# Sexual dimorphism from vertebrae: its potential use for sex estimation in an identified Portuguese sample

## Anabel Amores-Ampuero<sup>1</sup>, Joan Viciano<sup>2</sup>

- <sup>1</sup> Faculty of Law and Business, Francisco de Vitoria University, Madrid, Spain
- <sup>2</sup> Operative Unit of Anthropology, Department of Medicine and Ageing Sciences, 'G. d'Annunzio' University of Chieti-Pescara, Chieti, Italy

#### Acknowledgements

The authors thank the Department of Life Sciences of the University of Coimbra for granting access to the Coimbra Identified Skeletal Collection. Appreciation is also extended to Prof. Eugènia Cunha for her useful suggestions.

#### **Ethical statement**

All of the authors declare that they have no conflicts of interest. This study did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

## CRediT authorship contribution statement

**Anabel Amores-Ampuero:** Conceptualization, Methodology, Investigation, Validation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Joan Viciano:** Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing, Visualization.

#### **Abstract**

In archaeological and medicolegal contexts, sex estimation is a crucial parameter to personal identification. However, it can be a complex task if the skeletal remains are damaged or fragmented. For this reason, is important to establish new reliable methodologies and techniques using alternative sexually dimorphic anatomical regions than pelvic and skull such as vertebrae. The purpose of the current study was to evaluate the level of sexual dimorphism of first, second and seventh cervical and twelfth thoracic vertebrae from the Coimbra Identified Skeletal Collection of the University of Coimbra (Portugal) and to develop logistic regression equations for sex estimation based on metric data from these vertebrae. The sample comprised 73 individuals (38 males and 35 females) with a mean age of 50.10±18.34 years. Eleven multivariate logistic regression equations were developed with accuracy rates between 80.0% and 92.5%. The first cervical vertebra demonstrated to be useful for sex diagnosis when more sexually dimorphic anatomical regions (i.e., pelvis and skull) are not available or suitable for analysis.

## **Keywords**

Forensic Anthropology Population Data, sex diagnosis, vertebra, logistic regression analysis, ROC analysis

#### 1. Introduction

Sex estimation is an important starting point for establishing a biological profile of human remains. The examination of morphometric characteristics of the bony pelvis is considered the best reliable technique to estimate biological sex [1,2] followed by the skull [3–5]. The successful sex identification directly depend on the quantity and quality of the bone remains [6], so it becomes extremely complex when they are dispersed or fragmented [7,8]. The more bones available and in a better state of preservation, the greater probability to obtain reliable sexual evaluation results [6]. For these reasons is important to establish reliable methodologies and techniques that enable sex estimation using other skeletal structures when pelvis and skull are not available or damaged [1,9–11]; in these cases, long bones have provided high allocation accuracies for sex diagnosis [12,13], followed by ribs [14,15], vertebrae [16,17], sternum [14,18], patella [19,20] and the bones of hands and feet [21,22].

Several researchers have reported sexual dimorphism in different vertebrae of the spine. Specifically, various studies have analysed the first cervical vertebra (C1) [23,24], second cervical vertebra (C2) [9,25–29], seventh cervical vertebra (C7) [9,16,29,30], and twelfth thoracic vertebra (T12) [16,31–33], resulting in correct diagnosis of sex up to virtually 100%. The main reason for studying these specific four vertebrae is that, due to their anatomical characteristics, they are readily identifiable when they are found isolated from the rest of the spine [24,34]: the appearance of C1 is completely different from the other vertebrae because it is a ring of bone made up of two lateral masses joined by the anterior and the posterior arch; the C2 is easily identifiable due its odontoid process; the C7 has a distinctive long and prominent spinous process; the T12 have the convex inferior articular surfaces and directed lateralward.

The objectives of the present investigation were: (i) to evaluate the level of sexual dimorphism of C1, C2, C7 and T12 vertebrae measurements obtained from an identified Portuguese osteological collection and (ii) to develop logistic regression equations for sex estimation based on metric data from these vertebrae.

## 2. Material and methods

#### 2.1. Sample

This study was based on the Coimbra Identified Skeletal Collection, whose skeletal individuals are housed at University of Coimbra (Portugal). Among other data, reliable antemortem information about the sex, age at death, birth and death dates and cause of death are available for each individual [35].

The study sample comprised 73 adult individuals (38 males and 35 females) with an age at death ranged from 21 to 89 years (mean age =  $50.10 \pm 18.34$  years) (Table 1). Their death dates correspond to a time period between 1930 to 2015 (60.3% of the individuals dead from 1950 to 1977; thus, the sample was largely dated from third quarter of the 20th century).

#### 2.2. Collection of metric data

First, second and seventh cervical vertebra (C1, C2 and C7, respectively) and twelfth thoracic vertebra (T12) were selected for analysis. Only complete vertebrae with no morphologically altering pathological conditions (e.g., osteoporosis, trauma, metastatic disease to the bone) were included. Six measurements were collected for C1 and eight measurements for C2, C7 and T12. All measurements are described in Table 2 and were collected using a digital sliding calliper

rounding to the nearest 0.01 mm. For bilateral measurements, both sides were measured and the mean value was calculated and used in ulterior statistical analyses. Vertebral measurements were collected by the same examiner (A.A.A.), who repeated them after 15-day interval to conduct the intra-observer error analysis. For this purpose, all the vertebrae were re-measured.

