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ORIGINAL ARTICLE

Geographic Pattern of Cranial shape in Iranian Brown Bear *Ursusarctos* Linnaeus, 1758 using Geometric Morphometric approach

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ABSTRACT

The Iranian brown bear is distributed in north, west and northwest of Iran. This research was conducted to study the cranial shape variation of Iranian brown bear within their geographic distribution using Geometric Morphometric. Skulls were classified based on the geographical regions, age and gender. Sixty two skulls were studied based on species distribution in country. The skull morphology from three investigated area displayed phenotypic variation along its geographical distribution. Analysis of skull also indicated that the dorsal face is the best character to show cranial shape differences.

Key words: *Brown bear, Shape variation, Geometric Morphometric, Skull.*

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INTRODUCTION

The Iranian brown bear *Ursusarctos*is found broadly in the northern, western and northwest of the country associating with the Alborz, Zagros and Caucasia Mountains [11, 23, 30, 45]. These populations are being fragmented and roughly estimated 1700-2000 [15] for the whole country protecting by law and listed in "Protected" category [8, 9].

Cranium characteristics is applicable for studying phenotypic variation, as it is both genetically and functionally relevant and subjected to a substantial amount of selective pressure [2, 6, 42]. The cranial size differences among Alborz, Zagros and Caucasia populations have already been reported by [3].

Geometric morphometric (GM) is a quantitative method to analysis shape and widely applied to compare shape variations of biological structures [40]. Unlike traditional approach, in GM, data is obtained from the coordinates of landmark points [1], which are morphological points of specimens that are biological interest [34]. The landmark-based GM analysis also allows us to visualize differences among shapes [35]. With landmark-based GM a simplified shape of structures is determined and morphological changes and patterns of variation between and among samples can be evaluated [1].

This research was conducted to study the cranial shape variation of Iranian brown bear within their geographic distribution using the visualization techniques afforded by GM approach. Quantifying phenotypic differences among populations of a species may help to better understand of natural history across a species' geographic range, which would have implications for both theoretical and applied work in its ecology. Due to lacking enough female skulls in Zagros population, we used only male's skull to investigate geographical pattern of skull shape among three studied populations.

MATERIALS AND METHODS

Study area and sample collection: Based on the distribution of Iranian brown bear, they are divided into three populations including Alborz, Zagors and Caucasia.The Northern forests of Iran from Astara in the northwest to the eastern Golestan Province, support a larger population of brown bear [11, 17, 28]. Since various ecosystems can be found in this region, from Irano-Turanian landscapes in the south to highland alpine with altitude to 5600 m scrublands extending to deep hyrcanian forests adjust to the Caspian Sea. These forests are heavily covered by snow from December until March and are not accessible over that period. Therefore, it expected finding more samples in Alborz regions than two other [28]. The Zagros Mountains from south of Azerbaijan area to near Shiraz in the Fars Province, are in west with highest point about 4200 m is the largest mountain range in Iran. Iran's Caucasia Mountain in the northwest of country, is a part of the Lesser Caucasus system and contained the Azarbaijan and Ardabil Provinces (Figure 1). The distribution of bear in two latter regions has been reported to be sporadic [17].

The study was carried out from 2011 to 2013 with collecting the skulls from the museums of Iranian Department of Environment, private and hunter collections and bears perished in natures. In total 132 skulls were collected, selecting sixty two ones with specified gender and location (Table 1). The skulls were classified based on geographical regions, age and gender. Adult specimens were diagnosed based on the closure of tooth eruption suture in the skull [39] and wear and closure of the basioccipitale-basisphenodeum suture [4, 33].

Also, sex of samples was determined based on the external features of skull including size, relative width of the skull, and development of the sagittal crest [43]. According to [43] there is a distinct suture between frontal and parietal in immature bear (cubs to 3 years), the condyle-basal length is 150-285 mm and the canines reach up to 15 mm in length. In adult female from 4 years onwards, the condyle-basal length is 283-285 mm and the length and height of the sagittal crest is up to 3 and 1.5 cm, respectively. In adult male from 6 years, the condyle-basal length is up to 313 mm and length and height of sagittal crest are 9-11 and 1.5-2.7 cm, respectively [43]. ImageJ software (ver 1.45s) was used to measure the distances on the skulls.

