

Report

Sourcing elephant ivory from a 16th century

Portuguese shipwreck

Alida de Flamingh¹, Ashley Coutu^{2, 3, 4,*}, Judith Sealy², Shadreck Chirikure^{2, 5}, Armanda D.S. Bastos⁶, Nzila M. Libanda-Mubusisi⁷, Ripan S. Malhi^{1,8,9} & Alfred L. Roca^{1,9,10,11,*}

¹Program in Ecology, Evolution and Conservation Biology, University of Illinois at Urbana-Champaign (UIUC), Urbana IL, 61801, USA

²Department of Archaeology, University of Cape Town, Cape Town, 7700, RSA

³Pitt Rivers Museum, University of Oxford, Oxford, OX1 3PP, UK

⁴BioArCh, University of York, York, YO10 5DD, UK

⁵School of Archaeology, 1 South Parks Road, Oxford, OX1 3TG, UK

⁶Department of Zoology and Entomology, University of Pretoria, Pretoria, 0002, RSA

⁷National Museum of Namibia, Robert Mugabe Avenue, Windhoek, Namibia

⁸Department of Anthropology, UIUC, Urbana IL, 61801, USA

⁹Carl R. Woese Institute for Genomic Biology, UIUC, Urbana IL, 61801, USA

¹⁰Department of Animal Sciences, UIUC, Urbana IL, 61801, USA

¹¹Lead contact

*Correspondence:

ashley.coutu@prm.ox.ac.uk (AC) & roca@illinois.edu (ALR)

SUMMARY

The oldest known shipwreck in southern Africa was found in Namibia in 2008 [1–4]. Forty tons of cargo, including gold and silver coins, helped identify the ship as the *Bom Jesus*, a Portuguese *nau* (trading vessel) lost in 1533 while headed to India [4–6]. The cargo included >100 elephant tusks [7], which we examined using paleogenomic and stable isotope analyses. Nuclear DNA identified the ivory source as African forest (*Loxodonta cyclotis*) rather than savanna (*Loxodonta africana*) elephants. Mitochondrial sequences traced them to West and not Central Africa, and from ≥ 17 herds with distinct haplotypes. Four of the haplotypes are known from modern populations, others were potentially lost to subsequent hunting of elephants for ivory. Stable isotope analyses ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) indicated that the elephants were not from deep rainforests, but from savanna and mixed habitats. Such habitats surround the Guinean forest block of West Africa [8], and accord with the locations of major historic Portuguese trading ports [9,10]. West African forest elephants currently range into savanna habitats [11–13]; our findings suggest that this was not consequent to regional decimation of savanna elephants for their ivory in the 19th and 20th centuries. During the time of the *Bom Jesus*, ivory was a central driver in the formation of maritime trading systems connecting Europe, Africa, and Asia. Our integration of paleogenomic, archeological and historical methods to analyze the *Bom Jesus* ivory provides a framework for examining vast collections of archaeological ivories around the world, in shipwrecks and other contexts.

Keywords:

African forest elephant, *Loxodonta cyclotis*, maritime archeology, maritime history, mitochondrial genomes, paleogenomics, shipwreck cargo, species identification, stable carbon

isotopes, stable nitrogen isotopes

RESULTS AND DISCUSSION

Ivory on the *Bom Jesus* derived from forest elephants in West Africa

The shipwreck and its contents are well preserved (Figure 1) [1], and we were able to extract DNA successfully from 44 of 62 (71%) tusks available. Using targeted amplicon sequencing on the shipwreck ivory DNA, we examined three short unlinked chromosome segments for single nucleotide polymorphisms (SNPs) fixed between African savanna (*Loxodonta africana*) and African forest (*L. cyclotis*) elephants [14]. The character states detected were always diagnostic of forest and never of savanna elephants (Table S1). This indicated that the ivory derived from forest elephants which likely ranged across the entire Guinean and Congolian tropical forest blocks of West and Central Africa ([15]; Figure 2).

Female elephants remain with natal social groups (“herds”) in relatively restricted geographic ranges. Thus mitochondrial (mt)DNA, which is maternally transmitted, can often identify the geographic provenance of elephants [16,17]. A ~436 bp region of D-loop mtDNA was amplified for 44 shipwreck ivory samples (Table S2). African elephant mtDNA is geographically structured into 8 subclades [18](Figure 3A; [18]). In a network with published sequences representative of these 8 subclades (Figure 3B; Table S2; [17]), 23 of the 44 ivory samples grouped with the Western mtDNA subclade, carried only by elephants from West Africa. The other 21 grouped within the West-central subclade, carried by elephants in both West and Central Africa (Figure 3A). For 17 of 44 shipwreck ivory samples, complete mitogenomes were assembled (see STAR methods). Each of the 17 carried a distinct mitogenome haplotype, and when compared to 11 published mitogenomes (Table S2), the shipwreck ivory grouped with elephants from West Africa (Figure 3D).

Each mtDNA subclade consists of distinctive sequence haplotypes, many with very limited geographic distributions [18]. A 336 bp segment of the mtDNA D-loop control region of the shipwreck ivory was compared to the 37 published mtDNA sequences within the West-central (n=29) and Western (n=8) mtDNA subclades (Table S2; Figure 3C). The ivory exactly matched 3 haplotypes reported only from West Africa, with one exact match to a haplotype found in both West and Central Africa, and no matches to haplotypes carried exclusively by Central African elephants (Figure 3C). Additionally, 16 novel geographically-referenced elephant samples collected across Africa in the late 20th century were sequenced for the same mtDNA region as the ivory (Table S2). The shipwreck ivory haplotypes grouped with haplotypes of the newly sequenced elephants that were from West Africa (Figure S1).

The shipwreck ivory samples lacked haplotypes from Northern-savanna, Savanna-wide and Southeast-savanna subclades, present only among savanna elephants (see Figure 3A), consistent with the *Bom Jesus* ivory being exclusively from forest elephants. The shipwreck ivory carried no haplotypes from the North-central, East-central or South-central subclades, which would almost certainly have been present among tusks harvested in Central Africa (Figure 3A). Thus, all lines of evidence consistently indicated a West African forest elephant origin for all of the shipwreck ivory. The West African origin for the tusks also accords with historical records. Raw and carved ivories were exported from the Atlantic coast of Africa to Portugal from the mid-15th century [19,20]. Of three major regions believed to export ivory to Portugal [1,3,21], our genetic findings were consistent with an origin in Senegambia or the Gulf of Guinea (Figure 2) but rule out the Loango coast in Central Africa [22].

