ORIGINAL ARTICLE

Analysis of hair samples using microscopical and molecular techniques to ascertain claims of rare animal species

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Abstract

***Background*: An unidentified animal species named the Jenglot and claimed to be a rare living animal species was recently found in the deep jungle of Irian Jaya, Indonesia; brought to Kuala Lumpur, Malaysia by a businessman; and exhibited in a local museum. The owner of the Jenglot carcasses had made a request to perform DNA analysis on the Jenglot to ascertain its species.**

***Methods*: Because the muscle appeared very dry and recovery of DNA was extremely difficult, we therefore used the animals' hair for further analysis. Hair samples were collected from three different Jenglots that were different in colour and physical appearance. The samples were labelled as A, B, C and D, respectively.**

***Results*: Microscopic characteristics indicated that all four hair samples were of human origin, with a medullary index less than 1/3 and pigment distribution towards the periphery. The scale pattern on the hair samples was of the imbricate type, adding certainty to the hypothesis of human origin. A dried root sheath was found in samples B and C, which was contrary to expectations since the sample collection method left a few cm of hair on the body of the Jenglots. Sample D had black dye granules over the cuticular surface. Sequencing of the mitochondrial DNA (mtDNA) hypervariable segment I (HVS-I) region showed polymorphisms at positions 16140, 16182C, 16183C, 16189, 16217 and 16274 and heteroplasmy at positions 16112, 16232 and 16251, a human-specific mtDNA haplotype that was consistent across all the samples.**

***Conclusions*: Based on these findings, it was concluded that it is unlikely that the samples of Jenglot hair originated from an animal species.**

***Keywords*:** *Hair, mitochondrial DNA, microscopial analysis, health sciences*

Introduction

Morphological examination of hair samples is the first step in forensic hair comparisons. The main medico-legal concerns with hair examination include identification of the species of origin, ascertainment of the hair's provenance from the body and, finally, comparison of the control hair sample from the victim to the hair sample from the crime scene. Though it is not possible to definitely identify a sample of hair originating from a particular person's head, unequivocal determination of human origin can be established based on microscopic examination of the hair's cuticle, cortex, medulla and pigment granules (1).

Human and animal hairs show similarities in having an outer cuticle, cortex and medulla. The outer surface of the hair is covered by scales. Though there are similar morphological features, the scale pattern provides distinguishing characteristics between animal and human hairs. The scales of an animal's hair show many distinctions such as coronal (crown-like) and spinuous patterns, whereas in the case of humans the scale patterns are of the 'imbricate' type (flattened) (Figure 1) (2,3). Besides, the medullary index, which is the ratio of the medulla's width to the diameter of the hair, is 1/3 and below in humans compared to greater than 1/3 in animal hairs, due to the greater width of the medulla in animals (2,3).

With the advancement of forensic DNA typing, microscopic hair comparisons and DNA analysis can be complementary and provide information on the source of a hair. In this case analysis, hair

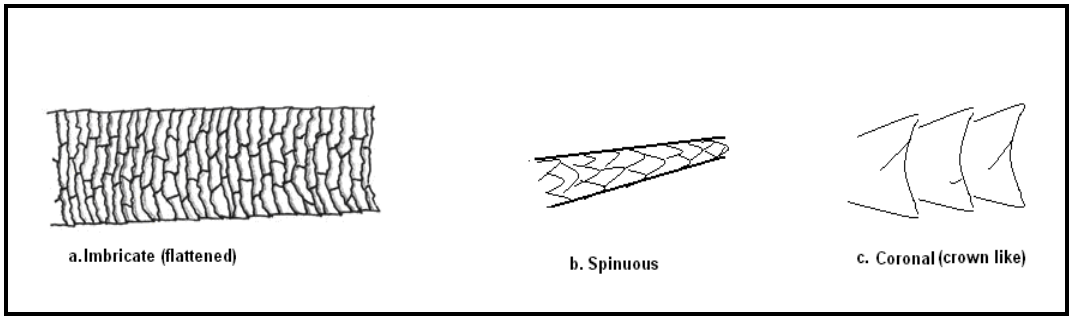

Figure 1: Representative diagram showing different scale patterns found on hairs: a, an imbricate scale pattern is unique to human; b, spinuous; c, coronal patterns are observed on animal hairs.

