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Lion Localizer: A Software tool for inferring the provenance of lions (*Panthera leo*) based on their mitochondrial DNA

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Abstract

28

29   The illegal poaching of lions for their body parts poses a severe threat to lion populations

30   across Africa. Poaching accounts for 35% of all human-caused lion deaths, with 51% attributed

31   to retaliatory killings following livestock predation. In nearly half of the retaliatory killings, lion

32   body parts are removed, suggesting that high demand for lion body parts may fuel killings

33   attributed to human-lion conflict. Trafficked items are often confiscated in transit or destination

34   countries far from their country of origin. DNA from lion parts may in some cases be the only

35   available means for examining their geographic origins. In this paper, we present the Lion

36   Localizer, a full-stack software tool that houses a comprehensive database of lion mtDNA

37   sequences sourced from previously published studies. The database covers 146 localities from

38   across the African continent and India, providing information on the potential provenance of

39   seized lion body parts. Lion mtDNA sequences of 350 bp or 1140 bp corresponding to the

40   cytochrome b region can be generated from lion products and queried against the Lion Localizer

41   database. Using the query sequence, the Lion Localizer generates a listing of exact or partial

42   matches, which are displayed on an interactive map of Africa. This allows for the rapid

43   identification of potential regions and localities where lions have been or are presently being

44   targeted by poachers. By examining the potential provenance of lion samples, the Lion Localizer

45   serves as a valuable resource in the fight against lion poaching. The software is available at

46   https://lionlocalizer.org.

47

48   Keywords: cytochrome b, forensic, 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). Lion body parts are removed in 48% of the retaliatory 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 about an expanding trade in lion body parts, both domestically within African countries and internationally, leading to unsustainable mortality rates and endangering the survival of lions (IUCN, 2006a, 2006b; Riggio et al., 2013; Bauer et al., 2016; Everatt et al., 2019). For example, TRAFFIC's Wildlife Trade and Information System (WiTIS) database 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, 2006b; 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

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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. 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 (Avise, 1995; Elliot, Cushman, et al., 2014; 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; Dietrich et al., 1975; Mosser & Packer, 2009; Sagamiko et al., 2015). Male lions disperse over long distances of 120-200 kilometers (Elliot, Valeix, et al., 2014; Funston, 2011; Morandin et al., 2014). In contrast, female lions typically remain within their natal range and rarely disperse unless recruited into another pride (Hanby & Bygott, 1987; Spong & Creel, 2001; Vanderwaal et al., 2009; Funston, 2011).

To enable the use of mitochondrial DNA for examining the provenance of confiscated lion products, we have developed 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 is therefore 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 in a table arranges the stored haplotypes 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 Africa continent shows all localities from which lion sequences have been reported, and specifically highlights localities from which sequences 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 none of the user-entered sequences or associated information are 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 DNA (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

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117   utilized to develop the backend, while ReactJS version 18.2.0 (ReactJS 2013**),** an efficient and

118   robust JavaScript library, was used for frontend development. The combination of React for

119   frontend development and Django for backend development is a popular web development stack,

120   offering a reliable and scalable solution for building modern web applications. The Lion

121   Localizer database was built using SQLite utilizing the Django Object-Relational-Model (ORM)

122   to store previously reported lion haplotypes as well as information about their corresponding

123   GenBank accession numbers, published journal articles that reported the sequences, and the

124   sample collection localities. To enable users to submit query sequences

125   (https://lionlocalizer.org/query) for comparison with haplotypes in the database, the Lion

126   Localizer employs Redux, a state management library for JavaScript applications (Redux 2015).

127   The entity-relationship diagram and the information stored in the database tables used for the

128   Lion Localizer are depicted in Supplementary Figure S1.

129

130   *Publications reporting sequences contained in the Lion Localizer Database*

131   The database of the Lion Localizer consists of previously published sequences of the

132   mitochondrial gene *MT-CYTB* (henceforth referred to as "cytochrome b" or "cyt b"). The cyt b

133   haplotypes of 1140 bp and all related information stored in the database (depicted in

134   Supplementary Figure S1) were compiled from previously generated and published sequences

135   for the species *Panthera leo*. We considered whether to incorporate the control region in addition

136   to cyt b; however, this was ruled out for two reasons. First, the control region, being prone to

137   indels (insertions or deletions), could vary in length, and this variability might affect the

138   alignments used for query comparison. Second, many published sequences of lion mitochondrial

139   DNA do not include the control region. Thus, the use of control region sequences in the database

140   would have reduced the total number of haplotypes and localities represented.

