



HAL
open science

Multiproxy evidence highlights exceptional heterogeneous social status in the middle neolithic in Europe

Gwenaëlle Goude, Sébastien Villotte, Domingo Salazar-García, Maïté Rivollat, Ana Arzelier, Marie-Hélène Pemonge, Marie-France Deguilloux, Aurore Schmitt

► **To cite this version:**

Gwenaëlle Goude, Sébastien Villotte, Domingo Salazar-García, Maïté Rivollat, Ana Arzelier, et al.. Multiproxy evidence highlights exceptional heterogeneous social status in the middle neolithic in Europe. *Archaeological and Anthropological Sciences*, 2025, 17 (12), pp.224. <10.1007/s12520-025-02321-4>. <hal-05362394>

HAL Id: hal-05362394

<https://hal.science/hal-05362394v1>

Submitted on 17 Nov 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC-ND 4.0 - Attribution - Non-commercial use - No Derivative Works - International License

1 **Multiproxy evidence highlights exceptional heterogeneous social status in the Middle Neolithic in**
2 **Europe**

3

4 Goude Gwenaëlle*[✉] 1, Villotte Sébastien 2,3, Salazar-García Domingo C. 4, 5, Rivollat Maité 6,7,8
5 Arzelier Ana 9, Pemonge Marie-Hélène 9, Deguilloux Marie-France* 9, Schmitt Aurore* 10

6

7 *These authors contributed equally to this work

8

9 1 – CNRS, Aix Marseille Univ, Minist Culture, LAMPEA, Aix-en-Provence, France

10 2 - UMR7206 Éco-Anthropologie, CNRS, MNHN, Université Paris Cité. Musée de l'Homme, Paris,
11 France

12 3- Quaternary environments & Humans, OD Earth and History of life, Royal Belgian Institute of
13 Natural Sciences, Brussels, Belgium

14 4 - Departament de Prehistòria, Arqueologia i Història Antiga, Universitat de València, València, Spain

15 5 - Department of Geological Sciences, University of Cape Town, Cape Town, South Africa

16 6 - CNRS, Bordeaux University, UMR 5199 PACEA, Pessac, France

17 7 - Ghent University, ArcheOs lab, Department of Archaeology, Ghent, Belgium

18 8 - Department of Archaeogenetics, Max Planck Institute for Evolutionary Anthropology, Leipzig,
19 Germany

20 9 - Bordeaux University, UMR 5199 PACEA, Pessac, France

21 10 - CNRS, Ministère de la Culture, Université Paul Valéry, ASM, Montpellier, France

22

23 [✉]Corresponding author: gwenaelle.goude@univ-amu.fr ; gwenaelle.goude@cnrs.fr

24 orcid.org/0000-0002-3008-3607

25

26 [✉]Corresponding author: gwenaelle.goude@univ-amu.fr ; gwenaelle.goude@cnrs.fr

27 orcid.org/0000-0002-3008-3607

28

29 **Abstract**

30 The contribution of multidisciplinary approaches to the study of the lifestyles of ancient populations is
31 now well established in the literature. Mobility, nutrition and physical activity are indicators that,
32 when considered together, can document social organisations such as patrilocality, gender practices or
33 social status. Our study is part of this dynamic scientific work and focuses on the Middle Neolithic
34 human remains from the site of Pontcharaud (excavation 1986; 4300-3900 BCE; Auvergne, France). It
35 aims to reconstruct patterns of behaviour, life history, and social organisation, particularly in light of
36 the site's unique funerary features, which remain a subject of debate. New data from
37 bioanthropological investigations (72 individuals), stable isotope measurements (bone collagen $\delta^{34}\text{S}$
38 from 55 individuals; $^{87}\text{Sr}/^{86}\text{Sr}$ from teeth enamel on 17 individuals) and genetic sex estimation (2
39 subadult individuals) were collated and integrated with contextual information, including funerary
40 treatments, previously published isotopic data ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$), and archaeological records. Stable isotope
41 data suggest that some females were exposed to more variable environments compared to males.
42 Strontium and sulphur isotope ratios also indicate differences in resource exploitation (water, plants,
43 and animals from different origin or growth conditions) and suggest a different provenance for at least
44 two females, one of whom was buried in a stone coffin. The seven adult and immature male
45 individuals from the site's only multiple grave yielded distinct stable isotope results (C, N, S and, to a
46 lesser extent, Sr), suggesting different origins and/or animal protein intake, compared to individuals
47 buried in single or double graves. Our multidisciplinary data reveal important variability in diet,
48 provenance, and organisation within the Pontcharaud funerary group. We propose that the seven males
49 from the multiple gathering may have been involved in a shared activity that exposed them to the same
50 environment—perhaps as individuals engaged in a common economic or social practice.

51

52 **Keywords:** Isotopes, Mobility, Diet, Funerary treatment, Economy, Social structuration

53

54 **Conflict of interest**

55 The authors declare that they have no known competing financial interests or personal relationships
56 that could have appeared to influence the work reported in this paper.

57

58 **Data availability statement**

59 The authors declare that the data supporting the findings of this study are all available within the
60 article and its supporting information (SI) files.

61 **Main text (2500-6000 words)**

62

63 **Introduction**

64 Multidisciplinary investigations aimed at reconstructing past human behaviours have proven
65 particularly effective in understanding the variability of mobility and dietary patterns during Prehistory
66 as well as in exploring gender roles and social status (e.g. Knipper et al 2017; Goude et al. 2019; Mittnik
67 et al. 2019; Cassidy et al. 2020; Masclans Latorre et al. 2021a). Several studies on Neolithic sites across
68 Europe have demonstrated differences in mobility patterns between females and males throughout their
69 life. The trend of a patrilocal system associated with female exogamy is frequently proposed, supported
70 by genetic evidence of exogenous origins and a broader range of strontium (Sr) values recorded in
71 females (e.g. Rivollat et al. 2023; Rey et al. 2019; Bentley et al. 2012; Bickle and Hoffmann 2007;
72 Goude et al. 2019; 2020a). However, early farmers' mobility is not solely restricted to females; rather,
73 it was part of a more complex social organisation within agropastoralist communities. This complexity
74 is increasingly evidenced by the growing dataset of strontium and sulphur (S) isotope analyses (e.g.
75 Rivollat et al. 2023; Bentley 2013; Hofmann 2020).

76 Differences in access to food resources between individuals, particularly between males and females,
77 have been well documented in Neolithic Europe through carbon (C), nitrogen (N) and sulphur stable
78 isotope analysis on bone collagen (e.g. Goude et al. 2020a). More recently, analysis of dentine collagen
79 has also revealed behavioural variation over the course of an individual's life (e.g. Goude et al. 2020b),
80 which may be related to "social ages" (e.g. Rey et al. 2021) and possibly to the sexual division of labour
81 across age categories (e.g. Villotte, Knusel 2014). For example, an isotopic study based on sequential
82 tooth sampling at the Neolithic site of Gurgy (Yonne, Paris basin, France) explored the possibility of
83 documenting sex-based roles during childhood and adolescence. It highlighted isotopic shifts (C, N, and
84 S) between females and males, first around the age of 9, and then again at approximately 14, mirroring
85 modifications in funerary treatment for the same age categories (Le Roy 2015; Rey et al. 2021). In
86 parallel, sexual division of labour among European Neolithic groups can be inferred from multiple lines
87 of evidence, including: stone tool morphology and use wear in association with the grave goods of the
88 deceased in burials (Masclans Latorre et al. 2021a), high humeral rigidity in females suggesting intense
89 manual labour (Macintosh et al. 2017), lateralized limb use in males possibly linked to throwing
90 behaviours (Villotte, Knusel 2014), and high-intensity upper limb activity in males, possibly related to
91 the practice of archery (Thomas 2014). Other authors have proposed that, rather than biological sex,
92 social status – particularly the social status of exogenous individuals – may also play a significant role
93 in the division of labour. This argument is supported by Goude et al. (2019) who compared multi-
94 element stable isotope data (C, N, S, Sr) and grave goods associated with the deceased (e.g. flint stone,
95 lamb foetuses buried with the deceased).

96 Beyond the mobility arguments, patrilocal patterns observed in European communities during the
97 Neolithic and throughout the Bronze Age (e.g. Knipper et al. 2017) highlight the importance of both
98 biological and possibly social kinship. The recent data from Gurgy (Yonne valley, Paris basin, France)
99 is particularly eloquent (Rivollat et al. 2023), as a body of genetic and isotopic data points to a paternal
100 rooting in the management of the territory, funeral ritual, and perhaps the family in the broadest sense.
101 The role of sex and social status in the social organisation of Prehistoric communities is also
102 demonstrated by the study of Mittnik et al. (2019), which compares genomic and isotopic data (Bronze
103 Age, Germany), as well as the study of Cassidy et al. (2020), which shows restrictive reproductive access
104 linked to the social elite and megalithic use in Neolithic Ireland. This brief synthesis of scenarios from
105 Prehistoric Western Europe highlights the complexity and diversity of social organisation patterns
106 regulated by at least sex and social status, but also perhaps by age and other factors that can arise in the
107 course of an individual's life (starvation, economy collapse, environmental constraints, interpersonal
108 violence, etc.).

109 In this context, the Middle Neolithic funerary site of Pontcharaud (Auvergne, Massif Central, France)
110 raises new questions. The Pontcharaud necropolis is characterised by the diversity of funerary deposits
111 and practices. There is no bias toward a particular age or sex for the deceased in the mainly individual
112 and double graves. However, its originality lies in the presence of a multiple gathering¹ of seven
113 individuals buried simultaneously. This deposit is exceptional for the Middle Neolithic period in France,
114 and its signification, as well as the social status of this particular funerary group, is still debated (e.g.
115 Testard 2004; Chambon and Leclerc 2007; Boulestin 2008; Schmitt and Saliba Serre 2014), with
116 interpretations ranging from warriors, accompanying dead, to sacrificed people. At the site level, a
117 previous study including stable isotope (carbon and nitrogen; summary in table 1 and SI 1) and
118 osteological records (Goude et al. 2013) pinpointed dietary differentiation among two categories of
119 individuals: adult females and the seven individuals found in the multiple gathering. Using a
120 multidisciplinary approach that includes osteobiography, biogeochemistry, ancient DNA, and a revised
121 reading of archaeological records, we aim to focus on this special osteological series to explore the role
122 of interrelated biological sex and social status in the formation of this community.

123

124 **Materials and Methods**

125 *Pontcharaud 2 site: a unique archaeological discovery*

126 The first excavation of the Pontcharaud site (figure 1) was conducted in 1986 under the supervision of
127 G. Loison (1998), uncovering 56 archaeological funerary structures (Schmitt et Saliba-Serre 2014)

¹ Multiple gathering is defined by Boulestin and Courtaud (2022) : if at least two people have been deposited in a unitary use, one will speak of gathering (*rassemblement*) and a gathering is termed 'multiple' (*multiple*) whenever it is possible to demonstrate that all the individuals have been gathered at the same time.

128 containing 72 individuals, dated to the Middle Neolithic chronocultural period (4300-3900 cal. BCE;
129 Goude et al. 2013). In 2019 and 2020, a new excavation was conducted adjacent to the former funerary
130 occupation (Delrieu 2022), discovering 50 new burials (Prodeo et al. 2024). The bioarchaeological
131 investigation of this new site is still ongoing². Besides, in 2000, another excavation in the same locality
132 yielded an additional ten Neolithic burials, which shared similarities in funerary treatment (site of Brézet
133 in Clermont-Ferrand Inrap Excavation, Vernet 2002). Thus, the history of fieldwork in this area suggests
134 the potential for division into sectors, indicating that the material investigated in the present paper,
135 centred on the 1986 remains, may only represent a specific part of the burial ground use by the same
136 population.

137 Among the structures excavated in 1986, individuals were found in different burial types, predominantly
138 within wooden coffins (a well-documented funerary practice in this area; Saintot et al. 2016), except for
139 one individual discovered within a stone coffin (individual 12). Overall, grave goods associated with
140 the deceased are infrequent (Loison 1998; Civetta et al. 2009). Most burials contain either one or two
141 deceased. However, one deposit in particular stands out as a multiple gathering (figure 2). This unique
142 structure contains a total of seven individuals (individuals 31 to 37) who were buried simultaneously.
143 Six of them were deposited in a prone position, oriented along a north-south axis and facing northward,
144 except for one skeleton (individual 36) lying southward. This individual (36), located at the eastern
145 extremity of the pit, was lying on his back, slightly rotated onto his right side. The first five bodies
146 deposited (33 to 37), in the north part of the pit, were aligned in a row, with their heads oriented along
147 an east-west axis. The last two (31, 32) were shifted to the south. The bodies were initially covered with
148 earth, followed by two limestone slabs of large dimensions and smaller stones covered the deposit, in
149 direct contact with the human remains. Notably, individual 37 was found with a flint arrowhead in an
150 upper thoracic vertebra, while individual 33 had received a blow to the head (Billard 2010). Both
151 traumas strongly suggest a violent death.

152

153 *The osteological collection*

154 A set of bioanthropological data (sex, age, stature, and pathologies) has been reported in various
155 previous publications (Civetta et al. 2009; Billard 2004; 2010; Schmitt and Saliba-Serre 2014). A
156 previous study (Goude et al. 2013) analyzed 58 human and seven animal bones, providing carbon ($\delta^{13}\text{C}$)
157 and nitrogen ($\delta^{15}\text{N}$) stable isotope compositions of bone collagen. This research highlighted (1) a diet
158 with significant animal protein intake for several individuals of both sexes, (2) a higher variability of
159 nitrogen isotope ratio values among females, and (3) a distinct isotopic cluster corresponding to the
160 individuals from the multiple gathering (figure 3; SI 1). These initial biochemical data allowed us to

² Osteological studies by I. Thomson and collaborators
<https://journals.openedition.org/arheopages/14238?lang=fr>

161 question the role of females and/or individuals with specific social statuses. The findings aligned with
162 other datasets indicating a high level of biomechanical stress recorded in young adults (high spinal
163 loads), possible sexual division of physical activities (based on long bone morphology), and varying
164 geographic origins among females (suggested by cranial shape) (Civetta 2009; Billard 2010). These
165 preliminary elements of discussion, combined with the well-preserved osteological collection of
166 Pontcharaud, encouraged us to pursue further bioarchaeological investigations using a multidisciplinary
167 approach. This included new isotopic analyses (S and Sr) to explore mobility patterns and social
168 structures. Additionally, as a first step, palaeogenomic analyses were conducted on two subadult
169 individuals from the multiple deposit to estimate their sex.

170

171 *Methods*

172 All the technical and laboratory information is available in Supplementary Information 2.

173 Osteological records

174 A control study was conducted on the 72 individuals to confirm the sex and age at death (SI 1).
175 Depending on growth stage, different methods were applied to estimate the age at death of subadult
176 individuals. For children under one year of age, stature was estimated from equations derived from
177 regressions proposed by Sellier (in Schmitt and Georges 2005) based on diaphyseal length. For
178 individuals older than one year, multiple methods were applied. Dental age proved to be a more reliable
179 indicator than bone age and was therefore preferred. Tooth eruption and mineralization were assessed
180 using Ubelaker's reference (1978). When teeth were absent or when the mineralization of permanent
181 teeth was complete, age was estimated based on the fusion of secondary ossification points (Birkner
182 1980). For adults, age-at-death estimation was based on the state of fusion of later-fusing secondary
183 centers of ossification, following Owings Webb and Suchey (1985) and Albert and Maples (1995), the
184 surface characteristics of the pubic symphyseal face, and the morphology of the sacroiliac surface of the
185 os coxae, following Schmitt's method (Schmitt, 2002, 2005). For sex assessment, the morphology and
186 morphometrics of the os coxae were employed as the primary means of diagnosis, following Brůžek
187 (2002) and Brůžek et al. (2017). For 9 individuals whose os coxae were not well preserved, we applied
188 a secondary sex diagnosis based on discriminant function analysis, a demonstrably reliable method
189 (Murail et al. 1999).

