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Study of Histological Skin Structure of *Python reticulatus* and *Varanus salvator*

(*Kajian Histologi Struktur Kulit Python reticulatus dan Varanus salvator*)

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A B S T R A C T

Reptile skin is covered with scales that form a protective barrier, making it waterproof and enabling life on land. The present study investigated the histological structure of the skin of the *Python reticulatus* and *Varanus salvator*. The samples used were *Python reticulatus* and *Varanus salvator* skin taken from the dorsal region. Preparations were made using the hematoxylin eosin (HE) staining method. The results showed that the histological structure of *Python reticulatus* skin consisted of two layers, epidermis and dermis. The epidermis was composed of stratum corneum, stratum granulosum, and stratum basal. The dermis consists of an outer layer called the stratum laxum (stratum spongiosum) and an inner layer called the stratum compactum. Meanwhile, the histological skin structure of *Varanus salvator* skin consists of epidermis which included oberhautchen, α-keratin layer, β-keratin layer, supra basal layer, and basal layer. The dermis consists of superficial dermis and deep dermis. There are differences between *Python reticulatus* skin that is distinguished by its ability to ecydis (skin shedding) the epidermis and *Varanus salvator* skin have osteoderm (OD) within their dermis layer.

A B S T R A K

Kulit reptil ditutupi sisik yang membentuk lapisan pelindung, sehingga tahan air dan memungkinkan kehidupan di darat. Penelitian ini menyelidiki struktur histologis kulit *Python reticulatus* dan *Varanus salvator*. Sampel yang digunakan adalah kulit *Python reticulatus* dan *Varanus salvator* yang diambil dari daerah punggung. Preparat dibuat dengan metode pewarnaan hematoxylin eosin (HE). Hasil penelitian menunjukkan bahwa struktur histologis kulit *Python reticulatus* terdiri dari dua lapisan yaitu epidermis dan dermis. Epidermis terdiri dari stratum korneum, stratum granulosum, dan stratum basal. Dermis terdiri dari lapisan luar yang disebut stratum laxum (stratum spongiosum) dan lapisan dalam yang disebut stratum compactum. Sementara itu, struktur histologis kulit *Varanus salvator* terdiri dari epidermis yang meliputi oberhautchen, lapisan α-keratin, lapisan β-keratin, lapisan supra basal, dan lapisan basal. Dermis terdiri dari dermis superfisial dan dermis dalam. Terdapat perbedaan antara kulit *Python reticulatus* yang dibedakan dari kemampuannya dalam melakukan ecydis (*skin shedding*) pada epidermis dan kulit *Varanus salvator* yang mempunyai osteoderm (OD) pada lapisan dermisnya.

Kata Kunci:
Dermis
Osteoderm
Kulit
Python reticulatus
Varanus salvator

1. Introduction

Reptiles are tetrapods that have two distinctive characteristics; scales and amniotic eggs (eggs with an internal fluid membrane), which are still of great importance for them (Rutland, Cigler, & Kubale, 2019). Reptiles have four orders; squamata (lizards, snakes, and worm lizards), crocodile (crocodile and alligators), chelonia (turtles and tortoises), and rhynchocephalia (tuatara) (Van Hoek, 2014).

Monitor lizards or the binomial *Varanus salvator* are reptiles that live wild and are found in abundance in Indonesia. *Varanus salvatores* generally inhabit the banks of rivers or waterways, lake shores, beaches and swamps, including mangrove swamps. In urban areas often found living in water channel culverts that empty into rivers. *Varanus salvator* can be used, especially their skin as jewelry, and their meat as food or medicine. According to Bhattacharya & Koch (2018) the lizards are one of those being hunted by human beings for several purposes as food and traditional medicine.

