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# Bioinformatics a Tool for Biosystematics Studies of *Lagenaria siceraria* (Mol.) Standl. Complex

**Fortune O. Awala*, Benjamin C. Ndukwu, Ikechukwu O. Agbagwa**

Department of Plant Science & Biotechnology, University of Port Harcourt, Port Harcourt, Nigeria
Email: *ihunnah.fortune@gmail.com, benjamin.ndukwu@gmail.com, ikechukwu.agbagwa@uniport.edu.ng

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## Abstract

Bioinformatics has been a major tool in the revolution of plant systematics in recent times. The diversity of fruit shapes in *Lagenaria siceraria* (Mol.) standl. species has been of great concern because of its fruit complexity. This study is based on the application of rubisco enzyme using *rbcL* marker because of its conservativeness and its ability to discriminate below the specific level hence its usage to sequence the chloroplast genome with ABI, PRISM 377 DNA sequencer. The sequences obtained were viewed using MEGA X software and subsequently subjected to validation through National Center for Biotechnology Information (NCBI) using Nucleotide Basic Local Alignment Search Tool (BLAST N). The result obtained showed that all the sequences belong to *Lagenaria siceraria* (Mol.) standl. with percentages ranging from 95% to 100% for query cover sequences and 98% to 100% for identity sequences. From the taxonomic report obtained sequence A, C, G, H, J, Q has the highest hits of 44 on *Lagenaria siceraria* out of 109 total value, sequence O and R has the highest hits of 44 on *Lagenaria siceraria* out of the total value of 111, sequence V has the highest hits of 44 on *Lagenaria siceraria* out of 119 total value and sequence X finally has the highest value hits of 44 on *Lagenaria sceriania* out of 105 total value, based on this report, phylogenetic tree was constructed to show the level of relatedness of the different fruit diversity of *L. siceraria* complex. This work therefore has aided in the molecular characterization of *Lagenaria siceraria* (Mol.) standl. landraces found in Nigeria.

## Keywords

Bioinformatics, Biosystematics, Phylogenetic, Diversity, *Lagenaria siceraria* (Mol.) Standl. Complex

## 1. Introduction

The species *Lagenaria siceraria* (Molina) Standl is a member of the Cucurbitaceae family, Cucurbitaceae subfamily and Benincaseae tribe [1]. The genus Lagenaria consists of five other wild species, namely *Lagenaria breviflora* (Benth) Roberty, *Lagenaria rufa* (Gilg) C. Jeffrey, *Lagenaria sphaerica* E. May, *Lagenaria abyssinia* (Hook F) C. Jeffrey, *Lagenaria guinensis* (G. Den) C. Jeffrey and *Lagenaria siceraria* (Molina) Standley is the most cultivated within the species of s*iceraria*; two morphologically distinct sub-species of bottle gourd have been recognized thus *Lagenaria siceraria* subspecies asiatica and *Lagenaria siceraria* subspecies *siceraria* [2] [3] [4]. It is universally agreed fact, that different species are systems of population which exhibit variation and not fixed entities, therefore no individual is identical as developed by [5]. Systematics as defined by [6], states that systematics is a natural science that deals with the study of individual, population and taxon relationship for the purposes of classification. Plant systematics is therefore based on the premise that in the enormous variation in the plant kingdom there exist discrete units called species that can be identified classified and named with further logical relationships developed among these units. Over the years, plant groupings have been redefined as more information is accumulated from newer approaches and different recent sources such as molecular systematics, and taximetrics. Variety of *Lagenaria* is known throughout West Africa where they serve different economic purposes but these cultivars are largely unrecorded in terms of micromorphological characteristics [7] and diversity of fruit shape and sizes continue to create serious taxonomic difficulties in the delimitation of the taxon *Lagenaria siceraria* (Mol) Standley below the specific level.

The emerging field of molecular systematics deals with the exclusive utilization of molecular data [6]. A molecular marker is a gene or DNA sequence with a known location on a chromosome that can be used to identify species, which detect variation at the nuclear, Mitochondrial, and chloroplast DNA. Molecular markers have been developed and utilized to provide the requisite landmarks for clarification of genetic variation [8]. DNA is more stable macromolecule than RNA and is found in all plant tissues hence, DNA based markers are preferred for precise identification of plant species [9]. An understanding of the extent of genetic diversity within and amongst the landraces of the gourd is important for strategic breeding and conservation [10].

Plants are generally more tolerant of variation, hence genetic variation may result from mutation or recombination [6] [11]. According to [12], effective utilization of plant genetic resources depends on a detailed understanding of their genetic variability. Interestingly, substantial variation exists in the ability of plants to tolerate gene imbalance both between different plant species and between varieties of the same species [13]. However, despite the widespread interest in molecular systematics, there is limited understanding of the molecular mechanisms that lead to phenotypic alterations in plant species as well as gene interactions involved in the global genomic level [14], went further to explain that the molecular systematic studies give deeper insight into genetic structures. It has performed an important role in molecular biologies, such as analyzing genetic diversity by classification of cultivars and germplasm collections. It has also aided in clearing phylogenetic relationships among groups or closely related species. It is further used to determine the sequence of nucleotides in the DNA of plants, conclusively, has increased significantly, understanding of plant evolution.

## 2. Materials and Methods

### 2.1. Study Map

The species were planted in the faculty of Agricultural science research farm, of the University of Port Harcourt (**Figure 1**).

### 2.2. Plant Material and DNA Isolation

Twenty-four landraces of *Lagenaria siceraria* (Mol.) Standl. were collected from different locations of Nigeria and planted at Agricultural Science Research Farm. The young fresh leaves from A African Bottle Gourd, B Kettle Gourd, C Caveman Club Gourd, D Base Ball Gourd, F Bushel Gourd, G Bird House Gourd, H Water Jug Gourd, I Cup Gourd, K Warted Bushel Gourd, L Extra Large Pawpaw Gourd, M Long Siphon Gourd, N Indian Gourd, O Chinese Bottle Gourd, P Mini Dipper Gourd, Q Goose Neck Gourd, S Swan Gourd, T Palm Wine Gourd, U Long Handle Dipper Gourd, V Powder Horn Gourd, W Snake Gourd and X Microphone Gourd were analyzed at Biotechnology Research Centre of the University of Port Harcourt in Partnership with International Institute of Tropical Agriculture (IITA) Ibadan in 2018. The fresh leave sample from 21-day-old seedlings was crushed using liquid nitrogen. Genomic DNA was extracted using a Zymo Quick Plant/Seed extraction method (2010) and stored at −20˚C. The quantity of the DNA was determined using Thermo Scientific™ NanoDrop 2000/2000C spectrophotometer, while the quality of DNA isolated was analyzed using 0.8% agarose gel electrophoresis and viewed with UV Transilluminator—BIOCOMdirect.

Polymerase chain reaction (PCR) analysis was carried out using standard PCR procedure. The amplification of the extracted DNA was done using Gene Amp® PCR System 9700 Dual 384—well sample block module, according to the protocol of Applied Biosystems (2002). The rbcl primer having a forward sequence of F (5’ ATGTCACCACAAACAGAAAC 3’) and reverse rbcl primer sequence of R (5’ TCGCATGTACCTGCAGTAGC 3’) with an expected size of 724 bp, 10 × PCR buffer 1.0, 50 mM $MgCl_2$ 0.4, 5 pMol forward primer 0.5, 5 pMol reverse primer 0.5, DMSO 0.8, 2.5 Mm DNTPs 0.8, Taq 5 u/μl 0.1, 100 ng/μl DNA 3.0, $H_2O$ 2.9 μl and a final volume of 10 μL. The amplification products were analyzed using a 1.5% agarose gel stained with EZ-Vision® in Gel solution. The samples are arranged A-X after the ladder and the ladder used is 50 bp from NEB (**Plate 1**).

Plate 1. Showing 21 days old *Lagenaria siceraria* (Mol.) Standl.

The amplification products from land races of *Lagenaria siceraria* (Mol.) Standl. were separated using 1.5% agarose electrophoresis and the expected size of bands were excised using scapel and purified by Spin Column Purification method. Purified amplicons were sequenced using *BigDye*® *Terminator v*3.1 *Cycle Sequencing Kit* protocol in ABI Prism 377 DNA Sequencer (Applied Biosciences, NY, USA).

### 2.3. Sequence Alignment and Phylogenetic Analysis

*Lagenaria siceraria* sequences in FASTA format were aligned and used in the analysis of protein content of the twenty three species, the process of sequence analysis was advanced through pair wise alignment, construction of a distance matrix using Mega X. The sequences were compared to the NCBI database using Nucleotide Basic Local Alignment Search Tool (BLASTN). The results of distance matrix were sent to Excel 2007, which was finally analyzed using PAST 3.14 software for the construction of phylogenetic tree showing similarity and distance relationship between the twenty-three species. The chloroplast sequences of *L. siceraria* from NCBI data base were analysed using CHLOROBOX.

## 3. Result

Molecular result of twenty-four samples of the landraces of *L. siceraria* is obtained below. The result of gel electrophoresis (**Figure 2**) shows the quality of genomic DNA after extraction. The bands is observed in A African Bottle Gourd, B Kettle Gourd, C Caveman Club Gourd, D Base Ball Gourd, F Bushel Gourd, G Bird House Gourd, H Water Jug Gourd, I Cup Gourd, K Warted Bushel Gourd, L Extra Large Pawpaw Gourd, M Long Siphon Gourd, N Indian Gourd, O Chinese Bottle Gourd, P Mini Dipper Gourd, Q Goose Neck Gourd, S Swan Gourd, T Palm Wine Gourd, U Long Handle Dipper Gourd, V Powder Horn Gourd, W Snake Gourd and X Microphone Gourd, showing the presence of the gDNA while in E Pot Gourd, J Pennis Shield Gourd and R Nigeria Rattle Gourd showed no band.

