

Lion Localizer: a software tool for inferring the provenance of lions (*Panthera leo*) using mitochondrial DNA

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Abstract

The illegal poaching of lions for their body parts poses a severe threat to lion populations across Africa. Poaching accounts for 35% of all human-caused lion deaths, with 51% attributed to retaliatory killings following livestock predation. In nearly half of the retaliatory killings, lion body parts are removed, suggesting that high demand for lion body parts may fuel killings attributed to human-lion conflict. Trafficked items are often confiscated in transit or destination countries far from their country of origin. DNA from lion parts may in some cases be the only available means for examining their geographic origins. In this paper, we present the Lion Localizer, a full-stack software tool that houses a comprehensive database of lion mitochondrial DNA (mtDNA) sequences sourced from previously published studies. The database covers 146 localities from across the African continent and India, providing information on the potential provenance of seized lion body parts. Lion mtDNA sequences of 350 bp or 1140 bp corresponding to the cytochrome b region can be generated from lion products and queried against the Lion Localizer database. Using the query sequence, the Lion Localizer generates a listing of exact or partial matches, which are displayed on an interactive map of Africa. This allows for the rapid identification of potential regions and localities where lions have been or are presently being targeted by poachers. By examining the potential provenance of lion samples, the Lion Localizer serves as a valuable resource in the fight against lion poaching. The software is available at <https://lionlocalizer.org>.

Keywords: cytochrome b, forensics, haplotype, poaching, lions, wildlife trafficking

Introduction

In the last 50 years, the number of lions (*Panthera leo*) has decreased by 75%, with their range declining by at least 71% (Riggio et al. 2013; Loveridge et al. 2022). This alarming downward trend is attributed to several factors, including poaching, habitat destruction, and prey depletion (Bauer et al. 2016; Loveridge et al. 2022). Currently, only an estimated 23,000-39,000 lions remain across Africa (Riggio et al. 2013; Bauer et al. 2016; Loveridge et al. 2022). The illegal poaching of lions for their body parts poses a severe threat to lion populations across Africa as it accounts for 35% of all human-caused lion deaths (Everatt et al. 2019). Moreover, the retaliatory killing of lions in response to livestock loss is responsible for 51% of human-caused lion deaths (Everatt et al. 2019), with lion body parts removed in 48% of these killings, indicating that the demand for lion body parts could also be encouraging the conflict-related killing of lions (Everatt et al. 2019).

There are concerns that an expanding trade in lion body parts, both domestically within African countries and internationally, could lead to unsustainable mortality rates and endanger the survival of lions (IUCN 2006a, b; Riggio et al. 2013; Bauer et al. 2016; Everatt et al. 2019). For example, the Wildlife Trade and Information System (WiTIS) database maintained by TRAFFIC records 57 seizures of lion body parts reported from Tanzania between 2010 and 2021, while 18 seizures were reported from Mozambique between 2010 and 2021, with teeth and claws being the most commonly confiscated lion products (Mole & Newton 2021). Lion body parts are often trafficked for medicinal purposes (IUCN 2006a, b; Riggio et al. 2013). The full scope of the illegal international trade of lion products and its effects on lion populations remains uncertain (Williams et al. 2015, 2017). Therefore, establishing tools that would help identify the provenance of confiscated lion products would be useful for understanding the geographic extent of illegal trafficking of the species.

The use of mitochondrial DNA (mtDNA) may be helpful for inferring the provenance of confiscated wildlife body parts (Ishida et al. 2013; Zhao et al. 2019). Because mtDNA is only transmitted maternally, it can be particularly useful for establishing geographic provenance for animal species in which female dispersal is much lower than male dispersal, as is the case for lions (Awise 1995; Elliot et al. 2014a; Funston 2011; Hanby & Bygott 1987; Ishida et al. 2013; Pusey 1987). Lion prides usually consist of many related females and their dependent offspring, as well as adult males that have dispersed from other prides (Bertram 1975; Mosser & Packer 2009; Packer & Pusey 1982; Sagamiko et al. 2015). Male lions disperse over long distances of 120-200 kilometers (Elliot et al. 2014b; Funston 2011; Morandin et al. 2014). In contrast, female lions typically remain within their natal range and rarely disperse unless recruited into another pride (Funston 2011; Hanby & Bygott 1987; Spong & Creel 2001; Vanderwaal et al. 2009).

To enable the use of mitochondrial DNA for examining the provenance of confiscated lion products, we here report development of the Lion Localizer (<https://lionlocalizer.org>), which is an interactive software that utilizes a database of lion mtDNA sequences compiled from previously published studies. Distinct mtDNA haplotypes may be limited to certain geographic regions, and can thus provide insights into the potential geographic origins of confiscated lion products. The Lion Localizer may be used to assist law enforcement and forensic laboratories in examining the geographic provenance of seized lion body parts. The Lion Localizer therefore has the potential to be a valuable resource for combating lion poaching, by rapidly identifying populations that are newly targeted, or that are being targeted most aggressively by poachers.

