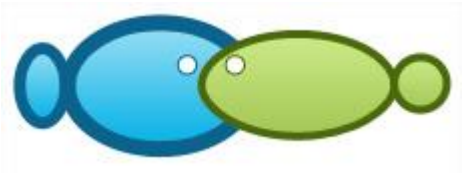

Genetic characterization required for morphological validation of *Oreochromis* sp. strains

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Abstract. This study was designed to characterize local (i.e., from Makurdi, Nigeria) and foreign (i.e., from Swansea) strains of *Oreochromis niloticus*. Truss network method was used to obtain morphometric data to understand the extent of phenotypic variation between the two strains. Mitochondria DNA cytochrome oxidase subunit I (COI) was however used to genetically confirm the identity of the strains. Result obtained revealed morphometric similarities and a wide overlap of the two strains as shown by the multivariate analysis revealed in the scattered plots/dendrogram plot. However, molecular characterization of the morphologically confirmed local strain showed a COI sequence of *Oreochromis aureus.* Although the phenotypic resemblance of the local strain to *O. niloticus* is not well understood, its genetic identity might have resulted from several generations of cross-fertilization between ♀ *O. aureus* with *Oreochromis* sp. since mitochondrial DNA is maternally inherited. The foreign strain, however, was affirmed to be *O. niloticus.* It is therefore concluded that morphometric characterization alone might not be sufficient to accurately identify Tilapia species/strains.
Key Words: environmental factors, morphometric parameters, Nile tilapia, Truss network.

Introduction. Tilapias are not just popular in many parts of the world; they are the second most produced fish group next to carp (FAO 2018). The Nile tilapia (*Oreochromis niloticus*) is hardy, easy to harvest, and possesses a white flesh that is appealing to consumers (Shahriar et al 2011; Hassan et al 2018). Although it is indigenous to Central Africa, North Africa, and the Middle East (Boyd 2004; Okomoda et al 2020), Nile tilapia culture is done in almost every part of the planet (Maclean et al 2002). Beyond aquaculture production, this species can now be found in natural and artificial water/ reservoirs because of their ease of acclimatization following their introduction in many countries (Kosai et al 2014). Its unique reproduction pattern (i.e., mouth-brooding) and hardy nature allow even the poor farmers with fewer resources to have the opportunity to rear the fish (Boyi 2019). As a result, *O. niloticus* alone contributes to almost 4% of the tilapia production resulting from aquaculture worldwide (Moushomi & Saha 2015; FAO 2014, 2018).

The growing interest in tilapia aquaculture has led to the emergence of studies on improvement of productivity through breeding programs, determination of its suitability for aquaculture, and improvement of the meat quality (Ponzoni et al 2005; Breves et al 2010; Chaijan 2011). Many studies on the morphometric characterization of tilapia have also been reported from different countries such as Nigeria (Olufeaga et al 2016), Bangladesh (Moushomi & Saha 2015), Sri Lanka (Samaradivakara et al 2012), Pakistan (Naeem et al 2011), Thailand (Kosai et al 2014), Philippines (Peligro & Jumawan 2016),

Colombia (Andrés et al 2019), to mention a few. However, no study has attempted comparing strains of tilapia across different countries.

Morphometric characterization is concerned with the study of variation and changes in the physical outlook of an organism as dictated by factors such as genetic and environmental conditions (Webster 2006; Webster & Sheets 2010; Kosai et al 2014). Morphological studies in tilapia are important because phenotypic plasticity is a frequently occurring phenomenon in fish, far more common than for any other vertebrate (Solomon et al 2015). This encompasses the capability to adjust body maintenance in reaction to changes in the ecosystem (Olufeagba et al 2015). More so, freshwater fishes exhibit an even far greater degree of morphometric plasticity than marine fishes, due to the frequent physiological and environmental variations in their habitat (Eklöv & Svanbäck 2005). This includes but is not limited to feeding habits, sexual dimorphism, behavioral and physiological state etc. (Langerhans 2008).

In aquaculture, the differences in morphology between different strains are of great concern for selective breeding because the shape is a commercially important trait that contributes to the market value of the product (Colihueque & Araneda 2014; de Oliveira et al 2016). However, morphological similarities between pure species and introgressed individuals are a source of misidentification and could constitute a problem in aquaculture. Molecular characterization is helping to correct this misidentification in species, especially where uncontrolled hybridization, genetic mix up (due to unplanned hybridization) and unethical species transfer have occurred (Nyingi et al 2009).

Tilapia species are known to have the ability to cross-fertilize easily with one another even under captive conditions (Hassan et al 2018). Introgressive hybridization of tilapia has also been reported in West African rivers by Anane (2018). More so, many crosses had been intentionally tested in the laboratory, and some are even fertile (Agnèse et al 1998; Hassan et al 2018; Okomoda et al 2020). The extensive polyculture system of rearing tilapia species together done in many farms (especially in sub-Saharan Africa) has been thought to be a source of genetic contamination and introgression of germplasm; however, there is a paucity of information in this regard (Boyi 2019). In addition to the morphometric characterization of *O. niloticus* from Makurdi in Nigeria and the foreign strain imported from Swansea, we attempted to confirm the strain's species using the mitochondrial DNA cytochrome oxidase subunit I (COI) in this study.