The ivory derived from forest elephants outside of deep rainforests

To further examine the geographic source(s) of the ivory, carbon and nitrogen stable

isotope ratios were measured (successfully for 97 of 100 tusks available) to determine the diets and habitats of the elephants. Stable carbon isotope ratios measured in ivory originate from the plant food the elephant consumed, which in turn is an indication of their habitats. Ivory has more positive $\delta^{13}\text{C}$ values than plant food due to further partitioning of carbon isotopes during digestion and tissue synthesis, with a diet-tissue enrichment of $\sim 5.5\text{‰}$ reported in modern and fossil proboscideans [23]. Published $\delta^{13}\text{C}_{\text{collagen}}$ for wild African elephants range from -27‰ for pure C_3 feeders in deep rainforests to -11‰ for savannah elephants consuming substantial quantities of C_4 grass [36, 44, 46, 49-50]. In the shipwreck ivory collagen, $\delta^{13}\text{C}$ values averaged $-20.4 \pm 1.2\text{‰}$, with a range from -22.2 to -17.1‰ (Figure 4; Table S3). Shipwreck ivory $\delta^{13}\text{C}$ values are more positive than those of modern elephants from Cameroon, Democratic Republic of Congo, and Liberia, which have ^{13}C -depleted values reflecting pure C_3 diets in deep forest/rainforest habitats with continuous tree canopy [24–27]. Shipwreck samples with the most positive $\delta^{13}\text{C}$ values fall within the range of modern comparative samples from open or shrub savanna environments, where elephants consume substantial proportions of C_4 grasses [28–30].

Variation of ^{15}N in elephants is driven primarily by nitrogen cycling in the soil, which is strongly influenced by moisture availability [31–34]. Published $\delta^{15}\text{N}_{\text{collagen}}$ values for wild African elephants range from 2‰ in moist areas to 17‰ in arid areas [29,30,35–40]. In the shipwreck ivory, $\delta^{15}\text{N}$ values averaged $6.8 \pm 0.8\text{‰}$, with a range from 4.8 to 9.0‰ (Fig. 4). Such values typically derive from mesic terrestrial environments, falling in the middle of the range of $\delta^{15}\text{N}$ values documented for African elephants, and encompassing the values for elephants living in habitats in Angola, Benin, Burkina Faso, Chad, Central African Republic, and Niger. The shipwreck ivory $\delta^{15}\text{N}$ are lower than those (above 10‰) of elephants in open, arid grassland savanna environments (Angola, Namibia, South Africa), or living in dense forests (Democratic

Republic of Congo) [28,30].

Values for the shipwreck ivory extend over 46% of the total variation in $\delta^{13}\text{C}$ and 49% of the total variation in $\delta^{15}\text{N}$ across African elephants (Figure 4). The broad range suggests that the ivory was sourced from elephants from different habitats with different rainfall regimes and vegetation, although not from deep rainforests or arid environments. For four tusks, we took additional incremental samples to examine within-individual dietary variation over time (Figure S2). The $\delta^{13}\text{C}$ values from the incremental sampling for all four of these tusks are indicative of elephants that lived in mixed savannah and woodland habitats during the time the tusks were growing. The longest sequence derives from tusk B6082: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values show a cyclical pattern of increasing and decreasing over a series of 22 growth increments, indicative of switching between C_3 browse and C_4 graze in the dry and wet seasons, respectively [28,29,41,42]. The range of $\delta^{13}\text{C}$ values (6‰) in this tusk is consistent with a habitat outside of the deep tropical forest, for which seasonal variation in $\delta^{13}\text{C}$ is typically $\leq 2\text{‰}$ [43,44].

The sourcing of ivory from West Africa to Portugal

In West Africa, dense tropical lowland rainforests of the Guinean forest block are surrounded by dry forest and thicket, and a mosaic of forest and savannah habitats (Figure 2; 39). Based on combined genetic and isotope analyses, the elephants hunted for the ivory cargo of the *Bom Jesus* originated from West African forest elephants in habitats outside the Guinean forest block. In 1482, the Portuguese built a fort at an established trading post in West Africa, São Jorge de Mina, or Elmina, on the south-western edge of the Dahomey (or Benin) Gap [9,10]. This is a long-established [42] area of savannah and drier type forest vegetation which provided an important corridor for transporting goods (including ivory) from the interior to the coast, thus avoiding travel through dense forest. Settlements near the Dahomey Gap then expanded as

139 Elmina became an important entrepôt.

140 Due to the difficulty of maneuvering large long-distance trading vessels and the dangers
141 of sailing close to the shore, outgoing ships on the India route typically did not tack along the
142 West African coastline, but would sail from Portugal southwest across the Atlantic and then
143 southeast on the trade winds [21,46]. Ivory from West Africa was frequently shipped to the
144 islands of Cape Verde and São Tomé, to be counted, weighed, and sent via smaller vessels to
145 Casa da Índia in Lisbon, the central clearing house for African and Indian imports to Portugal
146 [22,46–48]. Centralized loading of outgoing long-distance trading vessels (*naus*) in Lisbon
147 enabled tight control of the valuable cargo. Although the Great Lisbon earthquake and fire of
148 1755 destroyed many of the archives of the Casa da Índia, the consolidation of ivory from
149 different localities within West Africa accords with our findings: the range of isotope values
150 suggests multiple different habitats; as does the presence of 17 distinct mitogenome haplotypes,
151 indicating that the *Bom Jesus* ivory derived from at least 17 different “herds” of elephants.

152 **Implications for the ecology and conservation of West African elephants**

153 In West Africa, the historic range of savanna elephants was likely continuous across the
154 Sahelian/Sudanian savanna habitat belts north of the Guinean forest block [15]. The current
155 distributional range of forest elephants includes habitats both in tropical forest and in nearby
156 savannas [11–13,49]. Before our analyses, the recent distribution of forest elephants in West
157 Africa outside of tropical forest habitats could be attributed to decimation of savanna elephants
158 in West Africa in the 19th and 20th centuries [50,51]. Our combined genetic and isotope results
159 instead indicate that utilization of savanna habitats by forest elephants in West Africa preceded
160 the removal of savanna elephants, and dates back to at least the 16th century.

161 The *Bom Jesus* tusks are of varying length and size (from 2–33 kg), and the elephants

may have been hunted indiscriminately, both males and females, young and old alike [1,7]. Among the mtDNA haplotypes identified from the 16th century tusks, only four have been reported among contemporary populations (Figure 3C), likely reflecting the impact of the ivory trade and reduction of historic elephant range by 93% in West Africa [52]. Decrease in population size and genetic diversity are associated with negative conservation outcomes, such as expression of deleterious alleles, reduced reproductive fitness and increased risk of population extirpation [53]. Our geo-referenced sequences from modern elephants in the UCT collection, and the newly sequenced shipwreck ivory sequences, add substantially to the previously small body of isotopic and genetic information for West African elephants, with the potential to aid in the sourcing of confiscated illegal ivory [39,54]. Improving the ability to trace poached ivory can help guide optimal allocation of scarce law enforcement resources.

CONCLUSION

The *Bom Jesus* was one of 80 vessels that sailed from Lisbon on the India route between 1531 and 1540 [55]. This was one of the most strategic and lucrative commercial routes of the time; linking to established trading networks between Africa and Asia [56,57]. The large number of tusks recovered from the *Bom Jesus* is evidence of ivory acquisition and circulation driving the formative stages of globalization [58]. With a resolution not possible using any single approach, our interdisciplinary methodologies revealed the long-term genetic diversity and habitat use of the African forest elephant, helpful for conserving this iconic species [44]. To refine the sourcing of archeological and historical ivory, future work can utilize the combination of genetic and isotope methods presented here, and additional approaches in both fields as they are further developed.

