TAMIL NADU FOREST DEPARTMENT
ADVANCED INSTITUTE FOR WILDLIFE CONSERVATION
(RESEARCH, TRAINING & EDUCATION)

Annual Plan of Operations (APO) 2020-21

RESEARCH THEME 1:

APPLICATION OF FORENSIC SCIENCE IN WILDLIFE CRIME INVESTIGATIONS AND IN ENHANCEMENT OF SPECIES CONSERVATION

DEVELOPMENT OF MORPHOLOGY REFERRAL REPOSITORY OF ARTICLES IN WILDLIFE TRADE.

PROJECT COMPLETION REPORT

Tamil Nadu Forest Department

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

Project Completion Report
On

‘DEVELOPMENT OF MORPHOLOGY REFERRAL REPOSITORY OF ARTICLES IN WILDLIFE TRADE’

[Research theme 1: Application of forensic science in wildlife crime investigations and in enhancement of species conservation]

Annual Plan of Operations (APO) Project
(2020-21)

Submitted by
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August 2023

The content appearing in this report is the outcome of one year research that was funded by Tamil Nadu Government Forest Department under Annual Plan of Operations.

Published by Advanced Institute for Wildlife Conservation (AIWC) (Research, Training & Education), Vandalur, Chennai – 600 048, Tamil Nadu, India.

Printed by:

Office of the PCCF & Director: 044-29372331.

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Website: https://www.aiwc.res.in

ACKNOWLEDGEMENTS

I respectfully express my deepest gratitude to **Thiru. A. Udhayan, IFS**, PCCF & Director, AIWC, **Thiru. Nihar Ranjan, IFS**, Additional Director, AIWC, **Thiru. M.G. Ganesan,** Deputy Director (Technical), AIWC, **Dr. R. Kanchana, IFS,** Former Deputy Director (Administration), AIWC, for the endless support and encouragement during the project's tenure. I want to express my earnest gratitude to the Tamil Nadu Forest Department for funding support for completing this APO project (2020-21) successfully. I also wish to express my sincere thanks to all other officials & staff of AIWC for their support, especially **Dr. D. Vasanthakumari,** FVAS, the lab-in-charge, JRFs and Lab Assistant of Morphometry Lab, AIWC. I am also grateful to all officials & staff of Arignar Anna Zoological Park, Vandalur, Chennai, for providing reference specimens.

CONTENTS

MORPHOMETRY ANALYSIS

OBJECTIVE OF THE STUDY

- To Develop bio-physical referral repository of bones, tusks, antlers, horns, skins, scales etc., at Morphometry lab of AIWC.

The present study focused exclusively on the morphometric reference repositories of sambar deer and spotted deer antlers, as well as the canines and incisors of hippopotamus

1. Identification of Deer Antlers

Background of the study

Antlers are a distinguishing feature of deer, and identifying the particular species can help wildlife forensics investigators determine the origin of the antlers and potentially identify any illegal hunting or trafficking. Antlers of Sambar deer (*Rusa unicolor*) and Spotted deer (*Axis axis*) are majorly trafficked in India. Moreover, most of the cases received in AIWC for species identification are antlers of Spotted and Sambar deer.

Introduction

Antlers are unique characteristics of males of the family Cervidae and fully regenerate each year in relation to their sexual cycles (Berger *et al.,* 1995; Bubenik, 1968). It is mostly a secondary sexual characteristic of males except in genera *Rangifer*, where it is found in both sexes (Berger *et al.,* 1995). In males, it is principally used as weapons in intra-specific fighting or possibly as organs displaying strength (Chapman, 1881). These are bony outgrowth of the cranial frontal bones and differ from the keratinous horn of the Bovidae in those horns are not cast and re-grown annually. Bubenik (1968) reported that in Bovidae, Cervidae and Giraffidae, the horns and antlers were non-homologous in their development. Goss (2012) added that the most active zone of cell division is at the base of the horn, whereas in antlers, growth occurs at the tip. Antlers are branched and solid (Goss, 2012), whereas horns are un-branched and hollow.

