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Forensic Sexual Dimorphism from the First Cervical Vertebra (C1) in the Omani Population: A 2-Dimensional Geometric Morphometric Approach

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Abstract

Background: Accurate sex estimation from skeletal remains is essential in forensic identification, yet population-specific reference data for cervical vertebrae, particularly the first cervical vertebra (C1), are limited. This lack of data compromises the accuracy of sex estimation in forensic contexts where primary skeletal elements such as skull and pelvis are unavailable. Geometric morphometric (GM) analysis offers a robust alternative by quantifying shape variation independent of size, enabling precise assessment of morphological differences that conventional linear measurements may overlook. **Objective:** This study aimed to investigate the utility of C1 vertebra morphology for sex estimation among Omani adults using two-dimensional geometric morphometrics. **Methods:** A total of 320 lateral cervical radiographs (160 males, 160 females) were retrospectively collected from Sultan Qaboos University Hospital. Six anatomical landmarks on the atlas were digitized using TPSDig2, and shape analyses were performed using MorphoJ software. Statistical methods included Principal Component Analysis (PCA), Procrustes ANOVA, Canonical Variate Analysis (CVA), and Discriminant Function Analysis (DFA). Intra- and inter-observer reliability was also assessed. **Results:** While centroid size did not significantly differ between sexes ($p = 0.2432$), shape analysis revealed statistically significant sexual dimorphism ($p = 0.0457$). DFA correctly classified 77.19% of individuals by sex, with a cross-validated accuracy of 73.44%. Shape variation was most pronounced in the posterior tubercle and superior facets of the atlas. **Conclusions:** Two-dimensional geometric morphometric analysis of the first cervical vertebra (C1) reliably captures sexual dimorphism in the Omani population, providing a population-specific reference that enhances the accuracy and objectivity of forensic sex estimation.

Keywords: sexual dimorphism; first cervical vertebra (C1); geometric morphometrics; forensic anthropology; Omani population

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1. Introduction

Sex estimation is a foundational aspect of building a biological profile, serving as a critical first step in narrowing the pool of potential identities and facilitating positive identification. In practice, the pelvis and skull are regarded as the most reliable skeletal elements for this purpose due to their pronounced sexual dimorphism. However, in many forensic and medico-legal scenarios, these bones are often absent, fragmented, or incomplete, particularly in cases involving decomposition, trauma, or mass disasters. Such limitations underscore the need to identify alternative skeletal elements that can provide dependable indicators of sex, especially those that are more likely to be preserved [1].

The first cervical vertebra (atlas, C1) presents a potentially valuable yet underexplored structure for sex estimation. Its distinctive ring-shaped anatomy, consisting of two lateral masses connected by anterior and posterior arches, distinguishes it from other cervical vertebrae [2]. Its resilience and distinct anatomical features suggest that it may exhibit subtle but consistent sex-related differences arising from variations in biomechanics and overall skeletal robusticity [3]. Despite these factors, the atlas has not been as extensively studied as other skeletal regions, and its utility in sex estimation remains insufficiently established, particularly within specific populations. For comparison, studies on the second cervical vertebra (C2) have reported sex estimation accuracies ranging from approximately 68% to over 91% across populations such as Australia and India [2,4,5], highlighting the potential value of further investigation into C1.

Population specificity is a well-recognized factor influencing the accuracy of forensic anthropological methods [6]. Skeletal morphology is shaped by genetic, environmental, and lifestyle factors [7] leading to measurable differences across populations [8]. Consequently, methods developed in one population may not be directly applicable to another without validation. Within the Middle Eastern region, and specifically among the Omani population, there is a notable lack of research addressing sexual dimorphism of the cervical vertebrae. Challenges remain due to interpopulation differences and the potential for misclassification, emphasizing the importance of developing population-specific standards and continuous methodological refinement [9]. This gap limits the availability of appropriate reference standards for both forensic and clinical applications in the region.

