SYSTEMATICS OF LOGANIACEAE IN

WEST AFRICA

BY

ODUOYE, OLUSOLA THOMAS

B.Sc. (HONS.) BOTANY, LAGOS MATRIC NO: 020803050

A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph. D.) BOTANY

DEPARTMENT OF BOTANY, UNIVERSITY OF LAGOS, NIGERIA

MAY, 2014

SCHOOL OF POSTGRADUATE STUDIES UNIVERSITY OF LAGOS

CERTIFICATION

THIS IS TO CERTIFY THAT THE THESIS:

SYSTEMATICS OF LOGANIACEAE IN WEST AFRICA

SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, UNIVERSITY OF LAGOS FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY (Ph.D.) IN BOTANY

IS A RECORD OF ORIGINAL RESEARCH CARRIED OUT BY

ODUOYE, OLUSOLA THOMAS

IN THE DEPARTMENT OF BOTANY

ODUOYE, OLUSOLA THOMAS		05/05/14
AUTHOR'S NAME	SIGNATURE	DATE
1 ST SUPERVISOR'S NAME	SIGNATURE	DATE
2 ND SUPERVISOR'S NAME	SIGNATURE	DATE

DEDICATION

I want to dedicate this work to: the Alpha and Omega, the beginning and the ending, which is, which was, and which is to come, the Almighty. It goes also to those who started the journey; my late father, Evangelist Samuel Oduoye Abidoye and my mother, Mrs Deborah Oluwemi Oduoye. The work is yours my wife Mrs Oluwaseun Deborah Oduoye and my Children; Samuel Temiloluwa and David Opeoluwa. It also belongs to the indefatigable siblings of mine and optimistic mentors and helpers whom God has used to bring me this far.

ACKNOWLEDGMENTS

My profound gratitude goes to the Lord God Almighty; the Alpha and Omega, the first and the last, the beginning and the ending. He started this work and finished it successfully with me, may His name be praised for ever.

I want to express my appreciation to my Supervisors: Professor Oluwatoyin Temitayo Ogundipe and Professor James 'Dele Olowokudejo for their parental concern, support and guidance throughout the course of this study. Professor Ogundipe was my spiritual mentor, at the same time God used him wonderfully to bear a lot of costs in the course of the studies. I have received the treatment of a family member since the inception of the programme and have enjoyed the same privilege till date. The family sponsored both my national and international trainings. Professor Olowokudejo is just research ready all days. He asked a lot of questions that my attempt to supply him answers gave me directions in the course of the study. Incidentally, my two supervisors taught me from the undergraduate to my Ph. D. level. Both of you are wonderful, and may God reward all your efforts in folds.

My special appreciation goes to all my lecturers in the Department starting with Professor Adekunle, A. A. and Dr. (Mrs) Umebesse, C. E. These are my academic father and mother respectively from my year one in the University. They believed in me and gave me all needed encouragements that supported my stay in academics. Dr. (Mrs) Adekanmbi, O. H. inspired the immediate commencement of my Postgraduate studies after the undergraduate convocation. She discussed my case with the then Dean of Postgraduate Studies who eventually accepted to supervise me. I may not be able to enumerate the contributions of all my Lecturers in turn: Dr. Akinsoji, A. A., Dr. Shonubi, O. O., Dr. Ade-Ademilua, O. E., Dr. Odjegba, J. V., Dr. Kadiri, A.
B., Dr. Adesalu, T. A., Dr. Adeonipekun, A. P., Dr. Onuminya, O. T., Dr. Samuel, T. O., Prof.
Ilori, M. O. (Dean of Science), Dr. Ogunkanmi, L. A., Dr. Adekoya, K., they are all wonderful,
the Lord shall yet move you up and make your name great.

My sincere appreciation also goes to all funding bodies that gave financial supports during these studies: Science and Technology Education Post Basic (STEPB) Project (Cr: 4304-Uni), Innovators of Tomorrow Research and Technology Development Grant; Nigerian Conservation Foundation (NCF) Chief S. L. Edu Memorial Research Grant, 2011.

School of Postgraduate Studies, University of Lagos gave me Graduate Fellowship during the studies and I sincerely appreciate the gesture. The Redeemed Christian Fellowship of the University of Lagos, I am grateful to you all. The Lord shall lift you all in Jesus name. My colleagues are worthy of mentioning, I am grateful to you all for your unflinching supports.

