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Full Paper

Indonesian Bovidae hair characters: macroscopic and microscopic analyses

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Abstract: There are four wild bovid species in Indonesia, banteng (*Bos javanicus*), mountain anoa (*Bubalus quarlesi*), lowland anoa (*Bubalus depressicornis*) and Sumatran serow (*Capricornis sumatraensis*). The macro- and microscopic features of hair can be used to identify animals. Here, we identified the hair characteristics of Indonesian Bovidae in tropical environments. Hair samples of three wild bovid species, each with five individuals, were collected from the Museum Zoologicum Bogoriense, the National Research and Innovation Agency. Hair samples of domesticated cattle (*Bos taurus*) were collected from local livestock farms. The hair structure, cuticle scale, medulla structure and cross section were evaluated. The results show that hair structures of all four species vary slightly among species. *B. javanicus* and *B. taurus* were straight to curved, whereas *C. sumatraensis* and *B. quarlesi* were straight to wavy. The medulla structures of *B. javanicus, B. taurus* and *C. sumatraensis* were similar, with a lattice with multicellular composition and a continuous pattern. In *B. quarlensi* the structure was amorphous with a discontinuous and fragmented pattern. Therefore, the medullary index can be used for species differentiation.

Keywords: Bovidae, hair structure, *Bos javanicus, Bubalus quarlesi, Bos taurus, Capricornis sumatraensis*

INTRODUCTION

Indonesia has a high level of biodiversity, with 773 mammalian species found throughout the country, many of which are endemic [1]. Bovids are mammals that are widely distributed in Indonesia, with four endemic species: banteng (*Bos javanicus*), mountain anoa (*Bubalus quarlesi*), lowland anoa (*Bubalus depressicornis*) and Sumatran serow (*Capricornis sumatraensis*). The first three are categorised as endangered and the last one as vulnerable according to the International Union for Conservation of Nature Red List [2-4] and all of them are listed in the Convention on International Trade in Endangered Species of Wild Fauna and Flora Appendix II [5]. Indonesia also has a large population of domestic ungulates such as cows (*Bos taurus*), buffaloes (*Bubalus bubalis*), goats (*Capra hircus*) and sheep (*Ovis aries*) [1].

The wide distribution of Bovidae in Indonesia has allowed these animals to adapt to their environment. However, as a tropical area, it has a similar temperature range during the dry and wet seasons, with a few exceptions in a mountain landscape structure. Hair is part of the bodies of animals, and its function is to provide effective insulation and thermoregulation as part of their adaptation. Moreover, the behavioural adaptation of mammals to survival in their environment is also represented in their hair structure [6-8].

Scientifically, the hair structure of mammals is a useful material in zoology, morphology, phylogenetics, taxonomics, archaeology, forensics and other sciences. It also contributes to studying the evolution and domestication of various mammals. The macro- and microscopic features of hair can be used to identify species and analyse their important role in animal adaptation to the local environment [9, 10]. In this study, which is connected to our previous work [11], the hair characteristics of three Indonesian wild bovid species and a domesticated species were identified.

MATERIALS AND METHODS

Sample Collection and Preparation

Hair samples from three Indonesian wild bovid species, *Bos javanicus*, *Bubalus quarlesi* and *Capricornis sumatraensis*, each with five individuals, were obtained from the Museum Zoologicum Bogoriense, National Research and Innovation Agency. In addition, hair samples from five individuals of domesticated bovid, *Bos taurus* (cattle) were collected from a local livestock farm. Guard hairs were cut at the base of the skin in the dorsal region. The samples were labelled and stored in plastic zip-lock bags to prevent cross-contamination. In the laboratory the hair was washed with acetone for 5 min. to remove dirt.

