Preliminary Forensic Study of Hair Morphology and Micrometry of Small-Sized Dog Breeds

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ABSTRACT

Hong Kong, located in Southern China, is one of the world's most densely populated cities. Although dealing with the challenge of living in small spaces, more and more families are keeping pets. Pet ownership has undergone exponential growth as nearly 241,900 Hong Kong households (i.e., 9.4 % among all households) had pets at home, while the number of pet dogs and cats increased from 297,100 in 2005 to 405,200 in 2018. Dogs possess hair that is easily shed, thus has great chances to be found at a crime scene through primary or secondary transfer. As every contact leaves a trace, examination of dog hair to identify dog breeds may establish a link between suspect, victim and a crime scene as associative evidence. Previous studies on the morphology and micrometry of dog hair investigated large-sized dog breeds, such as Husky, Rottweiler and Doberman, while these dog breeds are not common in Hong Kong. This was the first study to investigate the morphological and micrometric differences among hair collected from small-sized dogs that are commonly found in homes with small living spaces. Hair samples were collected from popular small-sized dog breeds in Hong Kong such as Poodle, Shiba Inu, Miniature Schnauzer and Pomeranian. With a scanning electron microscope and a compound microscope, morphological (e.g., hair color, cuticle scale pattern and medullary pattern) and micrometric characteristics (e.g., hair shaft diameter, medulla diameter, medullary and cuticular indices) were determined. Distinctive cuticle scale pattern was observed: elongate petal in Shiba Inu and coronal in Poodle, while these arrangements were rarely found in large-sized dog breeds that were reported in previous studies. Hairs of Shiba Inu also showed the medulla to be uniserial ladder, which could be considered a distinctive characteristic of this breed. The findings from this preliminary study are useful for forensic investigations to differentiate and identify small-sized dog breeds through hair sampling. Further studies involving larger numbers of small-sized dog breeds and subjects will be carried out.

Keywords: Dog Hair, Forensic Animal Hair Analysis, Hair Identification, Morphology and Micrometry

I. INTRODUCTION

As a major international financial centre and the most important gateway to Mainland China, Hong Kong is one of the most densely populated cities in the world characterized by small apartments. Although Hong Kong had the smallest average apartment size of 484 square feet in the world [1], it is named as the most expensive property market for the past seven years [2]. The number of births has also dropped significantly over the past 7 years and in 2021, it hit a record low for the past 56 years with only 38,684 babies being born [3]. Put off by unaffordable housing prices, late marriage, a consistently low fertility rate, the high cost of childcare, as well as the physiological and psychological benefits that pets can bring to the owners [4, 5], more and more families are welcoming pets into their homes. A global survey published in 2016 showed that Hong Kong was ranked second in pet ownership, as 35% of the 27,000 online consumers declared that they were pet owners [6]. In 2018, it was estimated that nearly 241,900 Hong Kong households or about one in every 10 households kept pets at home [7]. Of which, 5.7% of households kept dogs, while the number of dogs owned by households increased over 10% from 197,700 in 2005 to 221,100 in 2018 [7].

In forensic investigations, hair is one of the most common types of trace evidence of great evidentiary value. As the principle of exchange states that every contact leaves a trace, hair can be readily shed from the skin of both humans and animals, and transferred from crime scene to object, victim, suspect or vice versa during the commission of a crime through direct or indirect contact [8]. Dogs are the most popular pet globally [6], while domestic dogs, stray dogs as well as dogs with different key canine roles such as guide dogs, mobility assistance dogs, military and police dogs are also very common to be found near humans. As dogs possess hair that is easily shed and is stable to withstand external conditions, dog hairs are frequently recovered from exhibits submitted to the forensic laboratory. While human hair is well-known for its morphological and micrometric features to permit a high degree of individualization, the microscopic analysis of dog hairs should also be studied in a forensic context in order to contribute to forensic investigations and trials by providing information such as breeds and establishing links between suspect, victim, and a crime scene as associative evidence.