The polymerase chain reaction product using *rbcl* primer (**Figure 3(a)**) shows a visible band at 850 bp in A African Bottle Gourd, C Caveman Club Gourd, D Base Ball Gourd, F Bushel Gourd, I Cup Gourd, K Warted Bushel Gourd, L Extra Large Pawpaw Gourd, M Long Siphon Gourd, N Indian Gourd, O Chinese Bottle Gourd, P Mini Dipper Gourd, R Nigeria Rattle Gourd, S Swan Gourd, T Palm Wine Gourd, U Long Handle Dipper Gourd, V Powder Horn Gourd, W Snake Gourd and X Microphone Gourd while B Kettle Gourd, E Pot Gourd, G

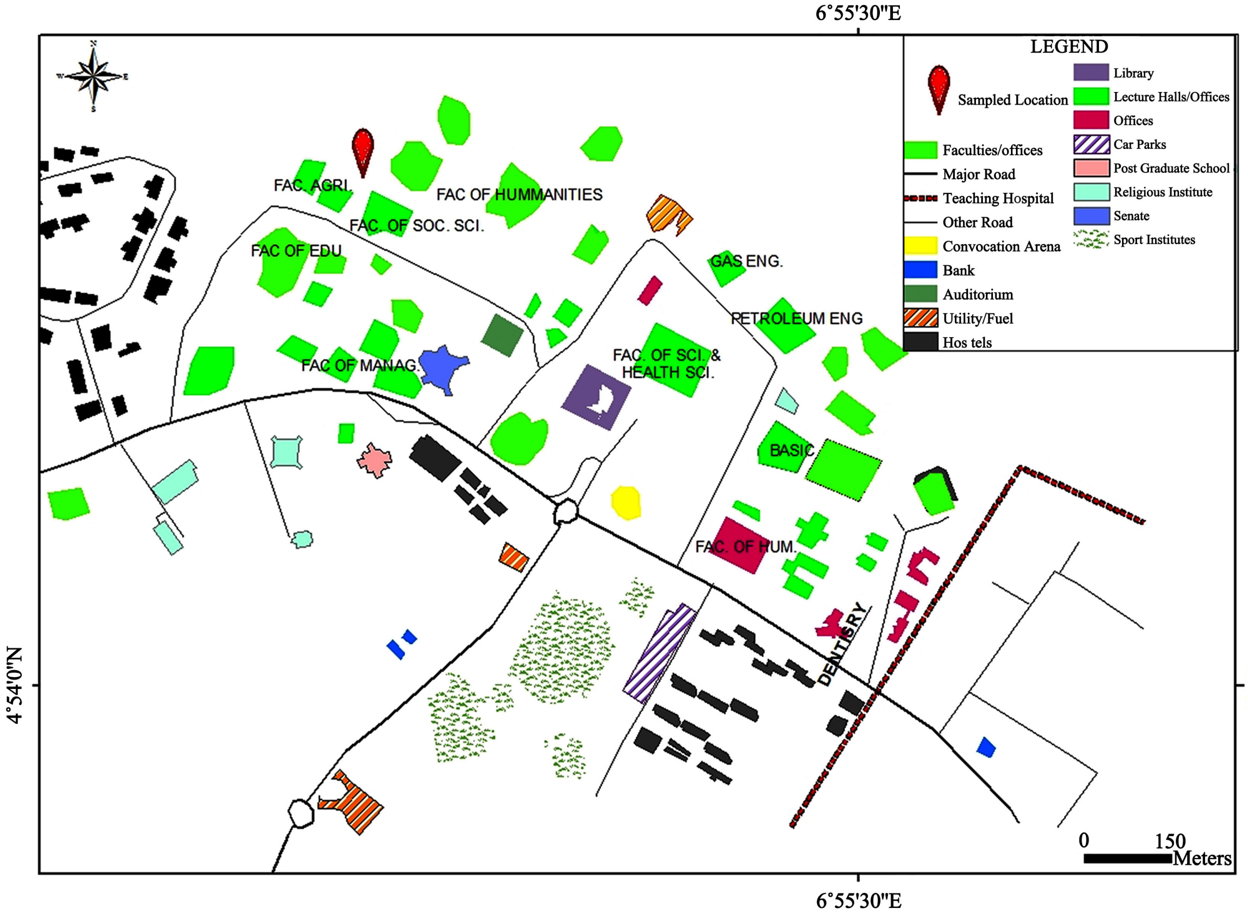



**Figure 1.** Map of the study area.

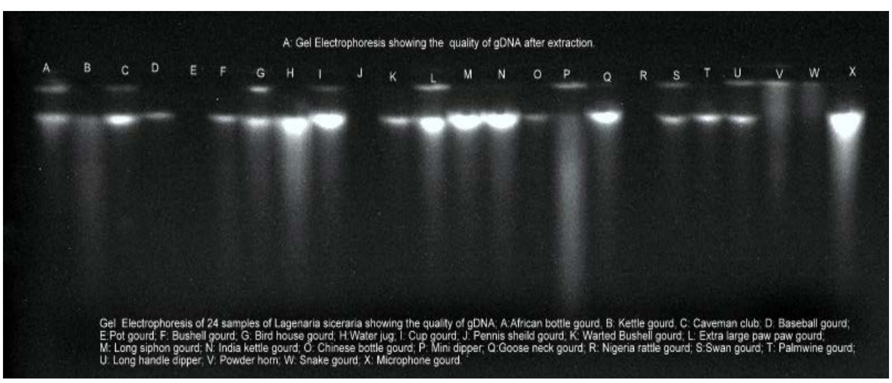
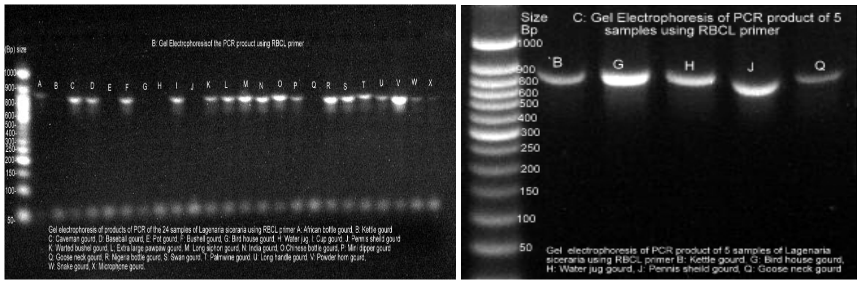



**Figure 2.** Gel electrophoresis: A: Showing Quality of gDNA extracted.




(a) (b)

**Figure 3.** (a) and (b), gel electrophoresis of PCR product using *rbcl* primer.

Bird House Gourd, H Water Jug Gourd, J Pennis Shield Gourd and Q Goose Neck Gourd showed no band on the agarose gel electrophoresis. In the repeat of the polymerase chain reaction (**Figure 3(b)**) it is observed that B Kettle Gourd, G Bird House Gourd, H Water Jug Gourd, J Pennis Shield Gourd and Q Goose Neck Gourd also showed band at 850 base pair.

The quantity of DNA extracted is read using spectrophotometer as shown in **Table 1**. The value ranges from 1.13 to 1.82

In **Table 2** the nucleotide sequence arrangement possesses an N character of 720. The sequence alignment for twenty-three landraces of *L. siceraria* shows great diversity and variability in the arrangement of the bases (**Table 3**).

The result observed from BLASTN (**Table 4**) shows that all the sequences belong to *Lagenaria siceraria* with percentages ranging from 95% to 100% for query cover sequences and 98% to 100% for identity sequences with an E-value of 0.00. From the taxonomic report, sequence F-Bushel Gourd, K-Warted Bushel Gourd, U-Long Handle Dipper Gourd, I-Cup Gourd, L-Extra, M-Long Siphon Gourd, S-Swan Gourd, T-Palm Wine Gourd and W-Snake Gourd has the highest hits of 44 on *Lagenaria siceraria* out of 109 total value, sequence O-Chinese Bottle Gourd, P-Mini Dipper Gourd and R-Nigeria Rattle has the highest hits of 44 on *L. siceraria* out of the total value of 111, sequence V-Powder Horn Gourd has the highest hits of 44 on *Lagenaria siceraria* out of 119 total value, sequence C-Caveman Club Gourd, G-Bird House Gourd, H-Water Jug Gourd, J-Pennis Sheild, Q-Goose Neck Gourd, obtained the highest hit of 45 out of the total value 104, while sequence A-African Bottle Gourd, B Kettle Gourd, D Base Ball Gourd, has a total taxonomic value of 104 out of which 44 were identified as *Lagenaria siceraria.* The total taxonomic value of N-Indian Gourd, was observed as 110 with the highest hit value being 44 on *Lagenaria siceraria* and sequence X-Microphone Gourd finally has a highest value hits of 44 on *Lagenaria siceraria* out of 105 total value.