samples were obtained from three Jenglots, which were claimed to be a rare living animal species only found in the deep jungle of Irian Jaya, Indonesia. Three Jenglots were bought from West Java by a Malaysian businessman for his personal collection and exhibited in one of the museums in Kuala Lumpur, where they have attracted numerous visitors. A request was then made by the owner of the Jenglot carcasses to the authors to perform DNA analysis so as to ascertain the veracity of the claim that they are a rare animal species. Hair samples were collected from three different Jenglots and were labeled as A, B, C and D. Samples A and B were taken from a medium-size Jenglot carcass, which was 32 cm tall. Sample C was collected from a 16.8-cm tall Jenglot while sample D was collected from the biggest Jenglot, which was 61.3 cm tall. All hair samples were collected from the head region of the Jenglots by cutting at the distal end of the hair.

Materials and Methods

Several strands of hair were placed in parallel on a microscope slide and two drops of water were added over the hairs in order to hold them in place. A cover slip was placed over the hairs and they were scanned along their length at 100x and 400x under a compound microscope to observe the morphological characteristics of the cuticle and medulla, and the distribution of pigment in the cortex. The medullary index and the diameter of the hairs were calculated using an ocular micrometer calibrated with a stage micrometer at 100x and 400x (4).

It is often very difficult to directly observe scale patterns from hair strands on a slide. Hence, a cast was made using nail polish to obtain the impression of the scales. A thin layer of nail polish was spread on a microscope slide and a hair was placed in the middle of the slide. It was allowed to stand for 15 minutes so that the nail polish could harden and the hair was then gently removed using forceps (4). The scale pattern was observed under a compound microscope at 100x and 400x.

DNA was extracted from each hair sample using the Promega hair and tissue extraction kit and Promega DNA IQ™ system (Promega, USA), following the manufacturer's protocols. Hair samples were thoroughly washed using soap solution and air-dried prior to the extraction to avoid contamination. The hypervariable segment I (HVS-I) region of human mtDNA was amplified using two sets of human-specific primers (L15996 and H16213; L16128 and H16432) (5) and sequenced. Polymorphisms were reported by aligning the HVS-I sequence from the hair samples with the Cambridge Reference Sequence (6).

Results and Discussion

Hair is an outgrowth of the skin produced from a structure called the hair follicle and found only in mammals. Humans develop hair follicles during foetal development and no new follicles are produced after birth. Hair is composed of the protein keratin and it is also the primary component of finger and toe nails (7).

Forensic analysis of hair centres on colour and structure, determined through microscopic magnification. The hair shaft has three forensically relevant layers: the cuticle, cortex and medulla. The cuticle has overlapping external scales, which helps in species identification. The scales of human hairs are imbricate whereas animal hairs show many distinct patterns such as coronal (crown-like) and spinuous scales (Figure 1) (1,2,4).

Within the hair cuticle is the cortex, made up of spindle-shaped cells that contain the colour pigments; the way the pigments are distributed helps to identify hairs from particular individuals. Hair colour is mostly the result of pigments and the

pigments of human hairs are distinguishable from those of other mammals. Human hairs are generally consistent in colour and pigmentation throughout the length of the hair shaft, whereas animal hairs may exhibit radical colour changes over a short distance—a phenomenon known as banding (4). The distribution and density of pigments in human and animal hairs can also be identifiable features. The pigmentation of human hairs is evenly distributed and denser toward the cuticle, whereas the pigmentation of animal hairs is more centrally distributed, i.e., denser towards the medulla (1,2,4,8,9).

At the centre of the hair shaft is the medulla, which is also valuable for species identification. Animals' medullary index (ratio of the medulla's diameter relative to the shaft's diameter) is greater than humans'. Humans have a medullary index of less than 1/3 while the medullary index of animals is greater than 1/3. However, the medulla is often fragmented or interrupted, which may result in differences in the identification of hairs from the same individual (1,2,4,8,9).

The results of the examination of the four hair samples labelled A, B, C and D are shown in Table 1. All hair samples were found to have a medullary index below 1/3, which is indicative of human origin (1,4,8,9). Moreover, moderate shaft diameter variations were observed in all four samples (Figure 2) and the scale pattern was of the imbricate type, indicating human origin (Figure 3) (1,4,8,9). The pigments were distributed more towards the cuticular margin, which is also a characteristic indicative of human origin (Figure 4) (1,4,7–10).

