Microstructure of Quills in Sunda Porcupine *Hystrix javanica* (F. Cuvier, 1823) [Mikrostruktur Duri Landak Jawa *Hystrix javanica* (F. Cuvier, 1823)]

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Received: March 2020, **Accepted**: May 2020

ABSTRACT

The purpose of this study was to identify the quills type, cuticle pattern, cross-section feature, and medulla structure of the quills in Sunda porcupine (*Hystrix javanica*). The second aim was to determine the nutritional content of the quills in Sunda porcupine. The specimens fixed in cacodylate buffer and glutaraldehyde, then dehydrated through graded series of alcohol, and freeze-dried. The specimens attached to the stubs by sticky tape, coated with gold and observed with Scanning Electron Microscope (SEM). Determination of cuticle pattern and medulla structure base on to the mammal's hair identification key by Teerink (1991). Nutritional content analysis using proximate. The study result showed that *H. javanica* has four types of quills; true, transitional, flat spine, and rattle quills. The SEM micrograph of the cuticle pattern showed characteristic variation in the shaft and base region, except the flat spine has no scaly feature in the base region. The cuticle of anogenital quill and hair in *H. javanica* has no specific feature or osmetrichia. The cross-sectional images of the three type quills of *H. javanica* revealed circular and alveolar arrangement. Only the flat spine has a quadriconcave feature. The true quills have multicellular and reverse cloisonné structure in the medulla, compare to the other three quills have no pattern. Nutritional content of the quills were water (89.93%), crude protein (93.66%), crude fat (0.44%), phosphorous (0.034%), calcium (0.2%), magnesium (0.01%), and sulphur (2,01%).

Keywords: Cuticle pattern, medulla structure, nutritional content, quill, *H. javanica*

ABSTRAK

Tujuan studi ini adalah untuk mengidentifikasi tipe duri, pola kutikula, irisan melintang, dan struktur medula duri Landak Jawa (*Hystrix javanica*). Tujuan kedua dari studi ini adalah untuk mempelajari kandungan nutrisi dalam duri Landak Jawa. Spesimen difiksasi dalam larutan buffer cacodylate dan glutaraldehyde, kemudian dehidrasi melalui alkohol bertingkat, kemudian dehidrasi dikering-bekukan menggunakan freeze dry. Spesimen ditempel pada stub dengan selotip, kemudian dilapisi emas, dan diamati dengan *Scanning Electron Microscope* (SEM). Penentuan pola kutikula dan struktur medulla berdasarkan kunci identifikasi rambut mamalia oleh Teerink (1991). Analisis kandungan nurtisi dalam duri landak menggunakan proksimat. Hasil studi menunjukkan bahwa Landak jawa memiliki empat jenis duri yaitu duri sejati, duri transisi, duri pipih, dan duri derak. Gambar SEM pola kutikula menunjukkan variasi karakteristik pada bagian ujung maupun pangkal duri, kecuali duri pipih tidak menujukkan pola kutikula pada bagian pangkalnya. Kutikula pada duri dan rambut bagian anogenital Landak Jawa tidak memiliki struktur khusus atau osmetrichia. Irisan melintang pada ketiga jenis duri landak jawa memiliki bentuk circular dan alveolar. Hanya duri pipih yang memiliki pola kuadrikonkaf. Duri sejati memiliki struktur medulla multiselular dan *reverse cloisonne,* sedangkan tiga tipe duri lainnya tidak memiliki pola. Kandungan nutrisi pada duri landak jawa meliputi air (89,93%), protein (93,66%), lemak (0,44%), fosfor (0,034%), kalsium (0,2%), magnesium (0,01%), dan belerang (2,01%).

Kata Kunci: Pola **k**utikula, struktur medulla, kandungan nutrisi, duri, *H. javanica*

INTRODUCTION

Hair is biologically crucial in mammals. Mammal hair plays a vital role in thermoregulation, body shape maintenance, waterproofing, and protection from pollution. The characteristics of hairs are different/distinct among species (Lee *et al.* 2014). In certain mammals, some hairs modified into spines or quills and have a thick, hard, tapered, and pointed tips (Everson 2015). Spines or quills found in four major groups of living mammals: hedgehogs, tenrecs, echidnas, and rodents, including porcupines (Vincent & Owers 1986; Chernova 2002; Moore *et al*. 2003; Cho *et al.* 2012).

