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Integrated morphological and morphometric approaches for the forensic study of seized claw samples

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Abstract
In recent times, there has been a growing number of reports involving the confiscation of claws and canine teeth, leading to increased requests for forensic examination to determine species identity (Nadarajan *et al.*, 2022). Determining species through DNA extraction and amplification from claw samples presents difficulties due to their high keratin content, which interferes with PCR processes (Kuffel *et al.*, 2021). Species determination from confiscated claws requires a comprehensive approach that incorporates morphological examination, radiographic analysis, morphometric measurements, and genetic testing (Driver, 2011). This multi-method strategy ensures reliable species identification, particularly when physical characteristics are unclear or deteriorated (Baker *et al.*, 2013; Ogden *et al.*, 2009). Such identification is crucial today given the escalating illegal wildlife trafficking that poses a significant danger to wild animal populations. The high market demand for wildlife products has also led to an increase in counterfeit items manufactured from domestic animal bones or hooves entering this illicit market (Sharma *et al.*, 2016). In this study, eight confiscated claw specimens underwent morphological analysis, revealing pale yellow coloration with pointed, curved tips. A distinctive thin curved ridge was visible along the entire curvature of the keratin covering. Radiographic examination showed varied density patterns, and morphometric data from this study were compared against established measurements for leopard and tiger claws documented by Sharma *et al.* (2016). DNA isolation from the seized claws was performed using the Qiagen QAamp® DNA mini Kit protocol. High-quality DNA was successfully obtained from six of the eight samples as confirmed by nanodrop analysis. Therefore, to effectively enforce the Wildlife (Protection) Act, 1972 of India and similar international legislation, it is crucial to combine morphological examination with DNA analysis. This integrated methodology enhances species identification accuracy and supports law enforcement efforts against illegal wildlife trade.

Keywords: Species identification, claws, DNA analysis, morphology, wildlife forensics, illegal trade

Introduction
Wildlife trade in India is a multidimensional phenomenon influenced by economic, cultural, social and environmental factors. This resulting in exploitation of wild animals and its parts by poaching and smuggling major threat for species to extinction (Saha R, 2025) [13]. The Wildlife (Protection) Amendment Act 2022, aims to strengthen the protection of endangered species like, tigers, leopards from schedule I and its body parts from illegal trade which remains a persistent concern. Even with increased surveillance and policing, the illegal trade goes on, due to connivance of crime syndicates and open access to the internet and borders, threatening the biodiversity of Southern India in a dangerous manner (Saha R, 2025, TRAFFIC India, 2024) [13]. Poachers are targeting wild Tigers and leopards for their body parts like skin, claws, canines as there is high demand in the illegal market. This resulting in habitat destruction and killing of felines species like tiger and Leopard. Feline claws are worn as to make pendants, charms against evil spirits, instilling courage in the wearer. Nowadays wildlife criminals making fake claws which are made from bone or horn or hoof or synthetic material giving them similar shape and size to meet high demand and earn more money. These fake items, often crafted and intended to deceive both buyers and law enforcement (Rajput *et al.* 2025) [12] These were frequently seized by enforcement agencies Chandewar NG. (2024) [3].

Seizer of claws constitute 3.8% of total articles related to wildlife crime received at Wildlife Institute of India, Dehradun for forensic examination (Sharma *et al.*, 2016) [14]. Recent years there is a increasing reports of claws and canine seizures, adversely impacting several significant species of mammals and increased demand for forensic inspection species identification (Nadarajan *et al.*, 2022) [8]. This study mainly focused on species identification from seized claws by morphology, x-ray imaging, morphometry and extracting DNA from these claws for further molecular studies. As extraction of DNA from seized biological sample is more critical stage in forensic genetic investigation studies (Ogden *et al.*, 2009) [9]. In the present study Qiagen QAamp® DNA mini Kit method is adopted and quality of DNA was assessed with the help of Nanodrop.

Materials and Methods

Source of Sample and cleaning

Eight seized claw sample without hood were submitted for species identification by forest officials of Hassan District (Karnataka) at Veterinary College, Hassan under KVAFSU, Bidar, Karnataka for species confirmation. The Seized samples are received with proper permission. Samples were cleaned in 70% ethanol and stored at room temperature. All the seized samples were free from claw hood or skin or hair attached to it (Fig. 1).

Radiographic imaging

The claws were subjected to radiographic imaging using a digital X-ray unit at Veterinary polyclinic. Radio density patterns were analysed and compared with images of known tiger and leopard claws.

Morphometric analysis

From all eight claws, two measurements were taken of each claw with the help of a digital Vernier calliper. 1) ac, distance from external coronary dermo-epidermal interface to the epidermis of the skin fold connecting the palmar flanges of the coronary horn. 2) bc, distance from claw tip to that base of claw. The ratio of two measurements (bc/ac) was calculated for each claw. The average value, Standard error (SE) and coefficient of variance (CV) where calculated and compared with reference values given by Sharma *et al.*, (2016) [14].

Extraction of DNA

Claw scrapings (5 mg) from the base of seized samples were collected with BP blade in a petri plate and made in to small pieces. These samples were allowed for Keratin digestion with 1M dithiothreitol (DTT) solution and Proteinase K solution overnight and one hour in hot water bath at 57 °C (Campos and Gilbert, 2012) [2]. The Qiagen QAamp® DNA mini Kit with minor modifications in the protocol was used for isolation. The quantity and quality of DNA was assessed using Nanodrop spectrophotometry.