Methodologically, most previous investigations into vertebral dimorphism have relied on conventional linear measurements [4,10]. While such approaches are straightforward and reproducible, they reduce complex anatomical structures into simplified dimensions, potentially overlooking subtle variations in shape. Geometric morphometric techniques offer an alternative by enabling the analysis of shape as a whole, preserving the spatial relationships among anatomical landmarks [11]. The use of two-dimensional geometric morphometrics (2D-GM) in the present study was primarily motivated by its practical applicability, accessibility, and compatibility with routinely acquired radiographic images in both forensic and clinical environments. Two-dimensional geometric morphometrics, in particular, provides high reliability, a comprehensive assessment of bone morphology, and consistency through the use of homologous landmarks across specimens, facilitating precise and reproducible analyses [12]. When applied to two-dimensional radiographic images, these methods provide a practical and non-invasive means of capturing morphological variation, making them particularly suitable for retrospective analyses of existing clinical data and potential use for forensic radiology. 2D-GM remains widely utilised in forensic anthropology due to its lower cost, reduced technical requirements, ease of standardisation, and applicability to retrospective radiographic datasets where advanced 3D imaging is unavailable [13–15]. Furthermore, when radiographic acquisition is standardised and anatomical landmarks are carefully selected, 2D approaches are capable of capturing

biologically meaningful shape variation and have demonstrated utility in sex estimation studies involving cranial and postcranial structures.

Given these considerations, a clear gap exists in the current literature. There is a need for population-specific studies that explore the potential of the atlas as a sex estimation tool using methods capable of capturing its complex morphology. The absence of such data is particularly evident for the Omani population, where no established models currently exist for sex estimation based on C1. This study aims to assess the utility of 2D geometric morphometric analysis of the C1 vertebra as a sex estimation tool in an Omani population. The results will contribute to developing region-specific forensic standards and enhance the application of forensic anthropology in Oman, offering a valuable tool for identification efforts when primary identifiers are unavailable.

2. Materials and Methods

2.1. Data Collection

This retrospective, cross-sectional study of lateral skull radiographs acquired from the Radiology and Molecular Imaging Department at Sultan Qaboos University Hospital (SQUH) between 2015 and 2021. Radiographic images were saved in Joint Photographic Experts Group (JPEG) and Digital Imaging and Communications in Medicine (DICOM) file formats to facilitate a comprehensive examination of the C1morphology. Ethical clearance for the study was provided by the SQUH Medical Research Ethics Committee (MREC) (MECID no: 2022119-10937).

X-rays came from regular patient visits, with head placement following clinic rules meant to match how people naturally hold their head. Still, because the study looked back at old records, it was not possible to confirm exact head angles every time, which counts as a weakness. To help balance differences in posture, readings used clear points on skull images taken from the side, even if tiny shifts might remain.

2.2. Study Population

In total, 320 Omani adults (160 males and 160 females), aged 18 to 65 years, who had cervical spine imaging at Sultan Qaboos University Hospital (SQUH) between 2015 and 2021 formed the study sample. All subjects were skeletally mature by age ($\geq$18 years). Only Omani nationals with clear documentation of their sex and good quality lateral radiographs showing adequate visualisation of the first cervical vertebra (C1) were included.

All radiographs in this study were acquired under standardized imaging conditions and protocol. The head was positioned in Natural Head Position (NHP), defined as the subject standing or seated upright with the visual axis aligned to a horizontal plane at eye level, allowing the craniovertebral junction to assume its physiological resting alignment. This approach was adopted because variation in head flexion, extension, or axial rotation can introduce projectional distortion of the atlas morphology, particularly affecting the apparent shape and spatial relationships of the lateral masses and anterior–posterior arches in two-dimensional radiographs.

The exclusion criteria were: (1) cervical spine trauma; (2) congenital abnormalities of the atlas; and (3) degenerative or pathological changes that can affect morphology of C1. Radiographs were retrieved retrospectively from the database of Radiology and Molecular Imaging Department, SQUH.

A biostatistician helped to estimate the sample size using a two-sample mean comparison with a 5% margin of error and 95% confidence interval, which resulted in a required sample size of 404 participants. However, due to data availability and strict inclusion criteria, a total of 320 eligible cases were included with equal proportion of males and females to minimise classification bias.

The sample was sex-balanced but derived from one tertiary care center and may not represent the demographic and regional diversity of the general Omani population. This potential limitation has been taken into account in the interpretation of the findings.

2.3. Materials

Landmark digitization was conducted using TpsDig2 software (Version 2.31; F.J. Rohlf, Stony Brook University, USA, 2015), and subsequent shape analyses were carried out via MorphoJ (Version 1.07a; C.P. Klingenberg, University of Manchester, UK, 2011) [16] and GraphPad Prism (Version 9; GraphPad Software, San Diego, CA, USA, 2021). Additional software applications, such as Inkscape, Microsoft Excel, and Notepad++, supported data organization and figure preparation.

