Scientific Research Publishing

American Journal of Plant Sciences, 2022, 13, 1335-1346
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

DNA Barcoding of Nigeria's Forest Species Listed in CITES and Other Endangered Plant Species of National Interest

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How to cite this paper: Onyia, C.O., Ilo, O.P., Obih, C.E., Ugbogu, O., Ojiego, B.O., Rufai, S.S., Onyemaechi, P.S. and Chukwuma, E.C. (2022) DNA Barcoding of Nigeria's Forest Species Listed in CITES and Other Endangered Plant Species of National Interest. *American Journal of Plant Sciences*, **13**, 1335-1346.
https://doi.org/10.4236/ajps.2022.1311090

Received: September 17, 2022
Accepted: November 18, 2022
Published: November 21, 2022

Abstract

Illegal trade is considered one of the greatest threats to the loss of biodiversity of endangered plants. Some of these plant species are often trafficked in processed forms, making it extremely difficult for taxonomic experts to identify them. In the past, illegal traders of endangered species have been arrested and prosecuted but eventually cleared due to a lack of conclusive evidence. DNA barcoding is a veritable tool to protect these endangered species from illegal trade. It identifies all stages of the species' life forms including processed products (milled or powdered animal and plant parts). The study utilised the *rbcL* gene as a single barcode region in the identification/authentication of 19 Nigeria's endangered forest species legislated under the CITES and other endangered species of national interest. The generated sequence barcodes were used to query NCBI-GenBank and BOLD databases. 57.89% of the samples were identified down to species level and 42.11% to genus level. Amongst the 19 samples, sample (S7) yielded a high-quality sequence for a single sequencing read (forward), sufficient to identify the sample with a 99.81% identity match on NCBI-GenBank and BOLD. The results reveal that the *rbcL* single barcode efficiently identified most of the sampled plants; this supports the potential utilisation of DNA barcoding in the accurate detection and conservation of CITES-listed plants in Nigeria. The study documented the CITES-listed plants and other essential plants endangered or threatened plants in Nigeria and provided the first chloroplast DNA reference dataset to support the utilisation of DNA barcoding to identify CITES-listed plant spe-

cies in Nigeria, which is significant for future studies.

Keywords

Annonaceae, Apocynaceae, BOLD, CITES, DNA Barcoding, NCBI, *rbcL*

1. Introduction

Land plants make up a large percentage of life diversity on earth, consisting of seed plants (angiosperms and gymnosperms), bryophytes, ferns and lilies, estimated at over 380,000 species [1] [2] [3]. It has been estimated that there are 352,000 species of angiosperms, 1300 species of gymnosperms and 13,000 species of bryophytes. During the last century, decreases in biodiversity have been increasingly observed. The Food and Agriculture Organization of the United Nations [4] reported the loss of 75% of the genetic diversity of crops, and according to Royal Botanic Gardens Kew [5], about one-fifth of known plant species are threatened with extinction. Nigeria is rich in bioresources and the distribution of biodiversity. This is shown by a broad range of habitats, starting from the Gulf of Guinea along the Atlantic coast, consisting of aquatic and non-aquatic organisms found in different ecological areas [6]. However, the country's biodiversity are being threatened by various factors, which include but are not limited to anthropogenic activities and climate change effects.

As reported in the 2013 IUCN's Red List, Nigeria's threatened species are 309. The taxonomic categories include twenty-six mammals, nineteen birds, eight reptiles, thirteen amphibians, sixty fishes, a mollusc, fourteen other invertebrates and one hundred and sixty-eight plants [7]. Also, Nigeria Federal Environmental Protection Agency [8] reported that Nigeria has over 5000 recorded species of plants. It is estimated that 0.4% of the plant species are threatened, and 8.5% are endangered and close to becoming extinct due to poaching, climate change, urbanisation and industrialisation. According to Borokini [9], 91 species of Nigerian plants are endemic, and they belong to 44 families, with Rubiaceae attributing to the majority. Conservation scientists opine that serious consideration should be aimed at rescuing the remnants of the significant zones for biodiversity in the nation. Furthermore, there is a common agreement on where the outstanding node of biodiversity exists in Nigeria. The first level of feat is proper identification and documentation (Federal Ministry of Environment [10]). How fast this can be achieved will depend on the county's capability, capacity and willpower to apply and deploy scientific advances such as DNA barcoding.

