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1 Far from home: a multi-analytical approach revealing the journey of 2 an African-born individual to imperial Rome

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34 **ABSTRACT**

35

36 Rome saw its number of foreign individuals increase considerably as the empire expanded. These
37 foreigners arrived as either free persons or slaves from the newly conquered provinces and near-
38 frontier zones and came to influence the whole life of the city. Yet relatively little is known about their
39 life histories. In this study, we bring direct evidence for the first example of an African-born migrant,
40 with an origin beyond the southern imperial border, discovered in Rome. Based on a multi-tissue
41 sampling strategy including molar teeth and mandibular cortical bone, a multi-analytical approach
42 including isotopic ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, $\delta^{34}\text{S}$, $^{87}\text{Sr}/^{86}\text{Sr}$), dental morphology (geometric morphometrics,
43 nonmetric traits) and ancient DNA (mitochondrial DNA, Y chromosome) analyses allows reconstructing
44 the journey and lifeway patterns of the individual US215/Mand1 buried in the mass grave from the
45 catacombs of Saints Peter and Marcellinus. The successful isotopic and dental morphology analyses
46 suggest that the individual was probably born in the vicinity of the Nile Valley or within the central
47 Sahara Desert. Results also suggest a diachronic change of residence in the area during their early life.
48 The way US215/Mand1 reached Rome is still hypothetical, although it seems likely that the individual
49 could have undergone forced migration as a slave to the capital.

50

51 **Keywords:** stable and radiogenic isotopes, dental morphology, ancient DNA, mobility, diet, Roman
52 period

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68 **1. INTRODUCTION**

69

70 During the last centuries of the Republic and the early ones of the Empire, foreign populations of
71 Rome increased very swiftly and, in many ways, came to influence the whole life of the city. Individuals
72 from the newly conquered provinces and from the near-frontier zones reached the Imperial capital,
73 and a large part of them arrived as enslaved individuals (Abrecht, 2019; de Ligt and Tacoma, 2016; La
74 Piana, 1927; Noy, 2000; Tacoma, 2016). Trans-Saharan slave trade and slave traffic across the southern
75 Egyptian border are well-attested during the 1st-3rd century AD, although little evidence is available
76 regarding the scale and the intensity of these imports (Kirwan, 1957; Law, 2009; Snowden Jr., 1947;
77 Wilson, 2012). Ascertaining the number of North African-born slaves and their descendants among the
78 Rome's population is almost impossible, despite the low number often suggested (George, 2003;
79 Tacoma, 2012). Dark-skinned or black Africans were surely a minority among the slave workforce, the
80 majority of which originated from northernmost and easternmost regions of the Roman world
81 (George, 2003; Gordon, 1924; Wilson, 2012). Africans who originated from outside the Empire, like all
82 other slaves in Roman times, may have gained wealth and autonomy as well as achieve a degree of
83 social status (George, 2003). However, most of African-born slaves were exploited in the domestic
84 households of the Roman elite (George, 2003). Romans were fond of exoticism. By exhibiting the
85 possession of such rare slaves, a slave-owner could display a level of sophistication among their *familia*
86 as well as exhibit their wealth and worldliness (George, 2003; McLaughlin, 2014). African slaves could
87 also have served in Rome's armed forces or worked for public institutions or temples (Lenski, 2006;
88 Silver, 2016; Weiss, 2004). Immigration of free men and women must not have been very large in
89 comparison with the importation of slaves. The city of Rome could not attract a large number of foreign
90 workers since most industries already used a servile population. However, the imperial Rome always
91 had a few wholesale merchants, shipowners, bankers, retail traders or adventurers, in pursuit of
92 success, and among them were many foreigners (Abrecht, 2019; de Ligt and Tacoma, 2016; La Piana,
93 1927; McLaughlin, 2014). Nevertheless, there is, to the best of our knowledge, little indications of such
94 individuals born beyond the southern imperial frontier and established in the capital.

95 The catacombs of Saints Peter and Marcellinus (hereafter: the SSPM catacombs), located at the
96 third milestone of the ancient Via Labicana, near the modern via Casilina in the south-east of Rome
97 (WGS 84: 41°52'43.4"N 12°32'54.6"E; Precision: exact) is a unique example to further discuss this topic.
98 Among our recent bioarcheological investigations of the SSPM assemblage, one individual, labeled
99 US215/Mand1, drew our attention. Their initial stable oxygen isotope values ($\delta^{18}\text{O}_{\text{sc(enamel)}}$ up to +2.7‰
100 – see Results) were very atypical for Rome and suggested a non-European origin, possibly African. The
101 objective of the present work is to refine the life history (dietary patterns, mobility, geographical origin,

102 and ancestry) of this individual in their population, social and geopolitical contexts, by a multi-
103 analytical approach using additional isotopic measurements on both bone and tooth ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$,
104 $\delta^{34}\text{S}$, $^{87}\text{Sr}/^{86}\text{Sr}$), dental morphology analyses (geometric morphometrics, nonmetric traits) and ancient
105 DNA investigation (mitochondrial DNA, Y chromosome) which are presented here.

106

107 **2. THE CATACOMBS OF SAINTS PETER AND MARCELLINUS**

108

109 In the early 2000s, several mass graves containing circa 3,000 skeletons were fortuitously
110 discovered in the oldest part of the SSPM catacombs (i.e. Region X), near the sanctuary of the
111 eponymous saints (Blanchard et al., 2007; Giuliani et al., 2007). The discovered area is characterized
112 by the presence of seven interconnected cavities of various shapes, dimensions and elevations (Fig. 1),
113 and distinct from the other burial chambers (i.e. *cubiculum*) or crypts commonly found in catacomb
114 systems (Blanchard et al., 2007). Archaeological excavations conducted from 2004 to 2010 have made
115 it possible to document the main characteristics of skeletal accumulations and to suspect their
116 relationship with one or more mortality crises (Castex et al., 2007, 2009, 2011). Skeletons were found
117 articulated and placed next to each other or in piles. The new corpses were deposited without
118 disturbing the old ones, and no filling was accumulated among individuals buried in the same level.
119 This evidence in conjunction with the relationship between the capacity of the burial chambers and
120 the volume of the bodies as well as the taphonomic evolution of all the layers of corpses together
121 suggested that several successive multiple inhumations were carried out (Castex et al., 2014; Kacki et
122 al., 2014). The very low frequency (< 5%) of traumatic lesions on the assemblage excludes the
123 hypothesis of interpersonal violence as the cause of death (e.g. massacres, martyrdom or human
124 sacrifice) (Castex and Blanchard, 2011). Altogether, the data argue for mortality events of epidemic
125 origin, occurring over a relatively short timeframe (Castex and Blanchard, 2011; Castex et al., 2014). A
126 significant number of the individuals were given highly elaborated funerary practices, never previously
127 recorded in Rome, including the use of gypsum-plastered textiles as body wrappings together with
128 precious and exotic foreign resinous substances (i.e. succinite, sandarac and frankincense) (Devièse et
129 al., 2017; Schotsmans et al., 2019). The high-level care offered to the deceased as well as the presence
130 of good-to-high quality textile remains (e.g. gold and probable silk threads, fineness of the weave)
131 suggested that at least part of these individuals belonged to the upper classes of the Roman society
132 (Blanchard et al. 2015; Devièse et al., 2017). Analysis of grave goods and ^{14}C data revealed that the
133 mass graves dated back to the early imperial period (1st-3rd c. AD) (Blanchard et al., 2015; Castex and
134 Blanchard, 2011).

135

136 **3. MATERIAL**

137

138 The individual US215/Mand1 was found in the chamber X83 during a rescue survey conducted
139 prior to the construction of a support pillar to secure the site (Fig. 2). Time limitation as well as
140 structural instability constrained investigators to remove human remains rapidly in layers of 20
141 centimeters in thickness at this location. In the prospect of a large-scale isotopic study in the SSPM
142 catacombs, a minimum number of individuals of 14 based on right hemi-mandibles with at least the
143 second molar embedded was estimated on this assemblage (Salesse 2015). Among them was
144 US215/Mand1, represented by only three lower right permanent molars in the mandible fragment
145 (Fig. 3).

146 Enamel of all teeth as well as a piece of mandibular cortical bone were sampled to isotopically
147 investigate diet and mobility of US215/Mand1. This multi-tissue sampling strategy allows exploiting
148 the differential growth timing of skeletal elements in order to reconstruct a detailed life history, at the
149 scale of the individual (see Supplementary File A). The isotopic results from US215/Mand1 have been
150 compared to those of the rest of the corpus (n = 129 individuals) from the Region X mass graves
151 (Salesse 2015) and then have been geographically recontextualized by comparisons with animal and
152 human populations from Italy or beyond (Buzon and Simonetti, 2013; di Lernia et al., 2013; Killgrove
153 and Tykot, 2013, 2018; Nitsch, 2012; O'Connell et al., 2019; Prowse et al., 2004; Rutgers et al., 2009;
154 Schrader et al., 2019; Sereno et al., 2008; Stojanowski and Knudson, 2011, 2014; Tafuri et al., 2006,
155 2018).

156 A small dataset (right-side mandible fragments with molars) from the mass graves of the
157 catacombs (n = 13, including US215/Mand1; Table 1), available at the time of this study, have been
158 used to explore biodistance through dental nonmetric traits. Further exploration of molar outline
159 shape was conducted on US215/Mand1 and a subset of individuals from the SSPM catacombs (n = 4,
160 Table 1) using geometric morphometrics.

161 Dentine of the US215/Mand1's second molar has been sampled for exploring the paternal and
162 maternal lineages through ancient DNA analysis. Teeth of a small batch of individuals (n = 5, Table 1)
163 randomly selected in the mass graves of the catacombs have also been investigated for comparative
164 purposes.

165

166 **4. METHODS**

167

168 The principles of the stable isotope analysis (including abbreviations), the dental morphology study
169 and the paleogenetic approach are set out in Supplementary File A.

170

171 4.1. Isotopic analysis

172

173 4.1.1. *Bone collagen extraction for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ analyses*

174

175 Bone preparation and chemical pretreatments were conducted at the stable isotope preparation
176 lab of UMR 7209 in the *Muséum National d'Histoire Naturelle* (MNHN) (Paris, France). Sampled bones
177 were cleaned using a tungsten carbide drill bit to retain only compact parts. Bone collagen was
178 extracted following the protocol of Longin (1971), modified by Bocherens et al. (1988, 1991). Bones
179 were crushed using a knife mill or a mortar and pestle. Powder samples (amount: \approx 400 mg; grain size:
180 0.3 to 0.7 mm) were decalcified in 40 ml of 1 M hydrochloric acid (HCl) at room temperature for 20
181 min. Gelatins were retrieved by filtration using a MF-Millipore membrane filter (pore size: 5 μm),
182 rinsed, and then soaked into 0.125 M sodium hydroxide at room temperature for 20 h to remove soil
183 organic matter. The samples were again filtered and rinsed. Gelatins were solubilized in 0.01 M HCl
184 ($\text{pH} = 10^{-2}$) at 100° C for 17 h and filtered a last time to trap possible impurities. Collagen samples were
185 freeze-dried at -87° C for at least 48 h and extraction yields (%Col) were calculated (expressed as a
186 weight percentage, wt.%). In modern bones, extraction yields are around 20.4 ± 3.9 wt.% (1SD)
187 (Bocherens et al., 1991; Salesse et al., 2019), and samples containing less than 1 wt.% of collagen are
188 considered unreliable (Dobberstein et al., 2009; Van Klinken, 1999).