Methods

Overview of the Lion Localizer

The Lion Localizer utilizes user-submitted queries to compare against lion haplotypes stored in a database. All identical sequences are stored as a single unique haplotype in the database. The Lion Localizer compares the query sequence to the stored haplotypes, and arranges the stored haplotypes in a table in descending order of the number of mismatches to the queried sequence. The geographic locations from which each haplotype has been reported are also stored in the database. A map centered on the African continent shows all localities from which lion sequences have been reported, and specifically highlights localities from which sequences exactly matching the query have been reported. The query sequence and associated user-defined information are also populated to the output page, which can be printed or saved by the user. However, it is important to note that the user-entered sequences and associated information are not saved in any way, to preserve confidentiality for users of the software. The initial design of the Lion Localizer took inspiration from the *Loxodonta* Localizer, which is an application that helps deduce the provenance of African elephants using their mitochondrial DNA haplotypes (Zhao et al. 2019). However, the software code for the Lion Localizer was developed completely independently. The Lion Localizer software is hosted at <https://lionlocalizer.org>, and the source code is accessible at <https://github.com/wesleyau/LionLocalizer>.

Programming of the Lion Localizer

The Lion Localizer is a user-friendly application developed with advanced technologies for both backend and frontend (full stack) development. The Django REST Framework (DRF) version 4.0.6 (Django Software Foundation 2018), a powerful and widely used framework, was utilized to develop the backend, while ReactJS version 18.2.0 (ReactJS

2013), an efficient and robust JavaScript library, was used for frontend development. The combination of React for frontend development and Django for backend development is a popular web development stack, offering a reliable and scalable solution for building modern web applications. The Lion Localizer database was built using SQLite utilizing the Django Object-Relational-Model (ORM) to store previously reported lion haplotypes as well as information about their corresponding GenBank accession numbers, published journal articles that reported the sequences, and the sample collection localities. To enable users to submit query sequences (<https://lionlocalizer.org/query>) for comparison with haplotypes in the database, the Lion Localizer employs Redux, a state management library for JavaScript applications (Redux 2015). The entity-relationship diagram and the information stored in the database tables used for the Lion Localizer are depicted in Supplementary Figure S1.

Publications reporting sequences contained in the Lion Localizer Database

The database of the Lion Localizer consists of previously published sequences of the mitochondrial gene *MT-CYB* (henceforth referred to as “cytochrome b” or “cyt b”) from the currently available literature. The cyt b haplotypes of 1140 bp and all related information stored in the database (depicted in Supplementary Figure S1) were compiled from previously generated and published sequences for the species *Panthera leo*. We considered whether to incorporate the control region in addition to cyt b; however, this was ruled out for two reasons. First, the control region, being prone to indels (insertions and/or deletions), could vary in length, and this variability might affect the alignments used for query comparison. Second, many published sequences of lion mitochondrial DNA do not include the control region. Thus, the use of control region sequences in the database would have reduced the total number of haplotypes and localities represented.

The *cyt b* sequences included in the database originate from a number of published sources; in all cases the sequences reported had to span the full 1140 bp *cyt b* region to be included in the database. Dubach and colleagues in 2005 published the sequences of 26 lions from 11 countries (AF384809-AF384818) (Dubach et al. 2005); Bertola and colleagues in 2011 sequenced 53 lions from 15 countries (GU131164–GU131185, AY781195–AY781210 and DQ018993–DQ018996) (Bertola et al. 2011); Dubach and colleagues in 2013 sequenced 75 lions from 8 different countries (KC495048-KC495058) (J. M. Dubach et al. 2013); Bertola and colleagues in 2015 sequenced 48 lions from 7 different countries (Bertola et al. 2015); and Barnett and colleagues sequenced 14 museum-preserved lions (MG792275-MG792277) (Barnett et al. 2018). These *cyt b* sequences from previous studies were filtered to consolidate duplicate sequences into a single haplotype within the Lion Localizer, as well as to ensure appropriate length (1140 bp), and to remove sequences with unknown or ambiguous sites (e.g., sequences were excluded if they contained “N” at one or more positions). After filtering, the Lion Localizer database currently contains 21 distinct haplotypes from 146 localities in 24 countries. Eleven (52%) of the haplotypes have been reported from lions from only one country (Supplementary Figure S2). The 146 localities include 12 of the “localities” consisting of only country names, i.e., there were lion specimens for which only the country of origin was available, and for which more precise collection information was not available. The other 134 localities correspond to lion specimens for which additional geographic information within a country was also available. The Lion Localizer provides the options to query this database using either the full 1140 bp *cyt b* sequence, or alternatively, laboratories have the option of using a 350 bp region within this sequence (**Figure 1**). This shorter 350 bp sequence will provide lower geographic resolution as it is less able to distinguish between some haplotypes.

Testing and Validation

To ensure the accuracy and reliability of the Lion Localizer database, rigorous and systematic testing was conducted, consisting of three sets of quality control assessments. The first involved checking the localities of the haplotypes included in the lion database that are shown on the output map (**Figure 2**) for accuracy. Every locality icon on the Google map on the Lion Localizer page had its resolution enhanced until the location names could be seen, and the location names were then confirmed to be accurate. If a location name was not found on the Google map, potential locations and GPS coordinates provided from the published papers were verified (using www.latlong.net). The localities on the map were also matched with the locations noted in the published papers from which sequences were obtained for the Lion Localizer database. This ensured that every locality on the Google map in the output page was referred to by at least one of the published papers. The locations listed in each published article were also double-checked, to verify that they appeared and were positioned properly on the Lion Localizer map, in order to avoid any potential error due to multiple places having the same name.

A second quality control assessment validated that the listing generated by the Lion Localizer for each haplotype was correct. The demonstration option in the query page was used to test each haplotype in the Lion Localizer database. The table listing of exact matches for each of the haplotypes was compared to the icons on the map to make sure that the locations in the table and in the map were the same. Each haplotype in the database was queried in GenBank using BLASTn (<https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Nucleotides>) (Altschul et al. 1990) to show that the output results for the Lion Localizer and those in GenBank matched exactly.

