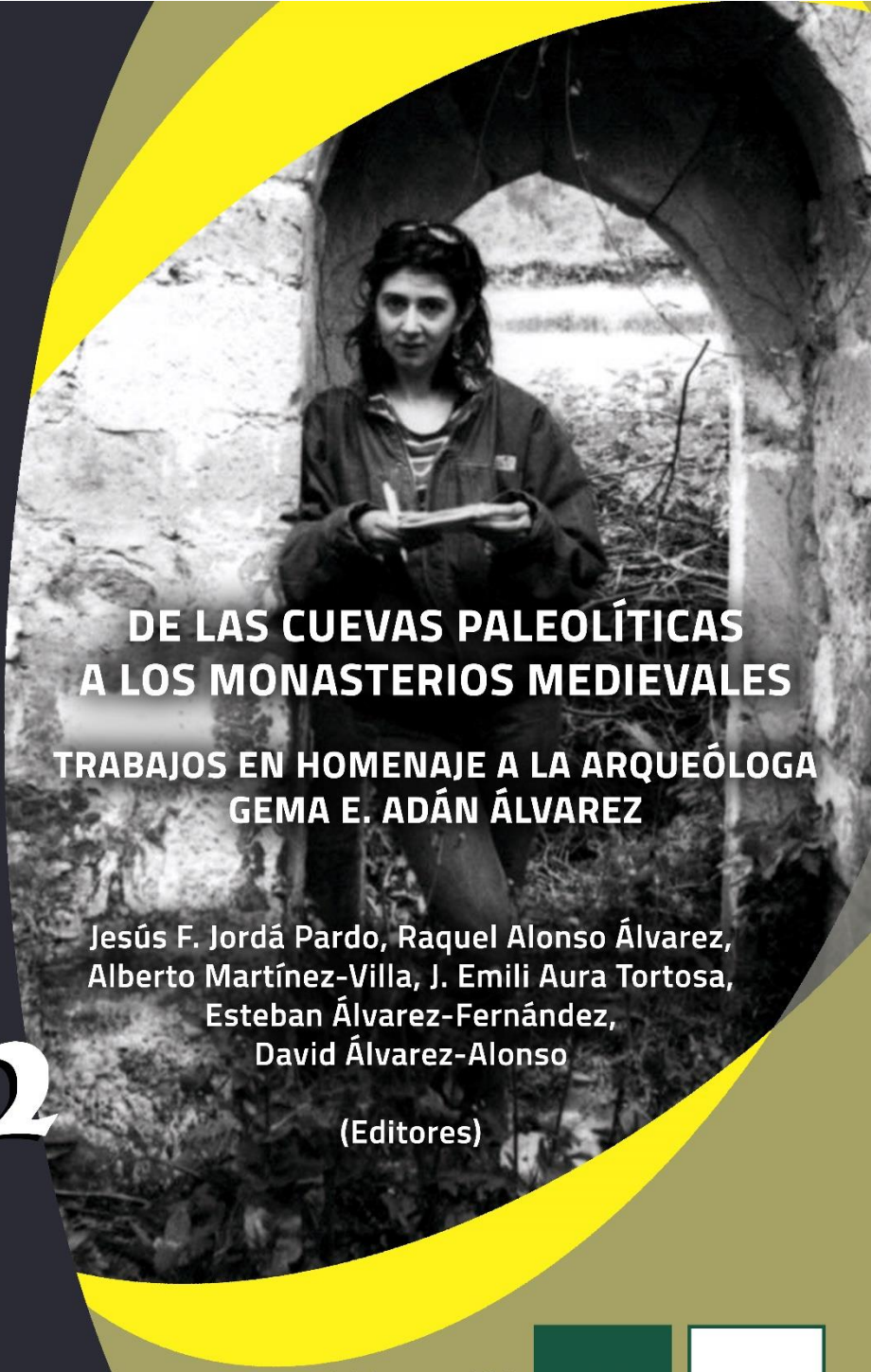
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DE LAS CUEVAS PALEOLÍTICAS A LOS MONASTERIOS MEDIEVALES

TRABAJOS EN HOMENAJE A LA ARQUEÓLOGA GEMA E. ADÁN ÁLVAREZ

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PALEODEMOGRAPHIC PROFILES OF THE POPULATIONS BURIED IN SAN PABLO CONVENT (BURGOS, SPAIN)

Perfiles paleodemográfico de las poblaciones enterradas en el Convento de San Pablo (Burgos, España)

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En memoria de nuestra querida Gema Adán.

Abstract: The Convent of San Pablo holds burials from the 13th-19th centuries in three areas: the Cloister, Church, and Chapter House. This paper compares methods for sex and age-at-death estimations in skeletal remains from the Cloister and Church (14th-18th centuries) and the Chapter House (17th-18th centuries). Sex estimates in adult individuals indicated male predominance in the Cloister and exclusively males in the Chapter House. Age-at-death estimation using TA3 software revealed individuals over 60 in all areas. The sex estimation in non-adults was particularly accurate in individuals older than 9. A new approach was proposed for non-adult age grouping based on the human pattern growth. Demographic profiles by area revealed unique trends and burial practices within the community.
Key words: *Sex estimation, age at death estimation, non-adults, growth, development.*

Resumen: El Convento de San Pablo alberga enterramientos desde los siglos XIII al XIX en tres áreas: el Claustro, la Iglesia y la Sala Capitular. En este trabajo se comparan distintos métodos para estimar de sexo y edad de muerte en restos esqueléticos del Claustro y la Iglesia (siglos XIV-XVIII) y la Sala Capitular (siglos XVII-XVIII). Las estimaciones de sexo en adultos indicaron un predominio masculino en el Claustro y exclusivamente varones en la Sala Capitular. La estimación de la edad

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de muerte mediante el software TA3 reveló individuos mayores de 60 en todas las áreas. La estimación de sexo en no adultos fue particularmente precisa en individuos mayores de 9 años. Se propuso un nuevo enfoque para la agrupación por edad en no adultos basado en el patrón de crecimiento humano. Los perfiles demográficos por área revelaron tendencias y prácticas funerarias únicas dentro de la comunidad.
Palabras clave: *Estimación de sexo, estimación de edad de muerte, no adultos, crecimiento, desarrollo.*

1 Introduction

Palaeodemography is the discipline dedicated to the study of past population dynamics (MCFADDEN 2021). It provides a crucial framework that brings various aspects of the past human experience, including aspects such as life expectancy, fertility, mortality risk and survival rates (BOLDSEN *et al*. 2022). The efficacy of paleodemographic analysis relies on the quality of the data, their analysis, and subsequent interpretation.

In bioarchaeological contexts, the data are derived from human skeletal remains, as a substantial amount of demographic information can be estimated from them. The most crucial parameters are the biological sex and age-at death. Achieving accurate estimations for these two parameters requires the use of reliable morphological indicators. Unfortunately, the estimation process is not always straightforward, as some parameters can be confidently estimated, while others cannot.

The accuracy of sexual estimation on adult individuals depends on the specific skeletal element under study. The pelvis and skull are the most reliable skeletal regions, with sexing methods based on them yielding accuracies of 98-100% (WASHBURN 1948; PHENICE 1969). However, achieving this level of accuracy requires the preservation of complete pelvis and skull, which is not always the case with archaeological specimens. In such instances, it is important to bear in mind that different parts of both the pelvis and skull may vary in reliability. Furthermore, even when both bones are intact, challenges may arise when morphological indicator of sex are not unequivocally reflective.

The other methods for sexing adult skeletons are based on postcranial and dental measurements. This kind of methods are based on predictive equations which classified the individual as male and female with a probability. A major drawback of these methods is their tendency to be sample-specific, resulting in decreased accuracy when applied to individuals from a population different from the one used in their development (ALBANESE *et al.* 2005). Thus, to ensure the highest accuracy possible, these equations should only be used on populations similar to that from which they were derived. When we have not direct way of knowing which reference sample is more appropriate in our study cases, the best choice is to conduct a sample-specific sex determination approach. Briefly, these methods consist of dividing a sample into two subsamples using the mean value as sectioning point, with values above the mean indicating males and those below the mean indicating females. Although high accuracies can be obtained with these approaches, it depends on factors such as the sample size and the sex ratio (ALBANESE *et al.* 2005). When the sample size is small and the

intersexual variability is modest, the recognition of bimodal distributions is hindered, making it challenging to estimate sex.

The accurate assessment of sex to non-adult skeletal material is generally considered problematic. Given the high accuracy of sex estimations in adults using skull and pelvis, these two regions have been investigated for developing a reliable method for sexing non-adult skeletons (WEAVER 1980; SCHUTKOWSKI 1993; MOLLESON *et al.* 1998; LOTH AND HENNEBERG 2001). Unfortunately, when these methods have been tested on other known sex non-adult samples, their overall performance is poor (MITTLER and SHERIDAN 1992; COQUEUGNIOT *et al*. 2002; SCHEUER 2002; SUTTER 2003; CARDOSO and SAUNDERS 2008; VLAK *et al*. 2008; GARCÍA-GONZÁLEZ *et al*. 2021). The low performance observed has been linked to inter-populational differences in both the pattern and degree of sexual dimorphism, as well as variations in the level of sexual dimorphism across different parts of the skeleton.

Another approach for sexing non-adult skeletons is based on the fact that sexual differences in long bone breadths are present at birth and persist due to males' greater muscle mass, resulting in relatively larger breadths. In light on this, Stull and Godde (2013) and Stull *et al.* (2017) have proposed two methods for sex estimation in non-adult individuals based on long bone dimensions. When one of these methods was tested on an external population, the results showed a low performance (LAMER *et al*. 2021). Although there are several statistical issues that may explain why this method fails, population differences in size and sexual dimorphism can also affect the applicability of this method.

In recent years, methods based on sexual dimorphism in dentition have gained acceptance for sexing non-adult individuals. Teeth are particularly suitable for sexing non-adult skeletons, as sexual differences in crown size and dental tissue proportions become apparent between birth and the age of 12 years (SIMPSON KUNOS 1998). Here, we should take into consideration the same precautions mentioned above for adult individuals, given the inter-populational variability.

