Sexual dimorphism in the caudal skull region of wistar albino rats: a geometric morphometric study

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Article Info

Article History
Received : 28.03.2025
Accepted : 05.06.2025
DOI: 10.33988/auvfd.1667635

Keywords
Cranium
Geometric morphometrics
Principal component analysis
Rattus norvegicus
Shape analysis

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How to cite this article: Yunus HA, Kılıçlı İB, Batur B, Ayvalı M, Bakıcı C (XXXX): Sexual dimorphism in the caudal skull region of wistar albino rats: a geometric morphometric study. Ankara Univ Vet Fak Derg, XX (X), 000-000. DOI: 10.33988/auvfd.1667635.

Abstract

Wistar albino rats are recognized as essential model organisms in biomedical research due to their anatomy and genetic homogeneity. Their relevance in morphological and developmental studies makes them ideal subjects for investigating skeletal variations, including sexually dimorphic traits. This study aimed to examine the shape variations in the caudal region of the skull in Wistar albino rats, and especially to investigate the morphological differences between the genders. A total of 60 rats (30 males and 30 female) were analysed using geometric morphometric techniques, with shape data collected with via landmark-based analysis using MorphoJ. Statistical analysis was performed with PAST software. Principal Component Analysis (PCA) identified significant sexual dimorphism, revealing distinct morphological differences between male and female rats. Comparative visual assessments showed that females had a wider foramen magnum and condylus occipitalis, a more dorsally positioned opisthocranion, and a laterally broader linea nuchae compared to males. Additionally, ANOVA confirmed the presence of significant sexual dimorphism, demonstrating that the morphological characteristics of the caudal skeleton varied distinctly between male and female Wistar albino rats. These results suggest that the caudal skull region can serve as a useful indicator of sexual dimorphism, with geometric morphometrics offering a reliable method for detecting sex-related skeletal differences. This study not only advances the understanding of sexual dimorphism in rodents but also provides a framework for further investigations in comparative anatomy and evolutionary biology.

Introduction

Wistar albino rats serve as essential model organisms in biomedical research due to their genetic consistency, rapid reproductive cycle, and adaptability to laboratory conditions. These rats, first standardized at the Wistar Institute in 1906, have been widely utilized in various disciplines (19). They are used in a wide variety of research areas in both veterinary and human medicine such as pharmacology (32, 39), toxicology (14), pathology (3), neurology (5), surgery (22) and endocrinology (18).

The skull is a critical structure that houses and protects the brain while providing attachment sites for various muscles and supporting essential sensory organs (26). Morphological variations in the skull have significant implications in forensic sciences, veterinary anatomy, and clinical applications. Knowledge of skull morphology is crucial for many applications such as taxonomic classification, evolutionary studies, and sex determination (13, 27, 42). Although skull shape analyses in rodents have been extensively conducted, previous studies have primarily focused on the ventral, dorsal, and lateral aspects of the skull, leaving the caudal region (CR) relatively unexplored (1, 4, 15, 20, 28, 41). The CR formed by the occipital bone, which contains important anatomical structures such as foramen magnum, plays a vital role in neurocranial stability and attachment of the suboccipital muscles (26). Despite its functional significance, this region has received limited attention in studies related to shape analysis, particularly in the context of sexual dimorphism. The investigation of sexual dimorphism has been demonstrated to provide valuable insights into the developmental processes of the skeleton, the biomechanical adaptations that occur, and the potential sex-related differences that may be present in rats (2, 4, 8, 23).

In rodent research, including studies on Wistar albino rats, shape variation is commonly analyzed for purposes such as sex determination, taxonomic classification, and the investigation of evolutionary trends (41, 42). Geometric morphometrics is a modern technique that examines the morphological properties of biological structures both visually and statistically. Based on the analysis of geometric data derived from cartesian coordinates, reference points, curves, and contours, this method reveals physical and functional differences between shapes (6, 10, 24). It has been successfully applied to various rodent species, such as Wistar albino rats, Sprague Dawley rats, and bandicoot rats, for identifying sex differences, classifying taxa, and studying evolutionary patterns (4, 28, 41). Notably, geometric morphometrics has significantly advanced comparative anatomy and contributed valuable clinical insights in veterinary medicine by uncovering subtle shape differences often missed by traditional morphometric approaches (24).

While several morphometric studies have analysed skull shape in rats, they have primarily concentrated on the ventral, dorsal, or lateral views, often overlooking the CR. In particular, research addressing sexual dimorphism in this region remains scarce. Given the functional and structural importance of the caudal skull housing critical neurocranial and musculoskeletal attachment sites, understanding its morphological variations is essential for comparative anatomy, evolutionary biology, and forensic applications. Although various geometric morphometric studies have examined skeletal sexual dimorphism in rats (1, 28), to our knowledge, no previous research has employed geometric morphometric methods to analyse the caudal skull region in Wistar albino rats or to evaluate sex-related shape variation in this specific area. This study aims to fill this gap by conducting a comprehensive geometric morphometric analysis of the caudal skull region in Wistar albino rats. Through both visual and statistical assessments, it seeks to quantify sexually dimorphic traits and identify potential factors contributing to these differences. By addressing this understudied anatomical region, the findings will enhance the current understanding of sexual variation in rodent cranial morphology and provide a foundation for future research in comparative anatomy, biomechanics, and developmental biology.