2.4. Landmarking Application

A geometric morphometric approach using two-dimensional coordinates derived from anatomical landmarks on the atlas (C1) was conducted. Six two-dimensional anatomical landmarks were manually placed on lateral skull radiographs utilizing TPS Dig2 (Version 2.31). Landmark selection was adapted from established morphometric protocols successfully applied in both traditional and geometric contexts [13,14,17]. Table 1 provides comprehensive landmark definitions, while Figure 1 visually illustrates their anatomical locations.

Table 1. Landmark definitions of cervical vertebra on lateral cervical radiograph.

Cervical Vertebra	Anatomical Landmark (L)	Definition	Landmark Type
First cervical (C1)	L1	The most anterior point of the vertebra	II
	L2	The most anterior and superior point of the vertebra	II
	L3	The most posterior and superior point of the vertebra	II
	L4	The most posterior point of the vertebra	II
	L5	The most posterior and inferior point of the vertebra	II
	L6	The most anterior and inferior point of the vertebra	II

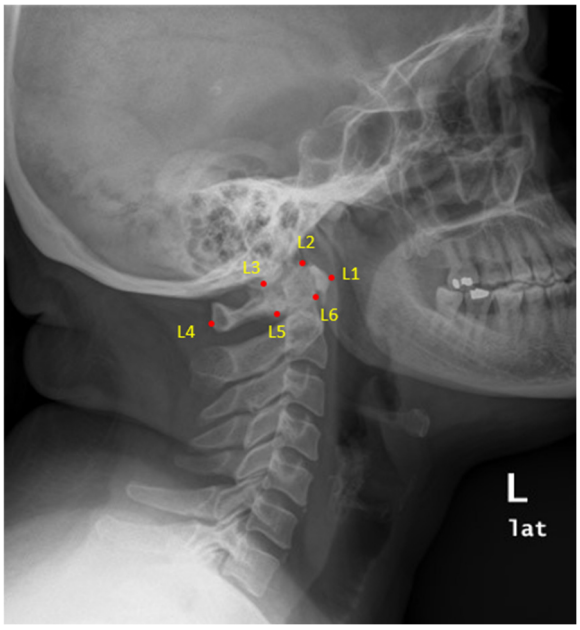


Figure 1. Lateral cervical radiograph illustrating anatomical landmarks of the C1 vertebra.

2.5. Intra- and Inter-Observer Analysis

To enhance measurement precision, all landmark placements were reviewed in collaboration with a radiologist [18]. Intra-observer repeatability was assessed using a pilot analysis, wherein the primary examiner performed landmark digitization on 40 radiographs on two separate occasions (2 weeks interval). Then, paired *t*-tests were employed to detect significant differences between repeated measures [18]. This washout period is consistent with best practices in morphometric research, ensuring that repeated measurements are independent and not influenced by recall of prior landmark placement. For inter-observer reliability, two independent assessors digitized landmarks on 40 radiographs, and outcomes were evaluated through independent samples *t*-tests.

2.6. Statistical Analysis

Geometric morphometric analyses were conducted using landmark coordinate data obtained from the two-dimensional radiographic images of the first cervical vertebra (C1). Two-dimensional geometric morphometric techniques were executed in MorphoJ (version 1.07a), including Generalized Procrustes Analysis (GPA), Principal Component Analysis (PCA), Procrustes ANOVA, Canonical Variate Analysis (CVA), and Discriminant Function Analysis (DFA). Prior to statistical analysis, landmark configurations were subjected to Generalized Procrustes Analysis (GPA) to remove non-shape variation associated with translation, rotation, and scale, thereby standardising all specimens into a common coordinate system. PCA was utilized to condense the dataset's landmark variables into principal components, elucidating primary sources of morphological variability [19]. Procrustes ANOVA assessed size and shape disparities among different age groups [16], while CVA examined the differentiation of shape characteristics within the sample population. To evaluate sexual dimorphism and classification performance, discriminant function analysis (DFA) was performed using the Procrustes shape coordinates as predictor variables. The classification procedure was designed to assess the ability of C1 morphology to correctly assign individuals to their respective sex categories based on geometric shape differences. To minimize overestimation of classification performance and improve generalizability of the model, cross-validation was applied using a leave-one-out cross-validation (LOOCV) approach, whereby each specimen was iteratively classified using a model generated from all remaining specimens excluding the test individual. Classification accuracy was therefore calculated based on cross-validated predictions rather than training data alone [15].

