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Full Paper

Hair characteristics of Indonesian Suidae: database for forensic identification

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Abstract: Animal hair examination may provide valuable information on forensic investigations. However, there are limited data on the hair histology of Indonesian mammals for serving as a database for identification. The present study provides histological analyses of the dorsal guard hairs of the Indonesian Suidae species *Sus scrofa, S. barbatus, S. verrucosus* and *S. celebensis*, which were examined from the collections of Museum Zoologicum Bogoriense, National Research and Innovation Agency. Comparative analysis was performed by light and scanning electron microscope. Different features of each animal species' hair were identified based on the morphology, cuticle scale pattern, type of medulla, pigmentation and cross-section characteristics. The results show that the cuticle scale pattern is an irregular wave in all species. The cuticle scale margin is rippled, and the distance between scales is close. The hair medulla is amorphous in all species. The hair pigmentation is yellowish-reddish in all species, but no pigmentation of hair is found in *S. verrucosus*. The cross-section shape of the hair shaft is different in all Indonesian Suidae species, which can be used for identifying the species of Indonesian Suidae.

Keywords: Suidae, Indonesia, hair, cross section, medulla, cuticle

INTRODUCTION

Mammalian hair acts as a protector and regulator of mammalian body temperature. There are two types of hair in mammals: guard hair and fine hair. Guard hair or protective hair has a thick and straight structure, and is generally located on the dorsal part of the hair. Fine hair is usually curly and thin. Each strand of hair has three parts: cuticle, cortex and medulla [1].

Mammalian hair is a biological material that is easy to collect, move and keep for a very long time without losing its quality or ability to reveal the owners' identities [2]. Because of their

Maejo Int. J. Sci. Technol. **2023**, *17*(03), 177-186

capability, hair can be used in many fields of study including archaeology, forensics, scat analysis, season analysis, geographic mobility assessment, and environmental toxicology [3-6]. Modern techniques widely used for hair identification are through biomolecular approach [7]; however, this method is pricey and takes a long time. Another method that can be used is the microscopic analysis method, which is cheap and fast to analyse, so it can save time and money [8]. Macroscopic and microscopic structures are widely used for examining hair and investigating the adaptation of animals to living conditions. Each animal has a different hair structure, size, shape, colour, medulla and cuticle. Hair cuticle scales have various forms and dimensions so they can be used as distinguishing characters in identification [9]. In forensic cases, a hair sample is sometimes the only evidence found at the crime scene. Without the presence of animals, animal hair can be used as evidence of hunting. Microscopic examination of hair can identify the type of animal being hunted [10].

One of Indonesia's most hunted ungulates is the Suidae, sold in traditional markets and sometimes adulterated as ‘halal‘(lawful) products to the muslim consumers. The identification of illicit hair-based products of Suidae origin is sometimes needed. The microscopic hair structure of some ungulates in Southern Europe has been reported [1]. However, there is not much information about the hair of tropical mammals [10-13], particularly Indonesian Suidae [14].In this study Indonesian Suidae hair was analysed through macroscopic and microscopic parameters to determine the character of each species and build the identification key for Indonesian Suidae.

MATERIALS AND METHODS

Sample Collection and Preparation

Dorsal guard hairs of Indonesian Suidae species, namely *Sus scrofa* (n= 5), *S. barbatus* (n= 5), *S. verrucosus* (n= 5) and *S. celebensis* (n= 5) were taken from the skin collection at the Museum Zoologicum Bogoriense, National Research and Innovation Agency, Indonesia. The skin collections ranged from 20 years to 40 years old. Only samples from adult specimens were collected as they provide reliable features [15]. Approximately 5-10 hair samples were cut up to the base of the skin. The collected hairs were immersed in acetone for 5 min. to remove dirt and sticky non-hair materials.

