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Morphological, morphometric and molecular characterization of seized pangolin scales for wildlife forensic identification

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Abstract

Illegal trafficking of pangolins and their body parts poses a major threat to the conservation of this endangered mammalian group. Reliable forensic identification of seized pangolin derivatives is essential for wildlife law enforcement and species conservation. The present study investigated the morphological, morphometric, and molecular characteristics of seized pangolin scales submitted for forensic confirmation. Seven pangolin scales comprising five scapular-shaped and two folded-shaped scales were examined. Gross morphology was assessed based on shape, curvature, groove pattern, surface texture, and basal configuration, while morphometric observations included weight, length, width, width-to-length ratio, and groove count. Scapular-shaped scales were broad, dorsoventrally flattened, and exhibited prominent transverse grooves, whereas folded-shaped scales were smaller with compact morphology and laterally inclined distal tips. Morphometric analysis revealed higher mean weight and width-to-length ratio in scapular-shaped scales compared to folded-shaped scales. Molecular analysis of pangolin scales yielded a mean DNA concentration of 49.11 ± 36.17 ng/ μ l, and PCR amplification success was observed in 44.44% of extractions using universal primers. The observed morphological and morphometric features corresponded with previously published descriptions of Indian pangolin scales. The study demonstrates that combined morphological, morphometric, and molecular approaches provide a reliable framework for forensic identification of seized pangolin scales and can support wildlife crime investigations and conservation efforts.

Keywords: Pangolin scales, Wildlife forensics, Morphometry, DNA isolation, PCR amplification, Illegal wildlife trade

Introduction

Pangolins are highly threatened mammals that are extensively targeted in illegal wildlife trade for their scales and meat. Pangolin scales are illegally traded for use in traditional medicines, ornamental products, and other commercial purposes in several countries, resulting in severe decline of wild populations (Soewu and Ayodele, 2009) [1]. Owing to increasing poaching and trafficking, all pangolin species are protected under wildlife conservation laws and international trade regulations. Identification of seized pangolin body parts is therefore essential in wildlife forensic investigations for effective implementation of wildlife protection laws and conservation of endangered species.

Pangolin scales are keratinized structures that exhibit characteristic variations in shape, size, groove pattern, and morphology depending on the species and anatomical region (Algewatta *et al.*, 2020; Suwal *et al.*, 2022) [2, 3, 8]. Hence, morphological and morphometric examination can provide important preliminary evidence for forensic identification. Previous studies have demonstrated the usefulness of standardized morphometric evaluation and scale characterization for forensic and taxonomic investigations (Perera *et al.*, 2020) [7]. However, visual identification alone may sometimes be insufficient, particularly in processed or degraded samples. Molecular techniques such as DNA isolation and PCR amplification are valuable tools for confirming species identity in wildlife forensic investigations involving keratinized tissues.

Materials and Methods

Seized and confiscated wildlife samples submitted by officials of the Forest Department were utilized in the present study following official permission and consent. The samples were received at the Department of Veterinary Public Health, Veterinary College, Hassan for forensic examination.

Morphological Examination

Gross morphological examination of pangolin scales was carried out by visual inspection and documentation using high-resolution digital photography. Morphological features such as shape, size, curvature, colour, texture, surface grooves, ridge pattern, basal margin, apex configuration, and dorsoventral flattening were recorded and compared with available standard descriptions from published literature.

Morphometric Analysis

Morphometric observations were recorded using a measuring scale and digital Vernier caliper (Advance®, accuracy 0.01 mm). Parameters including weight, length, width, width-to-length ratio, and groove count were measured for each seized scale. Descriptive statistical analysis including mean and standard deviation was performed.

DNA Extraction and PCR Amplification

DNA was extracted from pangolin scale samples using standard laboratory protocols. Three independent extractions were performed for each sample to assess DNA recovery efficiency. The quantity and purity of extracted DNA were evaluated spectrophotometrically using A260/A280 and A260/A230 absorbance ratios. PCR amplification was performed using universal primers targeting mitochondrial DNA regions commonly employed in wildlife forensic investigations. Amplified PCR products were analysed by agarose gel electrophoresis, and successful amplicons were subjected to sequencing and comparison with sequences available in the NCBI database for species confirmation.

Results and Discussion

A total of seven seized pangolin scales were subjected to morphological, morphometric, and molecular examination for forensic identification. Based on gross morphology, the scales were classified into two morphological types: scapular-shaped scales (n = 5) and folded-shaped scales (n = 2). The observed variation in scale morphology corresponds with previously described regional and structural variation in pangolin scales reported in Indian and Asian pangolins. The scapular-shaped scales were large, broad, and dorsoventrally flattened with a roughly triangular outline and a wide basal margin. The dorsal surface was distinctly

convex and exhibited prominent parallel transverse grooves extending across most of the scale surface, producing a ridge-like appearance. Groove depth was more pronounced in the central region and gradually reduced towards the margins. The apex was blunt to moderately pointed and showed evidence of natural abrasion. The ventral surface was slightly concave, and the inner layer appeared mildly glossy with shallow notching. These findings are consistent with the descriptions of broad rhombic or scapular-like scales reported by Soewu and Ayodele (2009) ^[1] and Algewatta *et al.* (2020) ^[2, 3], who identified broad rhombic scales as the dominant scale type in Indian pangolins. Similar broad trunk-associated scales with deep transverse ridges have also been described in Chinese pangolins by Suwal *et al.* (2022) ^[8].

