

# Geometric Morphometric Study and Cluster Analysis of Late Byzantine and Modern Human Crania

Senem Turan Ozdemir<sup>1</sup>, Ilker Ercan<sup>2</sup>, Guven Ozkaya<sup>2</sup>, N. Simsek Cankur<sup>1</sup> and Yilmaz Selim Erdal<sup>3</sup>

<sup>1</sup> Department of Anatomy, School of Medicine, Uludag University, Bursa, Turkey

<sup>2</sup> Department of Biostatistics, School of Medicine, Uludag University, Bursa, Turkey

<sup>3</sup> Department of Anthropology, School of Letters, Hacettepe University, Ankara, Turkey

## ABSTRACT

*Inter-population variation of cranial morphology, which plays an important role in human evolution studies and biological research, can be studied morphologically and metrically. Geometric morphometry compares body forms using specific landmarks determined by anatomical prominences. The aim of this study was to identify cranial shape differences between the crania of Byzantium period humans and modern humans. Variability in cranial shape was examined using the geometric morphometric technique based on landmark coordinates. Landmark coordinate data were collected from two-dimensional digital photogrammetry and were analyzed using generalized Procrustes analysis, hierarchical clustering and thin-plate spline analysis.*

**Key words:** cranium, shape, geometric morphometrics, generalized procrustes analysis

## Introduction

Craniofacial morphology is an important source of information for phylogenetic and population studies<sup>1</sup>. Cranial morphology is widely used to reconstruct evolutionary relationships, but its reliability in reflecting phylogeny and population history has been questioned<sup>2,3</sup>. Although interpopulation variation of craniofacial morphology has long been examined using traditional methods, in recent years, an alternative geometric morphometric method has been shown to provide new perspectives on previously studied material<sup>3-5</sup>. An analysis of craniofacial variation should take into account both the relative dimensions of the craniofacial skeleton and soft tissue and the arrangement of structures<sup>6</sup>.

The causes of craniofacial variations among human populations have been the subjects of controversy<sup>3</sup>. Many factors are responsible for craniofacial variation, including ecological variables (i.e., diet and climate), gene flow and genetic drift<sup>3</sup>. The causes of variations in craniofacial morphology are crucial to determining the characteristics of populations and establishing the differences and similarities between populations. Studies on human skeletons of different populations that occupied a single

area over different periods may be helpful in understanding the morphological characteristics of these populations.

Until 1333, Byzantines and Turks lived together in Iznik, which had been inhabited by Turks since 1075. Eyice<sup>7</sup> asserted that there were Turkish mercenaries inside the Byzantine army called »Turkopol«. Both the cohabitation of these communities and the presence of these mercenaries inside the army have been viewed as causes of the genetic heterogeneity in this community<sup>8</sup>. The formal comparison of present-day crania with the Byzantine crania obtained from excavations in Iznik that were inhabited largely by a heterogeneous community illustrates some of the similarities and differences between these two communities.

Geometric morphometrics is a landmark-based method that was developed to analyze form, and thus morphological changes. Landmark-based measurement, based on size and shape information, has a long history in anthropology and other fields. Landmark location analysis has been used in forensics<sup>9</sup>, computer assisted neuro-

surgery<sup>10</sup>, anthropological studies<sup>11–13</sup>, and MRI-based morphological analyses of the brain<sup>14,15</sup>. In recent years, studies of cranial morphology have utilized statistical shape analysis methods to examine craniofacial shape differences<sup>5,16–21</sup>. This methodology offers a new and promising direction for morphological analysis in anthropology<sup>22</sup> by permitting the multivariate and integrated study of morphological configurations, instead of linear measurements<sup>23–25</sup>. It reveals shape differences between different human populations<sup>25</sup>.

The aim of this paper is to investigate human cranial shape variation between the Byzantine and modern peri-

ods using statistical shape analysis. We used hierarchical clustering and the Thin Plate Spline (TPS) model to examine shape changes in the crania of two populations.

