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Application of Maturase K Primer to Determining Nomenclatural identities of Four Morphological Similar Mimosoid Clade Members.

Ebigwai JK,[1] Ubi GM[2], Akesa TM[1]

[1-] Department of Plant and Ecological studies, University of Calabar, Calabar, Nigeria
[2-] Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria
Corresponding author: ebijoe4@gmail.com

Abstract: The use of DNA barcode offers a better and more reliable alternative to morphology in plant species identification process. In this study, the chloroplast DNA region (*matk*) of the four morphologically similar Mimosoid species was studied. The objective was to evaluate its applicability in discriminating among these morphologically similar species. DNA samples obtained from their leafed specimens were studied in terms of DNA purity, PCR amplification, amplicons nucleotide, protein sequences, sequence authentication and phylogeny following standard methods of Extraction, Quantification, Amplification and Sequencing. The results showed similarities among the four species with respect to DNA purity, PCR amplification but revealed obvious differences between their amplicons in terms of nucleotide base pairs and amino acid residue. The nucleotide sequence of samples A, B, C and D had 755, 755, 724 and 779 bp respectively, with the corresponding 251, 251, 241 and 259 amino acid residues respectively. A total of 388 SNP was observed between samples A and B, 386 between B and C, and 386 between samples B and D. Others were 15, 14 and 9 bps between samples C and D, samples A and D, and samples A and C respectively. All the sequences were unambiguously identified; specimens A, C and D were identified as *Parkia biglobosa*, *Leucaena leucocephala* and *Prosopis africana* respectively; with 100 % identities while specimen B was identified as *Pentaclethra macrophylla* with 99.7% homology. A minimum inter-taxa barcode gap of 2 % was recorded between the study samples showing that *P. biglobosa*, *L. leucocephala*, *P. macrophylla* and *P. africana* are separate taxa. However, an 80 - 81 % barcode gaps were observed between *P. macrophylla* and other species, suggesting that *P. macrophylla* is distantly related to other species studied. The phylogenetic tree also suggested that *P. macrophylla* might represent a separate clade from *P. biglobosa*, *L. leucocephala*, *P. macrophylla* and *P. africana* based on their *matK* gene. This finding would serve the bedrock for a detailed phytochemical and morphological study to be conducted on each of the taxon to enhance rapid and cheap on field authentication process.
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1. Introduction

The emergence of DNA barcoding has had a positive impact on scientific enterprise and specifically nomenclatural practice (Gregory, 2005). DNA barcoding is a technique for characterizing species of organisms using a short DNA sequence from a standard and agreed- upon position in the genome (Hebert *et al*., 2003). DNA barcoding relies on finding different conserved regions in divergent species to produce a large-scale reference genome library (Watto *etal*.,2016). DNA barcoding is a recent and widely used molecular-based identification system that aims to identify biological specimens, and to assign them to a given species. However, DNA barcoding is even more than this, and besides many practical uses, it can be considered the core of an integrated taxonomic system, where bioinformatics plays a key role (El-Atroush*et al.,* 2015).

These barcodes are used for phylogenetic analysis, genetic diversity and species discrimination. These systems not only help to classify the organisms but also reveal genetic information to infer evolutionary history and speciation (Son *et al.,* 2003; Hebert *et al*., 2004). In plants, finding effective and robust conserved regions are more challenging because of high genome diversity (Kelchner, 2000).

Seven chloroplast genomic regions where evaluated by consortium for the barcode of life plant working group and finally, a combination of *matK* and *rbcl* was adopted as universal barcode for identification and authentication of flowering plant (Jumbo, 2011). Maturase k (known for short as *matK*)

has a high evolutionary rate, suitable length and obvious interspecific divergence as well as a low transition rate (Min and Hickey, 2007; Selvaraj *et al.,* 2008).

One of the challenges for plants barcoding is the ability to resolve sister species within a large geographical range. It is expected that system based on anyone, or small member of chloroplast gene will fail in certain taxonomic groups with extremely low amount of plastid variation while performing well in groups (Newmaster *et al*., 2008).

Mimosoid form one of the major groups of legumes and have been circumscribed as a clade in Caesalpinoideae within the family Fabaceae (LPWG, 2017). The clade comprised of about 80 genera, mostly tropical to subtropical in distribution, and major components of arid and semiarid regions throughout the world, where they are an important source of forage and fuel (Lucknow *et al*., 2003).

The morphological and nomenclatural ambiguities exhibited by diverse members of Mimosoidare clearly evident in the multiple of revisions so far conducted. Regrettably, most of the revisions aimed at proffering solutions were conducted either with non-DNA or single evidential tool.

Though several researchers have attempted the use of DNA barcoding in resolving taxonomic intricacies among plant taxa across the globe, there is still gross paucity of DNA barcode of plants in tropical and developing African nations. Also, chloroplast *matK* gene which has a better nomenclatural resolution power acrossseveral plant groups has not received the due attention in resolving taxonomic challenges among member of Mimosoid.

