

## Skin Histology and Microtopography of Papuan White Snake (*Micropechis ikaheka*) in Relation to Their Zoogeographical Distribution

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Papuan white snake (*Micropechis ikaheka*) is endemic to New Guinea (the region of the Papua and Papua New Guinea). Internal histology of skin layer and the microtopography structure on the surface scales of *M. ikaheka* were the aims of the study. This study also related to zoogeographical of the snake in Papua. Geographical skin color variation of *M. ikaheka* can be described in three groups, i.e. brown, yellow and black group. The three groups of the snake have specific zoogeography in the mainland of Papua and satellite islands to Papua New Guinea. Paraffin method used in this work showed approximately five layers i.e. *oberhautchen*, the beta ( $\beta$ )-layer, the *mesos* layer, the alpha ( $\alpha$ )-layer, and the dermis in the snake skin. Although the paraffin method cannot explain the arrangement of pigment cells, however, the dark color on the dermis might contain melanophores that might cause dark color of the snake body. Overlapping scales formed the hinge region were flexible to assist the snakes when they moved across substrate. Scanning electron microscopy (SEM) of the *oberhautchen* of all of *M. ikaheka* revealed no *microornamentation*. However, dorsal and ventral scales showed many follicles on the entire surface of the boundary scales.

Key words: Elapidae, *M. ikaheka ikaheka*, *M. ikaheka fasciatus*, skin coloration, *oberhautchen*, hinge

### INTRODUCTION

A scaly, keratinized integument is one of the distinctive features of reptilian, to make their skin airproof (Hildebrand & Goslow 2001). Snake scales, like other reptiles, grow from top layer or the epidermis (Abdel-Aal *et al.* 2011). In lepidosaurians (lizards, snakes, and sphenodontids) germinal layer of the epidermis spinosus-like keratinocytes alternate to hard (beta) and soft (alpha) layers (Toni *et al.* 2007; Chang *et al.* 2009). The whole skin of lepidosaurians is covered by overlapping epidermal scales that protect them from abrasion and dehydration. The epidermis of lepidosaurs is of particular complexity and interest (Hildebrand & Goslow 2001), moreover with very colorful scales and organized in an attractive pattern. The color of terrestrial reptiles can be diverse and their pigmentation complex, marked with colorful patches.

Reptilian skin has two principal layers, i.e. the dermis and the epidermis. The epidermis of snakes now consists of six main layers (Klein & Gorb 2012). From the outer scale surface towards the dermis are known the *oberhautchen* layer, ( $\beta$ )-layer, mesos-

layer, ( $\alpha$ )"layer, lacunar layer and the clear layer (Hildebrand & Goslow 2001; Abdel-Aal *et al.* 2011; Klein & Gorb 2012).

A number of the larger elapids (venomous snake) show great variation in the ground color as shown as a New Guinea elapid *Micropechis ikaheka*. These colour patterns are the main characteristics distinguishing populations of the distribution of *M. ikaheka*.

*Micropechis ikaheka* (local name "white snake"; English name "New Guinea or the small-eyed snake ikaheka") is endemic to New Guinea ranges from lowlands to about 1.500 m ASL in the mountains. The species is a venomous snake widespread across the mainland island of New Guinea as well as some of its smaller satellite islands (O'Shea 1996).

Two subspecies, *M.i. ikaheka* and *M.i. fasciatus*, have been recognised by some authors, but their distributional limits are unclear. Due to lack of diagnostic characters, the subspecific designations have often been ignored (O'Shea 1996). Therefore, for the further analysis on this research we mentioned the snake in species level. The most recent study by Krey (2009) on color variation and zoogeography of the species in Papua, we found three distinct colour patterns i.e. black, brown and yellow. A unique opportunity to study the histology and microtopography skin scales of Papuan white snake (*M. ikaheka*)

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since no basic research in this area. Therefore, this works were aimed to study the internal histology of skin layer and the microtopography structure on the surface of scales of *M. ikaheka*. It is also related to the existence of the snake on different zoogeographical locations in Papua.

## MATERIALS AND METHODS

**Study Specimen.** Field studies focus on the mainland of Papua and its satellite islands. *Micropechis ikaheka* was captured by hand and preserved in the field with 10% formalin, subsequently were stored in 70% ethanol. Field observation of the snakes was carried out during day and night on some habitats such as along a river, swamp, forest, and plantations as well. All field data such as locations of the snake and their altitude were recorded.

