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Bone maceration technique, gross anatomical and morphometrical studies of scapula, humerus, radius and ulnar bones of melanistic leopard (*Panthera pardus*)

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Abstract

Records on animal morphology are valuable additions to documenting natural history especially within zoological collection. In this research, bone maceration is an important technique to be recorded as it serves as preliminary works for bones articulation intended exhibition, research and documentation, and collection management. The observed differences and variation in the morphometric measurements of the bones from a melanistic leopard, *Panthera pardus* (Linnaeus, 1758) contribute significantly to the documentation record held at the Natural History Museum. Such measurements revealed minor asymmetries between the left and right bones with ranges of 0.1 cm – 0.5 cm upon measured from the cranial view, caudal view, median view, and lateral view.

Keyword: morphology, bone maceration, caudal, median, lateral, cranial

INTRODUCTION

The evolutionary emergence of limbs has shed significant light on the diverse ecosystems inhabited by tetrapod (Shubin *et al*., 1997). Tetrapods displays an extraordinary variety of body plans, including traits such as delicate wings, robust paws, slender hooved legs, and widely flattened flippers. Crucially, each limb type is functionally integrated yet evolutionary constrained (Raff, 1996). These adaptations and evolutionary traits have resulted in the different types of locomotion that exhibited across various animal taxa.

Felids are notoriously known for their carnivorous diet and species such as *P. pardus*, have anatomical characteristic almost similar to those domesticated felines with some species-specific parameters (Tomar *et al*., 2018). Such studies have been conducted by numerous researchers on both felines and canines respectively and some information on the osteological structures are available specifically for leopard (Kale *et al*., 1999; Kumar, 2008), panther (Patil *et al*., 1998), and Asiatic Lion (Pandey *et al*., 2004). Subsequently, the locomotion of the Felidae's family plays an important role in predatory behaviour (Meachen-Samuels

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and Van Valkenburgh, 2009) as their musculoskeletal structure facilitates the transmission of locomotor loads to the thorax, stabilises the shoulder and allows the mobility of forelimb which is pivotal in climbing and apprehension of prey (Fischer and Blickhan, 2006).

The melanistic leopard (*P. pardus*) are among feline species that is widely distributed in Southeast Asia region especially in Malaysia (Kawanishi *et al*., 2010). This wide-ranging species is adaptable and often comes into conflict with human populations especially in a heavily fragmented and disturbed area. Despite its widely distribution, there is a scarcity of morphological and morphometric data for the leopard's scapula, humerus, radius, and ulna in museums records in Malaysia. Therefore, this study aims to measure and collect data on the scapular, humerus, radius, and ulna of the leopard which would be an added value to the specimen record in Natural History Museum Malaysia.

According to the records in Natural History Museum of Malaysia, the lack of published bones maceration procedure and morphometric measurements of *P. pardus* as a zoological collection presses the need for this research to be conducted. Therefore, the aim of this paper is to provide an insight documentation of the procedure on bones maceration technique and its gross anatomical studies with morphometric measurements of *P. pardus*.

MATERIALS AND METHODS

This research was conducted on the scapula, humerus, radius, and ulna of the melanistic leopard (*P. pardus*) specimen that had died due to a roadkill accident and later, was donated to the Natural History Museum Malaysia by the Terengganu State Museum. This study includes maceration of bones prior to the articulation, followed by an analysis of bones morphology, and morphometrical measurements. Due to the roadkill nature of the specimen, the tibia and fibula parts of the specimen were broken while the rest of the bones possess no apparent skeletal disorders nor damages.

Numerous methods have been published on preparing and cleaning bones prior to articulation for taxidermy purposes (Hefti *et al.,* 1980; Husch *et al.,* 2021; Simonsen *et al.,* 2011; Uhre *et al.,* 2015). The method was modified in this research to accommodate the availability of resources. Firstly, the remaining flesh was scraped off and cleaned from the bones and each bone was then pierced using a small drill at both ends and later stored in a netting bag. Subsequently, the bones were then put into a simmering solution (90°C) made of 1%–3% sodium bicarbonate solution for 15–20 minutes. This process was repeated for a period of seven days, with the bones occasionally removed and the remaining flesh scraped off. After a period of seven days, the bones were then soaked with 30% hydrogen peroxide for another week in which the duration shall depends on the bone sizes.

