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Original Article

A Classification of Feline Skulls by Means of Geometric Morphometric Techniques

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ABSTRACT

Geometric morphometrics is a powerful tool for the study of morphological variation that possesses numerous advantages over more traditional approaches based on linear measurements. Here, the skull morphologies of 42 adult museum specimens of different species belonging to the family *Felidae* were analysed in order to assess the reliability of classifying two specimens labelled as *Panthera sp*. Using this technique. According to the results obtained, these specimens can be assigned to *Panthera pardus*. This is not just a contribution to the traceability of these museum pieces, but primarily an exemplification of the validity of geometric morphometric methods for classifying biological specimens.

Keywords: allometry, *Felidae*, landmark-based morphometrics, morphometry, *Panthera*.

INTRODUCTION

Comparison of anatomical characters between organisms has been a core element in comparative biology for centuries. Historically, taxonomic classification and understanding of biological diversity have been based mainly on morphological descriptions. In the early twentieth century, comparative biology entered a transition from a descriptive field to a quantitative science, where morphological analysis underwent a similar revolution of quantification. Based on this quantitative mathematical revolution, the study of morphology has gained in power by developing statistical shape analysis. This made possible the combination of multivariate statistical methods and new ways to visualize a structure. Geometric morphometrics (GM) can be defined as the quantitative representation and analysis of morphological shapes using geometric coordinates instead of measurements. See Bookstein (1991), Rohlf and Marcus (1993) and Monteiro and Reis (1999) for comprehensive descriptions. GM techniques have been used to analyse the variations in skulls of specimens, and they were shown to be objective and efficient compared to traditional methods (Rohlf, 1998). GM methods first quantify the form (size and shape) of each specimen according to the

location in space of a set of anatomical landmarks that are homologous among individuals. Shape and size are then separated using a Procrustes superimposition of landmarks, which translates the landmarks to a common origin, scales them to a common size, and rotates them to minimize their summed squared landmark distances. Procrustes superimposition thus enables one to quantify shape as the multidimensional deviation of a specimen's landmarks from a reference configuration, typically an average of the entire sample. An advantage of GM is that size is mathematically removed from the analysis to focus on pure shape.

Information about the structure of osteologic collections is of great importance for both the conservation and utilization of bone resources collected, so how to identify species and how to delineate them can be fundamental for classifying bone specimens. There is a need to be able to discriminate between species, as well as gain an understanding of the patterns of variation within and between species.

In this study, the authors try to classify two specimens labelled as *Panthera* spp. and deposited in the Natural History Museum of Barcelona using the techniques of GM. The purpose is not just to contribute to the traceability of these specific museum pieces, but primarily to test the goodness of geometric morphometric methods for classifying biological specimens.

MATERIAL AND METHODS

Material Examined

Forty-two adult specimens *(i.e.* individuals with fully erupted upper cheek teeth series) of different species belonging to the family *Felidae* (Fischer, 1817) were selected.The specimens are listed in Table 1. The taxonomy followed here is set out in the latest edition of Wozencraft (1993). His classification is used here for practical reasons, without prejudice, as it has been adopted by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the World Conservation Monitoring Centre (WCMC). Skulls were deposited in the Natural History Museum of Barcelona. Sex was not available for all specimens. Specimens referred to as 82-7141 and 82-7172 were labelled as *Pantherasp*.

Table 1: Specimens studied (N=42). The taxonomy followed here is set out in the latest edition of Wozencraft (1993)

	Vernacular name	Abbreviation	Males	Females	Unknown sex
Acinonyx jubatus (Schreber, 1775)	Cheetah	Ajub	1	0	0
Felis silvestris (Schreber, 1775)	Wildcat	Fsil	2	1	3
Leopardus pardalis (Linnaeus, 1758)	Ocelot	Lpar	0	0	1
Leptailurus serval (Schreber, 1776)	Serval	Lser	0	1	0
Lynx lynx (Linnaeus, 1758)	Eurasian lynx	Llyn	1	1	1
Panthera leo (Linnaeus, 1758)	Lion	Pleo	1	1	1
Panthera onca (Linnaeus, 1758)	Jaguar	Ponc	2	2	2
Panthera pardus (Linnaeus, 1758)	Leopard	Ppard	1	1	4
Panthera tigris (Linnaeus, 1758)	Tiger	Ptig	1	0	3
Panthera sp.	-	Sp.1 and Sp2	0	0	2
Profelis aurata (Temminck, 1827)	African golden cat	Paur	0	0	1
Puma concolor (Linnaeus, 1771)	Puma	Pcon	0	1	5
Uncia uncia (Schreber, 1775)	Snow leopard	Uunc	0	2	0

Image Acquisition

Image capture was performed with a Nikon® D70 digital camera (Nikon Inc., Tokyo, Japan) (image resolution of 2240 × 1488 pixels) equipped with a Nikon AF Nikkor® (Nikon Inc., Tokyo, Japan) 28 to 200-mm telephoto lens. The focal axis of the camera was parallel to the right lateral aspect of each skull. A ruler was used in this process. Fourteen homologous

and topologically equivalent landmarks were plotted on the skull in order to describe the size and shape of skull variations (Figure 1).