Geometric morphometric analysis: The ventral, dorsal and lateral sides of the skulls were photographed using a digital camera (Fuji HS10, 14 megapixels) installed on a tripod. A ruler was included in the images to allow the acquisition of a scaling factor. On the ventral, dorsal and lateral sides of cranium, 17, 13 and 16 landmark points were defined, respectively [10, 13, 23, 26, 44] (Figure 2) (Table 2). Then TpsUtil 1.33 and TpsDig 2.10 and [36, 37] software was used to digitize landmark points on 2D pictures. One side of skull adopted to digitize landmark point as used in previous studies [42]. This is a common method to reduce the time for data collection in symmetric structures [44].

The extracted data were superimposed using a Generalized Procrustes analysis (GPA) to remove non-shape variations [38]. In this analysis, shape coordinates were superimposed by standardizing each configuration to unit centroid size and minimizing differences in translation and rotation of all specimens using a least-square algorithm. Then, all data from three faces were analyzed using principal components analysis (PCA), canonical variate analysis (CVA) and MANOVA. For visualizing shape changes between groups, the consensus configurations in deformation grids were used. MorphoJ (ver. 1.05) and PAST (ver. 2.17) packages were used to analyze the morphometric data [18, 21].

RESULTS

Analysis of Geographic pattern Cranial Differences

Dorsal view: The morphospace defined by first two principal components (PCs) were included 60.51% of observed variations (PC1=44.23% and PC2=16.28%) (Figure 3). The PCA graph shows separation of three studied populations. CVA/MANOVA analysis revealed a significant differences between the dorsal view of three studied populations($P<0.05$). The main shape differences in the dorsal view found in the premaxilla, zygomatic arch and parietal bone (Figure 4). The deformation grids revealed that of Alborz population possesses a longer premaxilla region than the others and the zygomatic arch in the Caucasia bears is bigger than that of two others. In addition, the parietal bone (landmarks 8, 9 and 10) in the Zagros population was wider than the others.

Lateral view: The morphospace defined by first two PCs of the lateral view were included 46.21% of observed variations (PC1=26.95% and PC2=19.26%) (Figure 5). CVA/MANOVA analysis showed a significant difference between the Alborz and Caucasia populations ($P<0.05$). The results revealed that the Alborz population bears elongated sagittal crest and longer premaxilla than the others. In the Caucasia populations, the squamosal and frontal bone dorsally and ventrally has been displaced and therefor skull seems to be wider in lateral view (Figure 6).

Ventral view: The morphospace defined by first two PCs of the ventral view were included 42.85% of observed variations (PC1=30.56% and PC2=12.29%) (Figure7). CVA/MANOVA analysis showed a significant difference between Alborz and Caucasia populations in the ventral face (P<0.05). The deformation grids of ventral views in the Alborz and Caucasia populations displayed differences in the occipital bone, zygmatic arch and central axes. The zygomatic arch, occipital bone and palatine suture were wider in the Caucasia population (Figure 8).

Table 1: Number of skull analyzed

	Female			**Male**		
	Alborz	Zagros	Caucasus	Alborz	Zagros	Caucasus
Dorsal	12	0	2	22	15	9
Lateral	12	0	2	24	13	9
Ventral	12	0	2	24	13	9

Table 2: List of landmarks was used in morphometric analyses:

Dorsal View	
1	Edge of premaxillaseam in the septum
2	The edge of third incisor
3	Intersection between zygomatic arch and the maxilla
4	Highest point of the postorbital process
5	Squamosal-Jugal suture
6	The most inner point of zygomatic arch
7	The primary point of junction between squamosal and the braincase
8	Lowest point of the Squamosal curve
9	Lowest point of intersection of the Squamosal or parietal bone
10	Outline directly from landmark 8 and 9 to edge of parietal bone *
11	The highest point of the occipital crest
12	Line directly from landmark 9 to edge of sagittal crest*
13	Line directly from landmark 7 to Frontal suture*
14	Line directly from landmark 4 to Frontal suture*
15	Line directly from landmark 3 to nasal suture *
16	Midpoint the edge of nasal suture
17	Lowest point of the right nasal suture
Lateral View	
1	Front edge of the canine
2	Front edge of the fourth premolar
3	The end point of second molar
4	Highest point of the pterygoid
5	Highest point of the squamosal
6	Highest point of the external acoustic meatus
7	Innermost edge of the parietal bone
8	Outermost edge of the parietal bone
9	Line directly from landmark 6 to edge of sagittal crest*
10	Line directly from landmark 4 to central axis in frontal bone*
11	Highest point of the postorbital process
12	Midpoint between squamosal-jugal suture
13	Line directly from landmark 6 and 12 to frontal bone*
14	The earliest point of perforation from a socket
15	The end point of perforation from a socket
16	The end point of nasal bone suture
Ventral View	
1	The primary point of junction of incisor in premaxilla
2	Most posterior point of the third incisor
3	Front edge of the first premolar
4	The end point of the second molar
5	The midpoint between squamosal and jugal suture
6	Peak of the pterygoid bone
7	Peak of the para occipital bone
8	Peak of the occipital condyle

