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High level of fluctuating asymmetry in the Byzantine dogs from the Theodosius Harbor, Istanbul, Turkey

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Abstract: Asymmetry, the abnormality of an organism or a part of it from its perfect symmetry, is represented by three different categories: fluctuating asymmetry, directional asymmetry, and antisymmetry. Fluctuating asymmetry attributes to random developmental variation of a morphological character, whereas directional asymmetry attributes one of the body sides to be more prominent than the other. Antisymmetry appears whenever one body side of a biological body shows greater morphological appearance than the other. Since more environmental stress often produces greater effect of fluctuating asymmetry, it can be a good indicator of physiological stress in the morphological characteristic of a biological being. Applying, so far, the first geometric morphometric methods on any Byzantine fauna, this study aimed to determine the kind and direction of skull asymmetry occurred in Byzantine dog skulls. Aiming this, asymmetries in 16 adult Byzantine dog skulls unearthed form Yenikapı-Marmaray excavation (ancient Theodosius Harbor) in İstanbul, were compared with 39 adult skulls of modern pet dog breeds. Seventeen landmarks (3 midline and 14 bilateral) were selected on the digital pictures of the ventral aspect of each skull, and used for detailed analysis. The results showed a greater percentage of fluctuating asymmetry in the Byzantine dog skulls, suggesting them not to be the remains of pets or housed dogs but perhaps the labor or stray dogs in the Byzantine capital Constantinople.

Key words: Byzantine dog, geometric morphometrics, fluctuating asymmetry, Theodosius Harbor, *Canis familiaris*, Yenikapı-Marmaray excavation, İstanbul

1. Introduction

Geometric morphometric methods improve the morphometrics since they have unique abilities for measuring displacements, deformations, and rotations of objects [1], which enables researchers to quantify the qualitatively described morphological traits. In recent years, there are a rich number of zoological and archaeological studies carried out with the applications of geometric morphometrics methods [1-6]. In addition to their applications on various research questions, geometric morphometrics have also been applied to study the symmetry and asymmetry of shape [6]. Besides, particularly Mardia et al. (2000) and Klingenberg (2015) have combined the existing geometric morphometric methods for the study of symmetry and asymmetry in biological applications in general [7,8].

Asymmetry is defined as a deviation of a whole organism or a part of it from its perfect symmetry. It is composed of two different categories. Among different significant types of morphological asymmetries, the fluctuating asymmetry (FA) is the random developmental variation of a trait (or character) that is expected to be symmetrical on average [3]. It is a population-level measure of developmental instability [3,4,9]. The directional asymmetry (DA), on the other hand, occurs when one of the body sides shows stronger morphological structures or marks than the other [3,10]. Since the mammal body is bilaterally symmetrical in most the body parts at least at birth [11], DA, therefore, may develop as a consequence of genetic deformations and bone remodeling on the later-developed morphological traits [12,13]. The antisymmetry (AS) appears whenever one body side of a biological body shows greater morphological appearance than the other. It is notable that most of the internal organs such as heart, lungs, kidneys, and stomach as well as the brain are directionally asymmetric [14,15].

Among these asymmetries, the study of FA has been a useful tool to understand the developmental instability

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in a population level of distinct species [3,4,9]. However, compared to other skeletal parts, the skull has often been used to obtain the most elaborate result in the study of FA [4]. With the application of geometric morphometric techniques [1,6,16,17], this study primarily aimed to determine the type/s and directions of skull asymmetry in a group of well-preserved Byzantine dog skulls unearthed from the Yenikapı-Marmaray rescue excavations in İstanbul [18]. The obtained asymmetries were further compared to the asymmetries in the skulls of most common modern pet dog breeds in İstanbul, in an attempt to understand the apparent status of Byzantine dog population.

The site Yenikapı-Marmaray was known to be the ancient Theodosius Harbor of Byzantine capital [19]. Throughout the salvage excavations between 2004 and 2013, more than a hundred thousand complete bones of about 60 species were recorded from the site, and, among them, a large assemblage of dog remains were identified [18]. Being so far the richest canine assemblage recorded from any Byzantine site, the results of this study will add some new sheds of lights in the study of human-dog relationships as well as the status of dogs in the Byzantine capital Constantinople.