Specimens and locality data of *M. ikaheka* used in this study also consisted of museum specimen collection from (i) Museum Zoology Bogoriense at Cibinong, Bogor; (ii) Zoology Laboratory of the State University of Papua at Manokwari; and (iii) the collections of several herpetologists. For the purpose of analysis, specimens of *M. ikaheka* were chosen as representatives for variations of the existing groups. There are at least three groups-yellow, brown and black used in this study. Study sites have been surveyed as shown in Table 1.

Geographical location of *M. ikaheka* recorded with a Global Positioning System (GPS). Most coordinates of latitude and longitude plus altitude were recorded using GPS map 60csx (Garmin Co). The mapping of several locations without recording the coordinates (site number 14, 15, 16, 22, 23, 24) was carried out based on village names from the record of the specimens collected. The Map Sources 6.9 and Arcview 3.3 software were used for mapping the snake existence on different zoogeographical locations in Papua.

**Internal Skin Histology Preparation.** The skin histology of the *M. ikaheka* was analyzed for comparison with external surfaces of scales using paraffin method. All *M. ikaheka* specimens were preserved in 10% formalin for laboratory processing. Only one specimen from yellow groups was used in this work as a model and the basic assumptions for the other group of the *M. ikaheka*. There are two characteristics of the snake skin were studied, i.e. internal structure of the skin layer and pigmentation pattern.

Two skin positions were choice for initial examination based on the color pattern of the *M.*

*ikaheka* (yellow group). Position I is a representative for the dorsal caudal region, while position II represents of the dorsal mid-body region. The skins were cut into  $\pm 1/2$  cm pieces and fixed in FAAC solution for  $\pm 24$  hours. Fixative solution was removed from the tissues and washed several times with distilled water. The tissues were dehydrated in a graded series of ethanol: 30, 50, 70, 80, 95, and 100% each 15-30 minutes. After rarefaction in the xylene  $\pm 30$  minutes, the tissues were infiltrated and embedded in paraffin.

Skins were cut ( $1 \pm 2 \mu\text{m}$ ) with a rotary microtome in longitudinal planes. Several sections were deparaffined with xylene, subsequently were stained with double haematoxylin-eosin.

**Preparation of Snake Skin for Scanning Electron Microscope (SEM).** Three individuals of *M. ikaheka* representing for the black, brown, and yellow groups were chosen for SEM observations. The SEM method was used to study the microtopography structure of the *oberthoutchen* layer surface between the three groups of *M. ikaheka*. Skin characters between dorsal and ventral sides of the snake body were observed were: surface texture of scales, microornamentation and microtopography variation.

Three skin positions were identified for initial examination based on the color pattern of the skin scales. Position I is a representative for the dorsal mid-body region, position II represents of the dorsal caudal region, and position III represents the ventral mid-body region. The skin region from each of the chosen positions was examined at different magnifications (X35-X1000) in topographical mode using scan electron microscopy JEOL JSM 5310 LV. Several stages of preparation were taken before snake skin SEM observation i.e. cleaning, prefixation, fixation, dehydration, and drying. Dried samples were placed on the stub and then coated by Au using 5-8 mA ion current for 5 minutes, with 400-500 Å thickness using ion coater IB-2.

## RESULTS

**Distribution and Habitat of *Micropechis ikaheka* in Papua.** Field observations in Papua and specimens study were carried out very interesting specific pattern distribution of *M. ikaheka* (Figure 1). Three groups of the snake i.e. brown, yellow, and black have specific zoogeography in mainland of Papua and its satellite islands (such as Yapen Island, Batanta, and Waigeo Island). All altitude observations (Table 1) showed the species spread from 5 to 305 m ASL. However, specimen from Kelila and Baliem