My siblings are always available in the race. My eldest brother, Pastor Zacchaeus Oduoye was involved throughout and gave wonderful encouragements from my basic education to the tertiary levels. I owe a lot to him and I know the Lord shall reward him bountifully. Pastor I. O. Abidoye, Pastor A. O. Oduoye, Pastor E. O. Oduoye and Engnr. I. O. Oduoye are all good to me to the very end. My nephews and nieces Victor, Tolu, Israel, Opeyemi, Ifeoluwa, are all obedient and helpful since they come on board.

I also appreciate the entire work force of National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, starting from the Director/ CEO, Mr. W. Odofin, the Director (R&D), Dr. S. E. Aladele, up to the last in the cadre; thanks for your moral, financial support and teamwork privileges I enjoyed from you all.

TABLE OF CONTENTS

Caption	Page
TITLE PAGE	i
CERTIFICATION	ii
DEDICATION	iii
ACKNOWLEDGMENTS	iv
TABLE OF CONTENTS	vi
LIST OF TABLES	Х
LIST OF FIGURES	xi
LIST OF PLATES	xiii
ABSTRACT	xvi

Chapter One: INTRODUCTION

1.0	Introduction and Background of Study	1
1.1.0	Deoxyribonucleic Acid (DNA)	2
1.1.1	Molecular Systematics	6
1.2	Statement of Problems	10
1.3	Aim and Objectives	11
1.4	Significance of the Study	12
1.5.0	Operational definition of terms and abbreviations/Acronyms	13

Chapter Two: LITERATURE REVIEW

2.0	Systematic History of Loganiaceae	16
2.1.0	Classification of Loganiaceae	17
2.1.1	Systematic Positions of Some Genera Formerly Included in Loganiaceae	22
2.1.2	Buddlejaceae	22
2.1.3	Retzia	22
2.1.4	Plocosperma, Polypremum, Peltanthera and Sanango	23
2.2.0	Economic Importance of Loganiaceae	24
2.2.1	Anthocleista	24
2.2.2	Strychnos Species	25
2.2.3	Spigelia anthelmia	30
2.2.4	Usteria guineensis	30
2.3.0	Plant Identification and Taxonomic Key	31

Chapter Three: MATERIALS AND METHODS

3.1.0	Herbarium Study and Samples Collection	32
3.1.1	Herbarium Preparation	33
3.2.0	Biogeography	33
3.3.0	Morphological and Anatomical studies	34
3.3.1	Macromorphology	34
3.3.2	Micromorphology	34
3.4.0	Molecular Studies	35

3.4.1	DNA Extraction	36
3.4.2	Purification of Extracted DNA	37
3.4.3	Gel Electrophoresis	37
3.4.4	Loading of Samples and Running the Gel.	38
3.4.5	Cleaning and Quantifying PCR products	38
3.4.6	Spectrophotometry	39
3.4.7	Polymerase Chain Reaction (PCR) for DNA Amplification	39
3.4.8	Amplification of the Internal Transcribed Spacer (ITS) Region	39
3.4.9	Amplification of trnL-trnF spacer plus trnL intron	40
3.4.10	Cycle Sequencing	41
3.4.11	DNA Sequencing, Editing and Alignment	41
3.4.12	Sequencing of ITS region	42
3.4.13	Sequencing of trnL-trnF Spacer Plus trnL Intron	42
3.4.14	Phylogenetic Analyses	42
3.5.0	Data Analysis	42
3.6.0	Taxonomic Key	43

Chapter Four: RESULT ANALYSIS

4.0	Herbaria and field collection	44
4.1	Herbaria collection	44
4.1.1	Field collection	44
4.1.2	Herbarium preparation	46
4.2	Biogeography of Loganiaceae	63

4.3.0	Morphological and Anatomical Studies	79
4.3.1	Macro-morphology of the leaves of the species of Loganiaceae	79
4.3.2	Micro-morphology of the leaves of the species of Loganiaceae	101
4.3.3	Data Analysis	117
4.4	Molecular Analysis	150
4.4.1	Sample selection, DNA extraction and Gel – electrophoresis	150
4.4.2	Spectrophotometry	152
4.4.3	Amplification of gene regions and Sequencing	156
4.4.4	Phylogenetic Analysis	159
4.5.1	Taxonomic Keys for Loganiaceae species in West Africa	166