Sample Examinations

The morphological properties of hair, including colour, shape and length, were examined under a light microscope (IScope 1153-EPL, Euromex, Germany) at 100x magnification. The cuticle scale and medulla structure were evaluated based on Teerink [12]. For cross-section examination, hairs were cut transversely using a freezing microtome (Yamato RV-240, Japan) and analysed based on Dreyer [13]. The medulla was studied by mounting the hair on a glass slide with glycerin and covering it with a coverslip. Dark hairs were bleached using a commercial hair bleacher for one hour before observation. Images were captured using the light microscope at 400 x magnification. Hair length, hair diameter and medulla diameter were measured using ImageJ ver. 1.53 [14]. The medullary index (medullary thickness/hair thickness) was calculated. Data were analysed using one-way ANOVA in SPSS software ver.25.

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RESULTS AND DISCUSSION

Hair Morphology and Morphometry

The four Bovidae show different hair colouration. The hairs of *B. javanicus*, *C. sumatraensis* and *B. taurus* have a colour gradation, with *B. javanicus* hairs being black on males and brownish on females but both being lighter to the tip, while *B. taurus* and *C. sumatraensis* hairs being brownish but darker to the tip (Figure 1, Table 1). The results of this study are similar to those for other domesticated bovids: *Capra hircus* and *Ovies aries* [15]. In contrast, *B. quarlesi* was unicoloured (black). The pelage of *B. javanicus* adults is sexually dimorphic; therefore, male and female coat colours differ. Animal hair colour is determined mostly by genetics; nevertheless, certain exogenous or environmental influences can also play a vital role. Exposure to UV light, for example, can cause photobleaching of dark hair or photolysing of white hair, especially when combined with humid conditions and/or high temperatures [16]. The adaptation of animals to the environment is also influenced by coat colour. Animals with black coats absorb more thermal radiation and are more prone to heat stress than animals with lighter coats [17].

The hair structures of all four species were slightly different: *B. javanicus* and *B. taurus* were straight to curved, whereas *C. sumatraensis* and *B. quarlesi* were straight to wavy (Figure 1, Table 1). Straight and wavy (undulated) types of hair are one of the features of bovids, according to Koppikar and Sabnis [18] and De and Chakraborty [19]. This indicates a different adaptation between animals living at high and low altitudes. *B. javanicus* and *B. taurus* live at low altitudes between 2 to >600 m asl [20], while *B. quarlensi* lives at > 1000 m asl [2], and *C. sumatraensis* lives in mountain forests [21]. The long and wavy hair of highland bovid species represents an adaptation form of the animals to protect them from low temperatures at high altitudes. Longer hairs allow more air to be trapped in the hair coat, thereby providing higher insulation [22]. Moreover, dense hair can minimise heat exchange with the environment as a response to low temperatures [8]. Other factors such as diet, health and environment may influence hair length. Eurasian otter (*Lutra lutra*), which live in captivity with unvaried feed, has shorter hair than wild ones [23]. However, in this study *B. taurus*, the domestic cattle, has longer hair than wild *B. javanicus.* Although the environment is important for hair development, genetics is the most influential.