Table 1. Study sites of *Micropechis ikaheka* in Papua

Site number and locality	Latitude	Longitude	Biogeography region	Altitude (meter)	Collector
<b>Black group</b>					
Waifo	00° 05.970'	130° 45.642'	Waigeo Is.	50	Ps
Lopintol	00° 18.999'	130° 51.542'	Waigeo Is.	5	Ps
Urbinasopen	00° 20.219'	131° 15.544'	Waigeo Is.	15	Ps
Waimnir	00° 23'50.6"	130° 52'36.5"	Waigeo Is.	24	Ps
Wailebet	00° 53.744'	130° 38.498'	Batanta Is.	13	Ps
<b>Yellow group</b>					
Webya	00° 57.383'	130° 47.060'	Salawati Is.	25	Hc
Oransbari	01° 20'55.3"	134° 11'19.8"	Vogelkop	276	Ps
Saukorem	00° 44'45.3"	133° 23'33.9"	Vogelkop	34	Ps
Nuni	00° 46'09.2"	133° 58'57.0"	Vogelkop	24	LZU
Gunung Meja	00° 50'51.5"	134° 4'24.7"	Vogelkop	155	Ps
Andai	00° 55' 902"	134° 0' 557"	Vogelkop	36	LZU
Prafi	01° 00' 03.0"	134° 00'05.0"	Vogelkop	305	Ps
Tanah Merah	02° 28' 31.4"	133° 8'23.2"	Vogelkop	49	Ps
Manokwari	-	-	Vogelkop	-	LZU
Irian	-	-	Vogelkop	-	MZB
Jamursbamedi	-	-	Vogelkop	-	MZB
Terasai	02°01'54.0"	133°51'01.8"	Vogelkop	38	Ps
Sorong	00° 54' 40.3"	131° 18'57.6"	Vogelkop	40	Ps
<b>Brown group</b>					
Warironi	01° 50' 45.7"	136° 32' 59.4"	Yapen Is.	32	Hc
Noau	02° 04'57.1"	137° 27'32.1"	Northern	22	Hc
Kwerba	02°38'28.46"	138°24'48.35"	Northern	-	Hc
Cyclop	-	-	Northeastern	-	Hc
Kelila	-	-	Central	-	MZB
Baliem Valley	-	-	Central	-	MZB
Mindiptana	5° 38' 33"	141°	Southeastern	-	MZB
Kaimana	03°36' 16.8"	133°43'38.9"	Southwestern	64	Ps
Kuala Kencana	04°24' 11.3"	136°52' 12.4"	South	98	Ps

-: not available; Is.: Island; Ps: Present study; MZB: Muzeum Zoology Bogoriense; LZU: Zoology Laboratory Unipa; Hc: Herpetologist collection.

valley showed these snakes also live in the sea level to approximately 1700 m ASL.

*Micropechis ikaheka* that was found in the field observations confined to terrestrial rainforest areas. The snakes also found in the monsoon areas and the swamps. *M. ikaheka* was a nocturnal and secretive semi-fossorial snakes which inhabits leaf litter, loose soil or piles of decaying vegetation, husks of cocoa, coconut or palm oil. Several individuals were also observed in the hole of a fallen palm tree, under the tree buttresses and also in the rock crevice.

**Internal Skin Histology Analysis.** Position I: dorsal caudal region. The specimen *M. ikaheka* from yellow group showed a unique distinct color of skin scales in different parts of the snake body (Figure 2). Paraffin method used in this work could not explain the arrangement of pigment cells (chromatophore) in detail. However, some general information about *M. ikaheka* skin coloration and pigmentation might be described in these results. Dark colors on the entire surface of the dorsal caudal skin were due to the pigment (Figure 2-A1 and A2). The dark skin might contain melanophores, which only found within the dermis.

The other observation is the existence of overlapping scales and hinge regions (h) (Figure 2-B1 and B2). The melanin pigment is not evenly spread on all surfaces scales. There is a regular pattern of pigmentation in the *M. ikaheka* skin scales. The scales overlapping caused the skin surface region covered by other scales continued to the hinge region (h) to contain pigment (Figure 3).

Position II: dorsal midbody region. Approximately five layers were identified in microscope observation (Figure 4). These were the *oberhautchen*, the beta ( $\beta$ -layer, the *mesos* layer, the alpha ( $\alpha$ )-layer, and the dermis. The  $\beta$  and  $\alpha$  layer consisted of cells which become keratinized with the production of two types of keratin ( $\beta$  and  $\alpha$  keratin). The *oberhautchen* did not show smooth characteristics, followed the inner scale surface and hinge region (h) that composed of thin beta-layer.