As in the case of biological sex determination, methods for estimating age at death can be categorized for both adult and non-adult individuals. Unlike the methods used for sex determination in skeletons, those employed for age-at-death estimation in non-adult individuals tend to be more accurate compared to those for adults.

Age estimation for non-adult individuals is based on a range of ontogenetic processes, such as the development and eruption of the dentition, the length of long bones, fusion of epiphyses to diaphyses throughout the body, and maturity changes of epiphyses prior to fusion. Dental development is closely tied to chronological age and is commonly employed to estimate age in developing individuals (LIVERSIDGE 2008). However, where teeth are not preserved, age estimation primarily relies on the cranium and post-cranial skeleton. Growth standards based on the relationship between long bone lengths and chronological age are frequently used. There are several predictive equations derived from different modern samples with different growth patterns, body sizes and body proportions (RISSECH *et al*. 2008 2013; LOPEZ-COSTAS *et al*. 2012; CARDOSO *et al*. 2014; STULL *et al*. 2014). The main issue with these methods is their sample specificity. Bone lengths can be affected by stunted

growth attributable to starvation, malnutrition, or poor health, leading to significant variations in body sizes of individuals of the same age. This, in turn may result in under- or overestimating age when these methods are applied. This is due to the 'age mimicry', *i.e*, the tendency of these age estimates to mimic the age structure of the reference population. Thus, the main question here revolves around choosing the right equation for our sample. Regarding the standards of epiphyseal union, there is considerable variation in the sequence and timing of epiphyseal initiation and completion, adding complexity to age estimation process (COQUEUGNIOT and WEAVER 2007; CARDOSO 2008A, 2008B; CARDOSO *et al*. 2014B, 2014B). Moreover, the utility of standards charting the chronology of unions of epiphyses to diaphyses is limited to pre-adolescents and adolescents. In younger individuals, it is preferable estimate age from the onset of the ossification of the epiphyses and their subsequent changes in size and shape. Although there are several charts that describe the relationship between age and these changes on the knee and wrist (TODD 1937; PYLE AND HOERR 1955; GREULICH and PYLE 1959; ROCHE *et al*. 1975), they derived from radiographic studies conducted on living individuals and their application is difficult in skeletal material (KROGMAN and ISCAN 1987). Fortunately, some authors have adapted certain methods for their use in dry skeletal material (CONCEIÇÃO and CARDOSO 2011; GARCÍA-GONZÁLEZ *et al*. 2019). The application of these two adapted methods does not only improve our abilities to estimate age in non-adult skeletons, but also skeletal ages estimated in this way are able to offer a fair maturity index of the entire skeleton. Thus, it is important to recognize that in certain instances, the age determined through these methods serves more as an indicator of an individual's maturity stage than their chronological age.

In adults, age estimation becomes more complex, often relying on morphological changes linked to the degeneration of joints throughout the body and wear on the dentition. These processes are considerably variable, influenced by factors such as genetics, behavior, disease, and environmental conditions (BOLDSEN *et al*. 2022). For these reasons, although age-progressive changes can be obtained when examining anatomical structures such as the pubic symphysis or the auricular surface, classifying specimens is difficult because features distinguishing one stage from the next do not change consistently with advancing age in all populations. One of the main consequence is the misestimation of ages at death, with this bias being more prominent in older ages. Over the last 15 years, a methodological approach known as transition analysis (TA) has gained significance in paleodemographic research. This technique enables a more precise estimation of age, particularly in individuals older than 50 or 60 years at the time of death. It is based on scoring several traits from the entire skeleton.

In summary, while sex estimation of adult skeletons can yield reliable results, limitations may arise due to variations in the quality of sex indicators. Therefore, it is crucial to exercise caution during this process. Conversely, sex estimation in non-adult individuals is more challenging, primarily due to population differences in the pattern and degree of sexual dimorphism during ontogeny. Developing sample-specific methods can indeed be instrumental in addressing this issue. However, here we must take some cautions in order to consider age-related variability.

Regarding age-at death estimation in non-adult individuals, dental development offers high accuracy. However, when dentition is unavailable, selecting an appropriate age

estimation method requires careful consideration. In adult age estimation, problems related to misestimation appear to be resolved with TA, Nonetheless, understanding the performance improvement within the sample under study is necessary.

All of these issues related to biological sex and age-at-death estimations undoubtedly impacts the paleodemographic results, mainly affecting comparability of data. To ensure this comparability, estimations of sex and age derived from different methods should be compared. Comparing estimations across methods can also provide valuable insights into the accuracy and consistency of different approaches, contributing to a more comprehensive assessment of paleodemographic profiles and their interpretations. Ideally, this involves utilizing large samples that enable us to gain a clear understanding of the variability. Thus, the main objective of this paper is to conduct a comparative analysis of different methods commonly used for sex and age-at-death estimations within the extensive skeletal assemblage recovered from the Dominican Convent of San Pablo (Burgos, Spain). Once the most reliable estimations are established, we proceed to construct the paleodemographic profiles for the different populations buried in this Convent.

2 Materials and methods

The Convent of San Pablo was a Dominican convent located in Burgos (Spain) and founded in 1224 (ADÁN-ÁLVAREZ and CASILLAS 2005). During the Spanish War of Independence, French forces repurposed the convent into a military barracks and in 1835, during Mendizábal's Confiscation, friars were expelled, and the area of San Pablo saw diverse uses. In 1973 the convent was demolished. Between 2002 and 2003, an archaeological study leaded by the archaeologist Gema E. Adán Alvárez was conducted in conjunction with the construction of the Museum of Human Evolution. The burials of the Convent of San Pablo span a broad chronology, from the 13th century to the end of the 19th century (ADÁN-ÁLVAREZ 2003). These burials are distributed throughout three main areas: the Church, the Chapter House, and the Cloister area (Figure 1).

The most ancient burials, dating from the 13th century, are situated at the foundation base of the church and within the surveys conducted in the cloister corridors. During the Late Middle Ages burials began to take place inside the church and the cloister, where a vast accumulation of inhumations was found. These burial areas continued to be used until the 18th century. In the 16th century, burials started taking place in the Chapter House, and several crypts were constructed in the central aisle of the church. From the 19th century onwards, there are few documented burials due to the construction of modern cemeteries. The total number of individuals recovered from archaeological excavation in each of these areas, classified by chronology, is depicted in Table 1.

Due to the limited number of individuals recovered from the Cloister and the Church during both the 13th and 19th centuries, as well as in the Chapter House during the 16th and 18th centuries, we exclude them from the paleodemographic analysis.

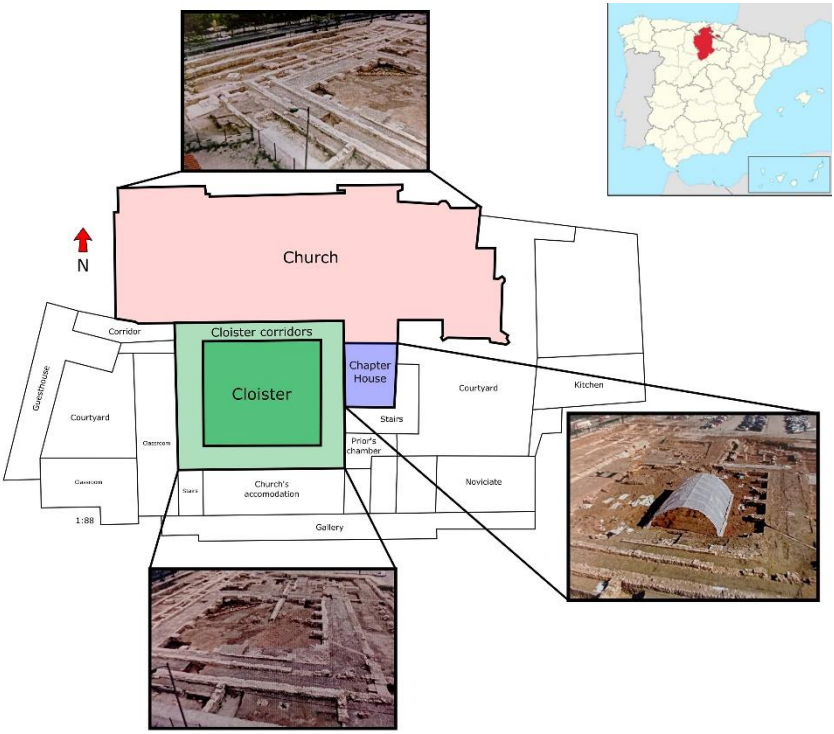


Figure 1. Map showing the three burial zones (Cloister, Church, and Chapter House) discovered during the excavations conducted in the Convent of San Pablo. Top-left: Map of Iberian Peninsula showing the location of Burgos.

Location	Chronology	Adults	Non adults	Total
Cloister	13th	5	3	8
	14th-18th	46	73	119
	19th	6	5	11
Church	13th	5	0	5
	14th-18th	185	39	224
	19th	0	6	6
Chapter house	16th-17th	12	0	12
	17th-18th	47	1	48
Total		306	125	**433**

Table 1: Number of individuals recovered from the archaeological excavation conducted at the Convent of San Pablo.

2.1 Sex and age at death estimation in adult individuals

As it was mentioned above, sex estimation in adult individuals proves more reliable than age-at-death estimation. Furthermore, all methods for estimating age at death hinge on a prior determination of the individual's sex. Hence, within this age group, we initially assessed the individual's sex and subsequently determined their age at death. We employed four distinct approaches for sex estimation: 1) considering pelvic shape differences, 2) skull sexual shape differences, 3) metric variances in the cranial and post-cranial skeleton, and 4) metric distinctions in cranial remains.