3. Results

3.1. Demographic Data

A total of 320 lateral cervical radiographs were retrieved from the database. The data comprises 160 male patients (50%) and 160 female patients (50%) of the Omani population (Table 2).

Table 2. Demographic characteristics of the study population (N = 320).

Sex	N (%)
Male	160 (50%)
Female	160 (50%)

3.2. Intra and Inter-Observer Analysis

There were no significant differences ($p > 0.05$) in terms of Procrustes coordinates of all six landmarks in either intra-observer or inter-observer analysis, manifesting the consistency of landmarking application by the author (Table 3).

Table 3. T-test results in the intra-observer and inter-observer error of the C1 vertebra (Note: $p > 0.05$).

Intra-Observer Landmarks	*p* Value	Inter-Observer Landmarks	*p* Value
L1	0.9689	L1	0.1185
L2	0.4220	L2	0.7423
L3	0.8054	L3	0.1453
L4	0.9506	L4	0.0514
L5	0.2379	L5	0.7726
L6	0.3752	L6	0.0993
L6	0.5927	L6	0.0977

3.3. Generalized Procrustes Analysis

Figure 2 shows a scatterplot of the superimposed landmark configurations, indicating the general C1 vertebra morphological shape from six landmarks of 320 cervical vertebral radiographs.

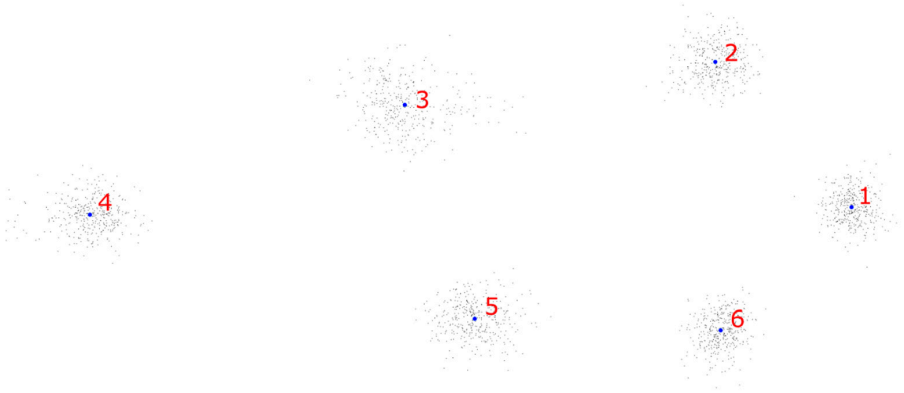


Figure 2. Generalized Procrustes analysis showing a scatter plot of the superimposed 6 sets of C1 landmark configurations; blue dots represent the mean landmarks, and small black dots represent landmarks of individuals from the samples.

3.4. Principal Component Analysis

PCA yielded eight principal components (PCs) that together accounted for 100% of the shape variation observed in the first cervical vertebra (C1). As illustrated in Figure 3, the majority of the variance was explained by the first three components. Specifically, PC1 contributed 36.36% of the total variation, followed by PC2 at 16.16%, and PC3 at 13.44%, resulting in a cumulative variance of 65.95%. These components were derived using MorphoJ software.

Lollipop plots displayed the relative displacement of landmarks from the average C1 configuration, represented by blue dots. Landmark 4 (posterior arch) exhibited the most noticeable variation in PC1, moving posteriorly, while landmarks 2 and 3 (superior anterior arc) showed slight superior displacement. In PC2, changes were primarily observed at landmark 4, shifting upward, and at landmark 1, showing anterior movement. PC3 variation was concentrated around landmarks 5 and 6 (inferior arc), suggesting lateral expansion.

Shape changes were also visualised using wireframe graphs. The light blue outline denoted the mean vertebra shape, whereas the dark blue outline represented variation along each principal component. Among the components, PC1 revealed the greatest degree of shape expansion in the posterior and superior regions of the vertebra, distinguishing it from PC2 and PC3 (Figure 4).