The total nucleotide composition of the species (**Table 5**) varies across the different landraces. A-African Bottle Gourd is observed to have a total nucleotide composition of 689, thymine having an average frequency of 20.1%, cytosine 20.0%, adenine 28.6%, and guanine 22.6%. The total nucleotide composition in B-Kettle Gourd is 686 with an average percentage frequency of 29.0% for thymine, 19.1% for cytosine, 28.9% for adenine and 22.4% for guanine. C-Caveman

**Table 1.** Nanodrop of *Lageneria siceraria* (Mol.) Standl.

| Sample Identity | Nucleic Acid | Unit | 260/280 |
|---|---|---|---|
| A-African Bottle Gourd | 58.3 | ng/µl | 1.3 |
| B-Kettle Gourd | 54.7 | ng/µl | 1.34 |
| C-Caveman Club Gourd | 57.2 | ng/µl | 1.29 |
| D-Base Ball Gourd | 19.6 | ng/µl | 1.82 |
| E-Pot Gourd | 30.6 | ng/µl | 1.23 |
| F-Bushel Gourd | 33.3 | ng/µl | 1.13 |
| G-Bird House Gourd | 94.8 | ng/µl | 1.36 |
| H-Water Jug Gourd | 78.1 | ng/µl | 1.47 |
| I-Cup Gourd | 47.2 | ng/µl | 1.23 |
| J-Pennis Shield Gourd | 86.7 | ng/µl | 1.46 |
| K-Warted Bushel Gourd | 58.2 | ng/µl | 1.31 |
| L-Extra Large Pawpaw Gourd | 41.3 | ng/µl | 1.16 |
| M-Long Siphon Gourd | 63 | ng/µl | 1.4 |
| N-Indian Gourd | 70.7 | ng/µl | 1.36 |
| O-Chinese Bottle Gourd | 53 | ng/µl | 1.48 |
| P-Mini Dipper Gourd | 30.5 | ng/µl | 1.2 |
| Q-Goose Neck Gourd, | 78.6 | ng/µl | 1.46 |
| R-Nigeria Rattle Gourd | 61.2 | ng/µl | 1.36 |
| S-Swan Gourd | 60.5 | ng/µl | 1.39 |
| T-Palm Wine Gourd | 35.4 | ng/µl | 1.2 |
| U-Long Handle Dipper Gourd | 35 | ng/µl | 1.25 |
| V-Powder Horn Gourd | 116.3 | ng/µl | 1.57 |
| W-Snake Gourd | 65.9 | ng/µl | 1.46 |
| X Microphone Gourd | 98.8 | ng/µl | 1.44 |

**Table 2.** Nucleotide sequence arrangement.

A-African Bottle Gourd,
TTAATTAAAATTAAGCCCTCCGCGACAAAAGAGGACAAAGGTCTCTGCCATCTTGGCAGC
ATTCCGAGTAACTCCTCAACCGGGAGTTCCACCTGAGGAAGCAGGGGCCGCTGTAGCTGC
TGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACCAGTCTTGATCG
TTACAAAGGACGATGCTATGGAATCGAGCCTGTTCCTGGAGAAGAAAATCAATATATTGC
TTATGTAGCTTATCCCCTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTC
CATTGTGGGTAATGTATTTGGATTCAAGGCTCTACGTGCTCTACGTCTGGAGGATTTGCG
AATCCCTACTGCTTATATTAAAACTTTCCAAGGCCCGCCTCATGGTATCCAGGTTGAAAG
AGATAAATTGAACAAGTATGGTCGCCCTCTATTGGGATGTACTATTAAACCAAAATTGGG
ATTATCCGCTAAGAATTATGGTAGAGCAGTTTATGAATGTTTACGCGGTGGACTTGATTT
TACCAAAGATGATGAAAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTCCT
ATTTTGTGCGGAAGCTATTTATAAATCACAGGCTGAAACAGGTGAAATCAAGGGACATTA
CTTGAATGCTACTGCAGTACACATGCAAA,
B-Kettle Gourd,
GGCAATTTAATTAAGCCCTCCTCGAATAAGAAACCAAAGATACTGATATCTTGGCAGCAT
TCCGAGTAACTCCTCAACCGGGAGTTCCACCTGAGGAAGCAGGGGCCGCTGTAGCTGCTG

AATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACCAGTCTTGATCGTT
ACAAAGGACGATGCTATGGAATCGAGCCTGTTCCTGGAGAAGAAAATCAATATATTGCTT
ATGTAGCTTATCCCCTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCA
TTGTGGGTAATGTATTTGGATTCAAGGCTCTACGTGCTCTACGTCTGGAGGATTTGCGAA
TCCCTACTGCTTATATTAAAACTTTCCAAGGCCCGCCTCATGGTATCCAGGTTGAAAGAG
ATAAATTGAACAAGTATGGTCGCCCTCTATTGGGATGTACTATTAAACCAAAATTGGGAT
TATCCGCTAAGAATTATGGTAGAGCAGTTTATGAATGTTTACGCGGTGGACTTGATTTTA
CCAAAGATGATGAAAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTCCTAT
TTTGTGCGGAAGCTATTTATAAATCACAGGCTGAAACAGGTGAAATCAAGGGACATTACT
TGAATGCTACTGCAGTAACATGCAAA,
C-Caveman Club Gourd,
AAAAATTTAGCCTTTCCCAGCGCAATATAGAGGAAACGGTCTCTGATATCTTGGCAGCAT
TCCGAGTAACTCCTCAACCGGGAGTTCCACCTGAGGAAGCAGGGGCCGCTGTAGCTGCTG
AATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACCAGTCTTGATCGTT
ACAAAGGACGATGCTATGGAATCGAGCCTGTTCCTGGAGAAGAAAATCAATATATTGCTT
ATGTAGCTTATCCCCTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCA
TTGTGGGTAATGTATTTGGATTCAAGGCTCTACGTGCTCTACGTCTGGAGGATTTGCGAA
TCCCTACTGCTTATATTAAAACTTTCCAAGGCCCGCCTCATGGTATCCAGGTTGAAAGAG
ATAAATTGAACAAGTATGGTCGCCCTCTATTGGGATGTACTATTAAACCAAAATTGGGAT
TATCCGCTAAGAATTATGGTAGAGCAGTTTATGAATGTTTACGCGGTGGACTTGATTTTA
CCAAAGATGATGAAAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTCCTAT
TTTGTGCGGAAGCTATTTATAAATCACAGGCTGAAACAGGTGAAATCAAGGGACATTACT
TGAATGCTACTGCAG
D-Base Ball Gourd,
AAATTTTTAATTTAGGCCTCCTCGAATATAGAAACCAAAGATACTGATATCTTGGCAGCA
TTCCGAGTAACTCCTCAACCGGGAGTTCCACCTGAGGAAGCAGGGGCCGCTGTAGCTGCT
GAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACCAGTCTTGATCGT
TACAAAGGACGATGCTATGGAATCGAGCCTGTTCCTGGAGAAGAAAATCAATATATTGCT
TATGTAGCTTATCCCCTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCC
ATTGTGGGTAATGTATTTGGATTCAAGGCTCTACGTGCTCTACGTCTGGAGGATTTGCGA
ATCCCTACTGCTTATATTAAAACTTTCCAAGGCCCGCCTCATGGTATCCAGGTTGAAAGA
GATAAATTGAACAAGTATGGTCGCCCTCTATTGGGATGTACTATTAAACCAAAATTGGGA
TTATCCGCTAAGAATTATGGTAGAGCAGTTTATGAATGTTTACGCGGTGGACTTGATTTT
ACCAAAGATGATGAAAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTCCTA
TTTTGTGCGGAAGCTATTTATAAATCACAGGCTGAAACAGGTGAAATCAAGGGACATTAC
TTGAATGCTACTGCAGTTAC
E-Bushel Gourd,
GTCAACTATTTATTGATTGTGTTAAGATTATAAATTGACTTATTATACTCCTGAATATGA
AACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTTCCACC
TGAGGAAGCAGGGGCCGCTGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTG
GACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTATGGAATCGAGCCTGT
TCCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTATCCCCTAGACCTTTTTGAAGA
AGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGATTCAAGGCTCT
ACGTGCTCTACGTCTGGAGGATTTGCGAATCCCTACTGCTTATATTAAAACTTTCCAAGG
CCCGCCTCATGGTATCCAGGTTGAAAGAGATAAATTGAACAAGTATGGTCGCCCTCTATT
GGGATGTACTATTAAACCAAAATTGGGATTATCCGCTAAGAATTATGGTAGAGCAGTTTA
TGAATGTTTACGCGGTGGACTTGATTTTACCAAAGATGATGAAAACGTGAATTCCCAACC
ATTTATGCGTTGGAGAGACCGTTTCCTATTTTGTGCGGAAGCTATTTATAAATCACAGGC
TGAAACAGGTGAAATCAAGGGACATTACTTGAATGCTACTGCAGGTACCATGCGAA
F-Bird House Gourd,
TAAAGGGATCACCTCGATTATCCTTAGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTT
CCACCTGAGGAAGCAGGGGCCGCTGTAGCTGCTGAATCTTCTACTGGTACATGGACAACT
GTGTGGACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTATGGAATCGAG
CCTGTTCCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTATCCCCTAGACCTTTTT
GAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGATTCAAG
GCTCTACGTGCTCTACGTCTGGAGGATTTGCGAATCCCTACTGCTTATATTAAAACTTTC
CAAGGCCCGCCTCATGGTATCCAGGTTGAAAGAGATAAATTGAACAAGTATGGTCGCCCT
CTATTGGGATGTACTATTAAACCAAAATTGGGATTATCCGCTAAGAATTATGGTAGAGCA
GTTTATGAATGTTTACGCGGTGGACTTGATTTTACCAAAGATGATGAAAACGTGAATTCC

