

RESEARCH ARTICLE

Sexual dimorphism of central midface skeleton: Geometric morphometrics approach on 3D models of human skull

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ABSTRACT

Morphological differences of the skeleton between the genders have long attracted the attention of disciplines concerned with sex determination, leading to the development of various approaches for gender assessment. Geometric morphometrics has recently been introduced as a method for identifying sex based on skeletal features through the application of statistical and mathematical tools. The study was conducted on 3D models of 210 human skulls (139 male and 71 female). Within the central midface skeleton, nine landmarks were selected and marked in the Landmark Editor software. After marking the landmarks, their spatial coordinates were extracted and then imported into the MorphoJ program to analyze shape and size variation between sexes. Principal component analysis, covariance matrix generation, and discriminant function analysis were carried out with sex as the classification variable. The findings indicated that sex estimation based on the size and shape of the central midface skeleton reached an accuracy of 73% for males and 70% for females. The shape of the central midface skeleton exhibited statistically significant sexual dimorphism independent of size. After excluding the influence of facial size and considering only shape variables, geometric morphometrics achieved predictive accuracy of 73% for males and 72% for females.

Keywords: Central midface skeleton, geometric morphometrics, sexual dimorphism, 3D models of human skulls

INTRODUCTION

Morphological differences in skeletal remains between males and females have consistently drawn the interest of scientific fields focused on sex determination, leading to the development of various approaches for establishing biological sex (Sarač-Hadžihalilović et al., 2020). Alongside conventional methods, geometric morphometrics has more recently been adopted for this purpose, relying on skeletal material to improve accuracy. Geometric morphometrics is a technique applied to quantify and evaluate the shape of biological structures, including skeletal elements, through advanced statistical and mathematical procedures (Ajanović et al., 2023a).

The application of geometric morphometrics gained momentum in the latter

half of the 20th century, when researchers began to recognize the limitations of conventional measuring techniques. Traditional approaches did not allow for a detailed exploration of complex morphological variation, as they were unable to precisely capture subtle shape differences. Unlike these earlier methods, geometric morphometrics provides a framework that can accurately describe shape variability, which is essential for understanding processes such as evolution, development, aging, and sexual dimorphism (Ivanović and Kalezić, 2013).

Geometric morphometrics introduced a novel framework where, instead of analyzing individual linear measurements, the spatial coordinates of landmarks are used to define shape with high precision (Hadžiomerović et al., 2023).

A landmark-based system is applied by marking specific, anatomically recognizable points on skeletal structures. Through multidimensional statistical techniques, this approach allows detailed shape assessment, supporting more accurate classification and evaluation of variation both across populations and at the level of individuals (Ajanović et al., 2023).

Geometric morphometrics relies on methods such as Generalized Procrustes Analysis, applied to two- or three-dimensional models. Techniques including Procrustes superimposition, Principal Component Analysis (PCA), and Euclidean Distance Matrix Analysis (EDMA) enable researchers to examine morphological differences related to age, sex, and population groups, independent of scale or orientation. Over time, geometric morphometrics has become an essential methodology in areas such as evolutionary biology and forensic anthropology (Ivanović and Kalezić, 2013).

The concept of digitizing skeletal remains to create three-dimensional models dates back to the early 1980s, but only in recent decades has it been more widely adopted, with a growing number of studies addressing these issues globally (Guzel et al. 2025; Hadžiomerović et al., 2025; Ocumura and Araujo, 2019).

Because of the crucial importance of accurate sex determination, many authors worldwide focus not only on analyzing individual skeletal regions but also on identifying methodological approaches that maximize accuracy in biological sex estimation.

In order to identify which regions of the viscerocranum

are most affected by sexual dimorphism, a study was performed on a dataset of 340 computed tomography (CT) scans of adult Bulgarians, including 156 males and 184 females (Toneva et al., 2022). The main outcomes indicated that male viscerocranial structures were significantly larger than those of females, with the most pronounced differences observed in the nasal, maxillary, and zygomatic areas. Among the regions, the orbital area displayed the greatest shape variation, while the nasal region showed the least. The accuracy of sex determination based solely on size reached 81.8%, whereas shape alone yielded an accuracy of 60–70% (depending on the region). The highest accuracy, 89.5%, was achieved when both shape and size were combined.

