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Biological sex *VS.* Archaeological Gender: Enamel peptide analysis of the horsemen of the Early Middle age necropolises of Campochiaro (Molise, Italy)

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Abstract

Sex estimation of human skeletons may present difficulties, especially in relation to their state of preservation or to weak sexual dimorphism. This leads to reliance on gendered grave goods, giving rise to potential issues related to the social role and condition of the individual and the relationship with the community. We aimed at examining skeletal remains from two VII century CE necropolises (Vicenne and Morrione) from Campochiaro in Molise (Italy), characterized by the presence of burials of “horsemen,” warriors buried with horse in the same pit, the analyses of which showed some uncertainty in sex estimation with the common morphological methods. Thus, we analyzed the tooth enamel peptides of fifteen individuals for the presence of sexually dependent variants of the protein amelogenin. Sex estimation was possible in all the individuals examined. Individuals with male gendered grave goods were all confirmed to be male, while individuals without gendered grave context had sex estimated for the first time. Biological sex, archeological gender, and the gendered division of society have been confirmed to be strongly linked in this particular community. Further studies should be undertaken to better understand the ethnic and migratory aspects of this relevant funerary context.

Keywords:

Sex diagnosis, Early Medieval necropolises, Palaeoproteomics, Gender, Cemetery and social organization

Abbreviations

No keyword abbreviations are available

1 Introduction

In bioarchaeological practice the examination of population structures of ancient societies poses two main problems: the first deals with methodological issues in reconstructing the biological profile of the individual (sex and age at death), while the second is the use of these biological features to infer cultural and social meanings (e.g. gender and social age) (Sofaer, 2006; Barbiera, 2012; Joyce, 2017).

In regards to sex estimation, the majority of published literature is based on classical and geometric morphometry, both in adults (Phenice, 1969; Lovejoy, 1985; Walker, 2008; Betti, 2014; Acsádi and Nemeskéri, 1970; Meindl and Lovejoy, 1989; Brooks and Suchey, 1990; Buikstra and Ubelaker, 1994; Loth and Henneberg, 1996; Brůžek et al., 2017; Lovejoy et al., 1985; Milella et al., 2021) and juveniles (Schaefer et al., 2009; Luna et al., 2017; Marino et al., 2021). However, practical anthropological applications of some of these methods may provoke difficulty in providing reliable sex attribution because of the subjectivity inherent in their observation, highly dependent on the expertise of the researcher (Langley et al., 2018). In addition, the state of preservation of skeletal remains, often highly fragmented or poorly represented in archaeological contexts, makes sex attribution even more problematic.

Among the biomolecular approaches, genomic and palaeoproteomic evidence-based studies in the archeological fields are increasingly common. In particular, palaeoproteomics is a rapidly expanding field of study, employed for a variety of purposes, with origins in food and medical science and now applied to forensics, archaeology, and paleontology (Buckley, 2019). In these fields, palaeoproteomic studies commonly focus on high-mineralized tissues (i.e. bones and teeth) as they are best preserved in the archeological record. Specifically, owing to mass spectrometry analyses, researchers were able to reconstruct diet (Hendy et al., 2018a), sex (Stewart et al., 2017) and taxonomy (Buckley et al., 2009) of faunal and human biological remains. In addition, proteins tend to be preserved at a much higher rate compared to other biological compounds, such as DNA, and present a lower chance of endogenous contamination (Hendy et al., 2018b). These approaches have solved some emblematic case studies where classical morphometrical analyses of biological profile reconstruction failed. One such case is the widely studied triple Gravettian burial of Dolní Věstonice (Moravia) (Klíma, 1988; Jelfnek, 1992; Pettitt, 2011; Trinkaus, et al., 2000; Trinkaus et al., 2001; Trinkaus et al., 2010) where genetic analyses revealed the sex of the central individual of the burial to be male, previously determined to be female (Fu et al., 2016), opening up new contextual interpretations (Belcastro and Mariotti, 2017 and references therein).

Another case refers to the “warrior-woman of Birka”, an X c. CE burial from Sweden containing a complete human skeleton along with silk garments, weapons, and the remains of two horses, considered for many years to belong to an elite male warrior. Ancient DNA analyses later confirmed the individual to be a biological female (Hedenstierna-Jonson et al., 2017).

In another recent study, chromosome-linked proteins found in dental enamel were studied in order to analyze the “Lovers of Modena;” two Late Antique individuals intentionally buried hand in hand (Lugli et al., 2019). Popular media had used this burial as evidence of *eternal love* in a male–female romantic relationship based on grave context alone; sex determination was ambiguous thanks to poor preservation. The study revealed that these two individuals were in fact biologically male, thus radically changing popular conception of the site.

These cases suggest that molecular and chemical analyses may be more reliable in defining biological parameters and that our cultural paradigms may influence the interpretation of archaeological contexts belonging to ancient cultures (Stewart et al., 2017; Parker et al., 2019; Buonasera et al., 2020).

All considered, the present study was aimed at verifying the biological sex of fifteen skeletons from a large Early Medieval (VII c. CE) funerary settlement from Campochiaro (Molise, Italy) organized in two coeval cemeteries (Vicenne and Morrione). Considering the presence of nineteen individuals identified as horsemen (12 from Vicenne and 7 from Morrione) (Ceglia, 2010), we aimed at ascertaining biological sex and comparing it with the archaeological context in an attempt to highlight their social identity. The poor preservation state of many horsemen and their underdeveloped sexually dimorphic traits prevented reliable sex assessment. We were also interested in diagnosing the sex of two juvenile individuals, one of them archeologically gendered as female and buried near a horseman. Thus, we applied palaeoproteomic analyses of enamel peptides (amelogenin) following the methods of the above mentioned study (Lugli et al., 2019).