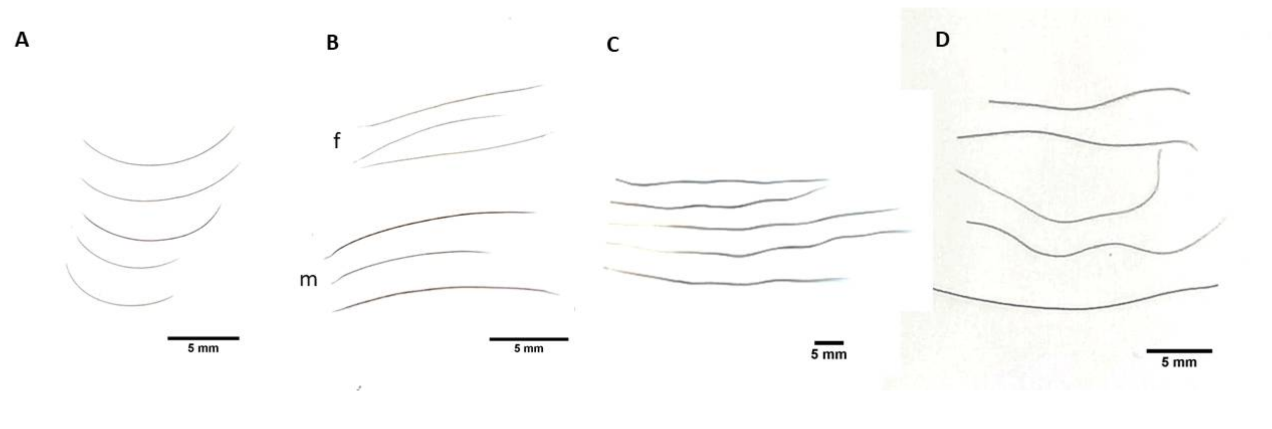

Figure 1. Macroscopic characters of hair: (A) *Bos taurus*, (B) *Bor javanicus*, f= female, m= male, (C) *Capricornis sumatraensis*, (D) *Bubalus quarlesi*. Scale bars = 5 mm.

Table 1. Hair morphology of Indonesian Bovid

Species	Morphological observation			
	Texture	Structure	Colour	Thickness (μm)
Bos taurus	Smooth	Curve	Base: Light brown Middle: Light brown Tip: Brown	Base: 47.5±13.3 Middle: 49.68±16.41 Tip: 50.9±15.0
Bos javanicus	Smooth	Straight	**Female:** Base: Dark brown middle: Light brown Tip: Beige **Male:** Base: Black Middle: Black Tip: Dark brown	Base: 88.4±19.7 Middle: 90.4±18.8 Tip: 64.4±15.1
Capricornis sumatraensis	Rough	Straight to wavy (undulated)	Base: White Middle: Dark brown-black Tip: Dark brown	Base: 141.5±22.9 Middle: 151.4±23.1 Tip: 114.3±22.6
Bubalus quarlesi	Smooth	Straight to wavy (undulated)	Black, no gradation	Base: 60.6±8.0 Middle: 54.91±8.06 Tip: 45.1±11.4

Yang et al. [24] reported that hair diameter significantly depends on species. *C. sumatraensis* has the largest hair diameter and *B. taurus* has the smallest (Table 1). Hair diameter is correlated with hair strength; animals in denser habitats tend to have larger hair diameters to protect them from friction. Moreover, hair diameter is influenced by body parts, sex and age. These correlate with testosterone production in adult male animals, which promotes secondary sexual characteristics in males to produce a greater hair diameter. Thick hair acts as a barrier against injuries sustained during physical conflict and male aggression [25].

Hair Cuticles

The cuticle design serves specific purposes and is engaged in the mechanical protection of the hair. The glossier the hair is, the less the mutual friction, and the softer the hair cover becomes, the less indented and smoother the hair scale profile [26]. All wild bovid species in this study show a regular-wave scale structure of cuticles with smooth-rippled margins, whereas *B. taurus* shows regular waves with smooth margins (Figure 2, Table 2). Regular and irregular wave structures are commonly found in ungulates such as the Cervidae and Suidae families [27, 28]. Domestication may change the hair structure, not only the length and diameter but also the cuticle structure. Meyer et al. [29] reported that domestication influences the important and distinctive properties of the hair shaft and indirectly the hair-producing follicle system. De Marinis and Asprea [30] reported that in wild animals the cuticle scale structure is influenced by age but not in domestic animals. One of the

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most evident results of domestication in various groups of animals is the retention of juvenile characteristics into adulthood.