190 The presence of external auditory exostosis (EAE), an asymptomatic bony growth caused by irritation
191 of the periosteum in the external meatus (Di Bartolomeo, 1979), was assessed following the criteria of
192 Standen et al. (1997) and Crowe et al. (2009). Repeated exposure to cold water is the main factor
193 triggering this irritation, and a high frequency of EAE in an archeological sample can be considered a
194 strong indicator of activities related to aquatic resource exploitation (for a review, see Villotte & Knüsel,

195 2016). The presence or absence of EAEs was recorded for 45 individuals (34 adults and 11 subadults
196 under 15 years of age). In most cases (82.2%), preservation allowed for bilateral assessment.

197 Enthesopathy at the humerus was recorded following Villotte' scoring system (2006, see also Villotte,
198 Castex et al., 2010). Data were collected for four entheses on each humerus, for all "adults. However,
199 results are discussed only for sexed individuals whose age at death was estimated to be under 50 years,
200 considering the relative strong influence of age on the apparition of enthesal changes in the skeleton
201 (e.g., Villotte & Santos, 2023). Fractured limbs were not scored, and individuals with systemic
202 pathologies were excluded from the study (see Villotte, Castex, et al., 2010 for exclusion criteria). The
203 final sub-sample consists of 15 females and 7 males. Skeletal markers associated with regular posture
204 (namely squatting and kneeling) were recorded. For squatting, the presence or absence of lateral facets
205 on the talus was assessed following Barnett (1954, stage F) and Boule (2001, lateral pressure facet) for
206 35 adults (in most cases (82.9%), both tali were examined bilaterally). For kneeling, given the variability
207 of skeletal changes associated with this posture (Ubelaker, 1979; Villotte, 2024), only changes on the
208 dorsal aspect of the distal part of the diaphysis of the first metatarsal were recorded. Only major, clearly
209 visible changes were considered. Nineteen individuals were included in this analysis, with bilateral
210 preservation of the first metatarsals in only five cases. These osteological data are integrated as part of
211 our holistic approach to the study of the living conditions of human groups. These bone indicators are
212 combined with other information to discuss the impact of physical activities and potential mobility on
213 access to and need for food resources (Masclans Latorre et al. 2021b).

214

215 Isotopic analyses

216 The protocols used for sample preparation and the equipment used for $\delta^{34}\text{S}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ analysis is
217 included in SI 2. Stable isotope analysis of sulphur ($\delta^{34}\text{S}$; figure 4) was performed on individuals
218 previously analysed for carbon and nitrogen isotopes, as well as on the limited number of available
219 animal remains (n=7) recovered from the burials (*Bos* sp., caprines and *Sus* sp.; Goude et al. 2013).
220 Specifically, 55 human bone samples selected based on the best collagen preservation from the previous
221 study (biological descriptions and data available in SI 1) were analysed to infer protein intake during
222 the last years of life (SI 2) (e.g. Szulc et al., 2000 ; Valentin 2003). Sulphur isotope analysis aids in
223 identifying food intake and assessing individual mobility (Nehlich, 2015). The spatial distribution of the
224 $\delta^{34}\text{S}$ values for mainland France, as reported by Bataille et al. (2021), ranges from approximately 4‰ to
225 8‰ for the Auvergne region. This range will serve as a reference for interpreting our data. Radiogenic
226 strontium isotope ratios ($^{87}\text{Sr}/^{86}\text{Sr}$) were measured in the tooth enamel of 17 individuals, selected based
227 on the availability and preservation of dental material, as well as the prior CN data (considering
228 individuals detected as isotopic outliers or based on their archaeological context) (SI 1). When possible,
229 two teeth per individual were sampled: for 11 individuals, both an M3 and an M2/P4 were sampled,

230 while for the remaining 6 individuals, only an M2/P4 was sampled. This sampling strategy enables the
231 assessment of potential changes in geological substrate and/or sources of supply (water, plant, animal)
232 between two life stages: childhood and early adulthood (SI 2). One of the challenges in the targeted
233 region is the highly variable lithology of the Massif Central (SI 2), which results in significant variations
234 in recorded $^{87}\text{Sr}/^{86}\text{Sr}$ values. Similar environmental complexities have been observed in Neolithic
235 contexts, such as at Gurgy (Rey et al. 2021; Rivollat et al. 2023). Recent $^{87}\text{Sr}/^{86}\text{Sr}$ values published by
236 Willmes et al. (2018) and Holt et al. (2021) for France indicate that within a 20km radius, and identically
237 within a 50 km radius, from the site, $^{87}\text{Sr}/^{86}\text{Sr}$ can range widely, from 0.704 to 0.721 (fig. SI2 – 1).
238 Additionally, geological investigations on granitic samples located at 75 km east of the site provide
239 $^{87}\text{Sr}/^{86}\text{Sr}$ values between 0.721 and 0.723 (Briot 2002). Ideally, we would have to include archaeological
240 microfauna teeth to establish a relevant local baseline, but there is none available at the site and it was
241 not possible to do a broader own bioavailable map for the region by using modern plant as recommended
242 by several authors (ex. Britton et al. 2020; Chevallier et al. 2025). .Given this context the current lack of
243 site-specific mapping, the intra-individual sampling approach proposed here will open the discussion on
244 the variation in lands traversed and used during an individual's lifetime.

245

246 Palaeogenomic analyses

247 Palaeogenomic analysis was conducted on both subadult individuals from the multiple gathering
248 (individuals 34 and 35) to assess their genetic sex, which could not be assessed through traditional
249 osteological methods (SI 2). Petrous bones were specifically targeted due to their superior DNA
250 preservation (Gamba et al., 2014; Pinhasi et al., 2015), and the sampling was carried out directly at the
251 osteological deposit of Les Milles at Aix-en-Provence, France. Details regarding DNA isolation, double-
252 stranded libraries construction with partial UDG treatment, and the use of unique index pairs are
253 provided in SI 2. A shallow shotgun sequencing of the libraries was performed to evaluate the
254 preservation of endogenous DNA and, if a sufficient number of human sequences were retrieved, to
255 estimate the genetic sex of the individuals. The endogenous DNA content and main characteristics of
256 the DNA libraries were determined using EAGER v2.4.5 (see SI 2 for details). We evaluated the sex of
257 the individuals using two independent methods developed by Skoglund et al. (2013) and Mittnik et al.
258 (2016) (SI2).

259

260 *Results*

261 Osteological records

262 A total of 23 subadults and 38 adults (>15 years) were identified, with both sexes present (17 males and
263 19 females). An overall underrepresentation of children was observed. The specific simultaneous
264 deposit of seven individuals contains exclusively adult males, along with the two subadults analysed for

265 aDNA analysis in this study. Endogenous DNA was successfully obtained from both individuals 34 and
266 35 (with respectively 23,47 and 1,28% of endogenous DNA; see Table 2 in SI 2). The retrieved human
267 sequences exhibited characteristics typical of degraded ancient DNA, including a noticeable percentage
268 of deamination at the 3' and 5' extremities of fragments, and short fragment lengths (Table 2 in SI 2).
269 Genetic sex estimation classified both subadults as males, based on Ry coefficients (0,0038 and 0,0142,
270 respectively; Skoglund et al. 2013), which indicated genotypes “consistent with XY but not XX”.
271 However, it is important to note that the reliability of the result for individual 35 is lower due to the low
272 coverage of the library (Skoglund et al 2013, Buonasera et al 2020). Nonetheless, the Y ratio calculated
273 using Mittnik et al.'s (2016) method (0,53 and 0,78, respectively) further confirmed the assignment of
274 XY genotypes for both individuals.

275 None of the studied individuals exhibit EAEs. This finding aligns with previous research indicating a
276 very low frequency of EAE in European Neolithic groups, possibly due to a moderate reliance on the
277 aquatic environment or a seasonal/temporary access to lakeside environments during life (Villotte,
278 Stefanović, & Knüsel, 2014).

279 Enthesopathies of the humerus are relatively rare among the young and mature adult individuals (i.e.,
280 those under 50 years old) from Pontcharaud (see table 1 in SI 2). Only 3 males (out of 7) and 3 females
281 (out of 15) display lesions, all of which are minor. This is expected, as major enthesopathic changes are
282 predominantly observed in older individuals or those with systemic diseases, and are likely unrelated to
283 biomechanical factors (Villotte, Polet, Colard, & Santos, 2022; Villotte & Santos, 2023). Most affected
284 individuals exhibit only a single enthesopathy, except for female subject 13, who presents two lesions
285 (one at the right lesser tubercle, and the other at the left lateral epicondyle). Given the very low number
286 of lesions and the overall small sample size, any inferences regarding a potential sexual division of labor
287 in this community remain limited. One can note, however, that two females (subjects 12 and 69, out of
288 8 examined) present a lesion on the right lateral epicondyle, whereas no males do. Additionally, only
289 one individual (male subject 1) displays a lesion at the right medial epicondyle, more precisely at the
290 attachment area of the medial collateral ligament. Lesions in this area have been associated with
291 activities implying a throwing motion (such as spear throwing or the use of axes and adzes, see Polet et
292 al. 2019; Villotte et al. 2010; Villotte & Knüsel, 2014).

293 None of the individuals display true squatting facets, as defined by Barnett (1954). However, if one
294 adopts Boule's (2001) perspective that the lateral pressure facet is an osteological feature associated
295 with ankle hyperdorsiflexion, it is highly likely that squatting was a common practice within this
296 community. Indeed, 94.3% of the individuals exhibit such changes, with only two exceptions (male
297 subjects 32 and 39). This high frequency aligns with observations in other Neolithic groups (Villotte,
298 unpublished data). In contrast, kneeling facets are rare in the sample. Only one individual (male subject
299 39, who notably lacks any pressure facet) displays one clear articular extension on the dorsal aspect of

300 the distal part of the left first metatarsal shaft (the right metatarsal was not preserved). This may be
 301 associated with the fact that this individual displays a tibiofibular ankylosis at the right side, maybe
 302 related to a traumatic event.

303

Burial Nb	Sex	Age category	$\delta^{13}\text{C}$ vs. PDB (‰)	$\delta^{15}\text{N}$ vs. Air (‰)	$\delta^{34}\text{S}$ vs. CDT (‰)	$^{87}\text{Sr}/^{86}\text{Sr}$ enamel/Childhood	$^{87}\text{Sr}/^{86}\text{Sr}$ enamel/Adolescence
3	F	>60 yr	-19.9	10.0	6.30		
4	F	20-49 yr	-20.3	11.6	5.3		
5	M	>30 yr	-19.8	10.3	7.9		
7		>40 yr	-20.1	10.6	8.8		
8		NA	-20.2	10.6	9.3		
9	F	20-29 yr	-20.2	9.4	15.4	0.710213161	0.710110231
10	M	20-49 yr	-19.7	10.4	10.6		
12	F	20-29 yr	-20.3	9.6	10.2	0.714077411	0.708570854
13	F	20-39 yr	-19.9	10.5	8.3		
14	F	>40 yr	-20.0	10.2	7.3		
15	M	>40 yr	-19.9	9.9	9.4		
16	M	20-49 yr	-19.8	10.4	10.6		
18	M	40-59 yr	-19.8	10.3	6.2		
23	F	20-29 yr	-20.4	10.7			
26	M	40-59 yr	-20.2	11.8	6.8		
27	M	20-39 yr	-19.8	10.6	8.8	0.711569206	0.711603592
29	F	20-29 yr	-19.9	10.0	5.1	0.71089182	0.711305131
30	F	20-39 yr	-20.1	8.7	6.9	0.711130363	0.710944735
31	M	40-59 yr	-20.4	9.4	10.4	0.71008511	0.709490971
32	M	20-29 yr	-20.9	9.7	11.2	0.708686067	
33	M	>30 yr	-20.7	9.4	11.3	0.709797635	
36	M	40-59 yr	-20.5	9.7	11.7	0.710601041	0.7094357
37	M	20-49 yr	-20.8	9.3	11.2	0.711256671	
38	F	NA	-20.2	9.8	6.7		
39	M	20-49 yr	-20.4	11.3	5.0		
40	F	20-49 yr	-20.2	5.3	7.0		
41	F	40-59 yr	-19.8	10.3	6.4		
45	M	>40 yr	-20.2	10.5	5.2	0.711571341	0.711756647
51	F	40-39 yr	-20.3	9.8	3.6	0.71112485	
52	F	20-29 yr	-20.7	11.3	7.3	0.710537298	0.71288097
53	F	20-29 yr	-20.3	9.9	6.0	0.710300926	0.71061283
55	F	20-39 yr	-20.3	9.7	5.6		
60	M	>40 yr	-20.1	10.5	5.6	0.710960294	0.71110326
61	F	20-49 yr	-20.4	10.6	5.9		
62	F	NA	-20.7	13.0	4.5		
63	M	20-49 yr	-20.1	10.1	5.1		

66	M	>40 yr	-19.9	10.6	6.5		
69	F	20-39 yr	-20.2	9.9	7.0		
50		15-17 yr	-20.1	9.5	5.2		
2HS		6-12 months	-19.4	11.0	4.2		
20		2-4 yr	-19.9	11.1	4.4		
22		4-5 yr	-20.5	10.0	5.0		
24		1-2 yr	-19.8	12.0	5.2		
25		3-5 yr	-19.7	11.9			
26bis		2-3 yr	-19.8	10.3	7.3		
34	M	7,5-12,5 yr	-20.9	9.7	8.3		
35	M	12-15 yr	-20.7	9.9	9.7	0.710229219	
42		5-9 yr	-19.7	9.5	5.6		
43		5-6 yr	-20.7	11.4	5.2		
46		6-8 yr	-20.1	10.4	7.4		
54		1-3 yr	-18.9	12.7	5.1		
56bis		5-11 yr	-20.7	10.9	7.0		
57		5-11 yr	-20.4	11.0	5.2		
58		16-19 yr	-20.3	10.3	5.1		
59		7-11 yr	-20.4	9.4		0.71066622	
64		14-17	-20.1	10.0	6.5		
65		0-1 ans	-19.7	12.1	4.2		
67		5-9 ans	-20.2	8.6			
68		16-19 yr	-20.1	9.8			
70		immature	-19.9	10.2	5.3		
71		immature	-19.4	12.2	4.4		

304

305

306 Isotopic data

307 All collagen samples analysed for sulphur isotope ratios provided enough S content (>0.15%; between
308 0.5 and 0.26% in this study; Nehlich and Richards 2009) supporting the reliability of the data
309 interpretation (SI 1; table 1). The $\delta^{34}\text{S}$ values of humans range from 3.6 to 15.4‰ (range = 11.8‰),
310 while animal data span from 3.7 to 12.7‰ (range = 9.0‰), indicating that both groups exploited
311 territories of similar isotopic variability (Figures 4a and 4b). Notably, the $\delta^{34}\text{S}$ values observed in
312 humans and animals exceed the expected range proposed by Bataille et al. (2021).. In humans, the most
313 pronounced outliers include two women and subjects from the multiple gathering individuals (figure
314 4b). However, the spatial distribution of these values (*ibid.*) may not fully reflect local isotopic diversity,
315 which is influenced by the significant geological variations in this area. Animal remains at Pontcharaud

316 are scarce within the funerary deposits, and their isotopic diversity could reflect a wide range of
317 geographical origin and trade. The selection of some animal remains associated with the humans in the
318 burial is probably more linked to the diversity of human life histories (pastoralism? exogamy?) and,
319 thus, a wide territory that is difficult to locate.

320 The $^{87}\text{Sr}/^{86}\text{Sr}$ data of human enamel range from 0.708 to 0.714 (SI 1). The 22 modern plant samples
321 from the IRHUM database (Willmes et al. 2014) used for bioavailable Sr mapping show a $^{87}\text{Sr}/^{86}\text{Sr}$ range
322 from 0.704 to 0.722. We also propose a range of values that would allow to identify individuals who
323 have frequented different environments compared with the rest of the group by using the mean \pm 2 SD
324 of all the data recorded on the enamel of human teeth (SI 1). The modern plants-soil samples (Willmes
325 et al. 2018; Holt et al. 2021) and mineral water (Négrel et al. 1997) from the area of the site (Limagne
326 basin and surrounding volcanic terrains) show $^{87}\text{Sr}/^{86}\text{Sr}$ within the same variability (ca. 0.704 to 0.721).
327 All archaeological human teeth strontium isotope ratio values fall within the broad range present nearby,
328 whether from close range or within 20km to 50km radius of the site (Figure 5a and Figure 5b).
329 Furthermore, when using the human enamel $^{87}\text{Sr}/^{86}\text{Sr}$ to provide a possible “local” range (ie. mean \pm
330 2SD), the range recorded is between 0.708 and 0.713 (SI 1) and encompasses almost all human data. In
331 this context, it is difficult to discuss on local *versus* non-local origin, notably due to the interlocking
332 geological substrates of sedimentary and volcanic origin surrounding the site. The female 12 is,
333 however, an exception and shows great variability in the environments used between childhood and
334 adolescence (SI 1 and Figures 5a and 5b).

