TAMIL NADU FOREST DEPARTMENT

ADVANCED INSTITUTE FOR WILDLIFE CONSERVATION

(Research, Training & Education)

PROJECT COMPLETION REPORT

DELINEATING FELINE CLAWS AND CANINE TEETH OF TIGER AND LEOPARD BY MORPHOMETRIC METHODS

ANNUAL PLAN OF OPERATIONS (APO - 2021-22)

Tamil Nadu Forest Department
ADVANCED INSTITUTE FOR WILDLIFE CONSERVATION
(Research, Training & Education)
Vandalur, Chennai – 600 048.

Project Completion Report
On

‘DELINEATING FELINE CLAWS AND CANINE TEETH OF TIGER AND LEOPARD BY MORPHOMETRIC METHODS’

Annual Plan of Operations (APO)
(2021-22)

Submitted By

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CONTENTS

1. INTRODUCTION

Illegal wildlife trade is a serious threat to the existence of wild animals. The Tiger (*Panthera tigris*) is listed as 'Endangered' and the Leopard *(Panthera pardus*) is listed as Vulnerable on the IUCN Red List of Threatened Species. Wild Tigers and Leopards are killed illegally to fuel the demand for their products, such as skin, claws, canines, etc. This demand for feline parts has led to poaching, habitat destruction, and other threats to the survival of these species (Sethi *et al.*, 2019). Conservation efforts to protect Tigers and Leopards must include addressing the issue of illegal wildlife trade. Tiger and Leopard's claws are worn as charms against evil spirits, instilling courage in the wearer. Many jewellery and artefacts are also made from their canines. The short supply and high demand for wildlife articles have caused an influx of many different forms of fake wildlife articles into this trade. In recent years, the prohibited trade of canines and claws has been going upward, adversely impacting several significant species of mammals. Increasing reports of canine and claw seizures demand forensic inspection and thorough investigation of evidence for species identification (Nadarajan *et al.*, 2022)

Species identification through DNA barcoding is challenging for samples like canine and claw as they are rich in calcium and keratin composition. Calcium is a Taq polymerase inhibitor, competitively binding to the polymerase in place of magnesium during PCR. As a result, it reduces amplification efficiency, and keratin is also a significant PCR inhibitor (Kuffel *et al.*, 2021).

This study mainly focused on species identification of the Tiger and Leopard using morphometric methods. Various methods have been followed for morphometric analyses based on the sample type and condition. This information can be used by conservationists and law enforcement officials to identify the species of wildlife products being sold in illegal wildlife markets and their genuineness. Additionally, studying the claws and teeth of Tigers and Leopards can provide insight into the behaviour and ecology of these animals. The size and shape of feline claws can reveal information about the type of prey an animal hunts (Stander, 1997). Morphometric analysis is a cost-effective and reliable method with various software-based measurements for species identification in our study.

Age is also a critical factor in debates over trophy carnivore hunting. Age-based trophy selection, whereby it is required that hunted animals meet or exceed a minimum age standard, is considered an essential component of the sustainable harvest of African lions (White *et al.,* 2017). Radiological analysis of canines and claws, such as age, species identification, and credibility of the sample, is used for various studies. Evidence shows that the pulp chamber's closure increases with the animal's age (White *et al.*, 2016). Scanning electron microscopy (SEM) is a powerful tool that can be used to examine the surface micro-structure and morphology of canines and claws. The differences in the claw and tooth structure and sharpness of Tigers and Leopard's canines, and these variations affect their hunting strategies and behaviour (DeLaurier *et al.*, 2006).

Scanning electron microscopy (SEM) is a powerful tool that can be used to examine the surface micro-structure and morphology of canines and claws. Using this technique, micro-level patterns of the structure, which are impossible for a person to mimic, can be observed in their fake replicas. The detailed morphology of Leopard and Tiger claws and canine structures is notoriously difficult to analyse because of their component tissues' extreme range of hardness. Hence, an approach using scanning electron microscopy was applied to study the morphology of the claws and canines of Tiger and Leopard (DeLaurier *et al.,* 2006).

The highly complex architecture of the claw sheath is generated by the living epidermis supported by the dermis and distal phalanx. The latter is characterized by an ossified unguicular hood, which overhangs the bony articular base and unguicular process of the distal phalanx and creates an unguicular recess. The dermis covers the complex surface of the bony distal phalanx but also creates unique structures, such as a dorsal dermal papilla that points distally and a curved ledge on the medial and lateral sides of the unguicular process (Fig.1) (Homberger *et al.,* 2009).

By delving into the intricacies of feline claws and canine teeth, it is possible to shed new light on these magnificent predators and provide a comprehensive resource for researchers to identify and ensure the accuracy, reliability and standard predictability of species identification.

Taxonomical Classification of study animals

Leopard	Tiger
Taxonomic Classification	**Taxonomic Classification**
Kingdom: Animalia	Kingdom: Animalia
Phylum : Chordata	Phylum : Chordata
Class : Mammalia	Class : Mammalia
Order : Carnivora	Order : Carnivora
Family : Felidae	Family : Felidae
Genus : *Panthera*	Genus : *Panthera*
Species : *pardus*	Species : *tigris*

The study animals Tiger (*Panthera tigris*) and Leopard (*Panthera pardus*) are of the same Felidae family and show many similarities in canine and claw morphology. This study reveals the key measurement variations and dissimilarities between these two species delineated for age identification.

2. OBJECTIVES

- ❖ To build the repository data for Leopard and Tiger canine and claws, which have high demand in illegal Wildlife trade market.
- ❖ To delineate the difference between Tiger and Leopard (species identification) and to find out genuinity of the sample obtained from seized products through morphometric measures alone.
- ❖ To estimate the approximate age for the forensic seizure

3. MATERIALS AND METHODS

3.1. Sample Source and Cleaning

The canine and claw samples, obtained from dead tigers and leopards after post-mortem, were sent to the AIWC repository by the field veterinary officers from various forest divisions of Tamil Nadu. The samples were received with proper permission. Samples were cleaned and stored in 70% ethanol.

Figure 3.1. Claw samples with hair and skin

- Claw samples with hairs (Fig. 3.1) and canines were air-dried for a week by separating them on a board (Fig 3.2)
- The canines were removed from the jaw bones and hairs in the claw were removed without damaging the samples.
- Finally, the samples were washed with soap oil and hot water and were stored in 70% ethanol.

Figure 3.2. Sample processing

Table 3.1. Total number of canine and claw samples examined

Sample	Animal	
	Tiger	Leopard
Canine	17	38
Claw	43	133

3.2. Visual examination of canine

Felid canines are conical-toothed and posteriorly recurved (Fig. 3.3); they have lateral grooves and two ridges that meet to form a lateral triangle and are not serrated (Martin, 1980). In the present study, various felid features of the canine, such as the presence of two grooves and a prominent calcified marginal rigid on both sides of the labial and lingual side of the maxillary canine, were examined and reported.

Colour of the canine tooth was examined as almost pale yellow in general but may vary from age to age. The lesser the age, the brighter the tooth. Young ones have white teeth as they undergo amelogenesis (enamel formation), and yellowing of teeth occurs with the increase of age.

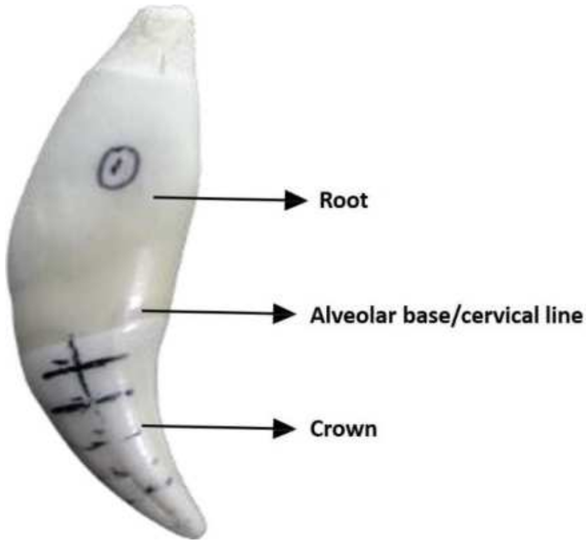


Figure 3.3. Canine Morphology

3.3. Visual Examination of Claw

Felid has retractile claws, and they are in a retracted position through most phases of locomotion, except possibly during the initial period of rapid acceleration. On the other hand, the claws are protruded during climbing and when grasping prey. Therefore, the retractile mechanism in felids is adapted for hunting and climbing (Gonyea and Ashworth, 1975).

The claws of felids are keratin-rich, with a hood attached to their paw. The presence of the unguicular process, which protrudes from the unguicular hood, is the main character of the claw to test its credibility (Fig. 3.4). In case a seizure contains a nail alone, the presence of a curve line along the curve of the keratinous claw sheath can be considered as a primary visual confirmation. The colouration of the claw nail is yellow and the appearance of a curved line is prominent.