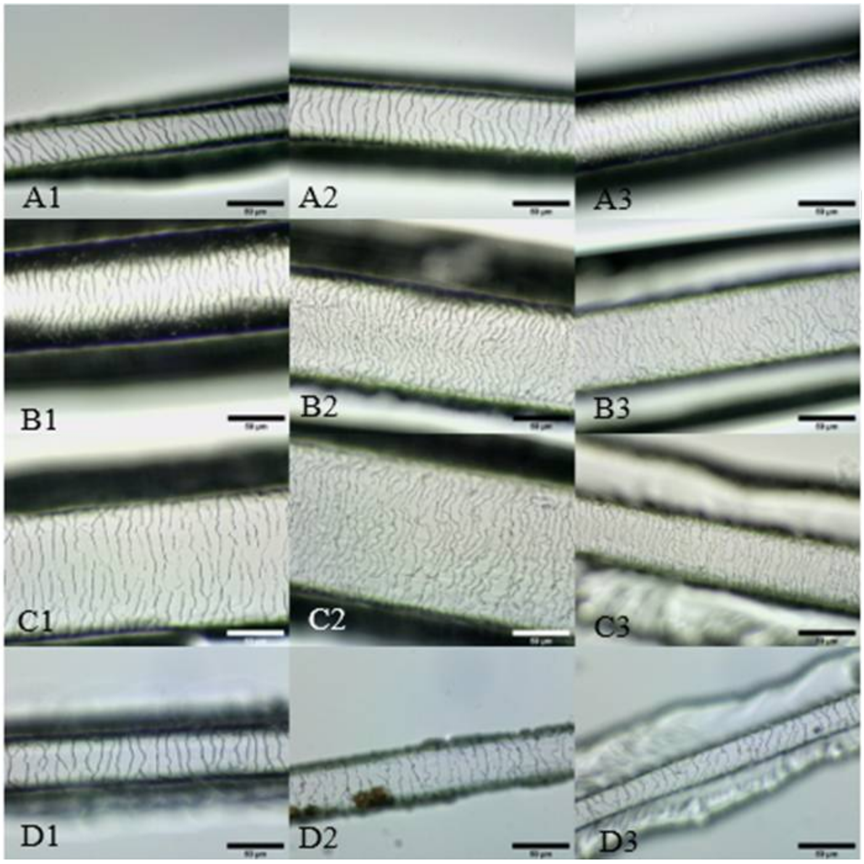

Figure 2. Scaled cuticle structure of hair: (A) *Bos taurus*, (B) *Bos javanicus*, (C) *Capricornis sumatraensis*, (D) *Bubalus quarlesi*; (1) Base, (2) Middle, (3) Tip. Scale bars = 50 µm.

Table 2. Hair cuticle characteristics of Indonesian Bovid species

Species	Cuticular characteristic				
	Scale position	Scale margin	Scale distance	Scale pattern	Scale count every 100 µm
Bos taurus	Transversal	Smooth	Near	Regular wave	Base: 11.5±1.4 Middle: 12.4±1.3 Tip: 15.2±2.1
Bos javanicus	Transversal	Smooth-rippled	Close	Regular wave - irregular wave	Base: 13.2±1.5 Middle: 15.3±1.7 Tip: 16.3±1.6
Capricornis sumatraensis	Transversal	Smooth-rippled	Close	Regular wave - irregular wave	Base: 10.7±1.5 Middle: 13.6±2.5 Tip: 17.0±2.5

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Table 2. (Continued)

Species	**Cuticular characteristic**				
	Scale position	**Scale margin**	**Scale distance**	**Scale pattern**	**Scale count every 100 µm**
Bubalus quarlesi	Transversal	Smooth – rippled	Near	Regular wave – irregular wave	Base: 11.5±0.9 Middle: 11.2±1.0 Tip: 11.6±0.5

Hair Medulla

The medulla is a hollow, cellular central core that runs through the centre of the cortex (Figure 3, Table 3). It may have a fragmented, continuous or discontinuous structure. The primary function of the medulla is to provide protection and temperature stability by creating internal air gaps. The medulla is also associated with adaptability. The adaptation mechanism through medulla structure was reported by Quadros et al. [31], who showed differences among neotropical marsupials in various living habitats. Species with the ladder medulla are often arboreal animals, associated with a reduction in fur width. Species with letter-like medullas are related to species living in forests, which may represent an adaptation related to heat absorption and thermoregulation.

