Histology of the Skin of Three Limbless Squamates Dwelling in Mesic and Arid Environments

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ABSTRACT

The skin of limbless squamates has an increased contact with the substrate compared with limbed counterparts. Comparatively, the contact with the substrate is intensified in fossorial species, where the whole circumference of the body interacts with the soil during underground locomotion. Although fossoriality in Squamata, specifically lizards and snakes, has been studied ecologically and morphologically (e.g., osteological changes), not enough detail is yet available regarding changes in organs critical for underground lifestyle such as the skin. Here we used histological and microscopical techniques (scanning electron microscopy and transmission electron microscopy) to uncover the structural detail of the epidermis and dermis in three limbless reptiles, the amphisbaenian *Diplometopon zarudnyi*, and two snakes, *Indotyphlops braminus* (Typhlopidae) and *Cerastes cerastes* (Viperidae). The skin of these taxa shows pronounced morphological diversity, which is likely associated to different environmental and functional demands upon these reptiles. Anat Rec, 299:979–989, 2016. © 2016 Wiley Periodicals, Inc.

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Living non-avian reptiles include a very heterogeneous vertebrate assemblage, as well as being one of the most species rich class of land vertebrates, surpassing 10,200 described taxa today (Daza, 2014; Uetz, 2015). Reptiles are the most abundant vertebrates in deserts, where they occupy almost every conceivable habitat available (Pianka, 1986, 1989). Diversification of lizards is high in arid tropical and subtropical regions worldwide, except in South America—Atacama and Patagonian deserts, while snakes are more abundant in forests of tropical latitudes, and extend into temperate latitudes north and south of the equator (Ditmars, 1931; Pianka and Vitt, 2003, Pincheira-Donoso et al., 2013).

There is a frequent misconception about the scaled integument of reptiles, which is commonly referred as an adaptive trait for water retention that allows for reptiles to flourish and adapt to a terrestrial life (e.g., Pough et al., 2013). Morphological studies considering the scale morphology of squamates dwelling in different environments have proposed that animals inhabiting warm-dry and warm areas have larger scales in smaller

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numbers than those dwelling in colder regions (Calsbeek et al., 2006; Wegener et al., 2014; Tulli and Robles, 2015), supporting the hypothesis that larger scales perform better as a heat exchanger and water conservation structure in arid environments. In contrast, it has been argued that the integument of some reptiles is a significant path for evaporative water loss, which even exceeds respiratory water loss (See revision in Lillywhite and Maderson, 1982). Consequently, reptile scales alone do not provide waterproofing; this quality is determined also by lipids, which provide an effective water barrier (Landmann, 1986; Lillywhite, 2006; Miller and Lutterschmidtt, 2014; Pough et al., 2016). Recently it has been found that some snakes also bear a microscopic lipid coating on both dorsal and ventral scales that function as a lubricant and prevent scale wearing especially on the ventral surface (Baio et al., 2015). This conclusion is reinforced by empirical data derived from experimental observations of one scaleless and one typical snake with scales, where no difference in water loss was observed between individuals of comparable age and size (Licht and Bennett, 1972). Physiological studies on lizards and snakes concluded that lipid content in the integument and differences in scale morphology are two nonmutually exclusive mechanisms by which organisms from arid environments can limit rates of water loss (Gunderson et al., 2011).

In squamates, the ultrastructure of the skin might play an important physiological function for water balance and protection. Early attempts to reveal the underlying anatomy of the skin in reptiles dates back to the 1800s (Dumeril et al., 1834), and although there are some modern studies that have elaborated with better optical devices and provide a generalized idea of the integument on squamates (e.g., Maderson, 1965; Gans, 1974; Alibardi and Toni, 2006; Abo-Eleneen and Allam, 2011), more anatomical detail is required to understand the effect of the environment on the cellular structure of the skin.