The third quality control assessment involved going through each publication that deposited sequences in GenBank that were used to make the Lion Localizer database. All lion

sequences mentioned in each paper were verified to be part of the Lion Localizer database (or to have been excluded due to one of the filters noted above). The relevant GenBank sequences, corresponding to each published paper, were trimmed and used to query the Lion Localizer for the 1140 bp and 350 bp regions that can be used as queries. The Lion Localizer results were compared to the information provided in the original papers for each haplotype sequence. This verified that the localities that the Lion Localizer shows for each paper for each haplotype, both on the output table and on the output map, all matched what had been reported in the original published papers.

Results (Including Software Description)

Query page and output page for the Lion Localizer

The Lion Localizer query page can be found at <https://lionlocalizer.org/query>. The query page includes two textboxes. One allows for entry of an optional id/description, which can include any information that the user considers relevant for identifying the source of the queried sequence. The second textbox allows entry of a user-generated 1140 bp or a 350 bp cytochrome b lion sequence, which comprises the query sequence. The sequence must be trimmed to the correct size and at the correct positions. Alternatively, as a demonstration sequence, one of the 1140 bp or 350 bp sequences stored in the database may be selected from a drop-down menu, which the user may use to examine the functionality of the Lion Localizer or to obtain a sequence of the correct length for use in trimming user-generated sequences. If a demonstration sequence is selected, the haplotype number and sequence will be automatically prefilled (**Figure 1**).

After the user submits their generated cyt b sequence (or a demonstration sequence), the Lion Localizer generates an output page that displays three sections: the query information, a table, and a map (**Figure 2**). To ensure a complete chain of custody, all query

information, including user-entered information that identifies the sample, as well as the mtDNA sequence, are populated to the output page by the Lion Localizer, which also shows the date and time that the output was generated (in Greenwich Mean Time). Beneath the query information, the output page shows a table that lists all of the haplotypes within the Lion Localizer database, with the haplotypes that most closely match the query sequence listed first, and all other haplotypes listed in decreasing order of match to the query sequence.

If the haplotype was reported from multiple localities, then the haplotype number repeats as a separate row for each of the localities. Additional information is listed next to each haplotype number on the table, including the number of bp mismatches to the query, number of bp matches, the country, the geographic locality (if any) within the country, the publication reporting the information for the haplotype, and GenBank accession number(s) for the previously reported sequences (<https://www.ncbi.nlm.nih.gov/genbank/>).

To the left of the output page, a Google map (<https://www.google.com/maps>) is displayed that is centered on the African continent (although the database also includes sequences of Asiatic lions). If sequences with zero mismatches to the query sequence were reported, a lion head icon is used to highlight the locality. The remaining localities from which lion sequences have been reported but did not exactly match the query sequence are indicated on the map using pins. Red pins indicate localities with some degree of geographic precision within-country. The location of lion sequences for which only country information is available are shown on the map using black pins. Clicking on any pin icon will open an information box that displays information for the haplotype at that location that most closely matches the query sequence that has the least number of mismatches (**Figure 3**).

Additional information accessible via the Lion Localizer

From any of the pages of the Lion Localizer platform, several tabs can be accessed by the user for pages with additional information. There is a page providing detailed instructions for using the Lion Localizer, and a dedicated page for laboratory protocols (<https://lionlocalizer.org/protocol>). The protocol provides guidance on generating lion mitochondrial DNA sequences (DNA extraction, amplification, Sanger-sequencing, trimming, and additional information). For trimming, we used a partial lion mitochondrial genome reference sequence, specifically targeting the cyt b region spanning positions 1-1140 out of a total length of 1461 base pairs. The reference, accession KJ652247.1, is available at [https://www.ncbi.nlm.nih.gov/nucleotide/KJ652247.1?report=genbank&log\\$=nuclalign&blast_rank=1&RID=D603T28F013](https://www.ncbi.nlm.nih.gov/nucleotide/KJ652247.1?report=genbank&log$=nuclalign&blast_rank=1&RID=D603T28F013). Other pages are designed to answer frequently asked questions (FAQs), outline the terms & conditions along with copyright details, and provide a list of scientific references that reported the sequences incorporated into the database. Additionally, a contact form is available for users to communicate suggestions or report any issues encountered with the software. Users can print the output page if they want to preserve the query information, table listing, and output map, as none of this information is stored by the Lion Localizer.

Discussion

We have developed the Lion Localizer software, which can be utilized to examine the geographic origins of lions by comparing their cyt b sequences to those reported in previously published studies (Barnett et al. 2018; Bertola et al. 2011, 2015, 2016; Dubach et al. 2005; J. M. Dubach et al. 2013). This method offers a number of advantages, including simplicity and speed for examining the provenance of confiscated lion products by using lion mitochondrial DNA. Notably, its use of mitochondrial DNA instead of nuclear DNA can be easier and

instrumental in establishing geographic provenance for animal species where female dispersal is considerably lower than male dispersal, such as lions (Awise 1995; Elliot et al. 2014a; Funston 2011; Hanby & Bygott 1987; Ishida et al. 2013; Pusey 1987). The Lion Localizer may be particularly informative in estimating the likely origins of confiscated body parts for lion haplotypes reported from only a single locality, a single country, or from a small number of neighboring nations. For example, Haplotype LEO0001 has only been reported once in the published literature, and only from Niokolo-Koba National Park, Senegal (**Figure 2**) (Bertola et al. 2015). Of the 21 haplotypes in the Lion Localizer database (each representing a distinct cyt b sequence), 11 (52%) have been reported from only one country (**Supplementary Figure S2**). In using the Lion Localizer, it may be useful to have the software show on the output map the geographic distribution of haplotypes with one or two mismatches to the query sequence, in order to assess the degree to which these similar haplotypes may cluster within a specific geographic area. The most reliable haplotypes for establishing provenance would be those with the most constrained geographic distribution, especially if such haplotypes are from a region that has been heavily sampled in terms of number of lions and numbers of localities.