Chapter Five: DISCUSSION

5.0 Discus	ssion	170
------------	-------	-----

Chapter Six: SUMMARY OF FINDINGS AND CONCLUSION

6.0	Summary of Findings	177
6.1	Conclusion	178
6.2	Contributions to Knowledge	179
	References	180
	Appendices	196

List of Tables

Tables	Title	Page Number
1	The summary of most recent classifications of Loganiaceae by different a	authors 18
2	Herbaria collections assessed for this study	47
3	Loganiaceae collections on the field	49
4A	Coordinates of collection and their decimal Maxent values	64
4B	West Africa nineteen environmental variables from Worldclim database	65
4C	Variable contributions to the Maxent model	78
5A	Morphological assessment of Loganiaceae	82
5B	Principal Component Analysis (PCA) showing communalities and component	onent
	matrix for Loganiaceae morphology	96
5C	Two principal components from PCA contributed about 6 4%	97
6A	Micro-morphological characters of the species of Loganiaceae	103
6B	Stomata distribution in Loganiaceae	107
6C	Trichome distribution in Loganiaceae	113
6D	Cuticular ornamentation and ergastic substances in Loganiaceae	114
7A	PCA of Loganiaceae micro-morphology four components up to 75 %	123
7B	PCA of Loganiaceae micro-morphology Showing Communalities	124
8	The Selected Samples for Sequencing at RBG, Kew	151
9	Spectrophotometric check of the DNA quality and quantity	153

List of Figures

Figure	s Title	Page Number	
1	A Section of DNA, Two Spiralling Strands	5	
2	Map of West Tropical Africa	21	
3	Strychnos spinosa showing dried fruit	28	
4A	Omission and predicted Area for Loganiaceae	67	
4B	The receiver operating characteristic (ROC) curve for Loganiaceae.	68	
4C	Species Distribution for Loganiaceae in West Tropical Africa	69	
4D	Jackknife of regularized training gain for Loganiaceae	70	
4E	Jackknife of test gain for Loganiaceae	71	
4F	Jackknife of Area Under Curve (AUC) for Loganiaceae	72	
4G	The marginal effect of changing exactly one environmental variable	73	
4H	Full view of response curves of Bio 10 and Bio 14.	75	
4I	The marginal effect of changing corresponding variables	76	
5A	Dendrogram showing relationship of Loganiaceae based on morphologica	l data 95	
5B	Principal Component Analysis (PCA) for Loganiaceae Morphology	98	
5C	Scatter plot of Leaf length (LL), Leaf width (LW) and Plant height	99	
5D	The scatter diagram for the first component obtained from PCA	100	
6A	Dendrogram of Loganiaceae based on adaxial micro-morphological data	119	
6B	Dendrogram of Loganiaceae based on abaxial micro-morphological data	120	
6C	Dendrogram based on adaxial and abaxial micro-morphological data	121	
6D	Dendrogram of Loganiaceae based on gross morphological data	122	
6E	PCA Scree Plot of Eigen values for Loganiaceae micro-morphological data	125	

List of Figures

Figures	Title	Page Number
6F	Scatter plot of epidermal cell number versus Stomata index	126
7A	Phylogenetic relationship of Loganiaceae based on ITS matrices	161
7B	Phylogenetic relationship of Loganiaceae combined matrices	162
7C	Phylogenetic relationship of Loganiaceae based on trnL E-F matrices	163
7D	Phylogenetic relationship of Strychnos based on combined matrices	164
7E	Phylogenetic relationship of Strychnos based on trnL E-F matrices	165

List of Plates

Plates	Title	Page Number
1	Photographs of Anthocleista species physiognomy	51
2	Photographs of Anthocleista and Mostuea species	52
3	Photographs of Spigelia anthelmia	53
4	Photographs of Strychnos and supports (hosts) in high forest	54
5	Photographs of Strychnos Physiognomy	55
6	Photographs of Strychnos Lamina	56
7	Photographs of Usteria guineensis	57
8	Photographs of Loganiaceae Inflorescence and Fruit	58
9	Photographs of Loganiaceae fruits and seeds	59
10	Photographs of Loganiaceae Diagnostic Features	60
11	Photographs of Voucher Specimens for Loganiaceae	61
12	Photographs of Strychnos tendril called hook	62
2A	Photomicrograph of the leaf surfaces of Anthocleista djalonensis	127
2B	Photomicrograph of the leaf surfaces of Anthocleista microphyla	128
2C	Photomicrograph of the leaf surfaces of Anthocleista nobilis	129
2D	Photomicrograph of the leaf surfaces of Anthocleista obanensis	130
2E	Photomicrograph of the leaf surfaces of Anthocleista procera	131
2F	Photomicrograph of the leaf surfaces of Anthocleista scandens	132
2G	Photomicrograph of the leaf surfaces of Mostuea brunonis	133
2H	Photomicrograph of Nuxia, Spigelia anthelmia and Strychnos aculeata	134
2I	Photomicrograph of Strychnos afzeli, S. barteri and S. boonei	135

