Forensic anthropology population data

A geometric-morphometric study of the cretan humerus for sex identification

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A B S T R A C T

Various scholars have pioneered sex assessment from different parts of the skeleton based on metrical or qualitative morphological characteristics. Lately though, a new technique which combines both traditional methods is becoming popular in forensic anthropology. Geometric-morphometrics is a field of multivariate statistical biometric analysis which allows the quantification of the shape and size components of morphological variation. The purpose of this investigation is to assess sex from the humerus in a contemporary Greek population using geometric-morphometric analysis of shape data derived from digital radiographs.

The study population consists of 97 well-preserved adult humeri from two cemeteries of Heraklion, Crete. The left humeri are radiographed using digital radiograph machine (TCA 4R PLUS). Assuming fragmentary patterns, the proximal and distal ends are studied separately. Five landmarks are selected on the radiograph of the proximal epiphysis and seven landmarks on the distal.

Generalised Procrustes analysis (GPA) and thin-plate splines are used to obtain the shape and size variables for statistical analysis. Then three discriminant function analyses were carried out: one uses the PC scores from Procrustes shape space, the second the centroid size alone and the third the PC scores of GPA residuals plus lnCS for analysis in Procrustes form space.

Results indicate the existence of shape differences between the sexes. In females the greater tubercle is smoother, with its superior border less pronounced. Additionally, females have a relatively squared distal epiphysis, while males exhibit a more rectangular shape. Shape differences between genders, for the cross-validated data, give slightly better classification results in the proximal humerus (73%) compared with the distal humerus (71%). Size alone performed better (86.5% for proximal and 85.6% for distal humerus). As anticipated, the classification accuracy improves (89.6% for proximal and 89.7% for distal epiphysis) when both size and shape are combined.

From the forensic standpoint, the usefulness of this study rests on the identification of sex based on shape differences observed on radiographs of fragmentary humeri that could not be assessed with traditional methods. The analysis of humeral radiographs by geometric-morphometric techniques offers an alternative way to identify the sex of unknown skeletal remains. Whether this is statistically better than simply using traditional osteometric methods is a question that needs to be tested in a meta-statistical approach.

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1. Introduction

When the entire skeleton is available, sex assessment is considered a relatively easy process [1]. However, in forensic investigations that is rarely the case, since the bones are usually recovered in a fragmentary state due to the effect of extreme environmental conditions and the activities of carnivores and/or other scavengers. Therefore, sexual assessment becomes more difficult given that the bones are incomplete and too fragile to be manipulated.

There are mainly two traditional approaches to estimate sex from skeletal remains. Qualitative morphological examination remains the quickest and easiest method and, in experienced hands, results in 95–100% accuracy when the whole skeleton is available [1]. Nevertheless, these methods present a certain number of limitations, such as inter- and intra-observer error or classification problems of the qualitative morphological characteristics, which make one sceptical considering their reliability [2]. Morphometric methods, on the other hand, are considered more advantageous in terms of objectivity, repeatability, data evaluation

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and applicability to both cranial and post-cranial skeleton [1,3]. However, some characteristics such as the prominence of the glabella or the external occipital protuberance are difficult to assess metrically.

Lately, a new technique which combines both morphometric and meristic characteristics is becoming popular. Procrustes-based geometric-morphometrics is a method that provides the means for quantifying shape differences in a two- or three-dimensional (2D or 3D) coordinate system [4–11]. As a research tool, it has been used to test a variety of hypotheses in a variety of disciplines using various different types of data sets [12], but it is only recently that it has been introduced in forensic anthropology. More specifically, sexual dimorphism has been studied on the greater sciatic notch, mandibular ramus flexure and the orbits [2], as well as in skulls and mandibles [13–17]. A recent study on anterior dentition [18] is worth mentioning here, which concluded that there are no two individuals with identical tooth morphology; this suggests the potential use of this methodology for positive identification in forensic cases.

The humerus is one of the strongest long bones of the skeleton which, even in a fragmented state, is likely to be recovered in a forensic case. Several studies using classical osteometric techniques confirm the existence of sexual dimorphism in the humerus [19–25]. Scholars agree that a population-specific study is required in order to have accurate results in sexing the skeleton for a given population [26,27].

The objective of this investigation is to discriminate sex from the humerus in a contemporary Greek population, with the application of geometric-morphometric techniques on digital radiographs. The study addresses population-specific morphological features for identification purposes in forensic investigation and thus provides potentially useful tools for modern medico-legal professionals.