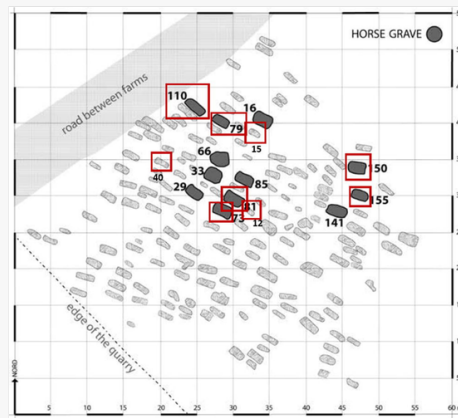
1.1 The context of the necropolises of Campochiaro

The two medieval (VII c. CE) necropolises (Vicenne and Morrione) of Campochiaro, discovered in the '80s and '90s of the previous century and located about 1 km from each other, are part of the same cultural context and are composed of 397 burials (Figs. 1 and 2). Grave goods display a combination of barbaro-germanic, romano-byzantine, and steppe-nomadic stylistic elements (Ceglia and Genito, 1991). Weapons are a common item in “male” burials, while jewellery is the most common find in “female” burials (Bóna, 2000; Ceglia, 2004; Ceglia, 2010; De Vingo, 2017; Fedele, 2017). Other common grave goods include coarse-ware ceramics, glass drinking vessels, and animal bones (Ceglia, 2010; Ceglia and Marchetta, 2012). The site has been assigned to the VII c. CE using relative dating methods, including burial organization, numismatics, and weapon (*scramasax*) typology (Arslan, 2004; Schmitt, 2007; Ceglia, 2010; Ceglia and Marchetta, 2012).



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Fig. 1



Schematic map of the Necropolis of Vicenne, Campochiaro. Dark shaded pits are horseman burials, those outlined in red are individuals studied in this article; to the northwest the burials have been disturbed by road construction (from De Vingo, 2017). 1.5 or 2 column image size. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)


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Fig. 2



Schematic map of the necropolis of Morrione, Campochiaro. Dark shaded pits are horseman burials and red outlined ones were studied individuals. A funerary chapel is located to the northwest (from De Vingo, 2017). 1.5 or 2 column image size. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The burials are organized in parallel rows with a west-east orientation, and are further grouped in a “nucleated” fashion. This burial pattern could possibly indicate “founder burials,” a ritual practiced by newly arrived migrant groups in order to create a sense of community identity, and to reinforce social structures and hierarchies once tentatively settled in new lands (Effros, 2003). The most unique aspect of these necropolises is the presence of nineteen contextual burials of horseman and horse (Genito, 1997; Ceglia, 2010; De Vingo, 2017; Ceglia and Marchetta, 2012), twelve and seven of those have been found in a central position at Vicenne and Morrione cemeteries, respectively (Figs. 1 and 2). The individual was interred, either in nude earth or in pits lined with stones or wooden supports, with the entire horse, grave goods, weapons, horse tack, stirrups, and often evidence of a “funerary feast” represented by drinking vessels and domesticated animal bones (Fig. 3) (Ceglia, 2010; De Vingo, 2017).

Fig. 3



the horseman burial of t. 102 from Morrione. Red indicates iron objects, blue is silver, green is bronze, and orange is bone (from Ebanista, 2014). 1 column image size. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The horseman burials of Campochiaro break sharply with previous imperial and italic cultures where either entire horses, horse skulls, or headless horses were buried in separate nearby pits to the interred individuals (Ebanista, 2011; De Vingo, 2017; Fedele, 2017) and is so far unknown from the rest of the Italian peninsula, with the exception of the inhumations of San Mauro in Cividale (Friuli, Italy). Here the inhumations differ slightly in burial organization: the warrior was placed in the bottom of the pit, a layer of flat stones was placed over them, and then the entire horse with tack was placed above the stone layer (Ahumada Silva, 2000; De Marchi, 2011). Horseman burials are much more common in the Carpathian Basin and Hungarian Plain, generally associated with the Pannonian-Avar culture of the VI to VIII centuries CE (Bóna, 2000; Bárdos, 2000). The presence of Avar-style stirrups at Campochiaro strengthens the hypothesis of a migration from Eastern Europe and Central Asia and anticipate to the VII c. the supposed entry of stirrups in Western Europe (Genito, 1991; Ceglia, 2010). The absence of any evidence of housing units reinforce the notions of a nomadic or seminomadic tradition of the community (Bóna, 2000; Ceglia, 2010; De Vingo, 2017).

