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Research Article

Experimental design of hair sampling: Preparation of hair specimens for taxonomic identification using microscopic morphology

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Abstract: Hair morphology provides important class-level information for mammalian species identification in forensic investigations, wildlife law enforcement, and museum-based taxonomy, particularly when biological material is limited or unsuitable for DNA analysis. This study presents a standardized experimental design for hair sampling and preparation to improve the reliability and reproducibility of microscopic hair examinations. The protocol evaluates detergent- and solvent-based cleaning methods, permanent slide mounting, cuticular cast preparation, and transverse cross-sectional analysis to examine diagnostic features of the cuticle, cortex, and medulla. Quantitative morphometric measurements were obtained from standardized mid-shaft regions, while cross-sectional analysis was used to assess internal structure and pigment distribution. Emphasis is placed on methodological standardization, defined measurement criteria, and interpretation within a class-level framework consistent with accepted forensic principles. The proposed workflow enhances transparency and comparability in hair morphology analysis. It provides a practical foundation for forensic casework, wildlife forensic investigations, and the development of reference collections, particularly in biodiversity-rich regions where non-invasive sampling is essential.

Keywords: Hair morphology; Forensic hair examination; Cuticle scale pattern; Medulla structure

1. Introduction

Hair grows from follicles located in the skin in regions containing hair-forming cells. Based on its attachment to the skin, hair can be divided into two main parts: the basal portion or hair root (papilla), which is embedded within the skin, and the hair shaft, which extends above the skin surface [1 & 2]. In terrestrial mammals, hair provides critical protection by serving as thermal insulation, contributing to thermoregulation, and reducing exposure to environmental stressors such as heat, cold, and humidity [3 & 4].

Mammalian hair is commonly classified into two fundamental types: guard hairs and fur hairs [5]. Guard hairs are particularly useful for taxonomic diagnosis and species identification because their morphological features—including shaft form, cuticle, cortex, and medulla—tend to be less variable than those of fur hairs [5 & 6]. Structurally, mammalian hair consists of three keratinized layers composed of dead cells: the outermost cuticle, the middle cortex, and the innermost medulla [1 & 7]. The medulla and adjacent tissues may contain pigment granules and other coloring substances that contribute to species-specific variation [8 & 9]. This study aims to develop and validate a standardized hair-preparation protocol that preserves diagnostic microstructural features and improves the reliability of mammalian taxonomic identification across forensic, wildlife, and museum-based applications.

2. Experimental Design

This study employs a controlled, comparative factorial design to evaluate how hair-cleaning and slide-preparation methods influence the visibility and integrity of diagnostic microstructures (cuticle and medulla) used for taxonomic identification. The workflow comprises three methodological components: (1) preparation of hair samples for taxonomic analysis, (2) preparation of permanent slides for external hair morphology and medulla examination, and (3) forensic-style cross-sectional analysis of cortex and medulla characteristics.

3. Preparation of Hair Samples for Taxonomic Analysis

3.1 Cleaning of Hair Samples

Hair samples were cleaned to remove surface contaminants, including lipids, soil particles, and dust adhering to the hair shaft, which may obscure diagnostic morphological features [6 & 10]. Two cleaning methods were evaluated.

3.2 Detergent Washing Method

Hair samples were washed or soaked in a 1–2% (v/v) diluted solution of dishwashing liquid or laboratory-grade laundry detergent prepared with distilled water, following established hair morphology protocols [5 & 9]. Samples were soaked for ≥2 h, with gentle agitation at regular intervals to facilitate removal of contaminants from the cuticle surface. Samples were then thoroughly rinsed with distilled water to remove residual detergent. Cleaned hairs were air-dried at room temperature or oven-dried at 40–50 °C to remove residual moisture (Figure 1).

Figure 1. Cleaning of hair samples: (a) soaking hair fibres in a detergent solution to remove lipid residues and dust particles; (b) rinsing the hair fibres with clean water to eliminate residual surfactant and contaminants; and (c) air-drying the cleaned hair samples at room temperature.

3.3 Xylene Soaking Method (Forensic Laboratory Protocol)

Xylene was used as an organic solvent to remove persistent surface contaminants from hair samples under controlled laboratory conditions, consistent with forensic hair examination practices [10 & 11]. When pigment distribution within the hair shaft was used as a diagnostic feature, prolonged exposure to xylene was avoided, as solvent treatment may extract pigment granules and alter taxonomically relevant characteristics [6 & 8]. For archival or long-stored samples, xylene treatment was omitted to minimise unnecessary chemical exposure. Laboratory personnel wore appropriate personal protective equipment, including nitrile gloves, laboratory coats, and eye protection.

Hair fibres were immersed in xylene for 30 min to 1 h, depending on the degree of surface contamination. Following solvent exposure, the samples were removed using solvent-resistant forceps and placed on inert, solvent-compatible surfaces. Hair samples were then air-dried at room temperature in the fume hood until complete solvent evaporation was achieved.