**Microtopography Structure of *M. ikaheka* Skin Scales.** Position I & II: dorsal midbody and caudal region. Based on SEM analysis of the *oberhautchen* on the dorsal outer scale surface of the mid-body and caudal of *M. ikaheka* were smooth and consisted none of *microornamentation* of pits/

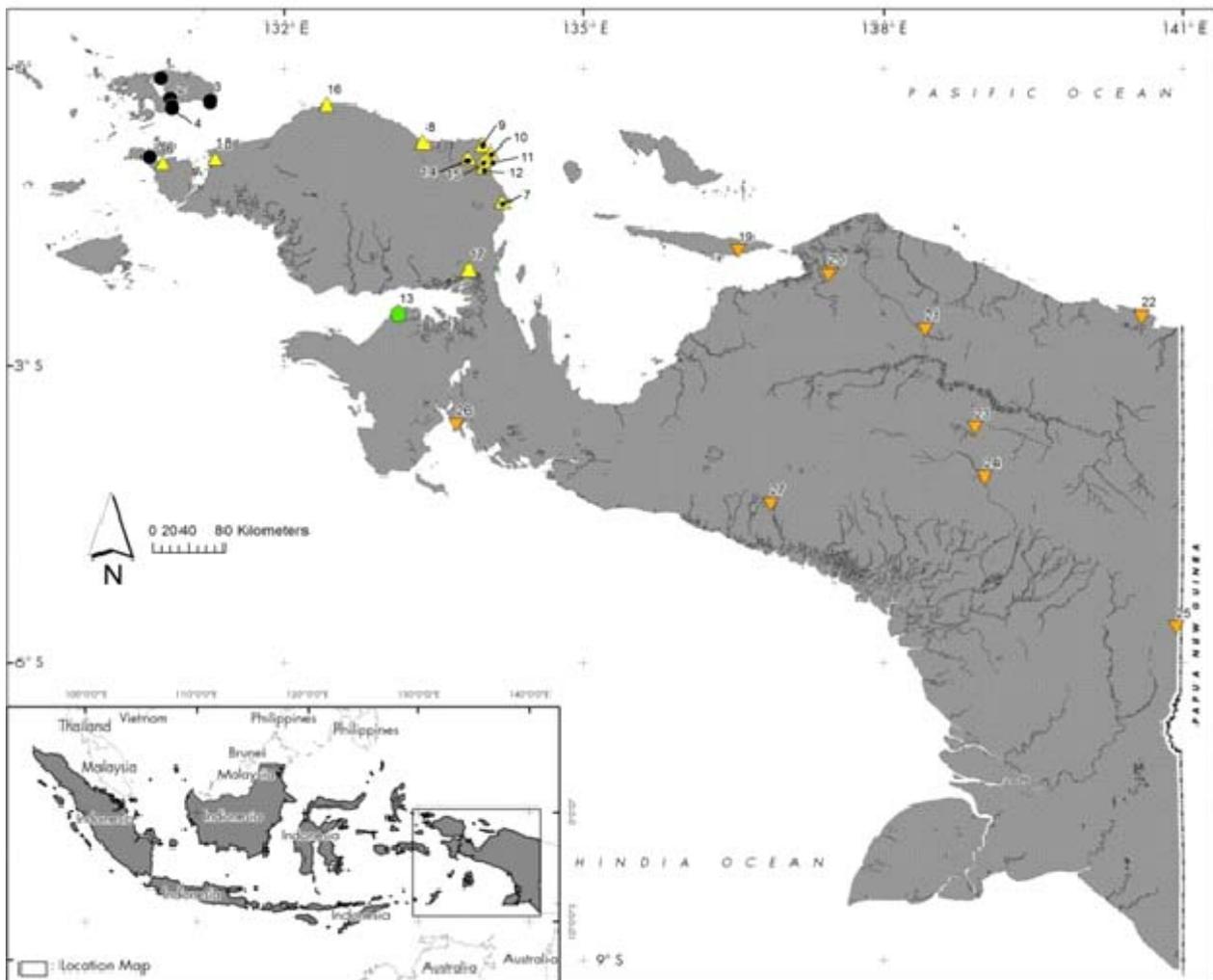


Figure 1. Map of distribution of *Micropechis ikaheka* in Papua, showing specific zoogeographical. The Yellow group (yellow triangle), brown group (reversed triangle), new group (black circle) and intermediate individual (green pentagon). Details of localities are shown in Table 1.