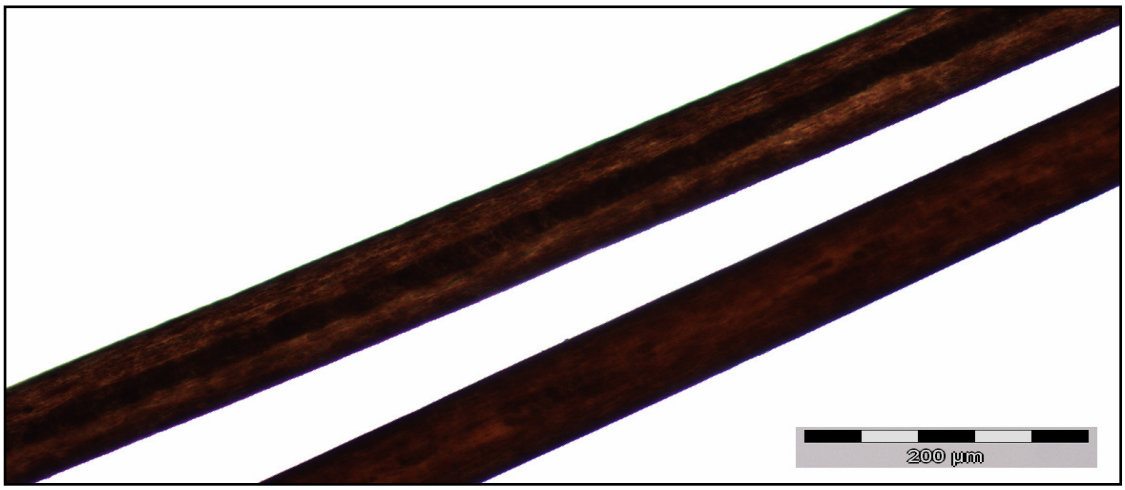

Figure 2: Representative hair from sample C (medullated) showing moderate shaft diameter variation (100x).

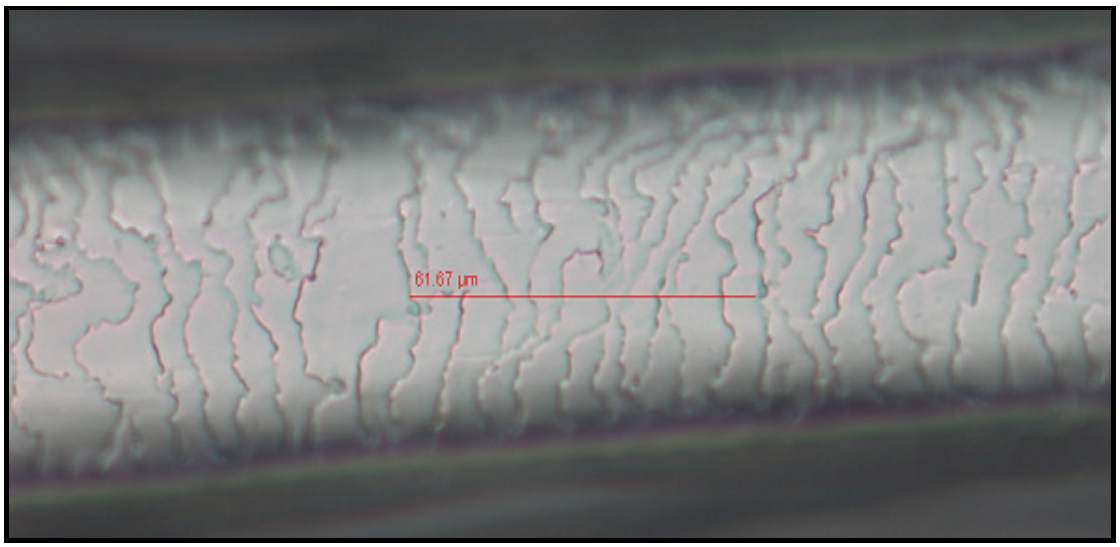

Figure 3: Scale impression cast showing the imbricate nature of the scales of the Jenglot's hair (400x).