335

336 *Discussion*

337 The results of this new study provide new insights to discuss the diversity of life histories encountered
338 in the Pontcharaud necropolis. This variability can be perceived at different levels, through various
339 parameters, and allows for the distinction of specific groups outlined hereafter, such as the social status
340 of specific individuals, both females and males, and their role within this Neolithic group.

341

342 Have females and males different life histories?

343 The new sulphur stable isotope data confirm previous findings based on carbon and nitrogen isotopic
344 records (Goude et al. 2013; summary in table 1 and SI 1), and a Kruskal-Wallis test indicates significant
345 difference (p-value = 0.003) between the female, the male and the individuals buried in the multiple
346 gathering. The description of the data also indicates that, in the context of similar funeral practices, some
347 females were exposed to more variable environments (range of $\delta^{34}\text{S}$: 11.2‰) compared to males (range
348 of $\delta^{34}\text{S}$ 5.6‰; excluding the individual of the multiple gathering; figures 4a and 4b). However, it can be

349 emphasized that this variability is mainly driven by the presence of a particular female with a high $\delta^{34}\text{S}$
350 (burial 9, 15.4‰; figure 4a). Even if more subtle, $^{87}\text{Sr}/^{86}\text{Sr}$ also tends to provide evidence of sex-based
351 differences in mobility or provenance during early life (i.e., from ca 2.5 to 14 years old). Figures 5a and
352 b indeed show that the female from burial 12 and, to a lesser extent, the female from burial 52 (at the
353 limit of the possible local range), acquired their food resources in different environments and/or
354 consumed resources that were not reared or farmed under the same geological conditions between
355 childhood and early adolescence. Also, it should be noted that females with no $^{87}\text{Sr}/^{86}\text{Sr}$ inter-age
356 variability have slightly lower values (from 0.7101 to 0.7113) than males (excluding the specific
357 multiple: 0.7109 to 0.7117). At Pontcharaud, adults of both sexes show greater $\delta^{34}\text{S}$ variability than
358 juveniles (range of $\delta^{34}\text{S}$: 3.2‰) buried here, who were probably less mobile, and particularly greater
359 than infants under the age of 3 years ($\delta^{34}\text{S}$ from 2 to 5.2‰; figure 4a), whose bone isotopic composition
360 included maternal milk. An extended territoriality or origin is not exclusive to females or adults. In a
361 way, the case of male 39 could testify to this extended territoriality and diversity of physical activities.
362 This individual shows the lowest $\delta^{34}\text{S}$ among males, one of the highest $\delta^{15}\text{N}$ values in the group, and the
363 unique kneeling facet associated with traumatic lesions on the right lower limb. The mobility of people
364 has already been proposed for other Middle Neolithic sites in relation to sex, age and perhaps social
365 status in France at Le Vigneau (Touraine, North-western France; Goude et al. 2019), and Gurgy (Rey et
366 al. 2019; 2021; Rivollat et al. 2023), in the Mediterranean (Goude et al. 2020b) and in Central Europe
367 (Bentley et al. 2002; Price 2006). In any case, the interpretation of the data (for both adults and subadults
368 of both sexes) is often difficult to link with specific patterns of activity or social organisation, as they
369 may be related to various forms of individual behaviour. In such cases, the combination of isotopic data
370 and activity indicators is crucial to disentangle life histories.

371

372 Can we identify females with specific life histories?

373 When comparing the isotopic dataset, a few females clearly distinguish themselves from the general
374 adult group (excluding the individuals from the multiple gathering) and may have had a distinct life
375 history.

376 Female 12 shows the highest difference in $^{87}\text{Sr}/^{86}\text{Sr}$ values between childhood and adolescence (to some
377 extent, same applies to female 52). Female 12 also has an isolated lesion on the lateral epicondyle,
378 possibly due to an intense mechanical stress on the right arm, and is buried in the unique stone coffin of
379 the site (Loison 1998). She has been given specific funeral treatment according to a social status yet to
380 be defined and/or a specific geographical origin (figures 6 and 7).

381 In contrast, female 52 does not present any specific biological or archaeological features that could help
382 explain the difference in strontium ratios recorded between childhood and early adolescence. However,

383 this female is one of the individuals with the highest $\delta^{15}\text{N}$, suggesting a potential status and/or mobility
384 related to specific protein intake.

385 In the case of female 53, the low mobility hypothesis is supported by the isotopic signals, which indicate
386 no significant change in the geological landscape between childhood and adolescence (figure 5). She
387 exploited resources with the same environmental conditions (water, geology) during the part of her life
388 targeted by our analyses and shares more similar $^{87}\text{Sr}/^{86}\text{Sr}$ values with all the males from the multiple
389 gathering than the other males..

390 Finally, for female 9, the scenario is more complex. Sulphur data clearly indicate a different origin
391 compared to the rest of the human group during the last years before death (bone remodelling
392 estimation). However, previous carbon and nitrogen isotopic ratios did not distinguish this individual
393 from the group (Goude et al. 2013), and the strontium ratios suggest a similar origin to most of the other
394 females during her childhood and early adolescence (figures 6 and 7). This individual may have spent a
395 significant part of her life outside the main Pontcharaud group's living territory, but within a geological
396 area where strontium and other environmental parameters are similar.

397 Like female 12, female 69 shows a lesion related to intense mechanical stress on the right arm. No
398 strontium data is available for this individual, and the C, N and S isotopic ratios do not distinguish her
399 from the rest of the group. The lack of automatic convergence between biogeochemical information and
400 traumatic lesions leads us to believe that there is great diversity in the life histories of these females,
401 compatible with the hypothesis of a diversity of origins and/or food access of several of them.

402

403 Who are the males from the specific multiple gathering?

404 The palaeogenomic study demonstrated that the two subadults (34 and 35) from the multiple gathering
405 were also males, making this burial exclusively composed of male individuals. From a biomolecular
406 perspective, the seven males from the multiple gathering share common characteristics, setting them
407 apart from the other individuals buried in the necropolis. The study of discrete traits across the entire
408 osteological series failed to distinguish them (Schmitt and Saliba-Serre 2014, Billard 2004), and
409 enthesopathy lesions do not show any clusters or patterns among the men found at the Pontcharaud
410 site. The five adult males provided different $\delta^{34}\text{S}$, which were higher (10.4 to 11.2‰) than most of the
411 other adults of both sexes (figures 4a, 4b, 6 and 7). This is consistent with previous findings based on
412 carbon and nitrogen isotope ratios, which differentiated them from the rest of the group. Their
413 respective carbon and nitrogen isotopic ranges were extremely homogeneous (mean $-20.7\text{‰} \pm 0.2$ for
414 $\delta^{13}\text{C}$ and mean $9.5\text{‰} \pm 0.2$ for $\delta^{15}\text{N}$) and statistically lower (Wilcoxon signed-rank tests, $p < 0,001$ for
415 C and $p = 0,003$ for N) than those measured for other Pontcharaud males, particularly for nitrogen
416 (figure 3; Goude et al. 2013). This previous result indicated different access to protein intake (animal

417 protein level and/or exploitation of different ecological environments). The $\delta^{34}\text{S}$ values of the two
418 subadults from the multiple gathering are also higher (8.3-9.7‰) than those of the other juveniles at
419 the site (4.2-7.3‰) (figures 4a and 4b), but lower than the values recorded for the adults from the
420 same deposit. This could indicate a possible ongoing change in the environment. Regarding $^{87}\text{Sr}/^{86}\text{Sr}$
421 data, the individuals from the multiple gathering show a wider intra-life range of values (0.708 to
422 0.711) compared to the other males (0.710 – 0.711). They possibly spent their childhood in areas with
423 a broader bioavailable Sr range (0.708-0.711 vs. 0.710-0.711 for other males). During adolescence, the
424 males from the multiple gathering were located in a different place ($^{87}\text{Sr}/^{86}\text{Sr}$ of M3: 0.709) compared
425 to the other males ($^{87}\text{Sr}/^{86}\text{Sr}$ of M3: 0.711). The immature individual from the multiple gathering
426 (indiv. 35, ca. 12-15 years of age) shows a similar $^{87}\text{Sr}/^{86}\text{Sr}$ value (0.710) to the immature buried in a
427 single deposit (indiv. 59, ca. 7-11 years of age). This value is within the range of female data, but
428 lower compared to the other males; this could suggest the exploitation of the same environment by
429 children, regardless of the mortuary treatment. When combining strontium and sulphur results (figures
430 6 and 7), the adults can be considered as a distinct cluster with a different life history in terms of
431 mobility/provenance and environment exploited. In short, the biogeochemical dataset of these seven
432 individuals confirmed that they shared similar environment, somewhat different from most of the other
433 Pontcharaud individuals. Adult 32 shows the lowest $^{87}\text{Sr}/^{86}\text{Sr}$ during childhood (ie. here between 4.5
434 and 8 years old), similar to female 12 during adolescence (ie. here between 8.5 and 17.5 years old).
435 The youngest of the two subadults (individual 34; 7.5-12.5 years old) may show mobility in progress,
436 with bone $\delta^{34}\text{S}$ almost reaching the values of the other subadult and adult individuals (figure 4). Social
437 ages have already been discussed based on isotopic and funerary data (cf. Le Roy 2015; Rey et al.
438 2021). For example, at Gurgy (Middle Neolithic, Yonne Valley, France), both the 8-9 and 14-15 age
439 groups (the ages of the two subadults here) appear to be possible transitional ages with mobility and/or
440 dietary modifications for both sexes. To conclude regarding isotope data, the seven members of this
441 group shared a common lifestyle and possibly engaged in similar economic activities dedicated to a
442 specific group of males within the community.

443 From its discovery, the multiple gathering has seemed to constitute a particular group, as its
444 characteristics indicate mortuary gestures that deviate from the current funerary practices observed in
445 the necropolis and the chrono-cultural context. The simultaneity of the multiple gathering being clearly
446 established, two hypotheses are retained to explain such an event according to B. Boulestin's hypothesis
447 (Boulestin 2008): either the dead are victims of a small or large mortality crisis (epidemics, famine, or
448 interpersonal violence), or they represent an event related to the accompanying dead. The accompanying
449 dead are individuals who kill themselves or are killed in connection with the death of a person holding
450 a major and public social role (Boulestin 2022). This practice is documented for the Neolithic period in
451 France, specifically in the 5th and 4th millennia (Lefranc and Chenal 2019; Schmitt 2023). This
452 assumption for the present site was supported by Testart (2004) and Boulestin (2008). The superposition
453 of the cadavers and the prone position of the six individuals, particularly unconventional for this area

454 and the chronological period, contrast with individual 36, who is lying on his back at the eastern
455 extremity of the pit. He could be the accompanied individual; however, his position is also
456 unconventional.

457 The second hypothesis of a mortality crisis should also be examined. Given that the deposit consists
458 exclusively of males, interpersonal violence becomes a more plausible scenario compared to famine or
459 epidemic, as both of the latter would likely affect the entire population without selective targeting based
460 on sex. Furthermore, individuals 33 and 37 likely died as a result of their injuries. Conflict and violence
461 during the Neolithic in Northwestern Europe are well documented nowadays (Fibiger et al. 2023), and
462 multiple gatherings related to remains from wartime acts are documented at this period across various
463 sites in France (Lefranc and Chenal 2019). Regarding war activities, no healed wounds were observed,
464 except for a mended small injury on the right ulna of individual 33. The use of archery in Neolithic
465 populations has been demonstrated through osteological data, using specifically the frequencies of upper
466 limb enthesopathies (Thomas 2014; Ryan-Despraz et al. 2023). In the studied group, no evidence of
467 archery was noted, but this activity cannot be excluded. Moreover, isotopic data show that this particular
468 group had access to a different diet. However, the carbon and nitrogen stable isotope data show a
469 possible lower trophic level than the other analyzed individuals, while an equivalent or even higher
470 consumption of animal proteins could be expected for warriors, which would result in a higher trophic
471 level (Clauzel et al. 2022). Therefore this hypothesis seems unconvincing but cannot be completely
472 rejected. The seven males could also have been the victims of a massacre³ (found together at the same
473 moment, in the wrong place?) while practicing their common activity. Activities related to pastoralism
474 and transhumance to high-altitude environments are not the preferred hypothesis for this group, due to
475 strontium isotope values that appear to be little affected by the use of volcanic soils (characteristic of
476 high-altitude environments in this region). Several multiple gatherings interpreted as massacres related
477 to interpersonal violence have been discovered in Europe (e.g. Alt et al. 2020; Meyer et al. 2018; Novak
478 et al. 2019; Schroeder et al. 2019). In these cases, the corpus was composed of both males and females,
479 and the individuals were not included in a cemetery.

480 Whether Pontcharaud individuals died in conflict as warriors or in a massacre, their bodies were
481 recovered where they died and buried in the community cemetery with funeral rituals specific to the
482 circumstances of their death and/or their social category.

483

484

485 *Conclusions and perspectives*

486 The site of Pontcharaud is a funerary complex and may reflect different expressions of the deceased
487 according to their life histories. It has been highlighted that the use of funerary places in Prehistory can

³ « killing by one group of people by another group of people, regardless of whether the victims are armed or not, regardless of age or sex, race [...] » (Dwyer and Ryan 2012).

488 be dedicated to a group with a strong biological, cultural or social affinity (e.g., male/female lineage,
489 nuclear families, high or low social status; e.g., Bentley 2013; Mittnik et al. 2019; Rivollat et al. 2023),
490 or even possible alloparenting relationships (Esparza et al., 2017). The Pontcharaud site sheds light on
491 a new bioarchaeological dataset, allowing the identification of very specific practices that make it
492 exceptional in several respects. The new isotopic study strengthens previous observation (Goude et al.
493 2013) and indicates more variability in environment exploited and/or diet among the females' cluster.
494 The hypothesis of diverse origins compared to males is possible but deserves further investigation (in
495 particular with aDNA). The seven males from the multiple gathering deposit share isotopic similarities
496 among themselves, and dissimilarities with other males and females of the necropolis. Perhaps their
497 gathering responds to their involvement in a particular activity For now, the previous hypothesis of
498 them being warriors whose cadavers were recovered from the battlefield is not completely supported
499 by osteological and isotopic data, although interpersonal violence towards the group is not discarded.
500 Moreover, the reason for this particular gathering still fits two other plausible scenarios:
501 accompanying dead or victims of a massacre. Further aDNA analyses of other buried individuals, as
502 well as the investigation of new parts of the necropolis (currently in progress), will certainly allow for
503 a better interpretation of this specific situation. This study also demonstrated the relevance of a
504 multidisciplinary approach, including a reasoned use of fragile osteological material and a careful
505 assessment of the gain/risk in the selection of samples and methods chosen. This approach has made it
506 possible to highlight an exceptional variability within this funerary complex and will certainly provide
507 relevant methodological support for future studies.