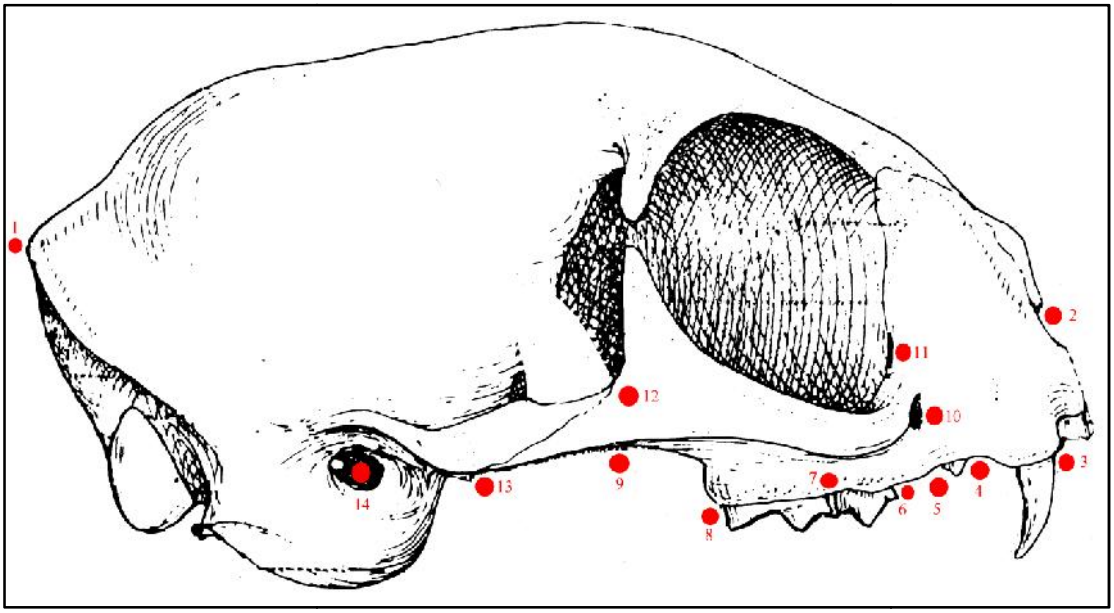

Figure 1: Landmark locations used (right lateral aspect of the skull), showing the locations of the 14 anatomical landmarks (numbered points) used to capture shape from the right lateral view of the skull. See text for anatomical locations of landmark definitions.

Landmarks used in this study were primarily chosen to describe major cranial and facial regions as well as regions of particular morpho-functional or sensory interest and they referred to the: (1) most nuchal part of the external occipital protuberance (*protuberantia occipitalis externa*); (2) most rostral part of the incisivo-nasal suture; (3) most rostral part of the base of the canine, at the maxillar bone; (4) most rostral part of the base of the 2nd premolar, at the maxillar bone; (5) most nuchal part of the base of the 2nd premolar, at the maxillar bone; (6) most rostral part of the base of the 3rd premolar, at the maxillar bone; (7) most rostral part of the base of the 4th premolar, at the maxillar bone; (8) most nuchal part of the base of the 1st molar, at the maxillar bone; (9) ventral part of the palatinum bone (*lamina perpendicularis ossis palatini)*; (10) midpoint of the infraorbital foramen (*foramen infraorbitale*); (11) midpoint of the fossa of lachrymal sac (*fossa sacri lacrimalis*); (12) dorsal part of the temporo-zygomatic suture at the zygomatic arch (*arcus zygomaticus*); (13) ventral part of the temporo-zygomatic suture at the zygomatic arch; (14) midpoint of the foramen on the timpanic foramen (*porus acusticus externus*).

Landmarks were digitized twice using tpsDig, v. 2.04 software and converted to scaled x and y coordinates and centroid size (CS, the square root of the sum of the squared distances among the landmarks in a configuration and their extracted centre of mass), and standardized after removing artefactual variation due to different positions of the specimens using CoordGen6f (H. D. Sheets, www.canisius.edu/sheets). Size information was retained as CS. A Mantel test between the two replicates reflected R=1, p << 0.00001, which suggested that the matrix entries were positively associated and thus the digitizing error was considered negligible.