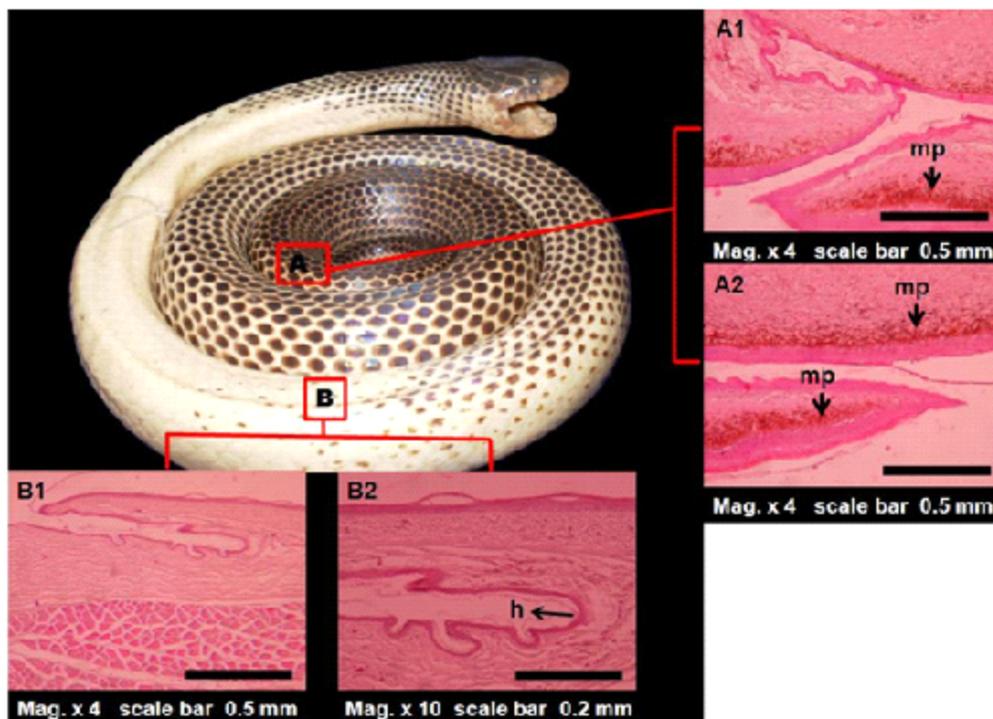


Figure 2. The arrangement and different color of *M. ikaheka* skin scales. A = skin samples from the dorsal caudal; B = skin samples from the dorsal midbody; mp = melanin pigment; h = hinge region.

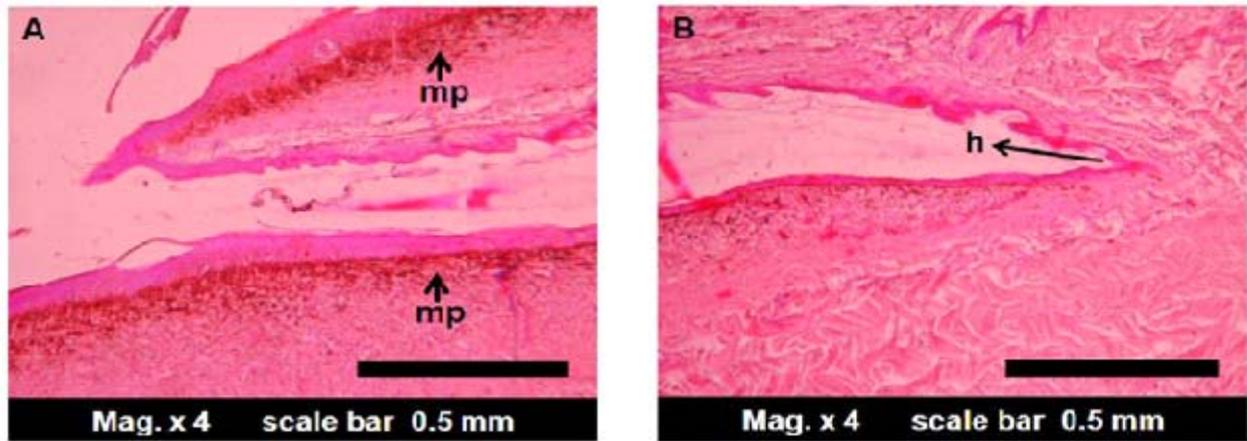


Figure 3. The scales overlapping and pigmentation pattern of the dorsal caudal region; mp = melanin pigment.