9	Most rostral point of the occipital condyle
10	Most posterior point of palatine bones suture
11	Intersection between palatine foramen and central axis prolong to the palatine suture*
12	Intersection between vertical line from landmark 3 and central axis of the skull*
13	The lowest point of the incisive foramen

Fig 1: Distribution of Brown Bear in Iran

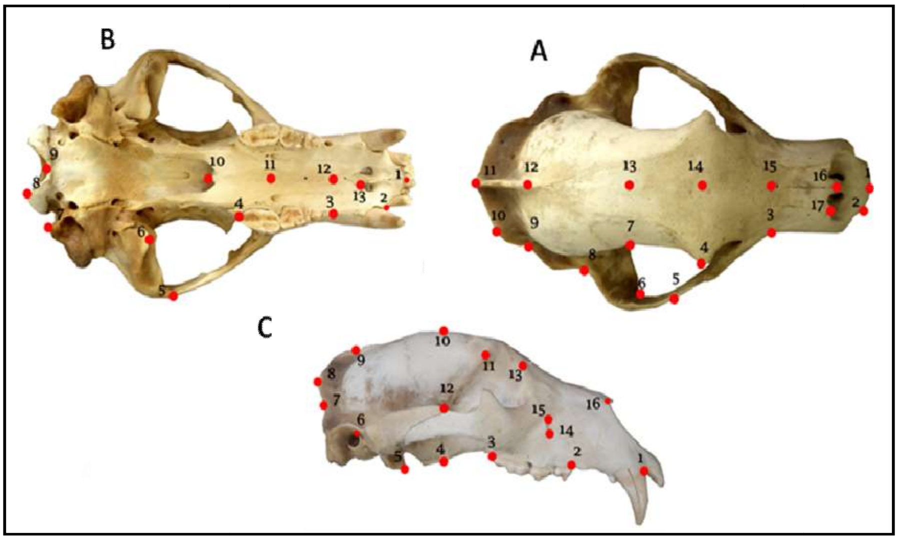

Fig 2: Landmarks used in the morphometric analysis of bear crania in dorsal (a), ventral (b), and lateral (c) views

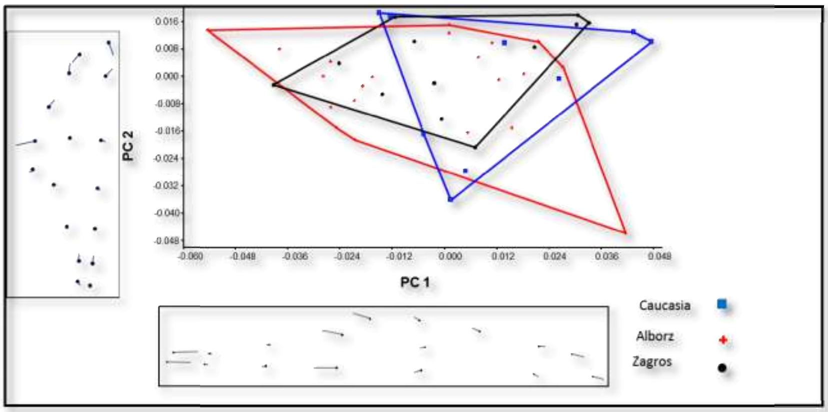

Fig 3: Morph space defined by PCs from analysis of crania in dorsal view

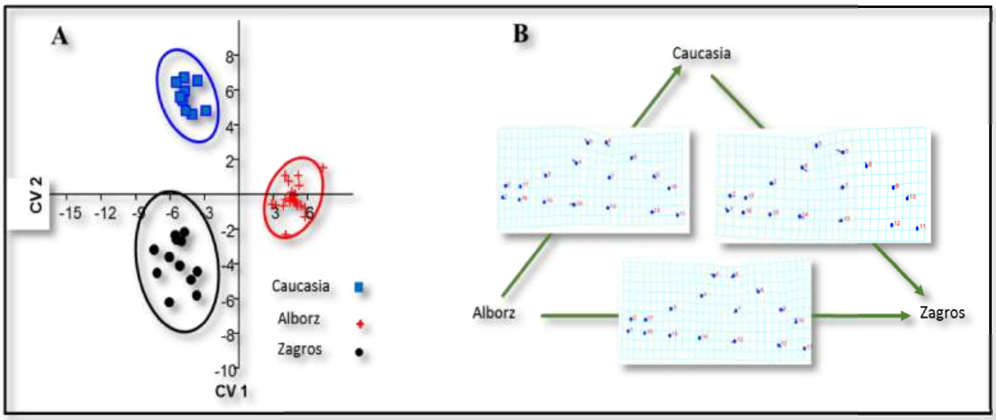

Fig 4: CVA analysis and differences in dorsal view between the Alborz, Zagros and Caucasia skulls are visualized through the deformation grid

