SUPPLEMENTARY INFORMATION

Loxodonta Localizer: a software tool for inferring the provenance of African elephants and their ivory using mitochondrial DNA

Supplementary Text

Additional information on the home and output pages of the Loxodonta *Localizer*

The home page provides information on the program, additional materials useful to the user, and allows input of a 316 bp mtDNA control region sequence that is used as a query against a database that contains African elephant sequences reported previously in the peer-reviewed scientific literature. There is a link to a file of frequently asked questions (FAQ) that provides additional assistance and references for studies that provided the African elephant mtDNA sequences in the database, and their geographic locations. The linked file also provides guidelines on the handling of sequences with ambiguous or unusual character states within the 316 bp control region sequence used as query to the LL, which may be due to heteroplasmy, indels or technical issues; on the handling of unprovenanced samples; and on the potential use of nuclear SNPs to identify the species of African elephant (though this last is not necessary to use the *Loxodonta* Localizer). The FAQ and other documents will be continuously updated, and current versions will be available through the LL website.

Finally, disclaimers are specified, notably that mtDNA patterns, unlike nuclear DNA patterns, do not reflect species barriers in elephantids due to strongly male-biased gene flow in elephants and ancient hybridization between elephant species (Roca et al. 2007; Petit and Excoffier 2009). For many hybridizing species pairs in which females are the non-dispersing sex, mito-nuclear discrepancies have been reported in which mtDNA patterns are unreliable for establishing species boundaries (Petit and Excoffier 2009). The transfer of mtDNA between recognized species, or the presence of mtDNA phylogeographic patterns that do not conform to nuclear DNA phylogegraphic patterns or to morphological distinctions have been commonly reported across living and extinct elephantids (Roca 2015). Discrepant mito-nuclear patterns exist not only between African elephant species (Debruyne 2005; Roca et al. 2005), but also within the Asian elephant species (*Elephas maximus*) (Fernando et al. 2000; Fleischer et al. 2001), within the woolly mammoth species (*Mammuthus primigenius*) (Gilbert et al. 2008; Roca 2015), between species of mammoths (genus *Mammuthus*) (Enk et al. 2011), and even between *L. cyclotis* and the now-extinct genus *Paleoloxodon* (Meyer et al. 2017).

Once a query sequence is submitted, the program checks the sequence length, and an error message is returned if the sequence is not 316 bp in length. This prevents the LL from processing an incorrect query sequence or providing a misleading result based on an improperly trimmed sequence. Likewise, if sequences have more than 7 mismatches between the query sequence and the closest matching African elephant sequence in the LL, an error message will indicate that the query sequence may not be from an African elephant or not be homologous to the required 316 bp mitochondrial control region sequence. Given the great diversity of African elephant sequences examined by previous studies sampling across their geographic range, it may be unlikely that an African elephant sequence with this degree of divergence from known sequences would be detected, and if such a sequence existed it may not provide reliable geographic information due to the high divergence. Such a sequence may indicate that the sample sequence is from an Asian elephant, a woolly mammoth, or a non-elephantid species.

If the query sequence is 316 bp in length and is similar to an existing African elephant sequence, then the LL generates output showing the geographic distribution of identical or similar sequences that have previously been published for African elephants, as is shown in Figure 1.