CAACCATTTATGCGTTGGAGAGACCGTTTCCTATTTTGTGCGGGAAGCTATTTATAAATC
ACAGGCTGAAACAGGTGAAATCAAGGGACATTACTTGAATGCTACTGCAGGTACATGCAA
A
G-Water Jug Gourd,
CAATTTCCCAGGAGTAAACTCCTCAACCGAGGAGTTCCACCTGAGGAAGCAGGGGCCGCT
GTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACCAGT
CTTGATCGTTACAAAGGACGATGCTATGGAATCGAGCCTGTTCCTGGAGAAGAAAATCAA
TATATTGCTTATGTAGCTTATCCCCTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATG
TTTACTTCCATTGTGGGTAATGTATTTGGATTCAAGGCTCTACGTGCTCTACGTCTGGAG
GATTTGCGAATCCCTACTGCTTATATTAAAACTTTCCAAGGCCCGCCTCATGGTATCCAG
GTTGAAAGAGATAAATTGAACAAGTATGGTCGCCCTCTATTGGGATGTACTATTAAACCA
AAATTGGGATTATCCGCTAAGAATTATGGTAGAGCAGTTTATGAATGTTTACGCGGTGGA
CTTGATTTTACCAAAAGATGATGAAAAACGTGAATTCCCAACCATTTTATGCGTTGGAGA
GACCGTTTCCTATTTTTGTGCGGAAAGCTATTTATAAATCACAGGGTGAAACAGGGGAAA
TCAAGGGAACATTTACTTGAATGCTACTGCAGGTACATGCAAAA
H-Cup Gourd,
GCACTACAGTAATAAATTGTGTTAAGATTATAAATTGACTTATTATACTCCTGAATATGA
AACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTTCCACC
TGAGGAAGCAGGGGCCGCTGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTG
GACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTATGGAATCGAGCCTGT
TCCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTATCCCCTAGACCTTTTTGAAGA
AGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGATTCAAGGCTCT
ACGTGCTCTACGTCTGGAGGATTTGCGAATCCCTACTGCTTATATTAAAACTTTCCAAGG
CCCGCCTCATGGTATCCAGGTTGAAAGAGATAAATTGAACAAGTATGGTCGCCCTCTATT
GGGATGTACTATTAAACCAAAATTGGGATTATCCGCTAAGAATTATGGTAGAGCAGTTTA
TGAATGTTTACGCGGTGGACTTGATTTTACCAAAGATGATGAAAACGTGAATTCCCAACC
ATTTATGCGTTGGAGAGACCGTTTCCTATTTTGTGCGGAAGCTATTTATAAATCACAGGC
TGAAACAGGTGAAATCAAGGGACATTACTTGAATGCTACTGCAGGTCCTTGCGA
I-Pennis Shield Gourd,
ACAAGGGGATTCACCCGATAATCCTTTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGT
TCCACCTGAGGAAGCAGGGGCCGCTGTAGCTGCTGAATCTTCTACTGGTACATGGACAAC
TGTGTGGACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTATGGAATCGA
GCCTGTTCCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTATCCCCTAGACCTTTT
TGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGATTCAA
GGCTCTACGTGCTCTACGTCTGGAGGATTTGCGAATCCCTACTGCTTATATTAAAACTTT
CCAAGGCCCGCCTCATGGTATCCAGGTTGAAAGAGATAAATTGAACAAGTATGGTCGCCC
TCTATTGGGATGTACTATTAAACCAAAATTGGGATTATCCGCTAAGAATTATGGTAGAGC
AGTTTATGAATGTTTACGCGGTGGACTTGATTTTACCAAAGATGATGAAAACGTGAATTC
CCAACCATTTATGCGTTGGAGAGACCGTTTCCTATTTTGTGCGGAAGCTATTTATAAATC
ACAGGCTGAAACAGGTGAAATCAAGGGACATTACTTGAATGCTACTGCAGGTACATGCAA
AAGAG
J-Warted Bushel Gourd,
GCCTTCCATGTAAAAATTTTTGTTAAGGATTATAATTGACTTATTATACTCCTGAATATG
AAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTTCCAC
CTGAGGAAGCAGGGGCCGCTGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGT
GGACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTATGGAATCGAGCCTG
TTCCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTATCCCCTAGACCTTTTTGAAG
AAGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGATTCAAGGCTC
TACGTGCTCTACGTCTGGAGGATTTGCGAATCCCTACTGCTTATATTAAAACTTTCCAAG
GCCCGCCTCATGGTATCCAGGTTGAAAGAGATAAATTGAACAAGTATGGTCGCCCTCTAT
TGGGATGTACTATTAAACCAAAATTGGGATTATCCGCTAAGAATTATGGTAGAGCAGTTT
ATGAATGTTTACGCGGTGGACTTGATTTTACCAAAGATGATGAAAACGTGAATTCCCAAC
CATTTATGCGTTGGAGAGACCGTTTCCTATTTTGTGCGGAAGCTATTTATAAATCACAGG
CTGAAACAGGTGAAATCAAGGGACATTACTTGAATGCTACTGCAGGAA
K-Extra Large Pawpaw Gourd,
TTCTGATCAAAGTAATGATGGTTGTTAAGATTATAATTGACTTATTATACTCCTGAATAT
GAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTTCCA
CCTGAGGAAGCAGGGGCCGCTGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTG
TGGACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTATGGAATCGAGCCT

GTTCCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTATCCCCTAGACCTTTTTGAA
GAAGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGATTCAAGGCT
CTACGTGCTCTACGTCTGGAGGATTTGCGAATCCCTACTGCTTATATTAAAACTTTCCAA
GGCCCGCCTCATGGTATCCAGGTTGAAAGAGATAAATTGAACAAGTATGGTCGCCCTCTA
TTGGGATGTACTATTAAACCAAAATTGGGATTATCCGCTAAGAATTATGGTAGAGCAGTT
TATGAATGTTTACGCGGTGGACTTGATTTTACCAAAGATGATGAAAACGTGAATTCCCAA
CCATTTATGCGTTGGAGAGACCGTTTCCTATTTTGTGCGGAAGCTATTTATAAATCACAG
GCTGAAACAGGTGAAATCAAGGGACATTACTTGAATGCTACTGCAGTACCATGGCGAAA
L-Long Siphon Gourd,
GTTCAACCAATGTTATGCGGTGTGTTAAGATTATAAATTGACTTATTATACTCCTGAATA
TGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTTCC
ACCTGAGGAAGCAGGGGCCGCTGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGT
GTGGACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTATGGAATCGAGCC
TGTTCCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTATCCCCTAGACCTTTTTGA
AGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGATTCAAGGC
TCTACGTGCTCTACGTCTGGAGGATTTGCGAATCCCTACTGCTTATATTAAAACTTTCCA
AGGCCCGCCTCATGGTATCCAGGTTGAAAGAGATAAATTGAACAAGTATGGTCGCCCTCT
ATTGGGATGTACTATTAAACCAAAATTGGGATTATCCGCTAAGAATTATGGTAGAGCAGT
TTATGAATGTTTACGCGGTGGACTTGATTTTACCAAAGATGATGAAAACGTGAATTCCCA
ACCATTTATGCGTTGGAGAGACCGTTTCCTATTTTGTGCGGAAGCTATTTATAAATCACA
GGCTGAAACAGGTGAAATCAAGGGACATTACTTGAATGCTACTGCAGTACCATGCGAA
M-Indian Gourd,
ATAGGGGGGGCCATTGCGGAACCCTGTAGCATTATAAATTGACTTATTATACTCCTGAAT
ATGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTTC
CACCTGAGGAAGCAGGGGCCGCTGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTG
TGTGGACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTATGGAATCGAGC
CTGTTCCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTATCCCCTAGACCTTTTTG
AAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGATTCAAGG
CTCTACGTGCTCTACGTCTGGAGGATTTGCGAATCCCTACTGCTTATATTAAAACTTTCC
AAGGCCCGCCTCATGGTATCCAGGTTGAAAGAGATAAATTGAACAAGTATGGTCGCCCTC
TATTGGGATGTACTATTAAACCAAAATTGGGATTATCCGCTAAGAATTATGGTAGAGCAG
TTTATGAATGTTTACGCGGTGGACTTGATTTTACCAAAGATGATGAAAACGTGAATTCCC
AACCATTTATGCGTTGGAGAGACCGTTTCCTATTTTGTGCGGAAGCTATTTATAAATCAC
AGGCTGAAACAGGTGAAATCAAGGGACATTACTTGAATGCTACTGCAGTACCATGCGAAA

N-Chinese Bottle Gourd,
GCCCTGCGTTGACTTATTATACTCCTGAATATGAAACCAAAGATACTGATATCTTGGCAG
CATTCCGAGTAACTCCTCAACCGGGAGTTCCACCTGAGGAAGCAGGGGCCGCTGTAGCTG
CTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACCAGTCTTGATC
GTTACAAAGGACGATGCTATGGAATCGAGCCTGTTCCTGGAGAAGAAAATCAATATATTG
CTTATGTAGCTTATCCCCTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTT
CCATTGTGGGTAATGTATTTGGATTCAAGGCTCTACGTGCTCTACGTCTGGAGGATTTGC
GAATCCCTACTGCTTATATTAAAACTTTCCAAGGCCCGCCTCATGGTATCCAGGTTGAAA
GAGATAAATTGAACAAGTATGGTCGCCCTCTATTGGGATGTACTATTAAACCAAAATTGG
GATTATCCGCTAAGAATTATGGTAGAGCAGTTTATGAATGTTTACGCGGTGGACTTGATT
TTACCAAAGATGATGAAAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTCC
TATTTTGTGCGGAAGCTATTTATAAATCACAGGCTGAAACAGGTGAAATCAAGGGACATT
ACTTGAATGCTACTGAG

O-Mini Dipper Gourd,
ACTTTTCCCCTTCGAAATATAGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTA
ACTCCTCAACCGGGAGTTCCACCTGAGGAAGCAGGGGCCGCTGTAGCTGCTGAATCTTCT
ACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGA
CGATGCTATGGAATCGAGCCTGTTCCTGGAGAAGAAAATCAATATATTGCTTATGTAGCT
TATCCCCTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGT
AATGTATTTGGATTCAAGGCTCTACGTGCTCTACGTCTGGAGGATTTGCGAATCCCTACT
GCTTATATTAAAACTTTCCAAGGCCCGCCTCATGGTATCCAGGTTGAAAGAGATAAATTG
AACAAGTATGGTCGCCCTCTATTGGGATGTACTATTAAACCAAAATTGGGATTATCCGCT
AAGAATTATGGTAGAGCAGTTTATGAATGTTTACGCGGTGGACTTGATTTTACCAAAGAT

GATGAAAACGTGAATTCCCAACCATTTATGCGTTGGAGAGACCGTTTCCTATTTTGTGCG
GAAGCTATTTATAAATCACAGGCTGAAACAGGTGAAATCAAGGGACATTACTTGAATGCT
ACTGAGTGCCCTTGCGAAA