2. Materials and methods

A total of 97 well-preserved adult humeri of Cretan origin were examined. The remains were selected from the exhumed skeletons of St. Konstantinos and Pateles Cemeteries, Heraklion, Crete. The study population consists of individuals who lived between the end of the 19th century and the beginning of the 20th and were buried in Crete. The mean age for males is 68.57 ± 13.52 (N = 50) and for females 72.98 ± 16.90 (N = 47). Of these remains, the left humeri were radiographed using a digital radiograph machine (TCA 4R PLUS). The standard orientation of the bones was achieved by letting the humerus balance on the horizontal plane, with the anterior surface facing the radiograph camera. The radiographic table was placed at a distance of 54 cm from the head of the camera.

Within the arbitrary 2D coordinate system created by this orientation, landmarks were defined as extreme points [28,29]. The epiphyseal ends were studied separately. In the first analysis, five landmarks were selected on the radiograph of the proximal humerus as defined in Table 1. The second analysis included seven landmarks on the radiograph of the distal epiphysis as described in the same table. Fig. 1a and b show the selected landmarks on the proximal and distal parts, respectively. Landmarks were digitalised using TPSDIG2 software [30]. Semi-landmarks were used to quantify the relative height of the caput humeri and slid in order to minimise bending energy following standard methods described elsewhere [31–33].

For the quantification of intra-observer variation, the standard procedure has been followed [34,35]. Five specimens were randomly selected and each of them was digitised five times. Principal components analysis was carried out, in order to test the relative position of the repeats with respect to each other and to the other individuals. This test evaluated the magnitude of error precision relative to the differences in shape between these five specimens and within the sample.

3. Geometric-morphometrics

Generalised Procrustes Superimposition GPA [8,36] and thin-plate splines [5,37] are used to obtain Procrustes shape coordinates and shape variables for different statistical analyses. Shape is defined by Kendall [7] as “all the information remaining when location, size and rotational factors are all removed.” More technical details about geometric-morphometric methodologies can be found in Rohlf and Slice [8], Bookstein [5], O’Higgins [10], Adams et al. [11], O’Higgins and Jones [35], Zelditch et al. [37] and Slice [38].

The metrics of the shape space is the Procrustes distance and is approximately the square root of the summed, squared inter-landmark distances of Procrustes registered specimens [4]. Size is measured as centroid size defined as the square root of the summed squared distances between each landmark and the centre of gravity (centroid) of each landmark configuration. It is an individual score obtained as a scaling factor during the partial

Table 1

| Proximal epiphysis | \( Lm1 \) | The projection of the medial and inferior part of the head |
| Distal epiphysis | \( Lm1 \) | The incision point between the medial epicondilus and medial part of the trochlea |

Fig. 1. (a) Landmarks selected on the radiograph of the proximal humerus and (b) landmarks selected on the radiograph of the distal humerus.
Procrustes superimposition [39]. In the absence of allometry, centroid size can be considered uncorrelated to shape [5,37].

4. Statistical analyses

Mean comparisons between males and females were carried out using a permutation model of multivariate analysis of variance (MANOVA) of Procrustes shape data [8]. In these analyses, the a priori assigned group membership was permuted by chance (N = 1000), and the frequency assessed as to how often a Procrustes distance equal or larger than that actually observed has been achieved between the means of the permuted group. This ratio gives a distribution-independent estimate of the significance of the observed mean shape differences between males and females and was performed using Morpheus and co-workers [40]. More methodological details can also be found in Fontaneto et al. [41]. The associated differences in female and male mean shapes are visualised using thin-plate splines transformation grids [5] transforming the female mean shape into the male or vice versa. In addition, to aid the identification of the morphological differences, thin-plate splines are used to warp the pixels of the digital radiograph images [42].

First, the image of the overall consensus is calculated using images and landmarks of the full sample. Then, consensus image and landmarks are unwarped into an exaggerated shape representing the female and the male mean shape, respectively. These warpings are calculated using tpsSUPER [42]. As a result of this, radiographs that visualise shape features of female and male epiphyses are obtained.

Then, three discriminant function analyses were carried out. One using the PC-scores from Procrustes shape space, a second using centroid size alone and a third using PC scores of GPA residuals plus lnCS for analysis in Procrustes form space [35,43,44].

In order to find the optimal combination of variables that best discriminate sexes, the all subset method was used. When P predictor variables are available to predict a dependent variable Y by regression, there are altogether $2^P$ such binary choices, making $2^P$ combinations. That includes the null regression that contains no predictors, and the full regression containing all P predictors. The optimal combination of P predictor variables can only be found by testing all $2^P$ combinations.