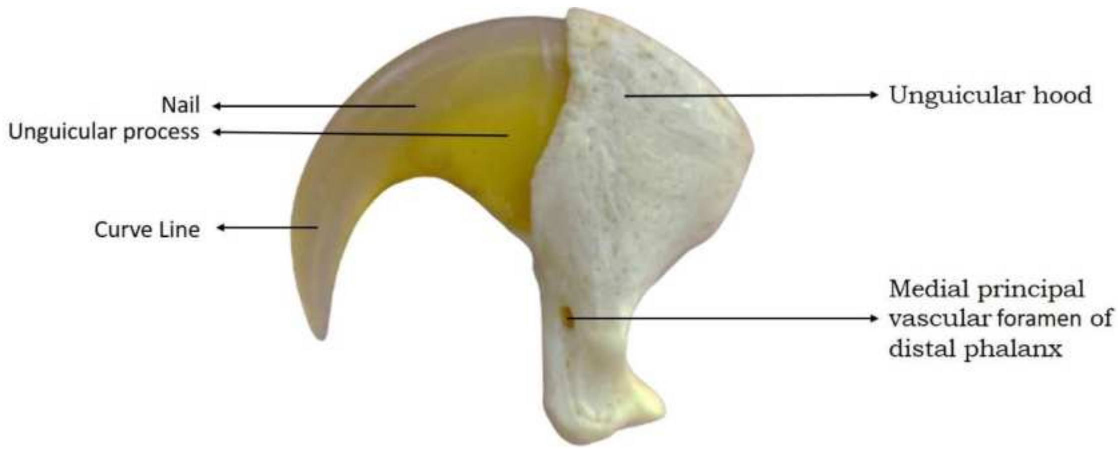


Figure 3.4. Claw Morphology

3.4. Morphometric measurements

Measurements can be taken either using a digital Vernier caliper or by using a landmark system of measurements in software by uploading a high-resolution photograph of the sample.

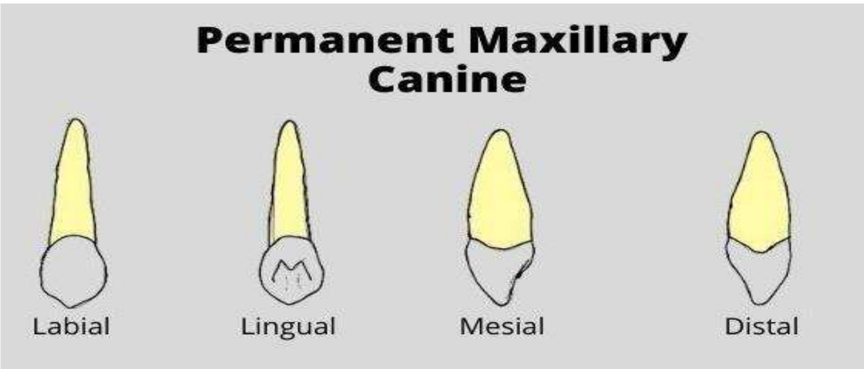


Figure 3.5. Canine anatomy

Around 19 various measurements were taken from one canine and 19 various measurements were taken from one claw of a Tiger and a Leopard and subjected to Descriptive statistical analysis (Tables 2, 3 &4) (Smuts *et al.,* 1977; Sharma *et al.,* 2016; Nadarajan *et al.,* 2022)**.**

Table 3.2. List of measurements taken in the canine teeth

Sl. No.	Measurements in mm		Procedure
1	Whole length	Labial	Whole length, root length and crown length of the canine is taken in labial, lingual, mesial and distal aspects (Fig. 3.5).
2	Root length	Lingual Mesial	
3	Crown length	Distal	
4	Crown breadth at various lengths		The breadth of the canine at different intervals of crown length is measured (90%, 70%, 60%, 45%, 30%, 15% and Alveolar base).
5	Grooves		Special characters like grooves may present in a few species like Felines. The number of grooves and their length can be taken
6	Mesio-distal length		Mesio-distal length of the root part will be taken
7	Canine geometry		Angle, radius and total length from to the tip of the canine are taken.

Table 3.3. List of measurements taken in the claw

Sl. No.	Measurements in mm	Procedure
1	AC (full)and AC (outer)– for hooded claw	Measurements were taken along the curve line of the claw.
2	BC (full) and BC (outer)– for hooded claw	Measurements were taken along the curve line of the claw.
3	AB (full) and AB (outer)– for hooded claw	Measurements were taken from the distal rim to the palmer flange
4	Outer total length (OTL)and inner total length (ITL) for-hooded claw	Measurements are taken from the nail tip to the claw tip.
5	Claw geometry - outer angle and radius (AG & R) and inner angle and radius (I-AG& I-R)-for hooded claw	Angulation and radius are taken for inner and outer curvature calculation
6	Nail width (N.W)/claw mid-point.	Nail width is taken along the mid-point of ITL to its claw end.

Table 3.4. List of measurements taken for hooded claw

Sl. No.	Measurements in mm	Procedure
1	Basal unguicular pleat (BUP)	Measurement is taken from base of the Palmar flange of coronary horn to its base
2	Total hood length (THL)	Measurement is taken from top to base if the unguicular hood is present.
3	Base of unguicular hood (BUH)	The basal region length of the unguicular hood was taken.
4	Top of unguicular hood (TUH)	The top region of the unguicular hood was taken
5	Dorsal distal phalynx pore (DDP)	Distance of medial principal vascular foramen of distal phalanx.

Tables 3.1, 3.2, 3.3 and 3.4 show a list of measurements performed in the claw and canine of Tiger and Leopard and the procedure to perform the same measurements in the future.

3.5. Age estimation and credibility assessment using X-ray analysis

Radiographic analysis is a diagnostic technique that involves X-ray imaging to examine the internal structures of claws and canines (Smuts *et al.,* 1977). Totally 55 canines and 176 claws were subjected to X-ray analysis at AAZP veterinary hospital, Vandalur (Fig. 3.6).

Figure 3.6. X-ray analysis of claw and canines of tiger and leopard at AAZP

3.5a. X-ray analysis of canine

The X-ray on the canine shows the variations in the pulp cavity, representing the age of the species. Closure of the pulp cavity is inversely proportional to age (Fig.3.7).

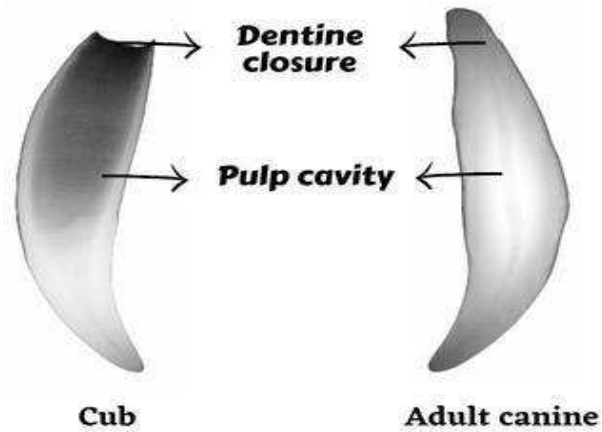


Figure 3.7. Pulp variation between cub and adult

Interpretationofdatathroughthepresenceofapulpcavityanditspercentagecanal sobeassessed.The width of the total canine and its pulp chamber was measured at 25%,50%and 75% (Fig3.8).

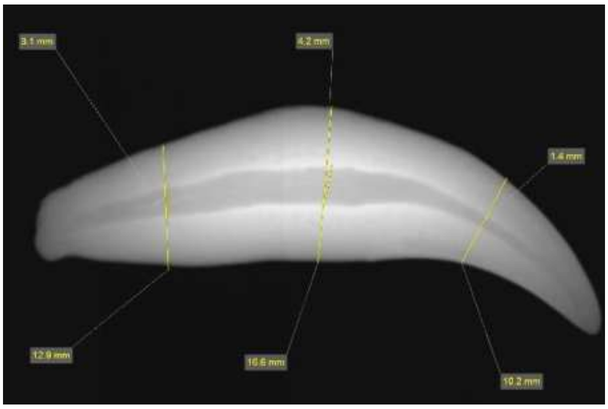
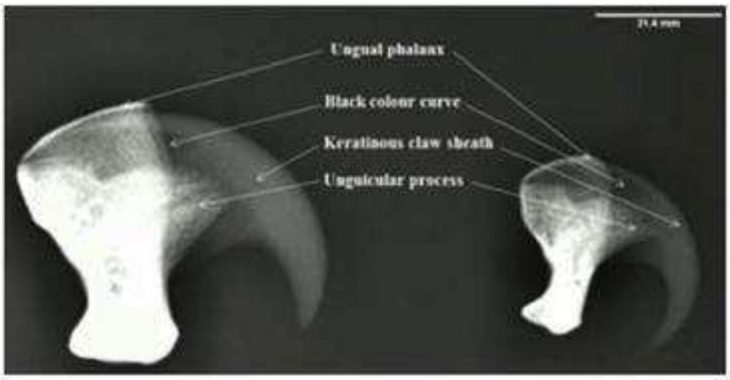


Figure 3.8. Pulp measurementson25%,50%and75%oftotalcaninelength

3.5b. X-ray analysis of claw

It has been reported that the keratin density in the claws of cats might have variations (Sharma *et al.,* 2016) (Fig. 3.90). By visual analysis of such density gradient of keratin content in claws using X-ray images, we can differentiate the available samples between original and replicas (fake). Presence of an unguicular process inside the nail can be clearly seen and this is a key factor for its originality. Analysis was done by using VD-viewer software with DICOM image.