P-Goose Neck Gourd,
GTCGCCTAATCCGTTTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTTCCACCTGAGG
AAGCAGGGGCCGCTGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCG
ATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTATGGAATCGAGCCTGTTCCTG
GAGAAGAAAATCAATATATTGCTTATGTAGCTTATCCCCTAGACCTTTTTGAAGAAGGTT
CTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGATTCAAGGCTCTACGTG
CTCTACGTCTGGAGGATTTGCGAATCCCTACTGCTTATATTAAAACTTTCCAAGGCCCGC
CTCATGGTATCCAGGTTGAAAGAGATAAATTGAACAAGTATGGTCGCCCTCTATTGGGAT
GTACTATTAAACCAAAATTGGGATTATCCGCTAAGAATTATGGTAGAGCAGTTTATGAAT
GTTTACGCGGTGGACTTGATTTTACCAAAGATGATGAAAACGTGAATTCCCAACCATTTA
TGCGTTGGAGAGACCGTTTCCTATTTTGTGCGGAAGCTATTTATAAATCACAGGCTGAAA
CAGGTGAAATCAAGGGACATTACTTGAATGCTACTGCAGGTTACATGCGAA

Q-Nigeria Rattle Gourd,
AAGCGAGGGCCCTTTGCCTAACCTGTTTAGCCTGTGATGTATAATTATCTTCCTGAATAT
GAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTTCCA
CCTGAGGAAGCAGGGGCCGCTGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTG
TGGACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTATGGAATCGAGCCT
GTTCCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTATCCCCTAGACCTTTTTGAA
GAAGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGATTCAAGGCT
CTACGTGCTCTACGTCTGGAGGATTTGCGAATCCCTACTGCTTATATTAAAACTTTCCAA
GGCCCGCCTCATGGTATCCAGGTTGAAAGAGATAAATTGAACAAGTATGGTCGCCCTCTA
TTGGGATGTACTATTAAACCAAAATTGGGATTATCCGCTAAGAATTATGGTAGAGCAGTT
TATGAATGTTTACGCGGTGGACTTGATTTTACCAAAGATGATGAAAACGTGAATTCCCAA
CCATTTATGCGTTGGAGAGACCGTTTCCTATTTTGTGCGGAAGCTATTTATAAATCACAG
GCTGAAACAGGTGAAATCAAGGGACATTACTTGAATGCTACTGAG

S-Swan Gourd,
GCCATCCGGCTTAAGGAAGTCCGTTAAAGATTATAAATTGACTTATTATACTCCTGAATA
TGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTTCC
ACCTGAGGAAGCAGGGGCCGCTGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGT
GTGGACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTATGGAATCGAGCC
TGTTCCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTATCCCCTAGACCTTTTTGA
AGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGATTCAAGGC
TCTACGTGCTCTACGTCTGGAGGATTTGCGAATCCCTACTGCTTATATTAAAACTTTCCA
AGGCCCGCCTCATGGTATCCAGGTTGAAAGAGATAAATTGAACAAGTATGGTCGCCCTCT
ATTGGGATGTACTATTAAACCAAAATTGGGATTATCCGCTAAGAATTATGGTAGAGCAGT
TTATGAATGTTTACGCGGTGGACTTGATTTTACCAAAGATGATGAAAACGTGAATTCCCA
ACCATTTATGCGTTGGAGAGACCGTTTCCTATTTTGTGCGGAAGCTATTTATAAATCACA
GGCTGAAACAGGTGAAATCAAGGGACATTACTTGAATGCTACTGCAGTACCATGCGAA
T-Palm Wine Gourd,
TTTCGGGTTAATACTTGTGTTAAGATTATAAATTGACTTATTATACTCCTGAATATGAAA
CCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTTCCACCTG
AGGAAGCAGGGGCCGCTGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGA
CCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTATGGAATCGAGCCTGTTC
CTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTATCCCCTAGACCTTTTTGAAGAAG
GTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGATTCAAGGCTCTAC
GTGCTCTACGTCTGGAGGATTTGCGAATCCCTACTGCTTATATTAAAACTTTCCAAGGCC
CGCCTCATGGTATCCAGGTTGAAAGAGATAAATTGAACAAGTATGGTCGCCCTCTATTGG
GATGTACTATTAAACCAAAATTGGGATTATCCGCTAAGAATTATGGTAGAGCAGTTTATG
AATGTTTACGCGGTGGACTTGATTTTACCAAAGATGATGAAAACGTGAATTCCCAACCAT
TTATGCGTTGGAGAGACCGTTTCCTATTTTGTGCGGAAGCTATTTATAAATCACAGGCTG
AAACAGGTGAAATCAAGGGACATTACTTGAATGCTACTGCAGTACCCTTGCGAA

U-Long Handle Dipper Gourd,
GCCTTCAATGGTAAAATTGTGTTAAGATTATAAATTGACTTATTATACTCCTGAATATGA
AACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTTCCACC
TGAGGAAGCAGGGGCCGCTGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTG
GACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTATGGAATCGAGCCTGT
TCCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTATCCCCTAGACCTTTTTGAAGA
AGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGATTCAAGGCTCT
ACGTGCTCTACGTCTGGAGGATTTGCGAATCCCTACTGCTTATATTAAAACTTTCCAAGG
CCCGCCTCATGGTATCCAGGTTGAAAGAGATAAATTGAACAAGTATGGTCGCCCTCTATT
GGGATGTACTATTAAACCAAAATTGGGATTATCCGCTAAGAATTATGGTAGAGCAGTTTA
TGAATGTTTACGCGGTGGACTTGATTTTACCAAAGATGATGAAAACGTGAATTCCCAACC
ATTTATGCGTTGGAGAGACCGTTTCCTATTTTGTGCGGAAGCTATTTATAAATCACAGGC
TGAAACAGGTGAAATCAAGGGACATTACTTGAATGCTACTGCAGTAACCTTGCGAAA

V-Powder Horn Gourd,
TTTGGGGGTCAATGCTGGTGTTAAGATTATAAATTGACTTATTATACTCCTGAATATGAA
ACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTTCCACCT
GAGGAAGCAGGGGCCGCTGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGG
ACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTATGGAATCGAGCCTGTT
CCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTATCCCCTAGACCTTTTTGAAGAA
GGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGATTCAAGGCTCTA
CGTGCTCTACGTCTGGAGGATTTGCGAATCCCTACTGCTTATATTAAAACTTTCCAAGGC
CCGCCTCATGGTATCCAGGTTGAAAGAGATAAATTGAACAAGTATGGTCGCCCTCTATTG
GGATGTACTATTAAACCAAAATTGGGATTATCCGCTAAGAATTATGGTAGAGCAGTTTAT
GAATGTTTACGCGGTGGACTTGATTTTACCAAAGATGATGAAAACGTGAATTCCCAACCA
TTTATGCGTTGGAGAGACCGTTTCCTATTTTGTGCGGAAGCTATTTATAAATCACAGGCT
GAAACAGGTGAAATCAAGGGACATTACTTGAATGCTACTGAG

W-Snake Gourd,
CATTCATTGTCTATAATTCTGTTAAGATTATAATTGACTTATTATACTCCTGAATATGAA
ACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTTCCACCT
GAGGAAGCAGGGGCCGCTGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTGG
ACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTATGGAATCGAGCCTGTT
CCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTATCCCCTAGACCTTTTTGAAGAA
GGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGATTCAAGGCTCTA
CGTGCTCTACGTCTGGAGGATTTGCGAATCCCTACTGCTTATATTAAAACTTTCCAAGGC
CCGCCTCATGGTATCCAGGTTGAAAGAGATAAATTGAACAAGTATGGTCGCCCTCTATTG
GGATGTACTATTAAACCAAAATTGGGATTATCCGCTAAGAATTATGGTAGAGCAGTTTAT
GAATGTTTACGCGGTGGACTTGATTTTACCAAAGATGATGAAAACGTGAATTCCCAACCA
TTTATGCGTTGGAGAGACCGTTTCCTATTTTGTGCGGAAGCTATTTATAAATCACAGGCT
GAAACAGGTGAAATCAAGGGACATTACTTGAATGCTACTGCAGTAAC

X Microphone Gourd,
GCATCAATGTGGGAGATTTCTTTGTGGGGTATGGTTGACTTATTATACTCCTGAATATGA
AACCAAAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTTCCACC
TGAGGAAGCAGGGGCCGCTGTAGCTGCTGAATCTTCTACTGGTACATGGACAACTGTGTG
GACCGATGGGCTTACCAGTCTTGATCGTTACAAAGGACGATGCTATGGAATCGAGCCTGT
TCCTGGAGAAGAAAATCAATATATTGCTTATGTAGCTTATCCCCTAGACCTTTTTGAAGA
AGGTTCTGTTACTAACATGTTTACTTCCATTGTGGGTAATGTATTTGGATTCAAGGCTCT
ACGTGCTCTACGTCTGGAGGATTTGCGAATCCCTACTGCTTATATTAAAACTTTCCAAGG
CCCGCCTCATGGTATCCAGGTTGAAAGAGATAAATTGAACAAGTATGGTCGCCCTCTATT
GGGATGTACTATTAAACCAAAATTGGGATTATCCGCTAAGAATTATGGTAGAGCAGTTTA
TGAATGTTTACGCGGTGGACTTGATTTTACCAAAGATGATGAAAACGTGAATTCCCAACC
ATTTATGCGTTGGAGAGACCGTTTCCTATTTTGTGCGGAAGCTATTTATAAATCACAGGC
TGAAACAGGTGAAATCAAAGGGACATTACTTGAATGCTACTGCAGGTACCATGCAGAAA