In squamates, the epidermis is characterized by an alternating vertical distribution of keratin types, the superficial part consists of cornified epidermis made of β-type keratin, while the underlying part is made of α-type keratin (Lillywhite and Maderson, 1982). The corneous β-proteins layers are thick on the outer surface and are represented by only one cell layer (Oberhaütchen) in the hinge region. Conversely, the α-keratin layer is fairly uniform in thickness over the entire body surface. This distribution of protein types can be explained as follows: the outer scale surface which is exposed to mechanical stress is strengthened by the stiff, resistant corneous β-proteins (Spearman, 1969; Alibardi, 2015), while the areas of the hinge regions, which facilitate movement of the body regions relative to each other, are covered by the elastic and pliable α-type keratin (Spearman and Riley, 1969). Because of rectilinear locomotion, the skin of limbless squamates has lost direct nonelastic connections with the skeleton and developed dermal mobility; the so called "liberation of the skin" occurs around the whole body in amphisbaenians, while in snakes only the ventral quadrants are detached (Gans, 1962).

The dermis of squamates (and other vertebrates) contains mainly fibrous collagen, secreted extracellularly by fibroblasts on connective tissue (Lange, 1931; Jones and Boyde, 1974; Abo-Eleneen and Allam, 2011). The superficial dermis is always relatively loosely packed with a much denser-packed deep dermis. The latter is attached to the muscle fascia by subcutaneous connective tissue (or hypodermis), the amount of which varies among body regions and species. In addition to fat cells, nerve axons, and blood vessels, the dermal matrix provides two cell types of particular significance in reptiles: the chromatophores and the scleroblasts. Chromatophores are prominent in anamniotes and lepidosaurians, where they form dermal chromatophore units that are responsible for both permanent and transient coloration patterns (Bagnara et al., 1979). Chromatophores have been shown to derive from the neural crest (Noden, 1980). Pigment cells have also been identified in the dermal skin of reptiles (Bagnara, 1998). Scleroblasts form a dermal skeleton in many different vertebrates and may also originate from the neural crest (Hall, 1980).

Interactions of skin with the soil surface, such as frictional resistance and adhesive properties affect the reptile's outer skin surface especially in limbless and strictly fossorial animals such as the majority of amphisbaenians (Gans, 1960). Dietary preference for large prey also can have some effect on the skin—the observable distensibility of snake skin facilitates accommodating and swallowing large food items (Gans, 1974). Likewise, morphological traits such as high numbers of scale rows in the skin in snakes might assist distension during swallowing (Pough and Groves, 1983).

In reptiles, the majority of scales are characterized by an expanded outer surface, a short inner surface, and a hinge region (Landmann, 1986). Scales also vary and differ according to the location in the body region (Maderson et al., 1998), or according to environmental impact (Allam and Abo-Eleneen, 2012). In snakes the scaled skin is formed by two regions that contrast drastically in histology, a region formed by a series of elevated, thickened horny epidermal scales and another region formed by thin inter-scale hinge regions (Maderson, 1965; Gans, 1974; Alibardi and Toni, 2006; Abo-Eleneen and Allam, 2011). Anatomical details on the epidermal scales of snakes have been studied, such as the carbohydrate histochemical distribution (*Natrix tessellata* and *Cerastes vipera*; Abo-Eleneen and Allam, 2011), mucous cells in the epidermal hinge region and carbohydrate distributions in the epidermal cells (*Xenochrophis piscator*, Banerjee and Mittal, 1978).

In this study we reviewed the histology of three limbless, burrower squamates with different microhabitats in Saudi Arabia and Egypt—the introduced *Indotyphlops braminus* (Fig. 1A, Typhlopidae) which burrows on mesic soils, *Diplometopon zarudnyi* (Fig. 1B, Trogonophiidae), and *Cerastes cerastes* (Fig. 1C, Viperidae) which burrow or dwell in dry and/or shifting sand environments (Said-Aliev, 1963). We focused on determining adaptations to microhabitat selection of the surface and structure of the skin using histological preparations, scanning electron microscope (SEM) and transmission electron microscope (TEM) images.

MATERIALS AND METHODS

Source of Specimens

Five adult specimens of each species were sampled in different localities in Egypt and Saudi Arabia. *I. braminus*

Fig. 1. Three limbless squamates included in this study. **A.** *Diplometopon zarudnyi* (Image courtesy of Steve Downer/ardea.com), **B.** *Indotyphlops braminus* (Image courtesy of Jeff Servoss), **C.** *Cerastes cerastes* (Image courtesy of Holger Krisp).