Currently, the skin trade has supported thousands of people, such as catchers in villages, collectors, processors, exporters, to the leather industry. No less than one million pieces of *Varanus salvator* skin were collected every year from various parts of the world (Kusuma, Alfiyanto, Srianto, Triakoso, & Legowo, 2017), with the most collected from Indonesia, particularly Kalimantan and Sumatera (Khoirunnisa', 2020).

In general, the skin of reptiles has two main layers consisting of the outermost layer epidermis and the dermis (Yenmiş & Ayaz, 2023). Each layer of skin has different anatomy and function. The layers of the epidermis include the stratum basal (the deepest portion of the epidermis), stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum (the most superficial portion of the epidermis) (Yousef, Alhajj, Fakoya, & Sharma, 2024).

Stratum basal, also known as stratum germinativum, is the deepest layer. The cells found in this layer are cuboidal to columnar mitotically active stem cells that are constantly producing keratinocytes. This layer also contains melanocytes. Stratum spinosum, 8 – 10 cell layers, also known as the prickle cell layer contains irregular. Stratum granulosum, 3 – 5 cell layers, contains diamond shaped cells with keratohyalin granules and lamellar granules. Stratum lucidum, 2 – 3 cell layers. The stratum corneum is the outermost layer of the epidermis which consists of 10 to 30 thin layers of dead keratinocytes that are continuously shed. The stratum corneum is also known as the "horn layer", because its cells are hardened like animal horns. The stratum corneum continues to peel off as new cells add. Within this layer, the dead keratinocytes secrete defenses which are part of our first immune defense (Yousef et al., 2024).

So far, this species study was still limited and available literature revealed that there is no information on the histological structure of the skin of the *Python reticulatus* and *Varanus salvator*. This species study was still limited to biological research (Natusch et al., 2019), body morphology (Janiawati, Kusrini, & Mardiastuti, 2016), reproductive biology (Al-Ma'ruf et al., 2021) and commercial harvesting. Therefore, the aim of this study was to investigate the histological features of the skin of the *Python reticulatus* and *Varanus salvator*.

2. Materials and Methods

2.1. *Study Period and Location*

This study was conducted from August to December 2023 at the Laboratory of Microbiology, Politeknik ATK Yogyakarta.

2.2. *Research Materials*

The tools used in this research were latex gloves, masks, labels, knives, stainless steel embedding molds (available in various sizes, namely 10 × 10 × 5 mm; 15 × 15 × 5 mm; 24 × 24 × 5 mm; 24 × 30 × 5 mm), rotary microtome, tissue water bath, disposable microtome, standard glass microscope slide 75 × 25 mm, laboratory oven (set at 37 °C), glass cover slip (25 × 60 mm). Materials used were fixative solution, ethyl alcohol, 96 % ethanol solution, 90 % and 70 % ethanol solution, clearing agent (xylene or, Histosol®, Neoclear®) 0.1 % gelatin in water (1g gelatin in 1 L distilled water), paraffin wax.

2.3. *Field Samples*

This study was conducted according to the regulations for Research in Animal Health of Indonesian Law on Livestock and Animal Health (UU/18/2009, article 80). The *Python reticulatus* and *Varanus salvator* samples were one piece of preserved skin from the leather industry.

2.4. *Research Methods*

The procedure for tissue fixation was to cut tissue/organ specimens to a size of approximately 4 mm. After the tissue has been fixed, the tissue is transferred into alcohol and processed as follows: 30 % ethanol for 45 minutes; 50 % ethanol for 45 minutes; 70 % ethanol for 45 minutes alcohol; 80 % ethanol for 45 minutes; 90 % ethanol for 45 minutes; 96 % ethanol for 45 minutes. The clearing process used toluene as a solvent aimed to replace ethanol originating from within the tissue. During infiltration, toluene will be replaced by paraffin. The ratio of ethanol and toluene is 1:1 (v/v) for 30 minutes and toluene for 60 minutes. This step was carried out by immersing the tissue in liquid paraffin in the incubator at a temperature of 60 °C for 60 minutes until the paraffin hardens to completely replace the xylene. The tissue immersion process was immersed in paraffin I for 2 hours, then transferred into paraffin II for 1 hour. The final tissue was placed in paraffin II for 2 hours. After the immersion process can be continued with blocking. Position the specimen as expected. After that, cool it briefly so that the position is not changed, then pour the liquid paraffin back up to the maximum level and label according to the samples (Jusuf, 2009).