There are about 29 species of porcupines distributed throughout most areas in the world. They were divided into two leading families: Old World porcupine (Hystricidae, African, or Indian crested porcupine) and New World (Erethizontidae). The porcupine is a rodent covered with keratinous quills that serve as a defence

against by piercing and wounding predators (Vincent & Owers 1986; Chpaman & Roze 1997). A thousand quills cover every inch of its body, except for its face, belly, and the undersides of its limbs and tail (Cho *et al*. 2012; Roze 2006). Porcupine quills are different lengths over different parts of the body, fall out quickly, and break readily when bent (Everson 2015). Generally, quills made of keratin protein and matrix, which makes it very rigid and sharp (Roze 1989).

The species member of Hystricidae has a different type of quills, for instance, Cape porcupines (*Hystrix africaeustralis*) has several quills: spines, true quills, tactile bristles, transitional quills, and rattle quills (Barthelmess 2006; van Weers 1983). Spines are sharp, stiff, flattened, and grooved bristle hairs that cover most of the body. True quills, the primary means of defence, are thickest of modified hairs and carry very sharp points. Relatively inflexible, true quills are circular in cross-section; diameter is widest in middle of the length of quill and tapers toward ends (Barthelmess 2006). Tactile bristles are very flexible. Like true quills, tactile bristles are circular in cross-section, but the diameter is greatest near the base of the hair and is less than the typical diameter of a quill. Transitional quills are intermediate between true quills and tactile bristles in length, diameter, and flexibility. Rattle quills also are circular in cross-section but are capsule-shaped, hollow, and open at the end. Rattle quills attach to tail by a thin stalk at the base of quills (van Weers 1983).

In Sunda porcupine, the front half of the body is covered with short dark spines. The short tail has both long pointed quill and rattle quill. The body densely covered with flattened spines which are smaller along the tail and are more flexible on the underparts. Coarse bristle-like hairs cover the feet. The type and structure of quill and spine of *H. brachyura* have reported by Raha *et al*. (2015), and the previous studies reported that the quills of *H. javanica* are categorized into four types; true quills, transition quills, rattle quills, and flat spine (Sheila 2011). However, in particular, it is no record of cuticle medulla structure of each quill. We conducted a research to study the cuticle pattern on the shaft region and medulla structure of the quills in Sunda porcupine. Sunda porcupine quills used for the therapeutic purpose by Asian people as a pain killer during bone fracture and toothache. In relation to those matter, the other purpose of this study was to investigate the nutritional content of the quills in Sunda porcupine.

MATERIALS AND METHODES

The samples were collected after natural shedding of Sunda porcupine quills (*H. javanica*). We were performed an investigation/research from/using a male and female individuals in captive facilities at Research Center for Biology, Indonesian Institute of Sciences. The quills that fall out naturally were collected every week for three months. Different types of quills were thoroughly washed with a soft soap solution to eliminate unwanted dirt. After collection, the materials were preserved in air-tight ziplock and kept under refrigeration for future work. The quills cut into small pieces then washed in water containing a detergent rinsed sequentially in water, distilled water and stored in 70% alcohol. The specimens for SEM examination were post-fixed in cacodylate buffer and glutaraldehyde, then dehydrated through graded series of alcohol, and freeze-dried. The specimens were attached to the stubs by sticky tape, coated with gold and observed with Scanning Electron Microscope (SEM) (JSM-5310LV-1000, Jeol, Japan). Micrographs were taken at different magnification depend on the sample type, and the cuticle pattern of the shaft and medulla classified according to terminology in Teerink (1991). Nutritional content analysed using Proximate. Protein content determined by Macro-Kjeldahl method, gross energy determined by Bomb Calorimeter, crude fat measured using Soxtec system HT 6, and the content was determined by gravimetric. The minerals sulfur, calcium, and phosphorus content measured using Atomic Absorption Spectroscopy (AAS) (David 1960; Van loon 1980; Butcher 2013).

