**[For SUPPLEMENTARY MATERIAL]**

**Historical and archaeogenomic identification of high-status Englishmen at Jamestown, Virginia**

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These supplementary materials provide detailed data related to the bioarchaeological and genetic analysis of the two skeletons referenced in the main article. OSM1 presents the results of previously conducted bioarchaeological investigations of the two skeletons. These studies followed standard data collection practices (Buikstra & Ubelaker 1994) and included isotopic testing following methods described by France *et al.* (2019) and Ubelaker & Owsley (2003). The heavy metal content in the bones was measured using inductively coupled plasma-mass spectrometry (ICP-MS) techniques as described in Little *et al*. (2014). Details of the process of genomic assessment are presented in OSM2 with Table S3 providing expanded information for both individuals.

**OSM1: Field and lab observations of JR2992C and JR170C**

**JR2992C**

This burial was second from the north end of the Chancel of the 1608 James Fort church (Givens *et al*. 2016). Although in use by 1608, recent research suggests church construction began in the winter of 1607 (Delano 2023). An adult male was buried in a supine position in an anthropomorphic coffin with the head in the west-end of the grave. This is one of only two excavated Jamestown burials in this style of coffin that has a box-like compartment framing the head, and is wider at the shoulders and narrower at the feet. The southernmost burial (JR170C) had the same style of coffin. The only associated artefacts were large nails used for the coffin’s construction. The cranium was face-up and slightly inclined to the right. The arms were positioned along the sides of the body. Both forearms had slight pronation and hand bones were on the sides of the pelvis. The knees and ankles were close together.

The individual is not markedly robust. The crown to heel measurement was 1.70m; breadth estimated at the shoulders was 0.34m; at elbows 0.46m; across the knees 0.20m; and across the ankles 0.16m.

Bone preservation was poor. The skeleton was represented by a broken cranium, including a nearly complete frontal, complete parietals, a nearly complete occipital, temporals, and partial maxillae. A nearly complete mandible was also present. The postcranial skeleton was partially represented by a right clavicle, fragments of the innominates, mostly complete shafts of the humeri, the proximal end of the right ulna diaphysis, a small fragment of the right radius, femora missing their necks and distal fourths, a left tibia missing its proximal end and distal third, a right tibia shaft that is mostly complete but missing its ends, and the distal third of the right fibula. At least three cervical vertebrae were recovered (C1, C2, C4), along with fragments of thoracic and lumbar vertebrae. Bones of the feet were present, but severely degraded.

The right maxillary dental arcade was complete, and all teeth were present in their sockets except the central incisor tooth and socket, which were not preserved. The right lateral incisor had one hypoplastic line. The right maxillary first molar had a periodontal abscess linked to complete carious destruction of the right second molar crown. A small perforation in the floor of right maxillary sinus indicated this abscess had ruptured into the sinus. The left maxillary dentition was represented by four loose teeth and four teeth in their sockets. The left first premolar had an interproximal carious lesion. Adjoining surfaces of the left second and third molars each had cervical carious lesions with root involvement. The mandibular dentition was complete except for antemortem loss of the left first premolar; its socket has resorbed. The right mandibular third molar had a small carious lesion. Moderate calculus deposits were present with slight to moderate alveolar resorption, most notably for the right maxillary molar.

*Age*

Several features suggested an age of 35 to 39 years. The endocranial and ectocranial sutures were mostly obliterated. Only the coronal suture exhibited slight retention of the suture line on the outer table. Shallow pacchionian depressions (or arachnoid granulations) were present on the inner table. The largest depression on the left parietal, near the sagittal suture, measured 4.3mm by 5.6mm in diameter and 1.8mm in depth. Slight meningeal artery impressions were visible on the inner table. Vascular channels were present on the ectocranial surface of the frontal. Tooth wear was moderate. Slight osteophyte formation was visible on the superior and inferior endplates of the cervical vertebrae. The femoral heads exhibited compact cancellous bone. The distal joint of the left first metatarsal had slight to moderate arthritic lipping.