Testing of the Loxodonta *Localizer*

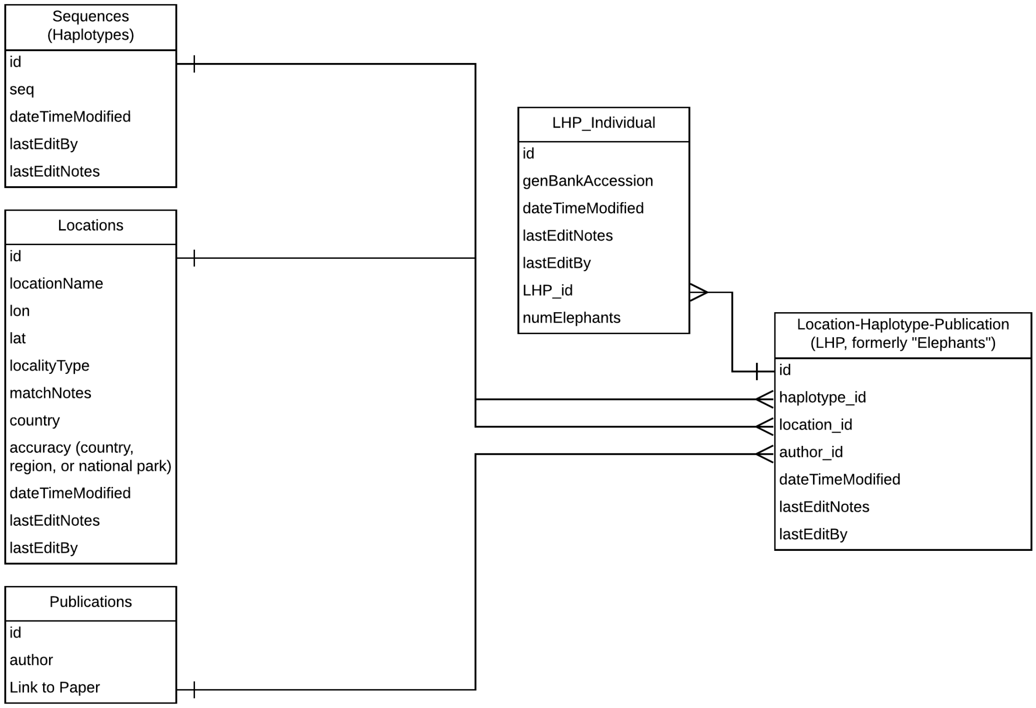
To ensure that errors and omissions were not present in the *Loxodonta* Localizer databases, systematic testing was conducted, notably including three major sets of tests. The first involved checking the locations shown on the output map (Figure 1) for accuracy and precision. First, the localities on the map were verified as correct. For each location icon on the Google map, the map resolution was increased until place names were visible, and the place name was verified as being present at the site of the icon. If the locality name did not appear on the Google map, potential locations were searched for using www.latlong.net. The map of localities was also compared to the places listed on maps published in papers that provided the GenBank entries used as haplotypes in the LL, to verify that there were no localities on the map that were not shown in at least one of the published papers. Likewise, the places referred to in each of the papers were verified as being present and at the right location on the LL map, in order to ensure that, for cases where the same name is assigned to more than one place, the correct one was accurately positioned on the LL map. For example, "Zambezi" may refer to a national park, a river that runs across several nations, and to political subdivisions in more than one country. Thus, we verified that each locality was accurately shown on the LL map, for each of the various published papers that generated mtDNA sequences.

A second major test was to confirm that every match found by the LL for each of the distinct sequences did not include any information that was in error. Each distinct haplotype in the LL pulldown menu was tested. The listing of exact matches for each LL was compared to the icons on the map, to make sure that the locations were consistent between the list and the map. Each distinct sequence was then queried against GenBank entries using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Nucleotides) (Altschul et al. 1990), with the results on the output page of the LL compared to the results listed in GenBank, to verify an exact correspondence between the two.

A third test looked for potential omissions by examining each paper that served as a source of sequence information for the LL. In this test, the listing of haplotypes was found in the paper. For each of the haplotypes in the paper, the locations at which they are reported by the paper were determined, then the GenBank entries were found for the paper. The relevant sequences obtained from GenBank were then trimmed and used to query the LL, with results compared to those listed for the corresponding haplotype in the paper (keeping in mind that the LL may use a shorter sequence than is reported in a paper, so that one LL haplotype may represent more than one haplotype in a paper, if the ones in the paper match exactly in the region that overlaps the LL sequence but had a mismatch outside that region that led to them being listed as distinct haplotypes in the paper). We verified that the localities that are listed for each paper for each haplotype on the LL output list, and on the LL output map matched what had been reported in the original paper. We would note that database coverage varies by range country, and that the haplotype distribution represents a minimum number of localities that a particular haplotype could have originated from.

