Folia Zool. – 51(3): 249–252 (2002)

Possibilities of RCH-microscopy in the reptilian research

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Received 14 September 2001; Acccepted 13 June 2002

A b s t r a c t . An entirely new method of optical microscopy in transmitted light "Relief Contrast after Hostounský" or RCH, Lambda Ltd. Praha, Czech Republic, was used to study of integument surface replicas of reptiles (microrelief adhesive method after Wolf) as well as reptilian sloughts. This equipment provides a three-dimensional image of high contrast and resolution. Compared to microscopy without phase or interference contrast, RCH-microscopy makes it possible to evaluate a three-dimensional organization of microrelief on reptilian scales. Results obtained from these microscopical observations can be used for both ecological and taxonomical studies on animals.

Key words: Reptilia, integument, replica, RCH-microscopy

Introduction

Optical microscopy remains a mainstream method for combined morphological and functional studies of both minute unicellular and bigger animals (when are appropriately prepared specimen is used). The most commonly used methods for biological observations in transmitted light include: bright field, dark field, phase contrast after Z e r n i k e (1935), differential interference contrast after Nomarski (B r o c k s c h 1994), and Hoffman's modulation contrast (H o f f m a n 1977).

An entirely novel mode of microscopical observation Relief Contrast after Hostounský or RCH-microcsopy (Ž i ž k a & H o s t o u n s k ý 1997), was investigated. The physical basis of the method is the interaction at a moveable lateral diaphragm (placed in a specially designed condenser) between monochromatic light and the specimen. It not only provides an enhanced contrast, but it also permits obtaining three-dimensional information about the object under investigation (Ž i ž k a et al. 1999, 2001).

Material and Methods

Separated sloughs or imprints (or pseudoreplicas) from the body surface of several reptile species were used for light microscopical examination, namely from *Elaphe longissima* (Colubridae), *Elaphe guttata* (Colubridae), *Natrix tessellata* (Colubridae), *Boaedon fuliginosus* (Colubridae), *Boa constrictor* (Boidae), *Eryx colubrinus* (Boidae), *Emys orbicularis* (Emydidae) and *Testudo hermanni* (Testudinidae). Replicas from the integument (our snakes being a resting stage according to M i t t a l & S i n g h 1987) we were prepared using a standard method after W o l f (1939).

The RCH microscope is based on a standard laboratory microscope (LAMBDA DN 45 - BH 51) fitted with a special condenser for RCH-microscopy (LAMBDA Ltd. Praha, Czech Republic – formerly MEOPTA Praha, Czechoslovakia) enabling a continuous adjustment of

both the relief effect and the wavelength and ordinary achromatic objectives or (in case of some replicas) AU objectives designed for specimens without a cover slip. Micrographs were taken using a Minolta X-300 S reflex camera completed to the microscope itself with a LAMBDA FS adapter. Negative colour film Agfacolor HDC 200 was used to take micrographs.

