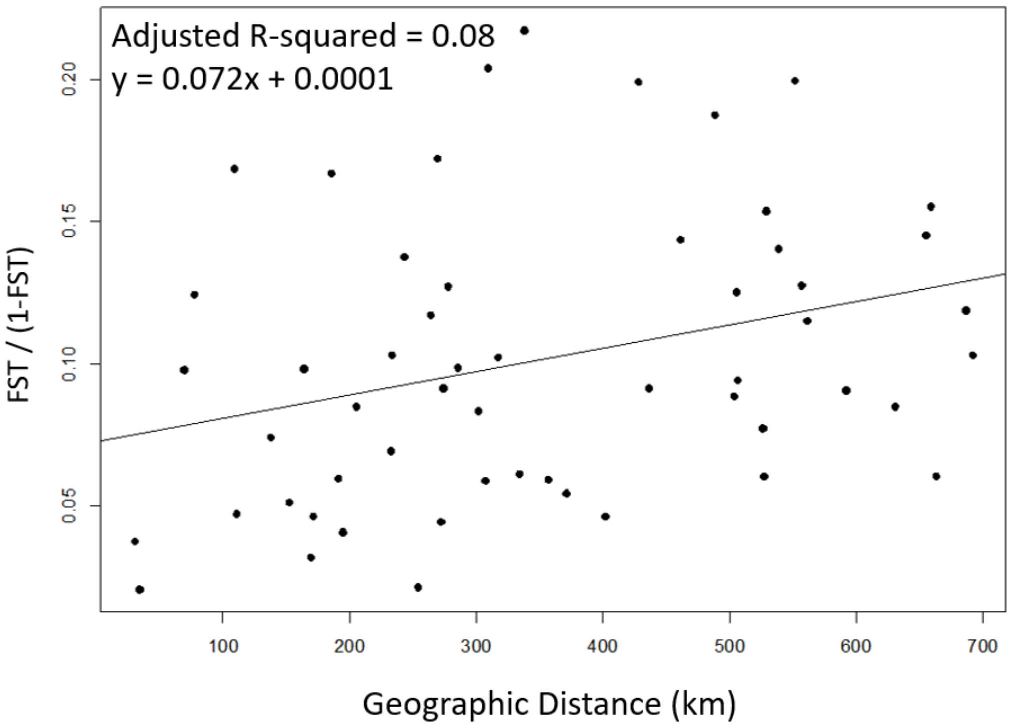

Figure 6. Relationship between genetic distance and geographic distance among wild *Oreochromis niloticus* collected from 11 sites in Ghana from December 2014 and July 2017.

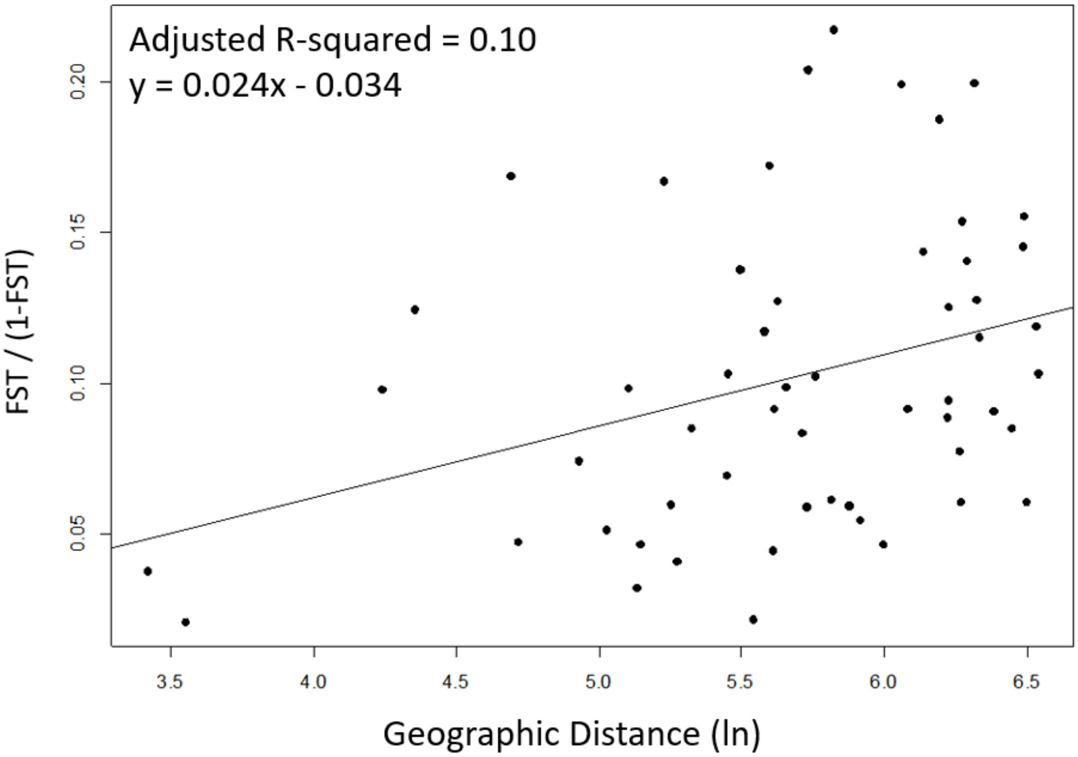

Figure 7. Relationship between genetic distances and logarithm of geographic distances among *Oreochromis niloticus* collected from 11 sites in Ghana from December 2014 and July 2017.

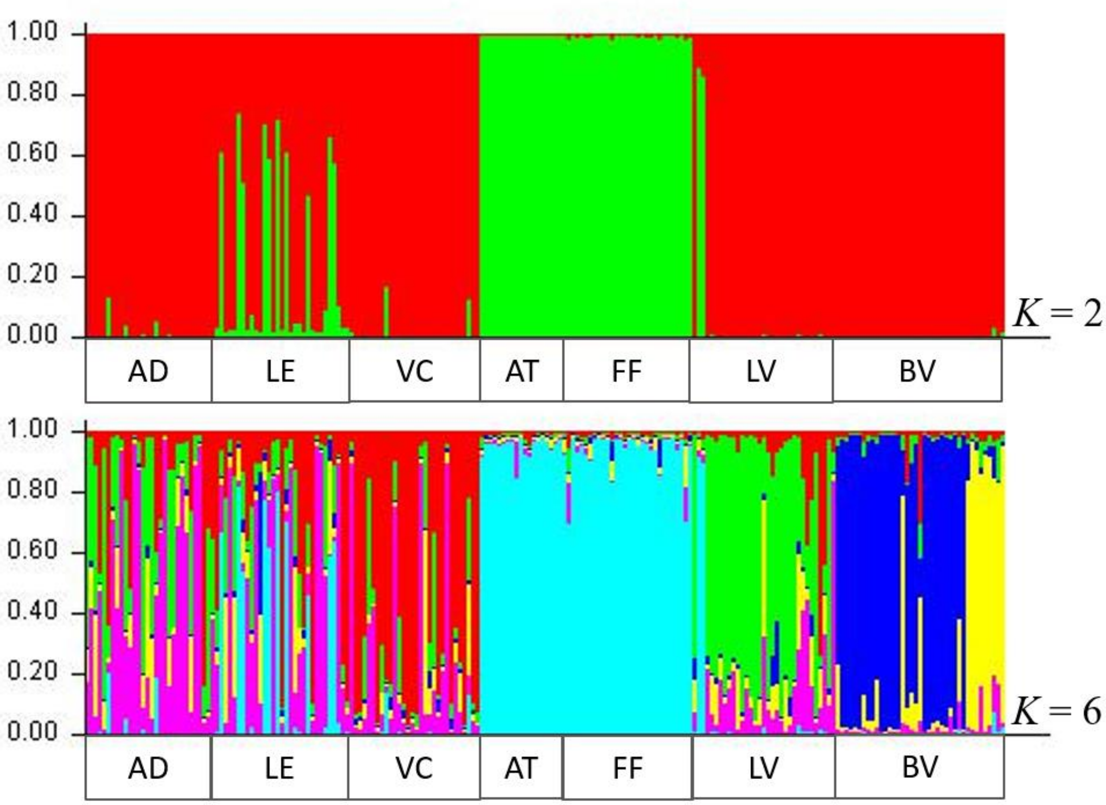

Figure 8. STRUCTURE results showing the proportion of each farmed and reference tilapia population's ancestry that was inferred to come from each of *K* = 2 (top plot) and *K* = 6 (bottom plot) clusters (MCMC = 500,000). Populations are on the *x*-axis and *Q* values are on the *y*-axis. AD = ARDEC, LE = Lee's Farm, VC = Volta Catch, AT = Akosombo Tilapia Farm, FF = Fujian Farm, LV = Lower Volta River, and BV = Black Volta River.

Table 1. Sample and site information for *Oreochromis* spp. collected in Ghana from December 2014 through July 2017.

Site Name	Sampling Location	Source	*N*
Afram River	Aframso	Wild	30
White Volta	Binaba	Wild	30
White Volta	Kulugu	Wild	30
Oti River	Sabare	Wild	30
Pra Pra	Twifo-Praso	Wild	30
Ankobra River	Ankobra	Wild	30
Tano	Asuhyea	Wild	30
Tano	Elubo	Wild	18
Juen Lagoon	Jehwi-Wharf	Wild	12
Black Volta	Kantu/Lawra/Talewona	Wild	39
Lower Volta River	Notreku-Akuse	Wild	33
ARDEC*	Akosombo	Farm	30
Lee’s Farm	Akosombo	Farm	32
Volta Catch	Asokwa-Kumasi	Market	29
Akosombo Tilapia	Ashaiman	Market	19
Fujian Farm	Asutuare	Farm	30

*ARDEC = Aquaculture Research and Development Center

Table 2. Technical details for amplification of ten microsatellite loci for *O. niloticus* from nine river basins and five farmed sites in Ghana.

