Chapter

Reptilian Skin and Its Special Histological Structures

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Abstract

Reptilian skin is covered with scales forming armor that makes it watertight and enables reptiles to live on land in contrast to amphibians. An important part of the skin is the horny epidermis, with thick \textit{stratum corneum} in which waxes are arranged in membrane-like layers. In lizards and snakes, the whole skin is covered in overlapping epidermal scales and in turtles and crocodiles in dermal scutes. The cornified part of the epidermis is strengthened by \( \beta \)-keratin and sometimes \( \alpha \)-keratin. In crocodiles and many turtles, the outer scale surface consists of \( \beta \)-keratin and the hinge region containing \( \alpha \)-keratin. In lizards and snakes, both keratins form continuous layers with the \( \alpha \)-keratin below the \( \beta \)-keratin. Some reptiles have developed a sensitive mechanosensory system in the skin. The colors of reptile skin are produced by melanocytes and three types of chromatophores: melanophores, xanthophores, and iridophores. The color patterns may be fixed or the chromatophores may provide rapid color change. Skin from different species of reptiles, turtles (red-eared slider (\textit{Trachemys scripta elegans})), snakes (Emerald tree boa (\textit{Corallus caninus}) and Burmese python (\textit{Python bivittatus})), Cuvier’s dwarf caiman (\textit{Paleosuchus palpebrosus}), lizards (Leopard Gecko (\textit{Eublepharis macularius})), and Green iguana (\textit{Iguana iguana}), were examined with histology techniques and compared.

Keywords: skin, histology, reptiles, special features

1. Reptiles

Reptiles are one of the six main animal groups together with amphibians, invertebrates, fish, birds, and mammals. They are tetrapods that diverged from ancestral amphibians approximately 340 million years ago. There are two characteristics that early reptiles had developed when diverging from amphibians: scales and amniotic eggs (eggs with an internal fluid membrane), which are still of great importance for them. Extant reptiles are represented by four orders: Squamata (lizards, snakes and worm-lizards), Crocodilia (alligators and crocodiles), Chelonia (tortoises and turtles), and Rhynchocephalia (tuatara) \cite{1}. Squamates are the most diverse from all of the groups and have exceptional skull mobility. The only exception to this exceptional skull mobility is the almost extinct tuatara, which only lives on a few New Zealand islands. This reptile has a skull which is not joined, the reptiles grow slowly and reproduce at a slow rate and have a prominent parietal eye on top of the head.

Snakes are carnivorous reptiles with highly mobile jaws, which enable them to swallow prey much larger than they are. They are legless (some species retain a pelvic girdle) and have an elongated body, this means that paired organs appear one in front
of other and they only have one functional lung. Some species have venom, used primarily to kill prey. Their skin is covered in scales and snakes are not slimy [2]. Lizards are quadrupedal squamates, except some legless, snake-like-bodied species. Often, they are territorial and have many antipredator strategies, such as camouflage, venom, reflex bleeding, and the ability to destroy and then regenerate their tails after destruction. They are covered in overlapping keratin scales, enabling them to live in the driest deserts on the earth [3, 4].

Crocodilians are the largest reptiles, and include the alligators, crocodiles, gharials, and caimans. They have elongated, structurally reinforced skulls, powerful jaw muscles, teeth in sockets, and a complete secondary palate; they are oviparous and, interestingly, adults provide extensive parental care to young.

Turtles are among the most ancient of the reptiles alive today and have changed little since they first appeared 200 million years ago. They have a protective shell that encloses their body and provides protection and camouflage. They have keratinized plates instead of teeth and a shell that consists of a carapace and plastron [5, 6].

2. Histology of reptile's skin

An integumental challenge for reptilian's terrestrial life was developing mechanisms in order to prevent water loss and to protect against ultraviolet irradiation, mechanical shields which offered protection and enabled evolution of different types of reptilian scales and scutes [3, 7].

Some skin histology features are similar between mammals and reptiles; on the other hand, they also have numerous differences. Reptiles have a reputation that they are “slimy” when we touch and hold them; however, they have dry skin, which has even fewer glands than mammals or amphibians. The main special feature of their skin is that the epidermis is heavily keratinized with a layer, which also prevents water loss. This feature reflects their greater commitment to a terrestrial existence. Scales are present but are fundamentally different from the dermal scales of fish. In reptiles, scales cannot be scraped off as in fish because they are an integral part of the skin. The reptilian scale usually lacks the bony under support of any significant structural contribution from dermis. It is a fold in the surface epidermis, an epidermal scale. The junction between adjacent epidermal scales provides a flexible hinge. If the epidermal scale is large and plate-like, it is also termed scutes. Epidermal scales in different species can be overgrown and skin protrusions can be formed in different regions, such as microornamentation, pits, sensory receptors, spines, horn-like processes, crests, scutes, plastron, carapace, and some others [7, 8]. These protrusions are essentially only of epidermal origin, without dermal participation [1]. In the perfect resting phase, the epidermis generally consists of four layers of dead but fully differentiated keratinocytes and basal live keratinocyte layer that form three main layers: stratum basale (germinativum), stratum granulosum, and stratum corneum.