was collected on soils of the Egyptian Nile Delta under dense vegetation in the proximity of an irrigation ditch. *D. zarudnyi* was collected in a desert habitat from Qatif Oasis, Saudi Arabia, and *C. cerastes* was collected from sandy desert areas in the Western, Eastern and Sinai Deserts. Detailed information on the distribution of the species examined in this study can be found elsewhere (Saleh, 1997; Uetz, 2015). We used an approved IACUC protocol, which follows the recommendations of The Animal Welfare Act. The study protocol (care and handling of experimental animals) was approved by the Animal Ethics Committee of the Zoology Department in the College of Science at King Saud and Beni-Suef Universities. To euthanize the specimens we used an injection of lidocaine (3%) using a mini-syringe in the abdominal area (Leary et al., 2013), and preserved 10% formalin solution, neutral buffered. Specimens were labeled and stored in the author's personal collection (AAA). The conservation status of the species used in this study is of "Least Concern" according to the International Union for Conservation of Nature (IUCN).

Scanning Electron Microscopy (SEM)

Small pieces of skin from the specimen's dorsal trunk region were fixed in 5% glutaraldehyde. The skins were then washed in 0.1 M cacodylate buffer and post-fixed in a solution of 1% osmium tetroxide at 37°C for 2 hr. This procedure was followed by dehydration, critical point drying and platinum-palladium ion-sputtering. The specimens were then studied under a scanning electron microscope (Jeol, JSM-5400LV).

Histology

Skin tissue samples from the dorsal trunk region were taken and fixed in 10% neutral formaldehyde solution; tissue was washed and dehydrated in ascending solutions of ethyl alcohol (75% to absolute). Tissue was cleared in xylene and embedded in paraffin wax. Serial 5 μm sections were cut and stained with Ehrlich's hematoxylin and eosin (Mallory, 1944). The periodic acid-Schiff's (PAS) method was used to stain the polysaccharides (McManus, 1946). In PAS, acetylation blocks the hydroxyl groups, forming acetyl esters, and deacetylation hydrolyzes the acetyl esters and unblocks the reactive hydroxyl groups. Bromophenol blue was used to stain the proteins (Mazia et al., 1953). These sections were examined and photographed using an Olympus microscope (UIS2 optical system) equipped with a U-TV0.5XC-3 mount adapter.

Transmission Electron Microscopy (TEM)

Samples of skin from the dorsal trunk region were cut into small pieces (1.0 mm^3), and fixed in fresh 3% glutaraldehyde-formaldehyde solution at 4°C for 18–24 hr (pH 7.4) and then post-fixed in isotonic 1% osmium tetroxide for 1 hr at 4°C.

Tissue was dehydrated in ascending solutions of ethyl alcohol in concentrations following this order: 50% for 30 min, 70% for 15 min (two changes), 80% for 15 min, 90% for 15 min, and absolute alcohol for 30 min (two changes). The skin samples were passed twice through propylene oxide solutions for 10 min.

Tissue was embedded in Spurrs' resin, following these steps: (1) the samples were immersed in propylene oxide; (2) 1:1 propylene oxide-resin mixture for 1 hr; (3) 1:3 propylene oxide-resin mixture overnight, (4) the samples were immersed in fresh pure resin at room temperature overnight. The next day, the specimens were transferred to capsules containing fresh resin and placed in an oven at 60°C for 1 day to ensure polymerization of the blocks. Semithin sections (1 μm) were cut from the blocks using a Reichert–Jung Ultra-cut 701701 Ultra Microtome (Vienna, Austria) with the aid of glass knives. The sections were stained with toluidine blue and examined on a light microscope. To detect the area of interest, ultrathin sections were then prepared using the ultramicrotome with glass blades, stained with uranyl acetate and lead citrate and examined with a Joel CX 100 transmission electron microscope operated at an accelerating voltage of 60 kV.

RESULTS

External Appearance

Diplometopon zarudnyi is a very divergent amphisbaenian with an elongated body that is triangular in cross section, the head is blunt and the tail is short and pointed (Vitt and Caldwell, 2014; Maisano et al., 2006). Eyes are rudimentary and external coloration is pink with scales arranged in rings or annuli that surround the body. *D. zarudnyi* has smooth, rectangular scales, which are juxtaposed in regular longitudinal and transverse rows (Fig. 2A,B). *Indotyphlops braminus* has a cylindrical, shiny brown or black body with a slightly lighter ventral side. Its eyes are vestigial and appear as black spots covered by cephalic scales. Dorsal and ventral scales are uniform, cycloid, and smooth. The scales are imbricated and arranged on oblique rows, two

