

THREE-DIMENSIONAL GEOMETRIC MORPHOMETRIC ANALYSIS OF THE VENTRAL SPHENOID IN DOMESTIC DOGS AND CATS

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This study aimed to evaluate size, shape, and allometric patterns of the ventral sphenoid region in domestic dogs and cats using a three-dimensional geometric morphometric approach. A total of 45 specimens were analyzed, including 21 dogs and 24 cats. Twelve anatomical landmarks were placed on the ventral sphenoid region and analyzed following Generalized Procrustes Analysis. Shape variation was assessed using principal component analysis and permutation-based multivariate models. Dogs exhibited significantly larger centroid sizes compared to cats. A clear shape differentiation between species was detected and remained significant after accounting for size effects, indicating that interspecific differences are not solely explained by scaling. While centroid size differences among cat subgroups were not statistically significant, shape differences persisted both before and after size correction. Pairwise comparison revealed that the Scottish Fold group showed the most pronounced and consistent divergence. Allometric relationships between size and shape were significant across the entire dataset, and the interaction between size and group suggested partially distinct growth-related shape trajectories in dogs and cats. Principal component analyses indicated that the major shape variation was associated with changes in midline elongation and lateral expansion, as well as three-dimensional repositioning of midline and lateral components. These findings demonstrate that the ventral sphenoid represents a biologically meaningful basicranial module capable of capturing both interspecific differences and size-independent shape variation among cat subgroups.

Keywords: Allometry, basicranium, shape variation, veterinary anatomy.

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INTRODUCTION

The cranial base constitutes a morphological and developmental bridge between the neurocranium and viscerocranium, playing a crucial role in determining craniofacial proportions and facial orientation. The sphenoid bone occupies a central position within this system; its basisphenoid and presphenoid components develop from cartilaginous precursors and are closely associated with growth patterns along the midline of the cranial base. Developmental studies have demonstrated that variations in the cranial base elongation can influence both the position and proportions of the facial region [1–3]. For this reason, the sphenoid and adjacent basicranial regions represent suitable targets for investigating interspecific and interbreed cranial differentiation [4,5].

Although cranial diversity in cats appears more limited compared to dogs, recent CT-based and geometric morphometric studies have revealed significant shape differences among cat breeds [6,7]. In particular, while centroid size variation among British Shorthair, Scottish Fold, and other cat groups tends to be weak, shape variation can be distinctly differentiated. Moreover, a considerable portion of caudal fossa variation in domestic cats has been reported to concentrate around the dorsum sellae of the basisphenoid, suggesting that the cranial base, especially the sphenoid region, carries relevant morphological information in the feline skull [7,8].

In this context, the aim of the present study was to quantitatively compare size and shape variation of the ventral sphenoid region in domestic dogs and cats using 12 three-dimensional anatomical landmarks. Additionally, differences among cat subgroups (British Shorthair, Scottish Fold, and domestic mixed-breed cats) were evaluated, and the allometric component of the observed variations was assessed. Although previous studies have demonstrated high cranial diversity in dogs and breed-related shape differentiation in cats, three-dimensional landmark-based analyses focusing specifically on the ventral sphenoid region remain limited [9–11]. Therefore, this study aims to provide a more targeted contribution to understanding species – and group – level patterns in basicranial morphology.

MATERIALS AND METHODS

Specimens

The computed tomography (CT) images used in this study were obtained retrospectively from the imaging archive of the Animal Hospital, Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa. A total of 45 skull scans were included, comprising 21 domestic dogs and 24 domestic cats. The cat group consisted of British Shorthair ($n = 4$), Scottish Fold ($n = 8$), and domestic mixed-breed cats ($n = 12$).

