

Macroscopic and Microscopic Study of the Integument and Accessory Organs of Malayan Pangolin (*Manis javanica*)

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บทคัดย่อ

การศึกษาทั้งทางมหากายวิภาค และ จุลกายวิภาคของอวัยวะห่อหุ้มร่างกายและองค์ประกอบ (เกล็ด เต้านม ต่อมทวาร ถุงของต่อมข้างทวาร และ แผ่นยึดที่ปลายหาง) ของตัวลิ่นชวา (*Manis javanica*) พบว่า ตัวลิ่นเตี้ยมีเกล็ดที่ส่วนหัว ลำตัว ขา และ หางแตกต่างกันทั้งขนาดและรูปร่าง ลิ่นแรกเกิดและ ลิ่นในครรภ์พบการพัฒนาารูปแบบของเกล็ดตั้งแต่ระยะตัวอ่อนที่ขนาดลำตัว 5 - 8 ซม. แต่ไม่พบในขนาด 2 ซม. การเกิดสีและลายที่ฐานเกล็ด และขนเส้นเล็กๆ ได้เกิดนั้น พบในตัวลิ่นแรกเกิดแต่ไม่พบใน ระยะในครรภ์ รูเปิดของต่อมรอบรูทวารพบที่ผิวหนังรอบรูทวาร การศึกษาโดยกล้อง SEM ที่แผ่นยึด ปลายหางพบว่า เป็นตุ่มปลายที่ขนาดเล็กๆ จำนวนมากปกคลุมผิวของแผ่นดังกล่าว หนังกำพร้าวของตัว ลิ่นเป็นเยื่อบุผิวแบบ keratinized stratified squamous epithelium มี keratohyalin granules จำนวนมากใน ชั้น stratum granulosum เกล็ดเป็นหนังกำพร้าวที่มีชั้น keratinized หนา ชั้นใต้หนังกำพร้าวของตัวลิ่นไม่พบ ว่ามีต่อมเหงื่อและต่อมไขมัน ในชั้น dermis ของเกล็ดพบว่ามี hair follicles ได้ขอบของเกล็ด รวมทั้งในชั้น dermis ของผิวหนังที่ไม่มีเกล็ดหุ้มจะพบ hair follicles กระจุกกระจายอยู่ด้วย ต่อมน้ำมันอยู่ใกล้รักแร้ ประกอบด้วยถุง alveoli แบบเซลล์ชั้นเดียว เซลล์ myoepithelium และ โครงร่างเนื้อเยื่อประสาน คล้ายสัตว์เลี้ยงลูกด้วยนมอื่นๆ ต่อมรอบรูทวารเป็นต่อมไขมันดัดแปลง มีรูเปิดเพื่อระบายสารคัดหลั่งสู่ ผิวด้านบน ถุงข้างรูทวารมีต่อมของมันฝังตัวอยู่ที่ผนังของถุงพบอยู่ข้างรูทวาร

คำสำคัญ: ตัวลิ่นชวา อวัยวะห่อหุ้มร่างกาย เกล็ด อวัยวะประกอบ การศึกษาทางสัตวศาสตร์

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ABSTRACT

The integument and accessory organs (scale, mammary gland, anal gland, anal sac and glandular pad of tail) of Malayan pangolins (*Manis javanica*) were studied by macroscopic and microscopic methods. In adult pangolins, scales from head, body, limbs and tail are different in the shape and size. In newborn and fetus of pangolins, the scale pattern is developed in fetus of crown-rump length of 5 cm. and 8 cm. but not in 2 cm. The coloration, the finely striated at the base of scale and the hairs underneath scale are developed in newborn pangolins but not in fetus. Openings of circumanal glands were located on the skin around anus. SEM study of glandular pad under the tip of the tail showed the entire surface was covered by numerous blunt papillae. Epidermis of pangolin's skin is keratinized stratified squamous epithelium with prominent keratohyalin granules in stratum granulosum layer. The scales are composed of very thick keratinized layer of epidermis. Neither sweat glands nor sebaceous glands were found in dermis of pangolin integument. The hair follicles were found underneath scale and the scattered hair follicles were also found in the area of skin uncovered by scales. The mammary glands located near axilla consist of single cell layer alveoli, myoepithelial cells and connective tissue stroma similar to other mammals. The circumanal glands, modified sebaceous glands, have several opening of excretory ducts for directly secreting to the superficial surface. The anal sac with glands of anal sac located at lateral side of the anus is similar to canine's anal sac glands.