The pyriform aperture represents the anterior bony opening of the nasal cavity and plays a key role in facial anatomy and the respiratory system. Sarač-Hadžihalilović et al. (2022) assessed the accuracy of sex estimation using the pyriform aperture. Their study included 211 skulls. The analysis showed that males were correctly classified in 64% of cases, and females in 70%, when both shape and size parameters were considered.

When size was excluded as a variable, classification accuracy decreased to 59.7% in males and 62.5% in females. In general, the male nasal aperture is taller and narrower, with a deeper nasal base, whereas in females it tends to be wider and bordered by straighter nasal bones.

Asghar et al. (2016), working on a sample of 40 skulls of unknown age and sex, aimed to broaden the understanding of sexual dimorphism in the morphology of the nasal opening. Their findings indicated that males exhibited significantly larger dimensions of the pyriform aperture compared to females, including height, width, and the pyriform index (calculated as width/height × 100). However, no statistically significant differences were detected in the size of the nasal bones between sexes. The most common shape of the pyriform aperture was described as triangular-oval, observed in 83.5% of cases.

The objective of our research was to investigate sex-related variation in the central midface skeleton, using geometric morphometrics applied to three-dimensional skull models from the Bosnian-Herzegovinian population.

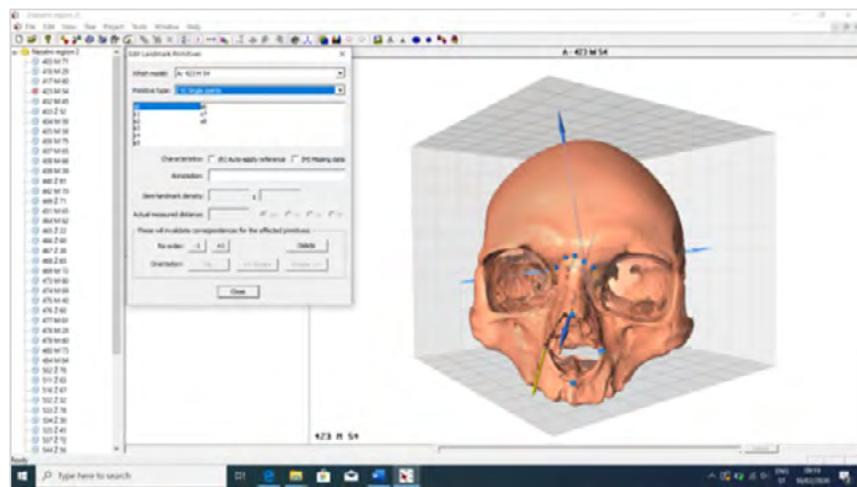


Figure 1 Landmarks marked on 3D models on central midface skeleton

MATERIAL AND METHODS

The research was conducted on three-dimensional models of 210 human skulls from Osteological Collection at Department of Human Anatomy, University of Sarajevo, Faculty of Medicine. All skulls were of known sex and age (139 male and 71 female). The 3D models were generated using a David Pro SLS 2 laser scanner.

On the reconstructed 3D models, nine landmarks were identified in the central midface skeleton using the Landmark Editor software. Each sample was marked with a total of nine points—three paired and three unpaired (Figure 1). The paired landmarks were: apertions—most lateral point on pyriform aperture, maxillonasofrontale—cross section of frontonasal, frontomaxillary and nasomaxillary sutures, and maxillofrontale—cross section of frontomaxillary suture and medial border of orbit. The unpaired landmarks were: akanthion—on the anterior nasal spine, rhinion on the top of pyriform aperture, and nasion—on cross-section of frontonasal and internasal sutures.

After the specific landmarks were identified, their spatial coordinates (x , y , z) within the coordinate system were exported as NTSYS files. These datasets were then imported into the MorphoJ software to perform analyses of shape and size differences between sexes (Klingenberg, 2011).