Materials and Methods

Ethics approval: The study was approved by the Ankara University Local Ethics Committee for Animal Experiments (Decision number: 2024-13-107).

Specimens: The study utilized a total of 60 (30 females and 30 males) Wistar albino rat skulls, all aged 10 months. Wistar rats at 10 months of age were selected for this study, as they are considered young adults that have reached full skeletal maturity, with peak bone mass typically formed by 12 months (31, 33). This ensured that the skulls used were fully developed and suitable for reliable morphometric comparison. All animals were raised under standardized conditions, including identical housing, feeding, and light cycles, to eliminate environmental variability. The skulls were minimize meticulously macerated using standardized methods to ensure preservation of structural integrity and were thoroughly cleaned without causing any damage. Subsequently, the caudal surfaces of all skulls were photographed under controlled conditions, maintaining consistent angles and distances.

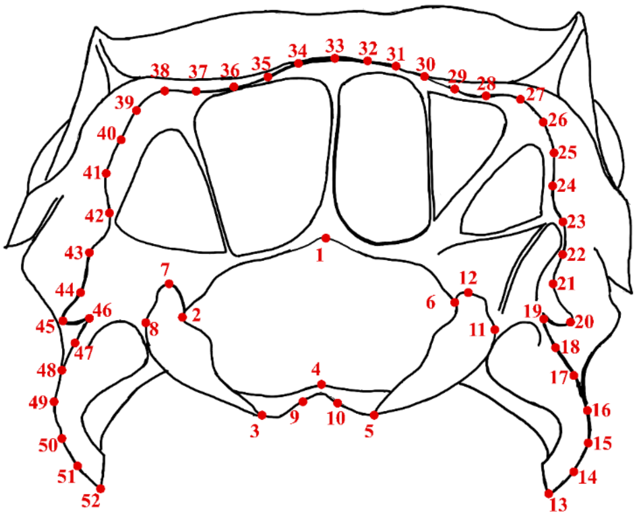

Figure 1. Anatomical landmarks determined for shape analysis.

Data Collection-Geometric Morphometrics: Before the landmarking process, the photographs in JPG format were converted to Tps files using TpsUtil (v. 2.32) (36). The landmark process was performed using TpsDig (v. 2.31) with the necessary image tools (37). Landmark procedure was performed by considering 3 different regions on the caudal surface. The three includes area 1 (A1) represents the foramen magnum, area 2 (A2) represents the condylus occipitalis, and area 3 (A3) represents the area where the linea nuchae line passes between the right and left processus paracondylaris and is drawn to include the opisthocranion (Figure 1). The region representing the whole head was represented as CR. A total of 52 landmarks were used in the CR. 6 landmarks were used in A1, 6 landmarks in A2 and 40 landmarks in A3. Landmarks marked according to specific anatomical regions were named Type I landmarks. Landmarks marked to better define specific regions on the skull were called Type III landmarks (4). The regions represented by landmarks are given in Table 1. In geometric morphometric studies, Type I landmark determination is usually done by taking into account previous studies. However, since there is no study focusing on geometric morphometrics in the caudal region of the skull, landmark points of previous studies could not be analysed in this study.

Table 1. Description of landmarks related to the anatomical region. FM: Foramen magnum, CO: Condylus occipitalis, (*) The indicated anatomical structure is observed to lie between the two landmarks.

Landmark	Anatomical Region
1	Midpoint on the dorsal margin of FM
2	Most left point of FM
3	Midpoint on the ventral corner of left CO
4	Midpoint on the ventral margin of FM
5	Midpoint on the ventral corner of right CO
7	Midpoint on the dorsal corner of left CO
8	Most lateral corner of left CO
9	Ventral end of the left CO
10	Ventral end of the right CO
11	Most lateral corner of right CO
12	Midpoint on the dorsal corner of rigft CO
7-2-3	Medial edge of left CO
12-6-5	Medial edge of right CO
13	Right processus paracondylaris
14-19*	Right processus jugularis
33	Opisthocranion
46-51*	Left processus jugularis
52	Left processus paracondylaris

MorphoJ (v. 1.08.01) was used for shape analysis. Firstly, the shape analyses were performed for the whole caudal region of the skull (25). Then, shape analyses were performed separately for A1, A2, and A3 areas representing caudal regions.

All analyses to be described were performed separately in four regions, namely CR, A1, A2, and A3. Generalised Procrustes Analysis (GPA) was applied to standardize all samples in a common coordinate system and Procrustes coordinates were generated. Principal Component Analysis (PCA) was performed to reveal general patterns of morphological variation and to show the relationship between them. Eigenvalues and eigenvectors were obtained from the matrix (11). Following this analysis, principal component (PC) values were obtained. PCA simplified the formal analysis and interpretation of the data obtained by reducing their dimensionality through the identification of principal components (PCs). PCs were visualised in MorphoJ using wireframe warp plots and transformation grids. Wireframe warp plots (Figure 2, 3, 4 and 5) show the shape variation between the represented components. The transformation grid (Figure 2, 3, 4 and 5) has a point represented by the landmark point, the magnitude and direction in which the shape variation of the point is represented, and the green lines below them are the grids in which the variations of the other points are added.In the Figures 2, 3, 4 and 5, the dots represent the mean shapes.