The variations in the keratin density were observed between tiger and leopard, which was already reported by Sharma *et al.* (2016).



A B

Figure 3.9. Keratin density variation between Tiger (A) and Leopard (B) claws (Sharma *et al.,* 2016)

3.6. Wear and Tear Measurements for canine

The natural process of tooth wear occurs over a time of more than 5-6 years in big cats. Canine teeth are sharp, pointed teeth located in the frontal region of the face and they are used for biting and tearing food, as well as for defence and aggression. An animal's approximate age can be estimated by analysing a canine's wear and tear percentage (Stander,1997). Tooth wear estimation was done based on Smith and Knight's standard tooth wear index system (1984) (Table 3.5). In this protocol tooth wear score was given to all the analyzed samples by visual examination (Tabel 3.5).

Table 3.5. Tooth Wear Index (Smith & Knight, 1984)

Tooth wear range	Score
No wear, no enamel loss	0
Loss of enamel (starting stage)	1
Dentine exposure less than 1/3ofcrown	2
Dentine exposure more than 1/3 of crown	3
Complete loss of enamel	4

3.7. Scanning Electron Microscopic Analysis

Scanning Electron Microscopic (SEM) images of canine and claw of Tiger, Leopard, Sloth bear and Jaguar were taken at IIT Madras (Fig. 3.10). SEM can give high-resolution images even in micro-level analysis. SEM can analyse challenging case samples (even a piece of canine) by comparing them with the representative sample at a certain point.

Figure 3.10. Scanning Electron Microscopic imaging at IIT Madras

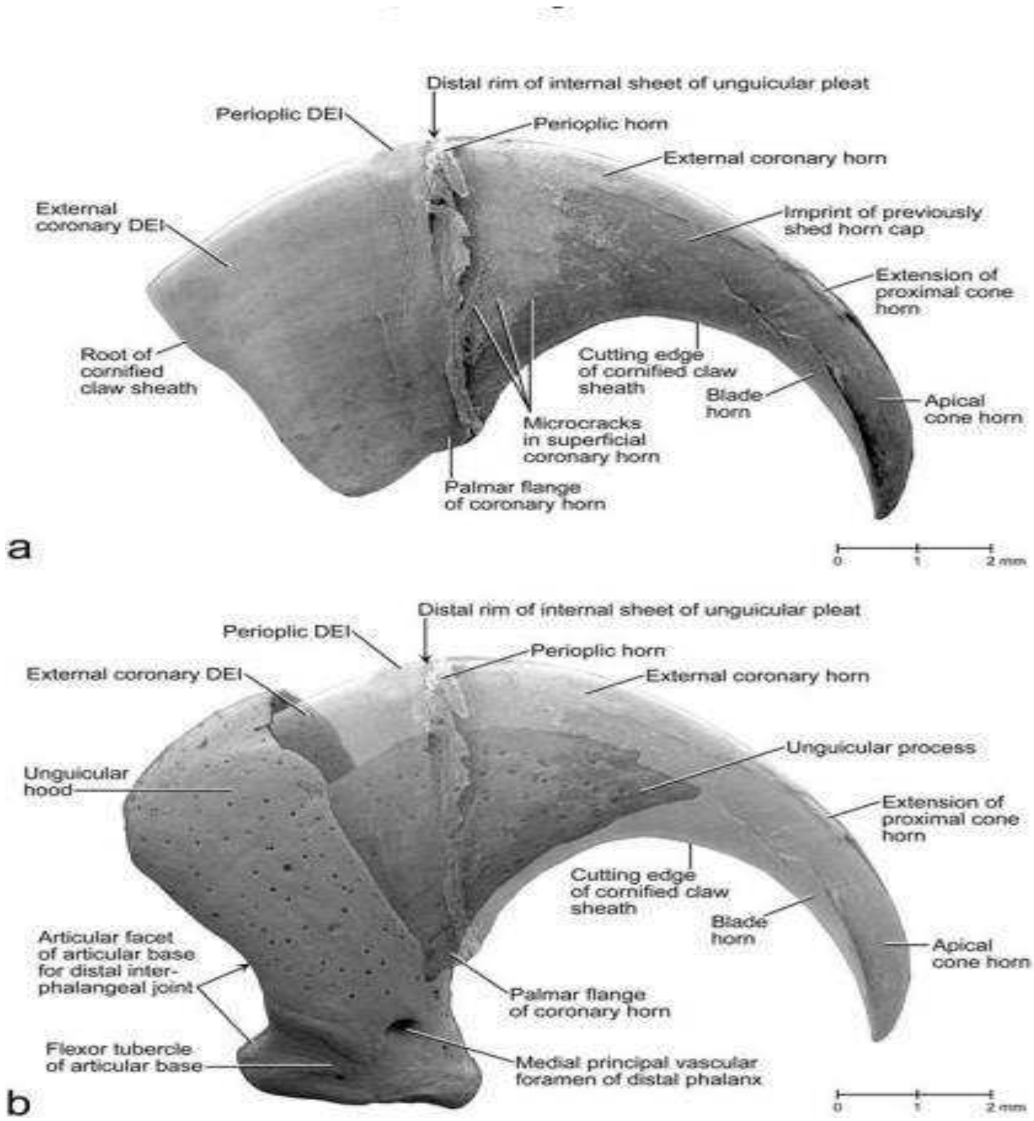


Figure 3.11. SEM image of Domestic Cat claw (Homberger *et al.,* 2009).

4. RESULTS

Morphometric parameters of canine and claw samples obtained from dead tigers and leopards were carefully studied. The samples used in this study were from animals of known age and body weight. All possible morphometric parameters were measured to delineate the canines and claws of Tiger and Leopard. Descriptive statistics of morphometric measurements were performed using SPSS statistical software. For the canine sample, whole length, root length and crown length were measured in labial, lingual, mesial and distal sides and mean values were calculated for labial and lingual sides (Labio-lingual). The same measurements were taken for the mesial and distal sides (mesio-distal). All the parameters were taken as such for claw, and the Excel sheet was fed into SPSS statistical analysis software for descriptive statistical analysis.

Canine morphology

In the mandibular canine, only one groove was present on the mesio-distal crown and a calcified margin on the lingual side was observed with a smooth labial side. The tip of the canine teeth was slightly inward in the maxillary canine. The size of the maxillary canine was a bit larger when compared with the mandibular canine. Mandibular canines were more curved than maxillaries. The root of the canine was larger than its crown part. A plane surface was seen in between the root and the crown called the Alveolar base. The roots of the canine teeth were highly uneven and patterned at the upper end (Fig.3.3).

Table 4.1 shows descriptive statistics of crown breadth length at 90%, 70%, 60%, 45%, 30%, 15% and alveolar base (cervical line) of canine of Leopard. The measurements were taken on a Millimeter scale.

Table 4.1. Descriptive statistics for crown breadth at various intervals in Leopard canine teeth (in mm)

Parameters	N	Range	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
90%	31	87.00	3.00	90.00	6.59	2.78	15.48	239.760
70%	31	65.17	4.83	70.00	8.43	2.05	11.45	131.125
60%	31	54.22	5.78	60.00	9.41	1.69	9.426	88.862
45%	31	38.00	7.00	45.00	10.56	1.16	6.48	42.034
30%	31	21.91	8.09	30.00	11.76	0.66	3.67	13.514
15%	31	6.66	9.11	15.77	12.73	0.30	1.67	2.804
Alveolar base	30	6.36	10.33	16.69	13.94	0.30	1.66	2.777

Table 4.2 shows descriptive statistics of crown breadth length at 90%, 70%,60%, 45%, 30%, and 15% of the alveolar base (cervical line) of the canine of Tiger. The measurements were taken on a millimeter scale.