## Material and Methods

### Sample

In order to examine the differences in cranial shape variation, 21 crania in the skeletal collections from the Late Byzantine and modern human periods were investigated. The material was stored in the osteological collection of the Department of Anatomy of Uludag University,

**TABLE 1**  
DESCRIPTION OF LANDMARKS

| No            | Name of landmark             | Description of landmarks  |
|---------------|------------------------------|---|
| VENTRAL IMAGE |                              |   |
| 1             | bregma                       | The point of intersection of the coronal and sagittal sutures   |
| 2             | trichion                     | A cephalometric point at the midpoint of the hairline at the top of the forehead.   |
| 3, 4          | frontal eminence             | Bony projection of the ectocranial surface of the frontal bone  |
| 5             | supraglabella                | A position above the glabella   |
| 6             | glabella                     | The most anterior midline point on the frontal bone, usually above the frontonasal suture   |
| 7             | nasion                       | The point of intersection between the frontonasal suture and the midsagittal plane.   |
| 8, 9          | maxillofrontale              | The anterior lacrimal crest of the maxilla at the frontomaxillary suture  |
| 10, 11        | supraorbitale                | A foramen in the supraorbital margin of the frontal bone at the junction of the medial and intermediate thirds.   |
| 12, 13        | frontotemporale              | The most medial point on the incurve of the temporal ridge, the points lie on the frontal bones just above the zygomaticofrontal suture.  |
| 14, 15        | frontozygomaticus            | The most lateral point on the frontozygomatic suture  |
| 16, 17        | zygomaxillare orbitale (zmo) | Zygomaxillary suture at the orbital margin  |
| 18, 19        | alare                        | Most lateral points on the nasal aperture in a transverse plane   |
| 20            | akanthion                    | The point where a line drawn between the inferiormost points of the nasal (piriform) aperture crosses the midsagittal plane.  |
| 21            | prosthion                    | The most anterior point in the midline on the alveolar processes of the maxillae  |
| LATERAL IMAGE |                              |   |
| 1             | mastoidale                   | The most inferior point on the mastoid process  |
| 2             | porion= auriculare           | The highest point on the superior margin of the external auditory meatus... Not a standard landmark as defined here. Instead it is defined as a point on the lateral aspect of the root of the zygomatic process at the deepest incurvature, wherever it may be |
| 3             | frontozygomaticus            | Most laterally positioned point on the fronto-zygomatic suture  |
| 4             | prosthion                    | The most anterior point on the alveolar border of the maxilla between the central incisors in the mid-sagittal plane.   |
| 5             | akanthion                    | The point where a line drawn between the inferiormost points of the nasal (piriform) aperture crosses the midsagittal plane. Note that this point is not necessarily located at the tip of the nasal spine. (top of the spina nasalis anterior)                 |
| 6             | nasion                       | The point of intersection between the frontonasal suture and the midsagittal plane.   |
| 7             | glabella                     | The most anterior midline point on the frontal bone, usually above the frontonasal suture   |
| 8             | ophryon                      | The craniometric point in the midline of the forehead immediately above the orbits  |
| 9             | supraglabella                | a position above the glabella   |
| 10            | pterion                      | Intersection of the frontoparietal and posterior part of the frontosphenoid suture  |
| 11            | bregma                       | The point of intersection of the coronal and sagittal sutures   |
| 12            | lambda                       | The point of intersection of the sagittal and lambdoidal sutures  |
| 13            | inion                        | The point of at the base of the external occipital protuberance   |

Turkey. Ten of the crania examined in the present study date back to the Late Byzantine period (13th century), and are part of the collection of bones excavated from a Roman amphitheater in Nicaea, Anatolia, between 1981 and 1985. Nicaea (present-day Iznik) is a small town in northwestern Turkey, on the eastern shore of Lake Iznik. It is the modern successor of the important Byzantine city where the first council of Christians was held in 325. All excavations and bone collections, as well as their assessment, were performed by archaeologists and anthropologists<sup>8,26–28</sup>. All skeletons were from males with an average age of 35 years (ranging between 25 and 50 years), and are believed to belong to soldiers of the Byzantine Emperor<sup>8–29</sup>, the bones of females and children found in the same site were excluded from the study. Unfortunately, many of the specimens excavated from the late Byzantium period are fragmentary, and hence the number of the Byzantium period specimens that can be morphologically analyzed is quite limited. In addition, 11 modern adult male crania stored at Uludag University were also included in the analysis for comparative purposes. The present-day crania were investigated by two anatomists according to the parameters set by Williams and Roger<sup>30</sup>. The anatomists concluded that the crania were indeed male. The craniofacial analyses required a preserved viscerocranium and neurocranium, which explains the low number of usable specimens for the two periods.

