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Comparative Histological Analysis of Caspian Thin-Toed Gecko (Reptilia, Squamata) and Persian sturgeon (Actinopterygii, Acipenseriformes) Skin

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Abstract
We carried out a comparative study on Caspian thin-toed gecko with Persian sturgeon. The skin plays a role in preventing damage to internal organs from mechanical stress and the harmful effects of sunlight, and is colored depending to the environment to protect against enemies. It also plays the role in the recognition and reproduction of members. The epidermis of *Tenuidactylus caspius* (Eichwald, 1831) resembles mammals and birds more than the epidermis of reptiles. The stratum corneum layer of the epidermis is the same thickness in the same body area of the Caspian thin-toed gecko representatives. *T.c. insularis*, which live by the seaside in horizontal position, have relatively few stratum corneum cells that cover the underside of the body, however, there are many air-filled cavities inside these cells. The stratum corneum underside of *T.c. caspius*, which are adapted to living in a vertical position, have a large number of cells, but they are flatter and have fewer air cavities. The stratum compactum layer of the dermis in the *T.c. caspius*, is denser and more compact than in the *T.c. insularis*. *Acipenser persicus* (Borodin, 1897) epidermis consist of outermost surface layer, middle layer and stratum germinativum. In sturgeon, the outer surface of skin is covered with a cuticle. Below it are mucous cells, taste buds and stratified flattened cells. The *stratum compactum* of *Acipenser persicus* is thick and covers the body like a shield.

Key words: *sturgeon, gecko, skin, epidermis, dermis, hypodermis*

1. Introduction

Caspian thin-toed gecko is widespread lizard in Azerbaijan (Hashimov et al. 2023). This species has a high adaptive capacity (Najafov et al. 2019). *Tenuidactylus caspius* (Eichwald, 1831) was first discovered in 1831 by Eichwald in the Baku mountains. In 1932 Menetrier caught this species in Baku and named them Uroma stix fasiata. At a time when it was assumed that only the subspecies of the *T.c. caspius* lives in Azerbaijan Peninsula, in 1978 Akhmedov found and studied the subspecies on the islands of Absheron *T.c. insularis.* At present, due to the development, the Caspian thin-toed gecko is distributed throughout. The morphological and histological structure of the *T.c. insularis* subspecies found in the Boyuk Zira of Absheron archipelago (40 ° 17′40 ″ N 49055′10 ″ N), Dash Zira (40 ° 17′27 ″ N 49 BC 58′40 ″ N) and in neighboring islands differs from the *T.c. caspius* living in other areas. And research on sturgeons was carried out within the framework of the project Azerbaijan Fish Farm-Baku State University.There are 6 species of sturgeon (*Acipenser persicus, Acipenser guldenstadti, Acipenser nudiventris, Acipenser stellatus, Acipenser ruthenus, Huso huso.*) in the Caspian Sea.

2. Materials and Methods

This research was carried out on the basis of a scientific work registered at BSU on April 04, 2021 under the number 3/262. Appropriate methodological approaches were used to study the histological structure of skin of *Tenuidactylus caspius* (Eichwald, 1831) and widespread in the territory of Azerbaijan. The research was conducted in 2022-2023. For this purpose, expeditions were organized to various territories of Azerbaijan. Population density of lizards was taken into account during the expeditions for histological studies. If the number of Caspian thin-toed geckos per 1000 m^2 was more than 10 individuals, then the lizards were taken from these populations and used for analysis. For histological studies 20 *Tenuidactylus caspius* (Eichwald, 1831) were used. Within the framework of the Azerbaijan Fish Farm-Baku State University project for research at the Yenikend Sturgeon Breeding Farm of "Azerbaijan Fish Farm" LLC, 10 medium-sized individuals of *Acipenser persicus* were caught for the purpose of research and joint research was carried out. The object was preliminarily fixed in 76% ethyl alcohol. Paraffin-embedded skin blocks were prepared from these samples and made 5 μm thick incisions. Paraffin is a water-insoluble substance (Hashimov et al. 2023). Since the tissue contains water, it is impregnated with paraffin. For water extraction from the tissue, we used ethyl alcohol. Firstly 76%, then 95%, and finally 100% ethanol are used during this process. After dehydration, toluene was used to obtain transparency. After skin clearing, it is embedded in paraffin, for microtome machine usage. The block is put on the stage of the microtome machine and incisions are made with a thickness of 5 μm. The slices are separated from the block and placed in distilled water prepared inside a container with a dark bottom at a temperature of 35°C. For solution preparation, add 5 ml of 95% etil alcohol for every 100 ml of its volume (Najafov et al. 2022). As soon as the cuts in the water take a straight shape, they are captured by the glass. Glass and mixture are dried at a temperature of 40-45°C. In this case, part of the paraffin melts and separates from the tissue, and provides adhesion of tissue to the glass (Najafov et al. 2021). The preparation of the dried glass is placed in two toluene baths for 5 minutes each. It is essential to remove paraffin and make the tissue transparent. Subsequently, the tissue is placed in containers with 100%, 95%, and 76% alcohol and aged for 4 minutes each. The sample is placed in distilled water for 10 minutes. The tissue is now ready to be dyed. Hematoxylin-eosin dyes are used to stain skin tissue. The tissue is kept in the hematoxylin-eosin dyed for 4 minutes, then placed under running water for 1-2 minutes (Najafov et al. 2022). Then the muscle tissue covered with a coverslip is examined under a light microscope.