Table 1: Results of morphological examination of hair samples

Characteristics examined	Sample A	Sample B	Sample C	Sample D
Colour	Black and grey hairs	Black and grey hairs	Brownish and grey hairs	Most hairs were dark with blackish dye granules over the cuticular surface; very few without dye granules
Medulla	Medullated and non-medullated	Medullated and non-medullated	Mostly non-medullated and a few with a medulla	Few hairs with a medulla - faintly visible
End morphology	Cut at one end and with a tapered tip	i. Mostly cut at one end and with a tapered tip ii. Few with dried hair root at one end and with cut markings at the other end	i. Mostly cut at one end and with a tapered tip ii. Few with dried hair root at one end and with cut markings at the other end	Cut at one end and with a tapered tip
Shaft diameter	Coarse and usually with little or no variation (80-110 μm)	Coarse and usually with little or no variation (80-120 μm)	Coarse and usually with little or no variation (80-110 μm)	Coarse and usually with little or no variation (80-120 μm)
Pigment distribution	Pigment was found to be more dense towards the periphery (cuticle)	Pigment was found to be more dense towards the periphery (cuticle)	Pigment was found to be more dense towards the periphery (cuticle)	Pigment was found to be more dense towards the periphery (cuticle)
Scales	Imbricate scales	Imbricate scales	Imbricate scales	Imbricate scales
Dye	-	-	-	Dye granules can be seen over the cuticular surface and were also found dissolved in acetone used to take scale impressions

Black dye granules were clearly visible under microscopic examination of the clear nail varnish imprints of the hair, indicating dyeing of the hairs in sample D (Figure 5). A few hairs from samples B and C also had an intact dried root at the other end of the cut tip, which indicated that the hairs were implanted upside down on the Jenglots' heads (Figure 6).

The mtDNA HVS-I region was successfully amplified using human-specific primers, supporting the morphological findings indicating the human origin of these hairs. Sequence analysis of the HVS-I fragment showed polymorphisms at positions 16140, 16182C, 16183C, 16189, 16217 and 16274 and heteroplasmy at positions 16112, 16232 and 16251. This haplotype was consistent across all the samples, suggesting a maternal relationship between the owners of the hairs or that all the hair samples might have originated from the same individual.

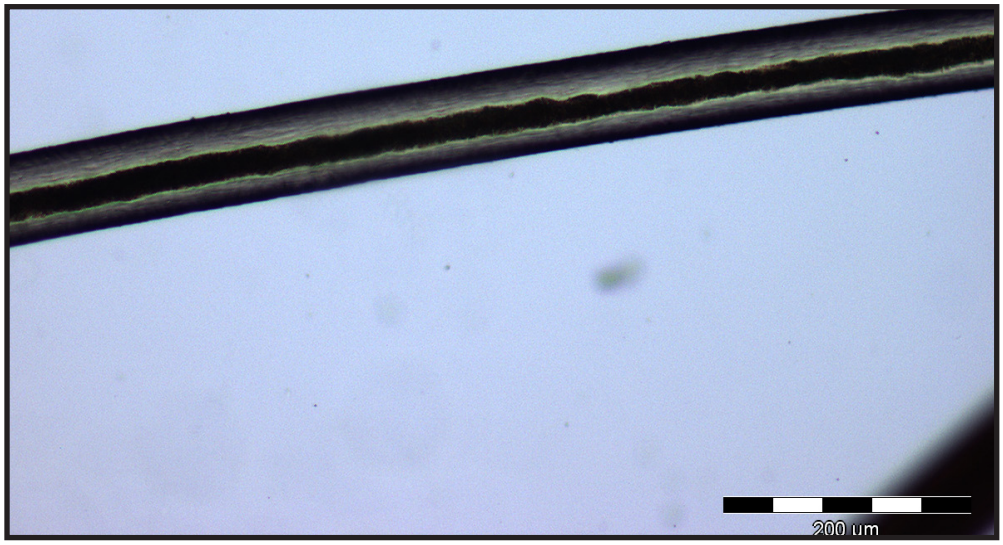

Figure 4: Medullated hair showing pigment granules more towards the cuticle (100x).