Staining procedure used is deparaffinization first with xylol (2 × 2 minutes). Hydration series with alcohol: alcohol 100 % (2 minutes); 95 % (2 minutes); 90 % (2 minutes); 80 % (2 minutes); 70 % (2 minutes); 80 % (2 minutes); 70 % (2 minutes); distilled water (3 minutes); distilled water (3 minutes). After that, it was incubated in a hematoxylin solution for 15 minutes. Wash with running water for 15 – 20 minutes. Observe under a microscope, if they are still too blue, wash them again with running air for a few minutes. If the color is sufficient, continue to the next step. Counterstaining in the Eosin solution works for 15 seconds to 2 minutes. Dehydrate in a series of alcohols with slowly increasing gradations from 70 % to 100 % over 2 minutes each. Remove and deappleize in xylol 2 × 2 minutes (Jusuf, 2009).

2.5. Data Analysis

The data of histological skin in *Python reticulatus* and *Varanus salvator* were analyzed descriptively and presented in histological figures.

3. Results and Discussion

3.1. Histological Structure of Python reticulatus ***Skin***

The histological sturctur of *Python reticulatus* skin with HE staining showed in figure 1. In this study *Python reticulatus* histological skin structure is composed of by the layers of epidermis and dermis. Figure 1A describes the histological analysis of *Python reticulatus*. The histology skin structure of *Python reticulatus* consists of the epidermis (epidermal stratum corneum, epidermal basal stratum).

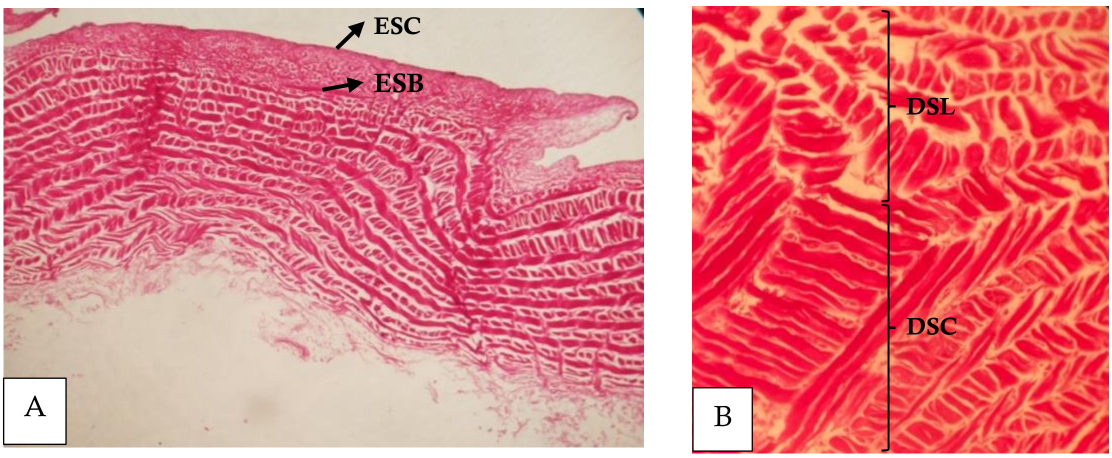

Figure 1. Micrograph *Python reticulatus* with Hematoxylin Eosin (HE) Staining. A = crossectional area of *Python reticulatus*, 40 ×; B = crossectional dermis area of *Python reticulatus*, 100 ×; ESC = Epidermal stratum corneum; ESS = Epidermal stratum spinosum; ESB = Epidermal stratum basal; DSL = Dermal stratum laxum; DSC = Dermal stratum compactum.