3. Results

Skin is spesialized, semi-transperent and elastic orqan. Geckos have dry skin, while fish have mucus. Skin consists of epidermis, dermis and hypodermis layers. Epidermis is specialized, semi-transparent and semi-elastic orqan. The epidermis of gecko consists of keratinized stratified squamous epithelium tissue. Upper part of the gekco skin is in the form of scales. Each scale of Caspian thin-toed gecko consists of an outer surface, an inner surface, and a hinge region. Scales of lizards have microornamentations. Scales do not allow keratinized skin to become an obstacle to movement. The epidermis of gecko resembles mammals and birds more than the epidermis of reptiles (Figure 1). The basal cells are located on the surface of the basal membrane and form a whole layer (*stratum germinativum*). These basal cells are cuboidal in shape and have large nuclei. Basal cells of *stratum germinativum* divide by mitosis to form A and B types basal cells. The A-type basal cells are stem cells that can remain in place and reproduce by mitosis. The B-type cells entered into the middle layer of epidermis and connected by desmosomes. Inside the cells of this layer, begin to form lamellar bodies. Lizard's tongue is covered with keratinocytes. The outer shape of the skin of the Caspian thin-toed gecko and Persian sturgeon adapted to remove harmful substances. Caspian thin-toed gecko cleans oil-contaminated skin with the help of sand. First, the gecko turned on the sand, allowing the sand to stick to its body. A few minutes later, the lizard repeated the same actions. After the sand absorbed the oil adhering to the lizard, when the lizard moved, the substance absorbed by the sand spilled onto the ground along with the sand. The cell is not found in the *oberhautchen* layer of the skin, which is constantly interacting with the exterior. It is a layer of hard substances with special fibers. The formation of this layer can be easily observed on the new skin during the ecdysis. Between the old and new gecko epidermis there is a wide lacunar layer and rectangular, transparent cells.