MorphoJ is a statistical platform that enables the evaluation of shape and size of specific regions. Within the program, Procrustes analysis is applied and principal component (PC) scores are recalculated to determine whether statistically significant differences between sexes exist, using geometric morphometrics techniques.

Geometric morphometrics provides detailed information about the shape of the studied structures, allowing the detection of morphological variation among them. For these purposes, the method relies on Generalized Procrustes Analysis (GPA). GPA functions by removing the effects of size, position, and orientation of the studied structures. Once these effects are excluded, only shape-related information remains, which can then be used for subsequent comparative analysis.

The effect of size is eliminated by scaling the configuration of landmarks to a unit centroid size, representing the fundamental geometric measure of the structure under investigation (Ivanović and Kalezić, 2013).

RESULTS

For analysis of sexual differences of central midface skeleton on 3D models of human skulls were used nine landmarks marked on 3D models represented on Figure 2.

In MorphoJ, Procrustes distances were calculated, after which sex was introduced as a classification variable to perform shape and size comparisons. A covariance matrix was then generated, and Principal Component Analysis (PCA) was applied. The results showed that the first two principal components (PC1 and PC2) accounted for 56.920% of the total variability in the central midface skeleton when both shape and size were considered (Table 1). Distribution of the examined skull sample based on the shape and size of the central midface skeleton in morphological space (skull variability by shape) defined by the first two principal components is presented on Figure 3.

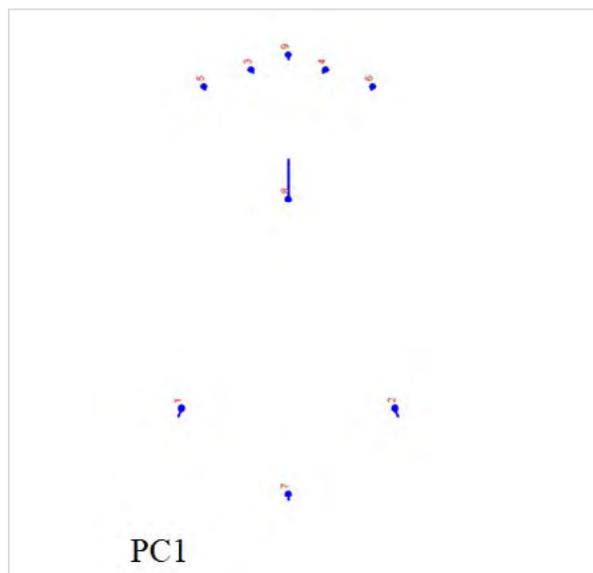


Figure 2 Patterns of shape variation in the central midface skeleton described by Principal Component 1. Blue circles represent the mean distribution of specific landmarks.

Blue lines indicate the direction and magnitude of changes in the mean positions of landmarks 1 and 2 – apertion, 3 and 4 – maxillonasofrontale, 5 and 6 – maxillofrontale, 7 – akanthion, 8 – rhinion, 9 – nasion

Table 1 Eigenvalues and percentage of shape and size variability of the central midface skeleton explained by eigenvalues obtained through Principal Component Analysis (PCA)

No of PCs	Eigenvalues	% Variance	Cumulative %
1.	0.00286803	35.755	35.755
2.	0.00169771	21.165	56.920
3.	0.00094943	11.836	68.756
4.	0.00079373	9.895	78.651
5.	0.00052518	6.547	85.198
6.	0.00039906	4.975	90.173
7.	0.00031326	3.905	94.079
8.	0.00025471	3.175	97.254
9.	0.00016515	2.059	99.313
10.	0.00003723	0.464	99.777