Supplementary Figure S1.

The entity-relationship diagram for the *Loxodonta* Localizer, along with a description of information included in the database's tables.



The software stores information in several tables, and in some cases the same information may be populated across more than one table.

id: An internally-generated unique identification number for each new entry, for each respective table

Update date: The date the information was entered, called "dateTimeModified"

GenBankAccession: If available, the accession number is provided for an entry. This is the number assigned to the sequence when it was entered in the GenBank database of sequences maintained by the US National Institutes of Health (NIH).

lastEditNotes: Notes included when information is entered into the table.

LHP_id: A foreign key linking a record from a table to the LHP table. Indicates a unique triad of location-haplotype-publication.

numElephants: This is the number of elephants to which the entry information applies. In some cases, the same published paper detected the same mtDNA sequence in more than one elephant from the same locality.

locationName: This is the name of the place from which the elephant specimens were originally collected within Africa. It is typically taken from the published paper that reported the sequences.

lon: This is longitude information assigned to the "locationName" and verified using information from the publication or from other sources.

lat: This is latitude information assigned to the "locationName" and verified using information from the publication or from other sources.

localityType: This is information about the location from which the elephant sample was originally collected, that is not indicated elsewhere in the dataset: for example, this may specify that only "country" information was available for some reported sequences.

matchNotes: Notes on how latitude and longitude information was obtained for the localities from which the elephants were collected. For example, the published article may specify a specific national park, but not provide latitude and longitude information for the park, yet this information can be obtained from other sources.

locality: This is locality information that appears as output in the *Loxodonta* Localizer.

accuracy: This indicates what quality of geographic information is available for the entry: 0 for country; 1 for region; 2 for a precise locality such as a specific national park.

oldPk: This is for indicated deleted records made within the table (not shown).

haplotype_id: The number assigned to each unique sequence in the database. The number begins with "LL" for "*Loxodonta* Localizer", and a new LL number is added only when a novel sequence is found not previously reported among elephants. Elephants that share the same 316 bp control region sequence are assigned the same haplotype_id, even if they are from different geographic regions or were reported by different studies.

location_id: Each distinct locality in Africa is assigned a separate number in this field.

author_id: Each peer-reviewed publication that reported sequences used as input to the *Loxodonta* Localizer is assigned a separate number.

author: The publication in which the sequence information was reported. The full citation is also provided in the webpage.

paperurl: A link to the URL for a copy of the published paper in which the sequence information was reported, shown as “Link to Paper” above.

seq: The actual 316 bp DNA sequence reported for the elephant(s) for each entry.

References for Supplementary Information:

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Meyer M, Palkopoulou E, Baleka S, Stiller M, Penkman KEH, Alt KW, Ishida Y, Mania D, Mallick S, Meijer T*, et al.* 2017. Palaeogenomes of Eurasian straight-tusked elephants challenge the current view of elephant evolution. *Elife*. 6.

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Roca AL, Georgiadis N, O'Brien SJ. 2005. Cytonuclear genomic dissociation in African elephant species. *Nature Genetics*. 37(1):96-100.

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Table S1. Elephant individuals sequenced, by region and country.

