

HITHERTO UNDESCRIBED ULTRASTRUCTURAL FEATURES IN THE EPIDERMIS OF TWO AFRICAN AMPHIBIANS

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The epidermis of *Hyperolius nitidulus* (Peters, 1875) (Hyperoliidae) consists of three strata: stratum corneum, stratum granulosum and stratum germinativum. The stratum corneum of both *H. nitidulus* and *Hemissus marmoratus* (Peters, 1854) (Hemisiidae), contains 1-2 replacement layers. The granular cells of *H. nitidulus* are arranged in 2-3 rows. No granular cells typical of the stratum granulosum could be seen in *H. marmoratus*. This is a unique situation for any amphibian. Very large germinative cells were observed in both anurans. In *H. nitidulus* a unique, long and slender 'pillar cell' is situated under the stratum corneum, extending through the stratum germinativum, and the basement membrane deep into the dermis. This cell contains abundant tonofilaments, and appears to function as a pillar supporting the frequently moulting stratum corneum.

INTRODUCTION

The ultrastructure of the amphibian epidermis cells has been described in about 12 anuran species. No epidermis of an African amphibian has previously been studied. A number of epidermal cell types have been described (Fox, 1986; Warburg *et al.*, 1994). Among these are cells, typical only of the larval stages, which ultimately disappear; others first appear near the completion of metamorphic climax, and persist in the juvenile and adult stages (Warburg & Lewinson, 1977; Rosenberg & Warburg, 1978, 1992, 1993, 1995; Warburg *et al.*, 1994).

Hyperolius nitidulus (D & B) (Hyperoliidae) is a unique frog in many ways (Linsenmair, 1998). It is a short-lived frog in which only the juveniles aestivate successfully, by clinging to vegetation exposed to the harsh ambient conditions persisting during the dry season. Two phases can be recognized in this frog: the wet season phase and the dry season phase. These phases differ in both their water balance and dermal skin structure (Geise & Linsenmair, 1986, 1988; Kobelt & Linsenmair, 1986, 1992). The dry season frog has a thin layer of desiccated mucus sealing the body surface and thus reducing water loss (Geise & Linsenmair, 1986). The main barrier against desiccation seems to be the stratum corneum (Geise & Linsenmair, 1988). Moreover, the dermis contains several layers of iridiophore filled with purine platelets that are partly arranged parallel to the surface and thus cause a high reflection (up to 70%) of the sun's radiation in the visible and infrared spectrum (Kobelt & Linsenmair, 1986, 1992).

In contrast, *H. marmoratus* is a long-lived, fossorial frog that rarely ventures onto the surface, and then only under very humid conditions. There is little published information on this frog (it is presently studied by one of us: KEL).

In the present study we describe some of the novel ultrastructural features in the ventral epidermis of these two African amphibian species: *H. nitidulus* and *Hemissus marmoratus* (Hemisiidae).

MATERIALS AND METHODS

Animals were collected in the Ivory Coast (by one of us: KEL) and dissected shortly after capture. Between capture and dissection they were kept under humid conditions and fed on either flies (*Hyperolius*) or ants (*Hemissus*). Pieces of ventral epidermis of three adult *H. nitidulus* and *H. marmoratus* were examined histologically and ultrastructurally using light and transmission electron microscopy. The methods are described in greater detail in Warburg *et al.*, (1994).

For light microscopy, tissues were fixed in Bouin's fluid for 24 hrs. Sections were stained with haematoxylin and eosin. Epon sections 2 µm thick, were stained with toluidin blue.

For Transmission Electron Microscopy (TEM), pieces of skin were fixed cold in 3% glutaraldehyde in 0.05 M cacodylate buffer for 2-4 hrs, and then rinsed in buffer containing 8% sucrose. Post-fixation was in 1% OsO₄ containing 1.5% potassium ferrocyanide for 1 hr, and dehydrated in graded ethanols. Finally, the material was embedded in Epon 812. Unstained sections 70-90A thick were mounted on copper grids 300 mesh, and were examined in a Jeol 100 B (TEM) operated at 60 KV.