Unworked elephant tusks, ivory working debris and finished objects made from ivory have been recovered from numerous archaeological contexts worldwide, including but not limited to shipwrecks with ivory cargo reported from the Mediterranean Sea and the Atlantic, Pacific, and Indian Oceans [59–63]. Our methods are applicable to the vast collections of historic and archaeological ivories in museums across the globe [64–66]. Analyzing historic and archaeological ivories affords a window into human-animal relationships across thousands of years, and can reveal the formative and changing patterns of exchange between people who lived oceans apart [30,67–71]. Our study on the largest archaeological cargo of African ivory ever found provides a framework for examining one of the world’s most important raw materials throughout human history.

ACKNOWLEDGMENTS:

We thank the Government of Namibia, Namibia National Museum, Namibia De Beers, Bruno Werz, Dieter Noli (shipwreck); Esther Goagoses, Fousy Kambombo, Henry Nakale, Dawid Kapule, Virimuje Kahuure, Eliot Mowa (sampling); Catherine Sole, Christian Pirk, Stokana Mahapa (UP sequencing); Madeline Zhu, Ian Newton and John Lanham (isotopes). Funding: USFWS African Elephant Conservation Fund AFE-1816-F18AP00819 (20th century sample sequencing; ALR); South African Research Chairs Initiative of the National Research Foundation and Department of Science and Technology of South Africa grant no 84407 (isotopes; JS); NRF UID78566 (UP facilities); USDA ILLU 875–952 and ILLU-538-939, PEEC and Clark Research Support Grants (AdeF). Claude Leon Foundation and the European Union (FP7- IOF -332165 –TEMBo; AC).

AUTHOR CONTRIBUTIONS:

Conception: ADF, AC, JS, SC, RSM, ALR. Samples and facilities: AC, JS, SC, ADSB, NMLB, RSM; Shipwreck curation: NMLB. Analyses: ADF, AC. Interpretation: ADF, AC, JS, SC, ADSB, RSM, ALR. Drafting: ADF, AC, JS, with contributions from all.

DECLARATION OF INTERESTS:

The authors declare no competing interests.

MAIN-TEXT FIGURE LEGENDS:

Figure 1. The *Bom Jesus* shipwreck cargo.

Top: Gold 10-*cruzado* coins (cross insignia) minted under the reign of King João III of Portugal in 1525, and withdrawn in the 1530s, helped to date and identify the ship [4]. Bottom: The shipwreck cargo included more than 100 unworked elephant tusks. (Images: Amy Toensing; National Geographic Image Collection license.)

Figure 2. Terrestrial vegetation types of West Africa.

West African terrestrial vegetation types (color-coded) include dense tropical lowland rainforests and mosaics of forests, as well as savannas [45]. In the early 16th century, Portuguese merchants traded at ports (red circles) along the West African coast (4): 1-Ceuta; 2-Azemour; 3-Safi; 4-Arguim & Cape Blanco; 5-Cape Verde; 6-Bezeguiche (Dakar), 7-Joal, 8-Sutuco & Gambia River; 9-Elmina (São Jorge de Mina); 10-São Tomé and Príncipe. Inset map shows Lisbon, and the shipwreck site in Namibia.

Figure 3. The shipwreck ivory originated in West Africa.

(A) African elephant mtDNA groups into 8 well-supported subclades (color-coded) [18]. (B) The shipwreck ivory mtDNA grouped with only the West-central subclade and Western subclades--dashed lines show distributions in (A) [18]. Subclades common only among savanna elephants or central African forest elephants were not detected in the shipwreck ivory. (C) Shipwreck ivory carried mtDNA haplotypes found only in West Africa (blue) or in both Central (orange) and West Africa, but never haplotypes found only in Central Africa. (D) For 17 of the ancient shipwreck ivory samples, complete mitogenomes grouped with modern elephants from West Africa. Bootstrap values are shown (asterisk indicates 100%). Panel A is modified from [18] (permission: <https://creativecommons.org/licenses/by-nc/3.0/legalcode>). Median joining network (B and C) cross-hatches indicate mutational differences; circles represent haplotypes.

Figure 4. Shipwreck ivory $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, with reference samples from elephants across Africa (33, 41, 43; Table S3 and S4).

Orange open icons represent elephants in drier, open savanna habitats; green open icons represent closed canopy forest habitats. Black icons represent elephants from mesic shrub and wooded savanna environments; the shipwreck ivory (black filled circles) cluster with these. The broad range of values for the shipwreck ivory suggests sourcing from multiple locations with different rainfall and vegetation patterns.

STAR*METHODS

RESOURCE AVAILABILITY

254 ***Lead Contact***

255 Further information and requests for resources and reagents should be directed to and will
256 be fulfilled by the Lead Contact, ALR (roca@illinois.edu)

257 ***Materials Availability***

258 This study did not generate new unique reagents.

259 ***Data and Code Availability***

260 Novel mitogenome sequences, ivory D-loop sequences, and 20th century reference
261 sample sequences generated during this study are available in the NCBI Sequence Read Archive
262 (mitogenomes) and GenBank (ivory D-loop and 20th century reference sequences). Mitogenome
263 bam files can be found under BioProject PRJNA668700 as part of the NCBI Sequence Read
264 Archive (<https://www.ncbi.nlm.nih.gov/sra/PRJNA668700>), ivory D-loop sequences can be
265 found under GenBank accession numbers MT576485-MT576528, and 20th century reference
266 sample sequences can be found under GenBank accession numbers MW115961 - MW115976.
267 Sequences generated in this manuscript were from ancient DNA templates, which are subject to
268 the effects of DNA damage (Figure S3).

269 Previously published reference sequences are available from GenBank (see Table S2 for
270 GenBank accession numbers).

271 The nuclear DNA SNP data generated during this study are available in Table S1.

272 The stable carbon and nitrogen isotope data generated during this study are available in Tables
273 S3 and S4.

274

275 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

276 ***Shipwreck ivory samples***

The elephant ivory samples were exported under Namibian export heritage permit
07/2013 and CITES permits 166360 and 152037.

METHOD DETAILS

DNA extraction

DNA was extracted from 62 ancient ivory samples using an ancient DNA extraction protocol previously developed and optimized by the Malhi Ancient DNA Laboratory [72] at the Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign (UIUC). All shipwreck ivory DNA extractions were conducted in this facility in a laboratory dedicated to the analysis of ancient DNA. DNA was extracted from 20th century geo-referenced samples from the University of Cape Town (UCT) collection in a dedicated Bio-Safety Level 2 laboratory at the University of Pretoria (UP), South Africa. The UP laboratory was decontaminated with DNA-off prior to the initiation of the project, and the entire laboratory was UV sterilized on a daily basis using a UV ceiling light for at least 1 hour. Using swabs, we sampled multiple surfaces in the laboratory and none of the surface samples yielded PCR amplicons for elephant mtDNA. No samples other than the 20th century historical reference samples were processed in this laboratory for the duration of this project. PCR amplification of both the shipwreck ivory and the 20th century historical reference samples was carried out in laboratories that were isolated from other laboratories in which DNA extractions were carried out. To ensure that external or cross-sample contamination was avoided, extraction and PCR-negative controls were included with each round of sample processing, and not more than 8 samples were processed at any one time.