List of Plates

Plates	Title	Page Number
2J	Photomicrograph of Strychnos campicola and S. camptoneura	136
2K	Photomicrograph of S. chromatoxylon, S. congolana and S. cuminodora	137
2L	Photomicrograph of Strychnos densiflora, S. dinklagei and S. floribund	a 138
2M	Photomicrograph of Strychnos gossweileri, S. icaja and S. innocua	139
2N	Photomicrograph of Strychnos johnsonii, S. longicaudata and S. lucens	140
20	Photomicrograph of S. malacoclados, S. memecyloides and S. nigritana	ı 141
2P	Photomicrograph of Strychnos nux-vomica, S. phaeotricha and S. soubr	ensis 142
2Q	Photomicrograph of Strychnos spinosa, S. splendens and Fagrae fragra	<i>ins</i> 143
2R	Photomicrograph of Strychnos staudtii, S. talbotiae and S. tricalysioides	5 144
2S	Photomicrograph of Strychnos urceolata, S. usambarensis and S. chryso	phylla 145
2T	Photomicrograph of Strychnos Indeterminate - SID 55, SID 56 and SID	57 146
2U	Photomicrograph of SID 58, SID 59 and SID 61	147
2V	Photomicrograph of <i>Strychnos</i> Indeterminate - SID 62, SID 63 and SID	64 148
2W	Photomicrograph of SID 65, SID 66 and Usteria guineensis	149
3A	Electrophorogram of genomic DNA of Loganiaceae species	155
3B	Electrophorogram of genomic DNA for 22 selected samples	155
3C	Electrophorogram of ITS3–ITS4 amplification	157
3D	Electrophorogram of ITS2–ITS5 amplification	157
3E	Electrophorogram of trnlC – trnlD amplification	157
3F	Electrophorogram trnlE-trnlF amplification	157
3G	Electrophorogram of ITS3– ITS4 purification	158

List of Plates

Plates	Title	Page Number
3Н	Electrophorogram of ITS2–ITS5 purification	158
3I	Electrophorogram of trnlC – trnlD purification	158
3J	Electrophorogram of trnlE -trnlF purification	158

ABSTRACT

Loganiaceae is a family of trees, shrubs and tendril-bearing lianas with 13 genera and about 350 species distributed mainly in the tropics, subtropics and a few in temperate regions of the world. The family was described by Von Martius in 1827 but has undergone numerous revisions that have expanded and contracted its circumscription, ranging from one genus at its smallest to 30 at its largest. This study addressed taxonomic problems of familial circumscription, generic delimitation and species identification that exist within the family. It utilized both gross morphology and molecular motifs for the delimitation of genera and species in the family. The taxonomy of the family was evaluated using macro and micro morphological characters with molecular studies. Environmental parameters adopted from the WorldClim database designed for West Africa revealed that the species distribution is affected mostly by the precipitation of coldest quarter of the year; precipitation of the driest month and least affected by precipitation of the warmest quarter of the year. A wide range of epidermal features provide taxonomically useful anatomical characters. The presence of stomata on both adaxial and abaxial surfaces (Amphistomata) distinguished Anthocleista djalonensis, Anthocleista procera and Strychnos spinosa from other species in the family. Anisocytic, anomocytic, paracytic and staurocytic stomata types occur in the family. Trichomes found in the family include simple unicellular, stellate, dendritic and conical types. There are different types of cuticular foldings, wax coatings and other ergastic substances in the family. Molecular studies involved four different gene regions which were sequenced across the selected 21 representative species from the family, for phylogenetic analysis. Sequences alignment and phylogenetic analyses revealed that 3 genera: Anthocleista, Mostuea and Nuxia which were originally circumscribed with the family should be separated from it. This is because of low bootstrap values below 40 % and the difficulty encountered in their alignment which showed that they are evolutionarily divergent and distant from the family. This study revealed that Loganiaceae is composed of 3 genera: *Spigelia, Strychnos* and *Usteria* with 39 species in West Africa as opposed to the 6 genera originally circumscribed in the family. In addition, one new species, *Strychnos* sp. (*species nova* - P01860082) was described for the first time while *Strychnos tomentosa* was also newly introduced and a taxonomic key for identifying the genera and species of the family was produced.