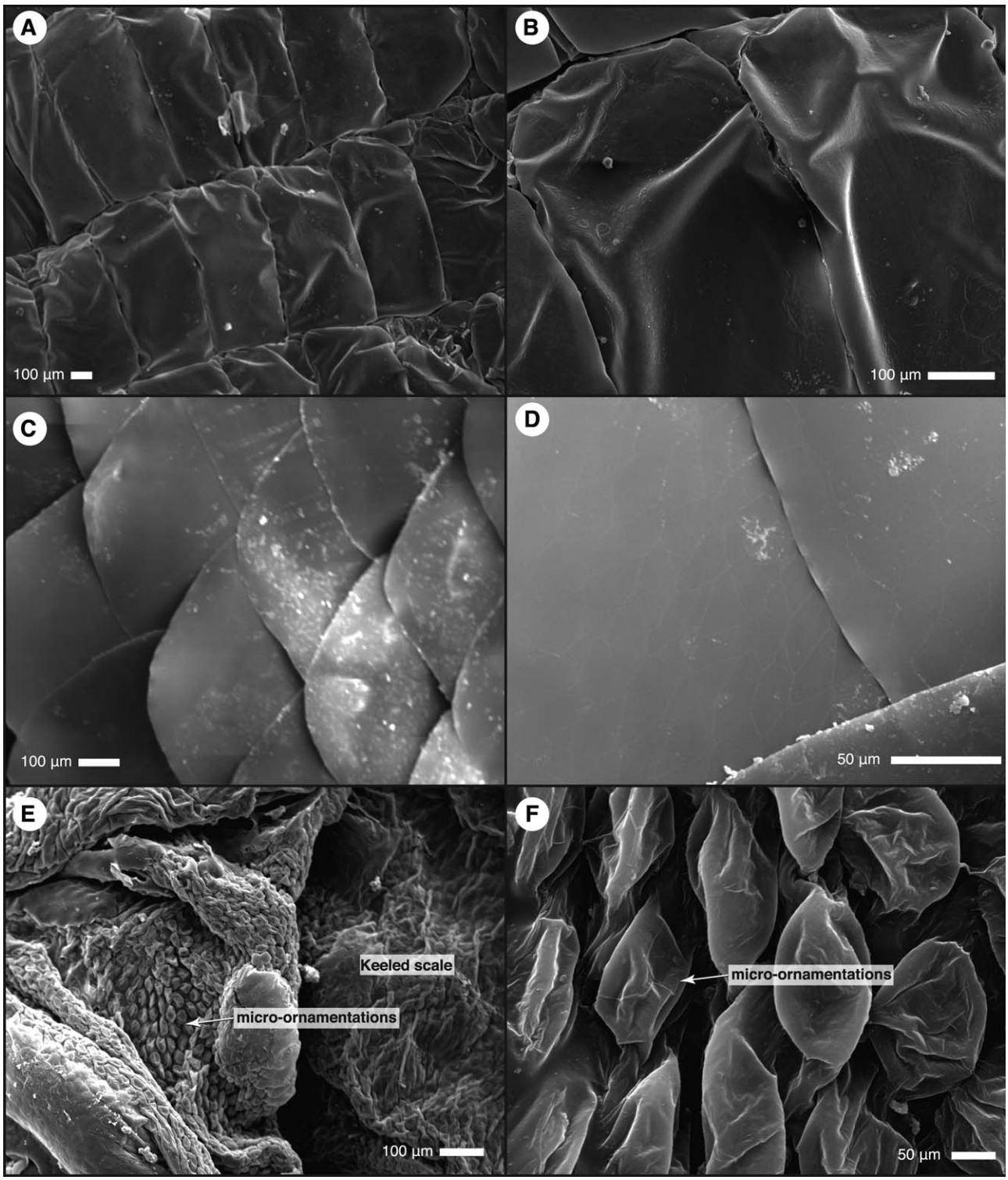

Fig. 2. Scanning electron micrograph of three types of dorsal body scales. **A,B**. *Diplometopon zarudnyi*, smooth rectangular scales. **C,D**. *Indotyphlops braminus*, smooth cycloid scales. **E,F**. *Cerastes cerastes*, keeled scales with micro-ornamentations.