According to Convention on Biological Diversity [11], the global conservation strategy deems genes, species and ecosystem as prospective tools for sustaining biodiversity ecosystems conservation. Species identification is essential in assessing the level of biodiversity that appraises species diversity for conservation and management efforts [12] [13]. Fast and accurate plant species identification is vital in almost all plant-based research, including biodiversity studies. To pro-

tect global biodiversity from poaching and international trade abuse, countries of the world signed a treaty on March 3 1973, known as the *Convention on International Trade on Endangered Species of Wild Fauna and Flora* (CITES), for regulating trade in endangered species across the border. Protected species are classified in CITES Appendices I, II, and III, in accordance with how a particular populace is being threatened by extinction (Convention on the International Trade in Endangered Species of Wild Fauna and Flora [14]). Species listed in CITES Appendix I are illegal for international trade except for research. However, foreign trade could be approved by appropriate agencies of the nations affected by the import and export. Species listed in Appendix II of CITES are not yet threatened by extinction but may soon become unless their trade is checked [6].

Nigeria has over two hundred plants and animal species listed in the CITES, almost half of which are plants and most of which belong to Orchidaceae, Fabaceae, Euphorbiaceae families and Cycadaceae [15]. Most of the endangered land plants are located in the country's various hard-to-reach protected forest reserves, from where some of the samples used in this study were collected. For adequate protection from illegal trade/over-exploitation for conservation purposes, there is a need for proper identification of the plants and authentication of same using DNA barcoding technique. The technique has been extensively utilised for several purposes and documented as a resurgence for taxonomy (Kress, 2017; Yu *et al*., 2021). DNA barcoding has also been applied in disease and pest control, market fraud detection and protection of endangered species from poachers and illegal international trade [16] [17]. Globally, there are ongoing initiatives to produce DNA barcodes for all assemblages of living organisms and the public availability of these information to comprehend, conserve, and use the world's biodiversity [18]. For instance, Pathak *et al.* [19] reported using DNA barcoding in identifying 29 medicinal plants in the kingdom of Bahrain, while Lv *et al.* [20] identified herbal plants in the Apocynaceae family using DNA barcodes.

A DNA barcode is a genetic signature occurring naturally within every living species' genome [21]. Mitochondrial cytochrome oxidase 1 gene (CO1) has been found effective in identifying animal groups, including birds, flies, butterflies and fish [22]; however, CO1 is ineffective for land plants. In the plastid region of land plants, the regions commonly used are two gene regions in the chloroplast, *matK* and Ribulose-1,5-bisphosphate carboxylase large subunit (*rbcL*), with two additional regions, *trnH-psbA* and internal transcribed spacer (ITS) or (ITS2) [23]. Jamdade *et al*. [24] assessed the ability of ITS2, *rbcL* and *matK* in classifying 20 key plant species of Caryophyllales and found that ITS2 was more effective in distinguishing between studied species. Lone *et al*. [25] investigated the use of ITS, *rbcL*, *trnH-psbA* and *matK* markers in identifying *Epimedium elatum*, a rare medicinal plant in the Himalayas, India and revealed highest polymorphic sites in ITS and *matK*. However, Negi *et al*. [26] identified *rbcL* as a potential marker to curb illicit trade of medicinal plants, *Aconitum heterophyl-*

lum and *Aconitum balfourii*. Also, Ho *et al*. [27], in comparing the effectiveness of *rbcL* and *matK* DNA barcodes in the classification of Jewel orchid in Vietnam, disclosed *rbcL* gene has higher characteristic potential than *matK*.

This paper presents the first report of Nigeria's Barcode of Wildlife—Plant Working Group (BWP-PWG) to protect the endangered flora species listed under CITES from poaching and illegal trading using DNA barcoding.

2. Materials and Methods

2.1. Sample Collection

Tools and materials used for the sample collection include Pair of secateurs, silica gel, machete and knife, polythene bag, hand lenses, a hardcover waterproof notebook, transparent rulers, plant presses, FluidX tubes, personal protective wear and a GPS device. Samples were collected as fresh as possible from the field and not touched with bare hands to avoid contamination. Young, fresh, flexible and non-waxy leaves, about 5 to 10 cm^2 in size, were chosen and stored in paper envelopes under relatively dry climatic conditions. The envelopes were assigned the same number as the plant specimen from which the leaves were collected, and the general rule is one plant per number. The number given to each plant is unique, written on a tag tied to the collected plant, and the replicates bear the same number. The samples were later transferred into FluidX tubes with unique barcodes containing silica gel. The ratio of the silica gel to leaf tissue was 5 - 10:1. Information, such as the GPS location, plant's taxonomic name, tissue barcode number and habitat, was recorded and presented in **Table 1**.