#### *Collection of two-dimensional craniofacial landmarks*

Anatomical landmarks are defined as biologically meaningful loci that can be unambiguously defined and repeatedly located with a high degree of accuracy and precision<sup>31</sup>. A fuzzy landmark represents a biological structure that is precisely delineated and that corresponds to a locus of some biological significance, but occupies an area that is larger than a single point<sup>32</sup>.

Standard anthropometric landmarks were chosen and marked on digital images as 2D coordinates for landmarks in the frontal (Figure 1) and lateral views (Figure 2). Images of the crania were obtained with a five me-

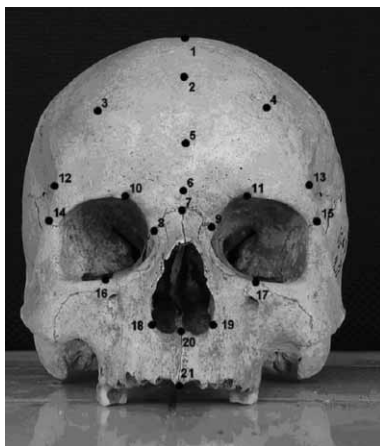


Fig. 1. Landmarks used in this study (frontal aspect).

gapixel digital camera from a distance of 1.5 meters (SONY DSC-W15). The landmarks were recorded using TPSDIG 2.04 software. For each cranial image, 21 anthropometric landmarks on the ventral view of the cranium, and 13 anthropometric landmarks on the lateral view of the cranium, were defined. These landmarks are described in Table 1.

#### *Statistical shape analysis*

Cluster analysis was used to classify the crania. Clustering methods are designed to create homogeneous groups of cases called clusters<sup>33</sup>. The Procrustes distance was used to compute similarity between individuals. The average linkage method (also known as UPGMA) was used for merging clusters.

The shape deformations of the crania of the Byzantine period from the crania of the modern period were evaluated using TPS analysis. Procrustes mean shapes were computed for TPS. In accordance with the TPS analysis, the points exhibiting the most enlargement and shrinkage were labeled as deformations.

To obtain the overall measures of shape variability for the modern and Byzantine periods, we compared the root mean square of Kendall's Riemannian distance ( $\rho$ ) to the mean shape<sup>34</sup>.

For frontal and lateral views, at the end of the cluster analysis, Fisher's exact test was used to test statistical differences in the distribution of cases.

In this study, the R, PAST 1.81, ClustanGraphics 8.00 and TpsSmall 1.20 software were used for statistical analysis.

#### *Landmark reliability*

We calculated the intra-rater reliability coefficient for a two-facet crossed design (»landmark pairs-by-rater-by-subject«,  $l \times r \times s$ ) based on the Generalizability Theory (GT)<sup>35</sup>. In GT, the reliability for relative (norm-referenced) interpretations is referred to as the generalizability (G) coefficient<sup>36</sup>.

Generalizability theory (GT) is an extension of classical measurement theory, and simultaneously takes into account all available error sources (facets), such as items, raters, test forms, and occasions, that influence the reli-



Fig. 2. Landmarks used in this study (lateral aspect).

ability for either relative (norm-referenced) or absolute (criterion-referenced) interpretations<sup>36,37</sup>. Classical test theory estimates only one source of error at a time, and provides estimates of reliability only for relative decisions. GT estimates multiple sources of measurement error separately in a single analysis. Generally, this is done by representing the overall error variance as a sum of variance components related to different sources of measurement error using statistical methods in the framework of analysis of variance (ANOVA)<sup>36,38</sup>.