Keywords: *Manis javanica*, integument, scale, accessory organs, morphological study.

INTRODUCTION

Pangolin is a mammal belongs to the Order Pholidota, under the Manidae family. *Manis javanica* is an Asiatic species. The scales mainly cover on the back and feet and serve as the protective armor. When disturbed, a pangolin protects itself by curling into a tight ball, the armored limbs and tail protecting the soft skin underneath. By this position, the sharp-edged scales are erected (Nowak, 1999). The other method of defense is a pungent spray of urine or rancid fluid from glands surrounding the anus (Murray, 1986). According to traditional Chinese medicine, the scales are believed to be a source of medicine for fever and skin disease (1). Due to the high demanded for scales

in traditional Chinese medicine (2), this trade is illegal but still goes on. To date pangolin is listed as an endangered species in the IUCN Red Book and on appendix II of the CITES.

The objective of this study is gross anatomical and microscopic study of the Malayan pangolin's integument and accessory organs; scale, normal skin, mammary gland, circumanal gland, anal sac, and also the glandular pad (the structure at the tip of the tail involving in curling process).

MATERIALS AND METHODS

Five adult and two baby pangolin carcasses from Khao Pratub-Chang Wild life Breeding center,

Thailand seized from smugglers were dissected for gross anatomical study of the following structures; Shape and pattern of scale on 4 regions; head, body, limb and tail, of adult, newborn and fetus pangolins, Mammary gland, Circumanal gland and anal sac, and Glandular pad at the tip of the tail.

Parts of glandular pad from adult and newborn pangolins were coated with gold and observed by scanning electron microscope (JOEL 35 CF).

Tissue samples of scale, normal skin, mammary gland, circumanal gland and anal sac taken from adult pangolin were fixed in 10% neutral buffer formalin, dehydrated in graded ethanol and embedded in paraffin. Tissue sections with 4 micrometer thickness were stained with Hematoxyline and Eosin. Under Olympus BX50[®] microscope, the sections were observed and photographed.

RESULTS AND DISCUSSION

A. Gross Anatomy of Malayan pangolin's integument and accessory organs

Scales

In adult pangolins, the scales completely cover from the head to the tip of the tail and limbs. There are lacking of scale on their snout, chin, lateral side of face, throat, belly and inner surface of four limbs. The base of scales attached to the thick skin underneath. Unlike Africa pangolins, Asian pangolins (*Manis javanica*) have some fine hairs beneath the scales (Lekagul and Jeffrey, 1988).

The coloration of scale is dark brown to yellow brown at head, body, limbs and tail. The color at the caudal end of the tail is paler than the

other parts of the body (Fig. 1A). The shape and size of the horny scales are different in difference areas of the body. Scales of the head are small flat horny scales with finely striated at the base (Fig. 1B). Scales of the dorsal part of the body and of the tail are large flat horny scales with finely striated at the base and the posterior sharp edge with obtuse angle (Fig. 1C-1D). The largest scales are located at the cranial end of the tail.

Scales of the lateral part of the body, lateral part of the tail and ventral part closed to the tip of the tail have strong keel in the middle of the scales and their posterior sharp edge are acute angle (Fig. 1E). Scales of the fore limb are small flat scale with finely striated at the base and their posterior sharp edge are obtuse angle the same as found in the head region. The scales close to the claw of fore limb are larger than the rest (Fig. 1F). Scales of the hind limb are small with little strong keel in the middle of the scale and their posterior sharp edge are acute angle the same as the lateral part of the body and lateral part of the tail (Fig. 1G). Even though hard scale of pangolin covered almost entire body surface, an external parasite infestation is possible. The adult stage of hard tick (Metastigmata: Ixodidae, identified by Assoc.Prof. Dr. Arkom Sangvaranond) was found at the interscalar area underneath the scale (Fig. 1H).

