

**Genetic Diversity of *Tilapia guineensis* (Bleeker, 1862) in Some  
Nigerian Coastal Waters**

By

**UKENYE Esther Adaku**

**Matric. No: 119072025**

**October, 2017**

**Genetic Diversity of *Tilapia guineensis* (Bleeker, 1862) in Some Nigerian Coastal Waters**

By

**UKENYE Esther Adaku**

B.Sc. Biochemistry (ESUT), M.Sc. Biochemistry (MOUAAU).

(119072025)

A thesis submitted for the award of Doctor of Philosophy (Ph.D.) in the  
Department of Cell Biology and Genetics, University of Lagos.

.....

Dr. I. A. Taiwo

*Supervisor*

.....

Professor B. O. Oboh

*Head of Department*

**SCHOOL OF POSTGRADUATE STUDIES,  
UNIVERSITY OF LAGOS,  
CERTIFICATION**

This is to certify that the thesis:

**Genetic Diversity of *Tilapia guineensis* (Bleeker, 1862) in Some Nigerian  
Coastal Waters**

Submitted to the School of Postgraduate Studies,  
University of Lagos

for the award of the degree of

Doctor of Philosophy (Ph.D.) is a record of original research carried out

By:

**UKENYE Esther Adaku**

In the Department of Cell Biology & Genetics

..... AUTHOR' NAME	..... SIGNATURE	..... DATE
..... 1 <sup>ST</sup> SUPERVISOR'S NAME	..... SIGNATURE	..... DATE
..... 2 <sup>ND</sup> SUPERVISOR'S NAME	..... SIGNATURE	..... DATE
..... 1 <sup>ST</sup> INTERNAL EXAMINER	..... SIGNATURE	..... DATE
..... 2 <sup>ND</sup> INTERNAL EXAMINER	..... SIGNATURE	..... DATE
..... EXTERNAL EXAMINER	..... SIGNATURE	..... DATE
..... SPGS REPRESENTATIVE	..... SIGNATURE	..... DATE

## AUTHOR'S STATEMENT

I hereby agree to give the University of Lagos through University of Lagos Library, a non-exclusive, worldwide right to reproduce and distribute my thesis and abstract (hereinafter "the Work") in whole or in part, by any and all media of distribution, in its present form or style or in any form or style as it may be translated for the purpose of future preservation and accessibility provided that such translation does not change its content.

By the grant of non-exclusive rights to University of Lagos through the Library under this agreement, I understand that the rights of the University of Lagos are royalty free and that I am free to publish the Work in its present version or future versions elsewhere.

## Warranties

I further agree as follows:

- i. That I am the author of the Work and I hereby give the University of Lagos the right to make available the Work in the way described above after a three (3) year period of the award of my doctorate degree in compliance with the regulation established by the University of Lagos Senate.
- ii. That the Work does not contain confidential information which should not be divulged to any third party without written consent.
- iii. That I have exercised reasonable care to ensure that the Work is original and it does not to the best of my knowledge breach any Nigerian law or infringe any third party's copyright or other Intellectual Property Right.
- iv. That to the extent that the Work contains material for which I do not hold copyright, I represent that I have obtained the unrestricted permission of the copyright holder to grant this license to the University of Lagos Library and that such third party material is clearly identified and acknowledged in the Work.
- v. In the event of a subsequent dispute over the copyrights to material contained in the Work, I agree to indemnify and hold harmless the University of Lagos and all of its officers, employees and agents for any uses of the material authorized by this agreement.
- vi. That the University of Lagos has no obligation whatsoever to take legal action on my behalf as the Depositor, in the event of breach of intellectual property rights, or any other right, in the material deposited.

_____ Author's Name	_____ Signature/Date	_____ Email
_____ Supervisor's Name	_____ Signature/Date	_____ Email
_____ Supervisor's Name	_____ Signature/Date	_____ Email

## DECLARATION

I declare that the work in this thesis titled “Genetic diversity of *Tilapia guineensis* in some Nigerian coastal waters” was carried out by me **UKENYE ESTHER ADAKU**, under the supervision of Dr. I. A. Taiwo and Dr. P. E. Anyanwu of the Department of Cell Biology and Genetics, Faculty of Science, University of Lagos, Nigeria and Nigerian Institute for Oceanography and Marine Research, Victoria Island, Lagos. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any other University.