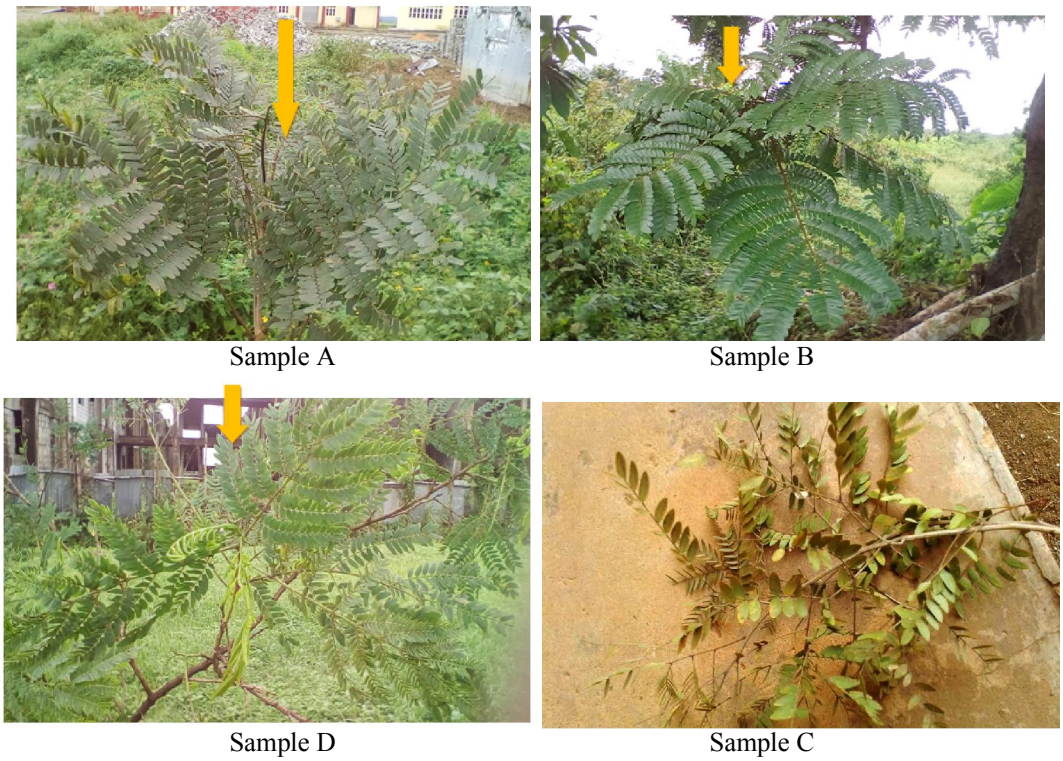
It is based on these drawbacks, that the study is aimed at employing DNA barcoding to resolving the morphological ambiguities among members of Mimosoid.

Methodology

Collection and authentication of specimens

Table 1. Location of Sample collection

Sample ID	Latitude (N)	Longitude (E)	Altitude (m)	Community
A	4.94992	8.35312	22	Unical
B	4.94584	8.35406	9	Unical
C	4.94518	8.35265	10	Unical
D	6.67419	8.79223	44	Ogoja



Sample A Sample B

Sample D Sample C

Plate1. Voucher specimens used for research

Fresh leaves specimens were obtained from four morphologically similar plant species growing naturally in Cross River State. The specimens A, B and C were obtained around University of Calabar, Calabar campus while specimen D (not found around Calabar) was obtained from Ogoja, Cross River State. The samples were confirmed to be members of Mimosoideae subfamily with the aid of taxonomic keys for Nigerian trees by Keay *et al*. (2011) and expert recognition by Dr. J. K. Ebigwai of the Department of Plant and Ecological Studies, University of Calabar. The specimens and their geographical information were presented in Table 3.1 and the voucher specimens shown in Plate 3.1.

DNA Extraction

Extraction was done using a Zymo plant/seed DNA mini prep extraction kit. One hundred and fifty milligrams (150 mg) of the plant leaves were transferred into ZR Bashing Bead Lysis tubes, 750 microliters of lysis solution were added to the tube. The tubes were secured in a bead beater fitted with a 2 ml tube holder assembly and processed at maximum speed for 5 minutes. The ZR bashing bead lysis tube was centrifuged at 10,000x*g* for 1 minute.

Four hundred microliters (400 µl) of supernatant was transferred to a Zymo-Spin IV spin Filter (orange top) in a collection tube and centrifuged at 7000 x*g* for 1 minute. One thousand two hundred microliters (1200 µl) of fungal/bacterial DNA binding buffer was added to the filtrate in the collection tubes bringing the final volume to 1600 µl, 800 µl was then transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10,000x*g* for 1 minute, the flow through was discarded from the collection tube.

The remaining volume was transferred to the same Zymo-spin and spun. Two hundred (200) µl of the DNA Pre-Was buffer was added to the Zymo-spin IIC in a new collection tube and spun at 10,000x*g* for 1 minute followed by the addition of 500 µl of fungal/bacterial DNA wash buffer and centrifuged at 10,000x*g* for 1 minute. The Zymo-spin IIC column was transferred to a clean 1.5 µl centrifuge tube, 100 µl of DNA elution buffer was added to the column matrix and centrifuged at 10,000x*g* for 30 seconds to elute the DNA. The eluted DNA was transferred into Zymo-spin IV-HRC column into a 1.5ml tube and spun at 10,000 xg for 1minute. The product was then stored at -20^{o}C for PCR.

DNA quantification

The extracted genomic DNA was quantified using the Nano drop 1000 spectrophotometer. The software of the equipment was lunched by double clicking on the Nano drop icon. The equipment was initialized with 2 ul of sterile distilled water and blanked using normal saline. Two microliters of the extracted DNA were loaded onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the "measure" button.

DNA Amplification

For amplification and sequencing of *matK* gene, primer pair: MatK-1RKIM-f and MatK-3FKIM-r were used following the method of Kuzmina *et al*. (2012). The DNA was amplified on an ABI 9700 Applied Bio systems thermal cycler at a final volume of 30 ul for 35 cycles. The PCR mix included: the X2 Dream Taq Master Mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), and the primers at a concentration of 0.5 uM and 25ng of the extracted DNA as template.