141   The cyt b sequences included in the database originate from a number of published

142   sources; in all cases the sequences reported included the full 1140 bp cyt b region stored in the

143   database. Dubach and colleagues in 2005 published the sequences of 26 lions from 11 countries

144   (AF384809-AF384818) (Dubach et al., 2005); Bertola and colleagues in 2011 sequenced 53

145   lions from 15 countries (GU131164–GU131185, AY781195–AY781210 and DQ018993–

146   DQ018996) (Bertola et al., 2011); Dubach and colleagues in 2013 sequenced 75 lions from 8

147   different countries (KC495048-KC495058) (Dubach et al., 2013); Barnett and colleagues

148   sequenced 14 museum-preserved lions (KJ545522-KJ545535) (Barnett et al., 2014); and Bertola

149   and colleagues in 2015 sequenced 48 lions from 7 different countries (Bertola et al., 2015).

150   These cyt b sequences from previous studies were filtered to consolidate duplicate sequences

151   into a single haplotype within the Lion Localizer, as well as to ensure appropriate length (1140

152   bp), and to remove sequences with unknown or ambiguous sites (e.g., sequences were excluded

153   if they contained "N" at one or more positions). After filtering, the Lion Localizer database

154   currently contains 21 distinct haplotypes from 146 localities in 25 countries. Among these, 12 of

155   the "localities" consist of only country information, i.e., there were lion specimens for which

156   only the country of origin was available, and for which more precise collection information was

157   not available. The other 134 localities represent lions for which additional geographic

158   information within a country was also available. The Lion Localizer provides the options to

159   query this database using either the full 1140 bp cyt b sequence, or alternatively using a 350 bp

160   region within this sequence.

161

162   *Testing and Validation*

163   To ensure the accuracy and reliability of the Lion Localizer database, rigorous and

164   systematic testing was conducted, consisting of three sets of quality control assessments. The

165   first involved checking the localities shown on the output map (Figure 1) for accuracy. Every

166   locality icon on the Google map on the Lion Localizer page had its resolution enhanced until the

167   location names could be seen, and the location names were then confirmed to be accurate. If a

168   location name was not found on the Google map, potential locations and GPS coordinates

169   provided from the published papers were verified (using www.latlong.net). The localities on the

170   map were also matched with the locations noted in the published papers from which sequences

171   were obtained for the Lion Localizer database. This ensured that every locality on the Google

172   map in the output page was referred to by at least one of the published papers. The locations

173   listed in each published article were also double-checked, to verify that they appeared and were

174   positioned properly on the Lion Localizer map, in order to avoid any potential error due to

175   multiple places having the same name.

176   A second quality control assessment validated that the listing generated by the Lion

177   Localizer for each haplotype was correct. The demonstration option in the query page was used

178   to test each haplotype in the Lion Localizer database. The table listing of exact matches for each

179   of the haplotypes was compared to the icons on the map to make sure that the locations in the

180   table and in the map were the same. Each haplotype in the database was queried in GenBank

181   using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Nucleotides) (Altschul et al.,

182   1990) to show that the output results for the Lion Localizer and those in GenBank matched

183   exactly.

184   The third quality control assessment involved going through each publication that

185 deposited sequences in GenBank that were used to make the Lion Localizer database. All lion

186 sequences mentioned in each paper were verified to be part of the Lion Localizer database (or to

187 have been excluded due to one of the filters noted above). The relevant GenBank sequences,

188 corresponding to each published paper, were trimmed and used to query the Lion Localizer for

189 the 1140 bp and 350 bp regions that can be used as queries. The Lion Localizer results were

190 compared to the information provided in the original papers for each haplotype sequence. This

191 verified that the localities that the Lion Localizer shows for each paper for each haplotype, both

192 on the output table and on the output map, all matched what had been reported in the original

193 published papers.

194

195 **Results (Including Software Description)**

196 *Query page and output page for the Lion Localizer*

197   The Lion Localizer query page can be found at https://lionlocalizer.org/query. The query

198 page includes two textboxes. One allows for entry of an optional id/description, which can

199 include any information that the user considers relevant for identifying the source of the queried

200 sequence. The second textbox allow entry of a user-generated 1140 bp or a 350 bp cytochrome B

201 lion sequence, which comprises the query sequence. Alternatively, as a demonstration sequence,

202 one of the 1140 bp or 350 bp sequences stored in the database may be selected from a drop-down

203 menu, which the user may use to examine the functionality of the Lion Localizer. If a

204 demonstration sequence is selected, any relevant information will be automatically prefilled

205 **(Figure 1)**.