2.2. DNA Extraction

According to the manufacturer's protocol, the extraction of genomic DNA from the samples was done using a modified DNA extraction kit (DNeasy plant mini kit, QIAGEN). Geno-grinder (2000, SPEX Inc.) was utilised to grind the lyophilised plant tissues, after which 20 mg of each plant tissue was put into distinctly labelled micro-centrifuge tubes. Approximately 400 µl of Buffer AP1 and 4 µl of RNase A stock solution (100 mg/ml) were added to each tube containing the powdered tissue. The mixtures were incubated in a water bath for 10 min at 65˚C. The tubes were homogenised 2 - 3 times by inverting tubes while incubating. Additionally, 130 µl of Buffer AP2 was added to the lysates, stirred, and incubated on ice for 5 min. Centrifuging of the lysates for 5 min at 14,000 rpm was made, and the supernatants were transferred to QIAshredder spin columns sitting in 2 ml collection tubes and centrifuged for 2 min at 14,000 rpm. The flow-through was moved to well-labelled micro-centrifuge tubes. About 1.5 volume of Buffer AP3/E was put into the cleared lysates and homogenised by pipetting.

Consequently, pipetting of 650 µl of the mixture into DNeasy mini spin columns sitting in 2 ml collection tubes was executed and centrifuged for 1 min at ≥6000 × g. Then, the filtrate was removed, and the spin columns returned to the

Table 1. Samples field collection data.

Sample ID	Taxonomic ID	Family	Tissue barcode	States collected	Locality	Decimal latitude	Decimal longitude
S1	*Funtumia elastica*	Apocynaceae	FR04499552	Ogun	Omo Biosphere Reserve, Etemi	6.975361	4.366111
S2	*Ficus exasperata*	Moraceae	FR04499555	Ogun	Omo Biosphere Reserve, Etemi	6.979333	4.371944
S3	*Kigelia Africana*	Bignoniaceae	FR04499557	Osun	Sasa	7.089556	4.350306
S4	*Diospyros mespiliformis*	Ebenaceae	FR04499559	Oyo	Ebuya, Old Oyo National Park	8.485583	3.758833
S5	*Lophira lanceolata*	Ochnaceae	FR04499560	Oyo	Ebuya, Old Oyo National Park	8.486889	3.758
S6	*Aframomum sceptrum*	Zingiberaceae	FR04499565	Oyo	Ebuya, Old Oyo National Park	8.486167	3.7565
S7	*Afzelia Africana*	Fabaceae	FR04499569	Oyo	Ebuya, Old Oyo National Park	8.487222	3.757444
S8	*Annona senegalensis*	Annonaceae	FR04499570	Oyo	Ebuya, Old Oyo National Park	8.487113	3.757417
S9	*Annona muricata*	Annonaceae	FR04499573	Oyo	Sepeteri	8.623306	3.645861
S10	*Albizia zygia*	Fabaceae	FR04499578	Osun	Abataju village, Oke-Odo, Ikire	7.383444	4.230056
S11	*Gongronema latifolium*	Apocynaceae	FR04499581	Osun	Abataju village, Oke-Odo	7.384222	4.230389
S12	*Funtumia africana*	Apocynaceae	FR04499582	Osun	Abataju village, Oke-Odo	7.384417	4.230528
S13	*Garcinia kola*	Clusiaceae	FR04499583	Osun	Abataju village, Oke-Odo	7.387944	4.229056
S14	*Lophira alata*	Ochnaceae	FR04499588	Edo	Arakwan, Okomu National Park	6.34225	5.361028
S15	*Khaya ivorensis*	Meliaceae	FR04499589	Edo	Arakwan, Okomu National Park	6.343	5.360167
S16	*Diospyros piscatoria*	Ebenaceae	FR04499595	Edo	Arakwan, Okomu National Park	6.349417	5.343083
S17	*Monodora tenuifolia*	Annonaceae	FR04499597	Oyo	Microbiology dept, University of Ibadan	7.442778	3.897139
S18	*Momordica charantia*	Cucurbitaceae	FR04499542	Oyo	FRIN arboretum	7.391694	3.85775
S19	*Entandrophragma angolense*	Meliaceae	FR04499544	Oyo	FRIN premises, opposite FHI	7.39175	3.863028