Locus	Primer sequences	Base-pair range	Annealing Temp (°C)	GenBank Accession number
UNH123	F: CATCATCACAGACAGATTAGA R: GATTGAGATTTCATTCAAG	171-245	54	G12276.1
UNH130	F: AGGAAGAATAGCATGTAGCAAGTA R: GTGTGATAAATAAAGAGGCAGAAA	164-242	58	G12283.1
UNH178	F: GTCACACCTCCATCATC R: AGTTGTTTGGTCGTGTAAG	114-144	58	G12330.1
UNH180	F: GCAACTAATCACACAATTTT R: GTTTAAGTTAAAAACAAATTCGTTT	121-187	58	G12332.1
UNH203	F: CACAAAGATGTCTAAACATGT R: GAATTTGACAGTTTGTTGTTTAC	65-97	56	G12354.1
UNH858	F: TTCAAACAGCTTCACGGTCA R: CTATGCCATGGCTAAAGTCAC	196-252	58	G68194.1
UNH898	F: GATGTCCCCACAAGGTATGAA R: TAATCCACTCACCCCGTTTC	214-292	58	G68215.1
UNH925	F: GTAGCTGCTGGGGTCTGAAG R: TAGCACTCTGCCACTTGTCC	172-252	58	G68234.1
UNH934	F: ACTGCAATGAAATGCTGCTT R: CCATTCCTCAGAGCACAACA	214-246	58	G68240.1
UNH991	F: AAGCCTTGCATAAAACAGCA R: AAAGTTTGCTGCCCTCAGTG	150-182	58	G68271.1

Table 3. Summary of genetic variation among eight microsatellite deoxyribonucleic acid (DNA) loci examined in wild tilapia populations from 11 sites (nine river basins) collected in Ghana from 2014 to 2017. N = number of individuals genotyped per locus, H_o = observed heterozygosity, H_e = expected heterozygosity, A = number of observed alleles per locus, Range = base-pair differences between the shortest and longest microsatellite alleles observed per locus, M-ratio = approximate ratio of A and Range, and F_{IS} = inbreeding coefficient. Values in bold are significantly different from each other ($P < 0.05$).

Population	Locus	N	H_o	H_e	A	Range	M-ratio	F_IS
Afram River	*UNH123*	30	0.90	0.86	10	24	0.40	-0.05
	UNH178	30	0.83	0.80	9	12	0.69	-0.04
	UNH180	30	**0.60**	**0.71**	7	15	0.44	0.16
	UNH203	30	0.63	0.56	4	15	0.25	-0.14
	UNH858	30	0.93	0.91	14	26	0.52	-0.02
	UNH898	30	0.93	0.82	15	24	0.60	-0.14
	UNH934	30	0.77	0.73	8	14	0.53	-0.05
	UNH991	30	0.43	0.45	5	10	0.49	0.04
	Mean	30.00	0.75	0.73	9.00	0.49	0.49	-0.03
	SD	0.00	0.17	0.15	3.674	0.125	0.13	0.10
White Volta-Binaba	*UNH123*	29	**0.66**	**0.83**	8	20	0.38	0.22
	UNH178	30	0.73	0.78	7	12	0.54	0.06
	UNH180	29	0.45	0.60	4	15	0.25	0.25
	UNH203	30	0.60	0.66	4	15	0.25	0.09
	UNH858	28	**0.86**	**0.93**	14	19	0.70	0.08
	UNH898	30	**0.83**	**0.79**	10	25	0.38	-0.06
	UNH934	29	0.45	0.48	6	13	0.43	0.06
	UNH991	29	**0.62**	**0.67**	6	15	0.38	0.08
	Mean	29.25	0.65	0.72	7.38	16.75	0.41	0.10
	SD	0.71	0.15	0.13	3.12	4.02	0.14	0.10

Table 3. Continued.

Population	Locus	N	H_o	H_e	A	Range	M-ratio	F_{IS}
White Volta-Kolugu	*UNH123*	30	**0.67**	**0.78**	11	20	0.52	0.15
	UNH178	30	**0.63**	**0.68**	7	11	0.58	0.06
	UNH180	30	0.53	0.62	5	14	0.33	0.14
	UNH203	30	0.60	0.52	4	16	0.24	-0.15
	UNH858	30	0.93	0.92	16	26	0.59	-0.02
	UNH898	30	**0.53**	**0.78**	11	25	0.42	0.32
	UNH934	30	0.60	0.69	7	14	0.47	0.13
	UNH991	30	0.57	0.61	6	15	0.38	0.07
	Mean	30	0.63	0.70	8	17.63	0.44	0.09
	SD	0	0.12	0.12	4	5.12	0.12	0.14
Oti River	*UNH123*	30	**0.77**	**0.79**	9	22	0.39	0.04
	UNH178	30	0.70	0.71	7	10	0.64	0.02
	UNH180	30	**0.63**	**0.77**	12	31	0.38	0.18
	UNH203	30	0.43	0.45	3	15	0.19	0.04
	UNH858	30	0.90	0.86	14	28	0.48	-0.05
	UNH898	29	0.83	0.84	13	26	0.48	0.01
	UNH934	30	0.60	0.57	6	14	0.40	-0.05
	UNH991	30	0.47	0.46	5	13	0.36	-0.01
	Mean	29.88	0.67	0.68	9	19.88	0.41	0.02
	SD	0.35	0.15	0.15	4	7.37	0.12	0.07
Pra	*UNH123*	30	0.80	0.76	7	17	0.39	-0.06
	UNH178	30	0.67	0.67	6	10	0.55	0.01
	UNH180	30	0.30	0.32	6	28	0.21	0.07
	UNH203	30	0.73	0.68	5	16	0.29	-0.08
	UNH858	30	0.97	0.88	13	19	0.65	-0.10
	UNH898	28	0.68	0.72	14	26	0.52	0.06
	UNH934	30	0.80	0.73	9	13	0.64	-0.10
	UNH991	30	0.43	0.41	7	15	0.64	-0.06
	Mean	29.75	0.67	0.65	8	18	0.46	-0.03
	SD	0.71	0.20	0.17	3	5.79	0.15	0.07

Table 3. Continued.

Population	Locus	N	H_o	H_e	A	Range	M-ratio	F_{IS}
Ankobra	*UNH123*	30	0.63	0.58	7	23	0.29	-0.10
	UNH178	30	**0.57**	**0.68**	7	9	0.70	0.17
	UNH180	30	0.20	0.19	3	14	0.20	-0.08
	UNH203	30	0.53	0.60	3	15	0.19	0.11
	UNH858	30	0.90	0.83	10	16	0.59	-0.09
	UNH898	30	0.50	0.48	9	26	0.33	-0.05
	UNH934	30	0.77	0.67	7	16	0.41	-0.15
	UNH991	30	0.67	0.66	7	15	0.44	-0.01
	Mean	30	0.60	0.58	7	16.75	0.39	-0.02
	SD	0	0.19	0.18	2	4.99	0.17	0.11
Tano-Asuhyea	*UNH123*	30	0.77	0.69	6	20	0.29	-0.12
	UNH178	30	**0.73**	**0.84**	7	11	0.53	0.12
	UNH180	30	0.27	0.29	3	9	0.30	0.09
	UNH203	30	0.80	0.61	3	15	0.19	-0.33
	UNH858	30	0.70	0.66	9	12	0.69	-0.06
	UNH898	30	0.80	0.80	10	27	0.36	0.01
	UNH934	30	0.43	0.49	3	16	0.18	0.12
	UNH991	30	0.73	0.74	6	15	0.38	0.01
	Mean	30	0.65	0.64	6	15.63	0.37	-0.02
	SD	0	0.18	0.17	3	5.34	0.17	0.15
Tano-Elubo	*UNH123*	18	1.00	0.89	11	35	0.31	-0.13
	UNH178	18	0.83	0.79	8	11	0.67	-0.06
	UNH180	17	0.82	0.70	5	11	0.42	-0.19
	UNH203	18	0.83	0.73	5	15	0.31	-0.14
	UNH858	18	0.89	0.90	13	23	0.54	0.02
	UNH898	18	0.89	0.88	12	24	0.48	-0.01
	UNH934	18	0.56	0.67	7	14	0.47	0.17
	UNH991	18	0.72	0.75	6	15	0.38	0.03
	Mean	17.88	0.82	0.79	8	18.50	0.45	-0.04
	SD	0.35	0.12	0.09	3	7.75	0.01	0.12

Table 3. Continued.

Population	Locus	N	H_o	H_e	A	Range	M-ratio	F_{IS}
Juen	*UNH123*	12	0.92	0.81	8	21	0.36	-0.14
	UNH178	12	0.83	0.87	8	14	0.53	0.04
	UNH180	12	0.25	0.24	3	9	0.30	-0.06
	UNH203	12	0.58	0.64	4	16	0.24	0.10
	UNH858	12	0.75	0.85	10	25	0.38	0.12
	UNH898	12	0.92	0.91	14	30	0.45	-0.01
	UNH934	12	0.75	0.80	6	15	0.38	0.07
	UNH991	12	**0.50**	**0.83**	8	15	0.50	0.41
	Mean	12	0.69	0.74	8	18.13	0.39	0.07
	SD	0	0.22	0.20	3	6.33	0.09	0.16
Black Volta	*UNH123*	39	**0.69**	**0.78**	11	31	0.34	0.12
	UNH178	39	**0.54**	**0.77**	10	12	0.77	0.30
	UNH180	26	**0.69**	**0.72**	6	27	0.21	0.04
	UNH203	39	**0.28**	**0.40**	3	12	0.23	0.30
	UNH858	39	**0.74**	**0.83**	16	22	0.70	0.10
	UNH898	37	**0.59**	**0.80**	12	30	0.39	0.26
	UNH934	39	**0.54**	**0.63**	10	16	0.59	0.15
	UNH991	39	0.72	0.72	6	16	0.35	0.01
	Mean	37.13	0.60	0.71	9	20.75	0.45	0.16
	SD	4.55	0.14	0.13	4	7.33	0.20	0.12
Lower Volta	*UNH123*	33	**0.58**	**0.74**	8	21	0.36	0.23
	UNH178	33	0.82	0.81	8	14	0.53	-0.01
	UNH180	31	**0.48**	**0.53**	3	9	0.30	0.09
	UNH203	33	0.67	0.68	3	16	0.24	0.02
	UNH858	30	0.97	0.90	10	25	0.38	-0.07
	UNH898	33	0.73	0.83	14	30	0.45	0.13
	UNH934	33	0.67	0.70	6	15	0.38	0.05
	UNH991	33	0.58	0.55	8	15	0.50	-0.05
	Mean	32.38	0.69	0.72	8	18.13	0.39	0.05
	SD	1.19	0.14	0.12	3	6.33	0.09	0.10

Table 4. Effective population size (N_e) estimates and 95% confidence intervals for wild *O. niloticus* population samples collected in Ghana. ∞ = infinite.