189 Carbon and nitrogen abundances and isotope compositions were measured (amount: 200-400 μg)
190 using a Costech Elemental Analyzer 4010 fitted with a zero-blank auto-sampler coupled via a ConFlo
191 IV to a ThermoScientific Delta V PLUS Isotope Ratio Mass Spectrometer at the environmental isotope
192 laboratory of the James Cook University's advanced analytical center (Cairns, Australia). Carbon and
193 nitrogen contents are expressed as percentages (%C and %N). In modern bones, %C and %N values
194 range from 15.3 to 47% and from 5.5 to 17.3%, respectively (Ambrose, 1990; Salesse et al., 2019).
195 Samples with %C and %N values below 13% and 4.8%, respectively, are recognized as severely altered
196 (Ambrose, 1990; Garvie-Lok, 2001; Iacumin et al., 1998; Reitsema, 2012; Van Klinken, 1999). Atomic
197 C:N ratios of modern bones vary between 2.9 and 3.6 (DeNiro, 1985; Salesse et al., 2019), and
198 archaeological samples with values below or above these thresholds indicate alteration or
199 contamination. The $\delta^{13}\text{C}_{\text{col}}$ and $\delta^{15}\text{N}_{\text{col}}$ values are reported as per mil (‰) difference relative to VPDB
200 and AIR, respectively. International and in-house standards (USGS-40: $\delta^{13}\text{C} = -26.4\text{‰}$ and $\delta^{15}\text{N} = -4.5\text{‰}$;
201 Taipan: $\delta^{13}\text{C} = -11.7\text{‰}$ and $\delta^{15}\text{N} = 11.8\text{‰}$; Chitin: $\delta^{13}\text{C} = -19\text{‰}$ and $\delta^{15}\text{N} = 2.2\text{‰}$) were analyzed for
202 quality control. During this study, analytical errors calculated from replicates of internal standards
203 were better than $\pm 5\%$ (1SD) for both %C and %N, $\pm 0.1\text{‰}$ (1SD) for $\delta^{13}\text{C}_{\text{col}}$ and $\pm 0.2\text{‰}$ (1SD) for $\delta^{15}\text{N}_{\text{col}}$.

204 Sulfur abundance and isotope composition were assessed (amount: 7-8 mg) using a Costech
205 Elemental Analyzer 4010 coupled with a ThermoScientific Delta V Advantage isotope ratio mass
206 spectrometer at the University of South Florida Stable Isotope Lab (Tampa, Florida, USA). Sulfur
207 content is expressed in percentages (%S). In modern bones, %S values as well as the atomic C:S and
208 N:S ratios range from 0.15 to 0.35%, from 300 to 900, and from 100 to 300, respectively (Bocherens et
209 al., 2011; Nehlich and Richards, 2009). Bone collagen samples with values outside these ranges are
210 considered unreliable. The $\delta^{34}\text{S}$ results are reported as per mil (‰) deviation and normalized to CDT
211 using certified and in-house reference materials (IAEA-S2: $\delta^{34}\text{S} = 22.7\text{‰}$; IAEA-S3: $\delta^{34}\text{S} = -32.3\text{‰}$; and
212 Elemental Microanalysis B2155: $\delta^{34}\text{S} = 6.7\text{‰}$). Measurements errors calculated from 46 replicates of
213 the B2155 standard were $\pm 0.11\%$ (1SD) for %S and $\pm 0.36\text{‰}$ (1SD) for $\delta^{34}\text{S}_{\text{col}}$.

214

215 4.1.2. Bone and tooth carbonate preparation for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ analyses

216

217 Bone carbonate preservation was investigated by Fourier transform infrared (FTIR) spectroscopy
218 in attenuated total reflection (ATR) mode before pretreatments (see Lebon et al. 2011 and Salesse et
219 al. 2014 for a presentation of the protocol used). The diagenetic trajectory of bone samples from the
220 SSPM catacombs has already been described in Salesse et al. (2014).

221 Bone and tooth carbonate samples were prepared following a revised version proposed by Salesse
222 et al. (2013) of the protocol of Balasse et al. (2002) at the MNHN. Sampled bones were crushed as
223 described above. Selected teeth were first cleaned and then sampled over the entire height of the
224 crown with a tungsten carbide drill bit. Powder samples (amount: ≈ 30 mg; grain size < 0.3 mm) were
225 treated with 1.5 ml of 2-3% sodium hypochlorite (NaClO) at room temperature for 48 h to remove
226 organic matter, and then with 1.5 ml of 1 M acetic acid (CH_3COOH) at room temperature for 1 h to
227 remove exogenous carbonates. NaClO and CH_3COOH solutions were renewed at least once during the
228 procedure. Samples were rinsed with distilled water between the two steps and at the end. Samples
229 were oven-dried at 65°C overnight. The purification process induced significant weight losses, up to
230 80%.

231 Carbon and oxygen isotope compositions were measured (amount: 580-630 μg) via a
232 ThermoScientific Kiel IV carbonate device interfaced with a ThermoScientific Delta V Advantage
233 isotope ratio mass spectrometer at the MNHN's isotope-ratio mass spectrometry service. The results
234 are reported as per mil (‰) deviation from VPDB reference standard scale. An in-house carbonate
235 standard (Marble LM: $\delta^{13}\text{C} = 2.13\text{‰}$ and $\delta^{18}\text{O} = -1.83\text{‰}$), normalized to the international standard NBS
236 19, was used for checking the accuracy of the measurements. Analytical precision calculated from 107
237 replicates of Marble LM were $\pm 0.03\text{‰}$ (1SD) for $\delta^{13}\text{C}$ and $\pm 0.07\text{‰}$ (1SD) for $\delta^{18}\text{O}$.

238

239 4.1.3. Tooth enamel sample preparation for $^{87}\text{Sr}/^{86}\text{Sr}$ analysis

240

241 Samples were processed in a clean room with filtered air and under laminar-flow hoods at the
242 *École Normale Supérieure* (ENS) (Lyon, France). Enamel samples were prepared as mentioned above.
243 Powder samples (amount: ≈ 30 mg; grain size < 0.3 mm) were ultrasonicated in 1 ml of 0.1 M CH_3COOH
244 for 5 min to eliminate diagenetic carbonates, rinsed with MilliQ water to neutral, and then freeze-dried
245 at -50°C for at least 12 h. Samples were then dissolved in 1 ml of 4 M of nitric acid (HNO_3). An aliquot
246 of 100 μl was taken from each sample for trace element concentration analysis.

247 Strontium was isolated following a similar protocol to that described in De Muynck et al. (2009).
248 Teflon ion exchange columns were filled with 2 ml of 50-100 μm bead size TrisKem Sr-Resin. Resin was
249 washed with MilliQ water and 0.05 M HNO_3 , and then pre-conditioned with 3 ml of 4 M HNO_3 . The
250 dissolved samples were loaded into the columns. Column blanks were prepared with 1 ml of 4 M HNO_3 .
251 Columns with samples were rinsed with 5 ml of 4 M HNO_3 to remove concomitant matrix elements,
252 and then with 6 ml of 0.05 M HNO_3 to strip off the purified strontium fraction. Retrieved strontium
253 samples were evaporated, and dried residues were finally dissolved in 1 ml of 0.05 M HNO_3 .

254 Strontium concentrations were determined using an Agilent 7500 CX inductively coupled plasma
255 mass spectrometry following the method of Balter and Lécuyer (2010), whereas strontium isotope
256 ratios were measured using a large-radius Nu 1700 multi-collector inductively coupled plasma mass
257 spectrometer, both at the ENS. Strontium isotope data were obtained at low-resolution in static mode.
258 Signal intensities were monitored on ^{83}Kr and ^{85}Rb and used to correct for interferences on m/z ratios
259 of 84 (Kr), 86 (Kr), and 87 (Rb). Ratios were corrected for mass bias fractionation using an internal
260 normalization to $^{86}\text{Sr}/^{88}\text{Sr} = 0.1194$. Gas flow instrumental mass fractionation was controlled with a
261 standard-sample bracketing approach involving the measurement of the standard NIST SRM 987
262 (recommended value of $^{87}\text{Sr}/^{86}\text{Sr} = 0.710248$; McArthur et al., 2001). Samples were randomized during
263 analysis and duplicates were measured to check for systematic errors. Repeated measurements of
264 NIST SRM 987 standard yielded an average value of $^{87}\text{Sr}/^{86}\text{Sr} = 0.71025 \pm 0.00002$ (2SD; 12 analyses).

265

266 4.1.4. Isotopic data processing

267

268 The Bayesian model FRUITS (Food Reconstruction Using Isotopic Transferred Signals, version 2.1.1;
269 Fernandes et al. 2014) was applied to evaluate the relative importance of plant and animal resources
270 in the US215/Mand1's diet as well as estimate their protein and carbohydrate/fat intakes. The model
271 parameters used are detailed in the Supplementary File B.

272 To reconstruct the $\delta^{18}\text{O}_{\text{dw (enamel)}}$ values of US215/Mand1, three steps were followed: 1) converting
273 $\delta^{18}\text{O}_{\text{sc [VPDB]}}$ into $\delta^{18}\text{O}_{\text{sc [VSMOW]}}$ values using the equation of Coplen (1988) (Eq. 1); 2) estimating $\delta^{18}\text{O}$ -
274 phosphate [VSMOW] from $\delta^{18}\text{O}_{\text{sc [VSMOW]}}$ values via the equation developed by Chenery et al. (2012) (Eq. 2);
275 and 3) calculating $\delta^{18}\text{O}_{\text{dw [VSMOW]}}$ from $\delta^{18}\text{O}_{\text{phosphate [VSMOW]}}$ values thanks to the formula proposed by Daux
276 et al. (2008) (Eq. 3).

277 Equation 1. $[\delta^{18}\text{O}_{\text{sc [VSMOW]} = 1.03091 \times \delta^{18}\text{O}_{\text{sc [VPDB]} + 30.91}]$

278 Equation 2. $[\delta^{18}\text{O}_{\text{phosphate [VSMOW]} = 1.122 \times \delta^{18}\text{O}_{\text{sc [VSMOW]} - 13.73}]$

279 Equation 3. $[\delta^{18}\text{O}_{\text{dw [VSMOW]} = 1.54 \times \delta^{18}\text{O}_{\text{phosphate [VSMOW]} - 33.72}]$

280 To reconstruct the $\delta^{18}\text{O}_{\text{dw (bone)}}$ values of US215/Mand1, two steps were followed: 1) converting
281 $\delta^{18}\text{O}_{\text{sc [VPDB]}}$ into $\delta^{18}\text{O}_{\text{sc [VSMOW]}}$ values using the equation of Coplen (1988) (Eq. 1); and 2) calculating
282 $\delta^{18}\text{O}_{\text{dw [VSMOW]}}$ from $\delta^{18}\text{O}_{\text{sc [VSMOW]}}$ via the equation proposed by Chenery et al. (2012) (Eq. 4).

283 Equation 4. $[\delta^{18}\text{O}_{\text{dw [VSMOW]} = 1,59 \times \delta^{18}\text{O}_{\text{sc [VSMOW]} - 48,634].$

284 These two procedures are recommended by Chenery et al. (2012) for individuals originating from
285 geographical regions characterized by a hot-arid climate and for individuals originating from the rest
286 of the world, respectively.

287 Finally, all the isotopic data, together with associated chronological and other supporting
288 information, from this study have been uploaded in the IsoArch database (Salesse et al., 2018, 2020).

289

290 4.2. Dental morphology analysis

291

292 4.2.1. *Nonmetric traits*

293

294 Dental traits (see list in Table 2) were recorded either as present/absent or as a grade from absent
295 to full expression, following the appropriate guidelines (i.e. Marado and Silva, 2016; Pilloud et al., 2018;
296 Scott and Irish, 2017). An interesting dental nonmetric trait, crenulation, was included in the
297 investigation. The trait is currently not part of the ASUDAS but has recently been explored further by
298 Pilloud et al. (2018) who observed highest frequencies (and scores) for the trait among modern
299 American Black and South African samples.

300 As recommended, each trait was represented by one tooth in the final analysis to prevent
301 redundancy (Irish, 2005; Nichol, 1990). To analyze the dental nonmetric data, a distance matrix was
302 created using Gower distances which measures the distance between pairs and then combines the
303 distances into a single value per record-pair (Gower, 1971). This allows for missing data and the use of
304 a mixed dataset of interval, ordinal and categorical data. However, the generated distance matrix is
305 non-Euclidean, requiring nonparametric tools for further data analysis. The distance matrix was
306 created using the function daisy in R package cluster (Maechler et al. 2019), allowing the user to define

307 symmetric and asymmetric binary values (e.g. in the case of rare traits). The morphological similarities
308 were further analyzed using the function `pam` in `cluster`, which partitions the data into clusters around
309 medoids represented by individual datapoints. Data was visualized using nonmetric multidimensional
310 scaling (NMDS) and t-distributed Stochastic Neighbor Embedding (t-SNE). Both are dimension
311 reduction techniques intended to condense data to a visually observable form. The former is a built-in
312 statistical tool in R (R Core Team, 2020), the latter was created using the R package `Rtsne` (van der
313 Maaten and Hinton, 2008).

314

315 4.2.2. *Geometric morphometrics*

316

317 Geometric morphometrics was utilized to explore shape change between a subset of individuals,
318 with the individual of interest as reference. To investigate only the shape of organism, size, location
319 and orientation must be excluded from the coordinates. The most common method to do this is the
320 generalized Procrustes superimposition where landmarks are rotated to best fit, centered and rescaled
321 to a size 1 (Webster and Sheets, 2010). The coordinates exist in Kendall's shape space (Kendall, 1984),
322 defined as a mathematical space induced by the shape coordinates (Mitteroecker and Gunz, 2009). To
323 treat the semi-landmarks, bending energy (Bookstein, 1997) which maps the 'path of least resistance'
324 between neighboring landmarks, was used.