RESULTS

The result showed that *Hystrix javanica* has four types of quill, namely true quills, transitional quills, rattle quills, and flat spines

(Figure1). Most of quills profile is a straight (Table 1). True quill is the primary means of defence; it was quite thick, very sharp, and inflexible (Figure1a). Transitional quills are the longest quills (Figure 1b). Rattle quills are resembling a cup glass shape (Figure 1c). Flat spines were sharp, stiff, and flattened that cover most of the porcupine body (Figure1d).

The SEM images of cuticle quills of Sunda porcupine showed almost similar in the shaft region, but it has characteristic differences in the base region. The cuticle of true quills and transitional quills on the shaft regions has the same pattern: irregular wave, rippled margin, and distance between margin is close (Figure 2A-B). Rattle quills have a transitional pattern, smooth margin, and distant (Figure 2C). Flat spine and anogenital hair showed regular wave pattern and smooth margin but with a distinction in the scaly arrangement (Figure 2D-E). The true quill on the base region has the most different pattern than that of other quills. It has a longitudinal position and elongates petal pattern (Figure 3A), while transitional quill has a regular wave pattern (Figure 3B). However, the flat spine does not elicit any scaly feature even at higher magnification (Figure 3C). The rattle quill has an irregular wave pattern (Figure 3D).

The cross-section images of quills showed circular shape, it has a channel and network of holes (Figure 4A-C) except the rattle quills without a network of holes (Figure 4D), and the

Table 1. Macroscopic characters of quills of Sunda porcupine.

Profile	General appearance (colour)	Length (cm)
Straight	White with black stripe or white with black white stripes.	12.59 ± 1.94
Straight and undulate	White with black and white stripes	13.27 ± 4.31
Straight	Black, brown, or brown-white.	4.12 ± 0.25
Straight	Cup glass shape and white.	3.77 ± 0.37
Straight	White	3.75 ± 1.29

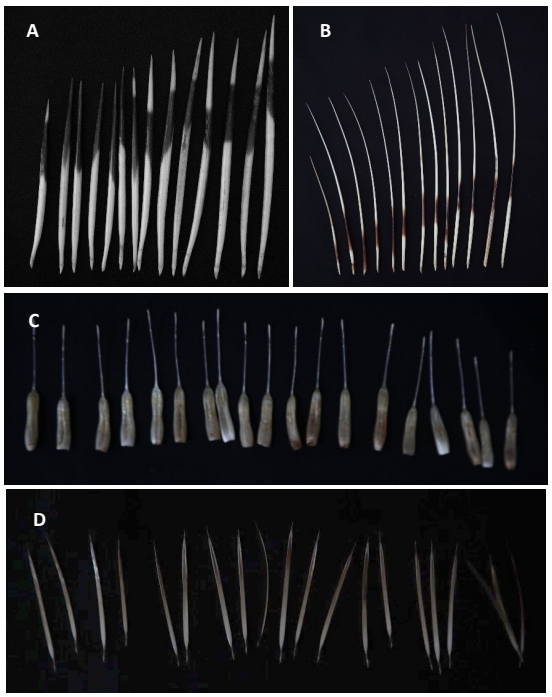

Figure 1. Quills feature of Sunda porcupine (*H. javanica*); true quills (A), transitional quills (B), rattle quills (C), and flat spines (D).