Antlers have been studied in detail on taxonomical, morphological, elemental, endocrinal and genetically and well documented by various scientists worldwide (Li, 2013; Nieto-Diaz *et al*., 2012). Bubenik (1969) reported that antlers were useful in visual characterization, distinguishing sex, individual and species. Antler characteristics have been used to determine the age class of male swamp deer and other deer species through the appearance of small bumps, spikes, length of the antler and distance from the pedicle to the tip.

The present study attempted to establish different parameters for the identification of antlers of spotted deer and sambar deer using morphometric analysis. Antler characterization based on measurements for identifying deer species explained by Yudha *et al.* (2019) was used as a reference for the present study.

Materials and Methods

The study utilized known reference antlers of sambar deer and spotted deer accessible at Morphology Lab, AIWC, sourced from Arignar Anna Zoological Park (AAZP), Vandalur. In total, 17 antlers of sambar deer and 11 antlers of spotted deer were employed. Different parameters of measurements, such as total length, BeHt (distance of bez branching to the base), BrHt (distance of brow branching to the base), *Bez* angle, Brow angle, the circumference of the main beam of the 3rd segment, the circumference of the base, etc., were measured and recorded. Most measurements were done using a measuring tape and *Bez* angle; the Brow angle was measured using a protractor.

Figure 1. Measuring the antler using a measuring tape

Results

Reference antlers of Spotted deer and Sambar deer.

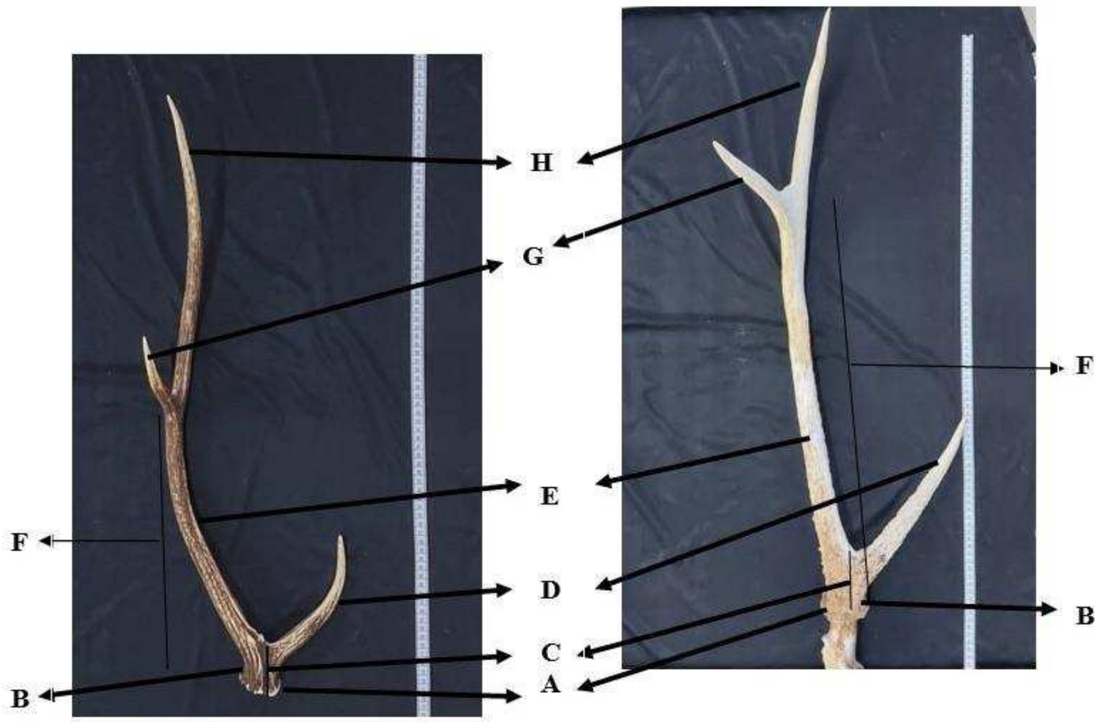

Figure 2.1. Reference antler of Spotted Deer at AIWC

Figure 2.2. Reference antler of Sambar Deer at AIWC

A- Blurr, B- Base, C- BrHt (Distance of brow branching to the base), D- Brow tine, E- Main beam 2nd Segment, F- BeHt (Distance to bez branching to the base), G- Bez, H- Main Beam 3rd segment (Yadha *et al.*, 2019)