In the first approach for sex estimation, we focus on sexual pelvic shape differences. These shape differences were evaluated in the greater sciatic notch, the preauricular sulcus and the anterior portion of the pubis (Figure 2). The scoring system proposed by (BUIKSTRA and UBELAKER 1994) is used to assess the greater sciatic notch. This scoring system consists of five different stages, ranging from broader sciatic notches to narrower ones. The greater sciatic notch tends to be broad in females (scores of 1 and 2) and narrow (scores of 4 and 5) in males. A score of 3 should be considered with cautions, as it can be observable in both males and females. The preauricular sulcus, more common in females, is evaluated using the method suggested by (IGARASHI *et al*. 2020), with implications for indicating female sex and potentially pregnancy and parturition. In line with this method, we assessed the various types of preauricular sulcus to refine our sex estimations. The pubis is examined following Phenice's method (1969) (PHENICE 1969), focusing on the ventral arc, subpubic concavity, and medial aspect of the ischiopubic ramus as indicators of sex, with distinct characteristics in males and females. The ventral arc and the subpubic concavity are only present in females. The medial aspect of the ischiopubic ramus is broad in males and notable narrow, resembling a crest, in females.

Regarding sexual estimation from the skull, we considered that sexual differences in this skeletal region are associated with greater robustness and size in males compared to females. We used the scoring system for sexually dimorphic cranial features proposed by Buikstra and Ubelaker (1994), who provides five aspects of skull morphology that are useful in determining sex. These are: the nuchal crest, the mastoid process, the supra-orbital margin, the glabella, and the mental eminence (Figure 2). In all these cases, a five-point scale is used, with the more gracile, female features at the lower end of the range.

Each scorable pelvic or skull trait was independently seriated following the progression expression for the trait in question. After scoring these skeletal nonmetric traits, we estimated the sex based on each of them separately. Subsequently, the final sex estimation was derived by considering all the nonmetric traits together.

For the sex estimation based on the metric variances of the skull and post-cranial skeleton we used the statistical software solution Fordisc ®. This software enables an analysis of sex, ancestry, and body height through flexible biometric approaches based on the availability of different measurements from the skeletons (OUSLEY and JANTZ 1996). The reference~~s~~ database is based on more than 1200 cases from the American Forensic Data Bank and more than 1700 skeletons from the Terry and Hamann Todd Collection. The measurements included for this analysis are those defined by Buikstra and Ubelaker (1994).

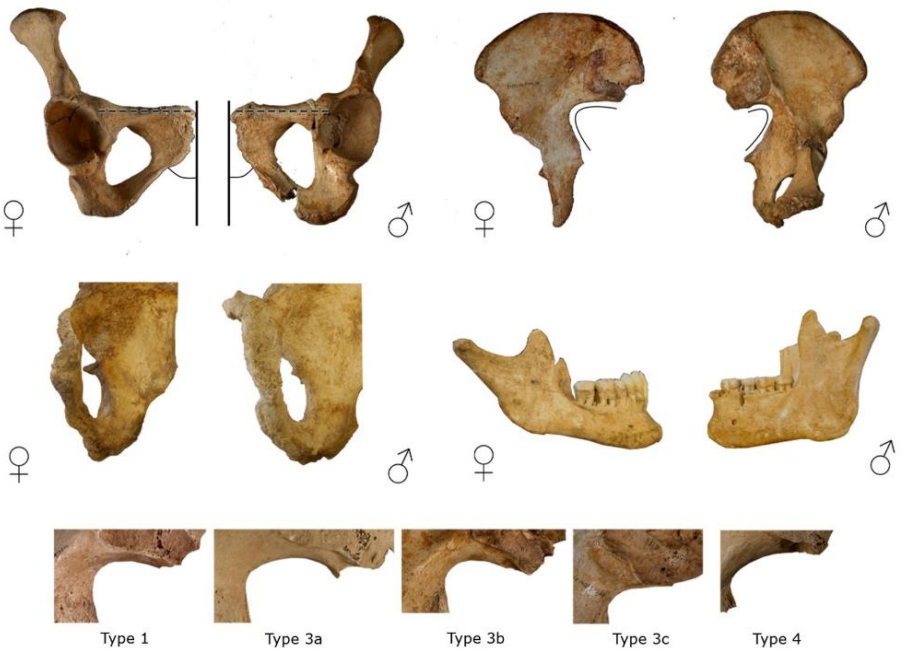


Figure 2. Some of the non-metric traits evaluated in the pelvic and the skull of adult individuals. Top left: subpubic angle, top right: greater sciatic notch, middle left: medial aspect, middle right; mental eminence, bottom: types of preauricular sulcus.

For the sex estimation based on the metric variances of the skull and post-cranial skeleton we used the statistical software solution Fordisc ®. This software enables an analysis of sex, ancestry, and body height through flexible biometric approaches based on the availability of different measurements from the skeletons (OUSLEY and JANTZ 1996). The references database is based on more than 1200 cases from the American Forensic Data Bank and more than 1700 skeletons from the Terry and Hamann Todd Collection. The measurements included for this analysis are those defined by Buikstra and Ubelaker (1994).

Regarding teeth, we employed an approach based on the sectioning point. To achieve this, we measured the bucco-lingual (BL) and mesio-distal (MD) diameters in all teeth. The adult overall mean of both measurements was uses as the discriminant criterion for determining sex.

The estimated sex derived from cranial and postcranial measurements, along with that obtained through the method of sectioning point for dental measurements, is compared to the estimated sex based on the morphological traits of the pelvis and cranium. Consistency was calculated as the percentage of correctly classified males and females using these approaches relative to the results obtained from sex determination based on pelvic and skull. Only measurements achieving an overall consistency level greater than 75% will be considered for application in other individuals.

Age at death in adult individuals was first estimated following the method proposed by Buckberry and Chamberlain (2002) (B&C) based on age-related changes of auricular surface. This method records age-related stages for different features of the auricular surface, which are then combined to provide a composite score from which an estimate of age is obtained. Subsequently, age-at-death was determined for adult individuals in the sample using TA3 software (OUSLEY *et al*. 2020). Although TA3 enables scoring traits from all the skeleton, we only scored those 19 variables related to the ilium and pubic bones, since methods based on this anatomical region have been reported to provide the highest accuracy. Consistent with the approach outlined by Ousley *et al*. (2020), ten variables were evaluated from the innominate, sacroiliac joint, and acetabulum, while four variables were considered from the auricular surface, and five variables were examined from the pubic symphysis. Bilateral traits were scored where applicable, resulting in a total possible score of 38 from a single skeleton. For the innominate and acetabulum, we recorded the iliac crest fusion stage and several traits describing the extent of exostosis, ossification, and lipping. In the case of the sacroiliac joint, we assessed the level of fusion with the sacrum. The auricular surface assessment involved recording four variables related to porosity and exostosis. Regarding the pubic symphysis, three out of the five components pertained to symphyseal face characteristics, while two referred to the ventral and dorsal margins along with immediately adjacent parts of the symphyseal surface. To test the extent to which TA3 improves the age- at death estimation obtained for Buckberry and Chamberlain (2002), we conducted a test in which the composite scores obtained from B&C's method were regressed against the mean age obtained from TA3 in a sub-sample of 132 individuals (60 females and 72 males) from the Cloister and the Church.

2.2 Sex and age at death estimation of non-adult individuals

Contrary to adults, where sex is more accurately estimated than age at death, in non-adults, age-at-death is more accurately estimated than sex. Moreover, methods for determining sex in non-adults based on nonmetric traits of the mandible and the ilium, rely on a prior age assessment. Thus, we first estimated age and subsequently determined sex. Age estimation was performed using three different approaches. For the first approach, we relied on dental development. To accomplish this, the specimens were scanned using a YXLON Compact (YXLON International X-Ray GmbH, Hamburg, Germany) industrial multislice computed tomography (CT) scanner, located at the University of Burgos. The mandibles were aligned along the long axis of the right mandible corpus. Scanner energy was set at 160 kV and 4 mA and the field of view was between 111.1 and 187.5 mm to encompass the entire mandibles. The Mimics™ (Materialize, Belgium) software program was used to visualize the CT images and make the virtual reconstructions. The mineralization stages of each tooth class were obtained by analysing the 3D volume renderings from these CT images and virtual reconstructions. The developmental stages of permanent dentition were scored using the method of (DEMIRJIAN *et al*. 1973) and were converted into age according to the adjusted data for prediction proposed by (LIVERSIDGE *et al*. 2006). The method developed by (LIVERSIDGE and MOLLESON 2004) was used for deciduous dentition.

For the second approach, we develop a sample-specific method based on the relationship between long bone lengths and age. To accomplish this, we construct a

regression of femoral length on estimated dental age in a sample of 34 dental aged individuals. We developed two models: inverse and classical calibration. In the inverse calibration model, the age is regressed on another variable (in this case, the femoral metaphyseal length) and thus, the age would be the dependent variable. However, it is the femoral metaphyseal length that depends on age, rather than the contrary. The alternative to inverse calibration is classical calibration. Based on this model, the femoral metaphyseal length (dependent) should be regressed on age (independent) followed by a solution for the age. While inverse calibration is preferred when the age distribution for the reference sample forms a reasonable prior, classical calibration is preferred if it is suspected that the estimated ages will be an extrapolation beyond the useful limits of the reference sample ages. We tested the performance of these two models calculating the error of the estimations relative to dental age.

In the third approach, we used the two methods developed by García-González *et al.* (2019) based on shape changes of the distal femoral epiphyses. For the first method, five maturity stages were coded based on morphological changes in the femoral distal epiphyses. A Bayesian statistical procedure was performed to produce a distribution of probabilities that an unknown individual belongs to a given age class based on the maturity it present. From these probability distributions, two different point estimates were calculated: the mode and the mean. The mode point estimate was calculated as the age with the highest posterior probability for each stage. The mean point estimate was calculated as these ages where the posterior probabilities were different from zero.

For the second method, the shape of the femoral distal epiphyses was quantified based on elliptical Fourier descriptors (EFDs). These EFDs were enter in a PCA and scores from the PC1 were regressed against age. Two different regression models were developed: inverse and classical regression (ICM and CCM respectively).