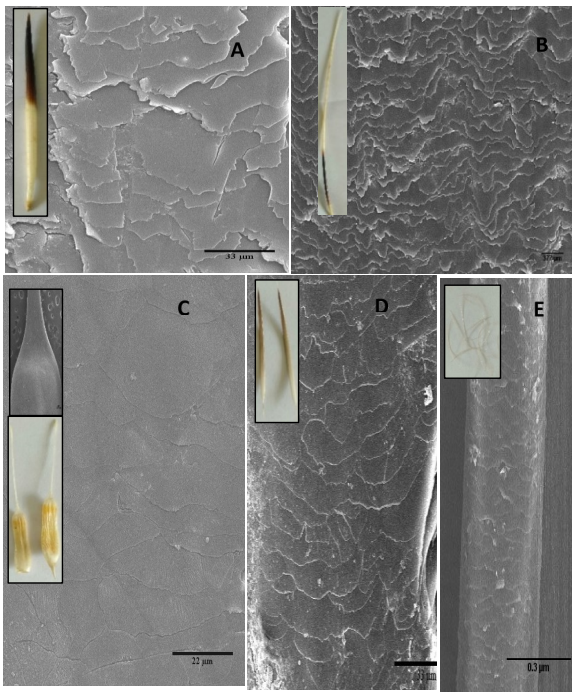

Figure 2. SEM micrographs of cuticle quills of the quill shaft of *H. javanica;* true quills (A), transitional quills (B), rattle quills (C), flat spine (D), and ano-genital quill (E).

flat spine showed quadri-concave shape (Figure 4E). The medulla structure showed multicellular and reverse cloisonne (Figure 5C) with longitudinal stringers. However, the medulla of both rattle quill and flat spine has a hollow shape (Figure 5B).

DISCUSSION

The cuticle microstructure of different type of quills reveals some variation. The variation of cuticle found on the quill shaft, the base (Tabel 2). However, no distinction on the microstructure and cross-sections of male and female of Sunda porcupine. The cuticle in porcupine quills points towards the root, which enhances the defensive function of the quill as a weapon (Rajaram & Menon 1986).

The cross-section showed that *H. javanica* quill has stiffeners attached to the cortex (Figure 6). Yang *et al.* (2013) reported that crested porcupine (*H. africaustralis*) also has the stiffener in the quill gradually extending towards the centre with foam filling the remaining area and surrounded by the cortex. The previous study also observed that cortex, stiffeners, and foam walls contain solid keratin (Yang *et al*. 2013).

The previous study reported that the quills of the lower back region in the North American porcupine (*Erethizon dorsatum*) has osmetrichia modifications for odour dispersal (Roze *et al*. 1991). Osmetrichia is a specialised scent hair for efficient storage pheromonal molecules (Müller-Schwarze *et al*. 1977; Raha *et al*. 2013). Osmetrichia found in African crested rat (Stoddart 1979). The

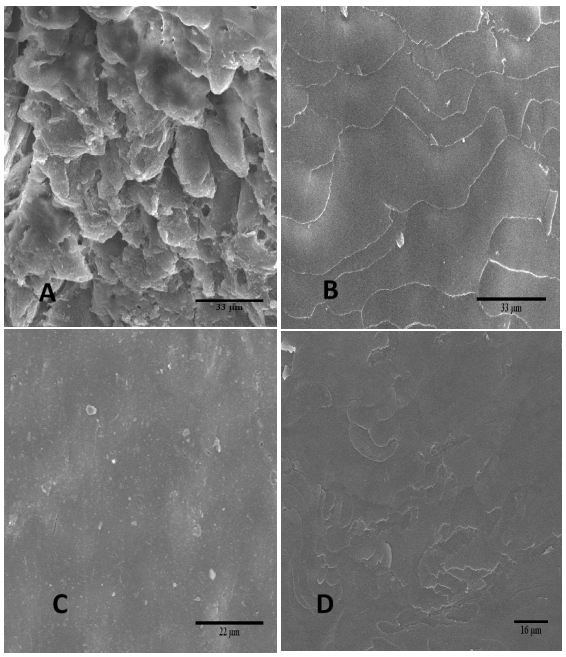

Figure 3. SEM micrographs of the cuticle of the quills base of *H. javanica;* true quills (A), transitional quills (B), flat spine (C) and rattle quills (D).