508 **Acknowledgements and authors contribution:** Authors thank the Ministry of Culture for sampling
509 authorizations (24/04/2015 and 04/07/2017), the Regional Services of Archaeology (SRA PACA and
510 SRA Rhone Alpes Auvergne) and Lola Bonnabel (INRAP, Minist. Culture) for their help regarding
511 the management of the osteological collection. Sébastien Villotte gratefully acknowledges the
512 financial support of the Fondation Fyssen. DCSG acknowledges funding from the Generalitat
513 Valenciana (CIDEAGENT/2019/061). We thanks S. Bédécarrats for his help in verifying statistical
514 tests. DeepL Pro software has also been used to improve English and we thanks R.C. Power for a
515 detailed proofread and significant corrections to the English. This study is part of a wider
516 multidisciplinary project for which other complementary data will be compared particularly to
517 document the place of females at the onset of farming. This research was funded by Institut Danone
518 France/Fondation pour la Recherche Médicale 2015 partnership (Women and diet at the beginning of
519 farming, 5th – 3rd millennium BC, France: a bio-anthropological approach; Dir. G. Goude 2016-2017;
520 [http://institutdanone.org/nos-prix/femmes-alimentation-les-premieres-societes-agropastorales-ve-iiiie-](http://institutdanone.org/nos-prix/femmes-alimentation-les-premieres-societes-agropastorales-ve-iiiie-millennaires-av-j-c-france-approche-bio-anthropologique/)
521 [millennaires-av-j-c-france-approche-bio-anthropologique/](http://institutdanone.org/nos-prix/femmes-alimentation-les-premieres-societes-agropastorales-ve-iiiie-millennaires-av-j-c-france-approche-bio-anthropologique/)). Anthropological material was studied by
522 ASc and SV. Isotope studies were conducted by GG and DCSG; they thank Petrus LeRoux, Kerryn
523 Gray and Fayrooza Rawoot for technical assistance at the University of Cape Town. This project has
524 received funding for the aDNA study from the French National Research Agency (ANR) and
525 Deutsche Forschungsgemeinschaft (DFG) under the Franco-German Call in Humanities and Social
526 Sciences project ANR-DFG INTERACT, grant ANR-17-FRAL-0010, DFG-HA-5407/4-1, 2018-2023,
527 coordinated by Marie-France Deguilloux and Wolfgang Haak. Genetic analyses were conducted by
528 MR, MFD, MHP and AA. GG, ASc, MFD wrote the manuscript with input from all the authors. We
529 sincerely thank the two reviewers and the editor for their relevant corrections and comments and for
530 helping improve this article.

531

532

533

534

535

536

537 **References**

- 538 Albert, A. M., Maples, W. R. (1995). Stages of epiphyseal union for thoracic and lumbar vertebral centra
539 as a method of age determination for teenage and young adult skeletons. *Journal of forensic*
540 *sciences*, 40(4), 623-633.
- 541 AlQahtani SJ, Hector MP, and Liversidge HM. 2010. Brief communication: The London atlas of human
542 tooth development and eruption. *American Journal of Physical Anthropology* 142(3):481-490.
- 543 Alt, K.W., Tejedor Rodríguez, C., Nicklisch, N. *et al.* (2020). A massacre of early Neolithic farmers in
544 the high Pyrenees at Els Trocs, Spain. *Scientific Reports* 10, 2131.
- 545 Bataille, C. P., Jaouen, K., Milano, S., Trost, M., Steinbrenner, S., Crubézy, É., Colleter, R. (2021).
546 Triple sulfur-oxygen-strontium isotopes probabilistic geographic assignment of archaeological remains
547 using a novel sulfur isoscape of western Europe. *PLoS One*, 16(5), e0250383.
- 548 Barnett, C. H. (1954). Squatting facets on the European talus. *Journal of anatomy*, 88(Pt 4), 509.
- 549 Bentley RA. 2006. Strontium Isotopes from the Earth to the Archaeological Skeleton: A Review. *Journal*
550 *of Archaeological Method and Theory* 13(3):136-187.
- 551 Bentley RA, 2013. Mobility and the diversity of Early Neolithic lives: Isotopic evidence from skeletons,
552 *Journal of Anthropological Archaeology*, 32: 303-312.
- 553 Bentley RA, T. Douglas Price, Jens Lüning, Detlef Gronenborn, Joachim Wahl, and Paul D. Fullagar.
554 2002. Human migration in early Neolithic Europe: strontium isotope analysis of early Neolithic
555 skeletons. *Current Anthropology* 2002 43:5, 799-804
556
- 557 Bentley RA, Chikhi L, and Price TD. 2003. The Neolithic transition in Europe: comparing broad scale
558 genetic and local scale isotopic evidence. *Antiquity* 77(295):63-66.
- 559 Bentley RA, Bickle P, Fibiger L, Nowell G, Dale C, Hedges R, Hamilton J, Wahl J, Francken M, Grupe
560 G et al. 2012. Community differentiation and kinship among Europe's first farmers. *PNAS* 109:9326-
561 9330.
- 562 Bickle, P., Hofmann, D. (2007). Moving on: the contribution of isotope studies to the early Neolithic of
563 Central Europe. *Antiquity*, 81(314), 1029-1041.
- 564 Billard M. 2004. Paléobiologie des populations du Néolithique moyen auvergnat : Résultats de l'étude
565 paléopathologique de la nécropole de Pontcharaud 2. In: Dartevelle H, editor. Auvergne et Midi -
566 Actualité de la recherche : actes de la cinquième session, Clermont-Ferrand (Puy-de-Dôme), 8 et 9
567 novembre 2002. Cressensac: Préhistoire du Sud-Ouest. p 91-98.

568 Billard M. 2010. Evolution des pathocénoses du Néolithique moyen à la Tène sur des séries ostéo-
569 archéologiques de Limagne d'Auvergne (Puy-de-Dôme). In: Beeching A, Thirault E, and Vital J, editors.
570 Economie et société à la fin de la Préhistoire Actualité de la Recherche Actes des 7e Rencontres
571 méridionales de préhistoire récente, Lyon-Bron (3-4 novembre 2006). Lyon: ALPARA. p 317-325.

572 Birkner (R.) 1980, L'image radiologique typique du squelette, Maloine, Paris.

573 Boulestin B. 2008. Pourquoi mourir ensemble ? A propos des tombes multiples dans le Néolithique
574 français. Bulletin de la Société préhistorique française 105(1):103-130.

575 Boulestin, B. (2022). The accompanying dead. In C. Knüsel & E. Schotsmans (Eds.) *The Routledge*
576 *Handbook of Archaeoethanatology* (pp. 89-205). Routledge, London.

577 Boulestin, B, Courtaud, P. (2022). Words between two worlds: collective burial and other questions of
578 grave terminology. In C. Knüsel & E. Schotsmans (Eds.) *The Routledge Handbook of*
579 *Archaeoethanatology* (pp. 55-68). Routledge, London.

580 Boulestin, B. (2023). Des morts privés de funérailles : cadre général de réflexion. In A. Schmitt A. & E.
581 Anstett E. (Eds) *Sans sépulture : modalités et enjeux de la privation de funérailles de la Préhistoire à*
582 *nos jours* (pp.1-21). Archaeopress, Oxford.

583 Boule, E.-L. (2001). Osteological features associated with ankle hyperdorsiflexion. *International*
584 *Journal of Osteoarchaeology*, 11(5), 345–349. <https://doi.org/10.1002/oa.572>

585 BRGM (2003). Carte géologique – Auvergne 1/1000000e. Bureau de Recherches Géologiques et
586 Minières Editions, Orléans.

587 Briot D. (2002). Datations Rb-Sr des granites du Massif central. Online document vs. 31 October 2024
588 <https://planet-terre.ens-lyon.fr/pdf/datations-massif-central.pdf> Britton, K., Le Corre, M., Willmes, M.,
589 Moffat, I., Grün, R., Mannino, M. A., ... & Jaouen, K. (2020). Sampling plants and malacofauna in
590 ⁸⁷Sr/⁸⁶Sr bioavailability studies: implications for isoscape mapping and reconstructing of past
591 mobility patterns. *Frontiers in Ecology and Evolution*, 8, 579473.

592 Brůžek J. (2002). A method for visual determination of sex, using the human hip bone. *American Journal*
593 *of Physical Anthropology: The Official Publication of the American Association of Physical*
594 *Anthropologists*, 117(2), 157-168.

595 Brůžek, J., Santos, F., Dutailly, B., Murail, P., & Cunha, E. (2017). Validation and reliability of the sex
596 estimation of the human os coxae using freely available DSP2 software for bioarchaeology and forensic
597 anthropology. *American journal of physical anthropology*, 164(2), 440-449.

598 Buonasera, T., Eerkens, J., de Flamingh, A., Engbring, L., Yip, J., Li, H., et al. (2020) A comparison of
599 proteomic, genomic, and osteological methods of archaeological sex estimation. *Scientific Reports* 10,
600 11897.

601 Cassidy LM, Maoldúin RÓ, Kador T, Lynch A, Jones C, Woodman PC, Murphy E, Ramsey G, Dowd
602 M, Noonan A et al. . 2020. A dynastic elite in monumental Neolithic society. *Nature* 582(7812):384-
603 388.

604 Chambon P., Leclerc J. (2007) – Les tombes multiples dans le Néolithique français, *Bulletin de la*
605 *Société préhistorique française*, 104, 2, p. 289-306.

606 Chevallier, S., James, H. F., Snoeck, C., Herrscher, E., & Peake, R. (2025). Dynamiques territoriales et
607 changements culturels à la fin de l'âge du Bronze: les nécropoles de Marolles-sur-Seine (vallée de la
608 Haute Seine). *Bulletins et mémoires de la Société d'Anthropologie de Paris. BMSAP*, 37(S).

609 Civetta A, Schmitt A, Saliba-Serre B, Gisclon J-L, and Loison G. 2009. Comparaison de deux «
610 populations » de la deuxième moitié du Ve millénaire avant notre ère : approche anthropométrique.
611 *Bulletins et Mémoires de la Société d'Anthropologie de Paris* 21(3-4):141-158.

612 Clauzel, T., Richardin, P., Ricard, J., Prilaux, G., Martin, J. E., Amiot, R., Fourel, F., Nguyen Van, A.,
613 Arnaud-Godet, F., Vinçon-Laugier, A., Flandrois, J.-P., & Lécuyer, C. (2023). Geographic origin and
614 social status of the Gallic warriors from Ribemont-sur-Ancre (France) studied through isotope
615 systematics of bone remains. *International Journal of Osteoarchaeology*, 33(1), 39–50.

616 Crowe, F., Sperduti, A., O'Connell, T. C., Craig, O. E., Kirsanow, K., Germoni, P., ... Bondioli, L.
617 (2009). Water-related occupations and diet in two Roman coastal communities (Italy, first to third
618 century AD): Correlation between stable carbon and nitrogen isotope values and auricular exostosis
619 prevalence. *American Journal of Physical Anthropology*, 142(3), 355–366.

620 Di Bartolomeo, JR. (1979). Exostoses of the external auditory canal. *Ann Otol Rhinol Laryngol*, 88.

621 Delrieu F. (2022). Le secteur de Pontcharaud à Clermont-Ferrand. *Anthropisation de la Grande*
622 *Limagne d'Auvergne du VIe à la fin du Ier millénaire avant notre ère. Archéopages, Hors-série 6 (1),*
623 346-353.

624 Dwyer, P. G., Ryan, L. (2012). Introduction: The Massacre and History. In P.G. Dwyer & L. Ryan (Eds)
625 *Theatres of Violence: Massacres, Mass Killings and Atrocity throughout History* (pp. xi-xxv). New
626 York, Berghahn Books.

627 Esparza, Á., Palomo-Díez, S., Velasco-Vázquez, J., Delibes, G., Arroyo-Pardo, E., and Salazar-García,
628 D. C. (2017) Familiar Kinship? Palaeogenetic and Isotopic Evidence from a Triple Burial of the Cogotas
629 I Archaeological Culture (Bronze Age, Iberian Peninsula). *Oxford Journal of Archaeology*, 36: 223–
630 242.

631 Fernandes, D.M., Cheronet, O., Gelabert, P., Pinhasi R. TKGWV2: an ancient DNA relatedness pipeline
632 for ultra-low coverage whole genome shotgun data. *Sci Rep* 11, 21262 (2021).
633 <https://doi.org/10.1038/s41598-021-00581-3>

634 Fibiger, L., Ahlström, T., Meyer, C., Smith, M. (2023). Conflict, violence, and warfare among early
635 farmers in Northwestern Europe. *PNAS*, 120 (4), e2209481119

636 Fu, Q. et al. Genome sequence of a 45,000-year-old modern human from western Siberia.
637 *Nature* 514, 445–449 (2014).

638 Gamba, C., Jones, E. R., Teasdale, M. D., McLaughlin, R. L., Gonzalez-Fortes, G., Mattiangeli, V.,
639 Domboróczki, L., Kóvári, I., Pap, I., Anders, A., Whittle, A., Dani, J., Raczky, P., Higham, T. F. G.,
640 Hofreiter, M., Bradley, D. G., & Pinhasi, R. (2014). Genome flux and stasis in a five millennium
641 transect of European prehistory. *Nature Communications*, 5, 5257.

642 Goude G., Salazar-García D.C., Power R. C., Rivollat M., Gourichon L., M.-F. Deguilloux, M.-H.
643 Pemonge, Bouby L., Binder D. 2020a. New insights on Neolithic food and mobility patterns in
644 Mediterranean coastal populations. *American Journal of Physical Anthropology*, 173: 218-235
645 doi.org/10.1002/ajpa.24089

646 Goude G., Dori I., Starnini E., Sparacello VS., Varalli A. 2020b. Multi-proxy dentine microsections
647 analyses reveal diachronic changes in life history adaptations, mobility, and tuberculosis-induced
648 wasting in prehistoric Liguria (Finale Ligure, Italy, northwestern Mediterranean). *International Journal*
649 *of Paleopathology*, 28 : 99-111.

650 Goude G, Salazar-Garcia DC, Power R, Terrom J, Rivollat M, Deguilloux M-F, Pemonge M-H, Le
651 Bailly M, André G, Coutelas A, Hauzeur A. 2019. A multidisciplinary approach to Neolithic life
652 reconstruction. *Journal of Archaeological Method and Theory*. 26(2) : 537-560.

653 G. Goude, Fontugne M., 2016. Carbon and nitrogen isotopic variability in bone collagen during the
654 Neolithic period: Influence of environmental factors and diet. *Journal of Archaeological Science*, 70:
655 117-131.

656 Goude G, Schmitt A, Herrscher E, Loison G, Cabut S, and André G. 2013. Pratiques alimentaires au
657 Néolithique moyen : Nouvelles données sur le site de Pontcharaud 2 (Auvergne, France). *Bulletin de la*
658 *Société Préhistorique Française* 110(2):299-317.

659 Goude G, Castroni F, Herrscher E, Cabut S, and Tafuri MA. 2012. First Strontium isotopic evidence of
660 mobility in the Neolithic of Southern France. *European Journal of Archaeology* 15(421-439).

661 Hofmann D. (2020). Not going anywhere? Migration as a social practice in the early Neolithic
662 Linearbandkeramik. *Quaternary International*, 560, 228-239.

663 Holt, E., Evans, J. A., Madgwick, R. (2021). Strontium ($^{87}\text{Sr}/^{86}\text{Sr}$) mapping: a critical review of methods
664 and approaches. *Earth-Science Reviews*, 216, 103593.

665 Knipper C, Mittnik A, Massy K, Kociumaka C, Kucukkalpci I, Maus M, Wittenborn F, Metz SE,
666 Staskiewicz A, Krause J et al. . 2017. Female exogamy and gene pool diversification at the transition
667 from the Final Neolithic to the Early Bronze Age in central Europe. Proceedings of the National
668 Academy of Sciences.

669 Korneliussen, T. S., Albrechtsen, A. & Nielsen, R. ANGSD: analysis of next generation sequencing
670 data. BMC Bioinform. 15, 356 (2014).

671 Le Roy, M. (2015). Les enfants au Néolithique: du contexte funéraire à l'interprétation socioculturelle
672 en France de 5700 à 2100 avant J.-C. Doctoral dissertation, Université de Bordeaux.

673 Lefranc, P. & Chenal F. (2019) Deposits of bodies in circular pits in the Neolithic period (mid-fifth to
674 the mid-fourth millenium BCE). *Human remains and violence: An interdisciplinarity journal*, 5 (1), 55-
675 69.

676 Liversidge HM. 2008. Timing of human mandibular third molar formation. *Annals of Human Biology*
677 35(3):294-321.

678 Loison G. 1998. La nécropole de Pontcharaud en Basse Auvergne. In: Guilaine J, editor. Sépultures
679 d'Occident et genèses des mégalithismes. Paris: Errance. p 189-206.

