



Faculty of Resource Science and Technology

**Adaptive Skin Morphology and Ecological Responses of Bornean Tree
Frogs (Family: Rhacophoridae)**

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Adaptive Skin Morphology and Ecological Responses of Bornean Tree Frogs
(Family: Rhacophoridae)

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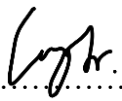
Faculty of Resource Science and Technology

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2025

DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.


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ABSTRACT

Previous studies on the skin of Bornean frogs have demonstrated that it functions as the first line of defense, protecting them against extreme heat, harmful substances, and prolonged ultraviolet radiation. However, detailed information about the skin's components, particularly the types and distribution of glands, remains limited, as does its relationship with environmental parameters and ecological adaptation. This study was conducted to examine variations in the skin structure of selected Bornean tree frogs through histological analysis and to investigate the correlation between their habitats and skin histology. Environmental parameters assessed included water pH, water turbidity, water temperature, and dissolved oxygen (DO). The skin was divided into four sections and preserved in 10% formalin before undergoing histological processing. Haematoxylin and eosin staining was used to examine the skin structures. Nine skin features, including the epidermis and glands, were studied in the Bornean tree frogs. The study found that the ventral epidermis is thicker than the dorsal epidermis, likely to provide extra protection against abrasion and pathogens due to frequent contact with surfaces. Species such as *Polypedates leucomystax*, *Polypedates ottilophus*, *Polypedates colletti*, and *Rhacophorus pardalis* have more mucous glands on the dorsal side, helping to protect the skin and retain moisture, reducing the risk of damage and dehydration. Seromucous glands were found throughout the skin of all studied species, suggesting their role in helping tree frogs adapt to drier habitats. *Polypedates ottilophus* has larger granular glands, likely responsible to such strong unpleasant odours which act as a chemical defence to deter predators. The results showed that *P. leucomystax* exhibited the most noticeable skin differences, with seven out of nine features varying significantly ($p < 0.05$), indicating more specialized skin adaptations. Spearman and Kendall correlations showed a significant positive correlation between seromucous glands and water pH ($p = 0.018$ and $p = 0.014$,

respectively), suggesting that these glands variations help the frogs adapt to environments with higher pH levels, possibly in habitat with less acidic water conditions. In conclusion, the findings contribute to a deeper understanding of the relationship between skin adaptations in Bornean tree frogs and the quality of their surrounding environment.

Keywords: Glands, histology, environmental parameters, tree frog

Morfologi Kulit Adaptif dan Tindak Balas Ekologi Katak Pokok Borneo (Famili: Rhacophoridae)

ABSTRAK

Kajian terdahulu mengenai kulit katak Borneo telah menunjukkan bahawa kulit berfungsi sebagai barisan pertahanan pertama, melindungi spesies ini daripada haba melampau, bahan berbahaya, dan pendedahan berpanjangan kepada sinaran ultraungu. Namun begitu, maklumat terperinci mengenai komponen kulit, khususnya jenis dan taburan kelenjar, masih terhad, begitu juga kaitannya dengan parameter persekitaran dan penyesuaian ekologi. Kajian ini dijalankan untuk meneliti variasi struktur kulit katak pokok Borneo terpilih melalui analisis histologi dan untuk mengkaji hubung kait antara habitat dan histologi kulit spesies tersebut. Parameter persekitaran yang dikaji termasuk pH air, kekeruhan air, suhu air, dan oksigen terlarut (DO). Kulit dibahagikan kepada empat bahagian dan diawet dalam formalin 10% sebelum melalui proses histologi. Teknik pewarnaan hematoksilin dan eosin digunakan untuk memeriksa struktur kulit. Sembilan ciri kulit, termasuk epidermis dan kelenjar, telah dikaji dalam spesies katak pokok Borneo. Kajian mendapati bahawa epidermis ventral adalah lebih tebal daripada epidermis dorsal, kemungkinan untuk memberi perlindungan tambahan terhadap lelasan dan patogen akibat kekerapan sentuhan dengan permukaan. Spesies seperti Polypedates leucomystax, Polypedates otilophus, Polypedates colletti, dan Rhacophorus pardalis mempunyai lebih banyak kelenjar mukus pada bahagian dorsal, yang membantu melindungi kulit serta mengekalkan kelembapan, sekali gus mengurangkan risiko kerosakan dan penyahhidratan. Kelenjar seromukus ditemui di seluruh kulit semua spesies yang dikaji, menunjukkan peranannya dalam membantu penyesuaian katak pokok terhadap habitat yang lebih kering. Polypedates otilophus mempunyai kelenjar granular yang lebih besar, yang berkemungkinan

menghasilkan bau yang kuat dan tidak menyenangkan sebagai pertahanan kimia untuk menghalang pemangsa. Hasil kajian menunjukkan bahawa P. leucomystax mempamerkan perbezaan kulit yang paling ketara, dengan tujuh daripada sembilan ciri berbeza secara signifikan ($p < 0.05$), menunjukkan penyesuaian kulit yang lebih khusus. Korelasi Spearman dan Kendall menunjukkan hubungan positif yang signifikan antara kelenjar seromukus dan pH air (masing-masing $p = 0.018$ dan $p = 0.014$), mencadangkan bahawa variasi kelenjar ini membantu katak menyesuaikan diri dengan persekitaran yang mempunyai tahap pH lebih tinggi, mungkin dalam habitat yang kurang berasid. Sebagai kesimpulan, penemuan ini menyumbang kepada pemahaman yang lebih mendalam tentang hubungan antara penyesuaian kulit katak pokok Borneo dan kualiti persekitaran habitatnya.

Kata kunci: Kelenjar, histologi, parameter persekitaran, katak pokok

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LIST OF ABBREVIATIONS

AES	Acoustic Encounter Surveys
ANOVA	Analysis of Variance
DC	Dorsal Centre
DH	Dorsal Head
DO	Dissolved Oxygen
DPX	Distyrene Plasticizer Xylene
FMHS	Faculty of Medical and Health Sciences
FRGS	Fundamental Research Grant Scheme
GPS	Global Positioning System
HCV	High Conservation Value
H&E	Haematoxylin and Eosin
HL	Hind Leg
IR	Infrared
µm	Micrometer
mm	Millimeter
NREB	Natural Resource and Environment Board
NMDS	Non-metric Multidimensional Scaling
Ph	Potential Hydrogen (measure of acidity or alkalinity)
PPT	Pusat Penyelidikan Tumbuhan
SPSS	Statistical Package for the Social Sciences
SVL	Snout-Vent Length
UNESCO	United Nations Educational, Scientific and Cultural Organization
UNIMAS	Universiti Malaysia Sarawak

VC	Ventral Centre
VES	Visual Encounter Surveys
VH	Ventral Head
WL	Wildlife
X	Xylene

CHAPTER 1

GENERAL INTRODUCTION

1.1 Study Background

Borneo is known for its highly varied frog fauna, with over 180 frog species documented across the island (Inger et al., 2017). Frogs belong to the Anura order of amphibians. Fundamentally, amphibians spend many of their lives in water before transitioning to a terrestrial adult or an animal that alternates between life in and out of the water (Duellman & Trueb, 1986). Frogs have no tail, prominent eyes, and strong, webbed hind feet that allow them to leap and swim. Many frog and toad populations live in small, impermanent ponds and the surrounding forested regions, which are often subjected to a variety of stressors such as industrial pollutants, climate change, and other factors.

Amphibians are a vital component of many ecosystems and play important roles in maintaining ecological balance. Frogs are often used as biological indicators due to their sensitivity to environmental changes. This sensitivity stems from their semi-permeable skin and life cycle that involves both aquatic and terrestrial stages, making them highly responsive to shifts in habitat quality, water chemistry, temperature, and pollution (Park & Do, 2023).

The microscopic study of plant and animal cells and tissues by staining, sectioning, and analysing them under a microscope is known as histology (Alturkistani et al., 2015). Histology aids in the comprehension of the structure-function relationship. Amphibian skin qualities are vital for retaining all the frog skin's functional functions, which work in tandem with the cardiac and respiratory systems. In frogs, there are three types of exocrine glands

seromucous gland, mucous gland, and granular gland (poison) (Mills & Prum, 1984). They also claim that all these glands differ in terms of cell type, size, and staining characteristics based on morphology. According to Zainudin et al. (2018), the frogs' vulnerability to their changing environment is determined by epidermis thickness, dermis layer, distribution, and number of glands.

Various anuran species have prominent colour or dorsal pattern polymorphisms, making them a great model for research on the evolution and upkeep of polymorphisms. Furthermore, compared to the evolutionary and developmental pathways that result in patterns, colour changes are probably governed by different mechanisms (Frost-Mason et al., 1994; Hoffman and Blouin, 2000). Colours can serve as a warning to predators that the frog might be toxic. Certain frogs possess the capability to alter the colour of their skin, enabling them to regulate their rate of heat absorption. This adaptive trait aids them in effectively controlling their body temperature. The coloration of certain frogs, like the blue dart frog, can serve as an indicator of their poisonous nature. The presence of blue hues in frogs acts as a visual signal indicating their inedibility. Studies demonstrate that specific brightly colored frogs possess a lethal toxin capable of causing harm or even fatality to humans (Summers et al., 1997).

1.2 Problem Statement

Frogs are distinguished by their unique morphological features, particularly their skin, which plays a critical role in their survival. The major components of frog skin, primarily lipids, are heavily influenced by their habitats (Li et al., 2024). While comprehensive research on the histological characteristics and ecological implications of frog skin in Borneo remains limited, existing studies have begun to shed light on this area.

Notably, Zainudin et al. (2018) conducted a histological analysis of Bornean frog, revealing variations in epidermal thickness and glandular structures that suggest possible morphological adaptations to environmental condition. Arboreal tree frogs, which rely heavily on moist microhabitats, are particularly vulnerable as their ecological niches are often confined to fragmented forest canopies and limited water sources. This study aims to address the gap in understanding how the structure and properties of frog skin support their ecological needs.

1.3 Hypothesis

Hypothesis 1:

- i. Hypothesis I (Null): There is no significant difference histological difference in the skin structure among the selected tree frog species.
- ii. Hypothesis II (Alternative): There is significant difference histological differences in the skin structure among the selected tree frog species.

Hypothesis 2:

- i. Hypothesis I (Null): There is no significant variation in epidermal thickness, dermis layer composition, or gland types and distribution across different skin regions among the selected tree frog species.
- ii. Hypothesis II (Alternative): There is significant variation in epidermal thickness, dermis layer composition, or gland types and distribution across different skin regions among the selected tree frog species.

Hypothesis 3:

- i. Hypothesis I (Null): There is no significant correlation between the skin histological characteristics of *Polypedates leucomystax* and environmental parameters (water pH, water turbidity, water temperature, and dissolved oxygen) across different locations in Sarawak.
- ii. Hypothesis II (Alternative): There is a significant correlation between the skin histological characteristics of *P. leucomystax* and environmental parameters (water pH, water turbidity, water temperature, and dissolved oxygen) across different locations in Sarawak.

1.4 Objectives

- i. To identify the skin structures of selected tree frog species (Family: Rhacophoridae).
- ii. To compare the skin structures across various skin regions among the selected tree frog species, focusing on epidermal thickness, dermis layers, and gland types.
- iii. To investigate the skin histology of *P. leucomystax* in connection with environmental parameters (Water pH, water turbidity, water temperature, and dissolved oxygen) at various locations in Sarawak.

CHAPTER 2

LITERATURE REVIEW

2.1 Tree Frog (Family: Rhacophoridae)

Borneo is home to an incredibly diverse range of frog species, with estimates suggesting over 180 species and counting, as new species continue to be discovered each year (Inger et al., 2017). Das et al. (2014) suggested Borneo's size, geography, and geologic record have all contributed to the island's high amphibian abundance and diversity. Past temperatures and land relations, in addition to current habitat conditions, are related to current diversity. Approximately 40 species of Rhacophoridae can be found in Borneo. The Family Rhacophoridae are referred to as 'tree frogs', and they are divided into eight genera. In the family Rhacophoridae, the eight genera are *Chiromantis*, *Feihyla*, *Kurixalus*, *Nyctixalus*, *Philautus*, *Polypedates*, *Rhacophorus*, and *Theloderma*.

Tree frogs (Anura: Rhacophoridae) are a vast family of arboreal frogs with about 320 species found in Sub-Saharan Africa, Philippines, China, Taiwan, Southeast Asia, Japan, and the Greater Sunda Islands (Orlov, 2012). Inger et al. (2017) stated that with the exception of a single genus found in Africa, the Rhacophoridae is primarily an Asian family. Most of the species reside in bushes or trees above ground, and the tips of their toes and fingers have been dilated into broad discs. Most of them reproduce in standing water bodies, and the eggs are laid above the water, in most species inside a constructed nest, rather than completely in the water. Tree frogs assume a posture in which the body plane is parallel to the surface, flex their legs and arms, expand their toes and fingers, and keep their hands and feet in the plane of the body whenever falling or jumping from their perching spot. They are able to navigate

in the air and minimize the velocity of descent by doing so. Some *Rhacophorus* species with widely webbed fingers and toes are even capable of gliding flights (Inger et al., 2017).

2.1.1 *Rhacophorus pardalis*

Rhacophorus pardalis, also known as the Flying Frog, primarily inhabits primary and secondary forests, often in proximity to water bodies such as ponds, slow-moving streams, and marshes (Figure 2.1). These frogs are arboreal, spending most of their time in the canopy. They are known for their ability to glide between trees using the webbing between their toes, which acts like a parachute. This adaptation helps them escape predators and move efficiently through their forest habitat. According to Inger et al. (2017), *R. pardalis* exhibit a bright green dorsal coloration with possible spots or patterns. Their ventral side is usually pale, often white or yellowish. One of the most distinctive features is the extensive webbing between their fingers and toes, which is brightly coloured, usually orange or yellow. This webbing is essential for their gliding ability. They have large, protruding eyes with horizontal pupils, which enhance their night vision, as they are primarily nocturnal. The skin of *R. pardalis* is generally smooth, aiding in reducing friction when gliding and allowing for a streamlined shape. Their skin contains mucous and seromucous glands that keep it moist, essential for their respiration and preventing desiccation. Granular glands also be present, which can secrete toxins as a defence mechanism against predators.



Figure 2.1: *Rhacophorus pardalis*

2.1.2 *Polypedates colletti*

Polypedates colletti, commonly known as Collett's tree frog, is a species that thrives in subtropical and tropical forests, often found near water bodies like ponds and slow-flowing streams. It is a semi-aquatic species that prefers areas with abundant vegetation, which provides both shelter and opportunities for breeding. Morphologically, this species has a slender build with long limbs adapted for climbing, aiding in its arboreal lifestyle. According to Inger et al. (2017), the skin is smooth on the dorsal side with a slightly granular texture around the flanks. The ventral side is softer and paler. The skin has a moist surface, typical of amphibians, allowing for efficient gas exchange and moisture retention. The colour and texture help the species blend into its surroundings, protecting it from predators. Most of this species display a distinct dark X-shaped or "hourglass" pattern on their back. The overall morphology and skin structure of *P. colletti* are well-suited to its arboreal, semi-aquatic environment, enhancing its survival and reproductive success.



Figure 2.2: *Polypedates colletti*

2.1.3 *Polypedates macrotis*

Polypedates macrotis also known as the large-eyed whip frog or the big-eyed tree frog, is a species of amphibian in the family Rhacophoridae. *Polypedates macrotis* is

typically found in tropical and subtropical moist lowland forests. It is arboreal, spending most of its time in trees and shrubs, often near bodies of water such as ponds, streams, or marshes. The species is known to be quite adaptable, sometimes inhabiting disturbed areas such as plantations or gardens, provided there is sufficient vegetation and water. The most distinctive feature of *P. macrotis* is its large, prominent eyes with horizontal pupils, which are adapted for nocturnal vision. It has a slender, elongated body with relatively long limbs, which aid in its arboreal lifestyle. The toes are webbed and equipped with adhesive pads that facilitate climbing on smooth surfaces. The frog's head is broad with a rounded snout, and the tympanum (eardrum) is visible and well-developed (Inger et al., 2017). The skin of *P. macrotis* is generally smooth, with a somewhat shiny appearance. The coloration is often brown or grey with darker markings for camouflage against tree bark or leaves, while the ventral surface is typically lighter, white or pale yellow and the dorsal skin may feature subtle granulations on the back and limbs to enhance blending with rough textures (Inger et al., 2017).



Figure 2.3: *Polypedates macrotis*

2.1.4 *Polypedates ottilophus*

Polypedates ottilophus, commonly known as the File-eared Tree Frog, is a species native to Southeast Asia, particularly found in regions like Borneo, Sumatra, and parts of the Malay Peninsula. These frogs are typically arboreal, meaning they live in trees, and they are often found in tropical rainforests, swamp forests, and lowland forests. They prefer habitats near slow-moving or stagnant water bodies like ponds, marshes, or water-filled tree holes, which are essential for their breeding. According to Inger et al. (2017), the File-eared Tree Frog has a distinctive morphology characterized by its broad, flattened body, and well-developed limbs. The most notable feature is the large, triangular, and fleshy protrusions or "file-ears" on the sides of their heads. Their limbs are long and slender, adapted for climbing, with adhesive pads on their fingers and toes that allow them to grip onto leaves and branches effectively. Their eyes are large, with horizontal pupils, providing them with excellent night vision, which is essential for their nocturnal lifestyle. The skin of *P. ottilophus* is smooth with a slightly granular texture in some areas, especially on the back. The dorsal surface is typically brown or grey with darker patterns, providing camouflage against tree bark and leaves. The ventral skin is lighter, often cream or pale yellow. The skin contains various glands, including granular glands that may play a role in defence by secreting toxic or noxious substances to deter predators (Inger et al., 2017). The epidermis of this species is relatively thin, facilitating gas exchange, which is crucial for amphibians.



Figure 2.4: *Polypedates otilophus*

2.1.5 *Polypedates leucomystax*

Polypedates leucomystax, commonly known as the Common Tree Frog or Four-lined Tree Frog, is a species of tree frog found throughout Southeast Asia. *Polypedates leucomystax* is a highly adaptable species with a broad ecological niche. It is commonly found in a variety of habitats, including lowland forests, montane forests, agricultural areas, gardens, and even urban environments. This frog is typically associated with moist environments and is often found near stagnant or slow-moving bodies of water, such as ponds, ditches, and rice paddies, which provide suitable breeding sites (Razali, 2017). *P. leucomystax* has a slender and elongated body typical of arboreal frogs, with long limbs that are well-adapted for climbing. The fingers and toes are equipped with adhesive pads that allow the frog to cling to vertical surfaces and navigate through the foliage with ease. The skin coloration is highly variable, ranging from light brown to greenish, often with darker markings or lines along the body. This species may also exhibit four narrow dark stripes running down its back or display scattered brown spots (Inger et al., 2017). This variability in coloration helps the frog blend into its environment, providing camouflage against predators. The skin of *P. leucomystax* is smooth and somewhat moist, which aids in cutaneous respiration, a significant mode of gas exchange for many amphibians. The dorsal

skin has small, scattered tubercles, while the ventral skin is smoother. The skin secretes mucus to maintain moisture, which is essential for the frog's survival in both its aquatic and terrestrial habitats. The skin also contains glands that produce toxic or noxious substances as a defence mechanism against predators.



Figure 2.5: *Polypedates leucomystax*

2.2 Frog Glands

Amphibians have a chemical defense mechanism that is mostly given by skin glands, primarily granular glands, which release chemical agents to combat microbial or parasitic infections (Rollins-Smith et al., 2005 & Sharma et al., 2012). Frogs and toads are two distinct groups of anurans, with significant differences in skin composition and gland presence. In frogs, there are three types of exocrine glands: seromucous gland, mucous gland, and granular gland (poison) (Mills and Prum, 1984). They also claim that all of these glands differ in terms of cell type, size, and staining characteristics based on morphology. The structure and distribution patterns of glands along the body differ between species (Toledo & Jared, 1995). The mucous gland protects the skin from water loss and acts as a chamber for the exchange of gases, while the serous or granular gland discharge includes poison that

is lethal to potential predators and germs on the skin surface (Zainudin et al., 2018). Toledo et al. (2011) found that macro glands associated with predator defense have distinct locations. According to Daly et al. (1987) amphibian skin's granular glands secrete a wide range of active biochemicals, including steroids, alkaloids, and peptides. The toad's parotid macro glands release venom over the dorsal head of the skin region, which is activated to discharge the venom after being bitten by a predator (Jared et al., 2009).

The morphology and function of serous glands as a source of volatile chemicals in the skin of the tree frog *Hypsiboas pulchellus* (Amphibia: Anura: Hylidae) were examined in a study investigating odorous secretions in anurans (Brunetti et al., 2016). Their findings revealed that mucous glands are evenly distributed throughout the body, whereas serous glands are more concentrated in the scapular region. Chemical analyses suggested a relationship between lipids and volatile compounds, implying that some of the volatiles in *H. pulchellus* are likely produced through fatty acid metabolism. Furthermore, the electron-translucent inclusions observed within the granules of serous glands were proposed as potential storage sites for volatile chemicals or their precursors. In five other species of hylid frogs belonging to the genus of *Cophomantini*, histochemical and glandular distribution analyses revealed that all of them had a high lipid content, whereas only the *H. pulchellus* group has a diverse distribution of serous glands. In conclusion, the odorous volatile secretions and the distribution pattern of serous glands in members of this species group are likely connected to defense functions.

A study was conducted on the distribution and average size of granular glands in the poisonous rock frog, *Odorrana hosii*, focusing on their defensive role and anatomical characteristics (Rasit et al., 2018). In comparison to other areas of the skin, the granular glands in *O. hosii* were significantly abundant in the dorsal head region, with the maximum

size in the dorsal central region. Because of the size and abundance of granular glands, the dorsal head and central skin area can be employed for peptide extraction. In conclusion, more research into the extraction of biochemical content from granular glands should concentrate on the skin over the dorsal head and central region, as it comprises the most granular glands and is the largest in size.

2.3 The Significance of Frog Skin

The thin skin of frogs assists them in both respiratory gas exchange and the absorption of water. Zainudin et al. (2018) elucidated that the functions of the frog skin collaborate with the cardiac and respiratory systems. Consequently, to facilitate effective cutaneous respiration and minimize water loss through evaporation, their skin undertakes osmotic reabsorption upon contact with water. Moreover, frogs also necessitate a moist body surface while in the air to facilitate the exchange of respiratory gases through the pharynx and lung development (Sungif, 2017). The reaction of frogs to environmental pollutants might provide insights into the effects on other vertebrates and even humans, given that chemical contamination is among the contributing factors to amphibian population declines. This also renders them vulnerable to environmental pollutants, specifically chemicals from pharmaceuticals, industry, and agriculture (Young et al., 2001; Roy, 2002).

Furthermore, it has been established for a considerable time that frog skin serves as an abundant reservoir of chemicals with the ability to eradicate bacteria, fungi, and viruses. According to Conlon et al. (2008), examining skin secretions from *O. hosii* that were stimulated by norepinephrine resulted in the identification of eight peptides with varying levels of antibacterial effects. Scientists have endeavoured to extract these infection-combatting compounds and modify them for potential use in creating novel antibiotics, given their tendency to harm human cells and vulnerability to elimination by specific substances

present in the bloodstream. Nonetheless, Conlon et al. (2010) have identified a method to modify the molecular composition of frog skin antibiotic compounds, rendering them less detrimental to human cells while enhancing their potency as agents that combat germs. They also identified additional adjustments that allowed the frog skin secretions to resist the assault of harmful enzymes within the bloodstream, leading to antibiotics that remain active for an extended period, thereby increasing their efficacy in combating infections.

A study was conducted to examine the diversity of structures and the distribution of host-defense peptides found in frog skin secretions (Conlon et al., 2011). Frogs from numerous species produce cationic peptides in their skin that take on an amphipathic α -helical shape when in an environment that mimics cell membranes. These peptides frequently exhibit bactericidal and fungicidal properties, aligning with the notion that they participate in the host's defense mechanism against harmful microorganisms. Although there is a prevalent misconception that antimicrobial peptides are produced in the skin of all anuran species, their distribution among species is irregular. This irregularity suggests that their synthesis might offer evolutionary benefits to the organism, but it's not an essential survival trait. The limited effectiveness of numerous antimicrobial peptides found in frog skin aligns with the theory that symbiotic bacteria on the skin's surface might serve as the primary defense against harmful microorganisms in the surroundings, while antimicrobial peptides could play a secondary role in certain species.

2.4 Histology for Frogs

Histology is the discipline that investigates the arrangement of tissue components, commencing with single cells and stretching across organs and organ systems. Histology also demonstrates evident connections with cell biology and anatomy; additionally, it establishes the foundational structure for comprehending function, serving as groundwork

for exploring irregularities in structure and function as well (Tamizhazhagan and Pugazhendy, 2017). According to Junqueira et al. (1998), with the progression of chemistry, pathology, immunology, and physiology along with the exploration of their interconnections, histology emerges as a means to enhance our comprehension of tissue biology. Moreover, the synthesis of insights from these domains gives rise to disciplines like histopathology, histophysiology, immunohistochemistry, and histochemistry which collectively contribute to a more comprehensive grasp of tissue biology. Histology investigation can involve a range of microscopy techniques, including light microscopy, electron microscopy, confocal microscopy, phase-contrast microscopy, polarizing microscopy, fluorescence microscopy, and scanning electron microscopy.

In the context of slide preparation for microscopic examination, histology investigations encompass a series of six primary stages. These stages encompass skin grossing, skin fixation, skin processing, skin embedding, skin sectioning, and finally, skin fishing. In the realm of histopathology, the Hematoxylin and Eosin (H & E) staining method stands out as the most prevalent technique. This method employs a mixture of two dyes, Hematoxylin and Eosin, to highlight the nucleus and cytoplasmic inclusions within clinical specimens. Acidic structures are imbued with a purplish-blue hue by hematoxylin, whereas eosin imparts a pinkish-red tint to basic structures (Young et al., 2010). Cardiff et al. (2014) stated that for optimal slides and outcomes, strict adherence to the protocol is imperative. Furthermore, careful attention must be paid to the thickness of the tissue section. This practice contributes to precise phenotyping and the clear depiction of tissue intricacies.

A histological analysis of the skin structure of the Bornean horned frog, *Megophrys nasuta* (Amphibia: Anura: Megophryidae), across different body regions was carried out to provide detailed anatomical insights (Zainudin et al., 2018). According to this research,

differences in skin traits could potentially signify their adjustments to the surrounding environment. The results revealed that the strata, epidermis, and dermis layers of *M. nasuta* skins exhibited consistent features across all regions of the body. Nonetheless, the thickness of the epidermal layer and the distribution of glands differ between the dorsal and ventral areas (which are the initial points of contact with the environment), implying a potential adaptive approach. The arrangement of serous and mucous glands across diverse skin regions could similarly mirror the behaviours of this species within its natural environment. These findings could offer insights into how the skin structure serves ecological requirements, particularly in response to the evolving conditions within fragmented habitats.

2.5 Ecological Implication of Frogs

The major threat to global biodiversity is the conversion of forested regions in Borneo to agricultural locations, such as oil palm plantations (Phalan et al., 2013). Agricultural expansion is the main cause of habitat loss in tropical forests, and it supports significantly less biodiversity than native forests (Yue et al., 2015). Many frog and toad populations live in small, transitory ponds and the surrounding forest region, which are often subjected to a variety of stressors, including climate change (Corn, 2005), industrial toxins (Sower et al., 2000), and urbanisation (Barrett et al., 2010). According to Berry et al. (2010), logging has a negative impact on the amphibian population. The impact of habitat fragmentation on frogs has been worse, as it affects dispersal, accessibility to breeding sites, and movement between neighbouring fragments (Becker et al., 2007).

Biomonitoring, or the use of organisms to measure environmental contamination, such as that of surrounding air or water, is becoming more widely used to analyse the impact of pollutants on specific ecosystems (Whitfield, 2001). Bornean amphibians are highly endemic and can be found in a range of environments, including sub-montane forests and

lowland dipterocarp forests (Zainudin et al., 2019). They provide effective model organisms for pollution research because their populations typically consist of large numbers of individuals, and they are good models of freshwater habitats (Burger & Snodgrass 1998). Frogs and toads can be used as ecological indicators to measure the impact of environmental influences on amphibian population decreases and they are susceptible to habitat change when are constantly modified and altered by human disturbances. They are extremely sensitive to their surroundings, particularly the quality of the water as they are highly dependent on water for reproduction, respiration and feeding. Changes in water quality have been linked to an increased rate of tadpole death and deformity. Skin, as the first organ to come into touch with environmental contaminants, will impact skin properties and functions, according to Haslam et al. (2014). For Bornean frogs, studies on the impact of ecological requirements on species assemblages (Zainudin et al., 2019; Zainudin et al., 2017) and frogs' glandular skin (Rasit et al 2017) have been done.

The significance of fragmented forests within oil palm plantations was explored through the analysis of frog assemblage structure (Amphibia: Anura), offering ecological insights into habitat disturbance and biodiversity (Zainudin et al., 2019). Species diversity changed dramatically between high conservation value areas (HCV) and plantation regions, according to the data. The locations with the largest percentage of species endemism and species of conservation value were found in the HCV. The anuran assemblages at the oil palm plantation were disjunctive, according to non-metric multidimensional scaling (NMDS) analysis, because the HCV regions provide separated assemblages for the anuran species, unlike the plantations. Water temperature, turbidity, salinity, and dissolved oxygen levels were found to have a substantial impact on the number of species. In comparison to plantation areas, the HCV lands support a higher diversity of species, including near

threatened and endemic species. In conclusion, these HCV sites in an oil palm plantation are functional and should be conserved as high-priority places for faunal conservation.

2.6 Environmental Quality

Environmental quality is a critical factor influencing the survival and health of amphibians, including tree frogs, as they are highly sensitive to environmental changes. Amphibians rely on water bodies for breeding, hydration, and maintaining skin permeability, which is essential for gas exchange and osmoregulation (Wells, 2010). Poor water quality, characterized by high levels of pollutants, low oxygen content, or pH imbalances, can lead to physiological stress, developmental abnormalities, and population declines (Hayes et al., 2010). Sarawak, located in Malaysian Borneo, is home to diverse amphibian species, including many endemic tree frogs. The region's tropical rainforest provides an abundance of streams, ponds, and wetlands that serve as critical habitats for these species. Since 1998, the Natural Resource and Environment Board (NREB) of Sarawak has been monitoring river water quality due to the declining trend caused by rapid population growth, urbanization, and industrialization. The rising levels of sedimentation and effluent discharge have further stressed the water bodies (Vivian, 2020).

The skin histology of *O. hosii* was examined in relation to water quality parameters across different sites in Sarawak to understand possible environmental influences on skin structure (Rasit et al., 2023). They focused on pH, turbidity, temperature, and DO levels. The study found that DO concentration was the most significant factor affecting amphibian skin health. Forested areas provided protection from direct sunlight. A significant difference in DO levels was observed in relation to the number of mucous and seromucous glands, with mucous glands showing a strong positive correlation and seromucous glands a strong negative correlation with DO. The skin of *O. hosii* from different locations showed variations

in gland numbers, reflecting environmental conditions. Mucous glands were identified as key indicators of water quality, emphasizing the importance of preserving natural habitats for this species.

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CHAPTER 3

MATERIALS AND METHODS

3.1 Study Site

Eight study sites in Sarawak were chosen for sample collection as shown in Figure 3.1. The study sites consisted of six unprotected areas, Komplek Sukan (West UNIMAS), Pusat Penyelidikan Tumbuhan (PPT, East UNIMAS), Ranchan (Serian), Bung Jagoi (Bau), Kampung Maludam and Kampung Long Iman (Mulu), and two protected areas, Sungai Rayu (Matang Wildlife) and Kubah National Park. This study was supported by FRGS/1/2020/SKK0/UNIMAS/01/1). Sample collection was conducted under Park Permit No. WL 40/2022, which was issued by the Sarawak Forestry Corporation. Approval of the research was granted by the Sarawak Biodiversity Centre with reference code SBC-2021-RDP-34-AHR.



Figure 3.1: Location of study sites in Sarawak.

Table 3.1: Location and coordinates of the study sites.

No.	Locality	Coordinates	
1.	Komplek Sukan (West UNIMAS)	1°27'39.8"N	110°26'07.1"E
2.	Pusat Penyelidikan Tumbuhan (PPT, East UNIMAS)	1°27'54.6"N	110°26'53.4"E
3.	Ranchan (Serian)	1°08'38.6"N	110°35'03.0"E
4.	Bung Jagoi (Bau)	1°20'25.9"N	110°02'04.5"E
5.	Kampung Maludam	1°39'17.0"N	111°01'34.7"E
6.	Kampung Long Iman (Mulu)	4°07'55.2"N	114°55'08.4"E
7.	Sungai Rayu (Matang Wildlife)	1°37'42.2"N	110°08'56.7"E
8.	Kubah National Park	1°36'45.6"N	110°11'48.9"E

3.1.1 Universiti Malaysia Sarawak (UNIMAS)

Universiti Malaysia Sarawak (UNIMAS), situated in Kota Samarahan within the state of Sarawak, Malaysia, is approximately 22 kilometers to the east of Kuching, the state capital. Two distinct sampling sites were chosen: Kompleks Sukan (West, UNIMAS) and Pusat Penyelidikan Tumbuhan (PPT, East UNIMAS). Kompleks Sukan (West, UNIMAS) is categorized as a lowland and non-peat swamp forest, positioned at coordinates 1°27'39.8"N and 110°26'07.1"E. Meanwhile, Pusat Penyelidikan Tumbuhan (PPT, East UNIMAS) represents the peat swamp forest, situated at coordinates 1°27'54.6"N and 110°26'53.4"E. PPT encompasses a diverse array of plant species, some of which are exclusive to peat swamp forests. Additionally, the area is home to numerous frog species, such as *R. pardalis*, *P. leucomystax*, among others.



Figure 3.2: Location of study sites in Sarawak Kompleks Sukan (West, UNIMAS).