The dog group included individuals from a variety of breeds representing a broad spectrum of cranial morphotypes, including brachycephalic, mesocephalic, and

dolichocephalic forms. Identified breeds included Chihuahua (n = 1), Cocker Spaniel (n = 2), English Setter (n = 1), French Bulldog (n = 1), German Shepherd Dog (n = 2), Golden Retriever (n = 5), Pug (n = 2), Yorkshire Terrier (n = 1), Spitz-type dogs (n = 1), shepherd-type dogs (n = 1), mixed shepherd-type dogs (n = 2), and mixed-breed dogs (n = 3). Due to the limited number of specimens per breed, dogs were analyzed as a single group in all statistical analyses. Only scans of adult individuals without visible cranial pathology, deformation, or fracture were included in the study.

Landmarks

After three-dimensional reconstruction of the ventral sphenoid region, landmark coordinates were collected through manual digitization using 3D Slicer (version 5.2.2). Landmarks were placed directly on the reconstructed three-dimensional models derived from CT data [12,13].

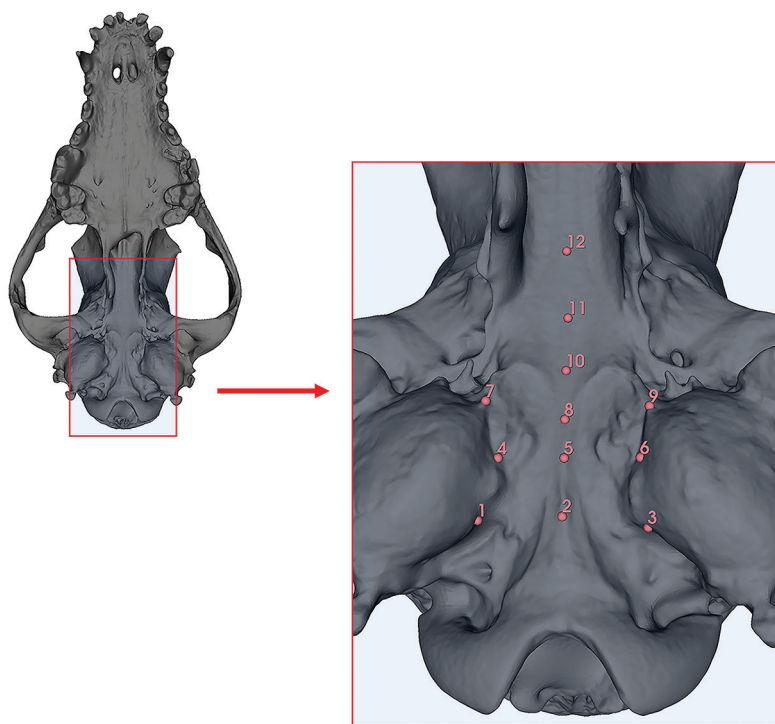


Figure 1. Landmarks

For each specimen, 12 homologous anatomical landmarks were defined to characterize the morphology of the ventral sphenoid. These landmarks were distributed across key anatomical regions, including the midline axis (basisphenoid–presphenoid region), bilateral lateral structures, and the anterior and posterior limits of the sphenoid area.

Only clearly distinguishable and anatomically consistent points were selected to ensure repeatability. To maintain consistency in landmark placement, all specimens were evaluated under standardized viewing orientations, with particular attention to ventral alignment.

The dataset consisted of the three-dimensional Cartesian coordinates of each landmark. No linear or angular measurements were used. The identical landmark scheme was applied to all specimens, allowing direct comparison of shape variation through subsequent geometric morphometric analyses.

Statistical Analysis

Landmark data stored as .fcsv files were imported into the R environment, and the x, y, and z coordinates of the 12 anatomical landmarks were organized into three-dimensional landmark arrays. To remove the effects of scale, translation, and rotation, all landmark configurations were aligned using Generalized Procrustes Analysis (GPA), and centroid size values were calculated for each specimen. Data processing, Procrustes superimposition, principal component analysis (PCA), and shape visualizations were conducted using the geomorph package [14,15].