The TpsSmall, v. 1.20 softwarewas used to assess the correlation between the 2D Procrustes distances to the Euclidean distances in tangent space for all skull shapes, and the relation was very close to linear for all of the data (r=0.999, Figure 2), suggesting that tangent space is an adequate approximation to Kendall, so it was used to proceed with the morphometric analyses.

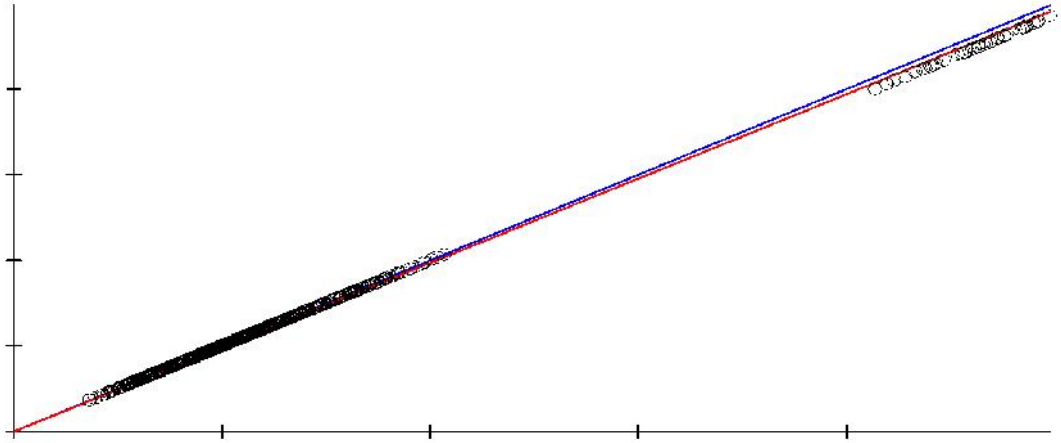

Figure 2: Scatter plot for all 2D data of Procrustes Distance against Euclidean tangent space distance (Tangent Distance) with best fitting lines through the origin for CS = 1; and an Orthogonal Projection, tangent at the consensus (Y-intercept: 0.000000, slope: 0.984, correlation (uncentred): 0.999, root MS error: 0.000481).

Statistical Treatment

Shape covariation was quantified using principal coordinates analysis (PCO), also known as Metric Multidimensional Scaling. This ordination method allowed the study of all felid specimens, grouped according to species and using Euclidean distances. The algorithm is from Davis (1986). The PCO routine finds the eigen values and eigenvectors of a matrix containing the distances or similarities between all data points, giving a measure of the variance accounted for by the corresponding eigenvectors (coordinates). A Minimal Spanning Tree (MST), the shortest possible set of lines connecting all points, was then used as a visual aid in grouping close points (specimens) based on the Euclidean distance measure of the original data points. A Non-Parametric MANOVA (NPMANOVA, also known as PERMANOVA) was done as a non-parametric test to compare form differences between these groups. A hierarchical clustering routine was then used to produce a dendrogram showing how average data per species could be clustered. Ward's algorithm was used (with this algorithm, clusters are joined such that increases in within-group variance are minimized). The measure of agreement between the original distances and the distances in the dendrogram was the cophenetic correlation coefficient (CPCC) (Sokal and Rohlf, 1962). Finally, K-means clustering, as a non-hierarchical clustering method, was used to assign each *Panthera* specimen. In an iterative procedure, the cluster assignment is initially random and items are then moved to the cluster that has the closest cluster mean, and the other cluster means are updated accordingly. This continues until items are no longer "jumping" to other clusters.

Statistical treatment was done using PAST- "Paleontological Statistics Software Package for Education and Data Analysis" (Hammer *et al.*, 2001) and MorphoJ (Klingenberg, 2011). All of the programs used in this study are available over the Internet by FTP from the "morphmet" directory at life.bio.sunysb.edu or via the WWW at http://life.bio.sunysb.edu/morph/.

RESULTS AND DISCUSSION

As a regression of shape (procrustes coordinates) versus size (ln CS) appeared non-significant (R^2=0.329, Wilk's λ=0.03, p<<<0.0001), so no allometric effect of size on shape was supported (only 6.97% of shape variation was due to size), PCO was performed with both size and shape. PCO appears in Figure 3. The first axis explained more than 97% of the total observed variance. The scatterplot coordinates 1 and 2 separated all *Panthera* specimens rather unambiguously and showed only minor partial overlap between *Panthera tigris* and

Panthera leo, and *Panthera pardus* and *Panthera onca*. Both *Panthera* sp. appeared in or very close to the *Panthera pardus* group.