Population	*N*	Linkage disequilibrium Method	Heterozygote excess method
AF	30	**43.3 (25.1, 102.6)**	551.3 (7.8, ∞)
WB	30	**21.4 (14.0, 36.2)**	∞ (∞, ∞)
WK	30	∞ (104.4, ∞)	∞ (129.5, ∞)
OT	30	**46.4 (23.7, 182.2)**	∞ (18.1, ∞)
PR	30	1184.4 (52.8, ∞)	22.5 (6.6, ∞)
AN	30	∞ (58.1, ∞)	∞ (6.7, ∞)
TA	30	∞ (120.3, ∞)	**53.9 (6.7, ∞)**
TE	18	**28.1 (15.8, 74.1)**	**24.4 (7.0, ∞)**
JU	12	50.1 (12.4, ∞)	∞ (15.0, ∞)
BV	39	**22.8 (14.9, 37.6)**	∞ (∞, ∞)
LV	33	178.2 (43.2, ∞)	∞ (22.2, ∞)

AF = Afram, WB = White Volta-Binaba, WK = White Volta- Kantu, OT = Oti, AN = Ankobra, TA = Tano-Asuhyea, TE = Tano-Elubo, JU = Juen, BV = Black Volta, and LV = Lower Volta

Table 5. Summary statistics and *p*-values for bottleneck tests conducted for wild *O. niloticus* populations. Tests with significant *p*-values (α = 0.05) are indicated in bold.

Population	mean_N	mean_k	mean_He	p_sign_IAM	p_sign_SMM	p_stdv_IAM	p_stdv_SMM	p_W_1t_IAM	p_W_2t_IAM	p_W_1t_SMM	p_W_2t_SMM
AF	60.00	9.00	0.731	0.309	0.058	0.183	**0.000**	0.156	0.313	0.990	**0.027**
WB	58.50	7.38	0.716	0.097	0.186	**0.035**	**0.027**	**0.020**	**0.039**	0.770	0.547
WK	60.00	8.38	0.699	0.580	**0.001**	0.292	**0.000**	0.156	0.313	1.000	**0.004**
OT	59.75	8.63	0.683	0.429	**0.001**	0.489	**0.000**	0.578	0.945	1.000	**0.004**
PR	59.50	8.38	0.647	0.407	**0.001**	**0.047**	**0.000**	0.727	0.641	1.000	**0.004**
AN	60.00	6.63	0.584	0.455	**0.010**	0.183	**0.000**	0.680	0.742	0.996	**0.012**
TA	60.00	5.88	0.640	0.252	0.426	0.065	**0.007**	**0.027**	0.055	0.809	0.461
TE	35.75	8.38	0.789	0.106	0.054	0.059	**0.031**	**0.010**	**0.020**	0.986	**0.039**
JU	24.00	7.63	0.743	0.595	0.052	0.463	**0.001**	0.527	1.000	0.986	**0.039**
BV	74.25	9.25	0.707	0.574	**0.001**	0.469	**0.000**	0.320	0.641	1.000	**0.004**
LV	64.75	9.75	0.718	0.173	**0.010**	0.303	**0.000**	0.680	0.742	0.994	**0.020**

AF = Afram, WB = White Volta-Binaba, WK = White Volta- Kantu, OT = Oti, AN = Ankobra, TA = Tano-Asuhyea, TE = Tano-Elubo, JU = Juen, BV = Black Volta, and LV = Lower Volta. N = mean number of genotypes, k = mean number of alleles, *He* = mean heterozygosity, sign = sign test, stdv = standard differences test, W_1t = Wilcoxon one-tailed test, W_2t = Wilcoxon two-tailed test. IAM = Infinite Allele Model, SMM = Stepwise Mutation Model.

Table 6. Pairwise F_{ST} values from nuclear microsatellite DNA sequences for wild *O. niloticus* populations sampled from 11 sites (nine river basins) in Ghana from December 2014 to July 2017.

	AF	WVB	WVK	OT	PR	AN	TA	TE	JU	BV	LV
AF	–										
WVB	0.032	–									
WVK	0.031	0.021	–								
OT	0.019	0.041	0.038	–							
PR	0.045	0.063	0.062	0.076	–						
AN	0.078	0.073	0.068	0.111	0.046	–					
TA	0.143	0.113	0.137	0.182	0.151	0.125	–				
TE	0.044	0.039	0.053	0.071	0.056	0.074	0.066	–			
JU	0.070	0.072	0.095	0.109	0.093	0.116	0.068	0.023	–		
BV	0.094	0.088	0.077	0.106	0.130	0.135	0.143	0.083	0.105	–	
LV	0.034	0.050	0.059	0.057	0.030	0.082	0.156	0.049	0.060	0.091	–

AF = Afram, WB = White Volta-Binaba, WK = White Volta- Kantu, OT = Oti, AN = Ankobra, TA = Tano-Asuhyea, TE = Tano-Elubo, JU = Juen, BV = Black Volta, and LV = Lower Volta.

Table 7. AMOVA for 8 nuclear DNA microsatellites loci in wild tilapia populations collected from 11 sites in Ghana from December 2014 to July 2017.

Source of Variation	d.f.	Sum of Squares	Percentage of Variation
Among populations	10	149.28	8.24
Within populations	613	1509.61	91.96
Total	623	1658.89	

Table 8. Genetic divergence statistics for each loci of a given sample size (n). Genic differentiation (*G*) from Fisher's exact test, Hendrick's $G_{ST'}$, Jost's *D* are compared with F_{ST} values.

Locus	F_{ST}	G_{ST} (Hendrick)	*D* (Jost)	*G* (Fisher's exact test) p-values
UNH123	0.069	0.220	0.185	0.000
UNH178	0.067	0.189	0.156	0.000
UNH180	0.197	0.377	0.250	0.000
UNH203	0.083	0.159	0.106	0.000
UNH858	0.084	0.484	0.454	0.000
UNH898	0.087	0.330	0.287	0.000
UNH934	0.073	0.147	0.106	0.000
UNH991	0.115	0.224	0.159	0.000

Table 9. Pairwise F_{ST} values from nuclear microsatellite DNA sequences for farmed *O. niloticus* populations sampled from 5 farm aquaculture facilities in Ghana in 2017. AD = ARDEC, LE = Lee's Farm, VC = Volta Catch, AT = Akosombo Tilapia Farm, and FF = Fujian Farm.

	AD	LE	VC	AT	FF
AD	–				
LE	0.039	–			
VC	0.046	0.047	–		
AT	0.184	0.120	0.202	–	
FF	0.192	0.119	0.210	-0.000	–

Table 10. AMOVA for 8 nuclear DNA microsatellites loci in farmed tilapia populations sampled in Ghana in 2017.

Source of Variation	d.f.	Sum of Squares	Percentage of Variation
Among populations	4	97.89	11.91
Within populations	275	789.814	88.09
Total	279	887.70	

Table S1. Allele frequencies (%) across 8 nuclear microsatellite DNA loci for wild *O. niloticus* populations sampled from 11 sites (9 river basins) in Ghana from 2014 to 2017. AF = Afram, WB = White Volta-Binaba, WK = White Volta- Kantu, OT = Oti, AN = Ankobra, TA = Tano-Asuhyea, TE = Tano-Elubo, JU = Juen, BV = Black Volta, and LV = Lower Volta. Private alleles are indicated in Bold.

Locus	Populations										
UNH123	AF	WB	WK	OT	PR	AN	TA	TE	JU	BV	VR
171	6.67	8.62	5.00	–	–	–	–	–	–	–	–
173	–	–	–	–	–	1.67	–	–	4.17	10.26	6.06
175	28.33	22.41	43.33	26.67	40.00	61.67	20.00	16.67	29.17	38.46	42.42
179	–	–	**1.67**	–	–	–	–	–	–	–	–
181	–	–	–	–	–	–	–	**2.78**	–	–	–
187	–	–	–	–	–	–	–	–	–	**1.28**	–
189	1.67	–	1.67	5.00	1.67	–	–	–	–	–	3.03
191	–	5.17	1.67	–	–	–	–	**11.11**	–	–	–
193	–	–	–	–	–	–	–	**2.78**	–	–	–
195	10.00	17.24	10.00	1.67	25.00	18.33	40.00	19.44	8.33	10.26	6.06
197	10.00	17.24	10.00	1.67	3.33	13.33	35.00	16.67	33.33	3.85	1.52
199	–	–	–	–	–	–	–	5.56			1.52
201	**6.67**	–	–	–	–	–	–	–	–	–	–
203	–	–	1.67	3.33	10.00	1.67	1.67	–	8.33	2.56	–
207	18.33	24.14	6.67	33.33	13.33	1.67	–	5.56	–	–	27.27
209	8.33	3.45	8.33	13.33	6.67	–	–	2.78	4.17	3.85	6.06
211	6.67	1.72	10.00	11.67	–	–	1.67	13.89		23.08	3.03
213	–	–	–	–	–	–	–	–	**4.17**	–	–
215	–	–	–	–	–	–	1.67	–	8.33	–	–
217	–	–	–	–	–	–	–	–	–	**1.28**	–
219	3.33	–	–	3.33	–	1.67	–	–	–	3.85	3.03
235	–	–	–	–	–	–	–	–	–	**1.28**	–
245	–	–	–	–	–	–	–	**2.78**	–	–	–

Table S1. Continued.

