PROCEEDINGS OF THE DNA BARCORDING TO COMBAT WILDLIFE CRIME WORKSHOP
HELD AT ARUSHA INSTITUTE OF ACCOUNTANCY, ARUSHA
19TH MAY 2016.

A COLLABORATIVE PROJECT BETWEEN NATIONAL MUSEUMS OF KENYA, SOKOINE UNIVERSITY OF AGRICULTURE, KENYA WILDLIFE SERVICES AND TANZANIA WILDLIFE INSTITUTE

SUPPORTED BY USAID UNDER THE USAID-PEER PROJECT NAMED "DNA BARCODING TO COMBAT WILDLIFE CRIME"

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1.0 BACKGROUND INFORMATION

Poaching for both meat and trophy has always been a major challenge in conservation history. Illegal trade in wildlife and its products affect the survival of magnitude number of species. The population of rhinos and elephants for instance has declined in recent years as a result of escalation in organized trade in their products. This has necessitated many states to take active measures to protect their biodiversity in recent years.However, wildlife criminals (poachers and traffickers) continue to develop new ways to circumvent detection and prosecution. Crime investigators on the other hand fail to hold these criminals responsible with confidence due to lack of reliable forensic tools admissible in courts of law. The prosecutors try to prove that the suspects have committed crimes on wildlife but fail because criminals tried to remove overtindicative morphological features specific to poached animals.

Over the recent years, this illegal wildlife poaching has turned into being a highly profitable business worldwide with remarkably low risks as trials of illegal wildlife traffickers are rare, largely because law enforcement officers, prosecutors, and judicial systems typically consider such crime a low priority. Large volumes of wildlife including those already at risk are being illegally poached and traded and if this trend is unabated it threatens future survival of some key species in East Africa region and beyond. To overt these challenges scientists are racing in arms to develop highly sensitive, accurate and high throughput DNA based techniques to mitigate these challenges.

One of the leading examples of this development is the institution of a standardized global DNA-based barcode identification system which provides a simple, universal tool for the identification of wildlife species and their products.DNA barcoding has now become an accepted and commonly used method for species identification practiced by taxonomists, ecologists, forensic scientists and other researchers. A Google-supported Barcode of Wildlife Project (BWP) hosted by the Smithsonian Institution in Washington,successively initiated these initiatives in Kenya since 2012. Recently, BWP as expanded these training and technical assistance to new participants in Tanzania through the recently funded USAID-PEER project since 2015. The new participating institutions are Sokoine University of Agriculture (SUA) and Tanzania Wildlife Institute (TAWIRI)

2.0 INTRODUCTION

Sokoine University of Agriculture (SUA) in collaboration with Tanzania Wildlife Institute (TAWIRI) organized a one-daysensitization workshop on 19th May 2016at the Institute of AccountancyArushaon the development of DNA barcodes for wildlife species in Tanzania. This is one of a milestone step in the use of standardized DNA forensic tools in its fight against wildlife crimes in the country. This sensitization workshop which was the first of its kind, brought together 36participants among others included the top officials from various wildlife Institutions in the country which areWildlife Division (WD) representing the Ministry of Natural Resources and Tourism (NMRT), Tanzania Wildlife Authority (TAWA), Tanzania National Parks (TANAPA),Ngorongoro Conservation Area Authority (NCAA), Tanzania Wildlife Research Institute (TAWIRI) and Sokoine University of Agriculture (SUA). Four participants for collaborating partner institutions from Kenya, namely National Museums of Kenya (NMK) and Kenya Wildlife Service (KWS) wereinvited to provide the training support.

The workshop was sponsored by the USAID-PEER program under the NAS-Sub-Grant award Agreement no 2000006232 and Sponsor Grant Award number AID-OAA-A-11-00012 entitled "DNA Barcoding to Combat Wildlife Crime" This project is further aimed at forging a closer cooperation as well as strengthen respective national institutions actively involved in wildlife crime investigations and providing forensic services within the region.