325 The mandible fragments were digitized using the R package `Stereomorph` (Olsen and Westneat,
326 2015), which created shape files for each sample, and analyzed with the sibling package `geomorph`
327 (Collyer and Adams, 2018). Due to attrition on the occlusal surfaces (and the subsequent loss of
328 landmark information), the analysis was constricted to an investigation of the outline created by the
329 three molars, using two landmarks as anchor and semi-landmarks to map the outline. The landmarks
330 were used only momentarily, the analysis focusing on semi-landmark-based outline due to heavy wear,
331 as `Stereomorph` requires them to create a start and end point for the semi-landmarks. To make sure
332 the outline resembles the tooth shape as well as possible, the landmarks were placed at the meeting
333 point of cusp 3 and 5 on LM1, as close to one another as possible, while the semi-landmarks were
334 placed around the tooth in the same direction. Before connecting the semi-landmarks, they were
335 redistributed around the shape in even distances using the package function `lapply`. Because we
336 wanted to create a closed outline of the three molars, the landmarks were omitted from the outline
337 to avoid duplication of the points (landmarks would correlate with the first and last semi-landmark
338 along the curve). Generalized Procrustes superimposition was then performed using function `gpagen`.
339 The differences between individuals were visualized using functions `plotTangentSpace`, performing
340 PCA, and `plotRefToTarget` which place the configuration on a grid and deforms it in locations where
341 change is observed.

342

343 4.3. aDNA extraction and analysis

344

345 Ancient DNA (aDNA) analyses were not anticipated before the excavation. The teeth were
346 therefore decontaminated, i.e. scraped, cleaned with bleach, and subsequently exposed to UV
347 radiation for 20 min on each side. All established aDNA guidelines were then followed to minimize
348 contamination during subsequent steps. Analyses were conducted in the aDNA facilities of UMR PACEA
349 at Bordeaux University (Pessac, France). The teeth were powdered and a sample of 100 mg for each
350 was incubated overnight in lysis buffer (0.5 M EDTA, pH 8, 25 mg/ml proteinase K, and 0.5% N-Lauryl
351 sarkosyl). The procedure of Allentoft et al. 2015 was then followed to extract the DNA (MinElute kit,
352 Qiagen). In order to assess maternal (mitochondrial DNA) and paternal (Y chromosome) lineages, a
353 combination of 18 mitochondrial and 10 Y chromosome SNPs were typed through one multiplex using
354 MALDI-TOF MS-based SNP genotyping (iPLEXTM Gold technology, Sequenom, Inc., San Diego, CA,
355 USA). All primers used for these experiments and procedure details are available in Rivollat et al.
356 (2015). This first set of analyses was also designed to test for the ancient DNA conservation in the
357 human remain and, in case of sufficient DNA preservation, was supposed to be followed by the
358 characterization of the mitochondrial first hypervariable region (HVR-I, nps 16,024–16,380) targeted
359 using four overlapping fragments (following the procedures described in Rivollat et al., 2015).

360

361 5. **RESULTS**

362

363 5.1. Mobility and dietary patterns of US215/Mand1 through stable isotopic data

364

365 Collagen was successfully extracted from the mandibular fragment of US215/Mand1. With an
366 extraction yield of 6.7 wt.%, it exceeds the minimum threshold of 1 wt.% indicating a satisfactory
367 sample preservation (Table 3). With 38.5%, 13.6% and 0.31% respectively, carbon, nitrogen and sulfur
368 contents fall into the reference ranges for well-preserved collagen samples (Table 3). The atomic C:N,
369 C:S and N:S ratios are 3.3, 331.2 and 100.3 respectively, which is well within the acceptable ranges of
370 variation for unaltered and uncontaminated collagen samples (Table 3). Therefore, the collagen extract
371 of this individual meets all the criteria for good-quality collagen. Regarding the FTIR indicators for bone
372 carbonate preservation, the mandible fragment of US215/Mand1 presents an IRSF value of 4.1, a
373 CO_3/PO_4 ratio of 0.46 and an Amide I/ PO_4 ratio of 0.24. These values differ significantly from those
374 measured on modern reference samples (see Salesse et al. 2014 and references therein). Compared
375 to the rest of the population (Salesse et al. 2014), US215/Mand1 falls into the group of bones

376 moderately recrystallized. It was, however, demonstrated that samples falling in this group had reliable
377 carbonate isotope signals (Salesse et al. 2014). Tooth preservation was not investigated since it is
378 widely recognized that enamel can survive most diagenetic regimes and preserve biogenic isotopic
379 signals, especially in European archaeological contexts as young as the Roman ones (e.g. Koch et al.,
380 1997; Wang and Cerling, 1994; Zazzo, 2014). The isotopic results obtained in this study can therefore
381 be considered reliable and used to explore mobility and diet.

382 US215/Mand1 exhibits $^{87}\text{Sr}/^{86}\text{Sr}_{\text{enamel}}$ ratios of 0.7085, 0.7086 and 0.7087 for M1, M2 and M3,
383 respectively (Table 4; Fig. 4). These $^{87}\text{Sr}/^{86}\text{Sr}_{\text{enamel}}$ ratios are within one standard deviation from both
384 the SSPM and the other Lazio population means (Fig. 4 and 5). Further, US215/Mand1 presents
385 $\delta^{18}\text{O}_{\text{sc (enamel)}}$ values of +1.3‰, +2.3‰ and +1.2‰ for M1, M2 and M3, respectively (Table 4). These
386 $\delta^{18}\text{O}_{\text{sc (enamel)}}$ values are significantly higher than the rest of the population from the region X of the
387 SSPM catacombs (Grubb's tests; $p < 0.00$) (Fig. 4), and other Roman individuals from Lazio (Grubb's
388 tests; $p < 0.00$) (Fig. 6). In contrast, with a $\delta^{18}\text{O}_{\text{sc (bone)}}$ value of -3.0‰ (Table 4), US215/Mand1 lies within
389 one standard deviation from the population mean (Fig. 4). The estimated $\delta^{18}\text{O}_{\text{dw}}$ values of
390 US215/Mand1 vary between +0.7‰ and +2.6‰ on teeth and it is +4.4‰ on bone (Table 4).

391 Breastfeeding and weaning could theoretically explain the high $\delta^{18}\text{O}_{\text{sc (enamel)}}$ values of
392 US215/Mand1. Breast milk is enriched in ^{18}O over the consumed drinking water, inducing elevated
393 $\delta^{18}\text{O}$ in infant tissues (Britton et al., 2015). Among modern humans living in non-industrialized and
394 traditional fertility societies, cessation of breastfeeding occurs in most cases between the age of two
395 or three (Alvarez, 2000; Britton et al., 2015; Kennedy, 2005; Sellen, 2001, 2007). Such a pattern was
396 also identified in Roman populations over the Empire based on isotopic but also written evidence (for
397 Rome, see Prowse et al., 2008; for Africa, see Dupras et al. 2001; Keenleyside et al. 2009). Among teeth,
398 only permanent first molars have their crown mineralized between birth and the age of three; the
399 other molars covering posterior age periods (Moorrees et al., 1963). Thus, breastfeeding processes
400 affect commonly only isotopic signals of M1s. These effects are, however, relatively limited, and lead
401 to an increase of $\delta^{18}\text{O}_{\text{sc (enamel)}}$ by approximately +0.7‰ (Herring et al., 1998; Knudson, 2009; Roberts
402 et al., 1988; Wright and Schwarcz, 1998, 1999). US215/Mand1 has a lower $\delta^{18}\text{O}_{\text{sc (enamel)}}$ value on M1
403 than on M2 (Fig. 4), which is an opposite pattern to what one can expect in case of a consumption of
404 breast milk and then a weaning during the first years of life. Moreover, US215/Mand1 M1 exhibits a
405 far higher value than the other SSPM population M2s (mean = $-3.7 \pm 1.1\%$) (Fig. 6). The $\delta^{18}\text{O}_{\text{sc (enamel)}}$
406 difference between US215/Mand1 M1 and the SSPM population M2s is 5‰, and greatly exceeds the
407 mean ^{18}O -enrichment of M1s caused by nursing.

408 Food and beverage processing could have influenced the $\delta^{18}\text{O}_{\text{sc}}$ signals recorded in US215/Mand1
409 enamel samples. Culture-specific culinary and dietary practices can be responsible of sizeable
410 modifications of the original stable isotope compositions of water in food and beverages (Brettell et

411 al., 2012; Britton et al., 2015; Daux et al., 2008; Warinner and Tuross, 2009). Recent experiments
412 highlighted that specific cooking processes may shift the $\delta^{18}\text{O}$ values of food resources up to +5.2‰
413 (e.g. Royer et al., 2017). In a lower degree, beverage production techniques and storage may increase
414 the $\delta^{18}\text{O}$ values of the initially used water up to +1.3‰ (e.g. Brettell et al., 2012; Spangenberg and
415 Vennemann, 2008). If food water and drinking liquids contribute together to the final oxygen isotopic
416 composition of consumer's body tissues, the former contributes much less than the latter to this signal.
417 Thus, the shifted $\delta^{18}\text{O}$ values of food would be mitigated by those of beverages. Both would be in turn
418 balanced by the $\delta^{18}\text{O}$ values of raw food and unprocessed drinking liquids (Brettell et al., 2012; Royer
419 et al., 2017). The final influence on the $\delta^{18}\text{O}_{\text{sc}}$ values, even in the hypothesis of a cumulative effect,
420 would be therefore well below the discrepancy observed between US215/Mand1 M1 and the mean
421 value of SSPM population M2s. Besides, culinary practices of Romans were assuredly diverse (André,
422 2009; Hilgers, 1969), and a mix of isotopic shifts in processed food and beverages would be expected,
423 which would necessarily reduce the effect of the most extreme offsets induced by certain cooking
424 methods. Furthermore, dietary habits are culturally mediated behaviors. Thus, the latter should be
425 shared by not only one individual but the entire group, which is clearly not the case in the SSPM
426 catacombs.

427 A different origin from the rest of the SSPM population appears therefore the most plausible
428 explanation for the atypical $\delta^{18}\text{O}_{\text{sc (enamel)}}$ values of US215/Mand1. Such high $\delta^{18}\text{O}$ values would indicate
429 that this individual is originated from a region characterized by a much warmer, drier climate than
430 Lazio. US215/Mand1 seems, however, to have moved around this region during their early life. While
431 M1 and M3 have similar $\delta^{18}\text{O}_{\text{sc (enamel)}}$ values, they both differ from M2 by about 1‰. This difference
432 suggests that US215/Mand1 either experienced a circular migration (between two different locations)
433 or had a complex migration trajectory (with several changes of residences) during their
434 childhood/adolescence in the above-described region. With a $\delta^{18}\text{O}_{\text{sc (bone)}}$ value falling within one
435 standard deviation from the population mean value, US215/Mand1 would have, however, inhabited
436 several years in Rome to record the local $\delta^{18}\text{O}$ signature in bone (Fig. 4). Furthermore, this result allows
437 us to assign an age to this individual, which was unknown so far. Based on the differences of $\delta^{18}\text{O}_{\text{sc}}$
438 values between teeth and bone, and taking into account the rhythms of bone turnover, it can be
439 established that US215/Mand1 was an adult. It can also be concluded that US215/Mand1 spent their
440 life in a geological zone similar to the area of Rome, or at least a region with analogous $^{87}\text{Sr}/^{86}\text{Sr}$ ratios
441 to the region of Rome, during the formation of the three molars.

442 US215/Mand1 presents $\delta^{13}\text{C}_{\text{sc (enamel)}}$ values of -12.9‰, -12.5‰ and -12.3‰ for M1, M2 and M3,
443 respectively (Table 3). Their mandible bone sample exhibits $\delta^{13}\text{C}_{\text{col}}$, $\delta^{15}\text{N}_{\text{col}}$, $\delta^{34}\text{S}_{\text{col}}$, and $\delta^{13}\text{C}_{\text{sc (bone)}}$ values
444 of -19.0‰, +12.5‰, +8.4‰, and -13.9‰, respectively (Table 3; Fig. 7 and 8). Taking into account the
445 average carbon offsets between a consumer's tissues and diet (Bocherens and Drucker, 2003;

446 Fernandes et al., 2012; Howland et al., 2003; Passey et al., 2005; Salesse, 2015; Warinner and Tuross,
447 2009), a $\delta^{13}\text{C}_{\text{diet}}$ value of about -26/-24‰ can be estimated.