The distributions of females and males in these statistical spaces of reduced dimensionality, as implied by the choice of different principal components analysis (PCA) axes, are explored via SPSS. Jackknife procedures [37,45] are carried out for cross-validation of the groupings.

5. Results

5.1. Digitalising error

The five repeats were submitted to a PCA, which showed that in all cases (proximal as well as distal epiphyses) the repeats are much closer to themselves than to other individuals or their repeats. The percentage of variance, which is explained by digitising error, was also calculated according to Cardini and Elton [46]. The ratio was computed for all repeats separately and the percentage varied from 0.75% to 3.1% for the proximal humerus an average of 1.9%) and from 3.3% to 8% for the distal humerus (an average of 4.8%).

6. Proximal humerus

6.1. Shape analysis

The PCA includes eight principal components that explain 100% of the shape variability in the proximal humerus. The first two principal components of this analysis are plotted in Fig. 2a. PC1 (horizontal axis) accounts for 48.6% of the shape variability while PC2 (vertical axis) explains 23.2% of the variability.

The MANOVA permutation test showed that the shape differences due to sex dimorphism are statistically significant at the level of $p < 0.044$.

The first six non-zero principal components of form space (accounting for 100% of variance) are used as independent variables.

![Fig. 2. Plots of the first two principal components of PCA in proximal humerus: (a) shape–space, (b) form–space and distal humerus, (c) shape–space and (d) form–space. Note that there is a clear separation of sexes in both proximal (b) and distal (d) end when form variables are used.](image-url)
in order to identify sex. Several different combinations were calculated according to all subset method and the best included four PCs (2, 3, 4 and 5) following a direct procedure (Wilks' lambda = 0.796, p < 0.0001). Classification accuracy was 75% for the original sample while leave-one-out classification yielded 73%.

Multiple regression of shape using all six PCs revealed that approximately 5% of the total variance is explained by sexual dimorphism.

Fig. 3a and b provides deformation grids for males and females. Observing the two grids one can note that the shape differences are mainly distributed between landmarks 2, 4 and 5. More specifically, in females there is an expansion of the grid between landmarks 2 and 4, which corresponds to the relative position of the great tubercle and the projection of the groove of the anatomical neck. Additionally, there is compression between landmarks 4 and 5, which indicates that the most superior point of greater tubercle is relatively closer to the axis defined by landmarks 1 and 5. Furthermore, there is an expansion on the grid between landmark 3 and the middle point between landmarks 1 and 2 on females compared with males indicating a relatively more voluminous caput in males.

Fig. 3 provides an average image for females (f), males (h) and the entire group (g) for the proximal end of the humerus.

6.2. Size analysis

In order to determine sex, a discriminant function analysis (DFA) using centroid size is performed (=156.183, Wilks’s lambda =0.375). Demarking point is 50.82. Therefore, values of centroid size greater than that indicate a male individual, while smaller values are assessed as female. Classification accuracy reaches 84% for males and 89.1% for females. The cross-validation procedure gives exactly the same results (Table 2).

Table 2
Classification accuracy using shape, form variables and centroid size for the proximal and the distal humerus.

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<thead>
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<tr>
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* PC 2, 3, 4 and 5.
* PC 1 and 4.
* PC 1, 6 and 8.
* PC 1, 3, 6 and 9.
6.3. Form (size and shape) analysis

The PCA of form space extracted nine principal components that explain 100% of the shape variability. Fig. 2b plots the first two principal components of these analyses. More specifically, PC1 (horizontal axis) accounts for 64.3%, while PC2 (vertical axis) explains 9.1% of the variability; in this subspace that accounts for most of the variation in the current study. There is a clear separation of the two groups in the direction of the horizontal axis, which indicates that sexual dimorphism is mainly based on size differences.

The first seven non-zero principal components of form space (accounting for 99.9% of variance) are used as independent variables in order to identify sex. Classification accuracy for direct analysis using all seven PCs is 90.6% while leave-one-out classification yields 88.5%. Using the all subsets method, PC1 and PC4 were selected as the optimal combination of variables, giving 89.6% of classification accuracy for both original and cross-validated data (Table 2).

7. Distal humerus

7.1. Shape analysis

The PCA includes 10 principal components that explain 100% of the shape variability. The first two principal components of this analysis are plotted in Fig. 2c. PC1 (horizontal axis) accounts for 31.78% of the shape variability while PC2 (vertical axis) explains 17.02% of the variability. Sexual dimorphism is not associated with either of these principal components and thus the two groups cannot be separated visually on scatter plots in this projection of shape space.