Based on Figure 1, stratum corneum was thinner than in scale regions, and the predominant keratins were α-keratins. In general, the histology structure of *Python reticulatus* consist of epidermal stratum, corneum, epidermal stratum granulosum, and epidermal stratum basal. Snake scales are found in the epidermis of the stratum corneum. In epidermal scale regions, the stratum corneum consists of a continuous layer of β-keratins and a thinner layer of α-keratins (Alibardi, 2016; Dubansky & Close, 2018). α-keratin is more flexible, while β-keratin provides strong and hard properties to reptile scales (Lazarus et al., 2021; Rutland et al., 2019). Beside the stratum granulosum, an intermediate layer of the skin, contains a lipid-rich film that serves as a significant component in establishing a water-permeable barrier. The basal layer in snakes serves to generate the granulosum (inner) and corneum (outer) layers, which replicate the granulosum and corneum layers, resulting in the displacement of the old epidermal layer when snake have ecidys (Rutland et al., 2019).

Dermis layer of *Python reticulatus* consisted of the dermal stratum laxum (also known as the stratum spongiosum) and the dermal stratum compactum (Figure 1B). Dermis structure of *Python reticulatus* is compact and dense. This is due to the acidic conditions during tanning cause the breaking of chemical bonds between collagen fibers in the skin, which contributes to the opening of the collagen fibers (Covington & Wise, 2019). Collagen fibers that have been opened will form cross-links with the tanning agent, making the fiber bonds more compact (Li, Wang, Li, & Shi, 2016; Xu, Wang, & Shi, 2022).

The dermal layer of the stratum laxum or spongiosum consists of loose connective tissue composed the bundle of thin collagen fibers and interspersed with capillary networks (Dubansky & Close, 2018). Meanwhile, the stratum compactum consists the bundle of thick collagen that are arranged in a regullar. Both layers of the dermis contain elastic fibers which function to return stretched integument to a resting position (Dubansky & Close, 2018).

Figure 1 shows there was no hypodermis layer. The absence of a hypodermis layer in *Python reticulatus* are intentional throughout the tanning process to avoid the presence of a fatty layer that may inhibit the penetration of chemicals and tanning agents (Kuria, 2023). Snakes are reptiles characterized by their scaly skin that covers their entire body. The scales are stacked in a pile that covers each other, with the mucosa layer on top and the subcutaneous tissue beneath; the scales keratinize and protect the snake from skin injury and dehydration (Rutland et al., 2019). The uniqueness of *Python reticulatus* is well-known by its ability to ecydis (skin shedding), which occurs simultaneously over the entire surface of the skin, removing the outer keratinized layer (Dubansky & Close, 2018).

3.2. *Histological Structure of Varanus salvator Skin*

The histological sturcture of *Valvanus salvator* skin with HE staining showed in figure 2. The skin was made up of two layers: epidermis and dermis. Hypodermis layer was not found in the micrograph, the absence of a hypodermis layer is intentional throughout the tanning process.