The inner layer, stratum germinativum, consists of cuboidal dividing cells that produce the protein keratin. The intermediate layer (stratum granulosum) has a lipid-rich film that plays a major role in providing water-permeable barrier in the skin. The outer stratum corneum is heavily keratinized in scales. Two forms of keratin are produced in reptiles: \( \alpha \)-keratin, which is flexible, and \( \beta \)-keratin, which provides strength and hardness and is unique to reptiles. \( \beta \)-keratin is found on the chelonian shells, whereas \( \alpha \)-keratin is found in the hinges or between the scutes [9–11]. It is in these weaker links that mites or infections can be present. The thick, keratinized skin of reptiles is at the expense of the cutaneous sensation. Reptiles have far less sensory feeling in their skin than birds or mammals, which is why they are more at risk from thermal burns in captivity (e.g., lizards and hot stones).
many reptiles, dermal bones (*gastralia*) are present especially in the abdominal area; however, they are not associated with scales.

The main layers of epidermis change prior to molting in the reptiles that slough large pieces of the cornified skin layer. In the turtles and crocodiles, sloughing of skin is modest, comparable to birds and mammals, in whom small flakes fall off at irregular intervals. But in lizards, and especially in snakes, shedding of the cornified layer, termed molting or ecdysis, results in removal of extensive sections of superficial epidermis. As molting begins, the *stratum basale*, which has given rise to the *strata granulosum* (inner) and *corneum* (outer), duplicates the deeper layers of granulosum and corneum, pushing up under the old layers. White blood cells invade the *stratum intermedium*, a temporary layer between old and new skin. These white blood cells are thought to promote the separation and loss of the old superficial layer of the skin [8]. Molting of different reptile species will be discussed in more detail later in the chapter.

The *dermis* in reptiles consists of fibrous connective tissue, blood and lymphatic vessels, nerves, and pigmentary cells. At the areas where dermal bones support the epidermis, bony plates called osteoderms, plates of dermal bone, are located under epidermal scales. They are present in crocodilians, some lizards, and some extinct species. Some bones of the turtle shell are modified osteoderms which have fused with the vertebrae to form a shell.

**Subcutaneous layer** (*hypodermis*, hypoderm, and *subcutis*) is the layer of tissue, which lies beneath dermis and mainly consists of fibroblasts, adipose cells, and macrophages. Subcutaneous fat is mostly poorly developed in reptiles in comparison to mammals. Some species are known for substantial subcutaneous fat pads, such as some species of geckos like the Mediterranean house gecko (*Hemidactylus turcicus*), and some of the snakes and lizards have paired abdominal “fat bodies” (*corpora adiposa*) that serve as the primary location for fat storage in adipose tissue. Also, the tail, especially in geckos, can be a large deposit of the subcutaneous tissue, however not in the snakes. These tail deposits include “inner fat” surrounding the caudal vertebrae, as well as subcutaneous caudal fat and are most concentrated near the base of a tail. These fat tissues could be for some animals the major adipose store in the body [12, 13].

**Integumental glands** of reptiles are usually restricted to certain areas of the body. In different species, there are some glandular-type tissues in different parts of the body, such as rows of femoral glands under femoral pores alongside the inner part of the thigh region of the hindlimb, which are observed especially in males. In some species of Crocodilians and turtles, scent glands are present. In male and female alligators, one pair of scent glands open in cloaca and another pair on the margins of the lower jaw. In some turtles, scent glands can produce quite pungent odors, especially when the animal is alarmed by handling. Precloacal pores are observed in some lizards and crocodiles. Most integumental glands in reptiles are thought to play a role in reproductive behavior or when predators are close, and their social role has not yet been well studied [8, 9, 11].

### 3. Reptile groups and their special skin features, especially their scales/scutes

The skin of reptiles reflects their greater commitment to a terrestrial existence as mentioned earlier in the chapter. Keratinization is extensive and skin glands are fewer than in amphibians. Scales are present, but these are fundamentally different from the dermal scales of fish. The reptilian scale usually lacks the bony under support or any significant structural contribution from the dermis. Instead, it is a fold in the surface epidermis, hence, an epidermal scale. The junction between adjacent epidermal scales is the flexible hinge (*Figure 1*). If the epidermal scale is large and
plate-like, it is sometimes termed a scute. Additionally, epidermal scales may be modified into crests, spines, or horn-like processes. Although not usually associated with scales, dermal bone is present in many reptiles. The gastralia, a collection of bones in the abdominal area, are examples of these. Where dermal bones support the epidermis, they are called osteoderms, plates of dermal bone located under the epidermal scales. Osteoderms are found in Crocodilians, some lizards, and some extinct reptiles. Some bones of the turtle shell are probably modified osteoderms.

Scales have many important functions, such as playing vital roles in skin permeability and providing protection from abrasion, and therefore tend to be thicker dorsally than ventrally. In some species, they form into large plates and shields on the head. In snakes, they are widened ventrally to form gastropeges, which are important for locomotion [1, 8].