Sample Examination

Dorsal guard hair samples were analysed by identifying the structure, colour, texture and length of the hair shaft based on Teerink [15]. Cuticle scales and cross sections were investigated using a scanning electron microscope (Jeol, Japan). Hair samples were divided into base, middle and tip parts. Each part of the hair was placed in a stub horizontally to identify the scale cuticle and vertically to identify the cross section, coated with gold, and analysed under a scanning electron microscope (JSM IT 200, Jeol, Japan) at 1000x magnification [16].

The collected hairs were placed on a glass slide, mounted with a drop of glycerin and covered by a coverslip. Images were acquired at 100x magnification using a light microscope (Euromex, Holland). In this study we analysed the hair medulla and cortex pigmentation. All measurements of length and width were done using ImageJ ver. 1.53 [17].

Maejo Int. J. Sci. Technol. **2023**, *17*(03), 177-186

RESULTS AND DISCUSSION

Hair Morphology

In the current study the dorsal guard hairs of the four Indonesian Suidae species are collected from the animal skin museum collection. Although the specimens are as old as 40 years, they can still be used for investigation [18]. The hair structures of all species are straight but curved with a rough texture and have branches at the tip part of the hair shaft in all samples (Table 1, Figure 1). There is no variation in the morphological structure of Suidae hair. In comparison, the Suidae dorsal hair colour varies depending on the species. *Sus scrofa* has two variations: the first, black gradation at the base and dark brown at the tip; the second, black at the base, white at the middle before the hair branch, followed by dark brown colour from the base of the hair branch to the tip of the hair shaft (Table 1, Figure 1A). The dorsal hair colour of *S. verrucosus* has four variations of hair colour combinations. There are black without gradation; red at the base, dark brown at the tip; white at the base, light brown at the tip; and dark brown at the base and black at the tip (Table 1, Figure 2A). *S. celebensis* has a consistent plain black colour (Table 1, Figure 1C), while *S. barbatus* has a hair colour that is at first glance similar to *S. scrofa*, with black gradation at the base and broken white from the middle to the tip. However, some are found to be dark brown at the tiny part of the tip of the hair branch (Table 1, Figure 1D). Compared to *S. scrofa*, the broken white colour of *S. barbatus* appears more dominant. The variation in hair colour determines the overall appearance of the pig's colour. For example, *S. celebensis* appears black while *S. barbatus* is black and white. Hair length shows that on average, the hair of *S. verrucosus* tends to be 57.4% longer than other Suidae hairs. However, the hair length of *S. barbatus* is 36.6% shorter than other Suidae hairs (Table 1).

The morphology of *S. scrofa* hair in this research is similar to that in earlier studies [1, 14]. Variations were found in hair colour, length and diameter. Some factors influence those parameters, including part of the body, habitat, temperature, hormones, age, sex and nutrition [19-23]. Therefore, a single characteristic is not accurate for investigation.

Hair Cuticle Scale

The outer part of the hair shaft is called the cuticle, composed of uncoloured layers of scale of various types and positions [24]. In the present study the cuticle scale positions are transversal and the scale types are irregular waves in all species. They ripple at the base to the tip of the hair shaft with close distances between scales (Table 1, Figure 2). No differences are found in the cuticle type, position, margin or distance between the scales in any Indonesian Suidae. A similar result on the Suidae hair cuticle was shown by Lee et al. [25], with the *S. scrofa* hair cuticle showing an irregular wave. Keogh [18] and Meyer et al. [26] reported that cuticle type was not influenced by sex but by body size as it correlated with the insulation process in the animal bodies. The present study shows that the distances between scales were close in all of the species studied. However, Nason [24] reported that numerous factors such as hair width, papilla cell activity and the drying process of skin-derived hair would influence the distance between scales. Thus, the distance between scale is insufficient for identification.