Material and Method. The current study was conducted at the Biotechnology Laboratory, in the Department of Fisheries and Aquaculture, located at the Federal University of Agriculture Makurdi (FUAM), from January 2019 to June 2019. Local samples of *Oreochromis niloticus* were collected from the Departmental farm of FUAM. This local strain has not been deliberately improved genetically through any conscious efforts. They have been long maintained in a polyculture system with other tilapia species too (spanning close to 5 years). The exotic samples, on the other hand, were gotten from Boboge farms in Makurdi. This farm had imported the strain of *O. niloticus* from Swansea into Nigeria since the year 2014. The Swansea strains are genetically improved strains with better growth performance characteristics. The collected samples were thereafter placed on ice and transported to the Biotechnology laboratory for morphological confirmation of the species and collection of truss network data.

The phenotypic identity of the fish samples was confirmed using the guide provided by Boulenger (1916). They were then tagged appropriately, and digital photographs of the fish were taken with the aid of a digital camera (Sony CyberShot camera of 16.2MP). The photos were then processed for measurement using the NIS element Basic Research software. The morphological data obtained is based on 12 homologous landmarks as shown in Figure 1. From this truss network, 30 linear measurements were obtained (Table 1). The experimental protocols used were scrutinized and approved by the Federal University of Agriculture Makurdi committee on research. Hence, all the protocols and methods involving the experimentation with animals were in line with both foreign and local guidelines.

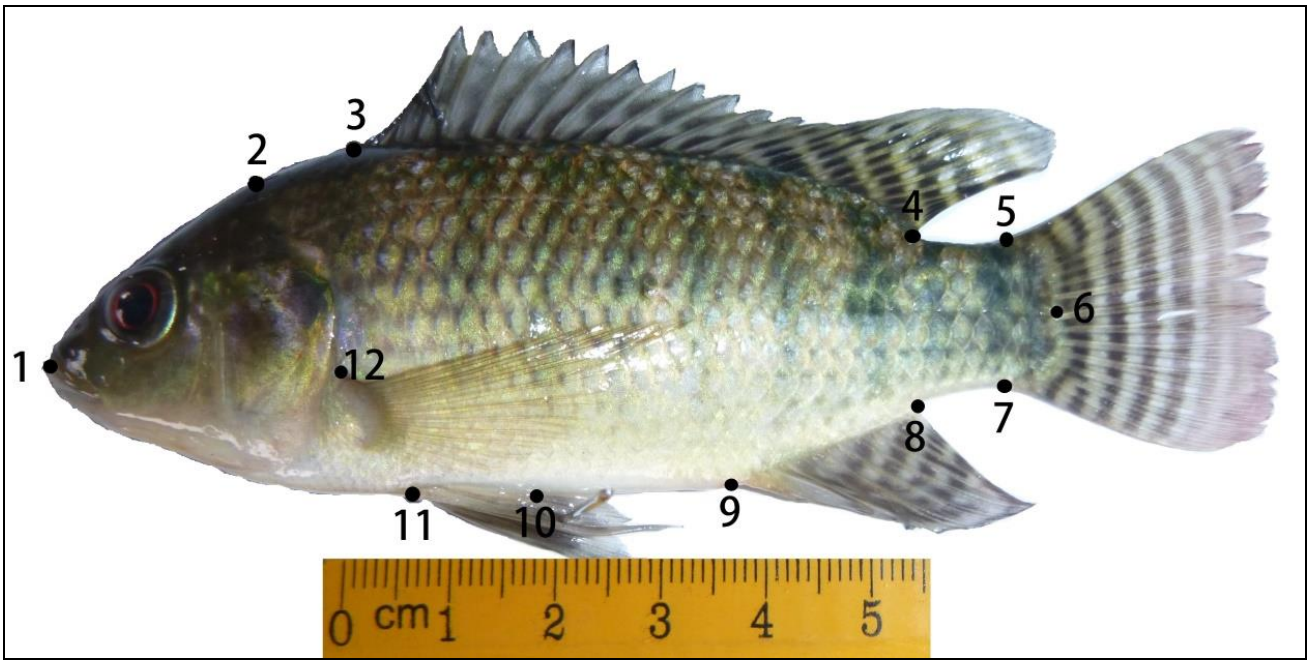

Figure 1. Illustration of tilapia showing twelve landmark points for constructing the truss network-based morphological measurements.

Before data analysis was done, the allometric formula proposed by Elliott et al (1995) was used to standardize the Truss network data and it is given below:

$$M_{adj} = M\ (Ls/L_o)^b$$

Where M = observed character measurement,
M adj = size-adjusted measurement,
Lo = standard length of the fish,
Ls = overall mean of the TL for all the progenies.
b = estimated for each character from the collected data as the slope of the regression of log M on log L_o, using all fish of all the progenies.

The transformed data obtained from the Truss network technique was analyzed with the principal component (PCA) and dendrogram with complete linkage plot using the computer software known as PAST. The sample centroids graph was generated allowing the determination of the morphological character that can be used to separate the fish samples into distinct strains should such distinction exist.

Table 1

Morphometric distances measured between landmark points of *Oreochromis* sp.