Size variation was assessed in two stages. Differences in centroid size between the main groups (dogs and cats) were evaluated using Welch's t-test based on log-transformed centroid size values. Differences among cat subgroups (British Shorthair, Scottish Fold, and domestic mixed-breed cats) were tested using one-way analysis of variance (ANOVA), followed by Tukey post hoc comparisons.

Shape variation was analyzed using Procrustes coordinates within permutation-based linear modeling frameworks. At the main group level, models were constructed as $\text{shape} \sim \text{main_group}$, and size-adjusted analyses were performed using $\text{shape} \sim \log\text{CS} + \text{main_group}$. For cat subgroups, analogous models ($\text{shape} \sim \text{cat_group}$ and $\text{shape} \sim \log\text{CS} + \text{cat_group}$) were applied. Pairwise comparisons among cat subgroups were evaluated using separate pairwise `procD.lm` models. All multivariate shape analyses were conducted using a randomized residual permutation procedure (RRPP) with 9,999 permutations [3].

To investigate allometric patterns, models were fitted as $\text{shape} \sim \log\text{CS}$ for the full dataset, $\text{shape} \sim \log\text{CS} \times \text{main_group}$ at the main group level, and $\text{shape} \sim \log\text{CS} \times \text{cat_group}$ for cat subgroups. Shape variation was visualized through PCA of Procrustes coordinates, and morphological changes along PC1 and PC2 axes were illustrated using ± 2 standard deviation deviations from the consensus configuration in inferior, coronal, and sagittal views. The PCA plot of dog breeds was interpreted as exploratory due to limited sample sizes per breed.

Statistical significance was set at $p < 0.05$ for all analyses.

RESULTS

Size Differences

Centroid size was significantly larger in dogs compared to cats (30.37 ± 6.05 vs. 23.06 ± 2.49). Welch's *t*-test performed on log-transformed centroid size values confirmed that this difference was statistically significant ($t = 5.01$, $p = 0.000025$). In addition to the higher mean values, dogs also exhibited a broader distribution of centroid size, indicating greater variability within the group (Figure 2).

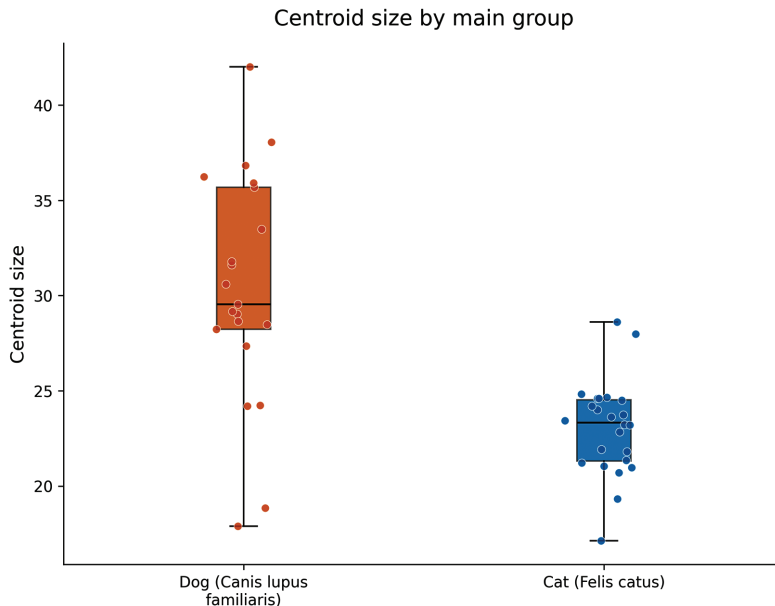


Figure 2. Distribution of ventral sphenoid centroid size among major groups

Among cat subgroups, centroid size was highest in Scottish Fold (24.38 ± 3.49), lowest in British Shorthair (21.06 ± 1.62), and intermediate in domestic mixed-breed cats (22.85 ± 1.34). However, these differences were not statistically significant (ANOVA: $F = 2.40$, $p = 0.115$). Post hoc Tukey tests revealed only a marginal trend between Scottish Fold and British Shorthair ($p = 0.098$), while all other pairwise comparisons were not significant.