Table1. Diagnostic characters of the antlers (Yudha *et al.*, 2019)

Sl. No.	Species	Characters
1.	**Sambar deer**	*Bez* grows inward
		Bez angle peripheral to main beam 3rd segment
		Brow angle intermediate
		High BrHt
		Extensions on branching (Especially on brow, forms are a connecting brow and main beam)
2.	**Spotted deer**	Relatively widely curved (Not significant in some individuals, especially young ones).
		BrHt intermediate (3–4 cm).
		Bez angle intermediate.
		*Bez*growsinward.
		Low *bez* angle (forms U-shape).

Comprehensive analysis of antler measurements at AIWC Morphometry Lab

A total of 17 known antlers of sambar deer and 11 known antlers of spotted deer available in AIWC Morphometry lab were used for the study. Different measurement based on different parts of the antler is given below:

Table 2. Measurement of Antlers of Sambar deer

Sl. No.	Total length (cm)	BeHt (cm)	BrHt (cm)	*Bez* angle	Brow angle	Circumference of main beam 3rd segment (cm)	Circumference of base (cm)
1	44	14	6	40^0	40^0	9.5	16
2	46	15	6	50^0	40^0	9.5	14.5
3	51	14	6.5	40^0	50^0	10	14.3
4	52	14	9	40^0	45^0	10.5	15
5	52	15.5	5	40^0	40^0	10	17
6	54	38.5	5	30^0	40^0	12	17.5
7	54	46.5	7.5	45^0	40^0	10.5	16
8	56	14	5	40^0	40^0	10.5	18
9	56	39.5	6.5	40^0	40^0	11	16
10	57	17.5	7	50^0	40^0	12	18.5
11	63	45	6	35^0	30^0	12.5	19
12	65	50	9.5	45^0	40^0	12	19.5
13	69	53	8.5	45^0	40.5^0	13.5	19
14	71	22	11	50^0	50^0	13	21
15	71	18	7.5	55^0	50^0	13.2	18
16	72	54	7	45^0	45^0	12	18.5
17	75	48	8	45^0	40^0	13.5	18

Table 3. Measurement of Antlers of Spotted Deer

Sl. No.	Total length (cm)	BeHt (cm)	BrHt (cm)	*Bez* angle	Brow angle	Circumference of main beam 3rd Segment (cm)	Circumference of base (cm)
1.	28	17.5	3.5	50^0	50^0	5	9.5
2.	67	33.5	5	40^0	50^0	8	13.5
3.	68	37	4.5	40^0	50^0	9	13.5
4.	69	41.5	4.5	40^0	50^0	7.8	13
5.	70	35	4.5	40^0	50^0	8	15
6.	72	34	5	40^0	50^0	14	8
7.	72	32	5	40^0	50^0	13	7.5
8.	73	34	5	40^0	50^0	13	8
9.	74	47	4	40^0	50^0	7.5	13
10.	75	38.5	4	40^0	50^0	7.5	13
11.	77	36	4	40^0	50^0	9	14

Table 4. Measurement Ranges for Antler Distinction

Sl. No	Species	Total length of antler	BrHt (distance of brow branching to the base)	BeHt (distance to *bez* Branching to the base)
1.	Sambar deer antler-reference	Range between 44-75cm (measurement of 17 reference antlers of Sambar in AIWC Morphometry lab)	Range between 5-11cm (measurement of 17 reference antlers of Sambar in AIWC Morphometry lab)	Range between 17.5-47 cm (measurement of 17 reference antlers of Sambar in AIWC Morphometry lab)
2.	Spotted deer antler-reference	Range between 28-77 cm (measurement of 10 reference antlers of Spotted deer in AIWC Morphometry lab)	Range between 3.5-5 cm (measurement of 10 reference antlers of Spotted deer in AIWC Morphometry lab)	Range between 29-39 cm (measurement of 10 reference antlers of Spotted deer in AIWC Morphometry lab)