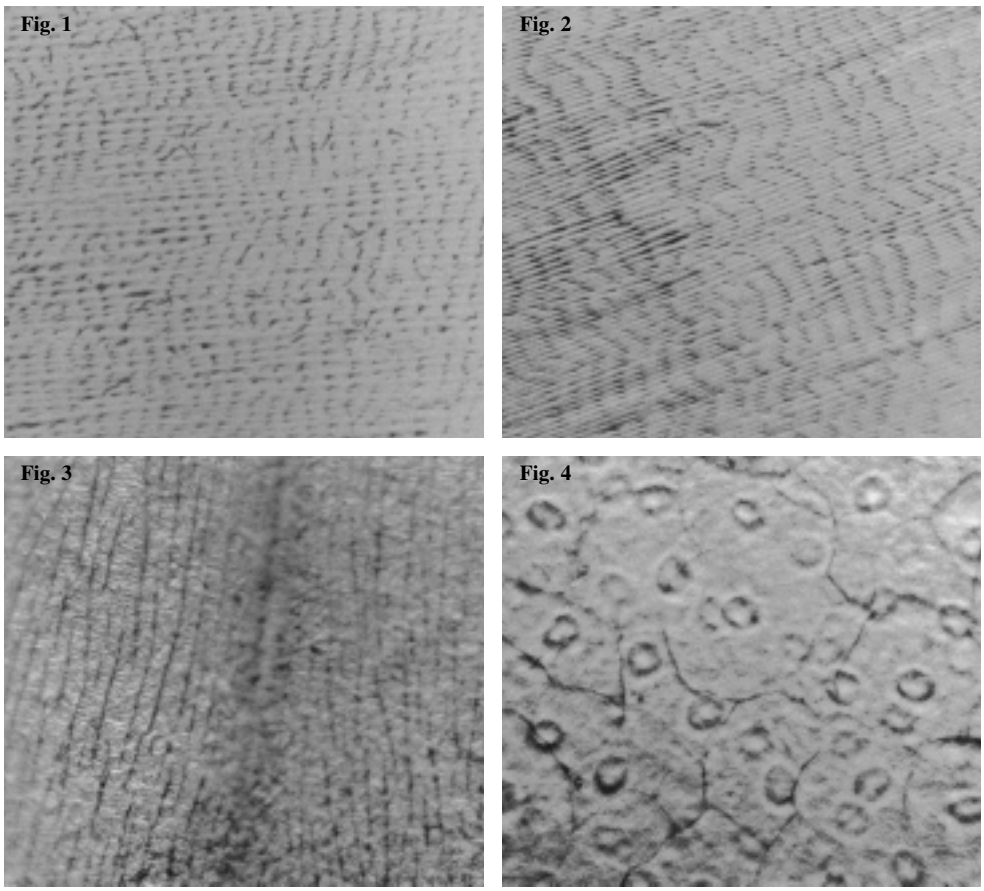

Fig. 1. Imprint of dorsalium from *Elaphe longissima*. Fine spikes at the edge of microrelief patterns of scale cells. Specific technical data: RCH model 107, objective AU 60×/0.85, eyepiece FO ×4, lateral diaphragm 1, aperture iris diaphragm 0.7, groundglass, filter 528–539 nm, exposure Auto 1–4 s, total magnification ×913.

Fig. 2. Imprint of dorsalium from *Boaedon fuliginosus*. Cells bear long needle-like extensions that become make longer towards to the centre of squama. Specific technical data: RCH model 107, objective AU 60×/0.85, eyepiece FO ×4, lateral diaphragm 1, aperture iris diaphragm 0.75, groundglass, filter 477 nm, exposure Auto 1–4 s, total magnification ×913.

Fig. 3. Slough of dorsalium from the hind part of the body of *Natrix tessellata*. A 3-dimensional arrangement of cells as well as the microrelief on the scale can be clearly seen. Specific technical data: RCH model 107, objective 10×/0.25, eyepiece FO ×4, lateral diaphragm 1, aperture iris diaphragm 0.6, groundglass, filter 516 nm, exposure Auto 1 s, total magnification ×152.

Fig. 4. Pseudoreplica of the top part of the head of *Emys orbicularis.* Surface cells (with nuclei) can also be seen, this being a rather rare observation. Specific technical data: RCH model 107, objective AU 40×/0.65, eyepiece FO ×4, lateral diaphragm 1, aperture iris diaphragm 0.65, groundglass, filter 477 nm, exposure Auto 1–4 s, total magnification ×617.

Results and Discussion

Two types of specimens were examined: (1) imprints (replicas) of body surface and (2) exuviae of reptiles. Among the most interesting findings were very fine spikes at the microrelief feature on scale cells of *E. longissima* (Fig. 1). Oval groups of cells (with extensions arranged very close to each other and without any fine spikes) were observed as well at the back of this snake. On the other hand, such spikes could not be seen in another colubrid snake *E. guttata*. An interesting result was obtained from an imprint from *E. colubrinus*; cells partly overlaped and formed a regular pattern. While overlapping cells could also be observed on imprints from dorsal squamae of another colubrid snake *B. fuliginosus,* they were considerably narrower with long needle-like extensions, which became longer towards to the centre of scale (Fig. 2), a rather uncommon feature on ventralia. Cells in dry exuviae of *N. tessellata* and *B. constrictor* gave the impression of three-dimenssional arrangement, including the microrelief of scales (Fig. 3). Carapace replicas of *T. hermanni* turtles indicated a movement of the animal on a non-uniform substrate (long straight grooves). These grooves were absent on *E. orbicularis* turtles but pseudoreplicas from the head featured epidermal cells with nuclei, which is fairly rare (Fig. 4).

The study presented here complements and expands the results presented in our earlier papers (Žižka & Pelc 1986, Žižka 1997), this time using a novel method of RCH-microscopy. Several other authors have studied the microstructure of snake scales. Bea (1986) concluded that (1) in species living in southern regions, a more complex architecture of the microstructures can be found, and that (2) sloughing of the old exuvia preserves the original micropattern (in new epidermis). Similarly Bea & Fontarnau (1986) postulated the micropattern is entirely stable. There are many reports on the possible effects of environment and living habits on the morphology of snake squamae, e.g. Price (1982). Our observations, although carried out only on a limited number of species, indicate that pattern are species – specific, although the effects of environment are not negligible (differences in structural patterns seen on scales in the family Colubridae).

Conclusions

The method of RCH-microscopy makes it possible to observe unstained (fresh) objects in which higher resolution (up to two-fold) and contrast are achieved even at lower magnification (e.g. objective ×40 or ×60 instead of immersion ×100). At the same time, three-dimensional (spatial) information is obtained about the object. In a large number of cases, image quality is comparable with that obtained by using much more complex and costly equipment (e.g. differential interference contrast after Nomarski).

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