UNH178	AF	WB	WK	OT	PR	AN	TA	TE	JU	BV	VR
114	–	–	–	–	–	3.33	–	–	4.17	–	1.52
120	33.33	40.00	50.00	50.00	51.67	50.00	10.00	41.67	20.83	19.23	36.36
122	–	–	–	–	–	–	–	–	**4.17**	–	–
124	5.00					1.67	–	8.33	–	3.85	9.09
126	16.67	8.33	23.33	3.33	8.33	15.00	8.33	11.11	–	5.13	12.12
128	3.33	16.67	3.33	6.67	5.00	23.33	5.00	5.56	12.50	3.85	3.03
130	13.33	1.67	1.67	5.00	21.67	5.00	11.67	11.11	4.17	16.67	15.15
132	21.67	11.67	3.33	11.67	11.67	1.67	16.67	2.78	25.00	1.28	9.09
134	–	–	–	15.00	–	–	–	–	–	2.56	–
136	**1.67**	–	–	–	–	–	–	–	–	–	–
138	–	–	–	–	–	–	–	–	–	**3.85**	–
140	3.33	15.00	16.67	8.33	1.67	–	23.33	5.56	12.50	41.03	12.12
142	–	–	1.67	–	–	–	25.00	13.89	16.67	–	–
144	1.67	6.67	–	–	–	–	–	–	–	2.56	1.52
UNH180	AF	WB	WK	OT	PR	AN	TA	TE	JU	BV	VR
121	–	–	–	–	1.67	–	–	23.53	–	–	–
125	10.00	1.72	–	5.00	–	–	–	5.88	–	11.54	4.84
127	1.67	–	1.67		–	–	–	2.94	–	19.23	–
129	–	–	–	**1.67**	–	–	–	–	–	–	–
135	1.67	–	–	1.67	–	–	–	–	–	–	–
137	46.67	39.66	20.00	41.67	81.67	90.00	83.33	47.06	87.50	17.31	66.13
139	6.67	–	6.67	16.67	–	–	–	–	–	–	–
141	–	–	–	–	–	–	–	–	–	–	**1.61**
143	25.00	8.62	15.00	3.33	1.67	8.33	3.33	20.59	8.33	46.15	–
145	–	–	–	**1.67**	–	–	–	–	–	–	–
149	–	–	–	–	**1.67**	–	–	–	–	–	–
155	8.33	50.00	56.67	18.33	11.67	–	13.33	–	4.17	3.85	17.74
165	–	–	–	–	–	**1.67**	–	–	–	–	–
169	–	–	–	1.67	–	–	–	–	–	–	3.23
171	–	–	–	**1.67**	–	–	–	–	–	–	–
177	–	–	–	5.00	1.67	–	–	–	–	–	4.84
179	–	–	–	–	–	–	–	–	–	**1.92**	–
187	–	–	–	1.67	–	–	–	–	–	–	1.61

Table S1. Continued.

UNH203	AF	WB	WK	OT	PR	AN	TA	TE	JU	BV	VR
65	38.33	33.33	23.33	30.00	18.33	28.33	43.33	41.67	54.17	15.38	25.76
67	55.00	45.00	65.00	68.33	38.33	55.00	45.00	27.78	20.83	75.64	33.33
79	–	–	–	–	–	–	–	**8.33**	–	–	–
81	–	–	–	–	–	–	–	–	–	–	**1.52**
85	–	–	–	–	–	–	–	**16.67**	–	–	–
89	–	–	–	–	–	–	–	–	–	**8.97**	–
91	3.33	1.67	–	–	–	–	–	–	–	–	–
93	–	–	–	–	**3.33**	–	–	–	–	–	–
95	3.33	20.00	10.00	1.67	38.33	16.67	11.67	5.56	20.83	–	39.39
97	–	–	1.67	–	1.67	–	–	–	4.17	–	–
UNH858	AF	WB	WK	OT	PR	AN	TA	TE	JU	BV	VR
196	1.67	–	3.33	1.67	–	–	–	11.11	8.33	–	–
198	–	–	**5.00**	–	–	–	–	–	–	–	–
200	–	–	–	–	–	–	–	–	–	**1.28**	–
204	1.67	–	–	1.67	–	–	–	–	–	2.56	–
208	3.33	–	1.67	–	1.67	–	–	–	4.17	2.56	10.00
210	11.67	8.93	3.33	10.00	–	–	–	–	–	3.85	–
212	–	–	–	–	3.33	5.00	–	5.56	–	–	15.00
214	–	3.57	–	–	–	–	–	–	–	1.28	–
216	1.67	–	–	–	1.67	–	8.33	2.78	4.17	11.54	3.33
218	5.00	–	3.33	1.67	18.33	–	1.67	8.33	4.17	6.41	5.00
220	11.67	7.14	5.00	30.00	–	–	–	2.78	12.50	1.28	–
222	1.67	–	–	–	3.33	1.67	8.33	11.11	–	–	1.67
224	–	5.36	5.00		16.67	26.67	56.67	25.00	33.33	1.28	–
226	–	10.71	16.67	1.67	–	–	6.67	5.56	–	–	–
228	–	–	–	–	–	3.33	–	8.33	–	1.28	1.67
230	–	3.57	3.33		5.00	6.67	1.67	2.78	–	–	3.33
232	8.33	7.14	11.67	5.00	–	–	3.33	–	–	1.28	1.67
234	–	1.79	–	3.33	–	–	–	–	–	–	1.67
236	–	–	5.00	–	1.67	6.67	–	5.56	–	6.41	–
238	–	5.36	–	–	10.00	26.67	6.67		4.17	15.38	15.00
240	10.00	14.29	3.33	10.00	3.33	3.33	6.67	2.78	20.83	6.41	18.33
242	15.00	8.93	8.33	15.00	11.67	3.33	–	8.33	4.17	35.90	11.67
244	10.00	5.36	16.67	1.67	20.00	16.67	–	–	–	1.28	6.67
246	13.33	7.14	6.67	11.67	3.33	–	–	–	4.17	–	3.33
248	5.00	10.71	1.67	5.00	–	–	–	–	–	–	1.67
252	–	–	–	**1.67**	–	–	–	–	–	–	–

Table S1. Continued.

UNH898	AF	WB	WK	OT	PR	AN	TA	TE	JU	BV	LV
214	–	–	–	–	–	–	–	–	–	**2.70**	–
232	1.67	1.67	–	–	–	–	1.67	8.33	4.17		7.58
234	16.67	16.67	13.33	1.72	1.79	1.67	–	2.78	–	4.05	1.52
236	1.67	25.00	30.00	20.69	–	1.67	–	–	–	–	–
242	–	1.67	–	–	7.14	1.67	–	19.44	29.17	39.19	27.27
244	36.67	35.00	35.00	29.31	51.79	71.67	36.67	25.00	4.17	13.51	28.79
246	5.00	–	–	–	–	–	16.67	2.78	–	10.81	4.55
250	5.00	1.67	–	–	–	–	–	–	–	–	–
252	–	5.00	3.33	–	–	–	–	–	–	–	3.03
254	–	–	–	1.72	–	–	–	2.78	–	–	1.52
256	1.67	–	–	–	–	3.33	3.33	–	4.17	6.76	–
258	–	–	1.67	1.72			1.67	–	4.17	–	–
260	–	10.00	1.67	1.72	3.57	3.33	11.67	5.56	–	1.35	6.06
262	–	–	–	–	–	–	–	–	4.17	1.35	–
264	1.67	–	–	–	1.79	–	–	5.56	8.33	–	–
266	3.33	–	–	1.72	3.57	–	–	–	4.17	–	–
268	1.67	–	1.67	1.72	1.79	–	13.33	–	8.33	12.16	4.55
270	1.67	–	–	–	–	–	1.67	5.56	–	1.35	3.03
272	1.67	1.67	1.67	5.17	1.79	–	–	5.56	4.17	5.41	–
274	11.67	–	3.33	15.52	5.36	11.67	11.67	13.89	8.33	1.35	1.52
276	–	–	**3.33**	–	–	–	–	–	–	–	–
278	1.67	–	–	–	1.79	–	–	–	–	–	–
280	8.33	–	–	5.17	5.36	–	–	2.78	8.33	–	6.06
282	–	1.67	–	12.07	3.57	–	–	–	–	–	3.03
284	–	–	5.00	–	1.79	1.67	–	–	4.17	–	–
286	–	–	–	1.72	8.93	3.33	1.67	–	–	–	1.52
292	–	–	–	–	–	–	–	–	**4.17**	–	–

Table S1. Continued.

