Use of DNA barcoding to combat wildlife crime

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Background: Wildlife-based tourism contributed c. 6.0% to Kenya's GDP in 2017, a significant contribution of which is related to her rich biodiversity. This biodiversity is under threat from wildlife crime. Currently, Kenya is a leading transit country for illegal wildlife products from Africa. In the past, wildlife criminals thrived partially due to the miniscule penalties previously associated with such offenses. However, with the harsh penalties spelt out in the new Wildlife Conservation and Management Act 2013 (WCMA 2013), and to avoid detection, wildlife criminals now debone bush meat and deal with it as butchered and processed meat, trade with eggs or juvenile stages of animals that are difficult to identify to species, and crush ivory into powder, thus removing the diagnostic morphological characteristics that would associate wildlife products to a particular wildlife species. It therefore becomes impossible for taxonomic experts to identify with confidence impounded wildlife materials as belonging to a particular (endangered) species. The National Museums of Kenya (NMK) in collaboration with partners (see above), has been involved in the development of capacity, personnel training and construction of DNA barcode reference library. DNA barcoding is a technique that uses DNA of an organism to identify it as belonging to a particular genetics laboratory at NMK by equipping it to be the center for construction of DNA barcode reference library, 2) to expand (from the predecesor project BWPK) the barcode reference library of endangered wildlife involved in wildlife crime, 3) use this DNA reference library to solve problems of wildlife crime, and 4) initiate DNA barcoding process in Tazania.

Approach:

	300 ₇ 287	A B C D E F G H I J K L M N 1 processingLab sequencingLab extractionPlateID extractionBarcode extractionWell tissueBarcode tissueRackBarcode tissuePosition	150 160 170 180 190 CGGCCGGGTCGAAGAAGGTGGTGTTAAGGTTGCGGTCGGT
		4 NMK-MGL Macrogen EWM_NMK_001 SA00429939 1032549362 C01 FB01885677 EVM_001 SA00214665 D06 muscle Nitrogen liquid NMK:HERP:60077 NMK 5 NMK-MGL Macrogen EVM_NMK_001 SA00429939 1032549363 D01 FB01885678 EVM_001 SA00214665 E06 muscle Nitrogen liquid NMK:HERP:60078 NMK 6 NMK-MGL Macrogen EVM_NMK_001 SA00429939 1032549364 E01 FB01885679 EVM_001 SA00214665 F06 muscle Nitrogen liquid NMK:HERP:60079 NMK 7 NMK-MGL Macrogen EVM_NMK_001 SA00429939 1032549365 F01 FB01885679 EVM_001 SA00214665 F06 muscle Nitrogen liquid NMK:HERP:60079 NMK 7 NMK-MGL Macrogen EVM_NMK_001 SA004299395 F01 FB01885679 EVM_001 SA00214665 A07 muscle Nitrogen liquid NMK:HERP:60080 NMK 7	
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		12 NMK-MGL Macrogen EVMNMK_001 N00429930 100224937 Co2 Fb0188568 EVM01 N00214665 A08 muscle Nitrogen liquid NMK-HER*00086 NMK 13 NMK-MGL Macrogen EVMNMK_01 SA00429939 1032549371 Do2 FB01885686 EVM01 SA00214665 A08 muscle Nitrogen liquid NMK-HER*00086 NMK 14 NMK-MGL Macrogen EVMNMK_01 SA00429939 1032549372 E02 FB01885687 EVM01 SA00214665 B08 muscle Nitrogen liquid NMK-HER*00086 NMK 15 NMK-MGL Macrogen EVMNMK_01 SA00429939 1032549373 F02 FB01885688 EVM01 SA00214665 D08 muscle Nitrogen liquid NMK:HER*06088 NMK 15 NMK-MGL Macrogen EVMNMK 001 SA00429939 1032549373 F02 FB01885688 EVM001 SA00214665 D08 muscle Nitrogen liquid NMK FMK 16 NMK-MGL Macrogen EVMNMK SA00429939 1032549374	698_BirdR1.ab1 CGGCCGGGTCGAAGAAGGTGGTGGTGTTAAGGTTGCGGTCGGT
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Fig 1: Research methods used to collect animal samples in the field and process them in the lab: a and b) some of the methods of trapping animals, c) number of samples collected from different animal species and taxa, d and e) databases (FIMS and LIMS) respectively, used to capture the collected samples metadata as well as record the lab day-to-day activities, f, g) DNA barcode submitted to GenBank

Results/Achievements:

1. The project has increased the capacity of the molecular genetics lab through acquisition of 16 state of the art equipment that have made it the center for construction of DNA barcode reference library. As a result, the efficiency of the lab has increased tremendously. For instance, the thermal cycler acquired using USAID PEER funds has two separate plates that takes 64 and 32 samples respectively; both can run two different PCR protocols simultaneously. The increase of thermal cyclers from one to three has increased the capacity of the lab to accomplishing specific analyses in a short time. Additionally, with the new GEL documentation system, a need for a dark room has been eliminated; we can run, view and document gels in the same procedure room. The lab now observes international standard practice of using separate equipment for separate procedures.