After a GPA, data are submitted to a MANOVA permutation test. The shape differences due to sex dimorphism are statistically significant at the level of $p < 0.003$. This means that of the 1000 permutations, for only two times was the Procrustes distance equal or larger than the observed distance.

The discriminant function analysis (DFA) for the distal humerus yields a significant difference of shape between the sexes using PC 1, 6 and 8 (Wilks’s lambda = 0.796, $p < 0.0001$). Ten males and 15 females were misclassified by the DFA; the classification accuracy for both groups reached 74.2% for original and 71.1% for cross-validated data (Table 2). Multiple regression of shape using all PCs indicates that approximately 5% of the total variance is explained by sexual dimorphism.

Fig. 4a and b provides deformation grids for males and females. There is a deformation of the grid of the lateral trochlea, which corresponds to the relative expansion of the grid between landmarks 3 and 4 in the male configuration. Additionally, a relative compression of the grid between landmarks 4 and 5 is observed, reflecting a relatively smaller capitulum with respect to the trochlea. Furthermore, the grid between landmark 6 and 7 is

![Deformation Grids](image-url)
expanded in the male configuration, suggesting a relative elongation of the distance between the two most lateral landmarks of the epiphysis in males. As a consequence of these relative changes, the female configuration is more square-shaped while the male configuration follows a more rectangular pattern.

Fig. 4 also provides an average image with the grid adjusted to the corresponding landmarks for females (c), males (e) and the entire group (d) for the distal end of the humerus.

7.2. Size analysis

DFA using centroid size is also applied for the distal end (F = 126.689, Wilks's lambda = 0.428). The demarking point is 55.87. Therefore, values of centroid size greater than that indicate a male individual, while smaller values are assessed as female individual. Classification accuracy reaches 80% for males and 91.5% for females. The cross-validation procedure gives exactly the same results (Table 2).

7.3. Form (size and shape) analysis

The PCA for form space extracted 13 principal components that explain 100% of the shape variability. Fig. 2d plots the first two principal components. PC1 (horizontal axis) accounts for 69%, while PC2 (vertical axis) explains only 9.1% of the variability. In this subspace, this accounts for most of the variation in the distal humerus. Again, there is a clear separation of the two groups in the direction of the horizontal axis which indicates that sexual dimorphism is mainly contributed by size differences.

The first 11 non-zero principal components of form space (accounting for 99.9% of variance) are used as independent variables in order to identify sex from the distal humerus. Classification accuracy for direct analysis is 89.7% while leave-one-out classification yields 87.6%. All subsets DFA revealed the 4 PCs (PC 1, 3, 6 and 9) that give the optimal group separation. Classification accuracy yielded 89.7% for the original and 88.7% for the cross-validated data (Table 2).

8. Discussion

The recovery of fragmentary skeletal remains, in forensic investigations, requires easy and rapid techniques for biological profiling and reconstruction of the scene history. The first and most vital biological characteristic under consideration is sex since it reduces the number of possible matches in the population by 50%. Although sex identification can be easily established when a complete skeleton is present, this is rarely the case in forensic investigations where mostly fragmented bony parts are recovered.

According to France [47], distal measurements are likely to reflect more sexual dimorphism in the humerus because this bone is subjected to greater functional or occupational stress. Scholars agree that epiphyseal structures tend to be more dimorphic than long [24,48]. Reviewing the current literature, one can note that the best discriminatory measurement varies in different samples. Proximal epiphysis has given more accurate results in populations from Guatemala [21], Germany [23], China [22] and South Africa [25]. On the contrary, studies of two different Japanese [22,24] populations and a Thai population [22] concluded that the distal part is more effective than the proximal. In all cases though, epiphyseal structures were included in the more effective three dimensions. The study of the osteometric data of the Cretan population concludes that proximal epiphysis is the most dimorphic part with classification accuracy of 89.9% while the distal epiphysis is ranked third along with length (85.1%) [49]. However, this is a very small difference which can be reversed by simply adding more specimens.

Apart from the classical osteometric studies, sexual dimorphism of the skeleton was also investigated by means of radiographs and computed tomography. Riepert and associates [50] studied sexual dimorphism in radiographs of the calcaneus, achieving 80% of correct group membership. Patil and Mody [51] accomplished sex identification from lateral cephalograms with an accuracy of 99%. A recent study on digital radiographs of the femur yielded classification accuracy up to 92.9% [52]. Additionally, Harma and Karakas [53] predicted sex with 84.6% accuracy by using computed tomography (CT) scans of femora derived from hospital patients. It seems that radiography can be quite successful in sex identification, apart from its acknowledged value in positive identification and age estimation [54–57]. Nevertheless, to our knowledge, no study deals with digital radiographs of the humerus.