2. Materials and methods

2.1. Sample

A sample of 55 complete canine crania was examined in this study. 16 of these specimens were selected from zooarchaeological assemblage of the Yenikapı-Marmaray excavation (ancient Theodosius Harbor), unearthed between 2004 and 2013 [18]. Although over 500 dog skulls were unearthed from the site, most of them were either partly broken or lacked the complete landmark spots. Besides, a large number of skulls belonged to young individuals and a considerable number of them showed different types of deformation marks. The skulls of young individuals or the broken skulls or with deformations were strictly excluded, considering the fact that they can introduce a significant amount of bias to the symmetry studies. Only the most complete skulls of adult individuals without any deformation and with complete landmark spots were most suitable for this study. Therefore, only 16 adult specimens of which the upper second molar (M^2) was fully erupted and without any sign of pathologic asymmetry were selected from the Byzantine assemblage. The other 39 specimens were the skulls of modern pet dogs –composed of mesaticephalic, brachycephalic and dolichocephalic dog breeds, collected from the Osteoarchaeology Research Center of İstanbul University-Cerrahpaşa. The breed distinctions within the modern pet dog skulls were not considered to be a primary concern; therefore, they all were analyzed as a single unit/group. Similarly, for the comparison with modern pet dog unit/group, the Byzantine dog skulls were also analyzed as a single unit/group.

2.2. Data acquisition and acquiring landmark data

Ventral side of the specimens was targeted and brought under examination in study, since the ventral side is the functional part of the skull (e.g., alimentary and respiratory pathways). In contrast, the consideration of the dorsal side would have reduced the availability of specimens, as some of them had fragmented calvaria. Besides, dorsal landmarks normally can only be lateral, with a coplanarity problem, and thus, posed the risk of losing complete biological information. Therefore, measurement of the ventral side was followed to obtain a more convincing result in comparison of the two dog groups.

High resolution photograph of the ventral side of each specimen was taken with a Nikon (D5100) digital camera and an AF-S DX Micro Nikkor 40mm f/1·2.8G lens. The camera was placed in a manner that the focal axis could be parallel to the horizontal plane and centered on the ventral aspect of the skull (Figure 1). Although focal distances varied between specimens, each specimen was placed on a stand alongside the ruler for the purpose of proper scaling.

The *x*, *y* coordinates of 17 landmarks (3 midline and 14 bilateral), on the ventral surface of the skull base, were used in this study (Table 1). The landmarks were chosen on the basis of adequately illustrating the anatomy of the basicranium (Figure 2). Most of the landmarks were obtained according to the guide of von den Driesch [20], which were sufficient and standard for summarizing the morphology of the ventral symmetric structures, contact points between bones, tips of processes, and points of maximum curvature. Coordinates for each landmark were extracted from the digital image of each specimen, using the digitalization software tpsDig v. 1.40 [21].

Coordinates also contained other components such as position, orientation and size, which were not related to shape. To remove these distortions, landmark configurations were superimposed by using the Generalized Procrustes Analysis, mainly based on a generalized least-squares minimization of the distance between corresponding landmarks [22]. By this superimposition, landmark configurations were translated to a common centroid position in the coordinate system, scaling them to unit centroid size (CS) and rotating them to minimize the distances between corresponding landmarks. Thus, by working on standardized landmark coordinates, superimposition methods allowed the shape analysis independent from the size [23]. The coordinates were previously projected in a Euclidean tangent space in order to test whether the shape variation was small enough to consider that the new tangent space was a good representation of the Procrustes data in a Euclidean space. To check it, the correlations between the tangent and