When pigment distribution within the hair shaft was used as a diagnostic feature for interspecific discrimination, prolonged exposure to xylene was avoided, as xylene may extract pigment granules and compromise taxonomically and forensically relevant characteristics (Figure 2). For archival or long-stored hair samples, the xylene soaking procedure was omitted to minimize unnecessary chemical exposure and reduce the risk of pigment or structural alteration.

All solvent waste was collected and disposed of in compliance with hazardous chemical waste regulations to ensure laboratory safety and evidentiary integrity.

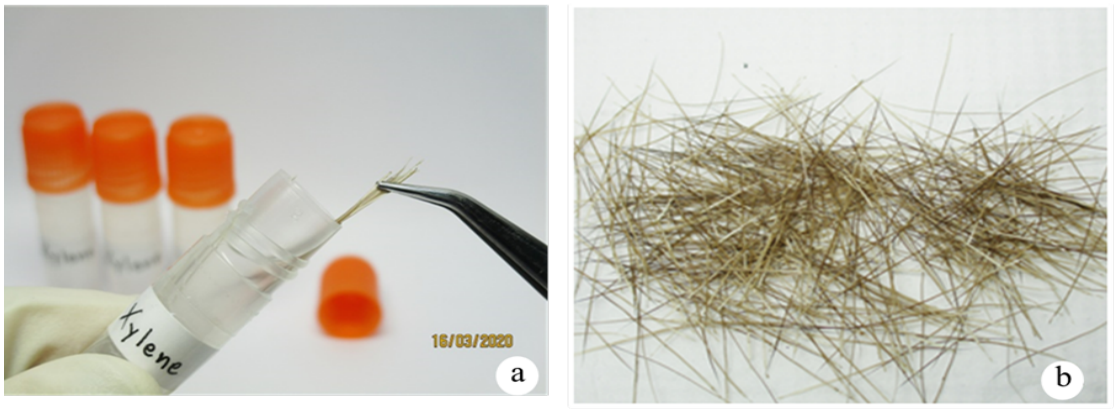

Figure 2. Cleaning of hair samples using xylene: (a) soaking hair fibres in xylene to remove surface contaminants; and (b) air-drying the hair samples after xylene treatment at room temperature within a fume hood.

Prepared hair samples were subsequently subjected to morphological examination, which included both external and internal structural analyses [5].

1. External hair characteristics were examined through macroscopic and microscopic assessment of physical hair morphology, including hair diameter, shape, and surface features, as well as detailed analysis of cuticle scale patterns (cuticle examination).

2. Internal hair characteristics were examined by analysing the structure of the medulla, including medullary pattern and continuity, and by assessing the structural components of the cortex, including pigmentation and internal composition.

4. Preparation of Hair Samples for Taxonomic Analysis

Prepared hair samples were mounted as permanent slides for microscopic examination following standard zoological and forensic microscopy protocols (Meyer et al., 2002; Robertson et al., 2019).

4.1 Preparation of microscope slides and mounting medium

Clean microscope slides and coverslips were prepared before mounting. A permanent mounting medium (Permount®) was used to promote long-term preservation, optical clarity, and structural stability.

4.2 Mounting of hair samples

Prepared hair samples were mounted as permanent slides for microscopic examination following standard zoological and forensic microscopy protocols [7 & 10]. Individual hair fibres were carefully placed onto the glass slide using fine forceps and oriented longitudinally to expose the mid-shaft region. A small drop of mounting medium was applied, and the hair was gently covered with a coverslip to minimise air bubble formation (Figure 3). The mounted slides were allowed to cure at room temperature for 24–48 h in a dust-free environment. After curing, slides were stored in labelled slide boxes at room temperature until microscopic analysis.

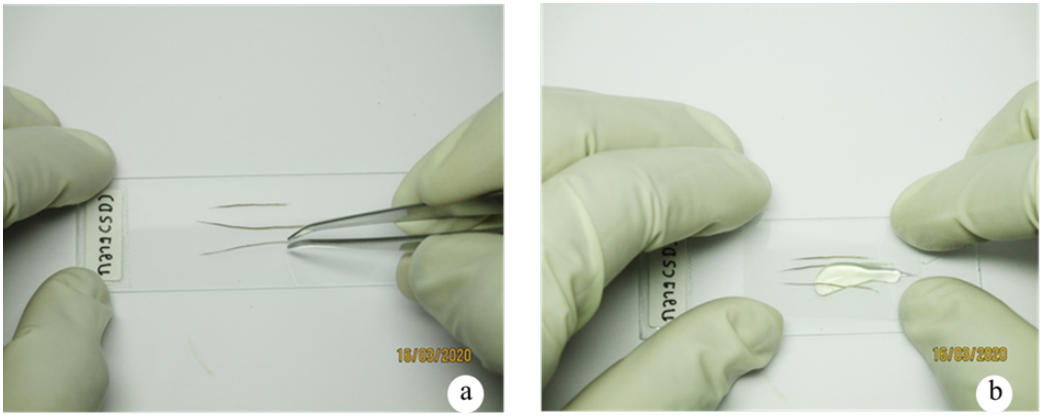

Figure 3. Permanent slide preparation procedure: (a) placement of a hair sample onto a glass microscope slide; and (b) application of Permount® mounting medium, followed by covering the hair sample with a glass coverslip.