In order to assess which model performed better in our sample, we compared the dental ages to the ages predicted by the different approaches for age estimation derived from the femoral epiphyseal shape (mode point estimate, mean point estimate, ICM and CCM). The statistical treatment is the Bland-Altman method (BLAND and ALTMAN 1999). Following it, we have calculated the absolute mean difference and standard deviation of the differences between age estimates using dental development and shape of femoral distal epiphysis. These two parameters allow us to estimate the 95% limits of agreement (an interval within which 95% of the differences between ages estimated by the two compared methods are expected to lie). Based on this approach, the two methods are considered inter-changeable when the absolute mean difference between them is not significantly different from zero and the limits of agreement are narrow enough. We used linear regression (differences between age estimates were regressed on the average of the estimates of the two methods) to test the null hypothesis that the mean differences between the two methods did not significantly differ from zero. If the slope differed significantly from zero, the null hypothesis should be rejected.

Regarding sex estimation in nonadult individuals, we performed two different approaches. The first of them is based on the non-metric sex-related traits for mandible and

ilium previously proposed by Weaver (1980), Schutkowski (1993), Molleson *et al.* (1998) and Loth and Henneberg (2001) (Table 2) (Figure 3). Each scoreable skeletal trait was independently seriated in order of progressive expression for the trait in question (*i.e.*, from the more female-like individual to the more male-like). After scoring of these skeletal nonmetric traits, we estimate the sex of individuals based on two criteria. Firstly, we assign a possible sex by considering the association of these nonmetric traits with each of the two biological sexes (Table 2). The final assignment to one sex was made based on the results obtained by García-González *et al*. (2021). These authors tested all these nonmetric traits in the Lisbon identified skeletal collection housed at the Bocage Museum (Lisbon, Portugal). They calculated the weighted probability of being a male or a female when a particular nonmetric is observed in the total sample, as well as within each of the four age groups in which they broke down the total sample.

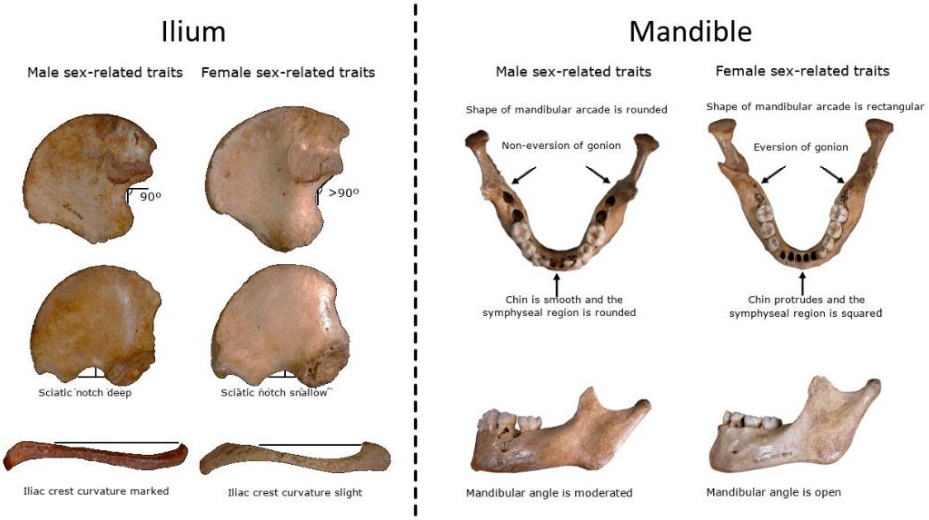


Figure 3. Non-metric traits evaluated in the ilium (left) and mandible (right) of non-adult individuals. Ilium. Top: angle of greater sciatic notch and arch criterion, middle: deep of the greater sciatic notch, bottom: S-shape of iliac crest. Mandible. Top: Eversion of gonion and shape of symphyseal region. Bottom: Mandibular angle.

Trait	Associated expression		Weighted probabilities for age group									
			0-1		2-5		6-10		>11		Overall	
	Females (F)	Males (M)	F	M	F	M	F	M	F	M	F	M
Auricular elevation[1]	Elevated	Flat	48	44	53	52	62	65	…	…	45	55
Sciatic notch angle[2]	>90º	≈ 90º	56	100	51	57	44	56	…	…	59	71
Depth of sciatic notch[2]	Shallow	Deep	56	100	43	0	71	56	…	…	55	59
Arc criteria[2]	Crosses	Bordering	56	100	43	31	44	56	…	…	52	55
Iliac crest curvature[2]	Marked	Slight	100	67	45	0	…	…	79	76	68	70
Mandibular protrusion[2]	Smooth	Protrudes	100	67	80	80	76	79	71	69	79	75
Mandibular arcade shape[2]	Rounded	Rectangular	100	100	71	100	62	71	63	71	71	85
Gonial eversion[2]	Absent	Present	50	0	67	75	67	100	58	100	57	80
Mandibular angle[3]	Moderated	Open	100	100	63	100	62	71	60	100	66	93
Mentum (outline)[3]	Smooth	Squared	0	50	82	89	62	71	73	86	75	79
Symphyseal shape[4]	Rounded	Squared	100	100	80	80	71	65	63	60	75	71

[1] proposed by Weaver (1980), [2] proposed by Schutkowski (1993), [3] proposed by Molleson *et al*. (1998) and [4] proposed by Loth and Henneberg (2001).

Table 2: Nonmetric sex-related traits of the ilium and mandible evaluated in this study along with the weighted probabilities of being a male or a female when a particular trait is observed derived from García-González et *al*. (2021)

For the second approach we considered sexual differences in long bone breadths. We developed our sample-specific model for sex estimation from femoral measurements. This methodological approach involves several steps. First, to validate the performance of our model, we developed it using a known-sex and known-age sample from the Luis Lopes collection, housed in the National Museum of Natural History in Lisbon, Portugal. The study sample includes 78 individuals, with 39 females and 39 males, ranging in age from birth up to 16 years old. We constructed relative growth trajectories of femoral proximal breadth (FPB), femoral midshaft breadth (FMSB), and femoral distal breadth (FDB) relative to femoral diaphyseal length (FDL) using log-transformed variables and Major Axis Regression (MAR). Given the strong correlation between femoral age and chronological age, we utilize femoral age as a surrogate age. Consequently, residuals from the three distinct regressions represent variations that are not accounted for by chronological age. As a result, we attribute this residual variability to sex-related factors. Residuals from these lines were used for SSM, with positive residuals indicating males and negative residuals indicating females. Sex differences were assessed using t-test on residuals. Accuracies were calculated as rate of success, and the residuals achieving the highest accuracy were selected for the subsequent step. In this way, we can detect which residuals are the most useful for sex estimation. The next step was to statistically compare relative growth trajectories between the Lisbon and SP samples. To do that, we tested if these fitted lines have a common slope and/or intercept following the method developed by Warton *et al.* (2006). If we do no detect differences between these relative growth trajectories, we can use the MAR developed for the Lisbon collection to calculate the residuals of San Pablo's sample. However, if significant differences are detected between the growth trajectories, then we should calculate the residuals for the San Pablo sample using its own regression model.

2.3 Paleodemographic profile

Once reliable estimates of sex and age-at-death were sought for as many individuals as possible, we proceeded to construct a distribution of sex and age at death for the populations buried in the Cloister, Church, and the Chapter House. Traditionally, paleodemographic studies divided the total population into 5-year age groups (ACSÁDI *et al*. 1970). However, here we follow a different approach for non-adult individuals. These individuals were divided into four groups following (BOGIN 1999). This author divided the growth period in infancy, childhood, juvenility, and adolescence. The infancy lasts the first years of human post-natal life. It is a period of rapid growth rate and during which the infants transited from a reliance on breast-feeding to the consumption of suitable weaning foods. Consequently, during this growth phase mortality and morbidity are closely related to an exposure to disease due to lack of maternal antibodies and/or heightens the risk of undernutrition resulting from inappropriate weaning foods. Childhood encompasses the ages about three to seven years. During this phase, individuals possess a relatively large, metabolically expensive brain (LEONARD *et al*. 2007). Although a great deal of the high energetic cost of human brain development is offset by slow and protracted corporal growth (KUZAWA *et al*. 2014), children still necessitate a nutrient-dense diet for proper brain growth. However, the skills necessary to provide such a diet for themselves are not yet developed at this stage, relying on the adults. Therefore, mortality and morbidity during this

growth stage may be interpreted as reflections of variations among populations in access to vital resources, different levels of parental care, nutrition, and health care. Juvenility is a period that typically spans from ages seven to ten for females, or until age 12 for boys characterized by important body composition changes, with one of the most notable being the 'adiposity rebound'. Brain and somatic tapper off during juvenility. Consequently, the energy allocation that was formerly associated with brain and somatic growth is temporarily stored as abdominal fat. This is crucial to support the energetically costly accelerating growth during the upcoming adolescence (BOGIN 1999). During this growth stage, individuals begin to forage much of their own food but also start competing with adults for food and space. Adolescence is the period beginning with puberty, during which growth spurt occurs and dimorphic secondary sexual characteristics start to develop. Given that individuals can meet the energetic requirements for their growth, we assume that mortality and morbidity during both juvenility and adolescence are related to the sociocultural environment and exposure to disease.

Adult individuals were classified as young adults (20-39 years), middle-aged adults (40-59), old adults (60-70) and senescent (>70 years).

3 Results

3.1 Sex estimation in adult individuals

Table 3 depicts the number of individuals available for sex estimation for each of the nonmetric traits of pelvic and skull. The greater sciatic notch is the trait most frequently assessed, while the glabella and supraorbital margin are the least assessed in the three burial areas.

Trait	Cloister 14^{th}-18^{th} cent.	Church 14^{th}-18^{th} cent.	Chapter house 17^{th}-18^{th} cent.	Total
Sciatic notch	70,4 (38/54)	70,3 (130/185)	68,1 (32/47)	69,9 (200/286)
Preauricular sulcus	68,5 (37/54)	68,6 (127/185)	68,1 (32/47)	68,5 (196/286)
Ventral arc	42,6 (23/54)	38,9 (72/185)	38,3 (18/47)	39,5 (113/286)
Subpubic concavity	31,5 (17/54)	20,0 (37/185)	38,3 (18/47)	38,7 (72/186)
Medial aspect	38,9 (21/54)	0	34,0 (16/47)	19,9 (37/186)
Nuchal crest	42,6 (23/54)	19,5 (36/185)	59,6 (28/47)	46,8 (87/186)
Mastoid process	40,7 (22/54)	49,7 (92/185)	55,3 (26/47)	75,3 (140/186)
Supra-orbital margin	22,2 (12/54)	25,4 (47/185)	42,6 (20/47)	42,5 (79/186)
Glabella	22,2 (12/54)	22,7 (42/185)	40,4(19/47)	39,5 (73/186)
Mental eminence	51,9 (28/54)	48,1 (89/185)	59,6 (28/47)	77,9 (145/186)

Table 3: Percentage the individuals in which each of the nonmetric traits for the pelvic and the skull regions can be assessed.