SN	*Landmark points*	*Character description*
1	1-2	Snout to the dorsal end of the head
2	2-3	Dorsal end of the head to the origin of the dorsal fin
3	3-4	Dorsal fin length
4	4-5	Posterior dorsal fin end to the dorsal attachment of the caudal fin to the tail
5	5-6	Dorsal caudal fin attachment to the tail to the midpoint of caudal peduncle
6	6-7	The caudal peduncle midpoint to the ventral attachment of caudal fin to tail
7	7-8	Ventral caudal fin attachment to the tail to the posterior anal fin end
8	8-9	Anal fin length
9	9-10	Origin of the anal fin to the posterior end of the pelvic fin
10	10-11	Pelvic fin length
11	11-12	Origin of the pelvic fin to the origin of the pectoral fin
12	1-3	Snout to the origin of the dorsal fin
13	1-4	Snout to the posterior end of the dorsal fin
14	1-5	Snout to the dorsal attachment of the caudal fin to the tail
15	1-6	Standard length
16	1-7	Snout to the ventral attachment of the caudal fin to the tail
17	1-8	Snout to the posterior end of the anal fin
18	1-9	Snout to the origin of the anal fin
19	1-10	Snout to the posterior end of the pelvic fin
20	1-11	Snout to the origin of the pelvic fin
21	1-12	Snout to the origin of the pectoral fin
22	2-12	Dorsal end of the head to the origin of the pectoral fin
23	3-12	Origin of the dorsal fin to the origin of the pectoral fin
24	4-12	Posterior end of the dorsal fin to the origin of the pectoral fin
25	5-12	Dorsal attachment of the caudal fin to the tail to the origin of the pectoral fin
26	6-12	The midpoint of the caudal peduncle to the origin of the pectoral fin
27	7-12	Ventral attachment of the caudal fin to the tail to the origin of the pectoral fin
28	8-12	Posterior end of the anal fin to the origin of the pectoral fin
29	9-12	Origin of the anal fin to the origin of the pectoral fin
30	10-12	Posterior end of the pelvic fin to the origin of the pectoral fin

Two samples of the exotic stain and three samples of local strain that have been morphometrically identified as *Oreochromis niloticus* were randomly selected and used for genetic confirmation of their species using the mitochondrial DNA COI. Genomic DNA was extracted from fin clip (pectoral fin) fixed in 10% formalin, using Easy Pure® FFPE Tissue genomic DNA kit (EE191) according to the instructions of the manufacturer. This genetic study was carried out at the Molecular Laboratory of the Covenant University, Ota, Ogun State, Nigeria. Amplification of the COI was done using the primer fish VF (5'-GTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC-3') forward and Fish R (5'-CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA-3') reverse primers (Trewavas 1982; Wu & Lang 2012).

A reaction volume of 50 µl was used containing 1X buffer, 100 µM dNTPs, 2 mM MgCl2, 10 pM of each primer, 3U of Taq DNA polymerase, and 100 ng genomic DNA. The reactions were performed using the BIO-RAD T 100™ Thermal Cycler (PCR Machine). PCR amplification was programmed as follows: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation, annealing and extension at 94°C, 63.5°C, and 72°C for 30 seconds, 30 seconds, and 45 seconds respectively. This was followed by a final extension at 72°C for 5 minutes and kept on the hold at a temperature of 4°C.

The PCR products were sent for sequencing at the Inqaba Biotechnical Industries (Pty) Ltd, Hatfield 0028, in South Africa (sequence was done using the Sanger method as adopted by Mizusawa et al 1986). The identity of the sequences was then confirmed at the NCBI (2019) website using the BLAST function. The sequence was also deposited at the Genbank, and the accession numbers were obtained (MK130700, MK130701, MK130703, and MK130704).

Results. Discriminant function analysis for the transformed homologous landmark distances of the *O. niloticus* strain is presented in Table 2. The Eigenvalue for the first three principal components was selected, based on Kaiser's (1961) recommendations of values above unity (i.e., 1). Results obtained revealed that the first principal component (PC1) accounted for 25.7% of the total variance with a mix of positive and negative coefficients. Principal component 2 (PC2) similarly had mixed sign coefficients and accounted for only 19.1% of the entire variance in both sample groups. In the same vein, PC3 accounted for only 12.3%. In summary, the three principal components cumulated to 57.2% of the variance for the tilapia strains from the local and exotic origins.

Table 2

Principal component analysis of transformed data from the landmark point of the *Oreochromis niloticus* strains (*n*=20 for each strain)

Variable	*PC1*	*PC2*	*PC3*
1-2	-0.25	-0.04	0.08
2-3	0.20	0.01	0.03
3-4	-0.04	-0.31	0.09
4-5	-0.01	0.06	-0.40
5-6	0.01	-0.02	-0.36
6-7	0.08	0.02	-0.23
7-8	-0.04	0.15	-0.28
8-9	-0.12	-0.17	-0.06
9-10	0.19	-0.07	0.18
10-11	-0.12	0.01	-0.36
11-12	0.00	-0.09	0.02
1-3	-0.16	-0.06	0.14
1-4	-0.14	-0.34	0.12
1-5	-0.16	-0.24	-0.11
1-6	-0.15	0.02	-0.18
1-7	-0.21	-0.14	-0.17
1-8	-0.18	-0.33	-0.01
1-9	-0.22	-0.16	-0.05
1-10	-0.29	0.03	-0.24
1-11	-0.30	0.00	0.16
1-12	-0.32	0.07	0.09
2-12	-0.28	-0.05	0.17
3-12	-0.26	-0.11	0.10
4-12	0.16	-0.35	0.03
5-12	0.10	-0.33	-0.15
6-12	0.23	-0.18	-0.22
7-12	0.09	-0.16	-0.06
8-12	0.16	-0.35	-0.04
9-12	0.16	-0.27	-0.05
10-12	-0.18	-0.01	-0.28
Eigen value	7.71	5.75	3.69
% of variance	25.69	19.16	12.30
Cumulative % variance	25.69	44.85	57.15

The scattered plot for the truss network data is presented in Figure 2. The biplot illustrated shows individual scores for (A) PC 1 versus PC 2; (B) PC 1 versus PC 3 and (C) PC 2 versus PC 3. The result obtained showed a wide overlap of the two strains without separation into distinct multivariate spaces. This overlap and non-separation into different multivariate space were further shown in the dendrogram plot depicted in Figure 3.