3.0 OFFICIAL OPENING REMARKS

Participants were introduced to the workshop, the session led by the workshop's facilitator Dr. Victor Kakengi (Principal Research Officer, TAWIRI). Thereafter, the workshop was officially inaugurated at 9:30 am by the Director of Research Development and Coordination for TAWIRI, Dr. Julius D. Keyyu who was representing the Director General of TAWIRI. He started by thanking all participants for devoting their valuable time to attend the workshop. He thanked the Principal Investigator (PI) from SUA and all other partners from Tanzania and Kenya for all the initiatives laid for this project. He pointed out that the project is highly timely as the illegal wildlife activities are a huge problem affecting a multitude number of species in the country and the region at large, and this promises to bring effective tools to combat the problem on hand. He extended by saying that DNA barcoding is currently being used in identification of plants, insects, studying gastrointestinal tract (GIT) populations. This project is going to develop for the first time DNA barcodes for wildlife species as a new forensic tool in fighting against wildlife crime in the country.He also pointed out that this project would help in equipping our forensic labs with new DNA tools as one step into solving the present and future challenges in wildlife sector in Tanzania.

4.0 PRESENTATIONS

The DNA barcoding project workshop had several presentations but the first presentation by the project PI, Prof. B. M. Mutayobawas used to guide the theme of the workshop. The synopsis of all the presentations and have been summarized below.

4.1.0. Session I Presentations Chaired by Prof. J. Kideghesho (SUA)

4.1.1. First Presentation

Forensic Science in Illegal Trade on Wildlife Products: Tanzania Experience- By Prof. B.M. Mutayoba, SUA

This presentation aimed at introducing and giving the background information about the project. The presenter started by explaining that the project is funded by USAID under the USAID-PEER program in collaboration with Kenya counterparts. To new beginners in forensics, he highlighted the principles of forensic science and as use of DNA (genomic and mitochondrial) in molecular forensics. He pointed out their roles in the fight against wildlife crime through identification, conviction and exoneration of suspects and convicts. He insisted on the importance of maintaining the chain of

custody, which involves proper recording of evidence from the time of discovery (at the crime scene), collections, storage, analysis, and transfer of evidence to the court.

Using genomic DNA, 16 elephant specific microsatellites have been developed and used to put in place anAfrican DNA reference library for *Loxodontaafricana* (both in forest and savannah elephants) with 2,000 unique reference samples from 81 locations in Africa. This was the result of B.M. Mutayoba's previous research work on wildlife forensics with Prof S.K. Wasser of the University of Washington, US, the tool extensively used internationallytodateto trace the source of origin of large seizures of ivory on transit from Africa to far East, Europe and other countries.

Mitochondrial DNA (MitDNA)on the other hand, has previously been used in Tanzania specifically at SUA for speciation of comprehended wildlife products submitted by investigative agencies within the wildlife sector using DNA markers targeting the D-loop and 16S genes.The use of cytochrome C oxidase subunit I (COI) as standardbarcoding system and speciation in animalsfor forensic analysis is thus a new tool at SUA and Tanzania in general. SUA has since year 2000, continued to serve as the only wildlife forensic center in the country but the evidences submitted for analysis have remained minimal compared to our Kenya counterparts.

There are several challenges facing the Tanzania wildlifesector that the country needs to address in order to put in place a unified standardized forensic capacity capable of mitigating the escalating level of wildlife poaching and trafficking seen over the recent years. Wildlife policing continues to be centralized within separate management institutions unlike our Kenya neighbors. The existing laws and policies are still weak and do not provide for harmonization of DNA forensic capacities. Moreover, systems of licensing and establishment of certified forensic centers are still missing and there is still very low level of understanding on matters relating to wildlife evidence handling, analysis and submission in courts. More importantly, there are poor internally funding initiatives to support DNA forensics development in order to reduce financial dependence to external donors. However, SUA, has since 2008 introduced a90 hrs Forensic Sciences course at undergraduate and postgraduate levels which continues to be improved and some of the graduates have alreadysecured forensic-related employments in Government Chemistry labs, police and wildlife institutions.