Shape Differences and Morphospace Patterns

Permutation-based RRPP analysis of Procrustes shape coordinates revealed a significant difference between dogs and cats ($R^2 = 0.145$, $F = 7.30$, $p = 0.0001$). This group effect remained significant after including size as a covariate ($R^2 = 0.106$, $F =$

5.69, $p = 0.0002$), indicating that the observed shape divergence cannot be explained solely by size differences.

In the PCA morphospace, cats were predominantly distributed along positive PC2 values, whereas dogs were mainly located along negative PC2 values. Despite this separation, partial overlap between the groups was observed along the PC1 axis (Figure 3).

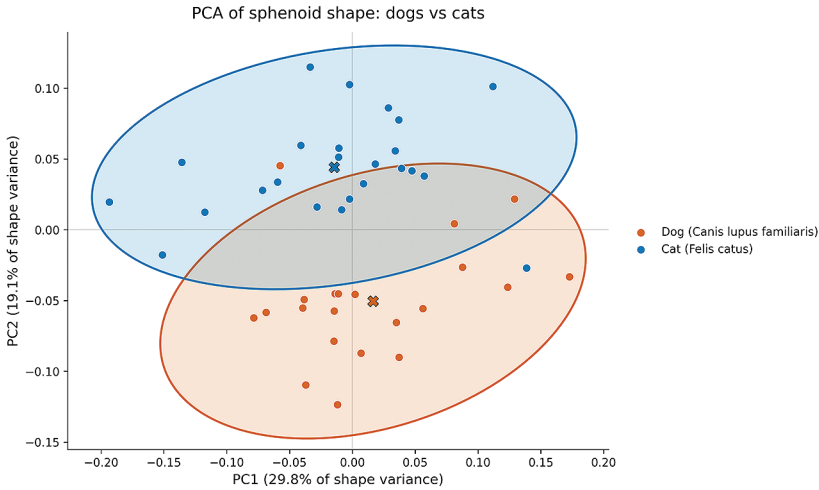


Figure 3. Main group PCA distribution of ventral sphenoid shape: comparison of dogs and cats

Significant shape differences were also detected among cat subgroups (raw shape model: $R^2 = 0.329$, $F = 5.16$, $p = 0.0001$). These differences persisted after size correction ($R^2 = 0.220$, $F = 4.01$, $p = 0.0002$). In the combined PCA distribution of all groups, Scottish Fold specimens tended to occupy negative PC1 values, British Shorthair specimens were positioned toward positive PC1 values, and domestic mixed-breed cats showed a tendency toward positive PC2 values. However, a degree of overlap between group distributions remained (Figure 4).

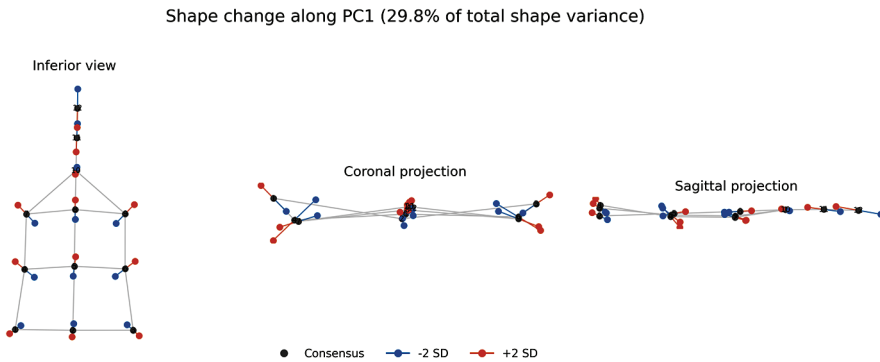


Figure 4. Ventral sphenoid shape changes along PC1 (-2 SD to +2 SD)

Pairwise comparisons based on raw shape data indicated significant differences among all cat subgroup pairs. After size correction, significant differences were retained for British Shorthair–Scottish Fold and Scottish Fold–domestic mixed-breed cat comparisons, whereas the difference between British Shorthair and domestic mixed-breed cats was no longer significant. This pattern suggests that shape differentiation among cat subgroups is primarily driven by the distinct morphology of Scottish Fold specimens.

