On the structural and optical properties of the Hungarian Meadow Viper (*Vipera ursinii rakosiensis*)

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ABSTRACT

Snakes optimize their body scales in terms of locomotion, thermoregulation, and conspicuousness for survival in their respective ecological niche. Here, we present our analysis of the scales of the Hungarian Meadow Viper (*Vipera ursinii rakosiensis*). Micro-fibril structures with nano-scale steps are observed on the ventral scales. These structures are oriented from head to the tail direction. Interestingly, a ridge like reticulate structure is observed on the dorsal scales. Spectacle scales are mostly flat and have polygonal cracks on the surface. High optical transmittance is measured on the ventral and spectacle scales. However, much reduced transmission is recorded on the dark dorsal scales which can be attributed to the presence of melanin within the scales. The scales are water hydrophobic; however, the contact angles are not high enough to allow for self-cleaning properties.

Keywords: Snake scales, spectacle scales, wettability, spectral transmittance, atomic force microscopy, scanning electron microscopy, optical spectroscopy

1. INTRODUCTION

Investigations on snake scales started more than a century ago with the discovery of surface structure on "Oberhäutchen" by Leydig [1]. In the 80's many studies have been conducted on the dorsal and ventral scales of various snake species [2-10]. Spike-shaped longitudinal ridge like structures are often found on the ventral scales [11-18]. It is widely accepted by the scientific community that such structures assist snakes in locomotion [11-13, 18, 19]. However, on the dorsal scales diverse ultrastructures are observed [2-4, 7-10]. Several concepts such as solar radiation reflection through light scattering, dark coloration enhancement, water harvesting and self-cleaning properties have already been proposed to explain the evolution of such structures [2, 20-24]. In general, it can be assumed that snakes optimize their scale ornamentation for adapting to their particular ecological niche [9].

Eyes of snakes are covered by hard integument called spectacles. These scales serve as mechanical protectors and UV filters for the eyeballs [25]. Optical properties of the spectacles were studied already in the ultraviolet and visible regime of the electromagnetic spectrum [25-28]. High transmittance was observed for the visible range; however, a cut-off in the ultraviolet regime was observed, too.

Snakes locomote in direct contact to the ground with unavoidable dust or other contaminants. Thus, another research question is how they clean their spectacles as they do not have any extremities for wiping. Therefore, it is interesting to check if the spectacles have self-cleaning properties comparable to the Lotus effect [29]. However, to our best knowledge no study has been conducted on the wetting properties of spectacles so far.

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Here, we present our analysis of the surface topography, wettability and optical properties of ventral, dorsal and spectacle scales of the Hungarian Meadow viper (*Vipera ursinii rakosiensis*) and study the multifunctionality of scales collected from different regions of its body. The exterior surface topography of all scales is imaged to get a detailed overview of the surface ornamentation. Furthermore, the water contact angle is measured on scales from different body parts to evaluate the relationship between scale ornamentation and the resulting wetting properties. Optical properties of the body scales are recorded in the range of 200 - 800 nm. The final comparison of the results provides an overview of the optimization of snake scales from different body regions for the adaption to the habitat of the Hungarian Meadow Viper.

2. MATERIALS AND METHODS

2.1 Sample preparation

All investigations are conducted on the ventral, dorsal and spectacle scales (from shed skin) of the Hungarian Meadow Viper (*Vipera ursinii rakosiensis*). This species is only found in lowland steppe grasslands of Hungary and western Romania (it is now extinct in Austria) [30]. The moulted snake scales were collected by G. Gomard, KIT. The scales were fixed on a glass substrate with two component glue for the SEM and AFM imaging. For the contact angle measurement, the scales were secured on a silicon substrate with conductive adhesive tape (Fotostrip, Tesa AG, Germany). Prepared samples were carefully cleaned with pressurized air. Temperature (21°C – 23°C) and humidity (50% - 70%) were well controlled during storage and measurements of all samples.

2.2 Characterization techniques

Surface topography of snake scales were imaged by scanning electron microscopy (SEM, SUPRA 60 VP, Zeiss, Germany). A thin silver layer of approximately 10 nm was sputter coated before imaging. The working distance between samples and SEM detector was kept within 5 – 7 mm and the acceleration voltage was set to 3 kV. Additionally, the topography of spectacle scales was imaged by AFM (Dimension Icon AFM, Veeco Inc., USA) using rectangular silicon cantilevers (All-in-One-Al, BudgetSensors).

The water contact angle (CA) measurements were conducted with an OCA 40 system and the CA was calculated with the corresponding SCA20 software (DataPhysics Instruments, Germany) with the Young-Laplace fitting method. The droplet volume was 1 µL and the dispensing speed was 0.5 µL/s. The resulting contact angles were averaged from 12 measurements and the corresponding standard deviation was calculated. Spectral transmittance was recorded with a LAMBDA 1050 Ultraviolet-Visible-Near Infrared (UV-VIS-NIR) spectrometer (PerkinElmer Inc., USA). The measured spectrum ranged from 200 to 800 nm. The resolution and beam spot size were set to 1 nm and approx. 2 mm, respectively.