The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 55ºC for 40 seconds; extension, 72ºC for 50 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 25 minutes and visualized on a blue light Tran's illuminator for a base pair product size. Each PCR reaction was repeated three (3) times on each sample and examined by electrophoresis on 1 % agarose gel, using DNA marker 1 kb ladder.

Amplicons sequencing and sequence analysis

The Amplicons were sequenced using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10 ul, the components included 0.25 ul BigDye® terminator v1.1/v3.1, 2.25ul of 5 x BigDye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100 bp.

The sequencing conditions were as follows 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4 min. The obtained sequences were edited using the bioinformatics algorithm Trace edit. The nucleotide sequences were aligned using *Clustal W* in MEGA7 (Kumar *et al*., 2016), to identify any parsimonial informative sites: Single nucleotides polymorphism (SNP).

Sequence identification

Sequence homology of the two samples was detected using Basic Local Alignment Tool (BLAST) for highly similar sequences from the National Center for Biotechnology Information (NCBI) non-redundant nucleotide (nr/nt) database. The query sequence was identified based on the percentage identity or similarity with a known sequence (Zheng *et al*., 2000).

The sample was said to be correctly identified when the highest BLAST % identity of the query sequence was from the expected species or the species belonging to the expected subfamily; ambiguous identification means that the highest BLAST %

identity for a query sequence was found to match several species of the study subfamily; incorrect identification means that the highest BLAST % identity of the query sequence was not from the expected subfamily (Shinwari *et al.,* 2014).

Phylogenetic tree method was used to resolve any ambiguous identification by BLAST. The *matK* sequences of other members of same genus were mined from NCBI in addition to those generated in this study. The genetic distances for the sequenced samples were equally evaluated. The inter specific and intra specific distances were computed using the Maximum Composite Likelihood model in MEGA7 (Kumar *et al*., 2016). The pairwise alignment of nucleotide sequence was done using *Clustal W* to identify any barcode gap.

Results

Results are presented for DNA quality, DNA Quantification, DNA Amplification, DNA sequencing and Phylogenetic analyses.

Table 2. Result on DNA quality

Sample ID	A_{260} (nm)	A_{280} (nm)	$A_{260/280}$
A	0.45	0.43	1.05
B	0.53	0.31	1.71
C	0.54	0.32	1.69
D	0.63	0.71	0.89

A260 nm is the absorbance wavelength for DNA
A280 nm is the absorbance wavelength for protein
$A_{260/280}$ is the purity index DNA

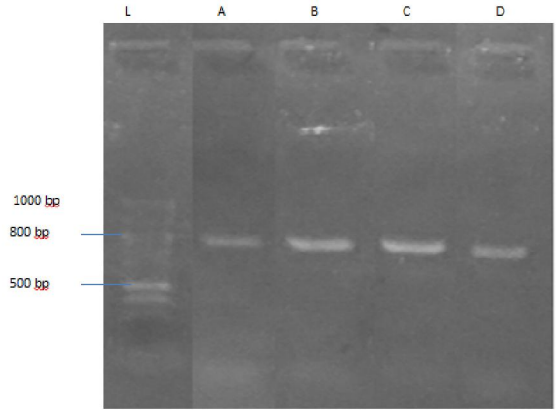


Fig. 1. Agarose gel electrophoresis showing the amplified mat-K gene of the respective plants, lane A-D showing the *mat-K* gene bands at 750-800bp while lane L represents the 100 bp molecular ladder.

Amplicons sequencing and barcode analysis

Result of the edited sequences of samples A, B, C and D was shown in Table 4.2. The pairwise sequence alignment result revealed the presence of 301 conserved codons and 25 residues in the nucleotide and amino acid sequence respectively, across the samples. Plate 4.1 and 4.2 show the pairwise alignment of nucleotide and amino acid sequences of the study samples respectively.

Result of the SNP between sample A, B, C and D was shown in Table 4.3. The Table revealed the highest SNP of 388 bp between samples A and C followed by the 386 bp between samples B and C, and 386 bp between samples B and D. Others were 15, 14 and 9 bps between samples C and D, samples A and D, and samples A and C respectively.

Table 3. *MatK* gene sequence of sample A, B, C and D

Sample ID	Nucleotide sequence	Amino acid
Sample A	755	251
Sample B	755	251
Sample C	724	241
Sample D	779	259

Table 4. Single Nucleotide Polymorphisms (SNPs) between samples A, B, C and D

	A_*MatK*-F	B_Ma*tK*-F	C_Ma*tK*-F	D_Ma*tK*-F
A_*MatK*-F				
B_Ma*tK*-F	388			
C_Ma*tK*-F	9	386		
D_Ma*tK*-F	14	385	15	

