CONVENTION ON INTERNATIONAL TRADE IN ENDANGERED SPECIES
OF WILD FAUNA AND FLORA

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LOXODONTA LOCALIZER: USING MITOCHONDRIAL DNA AS A TOOL FOR CITES ENFORCEMENT

1. This document has been submitted by Gabon, in relation to agenda item 69.1 on *Implementation of Resolution Conf. 10.10 (Rev. CoP17)* on Trade in elephant specimens, and agenda item 69.5 on *Closure of domestic ivory markets*.*

2. Methods for the determination of the geographic origin of ivory are necessary to control illegal poaching and smuggling. A DNA-based method to assign the provenance of ivory may be especially useful as a forensics tool.

3. The methods described in this document resulted from the scientific study '*Loxodonta* Localizer: a software tool for inferring the provenance of African elephants and their ivory using mitochondrial DNA' in Review.

* *The geographical designations employed in this document do not imply the expression of any opinion whatsoever on the part of the CITES Secretariat (or the United Nations Environment Programme) concerning the legal status of any country, territory, or area, or concerning the delimitation of its frontiers or boundaries. The responsibility for the contents of the document rests exclusively with its author.*

An enforcement tool for CITES

1. The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) encourages the forensic analysis of seizures of elephant ivory to help establish the geographic source of the ivory [1, 2]. A web-based software tool called the *Loxodonta* Localizer has been developed that uses African elephant DNA sequences to ascertain the geographic source of batches of ivory [3]. The web page includes a number of features that render it useful for examining confiscated ivory.

2. The database of the *Loxodonta* Localizer stores information on DNA sequences from 1,917 African elephant individuals across 24 range states [3]. The same sequenced region of DNA from newly confiscated ivory is used as a query. The *Loxodonta* Localizer compares the query to all of the stored sequences and provides a listing and map of matching sequences and the geographic regions from which they have been reported. Forensics laboratories in range, transit and destination countries can thereby readily ascertain the origin of ivory confiscated within their national borders. This precludes the necessity to further transport the confiscated ivory across international borders.

3. Analysis of the confiscated ivory can be conducted using laboratory methods that allow for quick turnaround times. The short mitochondrial (mt) DNA sequences that form the basis of the *Loxodonta* Localizer can be obtained quickly, because the methods used for extraction, amplification, sequencing, and sequence assembly are straightforward and well established in molecular laboratories. Typically, the time from the start of laboratory analysis to obtaining the sequence results has been *about one week*, and a report showing the provenance of ivory can be prepared for quick dissemination to source countries within the following days [3]. Rapid assessment of the geographic provenance of seized ivory is key to enabling law enforcement agencies to work collaboratively with colleagues from other countries of interest in relation to a particular seizure, and therefore help tackle the transnational nature of this illegal trade.

4. No prior information is needed regarding the potential source population of the ivory. After DNA samples of ivory are sequenced and submitted to the *Loxodonta* Localizer webpage, the software generates a map of Africa showing geographic locations from which identical or similar sequences have been reported. The map shows the localities with the closest matching sequences, and also displays all other geographic localities present in the database. The software thus provides an assessment of the locations in Africa from which elephants with identical or similar sequences to the ivory sequences have been reported.

5. In many cases, a sequence has been previously reported from reported from elephants in a single locality, region, or country, or from geographically adjacent countries. Such sequences with geographically restricted distributions would be the most helpful for ascertaining the provenance of a seizure of ivory. In many seizures, some tusks would have these informative DNA sequences. By focusing on these sequences with geographically restricted distributions, the *Loxodonta* Localizer allows for ascertainment of the regions or localities where elephants are being poached. The software would therefore be useful for quickly identifying elephant populations being newly or most targeted by poachers [3].

6. In order to add to the sequences of known provenance in the *Loxodonta* Localizer database, samples of elephants from each country can be collected in a non-invasive manner. Because mtDNA sequences can be obtained from dung, disturbance of living elephants can be avoided. Dung sampling would enable many laboratories to generate intensive and extensive surveys of African elephant mtDNA sequences, adding to the accuracy and precision of the *Loxodonta* Localizer.