680 Masclans Latorre A, Bickle P, and Hamon C. 2021a. Sexual Inequalities in the Early Neolithic?
681 Exploring Relationships Between Sexes/Genders at the Cemetery of Vedrovice Using Use-Wear
682 Analysis, Diet and Mobility. *Journal of Archaeological Method and Theory* 28(1):232-273.

683 Masclans, A., Tvrđý, Z., Pavúk, J., Cheben, M., & Bickle, P. 2021b. Exploring sexual division of labour
684 at “Nitra Horné Krškany” cemetery using stone tool use-wear analysis, physical activity markers, diet,
685 and mobility as proxies. *Archaeological and Anthropological Sciences*, 13(7), 109.

686 Meyer, C., Knipper, C., Nicklisch, N., Münster, A., Kürbis, O., Dresely, V. Meller, H. & Alt, K.W.
687 (2018). Early Neolithic executions indicated by clustered cranial trauma in the mass grave of
688 Halberstadt. *Nature Communications*, 9, 2472.

689 Macintosh, A. A., Pinhasi, R., & Stock, J. T. (2017). Prehistoric women’s manual labor exceeded that
690 of athletes through the first 5500 years of farming in Central Europe. *Science Advances*, 3(11),
691 eaao3893.

692 Mittnik A, Massy K, Knipper C, Wittenborn F, Friedrich R, Pfrengle S, Burri M, Carlichi-Witjes N,
693 Deeg H, Furtwängler A, Harbeck M, von Heyking K, Kociumaka C, Kucukkalpci I, Lindauer S, Metz
694 S, Staskiewicz A, Thiel A, Wahl J, Haak W, Pernicka E, Schiffels S, Stockhammer PW, Krause J.
695 Kinship-based social inequality in Bronze Age Europe. *Science*. 2019 Nov 8;366(6466):731-734.

696 Monroy Kuhn JM, Jakobsson M, and Günther T. 2018. Estimating genetic kin relationships in
697 prehistoric populations. *PLOS ONE* 13(4):e0195491.

698 Murail P, Brůžek J, Braga J. 1999. A new approach to sexual diagnosis in past populations. Practical
699 adjustments from Van Vark's procedure. *International Journal of Osteoarchaeology*, 9(1), 39-53.

700 Novak, M., Olalde, I., Ringbauer, H., Rohland, N., Ahern, J., Balen, J. et al. (2021). Genome-wide
701 analysis of nearly all the victims of a 6200-year-old massacre. *PLoS One*, 16 (3), e0247332.

702 Négrel, P., Fouillac, C., & Brach, M. (1997). Variations spatio-temporelles de la composition chimique
703 et des rapports $^{87}\text{Sr}/^{86}\text{Sr}$ des eaux minérales de la Limagne d'Allier. *Comptes Rendus de l'Académie des*
704 *Sciences-Series IIA-Earth and Planetary Science*, 325(2), 119-124.

705 Nehlich O, and Richards MP. 2009. Establishing collagen quality criteria for sulphur isotope analysis
706 of archaeological bone collagen. *Archaeological and Anthropological Sciences* 1(1):59-75.

707 Nehlich O. 2015. The application of sulphur isotope analyses in archaeological research: A review.
708 *Earth-Science Reviews* 142:1-17. Patterson N, Price AL, and Reich D. 2006. Population Structure and
709 Eigenanalysis. *PLOS Genetics* 2(12):e190.

710 Owings Webb P. A., Suchey J. M. (1985). Epiphyseal union of the anterior iliac crest and medial clavicle
711 in a modern multiracial sample of American males and females. *American Journal of Physical*
712 *Anthropology* 68: 457-466.

713 Pin C, Briot D, Bassin C, and Poitrasson F. 1994. Concomitant separation of strontium and samarium-
714 neodymium for isotopic analysis in silicate samples, based on specific extraction chromatography.
715 *Analytica Chimica Acta* 298(2):209-217.

716 Pinhasi, R., Fernandes, D., Sirak, K., Novak, M., Connell, S., Alpaslan-Roodenberg, S., Gerritsen, F.,
717 Moiseyev, V., Gromov, A., Raczky, P., Anders, A., Pietruszewsky, M., Rollefson, G., Jovanovic, M.,
718 Trinhhoang, H., Bar-Oz, G., Oxenham, M., Matsumura, H., & Hofreiter, M. (2015). Optimal Ancient
719 DNA Yields from the Inner Ear Part of the Human Petrous Bone. *PLOS ONE*, 10(6), e0129102.

720 Polet, C., Martiarena, M. L., Villotte, S., & Vercauteren, M. (2019). Throwing Activities Among
721 Neolithic Populations from the Meuse River Basin (Belgium, 4500–2500 BC) with a Focus on
722 Adolescents. *Childhood in the Past*, 12(2), 81–95.

723 Price, T., Wahl, J., & Bentley, R. (2006). Isotopic evidence for mobility and group organization among
724 Neolithic farmers at Talheim, Germany, 5000 BC. *European Journal of Archaeology*, 9(2-3), 259-284.

725 Prodeo, F., Georjon, C., Thomson, I. (2024) Aperçu de l'évolution des architectures et dépôts
726 funéraires au Néolithique à Clermont-Ferrand (63) « Pontcharaud ». In : Kerouanton I, Maitay C.,
727 Praud I, Soler L *La place des morts chez les vivants*. Architectures, mémoires et rituels de la fin du
728 Mésolithique à l'âge du Bronze. Actes des IVe Rencontres Nord-Sud de Préhistoire Récente (27-30 avril
729 2022, La Rochelle, France), Associations des Publications Chauvinoises, p. 235-254.

730 Rey L., Tacail T., Santos F., Rottier S., Goude G., Balter V. 2021. Disentangling diagenetic and biogenic
731 trace elements and Sr isotopes in fossil dental enamel and bone using laser ablation analysis. *Chemical*
732 *Geology*.

733 Rey L., Rottier S., Santos F., Goude G. 2021. Sex and age-related social organization in the Neolithic:
734 a promising survey from the Paris Basin. *Journal of Archaeological Science: Reports*, 38 : 103092.

735 Rey L, Salazar-García DC, Chambon Ph, Santos F, Rottier S, and Goude G. 2019. A multi-isotope
736 analysis of Neolithic human groups in the Yonne valley, Northern France: insights into dietary patterns
737 and social structure. *Archaeological and Anthropological Sciences* 11(10):5591-5616.

738 Rivollat M., Rohrlach A.B., Ringbauer H., Childebayeva A., Mendisco F., Barquera R., Szolek A., Le
739 Roy M., Colleran H., Aron F., Pemonge M.H., Späth E., Rey L., Goude G., Balter V., Krause J., Rottier
740 S., Deguilloux M.F., Haak W. 2023. Extensive pedigrees reveal the social organization of a Neolithic
741 community. *Nature*.

742 Rivollat M, Mendisco F, Pemonge M-H, Safi A, Saint-Marc D, Brémond A, Couture-Veschambre C,
743 Rottier S, and Deguilloux M-F. 2015. When the Waves of European Neolithization Met: First
744 Paleogenetic Evidence from Early Farmers in the Southern Paris Basin. *PloS ONE*.

745 Ryan-Despraz, J., Villotte, S., Desideri, J., & Besse, M. (2023). Multivariate assessments of activity-
746 related skeletal changes: Interpreting Bell Beaker specialized male archery and social organization in
747 Central Europe. *American Journal of Biological Anthropology*, 182(2), 237-263.

748 Saintot, S., Thomson, I., Bonnardin, S., Cabanis, M., Caillat, P., Franc, O., Garnier N., Surmely F.,
749 Vernet, G. (2016). L’habitat et la nécropole de Vertaizon dans le Puy-de-Dôme: Première présentation
750 d’un site du Néolithique moyen I en Auvergne. In J. Cauliez , I. Sénépart , L. Jallot , P.A.de labriffe ,
751 C. Gilabert , X. Gutherz (Dir.), *De la tombe au territoire*. AEP Toulouse, pp. 407-421.

752 Schmitt A., Saliba-Serre B., 2014. Paramètres biologiques et organisation spatiale de l’ensemble
753 funéraire néolithique moyen de Poncharaud 2 (Auvergne). *Anthropologie* LII/2: 153-169.

754 Schmitt, A., Murail, P., Cunha, N., Rougé, D. (2002) Variability of the pattern of aging on the human
755 skeleton: evidence from bone indicators and implications on age at death estimation. *Journal of Forensic*
756 *Sciences*, 47, 1203-1209.

757 Schmitt A. (2005). Une nouvelle méthode pour estimer l’âge au décès des adultes à partir de la surface
758 sacro-pelvienne iliaque. *Bulletins et Mémoires de la Société d’Anthropologie de Paris* 17 (1-2) : 89-101.

759 Schmitt A., Georges P. 2005 Quelle démarche suivre pour estimer l’âge au décès à partir du squelette ?
760 in : Charlier P. *Manuel Pratique de Paleopathologie Humaine*, De Boccard, Paris, pp.269-280.
761

762 Schmitt, A. (2022). Defining collective burials: three case studies. In C. Knüsel, E. Schotsmans, D.
763 Castex (Eds) *The Routledge Handbook of Archaeoethanatology* (pp. 2017-222), Routledge, London.

764 Schmitt A. (2023). Identifier des morts sans funérailles en contexte archéologique: des cas datés du
765 Néolithique. In A. Schmitt & E. Anstett E. (Eds) *Sans sépulture: modalités et enjeux de la privation de*
766 *funérailles de la Préhistoire à nos jours* (pp. 22-33). Archaeopress, Oxford.

767 Schroeder, H., Margaryan, A., Szmyt, M., Theulot, B., Włodarczak, P., Rasmussen, S., Gopalakrishnan,
768 S., Szczepanek, A., Konopka, T., Jensen, T.Z.T., Witkowska, B., Wilk, S., Przybyła, M.M., Pospieszny,
769 Ł., Sjögren, K.G., Belka, Z., Olsen, J., Kristiansen, K., Willerslev, E., Frei, K.M., Sikora, M., Johannsen,
770 N.N. & Allentoft M.E. (2019). Unraveling ancestry, kinship, and violence in a Late Neolithic mass
771 grave. *Proceedings of the National Academy of Sciences of the United States of America*, 116 (22),
772 10705-10710.

773 Szulc, P., Seeman, E., Delmas, P. D. (2000). Biochemical measurements of bone turnover in children
774 and adolescents. *Osteoporosis International*, 11, 281-294.

775 Standen, V. G., Arriaza, B. T., & Santoro, C. M. (1997). External auditory exostosis in prehistoric
776 Chilean populations: A test of the cold water hypothesis. *American Journal of Physical Anthropology*,
777 103(1), 119–129. [https://doi.org/10.1002/\(SICI\)1096-8644\(199705\)103:1<119::AID-](https://doi.org/10.1002/(SICI)1096-8644(199705)103:1<119::AID-AJPA8>3.0.CO;2-R)
778 [AJPA8>3.0.CO;2-R](https://doi.org/10.1002/(SICI)1096-8644(199705)103:1<119::AID-AJPA8>3.0.CO;2-R)

779 Testart A. (2004) – Les morts d’accompagnement : la servitude volontaire I, Paris, Errance, 261 p.

780 Thomas, A. 2014 Bioarcheology of the Middle Neolithic: Evidence for Archery Among Early European
781 Farmers. *American Journal of Physical Anthropology*, 154, 279-290.

782 Ubelaker D.H. (1978). *Human skeletal remains : excavation, analysis, interpretation*, Aldine, Chicago.

783 Valentin, J. (2003). Relative biological effectiveness (RBE), quality factor (Q), and radiation weighting
784 factor (wR): ICRP Publication 92. *Annals of the ICRP*, 33(4), 1-121.

785 Vernet G. 2002. Le Néolithique ancien (Epicardial) et le Néolithique moyen ancien sur le site du Brézet
786 à Clermont-Ferrand (Massif Central, France). In : Auvergne et Midi. Actualité de la recherche. Pré-
787 Actes des Cinquièmes Rencontres Méridionales de Préhistoire Récente, ClermontFerrand, p. 23-24.

788 Villotte, S. (2006). Connaissances médicales actuelles, cotation des enthésopathies: Nouvelle
789 méthode. *Bulletins et Mémoires de La Société d’Anthropologie de Paris, n.s.*, 18(1–2), 65–85.

790 Villotte, S. (2024). Activity-Related Skeletal Changes. In E. Nikita & T. Rehren (Eds.), *Encyclopedia*
791 *of Archaeology (Second Edition)* (pp. 892–900). Elsevier. [https://doi.org/10.1016/B978-0-323-90799-](https://doi.org/10.1016/B978-0-323-90799-6.00016-1)
792 [6.00016-1](https://doi.org/10.1016/B978-0-323-90799-6.00016-1)

793 Villotte, S., Castex, D., Couallier, V., Dutour, O., Knüsel, C. J., & Henry-Gambier, D. (2010).
794 Enthesopathies as occupational stress markers: Evidence from the upper limb. *American Journal of*
795 *Physical Anthropology*, 142(2), 224–234. <https://doi.org/10.1002/ajpa.21217>

796 Villotte, S., Churchill, S. E., Dutour, O., & Henry-Gambier, D. (2010). Subsistence activities and the
797 sexual division of labor in the European Upper Paleolithic and Mesolithic: Evidence from upper limb
798 enthesopathies. *Journal of Human Evolution*, 59(1), 35–43.
799 <https://doi.org/10.1016/j.jhevol.2010.02.001>

800 Villotte, S., & Knüsel, C. J. (2014). “I sing of arms and of a man...”: Medial epicondylitis and the
801 sexual division of labour in prehistoric Europe. *Journal of Archaeological Science*, 43, 168–174.
802 <https://doi.org/10.1016/j.jas.2013.12.009>

803 Villotte, S., & Knüsel, C. J. (2016). External auditory exostoses and prehistoric aquatic resource
804 procurement. *Journal of Archaeological Science: Reports*, 6, 633–636.
805 <https://doi.org/10.1016/j.jasrep.2015.05.013>

806 Villotte, S., Polet, C., Colard, C., & Santos, F. (2022). Enteseal changes and estimation of adult age-
807 at-death. *American Journal of Biological Anthropology*, 178(2), 201–204.
808 <https://doi.org/10.1002/ajpa.24458>

809 Villotte, S., & Santos, F. (2023). The effect of age on enteseal changes: A study of modifications at
810 appendicular attachment sites in a large sample of identified human skeletons. *International Journal of*
811 *Osteoarchaeology*, oa.3197. <https://doi.org/10.1002/oa.3197>

812 Villotte, S., Stefanović, S., & Knüsel, C. J. (2014). External auditory exostoses and aquatic activities
813 during the Mesolithic and the Neolithic in Europe: Results from a large prehistoric sample.
814 *Anthropologie*, LII(1), 73–89.

815 Willmes, M., Bataille, C. P., James, H. F., Moffat, I., McMorrow, L., Kinsley, L., Armstrong R.A.,
816 Eggins S., Grün, R. (2018). Mapping of bioavailable strontium isotope ratios in France for
817 archaeological provenance studies. *Applied Geochemistry*, 90, 75-86.

818 Willmes M, McMorrow L, Kinsley L, Armstrong R, Aubert M, Eggins S, Falguères C, Maureille B,
819 Moffat I, and Grün R. 2014. The IRHUM (Isotopic Reconstruction of Human Migration) database
820 – bioavailable strontium isotope ratios for geochemical fingerprinting in France. *Earth Syst Sci*
821 *Data* 6(1):117-122.

822

823

824

825

826

827 **Table caption - main text**

828

829 Table 1. Summary of the anthropological data and isotopic results (humans) of Pontcharaud (details
830 for both humans and animals in SI 1).

831

832

833 **Figure captions - main text**

834

835 Figure 1. Location of the site of Pontcharaud and other archaeological sites mentioned in the text.
836 Pictures of two different burial types: a stone coffin on the left and wooden coffins on the right. Photos
837 INRAP/G. Loison.

838

839 Figure 2. Photo and drawing of the individuals from the multiple gathering deposit (INRAP/G. Loison
840 modified by A. Schmitt).

841

842 Figure 3. A-Carbon and nitrogen stable isotope data from bone collagen of human and some animal
843 remains (Goude et al. 2013). B-Boxplots of nitrogen stable isotope composition distribution for females,
844 males and individuals of the multiple gathering deposit.