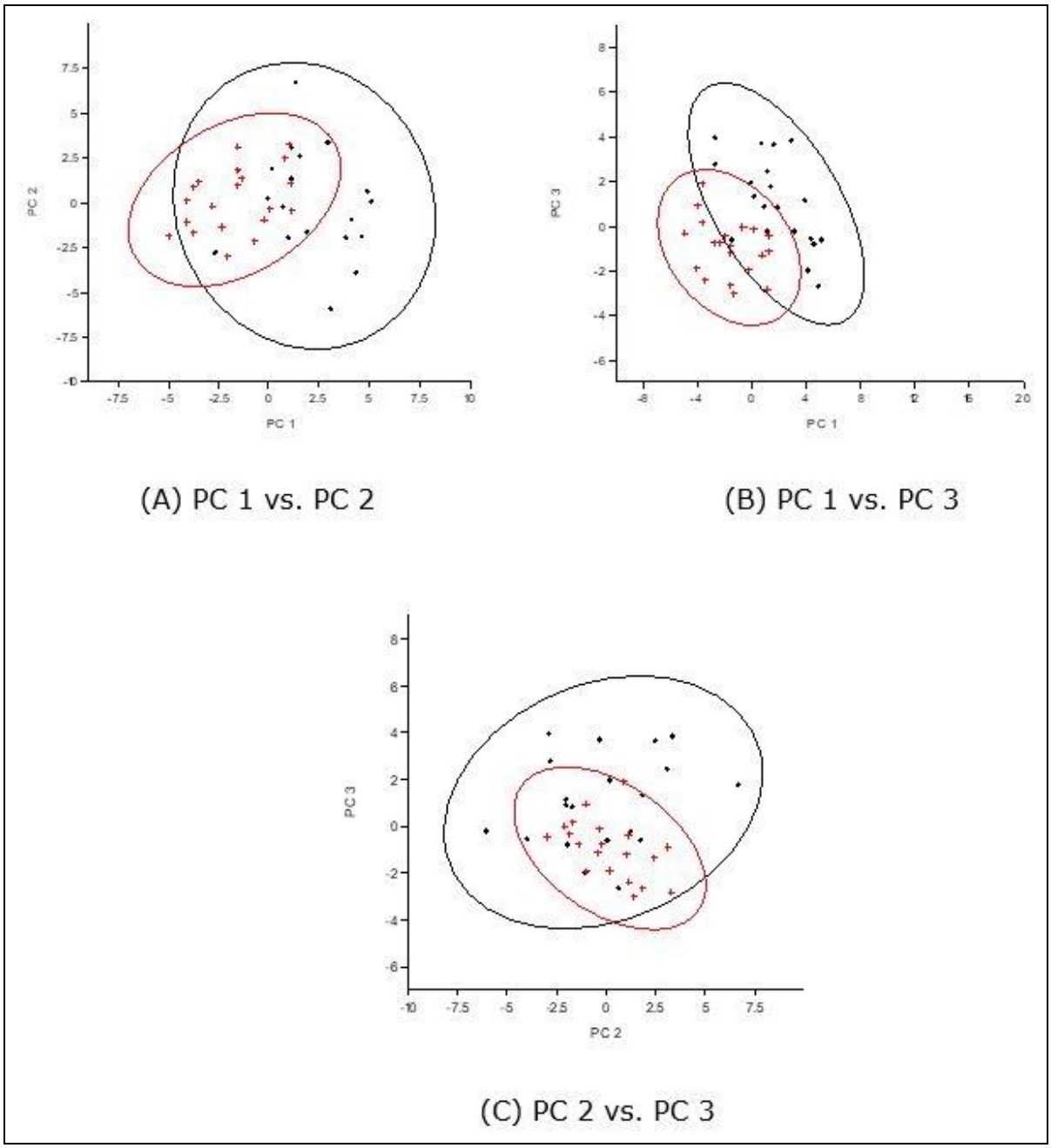

Figure 2. Principal component analysis of transformed morphometric data obtained from three strains of *Oreochromis niloticus* (*n*=20). Dot (black) = Makurdi strain (local strain); Cross (red) = Swansea strain (exotic strain).