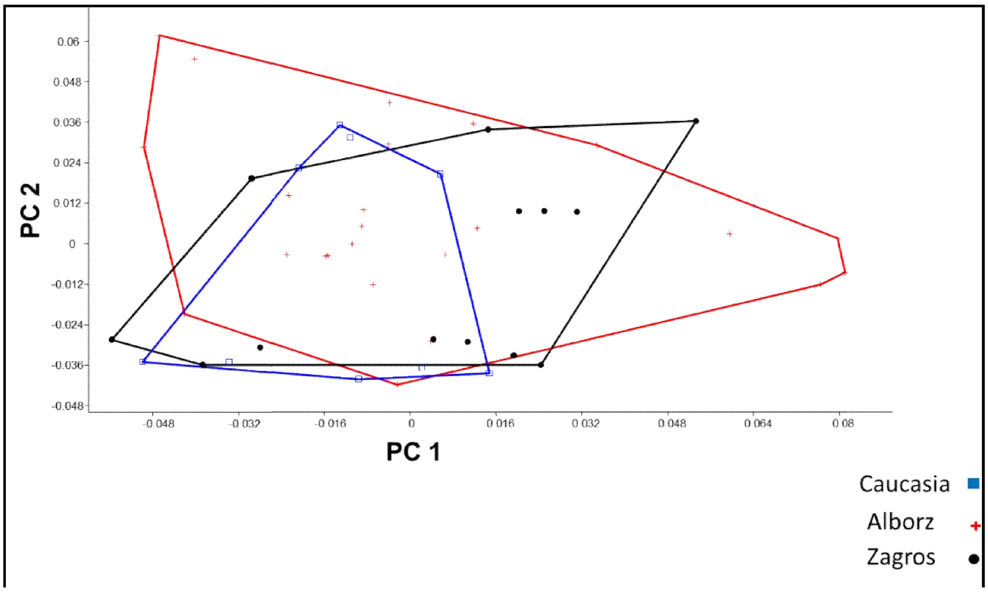

Fig 5: The morphospace defined by PCs from the analysis of crania in lateral view

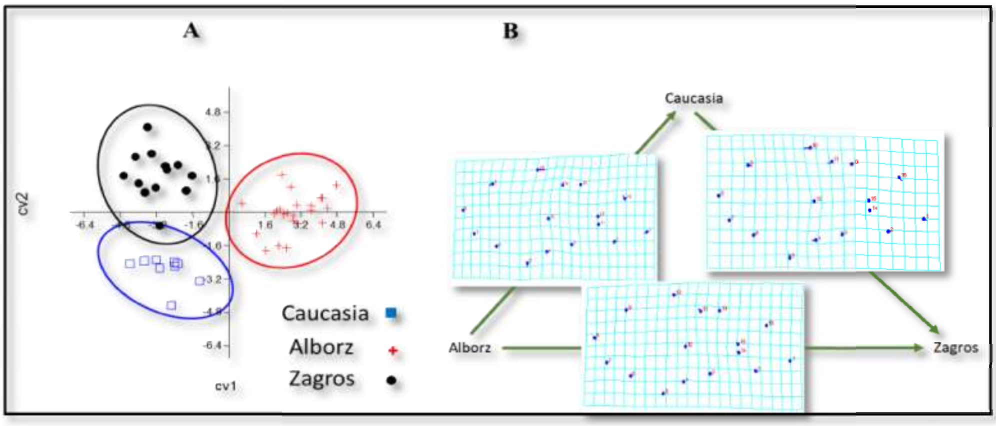

Fig 6: CVA analysis and differences in lateral view between the Alborz, Zagros and Caucasia skulls are visualized through the deformation grid