After PCA, Discriminant function analysis (DFA) was performed, and a comparative lollipop graph and a transformation grid were obtained between females and males (Figure 6). These graphs show in detail which anatomical regions in the CR and mean shapes between males and females. These differences indicated that the degree of separation between the groups was significant, and that relevant data were obtained.

Statistical Analysis: PAST (v. 4.03) was used for statistical analysis (17). ANOVA was performed to rigorously assess potential morphological differences between male and female rats and P values were obtained (Table 2). PC1, PC2, and PC3 were used as variables (shape) that affected the total variance in PCA for Wistar albino caudal shape. Statistical significance was determined when it produced a P-value below 0.05 ($P < 0.05$). Levene's test was used to assess the equality of variances between groups.

Table 2. Statistical data obtained through the PAST. The analysis was performed for the whole head region (CR area). (*) Statistically significant.

	Sum of Squares	Degrees of Freedom(df)	Mean Square	*F*	P value
Between Groups	4.59	103	0.0446	460	<0.05*
Within Groups	0.59	6136	0.0000961		**Permutation P (n=99999)**
Total	5.19	6239			<0.05*
Components of variance:					
Var(group): 74219.4			**Var(error):** 9702.63		
ICC: 0.8843			**Omega squared (ω^2):** 0.8834		
Levene´s test for homogeneity of variance, from means P:					>0.05
Levene´s test, from medians P:					>0.05

DOI: 10.33988/auvfd.1667635

After performing the ANOVA, residual plots were obtained (Figure 7). The proximity of each sample to the diagonal line was checked. The Shapiro-Wilk test was performed to test for excessive deviation from normality. In addition to the standard parametric ANOVA, a permutation test was also conducted to confirm the robustness of the results under fewer assumptions about the data distribution.

Results

Principal component values with varying degrees of variation were derived from four distinct regions (A1, A2, A3, and CR) using differing numbers of anatomical landmarks. Scatter plots of PC1 versus PC2 for the CR are depicted in Figure 2. In the entire caudal region, PC1, which has the highest variation, explains 26.377% of the shape variation, while PC2 explains 13.397%. PC1 and PC2 explain 37.774% of the total variation. Complementary visual data, including wireframe warp plots and transformation grids illustrating morphological changes corresponding to increasing PC1 and PC2 values, are also provided. The morphological variations associated with each PC, as identified through PCA, were analysed in detail. An increase in PC1 revealed inward morphological changes in the dorsal region encompassing the linea nuchae and opisthocranion, while the lateral region exhibited outward deformation. The processus paracondylaris was observed to shorten and shift dorsally, the condylus occipitalis enlarged and shifted ventrolaterally, and the foramen magnum showed expansion. Similarly, an increase in PC2 demonstrated dorsally oriented shape changes in the foramen magnum and condylus occipitalis. In contrast, the opisthocranion region displayed minimal variation, whereas the linea nuchae exhibited medial deformation towards the right processus jugularis and a corresponding change in the same direction towards the left processus paracondylaris. Analysis of scatter plots for the CR revealed a distinct separation between male and female individuals, particularly with increasing PC1 values.

A1: In the A1, PC1, which accounts the highest variation, explains 42.406% of the shape variation, while PC2 explains 24.206%. PC1 and PC2 explain 66.612% of the total variation. The PC1xPC2 scatter plots for the A1 are shown in Figure 3. In addition to these scatter plots, a wireframe warp plot and transformation grid were presented, providing visual data with the increase in PC1 and PC2 values. The morphological changes represented by each component obtained from the PCA analysis were detailed. With the increase in PC1, the dorsomedial point of the foramen magnum showed variation towards the ventral, the right and left lateral points towards the midpoint on the dorsal, and the ventromedial point with its entire base towards the ventral. With the increase in PC2, the dorsal points, the ventral points, and most lateral points showed variation towards the ventral, while other points showed partial dorsal placement. In addition, the narrowing in the right region of the foramen magnum was remarkable. When the scatter plot was examined, a distinction was seen between females and males with the increase in PC2 values, but in general, male and female scatter points showed close and complex placement.