New born pangolin scales do not harden until the second day of life (Nowak, 1999). The skin of fetus crown-rump length of 2 cm showing the beginning of scales pattern development with rough skin surface (Fig. 2A). Scale pattern had already developed in fetus crown-rump length of 5 cm, 8 cm and new born (Fig. 2B - 2E). The posterior sharp edge of fetus scales is acute angle without finely striated at the base of scale. The coloration

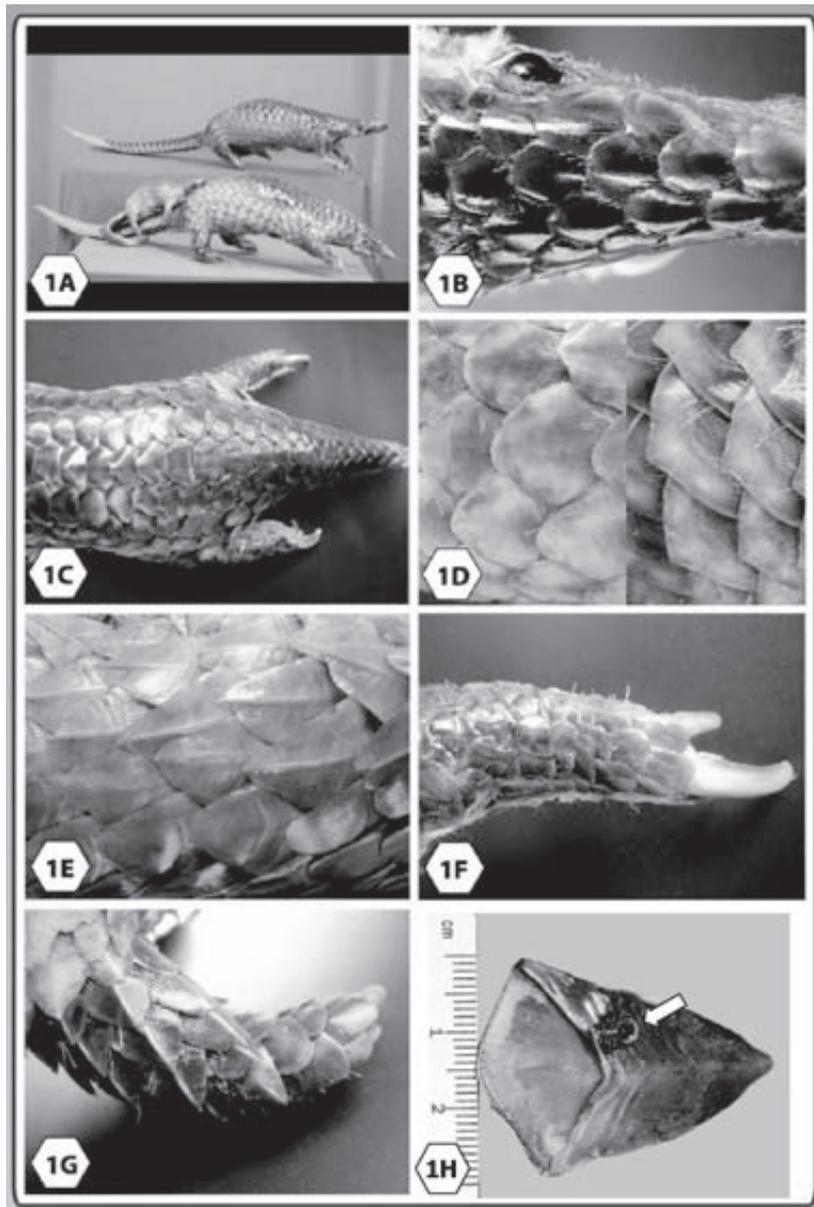


Figure 1A Pattern and coloration of Malayan pangolin's scale

Figure 1B Pangolin's head scale

Figure 1C-1D Pangolin's scale of the dorsal part of body

Figure 1E Pangolin's scale of the lateral part of body

Figure 1F Pangolin's scale of the fore limb

Figure 1G Pangolin's scale of the lateral part of hind limb

Figure 1H Hard tick (Metastigmata: Ixodidae) under Pangolin's scale (arrow)

of scale, the finely striated at the base and hairs beneath the scales are developed in new born but not fetus (Fig. 2E and 2F). Due to the difficulty to

know the age of fetus, the time of hair development beneath the scales and the coloration are not known.