RESULTS

In *H. nitidulus*, under the upper layer of the stratum corneum there are 2-3 replacement layers composed of flattened, tightly packed stratum corneum cells (Fig. 1A,B). In *H. marmoratus*, the epidermis appears to consist only of a stratum corneum with wide intercellular spaces in the ventral epidermis (Fig. 3A,B), and

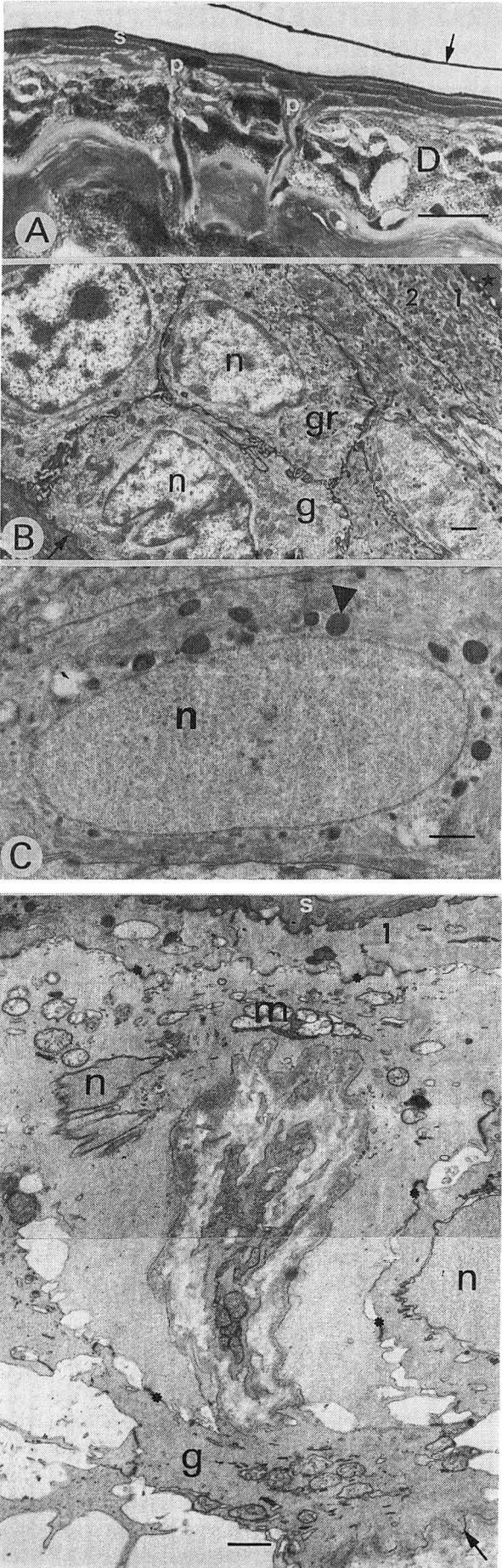


FIG. 1 (TOP LEFT). Sections through the epidermis of *Hyperolius nitidulus*. A, section through the entire depth of the ventral epidermis to the dermis (D) viewed in light microscope. Note the long pillar cells (p) situated perpendicular to the surface (arrow indicates moult) and stratum corneum (s), penetrating through the basement layer into the dermis (D)(x 420; scale bar=10 μ m). B, same as in A but an ultramicrograph. Note the several stratum corneum layers (asterisk,1,2), the granular cells (gr), and the large germinative cells (g) situated on the basement layer (arrows) (n-nucleus) (x 6060; scale bar=1 μ m). C, granular cell containing granules viewed in EM (arrowhead; n-nucleus) (x 10 500; scale bar=1 μ m).

FIG. 2 (BOTTOM LEFT). The large, slender pillar cell located perpendicular to the stratum corneum (s,1) and the basement layer (arrow), containing a nucleus (n) and mitochondria (m), is separated from neighbouring cells through tight junctions (flowers). A germinative cell (g) is situated on the basement layer (arrow) (x 9000; scale bar=1 μ m).