UNH934	AF	WB	WK	OT	PR	AN	TA	TE	JU	BV	VR
214	43.33	70.69	51.67	56.67	45.00	51.67	61.67	55.56	33.33	57.69	43.94
216	–	–	5.00	–	3.33	8.33	36.67	13.89	25.00	5.13	–
218	–	–	–	–	–	–	–	8.33	–	11.54	–
220	–	1.72	3.33	–	–	–	–	–	–	–	1.52
222	1.67	–	–	1.67	25.00	5.00	–	2.78	–	–	4.55
226	–	–	–	1.67	–	–	–	–	–	1.28	–
228	–	–	–	–	–	–	–	–	–	–	**3.03**
230	10.00	1.72	–	–	3.33	5.00	–	–	4.17	–	3.03
232	3.33	1.72	15.00	5.00	6.67	–	–	–	–	–	–
234	–	–	–	–	1.67	1.67	–	–	16.67	1.28	3.03
236	3.33	13.79	1.67	–	3.33	25.00	–	2.78		2.56	–
238	3.33	–	–	–	1.67	–	–	–	–	–	1.52
240	26.67	10.34	15.00	33.33	10.00	–	–	8.33	16.67	1.28	33.33
242	8.33	–	8.33	1.67	–	–	–	8.33		1.28	3.03
244	–	–	–	–	–	–	–	–	4.17	15.38	–
246	–	–	–	–	–	3.33	1.67	–	–	2.56	3.03

UNH991	AF	WB	WK	OT	PR	AN	TA	TE	JU	BV	VR
150	–	–	–	–	–	–	–	–	–	2.56	3.03
152	20.00	34.48	38.33	23.33	6.67	6.67	16.67	19.44	16.67	39.74	16.67
154	–	–	–	–	–	–	–	–	–	–	**1.52**
156	–	–	–	–	–	–	–	–	**4.17**	–	–
160	5.00	1.72	3.33	1.67	–	–	–	–	–	10.26	1.52
162	–	–	–	–	–	–	–	–	**8.33**	–	–
166	–	–	–	–	–	–	–	–	**8.33**	–	–
168	1.67	–	1.67	–	1.67	1.67	–	–	–	–	1.52
170	1.67	3.45	–	–	3.33	16.67	16.67	5.56	12.50	15.38	7.58
172	71.67	44.83	50.00	70.00	76.67	53.33	6.67	44.44	37.50	30.77	65.15
174	–	–	–	–	–	1.67	1.67	5.56	–	–	–
176	–	–	–	1.67	3.33	–	15.00	13.89	4.17	–	–
178	–	13.79	3.33	3.33	3.33	1.67	–	–	–	–	1.52
182	–	1.72	3.33	–	5.00	18.33	43.33	11.11	8.33	1.28	1.52

Table S2. STRUCTURE results with estimates for probable *K* clusters for *O. niloticus* from Ghana. *K* = 10 as the most likely number of clusters is indicated in bold.

Run #	K	Est. Ln prob. of data	Mean value of Ln likelihood	Variance of Ln likelihood
21	1	-9554.4	-9518.7	71.5
20	1	-9553.8	-9518.4	70.8
23	2	-9106.2	-9025.1	162.2
24	2	-9105.8	-9031.7	148.2
22	2	-9111.8	-9028.1	167.4
25	3	-8795.1	-8690.6	209
27	3	-8789.3	-8684.8	209
26	3	-8789.1	-8684.5	209.1
29	4	-8571.9	-8435.9	271.9
30	4	-8697.9	-8544.8	306.1
28	4	-8556.5	-8427.9	257.3
33	5	-8452.5	-8280	345
32	5	-8452.9	-8283.1	339.7
31	5	-8451.7	-8289.4	324.6
34	6	-8335.2	-8134.9	400.5
36	6	-8345.9	-8135.1	421.5
35	6	-8350.3	-8154.1	392.4
37	7	-8252.8	-8015	475.5
39	7	-8235.7	-8013.3	444.7
38	7	-8248.8	-8014.6	468.5
41	8	-8209.3	-7963.4	491.6
40	8	-8224	-7961.1	525.7
42	8	-8182.1	-7951.9	460.5
43	9	-8198.9	-7895	607.8
45	9	-8173.1	-7892.1	562
44	9	-8176.7	-7891.4	570.6
47	10	-8190.9	-7881	619.8
46	10	-8151.2	-7847.8	606.7
48	**10**	**-8118.8**	**-7830.1**	**577.5**
50	11	-8187.9	-7804.1	767.5
49	11	-8207.8	-7785.8	844
51	11	-8134.6	-7780.5	708.1

Chapter 4: Phenotypic variation for tolerance of high temperature in *O. niloticus* from the Volta River basin

Abstract

Fish farming in West Africa is dominated by outdoor culture systems such as ponds and cages, which often face high temperature and low dissolved oxygen conditions. However, the phenotypic variation displayed by different fish populations in response to these stressful environmental conditions is not well understood. This study was conducted to characterize the phenotypic variation of tolerance of high temperature in wild *Oreochromis niloticus* from the Volta basin (Afram and White Volta), in comparison with that of the selectively bred Akosombo strain widely cultured in Ghana. I found no statistically significant difference in the growth performance of all the stocks tested under the three temperature treatments (low– 26 ^{o}C, medium – 29 ^{o}C, and high – 32 ^{o}C). However, the results revealed that the Akosombo strain exhibited slightly better growth performance across the three temperature levels than the wild stocks. I found that the Afram population's performance mirrored that of the Akosombo strain, with most rapid growth recorded at the medium temperature level. The White Volta population on the other hand showed most rapid growth at both low and high temperature conditions, suggesting adaptation to high and wider temperature conditions. The results suggest that selective breeding experiments could focus on northern Ghanaian populations of *O. niloticus* to exploit high temperature tolerance due to their occurrence where water temperatures are relatively warm.

Introduction

Nile tilapia (*Oreochromis niloticus*) is the primary species contributing to the expansion of aquaculture in West Africa within the last two decades (FAO 2012). However, little is known about the performance of regional strains under West African conditions because most studies were conducted in Egypt or outside of Africa (Hulata et al. 1986; Elghobashy et al. 2000). Fish farming in West Africa is dominated by outdoor culture systems (ponds, tanks and cages) often faced with high temperatures and low dissolved oxygen (DO) levels. Hence, climate change and its potential effects on aquatic life may present significant challenges to the resilience of aquaculture in the region. Adaptation of *O. niloticus* to future climate conditions such as increase in temperature and decrease in DO will therefore be crucial to sustaining aquaculture in West Africa.

Selective breeding is a widely applied quantitative genetics approach that can be utilized to develop strains that are adapted to the current and future climate conditions of West Africa. Selective breeding is the only genetic improvement approach that could potentially provide permanent genetic gain that is transmittable from generation to generation (Hallerman 2000; Lind et al. 2012; Thoa et al. 2016). Growth rate is one of the most important breeding goals in selection experiments, and often can be improved using individual-based "mass" selection because it often has a sufficiently high heritability. Varying levels of success have been achieved from *O. niloticus* selective breeding experiments based on the mass selection approach. Hulata et al. (1986) and Huang and Liao (1990) both found little or no improvement over the base population using mass selection. However, later studies showed that mass selection can improve growth rate in *O. niloticus* significantly (Li et al. 2006). The earlier studies may have achieved no improvement in growth rate in *O. niloticus* because of previous bottlenecks in the founder populations, most likely as a result of conducting their experiments with small populations. Provided there is enough genetic variation within the population, such as in wild populations with no recent history of bottlenecks, mass selection can be useful in the early stages of a selective breeding program to improve growth rate (Eknath et al. 1993; Hallerman 2000; Attipoe et al. 2013).

Frimpong et al. (2016) provided valuable baseline information for designing an effective selective breeding program for West African strains of *O. niloticus*. Using a combination of field, laboratory growth trials and genetics studies, the authors reached conclusions that have the potential to change the prospects for tilapia aquaculture in Ghana and West Africa. A year-long sampling of three rivers, Afram, Oti and White Volta revealed water temperature variations along the latitudinal gradient in Ghana, with the Oti River exhibiting significantly higher water temperatures than the others (Frimpong et al. 2016). Although the authors noted that the results from the growth experiment need to be interpreted with caution due to variability in initial sizes of fish used and low replication, the combined results of the growth studies and the water quality sampling suggested that at least the Oti population might already be adapted to high temperature conditions. Frimpong et al. (2016) also found that the Akosombo strain, which was genetically differentiated from all the wild stocks studied, did not show superior growth performance under current or predicted future climate conditions. If confirmed, these results provide a compelling reason to begin discussions and ground work for developing alternative aquaculture strains from local stocks in Ghana.

Frimpong et al. (2016) also conducted a meta-analysis using results of 19 studies on the effects of temperature, DO, and salinity on the growth of *O. niloticus*. The authors found that temperature and DO had the most significant relationships with specific growth rate (Frimpong et al. 2016) and suggested that temperature and DO are the two most important environmental factors to consider in future growth trials. In general, the optimum temperature for *O. niloticus* growth is about 29 ^{o}C (Likongwe et al. 1996; Al-Asgah and Ali 1997; Baras et al. 2001; Moura et al. 2007, Azaza et al. 2008; El-Sayed and Kawanna 2008; Drummond et al. 2009; Xie et al. 2011; Frimpong et al. 2016). The importance of DO on growth, survival, and reproduction in *O. niloticus* is well established in the literature (Ishibashi et al. 2002; Evans et al. 2003; Delaney and Klesius 2004; Xu et al. 2006; Kolding et al. 2008; Tran-Duy et al. 2008). However, due to the negative correlation between temperature and DO in aquatic environments, it is challenging to estimate the separate effects of temperature and DO on the performance *O. niloticus* under experimental conditions (Frimpong et al. 2016). Hence, it may be more practical for future studies to focus on one factor at a time in characterizing the performance of *O. niloticus* under current and predicted stressful West African environmental conditions.

Some studies have been conducted for cold-tolerance and its heritability in *O. niloticus* (Li et al. 2002; Atwood et al. 2003; Cnaani et al. 2003; Charo-Karisa et al. 2005), but information on tolerance for high temperature is generally lacking. As far as West African populations of *O. niloticus* are concerned, little is known about the phenotypic variance for tolerance of high temperatures and low DO. This information is important for assessing whether selective breeding may prove useful for improving the performance of local strains of *O. niloticus* under current and expected increased temperature and decreased DO conditions in West Africa.