It is noteworthy that Paul the Deacon in his *Historia Langobardorum* (late VIII c. CE) indicated that the central deserted areas of the inner plateau among *Sepinum*, *Boianum*, and *Aesernia* were resettled by *Vulgares* (Avars/Bulgars/Huns) and that they still conserved their language during his own lifetime ([Instruction: This is the citation for the work of Paulus Diaconus/Paul the Deacon]*Historia Langobardorum* V.29; Bóna, 2000). This group was reportedly led by a Duke Alzeo who was tasked with settling those areas with his people to defend the boundaries of the Duchy of Benevento during the reign of Duke Romuald (671–687 CE)¹. This group was most likely also referenced by Fredegarius Scholasticus in his Chronicle (composed in the mid VII c. CE), though he writes of their activities in the early VII c. during the reign of Dagobert I in Austrasia (623–634 CE) ([Instruction: This is the citation for Fredegarius Scholasticus/Fredegar]*Chronicum cum suis continuatoribus* IV.72)². Here the duke is named Altioeus and is reported to have fled from the Franks and Bavarians to the lands of the Carantanian Slavs.

Thus, the funerary settlement of Campochiaro refers to the period shortly after the collapse of the Western Roman Empire in Late Antiquity, when many Eastern and Northern populations breached the empire as federates and mercenaries, eventually bringing the control of the Imperial Province of Italy into “Germanic” hands (Wickham, 1981;


Gasparri, 2012). Though the Longobard invasion and settlement of Northern and Central Italy, or *Langobardia Maior*, is rather well documented, the method of the initial conquest of peninsular Southern Italy (*Langobardia Minor*) is still unclear; in any case a *condottiero* by the name of Zotto is reported as having founded a duchy centered on the ancient Samnite capital of *Beneventum* (modern Benevento) in about 571 CE (*Historia Langobardorum* III.33; Christie, 1995). These Longobards were by no means a homogenous group of purely Germanic warriors, but also contained elements of provincial Roman (Pannonian and Norican) culture, as well as nomadic steppe peoples (Avars, Bulgars, Sarmatians, and Proto-Slavs) (*Historia Langobardorum* II.26; Kiszely, 1979; Christie, 1995).

A number of anthropological analyses have been undertaken in the last 20 years in order to reconstruct the biological features and lifestyle of this population (Belcastro, 2001; Belcastro and Facchini, 2001; 2004; Belcastro et al., 2001; 2002; 2003; 2007; Tanganelli et al., 2014). The presence of individuals of both sexes, distributed in all age classes from infant to advanced aged individuals, indicates a common demographic distribution. Infant mortality can be assumed to have been quite high (about ~ 30% of all the individuals from both sites, with the majority being under 8 years old) although it falls within typical ranges for rates of infant mortality for the Middle Ages in Italy (Tanganelli et al., 2014 and all references therein). Results of traumatic events, especially in males, due to interpersonal violence have been recorded, as well as the presence of infectious disease (leprosy) and other pathological anomalies (Giusberti, 1991; Belcastro et al., 2002, 2005; Donoghue et al., 2015; Schuenemann et al., 2018).

2 Materials

We selected fifteen individuals: nine from Vicenne and six from Morrione (Table 1). Nine of these were archaeologically classified as horsemen (Vicenne: tombs 73, 79, 81, 109/110³, 150, 155; Morrione: tombs 70, 134, 152). Among the Vicenne horsemen, only the individual of t. 109/110 could be confidently assigned as “male” based on skeletal characteristics, whereas the others (tombs 73, 79, 81, 155) were “probable males.” The horseman of t. 150 was “indeterminate” as were three other individuals of Morrione (two horsemen: tombs 134, 152; the individual of t. 171). Only one horseman from Morrione was classified as a “probable male” (t. 70). Among the juveniles we selected two individuals from Vicenne (tombs 12, 15). The child of t. 15, estimated as “female” based on grave goods, was interred in close proximity to the horseman of t. 16. A female control sample (Vicenne tombs 15, 40; Morrione tombs 68, 135) was also selected in order to verify the reliability of the palaeoproteomic analyses.

Table 1

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Sample composition from Campochiario. Vicenne (VCN), Morrione (MRN). Individuals are listed by tomb number. State of preservation was based on the above mentioned criteria. For sex diagnosis F, M and IND refer to Female, Male and Indeterminate; a question mark indicates probable sex. Grave goods and archaeological context from the tombs of Morrione are currently unavailable due to lack of published or archive documentation from the original dig. The last column reports the results of amelogenin-based sex determination using the same abbreviations as the sex diagnosis column.

| Site | Tomb | State of Preservation | Age at Death (osteological) | Sex (osteological) | Sex (archaeological) | Horseman Tomb? | Sex (amelogenin) |
|------|--------|-----------------------|-----------------------------|--------------------|----------------------|----------------|------------------|
| VCN | 12 | 3-0-2 | 6–8 | IND | IND | NO | M |
| VCN | 15 | 1-1-2 | 8–9 | IND | F | NO | F |
| VCN | 150 | 1-1-2 | 30–50 | IND | M | YES | M |
| VCN | 40 | 1-3-0 | >50 | F? | F | NO | F |
| VCN | 73 | 3-0-3 | 20–30 | M? | M | YES | M |
| VCN | 79 | 2-0-2 | 30–50 | M? | M | YES | M |
| VCN | 81 | 1-0-3 | 40–50 | M? | M | YES | M |
| VCN | 155 | 1-2-2 | 15–16 | M? | M | YES | M |
| VCN | 109/10 | 1-1-1 | 50–60 | M | M | YES | M |
| MRN | 134 | 2-0-3 | 30–40 | IND | – | YES | M |
| MRN | 152 | 2-0-3 | 20–30 | IND | – | YES | M |
| MRN | 171 | 1-0-3 | >50 | IND | – | NO | M |
| MRN | 68 | 1-2-1 | 20–30 | F | – | NO | F |
| MRN | 135 | 1-3-3 | 25–35 | F | – | NO | F |
| MRN | 70 | 1-1-2 | 16–18 | M? | – | YES | M |

Teeth were selected based on availability due to preservation, with a preference for the upper first permanent molar, due to a further use of the same samples for Sr isotope analyses. If not available, a lower first molar was selected. If this was also absent, upper or lower premolars were used.