Table 4.2. Descriptive statistics for crown breadth at various intervals in Tiger canine teeth (mm)

Parameters	N	Range	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
90%	17	3.18	4.38	7.56	6.46	0.23	0.97	0.947
70%	17	3.68	8.77	12.45	10.32	0.30	1.24	1.537
60%	17	4.42	10.40	14.82	12.23	0.34	1.44	2.078
45%	17	7.08	11.39	18.47	14.94	0.46	1.92	3.689
30%	17	7.16	12.60	19.76	17.02	0.47	1.94	3.784
15%	17	8.00	14.50	22.50	18.81	0.57	2.36	5.577
Alveolar base	17	7.72	16.30	24.02	20.88	0.50	2.08	4.357

Table 4.3 & 4.4 shows descriptive statistics of various parameters of canine teeth of leopards and tigers. The measurements were taken on a Millimeter scale.

Table 4.3. Descriptive statistics of Leopard canine teeth

Parameters	**N**	**Range**	**Minimum**	**Maximum**	**Mean**	**Std. Error**	**Std. Deviation**	**Variance**
Labio-lingual whole length	42	52.65	27.06	79.71	**61.08**	1.98	**12.87**	165.689
Mesio-distal whole length	42	52.17	25.94	78.12	**60.53**	2.01	**13.02**	169.711
Labio-lingual root length	42	30.16	17.59	47.76	**34.83**	1.17	**7.59**	57.593
Mesio-distal root length	42	29.59	16.33	45.92	**35.35**	1.12	**7.26**	52.726
Labio-lingual crown length	42	33.31	8.88	42.19	**25.28**	1.018	**6.60**	43.584
Mesio-distal crown length	42	32.42	8.58	41.00	**24.41**	1.05	**6.84**	46.794
Weight	42	13.95	0.71	14.66	**8.20**	0.62	**4.03**	16.276
Outer total length	42	50.71	25.26	75.97	**58.94**	1.83	**11.91**	142.000
Angulation	42	62.39	63.60	126.00	**96.39**	2.30	**14.92**	222.766
Radius	42	43.5467	21.6146	65.1613	**41.3555**	1.39	**9.03**	81.548
Root length	42	30.3800	16.2400	46.6200	**33.5553**	1.06	**6.90**	47.726
Mesio-distal breadth	42	11.4700	6.8300	18.3000	**13.8183**	0.43	**2.79**	7.794

Table 4.4. Descriptive statistics of Tiger canine teeth

Parameters	N	Range	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
Labio-lingual whole length	17	53.59	68.60	122.19	99.51	3.25	13.40	179.577
Mesio-distal whole length	17	63.67	62.44	126.11	97.96	3.83	15.79	249.496
Labio-lingual root length	17	42.78	30.65	73.43	59.21	2.43	10.02	100.508
Mesio-distal root length	17	44.67	30.18	74.86	58.11	2.58	10.64	113.215
Labio-lingual crown length	17	22.68	28.35	51.03	39.89	1.41	5.82	33.902
Mesio-distal crown length	17	23.40	28.05	51.45	40.23	1.61	6.64	44.166
Weight	17	41.30	19.20	60.50	34.84	2.90	11.96	143.067
outer total length	17	38.88	77.49	116.37	93.78	2.53	10.45	109.223
Angle	17	36.93	86.25	123.18	103.53	2.52	10.39	107.995
Radius	17	30.57	46.96	77.53	61.39	2.19	9.04	81.797
Root length	17	38.04	37.78	75.82	54.63	2.34	9.65	93.113
Mesio-distal breadth	17	7.81	18.43	26.24	22.19	0.55	2.27	5.177

Tables 4.5 and 4.6 show descriptive statistics of various parameters of canine teeth of Leopard and Tiger. The measurements are taken on a Millimeter scale.

Table 4.5. Descriptive statistics for Leopard claw

Parameters	N	Range	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
AC (full)	134	38.00	21.87	59.87	42.84	0.72	8.37	70.063
AC (OUT)	134	30.48	14.99	45.47	30.64	0.50	5.81	33.761
BC(full)	134	23.00	11.93	34.93	25.128321	0.39	4.58	21.043
BC(out)	134	17.36	9.47	26.83	19.30	0.32	3.70	13.737
AB(full)	134	9.58	10.44	20.02	15.95	0.18	2.07	4.314
AB(out)	134	15.55	4.15	19.70	14.61	0.19	2.18	4.744
I-TL	134	21.39	16.33	37.72	26.74	0.36	4.19	17.591
O-TL	134	28.20	19.13	47.34	32.85	0.41	4.78	22.906
Angle	134	60.27	71.14	131.4	106.81	0.92	10.72	114.915
Radius	134	18.86	6.85	25.71	17.23	0.22	2.53	6.398
Nail width	134	8.67	3.78	12.46	7.30	0.15	1.81	3.277
I-radius	134	6.33	4.74	11.07	7.47	0.09	1.09	1.194
I-angle	134	70.67	108.73	179.4	155.62	1.39	16.13	260.414
Weight	134	2.44	0	2.44	1.14	0.04	0.47	0.228

Table 4.6. Descriptive statistics for Leopard hood

Parameters	N	Range	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
Basal Unguicular Pleat	43	14.52	7.23	21.75	12.81	0.40	2.63	6.912
Dorsal Distal Phalynx	43	2.21	0.69	2.90	1.50	0.07	0.49	0.241
Top of Unguicular Hood	43	13.91	9.51	23.42	14.85	0.53	3.51	12.329
Total Hood Length	43	23.30	18.48	41.78	27.07	0.74	4.88	23.832
Base of Unguicular Hood	43	9.24	4.95	14.19	8.82	0.29	1.95	3.837

Tables 4.7 & 4.8 show descriptive statistics of various parameters of canine teeth of Leopard and Tiger. The measurements were taken in Millimeter scale.

Table 4.7. Descriptive statistics for Tiger claw

Parameters	N	Range	Minimum	Maximum	Mean	Std. Error	Std. Deviation	Variance
AC (full)	43	38.94	39.63	78.57	60.492	1.65	10.84	117.472
AC (OUT)	43	32.15	23.16	55.31	40.94	1.11	7.32	53.678
BC (full)	43	28.04	16.87	44.91	33.88	1.03	6.77	45.903
BC (out)	43	19.54	14.80	34.41	27.06	0.65	4.25	18.086
AB (full)	43	15.82	14.74	30.56	23.39	0.63	4.16	17.369
AB (out)	43	20.50	10.75	31.25	22.41	0.71	4.66	21.716
I-TL	43	20.47	22.54	43.01	32.90	0.84	5.55	30.817
O-TL	43	33.02	29.23	62.26	43.34	1.12	7.35	54.050
Angle	43	53.05	69.54	122.59	98.40	1.87	12.31	151.538
Radius	43	17.19	14.98	32.18	22.18	0.56	3.70	13.687
Nail width	43	8.08	4.53	12.61	8.40	0.33	2.18	4.748
I-radius	43	6.72	6.61	13.34	9.45	0.19	1.29	1.676
I-angle	43	53.57	125.35	178.92	154.54	2.44	16.03	257.011
Weight	43	7.04	0.70	7.74	3.36	0.26	1.73	2.987

Table 4.8. Descriptive statistics for Tiger hood

Parameters	N	Range (mm)	Minimum (mm)	Maximum (mm)	Mean (mm)	Std. Error	Std. Deviation	Variance
Basal Unguicular Pleat	26	18.45	10.62	29.07	17.16	0.81	4.16	17.320
Dorsal Distal Phalynx	26	2.96	0.90	3.86	1.86	0.15	0.78	0.617
Top of Unguicular Hood	26	24.55	14.14	38.69	23.20	1.47	7.52	56.655
Total Hood Length	26	33.37	27.62	60.99	39.42	1.78	9.06	82.216
Base of Unguicular Hood	26	10.05	10.67	20.73	14.82	0.60	3.06	9.404