#### 2.3. Statistical analyses

All statistical analyses were performed using the IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY, USA). Data were first assessed for normality in their distributions using Kolmogorov–Smirnov one-sample test and for homogeneity of variance using Levene's test. Next, a descriptive analysis was performed to calculate the sample size and the mean and standard deviation for each measurement. The differences between the mean values between males and females were analysed using the independent two-sample Student's t-test when normality and homogeneity of variance were fulfilled (p > 0.05) and otherwise (i.e., when the assumptions of Student's t-test are violated) with non-parametric Mann–Whitney U-test.

The differences between the mean values in all measurements collected at two different times (i.e., intra-observer error analysis) were evaluated by means of absolute and relative technical errors of measurement (TEM and rTEM, respectively) and the coefficient of reliability (R) following Ulijaszek and Kerr [36] as indicators of the repeatability of each measurement. TEM is an estimate of absolute precision expressed in the original measurement units (i.e., in mm). The lower the TEM obtained, the better is the precision of measurement. The coefficient of reliability represents the variance proportion exempt of measurement error. According to Ulijaszek and Kerr [36], R values greater than 0.95 are sufficiently precise.

Logistic regression analysis was performed to identify the most useful sex discriminant equations. To maximize the applicability of the technique for forensic and archaeological contexts in which the vertebrae are poorly preserver and/or incomplete, all possible combinations of measurements within each vertebra were determined. The -2 logit likelihood (-2LL) was calculated to determine the fits of the logistic regression models to the datasets (lower -2LL statistic, better fit).

Receiver operating characteristic (ROC) analysis was performed to compare the performance of the various individual measurements. The result of the ROC analysis is a curve that represents a plot of the male allocation on the *y*-axis (true positive proportion: 'sensitivity'), against the proportion of females mistaken for males on the *x*-axis (false positive proportion: '1 – specificity', where 'specificity' is the proportion of females correctly identified). The results allow comparison of different measurements through the comparison of the area under the curve (AUC). Higher the AUC value, better is the measurement to discriminate male from female. The same approach was applied to logistic regression equations developed to assess the goodness of fit of the models [37,38].

### 3. Results

#### 3.1. Intra-observer error analysis

Table 3 shows the differences between mean values for the repeated measurements. The TEM values were extremely low, varying between 0.00 and 0.02 mm. The results demonstrated that the different measurements can be collected with measurement error very close to 0, with a maximum error of 0.06%. Moreover, the high values of R in all variables (R > 0.95) also indicated an excellent precision of the measurements.

#### 3.2. Assessment of sexual dimorphism for vertebral dimensions

Descriptive statistics for males and females are presented in Table 4. The Kolmogorov–Smirnov test showed that 25 of the 30 vertebral measurements were normally distributed within the sex (p > 0.05). The Levene's test indicated that the sample was homogeneous in 28 of the 30 vertebral measurements (p > 0.05). Table 4 also shows the inferential statistics through Student's *t*-test and Mann-Whitney *U*-test, and the significance values for the differences between male and female measurements.

Data showed that the male vertebral measurements had higher means than the female for all measurements except for the SFT measurement for C1, and LFV and IFS measurements for T12. Significant differences ( $p \le 0.05$ ) between the sexes were observed for 20 of the 30 measurements.

Results of the ROC analysis for each individual measurement are reported in Table 5. Analysis of the AUC showed that all the variables could not estimate the sex with an accuracy >80%, except for LFV and IFT for C1 (AUC = 0.804 and 0.809, respectively), SFB for C2 (AUC = 0.809), LFV for C7 (AUC = 0.811) and SVB for T12 (AUC = 0.831). The threshold values presented in the Table 5 represent the ideal cut-off points for sex estimation for each measurement, with the respective values of sensitivity and specificity (ideal cut-off points are considered those that maximize the values of sensitivity and specificity). The sensitivity values provide an estimate of the proportion for each sex that would be expected to have a measurement value above (for males) or below (for females) the threshold value. Therefore, this value represents the proportion of a particular sex that would be expected to fall within the estimated range for the threshold value. The specificity values estimate the percentage of the opposite sex that could also have a measurement value in the range indicated by the threshold value. For those measurements with AUC >80%, the values of sensitivity ranged from 70.3% to 80.0%, and the values of specificity ranged from 71.0% to 80.0%.

#### 3.3. Logistic regression equations for a single vertebra

The logit equations and their allocation accuracy are presented in Table 6. The equations with a discriminant power <80% were excluded because they are of little utility for reliable sex estimation. The sex allocation accuracies ranged from 78.9% to 97.3% for males, and from 71.9% to 86.7% for females. Therefore, males were classified more accurately than females for the 11 logit equations developed. With sexes pooled, the overall sex allocation accuracies ranged from 80.0% to 92.5%.

The analysis of the logit equations obtained showed that them were developed with the combination of two or more measurements. Of the 11 logit equations, five were defined by two measurements ( $L_1$ ,  $L_2$ ,  $L_3$ ,  $L_8$  and  $L_{10}$ ), two were defined by three measurements ( $L_4$  and  $L_{11}$ ), one was defined by four measurements ( $L_5$ ), one was defined by six measurements ( $L_6$ ) and two were defined by eight measurements ( $L_7$  and  $L_9$ ). No logit equations were developed using only a single vertebral measurement.

