# TAXONOMIC REVISION OF THE GENUS ANNONA NATIVE TO AFRICA USING SELECTED BARCODES LOCI (MATK, TRNL AND RBCL)

BY

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# INTRODUCTION

- Traditional Taxonomy relies on morphology, making species identification challenging especially when they share similar traits (Lestari and Azrianingsih, 2019).
- Missing morphological traits can make identification difficult (Fitri, 2015).
- Different genera display variations, but species within a genus often look similar posing challenges for taxonomists using morphology alone.
- DNA barcoding reveals detailed information on variations of closely related species.
- DNA barcoding uses standardized Chloroplast markers to distinguish closely related species for conservation (Kang et al., 2017; Fatima et al., 2019).
- This study aims identify the species of *Annona* using chloroplasts markers such as matK, trnL and rbcL

# MATERIALS AND METHODS

- A total of 33 sequences were retrieved from the NCBI website (https://www.ncbi.nlm.nih.gov
- Sequence cleaning-MEGA 11
- Sequence alignment- Clustal W of the MEGA11 package
- Determination of match identification rate intraspecific and interspecific distances of each barcode- Taxon DNA software (Meier et al., 2008)
- Phylogenetic tree construction- Neighbour joining tree method with the Kimura 2-parameter substitution model (Kumaru et al., 2018, Onefeli, 2021)

# RESULTS

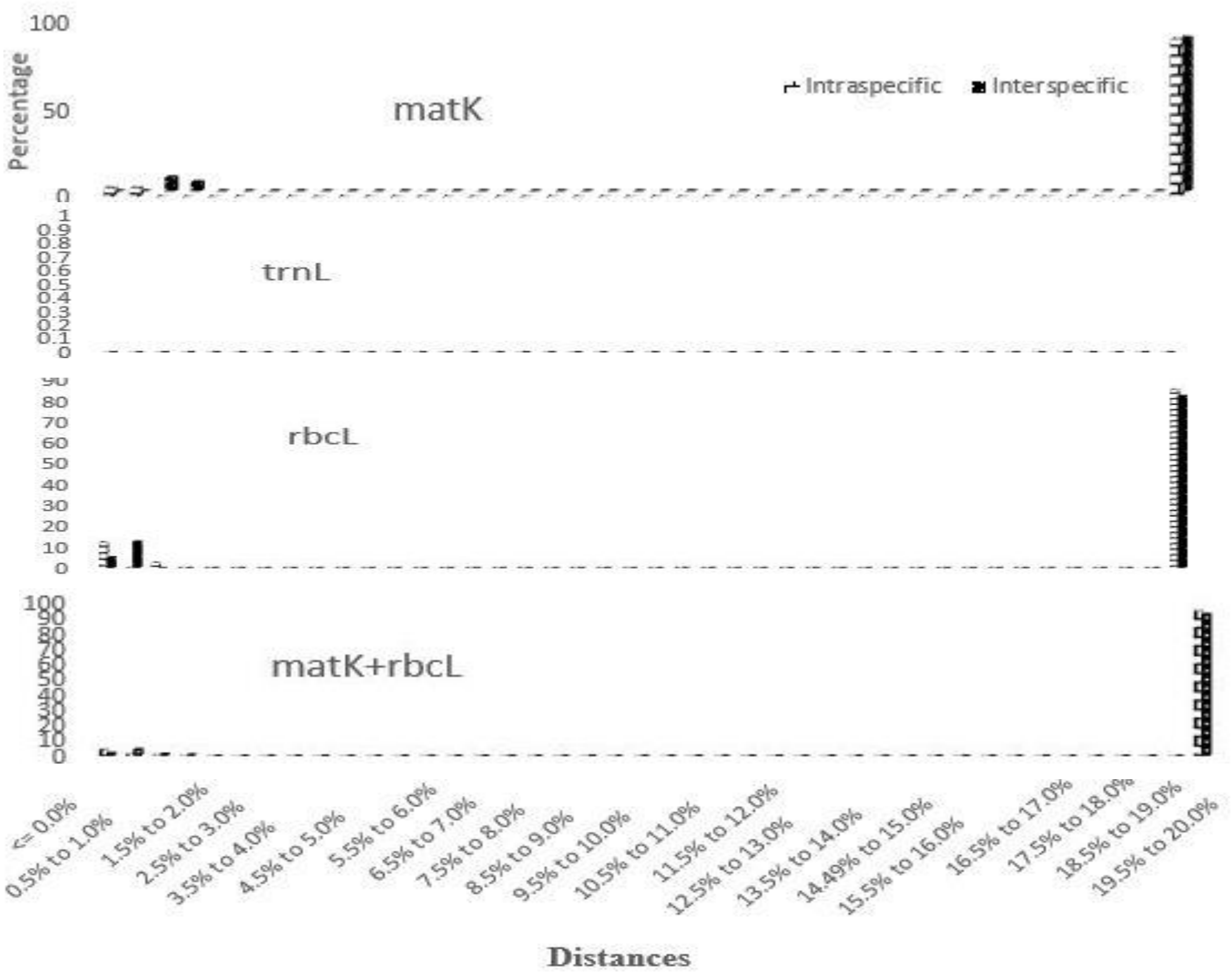
## Characteristics of single and concatenated barcode loci of the Genus *Annona*