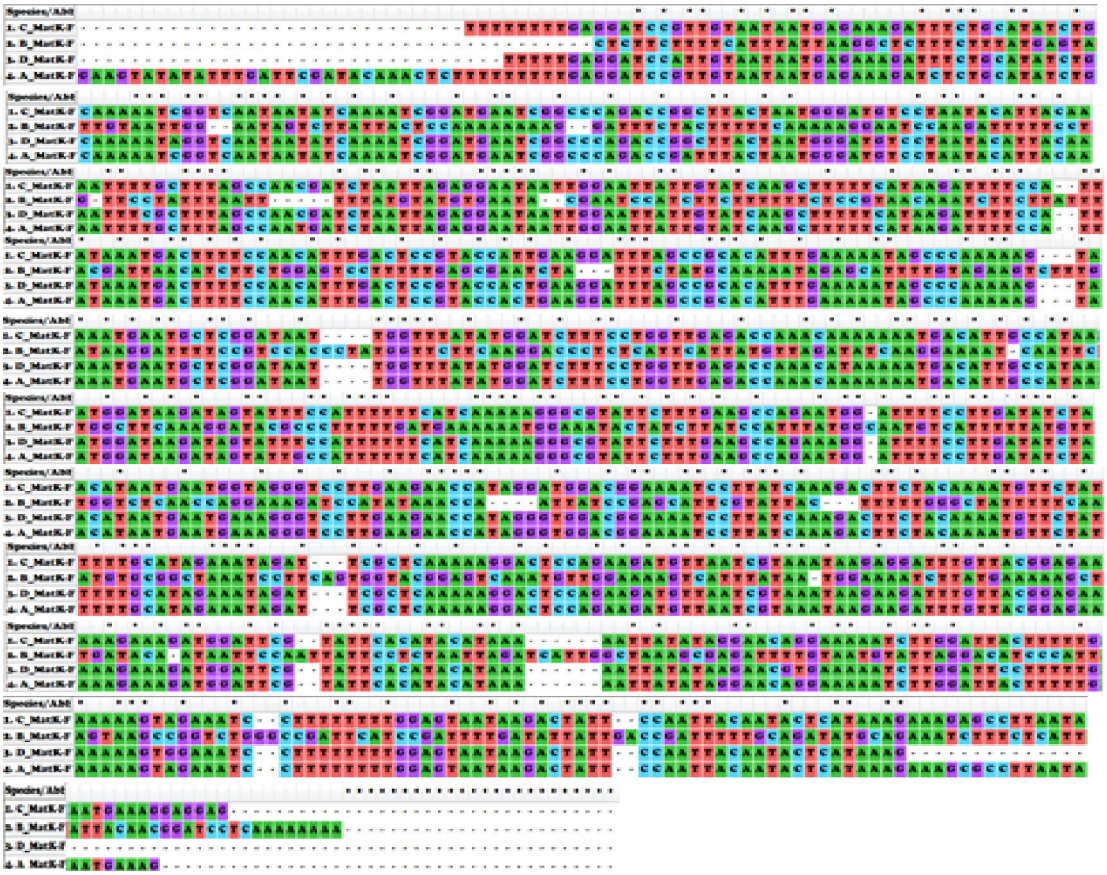


Plate 2. Pairwise alignment of *matK* nucleotide sequence of samples A, B, C and D
* represent the conserved regions
- represent Indel (deletion or insertion of base pair) while mismatch are loci of base substitution

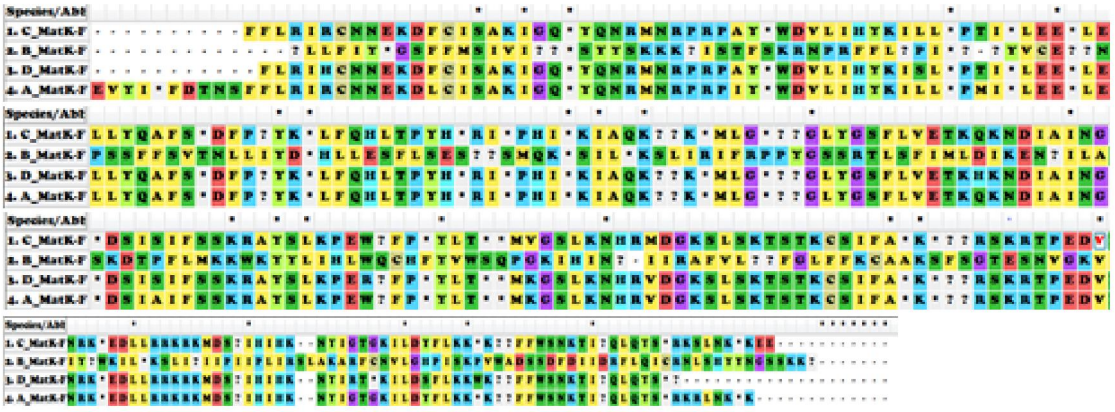


Plate 3. Aligned protein sequence of samples A, B, C and D
* represent the conserved regions
- represent deletion while mismatch represent amino acids substitution
? Represent non-transcribed segment

Sequence identification

The *matK* sequence of samples A, B, C and D was independently queried for highly similar sequence in the NCBI data base using BLASTn and the result is shown in Table 5. From the result; sample A, C and D exhibits maximum homology (100 %) with sequence of *Parkia biglobosa, Leucaena leucocephala* and *Prosopis africana* respectively, while sample B exhibited 99.7 % with *Pentaclethra macrophylla* in the NCBI data base.

The genetic distances between samples B and D in relation to NCBI sequences of the same taxon where evaluated. As shown in Table 5, sample B (*Pentaclethra macrophylla*) was significantly different from other three species studied with a genetic distance of 80 – 81 %. The distance between sample A (*Parkia biglobosa*), sample C (*Leucaena leucocephala*) and sample D (*Prosopis africana*) were within 2 %. Similar results were obtained between any pair of their sequences obtained from NCBS databank.

Fig 4.2 showed the Neighbor joining of the samples A, B, C and D in relation to their identical sequences obtained from NCBI databank. It was observed that samples A, B, C and D nested with *Parkia biglobosa, Pentaclethra macrophylla, Leucaena leucocephala* and *Prosopis africana* respectively with maximum bootstrap values of 100 % at each intraspecific node and 60 % inter generic node. It equally showed that *Parkia biglobosa, Leucaena leucocephala* and *Prosopis africana* nested on a common branch or clade while *Pentaclethra macrophylla* rather nested as an out group.