Results of the ROC analysis for each logit equation are reported in Table 7. The values of AUC ranged from 0.768 to 0.902. The values of sensitivity ranged from 74.3% to 85.7% and the values of specificity ranged from 71.1% to 94.7%. It can be concluded that the eleven logit equations perform well in predicting sex. Among these eleven logit equations developed,  $L_5$  (defined by four measurements of C1) and  $L_6$  (defined by six measurements of C1) were the most powerful to discriminate between sexes. In addition, they were the equations that had the highest percentage of sex allocation accuracy in the logistic regression analysis.

#### 4. Discussion

According to Christensen and Crowder [39], it is necessary to evaluate the reliability of the research methods and techniques when applied in medico-legal contexts to problems related to personal identification. In this sense, the intra-observer error analysis conducted in the present study demonstrates that the measurements are highly reproducible, which is an essential parameter for the reliability of any metric method. The results of reliability are comparable to previous studies [16,24,28].

The descriptive analyses of the present study indicated that males present higher mean values for vertebral measurements than females. The results show that 66.7% of the vertebral measurements show significant differences between the sexes, thus emphasizing the sexual dimorphism of human vertebrae according to previous studies [16,17,24–26]. The discriminating power of each vertebral measurement was assessed by estimating the AUC to determine those measurements that were more efficient to predict the sex among all the vertebrae. Only LFV and IFT for C1, SFB for C2, LFV for C7 and SVB for T12 showed AUC values equal or higher than 80%, indicating that they are the best variables for estimating sex. Our results agree with previous researches. Marino [23] highlight the utility of the articular surfaces and vertebral foramen measurements as predictors of sex for C1 vertebra. For Marlow and Pastor [26] and Wescott [25] SFB is the single best measurement for estimating sex in C2. Amores-Ampuero et al. [16] noted the LFV measurement as most efficient to sex estimation in C7. Amores-Ampuero et al. [16] and Hora and Sládek [17] identified the SVB measurement of T12 as a good predictor of sex.

The analysed vertebrae yielded sex allocation accuracies that ranged from 80.0% to 92.5%. A single measurement for each vertebra did not accurately estimate sex at the 80% accuracy threshold. The results are in line with previous studies [9,25,40]. According to Rozendaal et al. [24], Wescott [25] and Bethard and Seet [27], with every increase in the number of measurements used in a predictive equation for each vertebra, the accuracy for sex estimation also increases. Thus, the combination of vertebral measurements provides higher degree of accuracy than using only a single vertebral measurement. Several researches related to sexual estimation using the vertebrae, show different accuracies between them and the present study. The predictive accuracies ranged from 77% to 85% using the C1 [23], from 78% to 100% for the C2 [9,25–28], from 80% to 90% for the C7 [16,41], and from 80% to 94% for the T12 [16,31–33]. These interpopulational discrepancies may reflect that there are genetic, environmental and socioeconomic factors that can influence the levels sexual dimorphism in the size of vertebrae [24]. Moreover, these different accuracies could be also explained by the methodological procedures (e.g., analyses conducted in dry bones, CT scans or X-ray images) and statistical analyses used to evaluate the sexual dimorphism of vertebrae [9].

Despite being sexually dimorphic, vertebrae may be damaged by taphonomic processes and the diverse measurements cannot be collected. The spine is generally well represented and well preserved; however, the good preservation depends on each vertebra [42,43]. Observance in skeletal collections showed a better representation of cervical and lumbar vertebrae [42]. The deficient state of preservation of thoracic vertebrae has been associated to their low bone density [44], while the high preservation of lumbar vertebrae is associated with their shape and structural robusticity [42]. Cervical vertebrae are the best-preserved vertebrae of the spine, especially C1 and C2, because of they are protected by the cranium when the skeleton is articulated [26,42]. Despite of the generally well represented and well preserved of vertebrae in skeletal collections, the main limitation of the developed equations for sex estimation is that all of them (except for L<sub>10</sub> equation) require the collection of the LFV and/or WFV measurements. Therefore, it is

necessary that the vertebral arch is not separated from the vertebral body as a consequence of fractures of the pedicles. In such a case, these equations cannot be applied.

The results of the present study show that a single vertebra does have a strong potential in estimating sex. These findings are in agreement with studies of other researchers who have successfully estimated sex from a single vertebra [9,16,25,27,28,33]; while are contrary to those shown by Rozendaal et al. [24], whose results indicate that a single vertebra cannot accurately estimate sex with satisfactory accuracy. Specifically, our results indicate that C1 is the vertebra with the higher power for sex discrimination, resulting in correct diagnosis of sex up to 92.5% when all measurements are collected (equation  $L_6$ ). For all the equations for C1 vertebra (equations  $L_1$ –  $L_6$ ), the main measurements selected are related to articular facets and vertebral foramen. According to Marino [23] and Marlow and Pastor [26], the significant sexual dimorphism and the consequent discriminant power for sex estimation of C1 can be attributed to the functional relationship between the C1 and basicranium as load-bearing regions. Therefore, differences in size and weight of the skull (particularly, in the occipital condyles and foramen magnum) result in sexual dimorphism in the vertebral foramen and the superior and inferior articular facets of C1.