| Variable/Barcode loci | matK | rbcL | trnL | matK+rbcL | matK+trnL | rbcL+trnL | matK+rbcL+trnL |
|---|---|---|---|---|---|---|---|
| **Barcode length (bp)** | 1763 | 1463 | 903 | 1763 | 1774 | 1763 | 1763 |
| **Number of variable sites** | 17 | 12 | 2 | 743 | 129 | 600 | 1077 |
| **Parismony-Informative sites** | 8 | 2 | 0 | 408 | 586 | 125 | 630 |
| **Ranges of Intraspecific distances** | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Ranges of interspecific distances** | 0-0.015 | 0-0.010 | 0-0.009 | 0-1.480 | 0-1.550 | 0-1.640 | 0-2.340 |
| **Average intraspecific distances** | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Average interspecific distances** | 0.006 | 0.002 | 0.003 | 0.520 | 0.420 | 0.340 | 0.990 |

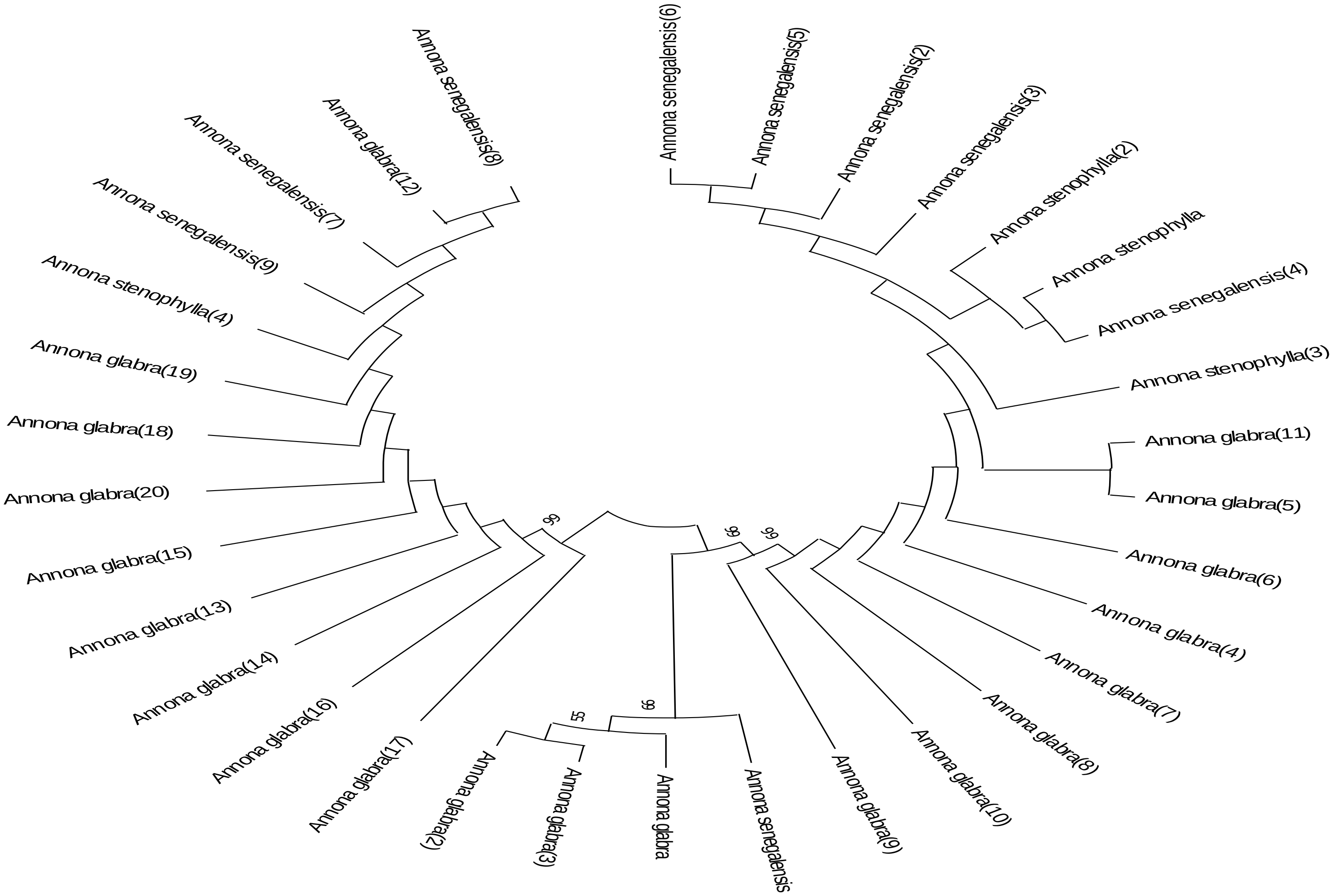
# BEST MATCH AND BEST CLOSE MATCH IDENTIFICATION FOR SINGLE AND CONCATENATED BARCODES OF THE GENUS *ISOLONA*