.....	.....	.....
Author’s Name	Signature	Date
Dr. I. A. Taiwo	.....	.....
(Senior Lecturer)	Signature	Date
Dr. P. E. Anyanwu	.....	.....
(Director of Research)	Signature	Date

## **DEDICATION**

I dedicate this work to God Almighty, my parents, my lovely husband and all those who contributed to the accomplishment of my academic goal and professional career.

## ACKNOWLEDGEMENTS

I wish to express my profound gratitude to my supervisor Dr Idowu Adewunmi Taiwo for his guidance, tireless effort, sound academic knowledge, constructive and objective criticism, patience, encouragements, valuable contributions and insight throughout the research. I also acknowledge his willingness and readiness to always attend to me; all the necessary corrections which contributed immensely to the successful completion of this research work were quite encouraging.

I am further obliged to appreciate Dr. Anyanwu, P. E. who co-supervised the work for her valuable advice, scientific contributions, encouragement, suggestions, motherly care and academic support during the period of study.

I would also like to thank Professor Bola O. Oboh, Head of Department, Cell Biology and Genetics for her leadership, Dr. Joseph Minari for his meaningful advice and suggestions. Dr. Bayo Ogunkanmi and D.r Khalid Adekoya, for their concern and encouragement, Dr. Kelechi Njoku for his significant contributions and advice. All the lecturers and entire staff of the department for their co-operation, provision of peaceful and conducive atmosphere all through my academic pursuit in the department of Cell Biology and Genetics. I appreciate your efforts.

I gratefully acknowledge the Agricultural Research Council of Nigeria Competitive Agricultural Research Scheme Grant (ARCN-CARGS) for the initial funding of this project. I appreciate Dr. G. R. Akande, the ED/CEO of NIOMR for his fatherly advice, encouragement and supports, to Dr. A. Oresgun my Head of Department, I say thank you. The assistance and support of Genomic Division of Biotechnology, Department of Nigerian Institute for Oceanography and Marine Research (NIOMR), Lagos, Nigeria under the supervision of Dr. O.R. Oguntade, the management team and the entire staff of NIOMR is well acknowledged. The cooperation of the Bioscience Laboratory staff of International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria and Nigerian Institute of Medical Research (NIMR) Lagos, Nigeria in conducting part of this research is highly appreciated.

I acknowledged in a special way; my lovely husband, children, siblings and father in-law. I would also like to thank Mrs Udoezika, Uchechukwu, a sister and a friend for her prayers encouragements and supports in the course of this programme. All my friends and well wishers, I appreciate you all for you are part of my success story in actualizing this vision.

Finally, I am very grateful to Almighty God for His infinite mercy, love, protection, provision, guidance, peace of mind, strength, good health, enablement and sound mind throughout the period of this study and my academic journey.

# TABLE OF CONTENTS

<b>Content</b>	<b>Page</b>
TITLE PAGE	i
CERTIFICATION	ii
DEDICATION	v
ACKNOWLEDGEMENTS	vi
TABLE OF CONTENTS	vii
LIST OF FIGURES	xiii
LIST OF TABLES	xiv
LIST OF APPENDICES	xvi
ABSTRACT	xvii
<b>Chapter One</b>	
1.0. INTRODUCTION	
1.1. Background of the Study	1
1.2. Statement of the Problem	5
1.3 Aim and Objectives of the Study	6
1.3.1 Aim	6
1.3.2 Specific Objectives	6



1.4.	Significance of Study	7
1.5	Definition of Terms and Abbreviations	8
1.5.1.	Terms	8
1.5.2.	Meaning of Abbreviations	9