3. TOPOLOGICAL ANALYSIS

3.1 Microornamentations on the Ventral and Dorsal Scales

A photograph of *V. u. rakosiensis* and SEM micrographs of its ventral and dorsal scales are presented in Fig. 1. The Hungarian Meadow Viper has a brown, black-margined zic-zac dorsal pattern. The ventral side has a brownish or greyish base color and dark (black or dark grey) ventral scales with small white spots. Iridescence observed on some scales (especially on the dark head and dorsal ones). The size and weight depend on age and sex of the viper [31]. Typically, females (as the specimen studied in this study) have a body size of 50-60 cm when adult and a weight of 100 to 150 g. A micron-sized fibril pattern is observed on the ventral scales of the species as shown in Fig. 1b). The fibrils are oriented from head to tail direction. Length and width of these fibrils are usually in the range of 8-9 µm and 1 µm, respectively. The height of these structures is around 60-70 nm as determined by the AFM. Besides, continuous nanoridges forming a reticulate pattern are found on the ventral scales. Similar to the microfibrils these nanoridges are oriented from head to the tail direction. In addition to microfibrils and nanoridges, shallow nano pores are also observed on the ventral scales. These pores are elliptical in shape with the long axis around 1.5 times of short axis.

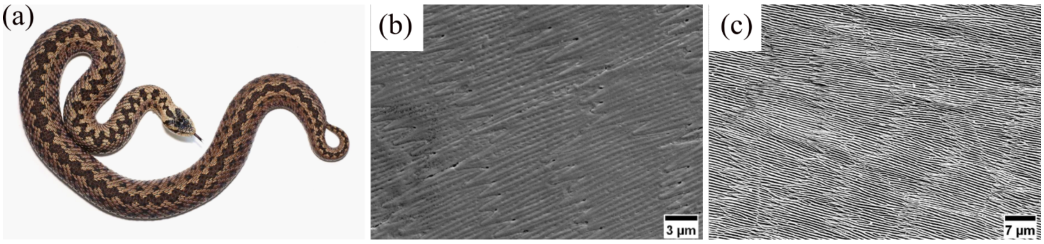

Figure 1. (a) Photograph of Hungarian Meadow Viper. SEM images of the (b) ventral (c) dorsal scales of *V. u. rakosiensis*. A microfibril like pattern oriented from head to the tail direction of the snake is observed on the ventral scales while a microfibril ridge like reticulate pattern is found on the scales of dorsal side.

In difference to the ventral scales reticulate structure with ridges is observed on the dorsal scales. These nanoridges are much more prominent on the dorsal side in comparison to the ventral scales.

3.2 Microornamentations on the Spectacle Scales

Spectacles, which are a hard integument layer protecting eyeballs from external injuries, are usually non -scattering. As snakes do not have eyelids nor any extremities for wiping their spectacles, they might have developed self-cleaning properties. Therefore, the spectacles were characterized with an AFM to verify if snakes feature any ultra-structure supporting self-cleaning. AFM images of the spectacles of *V. u. rakosiensis* are shown in Fig. 2. Different polygon border: narrow channels (black dents) and stitching boundaries (white boundaries) were observed. The narrow channels and stitching boundaries have depth and height in range of 15 nm and 20 nm respectively. Microfibrils similar to the ones found on ventral scales are observed on the stitching boundaries [32]. Alongside, these polygonal structures numerous nanopits were also found on the spectacles (Fig. 2b). These nanopits are extremely shallow with a diameter and depth around 100 nm and 10 nm, respectively. Besides, numerous traces of wear are also observed.

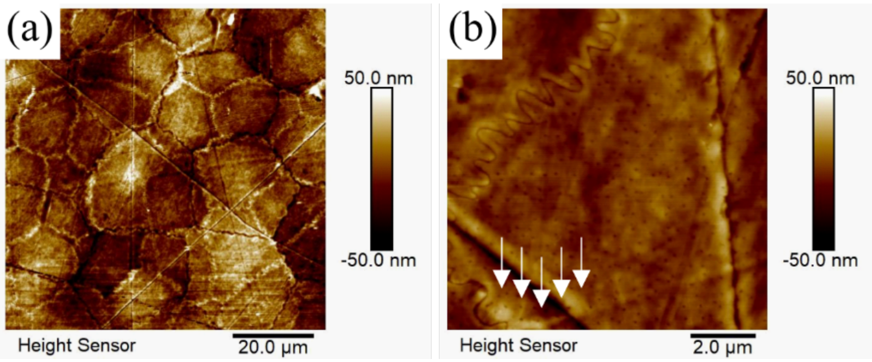

Figure 2. AFM images showcasing the microstructures on the exterior surfaces of spectacles. (a) Polygonal borders with channels and depth of 15 nm and 20 nm, respectively, are observed on the scales of *V. u. rakosiensis*. (b) An enlarged image of the same sample reveals a dense distribution of nanopits. Some of them are marked by white arrows. The scan sizes of images are (a) 80 µm and (b) 10 µm.