3 Methods

A general state of preservation of the analysed sample was assessed. State of preservation was based on three distinct skeletal sections using a skeletal inventory form (Fig. 4): skull-appendicular skeleton-limbs (including pelvic and shoulder girdles), where (0) indicates the absence, (1) when the section is complete or nearly complete, (2) partially fragmentary and (3) highly fragmentary.


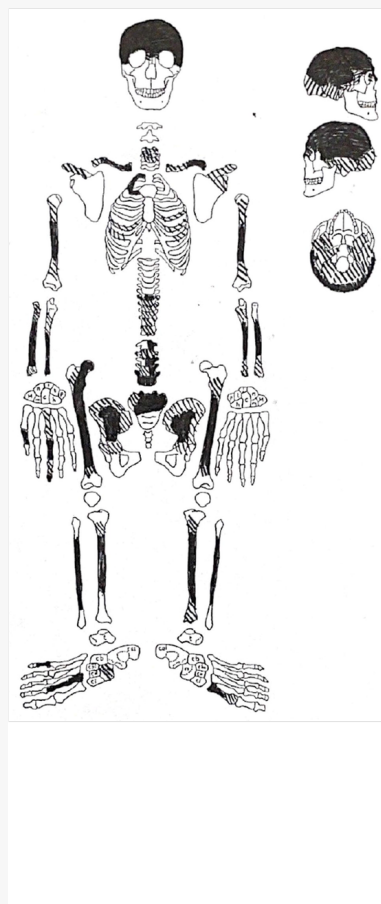
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Fig. 4




example of a skeletal inventory form for tomb 102, Morrione. Shaded sections indicate presence, hash marks indicate fragmentary status and white the missing parts. This individual displays a poor preservation state. 1 column image size.

From each of these individuals a single tooth specimen was manually extracted. Once removed, the teeth were placed into sterilized sample containers with individual teeth placed in separate closed sections. Enamel fragments (ca. 5 mg) were collected from each tooth specimen using a dentist drill and cleaned from adhering dentine residual. Samples were thus washed with MilliQ using an ultrasonic bath and leached for a few minutes using 5% HCl. Then, enamel peptides were extracted submerging enamel fragments in 200 μL of 5% HCl for 45 min. Supernatant was collected and purified through C_{18} functionalized silica spin tips (Thermo Scientific HyperSepTM SPE tips). Resin-bounded peptides were eluted using 20 μL of 60% acetonitrile in 0.1% formic acid. The whole laboratory protocol was performed at the palaeoproteomic facility of the BONES Lab (University of Bologna). Extracted peptides were dried and resuspended in a mixture of water:acetonitrile:formic acid 95:3:2, before the LC-MS/MS measures. Analyses were conducted using a Dionex Ultimate 3000 UHPLC coupled to a high-resolution Q Exactive mass spectrometer (Thermo Scientific, Bremen, Germany), housed at the Centro Interdipartimentale Grandi Strumenti of the University of Modena and Reggio Emilia, with a run time of 60 min per sample. An inclusion lists with the peptides of interest was included in the method ($[\text{M} + 2\text{H}]^{+2}$ 523.7748; 440.2233; 540.2796; 525.2975; 575.7533; 656.3528 m/z). More details on the analytical protocol are reported in [Lugli et al. \(2019; 2020\)](#). Ion chromatograms were manually inspected using XcaliburTM (Thermo Scientific) searching for specific AMELX and AMELY peptides, following Stewart and coauthors (2017) and [Lugli et al. \(2019\)](#). To refine sex estimation and possibly identify other endogenous tooth proteins, raw data was converted in Mascot generic format (MsConvert v. 3.0.10730, ProteoWizard tools) and searched against: Swiss-Prot (constrained to *Homo sapiens*) and cRAP (116 contaminant sequences). Search parameters were the same of [Lugli et al. \(2019\)](#), and include NQ deamidation and M oxidation as variable modifications We considered a protein as identified if at least two different significant peptides were observed. Raw data are available via ProteomeXchange with identifier PXD029559.

4 Results

The state of preservation of the examined sample is quite poor (Table 1). The previously estimated biological sex (Belcastro, 2001; Belcastro and Facchini, 2001; 2004; Belcastro et al., 2001; 2002; 2003; 2007; Tanganelli et al., 2014) and archeological gender are also reported in Table 1. Palaeoproteomic results are presented in Tables 1 and 2, and in Supplementary Figs. 1–15. Sex estimation was possible in all cases, i.e. amelogenin peptides were present in all the enamel samples. Twelve out of fifteen samples (80%) showed the presence of AMELY specific peptides (Fig. 5), indicating male sex. Three individuals were tentatively identified as females, due to the lack of AMELY. Indeed, the lack of AMELY specific peptides can be due to female sex or male false negative with undetectable AMELY (e.g. Parker et al., 2019; Buonasera et al., 2020; see also Parker et al., 2021 for additional discussion). Other dental proteins were identified thanks to database searches. In particular, we observed the presence of ameloblastin (15/15 samples, 100%), enamelin (7/15 samples, 46%) and collagen type I $\alpha 2$ (4/15 samples, 26%). A single sample also showed the presence of collagen type I $\alpha 1$. The presence of collagen is probably due to some residual dentine left during sample pre-treatment. No exogenous contaminants were identified through Mascot searches. Similarly, no proteins were found in experimental blanks (see also Supplementary Fig. 16).