The precision of measurements can be estimated in GT when two or more facets are taken into account<sup>36</sup>. Each landmark is located by each of several raters on each of several subjects, and the GT design has landmark pairs crossed with raters and subjects (»landmark pairs-by-rater-by-subject«,  $l \times r \times s$ ), hence it includes two facets, rater and subject. The total variance of observed scores is then a sum of the variance component for landmark pairs,  $\sigma_l^2$ , and error related variance components for (a) raters,  $\sigma_r^2$ , (b) subject,  $\sigma_s^2$ , (c) interaction »landmark pairs-by-rater«,  $\sigma_{lr}^2$ , (d) interaction »landmark pairs-by-subject«,  $\sigma_{ls}^2$ , (e) interaction »rater-by-subject«,  $\sigma_{rs}^2$ , and (f) interaction »landmark pairs-by-rater-by-subject«, confounded with other sources of error,  $\sigma_{lrs,e}^2$  (confounding occurs because there is only one observation for the within-cell error variance with the ANOVA design » $l \times r \times s$ «)<sup>36,37</sup>. (For calculating the G coefficient and the variance component, see also Ercan et al., 2008).

$$\sigma^2 = \sigma_l^2 + \sigma_r^2 + \sigma_s^2 + \sigma_{lr}^2 + \sigma_{rs}^2 + \sigma_{ls}^2 + \sigma_{lrs,e}^2$$

In this study, only one rater applied marks to the anatomical landmarks. The reliability of the rater was judged

using repeating landmarks on groups. The analysis of the rating indicated good repeatability for all groups  $G=0.9973$  for Byzantium (ventral view),  $G=0.9980$  for Byzantium (lateral view),  $G=0.9963$  for modern period (ventral view),  $G=0.9977$  for modern period (lateral view).

### Results

The overall measures of the shape variability of the frontal view for the modern and Byzantine periods are 0.0679 and 0.0718, respectively. The overall measures of the shape variability of the lateral view for the modern and Byzantine periods are 0.1222 and 0.0626, respectively.

In our study, which investigates the similarities between the frontal and lateral views of crania of the Byzantine and modern periods in general and landmark-based terms, the subjects could be split into two groups according to their similarity in terms of frontal and lateral views (Figure 3 and 4). Table 2 shows the distribution of the crania in our study according to the clusters.

When the crania were investigated according to their lateral views, two notable clusters were identified at the 0.082 dissimilarity level. Only one unit did not display clustering at this similarity level. Exactly half (5/10) of Byzantium crania and 36.4% (4/11) of those from the modern period fall into one cluster, which includes 42.9% (9/21) of the total subjects. The other cluster includes 40% (4/10) of Byzantium crania and 54.6% (6/11) of those from the modern period, comprising 47.6% (10/21) of the total objects (Table 2).

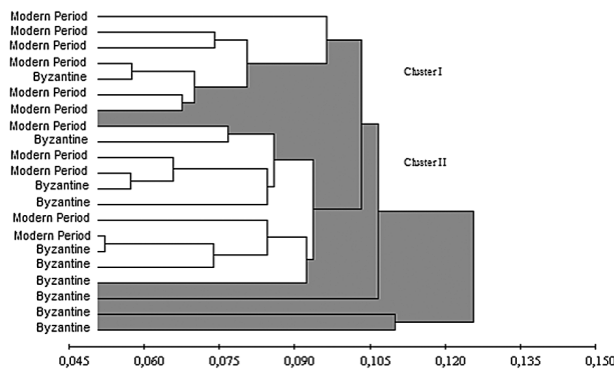


Fig. 3. Dendrogram indicating the classification of cases in terms of levels of Procrustes dissimilarity (frontal view).