In conclusion, when anthropologists are confronted with incomplete or poor preserved human skeletal remains, the vertebrae (especially the C1) can be used to correctly estimate the sex when other skeletal elements are not available or suitable for analysis. However, the main limitation of the equations developed is that they require the absence of fractures of the vertebral pedicles (i.e., that vertebral arch is not separated from the vertebral body) to measure the length and/or width of the vertebral foramen to be able to apply most of the developed equations.

#### References

- [1] M. Durić, Z. Rakočević, D. Donić, The reliability of sex determination of skeletons from forensic context in the Balkans, Forensic Sci. Int. 147 (2005) 159–164. https://doi.org/10.1016/j.forsciint.2004.09.111.
- [2] F. Santos, P. Guyomarc'h, R. Rmoutilova, J. Bruzek, A method of sexing the human os coxae based on logistic regressions and Bruzek's nonmetric traits., Am. J. Phys. Anthropol. 169 (2019) 435–447. https://doi.org/10.1002/ajpa.23855.
- [3] L. Luo, M. Wang, Y. Tian, F. Duan, Z. Wu, M. Zhou, Y. Rozenholc, Automatic sex determination of skulls based on a statistical shape model, Comput. Math. Methods Med. (2013) 251628. https://doi.org/10.1155/2013/251628.
- [4] B. Ramamoorthy, M.M. Pai, L.V. Prabhu, B.V. Muralimanju, R. Rai, Assessment of craniometric traits in South Indian dry skulls for sex determination, J. Forensic Leg. Med. 37 (2016) 8–14. https://doi.org/10.1016/j.jflm.2015.10.001.
- [5] C. Small, L. Schepartz, J. Hemingway, D. Brits, Three-dimensionally derived interlandmark distances for sex estimation in intact and fragmentary crania, Forensic Sci. Int. 287 (2018) 127–135. https://doi.org/10.1016/j.forsciint.2018.02.012.
- [6] H.M. Abo El-Atta, R.H. Abdel-Rahman, G. El-Hawary, H.M. Abo El-Al-Atta, Sexual dimorphism of foramen magnum: an Egyptian study, Egypt. J. Forensic Sci. 10 (2020) 1. https://doi.org/10.1186/s41935-019-0167-x.
- [7] S.S. Tambawala, F.R. Karjodkar, K. Sansara, N. Prakash, A.C. Dora, Sexual dimorphism of foramen magnum using Cone Beam Computed Tomography, J. Forensic Leg. Med. 44 (2016) 29–34. https://doi.org/10.1016/j.jflm.2016.08.005.

- [8] M. Jaitley, T. Phulambrikar, M. Kode, A. Gupta, S.K. Singh, Foramen magnum as a tool for sexual dimorphism: a cone beam computed tomography study, Indian J. Dent. Res. 27 (2016) 458–462. https://doi.org/10.4103/0970-9290.195610.
- [9] Y. Kaeswaren, L. Hackman, Sexual dimorphism in the cervical vertebrae and its potential for sex estimation of human skeletal remains in a white scottish population, Forensic Sci. Int. Reports. 1 (2019) 100023. https://doi.org/10.1016/j.fsir.2019.100023.
- [10] J. Viciano, A. Amores-Ampuero, Sex estimation in a contemporary Spanish population: cranial and dental anthropometry, Homo. (2020). https://doi.org/10.1127/homo/2020/1200.
- [11] S. López-Lázaro, I. Alemán, J. Viciano, J. Irurita, M.C. Botella, Sexual dimorphism of the maxillary postcanine dentition: a geometric morphometric analysis, Homo. (2020). https://doi.org/10.1127/homo/2020/1170.
- [12] M.K. Spradley, R.L. Jantz, Sex estimation in forensic anthropology: skull versus postcranial elements, J. Forensic Sci. 56 (2011) 289–296. https://doi.org/10.1111/j.1556-4029.2010.01635.x.
- [13] G.C. Krüger, E.N. L'Abbé, K.E. Stull, Sex estimation from the long bones of modern South Africans, Int. J. Legal Med. 131 (2017) 275–285. https://doi.org/10.1007/s00414-016-1488-z.
- [14] S. Peleg, R. Pelleg Kallevag, G. Dar, N. Steinberg, Y. Masharawi, H. May, New methods for sex estimation using sternum and rib morphology., Int. J. Legal Med. 134 (2020) 1519–1530. https://doi.org/10.1007/s00414-020-02266-4.
- [15] A.M. Kubicka, J. Piontek, Sex estimation from measurements of the first rib in a contemporary Polish population, Int. J. Legal Med. 130 (2016) 265–272. https://doi.org/10.1007/s00414-015-1247-6.
- [16] A. Amores, M.C. Botella, I. Alemán, Sexual dimorphism in the 7th cervical and 12th thoracic vertebrae from a Mediterranean population, J. Forensic Sci. 59 (2014) 301–305. https://doi.org/10.1111/1556-4029.12320.
- [17] M. Hora, V. Sládek, Population specificity of sex estimation from vertebrae, Forensic Sci. Int. 291 (2018) 279.e1-279.e12. https://doi.org/10.1016/j.forsciint.2018.08.015.
- [18] P. García-Parra, Á. Pérez Fernández, M. Djorojevic, M. Botella, I. Alemán, Sexual dimorphism of human sternum in a contemporary Spanish population, Forensic Sci. Int. 244 (2014) 313.e1-313.e9. https://doi.org/10.1016/j.forsciint.2014.06.019.
- [19] T.R. Peckmann, S. Meek, N. Dilkie, A. Rozendaal, Determination of sex from the patella in a contemporary Spanish population, J. Forensic Leg. Med. 44 (2016) 84–91. https://doi.org/10.1016/j.jflm.2016.09.007.
- [20] T. Michiue, A.M. Hishmat, S. Oritani, K. Miyamoto, M.F. Amin, T. Ishikawa, H. Maeda, Virtual computed tomography morphometry of the patella for estimation of sex using postmortem Japanese adult data in forensic identification, Forensic Sci. Int. 285 (2018) 206.e1-206.e6. https://doi.org/10.1016/j.forsciint.2017.11.029.
- [21] O. Ekizoglu, E. Inci, F.B. Palabiyik, I.O. Can, A. Er, M. Bozdag, I.E. Kacmaz, E.F. Kranioti, Sex estimation in a contemporary Turkish population based on CT scans of the calcaneus, Forensic Sci. Int. 279 (2017) 310.e1-310.e6. https://doi.org/10.1016/j.forsciint.2017.07.038.
- [22] P. Mastrangelo, S. De Luca, I. Alemán, M.C. Botella, Sex assessment from the carpals bones: discriminant function analysis in a 20th century Spanish sample, Forensic Sci. Int. 206 (2011) 216.e1-216.e10. https://doi.org/10.1016/j.forsciint.2011.01.007.