There are a number of potential limitations to the Lion Localizer, some of which could be addressed with further improvements. For instance, while many mtDNA sequences have been reported from a specific country or even a single locality, a considerable number of the 1140 bp haplotypes within the Lion Localizer have been reported from widespread geographic locations (**Figure 3**). Such wide-ranging haplotypes only provide a general indication of potential areas of origin for a lion in Africa but can exclude other geographic regions as unlikely to be the source of a confiscated lion specimen. The database of the Lion Localizer currently encompasses all unique published cyt b sequences for lions based on the current available literatures (Barnett et al. 2018; Bertola et al. 2011, 2015, 2016; Dubach et

al. 2005; J. M. Dubach et al. 2013). The lions and localities in the database represent the minimum of localities from which a specific haplotype might have originated, with the recognition that further research and subsequent publications could reveal additional localities in which a given or new haplotype may also be present. The accuracy of the Lion Localizer may in the future be improved by increasing sample sizes, adding more localities from future published lion mtDNA sequences, and the use of longer mtDNA sequences. These longer sequences may contain more variants that distinguishes haplotypes that are currently considered identical. Likewise, sequences from additional lions and additional localities would enhance the precision of the Lion Localizer. Increasing geographic coverage and the addition of more lions and longer sequences may identify rare haplotypes with recent mutations limited to a particular geographic region. It should also be noted that Lion Localizer does not take account of historic translocations that have taken place, and these may have led to localized disruptions in the existing patterns of genetic variation (Bertola et al. 2022).

Species other than lions, notably those with low levels of female dispersal, could potentially benefit from the use of mtDNA as a marker to examine the origin of confiscated wildlife products (Awise, 1995; Ishida et al., 2013; Kumar et al., 2019; Meiklejohn et al., 2021; Poniente et al., 2022). Several applications have already been developed for a number of taxa, e.g., to examine the geographic origin of African elephants (genus *Loxodonta*; <https://www.loxodontalocalizer.org>) (Ishida et al. 2013; Zhao et al. 2019) and to distinguish among taxa across a number of vertebrate groups (<https://dna-surveillance.auckland.ac.nz/>) (Ross et al. 2003). Similar software for other taxa would require construction of different databases and require intense quality control to identify the geographic distributions of unique haplotypes. The entity-relationship diagram for the Lion Localizer is provided as

Supplementary Figure S1, together with the table relationships and information included in the database tables, as a potential guide in the creation of such software.

The Lion Localizer represents an important advance in the utilization of DNA sequences for conservation and forensic purposes. By permitting examination of the geographic origins of confiscated lion body parts through the analysis of mtDNA haplotypes, it may offer insights helpful to law enforcement, wildlife forensics, and conservation efforts. The Lion Localizer offers ease of use, high speed, and the capacity to easily compare mtDNA sequencing results previously generated by different laboratories, aggregating them across studies to provide easy access to users of the software. Training in the Lion Localizer protocol has already been successfully completed in South Africa, Zambia, and Zimbabwe. Moving forward, increasing the number of lions and localities examined, and the lengths of mtDNA sequences generated, may further expand the potential of the Lion Localizer, and increase its impact on the conservation of lions.

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Lion Localizer

QUERY INSTRUCTIONS LABORATORY PROTOCOLS FAQ REFERENCES

Introduction

- Lion Localizer enables users to query an unknown mitochondrial cytochrome b (cytB) against a dataset of known geographic localities from which cytB sequences have been reported for lions, *Panthera leo*, using the dataset described in Bertola et al. 2016
- The outputs include a map showing the localities from which the cytB sequences have been reported
- In the box(es) below you may enter one long cytB sequence, or one short cytB sequence, as described [here](#)
- The description (optional) and sequence of the lion sample will be populated to the output page, but [neither are saved](#) by the lion localizer software

Input Query Sequences

1. Choose the type of cytB sequence that you will enter or choose a demo option

Demo: Select one long 1140 bp cytB lion sequence in the database ▾

Select an existing sequence in the Lion Localizer (LL) database:

LEO0021 ▾

2. Enter an ID and/or information on the lion sample that you are querying (optional), this will show up on the output page

Demo: LEO0021

3. Enter your lion DNA sequence and then click the submit query, the sequence will be populated on the output page. Instructions to generate these sequence(s) can be found [here](#)

```
ATGACCAACATTGAAATCAGCCCTTATCAAATATTAATCACTCATTGATCTTCCCACTCCACCAATATCTCAGCATGATGAAACTTTGGCTCCTTATAGGAGTATGTTA
ATCCTACAATCTCACCGGCTCTTTGTAGCCATACACACGAGACACAATAACGGCTTCTCATCAGTACCACATTTGCCGGATGAAACTATGGGTGAATATCCGGTACC
TACAGGCCAACGGAGCCTCCATATTTATCTGCTATACATGATGAGGACGAGGAATATACTATGGCTCCTATACTTTCTCAGAAACATGAAATATGGAAATCATATTTGCTCACAG
TTATAGCTACAGCCTTCTAGGATATGCTTACCCTGGGGCCAAATATCCTTTTGGAGTGAACCTGTAATCACTAATCTCCTATCAGCAATCCCATACATCGGAGCCGACCTAGTAGAGTG
GATCTGAGGAGGCTTCTCAGTAGACAAAGCCACCCTGACACGATTCTTTGCCCTCCACTTCCCTTCCATTATCATCTCAGCCCTAGCAGCAGTCCACCTCCTATTCCTCATGAAAC
```

SUBMIT QUERY

For printing purposes, the use of Chrome or Firefox are recommended, the printout may not be formatted as well by Safari or other browsers.