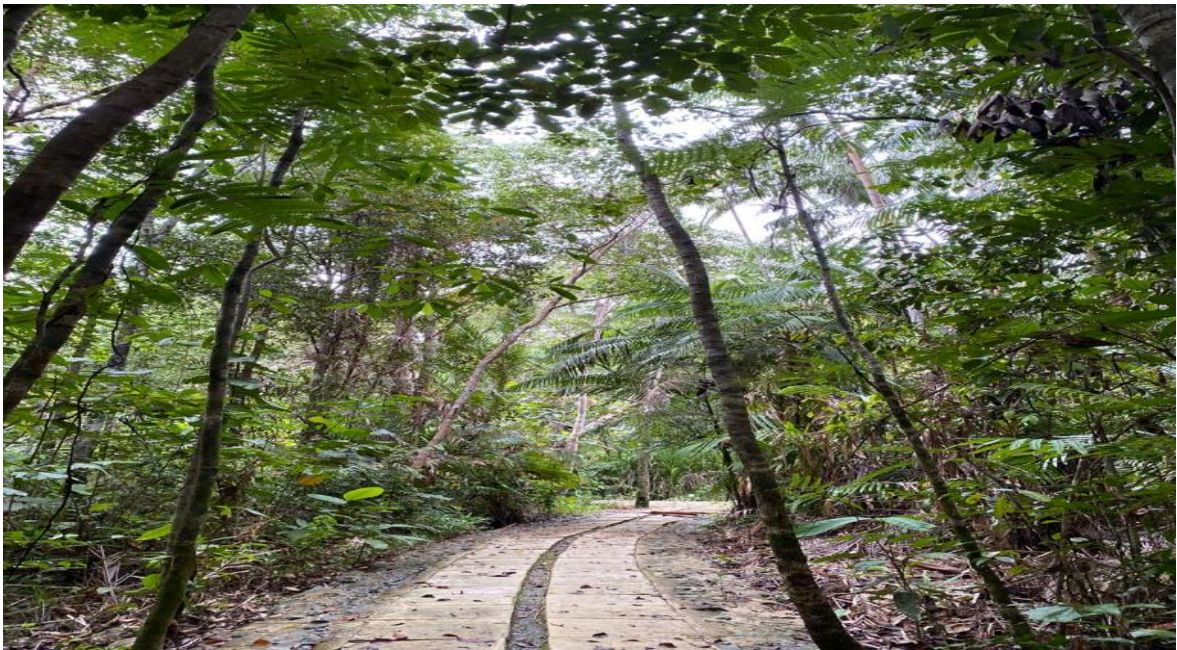


Figure 3.3: One of the trails at Pusat Penyelidikan Tumbuhan (PPT, East UNIMAS).

3.1.2 Ranchan, Serian

Ranchan Recreational Park is situated approximately 4 kilometers away from Serian town and the coordinates are 1°08'38.6"N and 110°35'03.0"E. The park boasts abundant

biodiversity, offering opportunities to encounter a diverse range of flora and fauna along its trails, such as native plants, amphibians, butterflies, birds, and small mammals. It also has beautiful crystal-clear waterfalls and streams. The park is also home to stunning waterfalls and streams, known for their crystal-clear beauty.



Figure 3.4: One of the waterfalls at Ranchan, Serian.

3.1.3 Bung Jagoi, Bau

Bung Jagoi is situated within the Bau District of Sarawak, Malaysia, renowned for promoting and exhibiting the vibrant heritage and traditions of Sarawak's indigenous communities. Traditional longhouses, emblematic of indigenous life in Sarawak, are featured in this area. Moreover, the natural surroundings of Bung Jagoi enhance their appeal. The fishpond located behind the longhouse was selected as the sampling site due to the presence of *P. leucomystax* observed in that vicinity.



Figure 3.5: Fishpond at the back of the longhouse.



Figure 3.6: *Polypedates leucomystax* spotted on a leaf of the plant.

3.1.4 Kampung Maludam

Kampung Maludam, positioned within the Betong Division of Sarawak, Malaysia, lies at coordinates 1°39'17.0"N and 111°01'34.7"E. Situated along the Maludam River, this rural community presents opportunities for a wide range of research endeavors, notably in fields such as rural development, cultural anthropology, and environmental studies.

Plantations featuring various flora within Kampung Maludam have been selected as sampling sites. These plantations boast diverse vegetation types, encompassing trees, shrubs, and ground cover, offering a multitude of microhabitats conducive to accommodating frogs with varying habitat preferences.



Figure 3.7: One of the plantations at Kampung Maludam.



Figure 3.8: Temporary pond found in the plantation at Kampung Maludam.

3.1.5 Kampung Long Iman, Mulu

Kampung Long Iman is a village situated in the Mulu area of Sarawak, Malaysia. It is located near Gunung Mulu National Park, a UNESCO World Heritage Site at coordinates 4°07'55.2"N and 114°55'08.4"E.



Figure 3.9: Kampung Long Iman, Mulu.

3.1.6 Sungai Rayu, Matang Wildlife

The Wildlife Centre, located near Kuching, the capital city of Sarawak, Malaysia, at coordinates 1°37'42.2"N and 110°08'56.7"E, is renowned as a conservation and research facility. Its primary focus is on the rehabilitation and preservation of wildlife, with a particular emphasis on endangered species native to Borneo, such as orangutans, sun bears, and hornbills. Matang Wildlife Centre boasts four jungle trails: the Pitcher trail, Sungai Rayu trail, Sungai Senduk trail, and Sungai Buluh trail. Among the species found within its confines were *Rentapia hosii*, *Phrynoidis asper*, *Rhacophorus pardalis*, *Ingerophrynus divergens*, and *Pulchrana bacamica*.



Figure 3.10: Sungai Rayu Trail at Matang Wildlife.

3.1.7 Kubah National Park

Kubah National Park is situated near Kuching, the capital of Sarawak, positioned at coordinates 1°36'45.6"N and 110°11'48.9"E. Within the park, a diverse array of flora and fauna thrives, including a multitude of amphibian species. Among its notable features are specially designed frog ponds aimed at attracting and providing habitats for various frog species. These ponds are particularly frequented by tree frogs primarily belonging to the *Polypedates* genus, such as *P. colletti*, *P. leucomystax*, *P. macrotis*, and *P. otilophus*. Additionally, *R. pardalis* sightings are common, with numerous individuals often observed directly within the park.



Figure 3.11: Frog Pond at Kubah National Park.

3.2 Field Technique

Sampling was conducted during the night, the period when frogs are most active and vocal, starting at 1800 hours and concluding at 2300 hours. Active sampling methods, including visual encounter surveys (VES) and acoustic encounter surveys (AES) (Zainudin et al., 2018), were employed to collect samples. Frogs were captured manually using bare hands, placed in plastic bags, and transported back to the base camp. Torch lights and headlamps were utilized to locate herpetofauna by detecting eye shine and individuals concealed beneath rocks, forest litter, and tree barks. Measurements of body mass, snout-vent length (SVL), and hind leg (HL) were taken for each frog. Species identification was performed using 'A Field Guide to The Frogs of Borneo (third edition) by Inger et al. (2017) as a reference. Only a maximum of three individuals per species per location were selected for histological study. Microhabitat characteristics, including vegetation, vertical and horizontal positions, and substrate, were noted for environmental assessment purposes (Zainudin et al., 2019). Frogs were individually bagged with details regarding capture time, date, microhabitat, GPS coordinates, and distance from the nearest water source.

Additionally, water quality parameters such as pH, turbidity, temperature, and dissolved oxygen were recorded. Water samples were collected and tested on-site using two instruments which are the Eutech pH 150 meter for measuring pH levels and the Eutech ECTN 100 IR waterproof handheld turbidimeter for assessing turbidity.



Figure 3.12: A - pH meter, Eutech pH 150 and B - waterproof handheld turbidimeter, Eutech ECTN 100 IR.

The devices were calibrated prior to use following the instructions in their respective manuals. Water samples were collected from the locations where the frog specimens were captured on-site and are documented in Appendix 2.

Table 3.2 presents a list of frog samples from the Rhacophoridae family collected across various localities in Sarawak, Malaysia, with a total of 24 individuals from five different species. *Polypedates leucomystax* was the most widely distributed species, found in six locations, with the highest number, 3 individuals recorded in Kampung Maludam, while *P. macrotis* was found in two sites with a total of 4 individuals. *Polypedates otitophus* and *P. colletti* were the least common, each represented by a single individual from Kubah National Park and Sungai Rayu Trail, respectively. *Rhacophorus pardalis* was present in

three locations, with the highest count (3 individuals) from Kubah National Park. The distribution suggests that *P. leucomystax* can thrive in diverse habitats, including human-modified areas, whereas species such as *P. ottilophus* and *P. colletti* appear more habitat-specific. The relatively low numbers of certain species may indicate habitat preferences or lower population densities, emphasizing the importance of habitat conservation to maintain biodiversity within the Rhacophoridae family.

Table 3.2: List of frogs' samples and the localities.

Family	Species name	Locality	Total individual/site
Rhacophoridae	<i>P. leucomystax</i>	Ranchan, Serian	1
		Kampung Maludam	3
		Kompleks Sukan (West UNIMAS)	2
		PPT (East UNIMAS)	1
		Kampung Long Iman, Mulu	2
		Bung Jagoi, Bau	2
	<i>P. macrotis</i>	Kubah National Park	1
		Sungai Rayu Trail, Matang Wildlife	3
	<i>P. ottilophus</i>	Kubah National Park	1
	<i>P. colletti</i>	Sungai Rayu Trail, Matang Wildlife	1
	<i>R. pardalis</i>	Kubah National Park	3
		Sungai Rayu Trail, Matang Wildlife	2
		PPT (East UNIMAS)	2
	Total individuals for all species collected		24

3.3 Histological Technique

Following approval from animal ethics authorities (UNIMAS/AEC/R/F07/072), the histological technique was conducted at the Histopathology Laboratory, Faculty of Medical and Health Sciences (FMHS), Universiti Malaysia Sarawak, to obtain frog skin samples. To preserve the samples, absolute ethanol was administered directly into the frog's heart for euthanasia, after which the tissues were extracted and preserved in absolute ethanol for DNA tissue preservation purposes. To protect the integrity of the skin structures, the dorsal and ventral skins were carefully and gradually excised using a scalpel and scissors. The removed skin was then positioned on filter paper with appropriate labelling. The skin was gently separated using forceps after applying a layer of distilled water and then fixed with 10% formalin. To achieve optimal results, it is necessary to proceed with the next step within 48 hours for the 10% formalin-fixed skin. Six steps are involved in preparing slides for histological examination: skin grossing, skin fixation, skin processing, skin embedding, skin sectioning, and staining.

The first stage of slide preparation involves skin grossing, where the skin is cut out and then positioned within the specimen container. The skin was divided into four sections, then placed inside the specimen container and subsequently immersed in 10% formalin for further fixation. The four sections are DH-dorsal head, DC-dorsal center, VH-ventral head and VC-ventral center. Fixation is a crucial phase in the histological specimen preparation process. Its purpose is to halt autolysis, thereby preserving the integrity of both the gross organs and microanatomy. This preservation helps prevent excessive bacterial growth postmortem, ensuring the tissues are adequately prepared for processing and subsequent staining reactions (Sungif, 2017). The physical structure and morphological details can be

effectively preserved when the tissue is adequately fixed. The optimal fixation duration for this study is 24 hours.

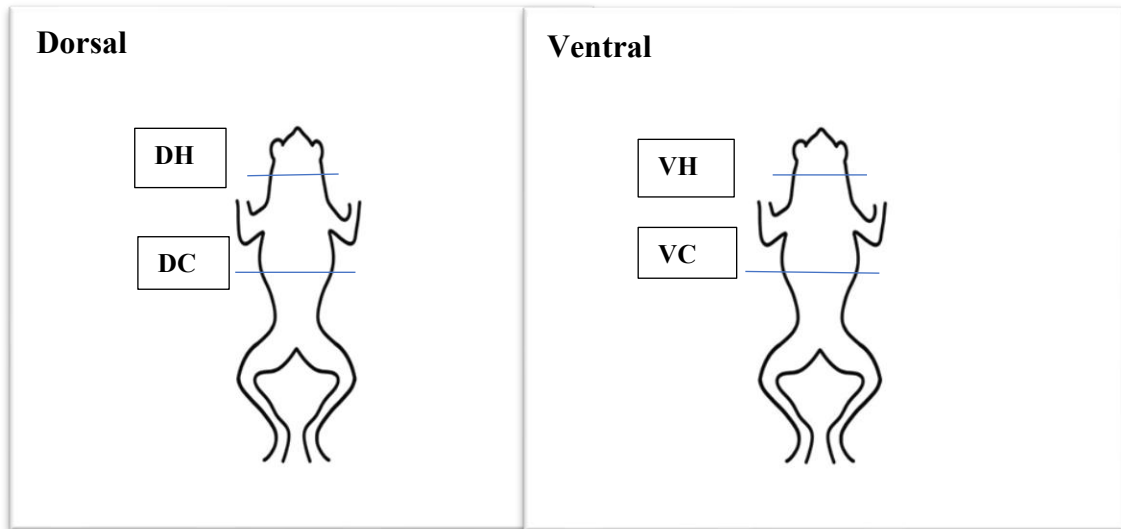


Figure 3.13: The dorsal and ventral skin areas of the tree frog were designated for histological examination. These areas were labelled as follows: DH for dorsal head, DC for dorsal center, VH for ventral head, and VC for ventral center.

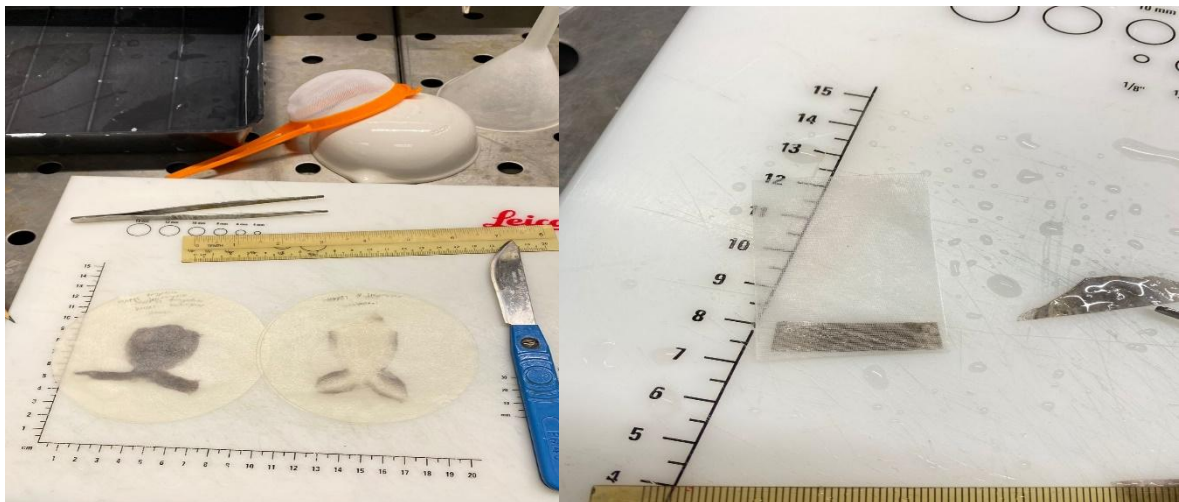


Figure 3.14: The process of skin grossing involves placing the skin sections within nylon mesh biopsy bags before placing them in the casket.



Figure 3.15: Skin fixation in 10% formalin.

Following skin fixation, skin processing occurred, involving placing the skins into the processor machine according to the standard protocol outlined in Table 3. Formalin was employed in the processor to prevent degradation of the skin. A high concentration of alcohol was utilized to eliminate wax and water from the skin. According to Junqueira (2005), Xylene was subsequently used instead of alcohol to impregnate the tissues with a paraffin-penetrating solvent.



Figure 3.16: Processor machine used for skin processing.

Table 3.3: Skin processing protocol.

Chemical	Time (Min)	Temperature (°C)
Formalin	60	-
Alcohol 70%	60	-
Alcohol 80%	60	-
Alcohol 95%	60	-
Absolute alcohol	60	-
Absolute alcohol	60	-
Absolute alcohol	60	-
Xylene	60	-
Xylene	60	-
Xylene	60	-
Paraffin	60	60
Paraffin	60	60
Paraffin	30	60
Paraffin	30	60

Next, during the skin embedding process, the samples were transferred to the Tissue Embedding Center, branded as 'Tissue-Tek®TEC®', for the preparation of skin blocks. The embedding process is important because improper embedding can lead to challenges in obtaining the desired ribbon during sectioning. The skin from the casket was transferred onto the stainless-steel embedding base mold, shaping the skin to preserve its preferred structure. The skins were fixed to create the block using wax heated to 65°C. The resulting block was then frozen using the Tissue Embedding Center (Cryo) machine, which is connected to the Tissue Embedding Center, at a temperature of -9°C. Paraffin was employed to embed the tissues for added rigidity.



Figure 3.17: Cryostat machine used for skin embedding.

After the paraffin had hardened, the microtome machine was employed for the trimming and sectioning process, slicing the block to obtain ribbons containing the skin and tissue structure. To optimize the trimming and sectioning process, it was essential for the tissue block to be cold enough. Before skin sectioning, it is necessary to perform the trimming process to ensure the paraffin block is flat and the tissue is adequately exposed for sectioning. The ribbons have a thickness of 3.5 micrometers.



Figure 3.18: Semi-automatic microtome machine used for trimming and sectioning.

The ribbon containing the skin tissue was immersed in hot water at 41-45 °C during the skin floating process (Sungif, 2017). This temperature effectively softens the wax so the section can relax and flatten without melting and minimizing wrinkles while preserving structural integrity. Temperatures that are too low can hinder proper spreading, whereas overly high temperatures risk distortion and loss of detail. It remained on the water's surface until it flattened. The ribbons containing the skin structure were positioned onto the labeled microscope slides. Subsequently, the slide was used to obtain the desired sample section and allowed to briefly dry to remove any excess water.



Figure 3.19: Floatation bath used for skin fishing along with the slides containing skin samples.

Observing unstained tissues under a microscope can be challenging due to their colorlessness, as noted by Junqueira (2005). Therefore, the Haematoxylin and Eosin (H & E) staining protocol, as modified by Sungif (2017), was employed. In this section, achieving a transparent appearance with pink and purple colour was deemed indicative of a satisfactory slide. Following the staining process, the coverslip was applied to the slide using Distyrene, Plasticizer, and Xylene (DPX) solution. The slide, containing the skin structure, was allowed to dry before proceeding with visualization. The coverslip serves to shield the microscope's objective lens from direct contact with the specimen and also enhances structural thickness during observation. This step requires careful handling to prevent the inclusion of air bubbles trapped between the coverslip, which could disrupt the viewing process.



Figure 3.20: Leica Auto stainer XL used for Haematoxylin and Eosin (H&E) staining.



Figure 3.21: The slides were mounted with Distyrene Plasticizer Xylene (DPX) and left at room temperature for drying purposes.

Table 3.4: Haematoxylin and Eosin (H & E) Protocol modified by Sungif (2017).

No.	Process	Time (mins & secs)
1.	Dewaxing with Xylene I	3 minutes
2.	Dewaxing with Xylene II	3 minutes
3.	Bring tissue section to water with Absolute alcohol I	3 minutes
4.	Bring tissue section to water with Absolute alcohol II	3 minutes
5.	Rinse with tap water	3 minutes
6.	Rinse with tap water	10 seconds
7.	Stain with Haematoxylin	20 minutes
8.	Rinse with tap water	3 minutes
9.	Decolourize with 1% acid alcohol (HCl)	2 seconds
10.	Rinse with tap water	3 minutes
11.	Bluing with 0.1% weak ammonia	2 minutes
12.	Rinse with tap water	3 minutes
13.	Counterstain with 1% Eosin	5 minutes
14.	Dehydration with 30% alcohol	2 seconds
15.	Dehydration with 50% alcohol	2 seconds
16.	Dehydration with 70% alcohol	2 seconds
17.	Dehydration with 95% alcohol	2 seconds
18.	Dehydration with Absolute alcohol I	2 seconds
19.	Dehydration with Absolute alcohol II	2 seconds
20.	Clearing with Xylene I	5 seconds
21.	Clearing with Xylene II	5 seconds
22.	Clearing with Xylene III	5 seconds
23.	Mount with Distyrene Plasticizer Xylene (DPX)	
24.	Cover the section with cover slip and press with forceps	
25.	Let the slide dry overnight	
26.	Remove DPX	
27.	Mark the slides	

3.4 Skin Structure Analysis

The light microscope Leica DM750 with 40x magnification was used to visualize and examine all slides. Furthermore, analysis was conducted on four skin regions: Dorsal Head (DH), Dorsal Centre (DC), Ventral Head (VH), and Ventral Centre (VC). Nine characters were constructed using three primary skin components: epidermis, dermis, and gland. Ten randomly selected skin slides from each region, encompassing both dorsal and ventral aspects, were observed. The summary of all nine skin parameters used for histological analysis is presented in Table 5. Each skin section was measured and analysed using ImageJ 1.15k software, with the extracted data saved in Microsoft Excel format. ImageJ, a widely accepted scientific image analysis tool, is utilized globally for displaying, editing, analysing, processing, and computing area and pixel value statistics of user-defined selections. This software was used to measure all the parameters under study and calculate the area of irregular glands.



Figure 3.22: Light microscope Leica DM750.

Table 3.5: Skin parameters for histological analysis. All measurements are in micrometer.

No.	Characters	Abbr.
1.	Thickness of epidermis	C1
2.	Thickness of stratum spongiosum	C2
3.	Thickness of stratum compactum	C3
4.	Number of granular glands	C4
5.	Number of seromucous glands	C5
6.	Number of mucous glands	C6
7.	Average size of granular glands present	C7
8.	Average size of seromucous glands present	C8
9.	Average size of mucous glands present	C9

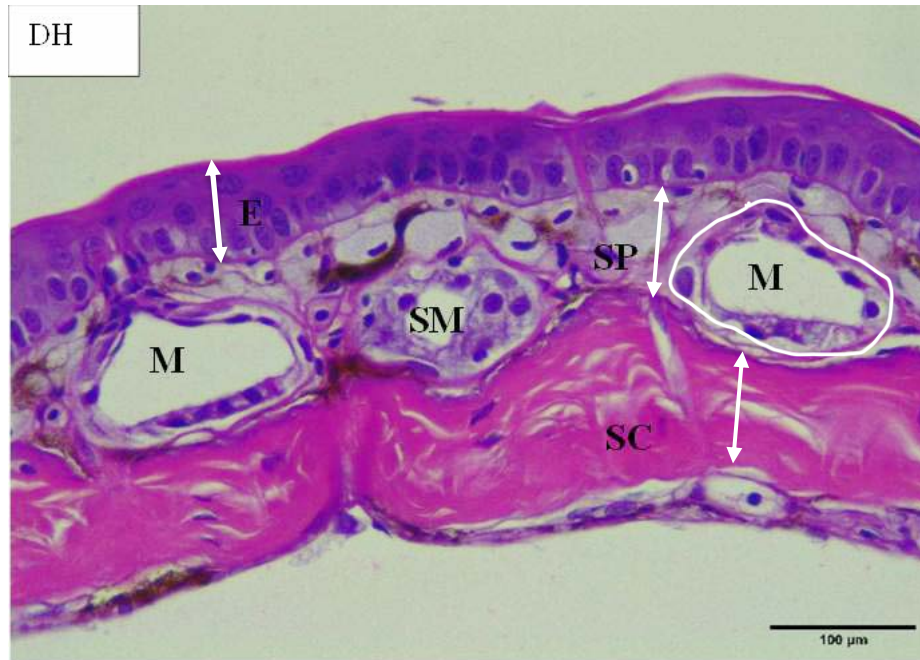


Figure 3.23: Skin structure of *Polypedates leucomystax* at Dorsal skin regions. Bar 100 μm . Magnification=40X (Note: DH, dorsal head; DC, dorsal centre; VH, ventral head; VC, ventral centre; E, epidermis; SP, stratum spongiosum; SC, stratum compactum; M, mucous gland; SM, seromucous gland).

Figure 3.23 presents a 40 $\times$ magnification of *Polypedates leucomystax* skin, displaying three distinct layers. The outermost layer is the epidermis, followed by the stratum spongiosum as the second layer and the stratum compactum as the third, along with the associated glands. The arrows illustrate the measurement points for each skin layer and the process of determining the area of irregular glands using the freehand selection tool in ImageJ software.

All data were analysed using the Statistical Package for the Social Sciences (SPSS) software. The Kruskal-Wallis test was selected to assess each parameter concerning the skin structure across various skin regions of tree frogs. This test, a non-parametric statistical method, was employed to ascertain if there exist statistically significant differences among three or more independent groups. Subsequently, statistical analysis was conducted using

ANOVA and post hoc tests to compare the sites and their water quality parameters. Additionally, the Pearson correlation test was utilized to examine the relationship between the histological findings of the selected tree frog species and the water quality parameters. The Pearson correlation test assesses if the correlation between two things is big enough to matter, suggesting there is a clear connection between them.

For water quality, a One-Way ANOVA was used to identify significant differences between each parameter, utilizing one grouped independent variable and one grouped dependent variable. This analysis examines the extremes of each parameter to determine whether there are any differences or similarities. The Spearman and Kendall correlation tests were used to analyse the relationship between water quality parameters (pH, turbidity, temperature, and DO) and the glands present on the skin of *Polypedates leucomystax* (mucous glands, seromucous glands, and granular glands). Both tests are designed for non-parametric data, as they do not assume linear regression or a specific data distribution. The Spearman correlation test measures the strength and direction of the association between two variables, while the Kendall correlation test assesses the dependency between two variables.

CHAPTER 4

RESULTS

4.1 Selected Bornean tree frogs

4.1.1 Introduction

The skin is a vital organ in multicellular organisms that serves as the primary barrier and first line of defence against harmful microbes, protecting the body from infections, pathogens, and environmental threats (Vivian, 2020). The skin is a complex functional structure composed of two main layers which are the epidermis and the dermis. The epidermis is the outermost layer of the skin that acts as a barrier between the frog's physical environment and its internal cellular environment. Most anurans possess mucous, granular, and seromucous glands, with granular glands also known as poison glands. These glands, located within the dermal layer, serve distinct functions and differ in both size and surface area. Histological analysis plays a crucial role in examining the cellular organization and biological condition of a frog's skin. This analysis focuses on the morphological structures of glands, providing insights into their functions and adaptations.

Understanding the histological characteristics of tree frog skin is crucial in determining how these amphibians adapt to their surroundings and how external stressors impact their survival. Despite the ecological importance of tree frogs, limited research has been conducted on the histological variations in their skin glands across different environmental conditions. This study aims to bridge this gap by providing a detailed histological analysis of the skin of tree frogs.

This chapter focuses on the histological examination of the skin from selected Bornean tree frogs. The study aims to compare the morphological features across different

skin regions of five frog species. The analysis employs the Kruskal-Wallis statistical test to identify significant differences, providing deeper insights into the variations in skin morphology and potential adaptations within these species. The study identified traits that showed significant differences across the skin regions of each species analysed. Furthermore, it sought to provide evidence highlighting the differences in skin characteristics among the species.

4.1.2 *Rhacophorus pardalis*

Histological examination of the skin of *R. pardalis* was carried out on seven ($n = 7$) individuals. The observations focus on the structural features of the skin and the variation of skin characters across four different skin regions. The results are presented in two parts: the general skin structure (Section 4.1.2.1) and the variation across regions (Section 4.1.2.2).

4.1.2.1 Skin Structure of *Rhacophorus pardalis*

The integument of *R. pardalis* is characterized by a relatively thin epidermis with an average thickness of $5.11 \times 10^1 \mu\text{m}$, ranging from $2.26 \times 10^1 \mu\text{m}$ to $8.92 \times 10^1 \mu\text{m}$, supported by a dermis that is clearly differentiated into the stratum spongiosum and stratum compactum. The stratum spongiosum exhibits a mean thickness of $1.04 \times 10^2 \mu\text{m}$, with values ranging between $0.47 \times 10^2 \mu\text{m}$ and $2.00 \times 10^2 \mu\text{m}$, while the stratum compactum demonstrates an average thickness of μm , varying from $0.24 \times 10^2 \mu\text{m}$ to $1.61 \times 10^2 \mu\text{m}$. The glandular distribution reflects distinct functional specialization: granular glands are sparsely distributed (mean 0.10 glands per site; maximum 2) but display the largest average cross-sectional area ($13.55 \times 10^3 \mu\text{m}^2$), consistent with their role in defensive secretion. In contrast, seromucous glands are more frequently encountered (mean 2.14 glands per site; maximum 5) with moderate size ($7.45 \times 10^3 \mu\text{m}^2$), whereas mucous glands are the least common (mean

0.07 glands per site; maximum 3) and exhibit the smallest average area ($4.83 \times 10^3 \mu\text{m}^2$). Collectively, these findings highlight a thin epidermal–dermal complex with heterogenous glandular architecture, suggesting adaptive morphological specializations that facilitate both physiological regulation and ecological defense in *R. pardalis*.

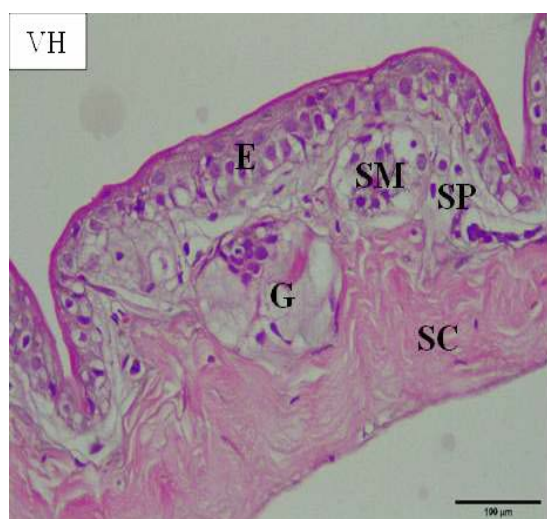
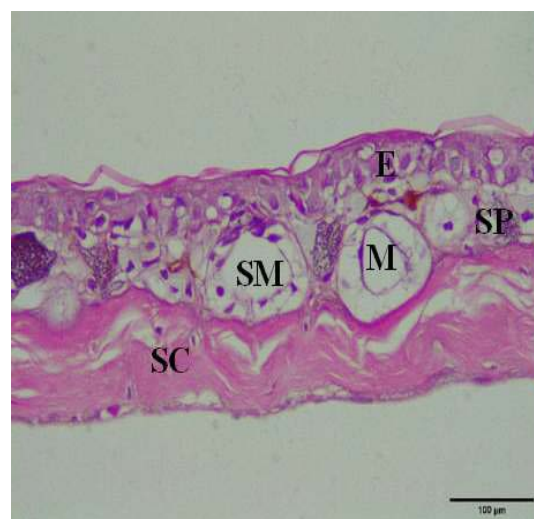
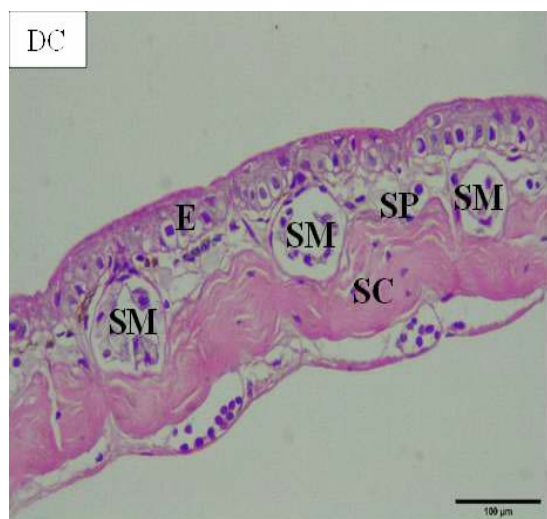
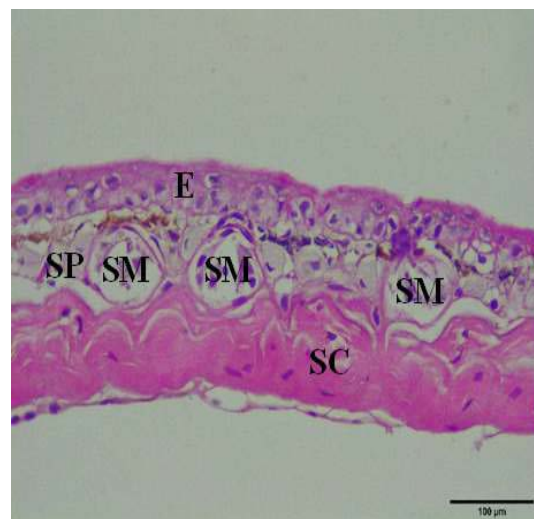
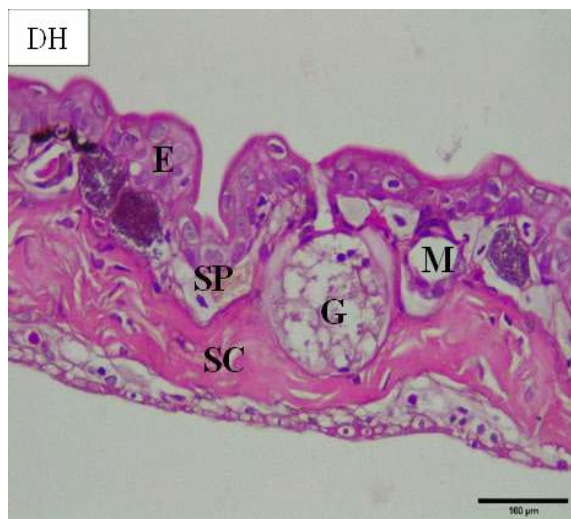
Table 4.1: Skin description of *Rhacophorus pardalis*.

Variables (individual = 7, 10 sites per skin region)	Descriptive statistics (n)			
	Maximum thickness (μm)	Minimum thickness (μm)	Average thickness (μm)	Notes: Average area of glands (in μm^2)
1. Epidermis				
(a) Thickness of epidermis (μm)	8.92×10^1	2.26×10^1	5.11×10^1	
2. Dermis				
a) Stratum spongiosum				
(i) Thickness of stratum spongiosum (μm)	2.00×10^2	0.47×10^2	1.04×10^2	
b) Stratum compactum				
(i) Thickness of stratum compactum (μm)	1.61×10^2	0.24×10^2	0.64×10^2	
	Maximum Number	Minimum Number	Average Number	Average area of glands (in μm^2)
c) Granular gland				
(i) Number of gland (n)	2	0	0.10	13.55×10^3
(ii) Area of gland				
d) Seromucous gland				
(i) Number of gland (n)	5	0	2.14	7.45×10^3
(ii) Area of gland				
e) Mucous gland				
(i) Number of gland (n)	3	0	0.07	4.83×10^3
(ii) Area of gland				

(ii) Area of gland				
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4.1.2.2 Variation of Skin Characters in Four Skin Regions (*Rhacophorus pardalis*)

Figure 4.1 showed the four regions of *R. pardalis*. Results were tabulated in Table 4.2 showed a comparison of skin characters of *R. pardalis* in four regions. Six characteristics were significantly different ($p < 0.05$); thickness of epidermis (C1), thickness of stratum spongiosum (C2), thickness of stratum compactum (C3), number of seromucous glands (C5), number of mucous glands (C6) and average size of seromucous glands present (C8). The cross section of the skin of *R. pardalis* showed clearer presentation of the mucous glands, seromucous glands and granular glands as well. The thickness of epidermis in ventral region is more compared to the dorsal regions. The ventral centre exhibited the thickest stratum spongiosum, measuring $1.06 \times 10^2 \mu\text{m}$. The stratum compactum is thicker in the ventral regions compared to the dorsal regions. The number of granular glands in this species is relatively low, with their average size being consistent across all regions. The number of seromucous glands is higher in this species compared to granular and mucous glands, with a greater presence in the ventral regions. In contrast, the number of mucous glands is higher in the dorsal regions than in the ventral regions, and they are absent in the ventral head. However, their average size remains consistent across all regions.