206   After the user submits their generated cyt b sequence (or a demonstration sequence), the

207   Lion Localizer generates an output page that displays three sections: the query information, a

208   table, and a map **(Figure 2)**. To ensure a complete chain of custody, all query information,

209   including user-entered information that identifies the sample, as well as the mtDNA sequence,

210   are populated to the output page by the Lion Localizer, which also shows the date and time that

211   the output was generated (in Greenwich Mean Time). Beneath the query information, the output

212   page shows a table that lists all of the haplotypes within the Lion Localizer database, with the

213   haplotypes that most closely match the query sequence listed first, and all other haplotypes listed

214   in decreasing order of match to the query sequence.

215   If the haplotype was reported from multiple localities, then the haplotype number repeats

216   as a separate row for each of the localities. Additional information is listed next to each

217   haplotype number on the table, including the number of bp mismatches to the query, number of

218   bp matches, the country, the geographic locality (if any) within the country, the publication

219   reporting the information for the haplotype, and GenBank accession number(s) for the previously

220   reported sequences (https://www.ncbi.nlm.nih.gov/genbank/).

221   To the left of the output page, a Google map (https://www.google.com/maps) is displayed

222   that is centered on the Africa continent (although the database includes sequences of Asiatic

223   lions). If sequences with zero mismatches to the query sequence were reported from a locality, a

224   lion head icon is used to highlight the locality. The remaining localities from which lion

225   sequences have been reported are indicated on the map using pins (if the lions with the closest

226   matching cyt b sequence in the locality did not exactly match the query sequence). Red pins

227   indicate localities with some degree of geographic precision within-country. For some lion

228   sequences, only country information was available, and these are shown on the map using black

229 pins. Clicking on any pin icon will open an information box that displays information (similar to

230 the information listed on the table) for the haplotype at that location that most closely matches

231 the query sequence **(Figure 3)**.

232

233 *Additional information accessible via the Lion Localizer*

234   From any of the pages of the Lion Localizer platform, several tabs can be accessed by the

235 user for pages with additional information. There is a page providing detailed instructions for

236 using the Lion Localizer, and a dedicated page for laboratory protocols

237 (https://lionlocalizer.org/protocol). The protocol provides guidance on generating lion

238 mitochondrial DNA sequences (DNA extraction, amplification, Sanger-sequencing, trimming,

239 and additional information). Other pages are designed to answer frequently asked questions

240 (FAQs), outline the terms & conditions along with copyright details, and provide a list of

241 scientific references that generated sequences incorporated into the database. Additionally, a

242 contact form is available for users to communicate suggestions or report any issues encountered

243 with the software. Users can print the output page if they want to preserve the query information,

244 table listing, and output map, as none of this information is stored by the Lion Localizer.

245

246   **Discussion**

247   We have developed the Lion Localizer software, which can be utilized to examine the

248 geographic origins of lions by comparing their cyt b sequences to those reported in previously

249 published studies (Barnett et al., 2018; Bertola et al., 2011, 2015, 2016; Dubach et al., 2005,

250 2013). This method offers a number of advantages, including simplicity and speed for examining

251 the provenance of confiscated lion products by using lion mitochondrial DNA. The Lion

252 Localizer may be particularly informative in estimating the likely origins of confiscated body

253 parts for lion haplotypes reported from only a single locality, a single country, or from a small

254 number of neighboring nations. For example, Haplotype LEO0001 has only been reported once

255 in the published literature, and only from Niokolo-Koba National Park (**Figure 2**) (Bertola et al.,

256 2015). Of the 21 haplotypes in the Lion Localizer database (each representing a distinct cyt b

257 sequence), 12 (57%) have been reported from only one country. In using the Loxodonta

258 Localizer, it may be useful to have it show the geographic distribution of haplotypes with one or

259 two mismatches to the query sequence on the map in order to assess the degree to which these

260 similar haplotypes may cluster within a specific geographic area. The most reliable haplotypes

261 for establishing provenance would be those with the most constrained geographic distribution,

262 especially if such haplotypes are from a region that has been heavily sampled in terms of number

263 of lions and numbers of localities.