collection tubes. A repeat of this phase was made for the residual. About 500 µl of Buffer AW was put into the DNeasy columns, inserted in new 2 ml collection tubes and centrifuged for 1 min at ≥6000 × g (≥8000 rpm). Then, the filtrate was thrown away, and 500 µl of Buffer AW was put into the DNeasy columns and centrifuged for 2 minutes at the highest rate for drying the membrane. Transfer of the DNeasy columns was made to 1.5 ml micro-centrifuge tubes, and 200 µl of preheated (65˚C) Buffer AE was put into the DNeasy membrane directly. They were incubated for 5 minutes at room temperature, after which centrifugation was done at ≥6000 × g (≥8000 rpm) for 1 minute for elution. The quality and concentration of DNA were determined using 2 µl of the diluted DNA sample on 1% agarose gel. DNA's quantity was measured with a Nanodrop spectrophotometer (2000/2000c, Thermo Scientific Inc.).

2.3. Polymerase Chain Reaction (PCR) Amplification

PCR was evaluated using a 25 µl cocktail made up of genomic DNA—100 ng, 10× PCR buffer—2.5 µl, 50 mM $MgCl_2$—1 µl, 2.5 mM dNTPs—2 µl, Taq polymerase—0.1 µl, DMSO—1 µl, forward and reverse primer—1 µl each and H_2O—11.3 µl. The gene region targeted and primer sequenced used are shown in Table 2. Amplification was performed using touch-down PCR as follows: an initial denaturation step of 5 mins at 94˚C, followed by nine cycles, each consisting of a denaturation step of 20 sec at 94˚C, an annealing step of 30 sec at 65˚C, and an extension step of 72˚C for 45 sec, this is followed by another 30 cycles each consisting of a denaturation step of 20 sec at 94˚C, annealing step of 30 sec at 55˚C, and an extension step of 72˚C for 45 sec. All amplification reactions were conducted in a GeneAmp PCR (Applied Biosystems). PCR amplicons were loaded on 1.5% agarose gel and ran at 100 volts for 2 hours.

2.4. Sequencing

The amplified products were used to select the amplicons with a single band and were purified according to the company's protocol (Cat. No.28106, QIAquick PCR Purification Kit). Afterwards, sequencing was executed using a sequencing kit (BigDye terminator cycle, Applied BioSystems). With ethanol EDTA solution,

Table 2. Gene region targeted and primer sequences used.

Gene and Region	Name of Primer	Primer Sequence 5' - 3'	Direction	Reference	Expected band size (bp)
rbcL	rbcLaf	ATGTCACCACAAACAGAGACTAAAGC	F	Levin *et al.* [28] modified from Soltis *et al.* [29]	750
	rbcLarev	GTAAAATCAAGTCCACCRCG	R	Kress and Erickson [30] modified from Fofana *et al.* [31]	

unincorporated dye terminators were cleaned and precipitated. Furthermore, the pellets were dissolved in HiDi formaldehyde buffer (Cat No. 4311320, Applied Biosystems). Finally, a Genetic Analyser (3130xl, Applied Biosystems) was used to complete the sequencing, and the obtained sequence trace files were uploaded to the open-access DNA Subway software, following the guidelines reported by Goff *et al*. [32] and Merchant *et al*. [33]. The DNA sequences were analysed on the Blue Line of the DNA Subway.

The sequences were trimmed and viewed, and the forward and reverse sequences were manually edited and then paired to construct a consensus sequence. In case(s) where the forward and reverse sequence could not be merged to generate a consensus sequence, only the readable barcode sequence was utilised in the subsequent analysis due to the poor quality. The consensus sequences were exported for downstream analysis on NCBI-GenBank [34] and BOLD [35]. The samples were recognised based on the maximum percentage identity score. In cases where NCBI GenBank and BOLD searches returned identical identifications, the samples were considered at least to the genus level. The generated sequence barcodes were translated to amino acid sequences using the EMBOSS Transeq web tool of the European Molecular Biology Laboratory's European Bioinformatics Institute (EMBL-EBI) to determine their suitable open reading frame. The sequences were later tendered to NCBI-GenBank, and their accession numbers were obtained, as shown in **Table 3**.

3. Results

This study tested the effectiveness of DNA barcoding to authenticate sampled CITES-listed land plants in Nigeria. Following the Nigerian plants CITES list created by the Barcode of Wildlife Nigeria-Plant Working Group in 2014, nineteen (19) plants in twelve (12) families belonging to Annonaceae and Apocynaceae families were sampled (**Table 1**). Extraction of DNA from all the samples was followed by PCR amplification of the *rbcL* barcode region. All the samples were successfully amplified. The PCR products, which were bidirectionally sequenced, produced a good sequence of at least 500 bp in length for both directions, with fewer nucleotide ambiguities. However, sample (S7) yielded a high-quality sequence for a single sequencing read (forward), sufficient to identify the sample with a 99.81% identity match on NCBI-GenBank and BOLD.