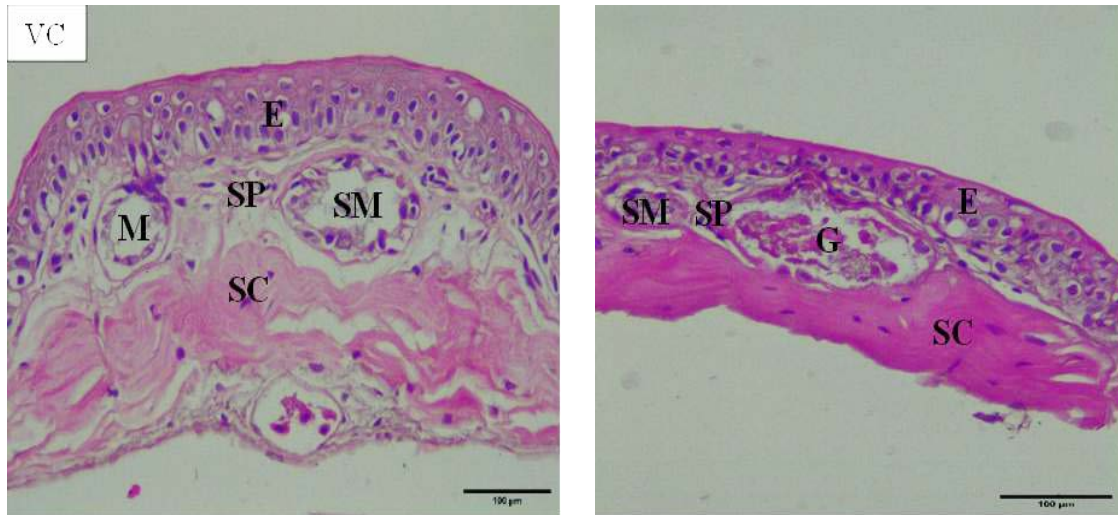


Figure 4.1: Skin structure of *Rhacophorus pardalis* at four skin regions. Bar 100 µm magnification=40X (Note: DH, dorsal head; DC, dorsal centre; VH, ventral head; VC, ventral centre; E, epidermis; SP, stratum spongiosum; SC, stratum compactum; M, mucous gland; G, granular gland; SM, seromucous gland).

Table 4.2: Skin measurement (Mean) of *Rhacophorus pardalis*

Character	C1* Thickness of epidermis (µm)	C2* Thickness of stratum spongiosum (µm)	C3* Thickness of stratum compactum (µm)	C4 Number of granular glands	C5* Number of seromucous glands
DH	$(4.32 \pm 1.05) \times 10^1$	$(1.01 \pm 0.27) \times 10^2$	$(5.87 \pm 1.77) \times 10^1$	$(6.00 \pm 23.40) \times 10^2$	1.97 ± 0.932
DC	$(4.29 \pm 0.88) \times 10^1$	$(0.97 \pm 0.26) \times 10^2$	$(5.67 \pm 1.68) \times 10^1$	$(0.160 \pm 0.404) \times 10^2$	1.91 ± 1.004
VH	$(5.83 \pm 1.46) \times 10^1$	$(1.12 \pm 0.29) \times 10^2$	$(7.07 \pm 2.20) \times 10^1$	$(9.00 \pm 28.20) \times 10^2$	2.66 ± 1.006
VC	$(6.01 \pm 1.42) \times 10^1$	$(1.06 \pm 0.24) \times 10^2$	$(6.90 \pm 2.60) \times 10^1$	$(0.100 \pm 0.302) \times 10^2$	2.03 ± 0.916
Character	C6* Number of mucous glands	C7 Average size of granular glands present (µm ²)	C8* Average size of seromucous glands present (µm ²)	C9 Average size of mucous glands present (µm ²)	
DH	0.17 ± 0.481	$(1.69 \pm 0.26) \times 10^4$	$(6.73 \pm 2.16) \times 10^3$	$(4.80 \pm 2.78) \times 10^3$	
DC	0.04 ± 0.204	$(1.33 \pm 0.35) \times 10^4$	$(7.29 \pm 7.09) \times 10^3$	$(5.27 \pm 3.34) \times 10^3$	
VH	0	$(1.37 \pm 0.52) \times 10^4$	$(7.68 \pm 1.92) \times 10^3$	0	
VC	0.07 ± 0.310	$(1.18 \pm 0.38) \times 10^4$	$(8.10 \pm 2.54) \times 10^3$	$(4.59 \pm 3.05) \times 10^3$	

(*) -p <0.05. (Note: DH, Dorsal Head; DC, Dorsal Centre; VH, Ventral Head; VC; Ventral Centre)

4.1.3 *Polypedates colletti*

A histological analysis of the skin of *P. colletti* was conducted on a single individual (n = 1). The examination focused on the structural characteristics of the skin and the variation of these features across four distinct skin regions. The findings are presented in two sections: the general skin structure (Section 4.1.3.1) and the regional variations (Section 4.1.3.2).

4.1.3.1 Skin Structure of *Polypedates colletti*

The skin of *P. colletti* demonstrates a relatively thin epidermal covering, with a maximum thickness of $1.19 \times 10^2 \mu\text{m}$, a minimum of $0.40 \times 10^2 \mu\text{m}$, and an average of $0.69 \times 10^2 \mu\text{m}$. The dermis is differentiated into the stratum spongiosum and stratum compactum. The stratum spongiosum records a maximum thickness of $2.12 \times 10^2 \mu\text{m}$, a minimum of $0.83 \times 10^2 \mu\text{m}$, and an average of $1.45 \times 10^2 \mu\text{m}$, while the stratum compactum ranges between $0.50 \times 10^2 \mu\text{m}$ and $1.70 \times 10^2 \mu\text{m}$, with an average thickness of $1.30 \times 10^2 \mu\text{m}$. In terms of glandular distribution, granular glands are infrequent (maximum of 1, minimum of 0, with an average of 0.325 glands) but occupy an average cross-sectional area of $1.92 \times 10^3 \mu\text{m}^2$. Seromucous glands are more abundant (maximum of 4, minimum of 0, and an average of 1.975 glands), with a larger mean area of $9.95 \times 10^3 \mu\text{m}^2$. By comparison, mucous glands are scarce (maximum of 2, minimum of 0, and an average of 0.200 glands) and smaller in area ($5.90 \times 10^3 \mu\text{m}^2$). Overall, the skin structure of *P. colletti* is characterized by a thin epidermal–dermal complex with relatively greater representation of seromucous glands, suggesting functional prioritization of mixed mucous–serous secretions over defensive or purely mucogenic output.

Table 4.3: Skin description of *Polypedates colletti*

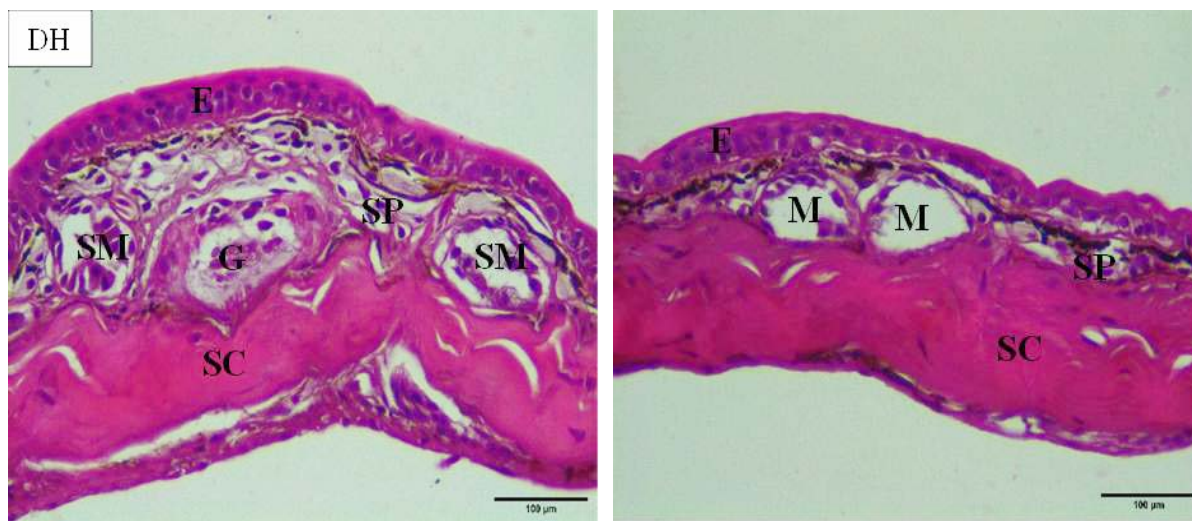
Variables (individual = 1, 10 sites per skin region)	Descriptive statistics (n)			
	Maximum thickness (µm)	Minimum thickness (µm)	Average thickness (µm)	Notes: Average area of glands (in µm ²)
1. Epidermis				
(a) Thickness of epidermis (µm)	1.19× 10 ²	0.40× 10 ²	0.69× 10 ²	
2. Dermis				
(a) Stratum spongiosum	2.12× 10 ²	0.83× 10 ²	1.45× 10 ²	
(i) Thickness of stratum spongiosum (µm)				
(b) Stratum compactum	1.70× 10 ²	0.50× 10 ²	1.30× 10 ²	
(i) Thickness of stratum compactum (µm)				
	Maximum Number	Minimum Number	Average Number	Average area of glands (in µm ²)
(c) Granular gland	1	0	0.325	1.92× 10 ³
(i) Number of gland (n) (ii) Area of gland				
(d) Seromucous gland	4	0	1.975	9.95× 10 ³
(i) Number of gland (n) (ii) Area of gland				
(e) Mucous gland	2	0	0.200	5.90× 10 ³
(i) Number of gland (n) (ii) Area of gland				

4.1.3.2 Variation of Skin Characters in Four Skin Regions (*Polypedates colletti*)

Figure 4.2 showed the four skin regions of *P. colletti*. Results were tabulated in Table 4.4 showed a comparison of skin characters of *P. colletti* in four regions. Five characteristics

were significantly different ($p < 0.05$); thickness of epidermis (C1), thickness of stratum spongiosum (C2), thickness of stratum compactum (C3), number of granular glands (C4), and average size of seromucous glands present (C8). The cross section of the skin of *P. colletti* showed clearer presentation of the granular, mucous and seromucous glands. The seromucous glands scattered throughout the upper part of stratum spongiosum and in all four skin regions. The granular glands were located below the seromucous glands within the stratum spongiosum.

The mucous glands were in stratum spongiosum and scattered in all regions except in ventral head. The thickness of epidermis, stratum spongiosum and stratum compactum in ventral region is more compared to the dorsal regions. The number of granular is higher in ventral regions with the highest mean count being 0.70 for ventral head. The average size of seromucous glands is bigger in ventral centre with a maximum of $2.29 \times 10^3 \mu\text{m}$. The granular gland appeared to be bigger and denser compared to mucous and seromucous glands.



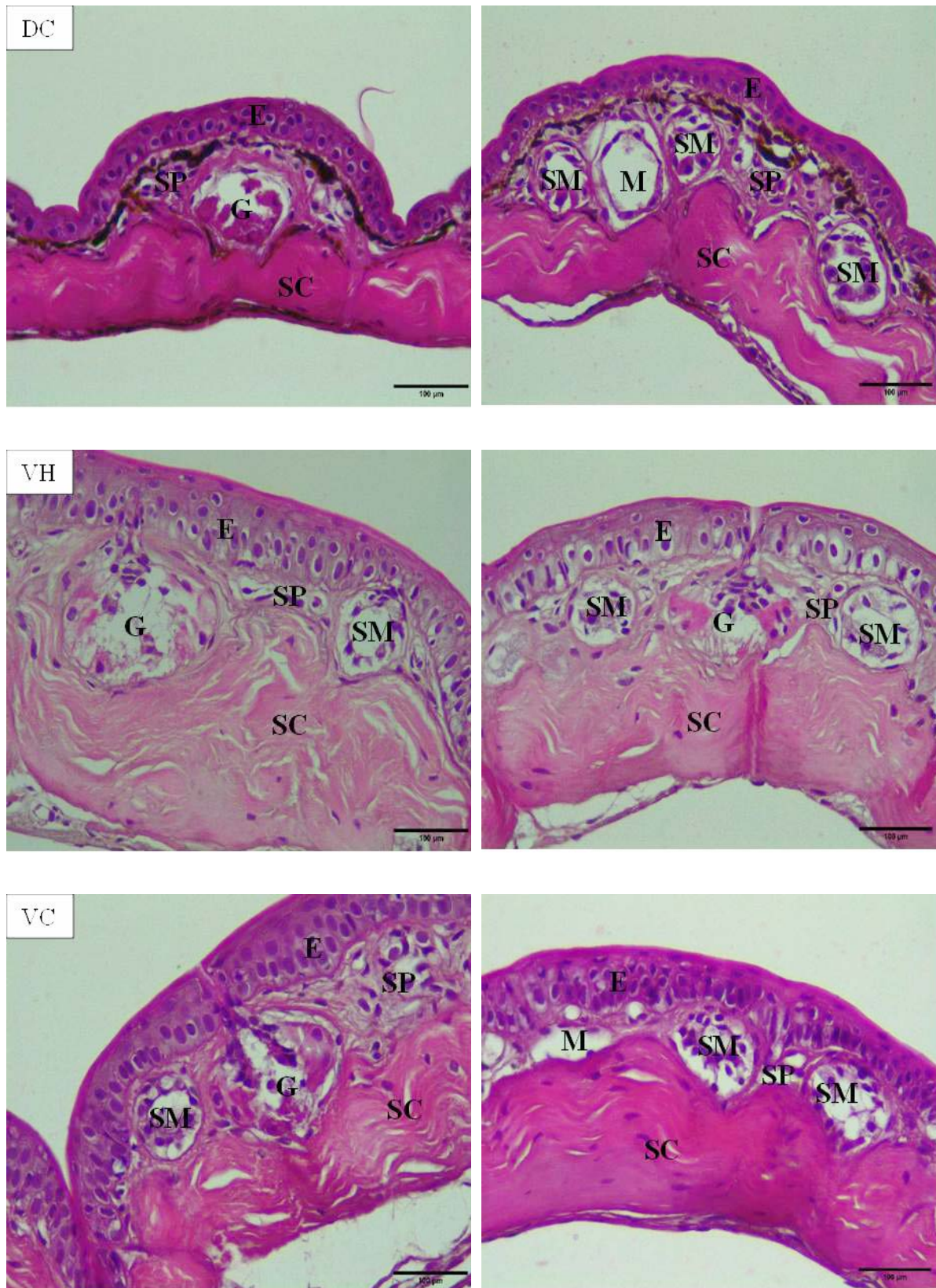


Figure 4.2: Skin structure of *Polypedates colletti* at four skin regions. Bar 100 µm. Magnification=40X (Note: DH, dorsal head; DC, dorsal centre; VH, ventral head; VC, ventral centre; E, epidermis; SP, stratum spongiosum; SC, stratum compactum; M, mucous gland; G, granular gland; SM, seromucous gland).

Table 4.4: Skin measurement (Mean) of *Polypedates colletti*

Character	C1* Thickness of epidermis (μm)	C2* Thickness of stratum spongiosum (μm)	C3* Thickness of stratum compactum (μm)	C4* Number of granular glands	C5 Number of seromucous glands
DH	$(5.00 \pm 0.90) \times 10^1$	$(1.36 \pm 0.30) \times 10^2$	$(1.01 \pm 0.21) \times 10^2$	0.10±0.32	1.90±0.88
DC	$(4.53 \pm 0.36) \times 10^1$	$(1.16 \pm 0.23) \times 10^2$	$(6.46 \pm 1.01) \times 10^2$	0.40±0.52	1.20±1.40
VH	$(8.28 \pm 1.83) \times 10^1$	$(1.65 \pm 0.24) \times 10^2$	$(1.20 \pm 0.24) \times 10^2$	0.70±0.48	2.40±1.06
VC	$(9.48 \pm 1.67) \times 10^1$	$(1.62 \pm 0.32) \times 10^2$	$(1.25 \pm 0.20) \times 10^2$	0.10±0.32	2.50±0.97
Character	C6 Number of mucous glands	C7 Average size of granular glands present (μm ²)	C8* Average size of seromucous glands present (μm ²)	C9 Average size of mucous glands present (μm ²)	
DH	0.30±0.66	$(1.87 \pm 0.00) \times 10^4$	$(8.86 \pm 0.57) \times 10^3$	$(7.19 \pm 1.48) \times 10^3$	
DC	0.30±0.48	$(1.59 \pm 0.12) \times 10^4$	$(8.25 \pm 1.61) \times 10^3$	$(6.19 \pm 2.00) \times 10^3$	
VH	0	$(2.03 \pm 0.44) \times 10^4$	$(9.82 \pm 2.39) \times 10^3$	0	
VC	0.20±0.42	$(2.46 \pm 0.00) \times 10^4$	$(1.21 \pm 0.22) \times 10^4$	$(4.19 \pm 0.61) \times 10^3$	

(*) -p <0.05. (Note: DH, Dorsal Head; DC, Dorsal Centre; VH, Ventral Head; VC; Ventral Centre)

4.1.4 *Polypedates macrotis*

Histological analysis of the skin of *Polypedates macrotis* was performed on four individuals (n = 4). The investigation examined the structural morphological traits of the skin and the variation of these characteristics across four distinct regions. The results are organized into two parts: the general skin structure (Section 4.1.4.1) and the regional variations (Section 4.1.4.2).

4.1.4.1 Skin Structure of *Polypedates macrotis*

The skin of *P. macrotis* is characterized by a moderately thin epidermis with a maximum thickness of $11.52 \times 10^1 \mu\text{m}$, a minimum of $2.73 \times 10^1 \mu\text{m}$, and an average of $6.26 \times 10^1 \mu\text{m}$. The dermis is differentiated into two layers: the stratum spongiosum, which ranges from $0.75 \times 10^2 \mu\text{m}$ to $2.51 \times 10^2 \mu\text{m}$ with an average thickness of $1.32 \times 10^2 \mu\text{m}$, and the stratum compactum, which varies between $0.45 \times 10^2 \mu\text{m}$ and $1.60 \times 10^2 \mu\text{m}$, averaging $0.98 \times 10^2 \mu\text{m}$. Glandular structures exhibit notable variation in abundance and size. Granular glands are rare, with a maximum of 1, minimum of 0, and an average of 0.09 glands, occupying a mean area of $1.90 \times 10^4 \mu\text{m}^2$. Seromucous glands are more frequent, with a maximum of 6, minimum of 0, and an average of 2.37 glands, but they display the smallest mean area ($0.85 \times 10^4 \mu\text{m}^2$) among the gland types. Mucous glands occur less frequently (maximum of 3, minimum of 0, and an average of 0.28 glands) with a mean area of $0.94 \times 10^4 \mu\text{m}^2$.

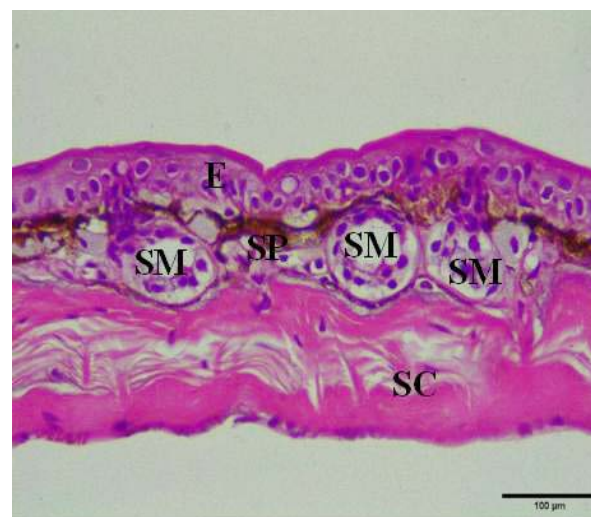
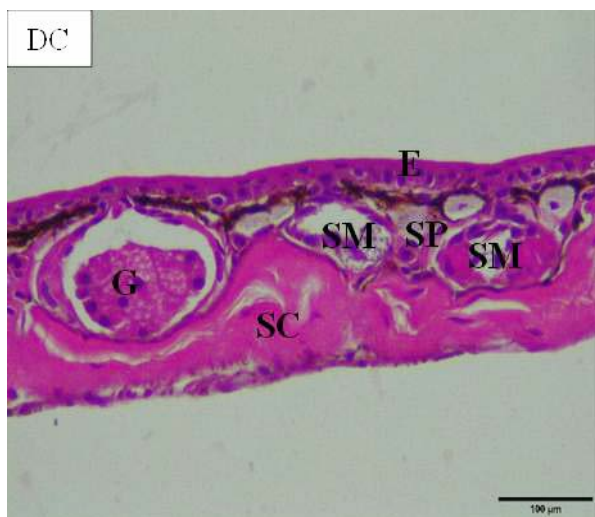
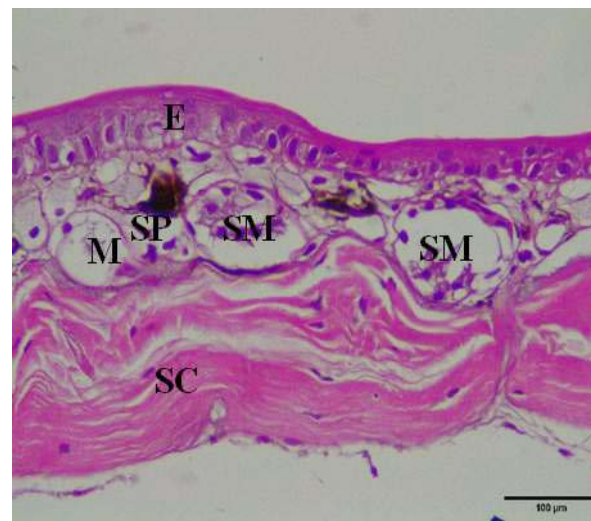
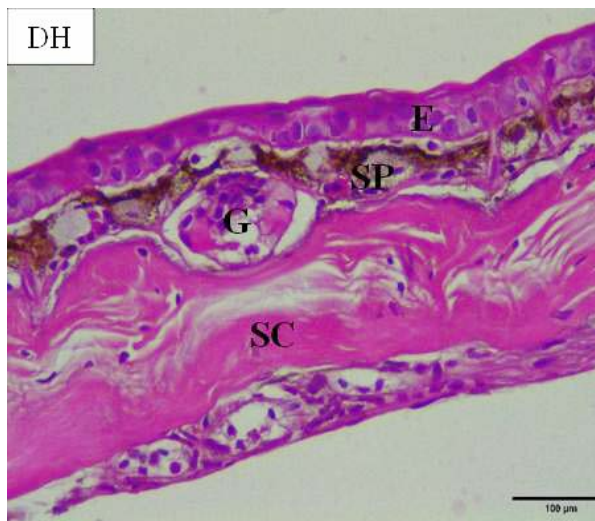
Table 4.5: Skin description of *Polypedates macrotis*

Variables (individual = 4, 10 sites per skin region)	Descriptive statistics (n)			
	Maximum thickness (µm)	Minimum thickness (µm)	Average thickness (µm)	Notes: Average area of glands (in µm ²)
1. Epidermis				
(a) Thickness of epidermis (µm)	11.52 × 10 ¹	2.73 × 10 ¹	6.2610 ¹	
2. Dermis				
a) Stratum spongiosum				
(i) Thickness of stratum spongiosum (µm)	2.51 × 10 ²	0.75 × 10 ²	1.32 × 10 ²	
b) Stratum compactum				
(i) Thickness of stratum compactum (µm)	1.60 × 10 ²	0.45 × 10 ²	0.98 × 10 ²	
	Maximum Number	Minimum Number	Average Number	Average area of glands (in µm²)
c) Granular gland				
(i) Number of gland (n)	1	0	0.09	1.90 × 10 ⁴
(ii) Area of gland				
d) Seromucous gland				
(i) Number of gland (n)	6	0	2.37	0.85 × 10 ⁴
(ii) Area of gland				
e) Mucous gland				
(i) Number of gland (n)	3	0	0.28	0.94 × 10 ⁴
(ii) Area of gland				

4.1.4.2 Variation of Skin Characters in Four Skin Regions (*Polypedates macrotis*)

Figure 4.3 showed four skin regions of *P. macrotis* and results were tabulated in Table 4.6. Four characteristics were significantly different ($p < 0.05$); thickness of epidermis (C1), thickness of stratum spongiosum (C2), thickness of stratum compactum (C3) and average size of seromucous glands present (C8). The cross-section of *P. macrotis*'s skin displayed a clearer

presentation of the granular, mucous, and seromucous glands. The epidermis in the ventral region is thicker compared to the dorsal regions. In contrast, the thickness of the stratum spongiosum and stratum compactum is greater in the dorsal region than in the ventral region. Although this species has a low number of granular glands, these glands are larger and denser compared to other glands. The number of seromucous glands is higher compared to the number of mucous glands but they are scattered all in these four regions.



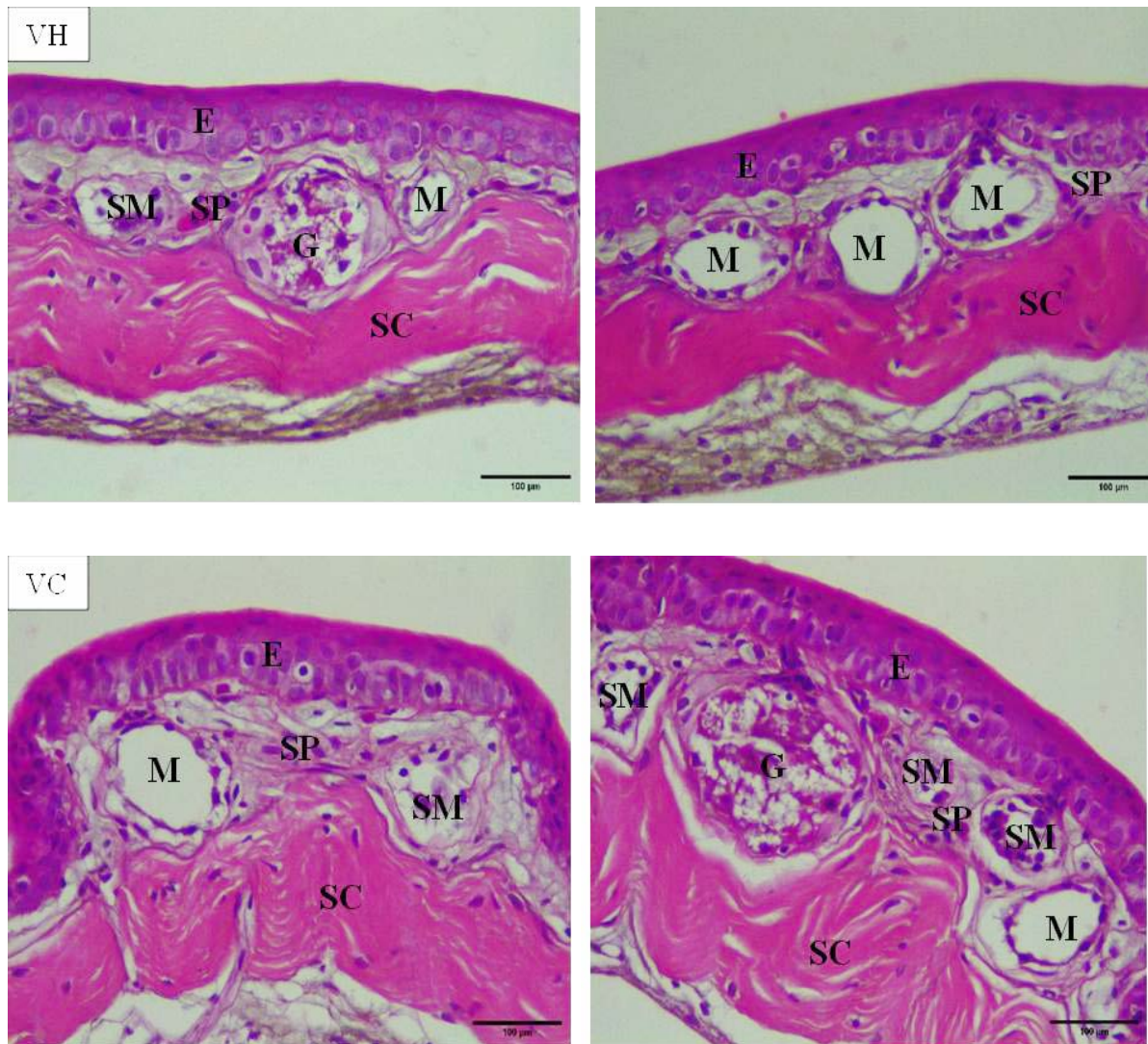


Figure 4.3: Skin structure of *Polypedates macrotis* at four skin regions. Bar 100 µm. Magnification=40X (Note: DH, dorsal head; DC, dorsal centre; VH, ventral head; VC, ventral centre; E, epidermis; SP, stratum spongiosum; SC, stratum compactum; M, mucous gland; G, granular gland; SM, seromucous gland).

Table 4.6: Skin measurement (Mean) of *Polypedates macrotis*

Character	C1*	C2*	C3*	C4	C5
	Thickness of epidermis (μm)	Thickness of stratum spongiosum (μm)	Thickness of stratum compactum (μm)	Number of granular glands	Number of seromucous glands
DH	$(5.22 \pm 0.78) \times 10^1$	$(1.40 \pm 0.26) \times 10^2$	$(1.08 \pm 0.19) \times 10^2$	0.05 ± 0.22	2.03 ± 1.00
DC	$(5.32 \pm 0.98) \times 10^1$	$(1.43 \pm 0.25) \times 10^2$	$(0.867 \pm 0.170) \times 10^2$	0.15 ± 0.36	2.43 ± 1.20
VH	$(6.71 \pm 1.43) \times 10^1$	$(1.09 \pm 0.22) \times 10^2$	$(0.925 \pm 0.165) \times 10^2$	0.13 ± 0.34	2.43 ± 1.32
VC	$(7.77 \pm 1.50) \times 10^1$	$(1.35 \pm 0.34) \times 10^2$	$(1.02 \pm 0.27) \times 10^2$	0.03 ± 0.16	2.60 ± 1.46
Character	C6	C7	C8*	C9*	
	Number of mucous glands	Average size of granular glands present (μm^2)	Average size of seromucous glands present (μm^2)	Average size of mucous glands present (μm^2)	
DH	0.15 ± 0.42	$(1.45 \pm 0.16) \times 10^4$	$(9.60 \pm 2.05) \times 10^3$	$(13.5 \pm 1.7) \times 10^3$	
DC	0.13 ± 0.46	$(2.20 \pm 0.79) \times 10^4$	$(8.80 \pm 2.79) \times 10^3$	$(9.74 \pm 1.09) \times 10^3$	
VH	0.40 ± 0.78	$(1.45 \pm 0.33) \times 10^4$	$(7.01 \pm 1.65) \times 10^3$	$(6.70 \pm 2.83) \times 10^3$	
VC	0.45 ± 0.85	$(3.22 \pm 0.00) \times 10^4$	$(8.44 \pm 2.01) \times 10^3$	$(10.0 \pm 2.8) \times 10^3$	

(*) -p <0.05. (Note: DH, Dorsal Head; DC, Dorsal Centre; VH, Ventral Head; VC; Ventral Centre)

4.1.5 *Polypedates otilophus*

Histological examination of the skin of *P. otilophus* was carried out on a single individual ($n = 1$). The analysis emphasized the structural features of the skin and the variation of these traits across four distinct regions. The results are presented in two parts: the general skin structure (Section 4.1.5.1) and the regional variations (Section 4.1.5.2).

4.1.5.1 Skin Structure of *Polypedates otilophus*

The skin of *P. otilophus* is characterized by a thin epidermis with a maximum thickness of $1.40 \times 10^2 \mu\text{m}$, a minimum of $0.58 \times 10^2 \mu\text{m}$, and an average of $0.90 \times 10^2 \mu\text{m}$. The dermis is organized into the stratum spongiosum and stratum compactum, with the stratum spongiosum ranging from $1.23 \times 10^2 \mu\text{m}$ to $2.47 \times 10^2 \mu\text{m}$ (average $1.86 \times 10^2 \mu\text{m}$) and the stratum compactum ranging between $0.50 \times 10^2 \mu\text{m}$ and $1.70 \times 10^2 \mu\text{m}$ (average $1.10 \times 10^2 \mu\text{m}$). Glandular distribution is variable, with granular glands occurring at low frequency (maximum 2, minimum 0, average 0.83) but occupying a relatively large mean area of $2.15 \times 10^4 \mu\text{m}^2$. Seromucous glands are more prevalent (maximum 4, minimum 0, average 1.50) with an average cross-sectional area of $1.33 \times 10^4 \mu\text{m}^2$, while mucous glands are rare (maximum 1, minimum 0, average 0.03) and smallest in area ($1.06 \times 10^4 \mu\text{m}^2$). Overall, the integument of *P. otilophus* demonstrates a simplified epidermal–dermal framework with a glandular profile dominated by seromucous and granular elements, indicating functional specialization toward secretory activity in maintaining physiological homeostasis and ecological adaptability.