Table 2

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Protein identification (significant peptides $n > 2$) in human teeth from Campochiaro. Vicenne (VCN), Morrione (MRN). AMELX = amelogenin X isoform 1 (*H. sapiens* Q99217); AMELY = amelogenin Y isoform 2 (*H. sapiens* Q99218); AMBN = ameloblastin (*H. sapiens* Q9NP70); ENAM = enamelin (*H. sapiens* Q9NRM1); CO1A2 = collagen type I $\alpha 2$ (*H. sapiens* P08123); CO1A1 = collagen type I $\alpha 1$ (*H. sapiens* P02452); AMELX and AMELY peptides were identified combining ion chromatograms and the database searches.

| Site | Tomb | AMELX | AMELY | AMBN | ENAM | CO1A1 | CO1A2 |
|------|---------|-------|-------|------|------|-------|-------|
| VCN | 12 | • | • | • | | | |
| VCN | 15 | • | | • | | | |
| VCN | 150 | • | • | • | | • | • |
| VCN | 40 | • | | • | • | | |
| VCN | 73 | • | • | • | | | |
| VCN | 79 | • | • | • | • | | • |
| VCN | 81 | • | • | • | • | | • |
| VCN | 155 | • | • | • | | | |
| VCN | 109/110 | • | • | • | • | | |
| MRN | 134 | • | • | • | • | | |
| MRN | 152 | • | • | • | | | |
| MRN | 171 | • | • | • | | | |
| MRN | 68 | • | | • | • | | |
| MRN | 135 | • | | • | • | | • |
| MRN | 70 | • | • | • | | | |


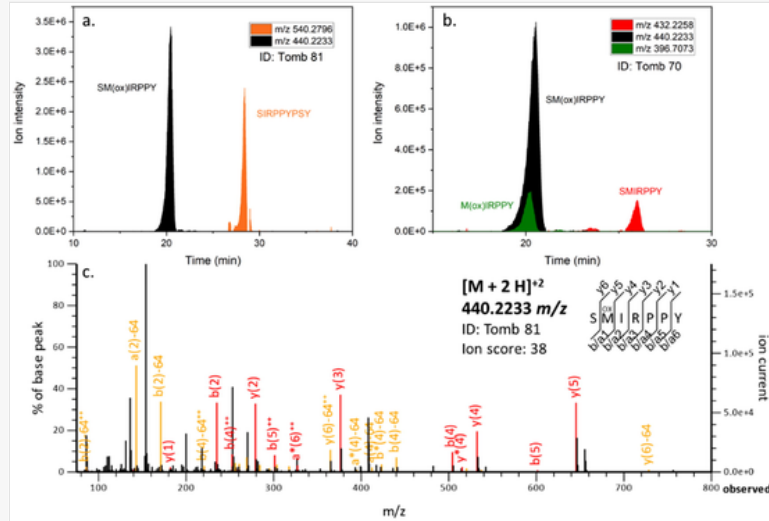
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Fig. 5



Examples of amelogenin-based sex estimation of Campochiario individuals; a) peaks corresponding to $[M + 2H]^{+2}$ 440.2233 and 540.2796 m/z and belonging to AMELY and AMELX respectively in ID VCN 81; b) confident identification of AMELY using a triple-peptide approach in ID MRN 70; c) Fragmentation spectrum of peptide SM(ox)IRPPY, as an additional evidence of AMELY presence in ID VCN 81. 2 column image size.

Thus, in detail, some individuals for which sex was undetermined have had sex assigned for the first time (tombs 12, 15, 150, 154, 132, 171), while for others that were considered probable (tombs 40, 70, 73, 79, 85, 155), the sex has been confirmed. In cases where the sex was archaeologically attributed (tombs 15, 40, 73, 79, 81, 109/110, 150, 155), it has been confirmed.

5 Discussion

The aim of this work was focused on defining the biological profile of some individuals (horsemen especially) of the Campochiario necropolises to compare the biological with the archaeological results, and possibly exploring their social identity. Palaeoproteomic analyses allowed us to remove previous uncertainty, strengthening its value in the understanding of the biological individual features and the structure of ancient communities. Even though aDNA analyses are useful to identify ancient individuals (Fu et al., 2016; Hedenstierna-Jonson et al., 2017), modifications and contaminations due to a variety of factors (processes of deposition, taphonomy, excavation, removal, laboratory activities, etc.) (Fairgrieve, 2008; Malmström et al., 2005) may strongly affect the outcomes. Palaeoproteomic analysis in sex diagnosis would seem to be a more reliable method in both archaeological (Lugli et al., 2019) and forensic settings as the amelogenin present in dental enamel is much less likely to be contaminated or degraded.