Mitochondrial DNA sequencing

A 436 bp fragment was amplified for 44 of 62 available ivory samples, and for 16 twentieth-century geographically-referenced samples from the UCT collection, using published mtDNA D-loop primers *Laf CR1* and *Laf CR2* [73]. Genomic libraries were constructed for 17 ancient ivory samples using the NEBNext® Ultra II™ DNA Library Prep kit and NEBNext® Multiplex Oligos (Unique Dual Indexes) for Illumina®. Libraries were pooled and shotgun sequenced on a HiSeq 4000 and NovaSeq 6000 platform at the UIUC Core Sequencing Facility.

Single nucleotide polymorphisms for nuclear DNA gene regions

To determine the species identity of the elephants from which the tusks had been harvested, we amplified short nDNA regions from three genes (*BGN*, *PHK*, *PLP*) that contain single nucleotide polymorphisms (SNPs) that show fixed character state differences between forest and savanna elephants [14] following the amplification procedure described in [14,72] (Table S1) and using primers *BGN-s2F-M13F*, *BGN-s2R2-M13R*, *PHK-s1F-M13F*, *PHK-s1R-M13R*, *PHK-s2F-M13F*, *PHK-s2R2-M13R*, *PLP-s1F-M13F* and *PLP-s1R2-M13R*.

Stable carbon and nitrogen isotope analysis

Elephant tusks grow continuously and incrementally [74]. To average possible seasonal and annual variation, we removed small pieces of ivory extending across multiple growth layers. Sampling attempted to minimise damage to the tusks. In the laboratory, sample surfaces were cleaned by sanding with a Dremel hand drill fitted with an emery disc. Collagen was extracted by demineralising in 0.3M HCl at room temperature for several days to 2 weeks, then rinsed with distilled water to neutrality. Acid was changed every few days. Samples were soaked in 0.1M NaOH overnight to remove base soluble contaminants and again rinsed with distilled water to neutrality. The samples were then put into pH 3, 0.01M HCl and heated to 70° C for 48 hours to denature the collagen ('gelatinization'), then filtered through 60-90 µm Ezee® filters and

lyophilized. Dentine collagen was successfully extracted from 97 of 100 shipwreck ivory samples.

We were allowed to sample four tusks incrementally, as these tusks had pieces which were damaged or broken and were therefore amenable to sampling a transverse section of the tusk along multiple growth layers. We sampled these four tusks from transverse sections, with incremental samples taken at every 1mm from the cementum – dentine junction inward towards the pulp cavity, following [29]. Approximately 10 milligrams of powder were drilled from each increment using a Microdrill. Collagen was extracted from each powder by demineralising in 0.3M HCl at room temperature overnight, then rinsing with distilled water to neutrality, centrifuging between rinses. Due to sample size constraints, only a single $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurement was obtained for each powder.

Approximately 0.5 milligrams of each extract were weighed into a tin capsule, placed in a Thermo Flash Elemental Analyser 2000, and combusted at $\sim 1650^\circ\text{C}$. The resultant CO_2 and N_2 gases were passed into a Delta V Plus mass spectrometer for measurement of carbon and nitrogen isotope ratios as well as elemental compositions (%C, %N). The results are expressed in the delta (δ) notation in parts per thousand (‰), relative to the international standards Vienna Pee Dee Belemnite (VPDB) for carbon and Ambient Inhalable Reservoir (AIR) for nitrogen. All samples other than serial samples were run in duplicate and the values averaged. The standard deviation of repeated measurements ($n=40$) of homogeneous standard materials was $\leq 0.2\text{‰}$ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. The $\delta^{13}\text{C}$ values of reference elephant samples have been corrected for depletion of ^{13}C in atmospheric CO_2 since the Industrial Revolution, due to burning of fossil fuels, to enable direct comparison with the shipwreck archaeological samples [75].

QUANTIFICATION AND STATISTICAL ANALYSIS

Mitochondrial DNA D-loop analyses

Mitochondrial D-loop sequences were compared to those of eight previously reported African elephant mtDNA subclades [18]. We downloaded three 4258-base pair (bp) reference sequences for each of the eight mtDNA subclades from the original dataset used by [18], aligned our shorter 436 bp sequences to the longer reference sequences using the program MUSCLE [76], and trimmed the sequences so that only regions present in both the reference sequences and the ivory sequences were included in the final alignment. The alignment was then used to construct a median-joining network in the software POPART (Population Analysis with Reticulate Trees) [77] that compared shipwreck ivory to mtDNA subclade reference sequences (Figure 3B), to novel twentieth-century geographically-referenced and published mtDNA sequences from West and Central African elephants (Figure S1). The genomic libraries used to assemble complete mitogenomes were constructed using multiple DNA extractions from the same samples as those used for the shorter D-loop analysis. For all DNA extractions per sample, the complete mitogenome and D-loop analyses resulted in consistent mtDNA subclade assignments for the sample.

A geographically-referenced database was compiled for haplotypes within the two mtDNA subclades that the ivory grouped with (Western and West-central mtDNA subclades) by downloading previously reported D-loop mtDNA elephant sequences from GenBank and coding them to reflect the geographic origin (West Africa or Central Africa) of those elephants. We aligned [76] the 436 bp ancient ivory sequences to the geographically-referenced database (336 bp), and trimmed the data so that only overlapping regions were present in the final alignment. The alignment was then used to infer a pairwise-distance based median-joining network [77]

(Figure 3C). It is important to note that some studies reporting reference sequences failed to list the frequency at which each haplotype was observed. We encourage future researchers to report both the haplotype sequence as well as the frequency at which haplotypes were observed in the population. However, the current study sought to determine only whether the ivory matched geographically-referenced haplotypes from West or from Central Africa, and thus the frequency at which haplotypes were observed in populations was not of concern.

Mitogenome analyses

All bioinformatic analyses were performed using the Biocluster2 supercomputer of the Carl R. Woese Institute for Genomic Biology. Reads were de-multiplexed and trimmed using AdapterRemoval [78] to have a minimum sequence length of 25 bp. Reads were aligned to the assembled African elephant genome (*Loxodonta africana* assembly Loxafr 3.0) and to a published forest elephant mitogenome [79] using bowtie2 [80] with the local alignment option, and capping fragment length at 1000 bp. Aligned sequences were transformed to BAM format in SAMtools v. 1.1 [81]. Using SAMtools, BAM files were filtered to remove unmapped reads and reads with a quality score less than 30, were sorted and indexed, and PCR duplicates were removed. Consensus sequences were generated from the de-duplicated alignment files in Geneious R7 (www.geneious.com) using a minimum read coverage of 3X and the “Highest Quality” algorithm that takes the relative residue quality into account when building majority consensus sequences. We were able to reconstruct mitogenomes for 17 ivory samples with 2.5-36.55 X average coverage (Table S2), and annotated them using the program GeSeq [82].