3.1 Lizards and their skin properties

3.1.1 Scales

The lizard’s skin is specific due to scales that form dense tight rows. Lizard scales vary in form from tubercular to plate-like or even largely overlapping each other in formation. The scales originate from the epidermal superficial layer of the skin and form keratinized wrinkles and may have bony plates underlying them (osteoderms). They are very close to each other, and links between them allow them to move in all directions. In lizards, scales can vary in form and be modified into crests, spines, or horns, depending on the type of lizard and on the body part of the lizard and are often of use in taxonomically differentiating species. On the head and on the ventral part, scales are plate-shaped. Scales are important to prevent water loss from the body, as well as to protect the body from injury, because the lizards touch the ground with the ventral surface of the body and thus damage the skin. The skin in the lizard does not follow the growth of the body, so they have to change it, which does not happen in one piece but in several smaller pieces [14].

In some lizards, it is characteristic that their fingers are covered with large scales. These scales serve them to move easier as in the case of the basilisk lizard (Basiliscus...
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*basiliscus*, especially on the water surface or are helpful in the sand skink (*Neoseps reynoldsi*) to move in the sand. Geckos (Gekkonidae) have flattened fingers, characterized by around 20 leaf-like formations on the ventral side of the toes, named lamellas, with a structure that enables animals to climb on the vertical and very smooth surfaces (Figure 2). Lamellas consist of setae (110 μm in length and 4.2 μm in width), which are similarly oriented and uniformly distributed in arrays [14]. Each seta branches to form a nanoarray of hundreds of spatular structures, which are 0.2 μm in length and width at the tip which then make adjacent contact with the surface. Gecko setae are formed primarily of β-keratin with some α-keratin components [15].

The skin glands are mostly restricted to certain parts of the body. Thus, in the medial side of the thighs of many lizards, for example, Green Iguana (*Iguana iguana*), there are femoral pores, beneath which femoral glands are located. These glands are larger and usually more developed in males. They secrete a waxed secretion that contains various pheromones relevant to the mating period or when the animal feels endangered. They also help to determine sex in these species [14].

The lizards do not have an external ear; however, in some species, they have a fold of the skin and tympanic membrane that can be seen from the side on the head in a shallow recess. This membrane is covered by a thin membrane in some species that is also changed in the process of ecdysis.

Some lizards such as Green Iguana (*Iguana iguana*) have partial third eye. This organ is a superficial parietal gland which also contains a lens, cornea, and retina, and is located immediately below the skin in the parietal opening between the parietal and frontal bones. The partial eye is a cavitary organ, which is constructed from epithelial cells that contain secretion glands and photoreceptors that convert light stimuli into neuroendocrine messages that can play an important role in thermoregulation but also in hormone production [16].

Lizard skin contains classical skin layers, which can vary in morphology at different positions. Here, we compare skin of the Leopard Gecko (*Eublepharis macularius*) (Figure 3) with the skin of the Green Iguana (*Iguana iguana*) (Figure 4), both sampled from the dorsal region. The most visible difference in the epidermis was seen in the level of keratinization, where the skin of the Green Iguana was keratinized to a higher extent and the outermost β-layer was much more pronounced. The second most prominent feature observed are the melanocytes, where in the Green Iguana the melanocytes are hardly seen at all. On the other hand, Leopard Geckos can vary very much in color and they exist in various color mutations

Figure 2.
The ventral view of a New Caledonian Giant Gecko (*Rhacodactylus leachianus*) climbing a vertical glass surface. On the ventral view of the foot of a New Caledonian Giant Gecko, a foot adhesive system is observed with adhesive lamellas which consist of microscale array of setae, which are together clustered in tetrads (Photography, Valentina Kubale).
(termed morphs). Our sample originates from the most common one, which is basic yellow in color with black spots, which also contains more melanophores. Other morphs include the high yellow (less black spots), hypomelanistic with ten or less dark spots on the body or on the other hand hypermelanistic, which has darker pigmentation but is not black in color. Blizzards are morphs that are completely pattern less. The lavender Gecko has light violet or lavender color included, the tangerine one has an orange color included in its coloration, the carrot tail has orange color on the tail and there are some more variations in color also present in different geckos [14].

3.1.2 The production of color

Especially important for camouflage (mimicry) is the skin color. Skin color is susceptible to changes depending on the amount of sunlight and it may be darker or lighter. Chromatophores are pigment-containing cells found in the dermis of the

Figure 3.
Dorsum skin histology of the Leopard Gecko (Eublepharis macularius) by H&E staining. The sample was taken in the resting stage of the epidermis, when the animal was not in the process of ecdysis. In epidermis, the most visible part is the basal layer with keratinocytes with nuclei, which are dividing by mitosis. In the figure is the part with overlapping scale. The cornified α-layer is very well visible. The intervening mature stratum (mesos) consists of a few layers of cells, which are often not very well seen under this magnification. Partially is also separated from the lower strata as well as the outermost β-layer. In the dermis, fibrous connective tissue, vessels, nerves, melanophores, and Merkel mechanoreceptor cells are observed. (A) 100× magnification, (B) 400× magnification.