Soon after, the bones were then washed with distilled water and were air-dried. The removal of muscles and ligaments along with the piercing of the bones are crucial as the bones must be degreased and whitened (Fages *et al*., 1994; Hussain *et al*., 2007). Piercing of the bones is important as it allows the stored grease in the marrow and medullary cavity to leach out and thus determining the neat appearance of bone material creation (Sandström, 1969). As for the bone's measurements, several parameters were measured accordingly and due to the morphological characteristics of the bones, measuring tape was used to measure as it was able to cover the surface irregularities of the bones.

RESULTS AND DISCUSSION

Table 1 summarize the morphometric measurements obtained from the right and left scapular bones of the melanistic leopard, P. pardus. Any slight, insignificant variation in the measurement can be attributed to the irregular surface topology of the scapular, which complicate complex and accurate coverage by a measuring instrument. Crucially, these observed variations are judged to have negligible impact on the inherent morphological function served by the bones.

Table 1. The morphometrical data of different parameters of scapula.

Parameters	**Right**	Left
Weight (g)	35	42
Maximum Length (Dorsal border to Glenoid cavity) (cm)	17.1	17.5
Maximum width (Cranial border to caudal angle) (cm)	12.1	12
Length of cranial border (cm)	15.1	15
Length of caudal border (cm)	14.2	14.7
Length of dorsal border (cm)	7.9	8.0
Length of scapular spine (cm)	13	12.5
Height of scapula spine from supraspinous fossa (cm)	3.3	3.5
Height of scapula spine from infraspinous fossa (cm)	3.6	3.5
Maximum width of supraspinous fossa (cm)	6.0	5.7
Maximum width of infraspinous fossa (cm)	7.3	7.2
Length of glenoid cavity (cm)	4.0	4.0
Width of glenoid cavity (cm)	3.1	3.0
Distance between glenoid cavity and acromion process (cm)	2.6	2.9

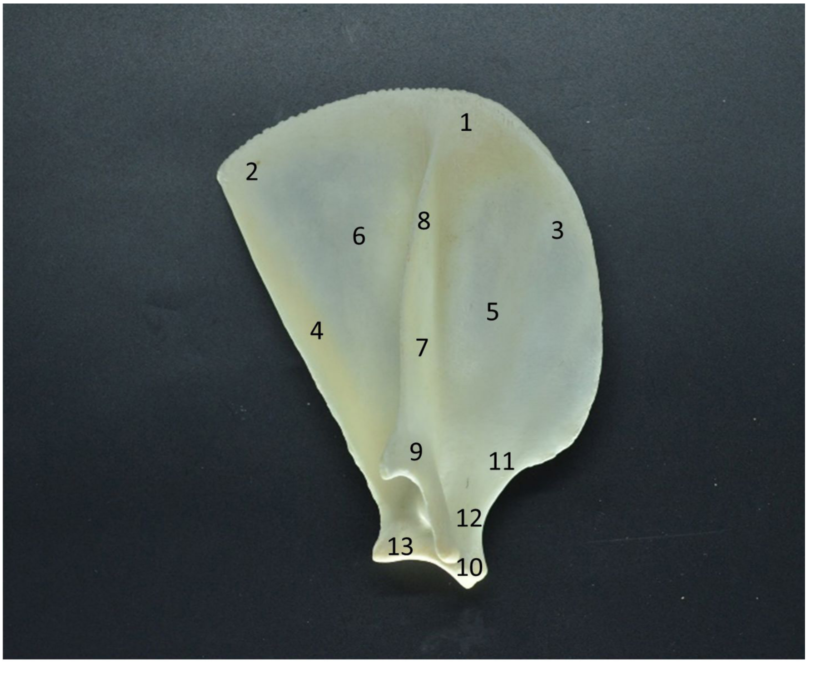


Figure 1. Lateral view of right scapula: 1, Cranial angle; 2, Caudal angle; 3, Cranial border; 4, Caudal border; 5, Supraspinous fossa; 6, Infraspinous fossa; 7, Scapular spine; 8, Tuberosity spine; 9, Suprahamate process; 10, Hamate process; 11, Scapular notch; 12, Coracoid process; 13, Glenoid cavity.