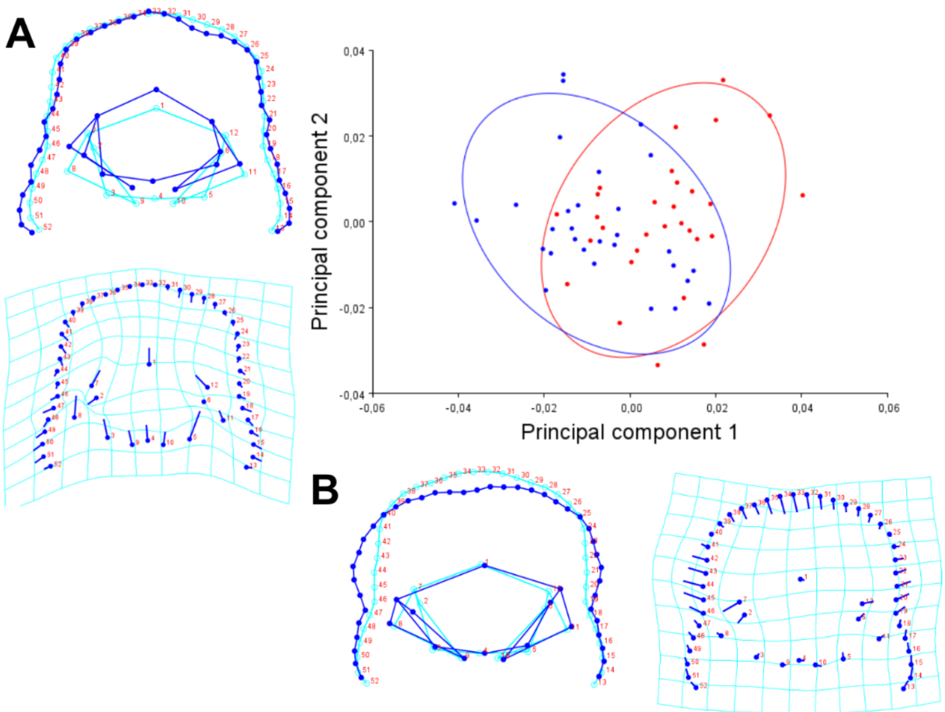

Figure 2. PCA scatter plot representing the whole head (CR area). Red dots represent females; blue dots represent males. A:Wireframe warp plot and transformation grid showing shape variations with increasing PC2. B: Wireframe warp plot and transformation grid showing shape variations with increasing PC1.

DOI: 10.33988/auvfd.1667635

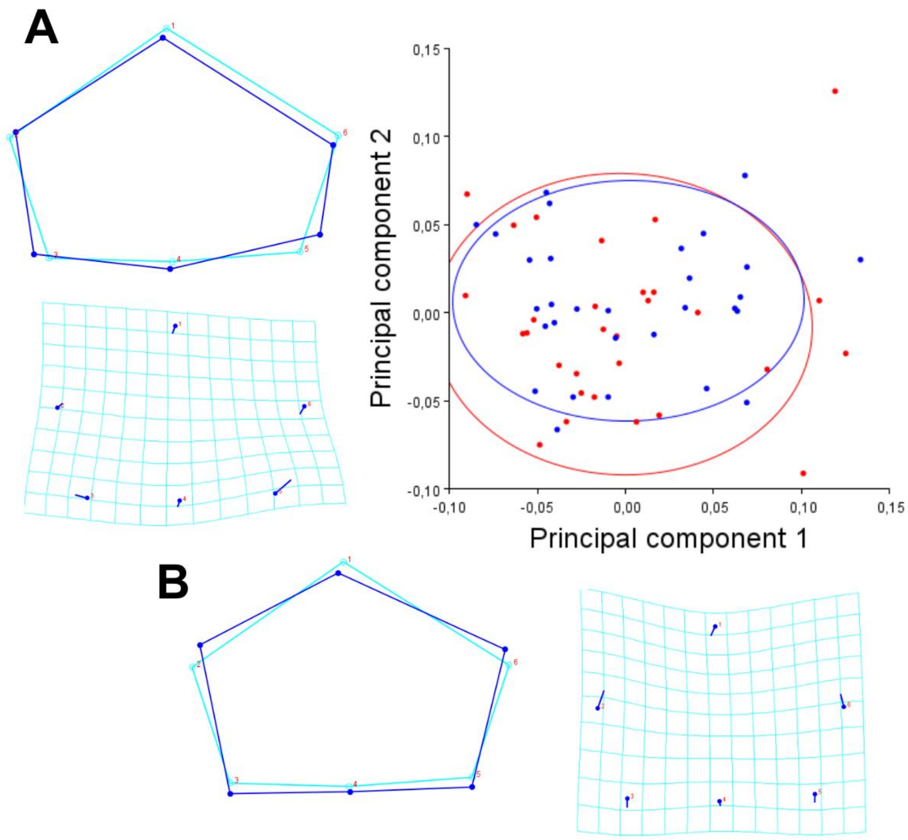

Figure 3. PCA scatter plot representing Area 1. Red dots represent females; blue dots represent males. A:Wireframe warp plot and transformation grid showing shape variations with increasing PC2. B: Wireframe warp plot and transformation grid showing shape variations with increasing PC1.