Region/ Country	Author	Elephant individuals (*N*)
Central Africa		
Cameroon		196
	Eggert et al. 2002	159
	Debruyne et al. 2003	2
	Ishida et al. 2013	35
Central African Republic		73
	Debruyne et al. 2003	1
	Johnson et al. 2007	10
	Ishida et al. 2013	54
	Mondol et al. 2015	8
Chad		1
	Debruyne et al. 2005	1
Republic of Congo		21
	Debruyne et al. 2003	2
	Johnson et al.2007	16
	Ishida et al. 2013	3
Democratic Republic of Congo (DRC		202
	Debruyne et al. 2003	9
	Debruyne et al. 2005*	4
	Ishida et al. 2013	20
	Mondol et al. 2015	169
Gabon		89
	Debruyne et al. 2003	2
	Johnson et al. 2007	70
	Ishida et al. 2013	17
Subtotal, Central Africa		582
Eastern Africa		
Eritrea		15
	Brandt et al. 2014	15
Kenya		452
	Eggert et al. 2002**	3
	Archie et al. 2006	236
	Ishida et al. 2013	157
	Nyakaana et al. 2002	56
Rwanda		40
	Mondol et al. 2015	40
Sudan		1
	Debruyne et al. 2005	1
Tanzania		2
	Debruyne et al. 2005	2
Uganda		107
	Nyakaana et al. 2002	104
	Debruyne et al. 2005	3
Subtotal, Eastern Africa		617
Southern Africa		
Botswana		120
	Debruyne et al. 2005	1
	Ishida et al. 2013	94
	Nyakaana et al. 2002	25
Mozambique		1
	Debruyne et al. 2005	1
Namibia		86
	Debruyne et al. 2005	2
	Ishida et al. 2013	60
	Nyakaana et al. 2002	24
South Africa		76
	Eggert et al. 2002	6
	Debruyne et al. 2003	2
	Debruyne et al. 2005	1
	Eggert et al. 2008	5
	Ishida et al. 2013	50
	Nyakaana et al. 2002	12
Zambia		1
	Debruyne et al. 2005	1
Zimbabwe		122
	Charif et al. 2004	5
	Debruyne et al. 2005	10
	Ishida et al. 2013	101
	Nyakaana et al. 2002	6
Subtotal, Southern Africa		406
West Africa		
Burkina Faso		74
	Mondol et al. 2015	74
Cote d'Ivorie		31
	Eggert et al. 2002	30
	Debruyne et al. 2005	1
Ghana		182
	Eggert et al. 2002	173
	Nyakaana et al. 2002	9
Liberia		1
	Debruyne et al. 2005	1
Mali		23
	Eggert et al. 2002	23
Sierra Leone		1***
	Debruyne et al. 2003	1
	Ishida et al. 2013	1
Subtotal, West Afica		312
Total elephant individuals across Africa		1917

Range state countries with no mtDNA sequences reported: Angola, Benin, Equatorial Guinea, Ethiopia, Guinea, Guinea Bissau, Malawi, Niger, Nigeria, Senegal, Somalia, Swaziland, Togo

Sequences in the *Loxodonta* Localizer as of May 30, 2019; details on the references are at the end of the Supplementary

*A sample listed as Luiza, Angola has coordinates within the DRC

**Elephants in North American zoos that originated in Kenya

*** The same Sierra Leone individual was sequenced by 2 studies

Table S2: Geographic assignment of ivory samples by nuclear and mitochondrial DNA

