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A New Approach to Protection and Conservation of Cites-Listed Species: DNA Barcoding of Parrots in Nigeria

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Abstract

Anthropogenic activities, coupled with climate change effects have led to biodiversity crisis and genetic erosion. The number of wildlife species being threatened and or endangered is on the increase. The Convention on International Trade on Endangered Fauna and Flora (CITES) has categorized these species under Appendices I, II, III, reflecting their level of endangerment as well as the protection level accorded each taxonomic group. DNA barcoding has over time, been identified as a tool for authenticating the taxonomic information of species at all stages of life, telling apart cryptic species, fighting fraud, poaching and prosecution of violators of CITES and for general conservation purposes. The national survey carried out to up-date the CITES list of Nigeria's endangered species revealed that some of the bird species such as the Hooded Vulture (*Necrosyrtes monachus*) and the grey parrot could hardly be found in the wild anymore. The International Union of Conservation of Nature (IUCN) in 2016, accorded the maximum level of protection to African Grey parrot by upgrading it to CITES Appendix I. This study, therefore, targeted CITES-listed parrot species that are fast disappearing through poaching and illegal trading belonging to the order-*Psittaciformes* and of genera-*Psittacula*, *Poicephalus* and *Psittacus* held in captivity in the orphanage of the National Parks Service Abuja, Nigeria. The aim of this initial project was to populate the GenBank with sequence libraries from bird species from this biogeographic region. Live birds were sampled and a set of primers-COI F and COI R tested were found effective in the amplification of the DNA of the samples. The primers were successfully used to amplify and sequence the genomic DNA, which sequences were deposited in the GenBank with their accession numbers obtained and published.

Keywords: *Psittacula; Poicephalus; Psittacus*; COI F; COI R

Background Information

DNA barcodes consist of a standardized short sequence of DNA between 400 and 800 bp long used for characterization and identification of all species. From results of several studies, the sequence diversity in a 648 bp region of the mitochondrial gene for cytochrome c oxidase I (COI) have proven to serve as a DNA barcode for the identification of animal species, including insects, fishes, big mammals and birds [1]. DNA barcoding has enabled scientists to readily and precisely recognize known species at all stages of life history, retrieve information about them and discover new species. More than one-third of the world's avian fauna (about 23,000 sequences from 3,800 species) were barcoded through the "All Birds Barcoding Initiative" (ABBI), and the initial North American avian species barcoding project [2]. Other groups have continued to study the avifauna species in their locations, thereby increasing the sequence libraries in GenBank [3,4].

Little work has been done in Africa, particularly in Nigeria with respect to DNA barcoding of her avian species, regardless of the fact that many of the species of birds are threatened, and some have gone into extinction. Some of the contributory factors to the dearth of data in generating genetic libraries of avifauna species are difficulties encountered in obtaining samples due to complete non-availability of frozen tissues or museum samples as is the case in other regions [5].

Nigeria possesses more than eight hundred and sixty-four (864) species of birds about 3% of these are either threatened or endangered [6]. Species in the CITES Appendix I list are prohibited for international trade on their specimen except for research purposes; although international trade may be authorized by the relevant authorities of the concerned countries by granting import and export permits. Species listed in CITES Appendix II are those not yet threatened with extinction but may soon become, if the trade is not checked.

The focus of this first report was on CITES-listed Avian species, belonging to the Order, Psittaciformes, hosted in the orphanage in National Park, Abuja (Figure 1).

Materials and Methods

Sample collection

Sampling was carried out in the orphanage located in the National Parks Services in Abuja, Nigeria (Figure 1) after the necessary permit was issued by the National Parks Services authority. The specimen was collected from live birds. Blood sampling from captive birds was laborious and stressful and sometimes the birds are sacrificed unintentionally.

Figure 1: Map of Nigeria showing geographical area (National Park Service) for collection of samples.

Figure 2: *Psittacula krameri.*

A blood sample was collected using a syringe, while skin and feather were taken using scalpels, and stored in the FluidX tubes containing EDTA. The samples were frozen at -20°C for further processing. It was difficult to obtain the desired high-quality digital photographs detailing important anatomical features due to the restiveness of the birds during sampling-the photographs of the sampled species are available in Figures 2-4. Details, such as the name of the sample, field number, GPS coordinates, sex, date, and name of collector for each voucher were recorded. Collection localities and other information about the specimens are available in Table 1 (Figure 2-4).