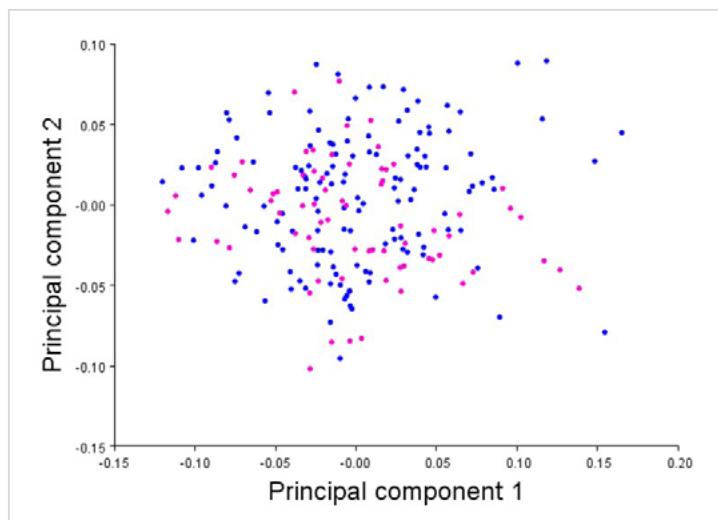


Figure 3 Distribution of the examined skull sample based on the shape and size of the central midface skeleton in morphological space (skull variability by shape) defined by the first two principal components

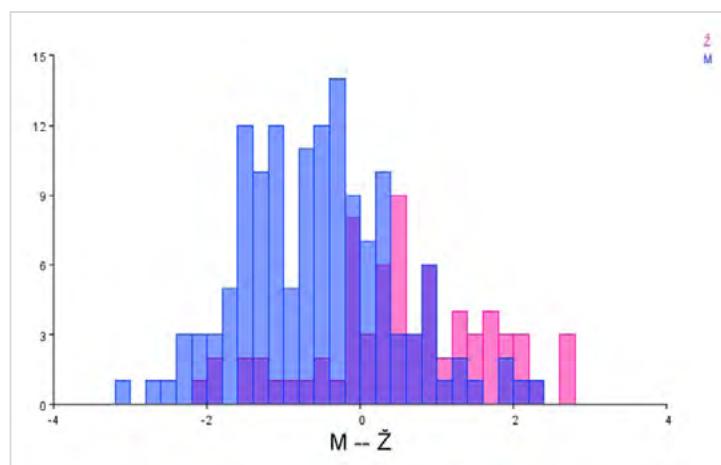


Figure 4 Discriminant functional analysis of the influence of shape and size of the central midface skeleton on sexual dimorphism (M- male, Ž- female)

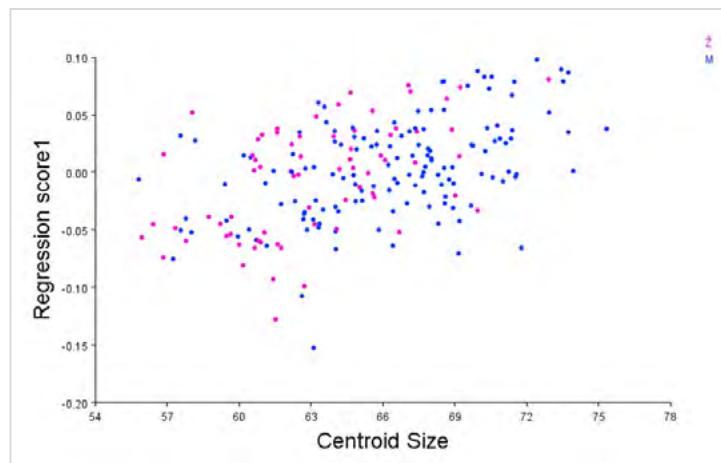


Figure 5 Effect of central midface skeleton size on sexual dimorphism of central midface skeleton shape

Discriminant functional analysis was used to compare the two groups. The analysis was performed by calculating the mean values for both groups using Procrustes and Mahalanobis distances. To determine whether statistically significant sex-related differences existed in the shape and size of the central midface skeleton, a classification accuracy test combined with discriminant functional analysis was conducted. The computed Procrustes distance was 0.0220, and the permutation test (with 1000 permutations) produced a p-value of less than 0.0001, confirming a statistically significant sexual dimorphism in both shape and size of the central midface skeleton which is presented on Figure 4.