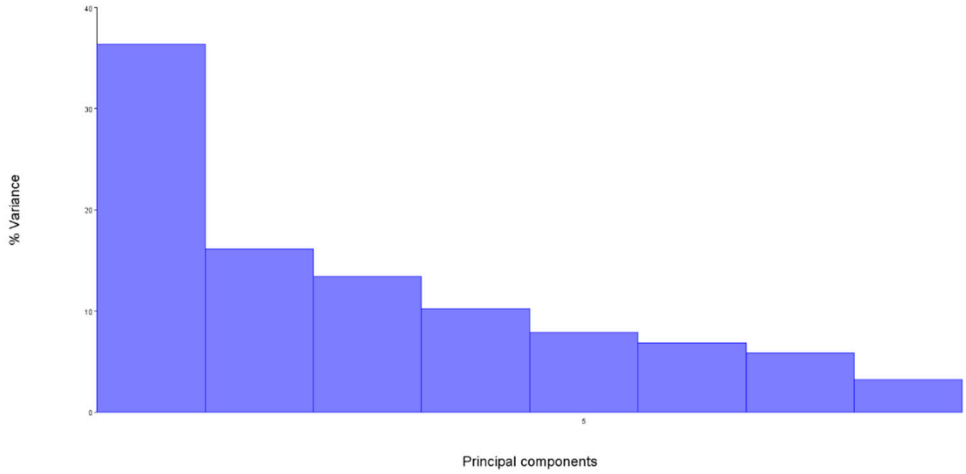


Figure 3. Histogram showing principal component distribution of C1.