Figures 4.1and 4.2 show range and distribution of Leopard and Tiger canines

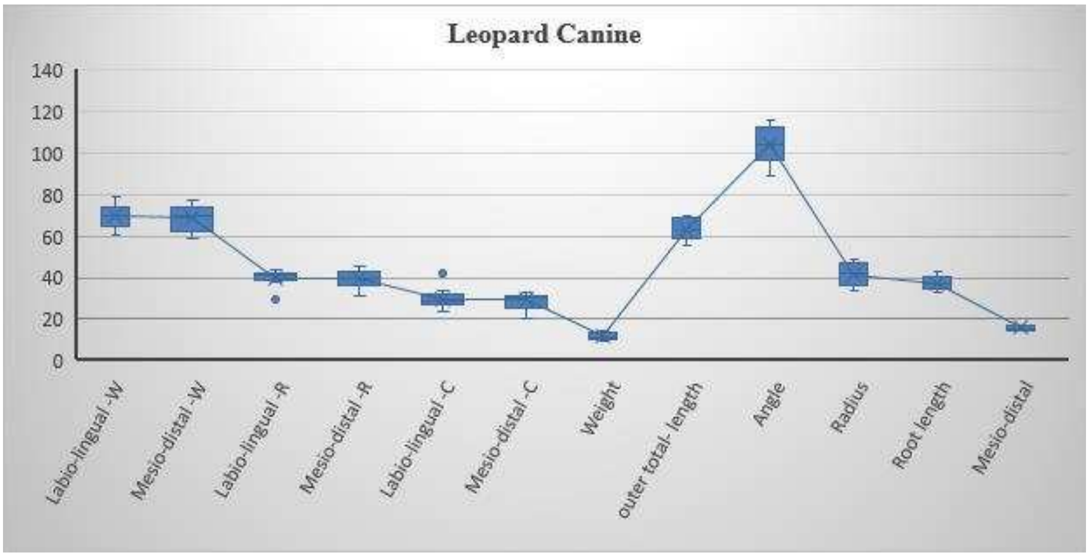


Figure 4.1. Distribution of Leopard canine

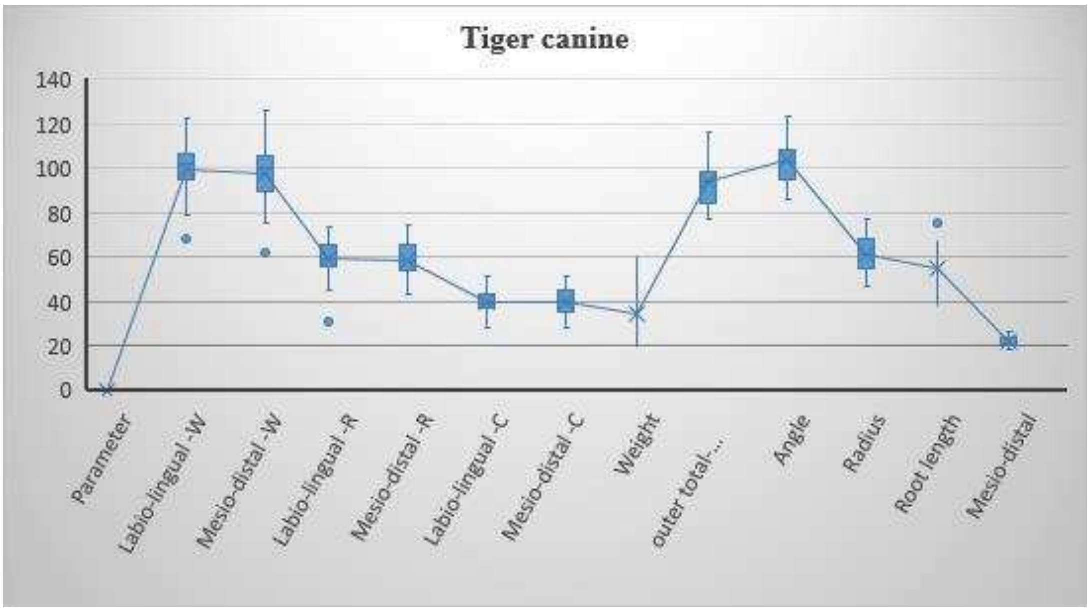


Figure 4.2. Distribution of Tiger canine

Figures 4.3 and 4.4 show range and distribution of Leopard and Tiger claws.