Shape Variation Along PC1 and PC2

PC1 accounted for 29.8% of the total shape variance, while PC2 explained 19.1%. Along PC1, the most pronounced displacements were observed in the posterior midline landmarks (particularly F11–F12) and bilateral lateral landmark pairs (F4/F6 and F7/F9). This axis represents a gradient between relative midline elongation and lateral expansion of the ventral sphenoid region (Figure 4).

Morphologically, negative PC1 values correspond to a configuration in which the midline is more elongated and prominent, while the lateral components are positioned closer to the midline, producing a relatively narrow and anteroposteriorly extended structure. In contrast, positive PC1 values are associated with a broader and more laterally expanded morphology, in which the bilateral elements are displaced outward and the midline appears comparatively shorter and more compact.

Along PC2, the most notable changes were concentrated in the lateral landmark pairs (F4/F7 and F6/F9), as well as in the lower midline landmarks (particularly around F2, F5, and F8). This axis more clearly captured the separation between dogs and cats observed in the PCA morphospace (Figure 5).

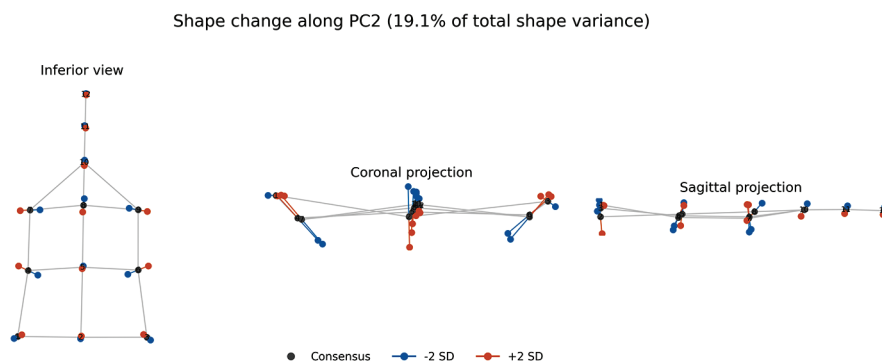


Figure 5. Ventral sphenoid shape changes along PC2 (-2 SD to $+2$ SD)

From a morphological perspective, PC2 reflects a coordinated three-dimensional reorganization between midline and lateral basicranial components rather than a simple size-related variation. At negative PC2 values, where dog specimens are predominantly located, the ventral sphenoid exhibits a more expanded and laterally

dispersed configuration, with lateral structures positioned further from the midline and slightly ventrally displaced. This results in a relatively wider and more open basicranial architecture.

In contrast, positive PC2 values, where most cat specimens are concentrated, are characterized by a more compact and centrally organized configuration. In these specimens, lateral elements are positioned closer to the midline and show a more aligned arrangement, suggesting a tighter and more integrated ventral sphenoid structure.

Taken together, these patterns indicate that the primary interspecific differences in ventral sphenoid morphology are not limited to overall size variation, but instead reflect differences in the spatial organization and relative positioning of midline and lateral components of the cranial base.

Allometry

When all specimens were considered together, a significant allometric relationship between centroid size and shape was detected ($R^2 = 0.113$, $F = 5.49$, $p = 0.0002$). In the main group model, both the group effect ($R^2 = 0.052$, $F = 2.94$, $p = 0.0091$) and the interaction between log-centroid size and group ($\log\text{CS} \times \text{group}$: $R^2 = 0.056$, $F = 3.19$, $p = 0.0056$) were significant. This indicates that dogs and cats not only differ in their mean shape, but also follow partially distinct allometric trajectories, with shape changing differently as size increases.