4.1.2. Second Presentation

Use of DNA Barcoding to Combat Wildlife Crime: Project overview by Beatrice Khayota, NMK

Wildlife criminals continue to invent new ways to avoid detection/prosecution such as trading or trafficking species as Butchered and processed meat products, eggs or juvenile stages, dried powders and other forms difficult to identify using morphological features even for taxonomic experts. DNA barcode provide handy technique to identify and assign materials to species.

The USAID-PEER project in Kenya is aimed at re-establishing the molecular biology laboratory at the National Museums of Kenya (NMK) by equipping it to be the centre for construction of the reference barcode library, expanding the barcode reference library of endangered and illegally-traded species, Initiating DNA barcode process in Tanzania and using DNA barcode library to assign impounded material to their species of origin to enhance prosecution of wildlife crime.

National Museums of Kenya (NMK) is the overall project coordinator. It has capacities for taxonomic expertise for species collection and identification, archival for voucher specimens and tissues, running /maintain the molecular lab facility for DNA analysis, DNA barcoding and bioinformatics expertise. The NMK is also a capacity building and support enforcement agency in Kenya.

USAID-PEER project has 6 other collaborating partners namely Kenya Wildlife Service (KWS, International Centre for Insect Physiology and Ecology (ICIPE), International Livestock Research institute (ILRI), Kenya Forest Service (KFS), Consortium for the Barcode of Life (CBOL), Sokoine University of Agriculture (SUA) and Tanzania Wildlife Research Institute (TAWIRI)

KWS is an expertise and lead agency for enforcement & prosecution in Kenya. It issues permits to conservation areas for sample/specimen collection and leads in immobilization and sample collection from large mammals. ICIPE on the other hand will be involved in the analysis using high resolution melting analyses (HRMA), sharing tissue samples and compliment bioinformatics expertise with NMK.ILRI will serve as home for sequencing services for Kenya partners. The other partner, KFS will serve to issue relevant permits for collection of proposed specimen for individual projects.CBOL will continue to provide technical support for laboratory processing of samples, data management and analysis, provide clarification on legal standards for use of DNA barcode for investigation and prosecution. CBOL will continue to raise funds for lab equipment and provide technical advice on selection, installation & calibration of equipment/instruments

SUA will provide a lead for activities to be done in Tanzania, organize outreach and workshop in Tanzania for government, enforcement agencies, CITES authorities, research institutions. It will lead in required training on DNA chain analysis while TAWIRI will serve as a key collaborating partner to SUA.

4.1.3. Third Presentation

*DNA barcoding data standards*by Mwaura, A, Ndithia, H. and Mwangi, E. (MNK)

Kenya is partner country to Barcode of Wildlife Data Standards (WBP**)** which creates high-quality public reference database of standardized DNA barcodes. In collaboration with Kenya, the USAID-PEER projects will build the same library in Tanzania using SUA and TAWIRI as project partners. The main purpose is to enhance easy and timely identification of wildlife crime exhibits aiding faster prosecution of wildlife crimes in the region. Standard Operating Procedures (SOPs) for sample collection developed by NMK taxa experts will be developed in Tanzania for library creation in line with their forensic expectations. Analytical standards developed by the Consortium for Barcode of Life (CBOL) will be used.

The collectors will be trained on expected meta data standards, identification of specimen by taxa experts and field data entry standards will be demonstrated using demo for collectors, using permanent label markers for labeling in the field. Tissue for DNA barcoding in the field will be preserved by various techniques including freezing, fast drying, and storage in liquid media (ie vapour phase liquid nitrogen (VPLN) using dry –shippers of cryotanks. Attempts to do DNA isolation in the field will reduce the above logistics. DNA tissue collection procedures following standard procedures might vary among different species of animals.