Figure 4.
Dorsum skin histology of the Green Iguana (Iguana iguana) by H&E staining. The sample was taken in the resting stage of the epidermis, when the animal was not in the process of ecdysis. In epidermis, different layers are observed. The stratum granulosum is not very clearly distinguishable with the nuclei. Cornified α-layer and the outermost β-layer are very visible. In the dermis, fibrous connective tissue, vessels, and nerves are observed. Fibers of the connective tissue are laid in a kind of pattern (A) 100× magnification, (B) 400× magnification.
skin and provide a large range of colors by changing the position of their granules. This ability is particularly significant for the chameleons, although it is also observed to a lesser extent in other types of lizards, such as the New Caledonian Giant Gecko (Figure 5), and in some species when light and temperature influence change of skin color to more pronounced such as in the Saharan Uromastyx (*Uromastyx geyri*) (Figure 6). The color of the lizard’s skin can also be affected by the environment and by the endocrine system [17, 18]. These pigment cells are not just confined to skin but can also occur in the peritoneum of some species, for example in turtles. Animals of the same species during breeding may, due to different mutations, change their basic color and thus produce offspring with new patterns, which are new morphs.

Chameleons are an extreme example group of lizards, and, of all the reptiles, they have the highest ability in relation to changing their skin coloration and pattern through combinations of pink, blue, red, orange, green, black, brown, light blue, yellow, turquoise, and purple [19]. Chameleons change skin color depending on the temperature of the surrounding area, their physical condition, intraspecies signaling and communication. Color change is also important for their camouflage. It signals a chameleon’s physiological condition and also shows its intentions toward other chameleons [19]. Chameleons tend to show brighter colors when displaying aggression to other chameleons, and darker colors when they signal they are not fighting [20].

Chameleons transform color by changing the space between the guanine crystals which are present in specialized cells named chromatophores. The color change is based upon the wavelength of light reflected off of the crystals. The skin of the chameleons is as in other reptiles consisting of epidermis, dermis, and hypodermis. The important features chameleons have regarding skin color are located in the dermis. It is within the dermis that the blood vessels, nerves, skin muscles, and special cells named chromatophores are present. The chromatophores contain guanine crystals and are subdivided into differing types including iridophores, xanthophores, erythrophores, guanophores, and melanophores (Figure 7).
The iridophores (granulophores) lie in the dermis. They are the most important for true color change, not just changing shades of the same color. They contain semi-crystalline nanocrystals of the amino acid guanine (the breakdown of uric acid) that reflects light. They are arranged in a network in a surface and in a deeper layer. In the deeper layer, iridophore crystals have a protective role for the organism against harmful rays. At the surface of the iridophore, smaller crystals are located, which can diffract different wavelengths of light, depending on their arrangement and density. The blue wavelengths are reflected more to produce a blue coloration in an effect called Tyndall scattering. When combined with the yellow carotenoids, they emit green color, which is a common camouflage in many reptiles [9]. Guanophores contain a colorless crystalline substance called guanin and reflect among others the
blue part of light. Xanthophores produce the pigments called pteridines and are
important for yellow shades of the skin. Erythrophores contain the pigment carotene, which they get from the other parts of the body and are important for shades of red. Both types of cells are located above the iridophores and when they cover each other they can form different color combinations. Green color is the consequence of the yellow pigment which covers the refracted blue color coming from iridophores. Melanophores produce the pigment melanin and they lie the deepest within the dermis. Pigment melanin-containing cells give rise to black, brown, yellow, and gray coloration. Albinism in reptiles is caused by lack of melanin. The carotenoid cells are found beneath the epidermis above the melanophores and produce yellow, red, and orange pigments [9], so albino reptiles are often yellow to orange color.

Dispersion of the pigment-containing organelles is only a partial mechanism [17]. Different chromatophores are arranged in two superimposed layers within their skin that control their color and thermoregulation. The top layer contains a lattice of guanine nanocrystals, and by exciting this lattice, the spacing between the nanocrystals can be manipulated, which in turn affects which wavelengths of light are reflected and which are absorbed. Exciting the lattice increases the distance between the nanocrystals, and the skin reflects longer wavelengths of light. Thus, in a relaxed state, the crystals reflect blue and green, but in an excited state, the longer wavelengths such as yellow, orange, green, and red are reflected [14, 20].