845

846 Figure 4a. Sulphur isotope composition of bone collagen from females, males, immatures, individuals
847 from the multiple gathering deposit, and animals.

848

849 Figure 4b. Boxplots (median, 25th -75th percentiles, minimum, maximum, and outliers) of $\delta^{34}\text{S}$ in bone
850 collagen from the different human groups and animals at Pontcharaud, and the range of values expected
851 for individuals from the Auvergne region, as proposed by Bataille et al. (2021).

852

853 Figure 5a. Strontium isotope composition from crown enamel of some females, males, and individuals
854 from the multiple gathering deposit.

855

856 Figure 5b. Boxplots (median, 25th -75th percentiles, minimum, maximum, and outliers) of $^{87}\text{Sr}/^{86}\text{Sr}$ in
857 dental enamel from the different human groups at Pontcharaud, mineral waters in the area of the site,
858 and the expected range of $^{87}\text{Sr}/^{86}\text{Sr}$ for individuals from the Limagne basin and volcanic terrains near
859 the site, as proposed by Holt et al. (2018).

860

861 Figure 6. Comparison between sulphur isotope composition of bone collagen and strontium isotope
862 composition from crown enamel grown during childhood for some individuals.

863

864 Figure 7. Comparison between sulphur isotope composition of bone collagen and strontium isotope
865 composition from crown enamel grown during early adolescence for some individuals.

866

867

868 **Supplementary Information captions**

869

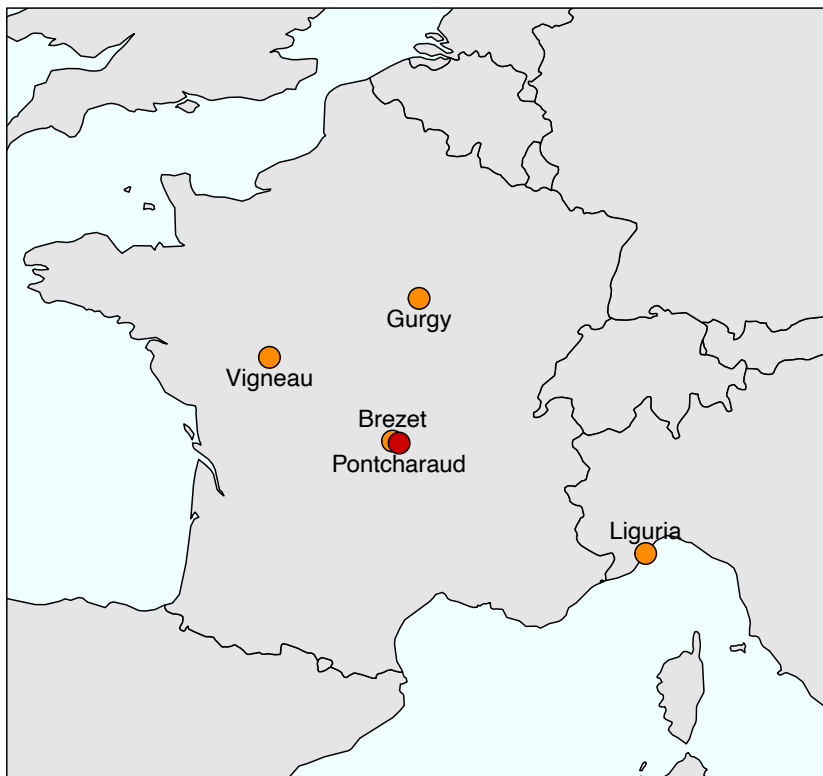
870 SI 1. Anthropological data and isotopic results (humans and animals) of Pontcharaud (Middle
871 Neolithic, Clermont-Ferrand, Auvergne, France)

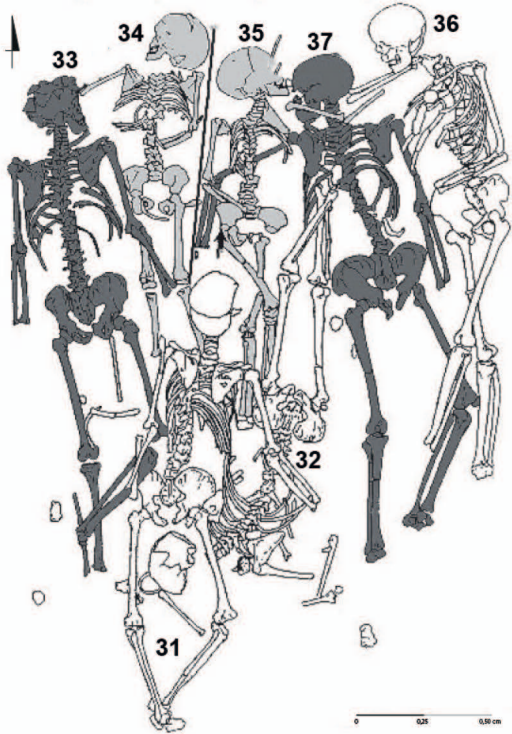
872

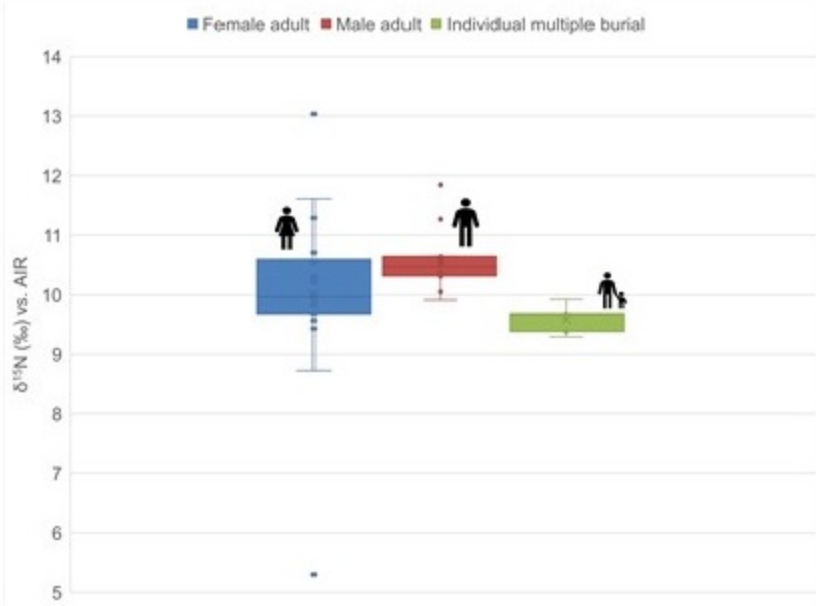
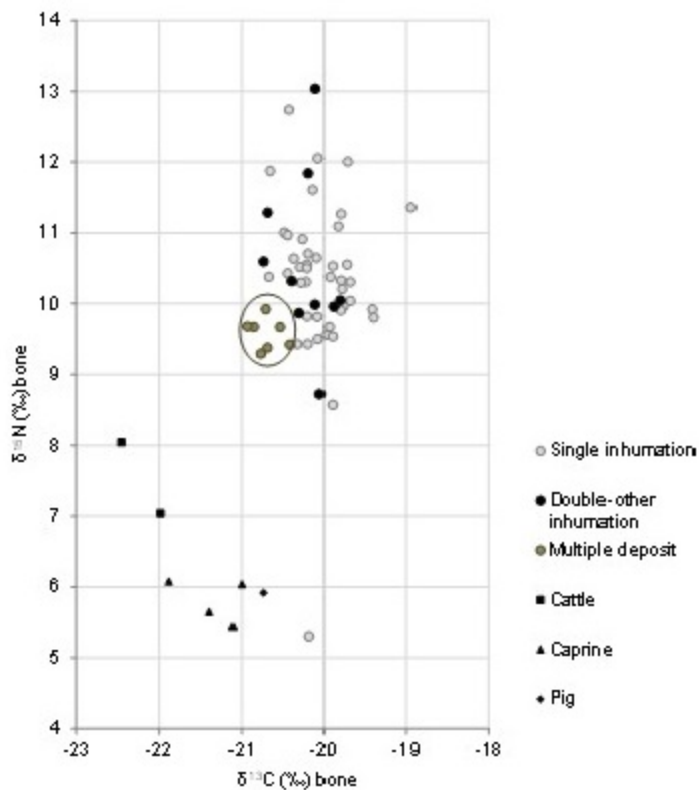
873 SI 2. Methodological and technical information