Table 5. *MatK* gene sequence of sample A, B, C and D

Sample ID	Identical sequence from NCBI	% homology
Sample A	*Parkia biglobosa*	100
Sample B	*Pentaclethra macrophylla*	99.7
Sample C	*Leucaena leucocephala*	100
Sample D	*Prosopis Africana*	100

Table 6. Analysis of barcode gaps in the matK gene sequence of *Parkia biglobosa, Leucaena leucocephala* and *Prosopis africana*

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1								
2	0.81							
3	0.02	0.80						
4	0.02	0.81	0.02					
5	0.00	0.81	0.02	0.02				
6	0.02	0.81	0.02	0.00	0.02			
7	0.81	0.00	0.80	0.81	0.81	0.81		
8	0.02	0.80	0.00	0.02	0.02	0.02	0.80	

Note: 1 = A_MatK-F, 2 = B_MatK-F, 3 = C_MatK-F, 4 = D_MatK-F, 5 = KX119401.1_*Parkia biglobosa,* 6 = KX119405.1_*Prosopis Africana,* 7 = KX302343.1_*Pentaclethra_macrophylla* and 8 = MH767953.1_*Leucaena_leucocephala*

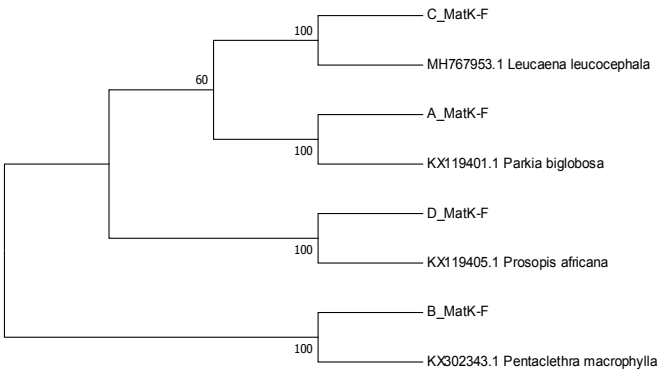


Fig. 2. Evolutionary relationships of *Parkia biglobosa, Pentaclethra macrophylla, Leucaena leucocephala* and *Prosopis africana*

The evolutionary history was inferred using the Neighbour-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The evolutionary distances were computed using the number of differences method and are in the units of the number of base differences per sequence. The analysis involved eight (8) nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All

positions containing gaps and missing data were eliminated. There was a total of 640 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

Discussion

Specimen identification to specific level is key to all biological or ecological research. The inherent uniqueness of each taxon DNA barcode has been exploited as a standard and rapid method for species identification (Ebigwai *et al.,* 2020, Ikram *et al*., 2015; Khan *et al*., 2015). The premium step in barcode-based species identification process is centred on isolation of pure DNA (Watto *et al*., 2016). DNA purity often measured as a ratio of a unit Ultra Violet (UV) absorbance power at 260nm and that of eight (8) parts of protein at 280nm; indicates the quality of DNA in the solution (Watto *et al*., 2016). Put succinctly, an $A_{260/280}$ ratio of 1.8 is indicative of a pure DNA solution. However, in practice, a range of 1.7 – 1.9 obtained in any extract is considered pure and hence devoid of significant concentrations of contaminations (Abdel-Latif and Osman, 2017).

In the present study, the DNA quality of samples A, B, C and D were 1.05, 1.71, 1.69, and 0.89 respectively. This implies that the DNA of all but B samples was below the purity limits, indicating contamination. Presence of polysaccharides, glycoproteins and phenolic members were identified by Watto *et al.* (2016) as significant sources of impurities during DNA extraction process. The degree of impurities in samples A, C and D as exemplified by the $A_{260/280}$ ratios is highly suggestive of samples with high concentrations of the aforementioned secondary metabolites when compared to that of sample B. Rvathi (2018), Abioye *et al.* (2013) and Badamasi et al. (2011) reports supported this assertion. Also, several sample matrixes such as degradation at primer binding sites (Newmaster *et al*., 2013) and DNA deterioration by the extraction chemicals (Schori *et al*., 2013) have been shown as other causal factors for low $A_{260/280}$ values. However, the purity level of any DNA extract does not impede PCR success (Abouseadaa *et al*., 2015) as contaminated samples have been reported to have been successfully amplified and sequenced (Ebigwai *et al.,* 2020).

In DNA-based species identification, PCR amplification of the target genome plays an important role in generating the barcode. PCR amplification of the four morphological ambiguous specimens (samples A – D) was 100 % successful. The PCR bands were clear and visible within 700 – 800 bp compared to the 1 kb molecular larder. Similar results with the amplification of *matK* gene in Fabaceae family has been reported by Ebigwai *et al*. (2020 in press) and Gao *et al.* (2011), suggesting the universality of the *matK* as a barcode region in Fabaceae family. The result however, contrasted previous report by Kuzmina *et al.* (2012) who recorded as low as 35% amplification in herbarium specimen and 45% of fresh specimen from 900 vascular plant specimen representing 312 species, 147 genera and 51 families and Sass *et al*. (2007) who recorded 24 % amplification success in *Cycas* using with the same marker, indicating that *matK* has low amplification rate.

Sequence recoverability is another criterion often considered in selecting genome region for barcoding (Hollingsworth, 2008). In the present study, all the samples were successfully sequenced, indicating 100 % sequence retrieval for *matK* gene in the studied taxa. The recovered sequences adequately satisfied the criterion of an appropriately short sequence length (300–800 bp) to be used as barcode (Kress *et al*., 2005). The present finding mirrored previous studies with *matK* (Tan *et al*., 2018; Nithaniyal *et al*., 2014; Hollingsworth *et al*., 2009; Chase *et al.,* 2007; Kress and Erickson, 2007). Similarly, the result is in support of Shinwari *et al.* (2014) and Gao et al. (2011) who recommended *matK* as a universal barcode region for Fabaceae family.