## **Chapter Two**

2.0.	LITERATURE REVIEW	
2.1.	Tilapia as a Fish	10
2.1.1	Tilapia Aquaculture Production Status	10
2.2	Importance of Tilapia	13
2.2.1	Vitamin and Mineral Content	13
2.2.2	Health Benefits of Tilapia fish	14
2.2.2.1	Disease Prevention	14
2.2.2.2	Growth and Development	14
2.2.2.3	Weight Loss	15
2.2.2.4	Bone Health	15
2.2.2.5	Prostate Cancer Prevention	15
2.2.2.6	Heart Health	16
2.2.2.7	Brain Health	16

2.2.2.8	Premature Aging	16
2.2.2.9	Immune System and Thyroid Function	17
2.3	Role of Tilapia in Food Security	18
2.4	Other Uses of Tilapia	18
2.5	Characterization of <i>Tilapia guineensis</i>	19
2.6	Seasonal Occurrence of the Fish species	19
2.7	Aquatic Physico-chemical Properties	20
2.8	Fish Morphology	22
2.8.1	Morphometric	24
2.8.2	Meristics	24
2.8.3	Truss Network System	24
2.9	Morphological Characteristics of Tilapia	25
2.9.1	Size and body shape	28
2.10	Morphometrics in Fish Population	29
2.11	Genetics of Tilapia	33
2.11.1	Genetics and Fisheries Management	33
2.12	Importance of Genetic Diversity to Fisheries	34
2.12.1	Adaptation and Survival	34
2.12.2	Fish Breeding	35

2.13	Genetic Improvement of Tilapia	37
2.14	Population Genetics of Fish	38
2.15	Genetic Diversity of Tilapia	39
2.16	Molecular Markers	42
2.16.1	Microsatellite	44
2.16.2	Microsatellites in Assessing Genetics Diversity	47
2.16.3	Microsatellites Population genetics	48
2.16.4	Microsatellite Amplifications	50
2.17	Genetic Diversity and Molecular Markers	51
2.18	Gel Electrophoresis	52
2.18.1	Polyacrylamide Gel Electrophoresis	53
2.19	The Relationship between Morphology and Genetics	54

### **Chapter Three**

3.0.	MATERIALS AND METHODS	
3.1.	Study Area/Sampling Site	56
3.2.	Physico-chemical Analysis	56
3.2.1	Physical Parameters	56
3.2.2	Dissolved Oxygen	59
3.2.3	Nutrient Content of Sampling Sites	59
3.3.	Fish Sample Collection	59
3.4	Morphometric Analyses	60
3.4.1	Morphometric Measurements	60
3.4.2	Meristic Measurements	61
3.4.3	Truss Measurements	61
3.5	Molecular analyses	63

3.5.1	Extraction of DNA	63
3.5.2	DNA Purity and Quantification	64
3.5.3	Agarose Gel Electrophoresis	64
3.5.4	Microsatellite Amplification by Polymerase Chain Reaction (PCR)	65
3.5.5	Polyacrylamide Gel Electrophoresis	67
3.6	Statistical Analysis	68

## **Chapter Four**

4.0	RESULTS	
4.1	Physico-chemical Analysis	69
4.2	Morphometric Analysis	72
4.2.1	Morphometrics	72
4.2.2	Meristic Analysis	79
4.2.3	Truss Tetwork System	84
4.3	Molecular Analyses	91
4.3.1	DNA Purity and Quantification Results	91
4.3.2	Agarose Gel Electrophoresis	92
4.3.3	The Result of PCR Amplification of Microsatellite	94
4.4	Genetic Variability among Microsatellite Loci	94
4.4.1	Allelic Diversity	94
4.4.2	Polymorphic Information Content (PIC) Value	94
4.5	Genetic Differences among Populations	95
4.5.1	Pairwise Genetic Dissimilarity	95
4.5.2	Phylogenetic Relationship	96