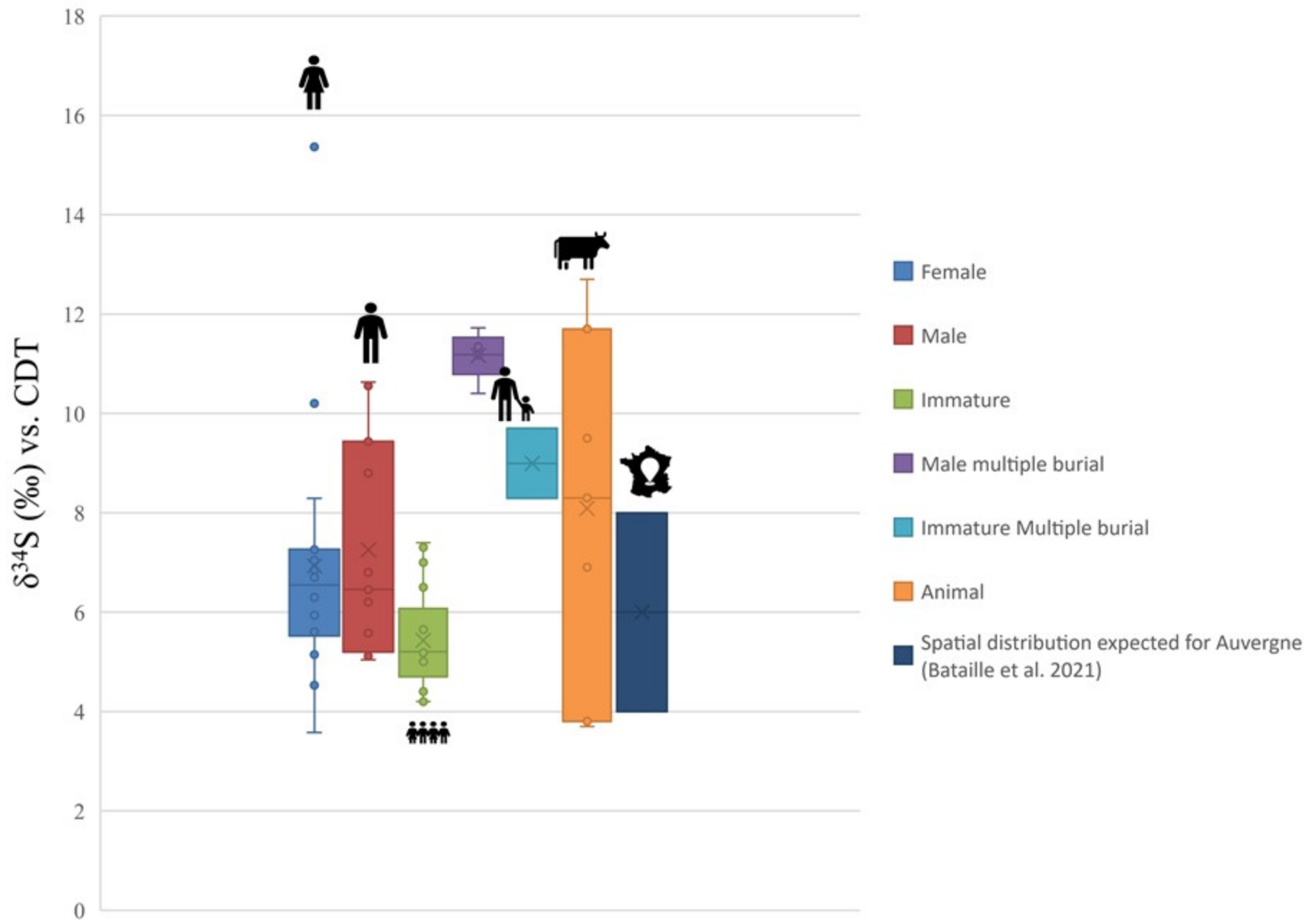
874

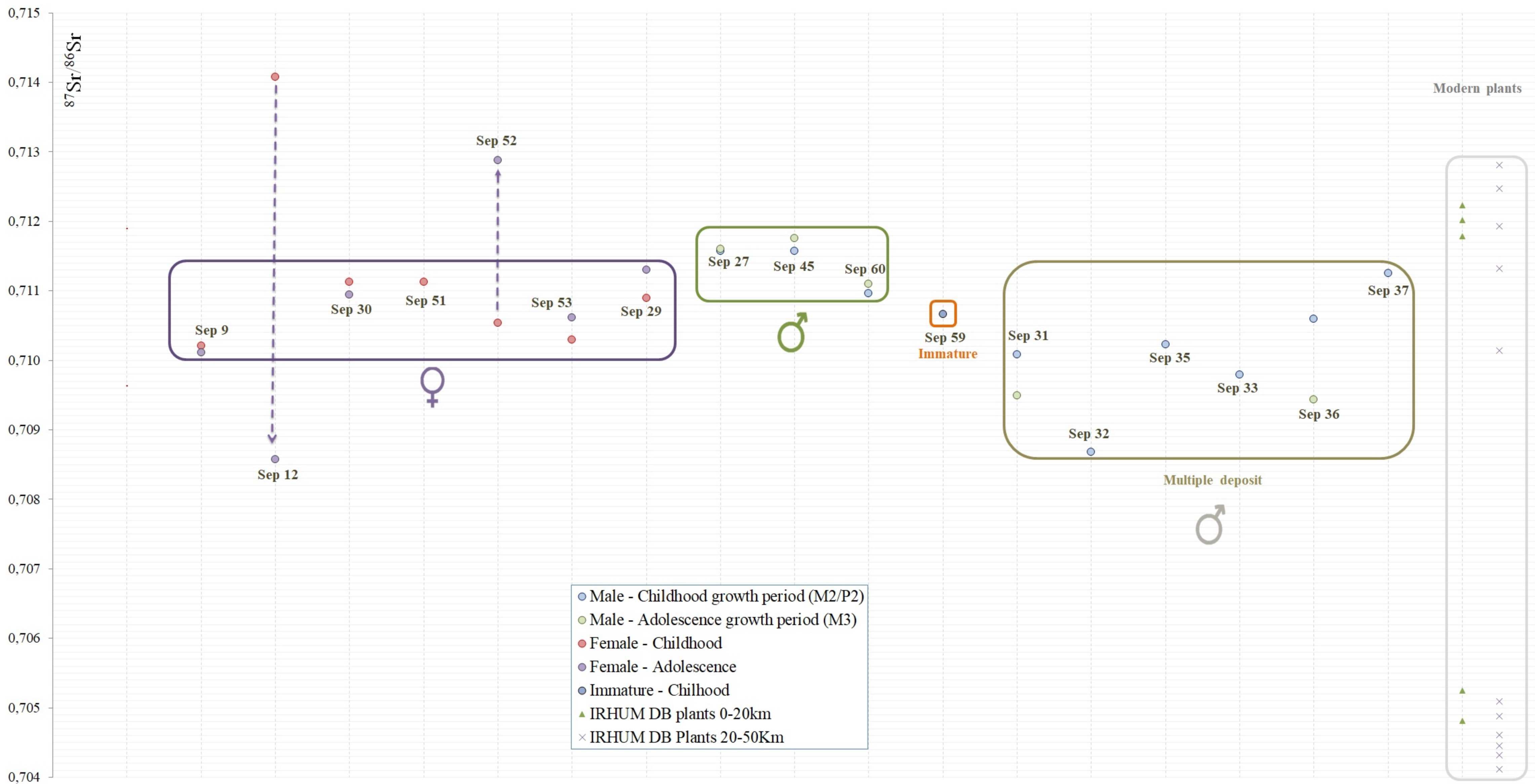
875











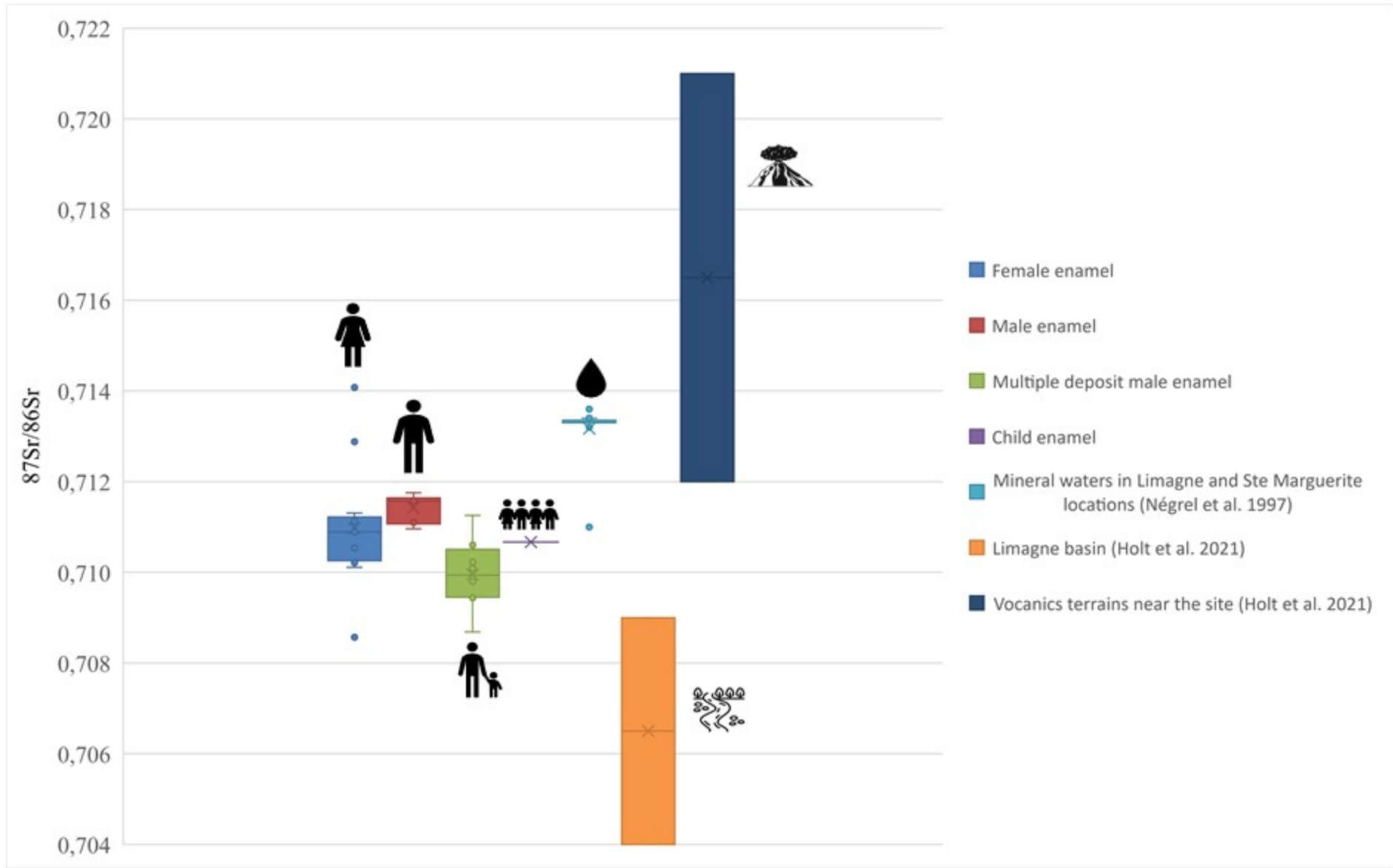
Group 1 (Purple box): Sep 9, Sep 30, Sep 51, Sep 53, Sep 29. Includes female symbols for Sep 51 and Sep 12.

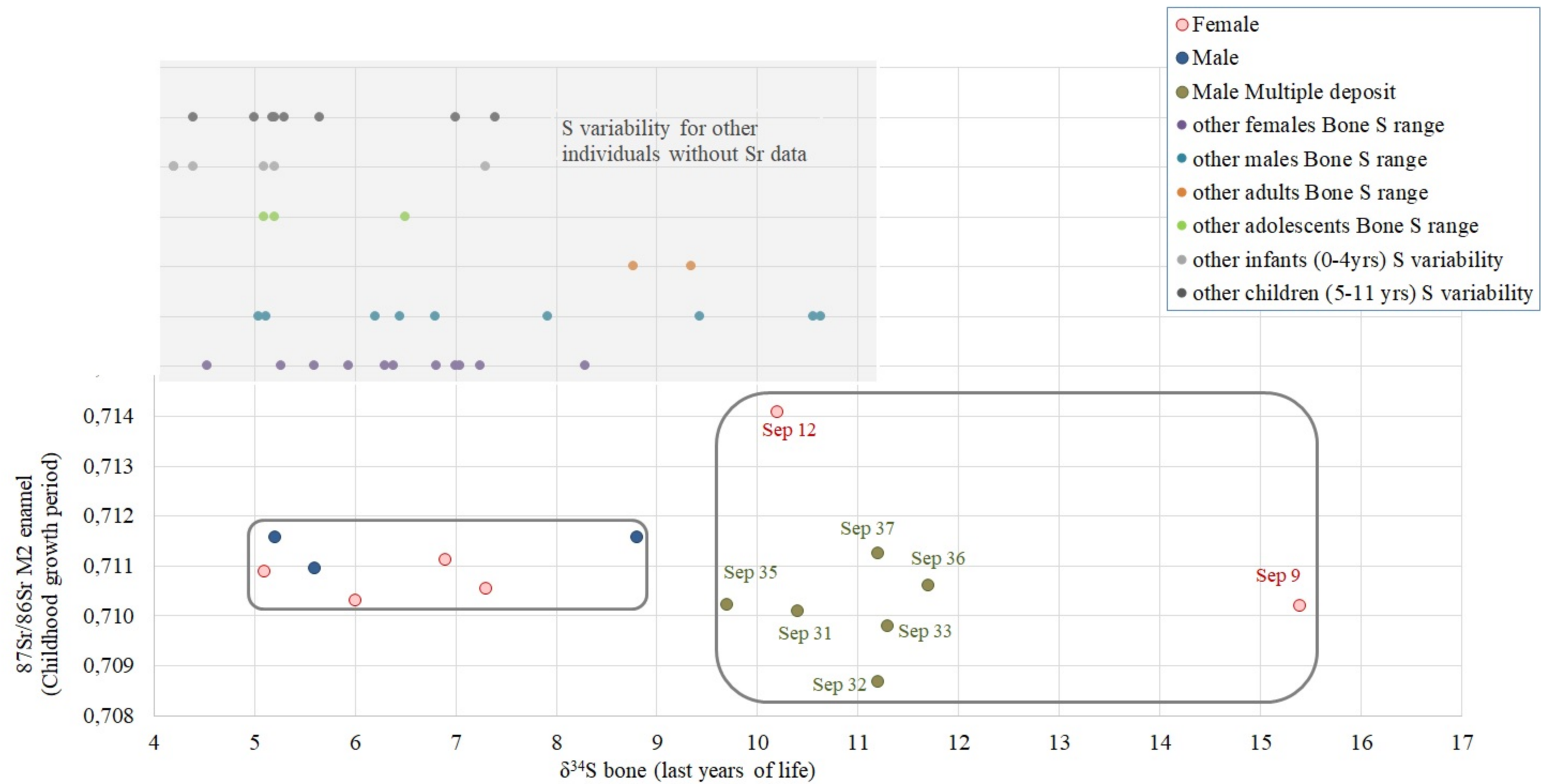
Group 2 (Green box): Sep 27, Sep 45, Sep 60. Includes male symbol for Sep 45.

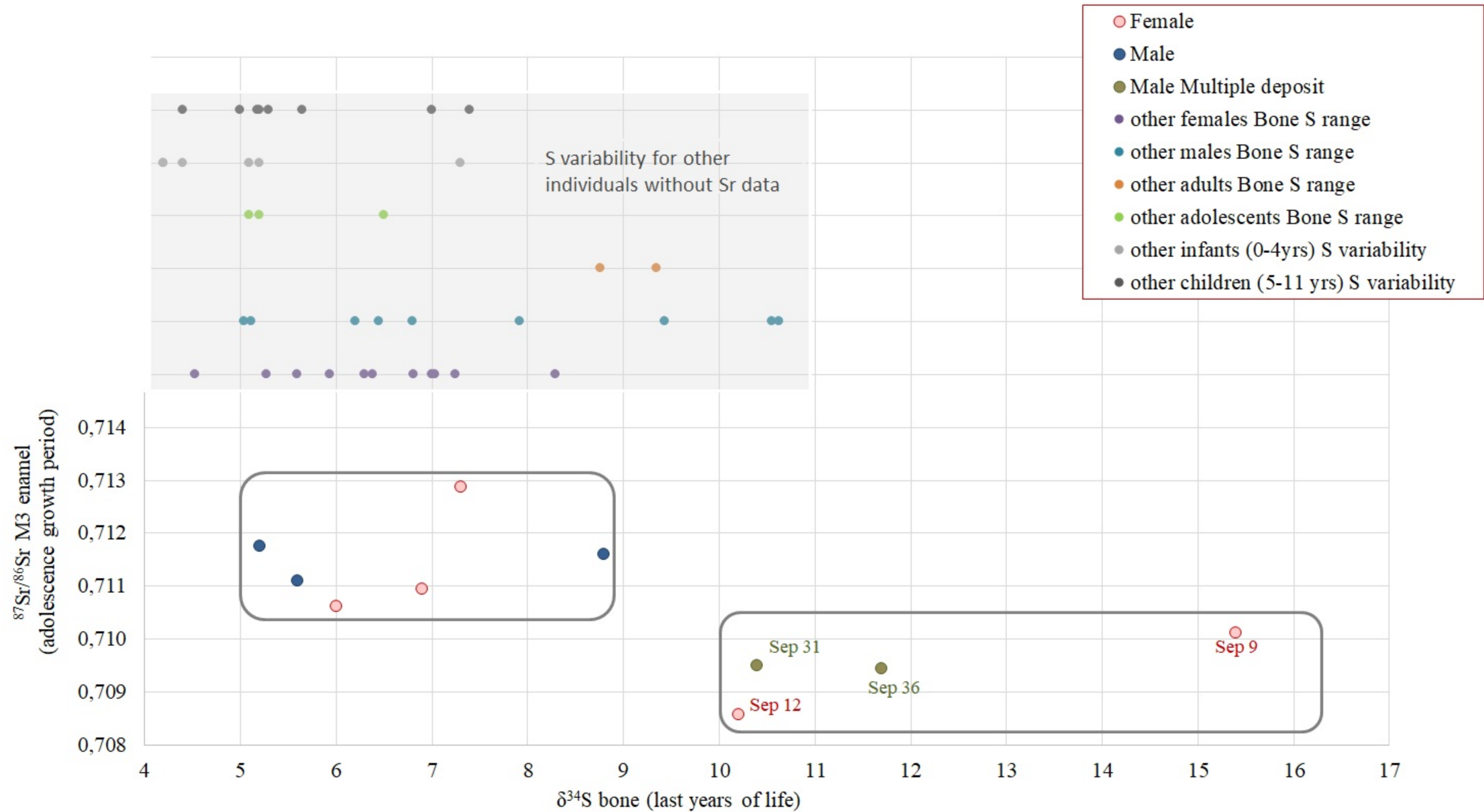
Group 3 (Orange box): Sep 59. Labeled "Immature".

Group 4 (Brown box): Sep 31, Sep 32, Sep 35, Sep 33, Sep 36, Sep 37. Labeled "Multiple deposit" with a male symbol.

Group 5 (Grey box): Modern plants. Includes IRHUM DB plants 0-20km (triangles) and IRHUM DB Plants 20-50Km (crosses).







Supplementary Information 2 – Methodological and technical information

Osteological analyses

S. Villotte, A. Schmitt

		Females		Males	
		Stage A	Stage B	Stage A	Stage B
Right side	Lesser tubercle	13	1	3	0
	Greater tubercle	12	0	4	0
	Lateral epicondyle	6	2	5	0
	Medial epicondyle	11	0	4	1
Left side	Lesser tubercle	11	0	6	0
	Greater tubercle	8	0	5	1
	Lateral epicondyle	9	1	3	1
	Medial epicondyle	10	0	4	0

Table 1. Frequencies of stages A (no lesions) and B (minor changes) for the entheses of the humerus according to side and the sex.

Biogeochemical analyses

G. Goude, D. C. Salazar-Garcia

Theoretical concepts

Sulphur isotope ratios are widely used additionally to carbon and nitrogen isotope ratios as it can be measured in the same protein fraction (collagen) and even sometimes together when analytical equipment allows it. Although $\delta^{34}\text{S}$ is more commonly used as a geographical marker (values differ depending on proximity to the sea, wind regime, and inland geological and fluvial landscape composition; e.g., Nehlich 2015; Zazzo et al. 2011), it also helps to detect the consumption of freshwater fish and marine food when significant in the diet (e.g. Nehlich 2009 ; Drucker et al. 2018). Recently, this proxy proved to be powerful to highlight mobility during infancy in Prehistoric communities (Goude et al. 2020b) as complementary to strontium isotope analysis. When combined with carbon and nitrogen isotope data, it helps to discuss the impact of mobility *versus* food choice in the collagen isotopic composition (e.g., Goude and Fontugne 2016).

As highly mineralized (ca. 96% of apatite), teeth are more resistant than bones and usually preferred for biogeochemical investigations of the mineral fraction (Budd et al., 2000; Hoppe et al., 2003). Importantly also, strontium isotopes do not show isotopic fractionation when incorporated through the foodweb (Bentley, 2006). This, together with the fact that enamel has no turnover during life (Nanci, 2007), allows to obtain locality information from a frozen time of its childhood and compare it to later stages of life. $^{87}\text{Sr}/^{86}\text{Sr}$ ratios depend on geology and show different values among different geologies, varying according to the bedrock age and the amount of ^{87}Rb . The heavier strontium isotope (^{87}Sr) is an unstable isotope that is formed from the radioactive decay of ^{87}Rb , while the lighter (^{86}Sr) is stable (Bentley, 2006). Different weather conditions like sea spray effect (Bentley, 2006), or the input of varied materials coming from the different environments like dust precipitation or alluvial deposition could influence and modify the estimated bedrock values (Sjögren et al., 2016). This is why bioavailable Sr values to be compared to the study individuals are useful and should be measured ideally from the surrounding areas of the study (Price et al., 2006). The bioavailable Sr background values for this study were selected from the IRHUM database of modern soils and plants (Willmes et al., 2014). Specifically, 22 points located in a radius of 50km around the site were chosen and reported together with the values from the archaeological material in Figure 5.

When possible we sampled for this study two teeth per individual from 11 individuals. Both an M3 and an M2/P4 were selected. From 6 individuals only an M2/P4 was sampled. This sampling strategy allows assessing if changes in geological substrate (SI 2) occurred between two periods of life estimated by using Alqhatani et al. (2010) and Liversidge (2008) crown growth pattern data: childhood (M2/P4:

Code de champ modifié

Code de champ modifié

Code de champ modifié

Code de champ modifié

Code de champ modifié

median age formation between 2.5 and 8 years old for crown) and adolescence (M3: median age formation between 8.5 and 14 years old) (AlQhatani et al. 2010).

Geological particularity of the Massif Central

The Pontcharaud site is located in the Limagne sedimentary basin, bordered by various geological terrains of volcanic origin: to the west, the Plateau des Dômes and the Chaîne des Puys, and to the east, the Monts du Forez (BRGM 2003; (fig. SI2-1 left). Analyses carried out on various mineral water springs by Négrel et al. (1997) in the Limagne sedimentary basin and 25 km south of Clermont-Ferrand show a variation in $^{87}\text{Sr}/^{86}\text{Sr}$ between 0.7110 and 0.7136. Data proposed in the literature by Willmes et al. (2018) and Holt et al. (2021) indicate $^{87}\text{Sr}/^{86}\text{Sr}$ values of plants and soils globally between 0.704 and 0.709 for sedimentary areas of the Limagne basin where the site is located, and globally between 0.712 and 0.721 for terrains of volcanic origin (fig. SI2-1 right).