Table 5. Antler Circumference measurements

Species	Circumference of main beam 3rd segment (cm)	Circumference of base (cm)
Sambar deer	9.5 to 13.5 cm	14-21cm
Spotted deer	5-14cm	8-14cm

Considering different measurement parameters for the antlers of Sambar deer and Spotted deer could help distinguish the species to solve wildlife crimes if the antlers retain their actual morphological structure. The total length of antlers of Sambar deer ranges from 44-75 cm and for Spotted deer ranges between 28-77cm. BrHt (distance of brow branching to the base) ranges between 5-11 cm and 3.5-5 cm for sambar deer and spotted deer, respectively. While considering the BeHt measurements, 14-54 cm and 17.5 to 47 cm for sambar deer and spotted deer correspondingly. *Bez* angle of 17 sambar deer antlers ranges from 35^o to $50^{o.}$ But a more repeated *Bez* angle was observed between 40^o to 45^o. On the other hand, *Bez* angle of 11 spotted deer antlers showed 40^o. Moreover, the brow angle of sambar deer was observed between 30^0-50^0, but more probability of 40^0-45^0. Nevertheless, a brow angle of 50^0 was observed in all antlers of spotted deer. Circumference of main beam of 3rd segment ranged from 9.5-13.5 cm and 5-14 cm were observed in sambar and spotted deer respectively. Circumference of base with 14-21 cm and 8-14 cm was observed in the antlers of sambar and spotted deer, respectively.

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Identification of Hippopotamus ivory

Introduction

Hippopotamus (*Hippopotamus amphibious*) have very large curved canines in front, slightly smaller incisors just behind the canines and molars in the back. The molars and incisors are used for chewing and it is speculated that once the molars lose their grinding effect due to wear the hippopotamus dies (Laws, 1968). The canines appear to be used for defensive purposes and fighting for dominance. The structure is similar to other mammalian teeth, with dentin as the largest fraction surrounded by a thin layer, enamel, or cementum. The interstitial zone is also known as the pulp cavity. The enamel of the hippo tooth has a density of 1.7 g/cm^3, had a compressive elastic modulus (enamel and dentin) of ~2.6 GPa and a hardness of 1.7 GPa for enamel and 0.3 GPa for dentin.

Tooth and tusk ivory can be carved into various shapes and objects. A few examples of tusks and teeth of elephants and hippopotami were carved as small statuary, netsukes, jewellery, flat ware handles, furniture inlays, and piano keys.

Materials and Methods

One pair of incisor and canine of suspected Hippopotomus (Fig. 3) were received in AIWC Morphometry Lab for analysis and marked as AIWC/CWF/10/21/0024 (c). Pair of incisor and canine was again marked as A & B. All samples were undergone for cross sectional study. Cross-section images and characteristics for Hippopotomus incisor and canine were referred from Locke (2008) and Chen *et al.* (2008) (Fig 4 & Fig 5).

Figure 3. Image of sample (case no. AIWC/CWF/10/21/0024(c))

Results

Figure 4. Reference image of the cross section of canine of Hippopotamus (Locke, 2008)