Maejo Int. J. Sci. Technol. **2023**, *17*(03), 177-186

Table 1. Morphology comparison of four Indonesian Suidae species

Feature	*S. scrofa*	*S. verrucosus*	*S. celebensis*	*S. barbatus*
		Morphology		
Colour	- black at the base, broken white at the middle, and dark brown at the tip - black at the base and dark brown at the tip	- black without gradation - red at the base and dark brown at the tip - white at the base and light brown at the tip - dark brown at the base and black at the tip	black without gradation	black at the base to the middle part, and broken white at the tip
Texture	rough	rough	rough	rough
Structure	straight, curve	straight, curve	straight, curve	straight, curve
Hair length (mm)	33.52 ± 6.93	48.23 ± 8.05	21.37 ± 3.97	19.39 ± 3.75
		Cuticle		
Scale position	transversal	transversal	transversal	transversal
Scale pattern	irregular wave	irregular wave	irregular wave	irregular wave
Structure of scale margin	rippled	rippled	rippled	rippled
Distance between scale margin	close	close	close	close
		Medulla based on compound microscope		
Composition	amorphous	amorphous	amorphous	amorphous
Structure	amorphous	amorphous	amorphous	amorphous
Margin	amorphous	amorphous	amorphous	amorphous
		Cross section		
Shape	circular-oval-triangular (tip)	circular-oval-triangular (tip)	circular-triangular (base, middle, tip)	circular-oval-triangular (tip)
Medulla shape	- base: none - middle: none - tip: flower shape	- base: none - middle: none - tip: oval	- base: circular-oval - middle: circular-oval - tip: circular-oval	- base: flower shape - middle: flower shape - tip: flower shape
		Hair diameter		
Size (µm)	- base: 311.08 ± 37.10 - middle: 328.09 ± 37.90 - tip: 322.69 ± 41.08	- base: 226.67 ± 30.68 - middle: 213.22 ± 18.82 - tip: 187.09 ± 30.29	- base: 218.46 ± 27.92 - middle: 251.71 ± 30.18 - tip: 252.02 ± 5.21	- base: 39.63 ± 4.67 - middle: 40.0 2 ± 4.68 - tip: 41.11 ± 5.21

Maejo Int. J. Sci. Technol. **2023**, *17*(03), 177-186

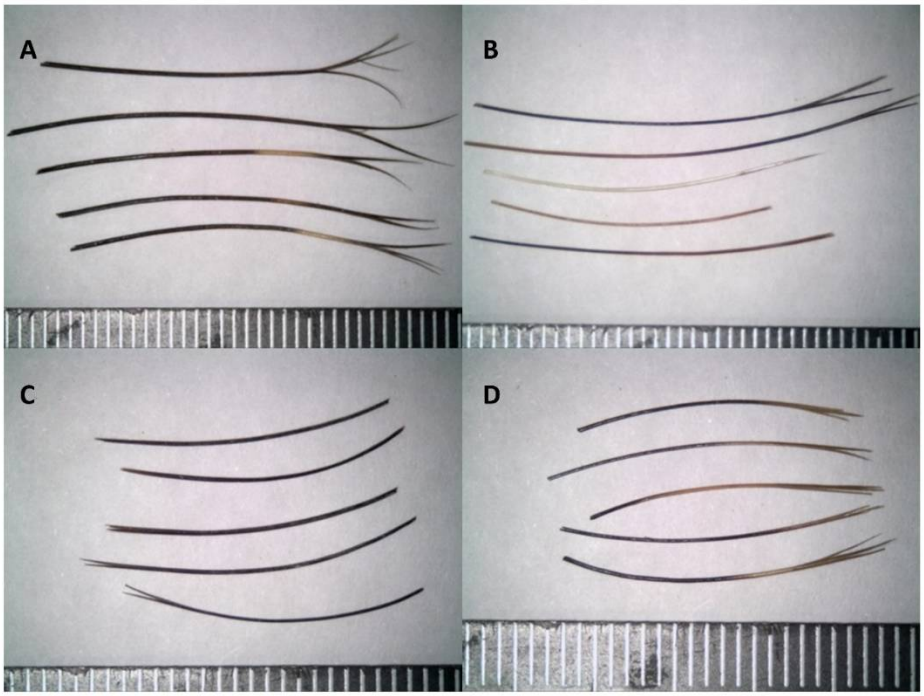

Figure 1. Morphology of Suidae hair. (A) *Sus scrofa*, (B) *S. verrucosus*, (C) *S. celebensis*, (D) *S. barbatus*. Scale ruler bars = 1,000 µm.