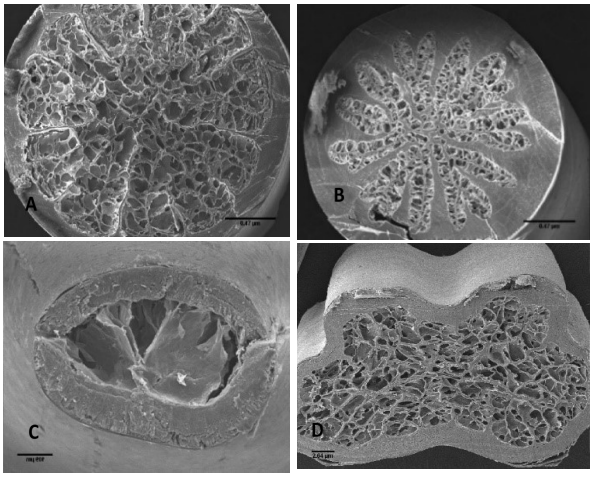

Figure 4. SEM micrograph of quills of *H. javanica* in cross-section; true quills, transitional quills (A) transitional quills (B), rattle quill (C), and flat spine (D).

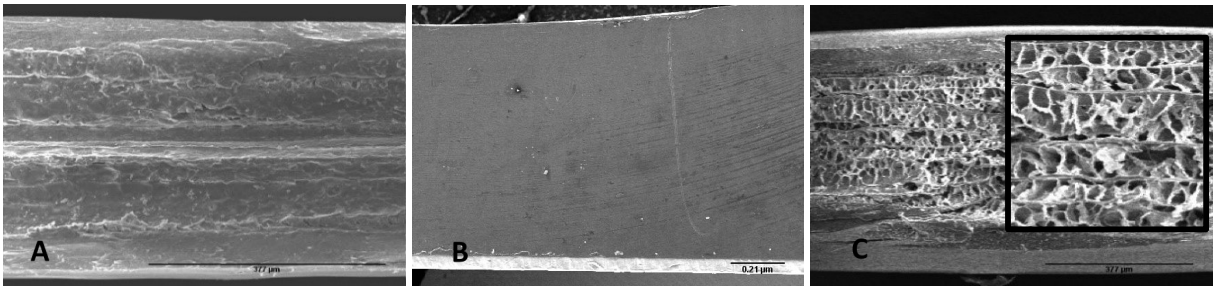

Figure 5. Cross-section of the quills medulla; transitional quills (a), rattle quills and flat spine (b), and true quills (c).

sternal gland hair of the male Brown antechinus (*Antechinus stuartii*) showed the arrangement of angular chambers (Toftegaard & Bradley 1999) (see Figure 7C). The tarsal hair tuft of black-tailed deer also has specialised scent hair with the large chamber between the cuticle scale (Müller-Schwarze *et al.* 1977) (Figure 7D). Other studies shared that some bat species has scent hair (Scully *et al.* 2000). However, the previous report shared that anogenital quill of *H. brachyura* from India subcontinent has no osmetricia (Raha *et al.* 2015) (Figure 7B). The present study result revealed that anogenital quill of Sunda porcupine has no osmetrichia feature as well (Figure 7A).

Toftegaard and Bradley 1999 revealed that the sternal hair surface of the male *Antechinus stuartii* modified into rough keratinized chambers, which act as reservoirs for the glandular secretions. These modifications structurally resemble the sebum storing tank gland hairs of the musk shrew, Suncus murinus viridescens (Balakrishnan 1987), is a crucial instrument in scent-marking in this species. The osmetrichia of deer are specialized for carrying secretions. The modified hair in the black-tailed deer also facilitates storage of sebum and its application to the substrate during scent-marking (Muller-Schwarz *et al.* 1977).

The arrangement of channels in the cross-sectional structure was different in four types of Sunda porcupine quill (Table 2). The arrangement of channels in the medulla of four type quills in *H. javanica* is alveolar and divided by septum as reported by Chernova & Kuznetsov (2001) that in *Hystrix*, the medulla is alveolar, which is divided by thick septae (Figure 8). The system

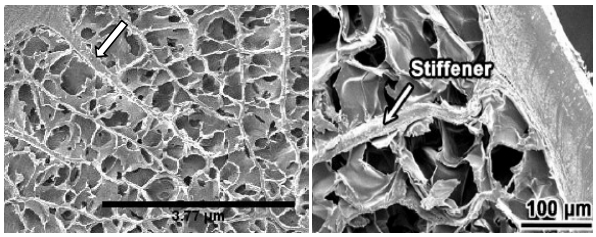

Figure 6. The transverse cross-section of the quills; the stiffener of *H. javanica* quill (A) and *H. africaaustralis* (B).