Citation

The citation for this software is pending.

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The Lion Localizer was made possible by the support of the American people through United States Agency for International Development (USAID). The views represented on this website do not necessarily reflect the views of the University of Illinois at Urbana-Champaign, USAID, or the United States government.

Figure 1. The Lion Localizer query page. A demonstration sequence (LEO0021) from the database is utilized as a query. For demonstration sequences, the ID (textbox 2) and sequence (textbox 3) are automatically prefilled for the user. Alternatively, the user can instead enter any information that can identify a confiscated lion product, along with the corresponding generated lion cyt b sequence. After entering the query information, the user clicks “submit query,” which prompts the Lion Localizer to compare the query sequence to the haplotypes in the database and to generate an output page.

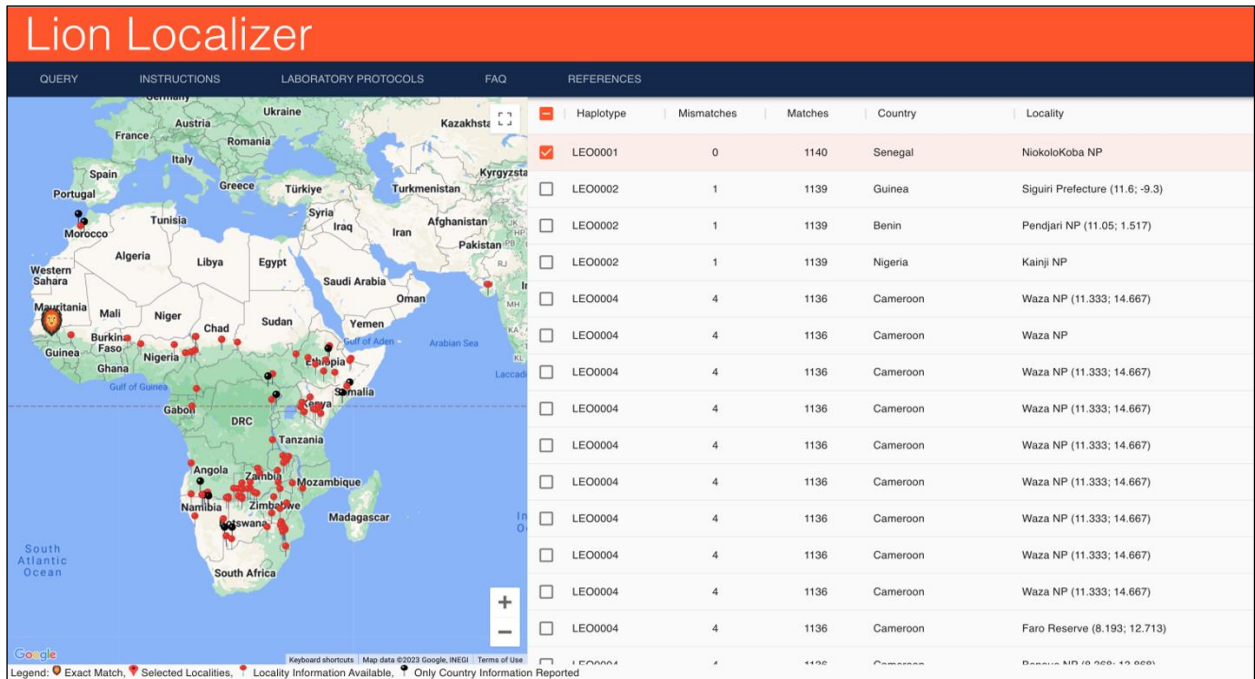


Figure 2. Output page for the Lion Localizer, which populates the information and sequence entered by the user in the query page (not shown), and the map and table depicted. The table (right) lists all haplotype sequences stored in the Lion Localizer database, in the order in which they match the query sequence. The table displays the haplotype ID, number of base pair mismatches and matches to the query, and the country, locality, scientific publication, and GenBank accession number(s) for all previously reported lion sequences. The map (left) shows localities across Africa from which lion sequences have been reported. Black pins indicate lion sequences for which only country information is available, whereas for sites shown using red pins more precise locality information is available. Localities with lion sequences that exactly match the query are indicated using the lion head icon. Large pins also appear on the map if a locality is selected in the table (not shown).