GenBank and BOLD comparison authenticated the identity of all the plant samples. Although DNA Subway's local nucleotide database allows a rapid search, it has limitations [36]. Based on this fact, GenBank and BOLD were employed in identifying the samples, of which 57.89% were identified at the species level, and 42.11% were identified only at the genus level. The top BLAST result was evaluated with the highest percent identity score for all sequences. In the case of a tie between two identity scores, the lowest e-value was used as a deciding factor. If there were identical e-values and identity scores for multiple species, the individuals were only identified to the genus level to avoid taxonomic misidentification.

Table 3. Blast output of the generated *rbcL* sequences of the sampled species.

Sample number	Taxonomic ID	GenBank ID	Percentage identity	BOLD ID	Percentage Identity	Accession number
S1	*Funtumia elastica*	*Funtumia elastic*	99.82	*Funtumia elastica*	100	MT385762
S2	*Ficus exasperate*	*Ficus hirta*	99.83	Ficus carica	99.83	MT385771
S3	*Kigelia Africana*	*Kigelia africana*	99.46	*Kigelia africana*	99.47	MT385758
S4	*Diospyros mespiliformis*	*Diospyros mespiliformis*	100	*Diospyros melanoxylon*	100	MT385755
S5	*Lophira lanceolata*	*Lophira lanceolata*	100	*Lophira lanceolata*	100	MT385763
S6	*Aframomum septrum*	Aframomum angustifolium	99.09	Aframomum sp.	99.63	MT385764
S7	*Afzelia Africana*	*Afzelia africana*	99.81	*Afzelia africana*	99.81	MT385760
S8	*Annona senegalensis*	*Annona senegalensis*	99.47	*Annona senegalensis*	99.46	MT385759
S9	*Annona muricate*	*Annona muricata*	99.24	*Annona muricata*	99.81	MT385765
S10	*Albizia zygia*	Albizia chevalieri	100	*Albizia lebbeck*	98.67	MT385766
S11	*Gongronema latifolium*	*Gongronema latifolium*	100	*Gongronema latifolium*	100	MF162300
S12	*Funtumia africana*	*Funtumia elastica*	99.44	*Funtumia elastica*	99.63	MT385760
S13	*Garcinia kola*	*Garcinia sp*	99.5	*Garcinia sp*	100	MT385770
S14	*Lophira alata*	*Lophira lanceolata*	100	*Lophira lanceolata*	100	MT385756
S15	*Khaya ivorensis*	*Khaya madagascariensis*	99.53	*Khaya nyasica*	99.35	MT385766
S16	*Diospyros piscatoria*	*Diospyros gabunensis*	98.99	*Diospyros gabunensis*	98.99	MT385757
S17	*Monodora tenuifolia*	*Monodora tenuifolia*	99.82	*Monodora tenuifolia*	99.82	MT385768
S18	*Momordica charantia*	*Momordica charantia*	100	*Momordica charantia*	100	MT385772
S19	*Entandrophragma angolense*	*Entandrophragma caudatum*	99.83	*Swietenia macrophylla*	98.5	MT385769

4. Discussion

Although no single barcode region has been reported to be suitable for accurate discrimination of all plant taxa [37], the results of this study found the *rbcL* single barcode as an adequate estimation of species' identification of most of the sampled tree plants. However, these findings would have to be tested with a

much more extensive sample collection. According to Yu *et al*. [17], although there have been remarkable developments in standardized genetic markers, DNA barcode markers are merged with other biotechnologies, such as spectrum technologies, to achieve improved characterisation outcomes.

One of the complicating factors and limitations in applying DNA barcoding in the fight against the illegal trade of CITES-listed plants is that most of the products are trafficked in parts (like timber) or processed forms, such as traditional herbal medicines composed of more than one ingredient. Such products may contain multiple plant species that can only be analysed accurately if several DNA barcode templates can be sequenced concurrently. Concurrent sequencing is presently effectively achieved by next-generation sequencing (NGS) technology. Coghlan *et al*. [38] highlighted the effectiveness of metabarcoding in their study of the CITES-listed species contained in diverse traditional Chinese medicine (TCM) samples formulated in capsules, powders, herbal tea and tablets. Their study revealed that some of the TCM products contained CITES-listed species, including Saiga antelope (*Saiga tatarica*) and the Asian black bear (*Ursus thibetanus*), as well as some non-listed ingredients and potentially harmful and allergic plants [39].