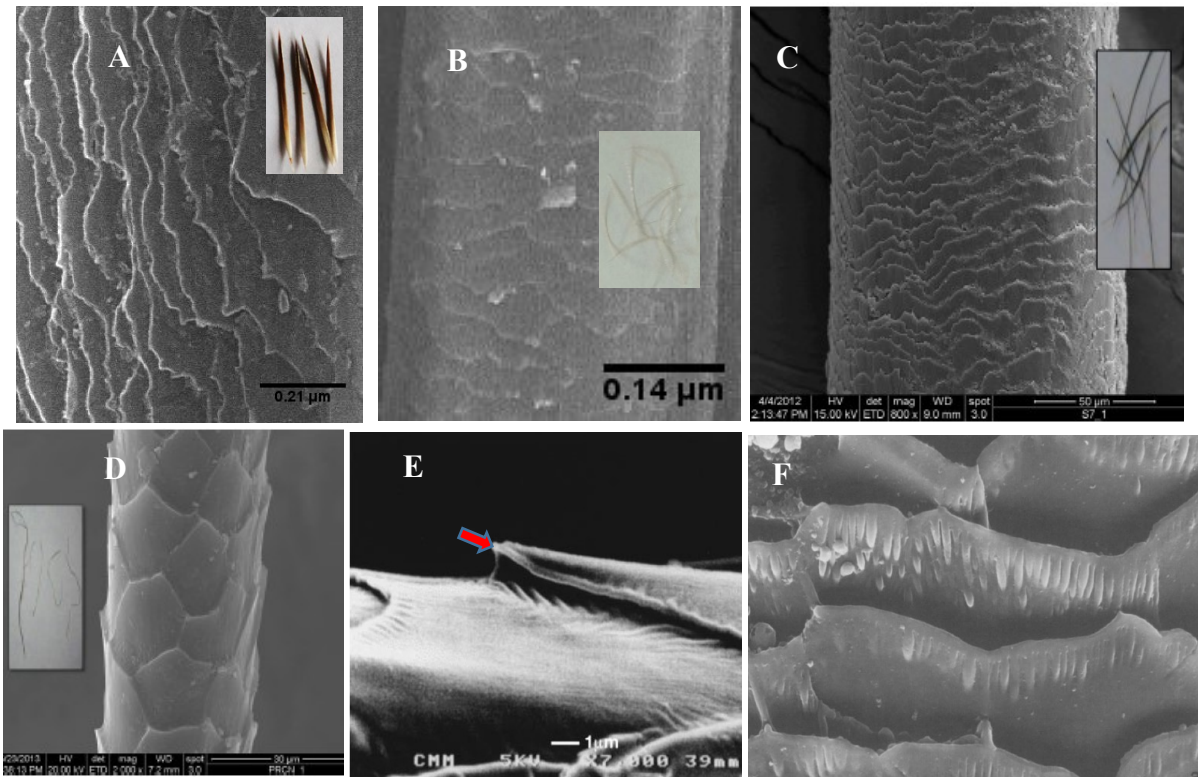

Figure 7. The cuticle of the anogenital spine and hair of *H. javanica* (A, B), anogenital quill and hair of *H. brachyura* (Raha *et al*. 2013), (C, D). Osmetrichia of the sternal gland hair of male *Antechinus stuartii* (Toftegaard and Bradley 1999), (E). The scent hair of the tarsal thuf of black-tailed deer (*Odocoileus hemionus)(*Muller-Schwarz *et al*. 1977) (F).

of internal septae of the quills provides for their mechanical strength. The medullary layers slightly truncated the cortex of transitional quill in *H. javanica* as reported by Raha *et al*. (2015) it occurs in the cortex of slender quill in *H. brachyura.*