			16 STR loci	LL mtDNA	16 STR loci	LL haplotype number**	LL mtDNA, geographic distribution of haplotype									
Original ID and seizure information			**Likely region of origin**	**Likely region of origin**	**Assigned country**		**Reported country/countries**									
LL shows mtDNA reported from a single country, or a few adjacent countries, with not more than one mismatch (asterisk indicates a country in the LL list that borders the country assigned using STRs)																
MYS1956	F-white	Gemas	Tridom	Tridom	Gabon	LL007	Cameroon*	CAR								
MYS0637	S-burn	Sungkai	West Africa	West Africa	Nigeria	LL013	Cameroon*									
MYS1268	F-burn	Sungkai	Tridom	Tridom	DRC	LL017	CAR*									
MYS0877	F-Letter	Sungkai	Tridom	Tridom	ROC	LL018	CAR*	Gabon*	ROC							
MYS0766	F-burn	Sungkai	Tridom	Tridom	Gabon	LL025	Gabon									
MYS1271	F-white	Gemas	Tridom	Tridom	Gabon	LL025	Gabon									
MYS0623	F-burn	Sungkai	Tridom	Tridom	ROC	LL033	Gabon*	CAR*	ROC	Cameroon*						
MYS0792	F-burn	Sungkai	Tridom	Tridom	ROC	LL033	Gabon*	CAR*	ROC	Cameroon*						
MYS0821	F-burn	Sungkai	Tridom	Tridom	Gabon	LL033	Gabon	CAR	ROC*	Cameroon*						
MYS1032	F-Orange	Sungkai	Tridom	Tridom	ROC	LL033	Gabon*	CAR*	ROC	Cameroon*						
MYS0833	F-burn	Sungkai	Tridom	Tridom	ROC	LL034	Gabon*	CAR*								
MYS0892	F-Letter	Sungkai	Tridom	Tridom	ROC	LL034	Gabon*	CAR*								
MYS0947	F-Letter	Sungkai	Tridom	Tridom	Gabon	LL034	Gabon	CAR								
MYS0748	F-white	Gemas	Tridom	Tridom	Gabon	LL034	Gabon	CAR								
MYS0960	F-Letter	Sungkai	Tridom	Tridom	ROC	LL034 (one mismatch)	Gabon*	CAR*								
MYS1129	F-Orange	Sungkai	Tridom	Tridom	ROC	LL035	Gabon*									
MYS0814	F-burn	Sungkai	Tridom	Tridom	ROC	LL036 (one mismatch)	Gabon*	CAR*								
MYS0631	F-burn	Sungkai	Tridom	Tridom	ROC	LL038	Gabon*									
MYS1895	F-white	Gemas	Tridom	Tridom	ROC	LL039	Gabon*									
MYS0800	F-burn	Sungkai	Tridom	Tridom	Cameroon	LL040	Gabon*									
MYS1884	F-Letter	Sungkai	Tridom	Tridom	Gabon	LL040	Gabon									
MYS0804	F-white	Gemas	West Africa	West Africa	Ghana	LL050	Cote d'Ivoire*									
MYS0022	S-white	Gemas	East Africa	East Africa	Kenya	LL070	Kenya	Uganda*								
MYS1431	S-tissue	Gemas	East Africa	East Africa	Uganda	LL071	Uganda	Kenya*								
MYS0011	S-white	Gemas	East Africa	East Africa	Tanzania	LL091	Tanzania									
LL shows mtDNA distributed across a broader geographic region																
MYS1157	F-W Afr, W	Gemas	West Africa	West Africa	Ivory Coast	LL015	Mali	Cameroon								
MYS1234	F-white	Gemas	Tridom	West Africa	Ocean	LL022	Ghana	Chad	Cameroon							
MYS1152	F-W Afr, T	Gemas	West Africa	West Africa	Ghana/Ivory Coast	LL022	Ghana	Chad	Cameroon							
MYS1165	F-W Afr, W	Gemas	West Africa	West Africa	Ghana	LL022	Ghana	Chad	Cameroon							
MYS1351	F-W Afr, W	Gemas	West Africa	West Africa	Ivory Coast	LL022	Ghana	Chad	Cameroon							
MYS1367	F-W Afr, W	Gemas	West Africa	West Africa	Ivory Coast	LL022	Ghana	Chad	Cameroon							
MYS1317	F-W Afr, B	Sungkai	West Africa	West Africa	Ghana	LL022 (1 mismatch)	Ghana	Chad	Cameroon							