7. The method encourages capacity building in African elephant range countries and in countries that serve as transit points or are the destination for smuggled ivory. The mtDNA sequences of elephants can be readily compared across laboratories, allowing for results to be combined across scientific studies. Thus collaboration across laboratories and countries would be enabled. Researchers within each African elephant range state could potentially collect samples locally, then extract, amplify and sequence mtDNA from elephants within their country. Surveys can be independently published by range state researchers before the sequences are incorporated into the *Loxodonta* Localizer.

A reference database of DNA sequences

8. The *Loxodonta* Localizer home page is shown in Figure 1 [3]. The home page links to detailed laboratory procedures on how to extract DNA from ivory, how to generate sequences from the extracted DNA, and how to trim the sequences to the appropriate length in order to query the software. As part of the queries to the software, additional information can be entered along with the sequence. This additional information may include descriptions of the samples, sample numbers, or other identifying information. This additional information will be reproduced by the *Loxodonta* Localizer on the output page, allowing the information describing the query sequence to be saved or printed with the output. Once the query sequence is submitted, the program will generate a map and a listing showing the closest matches to the query among the sequences included in the *Loxodonta* Localizer database.

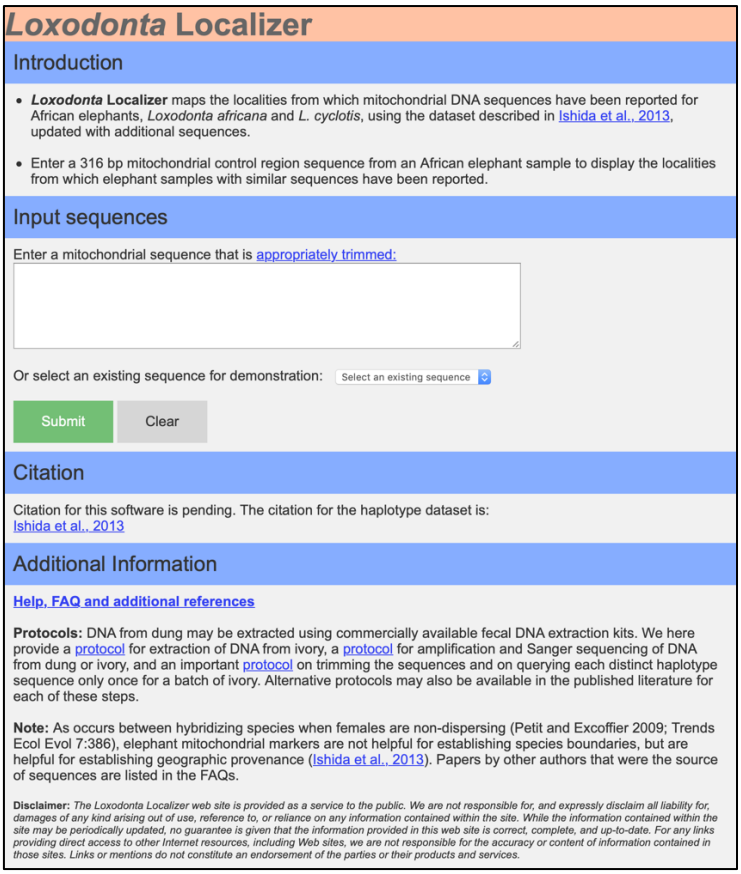

Fig 1. The *Loxodonta* Localizer home page.

9. The *Loxodonta* Localizer generates an output map that shows all of sub-Saharan Africa (Figure 2) [3], and thus includes all of the African elephant range states [4]. At location(s) at which the DNA sequences most closely matching the query have been reported, pins are shown on the map. The map also includes rectangles at all of the location for which sequences have been previously generated for African

elephants. Clicking on any of the map locations will provide information on the sequence(s) at each location that most closely match the query sequence.

10. A listing to the right of the map reiterates the query sequence and related information that was entered in the submission page, thus providing this information on the same page as the output, useful for forensic purposes. A timestamp that lists the date and time that the output was generated, and an alignment to the closest matching sequence are also shown. The reiterated query sequence and associated information, timestamp, map and listing may be saved or printed for each query.

11. The listing to the right of the map also shows database sequences that are exact matches to the query first, then shows database sequences with one mismatch to the query, then those with two mismatches, and so on. Every sequence stored in the database is shown in order of similarity to the query sequence. Additional information on each of the sequences in the database is also provided, to allow forensic scientists to readily access additional information within each peer-reviewed journal article that has reported each of the sequences.