The other study stated that almost same structural pattern in different types of the quills and the spines in cross-section of the genus Hystrix supports the view that in Hystricomorphs, these might have developed either from a group of the hair follicle of the same size or from one large follicle (Chernova 2006; Sokolov & Cehernova 1998)

In Asia, porcupine quills also possess antibiotic properties which can limit self-injury suffered in such falls (Roze *et al.* 1990). However, no report about the nutritional content of porcupine quills, especially in Indonesia. The quills of *H. javanica* consist of water, protein, fat, energy, sulfur, calcium (Ca), phosphor (P), magnesium and fibre (Table 3). This study showes that protein content in the quills is 93.66% higher than that of porcupine meat about 59.91% (Farida 2012). However, the fat in the quills is 0.44% lower than that in the porcupine meat (27.8%). Roze *et al.* (1990) revealed that the quills of American porcupine (*Erethizon dorsatum*) contain 18.6% of free fatty acid of the total fat in the quills. The study also reported that the antibiotic activity is associated with free fatty acids (but not neutral lipids) coating the quills. The extracts of quill fatty acids strongly inhibited the growth of six gram-positive bacterial strains. Further study of the fatty acid in Sunda porcupine quills is needed to extend our knowledge of potential antibiotic properties. Kakati & Daulo (2002) reported that spine of porcupine used for the therapeutic purpose such as pain relief. The report showed that spine of porcupine roasted and orally administered to reduce the fracture pain. Thus, further study needed to extend the knowledge about the potential of porcupine quill as the therapeutic purpose.

Table 2. The characteristic of quills microstructure of Sunda porcupine (*H. javanica*) base on the identification key of mammals by Teerink (1991) and Chernova and Kuznetsov (2001).

Quill type	The cuticle on the shaft region	The cuticle on the base region	Cross-section	Medulla structure
True quill	Irregular wave, rippled margin, the distance between margin is close	Longitudinal and elongate petal pattern	Circular and big channels	Alveolar, multicellular and reverse cloisonne
Transitional quill	Irregular wave rippled margin, and distance between margin is close	Regular wave pattern	A circular and small channel. The cortex truncated by medulla layer	Not found the pattern
Flat spines	Regular wave pattern and it has a smooth margin.	No scaly feature	Quadriconcave	Hollow shape
Rattle quill	Transitional pattern, smooth margin, and	Irregular wave	Circular without a network	Hollow shape
Anogenital hair	Regular wave and smooth margin	-	-	-

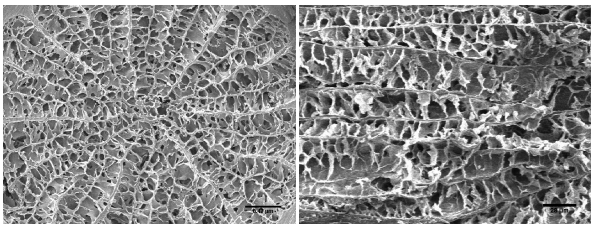

Figure 8. The arrangement of channels of the quills medulla of *H. javanica* .

Table 3. Nutrient content of *H. javanica* quills

Nutrient	Per cent (%)
Water	10.07
N-total protein	93.66
Crude fat	0.44
Gross energy (kal/g)	4579.2058
Sulfur	0.2
Calcium	0.21
Phospor	0.034
Magnesium	0.01
Crude fiber	6.94
Fatty acid	18.6

CONCLUSION

The cuticle pattern of the shaft and the base region of the quills of Sunda porcupine was different. However, the cross-section and medulla structure of four types of quills in *H. javanica*) almost similar. The quills of *H. javanica* has no specialised structure (osmetricia). The cuticle pattern and medulla structure of *H. javanica* presented in this study provides a piece of useful information for the identification key of Indonesian mammals hair and the nutritional composition present information for the potential therapeutic purpose.

ACKNOWLEDGEMENTS

We thank Mr Umar Sofyani for the kind help in samples collection, Mrs Yuni Apriyanti who assist during SEM preparation, and Mrs R. Lia Rahadian Amalia for the help during proximate analyses.

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