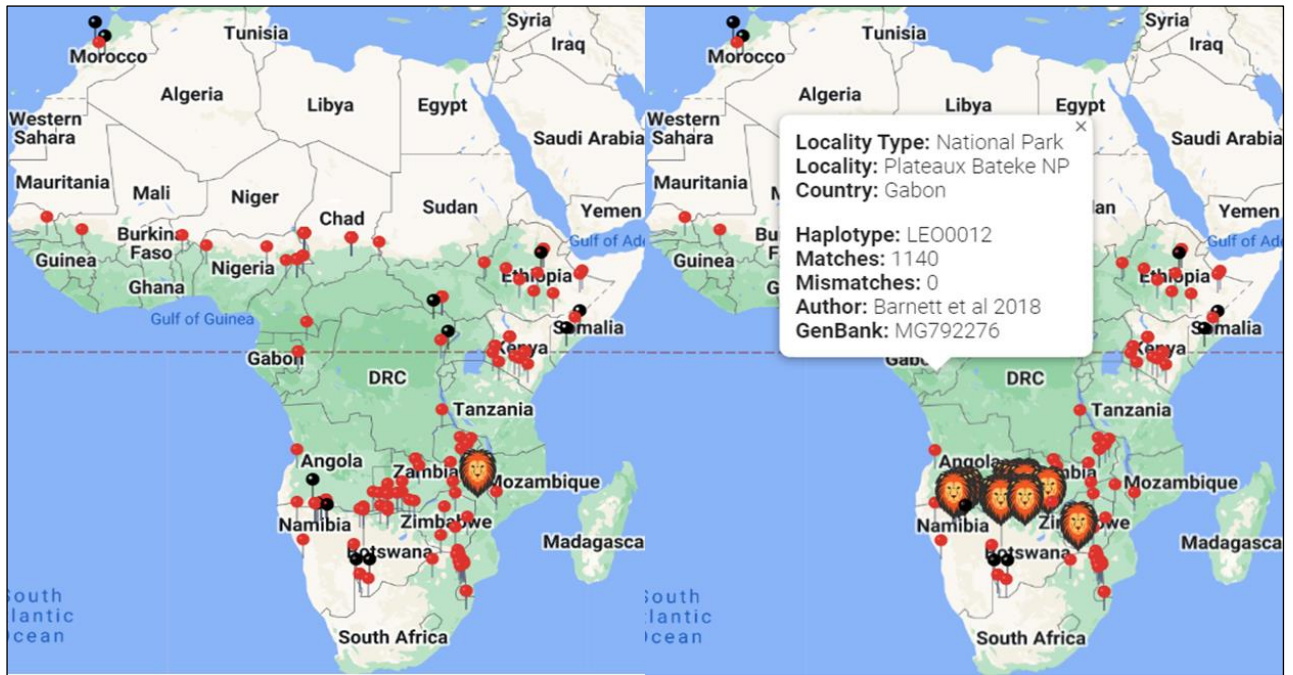


Figure 3. Output maps for sequences matching haplotypes LEO00011 (left) or LEO00012 (right) in the Lion Localizer database. The LEO00011 haplotype has previously been reported from a single locality, Changara National Park in Mozambique. Lion haplotypes with limited geographic distributions would be especially useful when examining the likely origin of confiscated body parts. The LEO00012 haplotype has previously been reported from a broad geographic region of southern Africa, and may primarily be useful for excluding other regions of Africa as being the likely source of confiscated lion body parts. Clicking on a locality in the map will display the box shown, which lists the locality name, type (country level or specific locality level) and country; and the haplotype in the locality that most closely matches the query sequence based on the mismatched numbers, the number of matches and mismatches versus the query, and the scientific publication and GenBank accession number for the closest matching sequence at that locality.

References

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Avise, J. C. (1995). Mitochondrial DNA polymorphism and a connection between genetics and demography of relevance to conservation. *Biology*, *9*(3), 686–690.
- Barnett, R., Sinding, M. H. S., Vieira, F. G., Mendoza, M. L. Z., Bonnet, M., Araldi, A., Kienast, I., Zambarda, A., Yamaguchi, N., Henschel, P., & Gilbert, M. T. P. (2018). No longer locally extinct? Tracing the origins of a lion (*Panthera leo*) living in Gabon. *Conservation Genetics*, *19*(3), 611–618. <https://doi.org/10.1007/S10592-017-1039-2/FIGURES/3>
- Bauer, H., Chardonnet, B., Scholte, P., Kamgang, S. A., Tiomoko, D. A., Tehou, A. C., Sinsin, B., Gebresenbet, F., Asefa, A., Bobo, K. S., Garba, H., Abagana, A. L., Diouck, D., Mohammed, A. A., & Sillero-Zubiri, C. (2020). Consider divergent regional perspectives to enhance wildlife conservation across Africa. *Nature Ecology & Evolution* *2020 5:2*, *5*(2), 149–152. <https://doi.org/10.1038/s41559-020-01343-6>
- Bauer, H., De Iongh, H. H., Princée, F. P. G., & Ngantou, D. (2003). Research needs for lion conservation in west and central Africa. *Comptes Rendus Biologies*, *326*(SUPPL. 1), 112–118. [https://doi.org/10.1016/S1631-0691\(03\)00047-7](https://doi.org/10.1016/S1631-0691(03)00047-7)
- Bauer, H., Packer, C., Funston, P. F., Henschel, P., & Nowell, K. &. (2016). *Panthera leo*. *The IUCN Red List of Threatened Species*. <https://doi.org/10.2305/IUCN.UK.2016-3.RLTS.T15951A107265605.en>
- Bauer, M. S., Damschroder, L., Hagedorn, H., Smith, J., & Kilbourne, A. M. (2015). An introduction to implementation science for the non-specialist. *BMC Psychology*, *3*(1), 1–12. <https://doi.org/10.1186/S40359-015-0089-9/TABLES/5>
- Bertola, L. D., Jongbloed, H., Van Der Gaag, K. J., De Knijff, P., Yamaguchi, N., Hooghiemstra, H., Bauer, H., Henschel, P., White, P. A., Driscoll, C. A., Tende, T., Ottosson, U., Saidu, Y., Vrieling, K., & De Iongh, H. H. (2016). Phylogeographic patterns in Africa and high resolution delineation of genetic clades in the lion (*Panthera leo*). *Scientific Reports* *2016 6:1*, *6*(1), 1–11. <https://doi.org/10.1038/srep30807>
- Bertola, L. D., Miller, S. M., Williams, V. L., Naude, V. N., Coals, P., Dures, S. G., Henschel, P., Chege, M., Sogbohossou, E. A., Ndiaye, A., Kiki, M., Gaylard, A., Ikanda, D. K., Becker, M. S., & Lindsey, P. (2022). Genetic guidelines for translocations: Maintaining intraspecific diversity in the lion (*Panthera leo*). *Evolutionary Applications*, *15*(1), 22–39. <https://doi.org/10.1111/EVA.13318>