| **Barcode loci** | **Best match** | | | **Best close match** | | | |
|---|---|---|---|---|---|---|---|
| | Correct (%) | Ambiguous (%) | Incorrect (%) | Correct (%) | Ambiguous (%) | Incorrect (%) | No match closer than 3% |
| **MatK** | 69.23 | 0.0 | 30.76 | 38.46 | 0 | 15.38 | 46.15 |
| **rbcL** | 31.25 | 31.25 | 37.50 | 31.25 | 25.00 | 25.00 | 18.75 |
| **trnL** | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **MatK+rbcL** | 48.27 | 20.68 | 31.03 | 31.48 | 13.79 | 20.68 | 31.03 |
| **matK+trnL** | 71.42 | 0 | 28.57 | 35.71 | 0 | 14.28 | 50.00 |
| **rbcL+trnL** | 35.29 | 29.41 | 35.29 | 29.41 | 23.52 | 23.52 | 23.52 |
| **matK+rbcL+trnL** | 53.33 | 16.66 | 30.00 | 33.33 | 13.33 | 20.00 | 33.33 |

# BARCODING GAP WITH THE DISTRIBUTION OF INTER- AND INTRA-SPECIFIC DISTANCES



# PHYLOGENETIC TREE



# DISCUSSION

- While matK loci provide good identification, studies (Mishra et al., 2017) indicate single barcodes may not sufficiently distinguish closely related species like Terminalia spp.“

- Combining trnL and matK chloroplast gene sequences is an efficient tool for plant species authentication and genotype identification (Awomukwu et al., 2015)."

- Barcodes with many variable sites (matk+rbcL, matK+trnL, rbcL+trnL, matK+rbcL+trnL) have low identification rates, aligning with Kavaliauskas et al. (2022) that highly variable loci don't ensure meaningful species discrimination."

- Research shows combining matK and other markers such as trnL provides higher resolution and better discriminatory performance than single markers (Li et al., 2019; Calleja-Cabrera et al., 2020).“

- High genetic diversity in phyletic groups, especially using the trnL barcode, may result from the open-pollinated nature of species (Rungis et al., 2019)."

# CONCLUSION

The use of single barcode matK, rbcL and trnL however proved efficient in discriminating the species of the genera *Anonna*, also the concatenation of the three barcodes provided a tree that shows that the result of this study can be applied to the classification of the genus *Anonna* most especially where identification using morphological characterization is abortive. Nevertheless, further research should be done if there would be a new identification of the species that existed in different phylogeny.

# THANK YOU

FOR

LISTENING