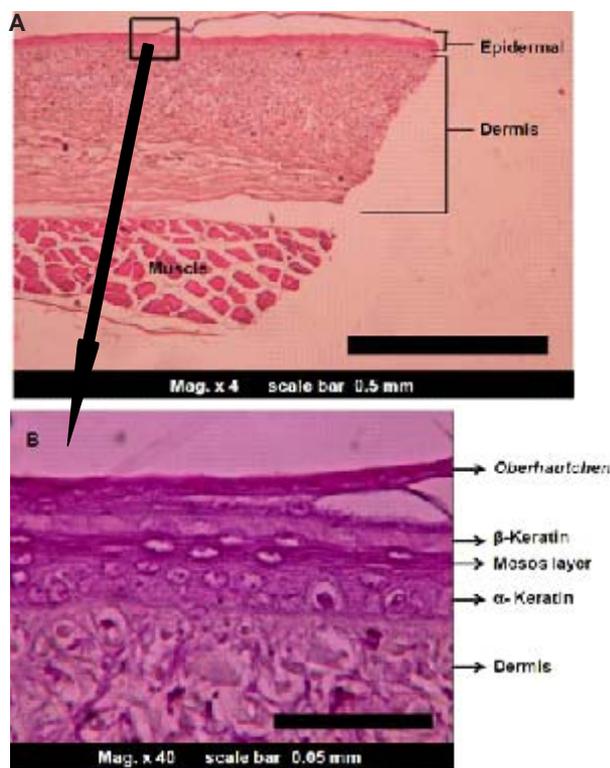


Figure 4. Photomicrographs of the dorsal midbody skin.

pores and ridges (Figure 5-A and C). These structures were common to all of specimen investigated in this work. However, dorsal scales with magnification of x1000 and x500 showed many follicles on the entire surface of the boundary scales (Figure 5-B and D).

The SEM observation of the dorsal scales surfaces also showed overlapping scales, which is common in the reptile taxon. The dorsal midbody scales were larger than those of dorsal caudal scales. Scales boundaries of the dorsal midbody scales were covered by overlapping of large scales.

Position III: ventral midbody region. In general, the ventral mid-body scales of *M. ikaheka* were larger than other body scales. Several ventral scales

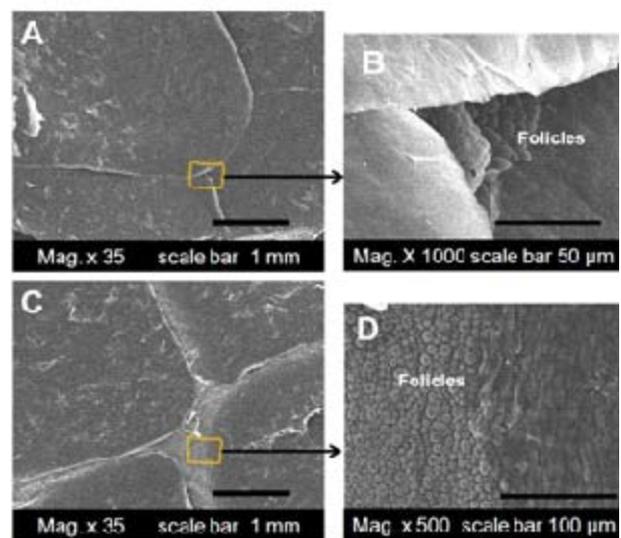


Figure 5. SEM images illustrating the microstructure of the oberhautchen on the dorsal outer scale surface of the mid-body (A,B) and caudal (C,D) scales.