Fig 2: Some of the equipment acquired by the USAID PEER project for the National Museums of Kenya's molecular genetics lab that have increased the efficiency of the lab, a) analytical balance for accurate measurements of chemicals, b) Gel doc for imaging and documentation of DNA and RNA suspended within polyacrylamide or agarose gels, c) Ice maker for making ice; ice condition is required for lab processes, d) water bath for use in the first stage of DNA extraction which involves shaking and digesting, e) Mupid gel electrophoresis, electrophoresis is a confirmatory procedure for every step in the lab analyses thus having a tank that could run multiple samples, f) Master cycler, which is able to set two PCR programmes

- 2. To expand the DNA barcode reference library, we have collected 287 animal samples (see Fig 1c) of six different taxonomic groups that are endangered, are in the CITES list and are involved in wildlife crime. These samples are at different stages of lab analyses (Fig 3a).
- 3. We have used the improved capacity of our lab to provide to our taxonomic expert at NMK, species identification and verification of impounded wildlife materials with limited diagnostic morphological characteristics (Fig 3b). KWS has afterwards used these exhibits in court.
- 4. The project has promoted strong partnerships between the different public and private institutions with different roles in the overall aim of fighting wildlife crime. This has had a positive impact in combating wildlife crime.
- 5. The PhD student in biotechnology (and also data analyst in this project) is at the tail end of field data collection on lovebirds. She is running the remaining data collection and lab analyses simultaneously (Fig 3c)
- 6. We have build capacity of 12 trainees from Tanzania on DNA chain analyses and informatics; post-training support is ongoing and trainees are preparing for proficiency test to be able to be qualified barcoding analysts (Fig 3 d).

Expected project impacts:

1. Provision of confident species identification for courtroom prosecution will lead to faster and increased number of successful prosecution and

conviction. This, together with the new WCMA 2013 which spells stiff penalties for dealing with endangered wildlife products, will ensure that cases related to wildlife crime are expedited, will provide deterrence to wildlife crime and would limit revenue streams to organized crime network usually associated with it . Previously court cases related to wildlife crime dragged in court for years due to contested species identification and lack of confidence evidence. So far, two successful convictions have been achieved using barcoding technology.

Fig. 3: a) number of samples from endangered animals of different taxonomic groups involved in wildlife crime in different stages of lab analyses and uploading to GenBank, b) some of the results of impounded wildlife materials with insufficient diagnostic morphological characteristics that provide challenge for confident species identification by the taxonomic expert at NMK submitted to our lab for verification/identification using DNA barcoding technology, c) the PhD student and data analyst in the USAID PEER project doing DNA lab analyses for her lovebirds project, d) training session by Kenyan DNA analysts to DNA barcoding trainees in Tanzania

- 2. Reduced wildlife crime will lead to reduced threat on endangered wildlife, leading to long-term increased population size of endangered/traded species with positive effects against wildlife crime in Kenya (and in Tanzania after the barcoding capacity has been fully established).
- 3. Tourism industry, a large proportion of which is dependent on Kenya's rich biodiversity, will flourish. This will lead to overall economic growth of the country, both from tourism income and from reduced incidences of terrorism attacks that usually negatively affect tourism and the entire economy. Terrorist network are usually linked to wildlife crime.

Challenges encountered:

- i. Small wild population of endangered species that makes it difficult to find enough (five) individuals of a species to collect samples from
- ii. Some species, e.g., Owls that are nocturnal, Turacos that are forest canopy species, are difficult to trap and require long time in the field and innovative ways to trap
- iii. Procurement challenges that delayed acquisition of equipment and lab supplies, eventual delayed lab analyses and delayed implementation of project activities
- iv. In Tanzania, challenges included withdrawal of trainees, trainees not meeting assignments deadlines, poor internet connectivity that affect communication and training progress
- v. challenges related to weather (too much rain that made some field sites inaccessible) and political climate (prolonged tension due to elections); both delayed field activities delayed project activities