448 The estimated $\delta^{13}\text{C}_{\text{diet}}$ value is close to the mean $\delta^{13}\text{C}_{\text{C}_3 \text{ plants}}$ value, suggesting that US215 had a diet
449 predominantly based on C_3 terrestrial plant and herbivorous animal resources. In addition, the $\delta^{15}\text{N}_{\text{col}}$
450 value (Fig. 7) is higher than the upper end of the common range for C_3 terrestrial consumers (Bocherens
451 and Drucker, 2003; Chisholm et al., 1982; Hedges and Reynard, 2007; Schoeninger et al., 1983),
452 indicating the inclusion of organisms with relatively high trophic levels, such as aquatic resources (Craig
453 et al., 2010; Drucker et al., 2005; Richards et al., 2015). With a $\delta^{34}\text{S}_{\text{col}}$ value falling into the range for
454 terrestrial/freshwater consumers (Fig. 7) (Nehlich, 2015; Peterson et al., 1985; Tsutaya et al., 2019), a
455 consumption of marine food sources can be discarded. Freshwater fish was most likely the aquatic
456 resource served at US215/Mand1's table. Thus, a mixed diet relying on the triad C_3 plants / C_3 herbivore
457 meat / freshwater fish products was consumed by this individual.

458 The US215/Mand1's dietary pattern is shared by a small batch of individuals from the Region X of
459 the SSPM catacombs (Salesse, 2015) but also by individuals from at least two other contemporaneous
460 populations from Rome [Catacombs of St. Callixtus (Rutgers et al., 2009); Tenuta des Duca (O'Connell
461 et al., 2019)] (Fig. 8). Moreover, if aquatic resources are considered regardless the environment from
462 which they originate, fish consumers are commonly identified in the Roman populations from Lazio.
463 Diets based on aquatic resources (only marine, both freshwater and marine, or without differentiation)
464 have been proposed as an explanation for individuals presenting high $\delta^{15}\text{N}_{\text{col}}$ values at Castellaccio
465 Europarco and Casal Bertone (Killgrove and Tykot, 2013), Gabii (Killgrove and Tykot, 2018), Casale del
466 Dolce and Osteria della Fontana (Nitsch, 2012), and SSPM catacombs (Nitsch, 2012; Salesse, 2015),
467 Isola Sacra and ANAS (Prowse et al., 2004), and finally Lucus Feroniae (Tafari et al., 2018) (Fig. 8). With
468 a $\delta^{15}\text{N}_{\text{col}}$ value of 12.5‰, US215/Mand1 stands among the top 10% of individuals with the most
469 elevated $\delta^{15}\text{N}_{\text{col}}$ values in Lazio ($n_{10\%} = 60$; $n_{\text{total}} = 603$) (Fig. 8). Apart a possible consumption of aquatic
470 resources, the individuals from this top 10% group are characterized by high protein intakes. Aquatic
471 resources, referring to fish in particular, contain generally more proteins than terrestrial animal source
472 food (FAO, 1989; Heinz and Heutzinger, 2007; Moharrery, 2007). A consumer of aquatic products, such
473 as US215/Mand1, has therefore a protein-rich diet.

474 Based on these findings, a dietary scenario including C_3 plants, C_3 terrestrial domesticated
475 herbivores and freshwater fish was tested through Bayesian modelling. The Bayesian approach
476 suggests that US215/Mand1 would have consumed $63 \pm 13\%$ of terrestrial plant cereals, $18 \pm 12\%$ of
477 meat of terrestrial animals, and $19 \pm 9\%$ of meat of freshwater fish. It estimates also that their diet
478 would have been composed of about 28% of proteins and 72% of carbohydrates/fats. However, due
479 to a limited baseline, this diet reconstruction must be considered as a tentative explanation, providing

480 orders of magnitude. Nevertheless, the Bayesian mixing model highlights that US215/Mand1
481 consumed terrestrial meat and aquatic resources in similar proportions and had a high protein intake.

482

483 5.2. Inferring the ancestry and origin of US215/Mand1 through dental morphology

484

485 The full list of recorded traits was analyzed for inter-trait correlation using Kendall tau-b, which led
486 to the exclusion of the C1-C2 crest and the mandibular pit tubercle on the second lower molar ($r =$
487 1.00 , $n = 8$, no p-value was generated). The entoconulid, metaconulid and distal fossa prevalence rates
488 exhibited no variation within the sample (0% present). An intra-observer error test by the data
489 collector ($r = 0.978$, $p < 0.001$, $n = 216$) has shown good agreement between observation events
490 (Maaranen et al., 2019).

491 The analysis was performed on the SSPM individuals (Table 1) using 11 traits, retained after the
492 inter-trait correlation test (Table 2). Though some of traits can be recorded in a scale, they were treated
493 as binary if present only in grade 1 (e.g. the protostylid, presented only as a buccal pit described by
494 Scott and Irish, 2017). Lower third molar absence, torsomolar angle and crenulation were only present
495 in one individual per trait, so they were treated as asymmetric binary variables. How traits were
496 treated is included in Table 1 and the full data and distance matrix in Tables C1 and C2.

497 A two-dimensional NMDS plot was created (Fig. 9). US215/Mand1 and US216/Mand2 resided far
498 from the centroid, the former falling beyond the 90% confidence level and the latter even beyond 95%.
499 Factor analysis indicated that most of the variation was contributed to enamel extension, anterior
500 fovea, hypocone size, crenulation and deflecting wrinkle (altogether circa 60%). A hierarchical cluster
501 analysis using complete-linkages also suggests the two individuals cluster away from the others (Fig.
502 10).

503 To explore the data further from a morphological perspective, the data was divided into clusters
504 using the function pam from the R package cluster. Silhouette method indicated an optimal number
505 of 5 clusters (Fig. C1). The clusters were used as group indicators in the second visualization technique,
506 t-SNE (Fig. C2), indicating that US215/Mand1 and US216/Mand2 formed their own morphological
507 groups. The data suggest that there is heterogeneity in the SSPM dataset, with US215/Mand1 and
508 US216/Mand2 even more morphologically different from the other individuals. Hierarchical clustering
509 places the two individuals close to one another, but the cluster analysis divides them even from each
510 other. It is pertinent to remember that the results are tentative, given the analysis was conducted
511 using three molars and altogether 11 variables (Table 2). Crenulation was observed on the lower
512 molars of US215/Mand1 but none of the other individuals in the SSPM sample.

513 Only five individuals from the SSPM catacombs were appropriate for the geometric morphometric
514 outline analysis (Fig. 11). Analysis on the molar outline did not produce clustering (Fig. 11) which is

515 probably due to small sample size. PC1 and PC2 explain 52% and 28% of the variation (cumulative
516 80%). Again, individuals US215/Mand1 and US216/Mand2 are separate but not significantly (ANOVA
517 effect size; $Z = 0.17$, $p = 0.46$), unsurprising given the distance between the individuals along PC1. Most
518 of the variation in the shape was captured in the lower third molar (Fig. C3), the most variable tooth
519 in the dentition. The inherent variation of this tooth could explain why each configuration was so
520 different, which coupled with the small sample size could not generate meaningful clusters.

521

522 5.3. aDNA, an attempt to address the provenance of US215/Mand1

523

524 No Y-chromosome SNPs could be characterized for any of the targeted samples, and very rare
525 mitochondrial SNPs were obtained (Supplementary File D). Mitochondrial results were conclusive only
526 for the sample US219/Mand1 from the chamber X83 to which replicable haplogroup H1 could be
527 assigned (Supplementary File C). The latter cannot be, however, specified with the type of investigation
528 applied here. Haplogroup H is the most common in today's Europe, and for example reach 38.99% in
529 Italy (Turchi et al., 2008). As the current repartition of the sub-haplogroup H1 covers Europe as well as
530 North Africa (Gleize et al., 2016), it cannot give a more precise information for the origin of individual
531 US219/Mand1, who could come from Italy as well as from other European or North African regions.
532 The other mitochondrial results were either non-replicable (Sq108 from X83), or inconsistent
533 (US215/Mand1 from X83), or too scarce (n°13/Mand1 from X81; Sq152 and US219/Mand2 from X83)
534 (Supplementary File C). The aDNA analyses clearly demonstrated a major DNA degradation in the
535 specific case of these individuals, which could easily be explained by the environmental conditions
536 inside the SSPM catacombs combined to specific funerary treatments (presence of lime) highly
537 detrimental to DNA preservation.

538

539 **6. DISCUSSION**

540

541 If regional-scale predictive $^{87}\text{Sr}/^{86}\text{Sr}$ maps for southern Europe, Near East and North Africa are still
542 missing, global and regional $\delta^{18}\text{O}$ isoscapes are available for the areas covered by the Roman Empire
543 at its greatest extent (i.e. during the 2nd century AD). Based on the OIPC maps
544 (http://wateriso.utah.edu/waterisotopes/pages/data_access/figures.html), three locations inside or
545 in the immediate vicinity of the Roman Empire can be identified as potential regions of origin for
546 US215/Mand1: the Arabian Peninsula, the Nile Valley and within the central Sahara Desert.

547 Measurements on modern rivers waters but also on archaeological human and faunal remains
548 sampled in these three regions corroborate the predictive $\delta^{18}\text{O}$ models. Upstream at Khartoum, Farah

549 et al. (2000) and Iacumin et al. (2016) determined mean $\delta^{18}\text{O}_{\text{water}}$ values for the main Nile of +2.5‰
550 and +1.8‰, respectively. Similarly, downstream at Cairo, Buzon and Bowen (2010) reported a mean
551 $\delta^{18}\text{O}_{\text{water}}$ value for the Nile River of +2.3‰. In a synthesis effort, Dufour et al. (2018) showed that the
552 $\delta^{18}\text{O}_{\text{water}}$ values could, however, vary a bit all along the river. Nevertheless, the $\delta^{18}\text{O}_{\text{water}}$ values for the
553 Nile would have remained broadly stable from the Late Period of ancient Egypt/the Meroitic Period in
554 Sudan to nowadays (Touzeau et al., 2013). This is mainly supported by the high and positive $\delta^{18}\text{O}$ values
555 displayed by human and animal samples during that time span (Buzon et al., 2019; Iacumin et al., 1996;
556 Iacumin et al., 2016; Touzeau et al., 2013). Also, oxygen isotope compositions of tooth enamel from
557 archaeological faunal specimens collected in the Libyan Sahara are in good accordance with the $\delta^{18}\text{O}$
558 values of modern precipitations (di Lernia et al., 2013). Besides, the variations of $\delta^{18}\text{O}_{\text{sc (enamel)}}$ values
559 suggest that US215/Mand1 spent their early life to change of residences in one of these three regions.

560 From a dental morphological perspective, US215/Mand1 differs from the rest of the SSPM
561 population (Fig. 9, 10, 11 and C2). Furthermore, the unique presence and high expression (grades ≥ 1
562 for M1 and M2; grade = 2 for M3) of crenulations on the US215/Mand1's molars are of particular
563 interest as they could be informative of a specific population history. This dental nonmetric trait has
564 notably been identified as being more common among African and African-derived populations
565 (Pilloud et al., 2018; Rhine, 1990). Especially, Pilloud and colleagues (2018) established that there was
566 a relationship between the presence of grades equal to or greater than 1 and the modern American
567 Black and South African samples they studied. Therefore, the complex molar surfaces as well as the
568 molar crenulation trait presence would support an African ancestry of US215/Mand1.