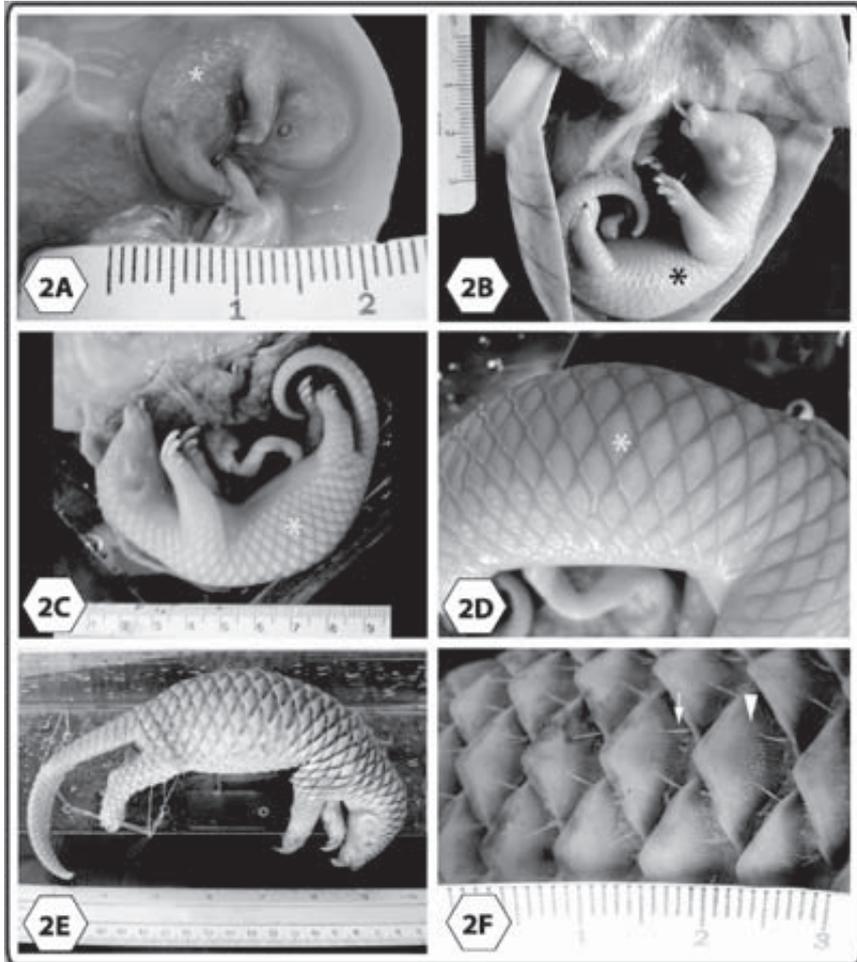


Figure 2A Pangolin fetus of Crown-Rump length of 2 cm. (*uneven skin surface)

Figure 2B Pangolin fetus of Crown-Rump length of 5 cm. (*scale pattern)

Figure 2C Pangolin fetus of Crown-Rump length of 8 cm. (*scale pattern)

Figure 2D Body scales without hair underneath the scale in fetus of Crown-Rump length of 8 cm.
(* scale without finely striated at the base of scale)

Figure 2E New born Pangolin

Figure 2F Body scale with finely striated at the base and hairs underneath the scale in new born Pangolin (arrow: Hairs underneath, arrow head: Scale with finely striated at the base of scale)

Mammary gland

Both adult male and female pangolins have single pair of pectoral mammary gland or axillary mammary gland (Fig. 3). During nursing the baby, the female pangolin lies on her back or side to nurse the baby. Weaning probably takes place after about 3 months (Lekagul and Jeffrey, 1988).