Within cat subgroups, the effect of log-centroid size remained significant ($R^2 = 0.080$, $F = 3.10$, $p = 0.0266$). However, neither the subgroup main effect ($p = 0.0896$) nor the interaction between size and subgroup ($\log\text{CS} \times \text{subgroup}$: $p = 0.0926$) reached statistical significance at the 0.05 level.

DISCUSSION

The results of the present study demonstrate that the ventral sphenoid region represents a structurally informative component of the cranial base, capturing both size-dependent and size-independent patterns of morphological variations (Table 1). A clear distinction between dogs and cats was observed not only in centroid size but also in shape, and importantly, shape differences remained significant after accounting for size effects. This indicates that interspecific variation in the ventral sphenoid cannot be attributed solely to scaling, but instead reflects differences in the spatial organization of basicranial structures.

In contrast, while size differences among cat subgroups were not statistically significant, shape variation was consistently detected both before and after size correction. This pattern suggests that morphological differentiation within cats is primarily driven by shape rather than size, highlighting the ventral sphenoid as a region sensitive to

subtle breed-related variation. Furthermore, the presence of significant allometric effects across the full dataset, combined with group-specific differences in allometric trajectories, indicates that growth-related shape changes contribute to, but do not fully explain, the observed morphological diversity.

Table 1. Summary of statistical results for size, shape, and allometry analyses

Analysis	Statistic	p-value	Interpretation
Size: dogs vs. cats	$t = 5.01$	0.000025	Dogs have significantly larger centroid size values than cats
Size: cat subgroups	$F = 2.40$	0.115	No significant size difference between cat subgroups
Shape: dogs vs. cats (raw)	$F = 7.30; R^2 = 0.145$	0.0001	Clear shape difference between main groups
Shape: dogs vs. cats (size-adjusted)	$F = 5.69; R^2 = 0.106$	0.0002	Shape difference persists after size correction
Shape: cat subgroups (raw)	$F = 5.16; R^2 = 0.329$	0.0001	Significant shape differences between cat subgroups
Shape: cat subgroups (size-adjusted)	$F = 4.01; R^2 = 0.220$	0.0002	Differences remain after size correction
Allometry: all samples	$F = 5.49; R^2 = 0.113$	0.0002	Significant relationship between size and shape
Allometry: logCS × main group	$F = 3.19; R^2 = 0.056$	0.0056	Dogs and cats show partially different allometric trajectories
Allometry: logCS × cat subgroup	$F = 1.68; R^2 = 0.086$	0.0926	Trend-level differences between cat subgroups

The persistence of shape differences between dogs and cats after size correction suggests that the ventral sphenoid reflects not only overall skull size but also species-specific organization of the cranial base. The cranial base plays a central role in determining craniofacial proportions and the spatial orientation of the facial skeleton, and its morphology is largely influenced by the endochondral development of components such as the basisphenoid and presphenoid [1–3]. Therefore, the observed interspecific

differences are biologically consistent with a three-dimensional reorganization of the cranial base rather than a simple consequence of overall skull size variation.

The broader size range and wider morphospace distribution observed in dogs further support this interpretation. Domestic dogs are known to exhibit an exceptional degree of cranial shape disparity, and previous studies have demonstrated that morphological distances among dog breeds can be comparable to interspecific differences within carnivores [9]. In addition, the fixation of brachycephalic head types in certain breeds indicates that the basicranium, together with the facial and neurocranial regions, can be strongly influenced by selective breeding.

Although the inclusion of multiple breeds in the present study likely increased within-group variability in dogs, the clear separation from cats despite this heterogeneity highlights the strong discriminatory power of the ventral sphenoid at the species level. This finding supports the view that the ventral sphenoid region represents a structurally informative component of the cranial base, capable of capturing biologically meaningful variations beyond simple size effects.