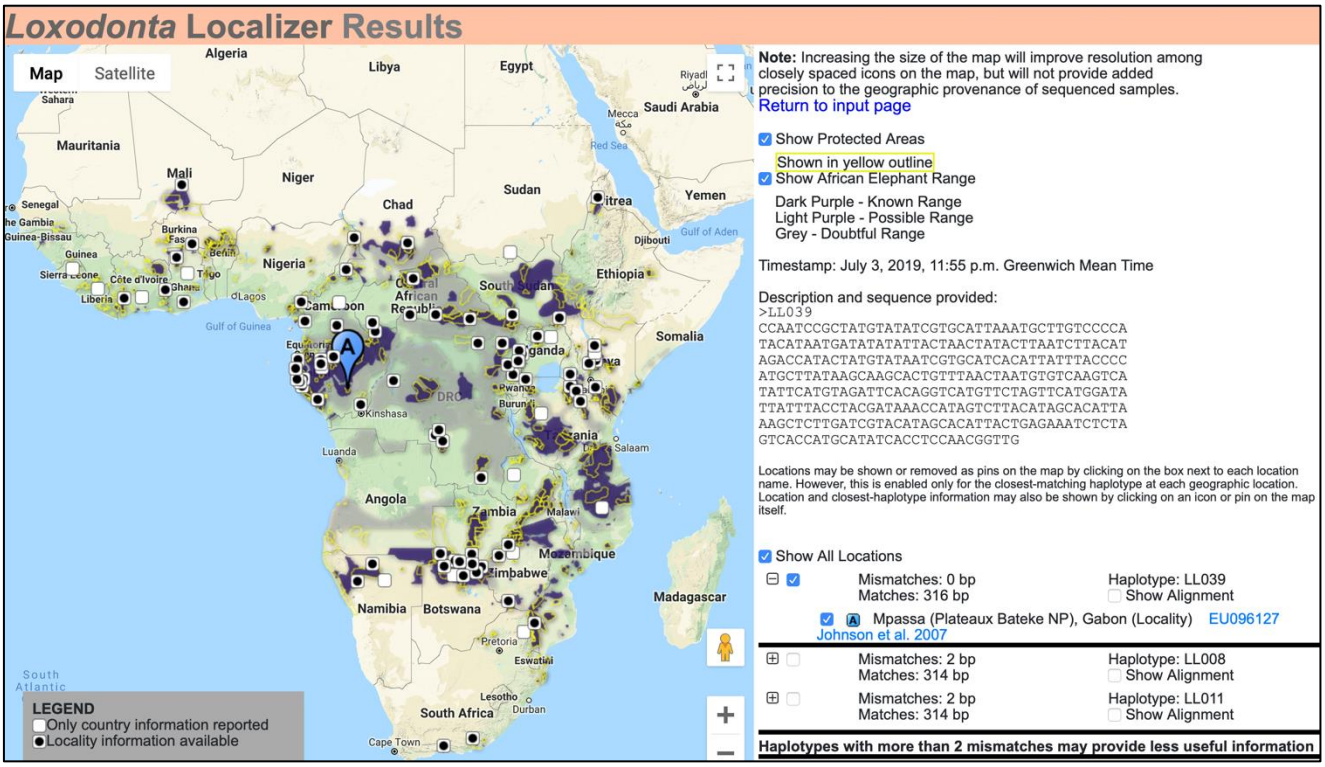

Fig 2. The *Loxodonta* Localizer output map and listing.

The utility of the *Loxodonta* Localizer for establishing the provenance of ivory

12. When the geographic distributions of sequences of African elephant mtDNA have been examined, most distinct sequences were found to have been reported from a single country or from geographically adjacent countries [5]. Thus most of the variations in mtDNA sequences are geographically localized [5]. The overall utility of mtDNA sequences for establishing the provenance of elephants is shown in Figure 3. This analysis of sequences (now present in the *Loxodonta* Localizer database) was conducted using

information from elephants from 81 localities in 22 countries, which were found to carry 101 distinct sequences (many elephants may share the same sequence). In this analysis, 62% of the distinct sequences were detected only within a single country (Fig. 3) [5]. This limited geographic distribution of most sequences indicated that mtDNA sequences would provide a useful tool for examining the provenance of seized ivory [5]. This information is incorporated in the map and listing that form the output page of the *Loxodonta* Localizer. Additional sequences from more elephants, and from additional range state countries, are being continuously added to the database.