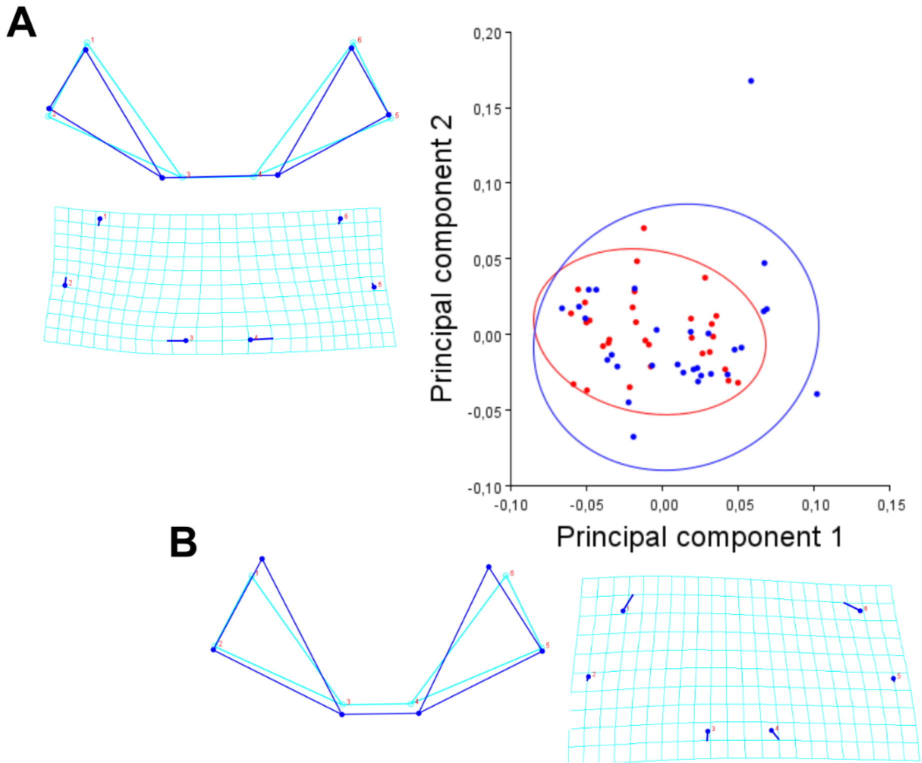

Figure 4. PCA scatter plot representing Area 2. Red dots represent females; blue dots represent males. A:Wireframe warp plot and transformation grid showing shape variations with increasing PC2. B: Wireframe warp plot and transformation grid showing shape variations with increasing PC1.

DOI: 10.33988/auvfd.1667635

A2: In the A2, PC1, which accounts the highest variation, explains 36.945% of the shape variation, while PC2 explains 26.989%. PC1 and PC2 explain 63.934% of the total variation. The PC1xPC2 scatter plots for the A2 are shown in Figure 4. In addition to these scatter plots, a wireframe warp plot and transformation grid were presented, providing visual data with increasing PC1 and PC2 values. The morphological changes represented by each component obtained from the PCA analysis were detailed. With the increase in PC1, the dots located in the midpoint on the dorsal corner showed dorsolateral variation. In addition, the dots in the midpoint on the ventral corner showed a ventrolateral shape variation. No significant variation was observed in the points located laterally. The right and left condylus occipitalis showed approximately symmetrical shape changes relative to each other across the median plane. With the increase in PC2, the dorsomedial point and the lateral point shifted ventrally, and the points at the midpoint on the ventral corner shifted laterally, symmetrical to the median plane. Condylus occipitalis was generally reduced from all borders. When the scatter plot was analysed, a separation was observed between males and females with increasing PC1 values, but in general, male and female scatter points were located close to each other.

A3: In the A3, PC1, which accounts the highest variation, explains 29.398% of the shape variation, while PC2 explains 18.684%. PC1 and PC2 explain 48.082% of the total variation. The PC1 × PC2 scatter plots for the A3 are presented in Figure 5, along with wireframe warp plots and transformation grids that provide visual representations of morphological changes associated with increasing PC1 and PC2 values. The morphological variations corresponding to each PC, as identified through PCA, were analysed in detail. With an increase in PC1, the dorsal region of the linea nuchae, including the opisthocranion, shifted inward, while the lateral region expanded laterally. The cranial structure exhibited flattening dorsally and widening laterally. Additionally, the processus paracondylaris was observed to shorten. With an increase in PC2, subtle morphological variations in the dorsal region involving the opisthocranion and linea nuchae became apparent, both laterally and medially. In the lateral region, the linea nuchae shifted dorsolaterally up to the processus jugularis and exhibited a pronounced midpoint on the dorsal shift from the processus jugularis to the processus paracondylaris. The analysis of the scatter plot revealed a clear distinction between male and female individuals, particularly as PC1 values increased.