Hair Medulla and Cortex Pigmentation

All species show an amorphous-type medulla with dark colour and unclear cell composition (Table 1, Figure 3). The margins of the medulla are irregular. The cortex pigmentation is yellowish-reddish and darker near the medulla. However, in *S. verrucosus*, several hairs are found to be unpigmented with a transparent cortex and white features on the hair shaft. High pigmentation of the hair shaft sometimes makes the medulla analysis more difficult. Pigmentation in the cortex expresses the hair colour and eventually the coat colour. This study shows that the naked eye can distinguish the hair shaft colour among Indonesian Suidae. Although the hair colours of *S. scrofa* and *S. barbatus* are similar, the position of the band of the hair shaft is different. Similar characteristics of the hair medulla and pigmentation in young and adult *S. scrofa* in southern Europe were reported by De Marinis and Asprea [1]. However, differences between European and Indonesian Suidae occur in the base part of the hair shaft, which shows a discontinuous pattern in the former but forms a continuous pattern in the latter. This correlates with the thermal insulation function of the medulla, where tropical animals might require a longer or wider medulla to adapt to the environment [27].

In all species, the medulla structure is crucial for identifying species at the family level [1, 27, 28] but not at lower levels such as subfamily, genus or species. The medulla structure does not give a good result at these levels due to the high resemblance among species. Therefore, it must be combined with other parameters.

Based on compound microscope results, Indonesian Suidae has an amorphous medulla structure, continuous throughout the hair shaft and has an irregular margin, similar to earlier study of *S. scrofa* by De Marinis and Asprea [1] and Desai et al. [29]. However, the finding contradicts that of Sharma et al. [11], which reported *S. scrofa* as having unicellular medulla with a straight margin. In contrast, Sari and Arpacik [30] reported that the structure of *S. scrofa* medulla was

Maejo Int. J. Sci. Technol. **2023**, *17*(03), 177-186

multicellular. On the other hand, Chernova [27] reported that Suiformes have no medulla. All of these contradicting results might be due to fact that the medulla is too dark and unclear to analyse. This has resulted in the medulla feature giving inaccurate identification of the Suiformes family.