Genomic DNA isolation

Genomic DNA from the animal tissues was extracted using the Qiagen Dneasy Blood and Tissue Kit (cat. 69506), according to the manufacturer's instructions. DNA quality and concentration were checked by running 2 µL of the diluted DNA sample on 1% agarose gel. Accurate DNA quantification was carried out using a NANODROP®2000 spectrophotometer (Thermo Scientific Inc.).

Figure 3: *Psittacus erithacus.*

Figure 4: *Poicephalus senegalus.*

PCR Amplification and DNA Sequencing

PCR was carried out in a total volume of 25 µL containing 100 ng of genomic DNA, 2.5 µL of 10 PCR buffer, 1 µL of 50 mm $MgCl_2$, 2µL of 2.5 mm $dNTP_s$ (Thermo Scientific), 0.1 µL Taq polymerase (Thermo Scientific), 1 µL of DMSO, 1 µL each of forward and reverse primer (COI F: 5'-TTCTCGAACCAGAAAGACATTGGCAC-3' and COI R: 5'- ACTTCTGGGTGGCCAAAGAATCAGAA-3' [7] and 11.3 µL of H_2O. Touch-down PCR was used for amplification as follows: initial denaturation step of 5mins at 94°C, followed by 9 cycles each consisting of a denaturation step of 20 sec at 94°C, 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 performed in a GeneAmp® PCR System 9700, Applied Biosystems. PCR amplicons were loaded on 1.5% agarose gel and run at 100 volts for 2 hours.

Sample IDs	Scientific name	English name	Identifier	Collectors	Date and time of collection	Location
1_NABDA_MAMMALS_1016	*Psittacula krameria*	Rose-ringed Parakeet	Yohanna Saidu	Yohanna Saidu, Rowaiye Adekunle, Onyia Christie	27-11-2014 Morning	National Park Abuja, Nigeria
2_NABDA_MAMMALS_1046	*Psittacus erithacus*	Grey parrot	Yohanna Saidu	Yohanna Saidu, Rowaiye Adekunle	15-12-2014 Morning	National Park Abuja, Nigeria
3_NABDA_MAMMALS_1048	*Poicephalus senegalus*	Senegal Parrot	Yohanna Saidu	Yohanna Saidu, Rowaiye Adekunle	15-12-2014 Morning	National Park Abuja, Nigeria

Table 1: Sample collection information.

The amplicons with single band were selected from the amplified products and purified using manufacturer's protocol (QIAquick PCR Purification Kit, cat. No.28106). Sequencing was performed by using a Big Dye terminator cycle sequencing kit (Applied Bio Systems), Unincorporated dye terminators were then purified and precipitated using ethanol EDTA solution. The pellets were then re-dissolved in HiDiformamide buffer (Applied Biosystems Cat No. 4311320). Sequencing was performed using 3130xl Genetic Analyser.

Data Analysis

The sequencing results generated were uploaded in the blue line of DNA Subway (https://dnasubway.cyverse.org/) which is an intuitive interface for analyzing DNA barcodes. Using the Blue Line, the assembled sequences were end-trimmed, paired in their respective forward and reverse sequences to build consensus sequences. Sequence alignment and percentage similarity searches were compared with GenBank database using NCBI web-based site, BLAST.

Results

The output of the BLAST query of the sequences produced significant hits and all three sequences were identified (Table 2). The percentage identity ranged from 98%-100%, total bit score obtained in all ranged from 904-1079. The query coverage spanned between 95% and 100% while the e-value for all sequence was zero.

The generated sequences were submitted to GenBank and the following accession numbers were assigned: MH844578, MH882517 and MH844579.

Sample IDs	Hit in NCBI database	Total score	Query coverage	E-value	% Identity	Accession No
1_NABDA_MAMMALS_1016	*Psittacula krameria*	904	100	0	98.07	DQ433147
2_NABDA_MAMMALS_1046	*Psittacus erithacus*	1053	100	0	100	KF381364
3_NABDA_MAMMALS_1048	*Poicephalus senegalus*	1079	95	0	98.84	KF381366

Table 2: BLAST outputs of the total score, query coverage, e-value, percentage identity and accession number obtained from different parrots species.