569 After excluding outliers (Grubb's tests), the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios display by humans vary from 0.70731
570 to 0.70807 in Egypt, from 0.70658 to 0.70912 in Sudan, and from 0.70975 to 0.71293 in central Sahara
571 Desert (Fig. 12) (Tafuri et al. 2006; Buzon et al., 2007; Sereno et al., 2008; Buzon and Simonetti, 2013;
572 di Lernia and Tafuri, 2013; di Lernia et al., 2013; Stojanowski and Knudson, 2014; Schrader et al., 2019).
573 Based on these data, neither the northern part of the Nile Valley nor the Saharan area appear to be
574 places from where US215/Mand1 could originate. Moreover, if at first glance the Third Cataract region
575 could be a possible origin for US215/Mand1 (core $^{87}\text{Sr}/^{86}\text{Sr}$ range = 0.70696-0.70912; Fig. 12), Buzon
576 and colleagues have suggested that individuals with $^{87}\text{Sr}/^{86}\text{Sr}$ values greater than 0.70783 did not
577 belong to this locality but were migrants from northern lands (Buzon and Simonetti, 2013; Buzon et
578 al., 2016). Among the latter stands the Second Cataract region. Because of its geological complexity,
579 this region presents a large local $^{87}\text{Sr}/^{86}\text{Sr}$ range, varying between 0.704 and 0.717 (Schrader et al.,
580 2019). Animal samples recovered near the Second Cataract exhibit a wide distribution of their $^{87}\text{Sr}/^{86}\text{Sr}$
581 values as well, ranging between 0.70667 and 0.70933 (after removing outliers via Grubb's test) (Fig.
582 12). This represents a pertinent area where US215/Mand1 could have spent their early life.
583 Additionally, cattle from Nubian sites show generally a broad range of $^{87}\text{Sr}/^{86}\text{Sr}$ values (Fig. 12), which

584 suggests that imports and/or transhumance of some animals may have occurred (Buzon and Simonetti,
585 2013; Schrader et al., 2019). Non-local cattle were most probably brought from surrounding regions
586 as tributes or traded with communities – either nomadic or not – from the arid desert edge (Buzon
587 and Simonetti, 2013; Iacumin et al., 2001; Thompson et al., 2008). Thus, cattle Sr variability would
588 indicate that various – unknown – localities from Sudan could have been possible places of origin for
589 US215/Mand1. Even though available $^{87}\text{Sr}/^{86}\text{Sr}$ values for humans from central Sahara Desert do not
590 fit with those of US215/Mand1, one could be intrigued by the isotopic composition of an equid
591 specimen recovered in the Messak Mountains (di Lernia et al., 2013). Of the two measurements taken
592 along its M3 crown, one is similar to the $^{87}\text{Sr}/^{86}\text{Sr}$ values exhibited by US215/Mand1 ($^{87}\text{Sr}/^{86}\text{Sr}_{\text{MK07/39C1-}}$
593 $_{09} = 0.7087$ in di Lernia et al., 2013) (Fig. 12). If this animal moved in that region, it also suggests that
594 the central Sahara Desert cannot be excluded as a potential origin of US215/Mand1.

595 Together these lines of evidence converge and argue for an origin beyond the African Roman
596 frontier for US215/Mand1, making this individual the first example of an African-born migrant revealed
597 by a multi-analytical approach including isotopic analysis and discovered in the Imperial capital.
598 Moreover, US215/Mand1 has experienced the second longest migration across the Roman Empire
599 highlighted by direct evidence, after the one discovered in Roman York (UK) discussed by Martiniano
600 et al. (2016) who suggested a Middle Eastern origin based on genomic and isotope data.

601 Fish, whether marine or freshwater, was a social marker at the time of the imperial era (André,
602 2009). While there are many indications that rich and poor alike had access to resources of the same
603 nature, they did have different dietary practices (André, 2009; Garnsey and Saller, 1987; Schirmer,
604 2014; Spurr, 1983). The quality but also the diversity of the food consumed, as well as the way it was
605 prepared, varied considerably between the low and high strata of Roman society (André, 2009; Purcell,
606 1995; Schirmer, 2014). Freshwater fish, in Rome as in other parts of the Empire, was consumed in large
607 quantities only by the most humble or ordinary people (André, 2009; Marzano, 2018). The wealthier
608 individuals and elites would occasionally consume it, but undoubtedly preferred marine fish. In
609 addition to their taste, marine fish also represented a potent sign of wealth. In short, fish, whether
610 marine or freshwater, was a social marker at the time of the imperial era (see André, 2009 and
611 Marzano, 2018 for thorough historical syntheses). Based on the isotopic results showing US215/Mand1
612 consuming freshwater fish in a significant proportion, one can suggest that US215/Mand1 had
613 probably a modest socio-economic status and belonged to the low strata of the Roman society.

614 These different lines of evidence support two storylines for US215/Mand1. The least likely would
615 be that US215/Mand1 was a free man or woman originated from the northern part of the African
616 continent, who would have settled in the city of Rome for specific business. The most likely would be
617 that after spending their early life moving in the vicinity of the Nile Valley or within the central Sahara
618 Desert, possibly due to a nomadic condition or a peregrine status, US215/Mand1 could have

619 experienced forced migration as a slave and was eventually shipped to Rome along trade routes. On
620 the basis of the evidence we have, it is unfortunately impossible to assess whether US215/Mand1
621 belonged to a private household or was owned by Rome's people, acting as a public slave. More
622 broadly, the presence of US215/Mand1 in the mass graves of the SSPM catacombs provides a better
623 understanding of the epidemic. As it seems that individuals from all strata of Rome were buried
624 together in these sepulchral spaces, the epidemic was not selective from a socio-economic point of
625 view. This would confirm the snapshot and the uncommon nature of this archaeological context.

626

627 **7. CONCLUSION**

628

629 Based on a multi-tissue sampling strategy including molar teeth and mandibular cortical bone, our
630 multi-analytical approach using isotopic ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, $\delta^{34}\text{S}$, $^{87}\text{Sr}/^{86}\text{Sr}$), dental morphology
631 (geometric morphometrics, nonmetric traits) and ancient DNA (mitochondrial DNA, Y chromosome)
632 analyses has refined the origin and life history of US215/Mand1 buried in the X83 mass grave from the
633 SSPM catacombs. Obtained lines of evidence suggest that this individual was born beyond the southern
634 *limes*, possibly in the vicinity of the Nile Valley or within the central Sahara Desert, and experienced
635 diachronic changes of residence during their early life, conceivably due to a nomadic condition or a
636 peregrine status. The way US215/Mand1 arrived in the imperial capital is still hypothetical, although it
637 seems likely that he/she could have undergone forced migration and eventually transported as a slave
638 to Rome. It cannot be totally excluded, however, that US215/Mand1 was a free man or woman
639 originated from North Africa, settling in the city of Rome for specific business affairs. This study
640 presents direct evidence of one of the unique cases of long-distance migration across the Empire of a
641 non-Roman. In particular, it highlights for the first time the journey of a North African-born individual
642 who died in the Eternal city. Enslaved or not, US215/Mand1 clearly illustrates and confirms the
643 cosmopolitan character of Rome. Finally, this study restates the importance and usefulness of multi-
644 proxy investigations for reconstructing the identities of past human individuals recovered in
645 archaeological contexts.

646

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648

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655 reviewers for their time and constructive comments on our manuscript.

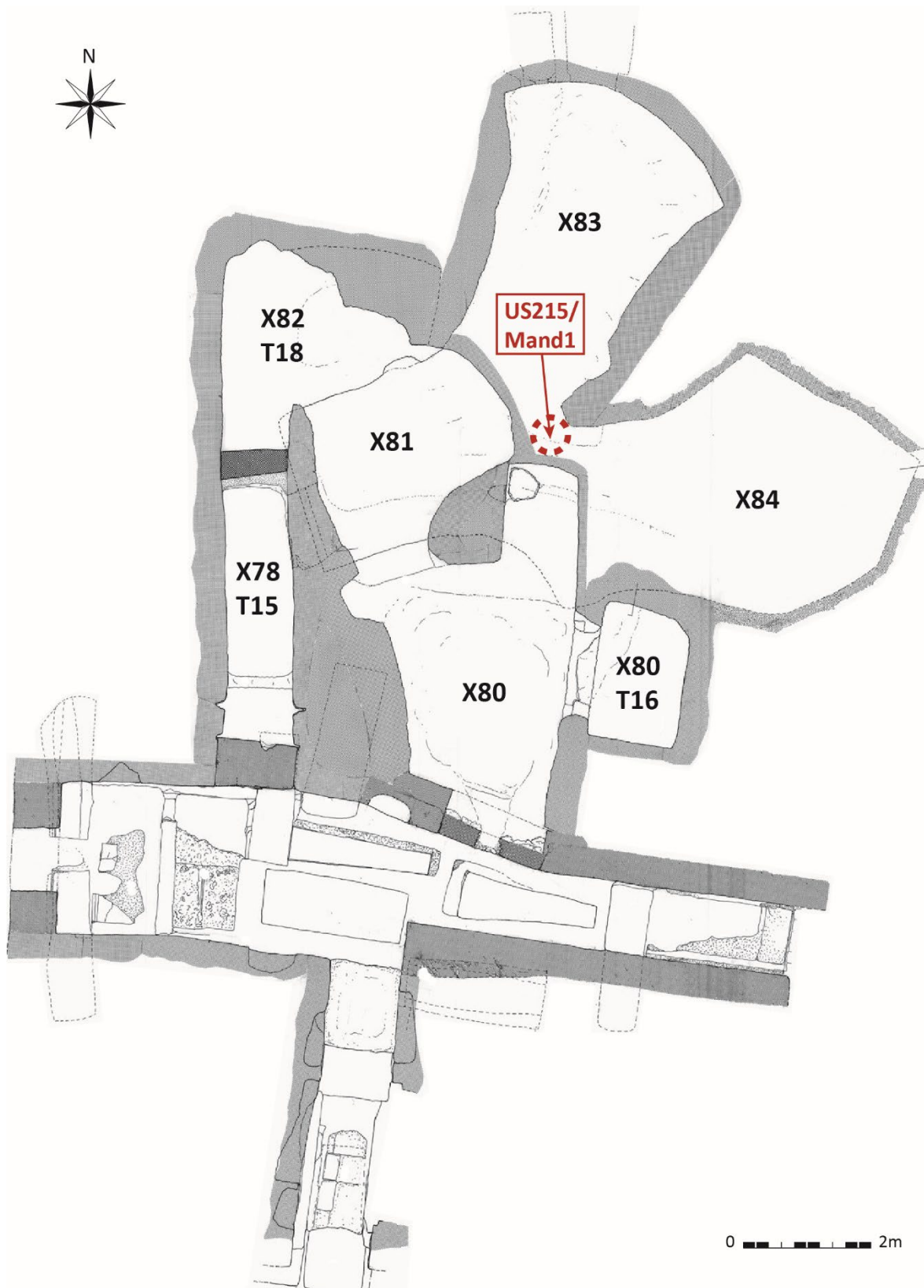
656

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658

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671

672 **Fig. 1.** Map of the burial chambers in the SSPM catacombs. Note: The dotted circle indicates the location where
673 the support pillar has been built and US215/Mand1 have been recovered. Modified after M. Ricciardi in Castex
674 and Blanchard 2011.



675

676 **Fig. 2.** Picture showing the support pillar newly built as well as the burials chambers X83 (left) and X84 (right) in
677 the background. Note: Picture of D. Glikzman/INRAP.