The folded-shaped scales were comparatively smaller and more compact. The basal region was narrow, and the distal tip was pointed and laterally inclined, producing a folded appearance. The dorsal surface was convex with shallow transverse grooves arranged in a similar orientation to the scapular-shaped scales but exhibiting a steeper profile. Comparable elongated and folded scale forms have been reported in transitional and caudal body regions of pangolins by Algewatta *et al.* (2020) ^[2, 3]. Previous geometric morphometric studies demonstrated that scale elongation, asymmetry, and curvature vary according to anatomical position and body region, particularly in adult pangolins where regional specialization is more pronounced (Moison *et al.*, 2025) ^[5].

Morphometric analysis demonstrated measurable differences between the two scale types. The scapular-shaped scales recorded a mean weight of 6.74±0.83 g, mean length of 5.72±0.96 cm, and mean width of 6.46±1.49 cm. In contrast, folded-shaped scales exhibited a lower mean weight of 4.88±0.63 g, mean length of 5.05±0.35 cm, and mean width of 4.95±0.35 cm. The width-to-length ratio was higher in scapular-shaped scales (1.13±0.15), indicating greater transverse expansion, whereas folded-shaped scales showed a comparatively lower and more uniform ratio (0.98±0.00). Groove counts ranged from 23 to 41 in scapular-shaped scales and from 28 to 32 in folded-shaped scales. The observed morphometric differences support the gross morphological classification and correspond closely with previous reports on Indian pangolin scales. Algewatta *et al.* (2022) ^[2, 3] reported that broad rhombic scales are generally larger and heavier than elongated or folded scales, reflecting their dorsal trunk association and broader transverse expansion. Similarly, Suwal *et al.* (2022) ^[8] observed that trunk scales in pangolins are broader and more rhomboidal, while caudal scales tend to be elongated and narrower. The present findings therefore suggest that the seized scales likely originated from different anatomical regions of the pangolin body.

Table 1: Morphometric characteristics of seized pangolin scales based on scale type.

Sl. No.	Type of pangolin scale	Weight of scale (grams)	Number of grooves	Length (cm)	Width (cm)	Width/Length ratio
1	Scapular-shaped scale	6.87	27	5.6	5.8	1.04
		7.57	36	7.2	8.3	1.15
		7.32	41	5.6	7.7	1.38
		6.48	24	5.7	5.8	1.02
		5.45	23	4.5	4.7	1.04
	Mean ± SD	6.74±0.83	-	5.72±0.96	6.46±1.49	1.13±0.15
	Mean±SE	6.74±0.37	-	5.72±0.43	6.46±0.67	1.13±0.07
2	Folded-shaped scale	4.43	28	5.3	5.2	0.98
		5.32	32	4.8	4.7	0.98
		Mean ± SD	4.88±0.63			
		Mean±SE	4.88±0.45			

Previous studies have emphasized the forensic significance of standardized morphometric evaluation of pangolin scales. Perera *et al.* (2020) [7] proposed standardized protocols for recording pangolin morphometry and scale characteristics to improve species identification and comparative forensic analysis. Although the present study involved a limited number of seized scales, the dimensional patterns observed were consistent with earlier published morphometric benchmarks for Indian pangolins. Growth-related variation and regional specialization in scale morphology have also been reported in African pangolins, indicating that scale dimensions are influenced by age and anatomical location rather than random variation (Morenikeji *et al.*, 2022) [6].

Molecular analysis of pangolin scales yielded a mean DNA concentration of 49.11 ± 36.17 ng/ μ l. However, the purity ratios were relatively low, with mean A260/A280 and A260/A230 ratios of 0.77 ± 0.31 and 0.56 ± 0.38 , respectively, indicating possible contamination or the presence of keratin-associated inhibitors. PCR amplification was successful in 4 out of 9 extractions, resulting in an amplification success

rate of 44.44%. The partial amplification success observed in the present study may be attributed to the highly keratinized nature of pangolin scales and degradation associated with environmental exposure and storage conditions of seized samples.

Similar challenges in DNA recovery from keratinized wildlife tissues such as scales, claws, horns, and hair have been reported in wildlife forensic investigations. Despite reduced purity and partial amplification success, successful PCR amplification from seized pangolin scales demonstrates the utility of molecular methods for forensic species identification. Mitochondrial DNA markers are particularly useful in wildlife forensic investigations because of their higher copy number and greater amplification success from degraded samples. The integration of gross morphology, morphometric analysis, and molecular confirmation in the present study therefore provides a reliable and non-destructive framework for forensic identification of seized pangolin scales.



Photograph of seized pangolin scales showing two morphological types: scapular-shaped scales (arrow) with broad triangular outline and prominent transverse grooves, and folded-shaped scales (starred) with elongated, curved morphology.



Photograph showing the ventral surface of a pangolin scale with a mildly concave profile and a notched, slightly glossy inner layer.

Conclusion

The present study demonstrated that morphological, morphometric, and molecular analyses are useful tools for forensic identification of seized pangolin scales. Distinct differences were observed between scapular-shaped and folded-shaped scales in their shape, groove pattern, and morphometric parameters, which were consistent with published descriptions of Indian pangolin scales. Although PCR amplification success was moderate due to the keratinized and degraded nature of the samples, successful DNA amplification confirmed the utility of molecular methods in wildlife forensic investigations. The combined approach provides a reliable framework for identification of

seized pangolin scales and can support wildlife conservation and law enforcement efforts.

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