anterior scales overlapping the base of the succeeding scale, creating a cycloid evolute pattern (Fig. 2C,D). *Cerastes cerastes*, similar to many vipers, has a broad triangular head and is easily identified by the presence of two supracoular horns. Dorsal scales are oval, juxtaposed (with some minor distal overlap), and develop a

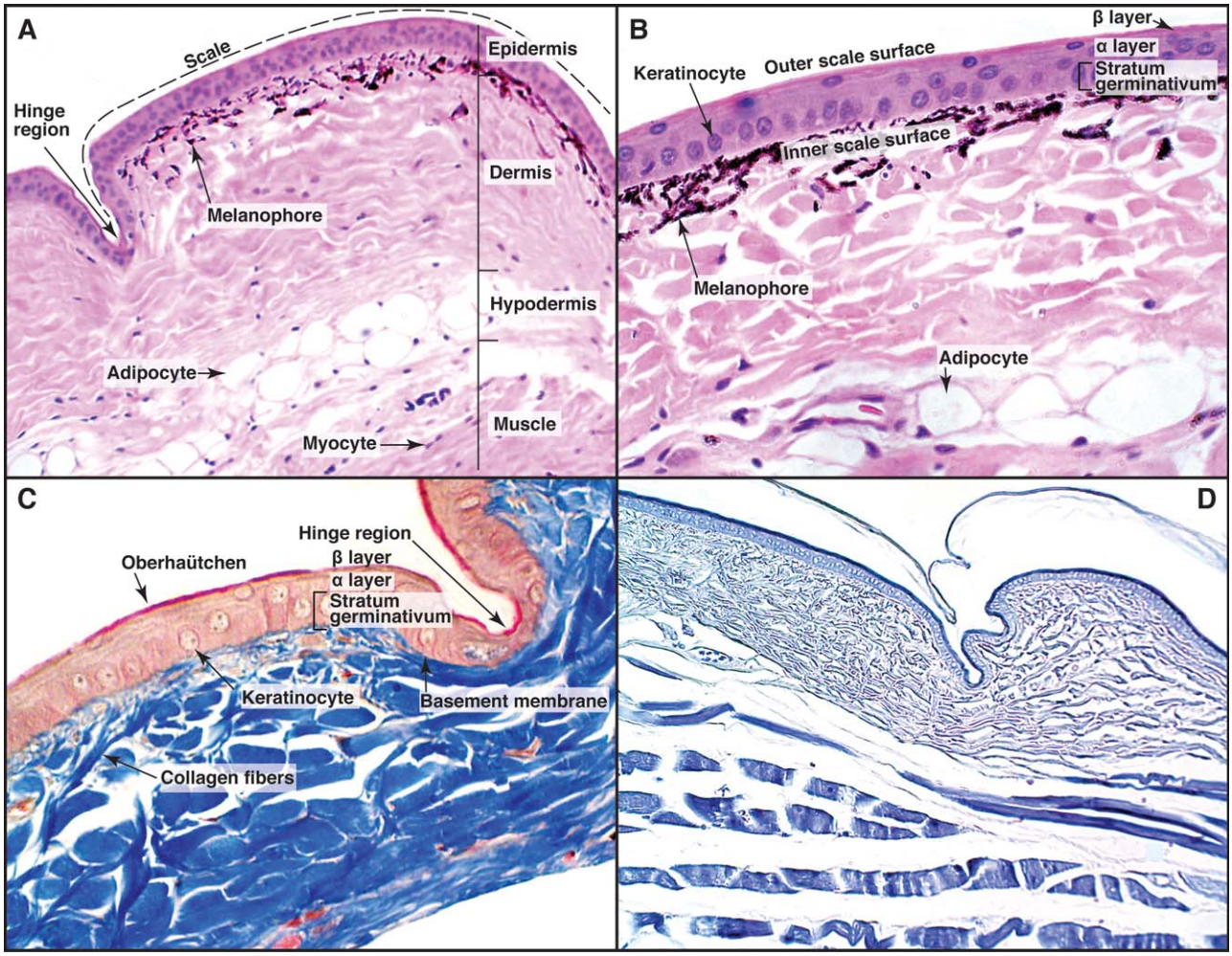

Fig. 3. The skin of *Diplometopon zarudnyi*. Photomicrographs of histological sections of the dorsal scales, **A.** H & E, ×100; **B.** Higher magnification showing details of the epidermis, dermis, subcutaneous tissue, and muscle tissue, H & E, ×400; **C.** Preparation showing distribution of collagenous fibers distribution, Masson's trichrome, ×400; **D.** Protein concentration, Bromophenol blue, ×40. Here and elsewhere α and β layer correspond to α-keratin and corneous β-protein layers, according to Alibardi et al. (2009) and Strasser et al. (2015).

longitudinal keel. On the dorsal surface of the scale, it develops multiple micro-ornamentations (Fig. 2E,F).