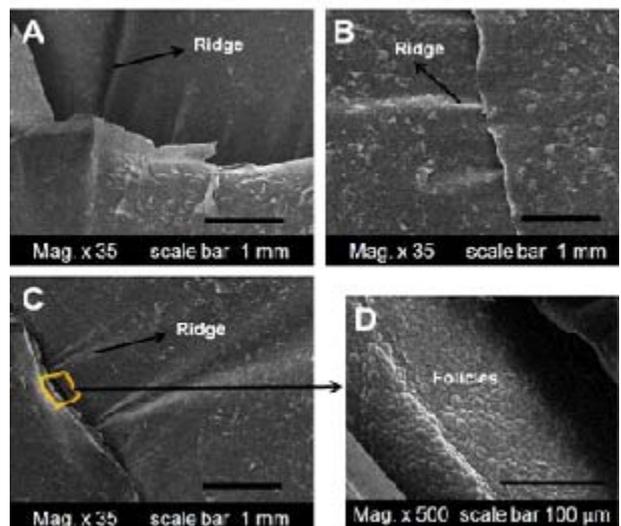


Figure 6. SEM images illustrating the microtopography of the oberhautchen on the ventral outer scale surface of the mid-body. Black group (A), brown group (B), yellow group (C), and ventral scales boundary (D).

of the snake have specialized ridge-like microornamentation (Figure 6-A, B, and C). There were at least two ridges on each ventral scales with irregular arrangement. As shown by the dorsal scales, the ventral boundary between scales showed many follicles as well (see Figure 6-D). However, the follicles on the ventral scale lay between the two scales, in contrast with the follicles on dorsal scales that were located at the base of the scales.

## DISCUSSION

**Zoogeographical Distribution of *M. ikaheka* in Papua.** According to the most generally accepted classification (O'Shea 1996), the yellow population is the subspecies of *M.i. ikaheka* with the distribution throughout Vogelkop region, including Salawati Island in north-western Papua. The brown group with dorsal banding pattern (*M.i fasciatus*) inhabits Yapen Island in northern Papua, and most of the New Guinea mainland: northern, southern and central region to Papua New Guinea. Surprisingly, an intermediate color appears between both groups (sub-species) in southwest Vogelkop, Tanah Merah, lowland forest of Bintuni, plus a new group (black group) from Batanta and Waego Island (Krey 2009). The intermediate individual might indicated the Bintuni as the hybrid zone of *M.i. ikaheka* from the Vogelkop region with *M.i. fasciatus* from the other regions.

Coloration of *M. ikaheka* indicated a variable locally. This study has no sufficient data to explain the variation in color pattern of the *M. ikaheka* in Papua. However, it explained the possibility of the current population of *M. ikaheka* founded following colonization events (dispersals) or the occurrence connections between formerly populations (vicarians). Yapen Island is a small fraction of the Van Rees Mountains on the mainland of New Guinea (Polhemus & Allen 2007), which led to the fauna include *M. ikaheka* on the island joined with the northern coast of Papua. Discontinuous local biogeography by Sagawin Strait that separates the Batanta and Salawati Island (approximately 5 km) result a fraction vicarian for paradise bird species (*Paradisaea minor* and *Cicinnurus magnivicus*) where both species does not exist in Batanta and also Waego Island (Beehler 2007).

### General Description of the Skin Scales.

Scales of various shapes and sizes cover the skin of *Micropechis ikaheka*. The number, arrangement, size and shape of the scales vary greatly from one species to the other, however they are genetically fixed for each species (Abdel-Aal *et al.* 2011). In this study, scales microtopography of all of *M.*

*ikaheka* specimen was similar. However, corresponding with their geographic distribution, they differ in color scales sharply. Therefore, the scales and color patterns might provide simple and accessible recognition of characteristics to classify *M. i. ikaheka* and *M. i. fasciatus* in New Guinea.

Similarity of the microtopography scale surface and there is no microornamentation in all specimen examined in this work, due to the snake occupy the same terrestrial habitat and use the same substrate as well (O'Shea 1996). However, Gower (2003) found six characters of microornamentation variation in 27 species of fossorial uropeltidae snake, such as cell shape, cell borders, pores/pits, cell length, denticulations and their length, denticulation length: cell length. Moreover, Klein and Gorb (2012) found difference in epidermis thickness, scale size and shape of the microstructure of the four snake species [*Lampropeltis getula californiae* (terrestrial), *Epicrates cenchria cenchria* (generalist), *Morelia viridis* (arboreal), and *Gongylophis colubrinus* (sand-burrowing)] correlated with a specific adaptation to factors such as habitat, substrate and locomotion.

Disparity between scales boundary and scales region (Figure 5) might be related to the scales contact intensity with substrate surface. Scales boundary might contain slightly thin keratin for relaxation maintenance when the snake moves. In addition to protecting the body, scales also aid in locomotion, assist retain moisture, and alter the surface characteristics (Abdel-Aal *et al.* 2011).