In the court of law, the significance and importance of dog hair examination and comparison were not well-established as human hair due to a perceived absence of morphological variation upon which to make a valid comparison [9]. Although the principles of human hair comparison are generally the same for dog hair, there were only limited studies on the morphology and micrometry of dog hair with the aim to differentiate and identify breeds [10-13]. In 2015, morphological and micrometrical differences in Labrador and German shepherd were reported when comparing to Husky, Bichon Maltese and Golden retriever [10]. However, the descriptive observations and quantitative data obtained by micrometry in this study could not lead to significant differences that allowed identification of one breed by simple hair examination [10]. A study in 2018 investigated dog hairs collected from 10 different common types of dog breeds such as Saint Bernard, French Mastiff, English Cocker Spaniel, Golden Retriever, Rottweiler, Caucasian Shepherd, German Shepherd, Pitbull, Chow Chow and Siberian Husky [11]. The medullary index ranged between 0.43 to 0.5 and the cuticular index ranged between 0.2 to 0.33 [11]. It was concluded that the thickness of hair, diameter of medulla and cortex, and other important microscopic characteristics were useful parameters to differentiate dog breeds [11]. In 2017, the medullary pattern of different dog breeds was reported to exhibit distinct patterns: lattice medulla pattern in Pomeranian, German Shepherd, Dalmatian and French Mastiff,

ladder medulla pattern in Doberman and Pug, simple continuous medulla in Golden Retriever, Saint Barnard and Labrador, and discontinuous medulla in Boxer [12]. However, a study in 2021 reported different findings on medullary pattern in which German shepherd, Golden Retriever, Labrador, Dalmatian, French Mastiff, Rottweiler, Doberman, and Saint Barnard showed uniserial ladder medulla pattern [13]. The above two studies also showed significant variations in the micrometry of some common breeds, such as the average diameter of shaft and medulla, while the medullary indices were the same [12, 13].

Recent research indicated that the popularity of dog breeds is dependent upon the region as different regions may have different beliefs, cultures, histories and lifestyles [14]. As Hong Kong is a heavily crowded city with small living spaces and growing populations, the most popular breed was found to be Shiba Inu, which is considered as a small-sized dog, weighing an average of 16 to 20 pounds [14]. A survey conducted in 2018 by a local pet food company also reported Poodle, Pug, Shiba Inu and Pomeranian as the most common dog breeds in Hong Kong [15], as these small-sized dogs are more suited to living in small apartments. Examination of dog hair is important and highly informative in many forensic investigations. However, most of the previous studies involving morphology and micrometry of dog hair were performed on large-sized dog breeds, such as Husky, Rottweiler and Doberman, while these dog breeds are not common in Hong Kong. Therefore, to make a significant contribution to the database of dog hair which is applicable to crowded cities with small living spaces, the aim of this study was to examine and compare dog hair of different small-sized dog breeds through morphological and micrometric analysis for differentiation and identification. In this study, hair was collected from Poodle, Shiba Inu, Miniature Schnauzer and Pomeranian, the most common dog breeds in Hong Kong. Using a scanning electron microscope (SEM) and a compound microscope, morphological characteristics (hair colour, cuticle pattern, medullary pattern, cuticle margin, medullary margin, and distance between cuticle margins) and micrometric characteristics (hair shaft diameter, medulla diameter, average cuticle scale length, medullary and cuticular indices) were determined. As dog hair provides strong basis for association, the findings from this preliminary study provided information of distinguishing characteristics that could be used to differentiate and identify common small-sized dog breeds, which is of very great value to forensic scientists. Further studies involving larger numbers of small-sized dog breeds and subjects will be carried out.

II. METHODS AND MATERIAL

Collection of hair sample

Hair samples from four different dog breeds (Poodle, Shiba Inu, Miniature Schnauzer, and Pomeranian) were collected randomly from neighbourhood. Written consent was taken from the owner of the dog before hair collection. All hair samples were collected from healthy dogs without any skin diseases. The age of the dog breed was between 6 months to 8 years to minimize the risks of infections and allergies. All hair samples were taken from the dorsal portion of the dog's body by the method of cutting. Hair samples were put in a labelled plastic zip-lock bag to avoid damages and contamination, and then stored at room temperature.

Laboratory examination

1. Compound microscope

A compound microscope with a magnification of eyepiece 10X and objective lens 10X was used to examine the overall hair morphology such as hair colour. Two to three hair samples from each dog were mounted on a microscope slide and a cover slip was then placed on top of the hair. Each mounted slide was labelled by marker and examined appropriately.

Microscopic characteristics such as medullary pattern, medullary margin and medulla diameter were determined.