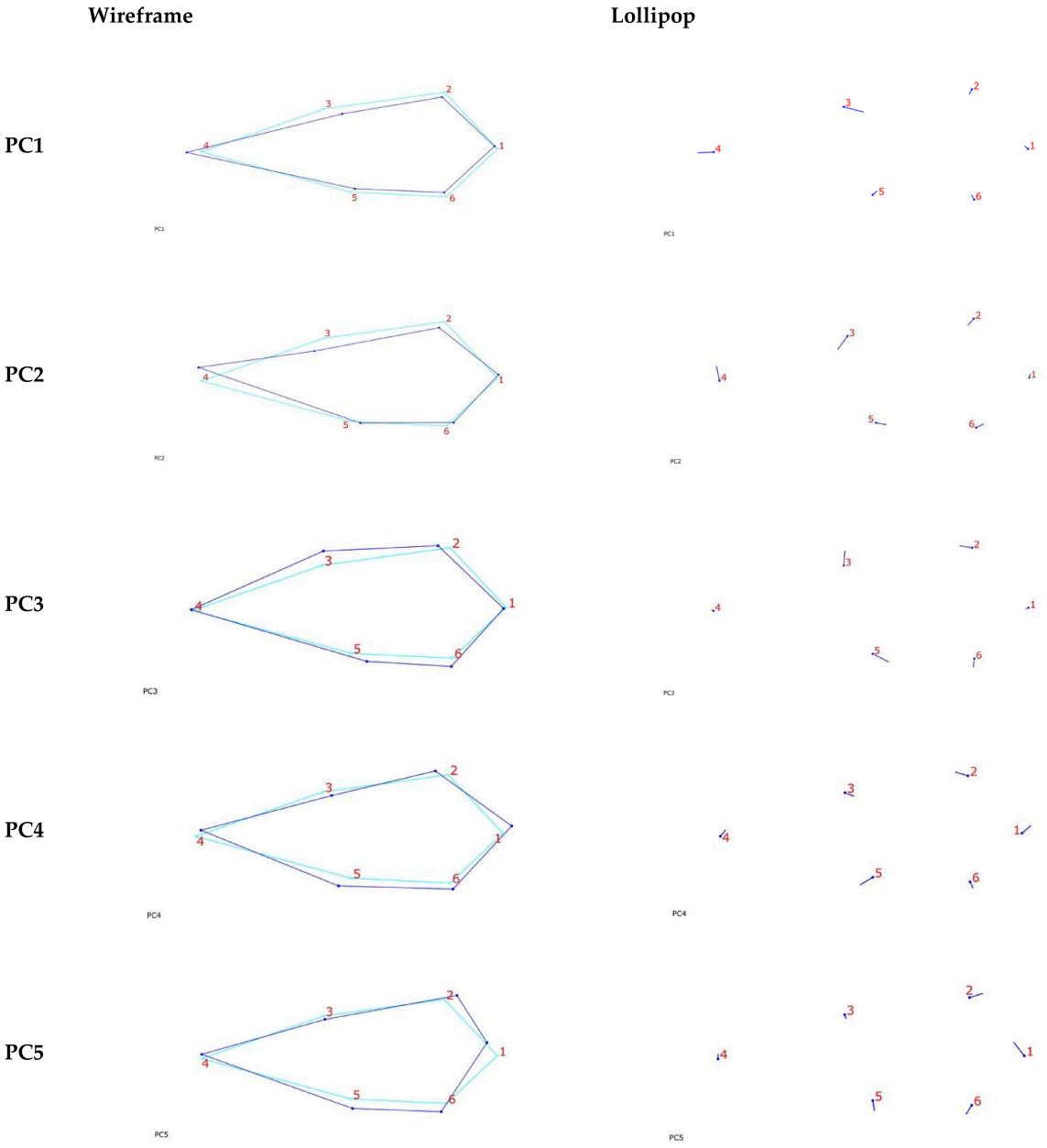


Figure 4. Lollipop (**right**) and wireframe (**left**) graphs of the first five PCs.

3.5. Procrustes ANOVA

In this study, there was no significant difference in the centroid size of the first cervical vertebra between males and females ($p = 0.2432$), with Goodall's F statistic indicating low variation ($F = 1.44$). However, there was a significant difference in the shape of the C1 vertebra between sexes ($p = 0.0457$, $F = 1.56$) (Table 4).

Table 4. The first cervical vertebra's centroid size and shape by sex.

Procrustes ANOVA	SS	MS	df	F	*p*-Value
Centroid size	3569.3376	3369.3356	1	1.44	0.2432
Shape	0.0162	0.00226	8	1.56	0.0457

Procrustes ANOVA; $p < 0.05$ is significant; SS is Sum of Squares; MS is Mean Squares; df is Degree of freedom; F is Goodall's statistic.

3.6. Discriminant Function Analysis

In this study, 77.19% of cases were accurately classified by sex, with the rate slightly decreasing to 73.44% after cross-validation. Among males, 78.13% were correctly identified, dropping to 71.88% following cross-validation. For females, classification accuracy decreased from 76.25% to 75.00% after cross-validation. These findings are summarized in Table 5. Figure 5 illustrates the discriminant function analysis comparing male and female groups after cross-validation.

Table 5. Discriminant function analysis with cross-validation of sex.

	Male		Female		Total	Original (%)	Cross Validation (%)
	Original	Cross Validation	Original	Cross Validation			
Male	125	115	35	45	160	78.13	71.88
Female	38	40	122	120	160	76.25	75.00
			Average (%)			77.19	73.44

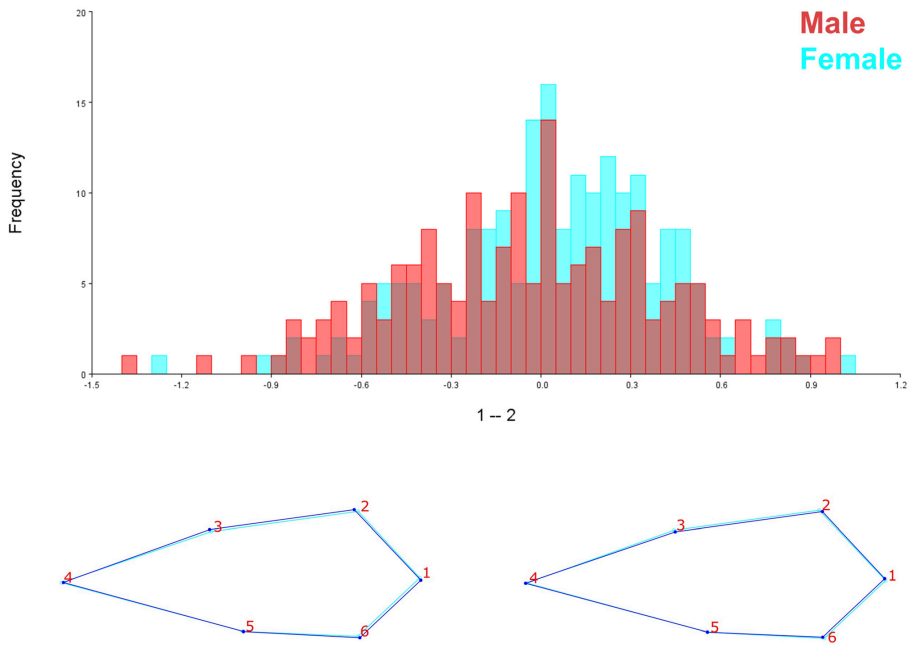


Figure 5. Histogram of DFA for pairwise comparison between males and females after cross-validation.