The Cloister more frequently exhibit a score of 3 for the greater sciatic notch, whereas a score of 4 is most commonly found in the Church and the Chapter House (Figure 4). In

contrast, a score of 1, representing the broader greater sciatic notch, is the less represented in the Cloister, Church, and Chapter House. The number of individuals with a score of five is higher in the Chapter House than in both the Cloister and the Church. Additionally, a score of 2 is more prevalent in the Cloister than in the other two burial locations.

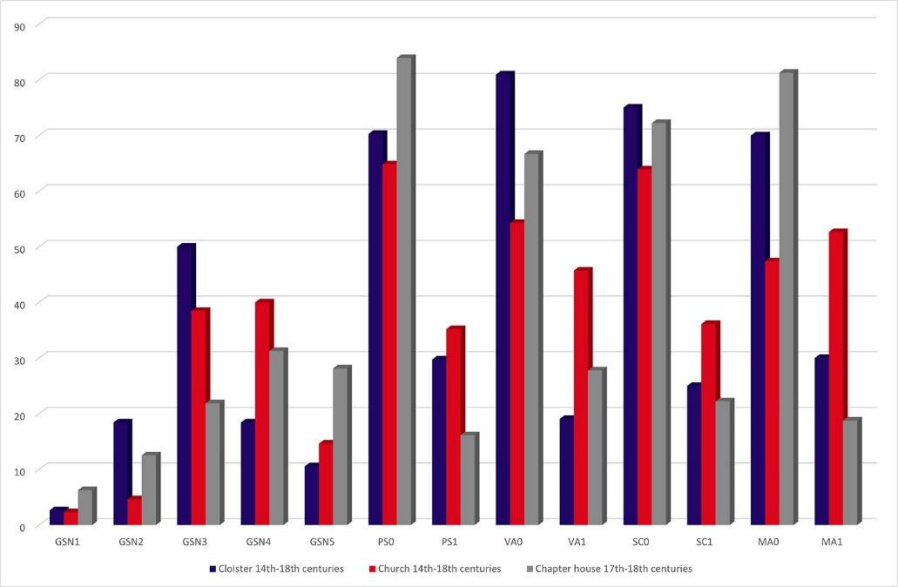


Figure 4. Distribution of the grades of the pelvic non-metric traits assessed in individuals from the Cloister, Church, and Chapter House. Blue: Cloister, Red: Church. Gray: Chapter House. GSN: greater sciatic notch, VA: ventral arc, SC: subpubic concavity, MA: medial aspect of the ischiopubic ramus, PS: preauricular sulcus. In the GSN a five-point scale is used with broad SCN at the lower end of the range and narrow at the upper end of the range. In VA, SC, MA, and PS only absence (0) and presence (1) was assessed.

In the case of the other pelvic nonmetric traits (preauricular sulcus, ventral arch, subpubic concavity, and medial aspect of the ischiopubic ramus) the state "absent" is more frequently assessed than the state "present" in all three burial zones (Figure 4). When these traits are absent, the Cloister and the Chapter House exhibit the highest frequencies. Specifically, in the Cloister, the highest incidence of absence is noted in the ventral arch and subpubic concavity, whereas in the Chapter House, it is observed in the absence of the preauricular sulcus and medial ramus. Conversely, where these traits are present, the Church show higher frequencies compared to the Cloister and the Chapter House (Figure 4).

Among individuals with preauricular sulcus, 30% and 12% in the Cloister and the Church, respectively, depict the type 4 (Figure 1), which is associated with females experienced childbirth.

Concerning the nonmetric traits of the skull, the lowest scores for all of them are more frequently found in the Cloister and the Church, while the highest ones are observed in the

Chapter House (Figure 5). A score of 1 is more frequently observed in all traits in the Church, except for the supraorbital margin and mental eminence, which show higher frequencies in the Cloister. Conversely, a score of five is most commonly found in the Chapter House, except for the supraorbital margin, where the highest frequencies are observed in the Cloister (Figure 5).

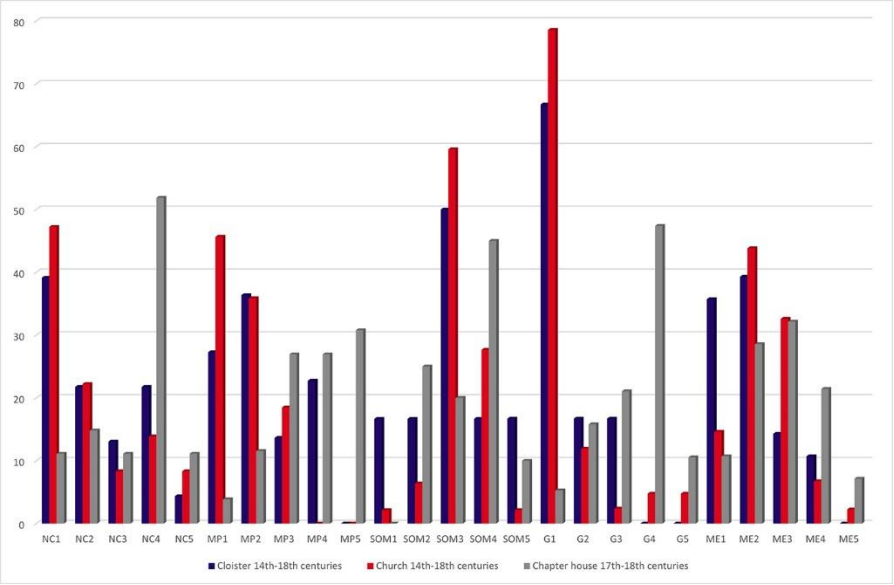


Figure 5. Distribution of the grades of the skull non-metric traits assessed in individuals from the Cloister, Church, and Chapter House. Blue: Cloister, Red: Church. Gray: Chapter House. NC: nuchal crest, MP: mastoid process, SOM: supra-orbital margin, G: glabella, ME: mental eminence (ME). In all of them, a five-point scale is used, with the more gracile features at the lower end of the range and the more robust at the upper end of the range.

Based on the scores for each nonmetric trait observed in the skull and pelvis, sex could be estimated for 78.72%, 77.66% and 76.0% of individuals from the Cloister, Church, and Chapter House, respectively. Among individuals from the Cloister where sex could be estimated, 67.60% are classified as male and 37.80% as female. In the Church, males constitute 51.37% of sexed individuals, while females make up the remaining 48.63%. Notably, in the Chapter House, all individuals in whom sex could be estimated are classified as male. Consequently, the sex estimation based on the metric methods was exclusively conducted on individuals from the Cloister and the Church.

Sex could be estimated from skull metrics in 68.52% and 93.51% of individuals from the Cloister and the Church, respectively. For postcranial measurements, these values increase to 92.6% and 98.38% for the same burial locations. Table 4 illustrates the consistency percentages between sex estimates derived from nonmetric traits of the pelvis and the skull, and those obtained from cranial and postcranial measurements using the

Fordisc software. These consistency percentages are higher when sex is estimated from postcranial measurements compared to estimates derived from cranial ones. Furthermore, whether considering both sexes together or separately, these percentages surpass 75%. Thus, we estimated the sex based on postcranial measurements using Fordisc software for individuals in whom we could not estimate this parameter from nonmetric traits. This was applicable to four individuals from the Cloister and six from the Church. Considering these newly sexed individuals, the percentage of individuals with sex estimation is 88.9% for the Cloister and 80.54% for the Church.

Burial place	Morphological sex	Sex classification using Fordisc	
		Cranial measurements	Postcranial measurements
Cloister	**Female**	60,0%	100%
	Male	80,0%	86,4%
	Total	72,0%	75,4%
Church	**Female**	84,9%	94,5%
	Male	50,0%	79,7%
	Total	68,3%	82,0%

Table 4: Consistency percentages between sex estimation from nonmetric traits of the skull and pelvis and estimated from cranial and postcranial measurements using Fordisc software.

Regarding sex estimation from dental metrics, the overall mean of each tooth was employed as the discriminant criterion for determining sex in the adult sample. These measurements were combined for both the Cloister and the Church. Table 5 provides descriptive statistics for each tooth measurement, considering both sexes together and separately for each sex established based on nonmetric traits. Table 5 also incorporates the consistency percentages between sex estimates derived from nonmetric traits of the pelvis and the skull and those obtained for the sample-specific method. The consistency percentages do not reach an acceptable level for sexing adult skeletons, neither for individual teeth nor dental dimensions. This holds true when considering both sexes together and separately. The combinations of measurements from the same and different teeth do not significantly increase the consistency percentages. Our findings depicted that the highest consistency percentages (72%) were those obtained using a combination of mesio-distal diameter of the lower second premolar and bucco-lingual diameter of the upper second molar. Considering that this consistency percentage is lower than 75%, we opted not to conduct additional sex estimations based on dental measurements.