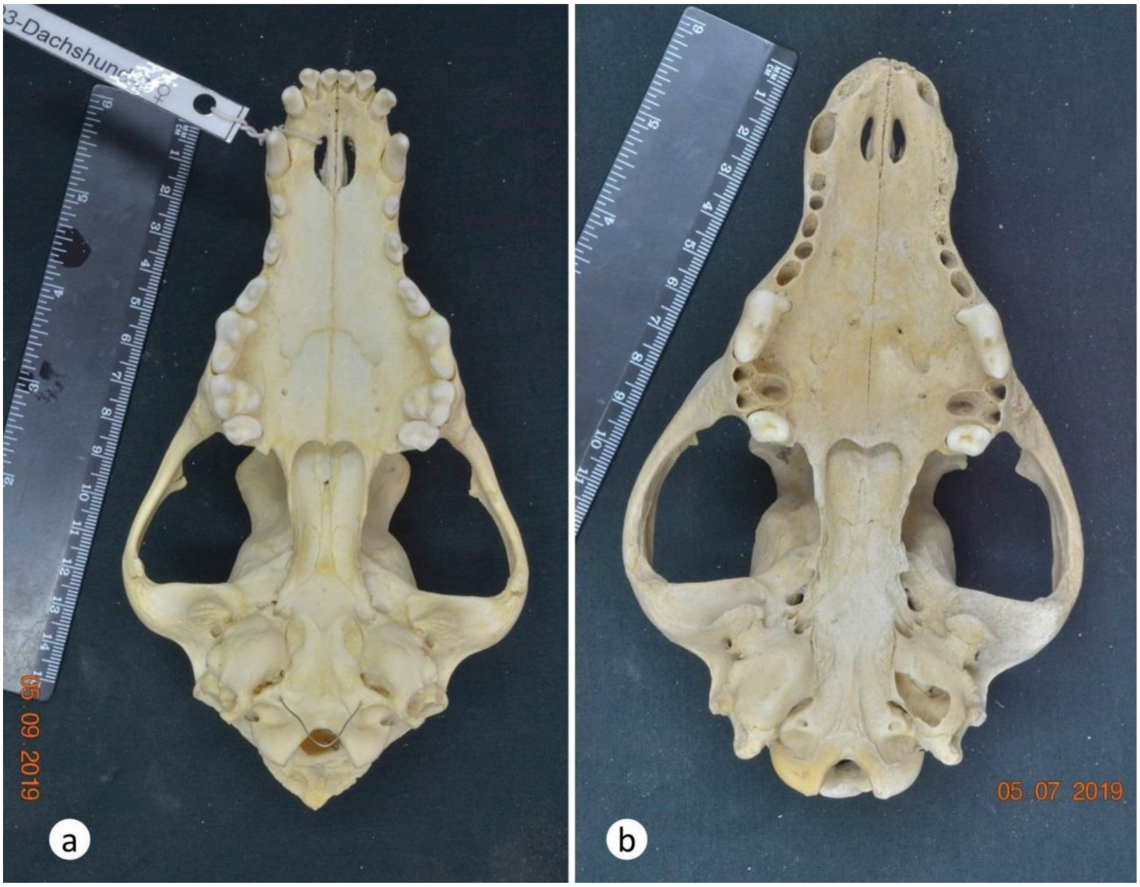

Figure 1. Examples of the pictures from the ventral aspect of the complete skull groups: a) a complete skull of modern adult pet dog; b) a complete skull of adult Byzantine dog from Yenikapı.

Table 1. List of ventral landmarks used in this study.

Landmark	Description
A	Sagittal most rostral point of the *corpus ossis incisivi*
B	Sagittal most caudal point of *lamina horizontalis ossis palatini*
C	Sagittal most rostral point of *foramen magnum*
1, 1’	Most rostral part of *fissura palatina*
2, 2’	Most caudal part of *fissura palatina*
3, 3’	Most rostral point of *processus temporalis ossis zygomatici*
4, 4’	Lateral part of suture between *processus temporalis ossis zygomatici* and *arcus zygomaticus*
5, 5’	*Foramen alare rostrale*
6, 6’	*Foramen ovale*
7, 7’	*Foramen palatinum majus*

Procrustes distances were computed by using tpsSmall v. 1.33 software [21]. The results of correlations (uncentered correlation = 0.999, root mean square error = 0.000136) confirmed that for both space distances were nearly identical.