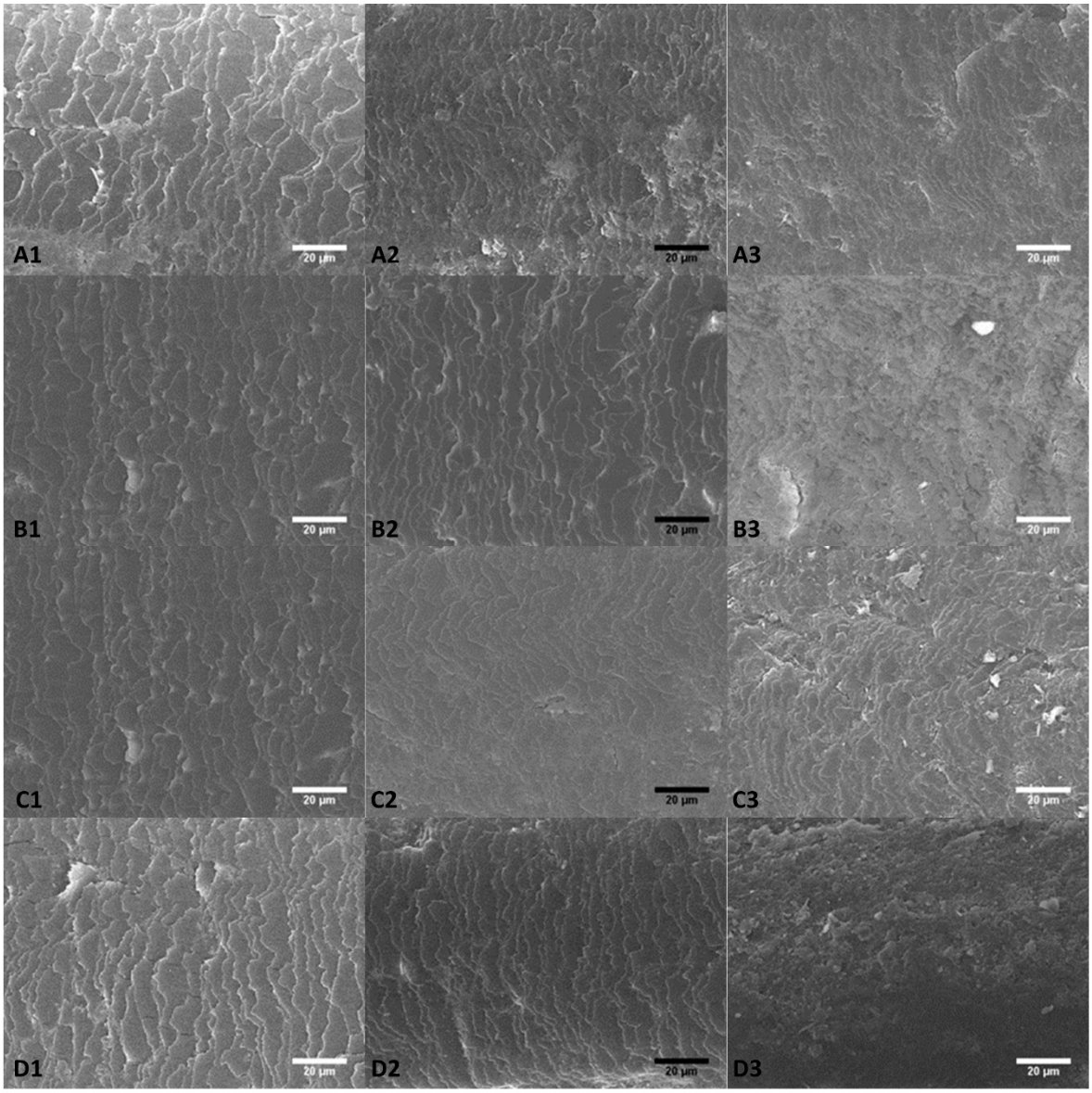

Figure 2. Hair cuticle of Indonesian Suidae hair species. (A) *Sus scrofa* (1. base, 2. middle, 3. tip), (B) *S. verrucosus*, (C) *S. celebensis*, (D) *S. barbatus*. Scale bars = 20 µm.