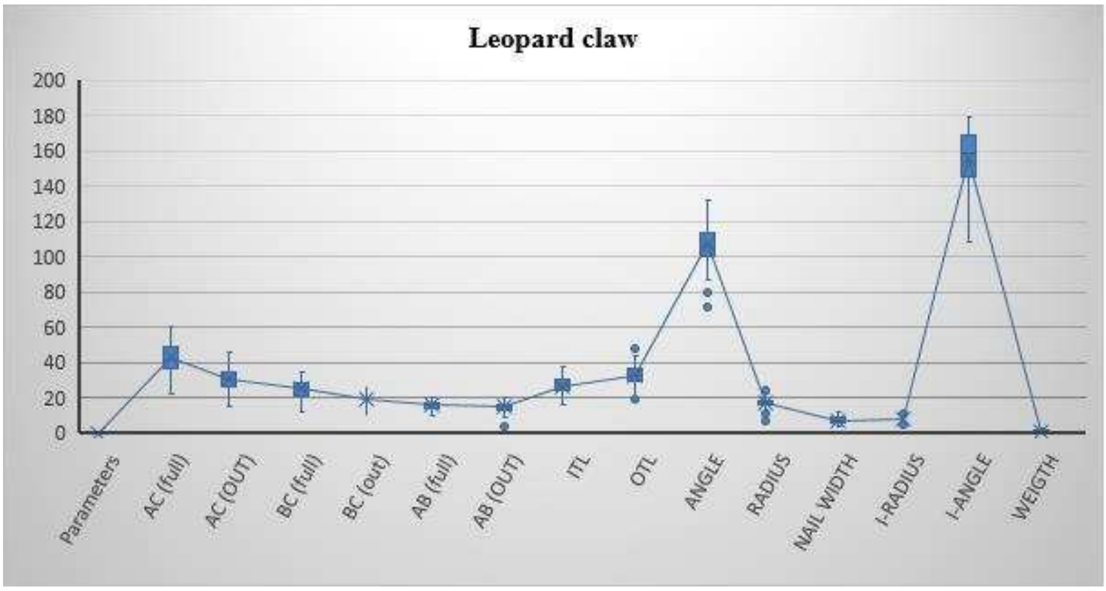


Figure 4.3. Boxplot of Leopard claw

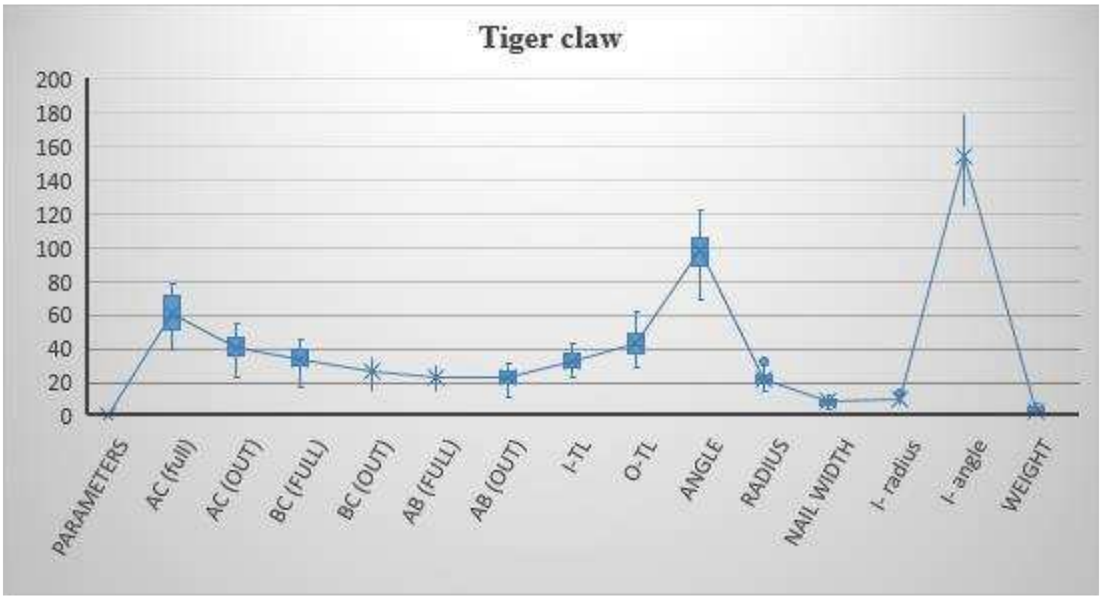


Figure 4.4. Boxplot of Tiger claw

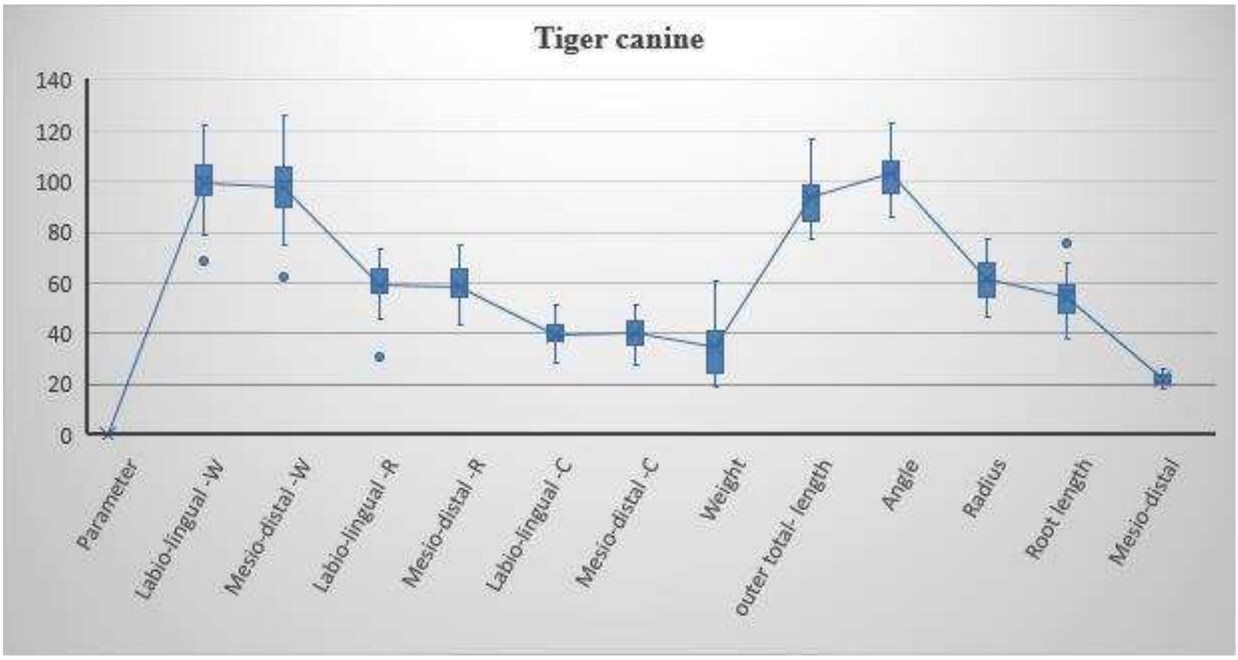


Figure 4.5. Bar diagram representation of canine mean comparison between Tiger and Leopard

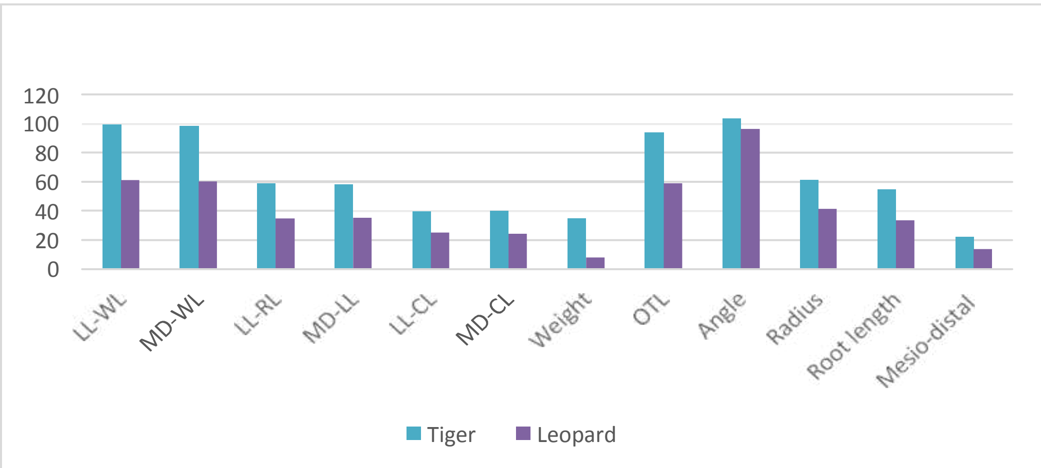
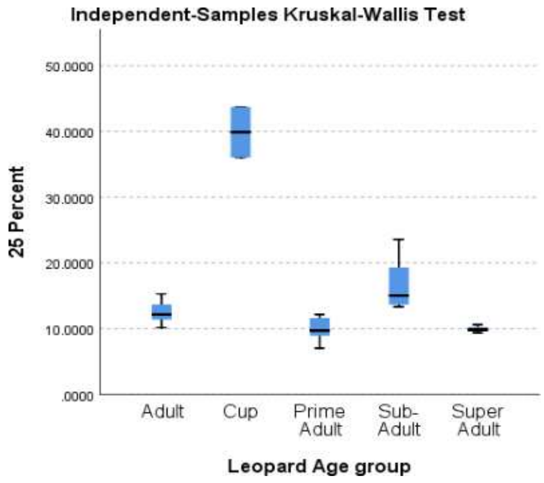
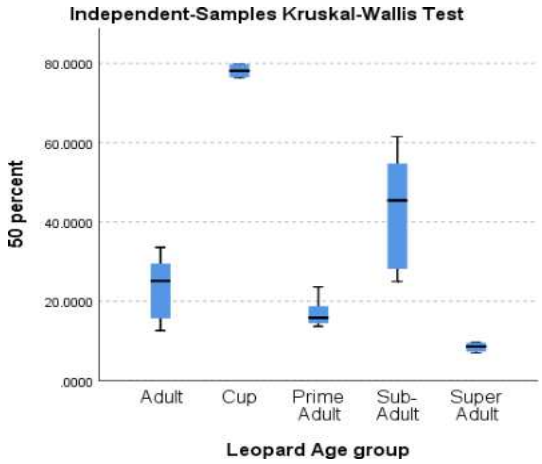


Figure 4.6. Bar diagram of canine mean comparison between Tiger and Leopard. LL-WL: labio-lingual whole length, MD-WL: mesio-distal whole length, LL-RL: labio-lingual root length, MD-LL: mesio-distal root length, LL-CL: labio-lingual crown length, MD-CL: mesio-distal crown length and OTL: outer total length. The measurements were taken on a Millimeter scale.

The pulp chamber and total chamber measurements were taken for all canines and x-ray was taken for all claw samples. The elucidation of the canine pulp chamber with five age groups in Leopard and two age groups in Tiger in radiographic analysis showed excellent statistical significance between the groups.

Kruskal–Wallis test was performed in SPSS for significance testing of independent-samples and the obtained results were interpreted below.





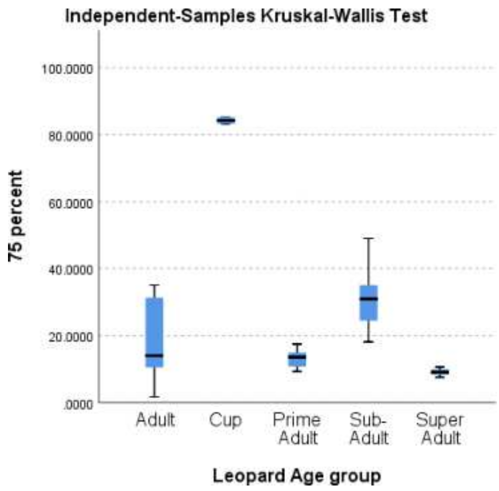


Figure 4.7. Boxplot of Pulp distribution across the age groups of Leopard at 25%, 50% and 75%.

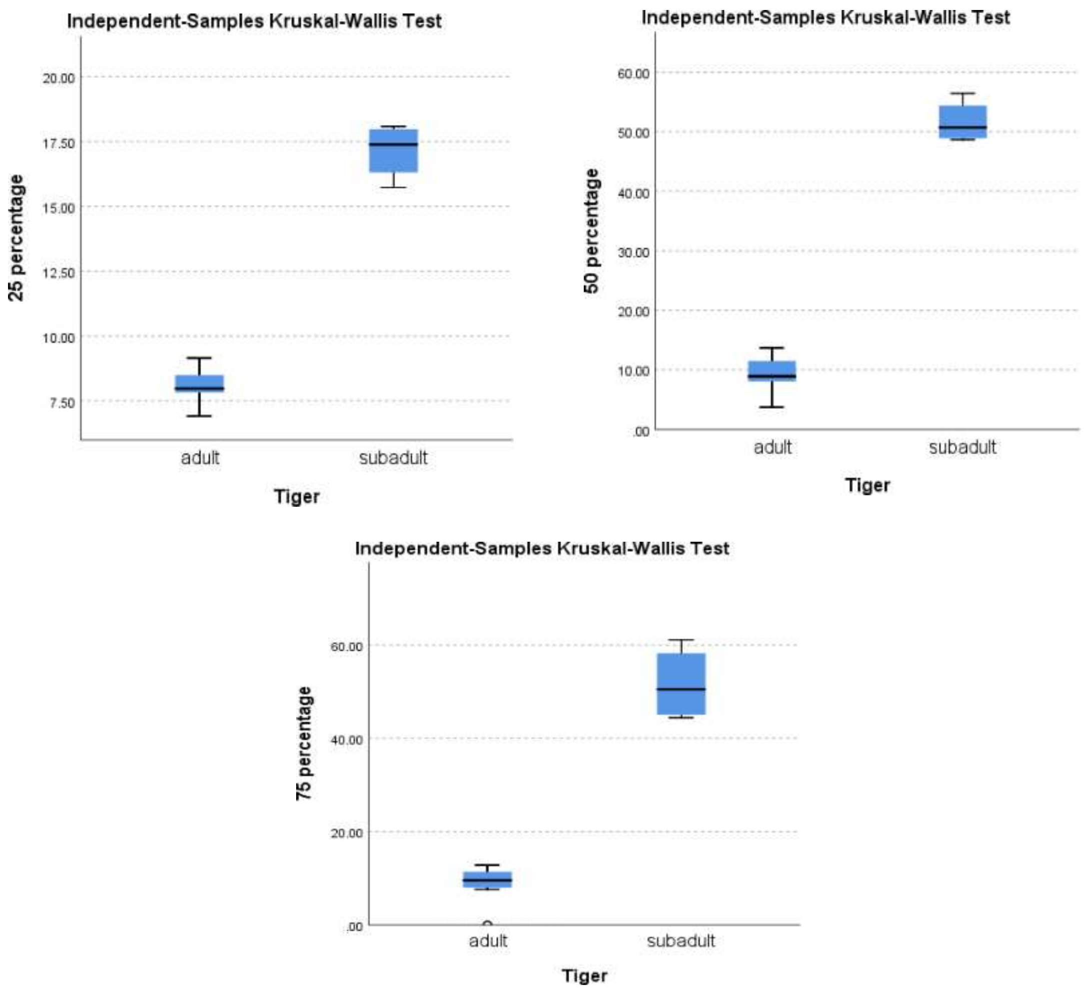


Fig 4.8. Boxplot of Pulp distribution across the two age groups of Tiger at 25%, 50 and 75%.