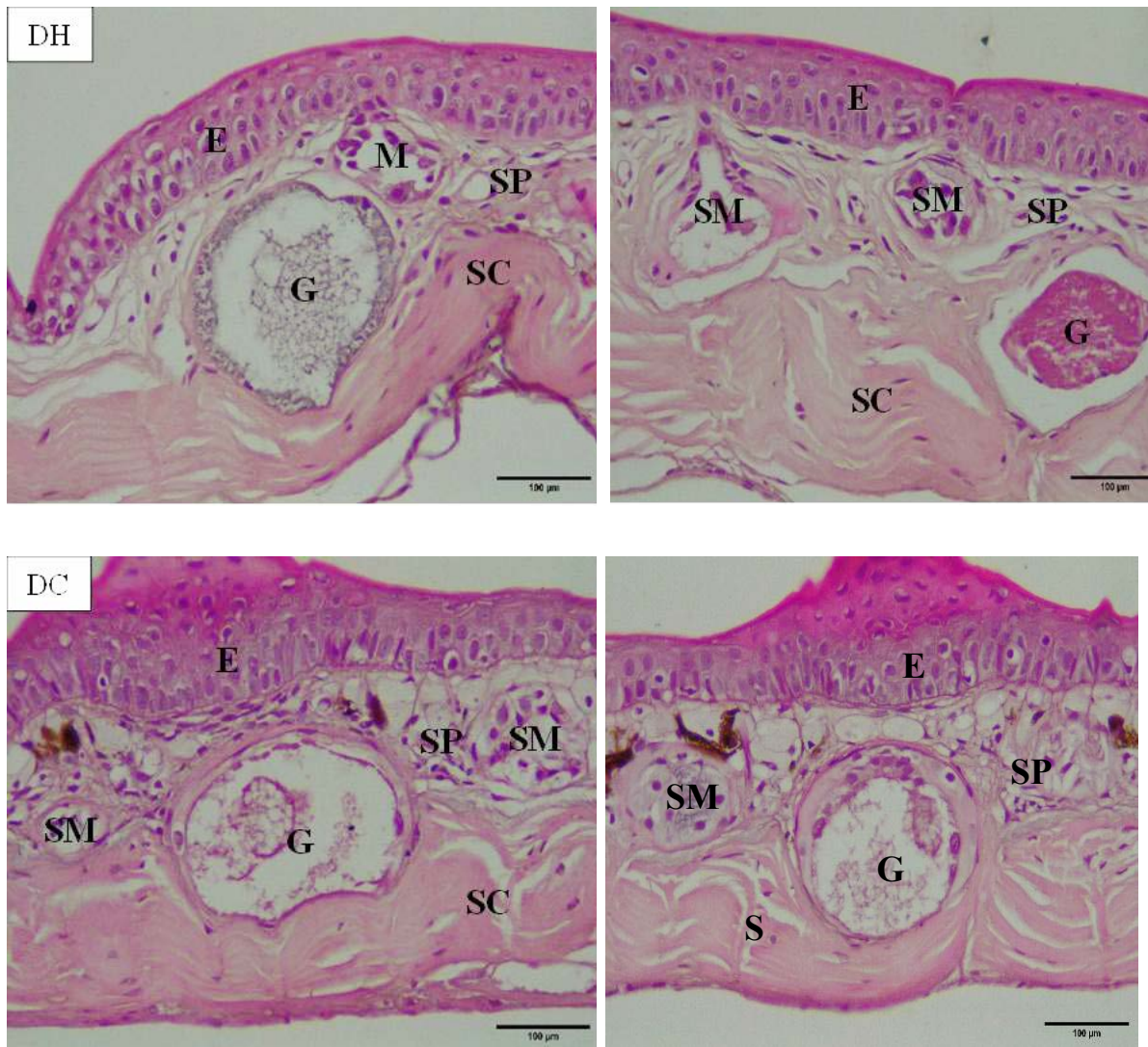
Table 4.7: Skin description of *Polypedates ottilophus*

Variables (individual 1=10 sites per skin region)	Descriptive statistics (n)			
	Maximum thickness (μm)	Minimum thickness (μm)	Average thickness (μm)	Notes: Average area of glands (in μm^2)
1. Epidermis				
(a) Thickness of epidermis (μm)	1.40×10^2	0.58×10^2	0.90×10^2	
2. Dermis				
(a) Stratum spongiosum				
(i) Thickness of stratum spongiosum (μm)	2.47×10^2	1.23×10^2	1.86×10^2	
(b) Stratum compactum				
(i) Thickness of stratum compactum (μm)	1.70×10^2	0.50×10^2	1.10×10^2	
	Maximum Number	Minimum Number	Average Number	Average area of glands (in μm^2)
(c) Granular gland				
(i) Number of gland (n)	2	0	0.83	2.15×10^4
(ii) Area of gland				
(d) Seromucous gland				
(i) Number of gland (n)	4	0	1.50	1.33×10^4
(ii) Area of gland				
(e) Mucous gland				
(i) Number of gland (n)	1	0	0.03	1.06×10^4
(ii) Area of gland				

4.1.5.2 Variation of Skin Characters in Four Skin Regions (*Polypedates ottilophus*)

Figure 4.4 showed four skin regions of *P. ottilophus* and results were tabulated in Table 12. Four characteristics were significantly different ($p < 0.05$); thickness of epidermis (C1),

thickness of stratum compactum (C3), number of granular glands (C4) and average size of seromucous glands present (C8). The thickness of epidermis and stratum compactum is thicker in ventral regions compared to dorsal regions. The granular glands and seromucous glands were distributed in all four regions. The presence of mucous glands is very low in this species, where only can be observed in dorsal head region. The number of granular glands is higher in this species compared to other species and it is observed more in ventral regions than in dorsal regions. The granular gland appeared to be bigger and denser. The average size of seromucous glands present in this species is bigger in dorsal regions compared to ventral regions.



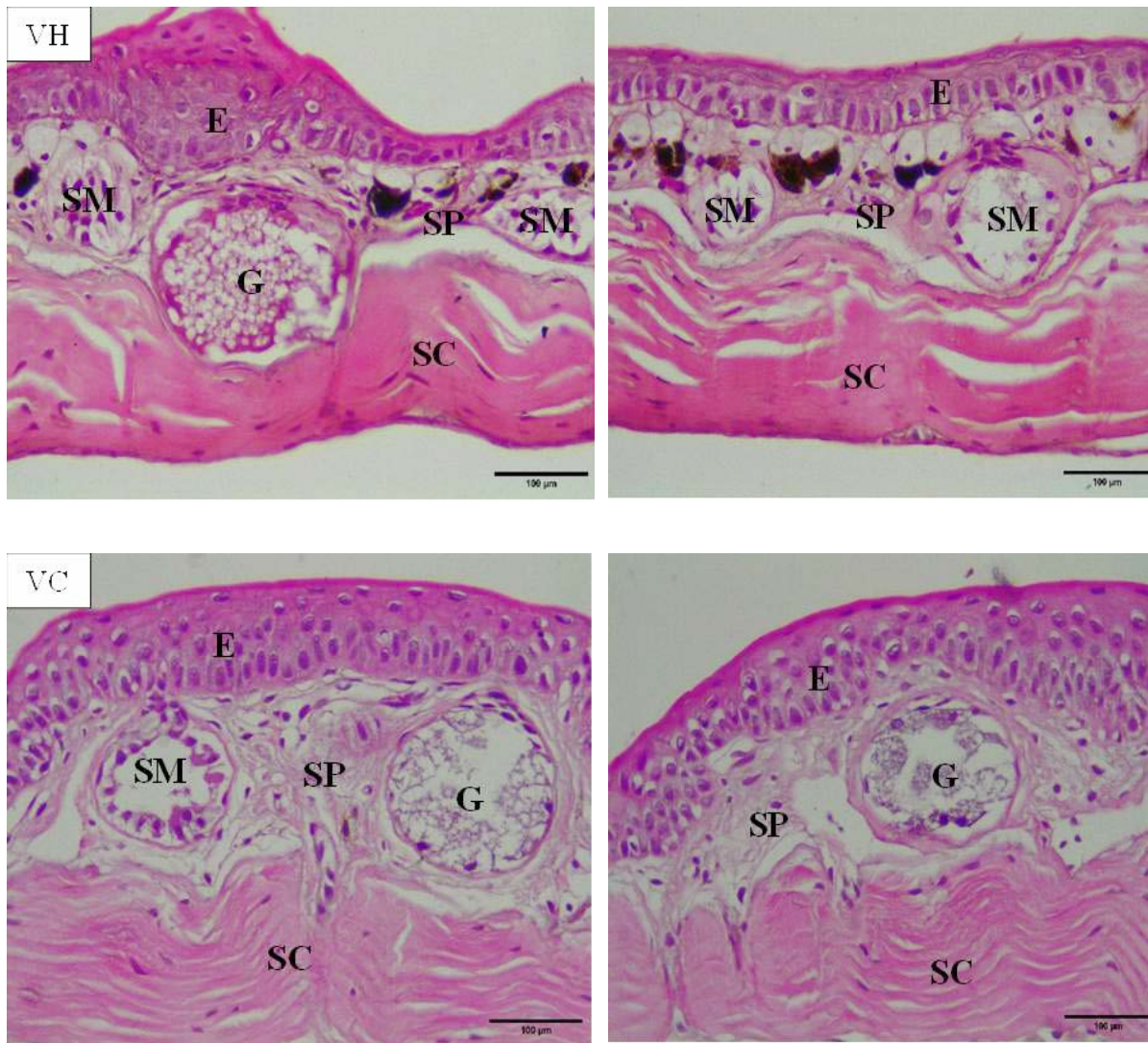


Figure 4.4: Skin structure of *Polypedates otilophus* at four skin regions. Bar 100 µm. Magnification=40X (Note: DH, dorsal head; DC, dorsal centre; VH, ventral head; VC, ventral centre; E, epidermis; SP, stratum spongiosum; SC, stratum compactum; M, mucous gland; G, granular gland; SM, seromucous gland).

Table 4.8: Skin measurement (Mean) of *Polypedates ottilophus*

Character	C1* Thickness of epidermis (µm)	C2 Thickness of stratum spongiosum (µm)	C3* Thickness of stratum compactum (µm)	C4* Number of granular glands	C5 Number of seromucous glands
DH	$(8.16 \pm 0.83) \times 10^1$	$(1.75 \pm 0.41) \times 10^2$	$(1.03 \pm 0.29) \times 10^2$	0.40 ± 0.52	1.60 ± 0.97
DC	$(9.00 \pm 2.09) \times 10^1$	$(1.94 \pm 0.28) \times 10^2$	$(0.89 \pm 0.16) \times 10^2$	0.97 ± 0.67	1.10 ± 0.88
VH	$(7.92 \pm 2.24) \times 10^1$	$(1.77 \pm 0.20) \times 10^2$	$(1.25 \pm 0.23) \times 10^2$	1.30 ± 0.82	1.10 ± 0.74
VC	$(10.7 \pm 1.5) \times 10^1$	$(1.99 \pm 0.29) \times 10^2$	$(1.23 \pm 0.26) \times 10^2$	0.60 ± 0.70	2.20 ± 1.14
Character	C6 Number of mucous glands	C7 Average size of granular glands present (µm ²)	C8* Average size of seromucous glands present (µm ²)	C9 Average size of mucous glands present (µm ²)	
DH	0.10 ± 0.312	$(2.42 \pm 0.84) \times 10^4$	$(1.47 \pm 0.46) \times 10^4$	$(1.06 \pm 0.00) \times 10^4$	
DC	0	$(2.19 \pm 1.08) \times 10^4$	$(1.40 \pm 0.41) \times 10^4$	0	
VH	0	$(1.98 \pm 0.49) \times 10^4$	$(1.04 \pm 0.14) \times 10^4$	0	
VC	0	$(2.15 \pm 0.34) \times 10^4$	$(1.51 \pm 0.27) \times 10^4$	0	

(*) -p <0.05. (Note: DH, Dorsal Head; DC, Dorsal Centre; VH, Ventral Head; VC; Ventral Centre)

4.1.6 *Polypedates leucomystax*

Histological examination of the skin of *P. leucomystax* was conducted on eleven individuals (n = 11). The analysis focused on the microstructural organization of the skin and the variation of these features across four distinct regions. The findings are presented in two sections: the general skin structure (Section 4.1.6.1) and the regional variations (Section 4.1.6.2).

4.1.6.1 Skin Structure of *Polypedates leucomystax*

The skin of *P. leucomystax* is defined by a relatively thin epidermis with a maximum thickness of $16.0 \times 10^1 \mu\text{m}$, a minimum of $3.11 \times 10^1 \mu\text{m}$, and an average of $7.83 \times 10^1 \mu\text{m}$. The dermis is stratified into the stratum spongiosum and stratum compactum, with the stratum spongiosum ranging from $0.47 \times 10^2 \mu\text{m}$ to $4.20 \times 10^2 \mu\text{m}$ (mean $1.34 \times 10^2 \mu\text{m}$) and the stratum compactum ranging from $0.28 \times 10^2 \mu\text{m}$ to $1.77 \times 10^2 \mu\text{m}$ (mean $0.88 \times 10^2 \mu\text{m}$). Glandular organisation is variable, with granular glands occurring infrequently (maximum 1, minimum 0, average 0.16) but occupying a relatively larger mean area of $2.79 \times 10^4 \mu\text{m}^2$. Seromucous glands are the most prevalent (maximum 6, minimum 0, average 2.46) with a mean area of $1.00 \times 10^4 \mu\text{m}^2$, while mucous glands occur rarely (maximum 3, minimum 0, average 0.17) and are smallest in size (mean area $0.56 \times 10^4 \mu\text{m}^2$).

Table 4.9: Skin description of *Polypedates leucomystax*

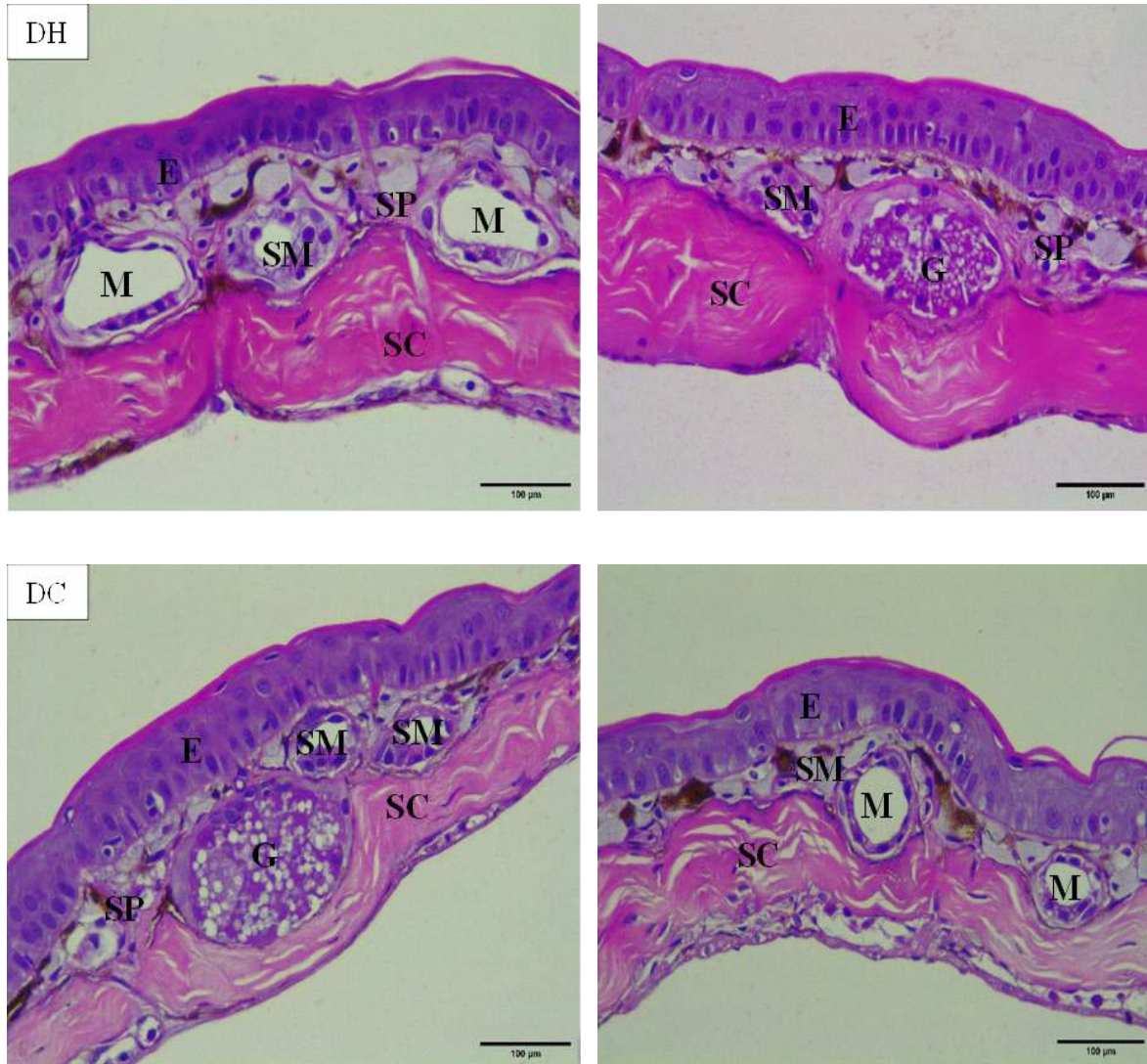
Variables (individual 11=10 sites per skin region)	Descriptive statistics (n)			
	Maximum thickness (μm)	Minimum thickness (μm)	Average thickness (μm)	Notes: Average area of glands (in μm^2)
1. Epidermis				
Thickness of epidermis (μm)				

	16.0×10^1	3.11×10^1	7.83×10^1	
2. Dermis				
(a) Stratum spongiosum				
(i) Thickness of stratum spongiosum (μm)	4.20×10^2	0.47×10^2	1.34×10^2	
(b) Stratum compactum				
(i) Thickness of stratum compactum (μm)	4.20×10^2	0.47×10^2	1.34×10^2	
	Maximum Number	Minimum Number	Average Number	Average area of glands (in μm^2)
(c) Granular gland				
(i) Number of gland (n)	1	0	0.16	2.79×10^4
(ii) Area of gland				
(d) Seromucous gland				
(i) Number of gland (n)	6	0	2.46	1.00×10^4
(ii) Area of gland				
(e) Mucous gland				
(i) Number of gland (n)	3	0	0.17	10^4
(ii) Area of gland				

4.1.6.2 Variation of Skin Characters in Four Skin Regions (*Polypedates leucomystax*)

Figure 4.5 showed four skin regions of *P. leucomystax* and results were tabulated in Table 4.10. Seven characteristics were significantly different ($p < 0.05$); thickness of epidermis (C1), thickness of stratum spongiosum (C2), thickness of stratum compactum (C3), number of granular glands (C4), number of seromucous glands (C5), number of mucous glands (C6) and average size of seromucous glands present (C8). This study shows that the thickness of epidermis, stratum spongiosum and stratum compactum is thicker in ventral regions compared to in dorsal regions. The number of granular glands is more in dorsal regions than in ventral regions. Next, the number of seromucous glands is more in ventral regions compared to dorsal regions while the number of mucous glands is in dorsal regions than in ventral regions. The

average size of seromucous glands present is bigger in ventral regions than in dorsal regions. The granular glands were situated beneath the seromucous glands within the stratum spongiosum. The granular appearance is clear in the granular glands due to the accumulation of secretory vesicles.



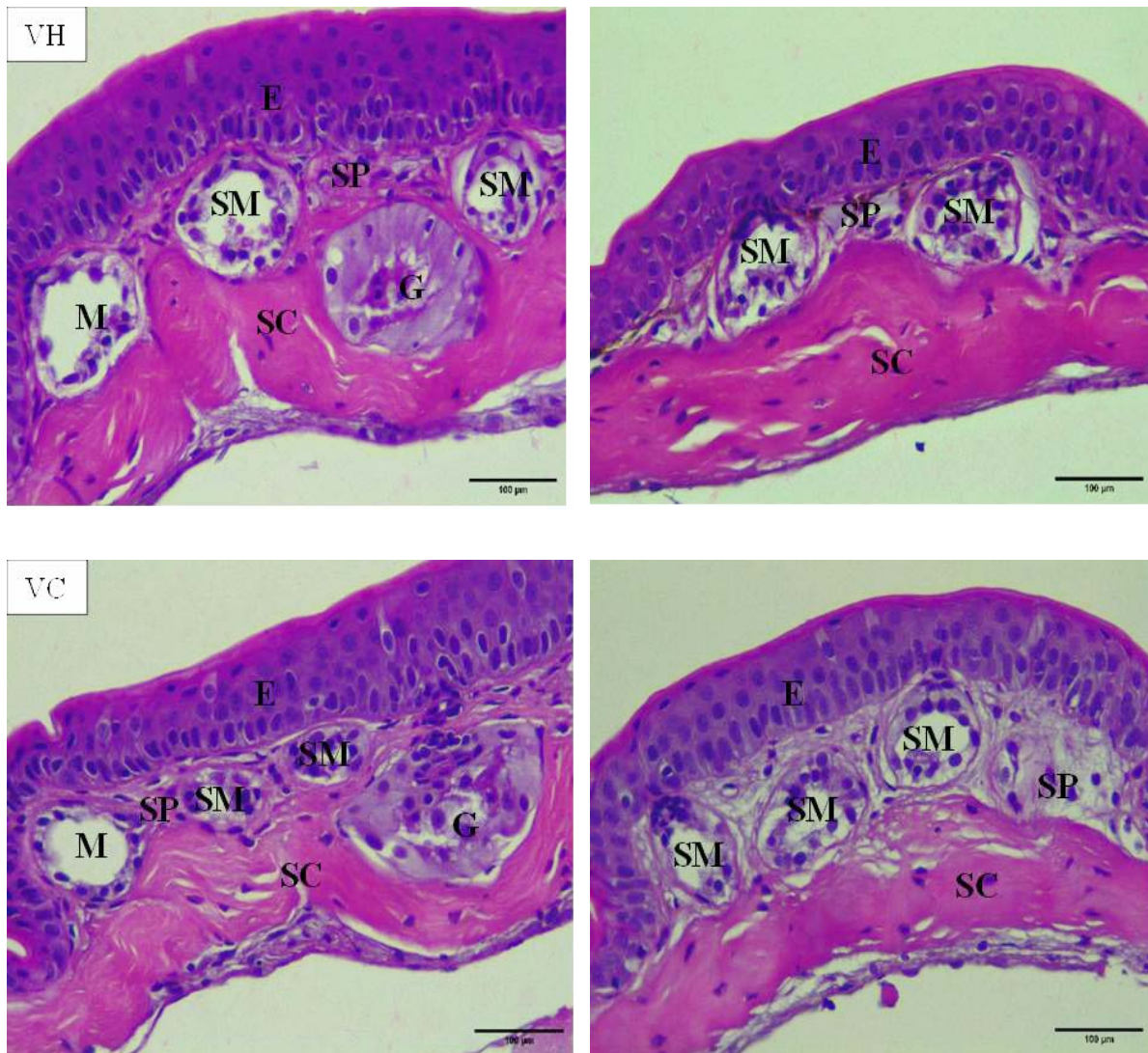


Figure 4.5: Skin structure of *Polypedates leucomystax* at four skin regions. Bar 100 µm. Magnification=40X (Note: DH, dorsal head; DC, dorsal centre; VH, ventral head; VC, ventral centre; E, epidermis; SP, stratum spongiosum; SC, stratum compactum; M, mucous gland; G, granular gland; SM, seromucous gland).

Table 4.10: Skin measurement (Mean) of *Polypedates leucomystax*

Character	C1* Thickness of epidermis (μm)	C2* Thickness of stratum spongiosum (μm)	C3* Thickness of stratum compactum (μm)	C4* Number of granular glands	C5* Number of seromucous glands
DH	$(6.45 \pm 1.89) \times 10^1$	$(1.34 \pm 0.57) \times 10^2$	$(8.76 \pm 3.04) \times 10^1$	0.18 ± 0.39	2.17 ± 0.91
DC	$(6.42 \pm 1.76) \times 10^1$	$(1.29 \pm 0.53) \times 10^2$	$(7.52 \pm 2.76) \times 10^1$	0.20 ± 0.40	2.08 ± 1.08
VH	$(9.05 \pm 1.94) \times 10^1$	$(1.37 \pm 0.47) \times 10^2$	$(9.08 \pm 2.98) \times 10^1$	0.18 ± 0.39	2.77 ± 1.20
VC	$(9.39 \pm 1.92) \times 10^1$	$(1.37 \pm 0.38) \times 10^2$	$(9.71 \pm 2.81) \times 10^1$	0.07 ± 0.26	2.80 ± 1.05
Character	C6* Number of mucous glands	C7 Average size of granular glands present (μm ²)	C8* Average size of seromucous glands present (μm ²)	C9 Average size of mucous glands present (μm ²)	
DH	0.17 ± 0.45	$(2.84 \pm 1.49) \times 10^4$	$(9.24 \pm 3.01) \times 10^3$	$(5.42 \pm 4.48) \times 10^3$	
DC	0.31 ± 0.65	$(2.59 \pm 1.57) \times 10^4$	$(8.61 \pm 2.98) \times 10^3$	$(5.58 \pm 2.87) \times 10^3$	
VH	0.09 ± 0.37	$(3.11 \pm 1.96) \times 10^4$	$(10.9 \pm 3.5) \times 10^3$	$(6.96 \pm 5.11) \times 10^3$	
VC	0.10 ± 0.36	$(2.57 \pm 2.40) \times 10^4$	$(11.2 \pm 3.7) \times 10^3$	$(3.25 \pm 1.48) \times 10^3$	

(*) -p <0.05. (Note: DH, Dorsal Head; DC, Dorsal Centre; VH, Ventral Head; VC; Ventral Centre

4.2 Environmental parameters of *Polypedates leucomystax*

4.2.1 Introduction

According to Razali (2017), *P. leucomystax*, commonly known as the four-lined tree frog, is an amphibian species sensitive to environmental changes, making it a useful bioindicator of ecosystem health. Environmental parameters such as pH, dissolved oxygen, temperature, and turbidity can influence its physiological functions, particularly skin histology, which plays a crucial role in respiration, osmoregulation, and protection. Variations in these parameters across different localities in Sarawak may lead to structural differences in the frog skin, potentially affecting the species' adaptability and survival (Rasit et al., 2023). However, there is limited research on how these environmental factors influence the skin morphology of *P. leucomystax*. This study aims to analyse the skin histology of *P. leucomystax* in relation to environmental parameters at different localities in Sarawak, examine variations in these parameters, investigate their relationship with skin structure, and determine the potential impact of habitat differences on the species' physiological adaptation.

Amphibian skin is a complex organ responsible for multiple vital functions, including respiration, osmoregulation, thermoregulation, defence, water absorption, and communication. Its structure and physiology are highly influenced by environmental conditions, which can lead to significant adaptations in skin morphology and function (Akat Çömnden et al., 2023). Studies have shown that environmental factors such as pH, temperature, and dissolved oxygen levels can significantly affect the diversity and composition of amphibian skin microbiota, which in turn can influence skin health and overall physiology (Lucia et al., 2024).

Research on the skin histology of *P. leucomystax* has revealed detailed structural characteristics, including the presence of various pigment cells that may play a role in adapting the frog to its environment (Iangrai & Hooroo, 2019). Given the sensitivity of amphibian skin to environmental changes, understanding the relationship between environmental parameters

and skin histology in *P. leucomystax* across different localities in Sarawak is crucial for assessing the species' adaptability and informing conservation efforts.

4.2.2 Analysis of Environmental Parameters Between Localities

The study assessed four environmental parameters: water pH, water turbidity, dissolved oxygen, and water temperature. All four environmental parameters which are water pH, water turbidity, dissolved oxygen, and water temperature exhibited significant variations across the different localities, with p-values less than 0.01 ($\alpha = 0.01$). This indicates that the differences observed were statistically significant, highlighting the influence of locality on these critical environmental parameters.

Table 4.11: Summarized ANOVA of environmental parameters between localities.

Parameters	df	F	Sig.	Mean±SE
pH	5	22.755	<0.01*	6.95±0.25
Turbidity	5	34.392	<0.01*	19.52±5.93
Dissolved Oxygen	5	132.744	<0.01*	28.68±0.90
Temperature	5	170.045	<0.01*	6.71±0.50

4.2.3 Post Hoc Test

Table 4.12 presents the results of a Post Hoc Tukey Pairwise Test analyzing water pH levels across six localities: Bau, West UNIMAS, Ranchan, East UNIMAS, Maludam, and Mulu. The localities are grouped into three subsets based on similar pH levels at a significance level of 0.05. Bau and West UNIMAS fall into the lowest water pH group, with values of 5.62 and 6.13, respectively. Ranchan overlaps between Subsets 1 and 2 with a pH of 6.64, while East UNIMAS (7.25) and Maludam (7.44) are in the middle range. Mulu, with the highest pH of 8.6000, forms its own subset. The significance values (0.06, 0.19, and 1.00) confirm notable differences between groups, indicating a significant relationship between water pH and localities.

Table 4.12: Homogenous subsets of Post Hoc Tukey Pairwise Test on water pH between localities.

Water pH				
Localities	N	Subset for alpha = 0.05		
		1	2	3
Bau	3	5.62		
West UNIMAS	3	6.13		
Ranchan	3	6.64	6.64	
East UNIMAS	3		7.25	
Maludam	3		7.44	
Mulu	3			8.60
Sig.		0.06	0.19	1.00

a. Uses Harmonic Mean Sample Size = 3.00.

Table 4.13 shows the results of a Post Hoc Tukey Pairwise Test for water turbidity across six localities: Ranchan, East UNIMAS, West UNIMAS, Mulu, Maludam, and Bau. The localities are grouped into two subsets based on similar turbidity levels at a significance level of 0.05. Subset 1 includes Ranchan (1.86), East UNIMAS (2.67), West UNIMAS (6.71), Mulu (15.97), and Maludam (19.53), indicating relatively low turbidity. Bau, with a turbidity of 70.37, forms its own group in Subset 2 due to significantly higher levels. The significance values (0.12 and 1.00) confirm notable differences between subsets, demonstrating significant variation in turbidity levels across the localities.

Table 4.13: Homogenous subsets of Post Hoc Tukey Pairwise Test on water turbidity between localities.

Turbidity			
Localities	N	Subset for alpha = 0.05	
		1	2
Ranchan	3	1.86	
East UNIMAS	3	2.67	
West UNIMAS	3	6.71	
Mulu	3	15.97	

Maludam	3	19.53	
Bau	3		70.37
Sig.		0.12	1.00

Uses Harmonic Mean Sample Size = 3.00

Table 4.14 displays the Post Hoc Tukey Pairwise Test results for temperature across six localities: Ranchan, Bau, Mulu, East UNIMAS, West UNIMAS, and Maludam. The localities are divided into four subsets based on similar temperature values at a significance level of 0.05. Ranchan (24.53) forms the first subset, while Bau (26.83), Mulu (27.22), and East UNIMAS (27.34) belong to the second subset. West UNIMAS (30.10) constitutes the third subset, and Maludam (36.03) represents the fourth subset, indicating the highest temperature. The significance values (1.00, 0.90, 1.00, and 1.00) suggest some variations in temperature levels, with distinct differences between subsets.

Table 4.14: Homogenous subsets of Post Hoc Tukey Pairwise Test on water temperature between localities.

Water Temperature					
Localities	N	Subset for alpha = 0.05			
		1	2	3	4
Ranchan	3	24.53			
Bau	3		26.83		
Mulu	3		27.22		
East UNIMAS	3		27.34		
West UNIMAS	3			30.10	
Maludam	3				36.03
Sig.		1.00	0.90	1.00	1.00

a. Uses Harmonic Mean Sample Size = 3.00.

Table 4.15 presents the Post Hoc Tukey Pairwise Test results for dissolved oxygen (DO) across six localities: Ranchan, Bau, Mulu, East UNIMAS, West UNIMAS, and Maludam. These localities are categorized into four subsets based on similar DO values at a significance level of 0.05. East UNIMAS, with a DO value of 2.37 mg/L ($p=1.00$), falls into

the first subset. West UNIMAS, with 6.73 mg/L ($p=0.350$), is placed in the second subset. Maludam (7.22 mg/L) and Mulu (7.24 mg/L) overlap between Subsets 2 and 3. Bau, with a DO value of 7.94 mg/L, forms the third subset. Lastly, Ranchan, with the highest DO value of 8.77 mg/L, constitutes the fourth subset.

Table 4.15: Homogenous subsets of Post Hoc Tukey Pairwise Test on water dissolved oxygen between localities.

Dissolved Oxygen (DO)					
Localities	N	Subset for alpha = 0.05			
		1	2	3	4
East UNIMAS	3	2.37			
West UNIMAS	3		6.73		
Maludam	3		7.22	7.22	
Mulu	3		7.24	7.24	
Bau	3			7.94	
Ranchan	3				8.77
Sig.		1.00	0.35	0.09	1.00

a. Uses Harmonic Mean Sample Size = 3.00.

4.2.4 Analysis of Correlation Tests

Two types of correlation tests, the Spearman Correlation Test and the Kendall Correlation Test, were employed to assess the strength and nature of relationships between gland characteristics and environmental parameters. These analyses examined how the number and area of different gland types, including mucous glands, seromucous glands, and granular glands, were associated with key environmental parameters indicators such as water pH, water turbidity, water temperature, and dissolved oxygen (DO).

Table 4.16 showed the correlation between number of glands present on *P. leucomystax* and environmental parameters while table 4.17 showed the correlation between number of glands present on *P. leucomystax* and environmental parameters using Kendall Correlation test. The analysis revealed no significant correlation between the number of glands (unit) and

environmental parameters as indicated by both the Spearman correlation ($p > 0.05$) and Kendall correlation ($p > 0.05$). This suggests that variations in the number of glands do not exhibit a consistent or ordinal relationship with environmental parameters metrics. These findings imply that the presence or quantity of glands may not directly influence environmental parameters, or that the relationship is mediated by other ecological or environmental factors not accounted for in this analysis.

Table 4.16: Spearman Correlation between number of glands (unit) and environmental parameters.

		Mucous	Seromucous	Granular	pH	Turbidity	Temperature	DO
Mucous	Correlation Coefficient	1.000	-0.406	0.316	0.462	-0.109	-0.044	-0.142
	Sig. (2-tailed)	0.00	0.215	0.343	0.153	0.750	0.898	0.678
	N	11	11	11	11	11	11	11
Seromucous	Correlation Coefficient	-0.406	1.000	0.247	0.111	-0.388	0.360	-0.231
	Sig. (2-tailed)	0.215	0.00	0.464	0.746	0.238	0.276	0.494
	N	11	11	11	11	11	11	11
Granular	Correlation Coefficient	0.316	0.247	1.000	-0.194	-0.370	-0.100	-0.322
	Sig. (2-tailed)	0.343	0.464	0.00	0.567	0.263	0.771	0.334
	N	11	11	11	11	11	11	11

*. Correlation is significant at the 0.05 level (2-tailed).

Table 4.17: Kendall Correlation between number of glands (unit) and environmental parameters.

		Mucous	Seromucous	Granular	pH	Turbidity	Temperature	DO
Mucous	Correlation Coefficient	1.000	-0.278	0.258	0.353	-0.078	-0.078	-0.118
	Sig. (2-tailed)	0.00	0.240	0.294	0.150	0.749	0.749	0.632
	N	11	11	11	11	11	11	11
Seromucous	Correlation Coefficient	-0.278	1.000	0.195	0.019	-0.289	0.327	-0.212
	Sig. (2-tailed)	0.240	0.00	0.422	0.937	0.233	0.177	0.382
	N	11	11	11	11	11	11	11
Granular	Correlation Coefficient	0.258	0.195	1.000	-0.144	-2.68	-0.062	-0.227
	Sig. (2-tailed)	0.294	0.422	0.00	0.567	0.288	0.806	0.368
	N	11	11	11	11	11	11	11

*. Correlation is significant at the 0.05 level (2-tailed).