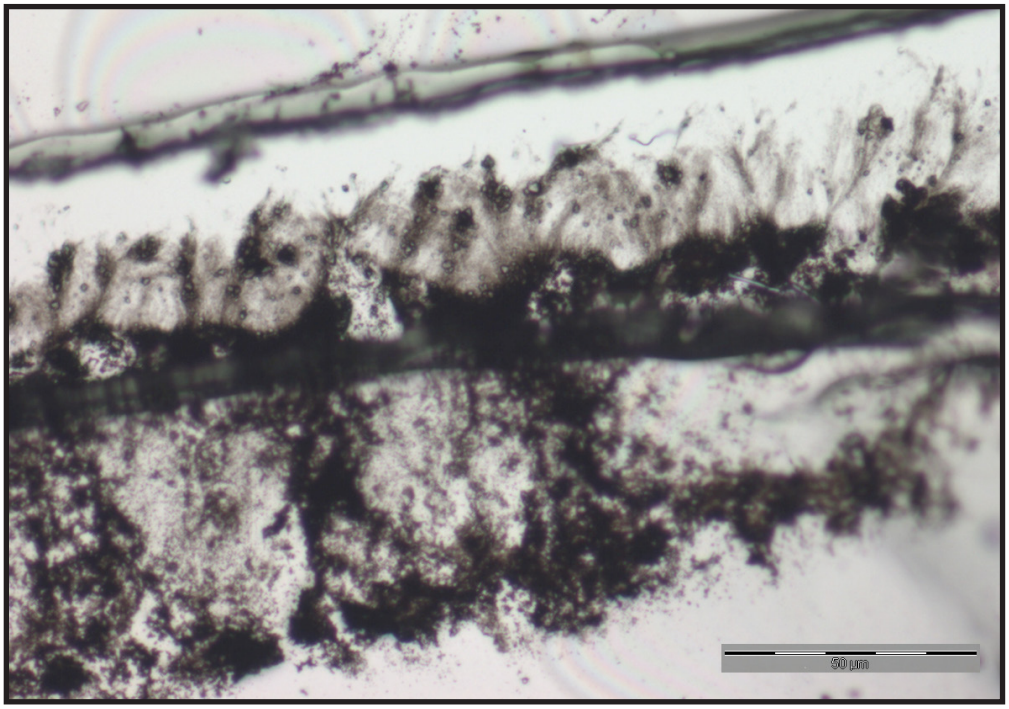

Figure 5: Black dye granules found when mounting a hair from sample D for scale casting in nail polish (100x).

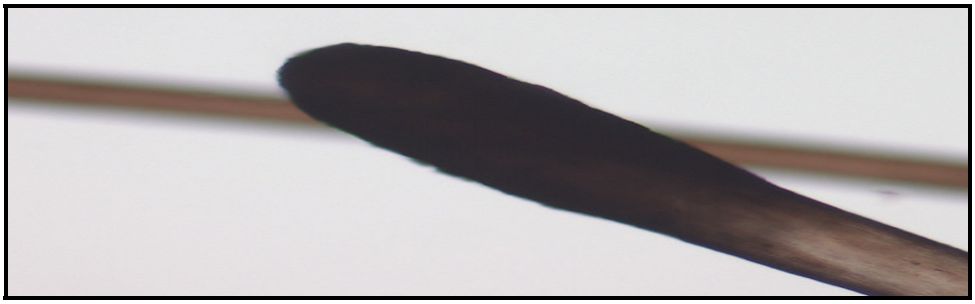

Figure 6: A hair from sample B with a dried-up hair root that appeared at the other end from the cut tip, which indicated that the hair was implanted upside down on the Jenglot's head (100x).

Conclusion

Classical morphological analysis of hair samples, jointly with mtDNA sequence-based analysis, was useful in disproving the claim of a rare animal species in this case. The morphological characteristics observed and the mtDNA sequence analysis proved that the hair samples are of human origin and had been implanted on the Jenglot carcasses' heads. This certainly disproves the claim and myth that the Jenglot is a rare animal species.

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Author's contributions

Conception and design, final approval of the article: ZZ
Data collection, assembly, analysis and interpretation; drafting of the article: SP, ZZ

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