- [23] E.A. Marino, Sex estimation using the first cervical vertebra, Am. J. Phys. Anthropol. 97 (1995) 127–133. https://doi.org/10.1002/ajpa.1330970205.
- [24] A.S. Rozendaal, S. Scott, T.R. Peckmann, S. Meek, Estimating sex from the seven cervical vertebrae: an analysis of two European skeletal populations, Forensic Sci. Int. 306 (2020) 110072. https://doi.org/10.1016/j.forsciint.2019.110072.
- [25] D.J. Wescott, Sex variation in the second cervical vertebra, J. Forensic Sci. 45 (2000) 462–466. https://doi.org/10.1520/JFS14707J.
- [26] E.J. Marlow, R.F. Pastor, Sex determination using the second cervical vertebra—A test of the method, J. Forensic Sci. 56 (2011) 165–169. https://doi.org/10.1111/j.1556-4029.2010.01543.x.
- [27] J.D. Bethard, B.L. Seet, Sex determination from the second cervical vertebra: a test of Wescott's method on a modern American sample, J. Forensic Sci. 58 (2013) 101–103. https://doi.org/10.1111/j.1556-4029.2012.02183.x.
- [28] I. Gama, D. Navega, E. Cunha, Sex estimation using the second cervical vertebra: a morphometric analysis in a documented Portuguese skeletal sample, Int. J. Legal Med. 129 (2015) 365–372. https://doi.org/10.1007/s00414-014-1083-0.
- [29] C.A. Miller, S.J. Hwang, M.M. Cotter, H.K. Vorperian, Cervical vertebral body growth and emergence of sexual dimorphism: a developmental study using computed tomography, J. Anat. 234 (2019) 764–777. https://doi.org/10.1111/joa.12976.
- [30] D. Ezra, Y. Masharawi, K. Salame, V. Slon, D. Alperovitch-Najenson, I. Hershkovitz, Demographic aspects in cervical vertebral bodies' size and shape (C3-C7): a skeletal study, Spine J. 17 (2017) 135–142. https://doi.org/10.1016/j.spinee.2016.08.022.
- [31] S.-B. Yu, U.-Y. Lee, D.-S. Kwak, Y.-W. Ahn, C.-Z. Jin, J. Zhao, H.-J. Sui, S.-H. Han, Determination of sex for the 12th thoracic vertebra by morphometry of three-dimensional reconstructed vertebral models, J. Forensic Sci. 53 (2008) 620–625. https://doi.org/10.1111/j.1556-4029.2008.00701.x.
- [32] W.B. Hou, K.L. Cheng, S.Y. Tian, Y.Q. Lu, Y.Y. Han, Y. Lai, Y.Q. Li, Metric method for sex determination based on the 12th thoracic vertebra in contemporary north-easterners in China, J. Forensic Leg. Med. 19 (2012) 137–143. https://doi.org/10.1016/j.jflm.2011.12.012.
- [33] F.M.M. Badr El Dine, M.M. El Shafei, Sex determination using anthropometric measurements from multi-slice computed tomography of the 12th thoracic and the first lumbar vertebrae among adult Egyptians, Egypt. J. Forensic Sci. 5 (2015) 82–89. https://doi.org/10.1016/j.ejfs.2014.07.005.
- [34] T.D. White, P.A. Folkens, The human bone manual, Academic Press, London, 2005. https://doi.org/10.1016/C2009-0-00102-0.
- [35] E. Cunha, S. Wasterlain, The Coimbra identified osteological collections, in: G. Grupe, J. Peters (Eds.), Skelet. Ser. Their Socioecon. Context, Verlag Marie Leidorf, Rahden, 2007: pp. 23–33.
- [36] S.J. Ulijaszek, D.A. Kerr, Anthropometric measurement error and the assessment of nutritional status, Br. J. Nutr. 82 (1999) 165–177. https://doi.org/10.1017/s0007114599001348.
- [37] D.W. Hosmer, S. Lemeshow, R.X. Sturdivant, Applied logistic regression, John Wiley & Sons, Hoboken, 2013.
- [38] D.T. Larose, C.D. Larose, Data mining and predictive analysis, John Wiley & Sons,