Results and Discussion

In the present study, collected samples analysed with burn test which produced a distinctive odour due to formation of volatile sulphur compounds (Sharma *et al.* 2016) [14], whereas a pungent smell is produced if it's made of bone or plastic as reported by Rajput *et al.*, 2025 [12]. Thus, it confirms the its originality of nail. Upon physical examination it is observed that claws were light yellowish in colour with sharp pointed tip with curvature. There was a thin curved line all along the curvature of keratinous sheath is present and is the main character of the claw to confirm its authenticity (Fig. 1) similar results were reported by Thunnisa, M. and Rajkumar, P., (2021) [15]. The unique keratin layering patterns observed in real carnivore claws due to their evolutionary adaptation to predation and climbing and are not replicated (Ethier *et al.*, 2010) [6]. Similar observations were also reported by Sharma *et al.* (2016) [14] and Pointed tip of claw and less worn shows that they are of young age group, this is confirmed by (Rajkumar *et al.*, 2021) [15] studies.

Fig 1: Images of seized claws, presence of thin curved lines along the curvature of claw.

X-ray image analysis

Radiological analysis of claws, such as age, species identification, and credibility of the sample, is used for various studies (White *et al.*, 2016) [16]. In the present study x-ray images where taken and heterogeneous radio density patterns were observed (Fig.2) and were analysed, compared with images of known tiger and leopard claws given by Sharma *et al.*, (2016) [14] and Pavithra *et al.*, (2025) [11].

Fig 2: Radiographic image of the seized claws.

Morphometric analysis

The morphometric measurements contribute to species identification and provide insights into the ecological roles (Parvej *et al.* 2014) [10]. In the present study claws were morphometrically studied using a Vernier calliper. Two lengths were recorded. At the base of the claw as (ac) and the length of the claw base to the tip of the claw (bc) in mm. The observations are the mean and standard error (SE) of claws 17.58±0.22(4%) mm in length and 14.03±0.36 (7%) mm in width. Their ratio is 1.25±0.04 (8%). The values in parentheses are the coefficient of variance. Present observations are compared with reference data given by Sharma *et al.*, 2016 [14]. Data of measurements of the tiger, leopard and seized claws are presented in table 1. There were significant variations in each in claw measurement within the group and in between groups. This may be because seized samples might have been altered while making articles, especially at the base (Fig. 3). There will be variation in the claws of forelimbs, which are longer than the hind limbs (Pavithra *et al.*, 2025) [11]. Leopards are opportunistic carnivores and prefer medium sized ungulate small birds, mammals and reptiles unlike tigers (Chaudhary *et al.*, 2023) [4]. Differentiation claws is a challenging task in wildlife forensics due to similarity in their morphology and anatomy and as each digit has varied claw size (Thunnisa and Rajkumar, 2021) [15]

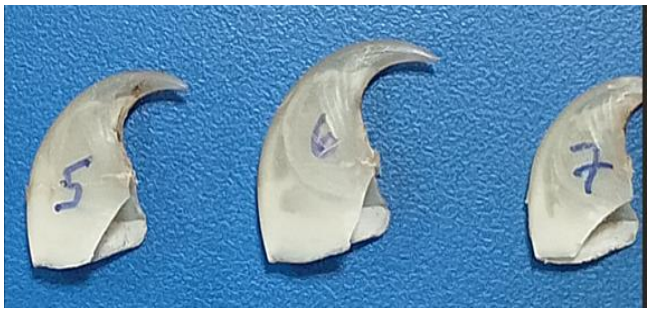

Fig 3: Seized claws damaged at base presented for species identification

Table 1: Claw measurements (mean ± SE) of Leopard, tiger and seized claws. Reference values by Sharma *et al.*, (2016) [14].

Claws	Number	Claw measurements (mean ± SE)		
		ac at base of claw (in mm)	bc length from base to tip (in mm)	bc/ac
Leopard	49	16.55±550.26 (11%)	15.45±45 (12%)	0.94±0.01 (10%)
Tiger	23	22.56±0.64 (14%)	22.64±0.50 (11%)	1.01±0.02 (8%)
Seized sample of present study	8	14.03±0.36 (7%)	17.58±0.22 (4%)	1.25±0.04 (8)

Extraction of DNA

For accurate identification of biological specimens like claws in wildlife crime is critical. In the present study for DNA extraction from claw sample were kept for enzymatic action in 1MM DTT solution overnight and 1 hours at 57°C. DNA is extracted from the claws with Qiagen QAamp® DNA mini Kit method. The quantity of DNA extracted ranged from 4.4 ng/µL to 31.2 ng/µL and purity evaluated at A260/A280 is 1.77 in Nanodrop spectrophotometry. In the present study we could get DNA from only 75% of total samples. This study shows that higher quantity of sample will yield good quantity of DNA however purity is not affected by initial quantity of a sample.

Conclusion

Identification of the species from seized material is important for the effective implementing wildlife protection laws. A hierarchical protocol need to be followed by synergetic application of burn test, visual examination, radiographic image analysis, morphometric measurements and DNA based analysis is crucial. Multidisciplinary approach will help wildlife species identification as most of the smuggled wildlife articles were altered its shape or size or damaged and sometimes fake ones were made from bone or hoof of other domestic animals. This method of assessment is more reliable, especially when samples with low DNA quality due to degraded samples. The development of morphometric reference database of known species with age, fore limb and hind limb claws from known sample and genetic database for determining the species involved in making seized claws.

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