Table 4.18 presents the correlation analysis between the area of glands (μm^2) and various environmental parameters. The Spearman correlation test revealed significant differences for the area of granular glands and seromucous glands, with a p-value of 0.013 ($\alpha = 0.05$), demonstrating a strong positive correlation at 0.718. Among the environmental parameters, pH showed a significant association with the area of seromucous glands, with a p-value of 0.018 ($\alpha = 0.05$). This relationship was characterized by a strong positive correlation, with 0.693. These findings suggest that the area of certain gland types, particularly granular and seromucous glands, is closely linked to environmental parameters characteristics, specifically pH levels. The strong positive correlations indicate that as the gland area increases, there is a corresponding improvement in pH, potentially highlighting an adaptive or physiological response of these glands to environmental conditions. This emphasizes the role of gland morphology in environmental interactions and its potential as a bioindicator for environmental parameters assessment.

Table 4.19 summarizes the Kendall correlation analysis between the area of glands (μm^2) and various environmental parameters. The test identified significant differences for the areas of granular glands and seromucous glands, with a p-value of 0.010 ($\alpha = 0.05$), indicating a strong positive correlation of 0.600. Additionally, the environmental parameters, pH showed a significant association with the area of seromucous glands, with a p-value of 0.014 ($\alpha = 0.05$). This association also demonstrated a strong positive correlation, with 0.597. These findings suggest a notable relationship between glandular area and environmental parameters, particularly with pH levels, highlighting potential adaptive or environmental interactions.

Table 4.18: Spearman Correlation between area of glands (μm^2) and environmental parameters.

		Mucous	Seromucous	Granular	pH	Turbidity	Temperature	DO
Mucous	Correlation Coefficient	1.000	0.418	0.136	0.356	-0.106	-0.189	-0.051
	Sig. (2-tailed)	0.00	0.201	0.689	0.283	0.756	0.577	0.882
	N	11	11	11	11	11	11	11
Seromucous	Correlation Coefficient	0.418	1.000	0.718*	0.693*	-0.249	0.499	-0.407
	Sig. (2-tailed)	0.201	0.00	0.013	0.018	0.459	0.118	0.215
	N	11	11	11	11	11	11	11
Granular	Correlation Coefficient	0.136	0.718*	1.000	0.388	-0.517	0.231	-0.139
	Sig. (2-tailed)	0.689	0.013	0.00	0.238	0.103	0.494	0.684
	N	11	11	11	11	11	11	11

*. Correlation is significant at the 0.05 level (2-tailed).

Table 4.19: Kendall Correlation between area of glands (μm^2) and environmental parameters.

		Mucous	Seromucous	Granular	pH	Turbidity	Temperature	DO
Mucous	Correlation Coefficient	1.000	0.309	0.127	0.250	-0.058	-0.135	-0.058
	Sig. (2-tailed)	0.00	0.186	0.586	0.302	0.812	0.578	0.812
	N	11	11	11	11	11	11	11
Seromucous	Correlation Coefficient	0.309	1.000	0.600*	0.597*	-0.173	0.366	-0.250
	Sig. (2-tailed)	0.186	0.00	0.010	0.014	0.474	0.131	0.302
	N	11	11	11	11	11	11	11
Granular	Correlation Coefficient	0.127	0.600*	1.000	0.250	-0.366	0.173	-0.135
	Sig. (2-tailed)	0.586	0.010	0.00	0.302	0.131	0.474	0.578
	N	11	11	11	11	11	11	11

*. Correlation is significant at the 0.05 level (2-tailed).

CHAPTER 5

DISCUSSION

5.1 Skin Descriptions of Selected Tree frog Species

Frogs are known to have a thin layer of skin that is moist and has a soft texture, playing a crucial role in their survival. Tree frog skin is highly permeable, allowing for efficient gas exchange and water absorption (Razali, 2017). The skin serves as the primary protective barrier for frog species, shielding them from extreme temperatures, chemical toxins, and excessive exposure to sunlight (Zainudin et al., 2018). As such, variations in skin characteristics often reflect their ability to adapt to different environmental conditions (Rasit et al., 2023). These adaptations may include differences in skin thickness and secretions, all of which contribute to the frog's ability to survive in diverse habitats, from humid rainforests to drier regions (Iangrai, & Hooroo, 2019). Given the threats of habitat fragmentation faced by tree frog species, we are investigating the skin structure of selected species across various body regions. Previous studies on frog epidermis have found that the outermost layer of their skin is typically thin and smooth (Varga et al., 2019). This epidermal layer is composed of distinct strata, including the stratum granulosum, stratum spinosum, and stratum germinativum, which contribute to the skin's protective and regenerative functions (Smoller & Hiatt, 2009). These layers work together to maintain the structural integrity and permeability of the skin, vital for the frogs' survival in various environmental conditions. The thinness of the skin also facilitates cutaneous respiration, a critical feature for amphibians. This present study reveals that frogs possess a thin epidermis, measuring less than $1 \times 10^4 \mu\text{m}$ and is composed of the stratum granulosum, stratum spinosum, and stratum germinativum. When compared to human skin, which ranges from 0.5 to 4 mm in thickness (Haslam et al., 2013), frog skin is significantly thinner, highlighting its specialized structure for cutaneous respiration and environmental adaptability.

These results support the statement that frog skin is indeed thin. This study demonstrates that across all six examined selected tree frog species, the epidermis in the ventral region is consistently thicker than in the dorsal region. The dorsal skin is usually involved in thermoregulation and may be more permeable for gas exchange. A thinner epidermis allows for better respiration through the skin, which is vital for amphibians (McDiarmid & Altig, 1999). Next, the dorsal side of frogs is often exposed to harsher environmental conditions, such as sunlight and potential abrasions from vegetation or surfaces compared to ventral side. A thinner epidermis can reduce weight and increase flexibility, aiding in movement and camouflage.

This variation in epidermal thickness is likely due to the need for improved protection against physical abrasion and pathogens, as the ventral side is frequently in contact with various surfaces. Furthermore, the increased thickness of the epidermis in this ventral side may also contribute to moisture retention, which is essential for maintaining hydration in terrestrial environments. These findings underscore the adaptive significance of epidermal structure concerning the ecological roles and habitats of tree frogs (Duellman & Trueb, 1994).

In tree frogs, the dermis is a highly specialized layer of skin that provides structural support, hydration, and various physiological functions, particularly as they inhabit moist or semi-aquatic environments. The dermis is divided into two primary compartments: the stratum spongiosum and the stratum compactum. The stratum spongiosum, the outer layer of the dermis, consists of loosely arranged connective tissue. The loose connective tissue supports the presence of various glands, including seromucous, mucous, and granular glands (Lim et al., 2024). This stratum spongiosum layer characterized by a spongy, porous structure that aids in storing water, which is essential for amphibians like tree frogs, given their permeable skin and reliance on skin-based respiration. It also has many blood vessels and lymph spaces that help

with nutrient exchange and keeping moisture, allowing tree frogs to stay hydrated and adjust to changing moisture levels in their environment. In these findings, the stratum spongiosum is greater on the ventral compared to dorsal for *P. leucomystax*, *P. otilophus*, *P. colletti*, *R. pardalis* except for *P. macrotis*. This is because the ventral side typically makes more contact with surfaces, where moisture and nutrient exchange are essential for hydration and environmental adaptability. A thicker stratum spongiosum on the ventral side helps these frogs absorb water effectively from their surroundings, which is particularly important in variable moisture conditions. However, *P. macrotis* does not follow this pattern, possibly due to differences in its habitat and behaviour compared with other species. While *P. leucomystax* is often associated with disturbed areas such as human settlements, agricultural landscapes, and roadside ditches, *P. macrotis* typically inhabits primary rainforests, secondary growth, and forest clearings surrounded by forest (Shahrudin, 2019). These ecological distinctions suggest that *P. macrotis* may rely less on ventral hydration through direct substrate contact and instead adapt to moisture availability in more closed forest environments, thereby influencing its dermal structural variation.

Situated below the stratum spongiosum and the stratum compactum layer is denser and provides structural integrity. This layer is primarily made up of tightly packed collagen fibres, which contribute to the skin's elasticity and durability. The compact arrangement of collagen fibres also serves as a physical barrier, offering protection against environmental stressors, pathogens, and potential predators (Sungif, 2017). The stratum compactum layer helps maintain the frog's shape and provides resistance against abrasions, which is particularly useful for tree frogs that engage in climbing and jumping (Lillywhite & Hillman, 1990). In species such as *P. leucomystax*, *P. otilophus*, *P. colletti*, and *R. pardalis*, the stratum compactum is thicker on the ventral side than on the dorsal side. This thicker layer on the ventral side provides extra support and protection, especially as these frogs often rest or move along surfaces, where

they encounter more environmental friction and potential injury. A thicker stratum compactum helps maintain durability and prevents damage from ground contact.

The present study revealed that the stratum spongiosum was consistently thicker than the stratum compactum across all tree frog species examined. This finding is consistent with earlier descriptions of amphibian skin, where the stratum spongiosum is generally more developed to facilitate hydration, gas exchange, and nutrient absorption (Duellman & Trueb, 1994; Wells, 2007). In the species studied, the thicker spongiosum likely reflects its high content of loosely arranged connective tissue, blood vessels, and lymphatic spaces, which are essential for maintaining water balance in moist and semi-aquatic environments. The observed thickness also corresponds with the presence of mucous and seromucous glands distributed within this layer, which have been previously reported to play important roles in moisture retention and cutaneous respiration (Sungif, 2017). In contrast, the stratum compactum was consistently thinner but more densely packed, functioning primarily as a supportive structural layer with tightly arranged collagen fibres, as described in earlier studies (Maderson, 1972). The relative differences in thickness between the two dermal strata therefore highlight the functional adaptations of tree frog skin, with the stratum spongiosum serving as the principal site of physiological exchange while the stratum compactum provides structural reinforcement.

Glands play a crucial role in the skin of tree frogs, and this study found that all species studied possess mucous glands. Mucous glands are typically tubular or acinar in shape. They can vary in size but are generally small and are composed of a cluster of secretory cells. These studies found that in species such as *P. leucomystax*, *P. otilophus*, *P. colletti*, and *R. pardalis*, the distribution of mucous glands is greater on the dorsal side than on the ventral side. The dorsal side is more exposed to environmental stressors, such as UV radiation, temperature fluctuations, and physical abrasion. A higher density of mucous glands in this area may help provide a protective mucus layer that can shield the skin from these factors, reducing the risk

of damage and dehydration (Duellman & Trueb, 1994). Moreover, although the ventral side plays a critical role in moisture absorption, the dorsal mucous glands also contribute to moisture retention. The secretion of mucus from the dorsal glands helps prevent desiccation in fluctuating humidity conditions (Wells, 2007). The dorsal side is more exposed to predators and potential mates. A thicker layer of mucus can help the frogs blend in with their surroundings, providing camouflage. Furthermore, the secretions might include pheromones or other chemical signals that are important for mating and social interactions (Mauricio et al., 2021). Furthermore, the specific habitats occupied by these species may necessitate a higher number of mucous glands on the dorsal side to cope with the unique challenges of their environments. For instance, tree frogs that live in more exposed or arboreal habitats may evolve to have greater mucus coverage to mitigate the risks associated with their lifestyles (Wu et al., 2022). In contrast, *P. macrotis* typically inhabits primary rainforests, secondary growth, and forest clearings surrounded by forest (Shahrudin, 2019). These habitat preferences suggest that this species is less dependent on ventral hydration through direct substrate contact and instead may adapt to the higher and more consistent moisture availability in closed forest environments, which could explain the different distribution pattern of its dermal structures. *P. otilophus* has the lowest distribution of mucous glands among the species studied where the mucous gland is not available at dorsal head. This reduced presence of mucous glands could be linked to its specific ecological adaptations and habitat preferences. For example, if *P. otilophus* lives in a relatively drier microhabitat or employs moisture-retaining strategies that differ from other tree frogs, it may have adapted via larger body size, which reduces its surface-area-to-volume ratio and thus limits water loss under desiccating conditions. Recent work on anuran allometry supports that frogs in drier environments tend to exhibit body shapes or sizes that help conserve water (Castro et al., 2021).

Frog skin contains seromucous glands, which play a key role in maintaining skin hydration and producing mucus. These findings show that the distribution of seromucous glands is higher in all studied species compared to that of mucous and granular glands. Additionally, seromucous glands are present throughout the skin structure across all species studied. Since seromucous glands are a combination of mucous and granular glands, they also secrete toxins that perform similar functions to those produced by granular glands (Lim et al., 2024). As seromucous glands contain mucous, they play a crucial role in maintaining skin moisture and creating a humid environment across all body regions (Sungif, 2017). This suggests that a higher presence of seromucous glands within the skin of tree frog could enhance their ability to endure and adapt to drier habitats for extended periods. By providing essential hydration, these glands may support the physiological demands of tree frog, aiding in thermoregulation and extending their ecological range to include environments with varying moisture levels. Although seromucous glands are distributed across all skin regions in the studied species, they are more concentrated on the ventral (underside) than on the dorsal (upper) surface. Since the ventral surface is often the first to meet the ground or bark, it is more susceptible to moisture loss and potential abrasion from these surfaces compared to the dorsal side. An increased presence of seromucous glands on this side may help retain moisture, creating a protective barrier that prevents dehydration (Razali, 2017).

The findings indicated that all studied species possessed granular glands in their skin regions. The granular glands secrete toxins that contain peptides, amines, and alkaloids, which provide protection against microorganisms present on their skin and are lethal to potential predators (Zainudin et al., 2018). According to Rasit et al. (2018), this gland also holds potential for medical applications due to its ability to produce secretions that contain peptides. These studies revealed that the distribution of granular glands is greater on the dorsal side compared to the ventral side in *R. pardalis*, *P. macrotis*, and *P. leucomystax*, except for *P.*

colletti and *P. otilophus*. The granular glands are most abundant in the dorsal regions of the skin, as these areas are usually exposed to the environment while both species are foraging, resting, or mating (Rasit et al., 2018). During activities like foraging, resting, and mating, the dorsal surface may be more active in terms of interactions with the environment. As such, an increased number of granular glands could provide additional biochemical defences to mitigate risks associated with these behaviours. The exceptions of *P. colletti* and *P. otilophus* may suggest unique adaptations or ecological niches that necessitate different distributions of glands. For example, these species might live in environments where the ventral side is more exposed to threats or where there is a greater need for moisture retention, resulting in distinct evolutionary strategies related to glandular distribution. Furthermore, it suggests that *P. otilophus* has larger granular glands than those found in other species, which may be responsible for emitting unpleasant odours. The production of these odours serves as a chemical defence mechanism, deterring potential predators and signalling danger (Razali et al., 2017). Larger granular glands could enhance the capacity to produce not only toxins but also potent volatile compounds, making *P. otilophus* less appealing to predators. This adaptation is particularly advantageous in their natural habitats, where olfactory cues play a significant role in predator-prey interactions (Eklöv, 2000).

The concentration of granular glands influences the rate of epithelialization in wounds of anuran skin (Rasit et al., 2018; Rasit et al., 2023). Further studies could explore how varying densities of these glands impact the healing process, potentially leading to insights into the mechanisms by which these secretions facilitate wound healing. Understanding the specific components and functions of the secretions from granular glands could also inform medical practices and the development of therapeutic agents for skin injuries in other species.

5.2 Comparison of Four Skin Regions of Selected Tree frog Species

Different regions of the skin can vary significantly in the thickness of their layers, including the epidermis and dermis. These areas also differ in the number and types of glands present, as well as the size of these glands. Accordingly, the study further investigated skin characteristics across four specific regions: the dorsal and ventral sections. These regions included the dorsal head (DH), dorsal centre (DC), ventral head (VH), and ventral centre (VC). The Kruskal-Wallis test was applied to determine if there were significant differences in skin characteristics across various skin regions within each tree frog species studied. The Kruskal-Wallis test was selected because it evaluates differences between multiple groups without requiring the data to follow a normal distribution. This makes it appropriate for comparing skin characteristics across various regions, particularly when the data may not meet the assumptions of normality or equal variances. The results demonstrated that *P. leucomystax* exhibited the most significant regional skin differences, with seven out of nine skin characteristics varying significantly ($p < 0.05$). This suggests that *P. leucomystax* may have more specialized adaptations across its skin regions than other species studied, possibly reflecting a broader range of ecological or behavioral adaptations (Iangrai & Hooroo, 2019). One possible reason for these significant variations could be that *P. leucomystax* occupies diverse habitats, requiring more adaptable skin features to handle changes in humidity, temperature, or other environmental factors (Razali et.al., 2017). For instance, differences in gland size or number might support more efficient moisture retention or thermoregulation, especially if the frog encounters both wet and dry conditions (Lim et al., 2024). *Rhacophorus pardalis* showed six significant differences, while *P. colletti* and *P. macrotis* each had five, and *P. otlophus* had four. The varying number of significant differences in skin characteristics among the tree frog species suggests that each species may have adapted differently to their respective environments or have distinct physiological needs. Overall, these findings highlight the idea

that skin characteristics can reflect each species' ecological adaptations and physiological requirements. Future research could explore how these variations in skin traits correlate with habitat type, behaviour, and environmental challenges, providing deeper insights into the evolutionary dynamics at play within these tree frog species.

5.3 Environmental Parameters of *Polypedates leucomystax*

Polypedates leucomystax was selected as the primary species of interest because it was recorded in six out of eight study locations, demonstrating the widest distribution among the species surveyed (Table 3.2). It also exhibited significant variation in seven out of nine examined skin traits across different skin regions (Table 4.10). Moreover, this species is capable of thriving in both natural and disturbed environments, indicating a high degree of ecological adaptability. The widespread distribution of *P. leucomystax* compared to other species studied suggests it may possess unique adaptive mechanisms, potentially linked to glandular functions that influence its survival across diverse habitats. These glands may play a critical role in maintaining physiological homeostasis and coping with environmental variations. Correlating the glandular activity or secretions in *P. leucomystax* with key environmental parameters such as pH, dissolved oxygen, turbidity, and temperature could provide valuable insights into its adaptability. Understanding how these factors influence gland function can help assess the species' ability to withstand environmental stressors and adapt to varying water conditions. Additionally, this relationship could be utilized as a bioindicator to monitor the health of aquatic ecosystems.

The pH levels measured across the six localities range from acidic (Bau, 5.62) to neutral or slightly alkaline (Mulu, 8.60). This variation reflects differences in environmental quality, likely influenced by local environmental factors such as pollution, surface runoff, or geological conditions (Müller et. Al., 2020). Significant variations in pH were observed across all localities, largely driven by intensive anthropogenic activities. Human actions such as

agricultural practices, deforestation, industrial discharges, and urban development are likely major contributors to these differences (Sandip et al., 2024). *Polypedates leucomystax*, a medium-sized rhacophorid in which females (SVL = 57–75 mm) are larger than males (37–50 mm), thrives across diverse environmental conditions and is frequently found in disturbed habitats such as agricultural areas, human habitations, gardens, roadside ditches, and forest edges (Berry, 1975; Ibrahim et al., 2008; Shahriza, 2016a). Its wide distribution, spanning from northeastern India through Indochina, the Malay Peninsula, and into Borneo, Java, and Sulawesi, underscores its exceptional ecological adaptability, making it a suitable species for assessing the influence of environmental stressors on amphibian skin traits (Shahrudin, 2019).

Turbidity is a key water quality indicator that plays a crucial role in the sustainable development of aquatic ecosystems and the preservation of biodiversity (Lin et al., 2023). It also reflects the presence of suspended particles, which can indicate pollution levels or habitat degradation. In unprotected areas, elevated turbidity levels, such as those observed at Bau (70.37 NTU), suggest poor water quality, likely due to sedimentation, runoff, or human-induced disturbances (Camara et al., 2019). Lower turbidity levels, like at Ranchan (1.86 NTU), may indicate comparatively better water quality. For *P. leucomystax*, glands play a crucial role in producing secretions that protect against desiccation, pathogens, and environmental toxins. The high turbidity at Bau is strongly associated with anthropogenic stressors, particularly legacy and ongoing small-scale gold mining, agricultural expansion (e.g., black pepper and oil palm cultivation), pesticide and fertilizer inputs, and deforestation in surrounding catchments. These activities increase sedimentation, introduce heavy metals, and accelerate surface runoff into rivers (Wan Hamid et al., 2019). Despite being an unprotected area, Ranchan Recreational Park exhibits better water quality compared to Maludam. This condition is supported by the presence of surrounding vegetation and comparatively lower

anthropogenic pressures, which help reduce erosion and surface runoff, resulting in the lower turbidity values observed (Nik Nurul Aizzah, 2014).

Water temperature determines the solubility of oxygen, with colder water holding more dissolved oxygen than warmer water. High temperatures can reduce oxygen availability, leading to stress or mortality for aquatic organisms, especially those sensitive to oxygen levels, such as amphibians and fish (Allan & Castillo, 2007). In unprotected areas, elevated water temperatures are often caused by human activities such as deforestation, removal of riparian vegetation (i.e. reduced canopy cover), and industrial runoff, which reduce shade and increase heat absorption by water bodies. For example, in tropical Borneo streams, the quality of riparian forest has been shown to significantly affect water temperature: streams with high riparian forest quality (and thus greater canopy cover) were nearly 4 °C cooler than ones in degraded or oil-palm dominated catchments (Luke et al., 2017). The unprotected sites exhibit water temperatures ranging from ~24.53 °C at Ranchan to ~36.03 °C at Maludam. Higher temperatures, such as those at Maludam, may indicate a decline in water quality due to reduced dissolved oxygen levels. Such elevated temperatures are often associated with human activities like deforestation and urbanization, which enhance heat absorption in water bodies. In contrast, lower temperatures, as observed at Ranchan, suggest better water quality with higher levels of dissolved oxygen, likely due to intact riparian vegetation and higher canopy cover (Luke et al., 2017).

Dissolved oxygen refers to the amount of oxygen present in the water, which fluctuates based on the rate of oxygen consumption (Vivian, 2020). Dissolved oxygen levels across unprotected sites ranged from 2.37 mg/L at East UNIMAS to 8.77 mg/L at Ranchan. The low DO concentration observed at East UNIMAS indicates poorer water quality, which may be attributed to factors like heightened organic pollution, nutrient loading, or water stagnation which conditions that increase the demand for oxygen. Conversely, the elevated DO levels

observed at Ranchan reflect a healthier aquatic ecosystem, signifying greater oxygen availability that enhances habitat suitability and supports the resilience of aquatic communities. These elevated oxygen levels are generally linked to cleaner water, enhanced water circulation, and effective aeration, all of which support the sustainability of aquatic life.

The glandular features of *P. leucomystax* highlight its exceptional ecological plasticity, enabling the species to persist under a wide spectrum of environmental conditions. This adaptability aligns with its broad distribution and frequent occurrence in both natural ecosystems and human-modified landscapes, such as forests, agricultural fields, and urban habitats (Berry, 1975; Ibrahim et al., 2008; Shahriza, 2016; Shahrudin, 2019). A key factor underpinning this resilience lies in the species' ability to withstand fluctuations in water quality parameters, including pH, temperature, and dissolved oxygen. *Polypedates* species have been shown to regulate metabolic processes and maintain homeostasis even under environmental stress, thereby ensuring survival without requiring significant morphological changes in glandular structures (Razali, 2017; Ingert et al., 2017). In *P. leucomystax*, the skin glands are particularly important, functioning efficiently across a range of conditions to maintain moisture balance and provide defense against pathogens. Their effectiveness across diverse environments suggests that gland numbers remain relatively stable even when external factors such as turbidity or oxygen concentration vary. Beyond physiological adaptations, the species also exhibits behavioral and reproductive strategies that further enhance its ecological success. For example, *P. leucomystax* deposits eggs in foam nests attached to vegetation overhanging water bodies where a strategy that shields embryos from aquatic predators and buffers them from fluctuating water quality, creating a stable microenvironment for development (Smith & Tan, 2022). Together, these morphological, physiological, and behavioral traits emphasize the remarkable adaptability of *P. leucomystax* and its capacity to thrive in diverse and dynamic habitats.

Additionally, *P. leucomystax* has developed defensive strategies to cope with environmental stressors. These strategies include behavioural adaptations such as seeking refuge in microhabitats that offer protection from extreme conditions, thereby reducing the need for significant morphological changes in glandular structures (Shahrudin, 2016).

For both Spearman and Kendall correlations show that only the seromucous glands show a significant positive correlation with pH $p=0.018$, Spearman correlation and $p=0.014$, Kendall correlation. This suggests that as pH increases, the area of seromucous glands tends to increase, while other glands do not show significant correlations with environmental parameters. The positive relationship between pH and seromucous glands indicates that these glands may adapt to environments with higher pH levels, which could be associated with less acidic water conditions. pH affects the solubility and biological availability of various substances in water, including nutrients and toxins (Scaramella et al., 2022). For amphibians like *Polypedates leucomystax*, higher pH levels may reduce acidity stress, allowing seromucous glands to expand and possibly enhance their protective function. These glands are known to secrete mucus with antimicrobial properties, which can protect the frog from pathogens (Sungif, 2017). In less acidic environments (higher pH), these glands might adapt to produce secretions optimized for such conditions. As the frogs are found in unprotected areas, they are likely exposed to various stressors like pollution, habitat disturbances, and changes in water quality. The significant relationship with pH suggests an adaptive mechanism where the seromucous glands enlarge or become more active to provide protection in variable conditions. Amphibians are sensitive to environmental changes due to their permeable skin (Rasit et al., 2023). The enlargement of seromucous glands in response to higher pH might reflect an evolutionary adaptation to maintain skin health and hydration, reduce pathogen susceptibility, and ensure survival in disturbed habitats.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The histological analysis of selected Bornean tree frog species (Family: Rhacophoridae) revealed distinct skin structures, reflecting specific adaptations to their ecological niches, environmental conditions, and survival strategies. Significant differences in the histology of various skin regions among the species were observed, indicating specialized functions such as water absorption, gas exchange, antimicrobial defense, and protection against environmental stressors. These variations highlight the structural diversity and functional specialization of tree frog skin as a critical evolutionary trait.

The study demonstrated the structural diversity of skin glands across species, offering new perspectives on how these adaptations facilitate the frogs' survival in distinct environments. Furthermore, the research effectively connected the frog's skin gland morphology environmental parameters, underscoring the importance of these glands in maintaining physiological homeostasis and providing protection from environmental stressors.

The skin glands correlate with environmental parameters focused on *P. leucomystax* due to its widespread distribution across various habitats in Sarawak, making it an ideal representative species for assessing environmental influences on amphibian skin structure. This species is known for its high ecological adaptability, which allows it to thrive in diverse environments, including areas with varying environmental quality conditions. In *P. leucomystax*, the investigation demonstrated a clear correlation between skin gland structure and environmental parameters across different locations in Sarawak, with seromucous glands showing a significant positive relationship with pH levels. Variations in environmental parameters including pH, turbidity, and dissolved oxygen, were associated with morphological

changes in gland size, suggesting an adaptive response to local environmental stressors. One key observation was the clear, positive correlation between the size of seromucous glands and pH levels, an aspect not previously documented in this species. This finding suggests that *P. leucomystax* has evolved specialized skin glands that adapt to fluctuating environmental conditions, which is crucial for its survival in areas with varying environmental conditions.

These findings underscore the sensitivity of *P. leucomystax* to environmental quality changes, reflecting its reliance on skin glands for maintaining physiological homeostasis and protection. This study also contributes to a better understanding of how amphibians, particularly tree frogs, are affected by environmental changes, which could inform conservation efforts in habitats subject to environmental quality degradation.

6.2 Recommendation

Future studies could expand on this research by investigating the molecular composition and biochemical properties of the secretions produced by different skin glands in tree frog species. This could provide deeper insights into their antimicrobial, hydrating, and defensive functions. Additionally, a comparative analysis of glandular structures across different amphibian species from varied ecological regions could help determine how environmental pressures influence gland development and function. Long-term studies monitoring changes in gland distribution and function in response to habitat degradation, pollution, and climate change would also be valuable for conservation efforts. Finally, investigating the role of tree frog skin as a bioindicator for assessing water quality and environmental health could offer practical applications in ecological monitoring and habitat conservation.

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APPENDICES

Appendix A: Datasheet of tree frog species captured.

ID	Species	Sex	Body size SVL (mm)	Vegetation type	Horizontal position	Vertical position	Substrate
Ranchan 25	<i>Polypedates leucomystax</i>	F	63	VA	HPE (Intermittent stream, on rock)	VPH (On rock)	SJ (Bank Rock)
Maludam 002	<i>Polypedates leucomystax</i>	M	49	VAgr (Agriculture)	HPL (Temporary Pond, on vegetation)	VPK (On seedling)	SA (Leaf)
Maludam 003	<i>Polypedates leucomystax</i>	M	40	VAgr (Agriculture)	HPL (Temporary Pond, on vegetation)	VPK (On seedling)	SA (Leaf)
Maludam 004	<i>Polypedates leucomystax</i>	M	31	VAgr (Agriculture)	HPL (Temporary Pond, on vegetation)	VPK (On seedling)	SA (Leaf)
UW55(West Unimas)	<i>Polypedates leucomystax</i>	F	54	VS (Marsh)	HPG (Distance from water body)	VPK (On seedling)	SA (Leaf)

UW53(West Unimas)	<i>Polypedates leucomystax</i>	M	46	VS (Marsh)	HPG (Distance from water body)	VPK seedling) (On	SA (Leaf)
PPT044 (East Unimas)	<i>Polypedates leucomystax</i>	M	44	VW (Peat Swamp)	HPL (Temporary Pond, on vegetation)	VPK seedling) (On	SB (Stem)
Mulu 087	<i>Polypedates leucomystax</i>	M	49	VAgr (Agriculture)	HPG (Distance from water body)	VPK seedling) (On	SA (Leaf)
Mulu 093	<i>Polypedates leucomystax</i>	M	48	VAgr (Agriculture)	HPG (Distance from water body)	VPK seedling) (On	SA (Leaf)
Bau 09	<i>Polypedates leucomystax</i>	M	39	VAgr (Agriculture)	HPL (Temporary Pond, on vegetation)	VPK seedling) (On	SA (Leaf)
Bau 12	<i>Polypedates leucomystax</i>	M	46	VAgr (Agriculture)	HPL (Temporary Pond, on vegetation)	VPK seedling) (On	SA (Leaf)
KNP1829	<i>Rhacophorus pardalis</i>	M	45	VE (Edge MDF)	HPR (Permanent Pond, distance from water)	VPK seedling) (On	SA (Leaf)
KNP1830	<i>Rhacophorus pardalis</i>	F	53	VE (Edge MDF)	HPR (Permanent	VPK seedling) (On	SA (Leaf)

					Pond, distance from water)		
KNP1831	<i>Rhacophorus pardalis</i>	M	47	VE (Edge MDF)	HPR (Permanent Pond, distance from water)	VPK (On seedling)	SA (Leaf)
MWC1569	<i>Rhacophorus pardalis</i>	F	55	VG (Secondary growth forest)	HPG (Distance from water body)	VPK (On seedling)	SA (Leaf)
MWC1617	<i>Rhacophorus pardalis</i>	F	58	VG (Secondary growth forest)	HPG (Distance from water body)	VPM (On tree)	SA (Leaf)
PPT035	<i>Rhacophorus pardalis</i>	F	49	VW (Peat Swamp)	HPL (Temporary Pond, on vegetation)	VPK (On seedling)	SB (Stem)
PPT043	<i>Rhacophorus pardalis</i>	F	49	VW (Peat Swamp)	HPL (Temporary Pond, on vegetation)	VPK (On seedling)	SA (Leaf)
KNP1838	<i>Polypedates macrotis</i>	M	32	VE (Edge MDF)	HPU (Permanent drainage, in plantation)	VPM (On tree)	SC (Twig)
MWC1561	<i>Polypedates macrotis</i>	F	58	VG (Secondary growth forest)	HPG (Distance from water body)	VPK (On seedling)	SB (Stem)

MWC1562	<i>Polypedates macrotis</i>	F	60	VG (Secondary growth forest)	HPG (Distance from water body)	VPK (On seedling)	SB (Stem)
MWC1570	<i>Polypedates macrotis</i>	F	57	VG (Secondary growth forest)	HPG (Distance from water body)	VPG (On surface of leaf litter)	SC (Twig)
KNP1832	<i>Polypedates otitophus</i>	F	58	VE (Edge MDF)	HPR (Permanent Pond, distance from water)	VPK (On seedling)	SA (Leaf)
MWC1612	<i>Polypedates colletti</i>	F	80	VG (Secondary growth forest)	HPG (Distance from water body)	VPM (On tree)	SD (Trunk)

Appendix B: Water quality data sheet.

Locality	pH	Turbidity	Temperature	Dissolved Oxygen (DO)
Ranchan	6.65	3.23	25.2	8.73
Ranchan	6.64	1.28	25.2	8.76
Ranchan	6.64	1.08	23.2	8.81
Maludam	7.52	19.65	35.68	7.25
Maludam	6.55	19.25	36.15	7.3
Maludam	8.25	19.68	36.25	7.1
West UNIMAS	6.1	6.65	29.8	6.42
West UNIMAS	5.8	6.67	30.45	6.68
West UNIMAS	6.5	6.8	30.05	7.1
East UNIMAS	7.4	2.5	27.5	2.4
East UNIMAS	7.25	2.86	26.78	2.6
East UNIMAS	7.1	2.65	27.75	2.1
Mulu	8.5	15.55	27.75	6.68
Mulu	8.7	16.67	26.65	7.45
Mulu	8.6	15.68	27.25	7.6
Bau	5.62	85.6	26.9	7.77
Bau	5.62	49.5	27.1	8.3
Bau	5.62	76	26.5	7.76

Appendix C: Histology measurement of tree frog skin.