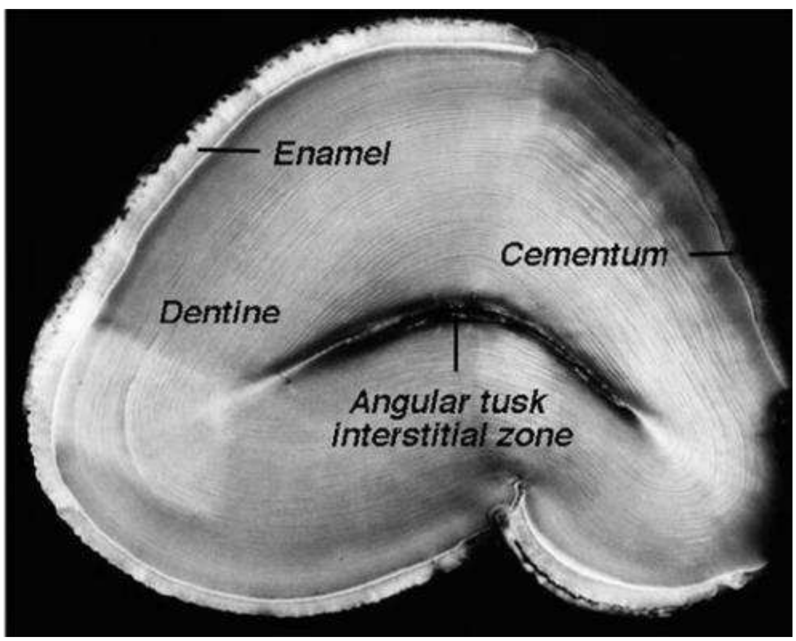

Figure 5. Reference image of the cross-section of canine of Hippopotamus (Chen *et al.,* 2008)