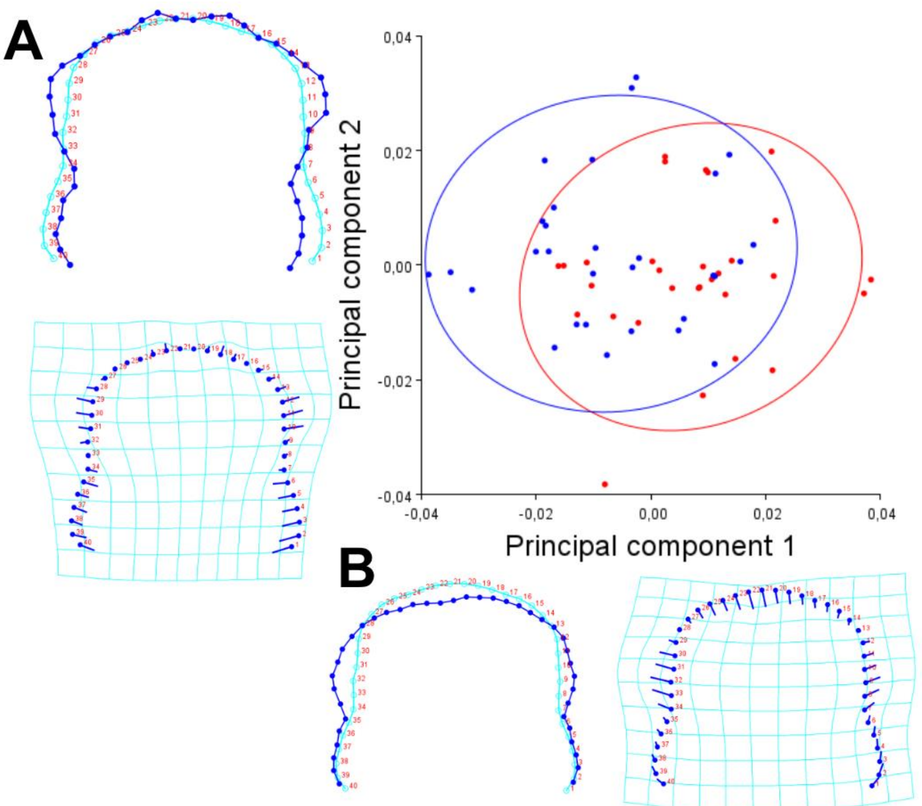

Figure 5. PCA scatter plot representing Area 3. Red dots represent females; blue dots represent males. A:Wireframe warp plot and transformation grid showing shape variations with increasing PC2. B: Wireframe warp plot and transformation grid showing shape variations with increasing PC1.

DOI: 10.33988/auvfd.1667635

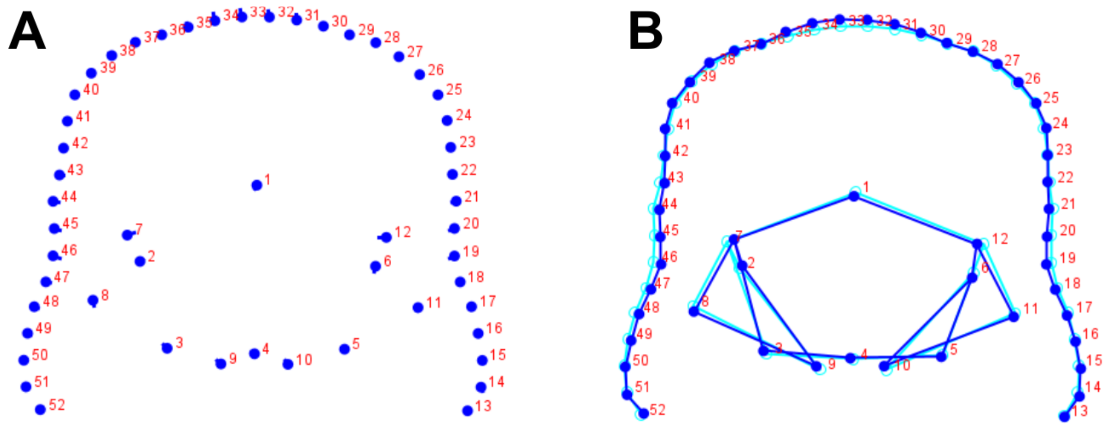

Figure 6. Mean shapes between male and female Wistar albino as a result of DFA. A: Lollipop graph, B: Wireframe warp graph, Green: Females, Blue: Males.

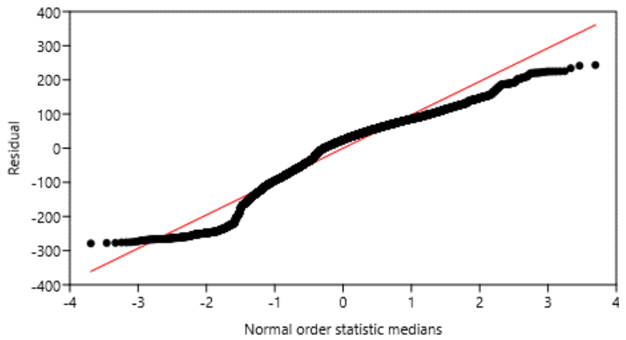

Figure 7. Residual graph taken from PAST. Shapiro-Wilk Test (W=0.9459, P>0.05). It shows a residuals graph generated from a Shapiro-Wilk test, where ANOVA test was conducted. X-axis: residual, Y-axis: Normal order statistic medians. Please refer to table 2 for detailed information.

Statistical Analysis: The gender-based statistical analysis conducted using ANOVA in PAST strongly supports the presence of significant sexual dimorphism in the caudal surface of Wistar albino rats (P < 0.05) (Figure 7, Table 2). The P-value underscores the existence of marked morphological differences in the caudal skeleton between male and female rats. Additionally, the high *F*-value indicates substantial differences between groups compared to the within-group variance. Anatomically, this finding aligns with expectations from studies on sexual dimorphism, highlighting significant shape differences in the caudal skull region between genders. This result further validates the robustness of the findings and reinforces the evidence of sexual dimorphism in the caudal region of the skull.