In addition, in archaeological contexts poor preservation of remains may prevent the use of morphometrical traits for sex assessment. Moreover, sexual dimorphism has varied from past to modern populations and it may show different grades of expression in different living populations as well (Borgognini Tarli and Repetto, 1986; Kanazawa and Novak, 2005; Scott et al., 2014). Even though sex is genetically determined, phenotypical expression may be affected by inner and outer environmental factors (Ruff, 1987; Piglucci, 1996; Monaghan, 2008; Haqq and Donahoe, 1998). Low sexual dimorphism is also a common factor for juveniles until they reach skeletal maturity (Schaefer et al., 2009; White et al., 2011), making sex estimation much more problematic for many archaeological and forensic skeletonized subadults.

Beyond biological sex, we may approach other aspects that concern the social sphere of ancient populations. Grave goods have traditionally been used as a marker of biological sex, especially in periods typified by rich grave assemblages. However, sex and gender enclose different concepts and meaning, the latter being a product of self-identification, socially and culturally constructed and considered as the communal interpretation and representation of biological differences (Sofaer, 2006; Barbiera, 2012; Fausto-Sterling, 2012; Joyce, 2017). In addition, the selection of grave goods may represent the necessity or desire of a community to identify and emphasize a specific role of the deceased, or to strengthen or create community identities. Thus, in ancient funerary contexts, reconstruction of the role of an individual in the community is complex due to the interaction between the identity of the deceased (in terms of age, gender, ethnicity, social status) and the signs and symbols left by those who preside over their funeral ritual and burial (Nizzo, 2015). In this regard, one of the main questions posited when studying archaeological funerary contexts is, “does the funeral display reflect an objective individual reality, or is it more symbolic and communal?” (Effros, 2003; Barbiera, 2012; Theuvs and Alkemade, 2000).

It can also be hypothesized that not all grave goods necessarily relate to the rituals of funerary performance. The individual of t. 109/110 (Vicenne), confirmed as male through palaeoproteomic analysis, was interred with a vitreous glass necklace incorporating the foot of a glass drinking vessel as a pendant. This object could be viewed in this

funerary context as being typically gendered as “female,” as vitreous glass necklaces are elsewhere absent from the tombs of adult males.

5.1 Sex and gender of the horsemen

The funerary ritual of contextual burials of horse and horseman can be seen as a strong marker of elite identity for said individuals within their community. Having confirmed that the studied horsemen were biological males and that sex corresponds to archeological gender, it is relevant to better define and understand the social structure of the population of Campochiaro. The results from amelogenin analyses support the hypothesis of male dominated communities. Female grave goods support the theory of a patriarchal society, albeit one in which feminine gender identity was important, displayed, and constantly reinforced through objects. Globular earrings and Byzantine fibulae could represent traditional signs of ethnic provenience, or merely high-status objects that were particularly in vogue within the sphere of the culturally influential Eastern Roman Empire (Ceglia 2012, Ceglia and Marchetta, 2012). This would seem to imply a strongly gendered division of society, entrenched through individual and subgroup signs and symbols, reflected in the funerary record.

Interpersonal violence, exposure to hazards and risks, and heavy use of weapons have left traces in many male skeletons (Belcastro and Facchini, 2004; Belcastro et al. 2001; 2005; Belcastro et al., 2002). In some cases, skeletal trauma can be linked to fatal injuries, with no signs of bone remodelling (Vicenne, t. 54). Whereas in other cases, such as the horseman of t. 102 from Morrione, the individual displays the result of a non-fatal traumatic head injury, most likely from a blunt-bladed weapon (Fig. 6). The individual lived for some time after the trauma as shown by diffuse bone remodelling, indicating probable attention and treatment of the wound by the community at large.


 Images are optimised for fast web viewing. Click on the image to view the original version.

Fig. 6



Supero-lateral right view of the t. 102 skull. Present on the frontal bone is the result of a healed traumatic lesion (4x7cm, depth 5 cm). The lesion is oriented latero-medially, beginning approximately 7 cm from the bregma on the coronal suture. Bone remodelling is diffuse at the deepest point. Image taken from a 3D model created from photogrammetry by an Author of the present work (AG). 1.5 column image size.

Thus, these skeletal markers seem to reflect the reality of a gender-organized bellicose warrior society. As previously stated, Paul the Deacon (*Historia Langobardorum* V.29) and Fredegar (*Chronicum cum suis continuatoribus* IV.72) refer to a group of mounted Bulgar/Avar warriors who came from beyond the Alps. The need for elite mounted warriors equipped with novel technology (stirrups) in the Duchy of Benevento is plausible, considering that Byzantines and Longobards frequently clashed to retain exploitative rights over the rich Southern Italian lands, specifically the plains of the Apulian hinterland (Rotili, 2010).

Regarding grave goods, the necropolises of Campochiaro also present many individuals accompanied by few objects, and in some cases said objects have no rigid gendered connotation (e.g. coins, small knives, combs; objects consistently found in burials of both sexes), such as younger juveniles. Some individuals even present no grave goods whatsoever, such as some peripheral tombs of Vicenne and the burials around the funerary chapel at Morrione (Figs. 1 and 2) (Ceglia, 2010; Ceglia, 2012). However, objects such as weapons and horse tack can be rigidly gendered as belonging to “males,” while jewellery as belonging to “females”. This reflects the necessity to distinguish in burial ritual the presence of both elite and low ranking individuals. In addition, the military elite status of the horsemen arise not only from their peculiar burial ritual but also from their central interment positions. These hierarchical and gender-organized communities were already inferred from the archaeological record and historical documents (Effros, 2003; Ceglia, 2004; Barbiera, 2012; De Vingo, 2017; Ceglia and Marchetta, 2012). Our data confirms the correspondence between the gender and biological sex of those horsemen.