Given this background, I conducted this study to characterize the tolerance to high-temperature conditions in two wild *O. niloticus* populations in Ghana to provide baseline information for a future selective breeding program.

Methods

Experimental location

I conducted the experiment in a wet laboratory located at the Faculty of Renewable Natural Resources Fish Farm, Kwame Nkrumah University of Science and Technology (KNUST), Ghana. The laboratory is equipped with over forty 16-gallon aquarium tanks and supplies, and was designed for conducting controlled laboratory growth trials. The Pilot Aquaculture Center (PAC) of the Fisheries Commission of Ghana provided concrete tanks of 10m^3 volume for holding parental stock, breeding of fish, and raising of fingerlings for experimentation at KNUST.

Experimental fish

O. niloticus parental stock were collected from the Afram River and White Volta in Aframso and Binaba townships, respectively, between December 2016 and January 2017. Fish were kept in concrete tanks at PAC, and fed high-protein diets for four to five months to produce approximately equal-aged experimental fish. The regionally important Akosombo strain of *O. niloticus* was obtained from PAC and served as a reference line.

Experimental design and conditions

I used a factorial design for the experiment with one factor (temperature) and three levels (low at 26 ^{o}C, medium at 29 ^{o}C, and high at 32 ^{o}C). High and medium water temperatures were obtained using individual aquarium heaters. Low temperature was the default room temperature, which was maintained with an air conditioner and fans. Twenty-one experimental tanks were used and each treatment had seven replicates. A pre-test was conducted for a month prior to the start of the experiment to refine the design and to ensure that the desired conditions were achieved. All experimental units were fitted with bubblers and water filters.

I stocked each of the twenty-one aquarium tanks with three fish, one fish from each of the three populations. Initial fish weights were recorded before stocking in tanks. Fish were fed ad libitum twice per day with commercial feed (Raanan Fish Feed, 48% crude protein) for the duration of the experiment, and the daily amount was recorded per tank. Prior to the experiment, fish were uniquely tagged by a fin-clip. Binaba stock (White Volta River) were clipped at the anal fin while the Akosombo strain were clipped at the dorsal fin. Aframso stock were not clipped because they appeared stressed at the beginning of the experiment. However, about two weeks into the experiment when all fish appeared stabilized, the surviving fish of the Aframso population were clipped at the caudal fin. The late tagging of the Aframso stock was necessary because it facilitated easy identification of fish from the three different populations, especially in the event of mortality. Fish were observed daily for signs of disease, and mortality recorded was used to compute fish survival at the end of the experiment.

Temperature, dissolved oxygen, and pH were measured twice daily with Sper Scientific and Hanna multiparameter meters. Water quality measurements were taken between 9:00 and 12:00 hrs, and between 18:00 and 19:00 hrs. Ammonia, nitrite and nitrate were measured thrice during the experiment with the API Freshwater Master Test Kit (Mars FishCare, USA). The experiment lasted for one month from May 25, 2017 to June 24, 2017, and final fish weights were recorded.

Statistical analysis

Data on the initial fish weights and the final fish weights were used to calculate growth in each experimental unit. Growth was estimated using specific growth rate [(Ln final weight - Ln initial weight) /time (duration in experiment) × 100]. A mixed-effects analysis of variance model accounting for fixed and random effects with two-factor interactions (temperature and strain) was used to analyze the data statistically, with Tank ID as a random block factor. Box-plots were used to graphically visualize results. All statistical analysis and plots were performed in R$^{©}$ statistical software version 3.5.1 (R Core Team 2018).

Results

Water quality measurements

The mean temperature recorded in the low treatment was 26.2^{o}C, medium was 29.4^{o}C, and high was 32.0^{o}C. As expected, the highest dissolved oxygen (DO) was recorded in the low temperature treatment (6.6 mg/L). The medium and high temperature treatments exhibited slightly lower DO values of 5.8 and 5.3 mg/L, respectively. The pH recorded was slightly acidic across treatments and ranged between 5.6 to 5.9. Nitrate (25.6 – 39.4 mg/L) and ammonia (0.71 – 1.22 mg/L) levels across treatments were higher than the levels recommended for fish (Hickey and Martin 2009; USEPA 2009). Nitrite (0.94 – 1.95 mg/L) was also high (Bhatnagar et al.

2004). However, both the ammonia and nitrite levels recorded were similar to those recorded in semi-intensive aquaculture operations in Ghana (Frimpong et al. 2014).

Effect of temperature treatment and strains on growth and survival

Overall, the Akosombo strain showed more rapid growth across all temperature treatments than the Aframso and Binaba populations (Figure 1). Growth was highest in the medium temperature level for both the Aframso stock and the Akosombo strain, while the Binaba stock recorded the least growth (Table 1). However, both populations also showed high variability in growth in the medium treatment. Binaba exhibited similar growth in both low and high treatment levels, suggesting a high temperature tolerance for the population. Overall, the Akosombo strain recorded the highest survival when averaged across treatments. Although all populations had varying degrees of infection in holding tanks prior to stocking, the Akosombo strain generally recovered more quickly than the wild stocks. In terms of treatments, the highest survival was recorded in the high temperature treatment and ranged from 93 to 100% (Table 1). The lowest survival was recorded in the low treatment level for the Binaba stock and the Akosombo strain, but the Aframso stock exhibited lowest survival in the medium treatment (Table 1).

The mixed-effects model showed no significant overall effect of temperature or population on fish growth ($\alpha = 0.05$) (Tables 2; Figures 2 and 3). Also, no significant interaction effect was observed between the temperature levels and the three different populations (Table 3).

Discussion

The results of this study showed that neither temperature nor population significantly affected fish growth. However, the study also revealed that, although not statistically significant, the Akosombo strain had better overall growth performance when all temperature levels were considered. These findings suggest that some genetic gains have indeed been achieved through selective breeding of the Akosombo strain, but also that the Akosombo strain may not necessarily be superior to wild stocks in terms of growth in current typical environments, corroborating the findings of Frimpong et al. (2016). Because laboratory conditions may not reflect culture conditions, it would be useful to repeat this experiment under typical rearing conditions to better compare the performance of the Akosombo strain to wild stocks. This information would guide the direction of future selective breeding programs in the region.

A key observation made in this study was the overall hardiness of the Akosombo strain. This study showed that the Akosombo strain had the highest overall survival across temperature treatments compared to both Aframso and Binaba stocks. This finding reflects the Akosombo strain's long-term acclimation to captive conditions and accompanying handling and transportation stress. Both the Aframso and Binaba stocks were prone to infection from injuries associated with travel from the sites where they were collected to the holding tanks at PAC. Additionally, the Akosombo strain recovered faster after infections compared to the wild stocks. Mortalities from similar infections resulted in the exclusion from this study fish from Sabare (Oti River), which Frimpong et al. (2016) previously recommend for future studies.

The gains in growth rate from the selective breeding program, coupled with the strain's hardiness, offers the Akosombo strain an advantage should development of the Akosombo strain be continued. However, given the likelihood that the Akosombo strain may be contaminated with the GIFT strain of *O. niloticus* (Chapters 2 and 3), it is imperative that the base population of the Akosombo strain is thoroughly screened using variable genetic markers to ascertain the extent of admixture with GIFT and to identify possible purebreds. Genetic screening of the Akosombo strain should be treated as a matter of urgency to avoid the unintended spread of GIFT and Akosombo strain hybrids to fish farmers across Ghana.

This study also revealed that the Akosombo strain's optimal temperature for growth is around 29^{o}C. This finding is important because it provides insight into the temperature tolerance of *O. niloticus* under West African culture conditions and shows similarity of *O. niloticus* temperature tolerance across Africa and Asia (Likongwe et al. 1996; Al-Asgah and Ali 1997; Baras et al. 2001; Moura et al. 2007; Azaza et al. 2008; El-Sayed and Kawanna 2008; Drummond et al. 2009; Xie et al. 2011; Frimpong et al. 2016). The finding that the optimal temperature for growth performance of *O. niloticus* is about 29^{o}C reflects how selective breeding programs have prioritized weight gain, but not temperature tolerance over the years.

This study aimed to assess the growth performance of the different strains at high temperature to provide insight into which resource strains could have high temperature tolerance, and prove useful for future selective breeding. The results of this study suggest that both the Akosombo strain and the Aframso stock may not be attractive candidate populations for selection for tolerance of high temperatures. On the other hand, the Binaba stock could be considered for future studies considering that its growth performance was similar across the three temperature levels. The Sabare stock (Oti River; not tested in this study) also should be included in future studies due to the possibility that the stock may already be adapted to high temperature conditions in its native environment (Frimpong et al. 2016). Future screening for potential resource stocks could also consider populations from the Black Volta River because of earlier findings that the Black Volta stocks may be more genetically diverse (Chapters 2 and 3).

Conclusion and Implications

This study characterized the phenotypic variation for tolerance of high temperature in two wild stocks of *O. niloticus* from the Volta basin (Aframso and Binaba), compared to that of the widely cultured Akosombo strain. I found no statistically significant difference in the growth performance among the populations tested under the three temperature treatments (low, medium, and high). However, the study revealed that the Akosombo strain showed a slightly higher growth across the three temperature levels than the wild stocks.

I also found that the Aframso stock's performance mirrored that of the Akosombo strain, with most rapid growth recorded at approximately 29 ^{o}C (medium temperature level). The Binaba stock, on the other hand, showed most rapid growth at both low (26 ^{o}C) and high (32 ^{o}C) temperature conditions suggesting that they could have a natural adaptation for high temperature tolerance. The results of this study suggest that future selective breeding experiments could focus on northern Ghanaian populations of *O. niloticus*. Focusing on northern populations would take advantage of the populations' potentially inherent high temperature tolerance because they occur at latitudes where water temperatures are relatively warm (Frimpong et al. 2016).