Structure of the Skin

General structure of the epidermis of squamates follows the known arrangement of several cell layers or strata. One of the basal layers is the stratum germinativum, which is the precursor of the overlying epidermal cell layers. On top of this layer, there are a series of layers, which in squamates alternate in the inner and outer generations (Landman, 1986). Overlying the stratum germinativum is the α-keratin layer, then a mucous layer (meso), then the corneous β-protein layer, covered by Oberhaütchen (which is generally the most superficial layer of the epidermis). This layer, together with the outer keratinized layer of the epidermis tends to be lost in histological preparations (Lang, 1989). Pigment cells termed melanocytes are located in the basal layer of the epidermis. These branched cells synthesize organelles (melanosomes) containing melanin pigment. In the majority of lizards, melanosomes are found in the α-keratin and corneous β-protein layers (Vitt and Caldwell, 2014), while in snakes, melanosomes are only present in the corneous β-protein layer (Landman, 1986).

Skin structure of *Diplometopon zarudnyi.* The scales have straight sides that are surrounded by narrow and deep grooves (hinge region), which define a tubercular shaped scale in profile (Fig. 3A–C). On the light photomicrograph images with different histological preparations some of these layers are clearly visible. The epidermis of *D. zarudnyi* is composed of several cell layers and below the basal cells (*stratum germinativum*) there is a small layer of abundant melanocytes. The *stratum germinativum* cells are cuboidal with spherical nuclei, whereas the cells overlying this layer, are flattened with oval nuclei (α-keratin, meso, and corneous β-protein layer; Fig. 4A). The transmission electron photomicrograph shows details of the most superficial layer, containing several layers of keratinized cells (ca. 15), which forms mainly the outer generation corneous