Table 4.9. Hypothesis Test Summary of Kruskal Wallis test

	Null Hypothesis	Sig.
1	The distribution of 25% is the same across categories of the Leopard and Tiger Age groups.	.000
2	The distribution of 50 per cent is the same across Leopard and Tiger Age categories.	.000
3	The distribution of 75% is the same across categories of Leopard and Tiger Age groups.	.000

The hypothesis test was carried out for the pulp cavity using the Kruskal-Wallis non-parametric test and the obtained significant value for each group at five Leopard categories was 0.000. Hence the null hypothesis is rejected and the alternative hypothesis "The pulp variation between the Leopard categories" was proved (Fig. 4.7).

Table 4.10. Pair-wise comparison using Dunn's test for Leopard age

Sample1-Sample2	Test Statistic	Sig.
Super Adult-Prime Adult	10.917	.089
Super Adult-Adult	16.800	.011
Super Adult-Sub-Adult	27.700	.000
Super Adult-Cup	35.000	.000
Prime Adult-Adult	5.883	.216
Prime Adult-Sub-Adult	-16.783	.000
Prime Adult-Cup	24.083	.005
Adult-Sub-Adult	-10.900	.028
Adult-Cup	-18.200	.034
Sub- Adult-Cup	7.300	.396

The pairwise hypothetical test was carried out using Dunn's non-parametric test. The result showed no significance between Super adult and prime adult and between sub-adult and cub. As they are closely related to each other, they failed to show significance and the remaining parametric categories showed excellent significance with each other.

Tooth wear calculation was taken for all canines and the score was given based on TWI by Smith & Knight (1984). Age is directly proportional to the occurrence of tooth wear. The tooth wear index was observed for all individual canines at three trials and the result was interpreted.

Table 4.11. Tooth wear index (TWI) score for Leopard canine

LEOPARD								
Parameters	**Individuals**							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Rt upper canine	1	1	2	0	0	0	4	0
Rt upper canine	1	2	2	1	0	0	4	0
Rt lower canine	1	1	2	0	0	0	4	0
Rt lower canine	0	1	2	0	0	0	4	0

Table 4.12. Tooth wear index (TWI score) for Tiger canine

TIGER				
PARAMETERS	**Individuals**			
	1	**2**	**3**	**4**
RT UPPER CANINE	4	1	2	0
LT UPPER CANINE	3	1	1	0
RT LOWER CANINE	2	2	2	0
LT LOWER CAINE	2	2	1	0

Scanning Electron Microscopy provides a detailed microscopic anatomy of canine and claw, which provides an insight into the micro characters study and authenticates its originality in case of complicated samples or even from a piece of sample (Fig. 4.9 & 4.10).

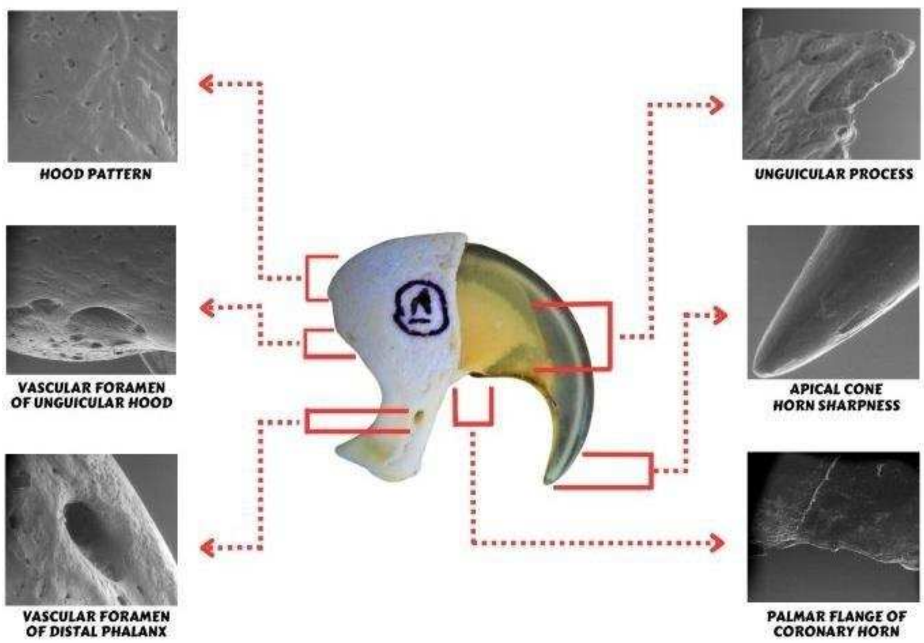


Figure 4.9. Scanning Electron Microscopic image of claw

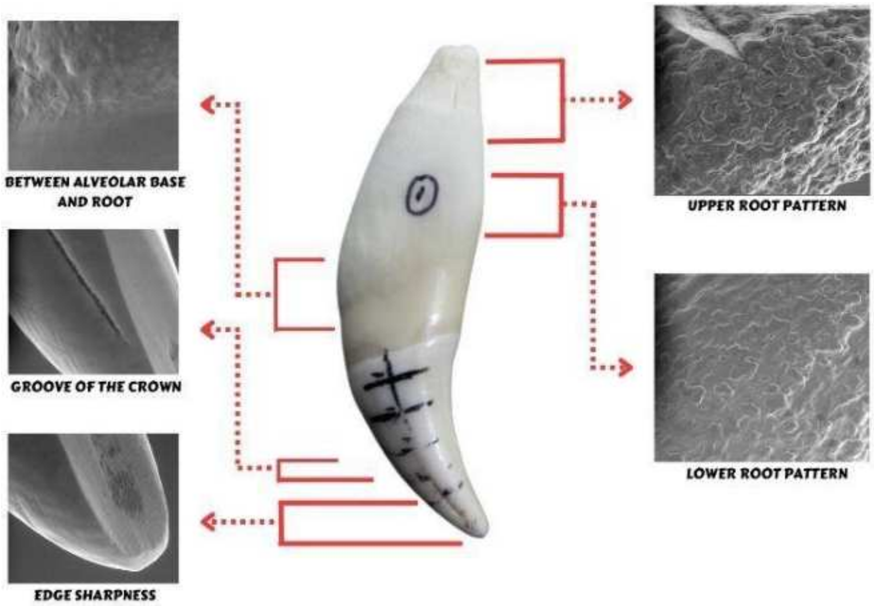


Figure 4.10. Scanning Electron Microscopic image of canine

5. Discussion

Conservation of Tiger and Leopards is vital for preserving biodiversity, ecological stability, and the cultural heritage associated with these iconic big cats. The decline in prey abundance and hunting by local people and its habitat destruction make large carnivores the most endangered species (Smith et al., 1999; Weins, 1993; Brodie, 2009). Efforts such as habitat protection, anti-poaching measures, community engagement, and international cooperation are crucial for their long-term survival.

Cats are the most specialized predators (Ewer 1973). These cats are digitigrade, with five toes on the forefeet but only four on the hind feet. Claws are retractile (Eaton 1974), and climbing capacitated (Harrison 1968). The most specialized predators (Ewer, 1973) and digitigrade, with five toes on the forefeet but only four on the hind feet. Claws are retractile (Eaton1974), and climbing capacitated (Harrison 1968).

It is important to note that these characteristics may vary slightly within the species due to factors such as age, gender, and subspecies. Therefore, a comprehensive examination of multiple features, including the claws, canines and body size, can help in accurately distinguishing between Tigers and Leopards.