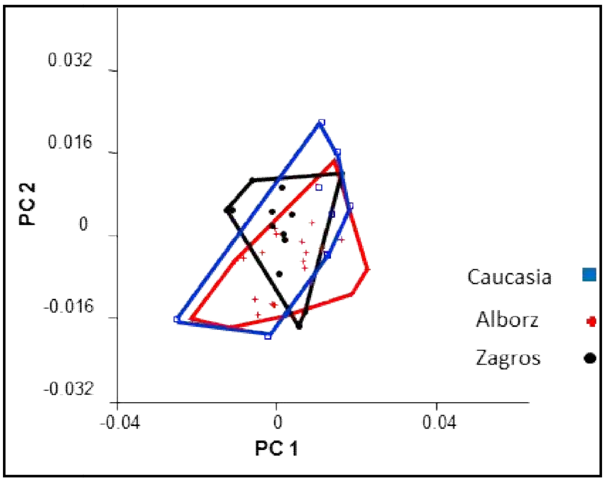

Fig 7: morphospace defined by PCs from analysis of crania in ventral view

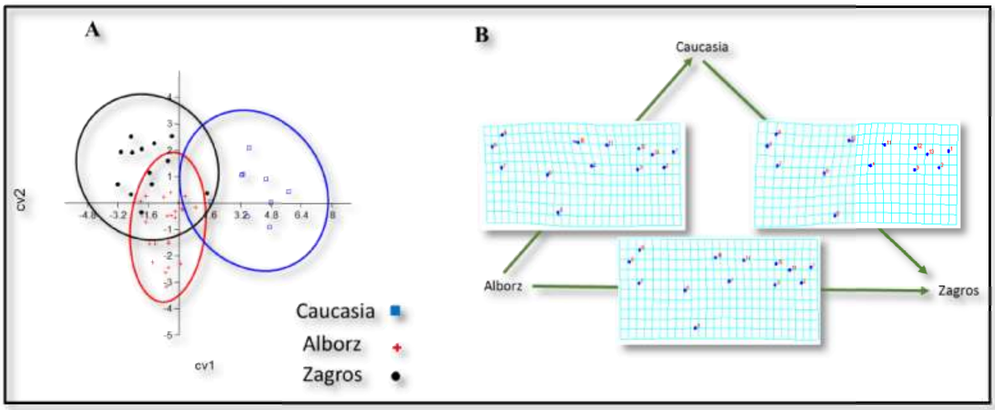

Fig 8: CVA analysis and differences in ventral view between the Alborz, Zagros and Caucasia skulls are visualized through the deformation grid

DISCUSSION

The results of this study on the skull of the Iranian brown bear confirmed the phenotypic variation along its geographical distribution range may be effected by the environmental parameters. The cranial shape differences between the Caucasia and Alborz populations were higher, despite little geographical distance. Many studies have demonstrated that animals with little geographical distance have more morphological similarity [19]. The morphological variation in bear's skull is possibly due to an early isolation or fragmentation. According to [7, 20, 25, 31] various environmental factors can influence the shape and size of skulls, in which the main factors can be geographical distance, climate fluctuation rate, food availability and population density.

Structural diversity of habitat, presence of predators and competitors may affect trends of variation as much or more than climate [19, 24]. Also there is a close association between cranial morphology and feeding behavior in bears [12, 14, 39]. The Iranian brown bear is a small size subspecies of brown bear and their habitat is mountainous areas [28]. This subspecies is generalist omnivores, especially consume plant material and are carnivore when animal pray available [29].It seems availability of food particularly meat, weather conditions and rainfall rate, landscape cover are the most important factors in morphological evolution.

Differences in the cranial shape of three studied population were related to the premaxilla, zygomatic arch, parietal bone, sagittal crest, squamosal, frontal and occipital bones. The Caucasia shows bigger zygomatic, squamosal, frontal and occipital bones. The zygomatic arch and occipital bone is the origin and supporter of temporalis and masseter muscles and reinforces those muscles [16, 27]. According to the [16, 22] enlarged frontal region also reinforce chewing temporal muscles. On the other hand, the sagittal crest of the Alborz population is posteriorly more projected than that of others. In addition, the parietal bone was wider in Zagros population. The wider parietal bone and a longer sagittal crest also supports more powerful muscles for the maxilla and premaxilla. Therefore, the results revealed that different strategies i.e. elongate of sagittal crest and enlargement of zygomatic arch have been evolved in three studied populations to increase muscle mass. Having a large muscle can help to feed a wider range of food [5, 32, 41].

The results also showed that only dorsal surface of skull in three studied populations are significantly different and no significant differences found in lateral and ventral faces of three studied populations. The controversial results of this study on the lateral and ventral faces may be related to unsuitability of these sides to compare the skull shape. Hence, results confirmed that the dorsal surface is the best face to study the cranial shape differences in the Iranian brown bears.

CONSIDERATION

Based on our results, there are morphological differences among three Iranian brown bear populations showing their progressive evolution. Hence, in protection program each population needs consideration as a separate evolutionary unit.

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