4. Snake's skin and scale features

In snakes, the skin is entirely covered with scales, specific to reptiles. The scales are set together as piles covering each other and are comprised of the upper part of mucosal layer of the skin with subcutaneous tissue below; they are keratinized and protect snakes from skin injuries and dehydration, basically to make it air-proof. When considering the position on the body, they have a different layout and shape. Scales, especially on the head, have an important role in determining the species of snakes. Smaller scales are found dorsally on the body and are placed in several rows. On the ventral, abdominal part of the body, scales are wide and transversally positioned. The shape and number of scales on the head, back, and belly are characteristic to each family, genus, and species. Scales have a nomenclature analogous to the position on the body. In “advanced” (Caenophidian) snakes, the broad belly scales and rows of dorsal scales correspond to the vertebrae, allowing scientists to count the vertebrae without dissection [14]. Scales protect the body of the snake, aid it in locomotion, allow moisture to be retained within and give simple or complex coloration patterns which help in camouflage and antipredator display. In some snakes, scales have been modified over time to serve other functions such as those of “eyelash” fringes, and the most distinctive modification—the rattle of the North American rattlesnakes. The snakes also use scales for different types of movement because they have lost their limbs through the evolution process [22]. With the abdominal part of their scales, snakes can resist the unevenness of the surface and move across bare terrain such as sand and roads, where they cannot push off rocks and branches (lateral undulation type of movement) and with their muscle strength push their body forward. Besides that, it is mathematically proven that snakes also rely on the frictional properties of their scales to slide [23]. The resistance of a snake’s belly scales is highest when its body is sliding sideways, rather than forward or backward. Snakes also seem to lift the parts of their bodies where friction is slowing movement the most, enabling them to slither faster. Snakes can move by folding themselves into pleats, contracting their bellies,
contorting into helices or slithering in an S-shape. Scientists suggested that the snakes’ belly scales, which can catch small bumps in the ground, might also aid movement. Behind the cloaca, scales are smaller and usually positioned in two lines. When closely observed, the border between the body and the tail is seen [23].

Snakes have pigmented scales; their color can change in certain species and some snakes are also albino snakes. Color of a young snake can be brighter than in adults and may also depend on the geographical position of the snake. In the cubs of green tree python (*Morelia viridis*), the color is yellowish to orange, however adult animals become green. The color of some boa species is also associated with the period of the day, where they are mostly darker at daylight and brighter at night time.

There is a polymorphism in snakes, but it is not common. It can be seen, for example, in the Turks Island Boa (*Epicrates chrysogaster*) and the Californian Royal Snake (*Lampropeltis getula californiae*), in which cells at the part of the snake may appear on individual animals forming a pattern and on the other part forming lines at the same time. During breeding of snakes, genetic mutations have emerged recently, and partial albinos are possible. During breeding, due to various mutations in the Ball Python (*Python regius*), color change may occur, and new species of morphs can be developed (Figure 8).

Snake skin contains pigmented cells and snakes use their color to camouflage (mimicry) or in order to give warning signs. A special form of mimicry is imitation of color, in which poor poisonous and non-lethal species of snake, such as the Arizona Mountain Kingsnake (*Lampropeltis pyromelana pyromelana*) (Figure 9), mimics an extremely poisonous painted coral snake (*Micrurus corallinus*) to protect themselves from predators [14].

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**Figure 8.**
A portrait of different patterns, colors, and morphs of Ball python (*Python regius*) (Photography, Leja Hrovatin, Nina Bajec, Nika Glavina, and Tilen Holynski). Very well-known and desired morphs are: Super Pastel Ivory morph (C), Piebald morph, Super Pastel Axanthic; Butter Pastel: Cinnamon, lesser (A), Super Pastel Spider; (D) Coral Glow/Banana in the comparison to normal ball python (B).
Snake’s skin is similarly as in lizard’s consisted of germinal layer of the epidermis spinosus-like keratinocytes that alternate to hard ($\beta$) and soft ($\alpha$) layers. Samples from two different species of snakes were observed histologically and skin samples were collected from different parts of the body. We have observed skin at the abdominal (ventral) part and the tail of the Burmese Python ($Python$ $bivittatus$) (Figure 10) and the lip part of the Emerald Tree Boa ($Corallus$ $caninus$) (Figure 11). From the outer scale surface toward the dermis are the oberhautchen layer, $\beta$-layer, mesos layer, $\alpha$-layer, lacunar layer, and the clear layer [1, 24, 25].

Iridescence is caused by the physical properties of light on the thin and transparent outer layer of the skin. When light strikes from an angle, the light spectrum is split into wavelengths of different colors. Depending on the color of the scales, this will cause iridescent effect when the snake moves. This feature is more obvious in black or dark snakes like the white-lipped python ($Liasis$ $albertisii$) (Figure 12) [14].

Snakes living among the leaves are most often green, and those living in the desert are often yellowish or reddish. Snakes have no skin glands other than cloacal glands. Some species can detect infrared light. Primitive boas have pronounced sensory receptors in the skin and can detect mice at a distance of 15 cm. Between the nostrils and the eye, in some snakes, there are special infrared receptors (pits) that allow them to feel hot-blooded animals and to attack them in the dark. These receptors are innervated with $n$. $mandibularis$, $n$. $maxillaris$, and $n$. $ophthalmicus$ (branch of $n$. $trigeminus$). Along the upper and lower lumbar scales, there are fewer pits, which are also innervated by the branches of $n$. $trigeminus$. These organs are extremely sensitive to temperature changes of as much as 0.002°C [14].