*Sex*

Features consistent with a male sex included a pronounced external occipital protuberance and large mastoid processes. The femora were also robust with well-defined lineae aspera.

*Stable isotope testing results*

Fragments of the cranium, left humerus, a right metatarsal, as well as the right maxillary third molar, were selected for isotope and heavy metals analysis (Tables S1 & S2). Smithsonian Museum Conservation Institute (MCI) Mass Spectrometry Laboratory followed these procedures for δ13C and δ15N sample processing:

Samples were run on a Thermo Delta V Advantage mass spectrometer in continuous flow mode coupled to a Costech 4010 Elemental Analyser (EA) via a Thermo Conflo IV. All calculations of raw isotope values were performed with Isodat 3.0 software. Approximately 0.5mg of tissue were weighed, packed into tin capsules, and introduced to the EA via a Costech Zero Blank Autosampler. Once the N2 and CO2 molecules were separated, sample gases and reference gas were introduced into the mass spectrometer and regulated by the Conflo IV. The CO2 peaks were quantitatively diluted by about 75% for animal tissues. All runs included a set of standards for every 10–12 samples. Standards include Costech Acetanilide and a urea (Urea-UIN3) standard, both of which are calibrated to USGS40 (L-glutamic acid) and USGS41 (L-glutamic acid). All standards were run with the same parameters and procedures as samples. Raw isotope values were corrected using a 2-point linear correction on the calibrated Costech Acetanilide and urea standards. The weight %N and weight %C values were calculated using a peak area calibration based on the homogeneous Costech Acetanilide standard. Reproducibility of standards was ≤0.2‰ (1σ) for both δ13C and δ15N. The error associated with all sample data points was ±0.2‰.

*taken from*: δ13C and δ15N Basic Mass Spec Procedures and Parameters (Instrument #1, Costech EA-IRMS),Smithsonian MCI Stable Isotope Mass Spectrometry Laboratory, Christine France 06/15/2015

**Table S1. Stable carbon (collagen) isotope values\*.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Burial**  **ID** | **Laboratory** | **Year of analysis** | **Sample** | **Collagen**  **yield (%)** | **δ13C**  **collagen (‰)** | **Weight % C** | **C:N** |
| **JR2992C** | Paleo-Isochem | 2014 | humerus | 0.6 | -18.1 | 43.8 | 3.3 |
| 2015 | cranial fragments | 0.7 | -19.0 | 6.1 | 3.5 |
| Smithsonian Museum Conservation Institute | 2014 | maxillary third molar | 3.2 | -19.6 | 36.7 | 3.5 |
| **JR170C** | Paleo-Isochem | 2014 | tibia and fibula fragments | 1.4 | -19.7 | 31.3 | 3.6 |
| Smithsonian Museum Conservation Institute | 2014 | mandibular first molar | 12.9 | -19.6 | 37.7 | 3.3 |

**\***Foradditionaldata on sample preparation see France (2015).

**Table S2. ICP-MS testing (Smithsonian Museum Conservation Institute). Values are in parts per million.**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Burial ID** | **Sample description** | **Cu** | **Zn** | **As** | **Rb** | **Sr** | **Cd** | **Ba** | **Pb** |
| JR2992C | right metatarsal fragments | 4.86 | 292 | 0.71 | 2.37 | 329 | 0.33 | 310 | 147.3 |
| JR170C | left tibia fragments | 3.53 | 148 | 0.42 | 1.99 | 289 | 0.25 | 155 | 126.8 |

\*Foradditionaldata on sample preparation see Little *et al*. (2014).

**JR170C**

The southern burial in the 1608 Church chancel was disturbed by an east-west boundary ditch that was filled in by the 1660s (Givens *et al*. 2016). The ditch overlaid the upper body and legs and damaged the pelvis. Centuries later, in 1938, a hand-dug utility trench severely damaged the skeleton’s right side, removing the right femur and shaving off the anterior left half of the skull. The anterior surface of the left patella was also shaved off.