**Table 3.** Sequence alignment for twenty-three (23) fruit shapes of *L. siceraria* (Mol.) Standl. Complex.

| S/N | TAXA | DNA SEQUENCE |
|---|---|---|
| 1 | A-African Bottle Gourd | TTAATTAAAATTAAGCCCTCCGCGACAAAAGAGGACAAAGGTCTCTGCCATCTTGGCAGC |
| 2 | B-Kettle Gourd | GGCAATTTAATTAAGCCCTCCTCGAATAAGAAACCAAAGATACTGATATCTTGGCAGCAT |
| 3 | C-Caveman Club Gourd | AAAAATTTAGCCTTTCCCAGCGCAATATAGAGGAAACGGTCTCTGATATCTTGGCAGCAT |
| 4 | D-Base Ball Gourd | AAATTTTTAATTTAGGCCTCCTCGAATATAGAAACCAAAGATACTGATATCTTGGCAGCA |
| 5 | F-Bushel Gourd | GTCAACTATTTATTGATTGTGTTAAGATTATAAATTGACTTATTATACTCCTGAATATGA |
| 6 | G-Bird House Gourd | TAAAGGGATCACCTCGATTATCCTTAGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTT |
| 7 | H-Water Jug Gourd | CAATTTCCCAGGAGTAAACTCCTCAACCGAGGAGTTCCACCTGAGGAAGCAGGGGCCGCT |
| 8 | I-Cup Gourd | GCACTACAGTAATAAATTGTGTTAAGATTATAAATTGACTTATTATACTCCTGAATATGA |
| 9 | J-Pennis Shield Gourd | ACAAGGGGATTCACCCGATAATCCTTTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGT |
| 10 | K-Warted Bushel Gourd | GCCTTCCATGTAAAAATTTTTGTTAAGGATTATAATTGACTTATTATACTCCTGAATATG |
| 11 | L-Extra Large Pawpaw Gourd | TTCTGATCAAAGTAATGATGGTTGTTAAGATTATAATTGACTTATTATACTCCTGAATAT |
| 12 | M-Long Siphon Gourd | GTTCAACCAATGTTATGCGGTGTGTTAAGATTATAAATTGACTTATTATACTCCTGAATA |
| 13 | N-Indian Gourd | ATAGGGGGGGCCATTGCGGAACCCTGTAGCATTATAAATTGACTTATTATACTCCTGAAT |
| 14 | O-Chinese Bottle Gourd | GCCCTGCGTTGACTTATTATACTCCTGAATATGAAACCAAAGATACTGATATCTTGGCAG |
| 15 | P-Mini Dipper Gourd | ACTTTTCCCCTTCGAAATATAGAAACCAAAGATACTGATATCTTGGCAGCATTCCGAGTA |
| 16 | Q-Goose Neck Gourd, | GTCGCCTAATCCGTTTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTTCCACCTGAGG |
| 17 | R-Nigeria Rattle Gourd | AAGCGAGGGCCCTTTGCCTAACCTGTTTAGCCTGTGATGTATAATTATCTTCCTGAATAT |
| 18 | S-Swan Gourd | GCCATCCGGCTTAAGGAAGTCCGTTAAAGATTATAAATTGACTTATTATACTCCTGAATA |
| 19 | T-Palm Wine Gourd | TTTCGGGTTAATACTTGTGTTAAGATTATAAATTGACTTATTATACTCCTGAATATGAAA |
| 20 | U-Long Handle Dipper Gourd | GCCTTCAATGGTAAAATTGTGTTAAGATTATAAATTGACTTATTATACTCCTGAATATGA |
| 21 | V-Powder Horn Gourd | TTTGGGGGTCAATGCTGGTGTTAAGATTATAAATTGACTTATTATACTCCTGAATATGAA |
| 22 | W-Snake Gourd | CATTCATTGTCTATAATTCTGTTAAGATTATAATTGACTTATTATACTCCTGAATATGAA |
| 23 | X Microphone Gourd | GCATCAATGTGGGAGATTTCTTTGTGGGGTATGGTTGACTTATTATACTCCTGAATATGA |

**Table 4.** Nucleotide basic local alignment search tool (BLAST N).

| S/N | NAME | FRUIT SHAPE | TOTAL TAXONOMY VALUE | HIGHEST HIT VALUE | QUERY % | IDENTITY % | E Value |
|---|---|---|---|---|---|---|---|
| 1 | Awala A | African Bottle Gourd | 104 | 44 | 91 | 99 | 0 |
| 2 | Awala B | Kettle Gourd | 104 | 44 | 97 | 99 | 0 |
| 3 | Awala C | Caveman Club Gourd | 104 | 45 | 93 | 100 | 0 |
| 4 | Awala D | Baseball Gourd | 104 | 44 | 97 | 99 | 0 |
| 5 | Awala F | Bushell Gourd | 109 | 44 | 97 | 99 | 0 |
| 6 | Awala G | Bird House Gourd | 104 | 45 | 96 | 99 | 0 |
| 7 | Awala H | Water Jug Gourd | 104 | 45 | 97 | 98 | 0 |
| 8 | Awala I | Cup Gourd | 109 | 44 | 97 | 99 | 0 |
| 9 | Awala J | Pennis Shield Gourd | 104 | 45 | 95 | 100 | 0 |

**Continued**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 10 | Awala K | Warted Bushel Gourd | 109 | 44 | 96 | 99 | 0 |
| 11 | Awala L | Extralarge Pawpaw Gourd | 109 | 44 | 97 | 99 | 0 |
| 12 | Awala M | Long siphon Gourd | 109 | 44 | 97 | 99 | 0 |
| 13 | Awala N | Indian Gourd | 110 | 44 | 95 | 99 | 0 |
| 14 | Awala O | Chinese Bottle Gourd | 111 | 44 | 98 | 100 | 0 |
| 15 | Awala P | Mini Dipper Gourd | 111 | 44 | 95 | 99 | 0 |
| 16 | Awala Q | Goose Neck Gourd | 104 | 45 | 97 | 99 | 0 |
| 17 | Awala R | Nigeria Rattle Gourd | 111 | 44 | 93 | 99 | 0 |
| 18 | Awala S | Swan Gourd | 109 | 44 | 96 | 99 | 0 |
| 19 | Awala T | Palm Wine Gourd | 109 | 44 | 96 | 99 | 0 |
| 20 | Awala U | Long Handle Dipper Gourd | 109 | 44 | 95 | 99 | 0 |
| 21 | Awala V | Powder Horn Gourd | 119 | 44 | 98 | 99 | 0 |
| 22 | Awala W | Snake Gourd | 109 | 44 | 96 | 99 | 0 |
| 23 | Awala X | Microphone Gourd | 105 | 44 | 95 | 99 | 0 |