In a later period (X c. CE) and in a North-Germanic/Viking context, the “elite warrior burial” from Birka (Sweden), previously attributed to a male, belonged to a female individual (Hedenstierna-Jonson et al., 2017), revealed by genetic

analyses. Although the individuals studied from Campochiaro display no such discrepancies, the above-mentioned case stresses the relevance of proper identification in reconstructing community ideals and beliefs of past societies.

5.2 Sex and gender of the juveniles

The palaeoproteomic analyses of the juveniles (tombs 12, 15) from Vicenne were able to predict sex in both cases, confirming the archaeological gender displayed in the grave goods of t. 15 (8–9 y., buried with an ornate bronze ring, vitreous glass necklace ornamented with bronze, bronze fibula, two bronze coins, and silver earrings) and assigning male sex to the previously indeterminate child of t. 12 (6–8 y., buried with an iron ring, small knife, bronze fibula, one gold coin, and coarse-ware vase) (Tanganelli et al., 2014). This is relevant considering the different meanings and value of childhood in the past and the difficulty of sex diagnosis in juveniles using osteological methods. Ambiguous gender identity emerges for children from ethnographic and historical data, defined later only in the course of development. This is particularly relevant especially in the projection and the social role of infants in Roman funerary practices and their perception as “non-individuals”. The newborn was led through an incremental process to the status of “person” beginning with the eruption of the incisors in the first year of life and was subject to a series of ritual treatments necessary for their social assimilation (Gowland, 2006; Gowland and Halcrow, 2020).

Furthermore, grave goods appear sporadically or not at all in Medieval burials in Southern and Western Europe (Alterauge et al., 2020). In the Campochiaro necropolises, as stated above, younger individuals were accompanied by few or no grave goods whatsoever, many without any strongly gendered display (Ceglia, 2010; Ceglia, 2012). In the case of the child from t. 15, the presence of gold coins and silver earrings indicates that she may have belonged to the community elite, such as the horsemen, as the donation of these objects would have been an economically significant act. Indeed, she was buried in close proximity to the warrior of t. 16, in a very central section of the Vicenne necropolis (Fig. 1). She could also be viewed as a potential relative of that horseman but this possible relationship can only be confirmed by additional aDNA analyses.

Thus, the spatial structure of the cemeteries of Vicenne and Morrione seems to reflect the social organization of this particular Early Medieval community. It is also worth noting that some individuals affected by leprosy were buried among the others without prejudice. This could indicate they were not shunned by society, or that the stigma and gravity of this disease were not yet recognized (Belcastro et al., 2005). In addition, an individual (t. 57) buried in a peripheral area of the Vicenne necropolis displays diverse skeletal pathologies (scoliosis, degenerative joint diseases) and the results of interpersonal violence. It would be reasonable to hypothesize a low social rank and role for that individual. The burial lacked the entire cranium even though there is no evidence of an ancient intentional removal (Bocchini and Belcastro, 2012). Further analyses of the structure of these medieval necropolises are required in order to infer the social organization of those communities.

6 Conclusion

The study was aimed at verifying and ascertaining biological sex of some horsemen and other individuals from a large Early Medieval funerary context from Campochiaro (Molise, Italy), comparing it with the archaeological data in an attempt to highlight their social identity. Considering their poor preservation state and scant sexually dimorphic traits that prevented reliable sex assessment, we applied palaeoproteomic analyses of enamel peptides that allowed outlining the biological profiles of those individuals, especially the horsemen. The clarification of the biological profile contributed to a better understanding of the social organization and beliefs of these VII c. CE “barbarian” communities, considering that the structure of the cemeteries may strongly reflect the social and cultural system of this ancient society.

In the necropolises of Campochiaro, clear social roles of individuals are emphasised by the organization and unique ritual of their burials. In particular, the central position of the rich and symbolically important tomb groups of elite warriors buried alongside their mounts seem to indicate a community hierarchically organized and highly gendered, even though they are not spatially isolated from the rest of the inhumations, both healthy and sick individuals. Grave goods can be understood to reflect social position and hierarchy in life in the majority of cases, with more ornate and more numerous objects representing higher status individuals. Considering the estimated sex, some skeletal lesions, and the grave goods of the horsemen, sex, gender, display, and identity in these communities have shown to be closely linked and based on social realities in life, transferred in an afterlife world. The close position of the remains of the young girl of t. 15 to the burial of the horseman of t. 16 in the necropolis of Vicenne and her rich grave goods likely shows the relevance of her social role, despite gender and age at death.

Considering the uniqueness of the cultural features of the funerary settlement of Campochiaro, with a burial ritual completely unrelated to other coeval contexts in the Italian peninsula, as well as innovative and culturally significant grave goods such as stirrups, possibly the earliest example of their introduction to Western Europe, further analyses through aDNA and strontium isotopes will be performed to better understand the ethnic composition of the community and its geographic provenience. Finally, even if thirty years have passed since these graves were first uncovered, the biological remains from the necropolises of Campochiaro still have a great wealth of information to help us better understand Early Medieval European societies, identities, and migrations.