In conclusion, this study documented the CITES-listed plants and other essential plants endangered or threatened plants in Nigeria. *rbcL* gene was employed as a single barcode region in the identification/authentication of some of Nigeria's endangered forest species legislated under CITES. In addition, the study provided the first chloroplast DNA reference dataset to support the utilisation of DNA barcoding to identify CITES-listed plant species in Nigeria, which is significant for future studies.

Acknowledgements

We thank the Consortium for Barcode of Life (CBOL) for supporting Nigeria's Barcode of Wildlife Project; specifically, Dr David Schindel for effective coordination of the CITES project globally.

Data Accessibility Statement

All data generated during this study are contained in this article. Raw sequence reads are deposited in NCBI GenBank with the following accession numbers; MT385762, MT385771, MT385758, MT385755, MT385763, MT385764, MT385760, MT385759, MT385765, MT385766, MF162300, MT385760, MT385770, MT385756, MT385766, MT385757, MT385768, MT385772, MT385769.

Author Contributions

Christie Oby Onyia: Conceptualization and Principal investigator; Christie Oby Onyia and Obianuju Patience Ilo: Writing—original draft; Chosen Ekene Obih: Investigation and Data analysis; Omokafe Ugbogu and Emmanuel Chukwudi

Chukwuma: Fieldwork and Curation; Beatrice Onyinye Ojiego and Paschaleen Soromtochukwu Onyemachi: Research design and methodology; Sunkanmi Saheed Rufai: Fieldwork. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Govaerts, R. (2001) How Many Species of Seed Plants Are There? *International Association for Plant Taxonomy* (*IAPT*), **50**, 1085-1090. https://doi.org/10.2307/1224723

[2] Scotland, R.W. and Wortley, A.H. (2003) How Many Species of Seed Plants Are There? *International Association for Plant Taxonomy* (*IAPT*), **52**, 101-104. https://doi.org/10.2307/3647306

[3] Thorne, R.F. (2002) How Many Species of Seed Plants Are There. *International Association for Plant Taxonomy* (*IAPT*), **51**, 511-512. https://doi.org/10.2307/1554864

[4] Food and Agriculture Organization of the United Nations (2004) What Is Agrobiodiversity (Building on Gender, Agrobiodiversity and Local Knowledge, Issue). https://www.fao.org/3/y5609e/y5609e02.htm#TopOfPage

[5] Royal Botanic Gardens Kew (2010) More than One-Fifth of World's Plants Face Threat of Extinction, New Analysis Finds. https://www.sciencedaily.com/releases/2010/09/100928193452.htm

[6] Onyia, C.O., Obih, C.E., Ilo, O.P., Ojiego, B.O., Iwu, V.C., Saidu, Y., Solomon, B.O., Amasiorah, V.I., Rowaiye, A.B. and Joshua, K.G. (2019) A New Approach to Protection and Conservation of Cites-Listed Species DNA Barcoding of Parrots in Nigeria. *Journal of Biodiversity & Endangered Species*, **7**, 1-4.

[7] Sedghi, A. (2013) Red List 2013: Threatened Species across the Regions of the World. *The Guardian*. https://www.theguardian.com/news/datablog/2013/nov/26/iucn-red-list-threatened-species-by-country-statistics#data

[8] Nigeria Federal Environmental Protection Agency (1991) Guidelines and Standards for Environmental Pollution Control in Nigeria. Federal Environmental Protection Agency (FEPA). https://books.google.co.za/books?id=fy8SAQAAIAAJ

[9] Borokini, T.I. (2014) A Systematic Compilation of Endemic Flora in Nigeria for Conservation Management. *Journal of Threatened Taxa*, **6**, 6406-6426. https://doi.org/10.11609/JoTT.o4010.6406-26

[10] Federal Ministry of Environment (2015) National Biodiversity Strategy and Action Plan 2016-2020. https://www.cbd.int/doc/world/ng/ng-nbsap-v2-en.pdf

[11] Convention on Biological Diversity (2007) Minutes of the Meeting of the Bureau of the Conference of the Parties to the Convention on Biological Diversity Held in Montreal on 14 October 2007. https://www.cbd.int/doc/meetings/cop-bureau/cop-bur-2007/cop-bur-2007-10-14-en.pdf

[12] Mace, G.M. (2004) The Role of Taxonomy in Species Conservation. *Philosophical Transactions of the Royal Society B*: *Biological Sciences*, **359**, 711-719. https://doi.org/10.1098/rstb.2003.1454

[13] Wilson, E.O. (2017) Biodiversity Research Requires More Boots on the Ground.