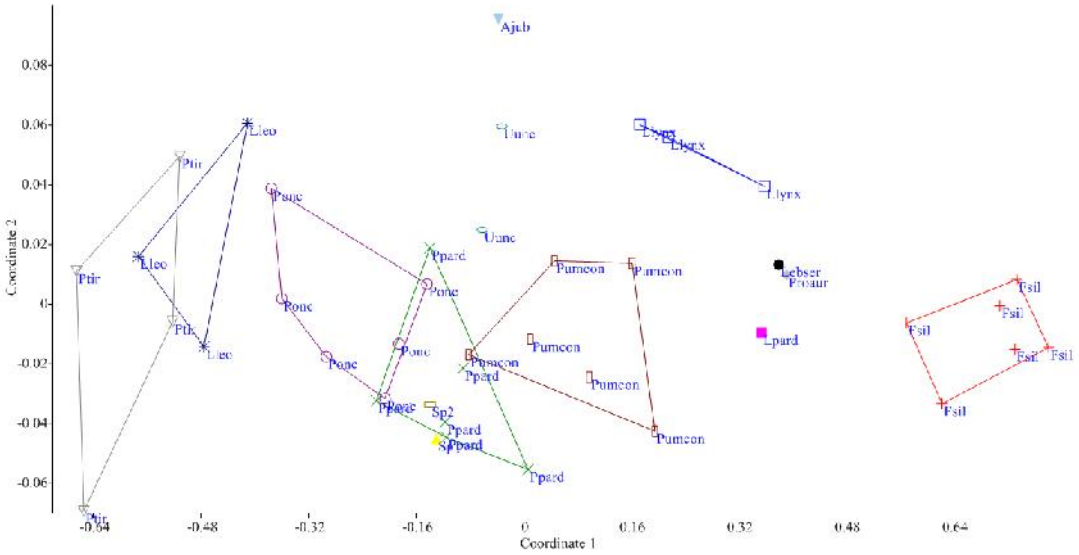

Figure 3: Scatterplot of principal coordinates analysis for the 42 specimensof different genera studied belonging to the family *Felidae*. The first axis explained more than 97% of the total observed variance. The scatterplot of PCO coordinates 1 and 2 separated all *Panthera* specimens rather unambiguously and showed only minor partial overlap between *Panthera tigris* and *Panthera leo*, and *Panthera pardus* and *Panthera onca*. Both *Panthera* spp appear in or very close to the *Panthera pardus* group. See table 1 for explanation of abbreviations.
***Panthera pardus* (Ppardus), *Panthera leo* (Pleo), *Panthera onca* (Pleo), *Panthera sp.*(Psp), *Panthera tigris*(Ptigris)**

The PCO of skull shape (procrustes variables alone) gave similar loads, but greatly reduced the discrimination (the first axis explained just 51.5 % of the total observed variance, figure not shown here) so groups were separated mainly because of their size. This underlines the fact that size was the most diagnostic cranial variable in terms of separating the *Panthera* species. No differences in form appeared between the non-species classified *Panthera* specimens and *Panthera onca* and *Panthera pardus* (Table 2).

Table 2: Results of Non-Parametric MANOVA test between *Panthera* groups (*Panthera onca Panthera leo, Panthera pardus*, and *Panthera tigris*). No differences appeared between the non-species classified *Panthera* specimens and the others. Non-significant results appear in bold.

	Panthera pardus	*Panthera onca*	*Panthera* sp.	*Panthera leo*	*Panthera tigris*
Panthera pardus	-	0.012	**0.794**	**0.012**	0.005
Panthera onca	0.012	-	**0.114**	**0.025**	0.005
Panthera sp.	**0.794**	**0.114**	-	**0.101**	**0.067**
Panthera leo	0.012	0.025	**0.101**	-	**0.251**
Panthera tigris	0.005	0.005	**0.067**	**0.251**	-

In the MST for *Panthera* specimens (Figure 4), *Panthera* sp. specimens showed the lowest separation to the *Panthera pardus* group. Ward's clustering clearly grouped the *pardus* and *Panthera* sp. specimens, with *tigris* and *leo* appearing in a separate cluster (Figure 5). The

cophenetic correlation coefficient was 0.897. Finally, k-means clustering assigned both *Panthera* sp. specimens to the *Panthera pardus* group.