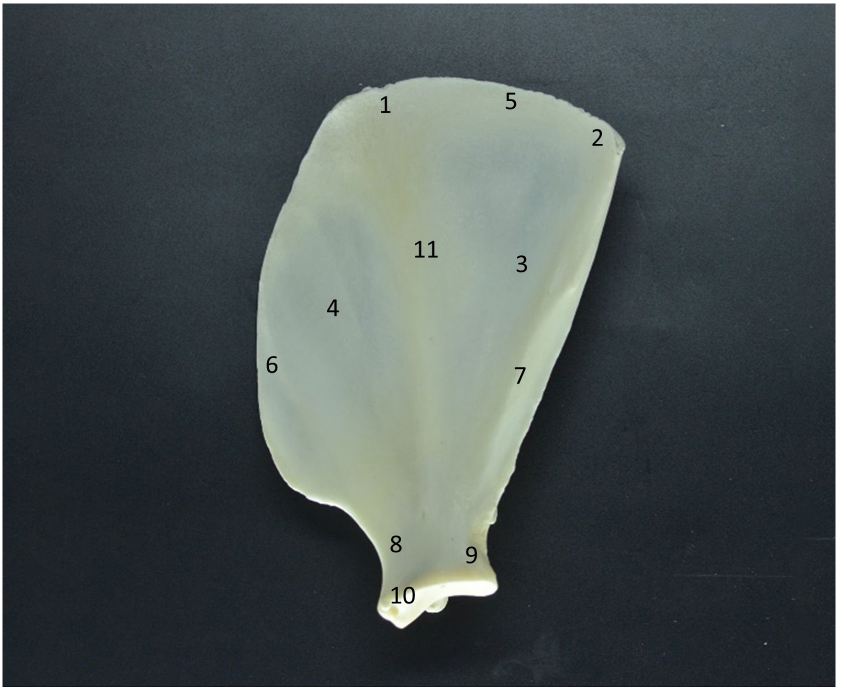


Figure 2. Medial view of right scapula: 1, Cranial angle; 2, Caudal angle; 3, Supraspinous fossa; 4, Infraspinous fossa; 5, Dorsal border; 6, Cranial border; 7, Caudal border; 8, Scapular notch; 9, Glenoid cavity; 10, Coracoid process; 11, Scapular spine.

Figure 3. Lateral view of left scapula: 1, Cranial angle; 2, Caudal angle; 3, Cranial border; 4, Caudal border; 5, Supraspinous fossa; 6, Infraspinous fossa; 7, Scapular spine; 8, Tuberosity of spine; 9, Suprahamate process; 10, Hamate process; 11, Scapular notch; 12, Coracoid process; 13, Glenoid cavity.

Figure 4. Medial view of left scapula: 1, Cranial angle; 2, Caudal angle; 3, Supraspinous fossa; 4, Infraspinous fossa; 5, Dorsal border; 6, Cranial border; 7, Caudal border; 8, Scapular notch; 9, Glenoid cavity; 10, Coracoid process.

The scapular of a tiger is in quadrangular shape in which it is similar to wild cat (Palanisamy *et al.,* 2018) and civet cat (Sarma *et al.,* 2017). Upon observation, the bone of the leopard itself showed a quadrilateral flat surface which consists of lateral and medial surface, three borders which are anterior, posterior, and dorsal border, and three angles which are cranial, dorsal, and glenoid angle which is also observed in other domesticated animals (Archana *et al*., 2016). These findings were also in accordance with the findings of (Pereira *et al*., 2016) in carnivore. As shown in Figures 1–4, the lateral surface of the scapular was then further divided into supraspinous fossa and infraspinous fossa. The supraspinous fossa is the flat area that is above the scapular spine and is more convex in shape which serves the origins of supraspinatus muscle. The infraspinous fossa is the flat area below the spine of the scapula and displays a convex shape in which it is the source of attachment for the infraspinatus muscle. Nevertheless, the inner surface displayed rough, pronounced lines due to the increased muscle attachment points on the scapula (KBD and Deka, 2019).

Table 2 shows the morphometrical data for different parameters of both left and right humerus of the leopard. As presented in the table, the measurement of humerus was taken according to its division which is the cylindrical shaft or diaphysis and two enlarged extremities, namely proximal and distal extremities. The humerus measured for a total length of 22.2 cm and 21.9 cm for both right and left respectively.

Table 2. The morphometrical data for different parameters of humerus.