Ancient DNA damage patterns were verified by aligning trimmed reads to the African forest elephant mitogenome with BWA [83] and quantifying damage in mapDamage2 [84] using a fragment size of 90 bp. The shipwreck ivory DNA showed damage patterns typical of ancient

DNA (Figure S3) with increased nucleotide misincorporations towards the terminal ends of the DNA molecules.

Consensus sequences of ancient ivory mitogenomes were compared to 11 previously published African elephant mitogenomes (Table S2) by inferring a maximum likelihood (ML) tree in RaXML [85]. Jmodeltest [86] indicated that the GTRGAMMA substitution model best fit the data. GTRGAMMA was therefore used in ML analysis which was repeated 1000 times with 100 bootstrap iterations for each run. The best ML tree was identified using the rapid bootstrapping algorithm in RaXML [85] and FigTree [87] was used to visualize the tree using a mid-point root. These reference sequences included published complete mitogenomes from two woolly mammoths (*Mammuthus primigenius*) with GenBank accession numbers EU155210 and EU153449 [88], two African savanna elephants (*Loxodonta africana*) with Genbank accession numbers NC000934 [89] and AB443879 [90], and seven African forest elephants (*Loxodonta cyclotis*) with Genbank accession numbers KY616976, KY616978-9, KJ557423-4, JN673263-4 [79,91,92].

GIS data for cartography

We used ArcMap (ESRI 2011, ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute) to create a geographic map of West Africa using the World Countries (Generalized) layer from ESRI and the UNESCO/UNEP vegetation layers [45]. Trading post locations are indicated with current country names (Figure 2).

LEGENDS FOR SUPPLEMENTARY INFORMATION:

Supplementary figures

Figure S1. A median-joining haplotype network (based on pairwise distances between haplotypes) compares shipwreck ivory mtDNA reference sequences (ivory colored) to newly sequenced 20th century reference samples (of known geographic origin) from the University of Cape Town collection (brown with black vertical stripes), and the 8 mtDNA subclades reported by Ishida et al. (2013). The shipwreck ivory haplotypes grouped with the haplotypes reported for 20th century elephants from West Africa, and not within mtDNA subclades common among savanna or central African forest elephants. Branch length is proportional to the number of mutational differences (indicated as cross-hatches) between haplotypes, circle size is related to the number of individuals carrying a haplotype.

Figure S2. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from successive growth increments for four of the shipwreck tusks (B6082, B6015, B6051, B6021) to explore variation in isotope values within one individual elephant. These four tusks were sampled from transverse sections, with incremental samples taken radially at every 1mm from the cementum – dentine junction inward towards the pulp cavity, following [29]. $\delta^{13}\text{C}$ values measured in the four tusks ranged from -24.0 to -17.1‰ and $\delta^{15}\text{N}$ values from 5.7 to 8.3‰. Sample B6082 is the only tusk with enough measurements to see cyclical patterns related to seasonal switching between browse and graze in dry and wet seasons, respectively, as documented in modern and historic African elephant populations that live in mixed savanna and woodland habitats [28,29]. Dashed vertical lines on the graph for B6082 suggest possible yearly cycles, which we have associated with the dips in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values at approximately every 6 incremental samples. This would mean that each incremental sample for B6082 represents approximately 8 weeks of growth and that all 22 samples together record approximately 176 weeks or 3.4 years of growth. If we apply this growth rate to the other three

tusks, it would explain why we do not see seasonal, cyclical patterns as we do for B6082: there are too few samples to record variation over multiple years of growth.

Figure S3. The shipwreck ivory DNA shows damage patterns characteristic of ancient DNA. The fragment misincorporation plots illustrated above are for a single individual (B6059 belonging to the Western mtDNA subclade – see Figure 1D) and are typical of the damage patterns observed in all of the ancient ivory samples. The smaller top four panels show the base frequency outside and inside the read sequence (the open grey box indicates the read span), and the bottom two plots are the base positions of substitutions from the 5' (left) and the 3' end (right). The bottom plot shows C to T substitutions in red, G to A substitutions in blue, and all other substitutions in grey. This figure was produced using the program mapDamage2 [84].

Supplementary tables

Table S1. Species diagnostic single nucleotide polymorphisms (SNPs) in regions of the nuclear genes *BGN*, *PHK*, and *PLP* which were sequenced in shipwreck ivory samples matched SNP character states found in African forest elephants (*Loxodonta cyclotis*). Species diagnostic SNPs are described by Ishida et al. (2011).

Table S2. Elephant mitochondrial sequences used in analyses.

Table S3. Shipwreck ivory UCT laboratory number, National Museum of Namibia sample number, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values as well as collagen quality indicators: %N, %C and C:N (atomic) ratios. Collagen extracts in this study had C:N ratios ranging from 3.1 to 3.4, with %C (by

weight) from 33.5 to 44.7 and %N from 11.8 to 16.1. C:N ratios between 2.9 and 3.6 indicate well preserved collagen [93,94]. In the last column, “x” indicates samples for which mtDNA was analyzed.

Table S4. Unpublished twentieth century reference samples from the University of Cape Town collections, including $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values as well as collagen quality indicators: %N, %C and C:N (atomic) ratios. In the last column, “x” indicates samples for which mtDNA was analyzed.

REFERENCES

1. Chirikure, S., Sinamai, A., Goagoses, E., Mubusisi, M., and Ndoro, W. (2010). Maritime archaeology and trans-oceanic trade: a case study of the Oranjemund shipwreck cargo, Namibia. *J. Marit. Archaeol.* 5, 37–55.
2. Hauptmann, A., Schneider, G., and Bartels, C. (2016). The shipwreck of Bom Jesus, AD 1533: fugger copper in Namibia. *J. African Archaeol.* 14, 184–207.
3. Mowa, E. (2018). Oranjemund shipwreck ivory: historical analysis on the prospective geographic origin. *Eur. Cent. Res. Train. Dev. UK* 6, 48–69. Available at: <internal-pdf://228.60.152.103/pnas-new.bst>.
4. Werz, B.E.J.S. (2010). Sub-saharan Africa’s oldest shipwreck: historical-archeological research of an early modern-era Portuguese merchantman on the Namibian coast. *Mar. Mirror* 96, 430–442.
5. Xavier, M. (1989). *Relações da carreira da Índia* (Publicações Alfa).
6. Academia Das Ciências De Lisboa (1979). *Memoria Das Armadas Que De Portugal*