At the ends of the tails of rattlesnakes ($Crotalus$ $sp.$), there is a special anatomical adjustment—a rattle. Also, their name derives from the Greek word krótalon, which means “rattle” or “castanet,” and refers to the rattle on the end of the tail which makes this group (genera $Crotalus$ and $Sistrurus$) very distinctive [26]. The rattle consists of up to 20 loosely interlocking hollow shells, each of which is at one point the scale covering the tip of the tail. Their number depends on the type and gender of the animal. In most other snakes, the tail tip is cone-shaped and not much thicker than the rest of the skin. It is shed along with the rest of molt. However, in rattlesnakes, it does not shed, and it also gets elongated, since younger specimens may shed three to four times per year, every time adding a new segment to the rattle. The end of the tail is much thicker and round shaped at the end, with one or two annular constrictions to prevent it from falling off. Before each shedding, a new button will
develop inside the last one and before the skin is shed, the tip of new button shrinks. This process continues, and an appendage consists of a number of interlocking segments that sound characteristically. The sound is generated by friction one button to another, especially when snake feels endangered. In grass snakes (*Natrix natrix*), pine snakes (*Pituophis spp.*), and kingsnakes (*Lampropeltis spp.*), similar sounds are produced as heard in rattlesnakes by shaking their tail or other body parts against the surface where they are [27].

Snakes periodically molt their scaly skins and acquire new ones. This permits replacement of old worn out skin, disposal of parasites, and is thought to allow the snake to grow. The shape and arrangement of scales are used to identify snake species [14].
5. Skin and scute features in crocodiles

In crocodiles and turtles, the dermal armor is formed from the deeper dermis rather than the epidermis and does not form the same sort of overlapping structure as snake scales. These dermal scales are more properly called scutes. Similar dermal scutes are found in the feet of birds and tails of some mammals and are believed to be the primitive form of dermal armor in reptiles [8].

The crocodile skin has horny plates, named scutes, in which shape, number, and position are important for the identification of the species. They can also become similar to bones and form an outer bone armor. The horny plates on the back are referred as the back shield, and below are dermal plates (4–10 longitudinal plates whose number varies depending on the animal species). On the abdominal side beneath the horny scutes, there are no bone plates. On the tail, scutes form rings with two rows continuing in one row of scutes by the end of the tail. The position of the horny scutes on the head is characteristic for each animal species. On the head beneath the horny scutes, bone plates are located. Unlike other reptiles, crocodiles do not shed their scutes, and they are renewed by scrubbing against different outer surfaces [28].
In the skin of the crocodile, pigmented cells are located that give a color that varies from green to light brown to gray. In most animals, the belly is lighter than the rest of the body. Scent glands in crocodiles open in the cloaca. Alligators of both sexes have one pair of scented glands.

Crocodiles can recognize the prey on land even when they are under water because their eyes are located dorsally on their heads. They have very well-developed hearing and vision. Their upper eyelids are more mobile than the bottom ones and there is a tarsal bone plate located in the lower eyelid, which can develop into bony structure in some years. The upper eyelids are used to close the eye. The crocodile has also developed a third eyelid containing a cartilage, covering the eye when the animal is under water. They have an external hole on the head that looks like a rasp to collect sounds from the environment and is closed with a fibrous moveable lid that closes the aperture when the animal dives [14].

A very interesting feature in the crocodilian skin is the higher density of “integumentary sensory organs” (ISOs) in their dermis, which are particularly dense in the mouth area and the facial part of the head. They contain multiple mechanoreceptors, which are innervated by the vast network of the peripheral nerves [29]. They are important for the detection of surface waves generated by the moving prey and important for regulating jaws closing, depending on the size of the prey [29, 30]. ISOs are observed as a common feature in the skin, observed as a lamellar body (Figure 13).

### 6. Turtles and their special skin features

In the turtle, there are free parts of the body, such as the head, legs, and tail, covered with scales. In a turtle, the skin’s appearance varies from smooth skin, where we can hardly see scales, to thick and crusty skin, which depends on the adaptation and the way of life. Toward the neck, the skin is wrinkled. Because of the adaptation of the land-based lifestyle in the Testudinidae family, the thicker skin is visible, and the scales are more pronounced. Changing the scales in turtles is periodic and individual and is more pronounced in aquatic turtles [14].

The turtle skin consists of the superficial part (*epidermis*) and the inner layer (*dermis*). Between these two layers, there is a basal lamina (BL). The surface layer...
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consists of three layers: stratum basale, stratum granulosum, and stratum corneum. In the stratum basale, new cells proliferate and replace old and dead cells and push them toward the surface of the skin. Epithelial cells, keratinocytes, which are found in the stratum corneum, produce the protein keratin, which plays a key role in reducing loss of water. On the parts of the body that are more exposed to mechanical pressure, the keratinized layer may be even thicker. There are no blood vessels in the skin epidermis, so the epidermis cells are fed by diffusion from the deeper layers of the skin through the BL. Apart from keratinocytes in the epidermis, melanocytes and Langerhans cells are also located there. Epidermis is developed from ectoderm, creates the BL, and has the function of retaining water in the body, as well as the protection against infections and harmful external influences. New cells created in stratum basale replace old and dead cells and suppress them at the surface of the skin. The skin dermis is derived from mesoderm and creates a reticular lamina (lamina reticularis). In this layer, there are many sensory nerves (nerve endings and mechanoreceptors) as well as glands, blood vessels, and lymph vessels. Subcutis is a fatty and slightly connective tissue (Figure 14).