4. Discussion

The present study evaluated sexual dimorphism of the first cervical vertebra (C1) in an Omani population using a two-dimensional geometric morphometric approach applied to lateral cervical radiographs. The findings demonstrate that the atlas exhibits measurable shape differences between males and females, supporting its potential utility as an alternative skeletal element for sex estimation when more commonly used structures are unavailable.

Procrustes ANOVA showed that there was a statistically significant difference in the C1 vertebra's shape between the sexes ($p = 0.0457$). This suggests that, after removing the effects of scale, orientation, and position, the configuration of anatomical landmarks on C1 retains sex-specific patterns. However, male and female centroid sizes did not differ significantly ($p = 0.2432$). The Procrustes ANOVA results indicate that sexual dimorphism in the atlas is expressed primarily through shape rather than centroid size. This is similar to what is published from the Greek population which shows a significant sex-based variation in the C1 vertebra [13]. However, size is shown to be a differentiating factor in the Malaysian and Brazilian populations [20,21]. This is an important observation, as it indicates that sexual dimorphism in C1 is not driven by general skeletal robusticity alone, but rather by more subtle morphological variations in form. Shape-based analyses, which capture the relative positioning and geometry of anatomical features, appear to be more sensitive in detecting sex differences in C1 of this population. This finding reinforces the value of geometric morphometric approaches, as such differences would likely remain undetected or underestimated using conventional linear measurements that emphasize absolute dimensions.

Sex was correctly classified in 73.44%. of cases using discriminant functional analysis (DFA) after cross-validation. From a forensic standpoint, an accuracy in the range of approximately 70–77% is considered acceptable for supplementary indicators. However, it is important to interpret these results within the appropriate forensic anthropological context, where skeletal analysis is inherently probabilistic rather than absolute. In forensic practice, particularly in disaster victim identification (DVI) and forensic casework involving commingled or fragmentary remains, the most sexually diagnostic skeletal elements such as the pelvis are frequently absent or severely compromised. Under such conditions, alternative anatomical structures, including the C1, become essential sources of biological information. Previous forensic anthropological studies have similarly demonstrated moderate classification accuracies for cervical vertebra-based sex estimation, reinforcing that such skeletal regions should be considered as complementary rather than primary indicators of sex. Similar to the Omani study, Marino's research indicated that the centroid size alone may not be a valid metric to estimate sex, attaining an accuracy of 77–85% with shape metrics of the first cervical vertebrae [22]. The considerable shape variability noted within the Omani study supports the findings of Sertel Meyvaci and colleagues who indicated an accuracy estimate of 78.8% with anatomical measurements of the first cervical vertebrae in the Turkish population [23]. Fauad and colleagues studied the Malaysian population with C1 classification accuracy of 80%, which is similar to a study on the Brazilian population with an accuracy of 81.2% [20,21]. Furthermore, DFA from European populations revealed a classification accuracy of 74% in males and 79.5% in females [17]. The observed variation in gender classification accuracy of cervical vertebrae could be a result of inter-population variation.

The findings of the present study are compared to both traditional morphometric approaches and three-dimensional geometric morphometric methodologies used in forensic sex estimation from C1 vertebrae. Conventional metric analyses of the atlas vertebra have primarily focused on linear dimensions, including transverse diameter, sagittal diameter,

vertebral canal dimensions, and measurements of the lateral masses, with reported classification accuracies generally ranging between approximately 70% and 90%, depending on the studied population, sample size, and statistical approach employed [24,25]. The classification accuracy observed in the present study (73–77%) is therefore comparable to several previously published cervical vertebra studies and falls within the expected range for morphometric analyses of non-pelvic skeletal elements. While traditional linear metric methods provide relatively simple and reproducible measurements, they may inadequately capture the complex spatial relationships and integrated shape characteristics of the craniovertebral region. In contrast, geometric morphometric approaches analyze the overall landmark configuration and preserve shape geometry, allowing a more comprehensive assessment of morphological variation beyond isolated dimensions [26]. Recent three-dimensional geometric morphometric (3D-GM) studies using computed tomography and surface scanning technologies have reported slightly higher classification accuracies, frequently exceeding 80% [27], likely due to their ability to capture complete spatial anatomy and depth-related morphological variation that may be lost in two-dimensional projections.