Following the classification accuracy test, a regression analysis was conducted in the MorphoJ software to

evaluate the impact of the central midface skeleton size on its shape. The mean values of central midface skeleton size, expressed as centroid size, indicated that size contributed 6% to shape variation. This effect was statistically significant ($p < 0.0001$, based on 10,000 permutations).

The influence of central midface skeleton size on overall shape, as well as the distribution of skulls in morphological space conditioned by central midface skeleton size, is illustrated in Figure 5.

After excluding the influence of size on the shape of the central midface skeleton, principal component analysis was recalculated. The results showed that the first two principal components accounted for 54.4% of the total shape variability in the central midface skeleton. (Table 2).

Table 2. Eigenvalues and percentage of shape variability of the central midface skeleton explained by eigenvalues obtained through Principal Component Analysis (PCA)

No of PCs	Eigenvalues	% Variance	Cumulative %
1.	0.00272333	36.883	36.883
2.	0.00129337	17.516	54.399
3.	0.00091176	12.348	66.747
4.	0.00078860	10.680	77.428
5.	0.00049098	6.649	84.077
6.	0.00039502	5.350	89.427
7.	0.00031078	4.209	93.636
8.	0.00025224	3.416	97.052
9.	0.00016250	2.201	99.253
10.	0.00003722	0.504	99.757

An analysis of sexual differences in the shape of the central midface skeleton, excluding the effect of size, was performed using the classification accuracy test, discriminant functional analysis. The difference between group means, expressed through Procrustes distance, was 0.0021. The permutation test with 1000 iterations yielded a p-value of less than 0.0001, confirming a statistically significant sex-related difference in the

shape of the central midface skeleton independent of size.

The classification accuracy test showed that, out of 139 male skulls, 101 were correctly identified as male, corresponding to an accuracy rate of 73% for the male group. For the 71 female skulls, 51 were classified as female, resulting in an accuracy of 71% for the female group (Table 3).

Table 3 Predictive accuracy of sex determination based on the shape of the central midface skeleton of the skull

Sex	Predictability of sex		Total
	Male	Female	
Male	101		139
Female		20	71
Total	121	89	210

The results of the discriminant functional analysis evaluating the influence of shape of central midface skeleton on cranial sexual dimorphism in the examined

sample are presented in Figure 6, while the interval of shape variation in the central midface skeleton is illustrated in Figure 7.

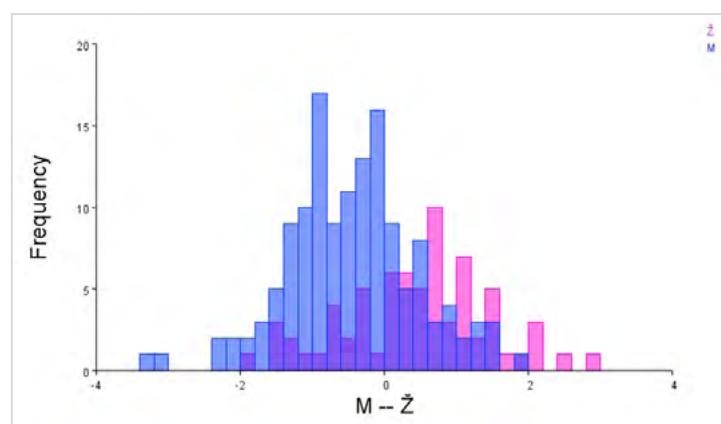


Figure 6 Discriminant functional analysis of the influence of central midface skeleton shape on cranial sexual dimorphism (M- male, Z- female)