In the turtle skin, horny plates are formed together with osteoderms. Dermal bones are found below in the inner part of the skin (dermis) and they grow together to gain more strength. Corneal scales are made of water-insoluble keratin, which are laid in the arrangement allowing a thin layer of skin between them that makes it easier for the animal to move. In tortoises, the osteoderms are grown together with the spine and ribs, thus forming the back of the armor, carapace. The back and abdomen of the armor, depending on the type of the turtle, consists of several bones (shields). Above the bones (osteoderms), there is a layer of skin (epidermis) which is in the turtles with soft shell (the genus Apalone, the genus of the turtle Dermochelys) “skinned.” In the other turtles, above the bony plates, there are also horny plates, which do not entirely match the shells’ strength and ability to regenerate [31].

Carapace is constructed from at least 38 corneal scutes, depending on the species of the turtle. In the middle of the carapace, along the back, there are vertebral or neural corneal scutes (mostly five). On the left and the right sides, the neural scutes have either bony or costal plates, and, laterally, there are marginal scutes. A series of smaller plates, which on the border with carapace and plastron,
are called inframarginal scutes. Cranial from the first neural scutes it is nuchal plate. Above the tail are two scutes named suprapygeal (supracaudal). In the intramarginal plates, Rathke’s pores are visible in sea turtles and similar structures can be observed in freshwater turtles. Below Rathke’s pores, Rathke’s glands are located, covered with fat tissue [31, 32]. The plastron is the nearly flat part of the shell structure of a turtle, which is basically the ventral surface of the shell. It also includes within its structure the anterior and posterior bridge struts and the bridge of the shell [32]. The plastron is made up of nine bones and the two epiplastra. The plastron usually consists of 12 plastral scutes, six on each side, which come together in the central line and their number depends on the shape of the shell and the type of turtle. Plastral formula is consisted from intergular, gular, humeral, pectoral, abdominal, femoral, and anal plastral scutes. The shape and mutual relationship of these scutes are of great importance in determining the species. In addition to the armor, turtles may also have specifically deployed jaw shells that may also be important in identifying the species. For example, in the sea turtles between the eyes, there are two horned shells that are characteristic of the Green sea turtle (Chelonia mydas), while in the other species there are more or only one. In the turtle, the dormant scutes are shed individually [14, 32].

In some turtles, fragrant glands are open in the cloaca, and in some species, they produce an intensive smell, especially when they feel endangered. For most skin glands, it is considered to play a major role in reproduction or defense against predators. In the terrestrial turtles, glands are located only on the thighs, while in the water turtles, the mucous glands are found along the skin. During the hibernation of turtles, gas exchange occurs through the skin, while being buried in the ground or for example at the bottom of the lake. The turtles have developed lacrimal glands (gll. lacrimalis) and Harder’s glands that, like lacrimal glands, produce tears and contain immunocompetent cells. In the sea turtles, the lacrimal gland has been altered and modified into the solitary gland. The turtles do not have nasolacrimal ducts (ductus nasolacrimalis) and tears are secreted by evaporation.

7. Ecdysis

The shedding of scales is called ecdysis, trivially mostly named molting or sloughing. Sloughing serves number of functions. Firstly, the old and worn skin is replaced and secondly it helps to get rid of parasites (mites and ticks). In the epidermis, which is generally consisted of stratum basale, stratum granulosum, and stratum corneum, during the process prior to molting in the reptiles that slough large pieces of cornified skin layer changes occur [8].

In snakes, the complete outer layer of skin is shed in one piece and layer. During molting, most animals also change their behavior, they prefer to hide or move to a safe place and refuse food. In snakes, a thin skin layer in the form of a thin transparent membrane (spectaculum) covers their eyes; therefore, before sloughing, their vision is the weakest, as they have cloudy eyes which become bluish in color. This affects their behavior, and because of their faint vision, they become more nervous. The snakes become restless and begin to rub on uneven surfaces [1]. Just before shedding, the color of the skin becomes dull, colorless, and dry looking and the eyes become cloudy or blue-colored. Skin sloughing depends on many factors, such as growth rate, season, hibernation, mating, etc. Molting is repeated periodically throughout a snake’s life. Wild animals slough two to four times a year (younger, still growing snakes may shed up to four times a year and later only twice), which in captivity can occur more often. The most common complications in captivity occur due to reduced humidity in the vivarium. The skin has no role in changing the gases
except in the marine snakes that alternate the gases through the skin. When sloughing, they help themselves by rubbing at different surfaces. The old skin breaks near the mouth and the snake wriggles out, aided by rubbing against rough surfaces. In many cases, the cast skin peels backward over the body from head to tail, in one piece like an old sock. A new, larger, and brighter layer of skin forms underneath [14].