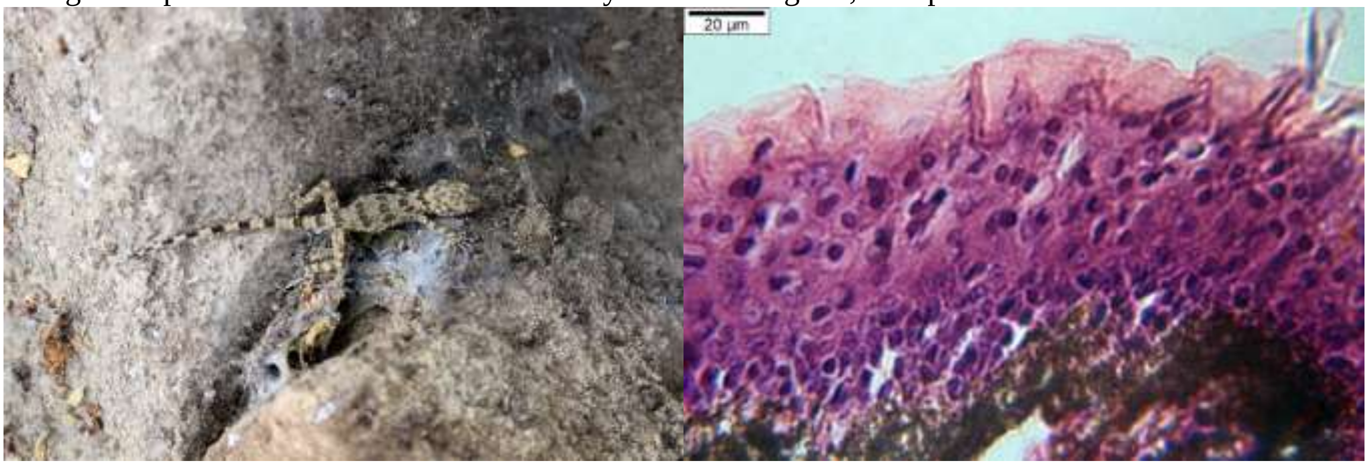

Figure 1. Caspian thin-toed gecko and histological structure of the epidermis

Acipenser persicus (Borodin, 1897) epidermis consist of outermost surface layer, middle layer and stratum germinativum. In sturgeon, the outer surface of skin is covered with a cuticle. Below it are mucous cells, taste buds and stratified flattened cells. The main cells of the middle layer of the epidermis are keratinocytes, which correspond to the stratum spinosum. Above these cells are flat cells with granules inside. But we did not notice that these cells form a whole layer. In the middle layer of the epidermis, a glandular duct can also be seen.The stratum germinativum consists of cuboidal or cylindrical cells located on the basement membrane. Melanocytes are also observed among these cells. The basement membrane separates the epidermis from the dermis.

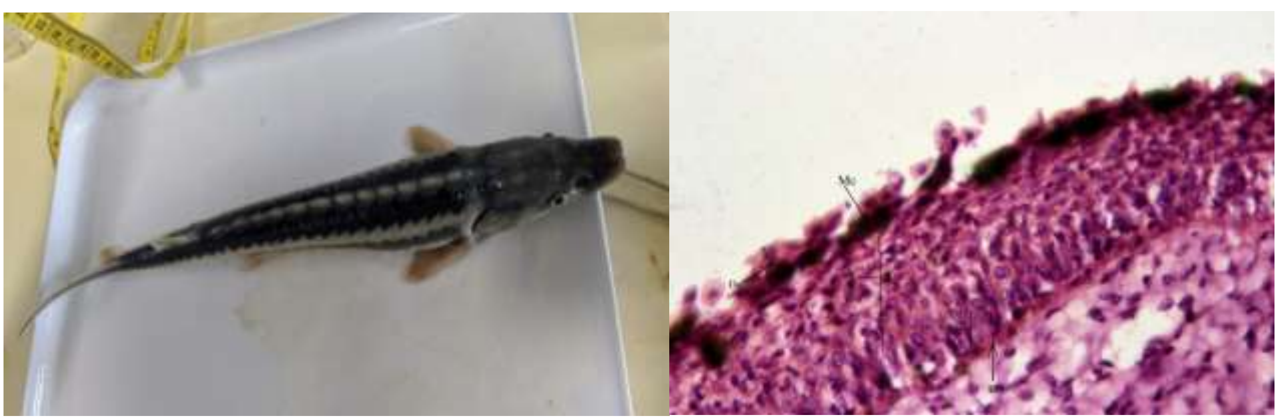

Figure 2. *Acipenser persicus* (Borodin, 1897) and histological structure of the epidermis of his skin (x40). Os-outermost surface layer, Ml-middle layer, Sg-stratum germinativum, Mc- mucous cells, Bm- basement membrane.

The dermis of lizard connects the epidermis to the hypodermis. This layer is full of collagen fibers in Caspian thin-toed gecko. The innermost part of the dermis (*stratum compactum*) in the *T.c. caspius* is denser composed and connective tissue elements assembled more compactly than in the *T.c. insularis*.

This layer is firmly joined to the bones and muscles in the gecko's tail and fingers. In the *T.c. caspius*, the binding of this layer to the bone is greater and denser than in the *T.c. insularis*. The *stratum laxum* in the *T.c. insularis* subspecies is thicker. In sturgeons, the dermis also can be divided into *stratum laxum* and *stratum compactum*. The stratum laxum, upper layer of the dermis, is also rich in elastic fibers and capillaries. Active fibroblasts are also observed in this layer. The *stratum compactum* of *Acipenser persicus* is thick and covers the body like a shield (Figure 3).