The individual was buried supine in an east-west aligned anthropomorphic-shaped coffin with the head to the west. The head primarily rested on its right side with the chin down. The iron coffin nails clearly define the anthropomorphic shape of the coffin’s head end. The nails were large and match those found with JR2992C.

The lateral profile of the cranium was defined during the excavation with only the posterior half of the left mandible well-preserved. Several loose teeth were present. A small portion of the distal right radius and ulna were present alongside the torso, indicating placement of the right arm along the side of the body. The left humerus was positioned along the skeleton’s side, as was the left ulna. The left radius was displaced when digging the utility trench.

Clearly visible in the vicinity of the distal left humerus and ribs were black stains. The discoloration reflected the presence of silver thread, the fragments of which were visible on the arm. A metal detector survey indicated that they are present over the chest, as were tiny, silver spangles. The chest region was removed *en block* for laboratory excavation. Following block removal and prior to laboratory excavation, the soil from the chest region was scanned using Xradia Versa 520 X-ray computed tomography instrumentation by Cornell University’s Biotechnology Resource Center. The non-destructive 3D-imaging technique recorded in detail the minute silver threads and spangles within the soil matrix (https://historicjamestowne.org/archaeology/chancel-burials/founders/william-west/). These were part of a bundled sash or scarf positioned over the chest of this individual.

The right femur was missing, and the left femur had postmortem breakage. Left hand proximal phalanges were resting on the anterior surface of the proximal left femur. The left patella was resting on the femur’s distal metaphysis. The anterior surface of the left patella was shaved off by the excavation of the utility trench. The tibiae and fibulae were present with the knees and ankles closely spaced.

Crown to heel in situ measurement length was 1.74m; shoulder breadth was 0.38m; leg length was 0.91m (Lt), and the femur length was 0.48m (Lt). The breadth across the knees as measured from the outsides of the tibiae metaphyses was 0.17m. The outside breadth at the ankles was 0.17m. This is the tallest individual of the four chancel men.

The skeleton removed from the field was highly fragmented and poorly preserved. However, the long bone cortices were not as degraded as observed for the other three burials. This condition may be a product of the individual’s younger age and thicker cortical bone. Vertebrae were present, but partially represented by centra and neural arches. Thoracic vertebrae eleven and twelve, and lumbar vertebrae one through five, were represented by articular facets only. Only one rib fragment was present. No joint surfaces were present for any of the long bones, except for the metatarsals and partial left femoral head. Green cuprous staining, confirmed using XRF analysis, was present on the internal surface of the right ilium.

All right maxillary teeth were present. The right first molar had a small occlusal carious lesion. No apical abscesses were present. The maxillary teeth showed slight wear. The left side maxillary dentition was represented by a loose third molar.

The mandible was nearly complete with postmortem breakage. The right mandibular dentition was represented by the canine, first premolar, and first, second and third molars. The right second premolar was lost antemortem and the alveolar socket had remodeled with slight posterior drift of the first premolar and slight medial drift of the first molar. Trace occlusal wear was noted, with the exception of the first molar, which had beginning dentin exposure. The left mandibular dentition was represented by the second premolar, and first, second and third molars. The anterior sockets were present but empty due to postmortem tooth loss.

*Age*

This skeleton was the youngest of the four chancel burials. An age of 22 to 25 years was based on tooth wear and tooth root formation. The left maxillary third molar had Apex-1/2 formation of the mesial buccal root. This opening could also be a remnant opening retained into the 20s. However, tooth wear was slight with no wear noted on the third molars. The C1 dens facet had a sharp margin. The C2 dens facet had no lipping. The cervical and first thoracic epiphyseal rings were fused. Remnant lines of union were still visible for some of the cervical epiphyseal rings. The cervical vertebrae had no arthritic changes. Epiphyses were fused where observations of epiphyseal formation were possible (e.g. left distal fibula and distal tibia). The partially preserved right auricular surface exhibited subtle retention of surface billowing at the superior margin. A left ilium fragment exhibited fusion of the iliac crest. The cortical bone was dense, and cortical surfaces were smooth. Cancellous bone spacing was compact (e.g. the left proximal tibia and left proximal and distal femur exhibited compact cancellous bone). The left femur and tibia had smooth external cortices, with only initial formation of longitudinal striae. The linea aspera, although defined, lacked rugosity that is more characteristic of older adults. No arthritic changes were observed on the calcanei, tali or left first metatarsal.