Maejo Int. J. Sci. Technol. **2023**, *17*(03), 177-186

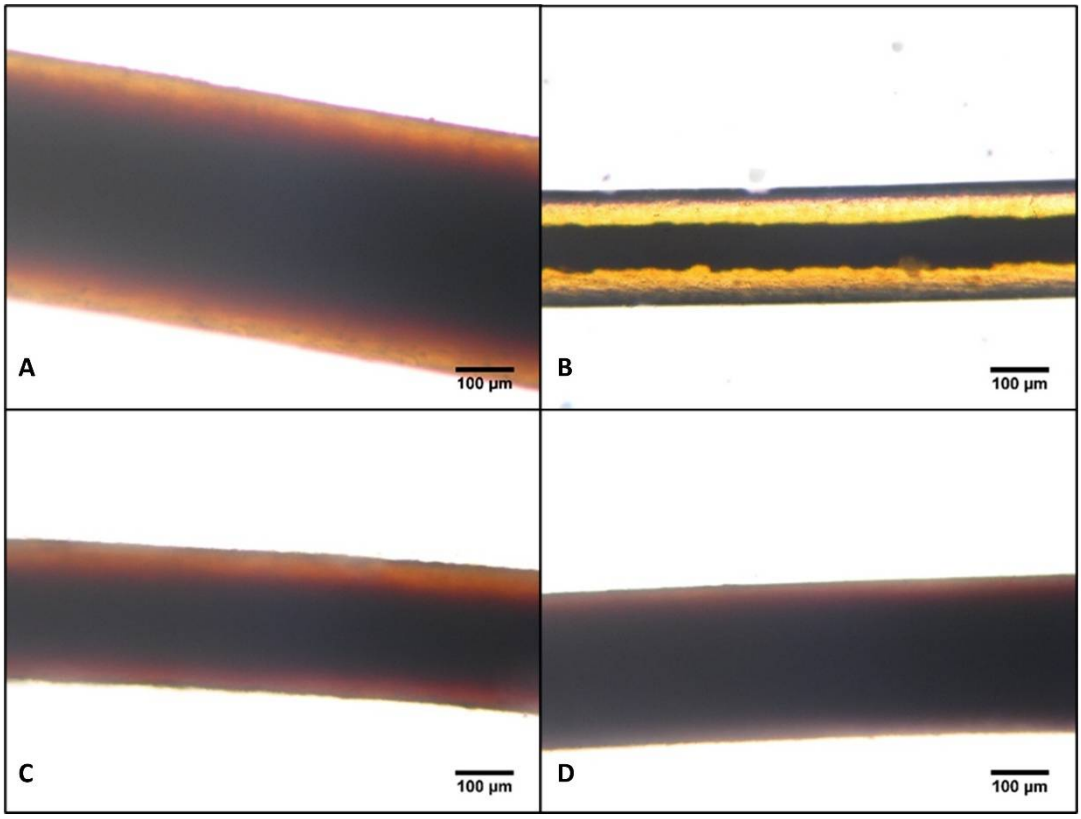

Figure 3. Hair medulla and cortex pigmentation of Suidae. A) *Sus scrofa*, B) *S. verrucosus*, C) *S. celebensis*, D) *S. barbatus*.

Hair Cross Section

The cross section of hair is rarely investigated in hair morphology studies. However, using the cross section of hair shaft in this study, the differences among Indonesian Suidae species are distinct. Chernova [27] reported that there was no medulla in Suiformes. However, through the cross section, our study shows that the medulla is present in all species with different shapes and parts of the hair shaft. The shape of the outer part of the hair is oval for *S. scrofa*, circular for *S. verrucosus* and *S. barbatus*, and triangular for *S. celebensis*. The outer and inner parts of the cross section (medulla) show a cavity with different shapes (Figure 4). Based on the cross-section data, there are no cavities in the base and middle parts of *S. scrofa* and *S. verucossus* hair shafts. *S. scrofa* has a flower-shaped cavity at the tip part of the hair shaft, whereas in *S. verucossus* it shows a circular to oval-shaped cavity. In comparison, *S. celebensis* and *S. barbatus* have cavities in all parts of the hair shaft. The *S. celebensis* hair shaft cavity is circular to oval in shape and *S. barbatus* has a flower shape cavity (Figure 4). Although the present results provide comparative data on hair morphology of four Indonesian Suidae species, almost all of them cannot be used for species-level identification. The only strong indicator is the cross section, which may be used to distinguish among Indonesian Suidae species.