Circumanal gland and Anal sac

Paired of Pangolin's anal sac are large oval structures located on each side of anus. They have the same size in both male and female pangolin. White spots of secretion from circumanal glands were seen at the elevation skin surface around the anus (Fig. 4A - 4D).

They used scent from the glands for marking around their territories (Lekagul and Jeffrey, 1988) and also when predators are near a repugnant odor are discharged from the gland around anus area (3).

Glandular pad

At the tip of pangolin's tail, the round area on the ventral surface of the tail with no scale covered called glandular pad (Fig 5A and 5B). It tightly holds the tail to the body when the pangolin curling to protected itself from any harm.

B. Microscopic structure of Malayan pangolin's integument and accessory organs

Skin and Scales

Pangolin's skin without covered scale on lateral side of body has very thick keratinized stratified squamous epidermis tightly attached to papillae of dense irregular connective tissue of dermis (Fig. 6A). The stratum granulosum is obviously filled with numerous keratohyalin granules (Fig. 6B). There is no any skin gland in dermis of this part of skin. The new born pangolin's scales

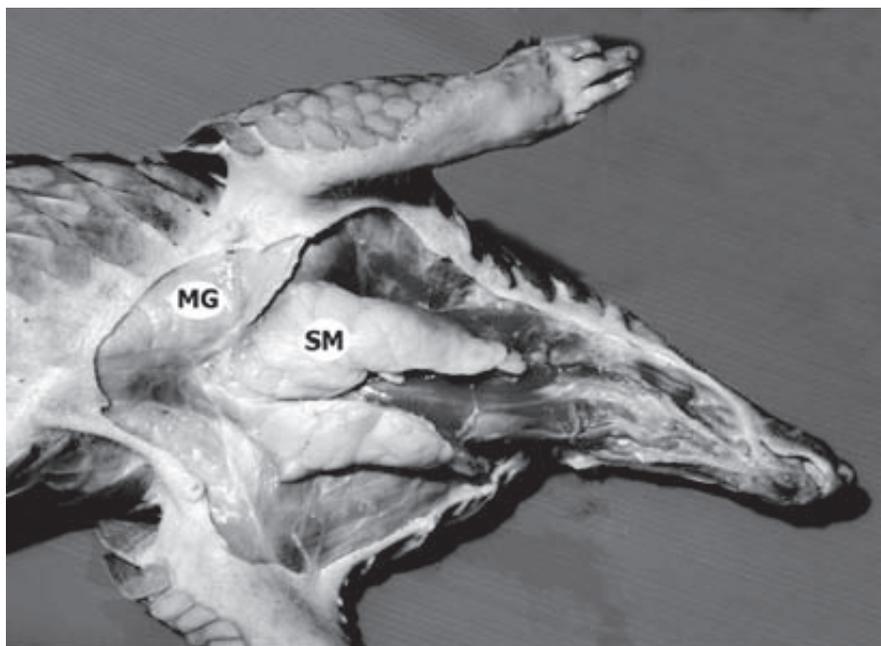


Figure 3 Mammary gland (MG) at the axilla of a female pangolin and submandibular gland (SM)

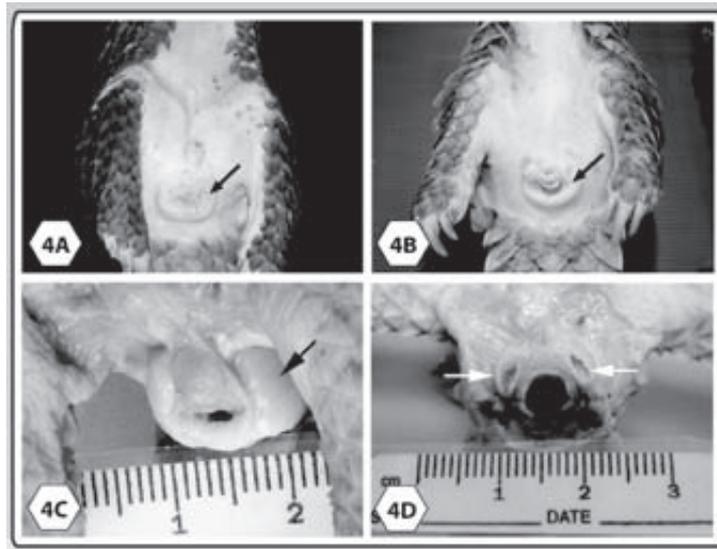


Figure 4A Anal gland (arrow) around a male Pangolin's anus

Figure 4B Anal gland (arrow) around a female Pangolin's anus

Figure 4C Newborn Pangolin's anal sac (arrow) (skin around anus was removed)