2.3. Measurement error analysis and antisymmetry (AS)

Measurement error is a confounding factor in the assessment of fluctuating asymmetry [1,24]; therefore, all skulls were digitized twice in order to estimate intra-observer error. A shape Procrustes ANOVA [24] was performed to analyze the total shape variation and to

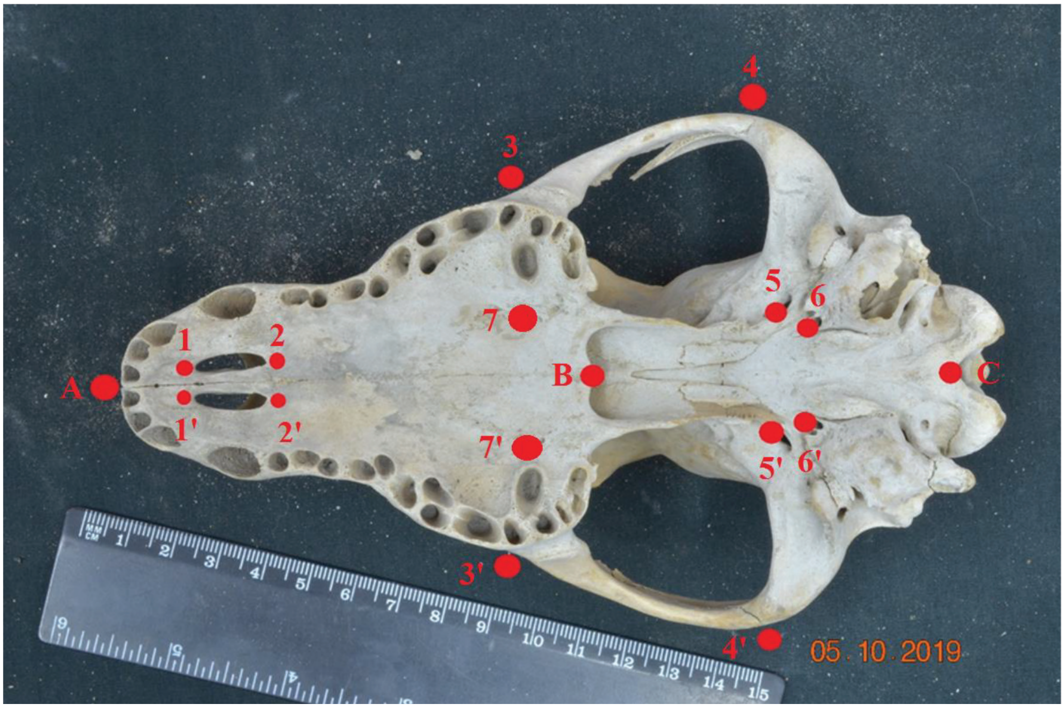

Figure 2. Fourteen paired (numbers) and three unpaired (letters) landmarks used on the picture (of each specimen) to describe basicranial shape.

examine the proportion of mean squares of measurement error with respect to overall variation. The AS was analyzed using scatterplots of the differences between the left and right side for each landmark. The formation of clusters of points in this distribution corresponded to a bimodal distribution in the differences between the left and the right sides, and therefore, to the presence of AS.

2.4. Symmetric and asymmetric variation

The total shape variation of the skull was partitioned into the symmetric and asymmetric components through Procrustes superimposition of the original landmark configurations and their mirror images. The asymmetry was quantified through the landmark deviations of the original configuration from the symmetric consensus of the original and mirror image [7]. The Procrustes fit with reflection of shape, and mapped the right and left shape configurations together. Procrustes distance was used as a measure of shape asymmetry between the right and left sides of the skulls. A Procrustes analysis of variance was performed to study the asymmetric component of shape, which allowed detecting the significance of different sources of variation such as inter-individual variation, FA and DA. Multivariate analysis of variance was used to determine the significance of asymmetry components for the shape variation (parametric) in covariate matrix. A final Canonical Variate Analysis (CVA) was performed for net asymmetry (NA), composed of the sum of FA and DA, between both groups by using a 10,000 permutation rounds on Procrustes distances on regression scores. Partitioning NA into its FA and DA components allowed determining the types of asymmetry accounts for the variation in non-directional asymmetry between the populations.