- 485 Passaram à Índia (Lisboa: Academia Das Ciências.).
- 486 7. Alves, F.J.S. (2011). The 16th century Portuguese shipwreck of Oranjemund, Namibia.
487 Rep. Mission. Carried out by Port. Team 2008 2009.
- 488 8. Whyte, F. (1983). The vegetation of Africa (Unesco, Paris).
- 489 9. Fritze, R.H. (2002). New worlds: the great voyages of discovery, 1400-1600 (Praeger
490 Publishers).
- 491 10. Blake, J.W. (1942). Europeans in West Africa 1450-1560, printed for the Hakluyt Society,
492 Vol. 1. I (London, 1942).
- 493 11. Grubb, P., Groves, C.P., Dudley, J.P., and Shoshani, J. (2000). Living African elephants
494 belong to two species: *Loxodonta africana* (Blumenbach, 1797) and *Loxodonta cyclotis*
495 (Matschie, 1900). Elephant 2, 3.
- 496 12. Groves, C.P., and Grubb, P. (2000). Do *Loxodonta cyclotis* and *L. africana* interbreed?
497 Elephant 2, 4.
- 498 13. Mondol, S., Moltke, I., Hart, J., Keigwin, M., Brown, L., Stephens, M., and Wasser, S.K.
499 (2015). New evidence for hybrid zones of forest and savanna elephants in Central and
500 West Africa. Mol. Ecol. 24, 6134–6147.
- 501 14. Ishida, Y., Demeke, Y., van Coeverden de Groot, P.J., Georgiadis, N.J., Leggett, K.E.A.,
502 Fox, V.E., and Roca, A.L. (2011). Distinguishing forest and savanna African elephants
503 using short nuclear DNA sequences. J. Hered. 102, 610–616.
- 504 15. Frade, F. (1955). Orde des Proboscidiens (Proboscidea Illiger, 1811). In Triaté de
505 Zoologie: Anatomie, Systématique, Biologie (ed P.-P. Grassé). Paris: Masson & Cie.
- 506 16. Archie, E.A., Hollister-Smith, J.A., Poole, J.H., Lee, P.C., Moss, C.J., Maldonado, J.E.,
507 Fleischer, R.C., and Alberts, S.C. (2007). Behavioural inbreeding avoidance in wild

- 508 African elephants. *Mol. Ecol.* 16, 4138–4148.
- 509 17. Fishlock, V., and Lee, P.C. (2013). Forest elephants: fission–fusion and social arenas.
510 *Anim. Behav.* 85, 357–363.
- 511 18. Ishida, Y., Georgiadis, N.J., Hondo, T., and Roca, A.L. (2013). Triangulating the
512 provenance of African elephants using mitochondrial DNA. *Evol. Appl.* 6, 253–265.
- 513 19. Afonso, L.U., and da Silva Horta, J. (2013). Afro-Portuguese Olifants with hunting scenes
514 (c. 1490-c. 1540). *Mand. Stud.*, 79–97.
- 515 20. Thornton, J.K. (1998). *Africa and Africans in the making of the Atlantic world, 1400-
516 1800* (Cambridge University Press).
- 517 21. Newitt, M. (2010). *The Portuguese in West Africa, 1415–1670: a documentary history
518* (Cambridge University Press).
- 519 22. Soares, M. de C. (2017). “Por conto e peso”: o comércio de marfim no Congo e Loango,
520 séculos XV–XVII. *An. do Mus. Paul. História e Cult. Mater.* 25, 59–86.
- 521 23. Cerling, T.E., Harris, J.M., and Leakey, M.G. (1999). Browsing and grazing in elephants:
522 the isotope record of modern and fossil proboscideans. *Oecologia* 120, 364–374.
- 523 24. Cerling, T.E., Hart, J.A., and Hart, T.B. (2004). Stable isotope ecology in the Ituri Forest.
524 *Oecologia* 138, 5–12.
- 525 25. Bonafini, M., Pellegrini, M., Ditchfield, P., and Pollard, A.M. (2013). Investigation of the
526 ‘canopy effect’ in the isotope ecology of temperate woodlands. *J. Archaeol. Sci.* 40, 3926–
527 3935.
- 528 26. Van der Merwe, N.J., and Medina, E. (1991). The canopy effect, carbon isotope ratios and
529 foodwebs in Amazonia. *J. Archaeol. Sci.* 18, 249–259.
- 530 27. CILSS - Comité Permanent Inter-états de Lutte contre la Sécheresse dans le Sahel (2016).

- 531 Landscapes of West Africa—A window on a changing world.
- 532 28. Cerling, T.E., Wittemyer, G., Ehleringer, J.R., Remien, C.H., and Douglas-Hamilton, I.
533 (2009). History of animals using isotope records (HAIR): a 6-year dietary history of one
534 family of African elephants. *Proc. Natl. Acad. Sci.* *106*, 8093–8100.
- 535 29. Codron, J., Codron, D., Sponheimer, M., Kirkman, K., Duffy, K.J., Raubenheimer, E.J.,
536 Mélice, J.-L., Grant, R., Clauss, M., and Lee-Thorp, J.A. (2012). Stable isotope series
537 from elephant ivory reveal lifetime histories of a true dietary generalist. *Proc. R. Soc. B*
538 *Biol. Sci.* *279*, 2433–2441.
- 539 30. Coutu, A.N., Lee-Thorp, J., Collins, M.J., and Lane, P.J. (2016). Mapping the elephants of
540 the 19th century East African ivory trade with a multi-isotope approach. *PLoS One* *11*,
541 e0163606.
- 542 31. Amundson, R., Austin, A.T., Schuur, E.A.G., Yoo, K., Matzek, V., Kendall, C., Uebersax,
543 A., Brenner, D., and Baisden, W.T. (2003). Global patterns of the isotopic composition of
544 soil and plant nitrogen. *Global Biogeochem. Cycles* *17*.
- 545 32. Aranibar, J.N., Otter, L., Macko, S.A., Feral, C.J.W., Epstein, H.E., Dowty, P.R., Eckardt,
546 F., Shugart, H.H., and Swap, R.J. (2004). Nitrogen cycling in the soil–plant system along
547 a precipitation gradient in the Kalahari sands. *Glob. Chang. Biol.* *10*, 359–373.
- 548 33. Murphy, B.P., and Bowman, D.M.J.S. (2006). Kangaroo metabolism does not cause the
549 relationship between bone collagen $\delta^{15}\text{N}$ and water availability. *Funct. Ecol.* *20*, 1062–
550 1069.
- 551 34. Murphy, B.P., and Bowman, D.M.J.S. (2009). The carbon and nitrogen isotope
552 composition of Australian grasses in relation to climate. *Funct. Ecol.* *23*, 1040–1049.
- 553 35. Van der Merwe, N.J., Lee-Thorp, J.A., Thackeray, J.F., Hall-Martin, A., Kruger, F.J.,