## **Chapter Five**

5.0.	DISCUSSION	102
5.1	Physico-chemical Condition of the Coastal Waters	102
5.2	Morphological Variation of Studied Populations	106
5.3	Genetic Variation among the Studied Populations	108
5.4	Morphological and Genetic Diversity of the Studied Populations	112
<b>Chapter Six</b>		
6.0.	Conclusion and Recommendation	114
6.1	Summary of Findings	115
6.2	Contributions to Knowledge	116
7.0	REFERENCES	117
	Appendices	161

## LIST OF FIGURES

<b>Figure</b>	<b>Title</b>	<b>Page</b>
1.	Morphological Characteristics of Tilapia.	27
2.	Map of Nigerian Coastal States Showing Sampling Stations	57
3.	Conventional Dimensions and Position of Truss Network Measured for Morphological Variation	62
4.	Principal Component Analysis of Morphometric data based on Location distribution of samples	77
5.	UPGMA Dendrogram Showing the Clustering of Morphometric data as Grouped by Location	78
6.	Principal Component Analysis of Meristic data based on Location Distribution	82
7.	UPGMA Dendrogram Showing the Clustering of Meristic data as Grouped by Location	83
8.	Principal Component Analysis of Truss Network on Location Distribution	88
9.	UPGMA Dendrogram Showing the Clustering of Trust Network data as Grouped by Location	88
10.	Agarose Gel Electrophoresis for DNA Confirmation	92
11.	Microsatellite Primers Optimization	93
12.	Polyacrylamide Electrophoresis of Amplified Microsatellite Loci	97
13.	UPGMA Dendrogram showing the Genetic Relationships among 12 Populations based on Nei's Genetic Distance	100
14.	Dendrogram based on Geographical Location using Longitudinal and Latitudinal Location of the Population	101

## LIST OF TABLES

Table	Title	Page
1.	Geographical Location of Sampling Stations	58
2.	SSR Primer Code, Sequences, Annealing Temperature and Band Size	66
3.	Physico chemical Parameters of Sampling Stations (Mean Values)	71
4.	Nutrient Result of Sampling Station (Mean Values)	71
5.	Principal Component Analysis of Morphometric Variables	73
6.	Descriptive Statistics for Mean Summary of Morphometric Characters of <i>T. guineensis</i> Studied	74-76
7.	Correlation Matrix between Different Morphometric Characters of <i>T. guineensis</i>	77
8.	Principal Component Analysis of Meristic Variables	80
9.	Descriptive Statistics for Mean Summary of Meristic Characters of <i>T. guineensis</i> Studied	81
10.	Correlation Matrix between Different Meristic Characters of <i>T. guineensis</i>	82
11.	Principal Component Analysis of Truss Network Variables	85
12.	Descriptive Statistics for Mean Summary of Truss Network Characters of <i>T. guineensis</i> Studied	86-87
13.	Correlation Matrix between Different Truss Network Characters of <i>T. guineensis</i>	88
14.	Principal Component Loadings for Morphometric and Meristic Characters of <i>T. guineensis</i> Populations	89
15.	DNA Concentration (ng/ $\mu$ l) and Ratio of Absorbance (260/280nm) of <i>T. guineensis</i> Populations.	91
16.	Characteristics of SSR Loci Analyzed	98
17.	Locus Specific Indices of Genetic Diversity in the cCombined Population	98
18.	Summary of the Genetic Diversity Level in the Twelve Studied Populations	99
19.	Nei's Genetic Distance between Twelve <i>T. guineensis</i> Populations Revealed By Nine Microsatellite Loci	99
20.	Distance Matrix based on Geographical (Longitude and Latitude) Location	100

## LIST OF PLATES

Plate	Title	Page
1.	<i>Tilapia guineensis</i>	62



## LIST OF APPENDICES

<b>Appendix</b>	<b>Title</b>	<b>Page</b>
I	Primers Sequence for Molecular Study	132
II	DNA Extraction Protocol	133
III	DNA Quantity and Quality Nanodrop Analysis	135
IV	Protocol for Agarose Gel Electrophoresis	136
V	Protocol for Polyacrylamide Gel Electrophoresis	137
VI	Descriptive Statistics for Mean Summary of Morphometric Characters	139
VII	Descriptive Statistics for Mean Summary of Meristic Characters	141
VIII	Descriptive Statistics for Trust Network Data	142