In this study all species have a letter-like medulla with heavy hair and are adapted to forest-living habitats. The medulla structure of *B. javanicus*, *B. taurus* and *C. sumatraensis* is lattice with a multicellular composition and a continuous pattern. This structure is commonly found in other Bovidae [15], whereas in the Cervidae family, the medulla is wider and more compact in composition [32]. The structure of *B. quarlensi* medulla is amorphous with a discontinuous and fragmented pattern (Figure 3, Table 3). Its structure and pattern are similar to those of another Bubalus species from Philippines, as Santiago-Flores et al. [33] reported for *Bubalus bubalis*. The variation in medullary structure among the three Bovidae families indicates that Bubalus has a different type of medulla from that of the other two genera. The medullary structure and cuticular pattern in domestic goats and sheep do not change with age. This shows that the medulla structure can be used for species identification.

The medullary index of Indonesian Bovidae is between 0.1-0.51, with the lowest value in *B. quarlensi* and the highest in *B. javanicus*. One-way ANOVA shows significant difference ($p < 0.05$) among the medullary indices of the four Bovidae species. In this study the medullary indices of *C. sumatraensis* and *B. taurus* are smaller than those of similar species in India [15]; it was reported that high-altitude species had a greater medullary index than low-altitude species. However, our study found the opposite result. *B. quarlesi* has a low medullary index although it lives at high altitudes and likes to wallow [34]. Sokolov [35] reported that the medulla provides a heat insulator to keep the animals warm under low-temperature conditions, including in water. However, other factors may influence heat insulation in each species.

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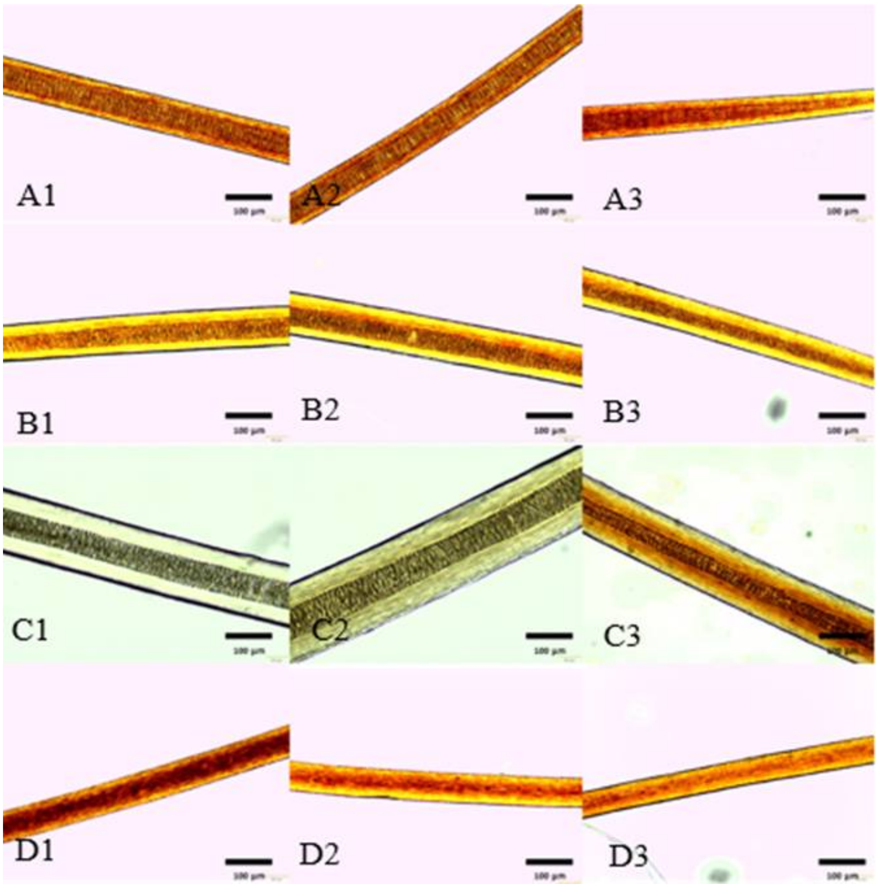

Figure 3. Structure of hair medulla: (A) *Bos taurus*, (B) *Bos javanicus*, (C) *Capricornis sumatraensis*, (D) *Bubalus quarlesi*; (1) Base, (2) Middle, (3) Tip. Scale bars = 100 µm.