Omega squared (ω^2), a measure of effect size, showed that the group factor had an exceptionally large effect on the morphological variation of the caudal skeleton in sexual dimorphism. The P-values for Levene's test showed that the variances between groups were not significantly different, indicating homogeneity of variances, which satisfies one of the key assumptions of ANOVA. The results of the Shapiro-Wilk and Levene's tests confirm the validity of ANOVA assumptions. Additionally, the permutation test (P < 0.05) further supports the robustness of the findings, confirming the presence of statistically significant shape differences between male and female rats.

Discriminant Function Analysis: Figure 6 presents mean shapes of the caudal region of the skull in female and male Wistar albino rats. Females exhibited a wider foramen magnum and condylus occipitalis, with expansions particularly prominent at the midpoint on the dorsal and rightmost lateral points of the foramen magnum, as well as at the midpoint on the dorsal and midpoint on the ventral corners of both the right and left condylus occipitalis. Additionally, the most lateral point of the left condylus occipitalis displayed noticeable widening. In males, the opisthocranion exhibited greater dorsal extension, and the right and left processus paracondylaris were symmetrically aligned relative to the median plane. Overall, female Wistar albino rats exhibited a narrower dorsal structure and a broader lateral structure in the CR compared to their male counterparts.

Discussion and Conclusion

This study highlights shape variations in the caudal region of the cranium in Wistar albino rats, with a particular focus on morphological differences between genders. Using geometric morphometric analyses, the research successfully identified distinct cranial morphological features between male and female rats. The regions showed pronounced shape variations, as indicated by increasing PC values, including the foramen magnum, condylus occipitalis, and linea nuchae. In the foramen magnum and condylus occipitalis, the observed shape variations were predominantly characterized by regional

DOI: 10.33988/auvfd.1667635

expansion. In contrast, the linea nuchae exhibited irregular shape changes without a consistent directional pattern. These findings contribute to a deeper understanding of sexual dimorphism in the caudal skull morphology of Wistar albino rats. Statistical analyses confirm geometric morphometrics as a powerful tool for detecting and measuring anatomical and inter-sex shape differences. This approach enhances the utility of geometric morphometrics in comparative anatomical studies.

Morphological differences between female and male Wistar albino rats have been thoroughly analysed using geometric morphometric techniques. The analyses show that female individuals have wider foramen magnum and condylus occipitalis regions, while male individuals exhibit a narrower and more compact morphology in these anatomical structures. In addition, the skulls of female individuals are more flattened from the top and wider from the lateral regions compared to males. These morphological differences may be influenced by a combination of genetic, hormonal, and biomechanical factors. Sexual dimorphism in cranial structures is often linked to reproductive roles, endocrine system regulation, and musculoskeletal adaptations required for sex-specific behaviours and physiological functions (40, 42). Hormonal influences, particularly variations in oestrogen and testosterone levels, play a crucial role in shaping skeletal morphology, as demonstrated in previous studies on mammalian cranial development (29, 30). Additionally, differences in muscle attachment sites and skull robustness may reflect biomechanical demands associated with sex-related behaviours, such as territoriality, mating strategies, and foraging patterns. For example, in rodents, the development of muscle attachment sites and the robustness of the skull can be linked to their ecological roles and behaviours (7, 21). In a broader context, geometric morphometric analyses have become a crucial tool in studying cranial shape variations in rodents, providing insights into taxonomy, functional adaptations, and phylogenetic relationships. This method allows researchers to assess shape-related morphometric variation more accurately than traditional linear measurements, which often focus on size rather than shape. By capturing and analysing shape differences with landmark-based approaches, geometric morphometrics enhances the ability to detect subtle sexual dimorphism and structural adaptations that might otherwise go unnoticed. These findings contribute not only to anatomical and forensic sciences but also to evolutionary biology by offering a deeper understanding of sex-related cranial differentiation (34).

In the Wistar albino rat study conducted by Gürbüz et al. (2024), PCA scatter plots generated from dorsal and ventral skull shape analyses revealed that shape variations were primarily concentrated in the neurocranium in dorsal images, while in ventral images, variations were more pronounced in the viscerocranium. Building upon this, our study focused on the caudal skull region and, to the best of the authors' knowledge, is the first of its kind in the literature identified a clear distinction between male and female Wistar albino rats in the CR and A3, particularly with increasing PC1 values. However, no distinct separation was observed in the A1 and A2. The distinction detected in the CR and A3 is likely attributable to the inclusion of the linea nuchae in the shape analysis. Given the morphological role of the linea nuchae in cranial structure and muscle attachment, its course and length appear to be key determinants of sexual dimorphism in the skulls of Wistar albino rats. These findings suggest that variations in this region contribute significantly to the observed differences between male and female individuals, reinforcing the importance of caudal skull morphology in sex-related skeletal differentiation.

In the comparative study by Ağaç et al. (2024), PCA scatter plots revealed distinct clustering patterns among three different rat strains, with the Wistar albino strain exhibiting a tendency to cluster toward the positive axis. The analysis further indicated that, as the PC1 value increased, the most caudal point of the right condylus occipitalis and the most rostral point of the foramen magnum converged, highlighting significant morphological differences in the Wistar albino strain compared to other strains. In our study, although a similar marked convergence between these two points was not observed in the CR, an increase in the PC1 value revealed that both points shifted to the right. Additionally, when the left condylus occipitalis was considered, the distance between these two points increased. This trend was consistent across both female and male individuals, with morphological differences between genders becoming more pronounced in a similar directional pattern. Thus, this study extends beyond intraspecific variations, providing a comprehensive assessment of sexual dimorphism in the CR of the skull in Wistar albino rats.