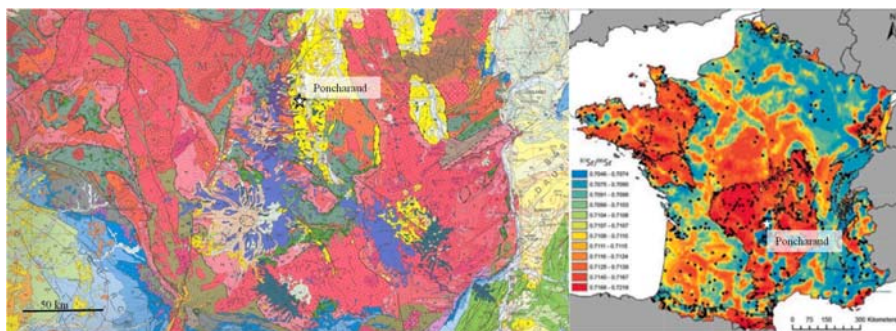


Figure SI2 – 1. Left: geological map of the BRGM (2003) with the location of the archaeological site (white star). Right: contour map using strontium ($^{87}\text{Sr}/^{86}\text{Sr}$) isotope data made by Holt et al. (2021) and Willmes et al. (2018), with the location of the archaeological site (white star).

Collagen extraction and sulphur stable isotope measurement

Collagen extraction was performed on chunk bones by using a modified protocol from Richards and Hedges (1999). Samples are demineralised in 0.5 M HCl at 5°C, then soaked in NaOH overnight at room temperature and solubilised with HCl pH 3 at 70°C during 48h. Solubilized collagen is filtered with EzeeFilter® device, frozen at -65°C in glass vials and then freeze-dried during 2 days. Elemental composition and stable isotope ratios of sulphur are measured by EA-IRMS (Europa Scientific elemental analyser coupled to Europa Scientific 20-20 IRMS; Iso-Analytical, Crewe, UK). Laboratory standards used are calibrated against IAEA international standard for all measurements; measurement error is 0.2‰.

Enamel preparation and strontium analysis

Sample preparation and analysis were carried out directly in the Clean Labs and Isotope Facilities of the University of Cape Town, South Africa. Prior to analysis, a chunk of enamel of ca. 20mg from each tooth was cut in a longitudinal way to reflect all mineralization stages of the dental piece. These pieces of enamel were cleaned by abrasion and dentine was removed completely, rinsed and ultrasonicated for 20 minutes in MilliQ water. The diamond drill bits used for this process were cleaned with ethanol and ultrasonicated in MilliQ water between samples to avoid cross-contamination (Budd et al., 2000). A subsample of each enamel piece was taken for Sr concentration in order to check for diagenesis. Following this, in a Clean Lab, the remaining chunks of cleaned enamel samples were digested with 2mL bi-distilled 65% HNO₃ in a closed Teflon beaker placed on a hotplate at 140 °C for an hour. After this, digested samples were dried and redissolved in 1.5 mL of bi-distilled 2M HNO₃ before being centrifuged at 4000 rpm for 20 minutes. The resulting supernatant was then collected for strontium separation chemistry. At this last step, a subsample from each sample was used to calculate the concentration with ⁸⁸Sr intensity (V) regression equation built with SRM987 standard from NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA). The isolation of strontium as carried out with 200µl of Eichrom Sr.Spec resin loaded in Bio-Spin Disposable Chromatography Bio-Rad Columns following the method described in Pin et al. (1994). The strontium fraction separated this way for each sample was then dried down dissolved in 2 ml 0.2% bi-distilled HNO₃ and diluted to 200 ppb Sr concentrations for the isotope analysis. A NuPlasma HR multicollector inductively-coupled-plasma mass spectrometer (MC-ICP-MS) was used to measure the ⁸⁷Sr/⁸⁶Sr ratios. Sample analyses were referenced to bracketing analyses of SRM987, using a ⁸⁷Sr/⁸⁶Sr reference value of 0.710255 from NIST. All strontium isotope data were corrected for isobaric rubidium interference at 87 amu using the measured signal for ⁸⁵Rb and the natural ⁸⁵Rb/⁸⁷Rb ratio. The instrumental mass fractionation was corrected using the measured ⁸⁷Sr/⁸⁶Sr ratio and the exponential law, as well as a true ⁸⁶Sr/⁸⁸Sr value of 0.1194. Results of repeated analyses of an in-house carbonate standard processed and measured with the batches of samples in this study are in agreement with long-term results for this in-house standard (⁸⁷Sr/⁸⁶Sr; 0.708915; 2 sigma 0.000047; n=125). Furthermore, one blank was added for every two batches in order to double check the cleanliness of the sample preparation.

References

- Bentley, A.R. (2006). Strontium isotopes from the earth to the archaeological skeleton: a review. *Journal of archaeological method and theory*, 13(3), 135-187.
- BRGM (2003). Carte géologique – Auvergne 1/1000000e. Bureau de Recherches Géologiques et Minières Editions, Orléans.

Briot D. (2002). Datations Rb-Sr des granites du Massif central. Online document vs. 31 October 2024 <https://planet-terre.ens-lyon.fr/pdf/datations-massif-central.pdf>

Budd, P., Montgomery, J., Barreiro, B., & Thomas, R. G. (2000). Differential diagenesis of strontium in archaeological human dental tissues. *Applied geochemistry*, 15(5), 687-694.

Drucker, D. G., Valentin, F., Thevenet, C., Mordant, D., Cottiaux, R., Delsate, D., & Van Neer, W. (2018). Aquatic resources in human diet in the Late Mesolithic in Northern France and Luxembourg: insights from carbon, nitrogen and sulphur isotope ratios. *Archaeological and Anthropological Sciences*, 10, 351-368.

Goude G., Dori I., Starnini E., Sparacello VS., Varalli A. 2020b. Multi-proxy dentine microsections analyses reveal diachronic changes in life history adaptations, mobility, and tuberculosis-induced wasting in prehistoric Liguria (Finale Ligure, Italy, northwestern Mediterranean). *International Journal of Paleopathology*, 28 : 99-111.

G. Goude, Fontugne M., 2016. Carbon and nitrogen isotopic variability in bone collagen during the Neolithic period: Influence of environmental factors and diet. *Journal of Archaeological Science*, 70: 117-131. [doi:10.1016/j.jas.2016.04.019](https://doi.org/10.1016/j.jas.2016.04.019)

Holt, E., Evans, J. A., Madgwick, R. (2021). Strontium ($^{87}\text{Sr}/^{86}\text{Sr}$) mapping: a critical review of methods and approaches. *Earth-Science Reviews*, 216, 103593.

Hoppe, K. A., Koch, P. L., & Furutani, T. T. (2003). Assessing the preservation of biogenic strontium in fossil bones and tooth enamel. *International Journal of Osteoarchaeology*, 13(1-2), 20-28.

Nanci, A. (2007). *Ten cate's oral histology-pageburst on vidualsource: development, structure, and function*. Elsevier Health Sciences.

Négrel, P., Fouillac, C., & Brach, M. (1997). Variations spatio-temporelles de la composition chimique et des rapports $^{87}\text{Sr}/^{86}\text{Sr}$ des eaux minérales de la Limagne d'Allier. *Comptes Rendus de l'Académie des Sciences-Series IIA-Earth and Planetary Science*, 325(2), 119-124.

Nehlich O. 2015. The application of sulphur isotope analyses in archaeological research: A review. *Earth-Science Reviews* 142:1-17. Patterson N, Price AL, and Reich D. 2006. Population Structure and Eigenanalysis. *PLOS Genetics* 2(12):e190.

Nehlich O, and Richards MP. 2009. Establishing collagen quality criteria for sulphur isotope analysis of archaeological bone collagen. *Archaeological and Anthropological Sciences* 1(1):59-75.

Price, T., Wahl, J., Bentley, R. (2006). Isotopic evidence for mobility and group organization among Neolithic farmers at Talheim, Germany, 5000 BC. *European Journal of Archaeology*, 9(2-3), 259-284.

Code de champ modifié

Sjögren, K. G., Price, T. D., & Kristiansen, K. (2016). Diet and mobility in the Corded Ware of Central Europe. *PloS one*, 11(5), e0155083.

Willmes, M., Bataille, C. P., James, H. F., Moffat, I., McMorrow, L., Kinsley, L., Armstrong R.A., Eggins S., Grün, R. (2018). Mapping of bioavailable strontium isotope ratios in France for archaeological provenance studies. *Applied Geochemistry*, 90, 75-86.

Willmes M, McMorrow L, Kinsley L, Armstrong R, Aubert M, Eggins S, Falguères C, Maureille B, Moffat I, and Grün R. 2014. The IRHUM (Isotopic Reconstruction of Human Migration) database – bioavailable strontium isotope ratios for geochemical fingerprinting in France. *Earth Syst Sci Data* 6(1):117-122.

Zazzo, A., Monahan, F. J., Moloney, A. P., Green, S., & Schmidt, O. (2011). Sulphur isotopes in animal hair track distance to sea. *Rapid Communications in Mass Spectrometry*, 25(17), 2371-2378.

Palaeogenomic analyses

MF. Deguilloux ; M. Rivollat ; MH. Pemonge ; A. Arzelier ; P. Justeau

Material

Samples targeted

Authorisation to perform destructive sampling for palaeogenomic analyses was granted by the DRAC Auvergne Rhône Alpes. Two subadults individuals originating from the Pontcharaud exceptional multiple gathering (individuals 34 and 35) were targeted in the present study in document their genetic sex.

Methods

DNA isolation

Sampling procedures were conducted in non-optimal conditions, in the Dépôt SRA d'Aix Les Milles. While work surfaces and tools could be decontaminated with diluted bleach, no samples decontamination steps could be followed, nor could the ambient air be monitored. Only petrous bones were targeted for palaeogenomic analyses according to their higher rate of DNA preservation (Gamba et al., 2014; Pinhasi et al., 2015). A layer of bone surface was abraded around the sampled area before low-speed drilling into the cochlea to retrieve between 20 and 100mg of bone powder.

Bone powder samples were then processed at the aDNA Laboratory of PACEA, University of Bordeaux.

DNA isolation and library construction

DNA was extracted from powder following the protocol described in Velsko et al. (2019). A total of 20mL of DNA template was used to build double-stranded libraries with unique index pairs (Aron et al., 2019; Stahl et al., 2020). We applied a partial UDG (half) protocol to remove most of the ancient DNA damage while preserving the characteristic damage pattern in the terminal nucleotides (Rohland et al., 2015).

We then performed shallow shotgun sequencing to permit assessments of DNA preservation and authenticity in the indexed libraries. Libraries were sequenced on an Illumina NextSeq 500 (2x75bp reads) at Institut de Recherches Biomédicales des Armées (Paris, France).

Bioinformatic analyses

Read processing, alignment and postmortem damage

Raw sequence data were processed using EAGER (Peltzer et al., 2016) including quality checking of FASTQ files with FastQC, and clipping and merging of paired-end reads with Clip&Merge. Autosomal

and sex chromosomal alignments were performed against the Human Reference Genome hs37d5 using BWA version 0.7.12 (with a lower read-length cut-off of 30 base pairs with MAPQ R 30 and disabling of seeding with -l 1000 being specified). Duplicate reads with the same orientation and same start and end positions were removed using DeDup v0.12.1. MapDamage v.2.0.6 was used to observe characteristic aDNA damage patterns, before trimming two bases at the ends of each read to remove residual deaminations with BamUtil (<https://genome.sph.umich.edu/wiki/BamUtil:trimBam>). A summary of results statistics is given in Table 2.

Code de champ modifié

Sample	Number of sequences obtained before merging (R1+R2)	Number of sequences after merging	Number of duplicates removed	Number of sequences mapped on human nuclear reference genome hs37d5	% of endogenous DNA	Mean coverage nuclear human genome	Number of sequences mapped on human mitochondrial reference genome rCRS	% deamination 1st Base 3'	% deamination 1st Base 5'	Average human sequences length
34	1 814 502	634 055	2 174	182 724	23,474	0,0033	67	0,0761	0,0799	56,59
35	2 108 978	701 373	99	9 308	1,279	0,0001	5	0,1014	0,1016	49,98

Table 2- Principal characteristics of the DNA libraries obtained for Pontacharaud individuals 34 and 35, determined using EAGER v2.4.5 (R1 and R2: reads obtained through paired-end sequencing; merging: merging of R1+R2 complementary reads; deamination: DNA sequences modification characteristic of ancient DNA).

Sex determination

Genetic sex was calculated using two different methods. The first one estimates a coefficient R_y representing the fraction of reads mapping to Y chromosome out of all reads mapping to either X or Y chromosomes and attributes a XX genotype if $R_y \leq 0,016$ while assigning a XY genotype if $R_y \geq 0,075$ (Skoglund et al., 2013). The authors recommend a minimum of 100,000 chromosome reads mapped on the reference genome. The second method calculates the coverage of each of the gonosomes relatively to the coverage of the autosomes (Mittnik et al., 2016). A threshold of Y ratio at $\leq 0,04$ is set for a XX genotype and at $\geq 0,4$ for XY.

References

Aron, F., Neumann, G., & Brandt, G. (2019). *Non-UDG treated double-stranded ancient DNA library preparation for Illumina sequencing v1*. <https://doi.org/10.17504/protocols.io.bakricv6>

Gamba, C., Jones, E. R., Teasdale, M. D., McLaughlin, R. L., Gonzalez-Fortes, G., Mattiangeli, V., Domboróczki, L., Kóvári, I., Pap, I., Anders, A., Whittle, A., Dani, J., Raczky, P., Higham, T. F. G., Hofreiter, M., Bradley, D. G., & Pinhasi, R. (2014). Genome flux and stasis in a five millennium transect of European prehistory. *Nature Communications*, *5*, 5257.

<https://doi.org/10.1038/ncomms6257>

Mittnik, A., Wang, C.-C., Svoboda, J., & Krause, J. (2016). A Molecular Approach to the Sexing of the Triple Burial at the Upper Paleolithic Site of Dolní Věstonice. *PLOS ONE*, *11*(10), e0163019.

<https://doi.org/10.1371/journal.pone.0163019>

Peltzer, A., Jäger, G., Herbig, A., Seitz, A., Kniep, C., Krause, J., & Nieselt, K. (2016). EAGER : Efficient ancient genome reconstruction. *Genome Biology*, *17*(1), 60. <https://doi.org/10.1186/s13059-016-0918-z>

Pinhasi, R., Fernandes, D., Sirak, K., Novak, M., Connell, S., Alpaslan-Roodenberg, S., Gerritsen, F., Moiseyev, V., Gromov, A., Raczky, P., Anders, A., Pietrusewsky, M., Rollefson, G., Jovanovic, M., Trinhhoang, H., Bar-Oz, G., Oxenham, M., Matsumura, H., & Hofreiter, M. (2015). Optimal Ancient DNA Yields from the Inner Ear Part of the Human Petrous Bone. *PLOS ONE*, *10*(6), e0129102.

<https://doi.org/10.1371/journal.pone.0129102>

Rohland, N., Harney, E., Mallick, S., Nordenfelt, S., & Reich, D. (2015). Partial uracil–DNA–glycosylase treatment for screening of ancient DNA. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1660), 20130624. <https://doi.org/10.1098/rstb.2013.0624>

Skoglund, P., Storå, J., Götherström, A., & Jakobsson, M. (2013). Accurate sex identification of ancient human remains using DNA shotgun sequencing. *Journal of Archaeological Science*, *40*(12), 4477–4482. <https://doi.org/10.1016/j.jas.2013.07.004>

Stahl, R., Warinner, C., Velsko, I., Orfanou, E., Aron, F., & Brandt, G. (2020). *Illumina double-stranded DNA dual indexing for ancient DNA*. <https://www.protocols.io/view/illumina-double-stranded-dna-dual-indexing-for-anc-bakticwn>

Velsko, I., Skourtanioti, E., & Brandt, G. (2019). *Ancient DNA Extraction from Skeletal Material v1*. <https://doi.org/10.17504/protocols.io.baksicwe>