Parameters	Right	Left
Weight (g)	114	104
Total length (cm)	22.2	21.9
Shaft Length (cm):		
Circumference of upper part	9.0	9.1
Circumference of middle part	8.0	7.6
Circumference of lower part	8.3	8.7
Circumference of head	11.8	12.0
Proximal extremity (cm):		
Circumference	15.5	15.5
Width	6.3	6.4
Distal extremity (cm):		
Circumference	14	14.3
Width	6.0	5.7
Depth of olecranon fossa	2.8	2.7

Figures 5–8 shows the cranial and caudal view of both right and left humerus. The proximal-third of the shaft appeared to be laterally compressed and distal third craniocaudally. The humerus has shown similarities with dogs (Hermanson *et al*., 2019) and lioness (Pandya et al., 2023) in terms of morphology as it possessed four surfaces which are medial, lateral, cranial, and caudal. The medial surface of the bones is nearly straight in its length with the presence of teres major tuberosity. As seen in the both of the diagram above, nutrient foramen can be observed in the distal third which is situated at the junction between the medial and caudal surfaces. The cranial surface was smooth and flattened distally, but triangular and rough proximally. It was narrow in the middle, forming the humerus crest. At the intersection of the proximal and middle thirds of the shaft, the humeral crest had a deltoid tuberosity in the shape of a low ridge. The results of (Künzel and Probst, 1998) in the cheetah supported the discovery of a very tiny elevation in this line dubbed teres minor tubercle, which is located just below the greater tubercle. The caudal surface, which extended from the humerus's neck, was smooth and flattened distally but rounded in the proximal two thirds. The lateral epicondyloid crest extended over the distal portion of this surface and these results are consistent with reports on felids and canids (Smith, 1999).

In the study of felid anatomy especially *P. pardus*, detailed morphometric analysis of skeletal elements is crucial for understanding their biomechanics, evolutionary adaptations, and comparative anatomy (Esteban *et al*., 2020). Table 3 presents the values of measurement on the radius bones of a leopard, focusing on various dimensions and comparison between the right and left sides. The data above offers a detailed values of the left and right bones including total length, proximal and distal extremities, and circumferences at different shaft sections. These measurements provide insights into the structural and functional asymmetries of the radius bones. The total length of the left radius is slightly longer than that of the right radius by 0.2 cm. This minor difference may reflect normal anatomical variation or slight asymmetries typical in wild animals that could be due to the random imprecisions during developmental process (Klingenberg, 2003). At the proximal extremity, the right radius exhibits a slightly larger circumference (by 0.1 cm) and width (by 0.1 cm) compared to the left.

Proximal Extremity

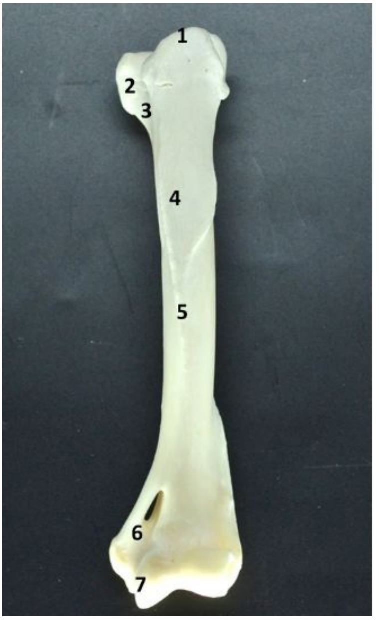
Shaft

Distal Extremity

Figure 5. Cranial view of right humerus: 1, Lesser tubercle; 2, Head; 3, Neck;4, Crest of lesser tubercle; 5, Shaft; 6, Medial epicondyle; 7, Lateral condyle; 8, Medial condyle; 9, Supracondyloid crest; 10, Deltoid tuberosity

Figure 6. Caudal view of right humerus: 1, Head; 2, Greater tubercle; 3, Neck; 4, Deltoid tuberosity; 5, Supracondyloid crest; 6, Olecranon fossa; 7, Medial supracondyloid foramen; 8, Medial epicondyle; 9, Lateral epicondyle

These differences are minor and likely fall within the range of normal variability observed in leopards. The distal extremity measurements show that the right radius has a larger circumference (by 0.3 cm) and width (by 0.2 cm) than the left. This increased robustness at the distal end of the right radius could be attributed to biomechanical stresses or slight differences in usage or loading patterns which corresponds to the behavioural strategy of this species that able to scale trees and leap skilfully (Nowak, 1999). The mid-shaft circumferences reveal small differences between the right and left radius bones whereby the right radius is slightly thicker at the upper and middle parts (by 0.1 cm each) and the left radius has a marginally greater circumference at the lower part (by 0.1 cm). The comparative analysis of the radius bones in *P. pardus* highlights minor asymmetries between the right and left sides. While the differences in measurements are generally small, they provide valuable insights into the structural variations of the radius bones. Overall, the observed measurements are consistent with typical anatomical variability in felids, and the differences between the right and left radius bones are within expected ranges.