The canine teeth, by their structure, shape, and position in the jaws, are well adapted to being wedged between the vertebrae of a prey animal's neck (Leyhausen, 1965 & 1979). Visual examination of Tiger and Leopard canines and claws can provide deep insights into their distinguishing characteristics. Leopard canines are relatively shorter and slenderer compared to those of Tigers. Tiger canines are larger and more robust than Leopards. They are longer and thicker, designed to deliver powerful bites to subdue their prey. The mesial side of the maxillary and mandibular canine have two grooves and the distal side has one groove in all feline families. The root length of the canine is comparatively longer than the crown. Claw features of the feline family are very similar to each other. Hence, no significant difference was seen.

Felines kill the prey and slice through its flesh (Ewer 1973). Seizing the prey is primarily a function of the forelimbs and biting is specialized for killing (Leyhausen 1965,1979). Nineteen different parameters of manual and software measurements were taken for canine and claw and the mean value showed a significant difference between Tiger and Leopard. Claw similarities are overcome by taking the angulation of the claw and a significant difference was seen between Tiger and Leopard, The result showed lower angulation and higher radius in Tiger than in Leopard.

Teethwereoftenusedasageindicatorsforbothbiologicalandforensicissues. Previous studies evidenced that the closure of the pulp chamber in big cats increases with age in canine teeth (Cameriere *et al.*, 2007). X-ray measurement on Claw shows varied keratin content in different individuals. Observation of the presence of an unguicular process inside the nail can be evidence of its originality. The presence of a pulp cavity is a key factor for its originality. The observation showed a wider pulp chamber in juvenile big cats, which gets narrowed due to dentine formation in adults. Age estimation was done by performance of pulp chamber percentage and concluded that the pulp chamber percentage was higher in cubs than adults.

It was also worth mentioning that dental examinations and analyses can provide a more comprehensive evaluation of tooth wear and dental health in these big cat species. The presence of tooth wear is higher in adult animals. Tooth wear in Tigers and Leopards can provide valuable information about their feeding behaviours, age, and overall dental health. A comparative study of tooth wear and age estimation between the species (Tiger/Leopard) provides clear and supportive data for X-ray analysis of age estimation. Several criteria have been used in existing indices, including depth of wear, nature of tooth wear and loss of surface contour (Hooper *et al.*, 2004). The index used in this study was designed to be simple to use but at the same time to allow a degree of sensitivity in the values recorded.

The study of Homberger *et al.* (2009) and De Laurier *et al.* (2006) confirms the determining role and surface profile of canine and claw of domestic cat. Scanning Electron Microscopic analysis of representative samples shows that the microporation and patter formation of the root part of the canine varied from species to species and age to age. Complicated samples that are difficult to confirm by the above methods will subjected to SEM analysis. Even a piece of sample from the seizure can also be used for the SEM analysis.

6.SUMMARY & CONCLUSION

- The present study focused on a novel attempt at species identification and age estimation of forensic seizures of canine and claw samples of Tiger and Leopard.
- Canine and claw samples obtained from post-mortem were processed and subjected to analysis. Various parametric comparative studies were conducted and the results were interpreted
- Visual examination of the canine and claw was crucial, which provided preliminary confirmation of the family to which the canine belonged.
- Morphometric measurements were carried out with 19 different aspects in both canine and claw, which provided a detailed comparative study on species-level identification.
- X-ray analysis was done to delineate age estimation and keratin density variation between Tiger and Leopard. The radiographic analysis provides insight into the age estimation, which is similar to the feline family.
- Tooth wear examination provided a deep understanding of age estimation and its ecological relativeness towards its habitat.
- Differentiation and identification of Royal Bengal Tiger (*Panthera tigris tigris*) and Indian Leopard (*Panthera pardus fusca*) claws and canine is a challenging task in wildlife forensics due to similarity in their morphology and anatomy and as each digit of felids has varied claw size.
- The present study focused on Species identification using the claw and canine of Tiger and Leopard by various morphometric methods. Interpretation of the result is given by a comparative study of proposed methodologies.
- Scanning Electron Microscopy provided a detailed anatomy of the microscopic structure of canine and claw, which provides an insight into character study and authenticated its originality in case of complicated samples or even from a piece of sample.

REFERENCES

Bhattarai, B. P., & Kindlmann, P. (2012). Interactions between Bengal tiger (*Panthera tigris*) and leopard (*Panthera pardus*): Implications for their conservation.Biodiversity andConservation, 21,2075-2094.

Cameriere, R., Ferrante, L., Liversidge, H.M., Prieto, J.L., & Brkic,H. (2008). Accuracy of age estimation in children using radiograph of developing teeth. Forensic scienceinternational,176(2-3), 173-177.

Cleuren, S. G., Hocking, D. P., & Evans, A. R. (2021). Fang evolution in venomous snakes: adaptation of 3D tooth shape to the biomechanical properties of their prey. Evolution,75(6),1377-1394.

DeLaurier, A., Boyde, A., Horton, M. A., & Price, J. S. (2006). Analysis of the surface characteristics and mineralization status of feline teeth using scanning electron microscopy. Journal of anatomy, 209(5), 655-669.

Eaton, R.L. (1974). The cheetah: the biology, ecology, and behavior of an endangered species.

Gonyea, W., & Ashworth, R. (1975). The form and function of retractile claws in the Felidae and other representative carnivorans. Journal of Morphology, 145(2),229-238.

Harrison, D. L. (1968). The Mammals of Arabia, Vol II Carnivora, Artiodactyla, Hyracoidea.pub. Ernest Benn Ltd., London, 349-365.

Homberger, D.G., Ham, K., Ogunbakin, T., Bonin, J.A., Hopkins, B.A., Osborn, M.L., & Bragulla, H.H. (2009). The structure of the cornified claw sheath in the domesticated cat (*Felis catus*): implications for the claw- shedding mechanism and the evolution of cornified digital end organs. Journal of Anatomy, 214(4), 620-643.

Hooper, S.M., Meredith, N., & Jagger, D.C. (2004). The development of a new index for measurement of incisal/occlusal tooth wear. Journal of Oral Rehabilitation, 31(3),206-212.

Kim, S.E., Arzi, B., Garcia, T.C., & Verstraete, F.J. (2018). Bite forces and their measurement in dogs and cats. Frontiers in Veterinary Science, 5, 76.

Lowry, M. S., & Folk, R. L. (1990). Sex determination of the California sea lion (*Zalophus californianus californianus*) from canine teeth. Marine Mammal Science, 6(1), 25-31.

Martin, L. D. (1980). Functional morphology and the evolution of cats.

Mweetwa, T., Christianson, D., Becker, M., Creel, S., Rosenblatt, E., Merkle, J.& Simpamba, T. (2018). Quantifying lion (*Panthera leo*) demographic response following a three-year moratorium on trophy hunting. PloS one, 13(5), e0197030.

Nadarajan, A., Prithiviraj, S., Kumari, V., Rangasamy, K., & Jana, D. (2022). Illegal trade of canines: Identifying suspected samples of Tigers (*Panthera tigris*) and Bears (*Melursus ursinus*).

Park, K., Ahn, J., Kang, S., Lee, E., Kim, S., Park, S. & Seo, K. (2014). Determining the age of cats by pulp cavity/tooth width ratio using dental radiography. Journal of Veterinary Science, 15(4), 557-561.

Saber, A.S.M., Cacec, T., Gummow, B., & Johns, K. (2016). Morphometric studies on the skull of the Australian domestic cat (*F. catus*) and its clinical implications for regional anesthesia. Journal of Veterinary Anatomy, 9(1),1-24.

Sethi, S., Goyal, S.P., & Choudhary, A.N. (2019). Poaching, illegal wildlife trade and bushmeat hunting in India and South Asia. International wildlife management: Conservation challenges in a changing world, 157.

Sharma, C.P., Sharma, S., Sharma, V., & Singh, R. (2019). Rapid and non-destructive identification of claws using ATR-FTIR spectroscopy–A novel approach in wildlife forensics. Science & Justice, 59(6), 622-629.

Sharma, V., Sharma, C.P., Kumar, V.P., & Goyal, S.P. (2016). Pioneer identification of fake tiger claws using morphometric and DNA-based analysis in wildlife forensics in India. Forensic science international, 266,226-233.

Smuts, G.L., Anderson, J.L., & Austin, J.C. (1978). Age determination of the African lion (*Panthera leo*). Journal of Zoology, 185(1), 115-146.

Stander, P. E. (1997). Field age determination of leopards by tooth wear. African Journal of Ecology, 35(2), 156-161.

White, P.A., Ikanda, D., Ferrante, L., Chardonnet, P., Mesochina, P., & Cameriere, R. (2017). Correction: Age estimation of African Lions *Panthera leo* by ratio of tooth areas. Plos one, 12(10), e0187003.

Kuffel, A., Gray, A., & Daeid, N.N. 2021. Impact of metal ions on PCR inhibition and RT-PCR efficiency. Int J Legal Med., 135(1):63-72.

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