The pair wise alignment of the recovered sequences revealed the presence of 301 conserved codons and 25 residues in the nucleotide and amino acid sequence respectively, across the samples. These correspond to 38.6 and 9.7 % reservation in their nucleotide and Amino acid sequences respectively. This indicates distant relationship among the studied sequences (Udensi *et al*., 2017). According to Wojciechowski *et al*. (2004), proteins in the same plant family shares at least 30 % similarities in their amino acid sequence. This implies that the studied taxa are related above family level.

All the studied specimens were unambiguously identified using BLASTn (Shinwari *et al*., 2014). Specimens A, B, C and D were identified as *Parkia biglobosa, Pentaclethra macrophylla, Leucaena leucocephala* and *Prosopis africana* respectively. This was similar to Gao *et al.* (2011) who conducted DNA barcoding of some members of Fabaceae family using the same marker gene. This confirmed that the plastid *matK* gene is an idle barcode for species discrimination in Fabaceae. Similar species recoveries with the marker gene have been reported across angiosperms notably by Kang *et al.* (2017), Mishra *et al*. (2017), Kim *et al*., (2016), Nithaniyal *et al*. (2014) and Kress *et al.* (2009). The present result however contradicted Tallei *et al.* (2016) who reported the paucity of barcode gaps in the *matK* gene of *Syzygium spp*.

Distance tree, based on the Neighbor-Joining method was used to evaluate the proportion of relatedness among the identified species, and the result

showed that *Parkia biglobosa, Leucaena leucocephala* and *Prosopis africana* are nested on a common branch while *Pentaclethra macrophylla* nested as an out-group. This implies that *Pentaclethra macrophylla* is genetically distant from the other species, and could account for the low conservation in the nucleotide and amino acid sequences of the studied specimens (Nithaniyal *et al*., 2014). The use of *matK* gene sequence for phylogenetic tree construction has been illustrated by de Melo Moura *et al.* (2019) and Kim *et al.* (2016). However, Phylogenetic tree-based methods are not appropriate for building phylogenetic relationships at lower levels of sequence variation (Saarela *et al.,* 2013).

The barcode gap between each pair of the studied sequence conformed to those between NCBI sequences of *Parkia biglobosa, Pentaclethra macrophylla, Leucaena leucocephala* and *Prosopis africana*. The interspecific barcode gaps in *Parkia biglobosa, Leucaena leucocephala* and *Prosopis africana* conformed to previous recommendation for species recognition while the intra specific distances equally satisfied the requirement for species retention according to Tallei and Kolondam (2015), Nithaniyal *et al*. (2014) and Purushothaman *et al*. (2014). However, the barcode gaps (80 - 81 %) between *Pentaclethra macrophylla* and other species studied were above the intra-familial threshold (Udensi *et al*., 2017; Wojciechowski *et al*., 2004; Kajita *et al*., 2001). In addition, the present result mirrored the 0.44 - 0.76 % inter species barcode recorded for *Agalii* (Pettengill and Neel, 2010), and the 0.38 - 1.55 % barcode gap for timber trees within the tropical dry evergreen forest of India (Nithaniyal *et al*., 2014). Generally, the performance of *matK* in the present study is in agreement with Gao *et al.* (2011) findings for the Fabaceae family.

Conclusion

The amplification and sequencing of conserved genome regions identified a novel sequence of *matK* in *Parkia biglobosa, Pentaclethra macrophylla, Leucaena leucocephala* and *Prosopis africana.* The findings resolved the nomenclatural identities of these four morphologically similar species.

Recommendation

This finding would serve the bedrock for a detailed phytochemical and morphological study to be conducted on each of the taxon to enhance rapid and cheap on field authentication process.

Corresponding author:
Dr Joseph Ebigwai
Department of Plant and Ecological studies, University of Calabar, Calabar, Nigeria. E-mail: ebijoe4@gmail.com

References

1. Gregory TR. DNA barcoding does not compete with taxonomy. Nature, 2005; 434:1067. doi:10.1038/4341067b.
2. Hebert P, Cywinska A, Ball SL, Dewaard J. Biological identification through DNA barcodes. Proceedings of the Royal Society of London B, 2003; 270. 313-321.
3. Wattoo, J, Saleem Z, Shahzad MS, Arif A, Hameed A, Saleem A. DNA Barcoding: Amplification and sequence analysis of RBCL and matK genome regions in three divergent plant species. Advancement in Life Sciences, 2016; 4.
4. El-Atroush H, Magdy M, Werner O. DNA Barcoding of two endangered medicinal Plants from Abou Galoom protectorate. Life Sci J 2015;12(9):101-109]. (ISSN:1097-8135). http://www.lifesciencesite.com. 14
5. Son YO, Kim J, Lim JC, Chung Y, Chung GH. Ripe fruits of Solanum nigrum L. inhibits cell growth and induces apoptosis in MCF-7 cells. Food and Chemical Toxicology, 2003; 41(10): 1421-1428.
6. Hebert PD, Penton EH, Burns JM, Janzen DH, Hallwachs W. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptesfulgerator. Proceedings of the National Academy of Sciences USA, 2004; 101(41): 14812- 14817.
7. Kelchner SA. The evolution of non-coding chloroplast DNA and its application in plant systematics. Annals of the Missouri Botanical Garden, 2000; 87(4): 482-498.
8. Jumbo U, Kato T, Ito M. Current progress in DNA barcoding and future implications for entomology. Entomological Science, 2011; 14(2): 107-124.
9. Min X, Hickey D. DNA Barcodes Provide a Quick Preview of Mitochondrial Genome Composition. PLoS one, 2007; 2. e325. 10.1371/journal.pone.0000325.
10. Selvaraj D, Sarma RK, Sathishkumar R. Phylogenetic analysis of chloroplast matK gene from Zingiberaceae for plant DNA barcoding. Bioinformation, 2008; *3*(1), 24–27. https://doi.org/10.6026/97320630003024.
11. Newmaster SG, Fazekas AJ, Steeves RAD, Janovec J. Testing candidate plant barcode regions in the Myristicaceae. Mol. Ecol. Resour. 2008; 8, 480– 490.