*Sex*

The mandible was large. The gonial angle was acute and the body was thick with a square mental eminence. The long bones were robust and large. The right auricular surface was not raised. All features were consistent with a male identification.

*Pathology*

The L4 exhibited an incomplete fracture along the inferior marginal rim of the left inferior facet. A piece of the rim measuring 7.7mm (transverse) by 4.5mm (superior-inferior) was partially detached, leaving a line of separation that had subsequently remodeled.

*Stable isotope testing results*

Fragments of left tibia and a left mandibular first molar were tested for isotopes and heavy metals (Tables S1 and S2).

**OSM2: Ancient DNA methods**

*Data generation*

Following UV irradiation and surface removal with a sanding disk to remove any contamination on the surface, ~75mg of bone powder was sampled from a petrous temporal of both individuals using a Dremel drill. Individual JR170C required additional sampling from a tooth extracted from the mandible due to extremely poor DNA preservation in the petrous. No analysable DNA was generated from the original petrous sample for this individual; therefore results are only reported from the second round of sampling (Table S3).

DNA extraction was performed according to a previously described protocol (Rohland *et al.* 2018) using binding buffer D. Library preparation was performed using either a single or double stranded preparation method with either no (Meyer & Kircher 2010; Kircher 2012) or partial- uracil–DNA–glycosylase (UDG) treatment (Rohland *et al.* 2015; Gansauge *et al.* 2020). Targeted enrichment capture was used to enrich for the entire mitochondrial genome and ~1.2 million sites in the nuclear genomes that are informative for population genetic analyses (Fu *et al.* 2013, 2015; Haak *et al.* 2015; Mathieson *et al.* 2015). Sequencing was performed on an Illumina NextSeq500 sequencer with 2×75 cycles in addition to 2×7 indexing cycles.

Sequencing reads were then merged by individual, matching the seven base pair indices at the 5’ and 3’ ends of each molecule and allowing for no more than one mismatch per index/barcode. Sequencing adapters were removed using the tool SeqPrep (https://github.com/jstjohn/SeqPrep). Paired end reads were then merged, requiring a minimum of 15 base pair overlaps, with up to one mismatch. Next, sequence reads were aligned to the Reconstructed Sapiens Reference Sequence (RSRS) mitochondrial genome (Behar *et al.* 2012) or to the hg19 human reference genome, using samse in BWA (v0.6.1) (Li & Durbin 2009). Duplicate sequences were then removed by filtering out reads with identical start and end positions, orientation and barcode pairs, retaining only the highest quality sequence for each duplicate. Since there was not sufficient coverage to call diploid genotypes, pseudo-haploid genotypes were called at each single nucleotide polymorphism (SNP) position for the ~1.2 million SNP positions targeted during enrichment capture. At each position, a randomly chosen sequence covering each targeted site was selected to represent the pseudo-haploid genotype after stripping either two or ten bases at each end of the molecule, for libraries prepared using a partial UDG or no UDG treatment, respectively, and restricting to sites with reads having a minimum mapping quality (MAPQ>10) and base quality (>20).

The ratio of Y-chromosome to X-chromosome sequences was used to determine molecular sex (Skoglund *et al.* 2013). Close genetic relatives (third degree or closer) were also identified using an approach that measures the relative rate of allele sharing between individuals, as described in Supplementary Information 6 in Olalde *et al.* (2019).