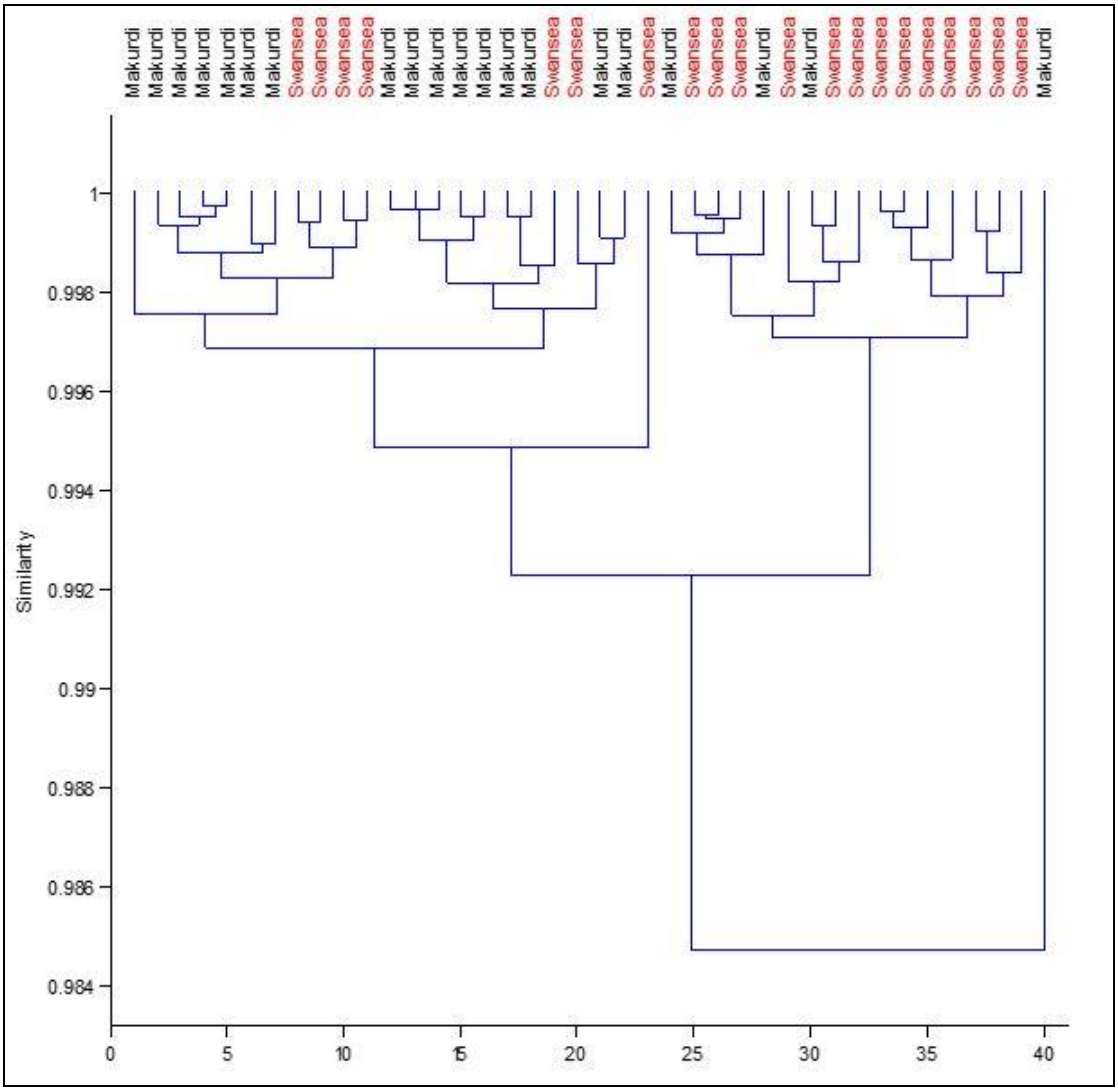

Figure 3. Dendrogram with complete linkage and Euclidean distance for data collected from the Trust network of three strains of *Oreochromis niloticus* (*n*=20). Dot (black) = Makurdi strain (local strain); Cross (red) = Swansea strain (exotic strain).

DNA band of approximately 750bp was obtained from the gel electrophoresis. For the genetic identification of the sequenced samples (Table 3), the BLAST of amplified sequences deposited in the Genbank revealed that the Local strains (i.e., MK130701, MK130703, and MK130704) had COI of the *Oreochromis aureus* and the similarity with other genes deposited in the Genbank ranging from 98-100%. The exotic strain (i.e., MK130700 and MK130702) were affirmed to be *O. niloticus* with a similarity of between 99-100% with other earlier deposited genes of the same species in the Genbank.

Table 3

Gene sequence blast of *Oreochromis* spp. with retrieved sequences from GenBank

Species	*Voucher*	*Accession No.*	*Similarity (%)*
O. niloticus	UAM: BPUJO:01	MK130700	(present study)
O. niloticus	UAM: BPUJO:03	MK130702	(present study)
O. niloticus	MOLOF1	KU565845	100
O. niloticus	OSPILURURS2	KU565846	100
O. niloticus	FAST2-FAC	KU565848	100
O. niloticus	EXCEL09-DAV	KU565849	100
O. niloticus	FAC20	KU565857	100
O. niloticus	EXCELB5-NFFTC	KU565859	100

O. niloticus	COLD1	KU565860	100
O. niloticus	BEST-I1	KU565867	100
O. niloticus	FAST3-FAC	KU565863	99.86
O. niloticus	JeliRed03	MF509597	99.72
O. aureus	UAM: BPUJO:02	MK130701	(present study)
O. aureus	UAM: BPUJO:04	MK130703	(present study)
O. aureus	UAM: BPUJO:05	MK130704	(present study)
O. aureus	BEST-I2	KU565830	100
O. aureus	OAUREUS2	KU565851	100
O. aureus	DK16-022	MF817704	100
O. aureus	DK16-028	MF817705	100
O. aureus	DK16-073	MF817706	100
O. aureus	DK16-X01	MF817707	100
O. aureus	GST2	KU565831	99.71
O. aureus	EXCEL05-DAV	KU565852	99.42
O. aureus	OAUREUS1	KU565844	98.69