4. WETTING PROPERTIES OF THE VENTRAL, DORSAL AND SPECTACLE SCALES

The hierarchical structures on the outer surface of dorsal scales suggest possible self-cleaning or water-collecting capability. Numerous studies have been conducted to investigate wet self-cleaning properties of lotus leaves [29]. When water droplets fall onto lotus leaves, they form spherical drops and roll of the surfaces collecting debris and other particles. Hierarchical micro and nanostructures on the leaf reduce the surface energy resulting in high contact (>150°,) and low roll of angles (<10°). Inspired by this self-cleaning effect, we studied the wetting properties of the ventral, dorsal and spectacles scales. Contact angles of 123 ± 2°, 106 ± 4° and 109 ± 1° were observed for the dorsal, ventral and spectacles surface of *V. u. rakosiensis*. The results show that the dorsal scale is more hydrophobic than the scales from other regions, but the spectacles scales are far from the superhydrophobic regime suggesting that wet self-cleaning properties are not present.

5. SPECTRAL TRANSMITTANCE

Spectral transmittance of the ventral, spectacle, and dark dorsal scales of *V.u rakosiensis* was measured in the ultraviolet and visible range of the electromagnetic spectrum. The results are summarized in Fig. 3. Interestingly, similar spectral transmittance patterns are observed for the ventral and spectacle scales. A relative high transmittance within the visible light spectrum with a cut off in the UV spectrum is observed. The transmittance of both ventral and spectacle scales reduced to 50% at the wavelength of 300 nm and drastically reduced to almost 0% at wavelength lower than 300 nm. Thus, it can be speculated that the scales act as a UV barrier. Melanin content on the dorsal scales is much higher in comparison with the ventral scales. Consequently, reduced transmission was recorded even in the visible regime.

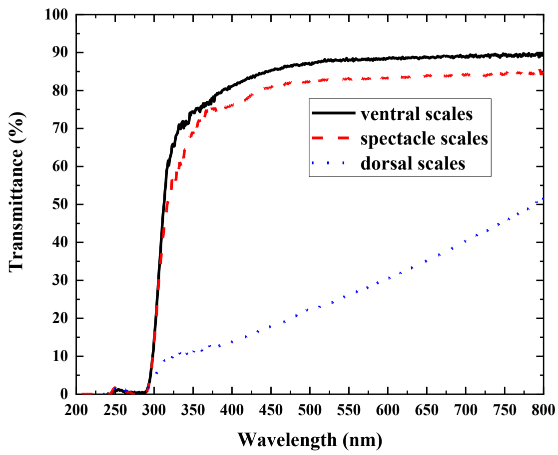

Figure 3. Spectral transmittance of the ventral, dorsal and spectacle scales of *V.u rakosiensis.* Optical high transmittance of the ventral and spectacle scales is comparable. However, the reduced transmission is found on the dorsal scales.

6. CONCLUSION

In this study, we summarized the surface morphology, wetting, and optical properties of ventral, dorsal and spectacle scales of the Hungarian Meadow Viper. A microfibril patterned structure oriented from head to tail direction is observed on the ventral scales. Interestingly, such structures were found only on the ventral side. A nanostructured ridge like reticulate pattern was observed on the dorsal scales. It further enlightens the fact that microfibril patterns on the ventral scales assists snakes in locomotion. As snakes do not have any extremities to wipe their spectacle scales; we speculated that these structures might show wet self-cleaning properties through the classical lotus effect. However, the structures found on the spectacles are quite shallow and did not show any outstanding superhydrophobic properties. Due to these low values, we conclude that snakes cannot self-clean their eyes with the classical lotus effect. Consequently, they have to clean their spectacles in other ways. Over a longer period, they have to renew their dirty or worn spectacles by the periodical molting process. Similar to the spectacle scales, the ventral scales showed very low values of contact angle. However, higher values were observed on dorsal scales which can be attributed to the scale ornamentation. Spectral transmittance of the ventral and spectacle scales is comparable, and it shows high transmittance for visible light. However, 50% transmittance was recorded at 300 nm which drastically reduced to 0% below 300 nm. Much reduced spectral transmittance was observed for the dorsal scales. This reduction can be attributed to the presence of melanin within the scale. It is widely accepted that, scale ornamentation on the ventral side often helps snake to locomote. However, reticulate structuring with nanoridges is found on the dorsal side. This difference of structuring on the ventral and dorsal scales indicate that snakes optimize their scale ornamentation to survive in their respective ecological niche. In continuation to this work, we have conducted topological, optical and wettability analysis on the ventral, dorsal and spectacle scales of twenty-seven snake species from different geographical locations and ecological niche, which will be published in our next communication.

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