4.1.4. Fourth Presentation

*Creation of a Reference Library For Wildlife Crime Enforcement by*Mwaura, A, Ndithia, H. and Mwangi, E. (MNK)

Requirements for DNA reference library database of DNA sequences involves, the generation of national priority species list, train laboratory analysts in sample collection, laboratory analysis, sequence analysis and GenBank upload.

(i) Generation of a priority species list is done by taxonomists. The species to appear in the priority species list should be among the threatened - illegal trade or other anthropogenic factors and of economic importance.

(ii) Training of Molecular analysts in DNA bacording project requires a person with prior molecular biology background and experience in sequencing data analysis.

(iii) Sample collectionfor every species: A minimum of 5 individuals should be sampled. The 5 individuals should represent different geographical localities (5 voucher/e-voucher specimens represent one species).The tissue samples collected should then be sub-sampled to bar-coded tubes for archival. This information together with DNA - aliquoted to barcoded tubes for archival should be fed into the FieldInformation Management System (FIMS) and uploaded to a database. The extraction process should be uploaded in the Laboratory Information Management System.

(iv) For the amplification of the DNA extracted, AccuPower RT PreMix from Bioneeris currently being used at the NMK. DNA barcoding primers target the Cytochrome C Oxidase Subunit I on the mitochondrial gene. The process should also be uploaded in the Laboratory Information Management System (LIMS) database.

(v) DNA amplicons should be purified before sequencing. The purification should be done following the manufacturer's protocol of the kit identified (i.e. GeneJet by Thermo Scientific).

(vi) The sequencing facility sends back ABI files that are assembled for analysis

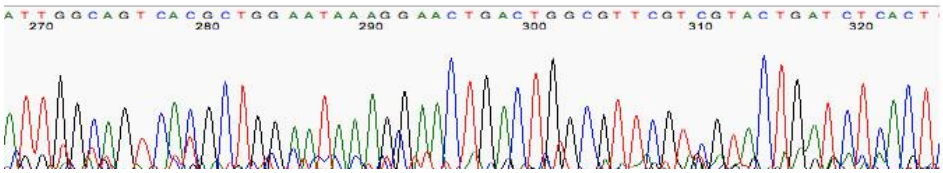

Fig 1. *ab 1 file*

(vii) Sequence analysis; Upload traces (ABI files) to the LIMS database for archival; In order to analyze the traces, download the traces from LIMS.Assemble the contig.Trim the contigs using the uploaded primers, Edit the sequences, Check the sequence quality with TranslatorX, MultiAlign.

(viii) GenBank Submission;Mark the sequences as pass or fail, annotate the sequences with LIMS/FIMS data, Download the GenBank Submission Plugin, Upload the

sequences to Genbank through the plugin, the sequences are marked with a "Barcode" tag in the GenBank database. The database is freely accessible.

4.1.5. Fifth Presentation

Wildlife Forensics and Law Enforcement, Kenya Experience byAntoinette Aluoch/Moses Otiende (KWS).

Illegal trade on wildlife and wildlife products is a major challenge for wildlife conservation in Kenya. Fighting wildlife crime using all available tools is necessary in preventing extinction of key endangered species and promoting biodiversity. In the past, Kenya Wildlife Service (KWS) has arrested and prosecuted many wildlife offenders and have lost cases due to inadequate scientific evidence to secure convictions.

The newly established wildlife forensic laboratory at KWS employs DNA barcode tools to identify and compare evidence from wildlife products. This is a method that is certified as accurate and has been tested in many other scientific settings and agreed upon as the best method for species identification. This is because it does not depend on morphology and can be used to analyze suspected wildlife exhibits including deboned/cooked/processed meat, horns, ivory, hides, etc. Priority species that are highly traded and critically endangered in Kenya have been barcoded and a reference library created. Recovered sequences from exhibits are screened against barcode records and report generated for onward transmission to court.