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Figures and Tables

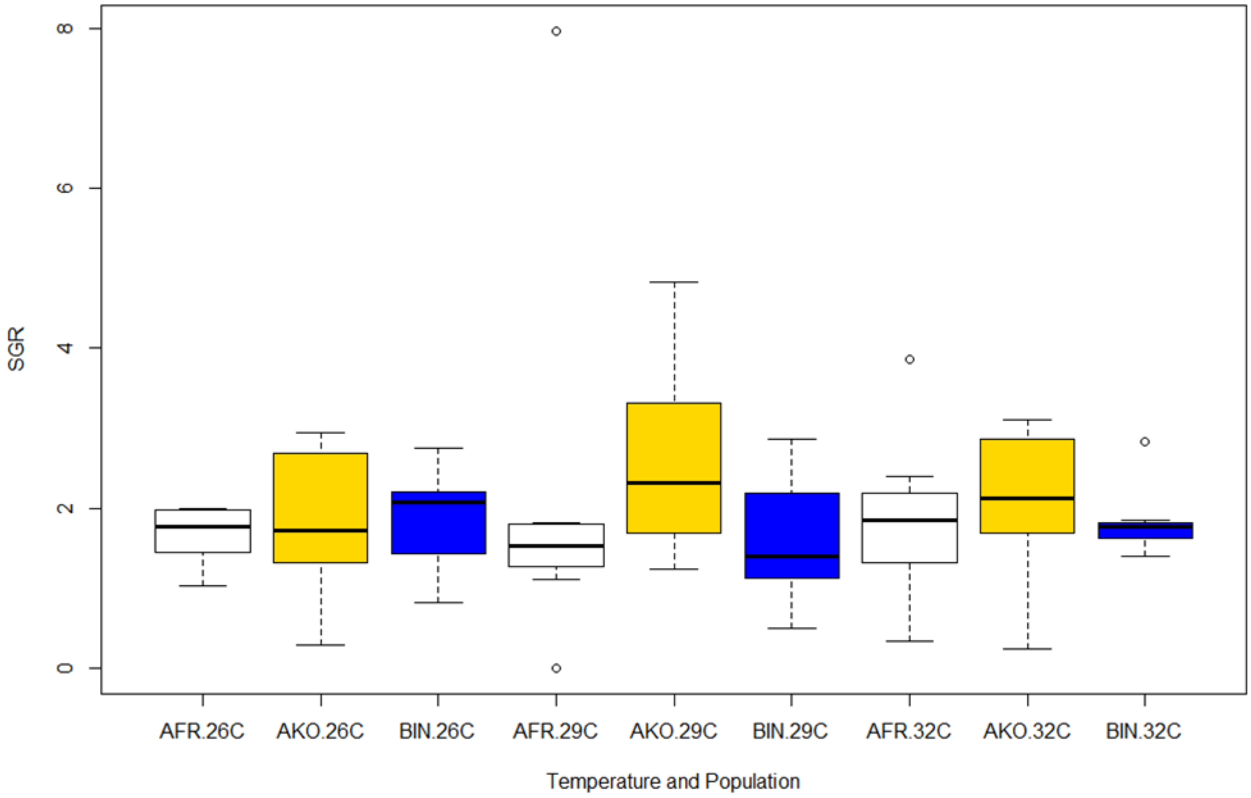

Figure 1. Comparison of growth of tilapia *Oreochromis niloticus* among three populations, (Afram and Binaba stocks, and Akosombo strain) and three treatment levels of temperature.

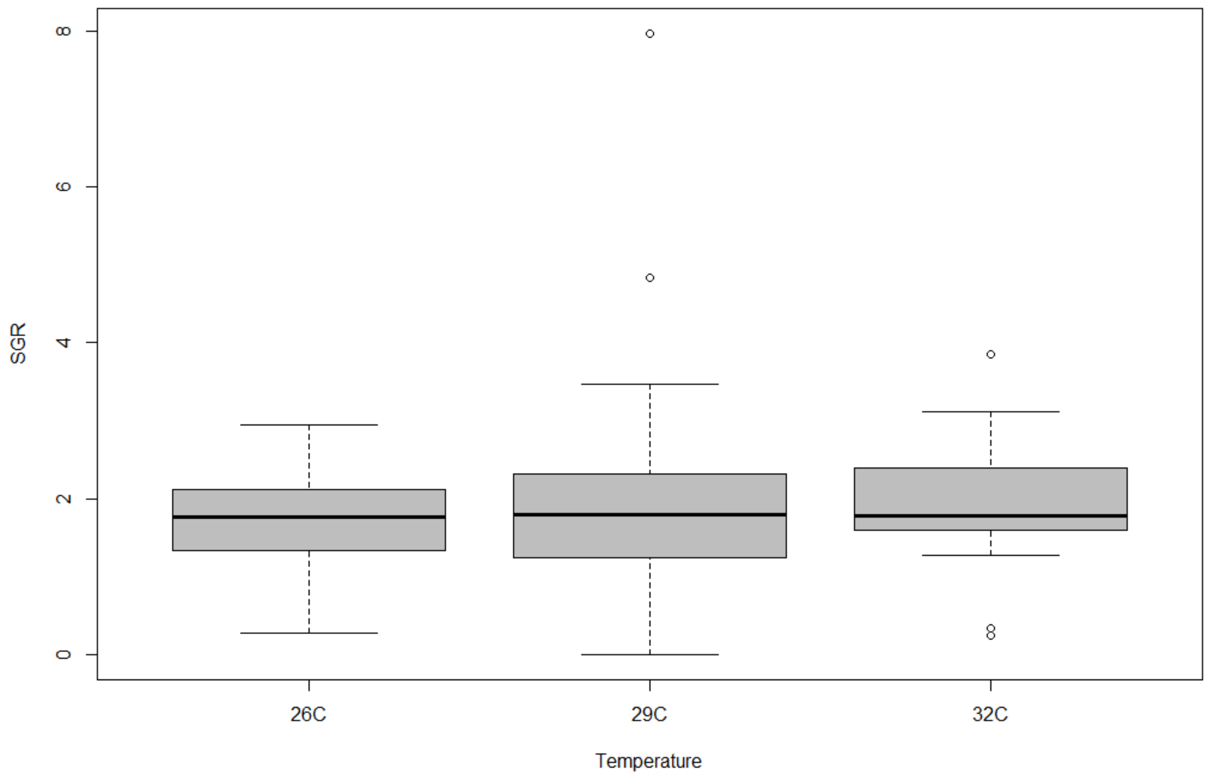

Figure 2. Comparison of growth of tilapia *Oreochromis niloticus* at three treatment levels of temperature for all populations (Afram and Binaba stocks, and Akosombo strain) combined.

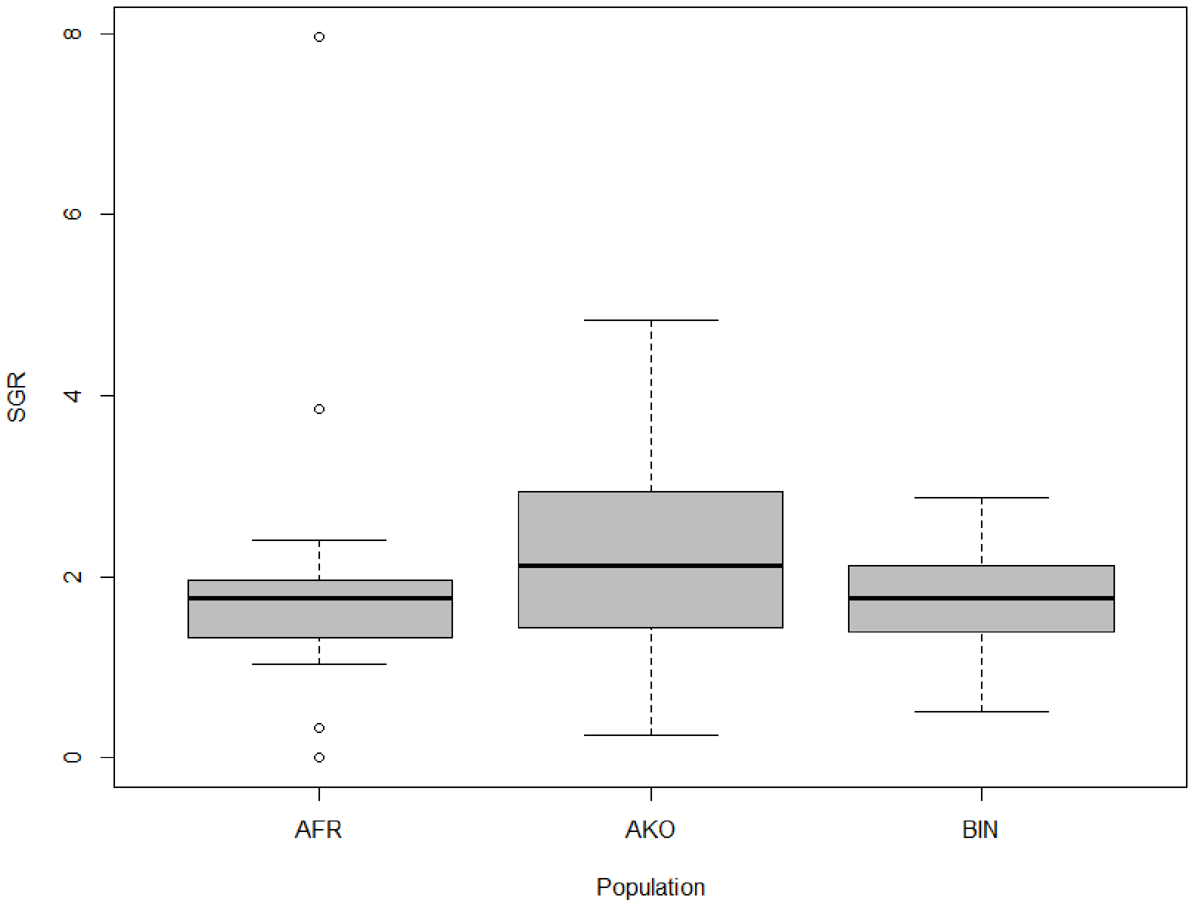

Figure 3. Comparison of growth of among three populations (Afram and Binaba stocks, and Akosombo strain) of Nile tilapia *Oreochromis niloticus.*

Table 1. Summary of treatment levels, growth and survival parameters measured for *O. niloticus*.