## Abstract

*Tilapia guineensis*, a typical estuarine cichlid species in the West Coast of Africa, is an important fish species in view of its immense contribution to the nutritional needs, economic growth, and development of many African nations including Nigeria. Knowledge of current level of diversity and genetic structure of *T. guineensis* populations in Nigeria is lacking. This knowledge will be useful for fishery management, aquaculture production, stock conservation, and fish improvement through selective breeding. In the present study, morphological and molecular techniques were used to characterize and investigate genetic diversity of this species for breeding and conservation purposes. Six hundred and twenty samples were collected from six coastal states namely Rivers, Delta, Lagos, Ondo, Akwa Ibom and Bayelsa in Nigeria. Two locations were selected per state to study intra and inter specific variations making a total of twelve locations. Three morphological methods (morphometric, meristic and truss network data) were used for determination of phenotypic variation while ten microsatellite markers were utilized for genetic diversity assessment. DNA was extracted and subjected to polymerase chain reaction (PCR) amplification and polyacrylamide gel electrophoresis (PAGE) for allelic separation and genetic differentiation. Physicochemical characteristics of sampling stations were determined to assess the environment of the organism. The physicochemical parameters investigated are water temperature, pH, conductivity, salinity, dissolved oxygen (DO) and biological oxygen demand (BOD). Physico-chemical samples were collected using standard procedures and samples analyzed using standard techniques. Physicochemical characteristics investigation reveals that all physicochemical parameters were within ranges of international permissible levels in water. This implies a satisfactory physicochemical regime during the study period, suggesting the water bodies still have a positive integrity to support aquatic life. Principal component analysis (PCA) revealed two principal components (PC-1 and PC-11) that accounted for 90.3% of observed variation in morphometric attributes; 58.1% and 58.8% in meristics and truss network system respectively. When compared to other locations, fish in Iwoama had the highest mean weight of  $0.29 \pm 0.006$  kg with a mean total length of  $0.24 \pm 0.002$  m. Truss network data showed that Brass location had the highest mean length of  $0.149 \pm 0.001$  m. Among the thirteen morphometric variables considered, pre-anal length (PAL) and standard length were the most correlated while dorsal fin count (DFC) and anal fin count (AFC) were the most correlated among the meristic variables. Cluster analysis revealed three clusters for meristic variables and two clusters for morphometric and truss network variables respectively while principal component loadings revealed that dorsal and caudal fin rays were the two most useful discriminating variables for differentiating *T. guineensis* populations in Nigeria. Molecular studies showed that all the loci were multi-allelic giving an average of 3.1 alleles per locus. The number of alleles ( $N_a$ ) ranged from 2 to 4 alleles per locus while the number of effective alleles ( $N_e$ ) ranged from 1.087 to 2.612. Buguma, Badagry and Brass populations had the highest genetic diversity as was revealed by heterozygosity and shannon index. However, genetic diversity was low in some studied populations of *T. guineensis* in Nigerian coastal waters. The longest pairwise genetic distance of 0.30 was between Brass in Bayelsa State and River Ethiope in Delta State. Clustering using simple sequence repeat (SSR) data gave four major clusters which did not concur with clustering based on geographical location. The present study established that meristics revealed more

variability than morphometrics and truss network system in differentiating the morphological stocks of *T. guineensis* and should be recommended for differentiating *T. guineensis*, especially when combined with the other two morphological methods. The study established that Buguma in Rivers state, Badagry in Lagos state and Brass in Bayelsa state populations have higher genetic diversity and are therefore identified as suitable areas for sourcing *T. guineensis* for fish improvement through appropriate breeding and conservation. However, Oron and Ibaka in Akwa Ibom state populations had the lowest biodiversity necessitating conservation efforts.

**Key words:** *Tilapia guineensis*, morphological, microsatellite, coastal rivers, Nigerian.