264   There are a number of potential limitations to the Lion Localizer, some of which could be

265 addressed with further improvements. For instance, while many mtDNA sequences have been

266 reported from a specific country or even a single locality, a considerable number of the 1140 bp

267 haplotypes within the Lion Localizer have been reported from widespread geographic locations

268 **(Figure 3)**. Such wide-ranging haplotypes may provide a general indication of potential areas of

269 origin for a lion in Africa, and can exclude other geographic regions as unlikely to be the source

270 of a confiscated lion specimen. Additionally, the lions and localities in the database represent the

271 minimum of localities from which a specific haplotype might have originated, as further

272 sampling may reveal other localities in which a given haplotype may also be present. The

273 accuracy of the Lion Localizer may in the future be improved by the use of longer mtDNA

274 sequences, as these longer sequences may show variation that distinguishes haplotypes that are

275 currently considered identical. Likewise, sequences from additional lions and additional

276 localities would enhance the precision of the Lion Localizer. Increasing geographic coverage and

277 the addition of more lions and longer sequences may identify rare haplotypes with recent

278 mutations limited to a particular geographic region.

279   Species other than lions, notably those with low levels of female dispersal, could

280 potentially benefit from the use of mtDNA as a marker to examine the origin of confiscated

281 wildlife products (Avise, 1995; Ishida et al., 2013). Several applications have already been

282 developed for a number of taxa, e.g., to examine the geographic origin of African elephants

283 (genus *Loxodonta*; https://www.loxodontalocalizer.org) (Ishida et al., 2013; Zhao et al., 2019)

284 and to distinguish among taxa across a number of vertebrate groups (https://dna-

285 surveillance.auckland.ac.nz/) (Ross et al., 2003). Similar software for other taxa would require

286 construction of different databases and require intense quality control to identify the geographic

287 distributions of unique haplotypes. The entity-relationship diagram for the Lion Localizer is

288 provided as Supplementary Figure S1, together with the table relationships and information

289 included in the database tables, as a potential guide in the creation of such software.

290   The Lion Localizer represents an important advance in the utilization of DNA sequences

291 for conservation and forensic purposes. By permitting examination of the geographic origins of

292 confiscated lion body parts through the analysis of mtDNA haplotypes, it may offer insights

293 helpful to law enforcement, wildlife forensics, and conservation efforts. The Lion Localizer

294 offers ease of use, high speed, and the capacity to easily compare mtDNA sequencing results

295 previously generated by different laboratories, aggregating them across studies to provide easy

296 access to users of the software. Moving forward, increasing the number of lions and localities

297   examined, and the lengths of mtDNA sequences generated, may further expand the potential of

298   the Lion Localizer, and increase its impact on the conservation of lions.

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315   **Figure 1**. The Lion Localizer query page. A demonstration sequence (LEO0021) from the

316   database is utilized as a query. For demonstration sequences, the ID (textbox 2) and sequence

317   (textbox 3) are automatically prefilled for the user. Alternatively, the user can instead enter any

318   information that can identify a confiscated lion product, along with the corresponding generated

319   lion cyt b sequence. After entering the query information, the user clicks "submit query," which

320   prompts the Lion Localizer to compare the query sequence to the haplotypes in the database and