Nature Ecology & Evolution, **1**, 1590-1591.
https://doi.org/10.1038/s41559-017-0360-y

[14] Convention on the International Trade in Endangered Species of Wild Fauna and Flora (2015) The CITES Appendices. https://cites.org/eng/app/index.php

[15] Barcode of Wildlife Project (2020) Enforcing Endangered Species Laws with DNA Barcodes. http://www.barcodeofwildlife.org

[16] Kress, W.J. (2017) Plant DNA Barcodes: Applications Today and in the Future. *Journal of Systematics and Evolution*, **55**, 291-307. https://doi.org/10.1111/jse.12254

[17] Yu, J., Wu, X., Liu, C., Newmaster, S., Ragupathy, S. and Kress, W.J. (2021) Progress in the Use of DNA Barcodes in the Identification and Classification of Medicinal Plants. *Ecotoxicology and Environmental Safety*, **208**, Article ID: 111691.
https://doi.org/10.1016/j.ecoenv.2020.111691

[18] de Vere, N., Rich, T.C.G., Trinder, S.A. and Long, C. (2015) DNA Barcoding for Plants. In: Batley, J., Ed., *Plant Genotyping*: *Methods and Protocols*, Springer, New York, 101-118. https://doi.org/10.1007/978-1-4939-1966-6_8

[19] Pathak, M.R., Mohamed, A.A.M. and Farooq, M. (2018) DNA Barcoding and Identification of Medicinal Plants in the Kingdom of Bahrain. *American Journal of Plant Sciences*, **9**, 2757-2774. https://doi.org/10.4236/ajps.2018.913200

[20] Lv, Y.-N., Yang, C.-Y., Shi, L.-C., Zhang, Z.-L., Xu, A.-S., Zhang, L.-X., Li, X.-L. and Li, H.-T. (2020) Identification of Medicinal Plants within the Apocynaceae Family Using ITS2 and psbA-trnH Barcodes. *Chinese Journal of Natural Medicines*, **18**, 594-605. https://doi.org/10.1016/S1875-5364(20)30071-6

[21] Onyia, C.O., Jideofor, O.P., Ojiego, B.O., Solomon, B.O., Ogundipe, O.T. and Ogbadu, L.J. (2014) Current Developments in the Application of DNA Barcoding to Solving Biodiversity Conservation Problems in Developing Countries. *Global Journal of Biology*, *Agriculture & Health Sciences*, **3**, 285-288.

[22] Habib, K.A., Neogi, A.K., Rahman, M., Oh, J., Lee, Y.H. and Kim, C.G. (2021) DNA Barcoding of Brackish and Marine Water Fishes and Shellfishes of Sundarbans, the World's Largest Mangrove Ecosystem. *PLOS ONE*, **16**, e0255110.
https://doi.org/10.1371/journal.pone.0255110

[23] Jones, L., Twyford, A.D., Ford, C.R., Rich, T.C.G., Davies, H., Forrest, L.L., Hart, M.L., McHaffie, H., Brown, M.R., Hollingsworth, P.M. and Vere, N. (2021) Barcode UK: A Complete DNA Barcoding Resource for the Flowering Plants and Conifers of the United Kingdom. *Molecular Ecology Resources*, **21**, 2050-2062.
https://doi.org/10.1111/1755-0998.13388

[24] Jamdade, R., Mosa, K.A., El-Keblawy, A., Al Shaer, K., Al Harthi, E., Al Sallani, M., Al Jasmi, M., Gairola, S., Shabana, H. and Mahmoud, T. (2022) DNA Barcodes for Accurate Identification of Selected Medicinal Plants (Caryophyllales): Toward Barcoding Flowering Plants of the United Arab Emirates. *Diversity*, **14**, 262.
https://doi.org/10.3390/d14040262

[25] Lone, S.A., Hassan, Q.P. and Gupta, S. (2019) Development of DNA Barcode for Rapid Identification of *Epimedium elatum* (Morren & Decne) from Northwestern Himalayas in India. *Journal of Applied Research on Medicinal and Aromatic Plants*, **13**, Article ID: 100205. https://doi.org/10.1016/j.jarmap.2019.100205

[26] Negi, R.K., Nautiyal, P., Bhatia, R. and Verma, R. (2021) rbcL, a Potential Candidate DNA Barcode Loci for Aconites: Conservation of Himalayan Aconites. *Molecular Biology Reports*, **48**, 6769-6777. https://doi.org/10.1007/s11033-021-06675-5