ID No.	Skin Part	Character	1	2	3	4	5	6	7	8	9	10	Total	Mean
		Thickness of epidermis (μm)	46.128	56.94	67.751	56.219	60.543	67.444	65.589	62.706	64.147	55.498	602.965	60.2965
		Thickness of stratum spongiosum (μm)	153.521	149.196	112.438	110.275	88.653	104.139	113.158	124.691	125.411	85.77	1167.252	116.7252
		Thickness of stratum compactum (μm)	86.587	102.594	111.326	100.675	127.333	122.624	147.561	111.326	98.956	118.602	1127.584	112.7584
		Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
RC25	DH	Number of seromucous glands	3	3	3	1	4	2	3	2	2	3	26	2.6
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0

		Average size of seromucous glands present (μm^2)	10587.499	12079.582	6404.067	8588.685	5498.629	9073.713	5228.179	8646.867	11197.521	7624.112	84928.854	8492.8854
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	31.139	35.484	58.658	86.176	72.417	71.693	61.555	65.175	38.381	42.726	563.404	56.3404
		Thickness of stratum spongiosum (μm)	138.317	94.866	131.799	109.35	121.661	135.42	152.992	126.006	104.281	90.521	1205.213	120.5213
		Thickness of stratum compactum (μm)	125.878	127.333	105.505	107.688	116.419	78.583	117.874	123.695	98.229	115.421	1116.625	111.6625
RC25	DC	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromuc	1	1	4	3	3	3	3	2	2	4	26	2.6

		ous glands												
		Number of mucous glands	0	1	0	0	0	0	0	0	0	0	1	0.1
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands present (μm^2)	13752.82	5279.192	6811.018	6957.754	6612.218	7791.14	7837.231	5982.502	7540.33	5936.469	74500.674	7450.0674
		Average size of mucous glands present (μm^2)	0	2338.752	0	0	0	0	0	0	0	0	2338.752	2338.752
		Thickness of epidermis (μm)	82.555	78.21	78.21	89.797	91.97	84.728	85.452	78.21	85.452	66.624	821.208	82.1208
		Thickness of stratum spongiosum (μm)	112.246	115.143	116.591	117.316	217.256	245.494	152.076	147.007	133.247	128.902	1485.278	148.5278

		Thickness of stratum compactum (μm)	149.89	134.659	147.707	141.158	88.77	73.49	127.333	146.251	130.971	141.158	1281.387	128.1387
RC25	VH	Number of granular glands	0	0	0	0	1	1	0	0	0	0	2	0.2
		Number of seromucous glands	2	2	2	3	1	3	4	3	4	2	26	2.6
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	0	0	0	0	36698.428	65065.871	0	0	0	0	101764.299	50882.1495
		Average size of seromucous glands present (μm^2)	9417.765	12290.35	11748.242	9960.698	8236.939	7253.237	13968.635	14463.103	10029.781	15079.145	112447.895	11244.7895
		Average size of mucous	0	0	0	0	0	0	0	0	0	0	0	0

		glands present (µm ²)												
		Thickness of epidermis (µm)	77.486	89.073	100.66	89.073	97.039	96.315	83.28	78.935	83.28	81.107	876.248	87.6248
		Thickness of stratum spongiosum (µm)	141.213	123.109	155.697	272.288	186.836	138.317	211.289	222.32	114.419	163.663	1729.151	172.9151
		Thickness of stratum compactum (µm)	132.427	137.52	108.415	101.867	115.691	133.9	110.598	110.598	106.232	104.05	1161.298	116.1298
RC25	VC	Number of granular glands	0	0	0	1	0	0	1	1	0	0	3	0.3
		Number of seromucous glands	4	5	3	3	3	4	2	2	4	4	34	3.4
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands	0	0	0	80214.531	0	0	44485.062	56482.056	0	0	181181.649	60393.883

		present (μm^2)												
		Average size of seromuc ous glands present (μm^2)	10998. 405	9448.985	14432.12 3	10066. 282	14950.89 4	10897.90 5	4859.15 9	797 6.53 3	102 70.8 41	13191.003	107092.13	1070 9.21 3
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thicknes s of epidermi s (μm)	41.916	54.589	53.614	59.463	43.866	41.916	38.755	38.0 17	55.5 64	41.673	469.373	46.9 373
		Thicknes s of stratum spongios um (μm)	98.455	85.782	95.53	76.034	112.102	104.304	104.547	89.1 94	115. 514	101.623	983.085	98.3 085
		Thicknes s of stratum compact um (μm)	93.135	100.411	112.781	110.59 8	112.053	89.509	82.949	73.4 9	95.3 18	88.77	959.014	95.9 014
		Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0

Malu dam 002	DH	Number of seromuc ous glands	2	1	2	2	2	1	3	4	3	1	21	2.1
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromuc ous glands present (μm^2)	8761.4 75	12397.13 9	6998.431	4729.9 2	7895.098	10278.70 6	6674.71 7	543 0.89 7	711 8.71 5	8097.26	78382.358	7838 .235 8
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thicknes s of epidermi s (μm)	43.135	45.328	44.597	42.404	48.253	53.37	51.908	53.3 7	43.8 66	49.006	475.237	47.5 237
		Thicknes s of	136.71 6	97.236	149.144	92.119	103.816	97.967	127.943	118. 438	94.3 12	97.967	1115.658	111. 5658

		stratum spongios um (µm)												
		Thicknes s of stratum compact um (µm)	57.482	75.672	69.851	65.486	87.314	60.392	70.579	67.6 69	93.1 35	69.851	717.431	71.7 431
Malu dam 002	DC	Number of granular glands	1	0	0	0	0	0	0	0	0	0	1	0.1
		Number of seromuc ous glands	2	1	3	2	3	3	2	2	4	2	24	2.4
		Number of mucous glands	0	0	0	1	0	0	0	0	1	0	2	0.2
		Average size of granular glands present (µm ²)	19191. 803	0	0	0	0	0	0	0	0	0	19191.803	1919 1.80 3
		Average size of seromuc ous glands present (µm ²)	6304.0 75	8392.131	8661.958	4655.8 91	5397.758	5581.51	10202.5 09	866 2.77	600 8.35 9	7239.197	71106.158	7110 .615 8

		Average size of mucous glands present (μm^2)	0	0	0	3330.519	0	0	0	0	2029.586	0	5360.105	2680.0525
		Thickness of epidermis (μm)	51.881	56.145	86.705	93.101	66.095	88.042	85.131	84.404	96.773	80.038	788.315	78.8315
		Thickness of stratum spongiosum (μm)	211.077	145.693	142.14	114.422	126.504	148.434	152.8	153.528	168.08	189.194	1551.872	155.1872
		Thickness of stratum compactum (μm)	130.971	93.135	88.042	93.135	89.497	103.322	102.594	75.672	88.77	106.96	972.098	97.2098
Maludam 002	VH	Number of granular glands	1	0	0	0	0	0	0	0	0	0	1	0.1
		Number of seromucous glands	4	4	4	4	5	4	4	2	2	3	36	3.6
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0

		Average size of granular glands present (μm^2)	16292.39	0	0	0	0	0	0	0	0	0	16292.39	16292.39
		Average size of seromucous glands present (μm^2)	6764.49	10805.332	10036.667	8014.282	7566.446	14342.598	13255.091	15496.313	22447.575	9900.233	118629.027	118629.027
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	86.608	107.688	86.587	114.964	89.497	107.688	89.497	116.419	88.77	87.314	975.032	975.032
		Thickness of stratum spongiosum (μm)	138.248	144.069	138.975	137.52	131.699	142.613	102.594	147.707	110.598	110.598	1304.621	1304.621
		Thickness of stratum compactum (μm)	96.898	79.31	67.669	73.49	93.135	87.314	83.676	106.96	86.587	101.872	876.911	876.911

		Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromucous glands	3	3	3	2	3	3	3	4	2	2	28	2.8
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
Malu dam 002	VC	Average size of seromucous glands present (μm^2)	12647.541	11978.283	11919.222	12377.532	10984.368	13813.168	12324.589	12222.115	10228.666	8324.249	116819.733	11681.9733
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of	48.75	50.933	70.579	70.579	58.937	62.575	66.941	45.84	58.937	54.571	588.642	58.8642

		epidermis (μm)												
		Thickness of stratum spongiosum (μm)	146.979	106.96	125.15	98.956	96.773	113.509	160.076	113.509	104.777	116.419	11183.108	118.3108
		Thickness of stratum compactum (μm)	60.392	89.497	79.31	110.598	94.59	91.68	90.228	105.505	82.221	136.792	940.813	94.0813
		Number of granular glands	1	0	0	0	0	0	0	0	0	0	1	0.1
Maludam 003	DH	Number of seromucous glands	3	3	2	3	2	3	2	3	1	2	24	2.4
		Number of mucous glands	0	0	1	1	0	0	0	0	0	0	2	0.2
		Average size of granular glands present (μm ²)	15346.396	0	0	0	0	0	0	0	0	0	15346.396	15346.396

		Average size of seromucous glands present (μm^2)	6558.161	7540.076	10352.817	7499.369	10136.369	6226.032	13375.33	7123.944	16057.949	7958.119	92828.166	9282.8166
		Average size of mucous glands present (μm^2)	0	0	2659.677	1326.044	0	0	0	0	0	0	3985.721	1992.8605
		Thickness of epidermis (μm)	77.245	67.348	91.97	78.935	65.175	78.21	61.555	59.382	89.797	75.314	744.931	74.4931
		Thickness of stratum spongiosum (μm)	210.495	84.728	94.866	81.107	94.869	112.971	139.765	98.487	85.452	91.245	1093.985	109.3985
		Thickness of stratum compactum (μm)	50.209	55.037	63.003	78.935	55.761	81.107	57.934	83.28	65.175	76.038	666.479	66.6479
Maludam 003	DC	Number of granular glands	1	0	0	0	0	0	1	0	0	0	2	0.2
		Number of seromuc	0	0	1	2	2	2	0	1	2	3	13	1.3

		ous glands												
		Number of mucous glands	1	2	1	1	1	0	0	0	2	1	9	0.9
		Average size of granular glands present (μm^2)	40352.958	0	0	0	0	0	17874.087	0	0	0	58227.045	29113.5225
		Average size of seromucous glands present (μm^2)	0	0	2643.267	7059.259	8656.477	8125.673	0	11231.919	5574.88	5120.933	48412.408	6916.05829
		Average size of mucous glands present (μm^2)	8008.989	6200.341	6251.822	2178.279	909	0	0	0	763.91	2043.327	26355.668	3765.09543
		Thickness of epidermis (μm)	94.142	107.901	102.832	102.832	91.248	123.109	96.315	84.728	105.005	97.039	1005.151	100.5151
		Thickness of stratum spongiosum (μm)	152.076	173.077	147.731	157.145	126.006	212.906	145.558	136.876	158.593	165.111	1575.079	157.5079

		Thickness of stratum compactum (μm)	115.867	70.969	89.085	70.245	96.315	57.209	93.418	78.21	73.141	80.383	824.842	82.4842
Maludam 003	VH	Number of granular glands	0	1	0	0	0	1	0	0	0	0	2	0.2
		Number of seromucous glands	4	4	4	2	3	2	4	4	3	3	33	3.3
		Number of mucous glands	0	0	0	1	0	0	0	0	0	0	1	0.1
		Average size of granular glands present (μm^2)	0	21669.686	0	0	0	41195.356	0	0	0	0	62865.042	31432.521
		Average size of seromucous glands present (μm^2)	11960.24	12224.885	14396.856	15807.51	12357.331	13812.692	14171.485	14144.958	15434.335	19745.869	144056.161	14405.6161
		Average size of mucous	0	0	0	2884.502	0	0	0	0	0	0	2884.502	2884.502

		glands present (µm ²)												
		Thickness of epidermis (µm)	94.59	90.952	87.314	119.33	127.333	118.602	152.323	129.516	106.96	96.046	1122.966	112.2966
		Thickness of stratum spongiosum (µm)	122.968	227.017	162.987	157.166	139.703	173.173	110.598	255.394	170.263	165.897	1685.166	168.5166
		Thickness of stratum compactum (µm)	101.867	117.874	134.61	91.68	120.057	125.878	54.571	76.4	58.21	85.131	966.278	96.6278
Maludam 003	VC	Number of granular glands	0	1	0	0	0	0	0	1	0	0	2	0.2
		Number of seromucous glands	3	2	6	4	3	3	3	4	3	2	33	3.3
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands	0	52553.464	0	0	0	0	0	35540.424	0	0	88093.888	44046.944

		present (μm^2)												
		Average size of seromucous glands present (μm^2)	12970. 434	16736.58 9	9991.374	13035. 804	15360.84 7	15161.40 9	13156.8 52	102 04.7 97	139 25.2 89	15887.12	136430.51 5	1364 3.05 15
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thicknes s of epidermi s (μm)	48.25 3	72.37 5	63.606	58.488	62.144	65.068	60.681	57.757	54.101	61.412	603. 885	60.3 885
		Thicknes s of stratum spongios um (μm)	136.7 16	106.0 1	117.70 7	116.245	192.27 9	180.208	103.816	123.528	107.472	126.48	131 0.46 1	131. 0461
		Thicknes s of stratum compact um (μm)	144.7 51	169.6 15	151.33 8	160.842	85.539	99.257	120.632	115.514	138.909	144.027	133 0.42 4	133. 0424
		Number of granular glands	0	0	0	0	1	1	0	0	0	0	2	0.2

Malu dam 004	DH	Number of seromuc ous glands	1	2	3	2	2	1	2	2	2	3	20	2
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	0	0	0	0	33858. 047	23580.707	0	0	0	0	574 38.7 54	2871 9.37 7
		Average size of seromuc ous glands present (μm^2)	17111 .736	10905 .119	7745.3 56	10471.36 6	6091.3 41	8589.364	9409.12 1	11045.42 8	10537.64 5	12502.932	104 409. 408	1044 0.94 08
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thicknes s of epidermi s (μm)	48.76 7	51.69 7	54.969	66.707	93.812	79.297	79.69	83.345	74.134	56.295	688. 713	68.8 713

		Thickness of stratum spongiosum (μm)	175.941	140.106	147.276	198.516	116.401	132.117	103.458	129.809	101.752	148.556	1393.932	1393932
		Thickness of stratum compactum (μm)	93.226	80.73	123.27	42.259	124.246	115.69	48.081	69.281	52.237	133.791	882.811	88.2811
Maludam 004	DC	Number of granular glands	1	0	0	1	0	0	0	0	0	0	2	0.2
		Number of seromucous glands	2	4	3	2	3	2	3	3	4	4	30	3
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	23620.083	0	0	26979.346	0	0	0	0	0	0	50599.429	25299.7145
		Average size of seromucous glands	6337.304	7637.049	9570.899	6595.382	7680.522	12403.909	7953.201	9676.315	8188.305	8998.812	85041.698	8504.1698

		present (μm^2)												
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thicknes s of epidermi s (μm)	86.96 4	84.10 5	68.185	81.155	86.27	89.925	73.11	75.303	65.068	60.699	770. 784	77.0 784
		Thicknes s of stratum spongios um (μm)	137.7 91	97.23 9	116.46 3	129.817	144.47 1	140.898	133.791	132.329	148.413	161.654	134 2.86 6	134. 2866
		Thicknes s of stratum compact um (μm)	94.90 2	103.0 85	98.049	109.777	118.84 8	116.302	160.438	130.867	106.02	125.018	116 3.30 6	116. 3306
Malu dam 004	VH	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromuc ous glands	1	2	3	4	3	3	4	2	2	3	27	2.7
		Number of	0	0	0	0	0	0	0	0	0	0	0	0

		mucous glands												
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands present (μm^2)	15143.322	8662.74	12627.69	10325.06	10887.233	12638.281	11558.065	13198.782	16087.797	13019.801	124148.771	12414.8771
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	100.203	93.652	114.389	79.256	91.39	62.807	62.251	81.896	55.607	61.941	803.392	80.3392
		Thickness of stratum spongiosum (μm)	143.303	169.076	122.113	137.511	117.762	144.373	98.33	168.891	133.062	87.268	1321.689	132.1689
		Thickness of stratum	158.026	148.529	154.278	129.39	139.211	139.642	151.463	121.418	145.489	84.394	1371.84	137.184

		compact um (μm)												
Malu dam 004	VC	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromuc ous glands	2	1	2	3	2	4	4	2	2	1	23	2.3
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm ²)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromuc ous glands present (μm ²)	10189 .384	15083 .516	12179. 06	8443.74	10019. 885	6768.622	6739.55 1	13776.67 4	7649.432	9398.609	100 248. 473	1002 4.84 73
		Average size of mucous glands present (μm ²)	0	0	0	0	0	0	0	0	0	0	0	0

		Thickness of epidermis (μm)	97.5	141.886	84.08	121.365	92.919	131.016	116.309	99.473	130.671	88.708	1103.927	110.3927
		Thickness of stratum spongiosum (μm)	326.803	189.212	298.075	226.642	336.891	249.562	239.182	306.335	420.085	246.488	2839.275	283.9275
		Thickness of stratum compactum (μm)	65.815	162.327	84.077	67.261	74.332	83.374	140.402	68.727	165.696	164.32	1076.331	107.6331
		Number of granular glands	1	0	1	0	1	0	1	1	1	1	7	0.7
UW55	DH	Number of seromucous glands	2	3	1	3	3	4	3	2	3	2	26	2.6
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm ²)	48849.925	0	48215.464	0	48275.329	0	39284.075	46103.267	44810.827	53927.75	329466.637	47066.6624

		Average size of seromucous glands present (μm^2)	15325.945	12171.339	13574.363	13070.084	16110.029	13095.266	14890.598	7080.626	17567.117	11444.438	134329.805	13432.9805
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	96.466	85.542	95.517	117.728	83.079	84.836	98.81	101.222	105.676	122.881	991.757	99.1757
		Thickness of stratum spongiosum (μm)	290.763	360.993	294.656	151.847	330.425	216.722	182.519	190.817	361.555	154.703	253.5	253.5
		Thickness of stratum compactum (μm)	60.857	83.518	91.461	134.763	70.189	134.558	177.37	167.436	55.564	103.005	1078.721	107.8721
UW55	DC	Number of granular glands	1	1	1	0	1	0	0	0	1	0	5	0.5
		Number of seromuc	1	3	1	3	3	2	2	3	2	2	22	2.2

		ous glands												
		Number of mucous glands	0	1	0	0	0	0	0	0	1	0	2	0.2
		Average size of granular glands present (μm^2)	45682.253	30888.737	49510.517	0	63521.989	0	0	0	58986.333	0	248589.829	49717.9658
		Average size of seromucous glands present (μm^2)	14931.182	14371.255	10042.335	14491.163	10799.851	17924.634	14394.387	13555.061	14739.323	11274.464	136523.655	13652.3655
		Average size of mucous glands present (μm^2)	0	8268.837	0	0	0	0	0	0	4600.688	0	12869.525	6434.7625
		Thickness of epidermis (μm)	115.867	94.935	110.112	129.839	100.67	104.321	126.678	118.757	82.555	80.604	1064.338	106.4338
		Thickness of stratum spongiosum (μm)	228.114	184.344	317.079	229.892	126.413	301.269	278.227	215.259	153.961	253.935	2288.493	228.8493

		Thickness of stratum compactum (μm)	86.903	134.791	77.517	119.031	124.566	117.325	87.508	108.601	86.949	63.007	1006.198	100.6198
UW55	VH	Number of granular glands	1	0	1	0	0	1	1	1	0	1	6	0.6
		Number of seromucous glands	2	4	2	5	5	2	3	2	4	4	33	3.3
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	54441.584	0	50051.813	0	0	60659.668	34627.132	49042.823	0	63249.269	312072.289	52012.0482
		Average size of seromucous glands present (μm^2)	12166.79	13743.76	20040.566	16002.817	9760.364	13650.121	14725.797	10300.543	16337.396	12104.063	138832.217	13883.2217
		Average size of mucous	0	0	0	0	0	0	0	0	0	0	0	0

		glands present (µm ²)												
		Thickness of epidermis (µm)	106.463	106.923	107.553	101.469	91.79	117.886	93.743	95.043	86.613	80.046	987.529	98.7529
		Thickness of stratum spongiosum (µm)	162.274	133.35	211.012	147.221	183.307	166.806	143.146	160.007	171.676	186.858	1665.657	166.5657
		Thickness of stratum compactum (µm)	97.935	115.625	135.82	125.457	108.664	119.578	117.882	124.768	118.751	138.742	1203.222	120.3222
UW55	VC	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromucous glands	3	2	4	3	2	4	5	3	4	3	33	3.3
		Number of mucous glands	0	0	0	1	0	0	0	0	0	0	1	0.1
		Average size of granular glands	0	0	0	0	0	0	0	0	0	0	0	0

		present (μm^2)												
		Average size of seromuc ous glands present (μm^2)	17279 .915	12371 .403	13996. 459	13915.91 6	19942. 586	19321.698	14579.1 95	12683.13 3	14257.40 3	20490.2	158 837. 908	1583 .790 8
		Average size of mucous glands present (μm^2)	0	0	0	4361.976	0	0	0	0	0	0	436 1.97 6	4361 .976
		Thickne ss of epidermi s (μm)	70.22 9	77.296	65.295	63. 575	58.141	63.3 2	60.829	69.551	62.418	66.63	657.284	65.7284
		Thickne ss of stratum spongios um (μm)	80.12 1	114.218	109.95	96. 995	92.828	88.0 42	125.735	102.729	131.338	89.759	1031.715	103.175
		Thickne ss of stratum compact um (μm)	38.29 5	66.273	69.886	45. 118	65.506	75.1 18	58.246	79.367	68.036	62.195	625.04	62.504
		Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0

UW53	D H	Number of seromuc ous glands	1	3	1	2	2	2	2	4	2	3	22	2.2
		Number of mucous glands	1	1	1	1	0	0	0	0	0	0	4	0.4
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromuc ous glands present (μm^2)	9986. 981	8603.347	9480.847	575 8.4 28	13418.831	859 8.99 4	9624.61 7	8498.156	10531.677	6840.7 51	91342.62 9	9134.26 29
		Average size of mucous glands present (μm^2)	799.9 68	959.503	1515.58	206 7.7 75	0	0	0	0	0	0	5342.826	1335.70 65
		Thickne ss of epidermi s (μm)	57.57 2	65.068	68.727	57. 404	70.189	62.1 44	69.516	72.596	58.966	66.534	648.716	64.8716

		Thickness of stratum spongiosum (μm)	79.297	108.944	92.122	103.909	173.665	182.952	91.461	86.486	91.484	140.379	1150.699	115.0699
		Thickness of stratum compactum (μm)	52.415	56.314	54.106	72.408	41.775	27.935	48.984	44.471	55.487	42.911	496.806	49.6806
UW53	D C	Number of granular glands	0	0	0	0	1	1	0	0	0	0	2	0.2
		Number of seromucous glands	3	1	4	3	2	1	2	1	2	2	21	2.1
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	0	0	0	0	21937.274	21778.168	0	0	0	0	43715.442	21857.721
		Average size of seromucous glands	6718.111	9196.387	7969.35	7048.041	5902.185	9860.246	7925.802	6539.467	8691.722	10880.71	80732.021	8073.2021

		present (μm^2)												
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickne ss of epidermi s (μm)	73.24 2	111.418	100.895	82. 277	90.258	104. 945	93.581	79.488	92.249	87.711	916.064	91.6064
		Thickne ss of stratum spongios um (μm)	104.7 11	96.884	88.499	85. 823	71.964	76.0 34	85.987	110.648	97.349	126.89 4	944.793	94.4793
		Thickne ss of stratum compact um (μm)	32.24 3	55.337	48.606	39. 729	51.619	74.1 59	41.415	47.087	51.862	53.809	495.866	49.5866
UW53	V H	Number of granular glands	1	1	0	0	0	0	1	0	0	0	3	0.3
		Number of seromuc ous glands	1	1	1	2	2	2	4	2	3	2	20	2
		Number of	0	0	0	0	0	0	0	0	0	0	0	0

		mucous glands												
		Average size of granular glands present (μm^2)	9940.6	9539.897	0	0	0	0	8833.812	0	0	0	28314.309	9438.103
		Average size of seromucous glands present (μm^2)	4560.243	2892.757	12062.893	8206.894	8457.608	9810.299	4995.214	13161.277	10944.109	13891.682	88982.976	8898.2976
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	85.573	117.058	117.728	105.121	115.514	114.894	124.461	114.971	131.698	120.303	1147.321	114.7321
		Thickness of stratum spongiosum (μm)	91.247	97.97	97.978	110.452	99.607	102.471	119.172	88.003	87.927	91.32	986.147	98.6147
		Thickness of stratum	40.653	87.111	91.399	94.606	63.753	67.297	70.323	66.438	85.938	55.799	723.317	72.3317

		compact um (μm)												
UW53	V C	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromuc ous glands	2	3	2	4	2	2	1	3	2	3	24	2.4
		Number of mucous glands	0	0	0	1	0	0	1	0	0	0	2	0.2
		Average size of granular glands present (μm ²)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromuc ous glands present (μm ²)	8969. 934	7696.498	8752.181	643 1.0 95	7094.702	674 4.15 4	16202.1 82	7165.197	7526.228	6364.7 42	82946.91 3	8294.69 13
		Average size of mucous glands present (μm ²)	0	0	0	116 1.4 86	0	0	4221.72 2	0	0	0	5383.208	2691.60 4

		Thickness of epidermis (μm)	83.358	51.13	39.432	31.148	37.187	40.579	46.347	55.213	46.623	49.504	480.521	48.0521
		Thickness of stratum spongiosum (μm)	179.45	154.988	151.561	73.156	103.556	130.359	136.899	149.905	138.47	122.145	1340.489	134.0489
		Thickness of stratum compactum (μm)	73.37	57.938	56.504	62.384	47.121	41.278	48.541	49.244	48.476	49.504	534.36	53.436
		Number of granular glands	0	0	0	0	0	1	1	1	1	0	4	0.4
PPT044	DH	Number of seromucous glands	4	4	2	1	1	1	0	2	1	4	20	2
		Number of mucous glands	0	0	0	1	1	0	0	0	0	0	2	0.2
		Average size of granular glands present (μm^2)	0	0	0	0	0	15491.458	20971.679	21211.34	23018.327	0	80692.804	20173.201

		Average size of seromucous glands present (μm^2)	17351.412	11164.676	13551.092	6760.688	4908.25	3549.354	0	7910.806	7775.271	11903.18	84874.729	9430.52544
		Average size of mucous glands present (μm^2)	0	0	0	6728.406	9949.18	0	0	0	0	0	16677.586	8338.793
		Thickness of epidermis (μm)	50.015	49.249	49.973	55.042	46.488	49.249	42.946	50.391	52.186	36.904	482.443	48.2443
		Thickness of stratum spongiosum (μm)	174.668	149.091	147.065	96.326	90.289	98.511	99.235	84.79	80.131	98.009	1118.115	111.8115
		Thickness of stratum compactum (μm)	51.462	51.665	60.848	48.541	44.764	71.028	47.071	38.49	70.457	74.705	559.031	55.9031
PPT044	DC	Number of granular glands	1	1	0	0	0	0	1	1	0	0	4	0.4
		Number of seromuc	2	2	4	3	2	2	1	0	3	3	22	2.2

		ous glands												
		Number of mucous glands	0	0	0	0	0	0	1	2	0	0	3	0.3
		Average size of granular glands present (μm^2)	26086.902	15170.686	0	0	0	0	14524.771	8418.156	0	0	64200.515	16050.1288
		Average size of seromucous glands present (μm^2)	7661.035	5318.408	8754.238	8529.159	8004.881	9475.189	9911.247	0	5946.259	8932.44	72532.856	8059.20622
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	9386.183	3888.859	0	0	13275.042	6637.521
		Thickness of epidermis (μm)	70.245	81.159	80.464	78.294	65.212	76.776	69.233	77.5	59.019	60.124	718.026	71.8026
		Thickness of stratum spongiosum (μm)	140.556	158.62	139.071	120.956	136.152	139.043	108.927	129.699	87.637	120.991	1281.652	128.1652

		Thickness of stratum compactum (μm)	55.803	107.187	110.857	102.172	75.536	90.568	86.949	91.981	73.897	77.517	872.467	87.2467
PPT044	VH	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromucous glands	2	6	3	1	1	4	2	4	3	6	32	3.2
		Number of mucous glands	0	0	1	0	1	0	0	0	0	0	2	0.2
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands present (μm^2)	15362.362	8379.514	9467.41	13979.954	14135.941	10424.162	11139.271	10715.712	8618.602	8500.995	110723.923	11072.3923
		Average size of mucous	0	0	1652.632	0	3944.885	0	0	0	0	0	5597.517	2798.7585

		glands present (µm ²)												
		Thickness of epidermis (µm)	78.72 2	88.41 4	80.386	73.979	84.054	82.218	93.418	81.552	91.524	109.436	863.703	86.3703
		Thickness of stratum spongiosum (µm)	143.483	193.994	121.941	124.696	130.006	137.388	127.528	107.006	108.973	127.505	1322.52	132.252
		Thickness of stratum compactum (µm)	111.254	94.911	104.441	103.559	100.051	70.304	89.844	95.043	94.935	86.204	950.546	95.0546
PPT044	VC	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromucous glands	4	2	2	2	3	3	1	2	2	3	24	2.4
		Number of mucous glands	0	0	0	0	0	0	0	0	1	0	1	0.1
		Average size of granular glands	0	0	0	0	0	0	0	0	0	0	0	0

		present (μm^2)												
		Average size of seromucous glands present (μm^2)	13488 .438	15317 .466	10614. 935	11854.84 6	10524. 122	9762.66	14608.2 71	9370.45	10684.94 5	10985.09	117 211. 223	1172 1.12 23
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	1923.759	0	192 3.75 9	1923 .759
		Thicknes s of epidermi s (μm)	67.26 5	67.36 1	68.008	68.118	74.558	73.635	65.105	77.265	64.341	71.83	697. 486	69.7 486
		Thicknes s of stratum spongios um (μm)	121.3 98	117.5 6	159.02 7	173.888	142.81 7	146.98	141.331	119.34	129.456	111.837	136 3.63 4	136. 3634
		Thicknes s of stratum compact um (μm)	97.41 2	100.6 53	79.074	80.997	94.493	78.504	83.886	86.93	65.072	78.036	845. 057	84.5 067
		Number of granular glands	0	0	1	1	0	0	0	0	0	0	2	0.2

Mulu 087	Dorsal Head	Number of seromuc ous glands	3	2	2	3	1	1	2	1	2	3	20	2
		Number of mucous glands	0	0	0	1	2	1	0	1	0	0	5	0.5
		Average size of granular glands present (μm^2)	0	0	18202. 311	15517.83 4	0	0	0	0	0	0	337 20.1 45	1686 0.07 25
		Average size of seromuc ous glands present (μm^2)	8094. 588	10245 .715	6914.3 94	8543.159	11718. 492	7041.073	12405.4 83	9018.217	8811.571	8905.971	916 98.6 63	9169 .866 3
		Average size of mucous glands present (μm^2)	0	0	0	10175.07 1	14366. 415	10783.697	0	10509.85 1	0	0	45,8 35	1145 8.75 85
		Thicknes s of epidermi s (μm)	72.61 9	81.21 5	50.578	50.467	63.083	66.963	78.067	69.408	69.919	62.354	664. 673	66.4 673

		Thickness of stratum spongiosum (μm)	119.909	113.481	132.361	104.639	103.248	106.533	155.475	101.568	178.899	123.662	1239.775	123.9775
		Thickness of stratum compactum (μm)	74.604	76.329	78.432	80.898	93.412	81.759	46.013	43.178	56.465	59.968	691.058	69.1058
Mulu087	DC	Number of granular glands	1	0	0	0	0	0	1	0	1	0	3	0.3
		Number of seromucous glands	1	4	3	3	2	0	3	2	2	0	20	2
		Number of mucous glands	0	0	0	0	1	2	0	0	0	2	5	0.5
		Average size of granular glands present (μm^2)	10527.668	0	0	0	0	0	25790.363	0	18945.455	0	55263.486	18421.162
		Average size of seromucous glands	6315.092	9654.995	9143.589	7983.984	5567.702	0	5304.753	7980.352	5079.072	0	57029.539	7128.69238

		present (μm^2)												
		Average size of mucous glands present (μm^2)	0	0	0	0	10161. 352	7033.144	0	0	0	6789.23	239 83.7 26	7994 .575 33
		Thicknes s of epidermi s (μm)	98.83 1	97.33 5	98.742	114.054	115.72 9	115.514	125.052	84.808	84.156	83.426	101 7.64 7	101. 7647
		Thicknes s of stratum spongios um (μm)	113.3 42	114.0 89	143.34 2	119.178	102.52 6	126.482	191.749	98.796	124.306	116.633	125 0.44 3	125. 0443
		Thicknes s of stratum compact um (μm)	90.88 3	76.03 8	72.275	112.931	84.561	91.531	65.4	67.996	102.137	76.71	840. 462	84.0 462
Mulu 087	VH	Number of granular glands	0	0	0	0	0	0	1	0	0	0	1	0.1
		Number of seromuc ous glandS	3	2	4	4	2	1	3	0	2	3	24	2.4
		Number of	0	1	0	0	1	3	0	1	0	0	6	0.6

		mucous glands												
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	23016.089	0	0	0	23016.089	23016.089
		Average size of seromucous glands present (μm^2)	11353.898	8995.055	11285.6	12841.627	10493.637	11021.375	13655.964	0	13292.054	12719.031	105658.241	11739.8046
		Average size of mucous glands present (μm^2)	0	14196.174	0	0	0	12363.782	0	11704.476	0	0	38264.432	12754.8107
		Thickness of epidermis (μm)	90.951	126.482	75.307	85.251	137.519	105.643	129.799	130.63	87.029	94.818	1063.429	106.3429
		Thickness of stratum spongiosum (μm)	161.747	146.227	157.626	121.886	140.603	129.809	156.712	179.857	144.627	143.661	1482.755	148.2755
		Thickness of stratum	55.918	106.901	133.951	79.72	108.472	120.063	87.638	50.784	86.579	77.765	907.791	90.7791

		compact um (μm)												
Mulu 087	VC	Number of granular glands	0	1	0	0	0	0	0	1	0	0	2	0.2
		Number of seromuc ous glands	4	2	4	4	3	4	2	2	2	4	31	3.1
		Number of mucous glands	0	0	0	0	0	0	0	1	0	0	1	0.1
		Average size of granular glands present (μm ²)	0	22930 .389	0	0	0	0	0	26557.38 2	0	0	494 87.7 71	2474 3.88 55
		Average size of seromuc ous glands present (μm ²)	12013 .065	10793 .942	13434. 233	12732.51 3	14878. 087	10902.046	16392.4 67	6087.6	14139.16	9828.086	121 201. 199	1212 0.11 99
		Average size of mucous glands present (μm ²)	0	0	0	0	0	0	0	11215.93 6	0	0	112 15.9 36	1121 5.93 6

		Thickness of epidermis (μm)	78.988	69.437	70.304	74.66	64.905	75.007	56.504	65.959	73.123	76.258	705.145	70.5145
		Thickness of stratum spongiosum (μm)	127.536	170.186	123.109	135.094	133.314	95.571	113.808	100.524	115.904	113.001	1228.047	122.8047
		Thickness of stratum compactum (μm)	118.029	99.341	87.771	101.89	93.227	84.756	89.202	78.865	74.59	73.123	900.794	90.0794
		Number of granular glands	1	1	0	0	0	0	0	0	0	0	2	0.2
Mulu 093	DH	Number of seromucous glands	2	2	3	3	3	3	2	2	3	1	24	2.4
		Number of mucous glands	0	0	0	0	0	0	0	0	0	2	2	0.2
		Average size of granular glands present (μm^2)	7211.05	21866.694	0	0	0	0	0	0	0	0	29077.744	14538.872