12. LPWG, Legume Phylogeny Working Group. A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny. Taxon, 2017; 66, 44 – 77.
13. Lucknow M, Miller JT, Murphy DJ, Livshultz, T.A phylogenetic analysis of the Mimosoideae (Leguminosae) based on chloroplast DNA sequence data. In: B.B. Klitgaard and A. Bruneau (editors). Advances in Legume Systematics, part 10, Higher Level Systematics, 2003; pp. 197-220. Royal Botanic Gardens, Kew.
14. Keay RWJ. *Trees of Nigeria*. Oxford, Clarendon Press, 2011; 476p.
15. Kuzmina ML, Johnson KL, Barron HR, Hebert PDN. Identification of the vascular plants of Churchill, Manitoba, using a DNA barcode library. BMC Ecology, 2012; 12, 25 – 25.
16. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Bio Evol. 2016; 33:1870–1874. https://doi.org/10.1093/molbev/msw054.
17. Zheng J, Shen W, He D, Kevin BL, Laird DM, Peter D. Prestin is the motor protein of cochlear outer hair cells. Nature. 2000; 405, 149-155.
18. Shinwari ZK, Jamil K, Zahra NB. Molecular systematics of selected genera of family Fabaceae. Pak. J. Bot., 2014; 46(2): 591-598.
19. Ebigwai JK, Udensi UO, Njoku KL, Damian A. Determining accurate nomenclature of five Ixoridae Sub family Members using DNA barcoding. Journal of Biological Sciences, 2020 (in press).
20. Ikram A, Zahra NB, Shinwari ZK, Qaiser M. Ethnomedicinal review of folklore medicinal plants belonging to family Apiaceae of Pakistan. Pak. J. Bot., 2015; 47(3): 1007-1014.
21. Khan MQ, Khalil AT, Rehman K, Shinwari ZK. Searching for DNA Barcodes in Plants. Global J. Biotechnol. Biochem, 2015; 10(1): 1-10.
22. Abdel-Latif A, Osman G. Comparison of three genomic DNA extraction methods to obtain high DNA quality from maize. Plant Method, 2017; 13, 1 – 9.
23. Revathi D. Phytochemical analysis of *Leucaena leucocephala* on various extracts. The Journal of Phytopharmacology, 2018; 7(6), 480-482 ISSN:2320-480.
24. Abioye EO, Akinpelu DU, Aiyegoro OA, Adeboye MF, Oni MO, Okoh AI. Preliminary phytochemical screening and antibacterial properties of crude stem bark extracts and fractions of *Parkia biglobosa* (Jacq.). Molecule, 2013; 18(7), 8485 – 8499 DIO: 10.3390/molecule18078485
25. Badamasi MZ, Adamu T, Abubakar U, Ladan MJ, Bala AY. Antimalarial activity and phytochemical analysis of the aqueous stem bark extract of *Prosopis africana* (Kirya) against Plasmodium species. Nigerian Journal of Parasitology, 2011; 32(2), 271-275 EISSN: 1117-4145.
26. Newmaster SG, Grguric M, Shanmughanandhan D, Ramalingam S, Ragupathy S. DNA barcoding detects contamination and substitution in North American herbal products. BMC Medicine, 2013; 11:222. doi:10.1186/1741-7015-11-222.
27. Schori, M, Appel M, Kitko A, Showalter AM. Engineered DNA polymerase improves PCR results for plastid DNA. Appl. Plant Sci., 2013; 1(2): 1-7.
28. Abouseadaa HH, Osman GH, Ramadan AM, Hassanein SE, Abdelsattar MT, Morsy YB, Alameldin HF, El-Ghareeb DK, Nour-Eldin HA, Salem R, Gad AA, Elkhodary SE, Shehata MM, Mahfouz HM, Eissa HF, Bahieldin A. Development of transgenic wheat (*Triticumaestivum* L.) expressing avidin gene conferring resistance to stored product insects. BMC Plant Biology, 2015; 15, 183 – 90.
29. Ebigwai JK, Akomaye FA, Ubi GM. Resolving taxonomic ambiguity between two morphological similar plant taxa using maturase K gene analysis. Journal of Biological Sciences, 2020; 20, 13-21 DOI: 10.3923/jbs.2020.13.21
30. Gao T, Sun Z, Yao H, Song J, Zhu Y, Ma X, Chen S. Identification of Fabaceae Plants Using the DNA Barcode *matK*. Planta Medica, 2011; 77, 92 - 94.
31. Sass C, Little DP, Stevenson DW, Specht, CD.DNA barcoding in the Cycadales: testing the potential of proposed barcoding markers for species identification of cycads. *Public Library of Science ONE*, 2007; 2, e1154.
32. Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA, Janzen DH. Use of DNA barcodesto identify flowering plants. Proceedings of National Academic of Science, United State of America, 2005; 102, 8369 – 8374.
33. Hollingsworth P. DNA barcoding plants in biodiversity hot spots: Progress and outstanding questions. Heredity,2008; 101, 1 – 2.
34. Tan SL, Luo YH, Hollingsworth PM, Burgess KS, Xu K, Li DZ, Gao LM. DNA barcoding herbaceous and woody plant species at a subalpine forest dynamic plot in Southwest China. Ecology and Evolution, 2018; 8, 7195 – 7205.
35. Nithaniyal S, Newmaster SG, Ragupathy S, Krishnamoorthy D, Vassou SL, Madasamy P. DNA Barcode Authentication of Wood Samples