Haplogrep2 (Weissenstiner *et al.* 2016) was used to determine mitochondrial haplogroups. The mitochondrial consensus sequence was generated from reads aligning to the RSRS mitochondrial genome by trimming either ten or two base pairs from the terminal ends of each read for libraries that underwent no damage correction (i.e. ‘minus’) or partial damage correction (i.e. ‘half’ or ‘USER’), respectively. Haplogroup assignments and quality scores are reported in Table S4. Y-chromosome haplogroups were determined following a procedure described in Supplementary Text 5 of Lazaridis *et al.* (2022) that considers all sequences that aligned to the Y-chromosome (Table S5). The Yfull 8.09 tree (https://www.yfull.com/) was used during calling and Y-chromosome haplogroup was denoted using the International Society of Genetic Genealogy notation (version 15.73, http://isogg.org).

Two standard methods were used to assess the authenticity of the sampled ancient DNA. First, contamMix (Fu *et al.* 2013) was used to determine the rate of matching to the consensus sequence in the mitochondrial genome. 97% was considered the minimum acceptable threshold of authenticity. Next, the rate of C-to-T substitutions at the terminal ends of DNA molecules was measured. Thresholds of 10% and 3% were considered to be the minimum threshold for authenticity in libraries prepared using either no or partial-UDG treatment, respectively (Rohland *et al.* 2015). There was not sufficient sequencing coverage to assess the rate of contamination in the X-chromosome for genetically male individuals, as is typically done using the tool ANGSD (Korneliussen *et al.* 2014), which requires a minimum of 200 SNPs sequenced on the X-chromosome.

*Analysis dataset*

The tool mergeit (Patterson *et al.* 2006) was used to combine the newly reported historical data with published data from 68 present-day populations (Patterson *et al.* 2012; Lazaridis *et al.* 2014, 2016; Biagini *et al.* 2019; Jeong *et al.* 2019). The resulting working dataset contained a total of 597 573 SNPs. All subsequent population genetic analyses were performed after restricting the sequence variants to transversion SNP positions to avoid biases caused by the mixed library preparation types.

*Principal component analysis*

Principal component analysis (PCA) was performed using the program *smartpca* (Patterson *et al.* 2006). The historical individuals were projected onto a PCA background produced using data of 1320 present-day individuals from 68 European and Near Eastern populations (Lazaridis *et al.* 2016) to explore their West Eurasian ancestry. Default parameters were used with the settings lsqproject:YES, shrinkmode:YES, and number outlier:0. Additionally, 95% confidence ellipses were calculated for the four historic individuals using ellconf: 0.95.

**Table S3. Expanded information on the ancient DNA analysis.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | | **Mitochondrial DNA** | | | **1.15 million autosomal targets** | | **Library information** | | | | |
| Lab ID | Skeletal element | Genetic sex | Y-chrom. haplogroup | Average coverage | haplogroup | Match to consensus (%) | Average coverage | Unique SNPs | No. libs. | Library ID | Library type\* | Fraction human (shotgun data) | Damage first nucleotide\*\* |
| I2096 | petrous | M | I1 | 17 | H10e | 98.0 ± 1.0% | 0.069 | 76449 | 6 | S4819.E1.L1 | ds.minus | 0.0059 | 0.162 |
| S2096.E1.L1 | ds.half | 0.0008 | n/a |
| S2096.E1.L2 | ss.USER | 0.00159 | 0.267 |
| S2096.E1.L3 | ss.USER | 0.00141 | 0.249 |
| S2096.E1.L4 | ss.USER | 0.00166 | 0.271 |
| S2096.E1.L5 | ss.USER | 0.00139 | 0.27 |
| I4652 | tooth | M | F | 161 | H10e | 99.8 ± 0.1% | 0.011 | 12657 | 3 | S4652.E1.L1 | ds.half | 0.0021 | 0.023 |
| S4652.E1.L4 | ss.USER | 0.00023 | 0.114 |
| S4652.E1.L5 | ss.USER | 0.00028 | 0.116 |

\* Library type: minus = no damage correction, half or USER = damage retained at last position, ds = double- stranded, ss = single-stranded)

\*\* Damage rate in first nucleotide computed on sequences mapping to the human genome