		Average size of seromucous glands present (μm^2)	7984.341	4696.471	11967.859	11689.895	9699.03	8282.913	11263.005	6734.816	9434.721	8897.537	90650.588	9065.0588
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	8974.394	8974.394	8974.394
		Thickness of epidermis (μm)	66.106	60.83	56.527	79.964	72.94	50.821	76.272	59.541	62.131	73.513	658.645	65.8645
		Thickness of stratum spongiosum (μm)	123.341	78.458	93.171	136.146	102.957	97.774	132.256	120.215	92.223	139.195	1115.736	111.5736
		Thickness of stratum compactum (μm)	73.602	65.212	61.559	63.793	71.543	95.766	60.916	67.348	82.901	82.584	725.224	72.5224
Mulu093	DC	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromuc	0	0	0	1	2	1	2	3	3	4	16	1.6

		ous glands												
		Number of mucous glands	1	3	3	1	1	1	0	0	0	0	10	1
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands present (μm^2)	0	0	0	14385.45	9921.822	8032.006	18784.485	10749.352	8420.254	12060.609	82353.978	11764.854
		Average size of mucous glands present (μm^2)	10103.185	4839.376	6200.837	8840.2	7172.01	7536.834	0	0	0	0	44692.442	7448.74033
		Thickness of epidermis (μm)	95.308	130.552	121.971	159.536	133.883	126.147	110.8	111.023	102.108	96.301	1187.629	118.7629
		Thickness of stratum spongiosum (μm)	97.373	154.276	135.49	210.189	133.956	150.525	108.396	103.701	140.758	100.995	1335.659	133.5659

		Thickness of stratum compactum (μm)	141.256	105.93	147.818	120.049	103.983	71.579	128.701	111.072	80.591	156.971	1167.95	116.795
Mulu093	VH	Number of granular glands	0	0	0	1	0	0	0	0	0	0	1	0.1
		Number of seromucous glands	3	3	1	1	3	3	3	3	1	2	23	2.3
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	0	0	0	28627.032	0	0	0	0	0	0	28627.032	28627.032
		Average size of seromucous glands present (μm^2)	10000.632	8965.945	14166.94	7508.631	8496.664	12876.043	11043.884	7574.864	13125.785	11480.233	105239.621	105239.621
		Average size of mucous	0	0	0	0	0	0	0	0	0	0	0	0

		glands present (μm^2)												
		Thickness of epidermis (μm)	111.072	84.728	96.448	107.726	97.902	93.698	92.694	94.944	101.037	94.891	975.14	97.514
		Thickness of stratum spongiosum (μm)	166.82	168.738	157.427	186.091	162.447	163.703	171.235	151.234	132.62	178.002	1638.317	163.8317
		Thickness of stratum compactum (μm)	114.469	152.16	109.388	87.917	103.293	100.974	84.016	150.907	119.981	105.85	1128.955	112.8955
Mulu 093	VC	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromucous glands	2	2	2	3	3	2	1	2	1	3	21	2.1
		Number of mucous glands	0	0	0	2	2	1	0	0	0	0	5	0.5
		Average size of granular glands	0	0	0	0	0	0	0	0	0	0	0	0

		present (μm^2)												
		Average size of seromuc ous glands present (μm^2)	17082 .994	18792 .089	16564. 515	16870.43 8	12746. 627	12530.74	15533.2 37	13510.79 9	15740.90 8	11877.581	151 249. 928	1512 4.99 28
		Average size of mucous glands present (μm^2)	0	0	0	2708.907	1992.9 53	3664.144	0	0	0	0	836 6.00 4	2788 .668
		Thicknes s of epidermi s (μm)	71.92	54.61 5	58.323	58.323	74.647	70.583	57.597	57.647	59.669	43.808	607. 132	60.7 132
		Thicknes s of stratum spongios um (μm)	97.41 4	112.0 91	114.82 8	108.418	121.51 2	133.226	131.488	96.817	120.804	46.59	108 3.18 8	108. 3188
		Thicknes s of stratum compact um (μm)	53.51 8	60.60 7	64.233	55.304	68.412	69.124	64.774	66.941	76.487	48.772	628. 172	62.8 172
		Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0

BJ12	DH	Number of seromucous glands	1	2	2	1	1	3	2	3	2	1	18	1.8
		Number of mucous glands	0	0	0	1	0	0	0	2	0	1	4	0.4
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands present (μm^2)	8164.861	6394.361	7206.859	10031.806	10749.36	8205.039	8168.567	5744.702	5970.817	2019.773	72656.145	7265.6145
		Average size of mucous glands present (μm^2)	0	0	0	2531.026	0	0	0	1525.639	0	1767.942	5824.607	1941.53567
		Thickness of epidermis (μm)	60.182	46.3	50.121	51.743	43.669	71.084	55.97	54.537	38.79	38.626	511.022	51.1022

		Thickness of stratum spongiosum (μm)	125.796	131.707	112.987	106.068	106.883	113.446	118.638	109.3	141.047	105.755	1171.627	117.1627
		Thickness of stratum compactum (μm)	76.563	84.115	102.659	94.616	69.707	78.005	79.441	75.061	64.59	79.244	804.001	80.4001
BJ12	DC	Number of granular glands	0	0	0	0	0	0	0	0	1	1	2	0.2
		Number of seromucous glands	2	1	3	2	1	2	2	1	2	0	16	1.6
		Number of mucous glands	0	0	0	0	1	0	0	0	0	0	1	0.1
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	11978.813	12033.226	24012.039	12006.0195
		Average size of seromucous glands	8768.617	12462.123	6373.154	6539.454	7599.431	9377.431	13221.972	11193.61	5388.974	0	80924.766	8991.64067

		present (μm^2)												
		Average size of mucous glands present (μm^2)	0	0	0	0	7896.7 94	0	0	0	0	0	789 6.79 4	7896 .794
		Thicknes s of epidermi s (μm)	74.97 6	90.65 6	68.836	102.776	75.728	80.421	84.258	91.197	67.026	63.824	799. 698	79.9 698
		Thicknes s of stratum spongios um (μm)	110.8 38	79.87 4	79.77	96.641	81.507	73.856	72.005	121.956	69.808	80.633	866. 888	86.6 888
		Thicknes s of stratum compact um (μm)	41.98 6	57.21 8	61.277	56.456	62.777	75.303	69.654	35.283	54.75	47.611	562. 315	56.2 315
BJ12	VH	Number of granular glands	1	0	0	1	0	0	0	0	0	0	2	0.2
		Number of seromuc ous glands	1	2	2	1	1	1	3	3	3	2	19	1.9
		Number of	0	0	0	0	0	0	0	0	1	0	1	0.1

		mucous glands												
		Average size of granular glands present (μm^2)	11196.337	0	0	9612.234	0	0	0	0	0	0	20808.571	10404.2855
		Average size of seromucous glands present (μm^2)	5690.193	5592.17	6808.948	6372.225	6734.087	6316.636	6400.673	7975.511	6194.115	5683.601	63768.159	6376.8159
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	1949.35	0	1949.35	1949.35
		Thickness of epidermis (μm)	56.223	65.803	80.769	96.785	80.981	98.427	82.014	74.45	73.928	59.291	768.671	76.8671
		Thickness of stratum spongiosum (μm)	70.262	131.212	80.951	115.127	106.613	115.625	76.936	78.156	90.032	97.459	962.373	96.2373
		Thickness of stratum	68.4	60.487	63.129	67.046	43.878	52.963	56.043	55.337	70.917	46.887	585.087	58.5087

		compact um (μm)												
BJ12	VC	Number of granular glands	0	1	0	0	0	0	0	0	0	0	1	0.1
		Number of seromuc ous glands	2	2	1	5	1	5	2	3	5	2	28	2.8
		Number of mucous glands	0	0	1	0	0	0	0	0	0	0	1	0.1
		Average size of granular glands present (μm ²)	0	16681 .992	0	0	0	0	0	0	0	0	166 81.9 92	1668 1.99 2
		Average size of seromuc ous glands present (μm ²)	3817. 901	9304. 684	9302.7 54	7384.132	9865.7 69	8490.931	7324.09 5	7065.165	4693.229	8861.25	761 09.9 1	7610 .991
		Average size of mucous glands present (μm ²)	0	0	2929.8 16	0	0	0	0	0	0	0	292 9.81 6	2929 .816

		Thickness of epidermis (μm)	40.145	55.918	46.024	71.74	59.451	55.112	69.688	52.641	69.643	61.749	582.111	58.211
		Thickness of stratum spongiosum (μm)	132.738	97.647	144.78	108.124	142.296	125.912	106.033	115.856	142.919	87.472	1203.777	120.3777
		Thickness of stratum compactum (μm)	49.403	39.439	56.605	91.313	76.397	77.613	68.477	83.856	66.433	57.052	666.588	66.6588
		Number of granular glands	1	0	0	0	0	0	0	0	0	1	2	0.2
BJ09	DH	Number of seromucous glands	1	3	2	3	2	1	2	2	2	0	18	1.8
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm ²)	13034.907	0	0	0	0	0	0	0	0	9673.265	22708.172	11354.086

		Average size of seromucous glands present (μm^2)	5511.008	5674.249	7535.634	6820.169	7293.656	10273.579	7583.018	9659.97	11529.386	0	71880.669	7986.741
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	56.889	66.727	64.105	56.031	65.088	85.961	75.269	49.478	50.621	58.685	628.854	62.8854
		Thickness of stratum spongiosum (μm)	98.121	97.959	111.304	135.646	105.527	133.858	142.956	93.467	83.55	66.085	1068.473	106.8473
		Thickness of stratum compactum (μm)	59.917	44.199	37.562	61.741	71.677	48.571	60.746	39.54	51.886	62.295	538.134	53.8134
BJ09	DC	Number of granular glands	0	0	0	0	0	0	0	1	0	0	1	0.1
		Number of seromuc	2	2	2	2	2	3	1	2	2	1	19	1.9

		ous glands												
		Number of mucous glands	0	0	0	0	0	0	0	0	1	0	1	0.1
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	6891.642	0	0	6891.642	6891.642
		Average size of seromucous glands present (μm^2)	7637.55	10130.133	6391.361	5911.963	7662.874	8104.977	9258.486	4448.266	3015.13	9335.959	71896.699	7189.6699
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	2669.56	0	2669.56	2669.56
		Thickness of epidermis (μm)	75.746	80.041	75.17	74.274	123.695	64.03	119.429	85.131	74.977	95.121	867.614	86.7614
		Thickness of stratum spongiosum (μm)	130.398	138.563	120.673	101.15	138.622	87.272	97.837	74.121	96.839	115.254	1100.729	110.0729

		Thickness of stratum compactum (μm)	56.046	94.951	72.328	65.683	131.49	79.31	78.613	78.559	72.733	71.477	801.19	80.119
BJ09	VH	Number of granular glands	1	0	0	1	0	0	0	0	0	0	2	0.2
		Number of seromucous glands	2	5	5	2	3	4	3	3	3	2	29	2.9
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	14403.305	0	0	14065.294	0	0	0	0	0	0	28468.599	14234.2995
		Average size of seromucous glands present (μm^2)	4844.809	8669.843	7606.525	6959.88	8619.779	5090.773	8371.574	6756.991	8108.918	9198.661	74227.753	7422.7753
		Average size of mucous	0	0	0	0	0	0	0	0	0	0	0	0

		glands present (μm^2)												
		Thickness of epidermis (μm)	74.88	90.73	86.347	58.493	80.131	65.526	73.099	89.928	74.134	55.419	748.687	74.8687
		Thickness of stratum spongiosum (μm)	87.078	111.473	78.993	76.626	95.24	94.595	93.584	112.335	85.339	94.088	929.351	92.9351
		Thickness of stratum compactum (μm)	70.921	98.297	67.736	68.821	64.511	65.265	88.942	103.458	89.332	86.949	804.232	80.4232
BJ09	VC	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromucous glands	2	3	3	4	4	3	3	3	1	2	28	2.8
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands	0	0	0	0	0	0	0	0	0	0	0	0

		present (μm^2)												
		Average size of seromuc ous glands present (μm^2)	5770. 815	6149. 118	6615.6 04	5287.931	6091.8 76	5964.633	4300.76 9	6883.749	6843.483	7465.828	613 73.8 06	6137 .380 6
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thicknes s of epidermi s (μm)	27.16 7	33.54 2	35.482	34.268	29.832	29.69	34.964	37.101	26.961	43.151	332. 158	33.2 158
		Thicknes s of stratum spongios um (μm)	72.09 3	65.81 2	80.091	52.469	69.867	74.573	67.825	66.277	64.035	65.828	678. 87	67.8 87
		Thicknes s of stratum compact um (μm)	35.68 3	29.97 4	44.146	39.399	32.775	46.533	37.78	47.435	37.899	31.766	383. 39	38.3 39
		Number of	0	0	0	0	0	0	0	0	0	0	0	0

		granular glands												
KNP 1829	DH	Number of seromucous glands	1	1	2	2	1	4	1	2	1	2	17	1.7
		Number of mucous glands	0	0	0	0	1	0	0	0	0	0	1	0.1
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands present (μm^2)	4102.726	3816.481	4838.368	3623.239	5571.892	3501.602	4558.211	2652.883	4572.329	4096.196	41333.927	4133.3927
		Average size of mucous glands present (μm^2)	0	0	0	0	893.677	0	0	0	0	0	893.677	893.677
		Thickness of	28.086	29.187	32.751	36.966	34.322	31.414	35.534	34.206	24.739	24.405	311.61	31.161

		epidermis (μm)												
		Thickness of stratum spongiosum (μm)	64.266	75.676	46.573	65.812	64.03	62.829	71.44	55.318	63.34	51.681	620.965	62.0965
		Thickness of stratum compactum (μm)	76.174	48.886	42.985	48.292	45.846	44.67	42.302	33.478	36.736	44.534	463.903	46.3903
KNP 1829	DC	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromucous glands	2	1	2	1	2	1	1	2	1	1	14	1.4
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm ²)	0	0	0	0	0	0	0	0	0	0	0	0

		Average size of seromucous glands present (μm^2)	7740.082	6652.634	3721.625	6838.641	3962.544	3909.837	5947.081	3485.94	6246.385	2920.686	51425.455	5142.5455
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	38.735	35.92	43.875	35.653	40.499	39.379	33.973	46.277	36.381	41.881	392.573	39.2573
		Thickness of stratum spongiosum (μm)	95.595	92.095	63.286	61.371	66.612	87.339	100.551	97.109	76.746	154.262	894.966	89.4966
		Thickness of stratum compactum (μm)	72.166	40.342	46.533	39.291	44.385	43.414	32.123	44.723	44.409	41.576	448.962	44.8962
KNP 1829	VH	Number of granular glands	1	0	0	0	0	0	0	0	0	1	2	0.2
		Number of seromuc	3	4	2	2	2	2	1	2	2	2	22	2.2

		ous glands												
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	6825.405	0	0	0	0	0	0	0	0	18881.573	25706.978	12853.489
		Average size of seromucous glands present (μm^2)	6678.165	6946.879	4458.149	5002.226	6233.444	6889.466	9000.654	7691.993	4889.546	5932.875	63723.397	6372.3397
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	47.835	49.59	68.801	58.942	41.27	38.011	43.151	38.571	53.196	49.483	488.85	48.885
		Thickness of stratum spongiosum (μm)	104.476	72.936	74.203	75.63	90.002	90.792	101.908	46.93	74.221	66.941	798.039	79.8039

		Thickness of stratum compactum (μm)	72.067	56.754	34.591	41.391	43.096	55.875	48.837	44.043	33.137	42.936	472.727	47.2727
KNP 1829	VC	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromucous glands	1	2	2	3	1	3	3	1	2	2	20	2
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands present (μm^2)	6981.587	5638.247	4459.208	4839.103	6928.055	7811.595	8337.867	3842.07	5714.574	5687.837	60240.143	6024.0143
		Average size of mucous	0	0	0	0	0	0	0	0	0	0	0	0

		glands present (μm^2)												
		Thickness of epidermis (μm)	52.891	53.36	45.84	47.295	43.681	51.089	48.111	61.12	57.316	58.25	518.953	51.8953
		Thickness of stratum spongiosum (μm)	143.254	95.493	101.994	104.141	98.239	106.772	104.324	119.748	94.984	74.392	1043.341	104.3341
		Thickness of stratum compactum (μm)	114.883	51.373	50.954	79.713	69.128	70.793	87.272	73.931	65.502	80.795	744.344	74.4344
		Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
KNP 1830	DH	Number of seromucous glands	3	3	2	1	1	3	3	3	3	1	23	2.3
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands	0	0	0	0	0	0	0	0	0	0	0	0

		present (μm^2)												
		Average size of seromuc ous glands present (μm^2)	8297. 984	7514. 271	6381.6 55	8834.766	7913.7 35	7116.513	5920.84 5	6878.465	5740.133	7814.556	724 12.9 23	7241 .292 3
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thicknes s of epidermi s (μm)	48.04 5	57.66 1	40.499	56.889	57.716	47.669	43.298	49.059	39.082	48.571	488. 489	48.8 489
		Thicknes s of stratum spongios um (μm)	92.10 9	93.59 5	105.17 3	100.068	117.04 7	108.051	84.115	114.239	90.535	99.407	100 4.33 9	100. 4339
		Thicknes s of stratum compact um (μm)	72.09 3	49.87 2	51.373	53.873	42.502	55.04	51.988	46.139	61.955	87.906	572. 741	57.2 741
KNP 1830	DC	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0

		Number of seromucous glands	2	1	3	1	2	1	3	2	3	2	20	2
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands present (μm^2)	6673.282	5194.939	6098.675	6550.631	8878.179	5525.655	6220.267	5314.06	4141.139	6423.127	61019.954	6101.9954
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	51.743	63.248	58.942	55.776	71.503	76.538	79.24	67.414	70.229	52.996	647.629	64.7629
		Thickness of	101.079	155.869	107.85	119.232	136.626	98.661	98.11	87.9	112.075	74.438	1091.84	109.184

		stratum spongios um (µm)												
		Thicknes s of stratum compact um (µm)	74.86	67.35 9	78.205	83.146	73.587	70.669	54.333	73.806	86.388	111.543	773. 896	77.3 896
KNP 1830	VH	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromuc ous glands	3	2	2	5	1	3	3	3	4	2	28	2.8
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (µm²)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromuc ous glands present (µm²)	6279. 092	9649. 205	6905.6 13	5702.308	7489.6 62	9456.14	6710.00 9	5093.582	7642.932	6425.068	713 53.6 11	7135 .361 1

		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	57.537	67.86	59.295	70.793	78.022	80.595	80.058	53.37	56.408	61.504	665.442	66.5442
		Thickness of stratum spongiosum (μm)	123.002	130.876	106.591	127.242	140.89	143.358	128.465	138.535	86.956	100.638	1226.553	122.6553
		Thickness of stratum compactum (μm)	104.07	89.34	106.085	114.002	73.931	88.808	93.866	90.675	127.04	135.282	1023.099	102.3099
KNP 1830	VC	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromuc	2	4	1	2	2	2	2	1	2	2	20	2

		ous glands												
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands present (μm^2)	7225.477	5369.224	10323.875	12002.343	10815.097	10029.306	8952.476	11221.964	6222.914	6929.438	89092.114	8909.2114
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	62.875	56.866	52.665	65.105	55.226	45.475	49.18	51.913	59.44	48.413	547.158	54.7158
		Thickness of stratum spongiosum (μm)	124.364	129.14	188.927	168.669	108.292	96.508	133.068	96.972	173.531	130.673	1350.144	135.0144

		Thickness of stratum compactum (μm)	98.101	74.762	38.714	35.831	55.952	62.892	51.198	67.996	40.024	57.386	582.856	58.2856
		Number of granular glands	0	0	1	1	0	0	1	0	1	0	4	0.4
KNP 1831	DH	Number of seromucous glands	2	1	3	0	1	3	0	3	2	4	19	1.9
		Number of mucous glands	0	3	0	1	0	0	1	0	0	0	5	0.5
		Average size of granular glands present (μm^2)	0	0	20677.082	17284.026	0	0	13313.701	0	16129.845	0	67404.654	16851.1635
		Average size of seromucous glands present (μm^2)	9457.375	8016.55	5905.006	0	9343.198	6404.652	0	5929.573	7412.912	8414.654	60883.92	7610.49
		Average size of mucous	0	5396.57	0	3847.388	0	0	4977.159	0	0	0	14221.17	4740.37233

		glands present (µm ²)												
		Thickness of epidermis (µm)	55.871	56.219	52.665	42.63	46.475	46.567	45.334	48.253	50.531	36.114	480.659	48.0659
		Thickness of stratum spongiosum (µm)	128.941	105.896	78.803	140.388	88.608	98.264	83.397	138.926	102.794	96.873	1062.89	106.289
		Thickness of stratum compactum (µm)	39.91	26.208	32.367	44.747	61.417	53.769	43.963	36.584	44.979	52.339	436.283	43.6283
KNP 1831	DC	Number of granular glands	0	1	0	1	0	0	0	1	0	0	3	0.3
		Number of seromucous glands	1	1	0	0	3	1	2	0	3	3	14	1.4
		Number of mucous glands	0	0	1	0	0	0	0	0	0	0	1	0.1
		Average size of granular glands	0	9522.08	0	11650.313	0	0	0	14268.51	0	0	35440.903	11813.6343

		present (μm^2)												
		Average size of seromuc ous glands present (μm^2)	14219 .514	3210. 255	0	0	6424.7 85	10017.925	7419.41 5	0	7539.769	6705.342	555 37.0 05	7933 .857 86
		Average size of mucous glands present (μm^2)	0	0	4042.1 27	0	0	0	0	0	0	0	404 2.12 7	4042 .127
		Thicknes s of epidermi s (μm)	51.51	48.23	65.758	71.461	69.766	48.787	80.743	65.084	65.068	75.732	642. 139	64.2 139
		Thicknes s of stratum spongios um (μm)	157.6 96	127.8 61	128.91	154.762	126.63 9	132.531	124.341	127.995	137.495	136.769	135 4.99 9	135. 4999
		Thicknes s of stratum compact um (μm)	120.4 54	106.3 74	89.952	86.319	70.315	80.633	81.913	101.752	65.068	75.53	878. 31	87.8 31
KNP 1831	VH	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0

		Number of seromucous glands	3	3	3	4	5	4	2	4	3	4	35	3.5
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands present (μm^2)	8071.624	9116.072	7683.789	7344.807	7006.65	7150.008	6005.998	8202.781	6775.719	7390.552	74748	7474.8
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	54.593	65.072	62.981	80.041	63.387	70.69	76.206	70.826	82.001	63.778	689.575	68.9575

		Thickness of stratum spongiosum (μm)	117.067	122.958	139.709	110.367	112.675	123.149	107.891	84.886	90.115	71.819	108.0636	108.0636
		Thickness of stratum compactum (μm)	52.324	65.563	56.413	41.26	47.572	63.298	52.72	76.542	49.72	52.731	558.143	55.8143
KNP 1831	VC	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromucous glands	3	4	3	1	2	3	2	1	3	0	22	2.2
		Number of mucous glands	0	0	0	0	0	0	0	0	0	1	1	0.1
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands	9273.712	7175.769	8011.007	11742.248	7988.161	9046.368	7818.336	7860.651	5530.256	0	744.46508	8271.83422

		present (μm^2)												
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	8611.813	861 1.81 3	8611 .813
		Thicknes s of epidermi s (μm)	46.81 3	34.39 3	43.726	57.031	61.482	42.03	44.17	56.678	47.858	37.742	471. 923	47.1 923
		Thicknes s of stratum spongios um (μm)	106.1 33	83.50 2	115.01 5	107.772	117.85 2	115.285	107.946	142.443	121.84	125.123	114 2.91 1	114. 2911
		Thicknes s of stratum compact um (μm)	59.95 5	83.15 9	73.971	108.324	68.185	59.977	96.508	70.732	75.629	64.237	760. 677	76.0 677
		Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
MW C156 9	DH	Number of seromuc ous glands	3	2	2	1	2	2	3	1	2	3	21	2.1
		Number of	0	0	0	1	0	0	0	0	0	0	1	0.1

		mucous glands												
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands present (μm^2)	6135.201	6000.831	7432.006	4770.839	5854.732	6673.866	7965.474	12307.935	8519.165	7098.918	72758.967	7275.8967
		Average size of mucous glands present (μm^2)	0	0	0	2357.002	0	0	0	0	0	0	2357.002	2357.002
		Thickness of epidermis (μm)	42.202	42.886	45.539	44.765	46.345	55.342	49.67	28.34	53.076	50.463	458.628	45.8628
		Thickness of stratum spongiosum (μm)	117.472	132.092	86.056	128.589	102.046	79.043	137.674	129.786	109.678	101.533	1123.969	112.3969
		Thickness of stratum	79.324	68.782	86.782	67.684	66.001	74.502	62.019	83.755	103.153	105.326	797.328	79.7328

		compact um (µm)												
MW C156 9	DC	Number of granular glands	0	2	0	0	0	0	1	1	0	0	4	0.4
		Number of seromuc ous glands	2	2	2	2	2	3	0	1	2	3	19	1.9
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (µm²)	0	9935. 774	0	0	0	0	18950.5 75	15016.73 8	0	0	439 03.0 87	1463 4.36 23
		Average size of seromuc ous glands present (µm²)	8140. 155	4008. 487	5034.6 1	7494.074	8932.3 58	4834.613	0	6949.821	6638.516	7940.305	599 72.9 39	6663 .659 89
		Average size of mucous glands present (µm²)	0	0	0	0	0	0	0	0	0	0	0	0

		Thickness of epidermis (μm)	50.295	52.201	51.432	52.766	57.873	69.038	89.197	71.131	78.351	61.69	633.974	63.3974
		Thickness of stratum spongiosum (μm)	120.785	140.192	90.765	102.482	105.301	133.841	123.077	92.593	111.562	105.585	1126.183	112.6183
		Thickness of stratum compactum (μm)	60.672	98.142	124.77	101.273	71.652	64.486	73.169	100.722	96.111	87.744	814.741	81.4741
MW C1569	VH	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromucous glands	3	3	2	2	2	3	2	3	2	3	25	2.5
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular	0	0	0	0	0	0	0	0	0	0	0	0

		glands present (µm²)												
		Average size of seromucous glands present (µm²)	7179.57	6968.914	6860.142	7742.347	6969.983	6759.506	11815.208	7305.713	10719.497	6854.589	79175.469	7917.5469
		Average size of mucous glands present (µm²)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (µm)	68.337	69.512	85.651	78.231	77.159	67.704	74.722	70.583	58.25	52.776	702.925	70.2925
		Thickness of stratum spongiosum (µm)	102.221	87.004	94.383	98.253	90.236	96.071	83.041	126.909	99.862	88.773	966.753	96.6753
		Thickness of stratum compactum (µm)	161.143	114.82	97.992	99.22	78.022	89.509	60.986	90.708	107.887	81.934	982.221	98.2221
MW C1569	VC	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0

		Number of seromucous glands	2	2	2	1	3	4	2	2	2	2	22	2.2
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands present (μm^2)	8106.792	8724.684	7346.455	9102.848	6828.17	6816.375	7180.476	8360.574	6425.068	5417.299	74308.741	7430.841
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	34.391	41.481	35.145	40.019	25.508	31.824	42.954	42.508	43.663	42.707	380.2	38.02
		Thickness of	79.15	111.551	87.919	67.229	58.036	64.758	100.414	93.274	101.96	103.568	867.859	86.7859

		stratum spongios um (µm)												
		Thicknes s of stratum compact um (µm)	56.49 7	72.88 5	77.838	62.193	53.295	45.817	56.754	53.716	55.222	49.67	583. 887	58.3 887
		Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
MW C161 7	DH	Number of seromuc ous glands	2	2	1	2	1	3	2	3	1	1	18	1.8
		Number of mucous glands	0	0	0	0	1	0	0	0	0	0	1	0.1
		Average size of granular glands present (µm ²)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromuc ous glands present (µm ²)	5509. 861	5188. 497	6638.6 93	4517.71	3205.1 66	3873.247	7894.64 6	4762.65	7590.313	6870.759	560 51.5 42	5605 .154 2

		Average size of mucous glands present (μm^2)	0	0	0	0	2981.923	0	0	0	0	0	2981.923	2981.923
		Thickness of epidermis (μm)	39.082	46.122	43.808	49.317	43.298	53.111	49.414	33.542	32.816	32.751	423.261	42.3261
		Thickness of stratum spongiosum (μm)	68.663	89.544	74.321	103.322	90.798	101.032	91.278	69.502	77.886	76.983	843.329	84.3329
		Thickness of stratum compactum (μm)	79.59	72.067	55.799	51.353	53.913	49.248	59.829	46.93	48.799	47.345	564.873	56.4873
MW C1617	DC	Number of granular glands	0	0	0	0	0	0	1	0	0	0	1	0.1
		Number of seromucous glands	3	2	1	2	2	2	0	2	4	3	21	2.1
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0

		Average size of granular glands present (μm^2)	0	0	0	0	0	0	10028.983	0	0	0	10028.983	10028.983
		Average size of seromucous glands present (μm^2)	4915.047	7723.405	4903.399	7441.837	7686.875	8440.694	0	5885.491	4522.078	4826.103	56344.929	6260.54767
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	48.75	42.508	56.759	63.407	66.941	62.002	69.506	76.925	63.286	56.408	606.492	60.6492
		Thickness of stratum spongiosum (μm)	108.437	92.614	111.518	89.627	82.234	91.706	107.454	79.31	84.209	82.658	929.767	92.9767
		Thickness of stratum compactum (μm)	73.403	72.093	37.137	62.643	56.102	59.736	56.328	58.955	61.865	67.825	606.087	60.6087

MW C161 7	VH	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromuc ous glands	2	2	2	4	4	2	3	4	4	2	29	2.9
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromuc ous glands present (μm^2)	9704. 06	8503. 079	7754.4 65	8036.916	6098.9 83	10191.723	5601.48 2	5879.932	6196.928	7773.26	757 40.8 28	7574 .082 8
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0

		Thickness of epidermis (μm)	60.58	74.296	77.282	77.159	45.118	50.621	43.517	42.227	40.125	45.892	556.817	55.6817
		Thickness of stratum spongiosum (μm)	134.137	81.96	97.675	110.837	112.063	135.365	119.445	134.641	134.269	129.78	1190.172	119.0172
		Thickness of stratum compactum (μm)	57.936	53.922	49.478	48.772	69.855	91.821	59.099	73.504	81.545	93.546	679.478	67.9478
MW C1617	VC	Number of granular glands	1	0	0	0	0	0	0	1	1	0	3	0.3
		Number of seromucous glands	1	3	2	4	3	2	2	1	1	2	21	2.1
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm ²)	20370.858	0	0	0	0	0	0	10127.633	12071.169	0	42569.66	14189.8867

		Average size of seromucous glands present (μm^2)	5260.294	9158.483	9362.431	7662.212	8829.001	10717.947	9995.54	7976.737	6576.632	8912.681	84451.958	8445.1958
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	22.568	29.841	35.534	44.385	31.498	40.96	37.456	23.834	39.298	38.591	343.965	34.3965
		Thickness of stratum spongiosum (μm)	83.755	84.454	99.607	84.782	93.888	95.703	77.641	90.798	84.604	86.108	881.34	88.134
		Thickness of stratum compactum (μm)	45.932	51.187	45.235	48.111	56.773	63.286	56.295	52.57	40.045	55.318	514.752	51.4752
		Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
PPT035	DH	Number of seromuc	1	2	2	2	3	2	2	1	3	1	19	1.9

		ous glands												
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands present (μm^2)	7743.612	6578.779	7313.627	6446.127	6188.384	6299.681	5399.387	6441.392	4914.635	7666.845	64992.469	6499.2469
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	40.85	33.754	33.668	29.975	40.948	36.621	40.942	49.763	30.055	26.36	362.936	36.2936
		Thickness of stratum spongiosum (μm)	115.774	70.639	88.907	91.153	88.339	99.026	80.461	69.516	77.717	110.212	891.744	89.1744

		Thickness of stratum compactum (μm)	38.591	71.34	92.754	40.376	46.343	49.72	58.506	38.755	70.247	65.803	572.435	572.435
PPT035	DC	Number of granular glands	1	0	0	0	0	0	0	0	0	0	1	0.1
		Number of seromucous glands	3	3	2	2	4	3	1	1	3	2	24	2.4
		Number of mucous glands	0	0	0	0	0	0	0	1	0	0	1	0.1
		Average size of granular glands present (μm^2)	9560.438	0	0	0	0	0	0	0	0	0	9560.438	9560.438
		Average size of seromucous glands present (μm^2)	5234.116	5468.889	61337.147	4633.501	5672.332	7342.891	4111.554	5167.444	6321.863	6488.837	111778.574	111778.574
		Average size of mucous	0	0	0	0	0	0	0	1937.235	0	0	1937.235	1937.235

		glands present (µm²)												
		Thickness of epidermis (µm)	27.935	44.89	44.164	40.323	51.619	47.409	76.038	47.522	46.996	34.486	461.382	46.1382
		Thickness of stratum spongiosum (µm)	107.931	109.991	84.334	89.341	66.687	106.289	128.748	71.221	81.267	79.811	925.62	92.562
		Thickness of stratum compactum (µm)	54.485	41.525	46.204	49.763	64.552	100.722	74.748	41.852	24.137	54.146	552.134	55.2134
PPT035	VH	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromucous glands	1	2	3	1	4	1	2	1	1	2	18	1.8
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands	0	0	0	0	0	0	0	0	0	0	0	0

		present (μm^2)												
		Average size of seromuc ous glands present (μm^2)	10179 .525	8695. 197	5886.2 98	9417.673	3059.2 11	11070.371	6728.11 8	5346.504	7918.022	4487.55	727 88.4 69	7278 .846 9
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thicknes s of epidermi s (μm)	48.83 7	50.39 5	39.707	50.98	56.173	30.879	51.089	52.247	55.799	31.591	467. 697	46.7 697
		Thicknes s of stratum spongios um (μm)	111.0 3	92.81 9	75.966	79.614	80.795	75.17	84.707	76.525	124.438	81.223	882. 287	88.2 287
		Thicknes s of stratum compact um (μm)	47.83 5	42.50 8	43.377	47.519	43.224	65.08	54.372	30.053	64.134	62.592	500. 694	50.0 694
PPT0 35	VC	Number of granular glands	1	0	0	1	0	0	0	1	0	0	3	0.3