Population	Temperature Level	IWGT (Mean ± SD)	FWGT (Mean ± SD)	SGR (Mean ± SD)	Survival (%)
AFR	Low	4.71 ± 0.69	7.51 ± 1.81	1.66 ± 0.38^{a}	87
AKO	Low	4.89 ± 0.99	8.47 ± 2.82	1.85 ± 0.98^{a}	86
BIN	Low	5.60 ± 1.82	8.70 ± 3.48	1.84 ± 0.66^{a}	76
AFR	Medium	5.00 ± 0.88	7.39 ± 2.19	2.24 ± 2.60^{b}	76
AKO	Medium	5.49 ± 0.43	11.01 ± 2.44	2.62 ± 1.28^{b}	92
BIN	Medium	5.21 ± 0.76	8.00 ± 2.19	1.63 ± 0.82^{b}	86
AFR	High	4.61 ± 0.81	8.41 ± 3.23	1.86 ± 1.02^{c}	100
AKO	High	5.19 ± 0.47	9.44 ± 2.84	2.08 ± 1.00^{c}	93
BIN	High	5.30 ± 1.25	9.13 ± 2.80	1.84 ± 0.46^{c}	95

IWGT = Initial fish weight, FWGT = Final fish weight, and SGR = Specific growth rate. SGR was as [Ln final weight - Ln initial weight) /time × 100], where time is the number of days each fish lasted in the experiment. Means with the same letter within treatment levels are not significantly different ($\alpha = 0.05$).

Table 2. Effect size and significance of fixed effects of temperature level and population of Nile tilapia *Oreochromis niloticus*. The dependent variable specific growth rate and Tank ID is the random effect.

Parameter	Estimate	Std. Error	*t*-value	*Pr*(>|*t*|)
(Intercept)	1.3806	0.4624	2.986	0.0042
PopAKO	0.2671	0.3627	0.737	0.4644
PopBIN	-0.1500	0.3627	-0.414	0.6807
TRT_TEMP29C	0.4638	0.3707	1.251	0.2160
TRT_TEMP32C	-0.1893	0.4657	-0.406	0.6860
TankID	0.0407	0.0359	1.134	0.2617

Table 3. Effect size and significance of fixed effects in temperature level and population of tilapia. The dependent variable is specific growth rate and Tank ID is the random effect.

Parameter	Estimate	Std. Error	*t*-value
(Intercept)	1.6614	0.4554	3.648
PopAKO	0.1900	0.6324	0.300
PopBIN	0.1829	0.6324	0.289
TRT_TEMP29C	0.5757	0.6441	0.894
TRT_TEMP32C	0.1986	0.6441	0.308
PopAKO:TRT_TEMP29C	0.1971	0.8944	0.220
PopBIN:TRT_TEMP29C	-0.7943	0.8944	-0.888
PopAKO:TRT_TEMP32C	0.0343	0.8944	0.038
PopBIN:TRT_TEMP32C	-0.2043	0.8944	-0.228

Chapter 5. General Conclusions and Implications

The importance of Nile tilapia *Oreochromis niloticus* in both global aquaculture production and commercial fisheries, especially in sub-Saharan Africa, cannot be overemphasized. As anthropogenic activities continue to threaten *O. niloticus* in their native environments, population decline is likely and this important genetic resource could be compromised. Understanding the genetic variation within and among wild populations is crucial for identifying potentially imperiled populations and for conserving them. This information also would aid in the purposeful selection of genetically diverse populations for sustainable aquaculture. The key findings from this study, which are discussed below, have implications for *O. niloticus* management and conservation in Ghana, and in Africa as a whole.

1) **Pure *O. niloticus* populations occur in the wild in Ghana, but some populations are naturally hybridized with *O. aureus*, which previously has not been documented.**
   The finding has several implications. First, it suggests that if *O. aureus* occur in Ghanaian rivers and natural hybridization continues, pure *O. niloticus* populations would be difficult to find in the wild in the foreseeable future. Indeed, the rate at which introgressed hybridization is being reported among *O. niloticus*, *O. aureus*, and *O. mossambicus* (e.g. Rognon and Guyomard 2003; D'Amato et al. 2007; the present study), suggests that pure populations of any one of these species may go "extinct" within the foreseeable future. This interspecific crossing could be evolutionarily significant because of the potential for speciation through hybridization. It would be useful to genetically characterize as many wild populations as possible across Africa to understand the extent of the hybridization among these species and to design measures to preserve pure germplasms for each of them.

   Second, the fact that introgressed hybrids of *O. niloticus* x *O. aureus* co-occurred with pure populations of *O. niloticus* in nearly all river basins in Ghana suggest two things: 1) the natural range of *O. aureus* has not been properly delineated, or 2) recent colonization into some West African rivers such as observed in this study has yet to be documented. These results warrant the design of a careful study, which involves extensively surveying all rivers in Ghana and neighboring countries to document the presence of *O. aureus* and to properly delineate its range.

Third, the identification of *O. niloticus* based primarily on the dark longitudinal bands on the caudal fin is no longer very useful in differentiating the species from closely related *O. aureus* and *O. mossambicus*. I found some morphological differences in terms of body shape and coloration in the tilapia samples collected in this study (Figure 1). However, all individuals sampled had the characteristic dark longitudinal bands on the caudal fins, even though there were slight variations in the closeness of the bands. Future work could focus on using as many morphometric and meristic characters as possible combined with genetic data to differentiate pure populations from hybrids.

2) **Several wild populations of *O. niloticus* have recently experienced genetic bottlenecks, evident by loss of genetic diversity and heterozygosity excess.**

This finding underscores the importance of proactively monitoring fisheries resources. Given that inland fisheries management is almost non-existent in Ghana, population declines such as those reported in this study could go unnoticed to the point of complete collapse of an entire fishery. The Black Volta River and the Tano River at Asuhyea populations appeared particularly vulnerable because they were genetically distinct from other populations and may be demographically isolated from other populations. None of these populations are actively managed by the inland fisheries department of the Fisheries Commission, yet fishing occurs daily in these rivers.

Effective inland fisheries management is required to ensure that we have thriving populations of *O. niloticus*. For example, at Ankobra, fishermen reported significant decline in fish catch to the extent that they imported fish from Half-Assini to trade at Ankobra. Ankobra also is one of the main rivers heavily impacted by illegal alluvial gold mining in Ghana. Overfishing coupled with the extensive habitat modification and destruction currently ongoing in freshwater systems in Ghana may contribute to extirpation of many *O. niloticus* populations. Conspicuously missing in the national dialogue of curbing illegal mining in Ghana is the voice of fisheries managers and scientists. The results from this study should be used to advocate for effective regulations, which would reduce the impact of mining on aquatic habitats and help recover declining populations of *O. niloticus*. A vibrant fishery is not only important for the sake of the species, but also for the many fishing communities whose sole livelihoods depend on a thriving fishery.

3) **At least two farms were growing non-native strains of tilapia, and some fish have escaped into at least the Lower Volta River and admixed with native populations.** As previously discussed, wild populations of *O. niloticus* in Ghanaian rivers may already be threatened by natural hybridization with *O. aureus*. The presence of non-native strains, some of which are hybrids with *O. mossambicus*, further threaten wild *O. niloticus* populations. My research showed that some wild populations, particularly in the Black Volta River, the White Volta and the Oti, may be unimpacted by interbreeding with non-native strains of tilapia. Hence, it is important to extensively characterize "pure" populations and to create sanctuaries or refuge zones to prevent future contact with non-native tilapia. My research findings also show the need to strictly monitor fish farmers across Ghana, since further importation of more non-native strains would undermine any effort to preserve native populations. Given that all the major basins studied are shared with at least one neighboring country, the activities of which are not regulated by Ghana, it will be prudent to develop a regional approach to conserve *O. niloticus* genetic resources for the benefit of all stakeholder countries.

4) **There are admixed individuals at the Aquaculture Research and Development Centre (ARDEC) due to a possible crossing of the Akosombo strain with the genetically improved farmed tilapia (GIFT) strain during experimentation with the two strains.** This finding warrants further screening of fish at ARDEC to ascertain purity of the Akosombo strain, especially since I also found signals of the GIFT in fish from the Pilot Aquaculture Centre (PAC). Given this possibility, it would not be advisable for the government to proceed with its plan to stock irrigation reservoirs in northern Ghana with *O. niloticus* fingerlings supplied by ARDEC, and the other two government hatcheries (PAC and the Ashaiman hatchery). Stocking these reservoirs would be one of the surest ways to contaminate the northern sub-basins of the Volta River with non-native strains. Even if the Akosombo strain is uncontaminated, it is still not advisable to conduct mass stocking of reservoirs without careful analysis of the genetic impact upon wild populations.

5) **The Binaba population may have adaptation for high water temperature.**
This finding is promising for a future selective breeding program focusing on selecting traits related to high temperature tolerance. Even though the Sabare population was not tested in this study, it is still an important population to consider with regard to high-temperature tolerance. The Black Volta populations also are potentially viable for aquaculture development and should be considered in future experiments.

References

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Figures and Tables

Figure 1. *Oreochromis* spp. collected from different sites in Ghana showing morphological variations.