Discussion. For a breeding program to be successful, stock discrimination is fundamental to its design and management (Begg & Waldman 1999; Wedemeyer 2001). Although fish is considered to undergo frequent morphological changes based on their susceptibility to environmental factors (Wimberger 1992; Solomon et al 2015), the current study observed overlapping of the two strains without individual unique separate clusters. Contrary to this finding, an earlier study by Swain et al (1991) had demonstrated significant morphological variation between wild and hatchery Coho salmon (*Oncorhynchus kisutch*) using landmark points of the Truss network system. Also, *Clarias gariepinus* and endangered *Tor putitora* had been reported to phenotypically differ for culture and wild strains as observed by Solomon et al (2015) and Patiyal et al (2014) respectively. Similar findings have also been reported by many authors for different tilapia species (Samaradivakara et al 2012; Naeem et al 2011; Kosai et al 2014; Moushomi & Saha 2015; Peligro & Jumawan 2016; Olufeaga et al 2016; Andrés et al 2019). Swain et al (1991) had opined that phenotypic variation observed mainly in research involving hatchery bred and wild strains of similar species reflects the differences in the rearing conditions. It may then be correct to hypothesize that the morphological similarities of the local and foreign strain may be linked to similar environmental and culture condition in which they were kept captive.

Molecular techniques have been applied in aquaculture worldwide, making it possible to effectively manage cultivated species and detect genetic introgression (Perez et al 1999; Gunnell et al 2008). The few samples sequenced in this study were insufficient to determine the phylogenetic interaction of the two strains. However, we observe that the phenotypically confirmed local strains were not pure *O. niloticus* but had COI of *O. aureus* instead. An earlier study by Rognon & Guyomard (2003) had reported introgressed *O. niloticus* samples from Senegal with mitochondrial DNA (mtDNA) identical to *O. aureus*. Morphological misidentification has been reported for introgressed hybrids between *Oreochromis niloticus* and *Oreochromis mossambicus* due to physical resemblance to *O. mossambicus* but having an mtDNA of *O. niloticus* (D'Amato et al 2007). The finding of this study, therefore, is suggestive of the fact that the local strain was an introgressed hybrid of *O. niloticus.*

Most mtDNA have conserved regions and have been used in the discrimination of not just different hybrid species but to know the direction of hybrid crosses (Moritz et al 1987; Okomoda et al 2019). This is simply because mtDNA is cytoplasmically housed in the cell and can only be inherited maternally (Moritz et al 1987; Wyatt et al 2006). Studies by do-Prado et al (2011) had used 16S mitochondrial genes for the discrimination of the cross combination of hybrids 'pintachara' and 'cachapinta'. In the same vein, the maternal parents of the hybrids between *Clarias gariepinus* and *Clarias batrachus* have been confirmed using the cytochrome b gene (Olufeagba & Okomoda 2016). COI had

also been employed by Waldbrieser and Bosworth (2008) in the discrimination of the reciprocal crosses of channel catfish *Ictalurus punctatus* and blue catfish *Ictalurus furcatus*. All these reported hybrids were however phenotypically distinct from their pure crosses. Although the phenotypic resemblance of the local strains to *O. niloticus* is not well understood, however, its COI identity might have resulted from several generations of introgressive hybridization between ♀ *Oreochromis aureus* with *Oreochromis* sp. The affirmation of the foreign strain as *Oreochromis niloticus* suggests the need for a more adequate monitoring of the genetic resources of the foreign strain than what is obtained for the local strain.

Conclusions. In the light of the genetic findings and the similarities in phenotypic characteristics of the strains, it is concluded that morphometric characterization alone might not be sufficient or accurate for the identification of tilapia species within an extensive polyculture system. However, powerful genetic tools such as genetic fingerprinting are recommended to have a holistic understanding of the genetic characteristics of the local strains.

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References

Agnèse J. F., Adepo B., Gourene L., Pouyaud L., 1998 Natural hybridization in tilapias. Genetics and Aquaculture in Africa. Éditions de l'Orstom Paris. in Agnèse, J-F. (ed). 95–103 pages.

Anane T. G. D., 2018 Characterization of the molecular genetic variation in wild and farmed Nile tilapia *Oreochromis niloticus* in Ghana for conservation and aquaculture development. Dissertation. Blacksburg, VA.

Andrés M., Cintia M., Ariel T., Martha O., James B., 2019 Body shape variation between farms of tilapia (*Oreochromis* sp.) in Colombian Andes using landmark-based geometric morphometrics. Latin American Journal of Aquatic Research, 47(1):194-200.

Begg G. A., Waldman J. R., 1999 An holistic approach to fish stock identification. Fisheries research, 43(1):35-44.

Boulenger G. A., 1916 Catalogue of the freshwater fishes of Africa in the British Museum (Nat History). Vols I – IV. London Trustees of the British Museum.

Boyd E. C., 2004 Farm-level issues in aquaculture certification: Tilapia. Report commissioned by WWF-US in 2004, Auburn University, Alabama.

Boyi P. U., 2019 Intraspecific hybridization and molecular characterization of local and exotic *Oreochromis Spp*. Master of Science in Fisheries thesis of the Federal University of Agriculture Makurdi, Nigeria. 102pp.