In the one-year it has been in operation, the laboratory has received and processed different exhibits mostly those involved in the bush meat trade and successfully identified them as belonging to wildlife species. This has enhanced prosecution and conviction of poachers and those involved in the illegal trade. Since inception, the laboratory has received 150 exhibits from poaching cases from different areas of Kenya. About 98% of the exhibits brought to the lab have been identified as having been obtained illegally from wildlife, both terrestrial and marine animals (including five Sea Turtles - Critically Endangered). To date we have successfully convicted five bush meat cases with the rest pending before the various courts awaiting determination.

4.1.6. Sixth Presentation

Wildlife DNA Barcoding Project in Tanzania: Dr Ernest MlingoEblate, TAWIRI

Tanzania is a key player in the contemporary illegal wildlife trade. The country transformed into a global conservation leader after resolving its poaching crisis in the 1980s. Over the past 30 years however, all indicators that raised the alarm in the 1980s have made a disturbing reappearance. For example, African elephant populations in Tanzania have declined 50% from 2010 levels; statistics for other animals are similarly grim. Additionally, there are currently zero white rhinos in Tanzania, reflecting a legacy of overexploitation and recent poaching crisis perpetuated by the illegal trade in rhino horn. Similarly, black rhino populations in Tanzania are estimated at 113 animals, and the African lion population has dropped 60% since 2005. Poaching for bushmeat has similarly escalated during the same period, threatening the survival of most large herbivores. Poachers are responsible for up to 90% of elephant deaths in Tanzania. When authorities do make a seizure the culprits are not

always caught and the judicial process does not always lead to successful prosecution. The prosecutors try to prove that the suspects have committed crimes on wildlife but fail because no morphological features available. The wildlife DNA Barcode Project is aimed to help solve these problems by providing enforcement officials with a reliable DNA-information on species identification and associate criminal to the crime scene.

Specific issues to be addressed in Tanzania by the project include (i) Establishing of DNA barcoding data standards and creation of reference library for wildlife crime enforcement (ii) Establishing the DNA Barcodes for endangered and most hunted wildlife species in National Parks, Game Reserves, Ngorongoro Conservation Area, Forest reserves, holding facilities, Confiscated wildlife materials. (iii) In collaboration with NMK and CBOL establish a public Barcode Database reference that will be used to aid the prosecution of illegal wildlife perpetuators in courts (iv) Training enforcement officials and other appropriate people on principles of chain of custody.

4.2 Session II:General DiscussionChaired by Prof. NdibalemaVedasto (SUA)

This section had three topics suggested for discussion namely (i) DNA barcoding, forensics and law enforcement,(ii) harmonization and involvement of key players in wildlife sector in Tanzania and (iii) policy issues regarding application of DNA barcoding in wildlife crimes in Tanzania. Due to the intertwining nature of the above topics, all these topics were discussed together and the following were observed and agreed.

(i) As wildlife DNA barcodes were observed to provide one of the most sensitive and standardized tools in the accurate assignment of the species of origin of wildlifeproducts including meat, bone, skins, etc, Tanzania government through its wildlife institutions should enumerate Kenya NMK and KWS examples to support the development and use of these tools in its wildlife legal system.

(ii) While the project will continue to establish these DNA barcodes for Tanzania wildlife species, the Ministry of Natural Resources and Tourism through its capacity will be required to support this project and start working on the modalities of their adoption in their prosecution cases

(iii) Adoption of DNA barcodes in wildlife prosecutions in Tanzania will undoubtedly require institutionalization of legally accepted standardized framework across all wildlife institutions (TANAPA, NCAA, WD-TAWA) on matters related to evidence collection, processing, storage, transport, analysis and submission in court.