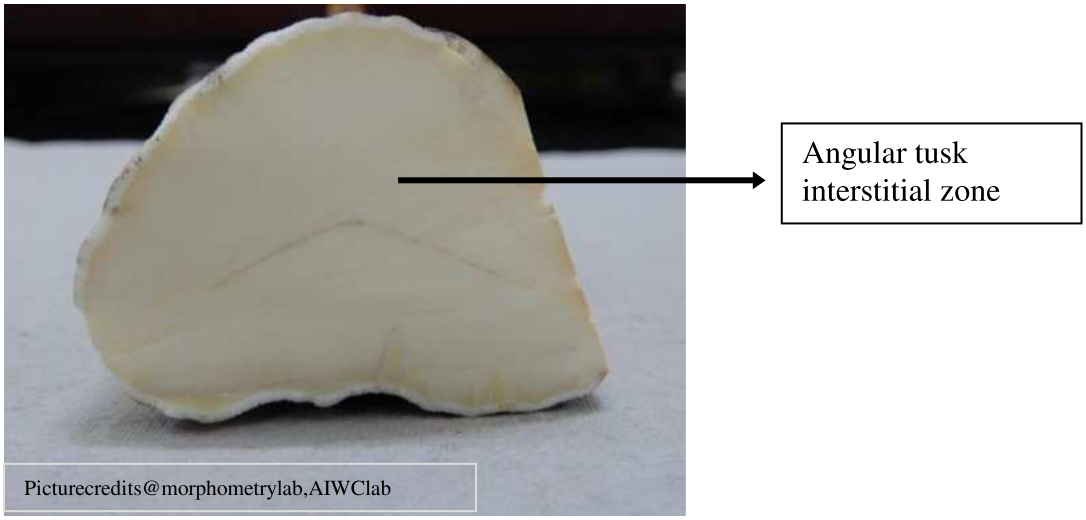

Figure 6. Cross section of Case sample Canine: AIWC/CWF/10/21/0024(c) A

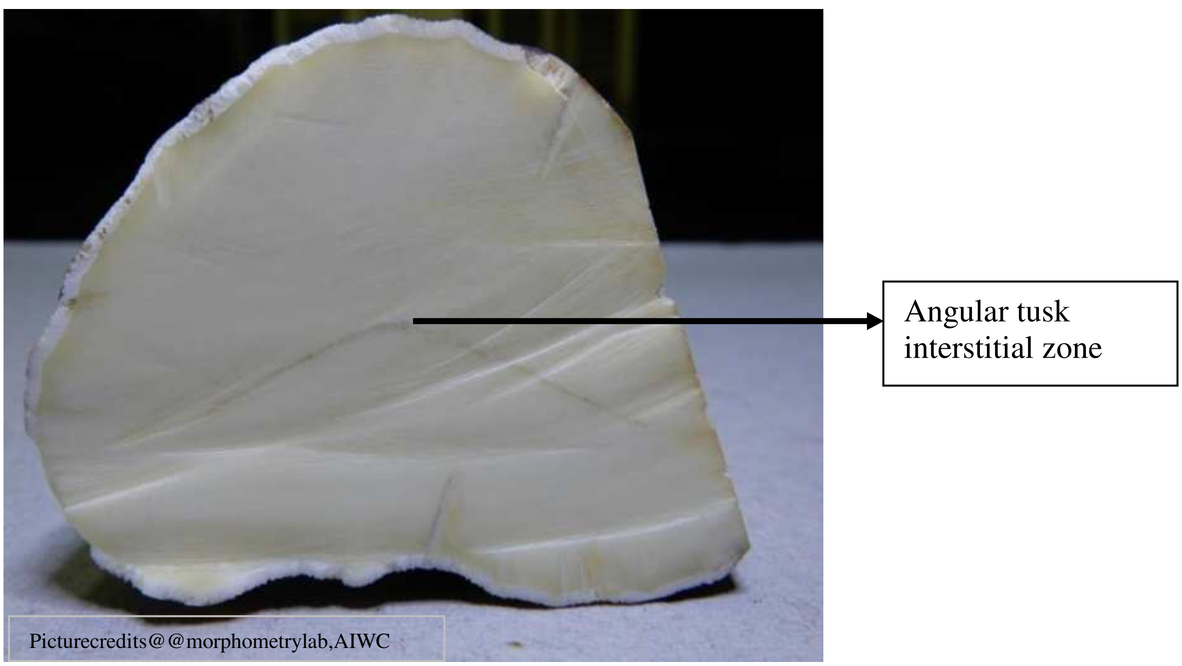

Figure 7. Cross section of Case sample AIWC/CWF/10/21/0024(c) B.

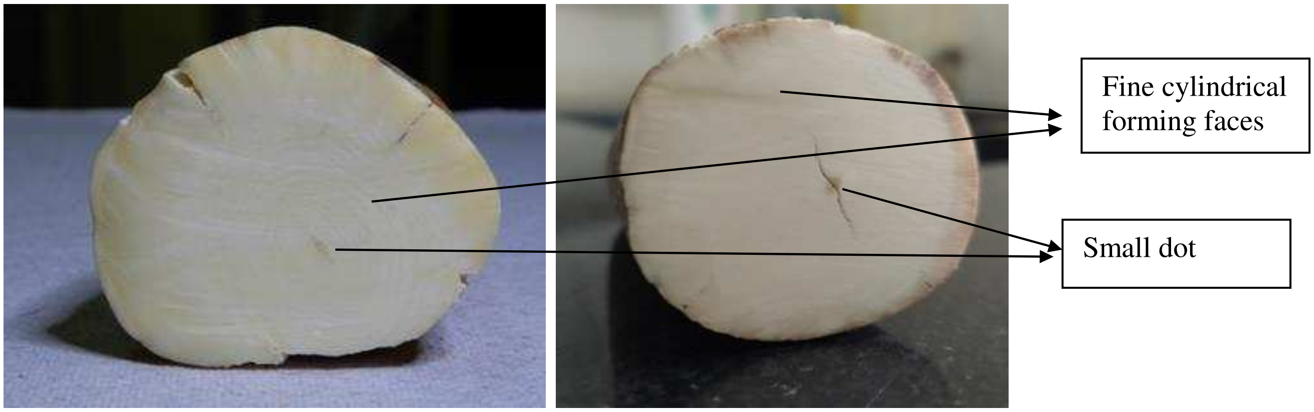

Figure 8.1. Cross section of Case sample Incisor Incisor-AIWC/CWF/10/21/0024(c)A

Figure 8.2. Cross section of Case AIWC/CWF/10/21/0024(c)B

(Picture credits@morphometrylab,AIWC)