Proximal Extremity

Shaft

Distal Extremity

Figure 7. Cranial view of left humerus: 1, Greater tubercle; 2, Lesser tubercle; 3, Neck; 4, Crest of lesser tubercle; 5, Shaft; 6, Supracondyloid foramen; 7, Medial condyle; 8, Lateral condyle

Figure 8. Caudal view of left humerus: 1, Greater tubercle; 2, Lesser tubercle; 3, Neck; 4, Body; 5, Olecranon fossa; 6, Medial epicondyle; 7, Lateral epicondyle

Table 3. The morphometrical data for different parameters of radius.

Parameters	**Right**	**Left**
Total length (cm)	17.5	17.7
Proximal Extremity (cm)		
Circumference	7.3	7.2
Width	2.8	2.7
Distal Extremity (cm)		
Circumference	9.8	9.5
Width	3.7	3.5
Circumference at mid shaft (cm)		
Circumference of upper part	5.3	5.2
Circumference of middle part	5.7	5.6
Circumference of lower part	5.9	6.0

Table 4 presents the measurement values showing differences between the right and left ulna of this particular species. The total length of the right ulna is slightly greater than that of the left ulna, indicating a minor asymmetry. These differences may be attributed to natural variations between limbs or measurement error. The proximal circumference of the left ulna is marginally larger than that of the right. This difference might reflect individual variability or developmental differences between the limbs.

Table 4. The morphometrical data for different parameters of ulna.

Parameters	Right	Left
Total length (cm)	23.0	22.7
Proximal extremity (cm)		
Circumference	9.3	9.5
Width	2.3	2.4
Distal extremity (cm)		
Circumference	6.1	5.9
Width	2.1	2.0
Circumference at mid shaft (cm)		
Circumference of upper part	7.4	7.2
Circumference of middle part	6.0	5.8
Circumference of lower part	4.9	4.9

Similarly, the width of the proximal extremity is slightly greater in the left ulna, further suggesting a degree of asymmetry and the distal circumference of the right ulna is slightly larger compared to the left, indicating another instance of asymmetry. The width measurements at the distal extremity follow a similar pattern, with the right ulna being marginally wider. The upper part of the mid-shaft circumference is greater in the right ulna, which might suggest a slightly more robust upper shaft in this limb. The middle part circumference is also slightly larger in the right ulna, reinforcing the observation of asymmetry. Interestingly, the circumference of the lower part of the mid-shaft is identical for both ulnae, indicating no detectable asymmetry at this specific location. The observed differences in ulnar measurements between the right and left limbs of the leopard specimens are relatively minor, but they provide significant insight into the natural variability of limb morphology. Asymmetry in limb dimensions is not uncommon in wildlife and can result from various factors, including genetic variation, environmental influences, or habitual behaviours (Coda *et al*., 2017).

The larger circumferences and widths in certain areas of the right ulna may suggest slight differences in mechanical load distribution or developmental adaptations. However, the identical measurements at the lower part of the mid-shaft indicate a level of consistency that could be linked to functional demands placed on the ulna during movement. The values of ulnar measurements in leopards highlights the natural variability in limb anatomy. While minor asymmetries are evident, they fall within the range of normal biological variation. Such studies are crucial for understanding the functional morphology of predators and can contribute to broader research on limb evolution and biomechanics in large carnivores.

CONCLUSION

This study has documented the procedure of bones maceration for the preparation of osteological collections in Natural History Museum of Malaysia. The maceration techniques, along with detailed gross anatomical and morphometrical analysis of the scapula, humerus, radius, and ulna of the melanistic leopard, has provided valuable insights into the structural adaptations and variations of these bones. The bone maceration process, when executed with precision, ensured the preservation of the skeletal integrity, allowing for accurate anatomical examination and morphometric measurements. The gross anatomical examination illuminated the distinct features and morphology of the scapula, humerus, radius, and ulna, contributing to a deeper understanding of the locomotor capabilities and musculoskeletal structure of this subspecies. Due to the nature of the species, there were no sufficient sample size due to the shortage of the specimen as only one specimen was donated to the museum. Nevertheless, the values obtained throughout this research may also serve as a baseline for a museum's documentation and thus serves as a baseline for comparative studies in other felid species, contributing to broader discussions on the evolution and adaptation of large carnivores.

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