Table 3. Hair medulla characteristics of Indonesian Bovid species

Species	Medullar characteristic				
	Medulla composition	**Medulla structure**	**Medulla pattern**	**Medulla margin**	**Medullary index**
Bos taurus	Multicellular	Lattice	Continuous	Scalloped, straight	Base: 0.50±0.09 Middle: 0.44±0.10 Tip: 0.40±0.03
Bos javanicus	Multicellular	Lattice	Continuous	Scalloped, straight	Base: 0.51±0.13 Middle: 0.51±0.13 Tip: 0.41±0.12
Capricornis sumatraensis	Multicellular	Lattice	Continuous	Scalloped, straight	Base: 0.41±0.10 Middle: 0.34±0.12 Tip: 0.24±0.06
Bubalus quarlesi	Amorphous	Amorphous	Discontinuous	Straight	Base: 0.10±0.02 Middle: 0.10±0.004 Tip: 0.24±0.06

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Hair cross section

The cross-sectional shape of hair, medulla and features of pigment granules in the hair cortex, including colour and distribution, are shown in the cross-sectional data (Table 4, Figure 4). The pigmentation of hair can express the coat colour of the animal's body [36]. In this study the cross section of Bovidae hair differs among species. The pigmentation gradation of hair shows different pigment distributions in every part of the hair. Although almost all samples show pigmentation concentrated at the tip part of the hair, the opposite is true for *B. javanicus*. An animal's surface colouration can affect heat exchange with its environment and thermoregulatory demand. However, it is unclear whether animals with dark or light coats absorb more solar heat. There is no straightforward relationship between coat colour and ambient temperature. A darker coat may absorb more or less solar heat depending on a complex set of organismal and environmental factors typically independent of pelage or feather colour. Many of these characteristics differ between species and may be under an individual's behavioural control [37].

Table 4. Hair cross-section characteristics of Indonesian Bovid species

Species	Cross section	
	Pigmentation	**Shape**
Bos taurus	Base: Light brown Middle: Brown Tip: Brown	Base: Oval Middle: Oval Tip: Circular
***Bos javanicus* (male)**	Base: Dark brown Middle: Dark brown Tip: Light brown	Base: Circular Middle: Circular Tip: Circular
Capricornis sumatraensis	Base: White Middle: Black Tip: Dark brown	Base: Circular Middle: Circular Tip: Oval
Bubalus quarlesi	Base: Black Middle: Black Tip: Black	Base: Circular Middle: Circular Tip: Circular