The ridge found in ventral scales might assist *M. ikaheka* when they moved on the surface of the substrate or channel. Gower (2003) found regular ridges in some uropeltid snake and suggests that the ridges microornamentation might offer performance advantages for life in soil. However, the functional significance of *M. ikaheka* ridges is difficult to ascertain. There are no data on the relative locomotors mechanics among *M. ikaheka* as yet.

Melanin pigments were identified in dorsal body with dark color (Figure 2 & 3). These findings suggested that of skin color was due to melanin richness. The staining method needed to distinguish the color of the different types of melanin in *M. ikaheka* in the future studies. Although this study only used one sample of the yellow group, one can predicted that the entire surface of the dark skin of the snake contained abundant melanin pigment. Kikuchi and Pfenning (2012) found black tissue in the skin of non-venomous scarlet kingsnake *Lampropeltis elapsoides* contained only melanophores, which were mostly large and found within the dermis.

Dark color might facilitate snakes to absorb heat during cooler weather (Tylor & O'Shea 2004).

The green python *Morelia viridis* is commonly yellow or red in juveniles but green in adult. The pythons are regionally variable in colour patterns found throughout the lowland rainforest of New Guinea and adjacent far northeastern Australia (Rawlings & Donnellan 2003). Gibson and Falls (1979) found that a melanic morph of a temperate zone snake maintained a higher body temperature when exposed to solar radiations than the lighter, striped morph snake. Therefore, one can conclude that colors are response with respect to age, seasons, and other environment factors. In this study, further ecology study of *M. ikaheka* is needed to explain the environmental factor drives color differentiation in *M. ikaheka* pattern.

In microscope investigation the skin of the snake were covered by keratinocytes. There are two main types of intermediate filament proteins in vertebrate keratinocytes: alpha ( $\alpha$ ) and beta ( $\beta$ ) keratins. The epidermal appendages of reptiles and birds are characterized by the presence of both alpha ( $\alpha$ ) and beta ( $\beta$ ) type keratin proteins (Sawyer *et al.* 2000). *Micropechis ikaheka* have both  $\alpha$  and  $\beta$ -keratins. These cells are thus being transformed into a hard protective layer called *oberhautchen* which constitutes the strongest outermost layer (Chang *et al.* 2009; Abdel-Aal *et al.* 2011). The  $\alpha$ -keratogenic tissue provides a barrier to water loss while an overlying  $\beta$ -keratogenic layer provides mechanical stiffness to the skin (Licht & Bannett 1972; Chang *et al.* 2009). The  $\beta$ -keratins are also responsible for the mechanical resistance of scales in reptiles (Toni & Alibardi 2007). Although  $\beta$ -keratin is important but the thickness of the layer is different in every scales region.

Overlapping scales and production of  $\beta$ -keratins provide strong protection (Wu *et al.* 2004).  $\beta$ -keratins are specific proteins utilized to make hard structures including scales, scutes, and claws (beak and feathers in birds) (Toni *et al.* 2007). In this study,  $\beta$ -keratin observed at the underside scale is thinner than the top surface (Figure 2-B2 and Figure 3) even much thinner on the hinge region (h). The  $\beta$ -layer arrangements of *M. ikaheka* are similar to the American anole *Anolis carolinensis*. Maderson *et al.* (1998) found the  $\beta$ -layer of the *A. carolinensis* was the thinnest, close to the sense organ and in the inner scale surface and hinge regions. The hinged region (h) that exists between scales should be flexible to assist the snakes when they moved across substrate.

This snake skin histology study showed that the thin hinged region can be the entry point for ectoparasites mite or other pathogens invade the host. McKenzie and Green (1976) found early skin lesions occurred in three captive carpet snakes (*Morelia spilotes variegata*) at hinge regions between scales. Simonov and Zinchenko (2010) also found common snake mite (*Ophionyssus natricis*) on the soft tissues around the eyes, under scales and shields on the underside of snakes head and more often in loreal pits of Siberian pit-viper (*Gloydius halys halys*). In the slive snakes of *M. ikaheka* we found some of mite on the underside of an overlapping ventral scales. The mite possessed a tiny sharp claw tarsus to stab the snake skin, especially in the hinge region (personal observations).

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