Figure 4D Newborn Pangolin's anal sac (arrow) (transverse section of anal sac)

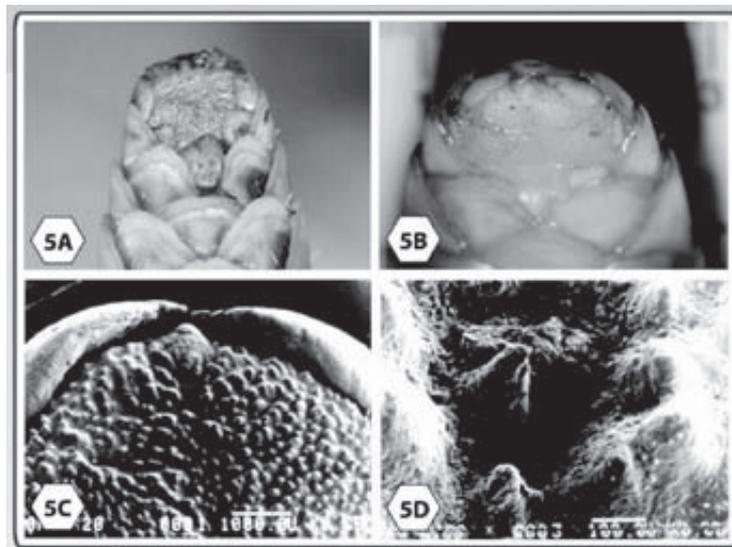


Figure 5A Pangolin's grandular pad at the tip of the tail

Figure 5B Pangolin's grandular pad at the tip of the tail

Figure 5C SEM of Pangolin's grandular pad shown blunt papillae

Figure 5D SEM of Pangolin's grandular pad shown blunt papillae

which are similar to the adult scales have the well development of hard keratinized layer of epidermis and no keratohyalin granule in the adjacent layer (Fig. 6C). The hypodermis under scales has abundance of skeletal muscle fibers (Fig. 6C).

However, epidermis at the interscalar area between 2 scales still has distinct keratohyalin granules in stratum granulosum (Fig. 6D). The dermis under scales has hair follicles which the hair shaft grew out to the rim of scales (Fig. 6C). The hair follicles