2.5. Allometry

A linear regression of the shape *versus* size was performed to detect how the asymmetric component of shape could change in relation to the size in both groups. Since the CS corresponds to the squared root of the sum of the squared distances from each landmark to the centroid point [23], the CS of the landmark configurations was used as a proxy for the size measurement. Symmetries of shape were analyzed in MorphoJ software v. 1.06c [25] by using object symmetry (i.e., the symmetry operator passing through the sagittal plane of the skull).

3. Results

3.1. Measurement error and variation of general sample

The total amount of measurement error for shape was less than 0.2% in both the Byzantine and modern pet dog group (Table 2-3). Therefore, measurement error was negligible, apparently random, and hence, not affecting the result of asymmetry analyses. Individual variation was statistically significant both in the shape (Table 2) and size (Table 3) measurement ($p < 0.01$). Scatter plot of the points of left-right differences for each landmark showed no clustering of points. Therefore, the size and shape AS was discarded, since the study was mainly focused on FA and DA.

3.2. Allometry

The regression of asymmetric component versus centroid size (CS) was only significant for the modern pet dog group ($p = 0.0021$), but not for the Byzantine dog group ($p = 0.197$). On the other hand, size explained a total of 7.25% observed asymmetric shape variation for the modern pet dog group (Figure 3).

3.3. Asymmetric component of shape

In Procrustes ANOVA analysis, statistically significative FA as well as DA ($p < 0.05$) was found in the modern pet dog group, while only a significative level of FA was observed in the Byzantine dog group (Table 2). However, the Pillai' trace criterion of asymmetry component shape variation (FA and DA) was significant on both groups (both $p < 0.02$). The level of nondirectional asymmetry was higher in the Byzantine dog group (12.4% vs 1.8%), and appearing that both groups were statistically differentiated on CVA ($p < 0.0001$) (Figure 4).

For the Byzantine dog group, shape variance associated with DA accounted for 14.3% of the total variance, while the variance associated with FA represented a total of 13.7% (Table 2). This strongly suggested that the Byzantine and modern pet dog groups did not have the same magnitude of differences in FA (Figure 5).

4. Discussion

In a biological feature, symmetry can be defined as the correct morphological arrangements of the repeating physical parts [7,17,26], whereas asymmetry is considered as a deviation from symmetry [8]. Alongside the study of different other morphological characteristics, asymmetry has been a significant characteristic in the

Table 2. Measurement error Procrustes ANOVA for shape and size of Byzantine (n = 16) and modern pet dog skulls (n = 39). Directional asymmetry (DA) represents the "side" effect and fluctuating asymmetry (FA) represents the "side*individual" interaction. Net asymmetry (NA) is composed of the sum of FA and DA. Sums of squares (SS) and mean squares (MS) are in units of Procrustes distances (dimensionless). The Pillai' trace criterion of asymmetry component shape variation (FA and DA) was significant on both groups.

Byzantine dog	SS	MS	Df	%	NA	F	P
Individuals	0.096289	0.000183	525	69.55		5.6	<.0001
DA	0.000705	4.7E-05	15	17.81	30.24	1.43	0.1266
FA	0.017207	3.28E-05	525	12.42		60.36	<.0001
Error	0.000261	5.43E-07	480	0.20			
Modern pet dog	SS	MS	Df	%	NA	F	P
Individuals	1.162414	0.002039	570	74.72		39.93	<.0001
DA	0.009538	0.000636	15	23.29	25.17	12.45	<.0001
FA	0.029112	5.11E-05	570	1.87		17.27	<.0001
Error	0.00346062	0.0000029578	1170	0.11			

Table 3. Measurement error for size analysis of Byzantine (n = 16) and modern pet dog group (n = 39). Net asymmetry (NA) is composed of the sum of FA and DA. Sums of squares (SS) and mean squares (MS) are in units of Procrustes distances (dimensionless).