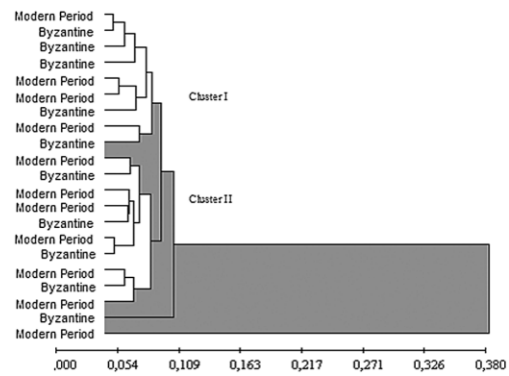


Fig. 4. Dendrogram indicating the classification of cases in terms of levels of Procrustes dissimilarity (lateral view).

TABLE 2  
COMPARISON OF THE CLUSTERS ACCORDING TO DISTRIBUTION OF THE PERIODS

|           | Frontal    |      |             |      | Lateral    |      |             |      |
|-----------|------------|------|-------------|------|------------|------|-------------|------|
|           | I. Cluster |      | II. Cluster |      | I. Cluster |      | II. Cluster |      |
|           | %          | n/N  | %           | n/N  | %          | n/N  | %           | n/N  |
| Byzantium | 10         | 1/10 | 60          | 6/10 | 50         | 5/10 | 40          | 4/10 |
| Modern    | 54.5       | 6/11 | 45.5        | 5/11 | 36         | 4/11 | 54.5        | 6/11 |
| p         | 0.063      |      | 0.669       |      | 0.669      |      | 0.669       |      |

Two notable clusters were identified at the 0.097 dissimilarity level when the crania were investigated according to their frontal views. Three units in Byzantium crania did not display clustering at this similarity level. One of these clusters comprises 10% (1/10) of Byzantium crania and 54.6% (6/11) of crania from the modern period, covering 33.3% (7/21) of the total subjects. The second cluster comprises 60% (6/10) of Byzantium crania and 45.5% (5/11) of crania from the modern period, covering (11/21) of the total subjects (Table 2).

For frontal and lateral clusters, there is no statistical difference in the distribution of cases across the periods.

According to the geometric morphometrics examination, there was no notable deformation in terms of both the frontal and lateral views between the Byzantium and modern crania. Figures 5 and 6 provide the Procrustes average shape coordinates of the Byzantium and Modern period crania.

Nevertheless, the most notable deformations of the frontal view are on the bregma, glabella, nasion, maxillofrontale, akantion, and prosthion of the midline; and on the frontozygomaticus and the nasion and bregma for the lateral view. Figures 7 and 8 show the deformations. While shape deformation is at a similar level for both the Byzantium and modern crania in the frontal view, in the case of the lateral view, deformation is at a higher level in the modern period.

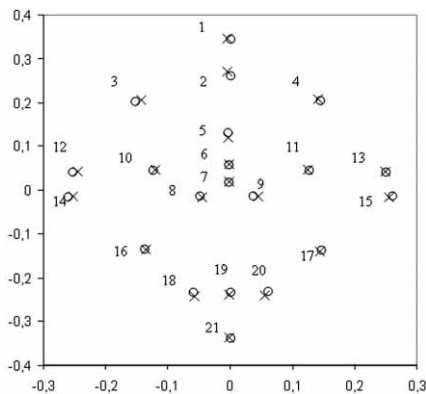


Fig. 5. The mean Procrustes shapes of frontal crania from the Byzantine (o) and modern (x) periods.

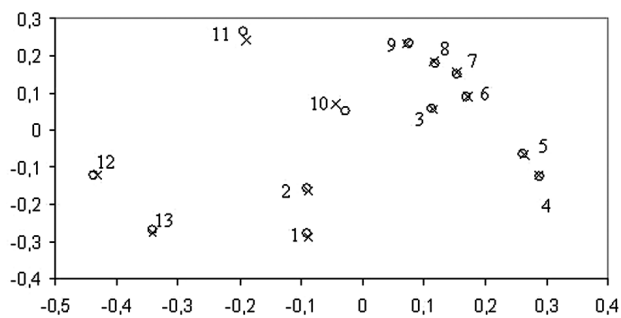


Fig. 6. The mean Procrustes shapes of the lateral crania from the Byzantine (o) and modern (x) periods.