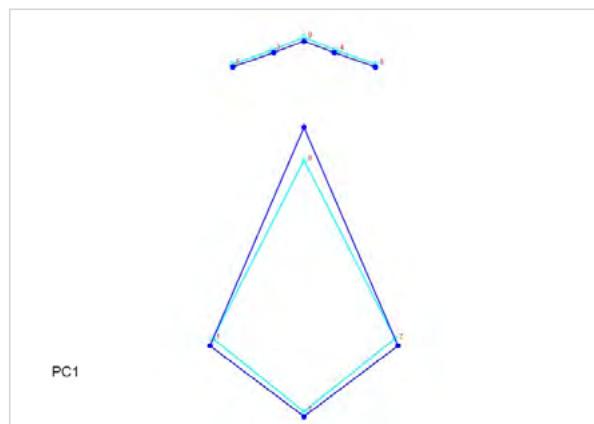


Figure 7 Range of shape variation of the central midface skeleton in the examined skulls

DISCUSSION AND CONCLUSION

The investigation of sexual dimorphism in the human skull is of great relevance for anthropology, evolutionary studies, and osteology. By reviewing previous studies dealing with sexual dimorphism of the midface skeleton, differences can be observed in the reported results, which may be population-specific. Moreover, authors employ various methodological approaches in their investigations, contributing to variability in findings.

In the study provided by Del Bove et al. (2023), a landmark-based technique was developed to enable automated assessment of dimorphic traits on the cranium. The research sample consisted of 228 individuals of known sex from various geographic backgrounds. The analysis revealed that the glabellar and supraciliary regions, the mastoid process, and the nasal area exhibited the strongest dimorphic differences. The accuracy of sex estimation using these regions was 73%, and when they were analyzed together, the accuracy increased to 77%.

In the study provided by Milella et al. (2021), the findings indicate that, consistent with Wainer's rule, male crania display greater overall variability in both size and shape, although statistical significance was confirmed only for total cranial size. The analysis showed that sexual differences were primarily explained by size, while shape contributes to a much smaller extent. Shape alone accounts for only a minor portion of the observed variability, and the cranial base revealed virtually no sexual dimorphism. Among the examined variables, the facial Procrustes form emerged as the most reliable

indicator for determining skeletal sex, providing the highest classification accuracy.

In the study provided by Holton et al. (2016), they analyzed 290 cephalometric radiographs from 38 individuals (20 males and 18 females) across nine age groups. The focus was on sex-related differences in nasal shape in relation to body growth, patterns of non-allometric variation, and the degree of integration between the nasal region and other parts of the facial skeleton. The results showed that both sexes shared similar patterns of variation, but males exhibited a disproportionately greater increase in nasal height with body growth compared to females. In addition, the male nasal region was found to be less integrated with adjacent facial structures than in females. It was concluded that developmental differences in the nasal region between males and females were linked to sex-specific differences in energy requirements (Holton et al., 2014).

The aim of the study of Cantin et al. (2009) was to assess the presence of sexual dimorphism in the dimensions of the pyriform aperture and to explore its possible association with skin color. Ninety skulls from the UNIFESP collection were examined, with available data on sex, age, and skin color. Measurements included the height, upper width, and lower width of the pyriform aperture. Male skulls showed larger values across all parameters, although statistical significance was confirmed only for height. When the sample was divided by skin color, the analysis revealed that male individuals consistently had significantly greater aperture height across all three groups (white, black, and brown). Among black individuals, significant differences were also observed in the upper width. Overall, aperture height emerged as the most reliable indicator of dimorphism, while the effect of skin color appeared minimal. These findings differ in part from previously published reports and highlight the need to reconsider traditional markers used for sex determination in specific populations.

The nasal index (NI) is recognized as a sensitive anthropometric indicator. It reflects sexual variation and has significant applications in forensic medicine and reconstructive surgery, serving as a valuable tool for sex estimation when identity is uncertain. In the study provided by Sharma et al. (2023), the aim was to evaluate nasal height (NH), nasal breadth (NB), and the nasal index (NI) in both sexes, using clinical and radiographic methods, and to explore their role in sexual dimorphism. They concluded that nasal measurements

were important anthropometric parameters for distinguishing between sexes. They provided reliable support for the assessment of sexual dimorphism, having practical implications in forensic medicine, anthropology, and nasal reconstructive procedures.