Byzantine dogs	SS	MS	Df	F	P
Individuals	24225322.9	692152.1	35	1059302	<.0001
Error	10.454468	0.653404	16		
Modern pet dogs	SS	MS	Df	F	P
Individuals	9588086	252318.1	38	121889.3	<.0001
Error	80.7323	2.070059	39		

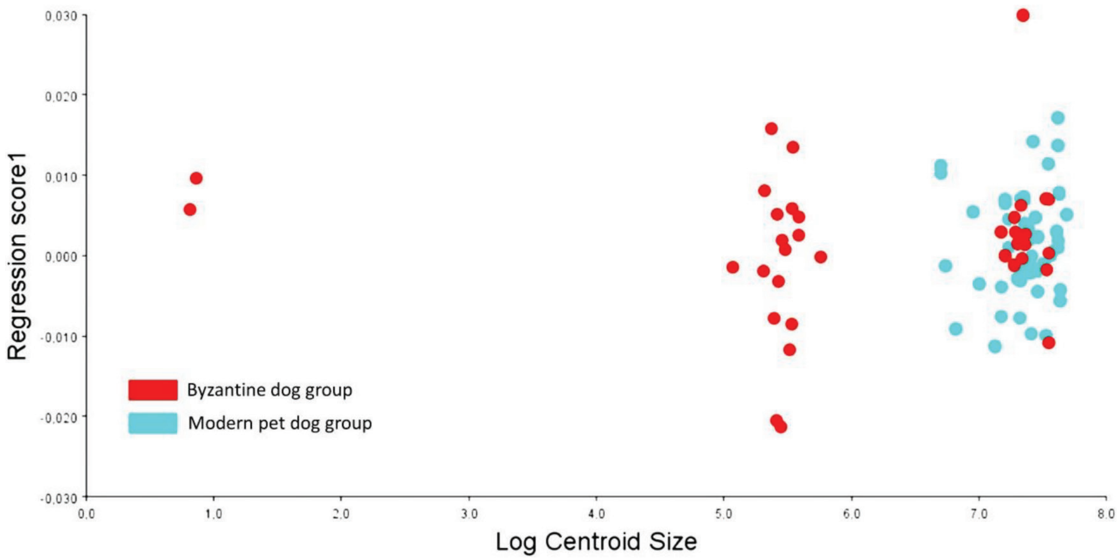

Figure 3. A linear regression of the asymmetric component showed a total of 7.25% shape variation in the modern pet dog group.

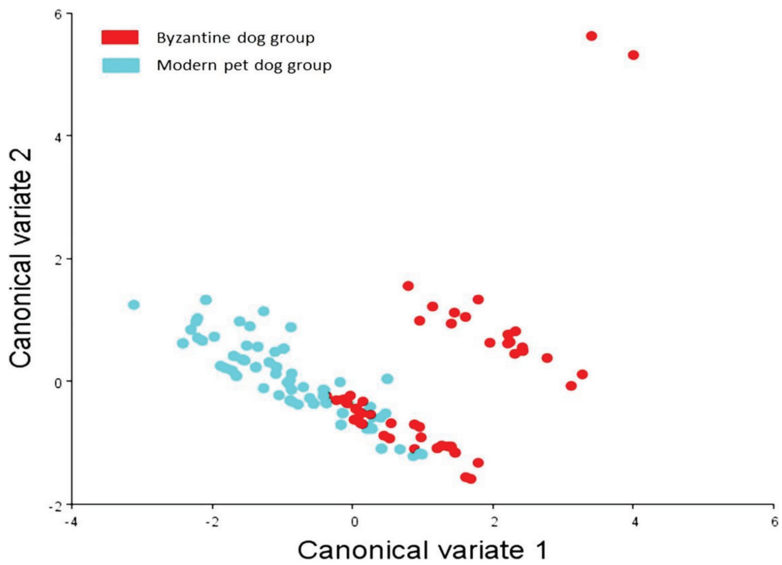

Figure 4. Canonical Variate Analysis of Byzantine dog group (n = 16) and modern pet dog group (n = 39) for asymmetric component of shape, showing statistical differences between them ($p < 0.0001$ from 10,000 permutation rounds for Procrustes distances among groups).