Hierarchical clustering and thin-plate spline analysis revealed that there is no significant difference between modern and Byzantium crania.

### Discussion

Metric and non-metric studies reflecting the population characteristics of skeletal and dental remains are fundamental to anthropological assessments and approaches. Metric studies with conventional morphometric methods are effective in displaying the characteristics of the skeletons. In recent years, numerous studies have focused on the assessment of the shape of an object, rather than its size, using the geometric morphometric technique. The technique has been found to reveal differences between human populations and additional subtle shape differences between males and females<sup>5,14,39</sup>.

The human skull is an important source of information for phylogenetic and population studies<sup>1</sup>. Craniometric traits (metric or non-metric) are regarded as useful tools in the study of the structures and histories of human populations<sup>1,40</sup>. Craniofacial anatomy has been well-studied for individual populations, but comparative studies have been conducted only recently. Human craniofacial traits have moderate to high degrees of population variation<sup>1</sup>. Relethford<sup>40</sup> reported that there is limited variation among major geographic or »racial« clusters in modern humans for both genetic and craniometric measures in his study. Many known factors, such as race, sex, climate, environmental and cultural features, influence skull morphology. Some cranial regions, particularly the face and neurocranium, are believed to be influenced by the environment and prone to convergence<sup>2</sup>. Others, such as the temporal bone, are believed to more accurately reflect phylogenetic relationships<sup>20</sup>. The head form is one of the typical »racial« expressions. Cranial

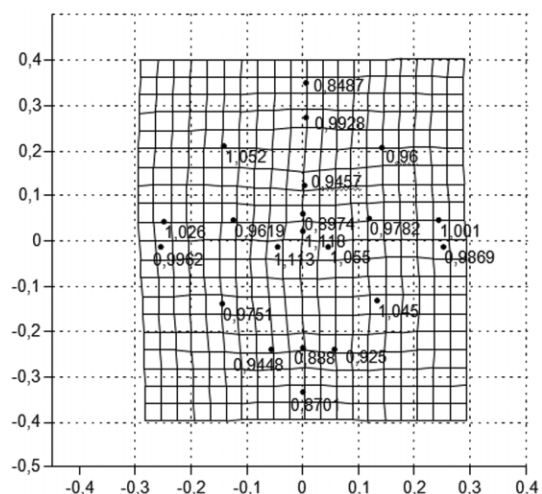


Fig. 7. Thin-plate spline demonstrating shape deformation in the average shape of the frontal crania between the Byzantine and modern periods. Expansion factors at the landmarks are shown numerically (expansion factors greater than one).

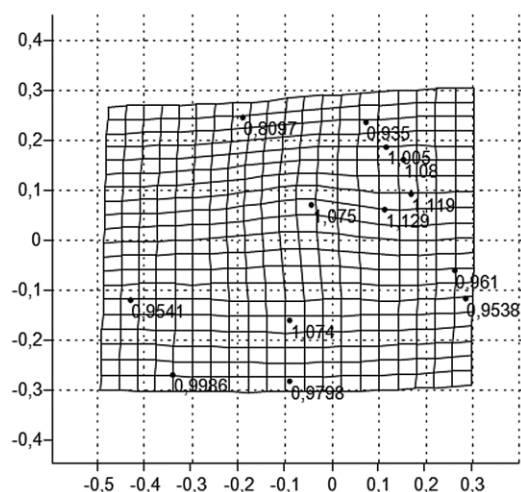


Fig. 8. Thin-plate spline demonstrating shape deformation in the average shape of the lateral crania between the Byzantine and modern periods. Expansion factors at the landmarks are shown numerically (expansion factors greater than one).

types, such as the dolichocephalic or brachycephalic morphological patterns, have long been recognized in biological and evolutionary anthropology. A long (dolichocephalic) head predominates among individuals from Europe, the Middle East and North America, whereas Asians have a tendency toward a round (brachycephalic) head. However, the comparison of results from different studies is controversial, as they are computed on very different kinds of samples (living humans or skeletal remains) from different geographical regions and using different statistical methods.