2. Scanning electron microscope

Cuticle features of hair such as hair shaft diameter, cuticle pattern, cuticle margin, distance between cuticle margins and cuticle scale length were investigated using a scanning electron microscope with high resolution. Two to three hair samples from each dog were mounted rigidly on a sample stub using a conductive adhesive tab. Samples were then sputter-coated with gold to increase the electrical conductivity and stability before performing examination. Magnification of 1000X to 3000X was used depending on the hair shaft diameter. Medullary index and cuticular index were then calculated for each hair sample.

III.RESULTS AND DISCUSSION

As a preliminary study, hair samples were collected from four small-sized dog breeds commonly found in Hong Kong. Using photographs taken with a scanning electron microscope and a compound microscope, microscopic and micrometric features of the hair samples were assessed, e.g., hair colour, medullary pattern, medullary margin and medulla diameter, hair shaft diameter, cuticle pattern, cuticle margin, distance between cuticle margins and cuticle scale length. Results of morphological examination of hair samples collected from the four different small-sized dog breeds are shown in Table 1. Micrometric measurements of the hair samples are also reported in Table 2. In this study, variations in medulla patterns and cuticle patterns were demonstrated between the four small-sized dog breeds. Hair samples from the same dog in the breed of Poodle and Miniature Schnauzer also showed different types of medulla pattern, which suggested further studies to be performed.

Medulla, the innermost layer of hair, can provide information of the family and species of animals. The diameter and pattern of medulla are also useful in species and breed identification and comparison. The average diameter of hair shaft was found to be highest in Pomeranian (64.75μm) and lowest in Poodle (13.54μm). To calculate the medullary index which is a big distinguishing feature between human and animal hair, the diameter of medulla is divided by the diameter of the hair shaft. Medullary index of the collected dog hair samples was found between 0.53 (Pomeranian) and 0.66 (Poodle). These micrometric measurements, which showed a very small variation, confirmed that the samples were animal hair as the measurements were over 1/3. Previous studies reported medullary index in some large-sized dog breeds: 0.58 (Saint Bernard), 0.59 (Doberman), 0.52 (Labrador Retriever), 0.54 (Dalmatian), 0.54 (Golden Retriever) and 0.55 (German Shepherd) [12]. Preliminary results showed that there was no statistical difference in the medullary index of dog hair samples collected from large and small-sized dog breeds. Distinct medulla patterns were also observed in the collected hair samples. A photomicrograph collection that shows the medulla patterns found in different dog breeds is shown in Figure 1. The most common pattern was interrupted as this pattern was observed in Poodle, Miniature Schnauzer and Pomeranian. Only hairs of Shiba Inu showed the medulla to be uniserial ladder, which could be considered a distinctive characteristic of this breed. In the breed of Poodle, medulla was absent in the hair sample collected from another dog (sample #3) due to the thin hair shaft with the lowest average diameter of hair shaft of 13.54μm. In the breed of Shiba Inu, medulla could not be observed in the hair sample collected from another dog (sample #5). This hair sample was opaque under optical microscope probably because of the melanin pigment present in the cuticle, cortex and medullar layers. Hence, depigmentation should be performed prior to microscopic analysis for better visualization.

Furthermore, hair samples from the same dog in the breed of Poodle and Miniature Schnauzer showed different types of medulla pattern. For example, the dog in the breed of Miniature Schnauzer had broken (interrupted) medulla in one hair sample, while another hair sample exhibited continuous type medulla. This added challenges in differentiating and identifying the dog breeds, which suggested further studies to be performed.

Cuticle, which is the outermost layer of hair shaft, has distinct pattern, margin and arrangement of scales that can be used for species characterization and identification. In the hair samples collected from four dog breeds, several distinct cuticle patterns were observed. Poodle exhibited coronal and regular wave patterns in cuticle, while Miniature Schnauzer and Pomeranian showed cuticle with irregular wave pattern, as shown in Figure 2. Elongate petal pattern was only demonstrated by hairs of Shiba Inu, while this cuticle pattern was rarely reported in previous studies. A study published in 2021 showed that elongate petal pattern in the cuticle was not observed among 42 dogs of different dog breeds [13]. Therefore, the elongate petal cuticle pattern observed in Shiba Inu in this study could be a distinctive feature of this breed. There was also a variation in the distance between cuticle margins between dog breeds. Two types of cuticle margin (near and distant) were observed, while close type was not observed. Distant margins were observed in Poodle, Miniature Schnauzer and Shiba Inu, whereas near margins were only observed in Pomeranian as shown in Figure 3.