study of the mammal skull morphology. Particularly, the study of fluctuating asymmetry (FA) can trace the small, completely random departures from bilateral symmetry [3,4,9], whereas the directional asymmetry (DA) can trace irregularities in the bilateral morphological traits, mostly resulted by genetic deformations [3,10]. Since it expresses small deviations from symmetry, FA has been an important criterion about genotype and environmental relationship in the evaluation of developmental instability [27], especially in geometric morphometric studies [8], assuming that it is an inverse measure of developmental stability [28]. It is also often used as a bio-indicator of environmental stress and therefore helps understand how an organism copes with external stress during its developmental process [28].

Different forms and aspects of environmental effects on FA have been discussed in a significant number of recent studies, many of them were carried out on modern animal populations [29-33] as well as archaeological samples [6,34]. It is important that studies on archaeofauna should consider the traits that bear witness to the living conditions

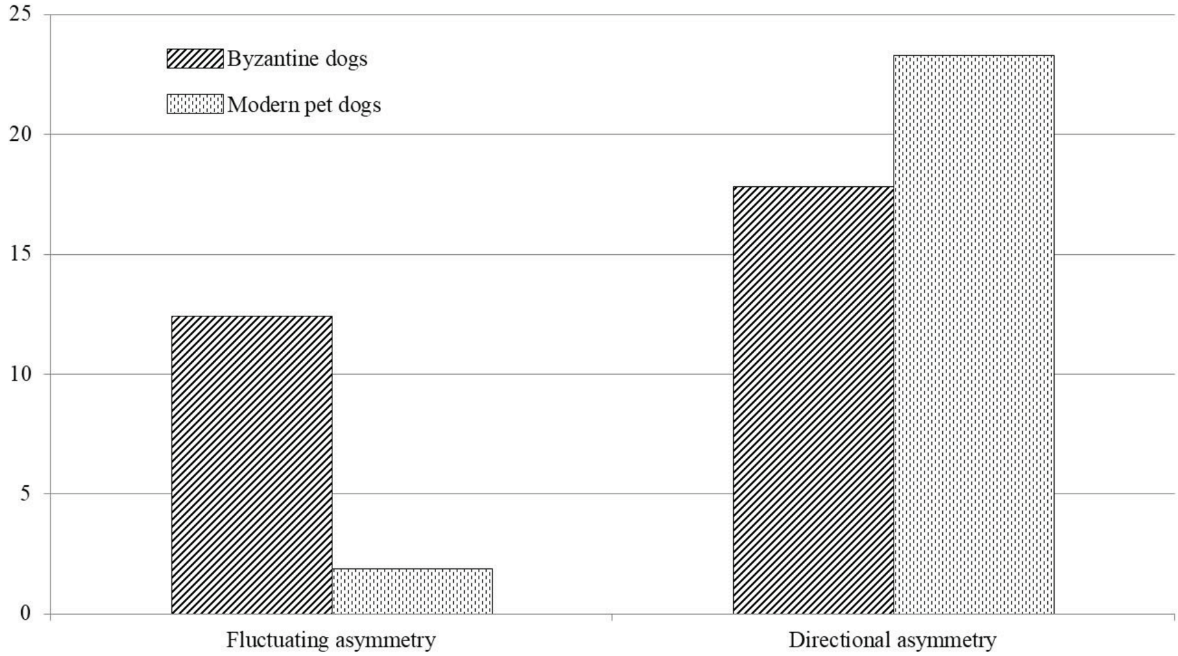

Figure 5. Differences of fluctuating asymmetry and directional asymmetry between two dog groups compared in this study: Byzantine dogs (n = 16), modern pet dogs (n = 39).

and development process of a particular animal population or individuals in their lifetime. For instance, these kinds of asymmetric traits occur in human populations due to their biological reaction to negative stimuli of the external environment such as malnutrition, inadequate hygienic conditions, and sick rate [3]. Hence, if a human or animal individual experiences such negative stimuli from the external environment in their lifetime, the asymmetric morphological features can often be visible on their skeletal remains from archaeological contexts.