Many studies have shown that both traditional morphometric and geometric morphometric methods can effectively detect sexual dimorphism in rats. Abdel-Rahman et al. (2009) examined sexual dimorphism in the skull in their study. In their study of African Nile and Tete Veld rats, geometric morphometric analysis revealed significant differences in skull shape between the sexes that could not be detected by linear methods. Afshan et al. (2013) found significant differences between male and female individuals using linear morphometric measurements in murid rodents. It was reported that males were consistently larger than females in all linear measurements. Berdnikovs et al. (2007) analysed differences in pelvic shape between the sexes using geometric morphometrics in an ontogenetic study of

DOI: 10.33988/auvfd.1667635

Sprague-Dawley rats showing how these differences change with development. In a study by Gürbüz and Demiraslan (2024), sex differences in the shape of the rat skull were statistically determined, and sexual dimorphism was found to be particularly pronounced in the oszygomaticum, palate and neurocranium regions. When these findings are evaluated in general terms, it can be seen that examining both size and shape differences in skeletal structures together provides a more comprehensive and accurate understanding of sexual dimorphism in rats. In this context, our study found statistically significant shape differences between male and female individuals in the caudal region of the rat skull using geometric morphometrics. In males, the changes observed in the foramen magnum and condylus occipitalis regions were mostly in the form of regional enlargement, whereas irregular shape changes were found in the linea nuchae region. In general, and in support of other studies, the caudal region was found to be wider in males. These results suggest that geometric morphometric methods are highly effective in detecting shape differences and provide a robust methodological framework for analysing sexual dimorphism in cranial morphology.

The observed shape changes in the Wistar albino rats' caudal cranial region likely result from a complex interplay of genetic inheritance, epigenetic modifications, and functional demands, as stated in the studies (12, 16, 34). Hallgrimsson et al. (2007) explored the impact of genetic and epigenetic factors on cranial morphology using three-dimensional (3D) geometric morphometrics in laboratory mice, offering a deep analysis of their influence on shape changes. Similarly, animal breeding studies by Lee and Yoo (2002) emphasize the inevitability of morphological variations among individuals, reinforcing the role of genetic factors in shaping cranial structures. On the other hand, Hallgrimsson et al. (2007) proposed that brain structure plays a crucial role in determining cranial shape variations. Supporting this, Scholz et al. (2016) utilized geometric morphometrics to analyse brain shape differences among three common mouse strains, identifying potential factors contributing to these variations. Additionally, Bonfili et al. (2022) investigated the morphological relationship between brain development and endocranial surfaces in mice, providing valuable insights into the interactions between the brain and skull through geometric morphometric analysis. Building on these findings, brain structure could be one of the reasons for the observed shape differences in the caudal region between male and female Wistar albino rats.

While this study identified significant morphological differences in the CR of the cranium of Wistar albino rats using geometric morphometrics based on two-dimensional (2D) images, this method has certain limitations. The functional and behavioural implications of the observed variations were not explored, and further research correlating morphology with outcomes like bite force or head movement could provide richer biological insights. Additionally, this cross-sectional analysis did not account for ontogenetic or age-related changes. Future longitudinal studies incorporating environmental factors would offer a more dynamic understanding of cranial shape changes. Finally, using 2D images presents a methodological constraint, employing 3D models derived from micro-computed tomography (micro-CT) or computed tomography (CT) scanning could yield more precise and comprehensive results (35).

This study highlights pronounced sexual dimorphism in the CR of the skull in Wistar albino rats, demonstrating significant morphological differences in various anatomical structures between genders. Notably, female rats exhibited a broader foramen magnum and occipital condyles, with the most pronounced differences observed at the midpoint on the dorsal margin and rightmost lateral points of the foramen magnum, as well as at the midpoint on the dorsal and midpoint on the ventral corners of both occipital condyles. This widening was accompanied by greater lateral expansion in females, in contrast to the more pronounced dorsal variation of the opisthocranion observed in males. These findings underscore the caudal skull region as a robust indicator of sexual dimorphism in this species. Overall, this study demonstrates the efficacy of geometric morphometrics in capturing sex-specific morphological variations and offers novel insights into the sexually dimorphic features of the caudal skeleton in rodents. The results contribute to a deeper understanding of sexual dimorphism in Wistar albino rats and establish a foundation for future research in comparative anatomy, functional morphology, and evolutionary biology.

Financial Support

This research received no grant from any funding agency/sector.

Ethical Statement

This study was carried out after the animal experiment was approved by Ankara University Local Ethics Committee (Decision number: 2024-13-107).

Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

CB, HAY, IBK conceived the idea, conceptualization and investigation. HAY and İBK planned the manuscript. BB, İBK and MA contributed to sample preparation. İBK and BB planned the methodology and formal analysis. HAY and CB have made significant scientific support and also contributed to the interpretation of the results. All authors provided significant contributions by giving feedback and help shape the manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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