Snake scales are not discrete but are extensions of the epidermis; hence, they are not shed separately but are ejected as a complete contiguous outer layer of skin during each molt, similar to a sock being turned inside out. During the sloughing of the snake, the *stratum basale* is creating new cells, doubling *stratum granulosum* and *stratum corneum*. Specific to snakes and other reptiles is the formation of a *stratum intermedium*, a temporary layer between old and new skin. In this part of the skin, white blood cells help to separate layers and get rid of the old layer of skin. Due to the pushing of old skin, the *stratum basale* is duplicated and after sloughing, the skin is up to 20% larger. Before the snakes shed the old skin, a new layer is already formed underneath it. The process of changing skin, takes about 2 weeks. As snakes grow, their skin cannot keep up with their growth, so they occasionally shed the skin (Figure 15).

In the case of lizards, this coating is shed periodically, usually coming off in flakes, but in some cases, such as lizards having elongated bodies, in a single piece. Some geckos will eat their own shed skin. Ecdysis is controlled by the thyroid gland. Changes in feeding behavior and activity occur prior to ecdysis and the reptiles become very susceptible to dehydration. Snakes tend to shed the whole skin, unlike lizards and chelonians which shed pieces, which makes them more vulnerable during ecdysis. In a healthy snake, the whole process can take up to about

![Figure 15](image-url)

*Figure 15.*

*Mechanism of the ecdysis in snakes (adopted from Kardong [8], designed by Pia Cigler).* In lizards and snakes, the shedding of the cornified layer is called molting or ecdysis and results in the removal of the superficial epidermis. As molting begins, *stratum basale*, which has given rise to the *strata granulosum* (inner) and *corneum* (outer), duplicates the deeper layers of *granulosum* and *corneum*, pushing up under the old layers. During ecdysis, the cells in the intermediate layer replicate to form a new three-layer epidermis. Once this process is complete, lymph and white blood cells diffuse into the area between the two layers, and enzymes are released to form cleavage zone at the separation line. At this phase, the snake becomes gray, and snakes and lizards without the eyelids are also blind, because *cornea* is also changed. The old skin is shed, and the new epithelium hardens, decreasing permeability to become a new skin. *GL*—germinative layer, *IG*—inner generation of epidermis, *OG*—outer generation of epidermis, *SL*—separation line, *S*—slough.
2 weeks [8, 32]. In turtles and crocodiles, sloughing of the skin arises to a lesser extent and it is comparable to that of birds and mammals, in whom small flakes fall off irregularly and it takes longer periods.

8. **Clinical importance of histology and anatomy knowledge for the dermatology of reptiles**

Reptile skin heals much slower than mammalian skin, often taking about 6 weeks to fully restore the defect. Malnourished animals are hypoproteinememic and unable to produce enough enzymes to form true cleavage zone, resulting in dysecdysis (failure to shed). Lack of moisture will also delay the process [11]. Skin permeability increases when skin is in contact with water, so water baths are a good way to rehabilitate sick reptiles and treat dysecdysis [11]. Wound healing is slow in reptiles, so stitches should be left at least 6 weeks [32]. It is best to leave stitches in place until ecdysis occurs since the increased activity in the dermis in epidermis promotes better healing and strength.

It should also be considered that during ecdysis, the skin becomes more permeable and more vulnerable to parasites and infection.

9. **Materials and methods for histology sections**

Samples of the skin tissue of different reptile species were preserved in 5% formaldehyde. Pieces of different types of tissue were included in the paraffin by using usual procedure with the apparatus Leica TP1020. Histological slides were prepared before use by the procedure which ensured that histological sections adhered to the slides properly and prevented sections from falling off the slides during further procedures. Tissue samples embedded in paraffin were cut by a hand microtome (Leica) into 5-μm-thick slices, which were transferred with brushes onto the smooth surface of warm water bath (40°C) and from there on the microscopic slides. Histological slides were dried in a thermostat (50°C). For classical histological staining with hematoxylin-eosin (H&E), samples were deparaffinized in xylene substitute (Neoclear; Merck) (2 × 5 minutes) and afterward rehydrated in decreasing concentrations of ethyl alcohol (100% for 2 × 5 minutes, 96% for 5 minutes, 75% for 5 minutes) and distilled water (2 × 5 minutes). In the following steps, samples were stained with either hematoxylin (Merc) (2 minutes), washed in running water (20 minutes), stained with eosin (1–2 minutes), and washed in distilled water (5 minutes) or Toluidine blue dye solution (20 minutes) and washed in distilled water (5 minutes) three times. Further, dehydration in ethyl alcohol with increasing concentration was performed (75% for approximately 5 minutes [depending on the sample; appropriate timing is observed during staining for intensity of reaction], 96% for 1× around 5 minutes, 100% for 2 × 5 minutes). Clarification of samples after drying and staining was carried out in xylene substitute (Neoclear) (3 × 5 minutes). At the end, a drop of Neo-Mount medium (Merck) was applied onto each tissue sample and the sample was covered with cover slide. Pictures were taken on Nikon FXA microscope with Nikon DS-F1 camera and transferred to program for image analysis by Lucia-G.

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