Discussion

In this paper, the effectiveness of COI in discriminating the avian species grouped as Nigerian parrots was tested and found successful (Table 2). The utility of barcoding relies on the assumption that genetic variation within a species is much smaller than the variation between species [4]. This assumption was verified in previous studies [1,8-10]. To bring greater reliability to the identification of species using short DNA sequences, a move should be made to supplement the mtDNA-based barcode with nuclear barcodes [4]. This would reduce the problem of reliance on a single character and help identify cases where mtDNA behaves differently from the nuclear genome. The present study provides an initial set of COI barcodes for three African parrots species hosted in Nigeria national park. More detailed sampling of COI sequences is needed for these parrot species, and barcodes need to be gathered for other more threatened CITES-listed bird species from Nigeria and other countries in West African.

This paper advocates the employment of DNA barcoding as a tool that can be used by CITES focal point in State Parties, to curb poaching and illegal trade in endangered species, especially for those species which parts are used for medicine, ornament or sold as bushmeat [11]. In research conducted in Nigeria, it recorded that hooded vultures are the most heavily traded species of vulture across West and Central Africa because of the medicinal value of its parts [12]. DNA barcoding is a tool that can be used to curtail this illegal trade since it has the capacity to trace all stages of species, from the egg to adult and in whatever form (Intact, part or processed). Hence the need to populate the GenBank with genetic libraries of such indigenous species from Afrotropical realm. However, for CITES to use of DNA barcoding in prosecution, appropriate chain of custody must be instituted to ensure total flow of actions from origin of the species to the scene of crime, which may likely to be the courtroom [13] also important is the establishment of national biodiversity forensic laboratory by the appropriate authority.

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Availability of Data and Materials

All data generated during this study are included in this published article. Sequence data were deposited in NCBI GenBank with the following accession numbers MH844578, MH882517 and MH844579.

References

1. Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004a) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. Proc Natl Acad Sci 101: 14812-14817.
2. Lijtmaer DA, Kerr KCR, MY Stoeckle MY, Tubaro PL (2012) DNA barcoding birds: from field collection to data analysis. Methods Mol Biol 858: 127-152.
3. Bilgin R, Ebeoğlu N, İnak S, Kırpık MA, Horns JJ, et al. (2016) DNA Barcoding of birds at a migratory hotspot in eastern turkey highlights continental phylogeographic relationships. PloS ONE 11: e0154454.
4. Yoo HY, Eah J, Kim JS, Young JK, Min M, et al. (2006) DNA barcoding of Korean birds. Mol Cells 22: 323-327. BirdLife International (2019). Partnership for nature and people-data zone-country profile: Nigeria.
5. Stoeckle M, Winker K (2009) A global snapshot of avian tissue collections: state of the enterprise. The Auk 126: 684-687.
6. BirdLife International (2019) Partnership for nature and people-data zone-country profile: Nigeria.
7. Dove CJ, Rotzel NC, Heacker M, Weigt LA (2008) Using DNA barcodes to identify bird species involved in bird strikes. J Wildlife Manag 72: 1231-1236.
8. Hajibabaei M, Janzen DH, Burns JM, Hallwachs W, Hebert PDN (2006) DNA barcodes distinguish species of tropical Lepidoptera. Proc Natl Acad Sci 103: 968-971.
9. Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM (2004b) Identification of Birds through DNA barcodes. PloS Biol 2: e312.
10. Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN (2005) Barcoding Australia's fish species. Phil. Trans R Soc B 360: 1847-1857.
11. McKean S, Mander M, Diederichs N, Ntuli L, Mavundla, et al. (2013) The impact of traditional use on vultures in South Africa. Vulture News 65: 15-36.
12. Buij R, Nikolaus G, Whytock R, Ingram DJ, Ogada D (2015) Trade of threatened vultures and other raptors for fetish and bushmeat in West and Central Africa. Oryx 50: 606-616.
13. Narayanaroa CA(2016) Forensic science-chain of custody: A brief note.