**Table S4. Mitochondrial haplogroup calls and mutations.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Lab ID** | **Assigned mitochondrial haplogroup** | **Quality** | **Mutations missing** | **Mutations present** | **Mutations remaining** |
| I2096 | H10e | 0.9366 | 1438G | 263G 750G 4769G 8860G 14470A 15326G 16093C 16221T | 3106d 16519C |
| I4652 | H10e | 1 |  | 263G 750G 1438G 4769G 8860G 14470A 15326G 16093C 16221T | 310d 311d 312d 313d 3106d 16519C |

**Table S5. Y haplogroup calls and mutations.**

|  |  |  |
| --- | --- | --- |
| **Lab ID** | **I2096** | **I4652** |
| **Assigned Y haplogroup** | I1 | F |
| **Mutations present** | I1:CTS11042(22914378T>C:C),CTS11126(22967741C>G:G),CTS1739(14042842A>G:G),Y1871(8349674C>A:A),Y1872(8349675G>C:C),Z2865(22948591A>G:G) | F:CTS3996(15323154A>G:G),F3512(23729951C>T:T) |
| **Mutations absent** | I-Y7282:Y7283(8873762C>T:C),I-Y18311:Y18316(19281094C>T:C),I-A8585:A8587(15499895A>G:A),I-Y15947:Y15947(6891284C>T:C),I-BY169301:BY170530(17368168G>T:G),I-Y3549:CTS11651(23203680C>G:C),I-Y31032:Y32101(8493063C>A:C),I-Y132292:Y87144(8511720G>A:G),I-PH497:BY86997(13649194G>A:G),I-Y139735:Y139736(16836773C>T:C),I-Y133595:BY34378(16771616C>T:C),I-PH2510:A14166(17314167C>T:C),A14173(23053434G>A:G),I-A5734:Y15882(15778437A>G:A),I-Y23119:Y23126(18163801C>G:C),I-A11537:A11537(17646827G>A:G),I-Y14344:Y14540(24469839G>A:G),I-FT110841:Y43625(21938186A>G:A),I-Y61868:Y36777(7638932C>T:C),I-FGC31738:FGC31738(13676739T>C:T),I-Y17387:Y17703(6636281A>G:A),I-Y7059:Y7060(6952551G>T:G),I-Y11539:BY190333(9384780A>G:A),I-Y29668:A11259(17003176C>T:C),I-Y40258:A6686(16954364C>T:C),I-Y61915:FT244584(22806967C>A:C),I-A13745:A13762(22516842C>T:C),I-FT50952:FT50086(14026457C>A:C),I-A21841:A21845(8524932G>A:G),I-Y73200:BY87414(13660615G>A:G),I-Y36041:A17347(9446004C>T:C),I-Y26079:BY173428(14320075G>A:G),Y26079(22058342G>A:G),I-Y56390:A9272(6932183T>A:T),I-Y22033:Y22033(17888972G>A:G),I-Y31033:Y31035(16760815T>C:T),I-Y4870:FGC36094(14028861G>T:G),I-Y21954:Y22408(17511419C>T:C),I-Y13046:Y13468(8598383C>T:C),I-Y37104:Y37104(8588402C>A:C),I-FT206938:FT206939(8674744A>G:A),FT207500(17309454C>A:C),I-Y15584:FGC23811(7286109A>C:A),I-A9239:A9244(9418013C>T:C),I-A8596:A8603(18727668G>A:G),I-PH4462:PH4523(19474975G>C:G),I-A12708:A12715(22677556A>G:A),I-A1550:A1550(7891602C>T:C),I-Y13025:A1515(21225357T>G:T),FGC23286(21251228C>T:C),I-Y6356:Y6361(15346238C>T:C),I-Y7279:A5924(7873679C>T:C),Y7396(15715950A>G:A),I-Y32666:A14325(8426470G>T:G),I-YP1081:FGC53720(17381683G>A:G),I-Y11205:FT34426(14677834G>T:G),I-A11062:A11063(7074059C>G:C),I-Y21293:A8283(2683462G>A:G),I-S2308:Z17931(8530294C>T:C),I-Y154876:A22348(16729579C>G:C) ||

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