In the study provided by Cappella et al. (2022), they concluded that while cranial morphology-based sex estimation methods were widely applied in forensic anthropology, their reliability had not been consistently validated across populations. Unlike craniometric techniques, which are better established, morphological approaches still require further investigation due to the influence of population variability on sexual dimorphism. In their research, the accuracy of existing regression models was assessed in a contemporary Italian population, and new logistic regression models were developed. These new models demonstrated improved precision and specificity compared to earlier approaches. The findings contribute to updating reference standards for this geographical area and emphasize the necessity of using validated methods when constructing biological profiles in forensic contexts.

In the study on the sample from Bosnian population (Sarač-Hadžihalilović et al., 2022), determination of gender based on form (size and shape) of pyriform aperture was of 64.03% accuracy for male and 70.83% accuracy for female gender, but based on the shape of pyriform aperture excluding effect of size, sex determination was possible with 59.71% accuracy for male and 62.5% accuracy for female.

Compared with this study, our results showed a higher percentage of correct gender determination based on the central midface skeleton.

The analysis of sexual dimorphism in the central midface skeleton confirms that this anatomical area represents

a valuable marker for distinguishing between males and females. Parameters of central midface skeleton consistently demonstrate sex-related differences, although the degree of dimorphism varies depending on the population studied and the methodological approach applied. These findings support the existence of sexual dimorphism but also highlight the importance of population-specific variability, emphasizing the need for reference standards tailored to specific geographic and ethnic groups. The application of modern techniques, including 3D morphometrics, logistic regression models, and validation of existing formulas, provides greater accuracy and reduces the risk of misclassification in forensic practice. Consequently, the central midface skeleton emerges as a reliable parameter in anthropometric, clinical, and forensic investigations, although its diagnostic value depends on appropriate methodological application and the consideration of population-specific variation.

In our study, the application of geometric morphometrics for sex assessment based on the central midface skeleton demonstrated predictive accuracy ranging from 70% to 73%.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – ZA, ED; Design – ZA, ED; Supervision – ZA, ED; Funding – ZA; Materials – ZA, UA; Data Collection and Processing – ZA, UA, FZ; Analysis and Interpretation – ZA, UA; Literature Search– MŠ, KD, GB, NK; Writing Manuscript – MŠ, KD, GB, NK; Critical Review– FZ, ED.

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Seksualni dimorfizam centralnog dijela skeleta sredine lica: Pristup geometrijske morfometrije na 3D modelima ljudske lobanje

SAŽETAK

Morfološke razlike skeleta između spolova već dugo privlače pažnju disciplina koje se bave određivanjem spola, što je dovelo do razvoja različitih pristupa za procjenu spola. Geometrijska morfometrija je nedavno uvedena kao metoda za identifikaciju spola na osnovu skeletnih obilježja putem primjene statističkih i matematičkih alata. Istraživanje je provedeno na 3D modelima 210 ljudskih lobanja (139 muških i 71 ženskih). Unutar centralnog dijela sredine lica odabранo je devet tačaka (landmarka) koje su označene u softveru Landmark Editor. Nakon označavanja tačaka, njihove prostorne koordinate su izdvojene i unesene u program MorphoJ radi analize varijacija oblika i veličine između spolova. Provedene su analiza osnovnih komponenti, generisanje kovarijacione matrice i diskriminantna analiza funkcija, pri čemu je spol korišten kao klasifikacijska varijabla. Rezultati su pokazali da je procjena spola na osnovu veličine i oblika centralnog dijela sredine lica postigla tačnost od 73% za muškarce i 70% za žene. Oblik centralnog dijela skeleta sredine lica pokazao je statistički značajan seksualni dimorfizam, nezavisno od veličine. Nakon isključivanja uticaja veličine lica i uzimanja u obzir samo varijabli oblika, geometrijska morfometrija je postigla tačnost predikcije od 73% za muškarce i 72% za žene.

Ključne riječi: Centralni dio skeleta sredine lica, geometrijska morfometrija, seksualni dimorfizam, 3D modeli ljudskih lobanja