Sexually dimorphic shape differences in the first cervical vertebra (C1) may represent ecological adaptations related to function and cranio-cervical relationships. One of the major functions of cervical vertebrae is to support the skull and allow for the head to move. Shape differences may thus reflect adaptations to different loads and movement patterns between the sexes. The morphology of the cervical spine is closely associated with cranial features, and therefore a source of dimorphism in males and females may arise due to evolutionary selective forces on head size and shape [28]. The degree of sexual dimorphism will likely vary among human populations as well, thus highlighting the importance of gaining population reference data when examining anatomical structures in a forensic scenario [1]. Additionally, population-specific morphology may further modulate these differences, as the Omani population may exhibit distinct C1 characteristics arising from genetic background, dietary patterns, and environmental influences. Age-related changes, including degenerative remodeling of articular surfaces, osteophytic development, and alterations in cortical thickness, may introduce additional non-sexual variability that can obscure dimorphic patterns, particularly in adult and older individuals. It should be noted, however, that the relative contributions of sexual dimorphism, population-specific morphology, and biomechanical loading to the observed shape variation cannot be definitively disentangled within the retrospective, cross-sectional design of the present study.

A key strength of this study lies in the application of geometric morphometrics to radiographic data as a means of improving sex estimation accuracy beyond what conventional linear measurements can achieve. By preserving the spatial relationships among anatomical landmarks, this method captures subtle, integrated shape differences that are often overlooked when relying solely on linear or angular measurements [29]. This is particularly important for anatomically complex structures such as the atlas, where variation is not confined to single dimensions but distributed across the entire configuration [28]. Furthermore, the use of lateral cervical radiographs enhances the practical and translational value of the study. Radiography is routinely performed in clinical practice, and in forensic contexts, it forms a core component of post-mortem examinations and Disaster Victim Identification (DVI) protocols, where imaging of skeletal structures is often captured before or during autopsy [30]. This allows forensic anthropologists to analyze skeletal morphology non-invasively. By integrating geometric morphometrics with forensic imaging, this approach offers a reliable, non-destructive, and widely applicable tool that bridges clinical practice and forensic identification, enhancing the potential for accurate sex estimation in both living and deceased individuals. In addition, in clinical and radiological contexts,

morphometric assessment of the upper cervical spine may contribute to the understanding of normal anatomical variation to assist in preoperative planning.

5. Limitations

Despite the strengths of this study, several limitations should be acknowledged. First, the use of two-dimensional lateral cervical radiographs inherently restricts the analysis to a single plane, which may not fully capture the three-dimensional complexity of the atlas. Subtle shape variations along other planes could therefore be missed, and future studies employing CT-based three-dimensional geometric morphometrics would provide a more comprehensive characterization of atlas morphology. Furthermore, the retrospective, cross-sectional radiographic design of this study does not allow us to disentangle the relative contributions of sexual dimorphism, population-specific morphology, and biomechanical loading to the observed shape variation. Future longitudinal or population-comparative studies are needed to address this. The sample size, while sufficient for exploratory analysis, may not fully represent the variability within the Omani population, limiting generalizability. The moderate classification accuracy achieved in this study (73–77%) indicates that 2D geometric morphometric analysis of C1 should not be used as a standalone forensic sex estimation tool. Rather, it should be applied as a supplementary indicator within a multi-element identification framework, particularly in cases where primary skeletal indicators such as the pelvis and skull are unavailable. Furthermore, the absence of external validation on an independent sample means that the reported accuracy estimates may not generalize beyond the present dataset. Future research should focus on increasing sample sizes and incorporating three-dimensional imaging modalities, such as CT scans, and validating the method on independent population samples to confirm its forensic applicability.

6. Conclusions

In conclusion, our results demonstrated a significant sexual dimorphism in the C1 vertebra morphology among the Omani adult population using a 2D geometric morphometric approach. Furthermore, the study supports a broader shift in forensic anthropology towards the use of digital and imaging-based datasets, which enable scalable, non-destructive, and population-specific analyses. Moving forward, efforts should be directed toward integrating this approach into comprehensive identification protocols, refining analytical models through larger and more diverse datasets, and exploring advanced computational techniques to improve classification performance. In this regard, the atlas should be viewed not as a standalone indicator, but as part of an evolving toolkit that leverages multiple skeletal and imaging markers to enhance the accuracy and reliability of forensic identification.

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Informed Consent Statement: Patient consent was waived due to the retrospective nature of the study.

Data Availability Statement: The data is available upon request from the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

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