MYS0341	S-letter/#	Sungkai	East Africa	East Africa	Tanzania	LL027	Tanzania	Uganda	DRC	Kenya						
MYS0026	S-tissue	Gemas	East Africa	East Africa	Uganda	LL027	Tanzania	Uganda	DRC	Kenya						
MYS0074	S-tissue	Gemas	East Africa	East Africa	Tanzania	LL027	Tanzania	Uganda	DRC	Kenya						
MYS0450	S-tissue	Gemas	East Africa	East Africa	Tanzania	LL027	Tanzania	Uganda	DRC	Kenya						
MYS0803	S-tissue	Gemas	East Africa	East Africa	South Sudan	LL027	Tanzania	Uganda	DRC	Kenya						
MYS0648	F-W Afr, B	Sungkai	West Africa	West Africa	Togo	LL045 (2 mismatches)	Ghana									
MYS0945	F-W Afr, B	Sungkai	West Africa	West Africa	Ghana	LL046	Ghana	Mali								
MYS0458	F-W Afr, W	Gemas	West Africa	West Africa	Ghana	LL046	Ghana	Mali								
MYS1307	F-W Afr, W	Gemas	West Africa	West Africa	Ghana	LL046	Ghana	Mali								
MYS0005	S-burn	Sungkai	East Africa	East Africa	Tanzania	LL062	Tanzania	Kenya	Uganda	South Africa	Eritrea	Botswana	Namibia	Zimbabwe	Rwanda	DRC
MYS0028	S-letter/#	Sungkai	East/Southern Africa	East/Southern Africa	Mozambique	LL062	Tanzania	Kenya	Uganda	South Africa	Eritrea	Botswana	Namibia	Zimbabwe	Rwanda	DRC
MYS0048	S-letter/#	Sungkai	East Africa	East/Southern Africa	Tanzania	LL062	Tanzania	Kenya	Uganda	South Africa	Eritrea	Botswana	Namibia	Zimbabwe	Rwanda	DRC
MYS0179	S-letter/#	Sungkai	East Africa	East/Southern Africa	Kenya	LL062	Tanzania	Kenya	Uganda	South Africa	Eritrea	Botswana	Namibia	Zimbabwe	Rwanda	DRC
MYS0400	S-letter/#	Sungkai	East/Southern Africa	East/Southern Africa	Malawi/Mozamibique	LL062	Tanzania	Kenya	Uganda	South Africa	Eritrea	Botswana	Namibia	Zimbabwe	Rwanda	DRC
MYS0153	S-tissue	Gemas	East Africa	East/Southern Africa	Tanzania	LL062	Tanzania	Kenya	Uganda	South Africa	Eritrea	Botswana	Namibia	Zimbabwe	Rwanda	DRC
MYS0371	S-tissue	Gemas	East Africa	East/Southern Africa	Tanzania	LL062	Tanzania	Kenya	Uganda	South Africa	Eritrea	Botswana	Namibia	Zimbabwe	Rwanda	DRC
MYS0449	S-tissue	Gemas	East/Southern Africa	East/Southern Africa	Mozambique	LL062	Tanzania	Kenya	Uganda	South Africa	Eritrea	Botswana	Namibia	Zimbabwe	Rwanda	DRC
MYS0476	S-tissue	Gemas	East Africa	East/Southern Africa	Kenya	LL062	Tanzania	Kenya	Uganda	South Africa	Eritrea	Botswana	Namibia	Zimbabwe	Rwanda	DRC
MYS0644	S-tissue	Gemas	East Africa	East/Southern Africa	Kenya	LL062	Tanzania	Kenya	Uganda	South Africa	Eritrea	Botswana	Namibia	Zimbabwe	Rwanda	DRC
MYS1586	S-tissue	Gemas	East/Southern Africa	East/Southern Africa	Mozambique	LL062	Tanzania	Kenya	Uganda	South Africa	Eritrea	Botswana	Namibia	Zimbabwe	Rwanda	DRC
MYS0012	S-white	Gemas	East Africa	East/Southern Africa	Uganda	LL062	Tanzania	Kenya	Uganda	South Africa	Eritrea	Botswana	Namibia	Zimbabwe	Rwanda	DRC
MYS0044	S-white	Gemas	East Africa	East/Southern Africa	Kenya	LL062	Tanzania	Kenya	Uganda	South Africa	Eritrea	Botswana	Namibia	Zimbabwe	Rwanda	DRC
MYS0482	S-white	Gemas	East/Southern Africa	East/Southern Africa	Zambia	LL062	Tanzania	Kenya	Uganda	South Africa	Eritrea	Botswana	Namibia	Zimbabwe	Rwanda	DRC