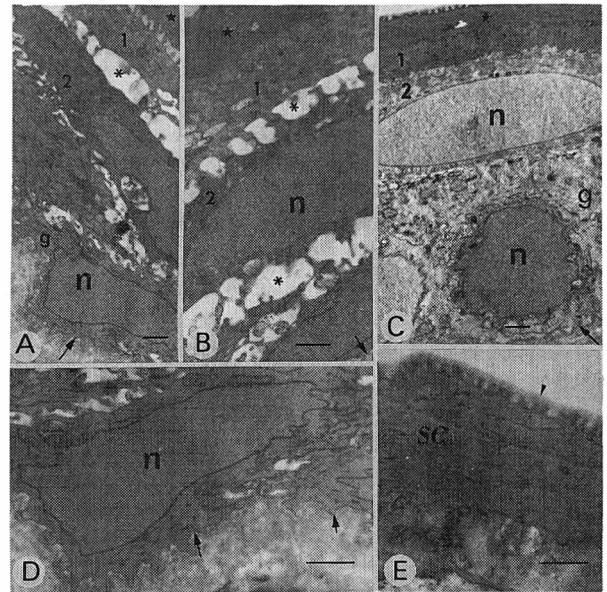


FIG. 3 (ABOVE). Sections through the ventral (A,B,D) and dorsal (C,E) epidermis of *Hemisus marmoratus*. A, section through the entire width of the ventral epidermis from the stratum corneum (asterisk), through its replacement layers (1,2) separated from each other by a wide intercellular space (flower), to a stratum germinativum cell (g) situated on the basement layer (arrow; n, nucleus). (x 7500; scale bar=1 μ m). B, as in A but enlarged. The stratum corneum (asterisk) and its two replacement layers (1,2) separated from each other and from the stratum germinativum by wide intercellular spaces (flowers). Arrow indicates the basement layer (x 10 500; scale bar=1 μ m). C, section through the entire width of the dorsal epidermis. Note the compact layers of stratum corneum (asterisk,1,2), and the stratum germinativum cell (g) situated on the basement layer (arrow; n-nucleus) (x 7500; scale bar=1 μ m). D, germinative cell situated on the basement membranes (arrows; n-nucleus). Note the abundance of tonofillaments (x 15000; scale bar=1 μ m). E, enlarged section through the dorsal stratum corneum (SC) showing the microvilli covered by fuzz (arrowhead) (x 15 000; scale bar= 1 μ m).

tightly packed cells in its dorsal epidermis (Fig. 3C,E), as well as a stratum germinativum (Fig. 3D). The stratum granulosum appears to be missing in this frog (Fig. 3A-C). This is the first time that a stratum granulosum could not be found in an amphibian. On the other hand, in *H. nitidulus* the stratum granulosum, which is situated under the stratum corneum, is composed of 1-2 rows of large, oval-shaped cells containing large granules around the large oval nucleus (Fig. 1B,C).

The stratum germinativum in both species is composed of large cells, fully packed with tonofilaments and mitochondria in a perinuclear arrangement, which are situated on the basement membrane (Figs. 1.B; 3 A-D).

In *H. nitidulus*, a unique new cell type was seen for the first time. This is a very long, slender, pillar-shaped cell, situated under the stratum corneum, with its root-shaped base penetrating through both the stratum germinativum and the basement membrane deep into the dermis (Figs. 1A; 2). This cell contains numerous tonofilaments, thus possibly indicating its function as a supporting cell. We suggest naming this cell "pillar cell" because of its pillar-like shape and its vertical position in the tissue, indicative of a putative function in supporting the stratum corneum. It seems to be unique to *H. nitidulus*, and was never previously observed in the epidermis of any other amphibian species.

DISCUSSION

Epidermal cell layers of amphibians increase in number until the completion of metamorphosis (Rosenberg & Warburg, 1978; Robinson & Heintzelman, 1987; Warburg *et al.*, 1994). Thus, in the juvenile *Pelobates syriacus* there are three epidermal cell layers, whereas in the adult between three and four layers. The epidermis of the two African anurans studied here differs in the number of layers. The epidermis of *H. marmoratum* appears to be composed of only two layers.

At metamorphic climax, the process of cell flattening and keratinization reaches its peak, culminating in the formation of the stratum corneum (Budtz, 1977; Warburg & Lewinson, 1977). There are a number of stratum corneum replacement layers found in *Hyperolius*. These may be related to the high frequency of moulting taking place in this species (Kobelt & Linsenmair, 1986). The replacement layer of the stratum corneum contains mucous granules (Fox, 1977). The presence of granules in epidermal surface cells appears to be a feature of the keratinization process (Budtz & Larsen, 1975). We have observed in *H. nitidulus* an outstanding richness in granules in these cells.

In the stratum granulosum of *Hyperolius*, the granular cells are most abundant. In *Rana ridibunda* these granules were apparently situated closer to the stratum corneum, possibly related to the process of keratinization, whereas in *Bufo calamita* they were

found in deeper layers, possibly indicating a role in water conservation (Navas *et al.*, 1980).

The large pillar cells described here for the first time in *Hyperolius*, may play a role in supporting the epidermis of this unique frog during its frequent moultings. These frequent and rather costly moultings are a means of preventing the formation of cocoon-like structures found in some other xeric-inhabiting arboreal frogs (Warburg, 1997). The reason could be that the formation of a cocoon will impede this frog's free functioning during the dry season (Linsenmair, unpublished).