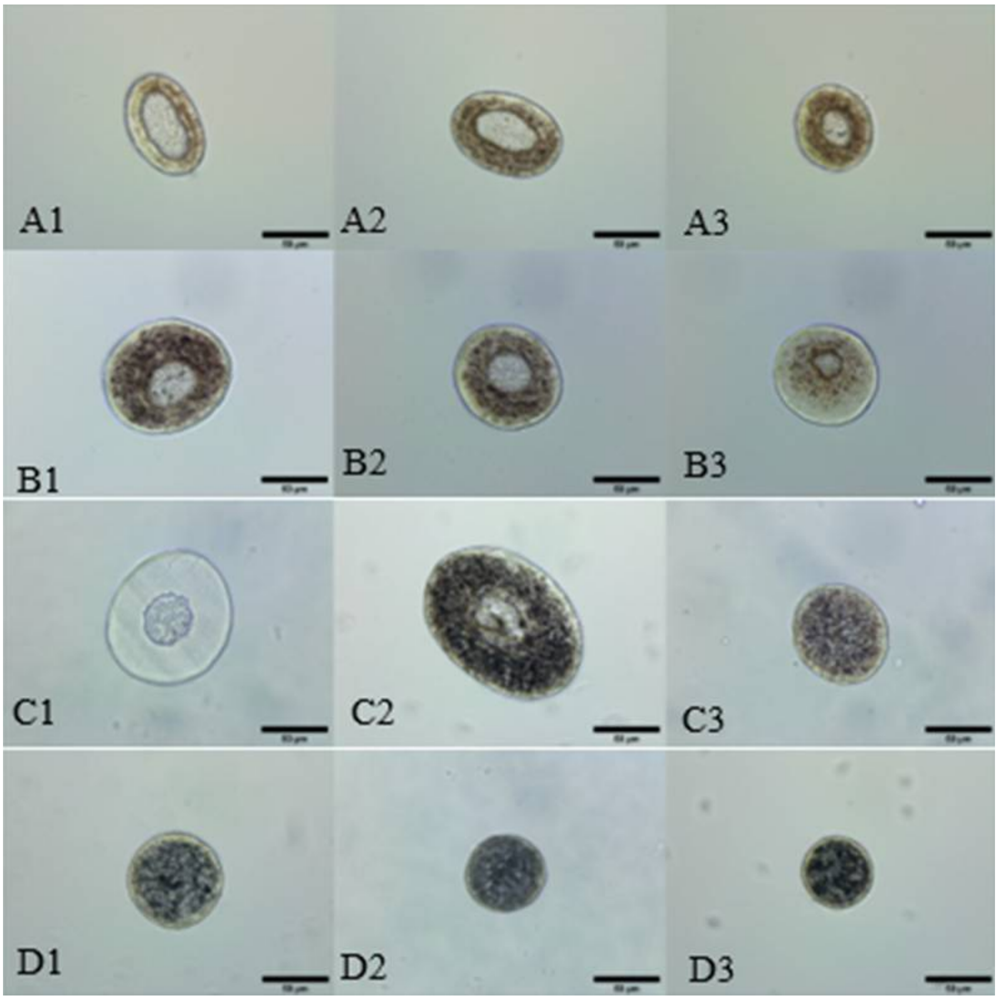

Figure 4. Cross section of hair: (A) *Bos taurus*, (B) *Bos javanicus*, (C) *Capricornis sumatraensis*, (D) *Bubalus quarlesi*; (1) Base, (2) Middle, (3) Tip. Scale bars = 40 µm.

The cross-sectional shape of mammalian hair was reported to be associated with animal behaviour. For example, in terrestrial mammals, the cross section of the hair shaft is commonly circular or oval, whereas in semi-aquatic mammals it is elongated [22]. In semi-aquatic mammals the elongated structure of hair cross section allows them to dry their hair rapidly. However, several terrestrial mammals show elongated hair cross sections, such as *Rusa timorensis*, *Gazella bennettii*, *Hemitragus hylocrius*, *Hemitragus jemlahicus*, *Nilgiritragus hylocrius* and *Ovis ammon* [15, 32, 38]. They might need flexible hair to support their daily life movements. Mohan et al. [39] reported that hair with elongated structure has higher flexibility than that with circular structure. In this study *B. taurus* has an elliptic cross section of hair shape in the base and middle parts of the hair, whereas *C. sumatraensis* has this hair shape in the middle part, which might be one of their adaptation forms to survive in their environment. *C. sumatraensis* also lives in rocky mountains and needs flexible hair to protect it from friction. However, genetics, evolution and environment can influence the hair characteristics of every species [40].

CONCLUSIONS

The hair characteristics of Bovidae species have significant implications for species identification. The species may be identified by their combination of macroscopic and microscopic hair characteristics. The morphological structure, morphometry and cross section of hair are adapted to the animals' environment and behaviour.

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