According to our preliminary study and observations, variations in medulla patterns and cuticle patterns were demonstrated between the four small-sized dog breeds. The most common medulla pattern was interrupted as this pattern was observed in Poodle, Miniature Schnauzer and Pomeranian. Only hairs of Shiba Inu showed the medulla to be uniserial ladder. For cuticle patterns, it was common to see coronal and regular wave patterns in Poodle, while Miniature Schnauzer, and Pomeranian showed cuticle with irregular wave pattern. Again, only hairs of Shiba Inu showed the cuticle to be elongate petal which was a pattern that was rarely reported in previous studies. Therefore, if a hair sample has uniserial ladder in medulla and elongate petal pattern in cuticle, there is a high possibility that the hair sample comes from a dog in the breed of Shiba Inu, as these mentioned morphological features could be considered distinctive characteristics of this breed. Overall, the data collected in this preliminary study could not be considered sufficient to distinguish different small-sized dog breeds because of the small sample size. Further studies involving larger numbers of small-sized dog breeds and subjects will be carried out.

Sample #	Breed (sex)	Hair color	Medullary pattern	Medullary margins	Cuticle pattern	Cuticle margins	Distance between cuticle margins
1	Poodle (F)	white	broken (interrupted)	fringed	coronal	smooth	distant
2	Poodle (F)	brown	Type 1: broken (interrupted) Type 2: broken (fragmented)	scalloped	regular wave	smooth	distant

3	Poodle (F)	white	absent	none	coronal	smooth	distant
4	Miniature Schnauzer (M)	light gray	Type 1: broken (interrupted) Type 2: continuous	fringed	irregular wave	crenate	distant
5	Shiba Inu (M)	black	cannot be observed	cannot be observed	elongate petal	dentate	distant
6	Shiba Inu (M)	brown and white	uniserial ladder	scalloped	elongate petal	dentate	distant
7	Pomeranian (M)	white	broken (interrupted)	fringed	irregular wave	crenate	near

Table 1: Results of morphological examination of hair samples collected from four different small-sized dog breeds in Hong Kong.

Sample #	Breed (sex)	Hair shaft average diameter (μm)	Medulla average diameter (μm)	Average cuticle scale length (μm)	Medullary index	Cuticular index
1	Poodle (F)	53.42	35.00	12.57	0.66	0.24
2	Poodle (F)	49.08	30.26	12.21	0.62	0.25
3	Poodle (F)	13.54	-	8.06	-	0.60
4	Miniature Schnauzer (M)	32.78	19.67	14.49	0.60	0.44
5	Shiba Inu (M)	18.95	-	17.49	-	0.92
6	Shiba Inu (M)	55.05	30.49	27.81	0.55	0.51
7	Pomeranian (M)	64.75	34.24	14.53	0.53	0.22