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- Budtz, P. E. (1977). Aspects of moulting in anurans and its control. *Symp. Zool. Soc. Lond* **32**, 317-334.
- Budtz, P. E. & Larsen, L. O. (1975). Structure of the toad epidermis during the moulting cycle. II. Electron microscopic observations on *Bufo bufo* (L.). *Cell. Tiss. Res.* **159**, 459-483.
- Fox, H. (1977). The anuran tadpole skin: changes occurring in it during metamorphosis and some comparisons with that of the adult. *Symp. Zool. Soc. Lond.* **39**, 269-289.
- Fox, H. (1986). Epidermis. In: *Biology of the Integument vol 2 Vertebrates*, 78-110. Bereiter-Hahn, J., Matoltsy, A. G., Richards, K. S. (Eds). Springer, Berlin.
- Geise, W. & Linsenmair, K. E. (1986). Adaptations of the reed frog *Hyperolius viridiflavus* (Amphibia, Anura, Hyperoliidae) to its arid environment. II. Some aspects of the water economy of *Hyperolius viridiflavus nitidulus* under wet and dry season conditions. *Oecologia* **68**, 542-548.
- Geise, W. & Linsenmair, K. E. (1988). Adaptations of the reed frog *Hyperolius viridiflavus* (Amphibia, Anura, Hyperoliidae) to its arid environment. IV. Ecological significance of water economy with comments on thermoregulation and energy allocation. *Oecologia* **77**, 327-338.
- Kobelt, F. & Linsenmair, K. E. (1986). Adaptations of the reed frog *Hyperolius viridiflavus* (Amphibia, Anura, Hyperoliidae) to its arid environment. I. The skin of *Hyperolius viridiflavus nitidulus* in wet and dry season conditions. *Oecologia* **68**, 533-541.
- Kobelt, F. & Linsenmair, K. E. (1992). Adaptations of the reed frog *Hyperolius viridiflavus* (Amphibia, Anura, Hyperoliidae) to its arid environment. VI. The iridiophores in the skin as radiation reflectors. *J. Comp. Physiol. B* **162**, 314-326.
- Linsenmair, K. E. (1998). Risk spreading and risk reducing tactics of West African anurans in an unpredictable and stressful environment. *Brit. Ecol. Soc. Symp.* (in press).
- Navas, P., Lopez-Campos, J. L. & Diaz-Flores, L. (1980). Diferencias epidermicas del estrato granular entre

- Rana ridibunda* y *Bufo calamita*. *Morfol. Normal Patol.* **4**, 313-321.
- Robinson, D. H. & Heintzelman, M. B. (1987). Morphology of ventral epidermis of *Rana catesbeiana* during metamorphosis. *Anat. Rec.* **217**, 305-317.
- Rosenberg, M. & Warburg, M. R. (1978). Changes in structure of ventral epidermis of *Rana ridibunda* during metamorphosis. *Cell Tiss. Res.* **195**, 111-122.
- Rosenberg, M. & Warburg, M. R. (1992). Ultrastructure and histochemistry of ventral epidermis in *Pelobates syriacus* (Anura; Pelobatidae) tadpoles. *Biol. Struct. Morphogen.* **4**, 164-170.
- Rosenberg, M. & Warburg, M. R. (1993). The ventral epidermis of *Pelobates syriacus* (Anura: Pelobatidae). *Isr. J. Zool.* **39**, 235-243.
- Rosenberg, M. & Warburg, M.R. (1995). Changes in structure and function of ventral epidermis in *Hyla arborea savignyi* Aud. (Anura; Hylidae) throughout metamorphosis. *Acta Zool.* **76**, 217-227.
- Warburg, M. R. (1997). *Ecophysiology of amphibians inhabiting xeric habitats*. Springer Verlag, Heidelberg, 182 pp.
- Warburg, M. R. & Lewinson, D. (1977). Ultrastructure of epidermis of *Salamandra salamandra* followed throughout ontogenesis. *Cell Tiss. Res.* **181**, 369-393.
- Warburg, M. R., Lewinson, D. & Rosenberg, M. (1994). Ontogenesis of amphibian epidermis. In: *Amphibian Biology vol 1 The Integument* 33-43. Heatwole, H. and Barthalmus, G.T. (Eds). Surrey Beatty & Sons, Sydney.

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