		Number of seromucous glands	0	1	1	2	3	2	1	3	1	2	16	1.6
		Number of mucous glands	0	0	0	0	0	0	0	0	1	0	1	0.1
		Average size of granular glands present (μm^2)	8884.533	0	0	9816.505	0	0	0	8430.341	0	0	27131.379	9043.793
		Average size of seromucous glands present (μm^2)	0	8468.754	7639.844	3864.482	3934.367	6265.445	6957.586	4642.155	3160.87	7152.504	52086.007	5787.33411
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	6442.627	0	6442.627	6442.627
		Thickness of epidermis (μm)	37.899	46.533	37.051	67.071	43.028	51.311	32.312	35.838	40.551	40.026	431.62	43.162

		Thickness of stratum spongiosum (μm)	107.71	94.203	145.067	120.464	116.93	100.74	96.948	102.852	101.084	91.057	107.7055	107.7055
		Thickness of stratum compactum (μm)	54.649	51.213	29.331	57.371	50.84	59.224	72.093	54.158	48.292	59.705	536.876	53.6876
		Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
PPT043	DH	Number of seromucous glands	2	2	1	3	1	4	2	2	1	3	21	2.1
		Number of mucous glands	0	0	1	0	1	0	1	0	1	0	4	0.4
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands	8967.918	9580.821	16557.907	8084.035	8632.524	7221.859	8142.978	8281.159	5215.057	8633.818	89318.076	8931.8076

		present (μm^2)												
		Average size of mucous glands present (μm^2)	0	0	1822.2 96	0	8992.3 6	0	8223.09 9	0	8478.813	0	275 16.5 68	6879 .142
		Thicknes s of epidermi s (μm)	49.48 3	40.75 3	48.028	46.71	51.743	49.973	44.48	56.773	47.02	43.929	478. 892	47.8 892
		Thicknes s of stratum spongios um (μm)	96.22 2	82.96 1	101.90 8	141.932	119.17 2	128.069	112.802	186.58	104.971	147.536	122 2.15 3	122. 2153
		Thicknes s of stratum compact um (μm)	42.98 5	61.86 5	63.507	48.756	63.32	63.407	68.074	29.912	71.31	47.435	560. 571	56.0 571
PPT0 43	DC	Number of granular glands	0	0	0	1	0	0	0	1	0	0	2	0.2
		Number of seromuc ous glands	3	3	1	1	3	3	2	3	0	3	22	2.2
		Number of	0	0	0	0	0	0	0	0	1	0	1	0.1

		mucous glands												
		Average size of granular glands present (μm^2)	0	0	0	15985.947	0	0	0	18472.677	0	0	34458.624	17229.312
		Average size of seromucous glands present (μm^2)	6094.38	7897.735	11029.251	5223.704	6748.814	7215.771	10101.073	5850.078	0	10113.338	70274.144	7808.23822
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	9832.917	0	9832.917	9832.917
		Thickness of epidermis (μm)	60.462	72.94	64.097	76.818	65.49	78.613	81.885	85.862	49.66	63.641	699.468	69.9468
		Thickness of stratum spongiosum (μm)	200.227	174.416	145.455	130.688	152.219	124.635	129.59	138.387	131.185	173.354	1500.156	150.0156
		Thickness of stratum	67.985	64.117	94.66	94.043	86.623	81.497	77.251	61.176	101.963	84.582	813.897	81.3897

		compact um (μm)												
PPT0 43	VH	Number of granular glands	1	0	1	0	1	0	0	0	0	1	4	0.4
		Number of seromuc ous glands	2	4	3	3	3	3	3	4	3	1	29	2.9
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm ²)	11120 .49	0	8605.3 47	0	16068. 714	0	0	0	0	20750.988	565 45.5 39	1413 6.38 48
		Average size of seromuc ous glands present (μm ²)	7649. 55	10504 .278	11276. 083	9187.151	7535.4 87	12114.582	12003.9 9	9817.431	11009.89 8	8887.003	999 85.4 53	9998 .545 3
		Average size of mucous glands present (μm ²)	0	0	0	0	0	0	0	0	0	0	0	0

		Thickness of epidermis (µm)	41.786	72.001	82.198	78.721	57.219	77.128	70.763	63.34	53.161	39.001	635.318	63.5318
		Thickness of stratum spongiosum (µm)	131.621	116.248	136.92	132.908	154.216	116.646	116.564	138.622	125.17	98.98	1267.895	126.7895
		Thickness of stratum compactum (µm)	57.316	59.616	62.057	60.001	57.941	56.773	50.954	82.379	75.76	54.547	617.344	61.7344
PPT043	VC	Number of granular glands	0	0	0	0	0	0	0	0	0	1	1	0.1
		Number of seromucous glands	2	3	3	2	2	1	1	3	3	1	21	2.1
		Number of mucous glands	0	0	0	0	0	2	1	0	0	0	3	0.3
		Average size of granular glands present (µm ²)	0	0	0	0	0	0	0	0	0	12983.552	12983.552	12983.552

		Average size of seromucous glands present (μm^2)	12193.643	10485.782	13486.981	11733.304	16819.269	11768.335	9237.133	13099.909	8922.24	8561.228	116307.824	11630.7824
		Average size of mucous glands present (μm^2)	0	0	0	0	0	2184.514	1137.744	0	0	0	3322.258	1661.129
		Thickness of epidermis (μm)	50.697	49.435	66.628	56.712	55.042	61.899	57.044	65.904	47.795	52.14	563.296	56.3296
		Thickness of stratum spongiosum (μm)	155.461	114.026	125.164	106.574	144.635	91.833	109.405	137.623	135.515	110.112	1230.348	123.0348
		Thickness of stratum compactum (μm)	90.452	88.423	104.343	109.503	96.535	91.561	98.365	85.501	118.049	82.872	965.604	96.5604
		Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
KNP 1838	DH	Number of seromuc	2	2	2	4	2	2	2	1	1	2	20	2

		ous glands												
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands present (μm^2)	8896.138	9705.148	7472.067	5880.647	9031.352	7513.497	6707.808	12061.906	9767.904	9224.078	86260.545	8626.0545
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	63.941	65.179	58.096	45.184	58.698	63.069	58.662	62.064	74.505	46.347	595.745	59.5745
		Thickness of stratum	133.509	155.353	123.867	152.554	124.045	165.495	147.228	128.634	168.794	123.143	1422.622	142.2622

		spongiosum (μm)												
		Thickness of stratum compactum (μm)	95.156	79.672	72.417	75.817	84.381	60.783	94.966	97.293	79.236	81.223	820.944	82.0944
KNP 1838	DC	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromucous glands	3	4	3	3	4	3	2	1	3	2	28	2.8
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands present (μm^2)	7066.834	8568.592	7657.276	7147.712	6621.264	10988.47	7705.144	9447.3307	6729.921	6878.945	78811.4887	7881.14887

		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	91.245	82.939	89.311	94.309	90.368	77.632	83.33	77.571	79.688	102.044	868.437	86.8437
		Thickness of stratum spongiosum (μm)	103.83	92.889	122.453	90.455	123.109	102.221	120.267	86.422	134.698	110.074	1086.418	108.6418
		Thickness of stratum compactum (μm)	87.145	95.922	97.29	78.548	105.441	97.098	81.571	111.525	96.706	93.645	944.891	94.4891
KNP 1838	VH	Number of granular glands	1	0	0	0	1	0	1	0	0	0	3	0.3
		Number of seromucous glands	2	3	3	3	2	4	0	1	2	2	22	2.2
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0

		Average size of granular glands present (μm^2)	10522.025	0	0	0	15776.22	0	14374.262	0	0	0	40672.507	13557.5023
		Average size of seromucous glands present (μm^2)	8097.355	5471.248	6356.008	5097.742	10598.678	5505.481	0	6442.712	8946.658	7967.502	64483.384	7164.82044
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	89.937	91.913	102.313	64.986	95.297	94.891	107.748	80.383	115.145	93.508	936.121	93.6121
		Thickness of stratum spongiosum (μm)	140.858	173.904	185.061	119.734	141.945	128.935	191.954	115.949	166.534	155.258	1520.132	152.0132
		Thickness of stratum compactum (μm)	114.915	124.349	142.463	111.985	119.602	89.085	141.221	104.371	126.035	96.383	1170.409	117.0409

KNP 1838	VC	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromuc ous glands	2	4	4	2	3	1	4	2	3	6	31	3.1
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromuc ous glands present (μm^2)	10892 .704	7619. 27	8758.9 87	8305.988	9707.0 32	9503.42	8565.35 8	8119.38	8311.057	8272.366	880 55.5 62	8805 .556 2
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thicknes s of	31.36 4	53.05 6	41.122	48.072	41.754	41.481	58.087	56.385	50.374	39.386	461. 081	46.1 081

		epidermis (μm)												
		Thickness of stratum spongiosum (μm)	120.848	127.473	120.218	136.986	122.741	141.278	137.275	133.921	108.445	147.986	1297.171	129.7171
		Thickness of stratum compactum (μm)	86.852	111.042	112.369	96.324	95.343	106.075	79.79	105.868	98.659	98.21	990.532	99.0532
		Number of granular glands	0	0	0	0	0	0	0	1	0	0	1	0.1
MW C1561	DH	Number of seromucous glands	3	2	1	2	1	2	1	0	1	2	15	1.5
		Number of mucous glands	0	0	0	0	0	0	0	0	1	0	1	0.1
		Average size of granular glands present (μm ²)	0	0	0	0	0	0	0	16081.597	0	0	16081.597	16081.597

		Average size of seromucous glands present (μm^2)	8024.994	10862.922	9885.507	10662.533	11678.332	10723.594	12125.347	0	8276.748	10721.741	92961.718	10329.0798
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	10246.931	0	10246.931	10246.931
		Thickness of epidermis (μm)	28.461	27.274	45.136	45.695	48.75	59.188	56.031	62.643	42.985	63.181	479.344	47.9344
		Thickness of stratum spongiosum (μm)	130.891	158.636	130.414	122.257	132.477	207.576	109.182	221.316	138.867	161.749	1513.365	151.3365
		Thickness of stratum compactum (μm)	44.67	68.709	57.776	98.476	109.957	88.117	107.808	57.556	95.654	100.748	829.471	82.9471
MW C1561	DC	Number of granular glands	1	1	0	0	0	1	0	1	0	0	4	0.4
		Number of seromuc	2	3	4	3	2	0	3	1	2	4	24	2.4

		ous glands												
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	19878.312	12412.121	0	0	0	37455.077	0	25810.393	0	0	95555.903	23888.9758
		Average size of seromucous glands present (μm^2)	8082.682	5158.408	6621.089	6947.801	6980.263	0	7155.063	4583.977	9133.011	8394.457	63056.751	7006.30567
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	50.933	64.529	62.613	63.37	59.776	65.251	55.652	56.773	52.277	75.001	606.175	60.6175
		Thickness of stratum	129.59	87.508	82.542	126.858	83.878	93.479	100.02	102.852	110.715	77.978	995.42	99.542

		spongiosum (μm)												
		Thickness of stratum compactum (μm)	109.165	92.639	95.329	63.32	88.069	73.125	82.321	101.267	89.544	83.762	878.541	87.8541
MW C1561	VH	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromucous glands	4	3	4	0	5	3	4	4	3	3	33	3.3
		Number of mucous glands	0	0	0	1	0	1	0	0	0	0	2	0.2
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands present (μm^2)	6517.145	6152.912	6151.309	0	5294.33	5106.308	5854.299	6141.338	8104.624	5478.536	54800.801	6088.97789

		Average size of mucous glands present (μm^2)	0	0	0	13284.09 2	0	6446.333	0	0	0	0	197 30.4 25	9865 .212 5
		Thickness of epidermis (μm)	73.20 1	88.05 4	74.345	74.231	69.262	66.409	66.249	75.672	57.316	48.374	693. 113	69.3 113
		Thickness of stratum spongiosum (μm)	110.3 42	119.5 82	166.01 5	120.787	110.96 6	147.751	110.486	128.729	155.753	136.893	130 7.30 4	130. 7304
		Thickness of stratum compactum (μm)	74.55 2	94.77 2	64.295	72.034	106.84 4	97.656	109.094	106.96	85.33	94.66	906. 197	90.6 197
MW C156 1	VC	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromucous glands	2	3	3	1	3	3	4	5	6	2	32	3.2
		Number of mucous glands	0	0	0	1	0	0	0	0	0	1	2	0.2

		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands present (μm^2)	8668.79	8479.774	10748.262	7726.67	8320.278	10870.619	6486.526	6433.309	7814.232	7113.591	82662.051	8266.2051
		Average size of mucous glands present (μm^2)	0	0	0	7045.118	0	0	0	0	0	13149.793	20194.911	10097.4555
		Thickness of epidermis (μm)	52.16	55.803	67.662	49.973	58.814	45.646	61.123	60.597	56.712	57.044	565.534	56.5534
		Thickness of stratum spongiosum (μm)	186.113	139.765	147.463	142.972	119.567	126.738	211.206	181.16	171.065	187.856	1613.905	161.3905
		Thickness of stratum compactum (μm)	91.981	109.35	89.014	119.49	94.153	102.131	133.35	147.007	117.325	102.896	1106.697	110.6697

		Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
MW C156 2	DH	Number of seromucous glands	3	2	4	3	2	2	3	3	4	3	29	2.9
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands present (μm^2)	10501.125	10037.166	8273.211	9447.132	6375.499	9623.95	11833.665	10732.785	9102.688	12615.619	98542.84	9854.284
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of	47.077	55.113	56.485	41.335	62.882	62.317	59.492	61.106	50.302	53.633	549.742	54.9742

		epidermis (μm)												
		Thickness of stratum spongiosum (μm)	133.53	176.699	136.175	136.868	118.766	148.716	142.056	151.353	186.35	148.022	147.8535	147.8535
		Thickness of stratum compactum (μm)	100.974	86.179	105.127	100.725	63.875	68.218	65.999	88.376	86.976	90.753	857.202	85.7202
MW C1562	DC	Number of granular glands	0	0	0	0	0	0	0	0	1	1	2	0.2
		Number of seromucous glands	3	1	2	2	3	4	5	2	1	2	25	2.5
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm ²)	0	0	0	0	0	0	0	0	19111.376	17452.625	36564.001	18282.0005

		Average size of seromucous glands present (μm^2)	9018.737	18413.776	11997.489	12273.336	8746.095	8813.192	8788.107	17226.075	10838.427	9080.56	115195.794	11519.5794
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	48.525	60.83	67.363	62.786	45.623	58.841	55.836	57.209	50.697	54.87	562.58	56.258
		Thickness of stratum spongiosum (μm)	98.618	110.883	104.772	88.352	74.59	77.972	88.935	101.756	103.655	140.663	990.196	99.0196
		Thickness of stratum compactum (μm)	75.314	71.693	99.959	86.325	68.891	72.71	75.983	81.43	68.318	135.608	836.231	83.6231
MW C156 2	VH	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromuc	3	2	3	3	0	2	1	2	3	4	23	2.3

		ous glands												
		Number of mucous glands	0	0	0	0	2	2	2	0	0	0	6	0.6
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands present (μm^2)	8977.23	9155.728	7577.273	6450.656	0	5212.154	4045.05	9066.896	5692.263	8061.519	64238.769	7137.641
		Average size of mucous glands present (μm^2)	0	0	0	0	5320.185	4686.419	8252.759	0	0	0	18259.363	6086.45433
		Thickness of epidermis (μm)	63.007	66.816	66.765	59.822	58.101	74.561	62.986	74.593	70.304	61.106	658.061	65.8061
		Thickness of stratum	102.924	100.701	116.971	104.504	84.032	113.805	89.809	108.647	89.844	92.976	1004.213	100.4213

		spongiosum (μm)												
		Thickness of stratum compactum (μm)	70.617	54.318	90.547	66.926	91.75	89.528	46.909	87.699	83.292	65.484	747.07	74.707
MW C1562	VC	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromucous glands	2	4	1	0	4	3	2	4	2	0	22	2.2
		Number of mucous glands	0	0	2	2	0	0	0	0	0	3	7	0.7
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands present (μm^2)	7272.262	5960.025	6684.471	0	5384.601	6232.186	6060.495	5585.63	7350.227	0	50529.897	6316.23712

		Average size of mucous glands present (μm^2)	0	0	8753.67	10052.404	0	0	0	0	0	5832.109	24638.183	8212.72767
		Thickness of epidermis (μm)	51.196	49.178	53.373	52.034	53.772	45.379	42.408	50.576	44.601	55.868	498.385	49.8385
		Thickness of stratum spongiosum (μm)	148.408	118.435	129.611	178.404	140.851	171.989	154.988	130.173	121.366	157.002	1451.227	145.1227
		Thickness of stratum compactum (μm)	105.527	105.276	142.656	139.279	160.43	122.043	139.179	137.106	108.319	101.815	1261.63	126.163
		Number of granular glands	1	0	0	0	0	0	0	0	0	0	1	0.1
MW C1570	DH	Number of seromucous glands	3	2	1	2	1	1	1	0	3	3	17	1.7
		Number of mucous glands	0	0	0	1	0	1	2	1	0	0	5	0.5

		Average size of granular glands present (μm^2)	12864.962	0	0	0	0	0	0	0	0	0	12864.962	12864.962
		Average size of seromucous glands present (μm^2)	6450.445	8927.157	7306.054	12735.831	15922.067	7963.795	8454.785	0	8326.574	11019.772	87106.48	9678.4978
		Average size of mucous glands present (μm^2)	0	0	0	13994.274	0	15224.064	14068.385	13752.698	0	0	57039.421	14259.8552
		Thickness of epidermis (μm)	45.214	57.14	44.162	51.18	52.157	55.527	57.796	44.691	54.835	42.098	504.8	50.48
		Thickness of stratum spongiosum (μm)	116.421	159.575	127.742	170.791	117.053	139.703	120.635	113.325	121.729	113.506	1300.48	130.048
		Thickness of stratum compactum (μm)	111.977	108.664	95.039	103.145	87.804	93.921	85.535	66.591	111.115	97.43	961.221	96.1221

MW C157 0	DC	Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
		Number of seromuc ous glands	0	0	4	3	2	2	2	3	3	1	20	2
		Number of mucous glands	1	2	0	0	0	0	0	0	0	2	5	0.5
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromuc ous glands present (μm^2)	0	0	5739.8 22	9597.992	8032.1 17	11961.281	8987.46 2	6903.428	9810.707	7584.151	686 16.9 6	8577 .12
		Average size of mucous glands present (μm^2)	10998 .809	9864. 776	0	0	0	0	0	0	0	8347.536	292 11.1 21	9737 .040 33
		Thicknes s of	48.52 1	77.49 7	66.724	59.505	84.807	63.699	62.876	61.662	69.64	53.448	648. 379	64.8 379

		epidermis (μm)												
		Thickness of stratum spongiosum (μm)	148.937	148.759	139.135	132.686	129.341	86.871	160.21	127.403	125.32	97.506	1296.168	129.6168
		Thickness of stratum compactum (μm)	116.187	101.786	122.097	99.151	125.032	83.354	81.909	103.835	104.543	103.835	1041.729	104.1729
MW C1570	VH	Number of granular glands	0	0	0	0	0	0	1	1	0	0	2	0.2
		Number of seromucous glands	2	3	3	0	3	0	1	1	4	2	19	1.9
		Number of mucous glands	0	1	0	3	0	2	1	0	0	1	8	0.8
		Average size of granular glands present (μm ²)	0	0	0	0	0	0	19874.213	11752.931	0	0	31627.144	15813.572

		Average size of seromucous glands present (μm^2)	9955.991	8048.833	8984.077	0	9340.799	0	8039.51	5865.153	6231.257	5304.862	61770.482	7721.31025
		Average size of mucous glands present (μm^2)	0	4095.025	0	9703.459	0	5665.978	3391.32	0	0	6194.558	29050.34	5810.068
		Thickness of epidermis (μm)	61.761	89.073	75.936	91.395	72.612	89.924	90.191	87.707	81.997	78.799	819.395	81.9395
		Thickness of stratum spongiosum (μm)	123.091	119.522	134.504	155.277	155.896	178.034	153.562	251.335	118.901	168.333	1558.455	155.8455
		Thickness of stratum compactum (μm)	90.049	155.647	112.826	126.646	160.479	113.713	101.534	106.116	145.186	124.513	1236.709	123.6709
MW C1570	VC	Number of granular glands	0	0	0	0	0	0	0	1	0	0	1	0.1
		Number of seromuc	0	3	2	1	1	1	3	3	3	2	19	1.9

		ous glands												
		Number of mucous glands	3	0	0	1	1	1	0	1	2	0	6	0.6
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	32172.47	0	0	32172.47	32172.47
		Average size of seromucous glands present (μm^2)	0	11057.481	12759.703	11403.217	13235.52	8944.171	10236.196	6047.76	6412.142	11108.106	91204.296	10133.8107
		Average size of mucous glands present (μm^2)	7145.063	0	0	14425.226	8908.718	12718.52	0	13132.013	9060.237	0	65389.777	10898.2962
		Thickness of epidermis (μm)	50.913	42.503	53.373	45.614	50.576	44.355	52.682	48.604	50.908	44.882	48.441	48.441

		Thickness of stratum spongiosum (μm)	134.011	111.569	128.855	148.926	124.103	118.519	131.692	125.015	119.897	98.659	1241.246	124.1246
		Thickness of stratum compactum (μm)	45.397	80.471	51.921	61.034	64.483	60.586	54.099	91.395	71.648	65.942	646.976	64.6976
		Number of granular glands	0	0	0	0	0	0	0	0	0	0	0	0
PPT028	DH	Number of seromucous glands	1	2	2	2	2	2	3	3	2	2	21	2.1
		Number of mucous glands	0	1	0	0	0	0	0	0	0	0	1	0.1
		Average size of granular glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of seromucous glands	9754.945	5531.205	10343.206	14148.554	9929.001	7997.644	6688.338	8248.958	7916.495	9418.949	89977.295	8997.7295

		present (μm^2)												
		Average size of mucous glands present (μm^2)	0	6935. 852	0	0	0	0	0	0	0	0	693 5.85 2	6935 .852
		Thicknes s of epidermi s (μm)	51.91 1	48.98 7	51.362	50.465	57.098	44.977	44.331	45.096	51.617	51.906	497. 75	49.7 75
		Thicknes s of stratum spongios um (μm)	152.0 69	157.7 92	188.75 7	138.905	164.57	187.296	143.164	155.818	143.743	123.186	155 5.3	155. 53
		Thicknes s of stratum compact um (μm)	59.23 4	72.37 6	43.864	74.601	46.788	37.292	57.042	48.697	55.068	72.376	567. 338	56.7 338
PPT0 28	DC	Number of granular glands	0	0	1	0	0	1	0	0	1	0	3	0.3
		Number of seromuc ous glands	1	3	1	2	2	1	2	3	1	3	19	1.9
		Number of	0	0	0	0	0	0	0	0	0	0	0	0

		mucous glands												
		Average size of granular glands present (μm^2)	0	0	12038.866	0	0	18118.159	0	0	14094.099	0	44251.124	14750.3747
		Average size of seromucous glands present (μm^2)	12304.136	11318.732	10760.441	10200.506	10663.08	9361.049	8436.346	9434.428	8594.813	8997.854	100071.385	10007.1385
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	62.42	64.309	72.379	80.417	93.91	92.219	60.789	82.273	74.357	81.703	764.776	76.4776
		Thickness of stratum spongiosum (μm)	192.909	163.087	135.474	123.868	163.974	119.173	135.374	135.319	121.377	143.099	1433.654	143.3654
		Thickness of stratum	55.039	72.376	74.037	75.897	54.693	57.028	78.959	71.274	63.215	68.988	671.506	67.1506

		compact um (μm)												
PPT0 28	VH	Number of granular glands	1	0	0	0	0	0	0	0	0	0	1	0.1
		Number of seromuc ous glands	2	3	2	4	3	3	2	1	3	3	26	2.6
		Number of mucous glands	0	0	0	0	0	0	0	1	0	0	1	0.1
		Average size of granular glands present (μm ²)	21939 .006	0	0	0	0	0	0	0	0	0	219 39.0 06	2193 9.00 6
		Average size of seromuc ous glands present (μm ²)	8072. 735	11069 .536	12614. 835	11555.53 7	11285. 932	11251.074	13906.9 79	13498.29 6	12693.87 6	14080.618	120 029. 418	1200 2.94 18
		Average size of mucous glands present (μm ²)	0	0	0	0	0	0	0	4026.436	0	0	402 6.43 6	4026 .436

		Thickness of epidermis (μm)	97.218	95.84	86.779	83.662	96.993	86.315	66.852	60.094	69.455	62.872	806.08	80.608
		Thickness of stratum spongiosum (μm)	157.711	135.095	139.402	121.498	174.785	153.531	191.023	123.689	113.344	158.926	1469.004	146.9004
		Thickness of stratum compactum (μm)	60.824	107.824	72.523	67.274	70.747	61.843	51.859	56.368	63.838	49.713	662.813	66.2813
PPT028	VC	Number of granular glands	0	0	0	0	1	0	1	0	1	1	4	0.4
		Number of seromucous glands	3	3	3	3	4	3	1	3	0	1	24	2.4
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	0	0	0	0	17435.655	0	14412.399	0	12487.812	15758.879	60094.745	15023.6862

		Average size of seromucous glands present (μm^2)	15886.437	10903.497	10412.805	8597.129	11433.547	9858.511	11869.443	7467.698	0	11434.928	97863.995	10873.7772
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	82.456	82.696	89.686	80.055	91.634	90.368	83.267	77.896	72.762	64.958	815.778	81.5778
		Thickness of stratum spongiosum (μm)	175.133	240.67	148.65	123.019	136.298	242.051	197.978	177.535	144.481	166.682	1752.497	175.2497
		Thickness of stratum compactum (μm)	121.939	49.787	127.367	93.274	119.622	64.86	102.636	142.621	118.604	88.054	1028.764	102.8764
		Number of granular glands	0	1	0	0	1	1	0	0	1	0	4	0.4
KNP 1832	DH	Number of seromuc	3	1	2	1	0	2	3	2	1	1	16	1.6

		ous glands												
		Number of mucous glands	0	0	0	0	0	0	0	0	0	1	1	0.1
		Average size of granular glands present (μm^2)	0	37658.495	0	0	15233.804	24102.63	0	0	19672.364	0	96667.293	24166.8232
		Average size of seromucous glands present (μm^2)	13352.349	8025.621	10594.59	11744.687	0	14221.888	15445.811	16288.339	17401.112	25161.312	132235.709	14692.8566
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	10614.708	10614.708	10614.708
		Thickness of epidermis (μm)	72.02	118.099	58.323	103.884	109.23	84.811	64.068	80.513	109.873	98.98	899.801	89.9801
		Thickness of stratum	161.085	217.764	162.422	204.348	223.409	160.151	184.907	207.669	239.497	181.271	1942.523	194.2523

		spongiosum (μm)												
		Thickness of stratum compactum (μm)	98.967	69.185	90.979	102.993	59.669	81.701	101.139	92.479	85.887	108.437	891.436	89.1436
KNP 1832	DC	Number of granular glands	2	1	0	2	1	1	1	1	1	0	10	1
		Number of seromucous glands	0	2	2	0	2	1	0	1	2	1	11	1.1
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	2225.099	35675.782	0	16386.46	32144.134	15642.523	15424.045	24530.409	33547.828	0	175576.28	21947.035
		Average size of seromucous glands present (μm^2)	0	7936.648	15829.588	0	8739.998	13241.384	0	16734.207	15388.133	20342.975	98212.933	14030.419

		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	79.713	140.478	70.953	66.945	82.59	68.412	79.441	73.099	67.245	63.449	792.325	79.2325
		Thickness of stratum spongiosum (μm)	153.072	203.302	162.977	182.741	138.886	176.108	186.442	181.679	185.297	197.523	1768.027	176.8027
		Thickness of stratum compactum (μm)	148.812	77.213	117.58	142.363	144.853	132.499	152.297	117.37	115.566	105.173	1253.726	125.3726
KNP 1832	VH	Number of granular glands	0	1	2	1	0	1	2	2	2	2	13	1.3
		Number of seromucous glands	2	2	0	1	2	1	1	1	0	1	11	1.1
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0

		Average size of granular glands present (μm^2)	0	31005.155	14366.716	21511.955	0	19844.429	15756.88	21209.651	17386.817	17180.869	158262.472	19782.809
		Average size of seromucous glands present (μm^2)	12820.047	7662.256	0	6237.032	12313.559	6135.558	10191.694	8323.161	0	8171.038	71854.345	8981.79312
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thickness of epidermis (μm)	126.719	96.379	101.877	93.161	116.794	100.79	96.948	131.109	114.966	86.599	1065.342	106.5342
		Thickness of stratum spongiosum (μm)	216.832	183.847	246.662	238.292	198.917	173.248	174.106	214.915	166.853	175.682	1989.354	198.9354
		Thickness of stratum compactum (μm)	92.41	138.341	82.272	110.887	125.254	116.077	132.229	114.35	149.162	170.246	1231.228	123.1228

KNP 1832	VC	Number of granular glands	0	1	0	1	0	0	1	0	1	2	6	0.6
		Number of seromuc ous glands	3	2	3	1	2	4	0	3	2	2	22	2.2
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	0	16561 .084	0	26599.77 2	0	0	23655.0 86	0	19898.07 8	20839.403	107 553. 423	2151 0.68 46
		Average size of seromuc ous glands present (μm^2)	17495 .409	10319 .463	17657. 591	16353.19 4	18055. 134	11556.475	0	15551.99 1	16104.98	13013.994	136 108. 231	1512 3.13 68
		Average size of mucous glands present (μm^2)	0	0	0	0	0	0	0	0	0	0	0	0
		Thicknes s of	43.24 9	40.342	58.25	58.933	46.41 4	48.028	52.57	39.533	67.146	45.42 8	499.893	49 .9

		epidermis (μm)												893
		Thickness of stratum spongiosum (μm)	193.021	86.366	170.487	123.909	151.32	138.64	127.458	126.363	108.144	129.72	1355.428	135.5428
		Thickness of stratum compactum (μm)	69.639	84.131	118.067	99.949	109.337	78.465	129.363	127.748	110.311	83.856	1010.866	101.0866
		Number of granular glands	1	0	0	0	0	0	0	0	0	0	1	0.1
MWC1612	DH	Number of seromucous glands	2	0	3	2	3	2	1	2	2	2	19	1.9
		Number of mucous glands	0	2	0	0	1	0	0	0	0	0	3	0.3
		Average size of granular glands present (μm^2)	18733.686	0	0	0	0	0	0	0	0	0	18733.686	1873.686
		Average size of seromuc	8990.595	0	9023.125	8132.654	9752.189	9138.306	8707.703	8477.431	7951.678	9574.82	71657.501	7961.9

		ous glands present (µm²)												4456
		Average size of mucous glands present (µm²)	0	5706.367	0	0	8675.055	0	0	0	0	0	14381.422	7190.711
		Thickness of epidermis (µm)	39.048	45.817	42.227	50.01	51.265	46.568	43.542	44.456	44.199	46.208	453.34	45.334
		Thickness of stratum spongiosum (µm)	119.73	146.123	83.299	105.062	107.316	92.029	116.351	158.628	110.121	117.823	1156.482	115.6482
		Thickness of stratum compactum (µm)	53.295	50.29	67.731	59.939	71.607	62.579	81.536	68.326	75.648	55.342	646.293	64.6293
MWC1612	DC	Number of granular glands	1	1	0	0	0	0	0	0	1	1	4	0.4
		Number of seromucous glands	2	0	0	3	1	0	1	4	1	0	12	1.2

		Number of mucous glands	0	0	1	0	1	1	0	0	0	0	3	0.3
		Average size of granular glands present (μm^2)	17832.95	15464.459	0	0	0	0	0	0	15345.513	14866.027	63508.949	15877.2372
		Average size of seromucous glands present (μm^2)	5796.194	0	0	8176.685	7884.617	0	11313.202	8350.912	7980.267	0	49501.877	8250.31283
		Average size of mucous glands present (μm^2)	0	0	4889.105	0	4661.097	9020.067	0	0	0	0	18570.269	6190.08967
		Thickness of epidermis (μm)	59.295	52.57	90.673	85.454	90.368	96.839	117.237	79.431	78.059	78.428	828.354	82.8354
		Thickness of stratum spongiosum (μm)	163.358	183.712	197.776	172.826	176.476	196.232	147.412	138.403	134.403	142.325	1652.923	165.2923

		Thickness of stratum compactum (μm)	65.683	87.556	107.776	135.849	169.685	139.415	135.394	113.697	121.065	126.614	1202.734	120.2734
MWC1612	VH	Number of granular glands	1	1	1	1	1	1	0	0	0	1	7	0.7
		Number of seromucous glands	3	1	3	1	2	1	3	3	4	2	23	2.3
		Number of mucous glands	0	0	0	0	0	0	0	0	0	0	0	0
		Average size of granular glands present (μm^2)	13385.742	16872.212	18833.218	21823.848	24550.351	27470.331	0	0	0	19379.884	142315.586	20330.798
		Average size of seromucous glands present (μm^2)	8788.412	14775.847	7805.202	7728.788	7197.241	10316.992	13369.643	8941.829	10776.625	8532.285	98232.864	9823.2864
		Average size of	0	0	0	0	0	0	0	0	0	0	0	0

		mucous glands present (μm^2)												
		Thickness of epidermis (μm)	107.476	90.955	84.517	112.819	100.79	118.682	89.352	82.427	99.468	61.848	948.334	948.334
		Thickness of stratum spongiosum (μm)	145.615	170.297	128.311	184.867	162.266	187.021	211.748	126.233	188.908	114.384	1619.65	1619.65
		Thickness of stratum compactum (μm)	133.156	109.23	123.208	101.233	167.468	141.188	125.564	124.527	99.652	127.414	1252.64	1252.64
MWC1612	VC	Number of granular glands	0	0	0	0	0	0	0	0	1	0	1	0.1
		Number of seromucous glands	3	3	2	4	2	3	3	3	1	1	25	2.5
		Number of mucous glands	1	0	1	0	0	0	0	0	0	0	2	0.2
		Average size of	0	0	0	0	0	0	0	0	24572.41	0	24572.41	2457

		granular glands present (μm^2)												2. 41
		Average size of seromuc ous glands present (μm^2)	10624 .905	12001.813	11945 .164	13280.78 3	13588 .161	14526.466	12769.6 43	9243.545	7844.733	15053 .092	120878.3 05	12 08 7. 83 05
		Average size of mucous glands present (μm^2)	3586. 002	0	4796. 631	0	0	0	0	0	0	0	8382.633	41 91 .3 16 5