- Hoboken, 2015.
- [39] A.M. Christensen, C.M. Crowder, Evidentiary standards for forensic anthropology, J. Forensic Sci. 54 (2009) 1211–1216. https://doi.org/10.1111/j.1556-4029.2009.01176.x.
- [40] M. Slaus, Z. Bedić, D. Strinović, V. Petrovečki, Sex determination by discriminant function analysis of the tibia for contemporary Croats, Forensic Sci. Int. 226 (2013) 302.e1-302.e4. https://doi.org/10.1016/j.forsciint.2013.01.025.
- [41] S.M. MacLaughlin, K.N. Oldale, Vertebral body diameters and sex prediction, Ann. Hum. Biol. 19 (1992) 285–292. https://doi.org/10.1080/03014469200002152.
- [42] S. Bello, P. Andrews, The intrinsic pattern of preservation of human skeletons and its influence on the interpretation of funerary behaviours, in: R. Gowland, C. Knüsel (Eds.), Soc. Archaeol. Funer. Remain., Oxbow Books, Oxford, 2006: pp. 1–13.
- [43] V. Sládek, J. Machácek, At the end of great Moravia: skeletons from the second church cemetery at Pohansko-Breclav (9th-10th century A.D.), British Archaeological Reports, Oxford, 2017.
- [44] P. Willey, A. Galloway, L. Snyder, Bone mineral density and survival of elements and element portions in the bones of the Crow Creek massacre victims, Am. J. Phys. Anthropol. 104 (1997) 513–528. https://doi.org/10.1002/(SICI)1096-8644(199712)104:4<513::AID-AJPA6>3.0.CO;2-S.
- [45] R. Martin, K. Saller, Lehrbuch der Anthropologie, Gustav Fischer, Stuttgart, 1957.

**Table 1.** Distribution of the sample by sex and age group

_				
Sex	21–40	41–60	>60	Total
Male	12	18	8	38
Female	12	9	14	35
Subtotal	24	27	22	73

Table 2. Definitions of the vertebral measurements and abbreviations used in this study

Vertebra	Abbreviation	Definition
All vertebrae	LFV	Length of vertebral foramen: the internal length of the vertebral foramen, measured at the inferior edge of the foramen in the median plane
	WFV	Width of vertebral foramen: the maximum internal width of the vertebral foramen, wherever it may occur, measured perpendicular to the median plane
	SFS	Superior facet sagittal diameter: the maximum sagittal diameter of the superior articular facet
	SFT	Superior facet transverse diameter: the maximum transverse diameter of the superior articular facet measured perpendicular to the sagittal diameter
	IFS	Inferior facet sagittal diameter: the maximum sagittal diameter of the inferior articular facet
	IFT	<i>Inferior facet transverse diameter</i> : the maximum transverse diameter of the inferior articular facet measured perpendicular to the sagittal diameter
Only for C2	SFB	Maximum width across the superior facets: the maximum width between the superior articular facets measured from the most lateral edges of the superior facets
	DTD	Dens transverse diameter: the maximum transverse (latero-lateral) diameter of the dens
Only for C7 and T12	SVB	Vertebral body sagittal diameter: the maximum sagittal diameter of the vertebral body
	TVB	Vertebral body transverse diameter: the maximum transverse diameter of the vertebral body

All measurements were previously defined by either Marino [23], Wescott [25] or Martin and Saller [45], though exact terminology and abbreviations may differ slightly.

**Table 3.** Intra-observer error analysis: technical error of measurement (TEM), relative technical error of measurement (rTEM) and coefficient of reliability (R) from two repetitions of measurements.

Vertebra	Measurement	N	TEM (mm)	rTEM (%)	R
C1	LFV	68	0.00	0.00	1.000
	WFV	70	0.02	0.06	1.000
	SFS	70	0.01	0.02	1.000
	SFT	69	0.00	0.00	1.000
	IFS	70	0.00	0.00	1.000
	IFT	70	0.00	0.00	1.000
C2	LFV	70	0.00	0.03	1.000
	WFV	70	0.01	0.04	1.000
	SFS	71	0.00	0.01	1.000
	SFT	70	0.00	0.00	1.000
	IFS	66	0.00	0.00	1.000
	IFT	61	0.00	0.01	1.000
	SFB	70	0.00	0.00	1.000
	DTD	71	0.00	0.01	1.000
C7	LFV	70	0.00	0.00	1.000
	WFV	70	0.00	0.00	1.000
	SFS	69	0.00	0.01	1.000
	SFT	67	0.00	0.00	1.000
	IFS	64	0.00	0.02	1.000
	IFT	67	0.00	0.00	1.000
	SVB	70	0.00	0.00	1.000
	TVB	70	0.00	0.00	1.000
T12	LFV	71	0.00	0.01	1.000
	WFV	71	0.00	0.01	1.000
	SFS	62	0.00	0.00	1.000
	SFT	62	0.00	0.00	1.000
	IFS	67	0.00	0.00	1.000
	IFT	66	0.00	0.00	1.000
	SVB	70	0.00	0.00	1.000
	TVB	70	0.00	0.00	1.000