5. Preparation of Hair Samples for Cuticle Examination

Cuticle scale patterns were examined using the cuticle cast method with clear nail polish, a widely applied and validated technique for mammalian hair identification [5 & 6]. More than 20 hair fibres per taxonomic unit were examined to account for intra-individual variation and improve classification reliability. Cuticle scale patterns were analyzed by producing cuticle casts using dried hair samples and a clear nail polish medium. The procedure was conducted as follows (Figure 4).

5.1 Preparation of the casting surface

A thin, even layer of clear nail polish was applied to a clean glass microscope slide to form a casting surface. The nail polish was allowed to partially dry until it reached a semi-tacky state. Adequate drying was assessed by gently pressing the edge of the nail polish film with a fingertip to ensure it was no longer fluid but still pliable.

5.2 Imprinting of hair cuticle patterns

Dried hair fibres were carefully placed onto the prepared casting surface. A transparent plastic sheet was positioned over the hair fibre, and gentle pressure was applied along the entire length of the hair using a glass rod. This step ensured uniform contact between the hair shaft and the semi-dried nail polish, thereby enabling consistent transfer of the cuticular scale pattern along the hair surface.

5.3 Removal of hair fibres from the cast

After imprinting, the slide was left undisturbed for approximately 5 min to allow the nail polish to set. The hair fibre was then removed using fine forceps, which gripped the proximal end of the hair and slowly lifted it away from the cast, in a motion similar to opening a book. This technique minimised the distortion or tearing of the cuticle impression.

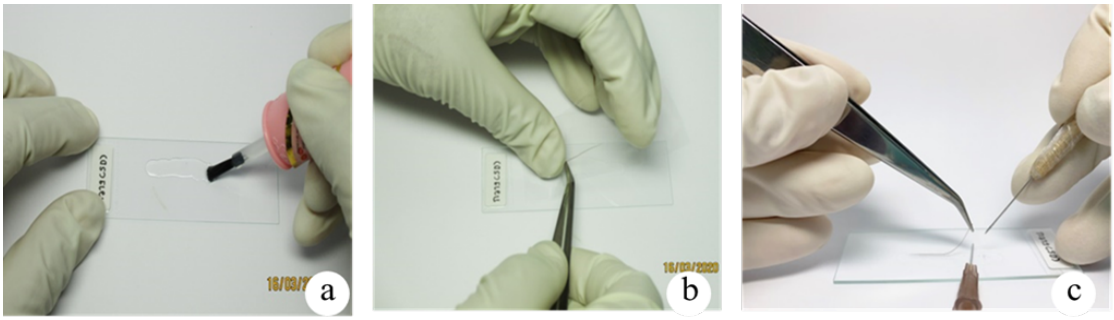

Figure 4. Procedure for producing hair cuticle casts: (a) application of a thin layer of clear nail polish onto a glass microscope slide; (b) placement of the hair fiber onto the casting surface, followed by gentle pressing along the hair shaft using a transparent plastic sheet; and (c) removal of the hair fiber from the cast by gently lifting the proximal end with a needle and fine forceps.

Methodological note: Accurate identification of cuticle scale morphology requires careful data collection and adequate sampling. To account for intra-individual and inter-hair variation, more than 20 hair fibres of varying diameters should be examined for each taxonomic unit. This approach improves the reliability and robustness of cuticle-based classification.

5. Forensic Examination of Cortex and Medulla Characteristics Using Cross-Sectional Analysis

Cross-sectional analysis was used to evaluate cortex and medulla morphology and pigment distribution, which provide class-level discriminatory information in forensic and taxonomic contexts [8 & 10]. Measurements were interpreted as class characteristics rather than as indicators of individualisation.

The cortex constitutes the internal region of the hair shaft situated between the medulla and the inner cuticle layer. This region commonly contains melanin pigment granules, which contribute to hair colouration and may provide discriminatory characteristics for forensic comparison. The distribution, density, and organisation of pigment granules within the cortex are known to vary among species and were therefore treated as class characteristics in the present examination.

