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Surface structure of the infrared sensitive pits of the boa *Corallus hortulanus*

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Abstract. Snakes of the subfamilies Boinae, Pythoninae and Crotalinae developed an infrared (IR) sense. The IR sensitive receptors (terminal nerve masses: TNMs) of most of these snakes lie in labial (boas and pythons) or loreal (pit vipers) pit organs. The surface structure of the epithelium in the IR receptor organs reveals a characteristic array of microscopic pores, which are associated with the IR sensitive TNMs. So far, only a few studies have been conducted to investigate these microscopic pores and none has been carried out for boa species with labial pits. In this ongoing investigation, the surface structure of one pit of the boa *Corallus hortulanus* was exemplarily examined. Scanning electron microscopy revealed the presence of two different types of microscopic pores in the pit fundus. This is the first record of microscopic pores in a boa with pits. Furthermore, one pore type has not been described yet.

Introduction

Infrared (IR) sensitive snakes occur in the family Boidae (Boinae and Pythoninae) as well as in the family Viperidae (Crotalinae). Snakes of all three subfamilies developed their facial infrared sensitive organs independently. Their IR receptors are terminal nerve masses (TNMs), which are connected via nerve bundles and the trigeminal nerve to the central nervous system. Crotalinae possess two loreal pits (e.g. *Crotalus atrox*), in which the IR sensitive receptors lie in a thin membrane suspended between an inner and outer chamber. In contrast to the Crotalinae, the boas and pythons possess their receptors in specialized labial scales, which differ in number, size and shape from species to species. The IR sensitive receptors lie either in labial pits (e.g. *Python reticulatus*) (Gopalakrishnakone 1984) or in the epidermis of the labial scales without specialized structures (e.g. *Boa constrictor*) (von Düring 1974). The surface structure of the epithelium in the IR receptor organs of Crotalinae, Boinae and Pythoninae reveals a characteristic array of pores, described as microscopic pores (Amemiya et al. 1995). The pores were only found in association with the IR sensitive TNMs. According to the family and snake species, differences in dimension and distribution of the pores exist. Amemiya et al. (1995) hypothesized the function of the microscopic pores to reflect visible light in order to enhance the resolution of the IR radiation. However, experimental evidence is lacking. Several morphological investigations on the IR sensitive organs of crotaline species and pythons have been carried out, whereas little is known about boas. Among boas, nothing is known about species that possess labial pits. The Amazon Tree Boa *Corallus hortulanus* appears to be a suitable experimental animal since it possesses large IR sensitive pits. As this snake hunts at dawn and night (Bartlett 2003) a good resolution of the IR detection would be of great advantage. According to the existing hypothesis, specialized surface structures in the pits, e.g. microscopic pores, are to be expected.

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Material and methods

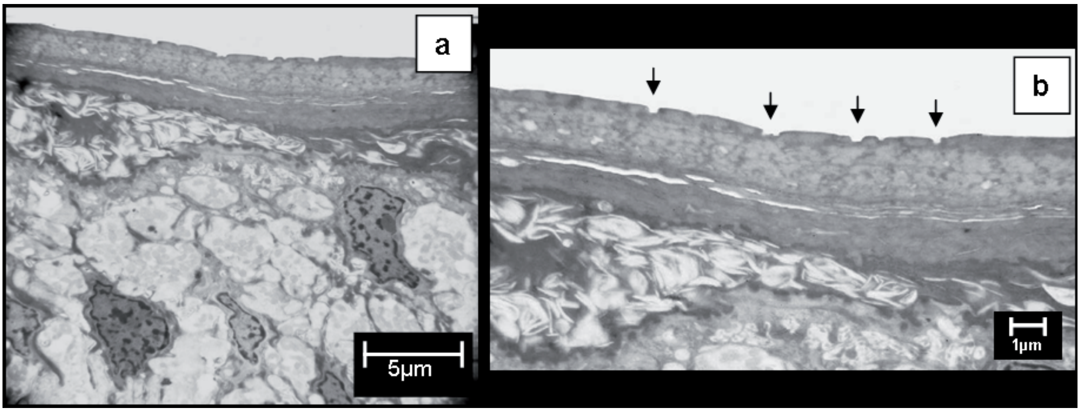
The labial scales of an anaesthetized and then dispatched juvenile *C. hortulanus* were dissected. The scales were dehydrated with ethanol and treated with hexamethyldisilizane (HMDS) for scanning electron microscopy (SEM). The HMDS treatment avoided shrinking or distortion of the tissue and thus well preserved the surface details. The labial scales were mounted on stainless steel stubs and sputter-coated with a gold layer (thickness: 30 nm) before the material was investigated with the SEM (Leo 440i, Leica, Nensheim).

For the transmission electron microscopy (TEM) examination, the dissected upper labial scales were kept in fixative (2.5% glutardialdehyde in 0.1M phosphate buffer) overnight. The material was washed with phosphate and cacodylate buffer, immersed in 2% osmium tetroxide for one hour, dehydrated in ethanol and embedded in Epon. Semi-thin sections (0.5 µm) stained with toluidine blue were used for light microscopy and for selecting characteristic areas for TEM. The ultra-thin sections for the TEM were stained with uranyl acetate and lead citrate and examined with a Zeiss 109 microscope.