This study revealed a greater percentage of FA in the Byzantine dog skulls. As their other skeletal parts did not show any potential genetic or biological deformation [18,19], it is likely that the high level of FA in the Byzantine dog skulls was probably resulted by their disadvantageous living condition, uneven environmental background, unhealthy feeding habit, or perhaps distant relationship with the urban human populations of Constantinople.

In morphometric studies, FA is considered to be a good indicator of physiological stress [3], since more environmental stresses often produce greater effects of FA [28]. As a result, skull fluctuating asymmetry can be explained by allometric scaling, indicating that this significative stress changed throughout different developmental stages of life. Since the stray dog populations, in general, often have a higher level of environmental stress than the household or pet dogs, and therefore present a higher level of fluctuating asymmetry, compared to the modern pet dogs in İstanbul, the far greater levels of FA in the Byzantine dogs from the Yenikapı-Marmaray may indicate that they were probably stray or street dogs with profound environmental stress.

Considerable percentage of DA was observed in the skulls of the modern pet dogs group from İstanbul. As it has been demonstrated in many other domestic mammals including cattle [13], horses [35,36], rabbits [26], small ruminants [37,38], and silver fox [39], the presence of DA in modern pet dog group could be a product of masticatory lateralization. The apparent absence of DA in Byzantine dog group, on the other hand, could merely be obscured by the similar percentage of directional asymmetry with net asymmetry (NA), this latter consisting of FA, antisymmetry and measurement error [40]. Therefore, since there is a continuum between the different types of asymmetry [41], it is likely that the absence of DA in the Byzantine dog group was merely statistical, and perhaps due to the presence of a very high level of FA.

Except only a few sites, canine remains were recorded from most of the Byzantine sites across Africa, Asia, and Europe. Yet, there is a lack of information about the status of dogs in the Byzantine world. It is argued that cats and dogs were often raised as pets in Byzantine cities [42]. Despite of having extremely exotic culinary practice and consumption of most of the other domestic mammals, so far no record of cynophagy was found from any Byzantine site. Nevertheless, dogs were also widely employed as shepherd dogs, guards, or hunting assistants [42]. With this general status of the dogs in the Byzantine Empire, it is possible to argue that there were also considerable numbers of starry dogs in Constantinople. The high level of FA in the dog skulls from Yenikapı-Marmaray excavations could stand for such claim. Apparently lived out of the human residence or without regular human care, the carcasses of these dogs were perhaps thrown into neighboring streams and channels of Constantinople, and, over time, deposited at the Theodosius Harbor area.

Being so far the first application of geometric morphometric techniques applied on canine remains from any of the Byzantine sites as well as a scientific observation indicating that the dog population from Yenikapı may not have high nutrition and cares like the pet dogs do, this study will bring some new sheds of light about the status of common dogs and their relationships with humans in the Byzantine capital Constantinople.

Conflict of interests

The authors declare no conflict of interests or any third-party interest regarding the data used in this study.

Ethics statement

The modern pet dogs used in this study were selected from the existing reference collections. No living dog was killed to obtain any of the specimens analyzed and compared. Hence, Ethics Committee agreement was not necessary to conduct this study.

Material and data availability

The Byzantine and modern pet dog skulls used in this study are housed in the collections of the Osteoarchaeology Research Center, İstanbul University-Cerrahpaşa. Byzantine specimens are available for further study or reanalysis via application through the General Directorate of Cultural Heritage and Museums, Ministry of Culture and Tourism, Republic of Turkey. The contents of statistical data are the sole responsibility of the second author. However, the datasets generated, analyzed, and used in this study will be provided by the corresponding author on request.

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