Sexual dimorphism of the humerus has been studied so far in terms of size. One must consider though that sex dimorphism is also expressed in shape. However, there is a lack of evidence for this concept [58]. An exception is a shape analysis of the humerus in a Portuguese sample using transformed indices derived from osteometric data [20]. The authors of this study conclude that excluding size (which explains 80% of the observed variability), men tend to have relatively shorter humeri with voluminous epiphyses while women have relatively longer shafts with smaller epiphyses, in the given population. This is consistent with the findings of our study (Fig. 3).

Another work by Lague and Jungers [58] deals with the shape of hominoid distal humeri using geometric-morphometrics. Although not the principal goal of this study, sexual dimorphism was mentioned in the results. It was found that the sexes of the American Whites and African-Americans showed a mixed pattern of affinities with the males of each group to be closer in shape to the females of the other group. Yet these results were not feasible in establishing shape criteria for the assessment of sex.

The objective of the current study is to validate the efficacy of the geometric-morphometric method in sex identification of humeral radiographs. The existence of sexual dimorphism in the humerus is well known and is mainly attributed to size differences [19,20,22,23,25,59]. This is consistent with our results. The existence though of shape differences is worthy of further investigation.

Observing the plots of the deformation of mean male and mean female proximal radiographs, one can note shape differences in the projection of the greater tubercle clearly and the superior border of the anatomical neck. In females, the greater tubercle is smoother with its superior border less pronounced. This observation could simply reflect the relatively weaker development of the supraspinatus muscle and consequently its insertion in females compared to males.

On the distal end, the male configuration is rectangular while the female configuration is square, probably due to the relatively wider epiphyseal breadth in males. A relative wider lateral trochlea, accompanied by a relatively smaller capitulum in males with respect to females, has also been observed (Fig. 3). These observations could be related to shape differences of the elbow articulation, but in order to confirm this interpretation further investigation is required.

Taking into account factors such as occupational stress and pathology, which could not be entirely controlled in this study, the humeral shape needs additional research. Furthermore, our study sample consists of individuals with high mean ages; thus age-related factors may affect differences in shape. Therefore, one must be cautious when anatomical interpretation is attempted.

Shape differences between males and females give slightly better classification results in proximal (75%) compared to the distal humerus (73%), which is opposite to France's results [47]. However, these differences are too small to lead to any definite
conclusion. As anticipated, classification accuracy improves when both size and shape are applied jointly. In a recent study of sexual dimorphism in American skulls, the authors concluded that the combination of size and shape gives more accurate results than shape alone as also the application classical osteometric techniques on the same population [13]. In our study, the combination of form variables performed well with classification accuracies reaching 90% for both epiphyses. Whether this is statistically better than simply using centroid size needs to be tested in a proper statistical approach.

The analysis of humeral radiographs by geometric-morphometric techniques offers an alternative way to identify the sex of unknown skeletal remains. Size differences between sexes have long been acknowledged and confirmed by the results of this study. Thus, the new conclusion derived from this investigation is the existence of shape differences between sexes as they are reflected in the radiographs of the humeral epiphyses. The combination of shape and size characteristics seems to better the results based on the analysis of each one of them independently. However, this is a method which requires a background in a complex statistical theory. Hence, its superiority compared to classical osteometric studies or the use of centroid size alone cannot be supported by the findings of this study without further meta-statistical analysis. A follow-up study using inter-landmark distances will allow the evaluation of the efficiency of this technique in forensics. Nonetheless, the current method can be applied successfully to approximate sex for forensic purposes and could also be applicable in the archaeological context.

9. Conclusion

From the forensic standpoint, the usefulness of this study rests on the estimation of sex from radiographs of fragmentary humeri. The use of radiographs instead of the actual bone allows the identification of semi-decomposed bodies without the need of special preparation (e.g., maceration), thus facilitating the whole medicolegal investigation. The application of geometric-morphometrics in humeral radiographs has proven to be successful, since it reveals shape differences that could not be assessed with conventional techniques and allows a combination of size and shape for the identification of sex. A follow-up study using inter-landmark distances is necessary to test whether Procrustes form space is indeed more advantageous in sex identification and worthy to be employed in similar studies.

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