Results

The 6^{th} upper labial scale was exemplarily examined with light and transmission electron microscopy. In the pit of this scale TNMs were found (Fig. 1a). These TNMs were densely packed with mitochondria (light grey matter) and several bundles of TNMs were interspaced with epithelial cells (dark grey matter). In addition, indentations (microscopic pores) were discovered in

Figure1. a: Electronmicrograph of the epidermal region of the 6th upper labial scale. b: Higher magnification of the surface of the Stratum corneum. Arrows indicate indentations.



the stratum corneum of the epidermis (Fig. 1b). The pores exhibit a diameter of less than 0.5 µm. In order to gain more information on the distribution and ultra structure of the pores, SEM studies were exemplarily undertaken with the 9th and 10th upper labial scales. The pit is situated between the scales and is one of the most pronounced pits of the caudal labial scales. The pit is covered with two different types of pores: i) pores with a diameter of about 0.3 - 0.5 µm and ii) small pores with a diameter of less than 0.2 µm (Fig. 2). The smaller pores are irregularly distributed over the pit fundus (Fig. 2b and c). The larger pores can only be found caudally in the pit of the 10th scale and are evenly distributed (Fig. 2d).

Discussion

The investigations of the labial pits of *C. hortulanus* revealed TNMs in the pits. Furthermore, this study was the first to demonstrate that the pits are covered with different types of microscopic pores. However, the exact TNM area size and congruence with the labial pits was only demonstrated for one upper labial scale so far, and

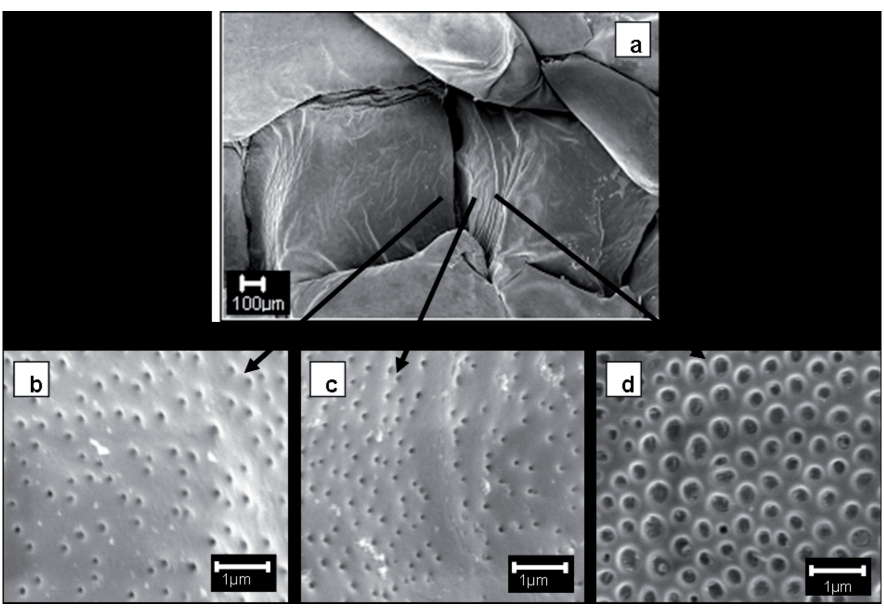


Figure 2. a: SEM picture of the labial pit between the 9th & 10th scale. The lower part leads towards the mouth, the upper part to the eye. b-d: Higher magnifications of the scale surface. Note the two different types of pores in the pit and their different distribution.

has to be thoroughly investigated for all labial scales in a forthcoming study. This information is necessary for an exact description of the IR sensitive areas and will serve as the base for the estimation of the sensitivity and resolution of the IR sense in this species.

The results of the SEM investigations revealed indentations in the Stratum corneum with diameters of less than 0.5 µm. These structures are similar to the microscopic pores of the crotaline and boid species examined so far (Amemiya et al. 1995). Amemiya and co-authors revealed only one pore type with a diameter of about 0.1 µm to 0.25 µm which was found in the pit fundus of the boid snakes *Python regius* and *P. molurus*, as well as on the membrane of the crotaline snake *Agkistrodon blomhoffi*. In these species the TNMs lie only in regions where pores are present. *Boa constrictor*, however, possesses larger pores (0.3 – 0.5µm) which are not confined to the IR sensitive areas of the labial scales (Amemiya et al. 1995). The larger pore type in the pits of *C. hortulanus* looks similar in size, shape and distribution to the pores found in *B. constrictor*. However, *C. hortulanus´* pit fundus is also covered by much smaller pores (about 0.2 µm in diameter), which have not been described so far.

The present study shows that boas with labial pits possess specialised surface structures (two different types of pores) in the IR sensitive regions. Therefore, these findings support the hypothesis that microscopic pores possibly enhance IR vision in snakes. Efficient absorption of IR radiation will be different between the loreal pits of crotaline snakes, the labial pits of boids and the IR sensitive scales of pitless boids. The form of the labial pits combined with the distribution of the pores and the TNMs probably allows and enhances a directional perception of the IR radiation. Further investigations of the differences and similarities of microscopic pores in different snake taxa will increase our knowledge on IR detection in snakes.

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