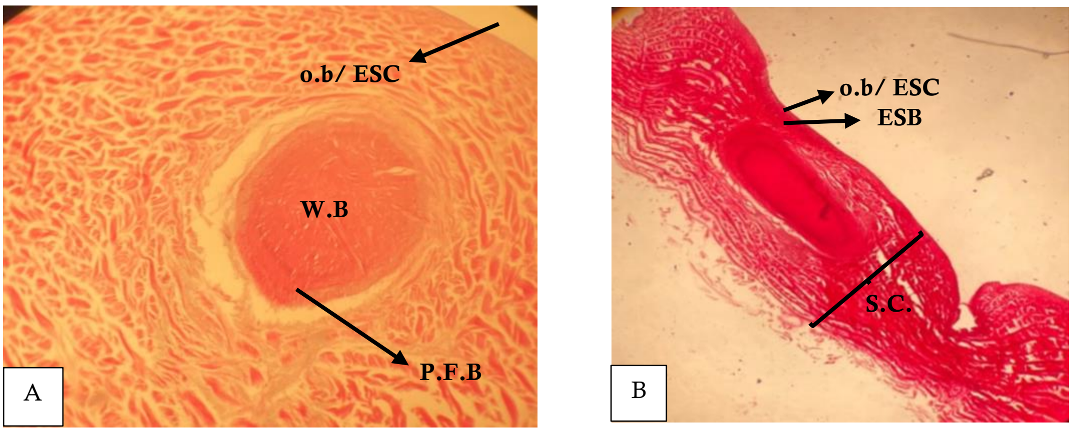

Figure 2. Micrograph *Varanus salvator* with Hematoxylin Eosin (HE) Staining. A = crossectional area of *Varanus salvator* 40 ×; B = crossectional area of *Varanus salvator,* 100 ×; o.b = Oberhautchen; ESC = epidermis stratum corneum; ESB = epidermis stratum basal; S.C = Stratum compactum; PFB = Paralel fibreb-bone; WB = Woven bone.

Figure 2A shows the oberhautchen epidermis layer (epidermis stratum corneum) in the top layer. Figure 2B shows the layer of oberhautchen epidermis layer and epidermal stratum basal. The study conducted by Boonchuay, Chantakru, Theerawatanasirikul, & Pongchairerk (2018) reveled that epidermis layer have epidermis layer's top layer (epidermis straturm corneum) and epidermal stratum basal. In general, the epidermis of lizards is composed of several layers, including the oberhautchen epidermis layer, α-keratin layer, β-keratin layer, supra basal layer, and basal layer. Furthermore, melanophores are distributed throughout the epidermis and superficial dermis layers.

Figure 2A dan 2B shows the dermis layer, from that micrograph revealed deep dermis or stratum compactum. In this study *Varanus salvator* have unique structure by the presence of osteoderm (OD) that consists of woven bone and parallel-fibred bone (Figure 2). According to Boonchuay et al. (2018) dermis has two distinct of layers: the superficial dermis layer and the deep dermis layer, also known as the stratum compactum. Furthermore, OD is a hard tissue organ found in vertebrates' dermis layer. In reptiles such as dinosaurs, alligators, and lizards, ODs are thought to develop spontaneously inside pre-existing dermal collagen structures, similar to tendon ossification (Kirby et al., 2020).

The cross-sectional micrographs of *Varanus salvator* skin in Kasmudjiastuti, Sutyasmi, & Murti (2015) showed a histological structure identical to this study, characterized by the presence of woven bone surrounded by parallel fiber bone in the dermis region. Woven bone is the OD's inner core, while parallel-fibred bone is its outside ring. The OD distribution in lizards is found in specific areas; throughout the body in varying arrangements as: non-overlapping overlapping mineralized clusters; as a continuous covering of overlapping plates; or as spicular mineralizations that thicken with age and exhibit a variety of structural designs; or not at all in lizard skin (Marghoub et al., 2022). Lizard ODs have a heterogeneous tissue composition, including woven bone, parallel-fibred bone, lamellar bone, and sharpey-fibre bone (Kirby et al., 2020; Maliuk et al., 2024).

The primary role of OD is to regulate body temperature through vascularization, provide structural support for the vertebrate spine, regulate metabolism and mineral levels, and serve as a framework for tendon attachment (Williams et al., 2022) . However, the functions of OD that have been proven are as lactate sequestration, locomotor support, thermo-regulation (Veenstra & Broeckhoven, 2022; Williams et al., 2022).

Figure 2A and 2B shows there was no hypodermis layer. The absence of a hypodermis layer in *Varanus salvator*s are intentional throughout the tanning process to avoid the presence of a fatty layer that may inhibit the penetration of chemicals and tanning agents (Kuria, 2023).