of Threatened and Commercial Timber Trees within the Tropical Dry Evergreen Forest of India. Public Library of Science ONE, 2014; 9, DOI: 10.1371/journal.pone.0107669.
36. Hollingsworth ML, Andra CA, Forrest LL, Richardson J, Pennington RT, Long DG, Cowan R, Chase MW, Gaudeul M, Hollingsworth PM. Selecting barcoding loci for plants: evaluation of seven candidate loci with species-level sampling in three divergent groups of land plants. Molecular Ecology Resources, 2009; 9, 439 - 457.
37. Chase MW, Cowan RS, Hollingsworth PM, van den Berg C, Madrinan S, Petersen G, Seberg O, Jorgsensen T, Cameron KM, Carine M, Pedersen N, Hedderson TAJ, Conrad F, Salazar GA, Richardson JE, Hollingsworth ML, Barraclough TG, Kelly L, Wilkinson M. A proposal for a standardised protocol to barcode all land plants. Taxon, 2007; 56, 295 – 299.
38. Kress WJ, Erickson DL. A Two-Locus Global DNA Barcode for Land Plants: The Coding rbcL Gene Complements the Non-Coding *trnH-psbA* Spacer Region. Public Library of Science ONE, 2007; 2, e508.
39. Udensi O, Edu N, Ikpeme E, Onung O, Emeagi L, Nwanze B, Ejiyere E, Chen L, Gerrano A. Genotyping of Pigeon Pea [*Cajanuscajan* (L.) Millsp.] Accessions Obtained from International Institute of Tropical Agriculture (IITA) Germplasm Using Random Amplified Polymorphic DNA. Journal of Experimental Agriculture International, 2017; 17, 1 – 12.
40. Wojciechowski MF, Lavin M, Sanderson MJ. A phylogeny of legumes (Leguminosae) based on analysis of the plastid *matK* gene resolves many well supported sub-clades within the family. American Journal of Botany, 2004; 91, 1846 – 1862.
41. Kang Y, Zhiyan D, Zang R, Long W. DNA barcoding analysis and phylogenetic relationships of tree species in tropical cloud forests. Scientific Report, 2017; 7, *12564* DOI:10.1038/s41598-017-13057-0.
42. Mishra P, Kumar A, Nagireddy A, Shukla AK, Sundaresan V. Evaluation of single and multilocus DNA barcodes towards species delineation in complex tree genus *Terminalia*. Public Library of Science ONE, 2017; 12, DOI: 10.1371/journalpone.0182836.
43. Kim WJ, Ji Y, Choi G, Kang YM, Yang S, Moon BC. Molecular identification and phylogenetic analysis of important medicinal plant species in genus Paeonia based on rDNA-ITS, matK, and rbcL DNA barcode sequences. Genetics and Molecular Research, 2016; 15, gmr.15038472.
44. Kress WJ, Erickson DLF, Jones A, Swenson NG, Perez R, Sanjur O, Bermingham E. Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. Proceedings of the National Academy of Sciences United State of America, 2009; 106, 18621 – 18626.
45. Tallei T, Irawan P, Kolondam B. DNA Barcoding Analysis of *matK* Gene of Some *Syzygium* Species. In: Bioinformatics Workshop 2016: Developing knowledge and skills in bioinformatics for young Indonesian scientists in improving research quality in life science and sustainable exploration of biodiversity in Indonesia, 13 – 15 September 2016, AI Azhar University Jakata, DOI: 10.13140/RG.2.2.10710.24641.
46. de Melo Moura CC, Brambach F, Hernandez KJB, Konstantin VK, Holger K, Sudarmiyati ST, Iskandar ZS, Gailing O. Integrating DNA Barcoding and Traditional Taxonomy for the Identification of Dipterocarps in Remnant Lowland Forests of Sumatra. Plants, 2019; 8, DOI:10.3390/plants8110461.
47. Saarela JM, Sokoloff PC, Gillespie LJ, Consaul LL, Bull RD. DNA barcoding the Canadian Arctic Flora: core plastid barcodes (*rbcL+matK*) for 490 Vascular Plant Species. *Public Library of Science ONE*, 2013; 8, 36.
48. Tallei TE, Kolondam BJ. DNA barcoding of Sangihe nutmeg (*Myristicafragrans*) using *matK* gene. HAYATI Journal of Biosciences, 2015; 22, 41-47.
49. Purushothaman N, Newsmaster SG, Ragupathy S, Stalin N, Suresh D, Arunraj DR, Gnanasekaran G, Vassou SL, Narasimhan D, Parani M. A tiered barcode authentication tool to differentiate medicinal Cassia species in India. Genetic Molecule Resources, 2014; 13, 2959 - 2968.
50. Kajita T, Ohashi H, Tateishi Y, Bailey CD, Doyle JJ. RbcL and legume phylogeny with particular reference to Phaseoleae. Millettieae and allies. Systematic Botany, 2001; 26, 515 – 536.
51. Pettengill JB, Neel MC. An evaluation of candidate plant DNA barcodes and assignment methods in diagnosing 29 species in the genus Agalinis (Orobanchaceae). American Journal of Botany, 2008; 97, 391 – 406.