321   to generate an output page.
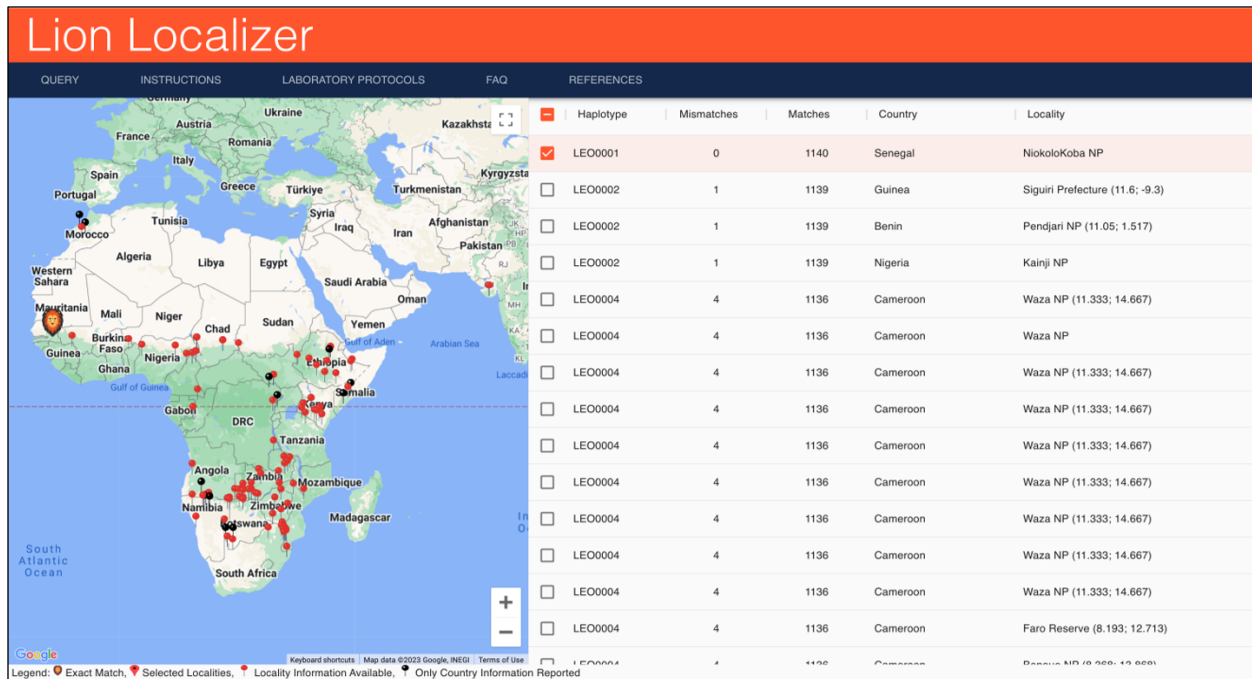
Figure 2. Output page for the Lion Localizer, which populates the information and sequence

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 number, 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).

334
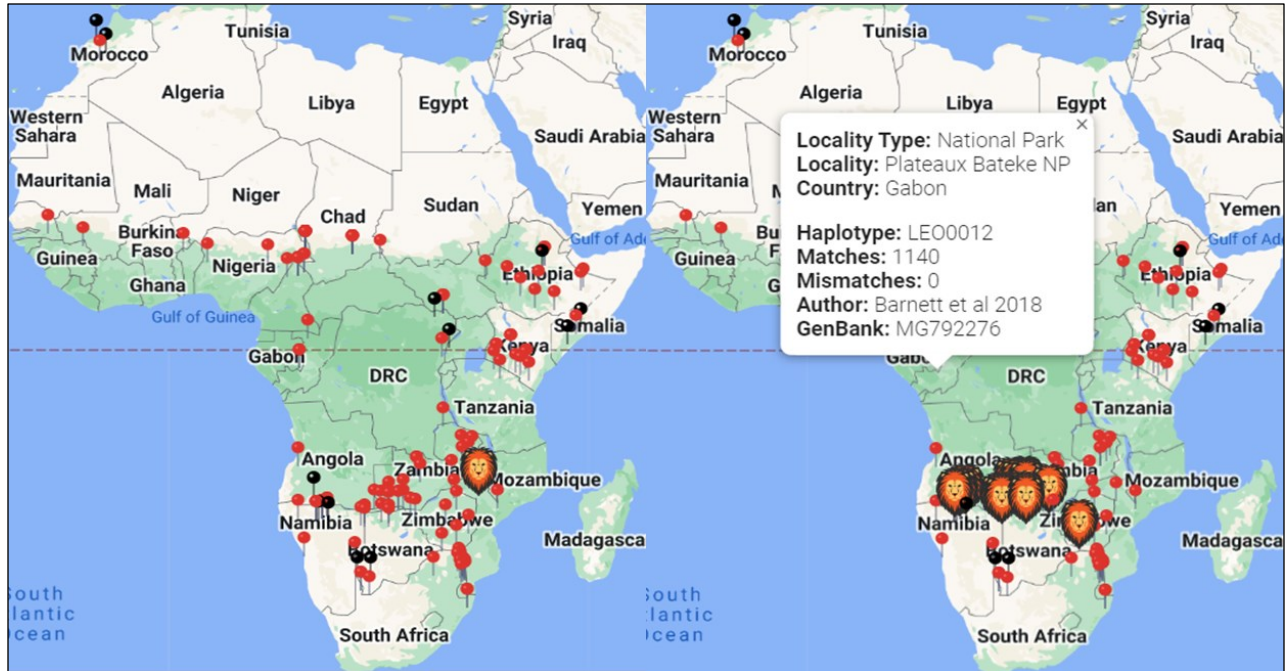
Figure 3. Output maps for sequences matching haplotypes LEO00011 (left) or LEO00012

(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 list the locality name, type and country; and the haplotype in

the locality that most closely matches the query sequence, 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.

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