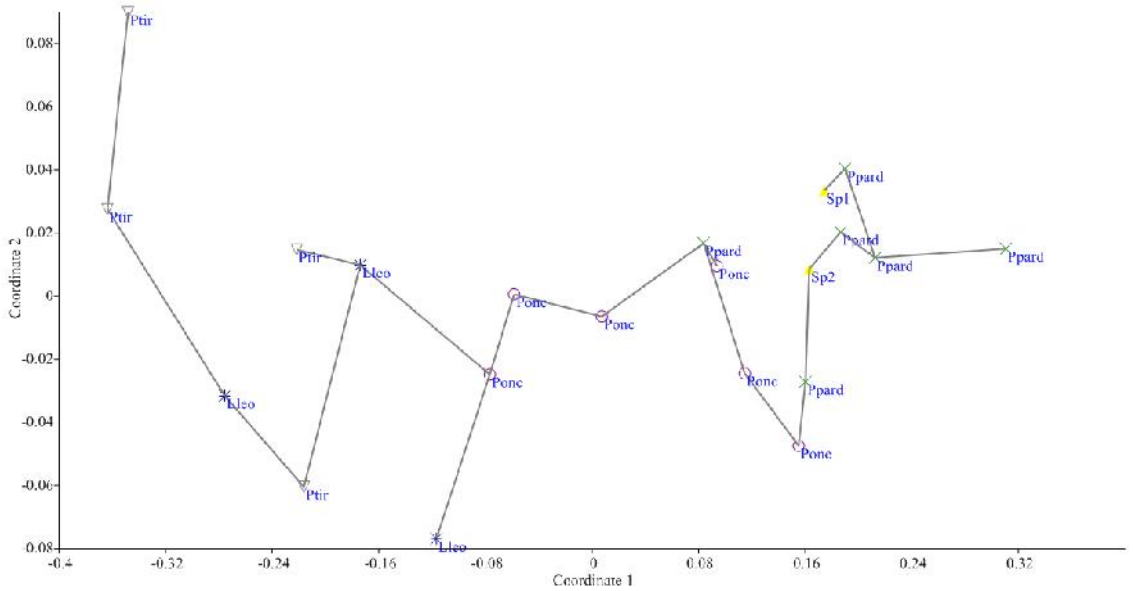

Figure 4: Minimum Span Tree for *Panthera* specimens, where the obtained tree has a weight less than or equal to the weight of every other spanning tree. See table 1 for explanation of abbreviations. *Panthera pardus* (Ppardus), *Panthera leo* (Pleo), *Panthera onca* (Pleo), *Panthera sp.*(Psp), *Panthera tigris*(Ptigris)

In conclusion, traditional multivariate analysis applied to geometric morphometric data provides an easy and effective way to classify species in felid skulls, although it is not a definitive method. Nevertheless, for the case presented here, there seems no doubt that both specimens referred to as 82-7141 and 82-7172 deposited in the Natural History Museum of Barcelona (Catalunya) belong to species *Panthera pardus*.

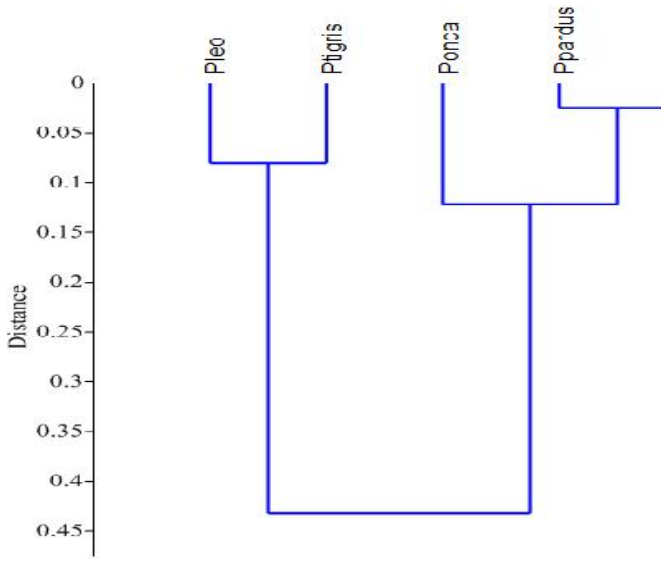

Figure 5: Ward's dendrogram showing how average data per species could be clustered. The cophenetic correlation coefficient was 0.897.
Panthera pardus (Ppardus), *Panthera leo* (Pleo), *Panthera onca* (Pleo), *Panthera sp.*(Psp), *Panthera tigris*(Ptigris)

CONCLUSION

According to the results obtained, unclassified specimens of *Panthera sp*. could be classified with certainty as *Panthera pardus*. This represents an exemplification of the validity of geometric morphometric methods for classifying biological specimens.

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