Table 2 : Micrometric measurements of hair samples collected from four different small-sized dog breeds in Hong Kong.
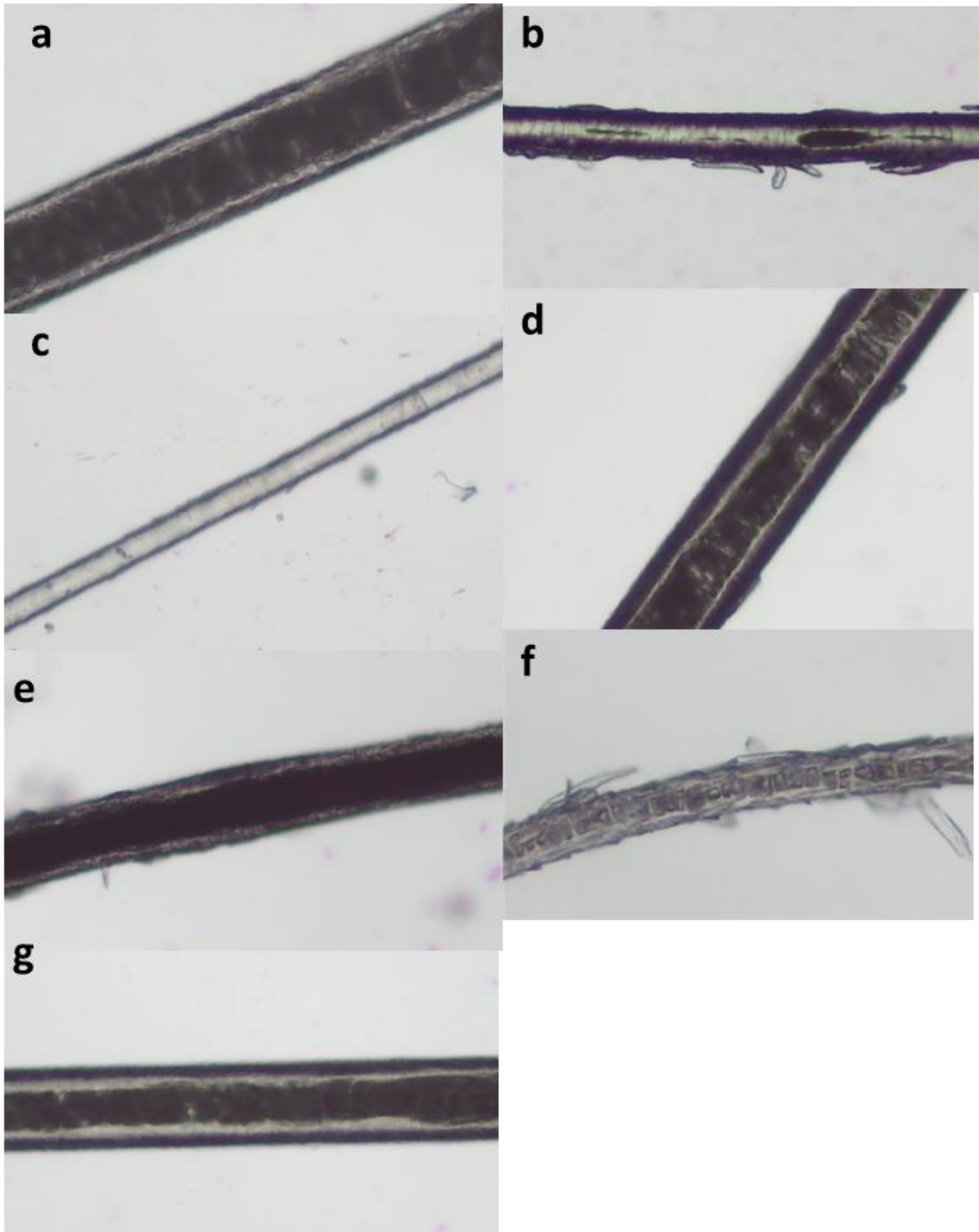
Figure 1. Medullary patterns in hair samples collected from four different small-sized dog breeds in Hong Kong using a compound microscope at 100X magnification.
(a) broken (interrupted) medulla (Poodle), (b) broken (fragmented) medulla (Poodle), (c) absent medulla (Poodle), (d) broken (interrupted) medulla (Miniature Schnauzer), (e) continuous medulla (Miniature Schnauzer), (f) uniserial ladder medulla (Shiba Inu), (g) broken (interrupted) medulla (Pomeranian)
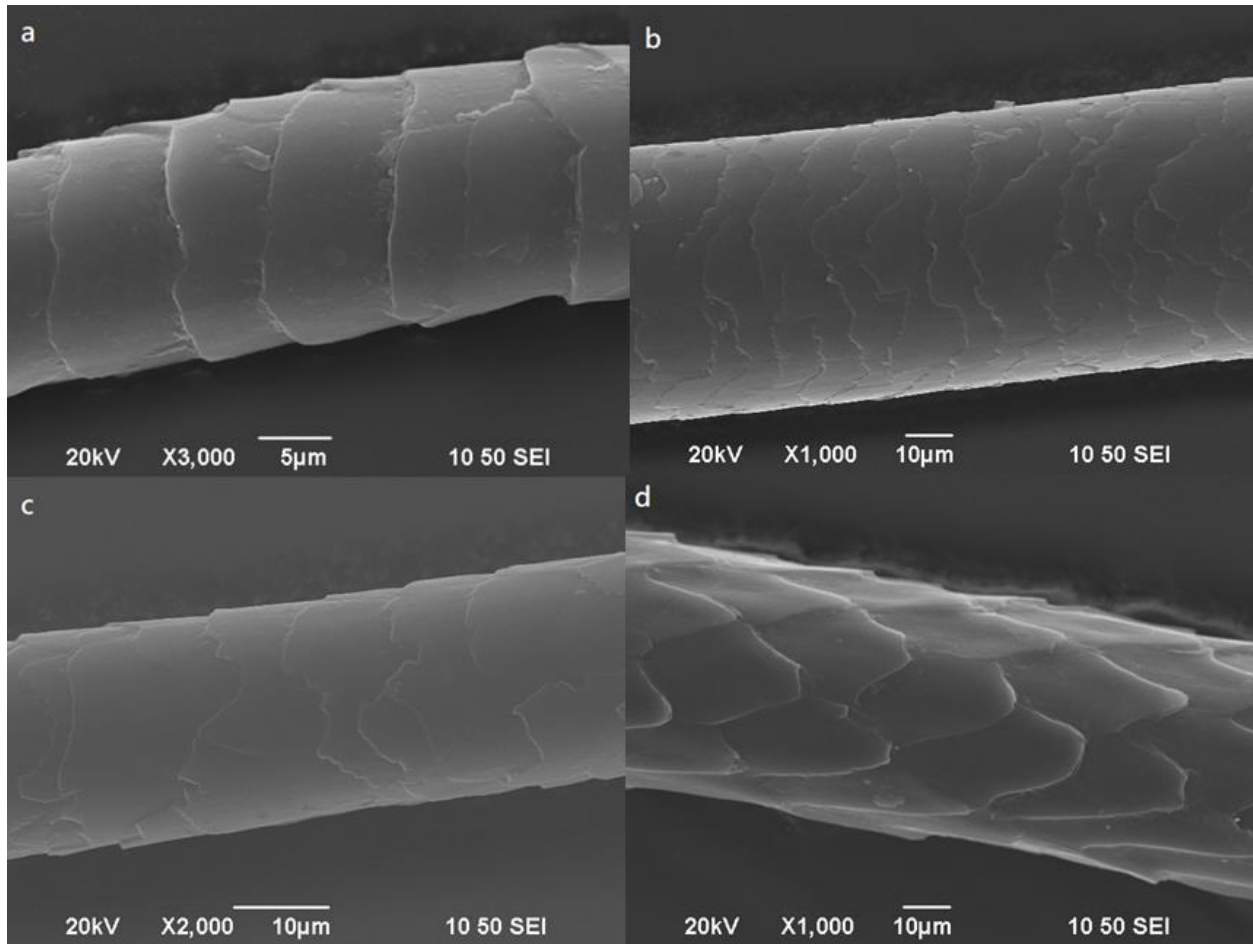