- Bertola, L. D., Tensen, L., Van Hooft, P., White, P. A., Driscoll, C. A., Henschel, P., Caragiulo, A., Dias-Freedman, I., Sogbohossou, E. A., Tumenta, P. N., Jirmo, T. H., De Snoo, G. R., De Iongh, H. H., & Vrieling, K. (2015). Autosomal and mtDNA markers affirm the distinctiveness of lions in west and central Africa. *PLOS ONE*, *10*(10), e0137975. <https://doi.org/10.1371/JOURNAL.PONE.0137975>
- Bertola, L. D., van Hooft, W. F., Vrieling, K., Uit de Weerd, D. R., York, D. S., Bauer, H., Prins, H. H. T., Funston, P. J., Udo de Haes, H. A., Leirs, H., van Haeringen, W. A., Sogbohossou, E., Tumenta, P. N., & De Iongh, H. H. (2011). Genetic diversity, evolutionary history and implications for conservation of the lion (*Panthera leo*) in west and central Africa. *Journal of Biogeography*, *38*(7), 1356–1367. <https://doi.org/10.1111/J.1365-2699.2011.02500.X>
- Bertram, B. C. R. (1975). Social factors influencing reproduction in wild lions. *Journal of Zoology*, *177*(4), 463–482. <https://doi.org/10.1111/J.1469-7998.1975.TB02246.X>
- Django Software Foundation. 2022. Django (Version 4.0.6). Lawrence (KS): Django Software Foundation. Available from: <https://djangoproject.com>
- Dietrich, G., Kalle, K., Krauss, W., Siedler, G., & Richardson, P. L. (1975). Allgemeine Meereskunde 3rd edn. In 5. *Madelain, F. & Kerut, E. G. Oceano/. Acta* (Vol. 593). Gebr. Borntraeger.
- Dubach, J. M., Briggs, M. B., White, P. A., Ament, B. A., & Patterson, B. D. (2013). Genetic perspectives on “Lion conservation units” in eastern and southern Africa. *Conservation Genetics*, *14*(4), 741–755. <https://doi.org/10.1007/S10592-013-0453-3/FIGURES/5>
- Dubach, J., Patterson, B. D., Briggs, M. B., Venzke, K., Flamand, J., Stander, P., Scheepers, L., & Kays, R. W. (2005). Molecular genetic variation across the southern and eastern geographic ranges of the African lion, *Panthera leo*. *Conservation Genetics*, *6*(1), 15–24. <https://doi.org/10.1007/S10592-004-7729-6/METRICS>
- Elliot, N. B., Cushman, S. A., Loveridge, A. J., Mtare, G., & Macdonald, D. W. (2014). Movements vary according to dispersal stage, group size, and rainfall: The case of the African lion. *Ecology*, *95*(10): 2860-2869., *95*(10), 2860–2869. <https://doi.org/10.1890/13-1793.1>
- Elliot, N. B., Valeix, M., Macdonald, D. W., Loveridge, A. J., Elliot, N. B., Valeix, M., Macdonald, D. W., & Loveridge, A. J. (2014). Social relationships affect dispersal timing revealing a delayed infanticide in African lions. *Oikos*, *123*(9), 1049–1056. <https://doi.org/10.1111/OIK.01266>
- Everatt, K. T., Kokes, R., & Lopez Pereira, C. (2019). Evidence of a further emerging threat to lion conservation; targeted poaching for body parts. *Biodiversity and*

Conservation, 28(14), 4099–4114. <https://doi.org/10.1007/S10531-019-01866-W/FIGURES/5>

Funston, P. J. (2011). Population characteristics of lions (*Panthera leo*) in the Kgalagadi Transfrontier Park. *https://Doi.Org/10.3957/056.041.0108*, 41(1), 1–10. <https://doi.org/10.3957/056.041.0108>

Hanby, J. P., & Bygott, J. D. (1987). Emigration of subadult lions. *Animal Behaviour*, 35(1), 161–169. [https://doi.org/10.1016/S0003-3472\(87\)80221-X](https://doi.org/10.1016/S0003-3472(87)80221-X)

Ishida, Y., Georgiadis, N. J., Hondo, T., & Roca, A. L. (2013). Triangulating the provenance of African elephants using mitochondrial DNA. *Evolutionary Applications*, 6(2), 253–265. <https://doi.org/10.1111/J.1752-4571.2012.00286.X>

IUCN. (2006a). Conservation strategy for the lion in west and central Africa.