Breves J. B., Hirano T., Grau E. G., 2010 Ionoregulatory and endocrine responses to disturbed salt and water balance in Mozambique tilapia exposed to confinement and handling stress. Comp. Biochem. Physiol. A; 155:294-300.

Chaijan M., 2011 Physicochemical changes of tilapia (*Oreochromis niloticus*) muscle during salting. Food Chemistry 129:1201-10.

Colihueque N., Araneda C., 2014 Appearance traits in fish farming: Progress from classical genetics to genomics, providing insight into current and potential genetic improvement. Frontiers in Genetics, 5:1-8.

D'Amato M. E., Esterhuyse M. M., van der Waal B. C. W., Brink D., Volckaert F. A. M., 2007 Hybridization and phylogeography of the Mozambique tilapia *Oreochromis*

mossambicus in southern Africa evidenced by mitochondrial and microsatellite DNA genotyping. Conservation Genetics 8:475–488.
de Oliveira C. A., Ribeiro R. P., Yoshida G. M., Kunita N. M., Rizzato G. S., de Oliveira S. N., Dos Santos A. L., Nguyen N. H., 2016 Correlated changes in body shape after five generations of selection to improve growth rate in a breeding program for Nile tilapia *Oreochromis niloticus* in Brazil. Journal of Applied Genetics, 57:487-493.
do-Prado F. D., Hashimoto D. T., Mendonca F. F., Senhorini J. A., Foresti F., Porto-Foresti F., 2011 Molecular identification of hybrids between Neotropical catfish species *Pseudoplatystoma corruscans* and *Pseudoplatystoma reticulatum*. Aquaculture Research, 42:1890-1894.
Eklöv P., Svanbäck R., 2005 Predation risk influences adaptive morphological variation in fish populations. The American Naturalist 167(3):440-452.
Elliott N. G., Haskard K., Koslow J. A., 1995 Morphometric analysis of orange roughy (*Hoplostethus atlanticus*) off the continental slope of southern Australia. Journal of Fisheries Biology 46(1):202-220.
Food and Agriculture Organization (FAO), 2014 Smallholders data portrait (available at www.fao.org/economic/esa/esaactivities/esa-smallholders/dataportrait/en/).
Food and Agriculture Organization (FAO), 2018 Fisheries and Aquaculture Information and Statistics Branch. Fisheries Global Information System (FIGIS). FI Institutional Websites. In: FAO Fisheries and Aquaculture Department. Rome. Accessed on 30 August 2018 (http://www.fao.org/figis/servlet/SQServlet?file=/usr/local/tomcat/8.5.16/figis/webapps/figis/temp/hqp_6915900564662940548.xml&outtype=html).
Gunnell K., Tada M. K., Hawthorne F. A., Keeley E. R., Ptacek M. B., 2008 Geographic patterns of introgressive hybridization between native Yellowstone cutthroat trout (*Oncorhynchus clarkia bouvieri*) and introduced rainbow trout (*O. mykiss*) in the South Fork of the Snake River watershed, Idaho. Conservation Genetics 9:49-64.
Hassan A., Okomoda V. T., Pradeep P. J., 2018 Triploidy induction by electric shock in Red hybrid Tilapia. Aquaculture. 495:823-830.
Kaiser H., 1961 A Note on Guttman's lower bound for the number of common factor. Multivariate Behavioral Research, 1:249-276.
Kosai P., Sathavorasmith P., Jiraungkoorskul K., Jiraungkoorskul W., 2014 Morphometric Characters of Nile Tilapia (*Oreochromis niloticus*) in Thailand. Agriculture Technology and Biological Sciences 11(10):857-863.
Langerhans R. B., 2008 Predictability of phenotypic differentiation across flow regimes in fishes. Integrative and Comparative Biology 48(6):750-768.
Maclean N., Rahman M. A., Sohm F., Hwang G., Iyengar A., Ayad H., Smith A., Farahmand H., 2002 Transgenic tilapia and the tilapia genome. Gene, 295:265-277.
Mizusawa S., Nishimura S., Seela F., 1986 Improvement of the dideoxy chain termination method of DNA sequencing by use of deoxy-7-deazaguanosine triphosphate in place of dGTP. Nucleic Acids Research, 14(3):1319-1324.
Moritz C., Dowling T. E., Brown W. M., 1987 Evolution of animal mitochondrial DNA: relevance for population biology and systematic. Ann Rev Ecol, Evolut Systematics, 18:269–292.
Moushomi R., Saha S., 2015 Landmark based shape variation of normal male, female and sex-reversed tilapia (*Oreochromis niloticus*) in Bangladesh Progressive Agriculture 26:168-172
Naeem M., Zuberi A., Salam A., Ali M., Riaz-ulHaq M., Khalid M., Mehreen M., Nasir M. F., Rasool S. A., Ishtiaq A., 2011 Some morphometric relationships of hatchery