Figure 2. Cuticle patterns in hair samples collected from four different small-sized dog breeds in Hong Kong using a scanning electron microscope at 1000X to 3000X magnification.
(a) coronal (Poodle), (b) irregular wave (Pomeranian), (c) irregular wave (Miniature Schnauzer), (d) elongate petal (Shiba Inu)
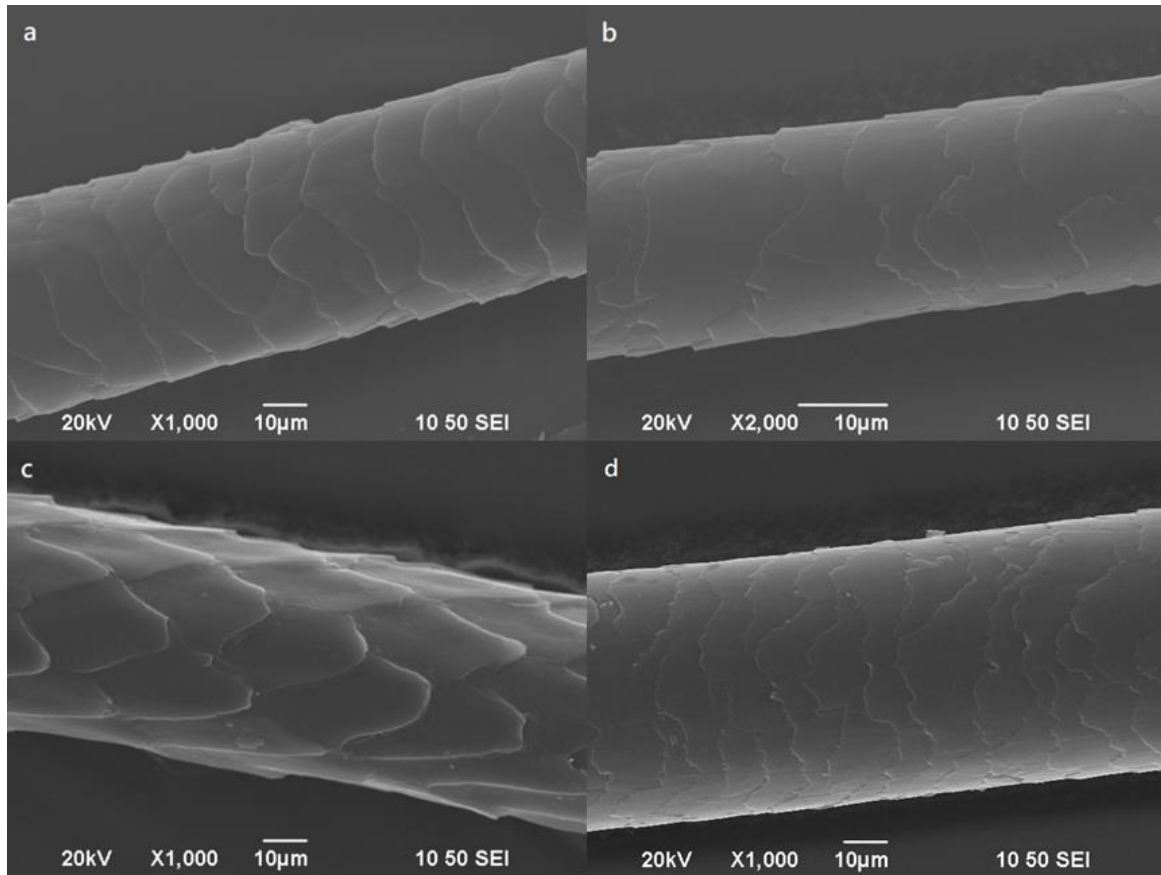
Figure 3. Distances between cuticle margins in hair samples collected from four different small-sized dog breeds in Hong Kong using a scanning electron microscope at 1000X to 2000X magnification. (a) distant margins (Poodle), (b) distant margins (Miniature Schnauzer), (c) distant margins (Shiba Inu), (d) near margins (Pomeranian)