IUCN. (2006b). Conservation strategy for the lion *Panthera leo* in eastern and southern Africa. www.felidae.org

Kumar, V., Chandra, K., Kundu, S., Tyagi, K., Amin Laskar, B., Singha, D., Chakraborty, R., & Pakrashi, A. (2019). Utility of mitochondrial DNA in wildlife forensic science: reliable identification of confiscated materials from eastern India. *Mitochondrial DNA Part B*, 4(1), 583–588. <https://doi.org/10.1080/23802359.2018.1561216>

Loveridge, A. J., Sousa, L. L., Cushman, S., Kaszta, Ž., & Macdonald, D. W. (2022). Where have all the lions gone? Establishing realistic baselines to assess decline and recovery of African lions. *Diversity and Distributions*, 28(11), 2388–2402. <https://doi.org/10.1111/ddi.13637>

Meiklejohn, K. A., Burnham-Curtis, M. K., Straughan, D. J., Giles, J., & Moore, M. K. (2021). Current methods, future directions and considerations of DNA-based taxonomic identification in wildlife forensics. *Forensic Science International: Animals and Environments*, 1, 100030. <https://doi.org/10.1016/J.FSIAE.2021.100030>

Mole, K., & Newton, D. (2021). Lions in Tanzania and Mozambique.

Morandín, C., Loveridge, A. J., Segelbacher, G., Elliot, N., Madzikanda, H., Macdonald, D. W., & Höglund, J. (2014). Gene flow and immigration: genetic diversity and population structure of lions (*Panthera leo*) in Hwange National Park, Zimbabwe. *Conservation Genetics*, 15(3), 697–706. <https://doi.org/10.1007/S10592-014-0571-6/TABLES/3>

Mosser, A., & Packer, C. (2009). Group territoriality and the benefits of sociality in the African lion, *Panthera leo*. *Animal Behaviour*, 78(2), 359–370. <https://doi.org/10.1016/J.ANBEHAV.2009.04.024>

- Packer, C., Pusey, A. Cooperation and competition within coalitions of male lions: kin selection or game theory?. *Nature* 296, 740–742 (1982).
<https://doi.org/10.1038/296740a0>
- Poniente, J. A., Pereda, J. M. R., Dela Peña, J. T., Ventolero, M. F. H., & Santos, M. D. (2022). Mitochondrial DNA-based species testing of confiscated aquatic wildlife in the Philippines. *Forensic Science International: Animals and Environments*, 2, 100051. <https://doi.org/10.1016/J.FSIAE.2022.100051>
- Pusey, A. E. (1987). Sex-based dispersal and inbreeding avoidance in birds and mammals. *Trends in Ecology & Evolution*, 2(10), 295–299.
[https://doi.org/10.1016/0169-5347\(87\)90081-4](https://doi.org/10.1016/0169-5347(87)90081-4)
- React. 2022. React (Version 18.2.0) Available from: <http://ReactJs.org>
- Redux. 2022. Redux (Version 4.2.1) Available from: <https://redux.js.org/>
- Riggio, J., Jacobson, A., Dollar, L., Bauer, H., Becker, M., Dickman, A., Funston, P., Groom, R., Henschel, P., de Iongh, H., Lichtenfeld, L., & Pimm, S. (2013). The size of savannah Africa: a lion's (*Panthera leo*) view. *Biodiversity and Conservation*, 22(1), 17–35. <https://doi.org/10.1007/S10531-012-0381-4/FIGURES/5>
- Ross, H. A., Lento, G. M., Dalebout, M. L., Goode, M., Ewing, G., McLaren, P., Rodrigo, A. G., Lavery, S., & Baker, C. S. (2003). DNA surveillance: web-based molecular identification of whales, dolphins, and porpoises. *Journal of Heredity*, 94(2), 111–114. <https://doi.org/10.1093/JHERED/ESG027>
- Sagamiko, T. D., Shaban, N., Nahonyo, C. L., & Makinde, O. D. (2015). Optimal control of a threatened wildebeest-lion prey-predator system in the serengeti ecosystem. *Open Journal of Ecology*, 05(04), 110–119.
<https://doi.org/10.4236/oje.2015.54010>
- Spong, G., & Creel, S. (2001). Deriving dispersal distances from genetic data. *Proceedings of the Royal Society B: Biological Sciences*, 268(1485), 2571.
<https://doi.org/10.1098/RSPB.2001.1835>
- Vanderwaal, K. L., Mosser, A., & Packer, C. (2009). Optimal group size, dispersal decisions and postdispersal relationships in female African lions. *Animal Behaviour*, 77, 949–954. <https://doi.org/10.1016/j.anbehav.2008.12.028>
- Williams, V. L., Loveridge, A. J., Newton, D. J., & Macdonald, D. W. (2017). Questionnaire survey of the Pan-African trade in lion body parts. *PLOS ONE*, 12(10), e0187060. <https://doi.org/10.1371/JOURNAL.PONE.0187060>
- Williams, V., Newton, D., Loveridge, A., & Macdonald, D. (2015). Bones Of contention: an assessment of the south African trade in African lion *Panthera leo* bones and other body parts A TRAFFIC & WildCRU Joint Report.

Zhao, K., Ishida, Y., Green, C. E., Davidson, A. G., Sitam, F. A. T., Donnelly, C. L., de Flamingh, A., Perrin-Stowe, T. I. N., Bourgeois, S., Brandt, A. L., Mundis, S. J., van Aarde, R. J., Greenberg, J. A., Malhi, R. S., Georgiadis, N. J., McEwing, R., & Roca, A. L. (2019). Loxodonta Localizer: a software tool for inferring the provenance of African elephants and their ivory using mitochondrial DNA. *Journal of Heredity*, 110(7), 761–768. <https://doi.org/10.1093/JHERED/ESZ058>

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