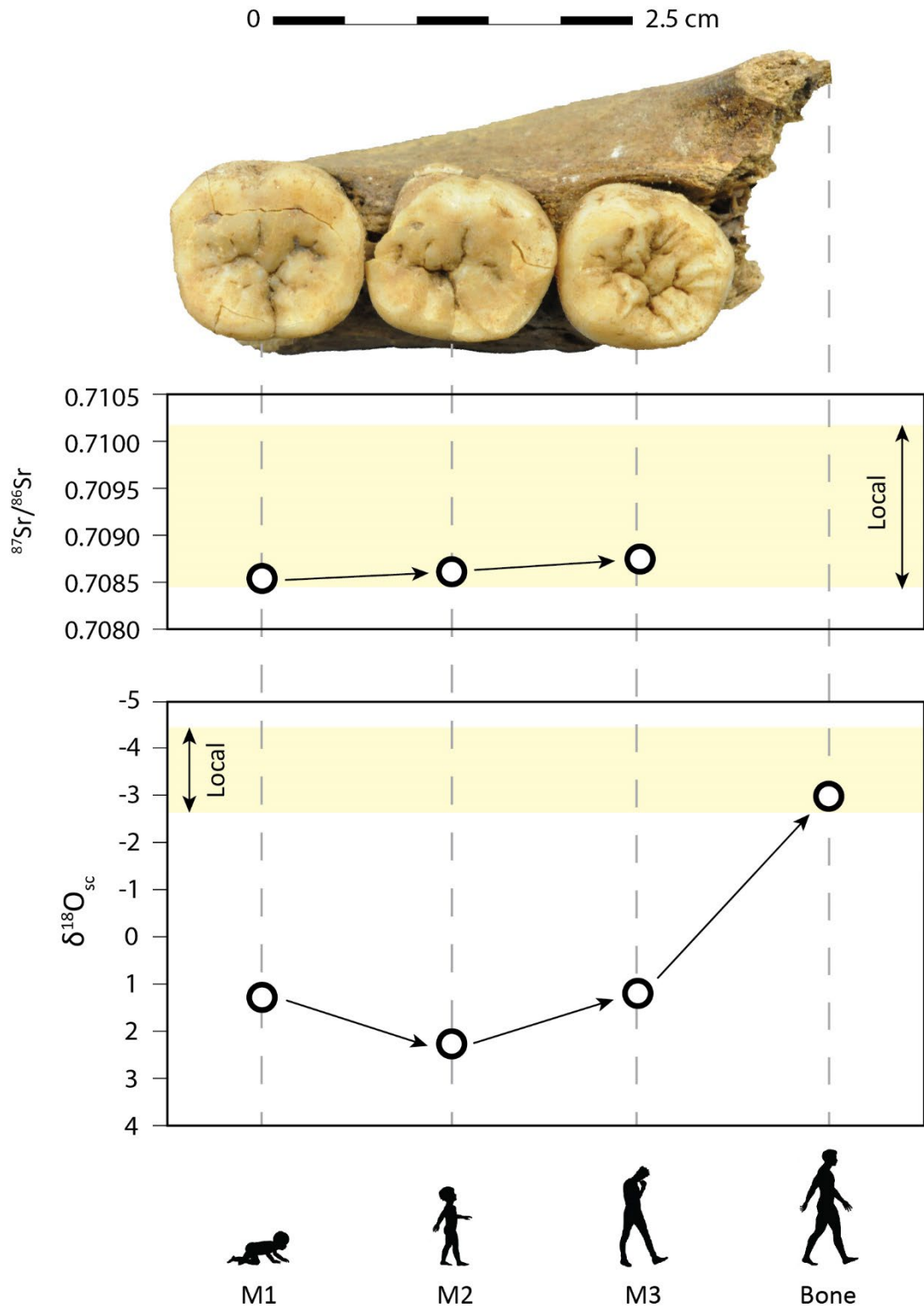
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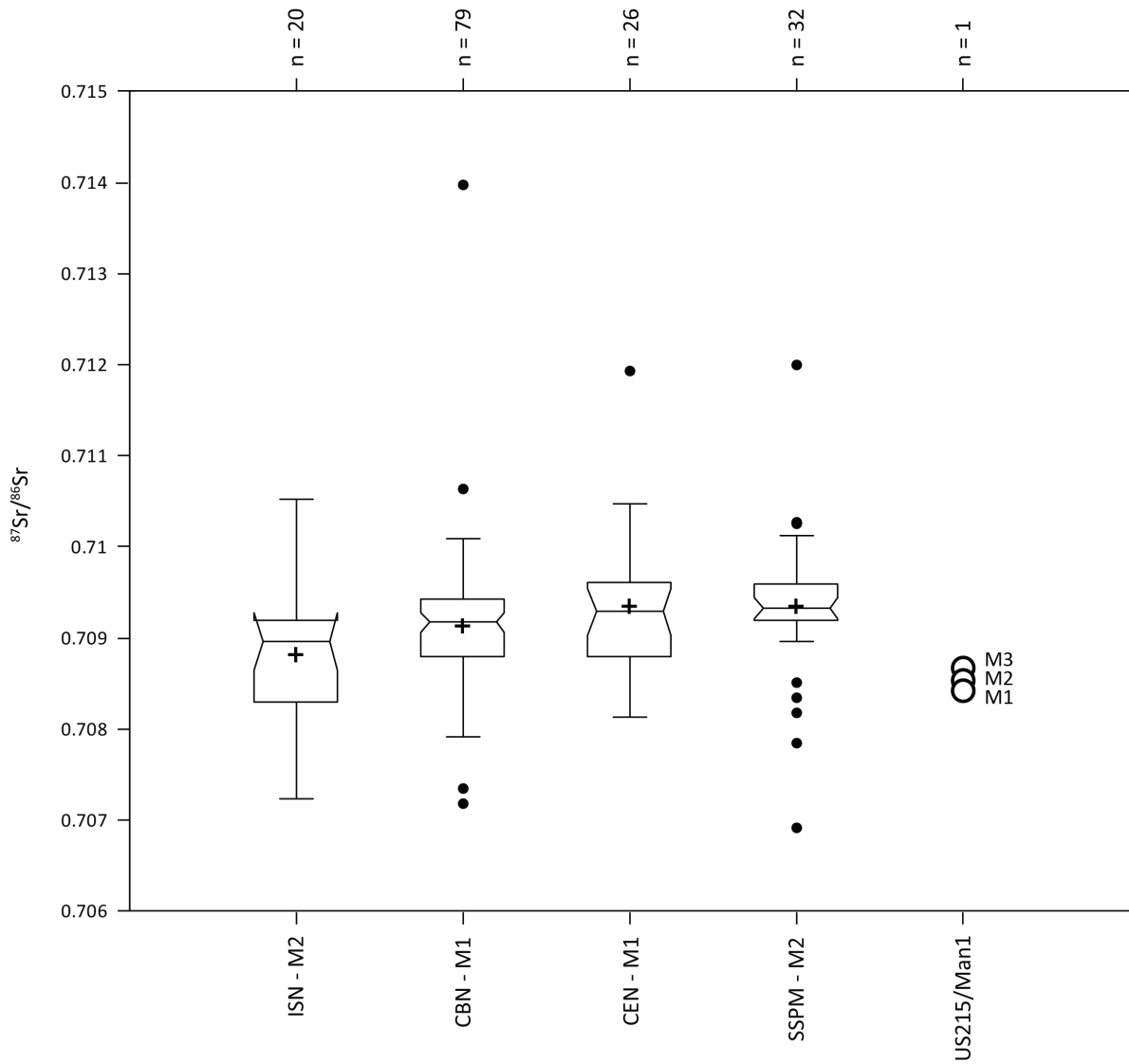
680 **Fig. 3.** Preserved right hemi-mandible fragment of US215/Mand1.

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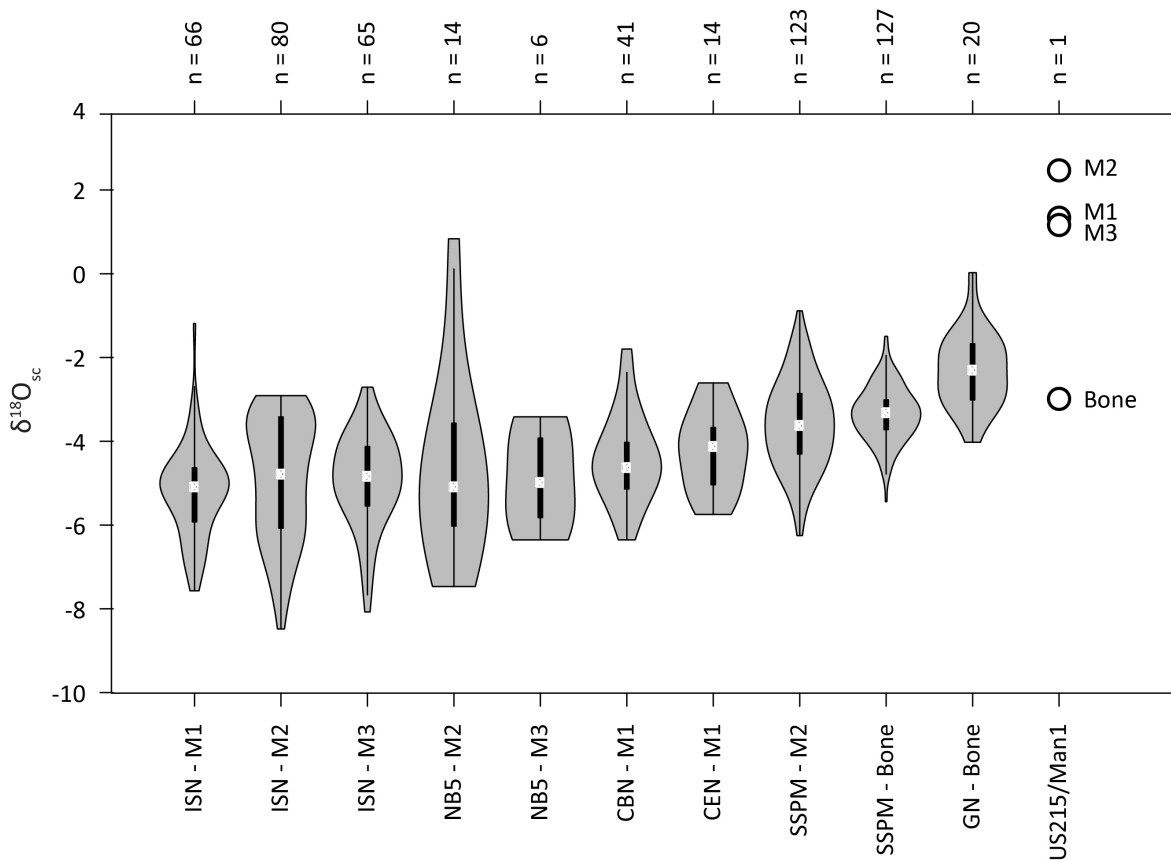
683 **Fig. 4.** Variation of the $^{87}\text{Sr}/^{86}\text{Sr}_{\text{enamel}}$, $\delta^{18}\text{O}_{\text{sc}(\text{enamel})}$ and $\delta^{18}\text{O}_{\text{sc}(\text{bone})}$ values in US215/Mand1. Note: 'M1', 'M2' and
 684 'M3' stands for permanent molars 1, 2 and 3, respectively. The yellow area corresponds to the local range defined
 685 as one standard deviation from the SSPM population mean. The standard deviation between replicates is inferior
 686 to size of dots.



687

688 **Fig. 5.** Inter-site comparison of the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios measured on human dental samples from the Rome's region.
 689 Note: 'M1' and 'M2' stands for permanent molars 1 and 2, respectively. 'ISN' refers to Isola Sacra Necropolis,
 690 'CBN' to Casal Bertone Necropolis, 'CEN' to Castellaccio Europarco Necropolis, and 'SSPM' to Saints Peter and
 691 Marcellinus catacombs. Bibliographic references: Salesse (2015), Killgrove and Montgomery (2016), Stark (2017).
 692 The boxes depict groups of data through their quartiles.