IV. CONCLUSION

There is a recent exponential growth of pet ownership in Hong Kong, probably due to unaffordable housing prices. Not only pet dogs, domestic dogs, stray dogs as well as dogs with different key canine roles such as military and police dogs are also very common to be found near humans. As a result, dog hairs are frequently recovered from exhibits submitted to the forensic laboratory. Examination of the dog hair is important and highly informative in many forensic investigations by providing information such as breeds and establishing links between suspect, victim, and a crime scene as associative evidence. This was the first study to investigate the morphological and micrometric differences among hair collected from small-sized dogs that are commonly found in homes with small living spaces. Although micrometric data did not offer significant differences among the four small-sized dog

breeds, variations in medulla patterns and cuticle patterns were demonstrated between the dog breeds and could be used to make distinction from large-sized dog breeds. Only hairs of Shiba Inu showed the cuticle to be elongate petal and the medulla to be uniserial ladder, whereas these patterns were rarely reported in previous studies. Therefore, these morphological features could be considered distinctive characteristics of this breed. As a preliminary study, the findings suggested that the morphology and micrometry of dog hair could provide information to differentiate and identify small-sized dog breeds through hair sampling, which is useful for forensic investigations and trials.

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Cite this article as :

Deejay S. Y. Mak, Wendy K. W. Lai, "Preliminary Forensic Study of Hair Morphology and Micrometry of Small-Sized Dog Breeds", International Journal of Scientific Research in Science and Technology (IJSRST), Online ISSN : 2395-602X, Print ISSN : 2395-6011, Volume 9 Issue 2, pp. 170-178, March-April 2022. Available at doi : https://doi.org/10.32628/IJSRST229226

Journal URL : https://ijsrst.com/IJSRST229226