The absence of significant size differences alongside the presence of clear shape differences among cat subgroups is particularly noteworthy. This pattern is consistent with previous geometric morphometric studies reporting limited variation in centroid size but pronounced shape differentiation among breeds such as British Shorthair and Scottish Fold [7,8]. In the present dataset, the fact that the Scottish Fold group consistently drives the observed separation in both raw and size-adjusted analyses suggests that this breed carries a more distinct cranial base signal at the level of the ventral sphenoid.

The persistent separation of Scottish Fold from the other cat groups may be linked to its breed-specific developmental background. The Scottish Fold phenotype has been associated with a dominant variant of the *TRPV4* gene, which is known to cause osteochondrodysplasia and to affect cartilage and bone development. Given that the ventral sphenoid region is closely related to endochondral ossification processes, a mechanistic link between this genetic background and the observed shape differentiation appears plausible. However, as the present study does not include genotypic or histological validation, this interpretation should be considered as a biologically reasonable hypothesis rather than direct causal evidence. In contrast, the disappearance of the difference between British Shorthair and domestic mixed-breed cats after size correction suggests that their divergence at the level of the ventral sphenoid is more subtle and partially influenced by scaling effects, rather than reflecting a fully independent shape differentiation.

The allometric results further support this interpretation. The presence of a significant relationship between size and shape across all specimens indicates that ventral sphenoid morphology is not independent of growth-related processes. However, the significant interaction between log-centroid size and group demonstrates that dogs and cats do

not respond to size increase in the same way, following partially distinct allometric trajectories.

In the PC1–logCS distribution, both groups showed a trend toward more negative PC1 scores with increasing size, suggesting that larger specimens tend to exhibit a relatively narrower and more elongated ventral sphenoid configuration. Notably, this trend appeared steeper in cats, indicating a stronger size-related shape response compared to dogs. These findings are consistent with comparative studies showing that cranial growth trajectories can diverge among domesticated forms [16].

Within cat subgroups, although the effect of log-centroid size was significant, the absence of a statistically significant interaction at the 0.05 level suggests that shape divergence among breeds cannot be explained solely by allometric scaling. In other words, the differentiation observed in Scottish Fold specimens is not merely a consequence of being larger or smaller, but rather reflects a partially size-independent reorganization of shape.

Several limitations of this study should be acknowledged. The relatively small sample size of the British Shorthair group ($n = 4$) may have reduced statistical power in some subgroup comparisons. In addition, although dogs were included as a single main group, the number of specimens representing individual breeds was generally low; therefore, the breed-level distribution presented in Figure 6 should be interpreted as exploratory rather than inferential. Furthermore, the analysis was restricted to 12 anatomical landmarks placed on the ventral sphenoid region. As a result, the broader structural relationships with the neurocranium, facial skeleton, and temporomandibular region were not directly assessed within this dataset. Despite these limitations, the present findings demonstrate that the ventral sphenoid represents a highly informative region within the domestic carnivore skull. Interspecific differences remained significant even after size correction, and among cat subgroups, a distinct size-independent shape signal, particularly associated with Scottish Fold specimens, was identified.

Ethical Approval

This study was conducted in accordance with national regulations governing animal research. Based on the evaluation of the Local Ethics Committee for Animal Experiments of Istanbul University-Cerrahpaşa, the study was classified as not requiring ethical approval, as it falls within the scope of diagnostic and clinical applications (Approval No: 2025/20; Decision date: February 28, 2025).