**Table 5.** Nucleotide composition frequencies of *Lageneria siceraria* (Mol.) Standl.

| | T(U) | C | A | G | Total | T-1 | C-1 | A-1 | G-1 | Pos #1 | T-2 | C-2 | A-2 | G-2 | Pos #2 | T-3 | C-3 | A-3 | G-3 | Pos #3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AWALA A 1F | 28.7 | 20.0 | 28.6 | 22.6 | 689 | 40.9 | 13.9 | 30.4 | 14.8 | 230 | 20.4 | 20.4 | 25.2 | 33.9 | 230 | 24.9 | 25.8 | 30.1 | 19.2 | 229 |
| AWALA B RCBL | 29.0 | 19.7 | 28.9 | 22.4 | 686 | 24.5 | 25.3 | 31.4 | 18.8 | 229 | 41.9 | 14.4 | 30.6 | 13.1 | 229 | 20.6 | 19.3 | 24.6 | 35.5 | 228 |
| AWALA C 1F | 29.3 | 19.7 | 28.0 | 23.0 | 675 | 24.9 | 25.3 | 30.7 | 19.1 | 225 | 42.7 | 14.2 | 28.9 | 14.2 | 225 | 20.4 | 19.6 | 24.4 | 35.6 | 225 |
| AWALA D 1F | 29.9 | 19.4 | 28.4 | 22.4 | 680 | 21.1 | 18.1 | 25.6 | 35.2 | 227 | 25.1 | 26.4 | 30.4 | 18.1 | 227 | 43.4 | 13.7 | 29.2 | 13.7 | 226 |
| AWALA F 1F | 30.3 | 18.9 | 28.6 | 22.2 | 716 | 44.8 | 12.6 | 28.9 | 13.8 | 239 | 22.6 | 18.0 | 25.5 | 33.9 | 239 | 23.5 | 26.1 | 31.5 | 18.9 | 238 |
| AWALA G RCBL | 29.2 | 19.7 | 28.0 | 23.1 | 661 | 23.1 | 20.4 | 24.0 | 32.6 | 221 | 24.1 | 22.7 | 30.5 | 22.7 | 220 | 40.5 | 15.9 | 29.5 | 14.1 | 220 |
| AWALA H RCBL | 29.2 | 19.1 | 28.7 | 23.0 | 644 | 22.3 | 21.4 | 27.0 | 29.3 | 215 | 26.0 | 20.0 | 29.8 | 24.2 | 215 | 39.3 | 15.9 | 29.4 | 15.4 | 214 |
| AWALA I 1F | 30.0 | 19.0 | 28.7 | 22.3 | 714 | 45.0 | 13.4 | 28.2 | 13.4 | 238 | 22.3 | 18.5 | 24.8 | 34.5 | 238 | 22.7 | 25.2 | 33.2 | 18.9 | 238 |
| AWALA J RCBL | 28.9 | 19.7 | 28.1 | 23.3 | 665 | 43.2 | 13.1 | 30.2 | 13.5 | 222 | 20.3 | 19.8 | 23.9 | 36.0 | 222 | 23.1 | 26.2 | 30.3 | 20.4 | 221 |
| AWALA K 1F | 30.2 | 18.9 | 28.7 | 22.2 | 708 | 23.7 | 25.4 | 31.4 | 19.5 | 236 | 44.5 | 12.7 | 30.1 | 12.7 | 236 | 22.5 | 18.6 | 24.6 | 34.3 | 236 |
| AWALA L 1F | 30.0 | 18.8 | 28.8 | 22.4 | 719 | 24.2 | 18.3 | 24.2 | 33.3 | 240 | 24.2 | 25.4 | 30.8 | 19.6 | 240 | 41.8 | 12.6 | 31.4 | 14.2 | 239 |
| AWALA M 1F | 29.9 | 19.1 | 28.6 | 22.4 | 718 | 43.8 | 13.3 | 29.2 | 13.8 | 240 | 22.6 | 18.4 | 23.8 | 35.1 | 239 | 23.4 | 25.5 | 32.6 | 18.4 | 239 |
| AWALA N 1F | 29.2 | 19.4 | 28.5 | 22.9 | 720 | 22.9 | 25.4 | 32.1 | 19.6 | 240 | 43.3 | 13.8 | 28.8 | 14.2 | 240 | 21.3 | 19.2 | 24.6 | 35.0 | 240 |
| AWALA O 1F | 29.7 | 19.6 | 27.9 | 22.7 | 677 | 23.5 | 26.5 | 31.4 | 18.6 | 226 | 44.7 | 13.3 | 27.9 | 14.2 | 226 | 20.9 | 19.1 | 24.4 | 35.6 | 225 |
| AWALA P 1F | 29.5 | 19.9 | 28.1 | 22.5 | 679 | 20.3 | 19.8 | 25.1 | 34.8 | 227 | 24.3 | 25.7 | 30.5 | 19.5 | 226 | 43.8 | 14.2 | 28.8 | 13.3 | 226 |
| AWALA Q RCBL | 29.5 | 19.8 | 27.3 | 23.3 | 651 | 24.9 | 24.9 | 29.0 | 21.2 | 217 | 43.3 | 13.8 | 29.0 | 13.8 | 217 | 20.3 | 20.7 | 24.0 | 35.0 | 217 |
| AWALA R 1F | 29.8 | 19.6 | 27.8 | 22.8 | 705 | 20.9 | 20.0 | 23.8 | 35.3 | 235 | 24.3 | 25.5 | 31.9 | 18.3 | 235 | 44.3 | 13.2 | 27.7 | 14.9 | 235 |
| AWALA S 1F | 29.4 | 19.5 | 28.7 | 22.4 | 718 | 42.9 | 13.8 | 29.6 | 13.8 | 240 | 21.8 | 18.4 | 24.7 | 35.1 | 239 | 23.4 | 26.4 | 31.8 | 18.4 | 239 |
| AWALA T 1F | 30.4 | 19.0 | 28.3 | 22.3 | 714 | 23.9 | 25.6 | 31.9 | 18.5 | 238 | 44.1 | 13.0 | 29.0 | 13.9 | 238 | 23.1 | 18.5 | 23.9 | 34.5 | 238 |
| AWALA U 1F | 30.0 | 19.0 | 28.9 | 22.2 | 717 | 43.5 | 12.6 | 29.7 | 14.2 | 239 | 22.2 | 18.4 | 25.1 | 34.3 | 239 | 24.3 | 25.9 | 31.8 | 18.0 | 239 |
| AWALA V 1F | 30.2 | 18.7 | 28.2 | 22.9 | 702 | 22.6 | 18.4 | 23.9 | 35.0 | 234 | 23.1 | 24.8 | 32.5 | 19.7 | 234 | 44.9 | 12.8 | 28.2 | 14.1 | 234 |
| AWALA W 1F | 30.6 | 19.1 | 28.6 | 21.8 | 707 | 22.5 | 18.6 | 25.0 | 33.9 | 236 | 25.4 | 26.3 | 30.5 | 17.8 | 236 | 43.8 | 12.3 | 30.2 | 13.6 | 235 |
| AWALA X 1F | 29.8 | 18.9 | 28.1 | 23.2 | 719 | 42.9 | 13.3 | 29.6 | 14.2 | 240 | 22.5 | 19.2 | 23.8 | 34.6 | 240 | 23.8 | 24.3 | 31.0 | 20.9 | 239 |
| Avg. | 29.7 | 19.3 | 28.4 | 22.6 | 695.0 | 30.2 | 19.0 | 28.4 | 22.4 | 231.9 | 29.3 | 19.3 | 28.1 | 23.3 | 231.7 | 29.5 | 19.6 | 28.6 | 22.2 | 231.3 |

Club Gourd have a nucleotide composition of 675 with an average percentage frequency of thymine 29.3%, cytosine 19.7%, adenine 28.0% and guanine 23.0%. The nucleotide composition of D-Base Ball Gourd consist of 680 nucleotides with an average percentage frequency of thymine 29.9%, cytosine 19.4%, adenine 28.4% and guanine 22.4%, in F-Bushel Gourd, the nucleotide composition is 716 while thymine 30.3%, cytosine 18.9%, adenine 28.6% and guanine 22.2%, G-Bird House Gourd has a total composition of nucleotide as 661, thymine 29.2%, cytosine 19.7%, adenine 28.0% and guanine 23.1%, the total composition of nucleotide in H-Water Jug Gourd is 664, thymine 29.2%, cytosine 19.1%, adenine 28.7% and guanine 23.0%, in I-Cup Gourd the total nucleotide composition is 714, thymine 30.0%, cytosine 19.0%, adenine 28.7% and guanine 22.3%, the total nucleotide composition is 665 in J-Pennis Shield Gourd, thymine 28.9%, cytosine 19.7%, adenine 28.1% and guanine 23.3%, also in K-Warted Bushel Gourd the total nucleotide composition is 708, thymine 30.2%, cytosine 18.9%, adenine 28.7% and guanine 22.2%, the total nucleotide composition of L-Extra Large Pawpaw Gourd is 719 were the percentage frequency of the bases are thymine 30.0%, cytosine 18.8%, adenine 28.8% and guanine 22.4%, M-Long Siphon Gourd has a total nucleotide composition of 718 and the percentage frequency of is thymine 29.9%, cytosine 19.1%, adenine 28.6% and guanine 22.4%, in N-Indian Gourd, the percentage frequency of thymine 29.2%, cytosine 19.4%, adenine 28.5% and guanine 22.9%, it possesses a total nucleotide composition of 720. O-Chinese Bottle Gourd has a total nucleotide composition as 677 while the percentage frequency of thymine is 29.7%, cytosine 19.6%, adenine 27.9% and guanine 22.7%, in P-Mini Dipper Gourd the total nucleotide composition equals 679 nucleotides while the percentage frequency of the nucleotides has thymine to be equal to 29.5%, cytosine 19.9%, adenine 28.1% and guanine 22.5%, Q-Goose Neck Gourd, the total composition of nucleotide is 651 while the percentage frequency of each bases are thymine 29.5%, cytosine 19.8%, adenine 27.3% and guanine 23.3%, in R-Nigeria Rattle Gourd, the total nucleotide composition is 705 and the percentage frequency of the nucleotide is thymine 29.8%, cytosine 19.6%, adenine 27.8% and guanine 22.8%. The total nucleotide composition of S-Swan Gourd is 718 having a percentage frequency as thymine 29.4%, cytosine 19.5%, adenine 28.7% and guanine 22.4%, T-Palm Wine Gourd the total nucleotide composition is 714 were the percentage frequency of the nucleotides are thymine 30.4%, cytosine 19.0%, adenine 28.3% and guanine 22.3%, in U-Long Handle Dipper Gourd the frequency of the nucleotides are thymine 30.0%, cytosine 19.0%, adenine 28.9% and guanine 22.4%, and a total nucleotide composition of 717. V-Powder Horn Gourd posseses a total nucleotide content of 702, with a percentage frequency of thymine 30.2%, cytosine 18.7%, adenine 28.2% and guanine 22.9%, in W-Snake Gourd the total nucleotide composition is 707 while the percentage frequency of the bases are: thymine 30.6%, cytosine 19.1%, adenine 28.6% and guanine 21.8%, and finally, X-Microphone Gourd is observed to have a total nucleotide composition of 719 with thymine as 29.8%, cytosine 18.9%, adenine 28.1% and guanine 23.2%, percentage frequencies.

Estimates of Evolutionary Divergence between Sequences are shown in the data matrix (**Table 6**). The number of base differences per site from between sequences is shown. Standard error estimate(s) are shown above the diagonal and were obtained by using analytical formulas. The rate variation among sites was modelled with a gamma distribution (shape parameter = 0.05). The analysis involved 23 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 720 positions in the final dataset. Evolutionary analyses are conducted in MEGA X [15].

The phylogenetic tree (**Figure 4**) shows great diversity and similarity in Long handle dipper -U and Microphone gourd-X are out group from the root, and meets at similarity scale of 0.7, while Kettle gourd-B and Caveman club gourd-C are out group from the second root of the tree meeting at a similarity scale of 1.0. The second root has two main clusters that meets at different similarity scale. The first cluster of the second root (R2a), African bottle gourd-A is seen as out group at 0.65 while Snake gourd-W and Powder horn gourd-V clustered together at 1.0 similarity scale. Swan gourd-S and palmwine gourd-T meets at 0.89 while Mini dipper gourd-P and Goose neck gourd-Q clustered at 1.0, Nigerian gourd-R is observed as an out group at 0.85 similarity scale. In the second cluster of the second root (R2b), Cup gourd-I was an out group at 0.85 while Baseball gourd-D, Bird house gourd-G, Water jug gourd-H, Penis shield gourd-J, Warted bushel gourd-K, Extra-large pawpaw gourd-L, Long siphon gourd-M, Indian gourd-N and Chinese gourd-O met at 1.0 similarity scale.