**Table 4.** Descriptive statistics for the vertebral measurements (in mm), and Student's *t*-test and Mann. Whitney *U* test for the mann differences between the gaves

Mann–Whitney *U*-test for the mean differences between the sexes. Male Female Statistics Vertebra Measurement Mean SD N Mean SD KS LUt Sig. C1 LFV 30.960 28.722 Y Y 0.000 \*\*\* 37 1.776 31 1.767 5.188 WFV \*\*\* 38 28.611 2.724 32 26.631 1.584 Y N 317.00 0.001 \*\*\* **SFS** 38 22.370 2.197 32 20.189 2.210 Y Y 4.127 0.000 **SFT** 38 11.143 1.317 31 11.406 1.666 Y Y -0.7320.467 38 15.985 32 Y Y 0.698 **IFS** 1.563 15.738 1.367 0.487 38 32 15.412 Y Y \*\*\* **IFT** 16.848 1.265 4.960 1.132 0.000C2 **LFV** 35 35 Y 447.00 17.128 2.889 15.890 1.433 N 0.052 \*\* 35 Y Y WFV 23.155 1.782 35 21.893 1.677 3.051 0.003 \*\* Y Y **SFS** 36 17.669 1.676 35 16.564 1.527 2.902 0.005 1.224 **SFT** 36 16.519 1.502 34 15.556 Y Y 2.933 0.005 \*\* 32 34 Y Y **IFS** 10.892 1.951 10.692 1.520 0.465 0.643 29 32 Y \* **IFT** 11.194 1.251 10.490 1.455 Y 2.018 0.048 \*\*\* 36 46.362 34 43.079 Y Y 5.522 0.000 **SFB** 2.391 2.583 35 DTD 36 10.577 0.863 9.923 0.662 Y Y 3.572 0.001C7 32 \*\*\* LFV 38 14.226 1.075 13.018 0.989 Y Y 4.858 0.000 WFV 38 23.680 1.924 32 22.996 1.655 Y Y 1.578 0.119 SFS 37 9.241 1.173 32 8.783 1.452 N Y 418.00 0.012 SFT 36 12.303 1.747 31 11.593 1.469 Y Y 1.784 0.079 **IFS** 34 10.750 1.515 30 10.276 1.966 N Y 344.00 0.026 **IFT** 36 12.269 1.990 31 11.310 1.748 Y Y 2.080 0.041 **SVB** 38 16.905 32 15.744 1.604 Y Y 3.173 0.002 \*\* 1.456 38 32 **TVB** 27.255 3.593 26.050 2.855 Y Y 1.531 0.130 T12 **LFV** 38 17.239 1.051 33 17.287 1.986 N Y 509.00 0.174 \*\* WFV 38 21.479 2.844 33 19.767 1.800 Y Y 2.976 0.004 Y 3.270 \*\* **SFS** 34 11.310 1.676 28 10.019 1.372 Y 0.002 33 9.576 29 Y SFT 8.775 1.163 Y 2.321 1.504 0.024 **IFS** 37 30 12.422 Y Y 12.146 1.639 1.570 -0.6990.487 **IFT** Y 36 9.093 1.198 30 8.909 1.073 N 496.50 0.575

N, number of individuals; SD, standard deviation; KS, Kolmogorov-Smirnov test (Y=yes, normality; N=no, no normality); L, Levene's test (Y=yes, homoscedasticity; N=no, no homoscedasticity); t, Student's t-test; U, Mann–Whitney U-test; p, p-value; Sig., significance at  $p \le 0.05$ , \*\* $p \le 0.01$ , \*\*\* $p \le 0.001$  level.