693



694

695 **Fig. 6.** Inter-site comparison of the $\delta^{18}\text{O}_{sc}$ values measured on human dental samples from the Rome's region.
 696 Note: 'M1', 'M2' and 'M3' stands for permanent molars 1, 2 and 3, respectively. 'ISN' refers to Isola Sacra
 697 Necropolis, 'NB5' to Navalvia Building 5, 'CBN' to Casal Bertone Necropolis, 'CEN' to Castellaccio Europarco
 698 Necropolis, 'GN' to Gabii Necropolis, and 'SSPM' to Saints Peter and Marcellinus catacombs. Bibliographic
 699 references: Prowse 2001, Prowse et al. 2007, Salesse (2015), Killgrove and Montgomery (2016), Stark (2017),
 700 Killgrove and Tykot (2018), O'Connell et al. (2019). The violins show the probability density of the data at different
 701 values, smoothed by a kernel density estimator. The boxes depict groups of data through their quartiles.
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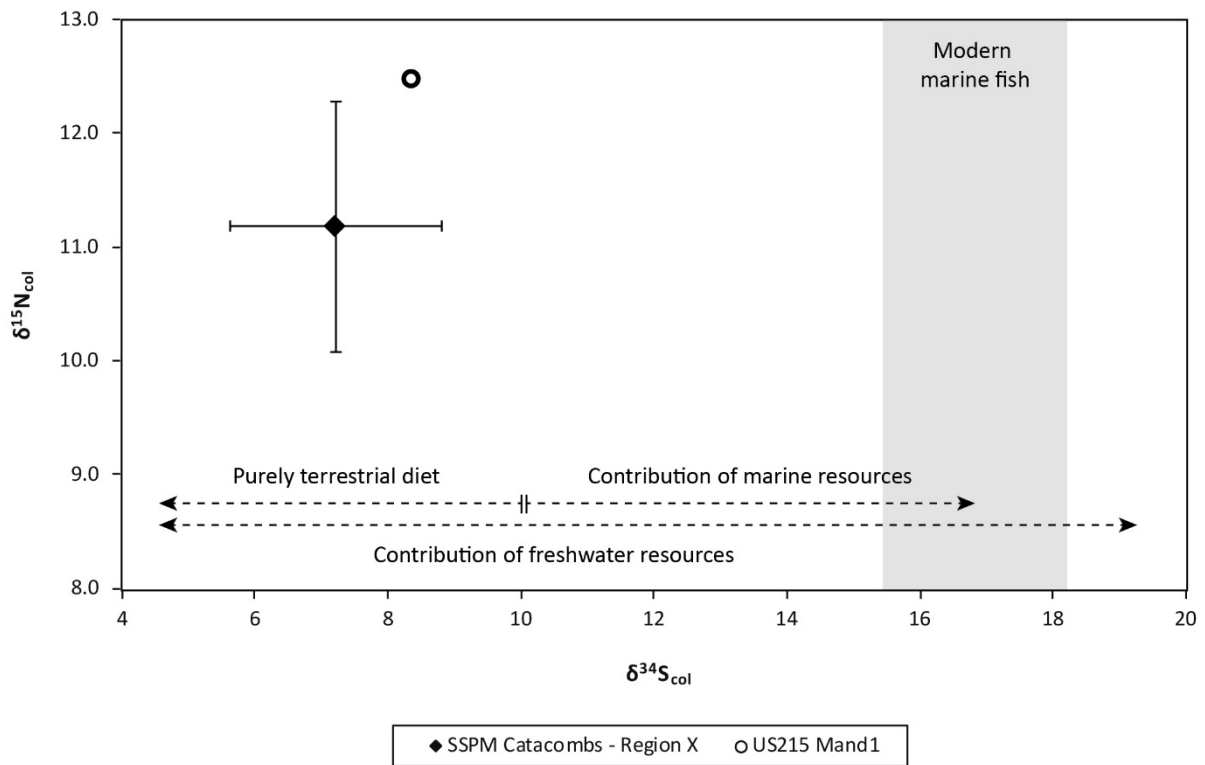
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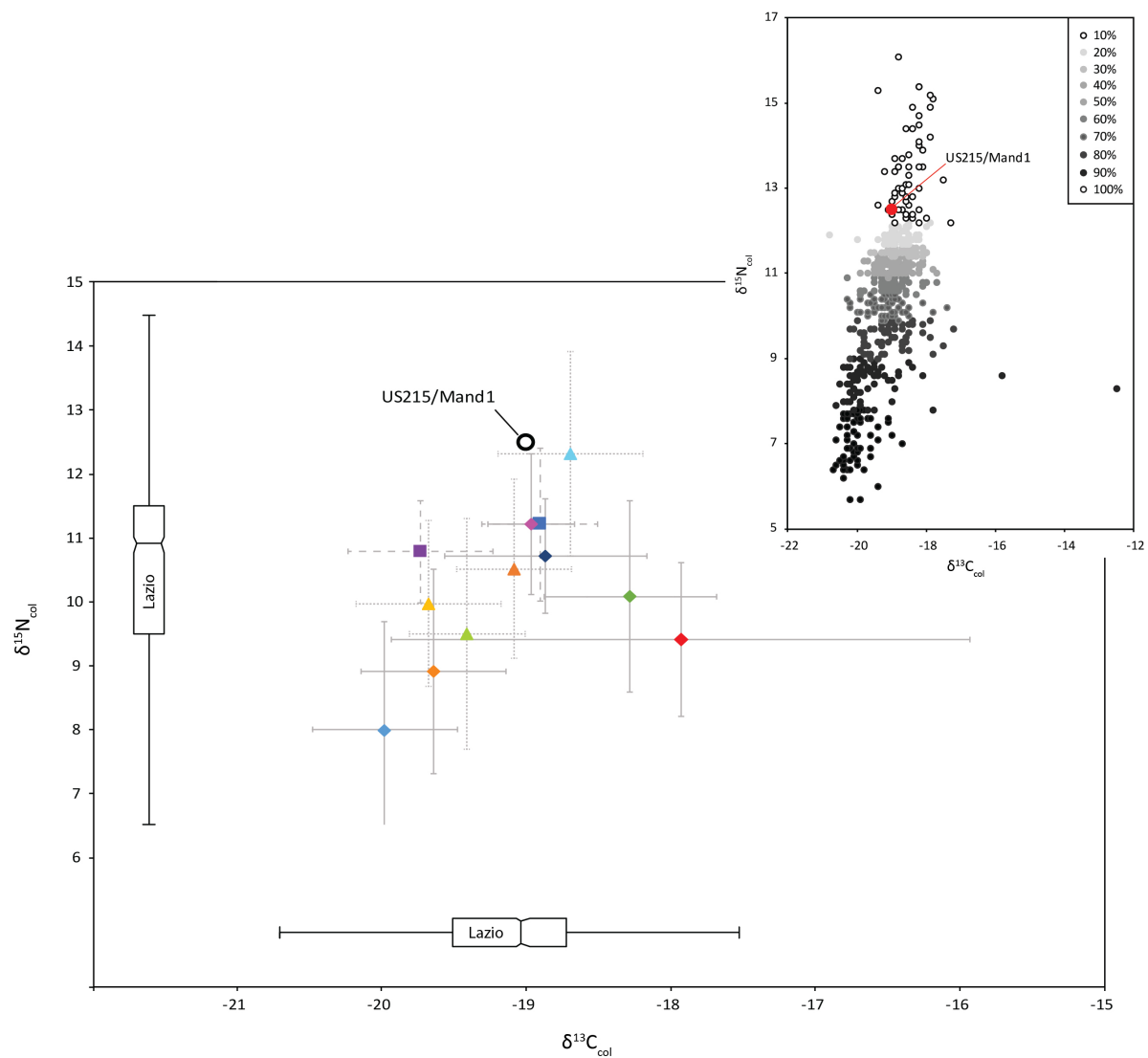
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714 **Fig. 7.** Biplot of the $\delta^{34}\text{S}_{\text{col}}$ and $\delta^{15}\text{N}_{\text{col}}$ values measured on bone samples from the SSPM individuals. Note: The
 715 shaded area corresponds to the range of $\delta^{34}\text{S}_{\text{col}}$ values for modern marine fish. The arrows indicate where
 716 individuals should stand according to their diet.



Contribution of Aquatic Resources:

- ◆ Castellaccio Europarco
- ◆ Osteria della Fontana
- ◆ SSPM Catacombs - Region X
- ◆ Casale del Dolce
- ◆ Casal Bertone
- ◆ Gabii

Contribution of Marine Fish:

- ▲ SSPM Catacombs - Region I
- ▲ Lucus Feroniae
- ▲ Isola Sacra
- ▲ ANAS

Contribution of Freshwater Fish:

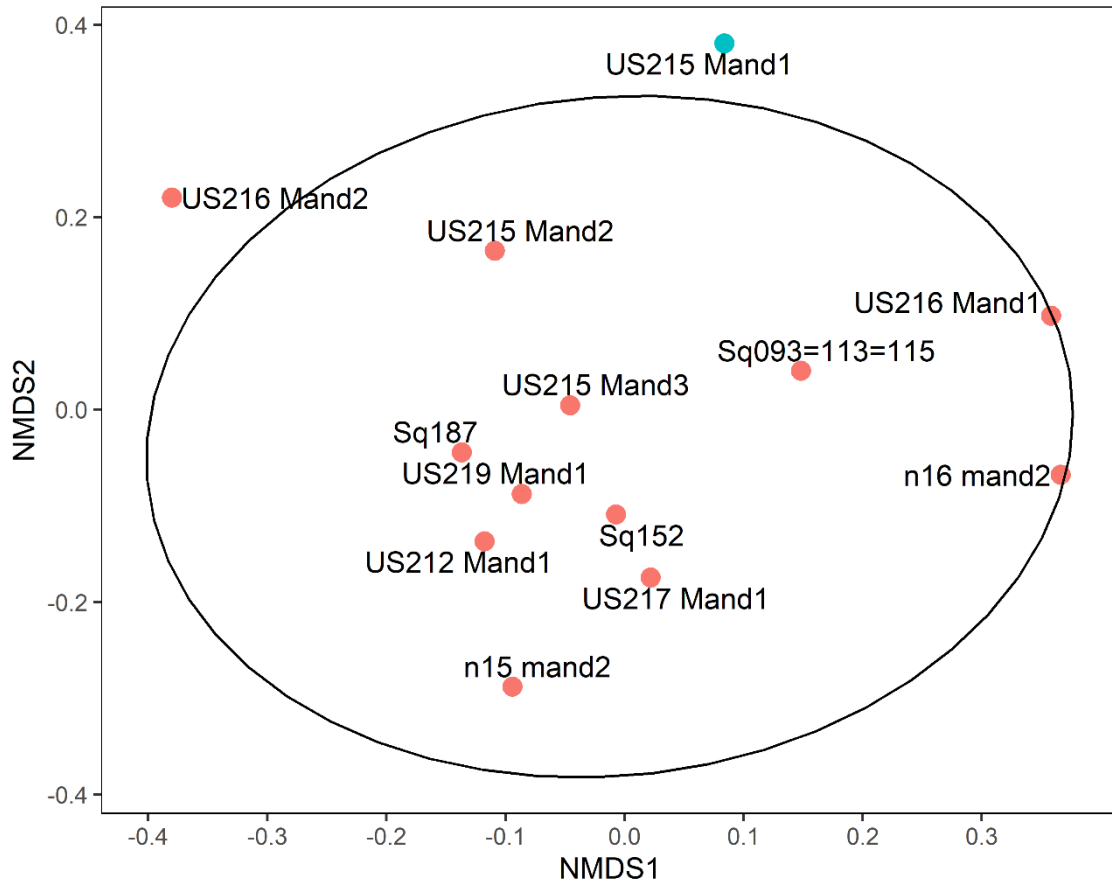
- St. Callixtus Catacombs
- Tenuta del Duca
- US215/Mand1

717

718 **Fig. 8.** Biplots of the $\delta^{13}C_{col}$ and $\delta^{15}N_{col}$ values (mean \pm 1 SD) measured on human bone samples from Lazio. Note:
 719 The classification of the populations is based on the interpretations proposed by Prowse et al. (2004), Rutgers et
 720 al. (2009), Nitsch (2012), Killgrove and Tykot (2013, 2018), Salesse (2015), Tafuri et al. (2018), O'Connell et al.
 721 (2019). The boxes show the regional variability of the isotopic values. Color gradient of the scatter plot in the top
 722 right corner is based on the $\delta^{15}N_{col}$ values.
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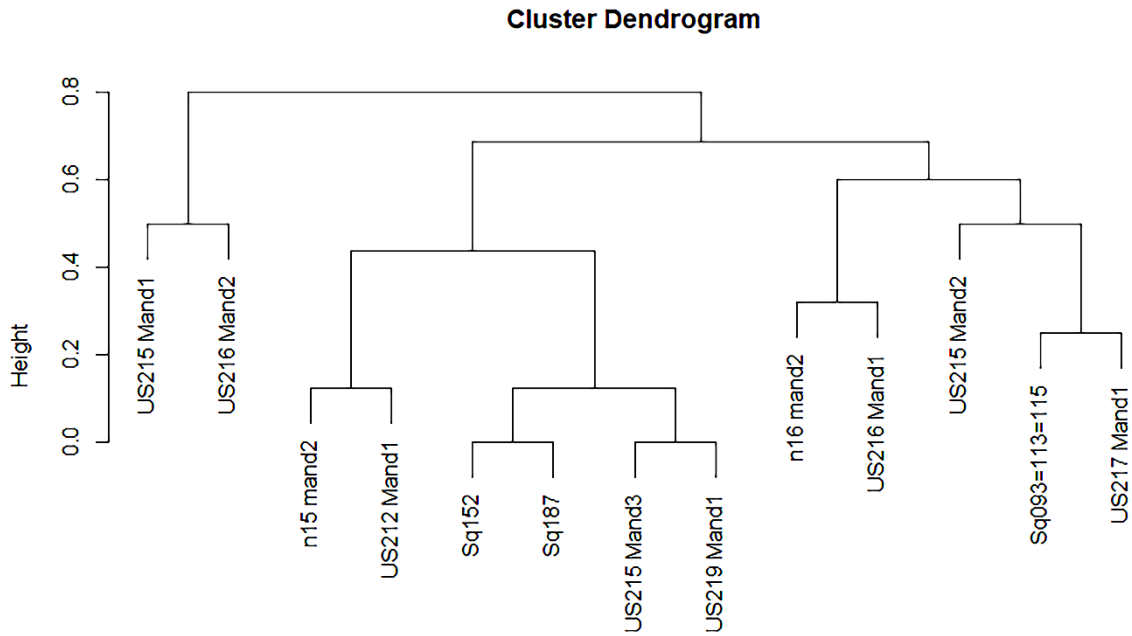
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727 **Fig. 9.** NMDS plot was created from the distance matrix (see Table C2) of the SSPM individuals. The ellipse marks
 728 the 90% confidence level for the sample. Both US215 Mand1 and US216 Mand2 fall far away from the rest of the
 729 individuals.