[27] Ho, V.T., Tran, T.K.P., Vu, T.T.T. and Widiarsih, S. (2021) Comparison of matK and rbcL DNA Barcodes for Genetic Classification of Jewel Orchid Accessions in

Vietnam. *Journal of Genetic Engineering and Biotechnology*, **19**, Article No. 93. https://doi.org/10.1186/s43141-021-00188-1

[28] Levin, R.A., Wagner, W.L., Hoch, P.C., Nepokroeff, M., Pires, J.C., Zimmer, E.A. and Sytsma, K.J. (2003) Family-Level Relationships of Onagraceae Based on Chloroplast rbcL and ndhF Data. *American Journal of Botany*, **90**, 107-115. https://doi.org/10.3732/ajb.90.1.107

[29] Soltis, P.S., Soltis, D.E. and Smiley, C.J. (1992) An rbcL Sequence from a Miocene Taxodium (Bald Cypress). *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 449-451. https://doi.org/10.1073/pnas.89.1.449

[30] Kress, W.J. and Erickson, D.L. (2007) A Two-Locus Global DNA Barcode for Land Plants: The Coding rbcL Gene Complements the Non-Coding trnH-psbA Spacer Region. *PLOS ONE*, **2**, e508. https://doi.org/10.1371/journal.pone.0000508

[31] Fofana, B., Harvengt, L., Baudoin, J.P. and Du Jardin, P. (1997) New Primers for the Polymerase Chain Amplification of cpDNA Intergenic Spacers in Phaseolus Phylogeny. *Belgian Journal of Botany*, **129**, 118-122. http://www.jstor.org/stable/20794388

[32] Goff, S.A., Vaughn, M., McKay, S., Lyons, E., Stapleton, A.E., Gessler, D., Matasci, N., Wang, L., Hanlon, M., Lenards, A., Muir, A., Merchant, N., Lowry, S., Mock, S., Helmke, M., Kubach, A., Narro, M., Hopkins, N., Micklos, D., Stanzione, D., *et al*. (2011) The iPlant Collaborative: Cyberinfrastructure for Plant Biology. *Frontiers in Plant Science*, **2**, Article No. 34. https://doi.org/10.3389/fpls.2011.00034

[33] Merchant, N., Lyons, E., Goff, S., Vaughn, M., Ware, D., Micklos, D. and Antin, P. (2016) The iPlant Collaborative: Cyberinfrastructure for Enabling Data to Discovery for the Life Sciences. *PLOS Biology*, **14**, e1002342. https://doi.org/10.1371/journal.pbio.1002342

[34] Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) Basic Local Alignment Search Tool. *Journal of Molecular Biology*, **215**, 403-410. https://doi.org/10.1016/S0022-2836(05)80360-2

[35] Ratnasingham, S. and Hebert, P.D. (2007) Bold: The Barcode of Life Data System. *Molecular Ecology Notes*, **7**, 355-364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

[36] Jensen-Vargas, E. and Marizzi, C. (2018) DNA Barcoding for Identification of Consumer-Relevant Fungi Sold in New York: A Powerful Tool for Citizen Scientists? *Foods*, **7**, Article No. 87. https://doi.org/10.3390/foods7060087

[37] Ballardini, M., Mercuri, A., Littardi, C., Abbas, S., Couderc, M., Ludena, B. and Pintaud, J.C. (2013) The Chloroplast DNA Locus psbZ-trnfM as a Potential Barcode Marker in Phoenix L. (Arecaceae). *Zookeys*, **365**, 71-82. https://doi.org/10.3897/zookeys.365.5725

[38] Coghlan, M.L., Haile, J., Houston, J., Murray, D.C., White, N.E., Moolhuijzen, P., Bellgard, M.I. and Bunce, M. (2012) Deep Sequencing of Plant and Animal DNA Contained within Traditional Chinese Medicines Reveals Legality Issues and Health Safety Concerns. *PLOS Genetics*, **8**, e1002657. https://doi.org/10.1371/journal.pgen.1002657

[39] Staats, M., Arulandhu, A.J., Gravendeel, B., Holst-Jensen, A., Scholtens, I., Peelen, T., Prins, T.W. and Kok, E. (2016) Advances in DNA Metabarcoding for Food and Wildlife Forensic Species Identification. *Analytical and Bioanalytical Chemistry*, **408**, 4615-4630. https://doi.org/10.1007/s00216-016-9595-8