Tooth	Vble	Females			Males			Total			Consistency percentage		
		N	Mean ± SD	CV	N	Mean ± SD	CV	N	Mean ± SD	CV	F	M	T
UI1	BL	30	6,74 ± 0,47	6,96	35	7,05 ± 0,44	6,32	65	6,90 ± 0,48	6,93	63,3	65,7	64,6
	MD	30	8,01± 0,79	9,89	35	8,09 ± 0,67	8,33	65	8,05 ± 0,09	9,01	43,3	68,6	59,2
UI2	BL	28	6,01 ± 0,54	9,07	27	6,40 ± 0,61	9,55	55	6,20± 0,61	9,78	64,5	59,5	61,8
	MD	28	6,28 ± 0,55	8,82	27	6,38 ± 0,57	8,98	55	6,33 ± 0,56	8,85	61,3	37,8	48,5
UC	BL	32	7,65 ± 0,57	7,47	16	7,74 ± 0,51	6,60	48	7,68 ± 0,55	7,14	43,7	62,5	50,0
	MD	32	7,11 ± 0,48	6,70	16	7,21 ± 0,55	7,69	48	7,15 ± 0,50	7,00	56,2	37,5	50,0
UP3	BL	37	8,31 ± 0,67	8,04	45	8,63 ± 0,45	5,18	82	8,49 ± 0,58	6,81	51,4	62,2	57,3
	MD	37	6,39 ± 0,47	7,34	45	6,47 ± 0,48	7,44	82	6,43 ± 0,47	7,38	59,5	55,6	57,3
UP4	BL	40	8,55 ± 0,51	6,02	38	8,83 ± 0,53	6,05	78	8,68 ± 0,54	6,22	55,0	57,9	56,4
	MD	40	6,19 ± 0,45	7,22	38	6,44 ± 0,46	7,13	78	6,31 ± 0,47	7,40	67,5	52,6	60,3
UM1	BL	20	10,62 ± 0,63	5,94	23	10,77 ± 0,64	5,96	43	10,70 ± 0,63	5,92	45,0	65,0	51,2
	MD	20	9,70 ± 0,61	6,35	23	9,90 ± 0,60	6,08	43	9,81 ± 0,61	6,22	55,0	85,0	65,1
UM2	BL	30	10,50 ± 0,55	5,26	34	10,95 ± 0,74	6,76	64	10,74 ± 0,69	6,44	63,3	64,7	64,1
	MD	30	9,08 ± 0,54	5,96	34	9,03 ± 8,85	8,23	64	9,06 ± 0,65	7,20	50,0	47,1	48,4
LI1	BL	39	5,72 ± 0,51	8,87	43	5,89 ± 0,41	6,96	82	5,81 ± 0,46	7,97	46,2	53,5	50,0
	MD	39	5,05 ± 0,72	14,21	43	4,96 ± 0,54	10,85	82	5,00 ± 0,63	12,54	59,0	58,1	58,5
LI2	BL	31	5,96 ± 0,58	9,72	37	6,34 ± 0,59	9,38	68	6,17 ± 0,61	9,95	62,5	48,9	55,9
	MD	31	6,22 ± 0,56	8,98	37	6,25 ± 0,67	10,72	68	6,24 ± 0,62	9,90	97,9	11,1	55,9
LC	BL	41	6,91 ± 0,39	5,66	17	6,97 ± 0,39	5,62	58	6,93 ± 0,39	5,61	33,9	85,7	58,1
	MD	41	6,08 ± 0,33	5,50	17	6,20 ± 0,32	5,23	58	6,12 ± 0,33	5,44	46,4	83,7	63,8

Tooth	Vble	Females			Males			Total			Consistency percentage		
		N	Mean ± SD	CV	N	Mean ± SD	CV	N	Mean ± SD	CV	F	M	T
LP3	BL	50	7,07 ± 0,46	6,51	52	7,45 ± 0,54	7,23	102	7,26 ± 0,53	7,36	64,0	57,7	60,8
	MD	50	6,49 ± 0,43	6,66	52	6,64 ± 0,41	6,20	102	6,56 ± 0,43	6,50	48,0	59,6	53,9
LP4	BL	46	7,69 ± 0,08	7,48	48	7,90 ± 0,52	6,61	94	7,80 ± 0,55	7,13	50,0	62,5	56,4
	MD	46	6,71 ± 0,47	6,98	48	6,69 ± 0,43	6,39	94	6,70 ± 0,44	6,67	45,7	50,0	47,9
LM1	BL	35	10,02 ± 0,47	4,72	38	10,18 ± 0,50	4,93	73	10,10 ± 0,49	4,87	48,6	65,8	57,5
	MD	35	10,53 ± 0,59	5,60	38	10,62 ± 0,12	6,74	73	10,58 ± 0,65	6,19	57,1	52,6	54,8
LM2	BL	39	9,53 ± 0,47	9,52	47	9,80 ± 0,48	4,88	86	9,68 ± 0,49	5,09	59,0	59,6	59,3
	MD	39	10,19 ± 0,56	5,45	47	10,21 ± 0,58	5,73	86	10,20 ± 0,57	5,58	51,3	53,2	52,3

U: Upper, L: Lower: I1: Central incisor, I2: Lateral incisor C: Canine, P3: first premolar, P4; second premolar, M1; first molar, M2: second molar, BL: Bucco-lingual diameter, MD: Mesio-distal diameter. SD: Standard deviation, CV: Coefficient of variation. Vble: Variable, F: Females, M: Males, T: Total sample.

Table 5: Summary statistics for each of dental variables measured in the dentition, including the percentage of consistency with sex estimations derived from morphological traits of the pelvic and skull.

3.2 Age estimation in adult individuals

In Figure 6, the relationship between the sum of scores following B&C's method and the mean age estimated by TA3 is shown. The observed pattern suggests that B&C's method underestimates age up to around 60 years and overestimates it thereafter. Consequently, this discrepancy notably affects the age estimation of older individuals.

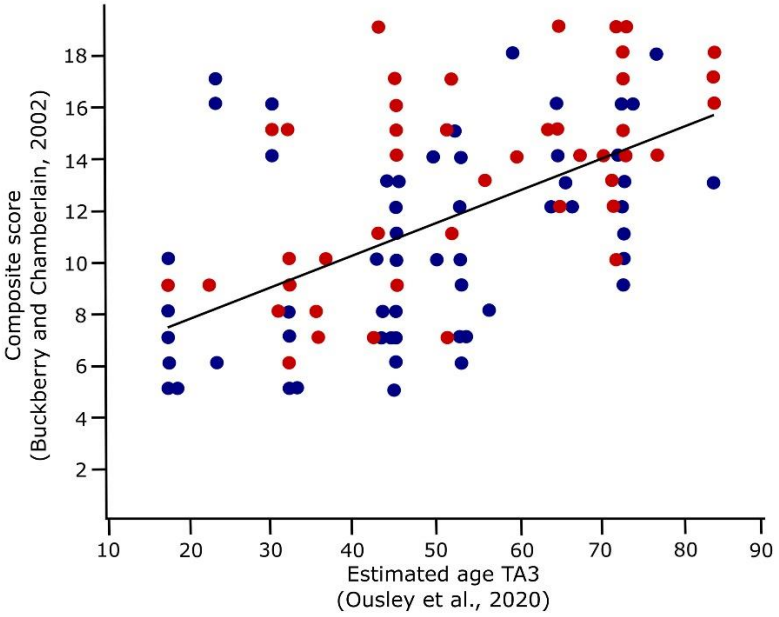


Figure 6. Comparison between composite scores from BUCKBERRY and CHAMBERLAIN (2002) and ages-at-death derived from TA3. Blue circles: males, Red circles: females.

We estimated age-at-death using Transition Analysis in a total of 212 individuals: 41 from the Cloister (comprising 89.1% of the total adult sample) 133 from the Church (constituting 71.9% of the total adult sample), and 38 from the Chapter House (representing 80.8% of the total adult sample). When separating these individuals by sex, we found 24 males and 15 females in the Cloister, and 60 males and 53 females in the Church. Additionally, 2 individuals in the Cloister and 20 individuals in the Church could not have their sex estimated. In the Chapter House, the number of individuals for whom sex estimation was not possible is 3. However, as we mentioned above, all three can be considered as males. The most notable result of the age-at-death estimations is the identification of individuals aged over 60 at the time of death. In the Cloister, we detected 8 individuals older than this age (7 males and 1 female). In the Church, this number is 22 (10 males, 11 females and 1 with undetermined sex). In the Chapter House, the count of individuals older than 60 years is 18.

3.3 Age estimation in nonadult individuals

Age-at-death from dental development was assessed in 50 non-adult individuals from the Cloister and 22 from the Church. To evaluate the accuracy of age estimation from femoral length, we compared the performance of two models (inverse and classical calibration) by calculating the error relative to dental age.

The inverse calibration model is:

Age = 4.64+ 0.04xFemoral diaphyseal length (R^2=0.78, Error: 1.56)

The classical calibration model is:

Age = (Femoral diaphyseal length-133.05)/17.34 (R^2=0.78, Error= 1.23)

In both models age is in years and Femoral diaphyseal length in mm.

The classical calibration model exhibited the lowest overall error, leading to employ this model for predicting age based on femoral length. Consequently, we estimated age from length in 12 individuals from the Cloister and 4 from the Church. By considering both dental-aged and femoral-aged individuals, we were able to estimate age at death in 84.9% and 71.8% of nonadult individuals from the Cloister and the Church, respectively.

The following step to try estimate age in the rest of the individuals was to apply the method proposed by García-González *et al*. (2019). Previously to do that, we performed an analysis of the performance of this method in our sample. Table 6 depicts the agreement between the two models based on femoral distal shape and those with dental age estimation in nonadult individuals from the Cloister and the Church. Comparisons between shape models proposed by García-González *et al*. (2019) and those using dental development show that the mean differences between them significantly differ from zero. Moreover, the limits of agreement are extremely wide. As a result, ages estimated from femoral distal epiphyses do not align with dental age estimates. In our sample, the ages estimated from femoral distal epiphyses function more as an indicator of an individual's maturity rather than an accurate age estimation. Consequently, some cautions are needed when this method is applied for age estimation.

Model	**Differences**			**Slope**	
	Mean	**SD**	**Limits of agreement**	**Coeff.**	**Probability**
Mode	1.82	1.10	(-0.33,3.98)	-0.10	0.16
Mean	1.47	0.87	(-0.24,3.17)	-0.28	0.00
ICM	1.15	0.81	(-0.45, 2.75)	-0.21	0.00
CCM	1.92	1.26	(-0.55, 4.40)	-0.45	0.00

Mode: age estimates based on mode point estimates derived from the probabilistic approach. Mean: age estimates based on weighted mean point estimates derived from the probabilistic approach. ICM: age estimates based on the inverse calibration model. CCM: age estimates based on classical calibration model.