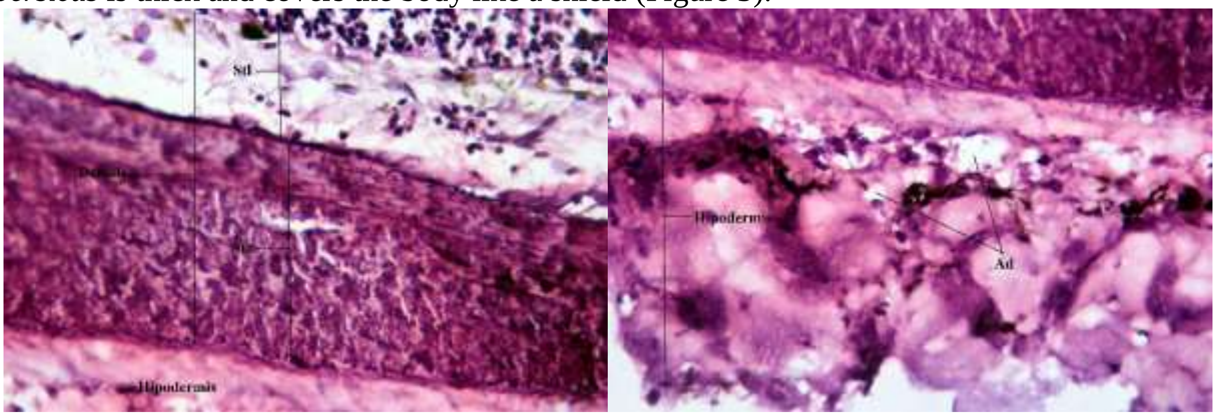

Figure 3. Histological structure of dermis (left, x40) and *hipodermis* (right, x90) of *Acipenser persicus* (Borodin, 1897) skin. Stl- *stratum laxum*,Stc- *stratum compactum*, Ad- adipose cells

The chromatophores, which are more common on the skin of the upper part of the body, provide camouflage and protection from harmful sun rays. Melanophore cells accumulates dark pigment (Figure 4). Depending on the amount of pigment around the nucleus or in the pseudopodia of the melanophores, the upper part of the reptile's skin becomes light or dark. The melanophores have also been observed in *stratum germinativum*, *stratum spinosum* and *stratum granulosum* layers of the epidermis. Lipophores turn yellowish because they collect lipochrome droplets. Although guanophores cells do not accumulate pigment, since guanophores have platelets they reflect light (Hashimova et al. 2018).