(iv) Though DNA is the most powerful tool in forensics to date, it however poses a potential of being manipulated easily. In that regard, the use of DNA in legal matters will requirethe establishment within the wildlife legal framework, an institution mandated to license labs, handling forensic DNA matters and all matters related to sample collection, confidentiality in analysis, and handling of matters related offences and penalties in DNA evidence abuses.

5.0 CONCLUSIONS AND WAY FORWARD

Participants noted that with the current level of poaching escalating across all major wildlife protected areas, there is a need for the government to take a leap in supporting the individuals and institutions involved in developing DNA forensic tools for this country. The wildlife institutions including the WD and TAWA should help to put into place the necessary framework required for their fast uptake in

wildlife legal setup. The Zanzibar Wildlife Institutions should also be taken on board on issues related to changes in the existing wildlife Act if necessary to accommodate these developments. The participants representing the Wildlife Division promised to start working on these recommendations.

6.0 CROSSING REMARKS

-By Dr Nebbo J. Mwina (Assistant Director- Training, Research and Statistics, WD, MNRT)

The workshop was officially closed at 15:00 hrs by Dr NebboMwina, Assistant Director-Training, Research and Statistics, Wildlife Division. In her remarks, she thanked the USAID through the USAID-PEER project for sponsoring the workshop and supporting the DNA barcoding project in Tanzania. She congratulated and appreciated the work done by the researchers both in Tanzania and Kenya on their effort to conserve the country's biodiversity. She thanked TAWIRI/SUA for organizing this successful and eye opener workshop to Tanzania wildlife stakeholders who attended the workshop. Indeed, she emphasized the need for Tanzania to start moving faster in supporting the institutionalization of the upcoming DNA capacities in its wildlife legal framework. She also thanked the Kenya invitees for putting up enumerative presentations. Lastly, she promised to make a close follow-up on all issues requiring immediate ministerial and governmental interventions.

7.0 APPENDICES

7.1 APPENDIX I: DNA Barcoding to combat Wildlife Crime Workshop Timetable

Moderator – Dr. Kakengi Victor, TAWIRI

Time	Event	Responsible
8.30 –9.00 am	Arriving to the Venue and Registration	All participants
9.00 – 9.20	Communication from the Chair and self introductions	All
9.20 – 9.30	Welcome remarks	DG - TAWIRI
9.30 – 9.40	Remarks from Project Funding Agency	USAID Representative
9.40 – 10.30	Group Photo and TEA break	All participants
Session I	Presentations – Chair Prof. Kideghesho (SUA) Rapatours – ChumaIdrissa and Shedrack Kitimu	Chairperson
10.30 – 10.50	Forensic science in illegal trade on wildlife products: Tanzania experience	B.M Mutayoba - SUA
10.50 – 11.10	DNA Barcoding Project overview	Beatrice Khayota – NMK
11.10 – 11.30	DNA barcoding data standards	Ann Mwaura / Esther Mwangi /RugaMwangi- NMK
11.30 – 11.50	DNA barcoding and creation of reference library for wildlife crime enforcement	Ann Mwaura / Esther Mwangi- NMK
11.50 – 12.10	Wildlife Forensics and law enforcement: Kenya experience	Antoinette Aluoch/Moses Otiende - KWS
12.10 – 12.30	Overview of the Wildlife Barcoding Project in Tanzania	Ernest Eblate - TAWIRI
12.30-1.00	Plenary session	All presenters
1.00-2.00	Lunch	
Session II	General Discussions – Chair Prof. NdibalemaVedasto (SUA) Rapatours – IddiLipende and Edson Kinimi	Chair
2.00 – 4.00	Suggested Topics 1. DNA BARCODING, Forensics and Law Enforcement 2. Harmonization and Involvement of key players in wildlife sector in Tanzania 3. Policy issues regarding application of DNA BARCODING in Wildlife crimes	All
4.00-4.15	Closing Remarks	TAWA / Wildlife DIVISION

7.2 APPENDIX II: List of participants

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7.3 Photo illustrations during the workshop