Table 6: Agreement between the models proposed by García-González *et al.* (2019) based on femoral distal epiphysis shape and those with dental age estimation in San Pablo.

3.4 Sex estimation in nonadult individuals

The sample available for sexing non-adult adults based on non-metric traits of ilium and mandible consist of 38 ilia and 35 mandibles. Both in the Cloister and the Church, individuals exhibit an elevated auricula more frequently, along with a shallow sciatic notch with an angle higher than 90º. Additionally, the curved line that extends ventrally from the lateral rim of the auricula surface do not overlap with the curved line that extends dorsally from the anterior rim of the sciatic notch (the arch criterion). In the case of mandibles, the female morphology is also more frequent. The exception are the mandibular protrusion and arcade shape and the symphyseal shape.

Considering the weighted probabilities of being male or a female when a particular trait is observed for each age group, as provided in Table 2, we have assigned sex in 35 non-adult individuals (comprising 64.8% of total sample). Of these, 18 individuals (33.3% of total sample) have been assigned to the male sex category, while 17 individuals (31.5% of total sample) have been assigned to female sex category.

Regarding the SSM based on femoral long bone dimensions, in Table 7 are depicted the values for the slope, y-intercept and R^2 for Major Axis regressions in Portuguese and San Pablo sample. T-test between residuals from the Portuguese regressions show that sexual differences are statistically significant. Consequently, they are suitable to perform a sexual determination.

Variables	Population	Intercept	Slope	R^2
FVDP versus FDL	Portuguese	-0.33 (-0.42, -0.24)	0.73 (0.69, 0.77)	0.94
	San Pablo	-0.62 (-0.75, -0.49)	0.85 (0.80, 0.91)	0.94
FMSB versus FDL	Portuguese	-0.55 (-0.66, -0.44)	0.73 (0.68, 0.78)	0.94
	San Pablo	-0.47 (-0.59, -0.32)	0.70 (0.64, 0.75)	0.89
FDB versus FDL	Portuguese	-0.07 (-0.19, 0.04)	0.74 (0.69, 0.79)	0.94
	San Pablo	0.11 (0.00, 0.21)	0.66 (0.62, 0.72)	0.93

FPB: femoral proximal breadth, FDL: femoral diaphyseal length, FMSD: femoral midshaft breadth, FDB; femoral distal breadth. Values between parenthesis for intercept and slope represent the 95% confidence interval for these parameters.

Table 7: Values for slope, y-intercept, and R2 for Major Axis regressions in Portuguese and San Pablo samples.

In Table 8 are depicted the accuracies of sex estimation, calculated as rate of success, for each age group and the total sample in the Lisbon collection. Males consistently had higher sex estimation accuracies when all sample is considered. This holds true when the different age groups are considered, except for 4-9 years group, where females achieved an accuracy of 100%. While accuracies for FMSB and FDB from 9 years achieved the minimum acceptable level for sexing non-adult skeletons in forensic contexts, this level of accuracy was reached only for males in the first age group. Nonetheless, these accuracies are equivalent or even higher than those obtained using non-metric traits of ilium and mandible in similar age groups of the same collection (GARCÍA-GONZÁLEZ *et al*. 2021). Therefore, we

considered that this sample specific method is appropriate for sexing non-adult skeletons using the residuals from FMSB and FDB, overall, in individuals aged over 9 years. The statistically comparisons between relative growth trajectories between Portuguese and San Pablo sample show differences in the slope. Thus, for sexing non-adult skeletons from San Pablo we use the residuals extracted from these own regressions. In this way, we estimated the sex in 35 non-adult individuals. We assigned a sex to an individual only when the two different regressions estimate the same sex. Of these, 20 individuals have been assigned to the male sex category, while 23 have been assigned to female sex category.

Age group	Sex	FPB	FMSB	FDB
0-2 years	F	16.7 (1/6)	57.1 (4/7)	71.4 (5/7)
	M	85.7 (6/7)	85.7 (6/7)	71.4 (5/7)
	F+M	53.8 (7/13)	71.4 (10/14)	71.4 (10/14)
2-4 years	F	66.7 (4/6)	16.7 (1/6)	50.0 (3/6)
	M	50.0 (4/8)	50.0 (4/8)	85.6 (6/7)
	F+M	57.1 (8/14)	35.7 (5/14)	69.2 (9/13)
4-9 years	F	71.4 (5/7)	**100 (4/4)**	**75.0 (3/4)**
	M	66.7 (8/12)	62.0 (5/8)	**87.5 (7/8)**
	F+M	68.4 (13/19)	**75.0 (9/12)**	**83.3 (10/12)**
9-15 years	F	60.0 (12/20)	**75.0 (6/8)**	**75.0 (6/8)**
	M	58.3 (7/12)	**75.0 (6/8)**	50.0 (4/8)
	F+M	59.4 (19/32)	**75.0 (12/16)**	**62.5 (10/16)**
Total	F	57.5 (22/39)	57.7 (15/25)	69.2 (17/25)
	M	65.0 (25/39)	78.1 (21/31)	71.0 (22/30)
	F+M	61.0 (47/78)	69.0 (36/56)	70.2 (39/55)

F: females, M: males, FPB: femoral proximal breadth residuals, FMSD: femoral midshaft breadth residuals, FDB; femoral distal breadth residuals. In bold are marked these accuracies which attain the minimum acceptable for sexing non-adult skeletons.

Table 8: Total, female, and male accuracies for residuals from each growth trajectory separated for age groups.

Interestingly, comparing those individuals in which we could estimate sex based on non-metric traits of the ilium and mandible and the femoral relative dimensions, we obtained a 100% of agreement.

3.5 Paleodemographic profile of San Pablo sample

Based on all the aforementioned results, we constructed the paleodemographic profile for the three different burial zones using individuals for whom age could be estimated (Figure 7). Age estimation was possible for 103 individuals from the Cloister, 164 from the Church, and 30 from the Chapter House. Sex could not be estimated for 28 aged individuals from the Cloister and 34 from the Church. Among the youngest age group (0-3 years) the Cloister stands out with the highest number of individuals, primarily males, indicating a notable presence in this age group. Similarly, in the 3-7 age range, the Cloister maintains its

prominence, but in this case, showcasing a higher number of females compared to males. The prominence of males from the Cloister is maintained in the 7-12 age group. As the age brackets progress, there's a noticeable shift in distribution. Notably, the Church exhibits a significant presence in the 20-39 and 40-59 age categories, showing a balanced distribution between males and females. Given the distinct characteristics of the burials in the Chapter House, this area demonstrates a distinct trend, notably harboring individuals aged over 70 years.

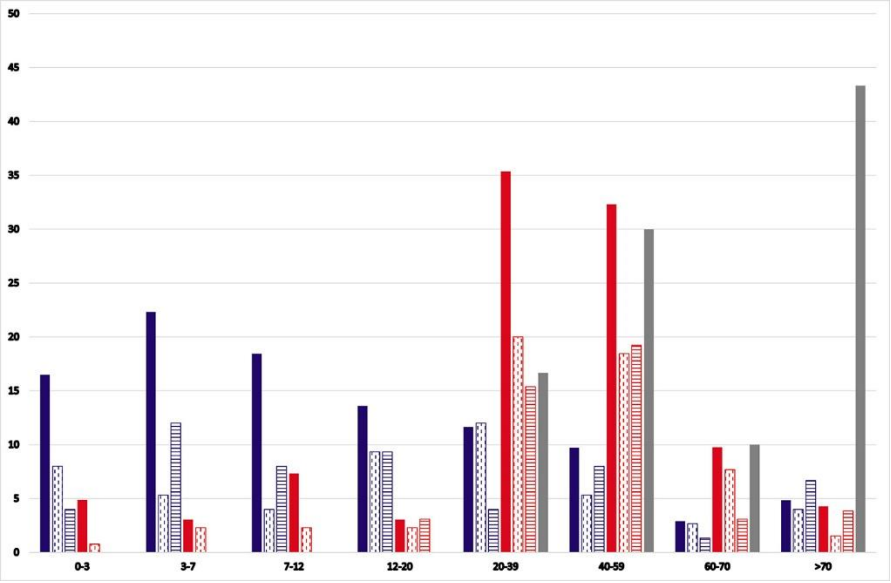


Figure 7. Paleodemographic profiles of the Cloister, Church, and Chapter House of the Convent of San Pablo. Solid blue: Cloister pooled-sex, Solid red: Church pooled sex. Solid gray: Chapter House males, Vertical blue tripes: Cloister males, Horizontal blue stripes: Cloister females, Vertical red stripes: Church males, Horizontal red stripes: Church females.

4 Discussion

In this study, we conducted a comprehensive analysis of sex and age-at-death estimations derived from different indicators in the large skeletal collection buried in the Cloister (14th-18th centuries), Church (14th-18th centuries) and Chapter House (17th-18th centuries) of the Convent of San Pablo. This allowed us to compare the different estimations for each of two parameters, obtaining reliable paleodemographic profiles.

In adults, sex estimation has primarily relied on pelvic and skull non-metric traits. The sciatic notch is the most frequently assessed pelvic trait (Table 3). Our observations revealed a high prevalence of narrow sciatic notches in the Church and the Chapter House, while an intermediate morphology is more common in the Cloister (Figure 4). Conversely, a wide sciatic notch is more prevalent among individuals in the Cloister. Since the female sciatic

notch tends to be wider than that of males, we could reliably assign male sex to more individuals from the Church and the Chapter House, and female sex to those from the Cloister. However, challenges emerged in sex estimation for individuals with an intermediate morphology of the sciatic notch. In such cases, we focused on assessing the ventral arc, subpubic concavity, and medial aspect of the ischiopubic ramus. This approach allowed us to assign male or female sex in instances where the sciatic notch morphology was intermediate.