- 554 Coetzee, H., Bell, R.H. V, and Lindeque, M. (1990). Source-area determination of
555 elephant ivory by isotopic analysis. *Nature* 346, 744.
- 556 36. Vogel, J.C., Talma, A.S., Hall-Martint, A.J., and Viljoen, P.J. (1990). Carbon and nitrogen
557 isotopes in elephants. *Am. J. Psychiat* 142, 163–170.
- 558 37. Heaton, T.H.E., Vogel, J.C., von La Chevallierie, G., and Collett, G. (1986). Climatic
559 influence on the isotopic composition of bone nitrogen. *Nature* 322, 822.
- 560 38. Ishibashi, H., Takeuchi, T., Whyte, I., and Koike, H. (1999). $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$
561 measurements from the African elephant, *Loxodonta africana*, used for ivory sourcing.
- 562 39. Ziegler, S., Merker, S., Streit, B., Boner, M., and Jacob, D.E. (2016). Towards
563 understanding isotope variability in elephant ivory to establish isotopic profiling and
564 source-area determination. *Biol. Conserv.* 197, 154–163.
- 565 40. Cerling, T.E., Omondi, P., and Macharia, A.N. (2007). Diets of Kenyan elephants from
566 stable isotopes and the origin of confiscated ivory in Kenya. *Afr. J. Ecol.* 45, 614–623.
- 567 41. Coutu, A.N. (2019). Historic molecules connect the past to modern conservation. In *The*
568 *Oxford Handbook of Historical Ecology and Applied Archaeology*, Daryl Stump and
569 Christian Isendahl, ed. (Newcastle University), pp. 208–225.
- 570 42. Codron, J., Kirkman, K., Duffy, K.J., Sponheimer, M., Lee-Thorp, J.A., Ganswindt, A.,
571 Clauss, M., and Codron, D. (2013). Stable isotope turnover and variability in tail hairs of
572 captive and free-ranging African elephants (*Loxodonta africana*) reveal dietary niche
573 differences within populations. *Can. J. Zool.* 91, 124–134.
- 574 43. Coutu, A.N. (2012). Tracing the links between elephants, humans, and landscapes during
575 the nineteenth-century East African ivory trade: A bioarchaeological study. *Azania*
576 *Archaeol. Res. Africa* 47, 242.

- 577 44. Coutu, A.N. (2019). Historic Molecules Connect the Past to Modern Conservation. In The
578 Oxford Handbook of Historical Ecology and Applied Archaeology, Daryl Stump and
579 Christian Isendahl, ed. (Newcastle University), pp. 208–225.
- 580 45. White, F. (1983). The vegetation of Africa: a descriptive memoir to accompany the
581 UNESCO/AETFAT/UNSO vegetation map of Africa by F White Natural Re. (Unesco,
582 Paris).
- 583 46. de Castro, F.V. (2005). The pepper wreck: a Portuguese Indiaman at the mouth of the
584 Tagus river (Texas A&M University Press).
- 585 47. Garfield, R. (1992). A history of São Tomé Island, 1470-1655: the key to Guinea (Edwin
586 Mellen Pr).
- 587 48. de Alencastro, L.F. (2007). The Economic Network of Portugal's Atlantic World',
588 Francisco Bethencourt en Diogo Ramada Curto. Port. Ocean. Expans. 1400-1800, 109–
589 137.
- 590 49. Tchamba, M.N., and Seme, P.M. (1993). Diet and feeding behaviour of the forest elephant
591 in the Santchou Reserve, Cameroon. Afr. J. Ecol. 31, 165–171.
- 592 50. Michelmores, F., Beardsley, K., Barnes, R.F.W., and Douglas-Hamilton, I. (1994). A
593 model illustrating the changes in forest elephant numbers caused by poaching. Afr. J.
594 Ecol. 32, 89–99.
- 595 51. Maisels, F., Strindberg, S., Blake, S., Wittemyer, G., Hart, J., Williamson, E.A., Aba'a,
596 R., Abitsi, G., Ambahe, R.D., and Amsini, F. (2013). Devastating decline of forest
597 elephants in Central Africa. PLoS One 8, e59469.
- 598 52. Roth, H.H., and Douglas-Hamilton, I. (1991). Distribution and status of elephants in West
599 Africa. Mammalia 55, 489–528.

- 600 53. Allendorf, F.W., Luikart, G., and Aitken, S.N. (2013). Conservation and the genetics of
601 populations 2nd Editio. (West Sussex, UK: Blackwell Publishing, Wiley and Sons).
- 602 54. Wasser, S.K., Torkelson, A., Winters, M., Horeaux, Y., Tucker, S., Otiende, M.Y., Sitam,
603 F.A.T., Buckleton, J., and Weir, B.S. (2018). Combating transnational organized crime by
604 linking multiple large ivory seizures to the same dealer. *Sci. Adv.* 4, eaat0625.
- 605 55. Schwartz SB (2007). Portuguese oceanic expansion, 1400-1800 C. D. The Economy of the
606 Portuguese Empire. In: Bethencourt F, ed. (Cambridge: Cambridge University Press; pp.
607 19–48).
- 608 56. Chirikure, S. (2014). Land and sea links: 1500 years of connectivity between southern
609 Africa and the Indian Ocean rim regions, AD 700 to 1700. *African Archaeol. Rev.* 31,
610 705–724.
- 611 57. Wynne-Jones, S., and Laviolette, A. (2017). *Swahili World* (Taylor & Francis Limited).
- 612 58. Chaiklin, M. (2010). Ivory in world history—early modern trade in context. *Hist. Compass*
613 8, 530–542.
- 614 59. de Santiago, R. (2016). *Across Three Oceans: Shipwrecks as Early Modern Globalism*.
- 615 60. Lane, P.J. (2012). Maritime and shipwreck archaeology in the western Indian Ocean and
616 southern Red Sea: An overview of past and current research. *J. Marit. Archaeol.* 7, 9–41.
- 617 61. Tripathi, S. (2015). *Shipwrecks Around the World: Revelations of the Past* (Delta Book
618 World).
- 619 62. Tripathi, S., and Godfrey, I. (2007). Studies on elephant tusks and hippopotamus teeth
620 collected from the early 17th century Portuguese shipwreck off Goa, west coast of India:
621 Evidence of maritime trade between Goa, Portugal and African countries. *Curr. Sci.*, 332–
622 339.

- 623 63. Green, J. (2015). The wreck of the VOC'retourschip Zeewijk': An archaeological and
624 historical puzzle. *J. Australas. Inst. Marit. Archaeol.* 39, 9.
- 625 64. Lane, P.J. (2015). Introduction: archaeological ivories in a global perspective.
- 626 65. Coutu, A.N. (2015). The elephant in the room: mapping the footsteps of historic elephants
627 with big game hunting collections. *World Archaeol.* 47, 486–503.
- 628 66. Good, C., Tyrrell, P., Zhou, Z., and Macdonald, D.W. (2019). Elephants never forget,
629 should art museums remember too? Historic ivory collections as ambassadors for
630 conservation education. *Biodivers. Conserv.* 28, 1331–1342.
- 631 67. Steguweit, L. (2015). Rotten ivory as raw material source in European Upper Palaeolithic.
632 *Quat. Int.* 361, 313–318.
- 633 68. Guérin, S.M. (2010). Avorio d'ogni ragione: the supply of elephant ivory to northern
634 Europe in the Gothic era. *J. Mediev. Hist.* 36, 156–174.
- 635 69. Seaver, K.A. (2009). Desirable teeth: the medieval trade in Arctic and African ivory. *J.*
636 *Glob. Hist.* 4, 271–292.
- 637 70. Coutu, A.N., Whitelaw, G., le Roux, P., and Sealy, J. (2016). Earliest evidence for the
638 ivory trade in southern Africa: Isotopic and ZooMS analysis of seventh–tenth century AD
639 ivory from KwaZulu-Natal. *African Archaeol. Rev.* 33, 411–435.
- 640 71. Gillman, M.E. (2017). A tale of two ivories: elephant and walrus. *Espac. Tiempo y Forma.*
641 *Ser. VII, Hist. del Arte*, 81–105.
- 642 72. Cui, Y., Lindo, J., Hughes, C.E., Johnson, J.W., Hernandez, A.G., Kemp, B.M., Ma, J.,
643 Cunningham, R., Petzelt, B., and Mitchell, J. (2013). Ancient DNA analysis of mid-
644 holocene individuals from the Northwest Coast of North America reveals different
645 evolutionary paths for mitogenomes. *PLoS One* 8, e66948.