MYS0013	S-tissue	Gemas	Tridom	East/Southern Africa	DRC	LL062	Tanzania	Kenya	Uganda	South Africa	Eritrea	Botswana	Namibia	Zimbabwe	Rwanda	DRC
MYS0177	S-letter/#	Sungkai	East Africa	East/Southern Africa	Tanzania	LL062 (one mismatch)	Tanzania	Kenya	Uganda	South Africa	Eritrea	Botswana	Namibia	Zimbabwe	Rwanda	DRC
MYS0043	S-letter/#	Sungkai	East Africa	East/Southern Africa	Tanzania	LL066	Tanzania	Kenya	Botswana	Zimbabwe	South Africa					
MYS0332	S-letter/#	Sungkai	East/Southern Africa	East/Southern Africa	Mozambique	LL066	Tanzania	Kenya	Botswana	Zimbabwe	South Africa					
MYS2305	S-letter/#	Sungkai	East/Southern Africa	East/Southern Africa	Zambia	LL066	Tanzania	Kenya	Botswana	Zimbabwe	South Africa					
MYS0655	S-tissue	Gemas	East Africa	East/Southern Africa	Kenya	LL066	Tanzania	Kenya	Botswana	Zimbabwe	South Africa					
MYS1788	S-tissue	Gemas	East/Southern Africa	East/Southern Africa	Zambia	LL066	Tanzania	Kenya	Botswana	Zimbabwe	South Africa					
MYS0014	S-white	Gemas	East/Southern Africa	East/Southern Africa	Mozambique	LL066	Tanzania	Kenya	Botswana	Zimbabwe	South Africa					
MYS1650	S-white	Gemas	East/Southern Africa	East/Southern Africa	Zambia	LL066	Tanzania	Kenya	Botswana	Zimbabwe	South Africa					
MYS0099	S-letter/#	Sungkai	East Africa	East/Southern Africa	Zambia	LL066 (with a deletion)	Tanzania	Kenya	Botswana	Zimbabwe	South Africa					
MYS0394	S-letter/#	Sungkai	East Africa	East/Southern Africa	Tanzania	LL066 (with a deletion)	Tanzania	Kenya	Botswana	Zimbabwe	South Africa					
MYS2257	S-letter/#	Sungkai	East/Southern Africa	East/Southern Africa	Malawi	LL066 (with a deletion)	Tanzania	Kenya	Botswana	Zimbabwe	South Africa					
MYS0998	S-tissue	Sungkai	East/Southern Africa	East/Southern Africa	Mozambique	LL066 (with a deletion)	Tanzania	Kenya	Botswana	Zimbabwe	South Africa					
MYS0110	S-white	Gemas	East Africa	East/Southern Africa	Tanzania	LL066 (with a deletion)	Tanzania	Kenya	Botswana	Zimbabwe	South Africa					
MYS0199	S-white	Gemas	East Africa	East/Southern Africa	Tanzania	LL066 (with a deletion)	Tanzania	Kenya	Botswana	Zimbabwe	South Africa					
MYS0636	S-tissue	Gemas	East Africa	East/West Africa	Kenya	LL069	Kenya	Cameroon	Uganda							
MYS0451	S-white	Gemas	East Africa	East/West Africa	Southern Sudan	LL069	Kenya	Cameroon	Uganda							

Notes: *In the first section of the table, a single asterisk indicates countries that border the country assigned using nuclear short tandem repeat (STR) loci; **Results are reported by *Loxodonta* localizer (LL) haplotype number; tusks with the same closest LL sequence are boxed together. Gray indicates sample(s) for which the 16 STR assignment reported is unclear. CAR is the Central African Republic; DRC is the Democratic Republic of Congo; ROC is the Republic of Congo. LL refers to the *Loxodonta* Localizer mtDNA-based results. The 16 STR (short tandem repeat) loci results were reported in Cerling et al. 2016; Proc Natl Acad Sci U S A 113(47):13330.