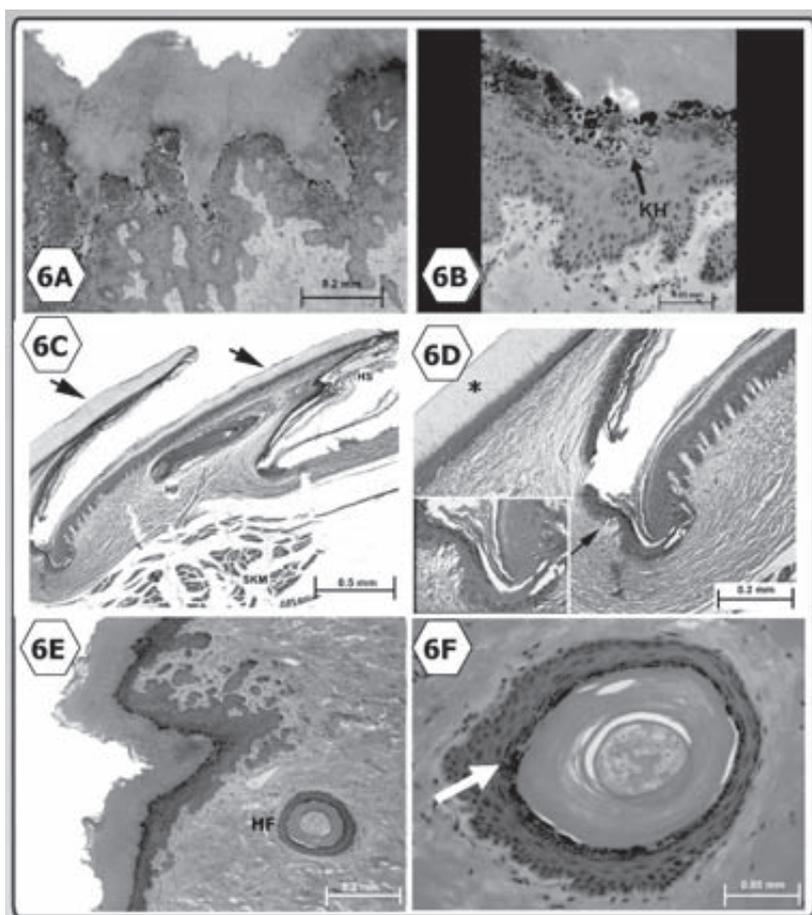


Figure 6A Pangolin's skin without scale on the lateral side of body (H&E, X10)

Figure 6B Pangolin's skin, KH= Keratohyalin granules (H&E, X40)

Figure 6C New born pangolin's skin covered by scale, scales (arrows) with hair follicle (HF) and hair shaft (HS) under the scale, SKM = Skeletal muscles in hypodermis (H&E, X4)

Figure 6D New born pangolin's scale, * = Keratinized layer (H&E, X20); The inset shown X20 magnification of interscalar area (arrow).

Figure 6E Pangolin's skin on the ventral of body, Hair follicle (HF) (H&E, X40)

Figure 6 Hair follicle, inner root sheath with keratohyalin granules (arrow) (H&E, X40)

also were found at the dermis of skin at the ventral side of the body (Fig. 6E). The inner root sheath of hair follicle is filled with keratohyalin granules similar to stratum granulosum of epidermis (Fig. 6F). The microscopic structure of pangolin scales is comparable to the claw of canine which has hard keratin layer of epidermis and lack of a granular layer (Müller et al., 1993; Müller, 1999). Even though both pangolins and hedgehogs are the mammals having modified skin appendage, pangolin scales and hedgehog spines develop in different way (Don and Reeder, 2005). Comparing with spines of the hedgehog, pangolin scales are not the modified hairs and do not grow out from the follicles as in hedgehogs (4). The hair follicles in the dermis under pangolin scales are the events

to support the difference between these two structures.

Mammary gland

Inactive mammary gland tissue of female pangolin is similar to other mammals. The small lobules are separated by interlobular connective tissue (Fig. 7A and 7B). The ducts and secretory acini are covered with cuboidal cells. In lactating mammary gland, enlarge interlobular ducts and proliferation of alveolar cells are notified (Fig. 7C and 7D).

Circumanal gland and Anal sac gland

There are two types of gland around the anus of pangolin, the circumanal gland and the