7 Data availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al., 2019) partner repository with the dataset identifier PXD029559.

Uncited ref [Instruction: This source is credited in the text. Paulus Diaconus is the original latin naming convention of Paul the Deacon in his life. In the references list it should not be listed as Diaconus, P., but rather Paulus Diaconus, as it is an ancient primary source. In text citations are listed as *Historia Langobardorum* with book and chapter number, as is the norm for ancient sources] references

Diaconus (1992).

CRedit authorship contribution statement

Anthony Gasparini: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Federico Lugli:** Methodology, Data curation, Formal analysis, Writing – review & editing, Funding acquisition. **Sara Silvestrini:** Data curation. **Isabella Marchetta:** Data curation. **Stefano Benazzi:** Funding acquisition. **Maria Giovanna Belcastro:** Conceptualization, Data curation, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Supervision.

Declaration of Competing Interest

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

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Appendix A Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jasrep.2021.103337>.

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Footnotes

Text Footnotes

[1] *Per haec tempora Vulgarum dux Alzece nomine, incertum quam ob causam, a sua gente digressus, Italiam pacifice introiens, cum omni sui ducatus exercitu ad regem Grimuald venit, ei se servituum atque in eius patria habitaturum promittens. Quem ille ad Romualdum filium Beneventum dirigens, ut ei cum suo populo loca ad habitandum concedere deberet, praecepit. Quos Romualdus dux gratanter excipiens, eisdem spatiosa ad habitandum loca, quae usque ad illud tempus deserta erant, contribuit, scilicet Sepinum, Bovianum et Iserniam et alias cum suis territoriis civitates, ipsumque Alzeconem, mutato dignitatis nomine, de duce gastaldium vocitari praecepit. Qui usque hodie in his ut diximus locis habitantes, quamquam et Latine loquantur, linguae tamen propriae usum minime amiserunt.*

[2] *Eo anno in Abarorum, cognomento Chunorum, regno in Pannonia surrexit vehemens intentio, eo quod de regno certarent, cui deberetur ad succedendum, unus ex Abaris et alius ex Bulgaris; collecta multitudine uterque in invicem pugnarunt. Tandem Abari Bulgaros superant. Bulgaris superatis, novem millia virorum cum uxoribus et liberis de Pannonia expulsi, ad Dagobertum expetunt, petentes ut eos in terra Francorum ad manendum reciperet. Dagobertus jubet eos ad hiemandum Bajoarios recipere, dummodo pertractaret cum Francis quid exinde fieret. Cumque dispersi per domos Bajoariorum ad biemandum fuissent, consilio Francorum Dagobertus Bajoariis jubet ut Bulgaros illos cum uxoribus et liberis unusquisque in domo sua in una nocte Bajoarii interficerent, quod protinus a Bajoariis est impletum. Nec quisquam ex illis remansit Bulgaris, nisi tantum Altioeus cum septingentis viris, et uxoribus cum liberis, qui in marca Winidorum salvatus est. Post haec cum Walluco duce Winidorum annis plurimis vixit cum suis.*

[3] The number of tomb 109/110 refers to the confusion in the documentation and to only one skeleton. T.110 is not present in the collection housed in the Laboratory of Physical Anthropology of the University of Bologna. The 109 has been marked as being a horseman and it is likely that tt. 109 and 110 represent the same individual, though it is nearly impossible to prove unless t. 110 is found. In addition, t.109 was equipped with horse harnesses and weapons, though in previous publications t. 110 is always reported as a horseman.

Highlights

- Unique burial context of the Early Medieval necropolises of Campochiaro (Italy)
 - Comparison between biological sex and archeological gender.
 - Poor preservation of human skeletons and sex estimation.
 - Amelogenin analysis and sex estimation.
 - Cemetery structure and social organization.
-

Appendix A Supplementary data

The following are the Supplementary data to this article:

 [Multimedia Component 1](#)

Supplementary data 1

Queries and Answers

Q1

Query: Please review the **given names and surnames** to make sure that we have identified them correctly and that they are presented in the desired order. Carefully verify the spelling of all authors' names as well. If changes are needed, please provide the edits in the author section. /

Answer: Reviewed

Q2

Query: Your article is being processed as a regular item to be included in a regular issue. Please confirm if this is correct or if your article should be published in a special issue using the responses below. /

Answer: Yes

Q3

Query: Ref(s). 'Belcastro et al., 2002' is/are cited in the text but not provided in the reference list. Please provide it/them in the reference list or delete these citations from the text. /

Answer: this is provided in reference list, it is: Belcastro, M.G., Facchini, F., Fulcheri, E. 2002. "Dismorfosi Cranio-facciale della Tomba 112 della Necropoli Altomedievale di Vicenne-Campochiaro (CB, Molise)." in E. Pacciani, R. Boano, M. Micheletti (eds). *Antropologia del Medioevo: Biologia e cultura: le Alpi e la Penisola*. Arezzo: L.P. Grafiche. pp. 56-59.

Q4

Query: The **Uncited References** section comprises references that occur in the reference list but are not available in the body of the article text. Please cite each reference in the text or, alternatively, delete it. Any reference not dealt with will be retained in this section. /

Answer: Not done, comment provided

Q5

Query: Correctly acknowledging the primary **funders and grant IDs** of your research is important to ensure compliance with funder policies. Please make sure that funders are mentioned accordingly. /

Answer: Reviewed