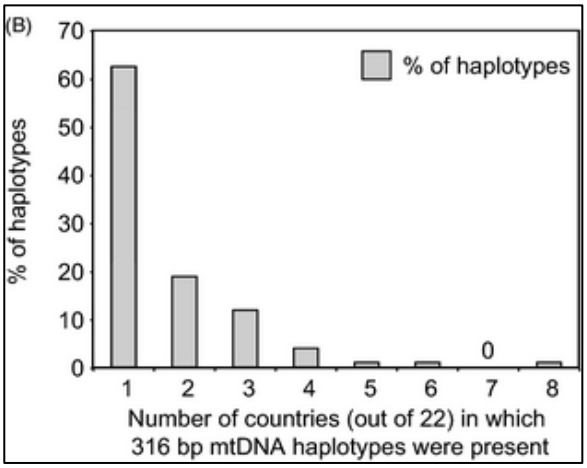

Fig 3. The geographic distribution across countries of 101 distinct DNA sequences ("haplotypes") in African elephants

13. Most useful for establishing the provenance of ivory are the mtDNA sequences that have only been reported from a small number of adjacent countries, or from a single country, or even from only a single locality within a country. When DNA from a batch of confiscated ivory is sequenced, the samples that have sequences of geographically limited distribution would be the especially helpful for assessment of provenance. The sequences from the samples can be grouped as two sets, one for more narrowly distributed and the other for more widespread sequences. Then the set of sequences that is geographically narrowly distributed can be examined to more precisely ascertain the geographic provenance of the sequenced ivory.

14. The LL could be used to examine and compare sequences within or between seizures for further insights into the origins of the ivory. Within a seizure, one may examine whether the geographic distributions of sequences are consistent with a common provenance for the tusks. If two different seizures of ivory do not overlap in mtDNA sequences, this would establish that the two sets of ivory are most likely to have different geographic origins. When two seizures of ivory show no overlap in mtDNA sequences, this would also preclude the necessity for further analyzing the two seizures to determine whether two tusks in the different shipments were from the same individual elephant.

15. Even if some sequences are shared between ivory seizures, one could separate the sequences into two categories: those with a geographically widespread distribution, and those with a geographically limited distribution. Comparing, between seizures, only those sequences that have a geographically limited distribution would also give an indication as to whether the ivory in each seizure may have originated in the same geographic region.

16. If two different ivory seizures both had tusks with the same rare mitochondrial sequence, the two tusks could be specifically targeted for further DNA analysis to determine whether they are from the same individual. The *Loxodonta* Localizer would facilitate an initial screening of ivory to determine which tusks, found in two different seizures of ivory, may potentially represent the same individual (right and left tusks from the same elephant). Additional laboratory analyses (using other genetic markers) could then be conducted to determine whether the two tusks were from the same individual. If tusks from the same individual are present in two different seizures of ivory, the two seizures can be linked to the same smuggling network.

17. The sequences from seized ivory could be compared to sequences obtained from carcasses in the field. Should an uncommon sequence be shared between a confiscated tusk and a carcass, this would suggest whether further laboratory analyses (using other genetic markers) may be worth conducting to establish that the confiscated tusk originated in a carcass found in a particular country, potentially useful for prosecutions in the source country.

18. Overall, the *Loxodonta* Localizer enables the origin of confiscated ivory to be examined within days, using broadly available DNA-based methods. It empowers a large number of forensics laboratories world-wide to conduct molecular analyses of confiscated ivory. This software tool allows rapid identification of potential regions or localities from which elephants are being poached, enabling conservation measures to be implemented in populations newly or consistently targeted by poachers.

References

[1] CITES, *SC69 Doc. 51.1: Elephant Conservation, Illegal Killing and Ivory Trade*. 2017, Geneva: Convention on International Trade in Endangered Species of Wild Fauna and Flora.

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[5] Ishida, Y., N.J. Georgiadis, T. Hondo, and A.L. Roca, *Triangulating the provenance of African elephants using mitochondrial DNA.* Evol Appl, 2013. **6**(2): p. 253-65.