- 646 73. Nyakaana, S., and Arctander, P. (1999). Population genetic structure of the African
647 elephant in Uganda based on variation at mitochondrial and nuclear loci: evidence for
648 male-biased gene flow. *Mol. Ecol.* 8, 1105–1115.
- 649 74. Fisher, D.C., Cherney, M.D., Newton, C., Rountrey, A.N., Calamari, Z.T., Stucky, R.K.,
650 Lucking, C., and Petrie, L. (2014). Taxonomic overview and tusk growth analyses of
651 Ziegler Reservoir proboscideans. *Quat. Res.* 82, 518–532.
- 652 75. Hellevang, H., and Aagaard, P. (2015). Constraints on natural global atmospheric CO₂
653 fluxes from 1860 to 2010 using a simplified explicit forward model. *Sci. Rep.* 5, 17352.
- 654 76. Edgar, R.C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high
655 throughput. *Nucleic Acids Res.* 32, 1792–1797.
- 656 77. Leigh, J.W., and Bryant, D. (2015). Popart: full-feature software for haplotype network
657 construction. *Methods Ecol. Evol.* 6, 1110–1116.
- 658 78. Lindgreen, S. (2012). AdapterRemoval: easy cleaning of next-generation sequencing
659 reads. *BMC Res. Notes* 5, 337.
- 660 79. Brandt, A.L., Ishida, Y., Georgiadis, N.J., and Roca, A.L. (2012). Forest elephant
661 mitochondrial genomes reveal that elephantid diversification in Africa tracked climate
662 transitions. *Mol. Ecol.* 21, 1175–1189.
- 663 80. Langmead, B., and Salzberg, S.L. (2012). Fast gapped-read alignment with Bowtie 2. *Nat.*
664 *Methods* 9, 357.
- 665 81. Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis,
666 G., and Durbin, R. (2009). The sequence alignment/map format and SAMtools.
667 *Bioinformatics* 25, 2078–2079.
- 668 82. Tillich, M., Lehwark, P., Pellizzer, T., Ulbricht-Jones, E.S., Fischer, A., Bock, R., and

669 Greiner, S. (2017). GeSeq—versatile and accurate annotation of organelle genomes.
670 Nucleic Acids Res. 45, W6–W11.

671 83. Li, H., and Durbin, R. (2010). Fast and accurate long-read alignment with Burrows–
672 Wheeler transform. Bioinformatics 26, 589–595.

673 84. Jónsson, H., Ginolhac, A., Schubert, M., Johnson, P.L.F., and Orlando, L. (2013).
674 mapDamage2. 0: fast approximate Bayesian estimates of ancient DNA damage
675 parameters. Bioinformatics 29, 1682–1684.

676 85. Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-
677 analysis of large phylogenies. Bioinformatics 30, 1312–1313.

678 86. Darriba, D., Taboada, G.L., Doallo, R., and Posada, D. (2012). jModelTest 2: more
679 models, new heuristics and parallel computing. Nat. Methods 9, 772.

680 87. Rambaut, A., and Drummond, A. (2009). FigTree v1. 3.1 (Institute of Evolutionary
681 Biology, University of Edinburgh).

682 88. Gilbert, M.T.P., Drautz, D.I., Lesk, A.M., Ho, S.Y.W., Qi, J., Ratan, A., Hsu, C.-H., Sher,
683 A., Dalén, L., and Götherström, A. (2008). Intraspecific phylogenetic analysis of Siberian
684 woolly mammoths using complete mitochondrial genomes. Proc. Natl. Acad. Sci. 105,
685 8327–8332.

686 89. Hauf, J., Waddell, P.J., Chalwatzis, N., Joger, U., and Zimmermann, F.K. (2000). The
687 complete mitochondrial genome sequence of the African elephant (*Loxodonta africana*),
688 phylogenetic relationships of Proboscidae to other mammals, and D-loop heteroplasmy.
689 ZOOLOGY-JENA- 102, 184–195.

690 90. Murata, Y., Yonezawa, T., Kihara, I., Kashiwamura, T., Sugihara, Y., Nikaido, M.,
691 Okada, N., Endo, H., and Hasegawa, M. (2009). Chronology of the extant African

elephant species and case study of the species identification of the small African elephant with the molecular phylogenetic method. *Gene* 441, 176–186.

91. Meyer, M., Palkopoulou, E., Baleka, S., Stiller, M., Penkman, K.E.H., Alt, K.W., Ishida, Y., Mania, D., Mallick, S., and Meijer, T. (2017). Palaeogenomes of Eurasian straight-tusked elephants challenge the current view of elephant evolution. *Elife* 6, e25413.
92. Finch, T.M., Zhao, N., Korkin, D., Frederick, K.H., and Eggert, L.S. (2014). Evidence of positive selection in mitochondrial complexes I and V of the African elephant. *PLoS One* 9, e92587.
93. Ambrose, S.H. (1990). Preparation and characterization of bone and tooth collagen for isotopic analysis. *J. Archaeol. Sci.* 17, 431–451.
94. Van Klinken, G.J. (1999). Bone collagen quality indicators for palaeodietary and radiocarbon measurements. *J. Archaeol. Sci.* 26, 687–695.
95. Debruyne, R. (2005). A case study of apparent conflict between molecular phylogenies: the interrelationships of African elephants. *Cladistics* 21, 31–50.
96. Debruyne, R., Van Holt, A., Barriel, V., and Tassy, P. (2003). Status of the so-called African pygmy elephant (*Loxodonta pumilio* (Noack 1906)): phylogeny of cytochrome b and mitochondrial control region sequences. *C. R. Biol.* 326, 687–697.
97. Eggert, L.S., Rasner, C.A., and Woodruff, D.S. (2002). The evolution and phylogeography of the African elephant inferred from mitochondrial DNA sequence and nuclear microsatellite markers. *Proc. R. Soc. London. Ser. B Biol. Sci.* 269, 1993–2006.
98. Nyakaana, S., Arctander, P., and Siegmund, H.R. (2002). Population structure of the African savannah elephant inferred from mitochondrial control region sequences and nuclear microsatellite loci. *Heredity (Edinb)*. 89, 90.