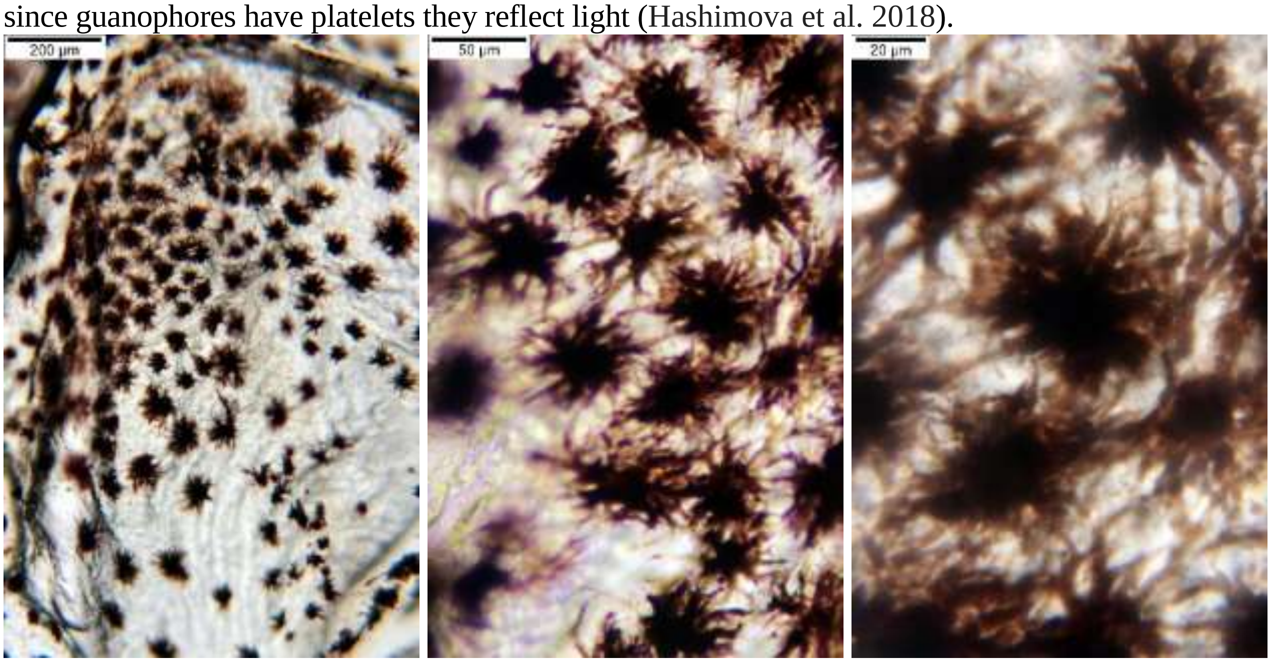

Figure 4. Structure of the Caspian thin-toed gecko's melanophores

Physiological color changes are also observed in Caspian thin-toed geckos (Najafov et al. 2022). This process takes place slowly. The physiological change in skin color is due to displacement of some chromatophores towards each other, and the thickness of guanophores and lipophore cells across the width of the skin. In geckos living in a vertical position, the pseudopods of melanophore cells are darker than those around the nuclei. The lipophores are thin and the quanophores are cylindrical during cross-sectional viewing. It protects these animals from their enemies during hunting and at night among rocks and stones (Najafov et al. 2017). Melanophores of thin-toed geckos found on islands and in sandy areas have a lot of melanin around the nucleus, and relatively little pigment in pseudopods. The lipophores are relatively high in these animals, and guanophores are noticeable in the form of flattened on the surface. This shows the color of the upper skin of the lizard in a dusty form. The presence of this color is a form of its concentration on the environment in which it lives (Hashimov et al. 2023).

The hypodermis is located under the dermis and contains fibroblasts, fat cells, and macrophages. Fat cells under the skin are poorly developed. These cells are of mesenchymal origin. Mesenchymal cells transform into fibroblasts and lipoblasts. The lipoblasts are formed by the accumulation of fat in mesenchymal cells. During the fat accumulation, mesenchymal cells lose their protrusions and form round shapes. Rounded lipoblasts transform into adipocytes (Figure 3). Lipid-storing cells have two forms: brown-colored and yellowish adipose cells. At the end of autumn, the number of brown adipose cells in the Caspian thin-toed geckos increases. Cytological analysis of these cells showed that the cytoplasm of adipose cells contains a large number of mitochondria. It is mainly observed in the abdomen under the skin and around the tail of gecko. In geckos, spare food is mainly collected in the tail. In summer, the starving lizard first loses weight from the tail. As a result, ecdysis occurs on the lizard's tail. It is assumed that the accumulated lipids are used during the brumation period. Yellowish adipose cells are more common in the *T.c. insularis* and in the older *T.c. caspius* which we keep in a special terrarium. The nuclei inside these cells are compressed towards the membrane and large lipid droplets are observed. During the second half of April, the examined lizards lacked any yellow or brown adipose cells. Instead of these cells, very few lipoblast cells were found. The hypodermis is an elastic layer that connects to the organs in the bottom layer of the skin. The elasticity of this layer allows the skin to slide over the body and return to its previous position easily.

4. Discussion

An integumental challenge for gecko's terrestrial life was developing mechanisms (Hashimov, 2022) in order to prevent water loss and to protect against ultraviolet irradiation (Huseynova 2021, 2022), mechanical shields which offered protection (Akat et al. 2022). Some skin histology features are similar between fishes and lizards Geckos have dry skin, which has even fewer glands than fishes or amphibians (Williams et al. 2016). The main special feature of their skin is that the epidermis is heavily keratinized with a layer, which also prevents water loss (Ligon et al. 2016). In the perfect resting phase, the epidermis of geckos generally consists of four layers of dead (Mäthger et al. 2007) but fully differentiated keratinocytes and basal live keratinocyte layer that form three main layers: stratum basale (germinativum), stratum granulosum, and stratum corneum (Rutland et al. 2019). The dermis in reptiles consists of fibrous connective tissue, blood and lymphatic vessels, nerves, and pigmentary cells (Krey et al. 2013). Hypodermis lies beneath dermis and consists of fibroblasts, adipose cells, and macrophages. Subcutaneous fat is mostly poorly developed in reptiles in comparison to fishes (Price 2017). Geckos have paired abdominal corpora adiposa that serve as the primary location for fat storage in adipose tissue (Herskovitz et al. 2016). The tail of geckos, can be a large deposit of the subcutaneous tissue, however not in the fishes (Haslam et al. 2014).

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