3.3. Characterictic Histology Structure of Python reticulatus and Varanus salvator

Based on this study *Python reticulatus and Varanus salvator have* differences characteristic structure, these reptils have uniqness. *Python reticulatus* well-known by its ability to ecydis (skin shedding), which occurs simultaneously over the entire surface of the skin, removing the outer keratinized layer, meanwhile *Varanus Salvator* have osteoderm (OD) within their dermis layer. Table 1 explained the characteristic of these two reptiles.

Table 1. Characteristic histology structure of *Python reticulatus* and *Varanus Salvator*

Histology structure	*Python reticulatus*	*Varanus Salvator*
Epidermis	Consisting of stratum corneum, stratum granulosum, and stratum basal	Oberhautchen, supra basal layer, and basal layer
Dermis	consists of stratum spongiosum and stratum compactum	deep dermis
Hypodermis	Absence of hypodermis layer throughout the tanning process	Absence of hypodermis layer throughout the tanning process
Uniqueness	ability to ecydis (skin shedding), which occurs simultaneously over the entire surface of the skin, removing the outer keratinized layer	*Salvator* have osteoderm (OD) within their dermis layer

Source: Primary data from research results (2023).

Reptiles' histological structure is mostly composed of the epidermis, dermis, and hypodermis (Rutland et al., 2019). The distinctive characteristics of reptile skin is epidermis layer, which is rich in keratin and functions to prevent water loss. The integument in reptiles consists of scales that are strong and non-stretchable. Scales provide mechanical protection against abrasion, while inter scale skin gives movement of the animal and allows the movement of underlying skin structures such as muscles and skeletal elements (Dubansky & Close, 2018). Aside from that, reptile scales are not the same as fish scales; reptile scales are part of the skin. Reptile scales have no bones and make a considerable structural contribution to the dermis (Dhouailly, 2023; Rutland et al., 2019).

Python reticulatus and *Varanus salvator*s are reptiles with unique skin, yet they have different skin structures. *Python reticulatus* have a skin structure of the epidermis, consisting of stratum corneum, stratum granulosum, and stratum basal, as well as the dermis, which consists of stratum spongiosum and stratum compactum (Dubansky & Close, 2018; Rutland et al., 2019). Furthermore, Lizards have epidermis (oberhautchen, α-keratin layer, β-keratin layer, supra basal layer, and basal layer) and dermis (superficial and deep dermis) (Boonchuay et al., 2018). The absence of a hypodermis layer in *Python reticulatus* and *Varanus salvator*s are intentional throughout the tanning process to avoid the presence of a fatty layer that may inhibit the penetration of chemicals and tanning agents (Kuria, 2023).

Python reticulatus is well-known by its ability to ecydis (skin shedding), which occurs simultaneously over the entire surface of the skin, removing the outer keratinized layer (Dubansky & Close, 2018). Moreover, lizards have osteoderm (OD) within their dermis layer. The primary role of OD is to regulate body temperature through vascularization, provide structural support for the vertebrate spine, regulate metabolism and mineral levels, and serve as a framework for tendon attachment (Williams et al., 2022). However, the functions of OD that have been proven are as lactate sequestration, locomotor support, thermo-regulation (Veenstra & Broeckhoven, 2022; Williams et al., 2022).

4. Conclusion

There were differences in the histological structure of the skin between the reptile species. Histology structure of *Python reticulatus* skin consisted of epidermis (stratum corneum, stratum granulosum, and stratum basal), as well as the dermis (Stratum spongiosum and stratum compactum). Meanwhile, *Varanus salvator*s skin have epidermis (oberhautchen, α-keratin layer, β-keratin layer, supra basal layer, and basal layer) and dermis (deep dermis). *Python reticulatus* skin was distinguished by its ability to ecydis (skin shedding), and *Varanus salvator* skin have osteoderm (OD) within their dermis layer.

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