In the case of non-metric traits of skull, they are more frequently assessed in the Chapter House than in the Cloister and the Church. Moreover, scores for each of these traits varied across the burial locations. For instance, a score of 4 for nuchal crest was more prevalent in the Chapter House (51.85%), while scores of 1 and 2 were more common in the Cloister and Church. Mastoid process scores varied too, with low scores more frequent in the Church, and intermediate one predominant in the Chapter House. The distribution of supra-orbital margin scores showed variability, with scores of three more common in the Church and Chapter House. Glabella scores differed as well, with a score of 1 being the most common overall and score of four notably higher in the Chapter House. Mental eminence scores also displayed differences, with a score of 2 more prevalent in the Church and Chapter House. Thus, scores related to male sex are more common among individuals from the Chapter House, which allow to sex males in this burial place reliably. Given that the Chapter House served as the designated burial site for monks, and every individual for whom sex could be estimated was classified as male, we can infer that all individuals belonged to this sex. In contrast, scores related to females are commonly observed in the Church and the Cloister. However, sex estimated for individuals from the Cloister and the Church from the skull are congruent with those estimated from the pelvic.

In comparing metric analysis using Fordisc® with morphological determination of sex, consistency percentages vary based on cranial and postcranial measurements, as well as the origin of individuals from either the Cloister or the Church (Table 4). The highest consistency percentages at both burial locations are attained for postcranial measurements, both considering the total sample as sexes separated. These results contrast with those reported by Ubelaker *et al.* (2002) in a Spanish bone collection from the 16th and 17th centuries, where they found significant discrepancies between the morphological method and the use of Fordisc® software. These discrepancies, along with other findings, suggest that the use of Fordisc® for sex estimation in European individuals may not be advisable (RAMSTHALER *et al.* 2007). The reasons underlying this were attributed to differences in the population profile and secular changes in body size. Thus, the good agreement between sex estimation using Fordisc® and morphological sex seems to indicate that there are not differences in body size between San Pablo sample and the US American population used as reference sample in this software.

In contrast to postcranial measurements, dental diameters show low consistency percentages. This could be due to a low level the sexual dimorphism in dental dimensions. Several authors have noted that sexual dimorphism is greater in BL diameters. Additionally, although there is a great deal of population variation in the tooth type that is most sexually dimorphic, the lower canine is generally the most dimorphic tooth (GUATELLI-STEINBERG *et al*. 2008). The coefficient variation (CV), a statistic which measures the variation inside a

sample, is considered to be very highly correlated with sexual dimorphism because with increasing sexual dimorphism, the difference between male and female means increases, causing a proportional increase in the standard deviation of the pooled-sex sample. In our sample the CV of the bucco-lingual diameter of the lower canine is lower than in other teeth (Table 5). Although at first glance it can be interpreted as that in the San Pablo sample the canine is not the most dimorphic tooth, there are other factors to consider. Crown size variability is usually interpreted as a direct result of sexual dimorphism, but other factors such as maternal health status or the presence of hypoplasias may account for much of dental size differences among individuals. Garn *et al.* (1979) show that maternal and fetal determinants of permanent tooth dimensions may account for as much as half of crown-size variability. These effects are mainly noted in the incisors and the first molar, as they are the permanent teeth forming in prenatal period. This could be related to the fact that in some case, such as the LI2 and UI1 and UI2, despite showing the highest CV values, the consistencies percentage with sex estimation from morphological traits of the skull and pelvis slightly surpass the 50%. Another aspect to consider is that enamel hypoplasia significantly reduced the crown dimensions (MCKEE and LUNZ 1990). The prevalence of hypoplasias is very high in San Pablo sample, being higher in males than in females (SÁNCHEZ-PUENTE 2022). Thus, we cannot discard the possibility that in males, the reduction in dental size is greater in females. This may result in a decrease in sexual differences in dental dimensions and, thus, low performance for sex estimation. In any case, dental diameters have proven unsuccessful for sex estimation in adult sample from San Pablo. Therefore, we did not conduct an approach to try sex non-adult individuals from the dentition.

When estimating sex of non-adult individuals, we employ a meticulous analysis utilizing two distinct approaches. In the first approach, we categorize individuals as male, or female based on the assessment of nonmetric traits observed in the ilium and skull. However, this categorization is only considered reliable when the probabilities of being male or female are sufficiently high to ensure accurate sex estimation. For the second approach, we rely on a sample-specific method considering that sexual size differences are already present at birth. Again, we follow a careful protocol, assigning the sex only in cases where residuals from different regressions estimate the same sex. The consistency percentages between sex assigned for nonmetric traits and for femoral relative size of 100% suggest that our sex estimations of non-adult individuals are highly reliable. This undoubtedly improves our ability to reconstruct sex-specific child mortality and morbidity rates, sex-specific growth patterns and the construction of demographic profiles.

Age at death in non-adults was estimated in a high percentage of individuals based on dental development. In a comprehensive study about the performance of several dental age methods, (LIVERSIDGE *et al.* 2010) showed that most methods can estimate age with errors less than 1 year. Thus, we can assume that our age estimates are highly precise. In cases where age at death was estimated based on femoral length, we utilized a sample-specific method. Thus, we can also rely on these estimations, which are not influenced by the effect of age mimicry using methods developed from different populations. However, this is not the case of ages estimated from shape and morphological changes of femoral distal epiphysis. The reference sample used for developing this method represents the lower-to-

middle social classes in the city of Lisbon (CARDOSO 2006). Maturational rate is known to depend on large number of factors such as nutritional intake and health status (EVELETH and TANNER 1976). Indeed, the Lisbon sample demonstrates a delay in skeletal maturation (CONCEIÇÃO and CARDOSO 2011). Thus, it is possible that the incongruence between dental ages and those estimated from the different models based on shape changes of femoral distal epiphysis reflects an 'age mimicry' phenomenon associated with the latter method. However, some evidence point out that children from the Cloister and the Church of San Pablo also experienced in general a delay in skeletal development. First, in a preliminary analysis, we observed that many non-adult individuals exhibit nonspecific stress indicators, such as dental hypoplasia, cribra orbitalia, and periostitis. These indicators suggest that these individuals may have experienced nutritional deficiencies or illness, which could contribute to stunted growth and delayed skeletal development. Indeed, the growth trajectory of femoral length in a pooled San Pablo sample (Cloister plus Church) does not differ to that from the Lisbon sample (GARCÍA-GONZÁLEZ 2013). García-González *et al*. (2019) found a negative correlation between femoral length and skeletal ages predicted from femoral distal epiphysis shape in the Lisbon sample. Femoral length is the best surrogate of stature. Generally, individuals showing an advanced skeletal development, while tall for their chronological age, tend to be short for their skeletal ages and, individuals showing a delayed skeletal development, are short for their chronological age but tall for their skeletal age (BAYLEY 1946). Thus, this negative correlation suggests that individuals with a delay in skeletal development are taller than other individuals with the same skeletal age. The same negative correlation has been found in the San Pablo sample. Hence, it is reasonable to assume that the majority of non-adult individuals exhibit delayed skeletal development. Skeletal ages derived from femoral distal epiphysis may therefore be more suitable indicators of maturity stages in these individuals than chronological age.

The use of TA3 software has enabled us to identify individuals older than 60 years old among adults. The significant presence of these older individuals in the Chapter House, which served as the burial place for Monks, suggests the reliability of these estimations. Monks enjoyed better life conditions compared to many others buried in the Cloister and the Church, making it reasonable to believe that they attained older ages.

The careful and comprehensive estimation of sex and age-at-death has enabled us to construct a reliable paleodemographic profile. The main conclusion drawn from this profile reveals nuanced demographic patterns across various age groups and burial zones, highlighting variations in population dynamics and burial practices within the studied community. Differences in burial practices are underscored by the distinct age structure observed in the Chapter House compared to both the Cloister and the Church. While in the Chapter House were buried the monks, the Cloister and the Church were used for ordinary individuals.

One of the most interesting aspect related to population dynamics is the difference between the number of non-adult individuals (overall infants and children) buried in the Cloister and in the Church, being considerably higher in the Cloister. A higher proportion of skeletons from non-adult individuals and young adults are usually related to an increase in the fertility rate (BOLDSEN *et al*. 2021). In populations where fertility rates increase significantly, maternal mortality rates will notably rise. One way to estimate maternal

mortality is based on the number of young women aged between 20 and 30 years (the age at which they are assumed to have their first pregnancy). In samples where there is an excess of women in this age group, this is often interpreted as evidence of high maternal mortality. However, the lower number of young females buried in the Cloister compared to the Church may initially seem inconsistent with high fertility rates. At this juncture, it is crucial to consider that high maternal mortality rates are not solely linked to increased fertility but also to the presence of obstetric problems. Based on this, the high number of females found in the Church, could be related to these shortcomings. The maternal death caused by deliveries and complications of pregnancy is related to variation in sexual stature dimorphism across populations (GUÉGAN *et al*. 2000). However, there are not differences neither between females from the Cloister and the Church nor in the stature sexual dimorphism between them (RODRIGUEZ *et al*., 2024). At this point, it is important consider that the assumption that an excess of deceased young women indicates high maternal mortality has not yet been corroborated (BOLDSEN *et al*. 2021). Thus, it is possible that the higher number of infants and children in the Cloister compared to the Church could be attributed to changes in fertility rates. Nonetheless, other factors such as a different access patterns to the Church and the Cloister cannot be ruled out.

Another important aspect is the difference between the number of males and females during infancy and childhood buried in the Cloister. Many of the infants buried in the Cloister are males. Given the more rapid and protracted growth, greater allocation of energy to brain and lean muscle development, and relatively reduced levels of adipose tissue of males compared to females, the former are more sensitive to environmental or nutritional deprivation during early life (STINSON 1985). In contrast, females are more frequent during childhood period than males. This may be related to cultural factors that could contribute to creating differential nutritional or environmental exposures for males compared to females during growth, potentially compensating for the greater male environmental sensitivity (GUATELLI-STEINBERG and LUKACS 1999). These differential factors seem to be maintained during the juvenile phase.

5 Conclusions

We conducted a comprehensive study of sex and age-at-death estimation in adult and non-adult individuals recovered from the Cloister, Church, and Chapter House of the Convent of San Pablo. This allowed us to obtain highly reliable estimates, from which we conducted a paleodemographic analysis. Our findings show variations in population dynamics and burial practices within the studied community.

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