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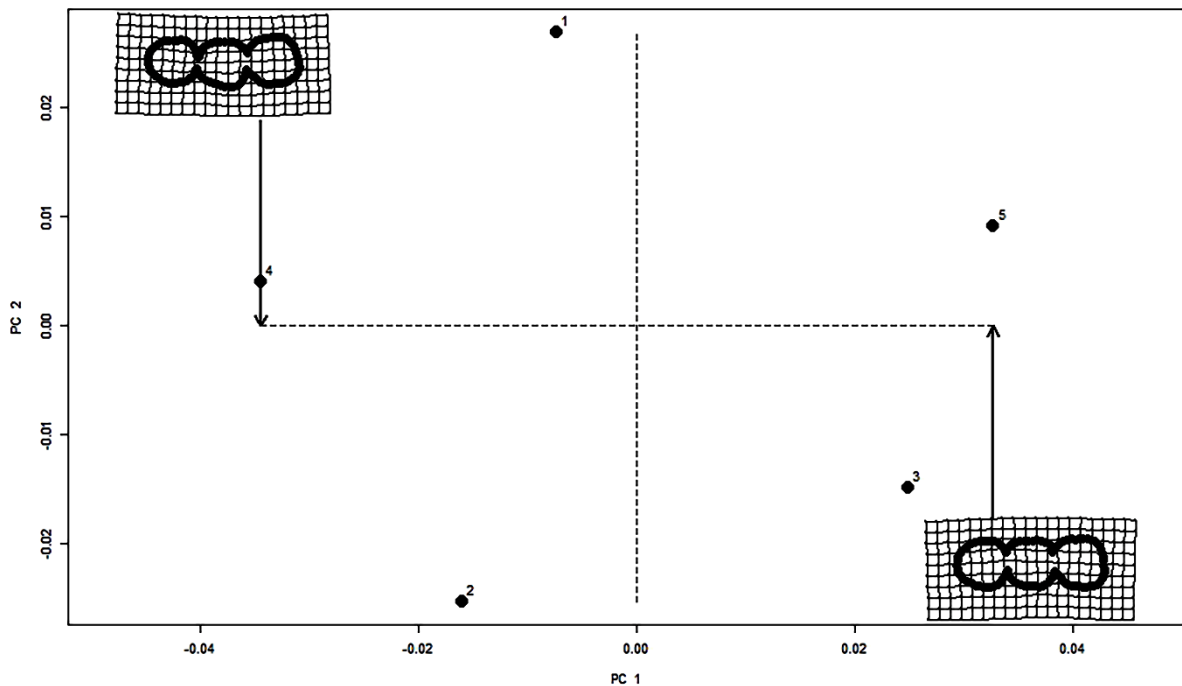
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734 **Fig. 10.** Hierarchical dendrogram generated from the SSPM non-metric dental dataset. Note: The agglomerative
 735 method complete linkage was used. US215/Mand1 (ID 206) and US216/Mand2 (ID 210) are separated from the
 736 rest.

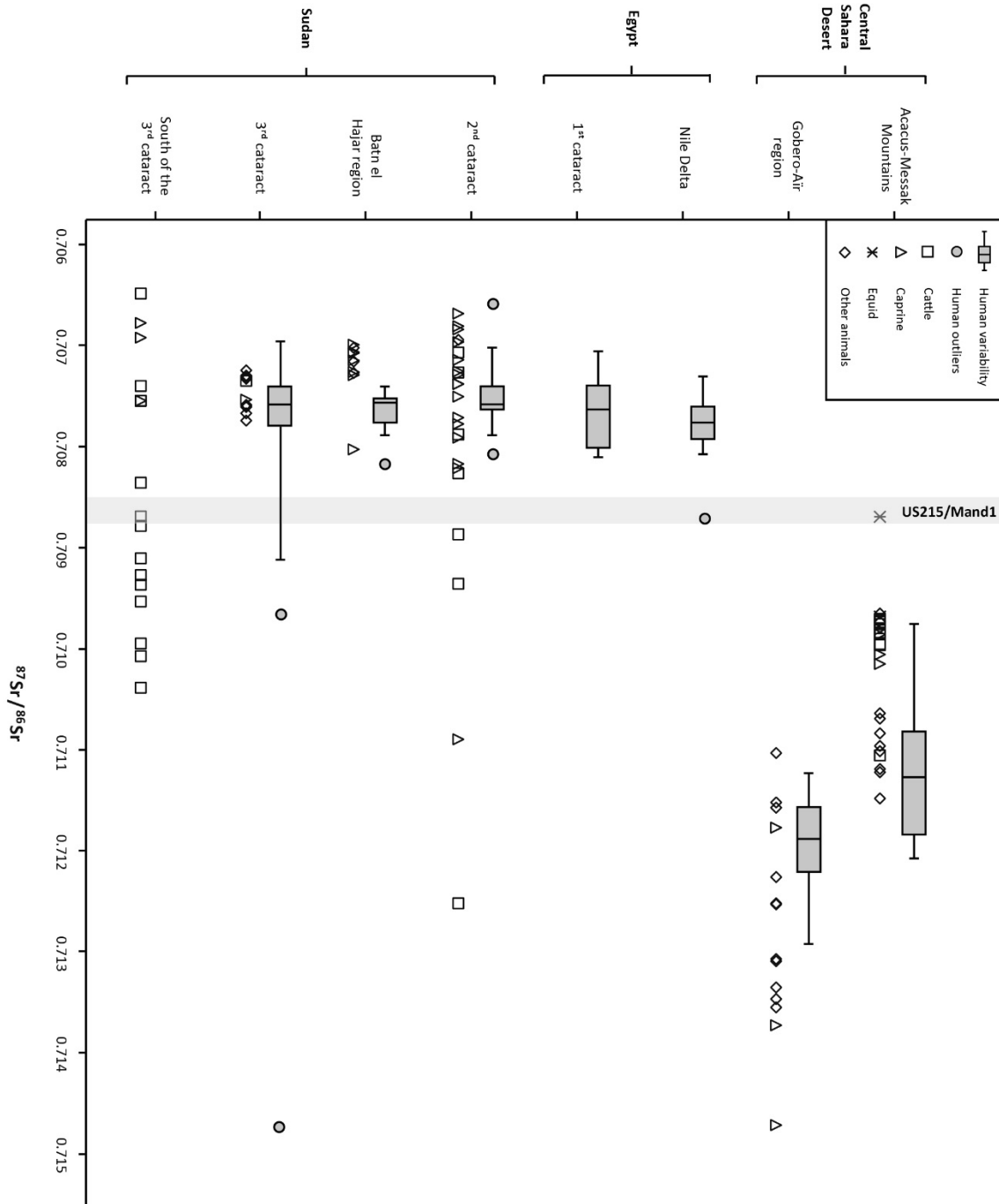
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739 **Fig. 11.** PCA plot performed on the geometric morphometric data obtained on the SSPM individuals. Note: PC1
 740 explains 52% of the shape variation, and PC2 28%. ID1=X81_n°14/Mand2, ID2=X83_US215/Mand1,
 741 ID3=X83_US216/Mand2, ID4=X83_US217/Mand1, and ID5=X83_US219/Mand1.

742



743

744 **Fig. 12.** Variability of the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios measured on human and faunal remains from North African region.
 745 Bibliographic references: Tafuri et al. (2006), Sereno et al. (2008), di Lernia and Tafuri (2013), di Lernia et al.
 746 (2013), Stojanowski and Knudson (2014) for Central Sahara Desert; Buzon et al. (2007), Buzon and Simonetti
 747 (2013), Schrader et al. (2019) for Egypt-Sudan. Note: Populations dates from different times periods. Boxes
 748 represent the range of variation of human values. Outliers have been identified through Grubb's tests. The
 749 shaded area corresponds to the range of $^{87}\text{Sr}/^{86}\text{Sr}$ values exhibit by the molars of US215/Mand1.

750 **TABLES**

751

752 **Table 1.** Individuals used in the biodistance (based on dental traits (DT)), geometric morphometrics (GM) and
753 DNA analyses.

DT ID	GM ID	DNA ID	Room/Site	Individual	Sex	Age
-	-	1	X81	n°13/Mand1	U	11.8+
-	1	-	X81	n°14/Mand2	U	16.4+
101	-	-	X81	n°15/Mand2	U	-
102	-	-	X81	n°16/Mand2	U	11.8+
-	-	2	X83	Sq108	F	15-19
203	-	-	X83	Sq093=113=115	U	20+
204	-	3	X83	Sq152	U	20+
205	-	-	X83	US212/Mand1	U	8.7+
206	2	4	X83	US215/Mand1	U	16.4+
207	-	-	X83	US215/Mand2	U	16.4+
208	-	-	X83	US215/Mand3	U	16.4+
209	-	-	X83	US216/Mand1	U	14.8+
210	3	-	X83	US216/Mand2	U	16.4+
211	4	-	X83	US217/Mand1	U	16.4+
212	5	5	X83	US219/Mand1	U	16.4+
-	-	6	X83	US219/Mand2	U	11.8+
313	-	-	X84	Sq187	F?	15-19

754

755

756 **Table 2.** Traits used to investigate biodistance.

Trait	Abbr.	Tooth	Reference	Type	n
Protostylid	PR	LM1	Scott and Irish, 2017	Binary	5
Deflecting wrinkle	DW	LM1	Scott and Irish, 2017	Ordinal	9
Hypoconulid	C5	LM2	Scott and Irish, 2017	Ordinal	10
Groove pattern (present=y)	GP	LM2	Scott and Irish, 2017	Binary	13
Anterior fossa	AF	LM2	Scott and Irish, 2017	Ordinal	12
Enamel extension	EE	LM2	Scott and Irish, 2017	Ordinal	13
Root number	R	LM2	Scott and Irish, 2017	Ordinal	8
Crenulation	C	LM2	Pilloud et al., 2018	Binary	8
Lower third molar absence	UM3V	LM3	Scott and Irish, 2017	Binary	12
Mandibular molar pit tubercle	MPT	LM3	Marado and Silva, 2016	Ordinal	8
Torsomolar angle	TA	LM3	Scott and Irish, 2017	Binary	9

757 Note: 'Abbr.' stands for abbreviation. 'Tooth' refers to teeth selected to represent the trait. 'Type' refers to the
758 way the trait was coded into the software. 'n' indicates the overall number of observations from the SSPM
759 individuals.

760

Table 3. Stable isotope results for reconstruction dietary patterns of US215/Mand1.

US215/Mand1 - Diet														
Molar teeth			Mandibular bone											
M1	M2	M3	%Col	%C	%N	%S	C:N	C:S	N:S	$\delta^{13}\text{C}_{\text{col}}$	$\delta^{15}\text{N}_{\text{col}}$	$\delta^{34}\text{S}_{\text{col}}$	$\delta^{13}\text{C}_{\text{carb}}$	
$\delta^{13}\text{C}_{\text{carb}}$	$\delta^{13}\text{C}_{\text{carb}}$	$\delta^{13}\text{C}_{\text{carb}}$												
-12.9	-12.5	-12.3	6.7	38.5	13.6	0.31	3.3	331.2	100.3	-19.0	+12.5	+8.4	-13.9	

761

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764

Table 4. Stable isotope results for discussing the mobility of US215/Mand1.

US215/Mand1 - Mobility											
Molar teeth									Mandibular bone		
M1			M2			M3					
$\delta^{18}\text{O}_{\text{carb}}$	$\delta^{18}\text{O}_{\text{dw}}$	$^{87}\text{Sr}/^{86}\text{Sr}$	$\delta^{18}\text{O}_{\text{carb}}$	$\delta^{18}\text{O}_{\text{dw}}$	$^{87}\text{Sr}/^{86}\text{Sr}$	$\delta^{18}\text{O}_{\text{carb}}$	$\delta^{18}\text{O}_{\text{dw}}$	$^{87}\text{Sr}/^{86}\text{Sr}$	$\delta^{18}\text{O}_{\text{carb}}$	$\delta^{18}\text{O}_{\text{dw}}$	
+1.3	+0.9	0.7085	+2.3	+2.6	0.7086	+1.2	+0.7	0.7087	-3.0	-4.4	

765

Note: See section 4.1.4 for the conversion procedures to estimate the $\delta^{18}\text{O}_{\text{dw}}$ values.

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