Cortical and medullary features were examined using transverse cross-sectional analysis prepared as temporary wet mounts, a method commonly applied in forensic hair examinations when permanent sectioning is not required. Hair samples selected for study were sectioned into thin transverse slices (cross sections; X-S) using the following standardised procedure:

1. A clean glass microscope slide and cover slip were prepared and inspected to ensure the absence of extraneous material.
2. A thin hair cross-section was placed onto the slide using fine forceps.
3. One to two drops of distilled water or an appropriate staining solution were applied to the specimen.
4. The cover slip was applied by placing one edge in contact with the liquid at approximately a 45° angle and slowly lowering the opposite edge to minimise air entrapment. Fine needles were used as needed to ensure proper specimen positioning.

Cross-sectional examinations were conducted on samples from five standardised body regions (head, shoulder, back, hip, and abdomen). From each region, five hairs were randomly selected to represent intra-individual variation. Proximal and distal ends were excluded, and only the mid-shaft region was analysed to reduce variation attributable to hair growth phase and wear. For each region, three cross-sectional replicates were examined.

Morphometric measurements were obtained using AxioVision Rel. 4.8. Four parameters were recorded and averaged per region: (a) maximum transverse hair diameter, (b) minimum transverse hair diameter, (c) thickness of the right medullary boundary, and (d) thickness of the left medullary boundary (Figure 5). All observations and measurements were documented using calibrated imaging systems.

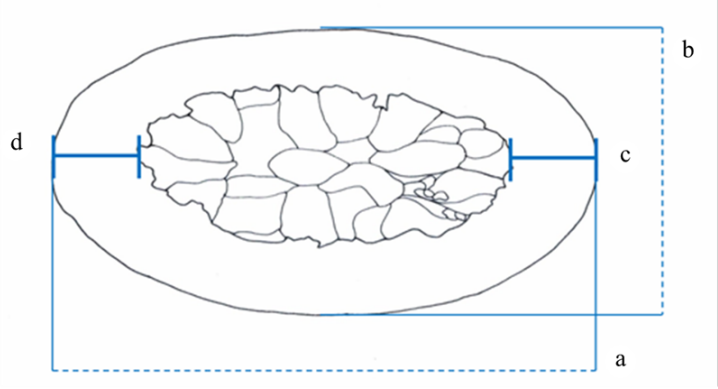

Figure 5. Cross-sectional measurements of a hair fibre showing four measured parameters: (a) maximum transverse diameter, (b) minimum transverse diameter, (c) thickness of the right medullary boundary, and (d) thickness of the left medullary boundary.

6. Conclusions

Hair morphology remains a valuable source of class-level information in forensic science, wildlife investigations, and museum-based taxonomy, particularly when biological material is limited, degraded, or non-invasively collected. This review highlights standardized approaches for hair sample preparation, microscopic examination, and cross-sectional analysis that improve the reliability and reproducibility of morphological assessments. Emphasis on controlled cleaning procedures, consistent slide preparation, and clearly defined measurement criteria is essential for preserving diagnostically relevant features of the cuticle, cortex, and medulla.

The integration of quantitative morphometric measurements, such as hair diameter and medullary indices, with qualitative pattern-based assessment enhances transparency and supports more robust comparative evaluations. Cross-sectional analysis further contributes critical information on internal hair structure and pigment distribution, complementing longitudinal observations. Importantly, interpretation of hair evidence must remain within the framework of class-level association, with explicit recognition of methodological limitations and sources of intra- and interspecific variation.

7. Future Perspectives: Wildlife Applications in Thailand

In Thailand, where biodiversity is exceptionally high, and many wildlife species are protected, hair-based morphological analysis represents a valuable non-invasive tool for wildlife monitoring and forensic enforcement [12 & 13]. The development of regional reference databases, integration with molecular approaches, and interlaboratory validation will be critical to strengthening wildlife forensic capacity and biodiversity management in Thailand.

Future research should priorities the development of region-specific reference databases on hair morphology for Thai wildlife, particularly for mammals of conservation concern, such as small carnivores, ungulates, bats, and rodents. Establishing standardized reference collections linked to voucher specimens housed in national museums and research institutions will enhance the accuracy and credibility of species identification.

Integration of hair morphology with complementary analytical approaches, including DNA-based methods, isotopic analysis, and advanced imaging techniques, will further strengthen forensic and ecological applications. Additionally, inter-laboratory validation studies and capacity building among forensic and wildlife laboratories in Thailand are essential to promote methodological consistency and support wildlife law enforcement.

As wildlife trafficking and human–wildlife interactions continue to pose challenges in the region, standardized hair morphology analysis applied within clearly defined evidentiary limits has the potential to become a practical and cost-effective tool for conservation research, wildlife forensics, and biodiversity management in Thailand.

8. Patents

Author Contributions: S.H. and K.B. conceptualized and conducted the study, performed data curation, formal analysis, and prepared the original draft. S.H supervised the research and provided critical review and editing. All authors have read and agreed to the published version of the manuscript.

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