Upper and lower canines and incisors are the most common sources of hippo ivory (Krzyszkowska, 1988). Each type of tooth has a distinctive gross morphology. The hippo's curved upper canines are oval to rounded in cross-section. In the unprocessed state, a deep longitudinal in dentation extends for the tooth's length on the inner surface of the curve. A broad longitudinal band of enamel covers approximately two-thirds of the surface area of the tooth. This enamel band is frequently removed during the carving process. The surface not coated with enamel displays a very thin layer of cementum. This may also be removed during processing. The upper canine's interstitial zone is a curved or broadly arched line. The lower canines are the hippo's largest teeth. They are strongly curved. In cross-section, the lower canines are triangular. Raw lower canines will display a faint longitudinal indentation, a marked rippling of the surface and an approximate two-thirds coverage with enamel. Like the upper canine, a thin layer of cementum exists in the areas not covered with enamel. And, as with the upper canines, these surface characteristics are frequently removed during processing (Edgard & Mary-Jacque, 1992). The interstitial zone in the lower canine is a broadly arched line. Hippo incisors can be described as peg shaped. Enamel is found on the tooth crown. The center of the tooth in cross-section shows a small dot.

Most hippopotamus ivory, such as that in the canines had wide (100–300 lm) easily resolvable bands that were continuous with the faint growth layers described earlier when both were present. These canines were triangular in cross section with Y-shaped forming faces (angular tusk intestinal zone) (Fig 6 & Fig 7) unlike the incisors that had circular cross sections and cylindrical forming faces. The wide growth bands from canines differed from the faint layers of incisors in several respects. 1) The wide bands met (or fused) with one another in both transverse and radial profiles. 2) They had more of the glistening white component indicating calcification. 3) They lay at an angle of 108^0–258^0 to the forming face (Locke, 2008).

This particular Y shape (Fig 8.1 & Fig 8.2) in canines and cylindrical forming faces & small dot in the centre in incisor (Fig 8.1 & Fig 8.2) were observed in both pair of canines and incisors received in morphometry lab, AIWC and confirming their origin as being from a Hippopotamus.

Conclusion

The illegal wildlife trade significantly threatens many extant wild animal species worldwide. To combat the illicit wildlife trade, species-level identification of seized wildlife parts or products is required for the legal prosecution of the accused/s in a court of law. The morphometric method plays a paramount importance in the field of wildlife forensics. It is non-invasive and does not require destroying the specimen being examined. This is especially important in wildlife forensics, where preserving evidence for legal purposes is essential. It allows investigators together valuable information without compromising the sample's integrity.

Morphometric analysis can yield rapid results, making it particularly useful when quick assessments are needed, such as at customs checkpoints, in wildlife trafficking investigations, or in wildlife crime scenes. Timely identification can lead to more effective law enforcement actions. Wildlife articles such as ivory, skin, bones, antlers, scales *etc*. can be identified using this approach. This approach could be more feasible only the wildlife article maintains its actual shape. Moreover, it can complement genetic analysis by providing a preliminary identification of specimens. While genetic analysis offers the highest level of precision, it is often more time-consuming and costly. Morphometric analysis can help prioritize which samples should undergo further genetic testing. It grounded in scientific principles and statistical analysis. This adds credibility to the identification process and strengthens the evidentiary value of findings in a court of law. It provides a solid scientific basis for legal actions against wildlife traffickers. Morphometric analysis provides a highly accurate means of distinguishing between different deer species, enabling forensic experts to determine precisely which species the antlers Belong to. This is crucial for enforcing wildlife protection laws and ensuring the appropriate legal actions are taken.

In conclusion, the morphometric methods for wildlife article are a powerful tool in wildlife forensics. Its accuracy, non-invasive nature, speed, scientific credibility, and role in supporting conservation efforts make it indispensable in combating illegal wildlife trade and ensuring the conservation of wildlife.

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