Geometric morphometric methods are useful tools for quantitating the shape differences between crania of different populations. Some studies have assessed the shape dissimilarities in the crania of different populations with geometric morphometric methods<sup>5,19–20</sup>. Franklin et al.<sup>5</sup> examined cranial variation in 12 modern human populations from southern Africa, applying geometric morphometric methods. They demonstrated population-specific features, reporting that the crania of more southerly populations are characteristically more brachycephalic and less prognathic. Ogihara et al.<sup>20</sup> examined temporal variation in human crania excavated from the Himrin Basin and neighboring areas in northern Iraq using geometric morphometric methods. They reported that cranial shape in the pre-Islamic period was relatively dolichocephalic, whereas crania from the Islamic period were

more diverse, with both dolichocephalic and brachycephalic populations present. Ozbek<sup>29</sup> and Erdal<sup>28</sup> assessed the cranial index ( $X: 81.5$ ) of Byzantine crania obtained from the excavations at the same site (Iznik) and identified brachycephalic crania as the main cranial type (55%). The remaining 45.5% of the crania were identified as mesocephalic and dolichocephalic<sup>28</sup>.

In the present analysis, the modern population was included for comparison to provide a better picture of craniofacial characteristics of the Byzantine population. It was observed that there are two groups of crania within both the frontal and lateral views according to hierarchical cluster analysis. However, it was concluded that group differences were not due to a periodical dissimilarity. The most notable deformation of the frontal view was on the midline landmarks of the frontal and nasal bone. For the lateral view, there was deformation in similar landmarks.

Ozbek<sup>29</sup> presented a complex table representing the ratios of the different genetic compositions encountered during the Iznik excavations. The studies on cranial morphometry indicated that the Iznik population had a heterogeneous genetic structure. In addition, according to the frequency of the protostylid, the shovel shaped incisor and carabelli tubercles on the dentition, the Iznik population showed both European and Asian genetic properties. The presence of Asian genetic properties is supported by the values of high incisive breadth index (0.81) and low canine breadth index (0.88)<sup>8</sup>. DNA studies of living people show that modern Turkey is also characterized by genetic heterogeneity<sup>41</sup>. In the present study, although the crania are clustered in two groups, each group contains crania from both periods. This may indicate both genetic and morphological similarities between the periods.

## Conclusion

Although many studies have analyzed this set of skeletal remains from the Byzantium period<sup>42,43</sup>, this study is the first attempt to apply a geometric morphometric technique to the analysis of cranial variations. In contrast to conventional morphometric studies, the geometric morphometric method has the advantage of providing a size-free analysis. The limited amount of complete material available for analysis is the major shortfall of this study. However, the study has integrity, as the crania from the late Byzantium period date back 800 years.

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### I. Ercan

Department of Biostatistics, Uludag University, Gorukle Campus, 16059, Bursa, Turkey  
e-mail: [ercan@uludag.edu.tr](mailto:ercan@uludag.edu.tr)

## GEOMETRIJSKA MORFOMETRIJSKA STUDIJA I KLASTER ANALIZA KASNOBIZANTSKIH I MODERNIH LJUDSKIH KRANIJALNIH OBLIKA

### SAŽETAK

Unutarpopulacijska varijacija kranijalne morfologije, koja ima važnu ulogu u studijama o ljudskoj evoluciji i biološkim istraživanjima, može se proučavati morfološki i metrički. Geometrijska morfometrija uspoređuje tjelesne oblike koristeći specifične oznake određene anatomskim ispupčenjima. Cilj ove studije bio je identificirati razlike kranijalnih oblika između ljudi iz bizantinskog vremena i modernih ljudi. Varijabilnost kranijalnih oblika proučavana je koristeći se geometrijskom morfometrijskom tehnikom temeljenom na koordinatama oznaka. Podaci o koordinatama oznaka sakupljeni su pomoću dvodimenzionalne digitalne fotogrametrije te su analizirani koristeći se generaliziranom »procrustes« analizom, hijerarhijskim klastiranjem i »thin-plate spline« analizom.