26.386

37.852

1.808

3.180

33

33

Y

Y

N

3.978

192.50

\*\*\*

\*\*\*

0.000

0.000

**SVB** 

**TVB** 

37

37

29.654

41.957

3.439

5.108

**Table 5.** ROC analysis for vertebral measurements

Vertebra	Measurement <sup>†</sup>	AUC	Threshold	Sensitivity	Specificity
C1	LFV	0.804	29.79	0.730	0.733
	WFV	0.733	27.11	0.649	0.667
	SFS	0.786	21.41	0.757	0.800
	SFT	0.435	11.16	0.514	0.433
	IFS	0.515	15.76	0.514	0.500
	IFT	0.809	16.32	0.703	0.800
C2	LFV	0.611	16.13	0.586	0.548
	WFV	0.724	22.55	0.655	0.677
	SFS	0.677	17.05	0.690	0.613
	SFT	0.672	16.47	0.586	0.677
	IFS	0.501	10.64	0.517	0.516
	IFT	0.642	10.86	0.621	0.613
	SFB	0.809	44.82	0.724	0.710
	DTD	0.733	10.07	0.724	0.677
C7	LFV	0.811	13.85	0.750	0.793
	WFV	0.656	23.68	0.594	0.621
	SFS	0.626	8.60	0.625	0.552
	SFT	0.586	11.82	0.563	0.517
	IFS	0.699	9.87	0.750	0.724
	IFT	0.606	11.64	0.688	0.586
	SVB	0.722	16.22	0.625	0.690
	TVB	0.682	27.18	0.688	0.655
T12	LFV	0.661	17.12	0.667	0.680
	WFV	0.728	20.00	0.800	0.600
	SFS	0.758	10.06	0.733	0.720
	SFT	0.681	8.88	0.633	0.600
	IFS	0.487	12.00	0.533	0.520
	IFT	0.530	8.55	0.567	0.480
	SVB	0.831	27.75	0.800	0.800
4	TVB	0.789	40.45	0.733	0.720

<sup>†</sup> See Table 2 for abbreviations of the vertebral measurements. AUC, area under de ROC curve.

**Table 6.** Binary logistic regression equations and assessment of the fit of each logit equation<sup>†</sup>

				Male correct		Female	Female correct	
Vertebra	Logit equation	N	-2LL	n/N	%	n/N	%	Total
<b>C</b> 1	$L_1 = 31.760 - 0.749(LFV) - 0.449(SFS)$	68	60.034	30/37	81.1	25/31	80.6	80.9
	$L_2 = 36.971 - 0.683(LFV) - 1.035(IFT)$	68	55.652	31/37	83.8	24/31	77.4	80.9
	$L_3 = 23.520 - 0.505(WFV) - 0.461(SFS)$	70	68.262	30/38	78.9	26/32	81.3	80.0
	$L_4 = 38.885 - 0.944(LFV) + 0.714(SFT) - 1.176(IFT)$	67	48.125	31/37	83.8	24/30	80.0	82.1
	$L_5 = 48.510 - 1.128(LFV) - 0.536(SFS) + 1.052(SFT) - 0.957(IFT)$	67	40.470	32/37	86.5	26/30	86.7	86.6
	$L_6 = 56.929 - 0.773 (LFV) - 0.635 (WFV) - 0.677 (SFS) + 1.290 (SFT) + 0.817 (IFS) - 1.847 (IFT)$	67	31.353	36/37	97.3	26/30	86.7	92.5
C2	$L_7 = 29.893 - 0.073 (LFV) - 0.305 (WFV) - 0.071 (SFS) - 0.110 (SFT) + 0.194 (IFS) + 0.004 (IFT) - 0.294 (SFB) - 0.759 (DTD) + 0.004 (IFT) - 0.004 (IFT) -$	60	57.637	24/29	82.8	24/31	77.4	80.0
C7	$L_8 = 33.574 - 1.573(LFV) - 0.748(SVB)$	70	62.571	33/38	86.8	23/32	71.9	80.0
	$L_9 = 54.005 - 1.664(LFV) - 0.452(WFV) + 0.287(SFS) + 0.014(SFT) - 0.412(IFS) - 0.102(IFT) - 0.972(SVB) - 0.079(TVB) + 0.014(SFT) - 0$	61	44.703	27/32	84.4	24/29	82.8	83.6
T12	$L_{10} = 17.479 - 0.513(SFS) - 0.437(SVB)$	61	59.783	27/33	81.8	23/28	82.1	82.0
	$L_{11} = 31.093 - 0.455(WFV) - 0.598(SFT) - 0.595(SVB)$	55	44.189	26/30	86.7	19/25	76.0	81.8

<sup>&</sup>lt;sup>†</sup> Only logit equations with correct allocation rates >80% are presented.

N, indicates the total number of individuals used to develop the logit equations; -2LL, -2 log likelihood value; n, indicates the number of individuals correctly classified compared with the total of individuals used for the classification.

**Table 7.** ROC analysis for logistic regression equations

					95% Confide	ence Interval		_
Vertebra	Logit equation	AUC	SE	p	Lower	Upper	Sensitivity	Specificity
C1	$L_1$	0.809	0.053	0.000	0.704	0.914	0.829	0.789
	$L_2$	0.808	0.054	0.000	0.703	0.913	0.800	0.816
	$L_3$	0.809	0.053	0.000	0.704	0.914	0.829	0.789
	$L_4$	0.808	0.054	0.000	0.703	0.913	0.800	0.816
	$L_5$	0.850	0.049	0.000	0.754	0.945	0.857	0.842
	$L_6$	0.902	0.041	0.000	0.822	0.982	0.857	0.947
C2	$L_7$	0.780	0.056	0.000	0.670	0.891	0.771	0.789
C7	$L_8$	0.806	0.054	0.000	0.699	0.912	0.743	0.868
	$L_9$	0.809	0.053	0.000	0.704	0.914	0.829	0.789
T12	$L_{10}$	0.784	0.056	0.000	0.675	0.893	0.857	0.711
	$L_{11}$	0.768	0.057	0.000	0.656	0.881	0.800	0.737

AUC, area under the ROC curve; SE, standard error; p, statistical significance at  $p \le 0.05$ .