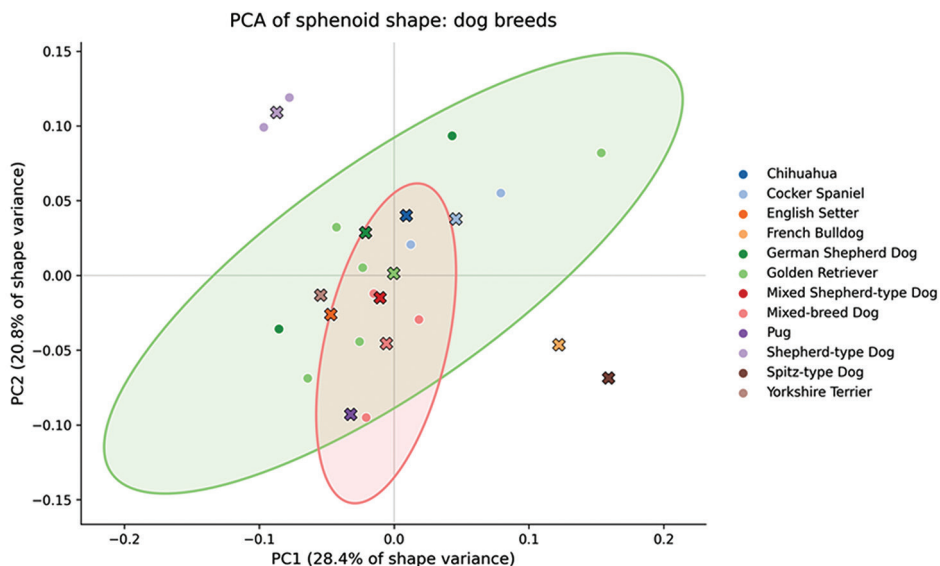


Figure 6. PCA distribution of dog samples according to standardized breed labels (exploratory visualization)

Authors' contributions

BCG contributed to conceptualization, methodology, resources, and writing original draft. SD contributed to methodology and writing original draft. NM contributed to software, methodology, and writing review and editing. OG contributed to investigation, supervision, and writing review and editing. SA contributed to visualization and writing review and editing. MD contributed to methodology and writing review and editing.

Declaration of conflicting interests


The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Informed Consent

The owner understood procedure and agrees that results related to investigation or treatment of their companion animals, could be published in Scientific Journal Acta Veterinaria-Beograd.

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TRODIMENZIONALNA GEOMETRIJSKA MORFOMETRIJSKA ANALIZA VENTRALNOG SFENOIDA KOD DOMAĆIH PASA I MAČAKA

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Ova studija je imala za cilj da proceni veličinu, oblik i alometrijske obrasce ventralnog sfenoidnog regiona kod pasa i mačaka korišćenjem trodimenzionalnog geometrijskog morfometrijskog pristupa. Analizirano je ukupno 45 uzoraka, uključujući 21 psa i 24 mačke. Dvanaest anatomskih orijentira postavljeno je na ventralnom sfenoidnom regionu i analizirano prateći generalizovanu Prokrustovu analizu. Varijacija oblika procenjena je korišćenjem analize glavnih komponenti i multivarijantnih modela zasnovanih na permutacijama. Psi su pokazali značajno veće veličine centroida u poređenju sa mačkama. Otkrivena je jasna diferencijacija oblika između vrsta i ostala je značajna nakon uzimanja u obzir efekata veličine, što ukazuje da se međuspecifične razlike ne objašnjavaju isključivo skaliranjem. Iako razlike u veličini centroida među podgrupama mačaka nisu bile statistički značajne, razlike u obliku su se nastavile i pre i posle korekcije veličine. Poređenje parova je pokazalo da je grupa mačaka rase "Scottish Fold" pokazala najizraženiju i najkonzistentniju divergenciju. Alometrijski odnosi između veličine i oblika bili su značajni u celom skupu podataka, a interakcija između veličine i grupe sugerisala je delimično različite putanje oblika povezane sa rastom kod pasa i mačaka. Analize glavnih komponenti pokazale su da je glavna varijacija oblika povezana sa promenama u izduženju srednje linije i lateralnom širenju, kao i sa trodimenzionalnim repozicioniranjem srednjih i lateralnih komponenti. Ovi nalazi pokazuju da ventralni sfenoid predstavlja biološki značajan bazični kranijalni modul sposoban da obuhvati i međuspecifične razlike i varijacije oblika nezavisne od veličine među podgrupama mačaka.