Maejo Int. J. Sci. Technol. **2023**, *17*(03), 177-186

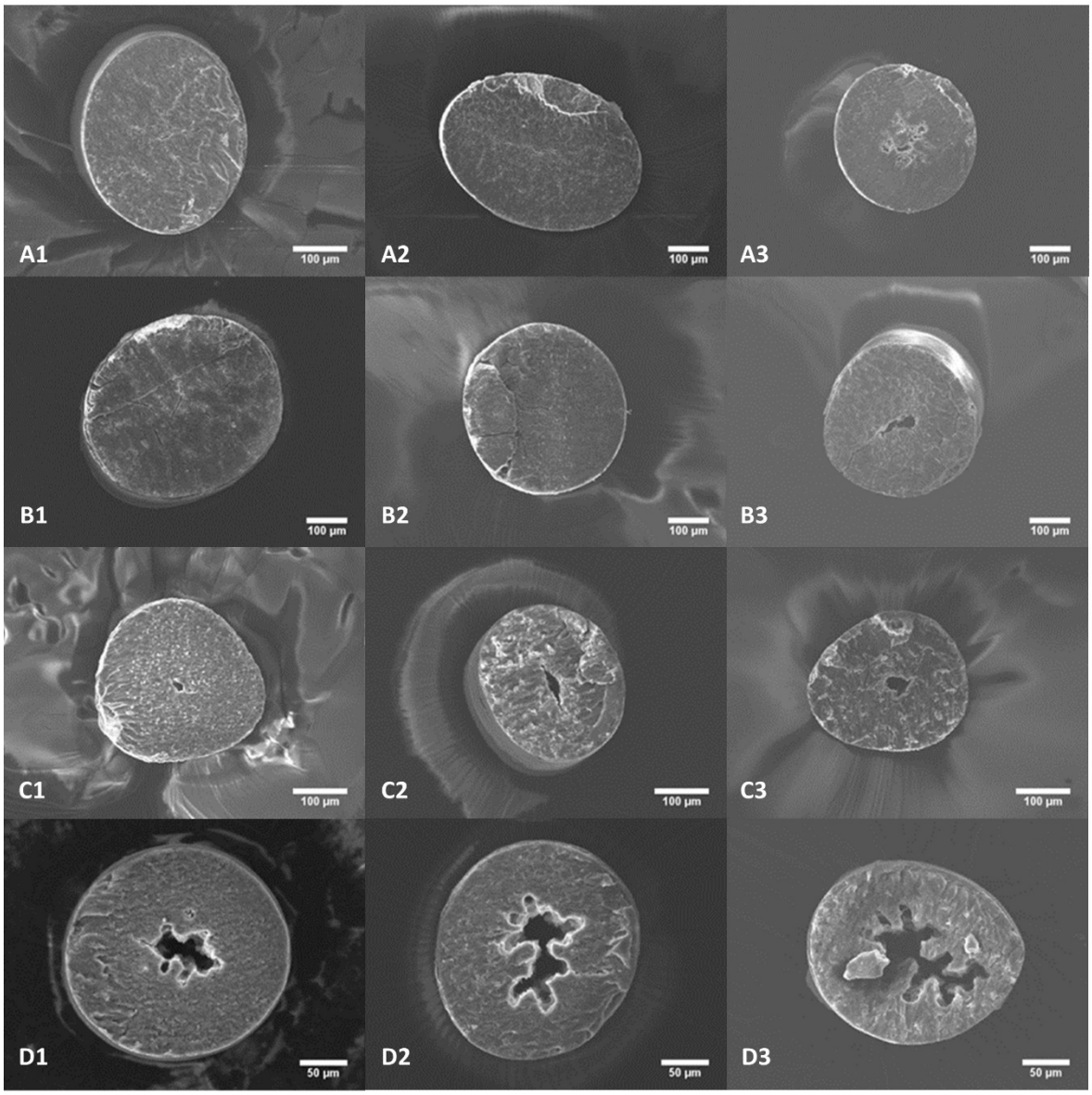

Figure 4. Cross section of Indonesian Suidae hair shaft. (A) *Sus scrofa* (1. base, 2. middle, 3. tip), (B) *S. verrucosus*, (C) *S. celebensis*, (D) *S. barbatus*. Scale bars A-C = 100 µm, D = 50 µm.

CONCLUSIONS

The morphological structures of the four species of Indonesian Suidae are similar to one another. However, differences are found in the cross section of the hair shaft. Observing several features to differentiate among the hair of the four Suidae species is essential to obtain specific characteristics at the species level.

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