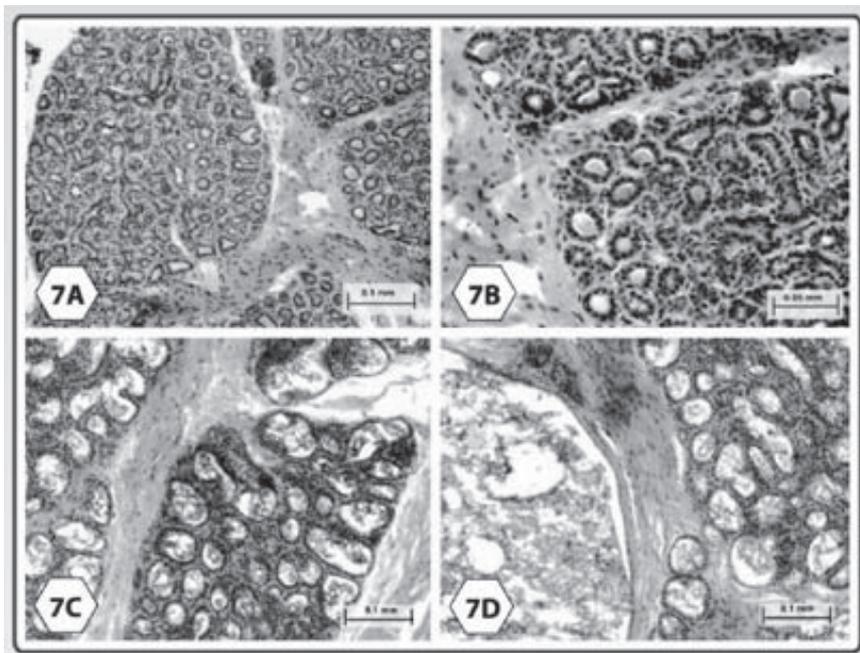


Figure 7A Pangolin's mammary gland (inactive) (H&E, X20)

Figure 7B Pangolin's mammary gland (inactive) (H&E, X40)

Figure 7C Pangolin's mammary gland (lactation) (H&E, X20)

Figure 7D Pangolin's mammary gland (lactation) (H&E, X20)

anal sac gland. The small and numerous circumanal glands are modified sebaceous gland having opening of excretory duct to the root of hair follicles and also to the cutaneous zone of anus (Fig. 8A and 8B). This type of gland in canine consists of superficial portion, typical sebaceous glands, and deep portion, ductless with compact masses hepatoid lobular gland. It was reported that this gland in canine is not exocrine (Konig et al., 1985). Anal sac glands were located around the large anal sac which is the storage site of secretion from the gland. Anal sac of dogs was explained as the paired structure which each sac has a duct for draining the content in the sac to the opening near the cutaneous zone of anus (Evan, 1993) However,

we were unable to trace the opening of anal sac. Anal sac glands of dog are apocrine sweat glands whereas in cat are sebaceous and apocrine sweat glands (Dellmann, 1998). In pangolins, only modified sebaceous gland of anal sac gland was found (Fig. 8C and 8D).

Glandular pad

SEM study of glandular pad under the tip of the tail showed the entire surface was covers by numerous blunt papillae (Fig. 5C and 5D).

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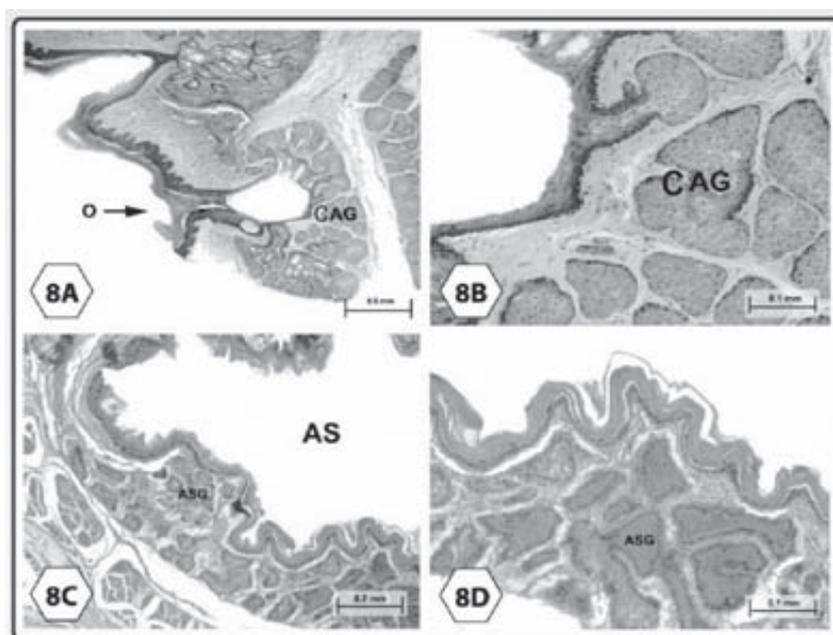


Figure 8A Pangolin's Circumanal gland (CAG) with the opening (O) (H&E, X4)

Figure 8B Pangolin's Circumanal gland (CAG) (H&E, X20)

Figure 8C Pangolin's anal sac gland (ASG) & anal sac (AS) (H&E, X10)

Figure 8D Pangolin's anal sac gland (ASG) (H&E, X20)

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