## 4. Discussion

According to [6], the concentration of macromolecular studies was geared towards DNA and RNA hence, molecular systematics deals with the utilization of nucleic acid data. Genetic materials have been used in the understanding of the

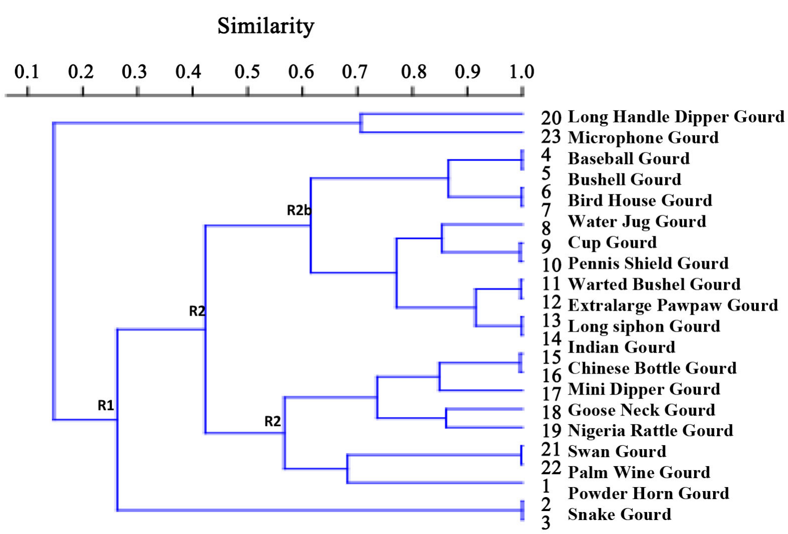



**Figure 4.** Phylogenetic tree of *Lageneria siceraria* (Mol.) Standl.

**Table 6.** Distance matrix of *Lageneria siceraria* (Mol.) Standl.

| AWALA_A_1F | | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AWALA_B_RCBL | 0.75 | | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| AWALA_C_1F | 0.76 | 0.04 | | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| AWALA_D_1F | 0.69 | 0.69 | 0.71 | | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| AWALA_F_1F | 0.74 | 0.73 | 0.73 | 0.77 | | 0.02 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.00 | 0.02 | 0.02 | 0.01 |
| AWALA_G_RCBL | 0.77 | 0.77 | 0.77 | 0.73 | 0.75 | | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| AWALA_H_RCBL | 0.72 | 0.76 | 0.76 | 0.74 | 0.78 | 0.70 | | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| AWALA_I_1F | 0.74 | 0.73 | 0.74 | 0.77 | 0.02 | 0.75 | 0.78 | | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.01 | 0.02 | 0.02 | 0.01 |
| AWALA_J_RCBL | 0.74 | 0.73 | 0.73 | 0.77 | 0.72 | 0.62 | 0.75 | 0.72 | | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| AWALA_K_1F | 0.77 | 0.72 | 0.73 | 0.73 | 0.70 | 0.76 | 0.75 | 0.70 | 0.76 | | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| AWALA_L_1F | 0.73 | 0.78 | 0.78 | 0.72 | 0.76 | 0.71 | 0.72 | 0.76 | 0.75 | 0.71 | | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| AWALA_M_1F | 0.72 | 0.74 | 0.75 | 0.78 | 0.72 | 0.77 | 0.76 | 0.73 | 0.71 | 0.77 | 0.69 | | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| AWALA_N_1F | 0.78 | 0.76 | 0.76 | 0.75 | 0.77 | 0.75 | 0.74 | 0.76 | 0.75 | 0.73 | 0.77 | 0.72 | | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| AWALA_O_1F | 0.72 | 0.73 | 0.73 | 0.77 | 0.77 | 0.75 | 0.74 | 0.77 | 0.78 | 0.72 | 0.78 | 0.73 | 0.72 | | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| AWALA_P_1F | 0.76 | 0.74 | 0.75 | 0.72 | 0.72 | 0.74 | 0.73 | 0.72 | 0.78 | 0.73 | 0.71 | 0.73 | 0.75 | 0.76 | | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| AWALA_Q_RCBL | 0.75 | 0.71 | 0.71 | 0.74 | 0.75 | 0.78 | 0.78 | 0.76 | 0.76 | 0.71 | 0.76 | 0.74 | 0.74 | 0.70 | 0.78 | | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| AWALA_R_1F | 0.74 | 0.78 | 0.77 | 0.72 | 0.77 | 0.70 | 0.73 | 0.77 | 0.75 | 0.72 | 0.06 | 0.72 | 0.75 | 0.77 | 0.71 | 0.76 | | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| AWALA_S_1F | 0.72 | 0.74 | 0.75 | 0.78 | 0.72 | 0.76 | 0.76 | 0.73 | 0.71 | 0.76 | 0.70 | 0.03 | 0.71 | 0.73 | 0.73 | 0.74 | 0.72 | | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| AWALA_T_1F | 0.74 | 0.73 | 0.74 | 0.77 | 0.77 | 0.75 | 0.75 | 0.76 | 0.73 | 0.73 | 0.76 | 0.76 | 0.72 | 0.68 | 0.78 | 0.73 | 0.76 | 0.77 | | 0.02 | 0.02 | 0.02 | 0.02 |
| AWALA_U_1F | 0.73 | 0.73 | 0.74 | 0.77 | 0.02 | 0.75 | 0.78 | 0.02 | 0.72 | 0.70 | 0.76 | 0.73 | 0.77 | 0.77 | 0.72 | 0.76 | 0.78 | 0.72 | 0.76 | | 0.02 | 0.02 | 0.01 |
| AWALA_V_1F | 0.78 | 0.78 | 0.78 | 0.74 | 0.71 | 0.72 | 0.77 | 0.72 | 0.75 | 0.76 | 0.72 | 0.76 | 0.77 | 0.75 | 0.72 | 0.72 | 0.72 | 0.76 | 0.70 | 0.72 | | 0.01 | 0.02 |
| AWALA_W_1F | 0.78 | 0.78 | 0.77 | 0.73 | 0.70 | 0.73 | 0.75 | 0.70 | 0.76 | 0.76 | 0.72 | 0.76 | 0.77 | 0.75 | 0.71 | 0.72 | 0.72 | 0.76 | 0.71 | 0.70 | 0.04 | | 0.02 |
| AWALA_X_1F | 0.73 | 0.73 | 0.74 | 0.77 | 0.08 | 0.75 | 0.78 | 0.07 | 0.71 | 0.68 | 0.74 | 0.73 | 0.76 | 0.77 | 0.73 | 0.76 | 0.76 | 0.73 | 0.76 | 0.07 | 0.72 | 0.72 | |

evolutionary relationship. The quality of the chloroplast DNA was very visible at 850 bp across the landraces of *L. siceraria* using agarose gel electrophoresis (**Figure 2**; **Figure 3**). The quantified DNA (**Table 3**) had an absorbance ratio of 1.2 to 1.8 showing the purity of the DNA, which is in line with the work of [16] [17]. The conserved *rbcL* gene has been utilized in PCR amplification of chloroplast gene sequences for determining and ratifying phylogenies [18] [19]. The sequence alignment of the twenty-three landraces from Nigeria shows great variation in the arrangement of the nucleotide bases (**Figure 4**), which is due to gene recombination and mutation.

The sequences were subjected to validation through National Center for Biotechnology Information (NCBI) using Nucleotide Basic Local Alignment Search Tool (BLAST N). The result obtained (**Table 4**) proves that all the sequences belong to *Lagenaria siceraria* (Mol.) standl. with percentages ranging from 95% to 100% for query cover sequences and 98% to 100% for identity sequences with an E-value of 0.00. From the taxonomic report obtained sequence F-Bushel Gourd, K-Warted bushel gourd, U-Long handle dipper, I-Cup gourd, L-Extra-large pawpaw gourd, M-Long siphon gourd, S-Swan gourd, T-Palmwine gourd, W-Snake gourd has the highest hits of 44 on *Lagenaria siceraria* out of 109 total value, sequence O-Chinese gourd, P-Mini dipper gourd and R-Nigeria rattle gourd has the highest hits of 44 on *L. siceraria* out of the total value of 111, sequence V has the highest hits of 44 on *Lagenaria siceraria* out of 119 total value, sequence C-Caveman club gourd, G-Bird house gourd, H-Water jug gourd, J-Pennis shield gourd, Q-Goose neck gourd obtained the highest hit of 45 out of the total value 104, while sequence A-African bottle gourd, B-Kettle gourd, D-Baseball gourd has a total taxonomic value of 104 out of which 44 were identified as *Lagenaria siceraria.* The total taxonomic value of N-Indian gourd was observed as 110 with the highest hit value being 44 on *L. siceraria* and sequence X-Microphone gourd finally has a highest value hits of 44 on *Lagenaria siceraria* out of 105 total value.

Thus the phylogenetic tree (**Figure 4**) was constructed to show the relatedness of the landraces (biotypes). The Long handle dipper and Microphone gourd are out group from the root, and meets at similarity scale of 0.7, while Kettle gourd and Caveman club gourd are out group from the second root of the tree meeting at a similarity scale of 1.0. The second root has two main clusters that meets at different similarity scale. The first cluster of the second root (R2a), African bottle gourd is seen as out group at 0.65 while snake gourd and powder horn gourd clustered together at 1.0 similarity scale. Swan gourd and palmwine gourd meets at 0.89 while Mini dipper gourd and Goose neck gourd clustered at 1.0, Nigerian gourd is observed as an out group at 0.85 similarity scale. In the second cluster of the second root (R2b), Cup gourd was an out group at 0.85 while Baseball gourd, Bird house gourd, Water jug gourd, Penis shield gourd, Warted bushel gourd, Extra-large pawpaw gourd, Long siphon gourd, Indian gourd and Chinese gourd met at 1.0 similarity scale. The phytogenetic studies, have therefore aided in proper understanding of the gene pool and genetic variability of the landraces (biotypes) of *L. siceraria* found in Nigeria.

## 5. Conclusion

The use of bioinformatics tools in biosystematics studies of *Lagenaria siceraria* landraces (biotypes) found in Nigeria has served as a tool in resolving the quagmire in the diversity of *L. siceraria* complex and the classification of the Landraces into proper taxa. Hence molecular systematics of the species demonstrates differences in sequence arrangement that is due to gene recombination and the functional effect of heterologous genes expressed phenotypically on the fruit shape thereby resulting in the diversity of in fruit shapes of *Lagenaria siceraria* (Mol.) Standl. found in Nigeria.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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