reared male population of *Oreochromis mossambicus* from Pakistan. Afr. J. Biotechnol. 10:17362-6.
National Center for Biotechnology Information (NCBI), 2019 – cited 2019 June 30. Available from: https://www.ncbi.nlm.nih.gov/.
Nyingi D., De Vos L., Aman R., Agnèse J. F., 2009 Genetic characterization of an unknown and endangered native population of the Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) (Cichlidae; Teleostei) in the Loboi Swamp (Kenya). Aquaculture 297:57–63.
Okomoda V. T., Pradeep P. J. Oladimeji A. S., Abol-Munafi A. B., Alabi K. I., Ikhwanuddin M., Martins C. O., Umaru J. A., Hassan A., 2020 Effect of Electric induced Triploidization on Sex ratio, Growth and Gonad Histology of Red hybrid Tilapia. Aquaculture. 520:734991.
Okomoda V. T., Koh I. C. C., Hassan A., Amornsakun T., Shahreza M. S., 2019 Parentage Analysis of the Progenies of the Reciprocal Crosses of *Pangasianodon hypophthalmus* (Sauvage, 1878) and *Clarias gariepinus* (Burchell, 1822) using Cytochrome b Gene. Jordan Journal of Biological Sciences 12(1):107–117.
Olufeagba S. O., Aladele S. E., Okomoda V. T., Okere A. U. Oduoye O. T., Hassan T., 2015 Morphological Variations of Cultured and wild *Oreochromis niloticus* from Ibadan and Kainji in Nigeria. Nigerian Journal of Fisheries. 12(1):828-836.
Olufeagba S. O., Okomoda V. T., 2016 Parentage determination of the hybrids between Clarias batrachus and *Clarias gariepinus* using Cytochrome B. Banat's J Biotechnol., 14(1):55-59.
Olufeagba S. O., Okomoda V. T., Shaibu G., 2016 Embryogenesis and early growth of pure strains and hybrids between *Clarias gariepinus* (Burchell, 1822) and *Heterobranchus longifilis* Valenciennes, 1840. North American Journal of Aquaculture 78(4):346–355.
Patiyal R. S., Mir J. I., Sharma R. C., Chandra S., Mahanta P. C., 2014 Pattern of meristic and morphometric variations between wild and captive stocks of endangered *Tor putitora* (Hamilton 1822) using multivariate statistical analysis methods – Proc. Natl Acad. Sci., India, Sect. B Biol. Sci. 84:123-129.
Peligro V. C., Jumawan J. C., 2016 Assessment of fluctuating asymmetry in the body shapes of Nile tilapia (*Oreochromis niloticus*) from Masao river, Butuan city, Philippines. AACL Bioflux 9(3):604-613.
Perez J., Martinez J. L., Moran P., Beall, E., Garcia Vazquez E., 1999 Identification of Atlantic salmon X brown trout hybrids with a nuclear marker useful for evolutionary studies. Journal of Fish Biology 54:460-464.
Ponzoni R. W., Lind C. E., Safari A., Agyakwah S. K., Attipoe F. Y. K., El-Naggar G. O., Hamzah A., 2005 Differences in sexual size dimorphism among farmed tilapia species and strains undergoing genetic improvement for body weight. Aquaculture Reports*,* 1:20–27.
Rognon X., Guyomard R., 2003 Large extent of mitochondrial DNA transfer from *Oreochromis aureus* to *O. niloticus* in West Africa. Molecular Ecology 12:435–445.
Samaradivakara S. P., Hirimuthugodal N. Y., Gunawardana R. H. A. N. M., Illeperuma R. J., Fernandopulle N. D., Silva A. D. D., Alexander P. A. B. D., 2012 Morphological variation of four tilapia populations in selected reservoirs in Sri Lanka. Trop. Agri. Res 23:105-116.
Shahriar N. K. M., Mamun A. A., Sarker B. S., Tonny U. S., 2011 Morphological variability of the 11th generation strain of Nile tilapia, (*Oreochromis niloticus*) and traditional genetically improved farmed tilapia. J. Bangladesh Agri. Univ. 9:345-9.

Solomon S. G., Okomoda V. T., Ogbenyikwu A. I., 2015 Intraspecific morphological variation between cultured and wild *Clarias gariepinus* (Burchell) (Clariidae, Siluriformes). Archives of Polish Fisheries 23(1):53-61.
Swain D. P., Ridell B. E., Murray C. B., 1991 Morphological differences between hatchery and wild populations of coho salmon (*Oncorhynchus kisutch*): environmental versus genetic origin – Can. J. Fish. Aquat. Sci. 48:1783-1791.
Trewavas E., 1982 Generic groupings of Tilapia used in aquaculture. Aquaculture 27:79–81.
Waldbrieser G. C., Bosworth B. G., 2008 Utilization of a Rapid DNA-Based Assay for Molecular Verification of Channel Catfish, Blue Catfish, F1 Hybrids, and Backcross Offspring at Several Life Stages. North Am J Aquaculture, 70:388–395.
Webster M., 2006 Introduction to Geometric Morphometrics. Department of the Geophysical Sciences, University of Chicago.
Webster M., Sheets H. D., 2010 A practical introduction to landmark-based geometric morphometrics. Quantitative methods in paleobiology 16:168-188.
Wedemeyer G. A., 2001 Fish hatchery management, second edition. American Fisheries Society, Betesda, Maryland.
Wimberger P. H., 1992 Plasticity of fish body shape, the effects of diet, development, family and age in two species of *Geophagus* (Pisces: Cichlidae) – Biol. J. Linn. Soc. 45:197-218.
Wu L., Lang J., 2012 Identifications of Captive and Wild Tilapia Species Existing in Hawaii by Mitochondrial DNA Control Region Sequence. PLoS One 7(12):e51731.
Wyatt P. M. W., Pitts C. S., Butlin R. K., 2006 A molecular approach to detect hybridization between bream *Abramis brama*, roach *Rutlius rutilus* and rudd *Scardinius erythrophthalmus*. Journal of Fish Biology 69:52–71.

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