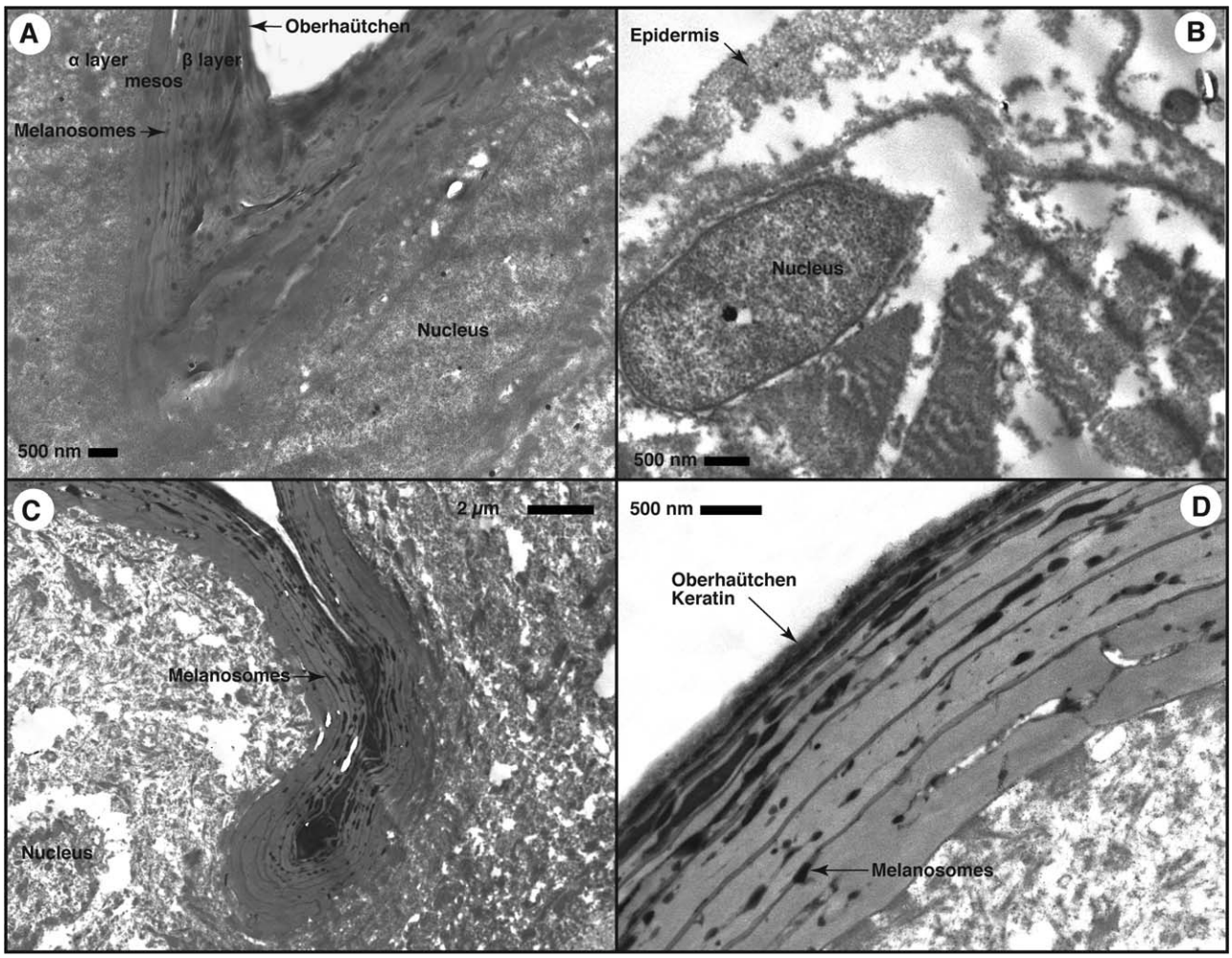

Fig. 4. Transmission electron micrographs. **A.** Superficial layer of the epidermis of *Diplometopon zarudnyi*; **B.** Superficial layer of the epidermis of *Indotyphlops braminus*. **C**,**D.** Superficial layer of the epidermis of *Cerastes cerastes*.

β-protein layer, and contains densely distributed melanosomes (Fig. 4A).

Below the epidermis, the dermis is at least four times as thick, and contains abundant collagen fibers arranged in a reticular pattern. The best contrast between the dermis and the epidermis was observed with the Masson's trichrome staining (Fig. 3C). The hypodermis is composed mainly of a layer of abundant subcutaneous adipose tissue and overlies a deep fascia and skeletal muscle fibers (Fig. 3A,B). The PAS-reaction on the skin of *D. zarudnyi* revealed a moderate amount of polysaccharides in the epidermis and a low amount of polysaccharides in the dermis (Fig. 3A,B). Bromophenol blue staining of the skin of *D. zarudnyi*, revealed that proteins were scattered in the epidermis and dermis (Fig. 3D).

Skin structure of *Indotyphlops braminus*. On the light photomicrograph images the scales appear smooth; they have round edges and are partially overlapped (Fig. 5A–C). In lateral view the scales look smooth, spiny, and each scale has a free outer and an inner scale surface. The shedding outer generation layer on the Oberhaütchen shows regular transverse divisions, conferring the appearance of incipient serrations (Fig. 5C). Cells of the *stratum germinativum* are not distinct as they are obscured by melanocytes (Fig. 5B). Melanosomes are concentrated towards the outer scale surface. In both sides of the scale, there is a distinguishable layer of flattened cells with oval nuclei forming the α layer (Fig. 4B). Masson's trichrome staining (Fig. 5C) clearly distinguishes the epidermis, dermis and muscle tissue. The collagen fibers of the dermis are highly packed. PAS reaction revealed a high amount of polysaccharides in the epidermis, and a low amount of polysaccharides in the dermis (Fig. 5A,B). Bromophenol blue staining revealed a high content of proteins in the epidermis and dermis (Fig. 5D).

Skin structure of *Cerastes cerastes*. The scales are highly complex and in lateral view they look more finger-like than spike-like as in *I. braminus*; the outer scale surface bears numerous micro-ornamentations (Figs. 2E,F and 6A). These microstructures differ from the pits and spinules reported in some xenodontine

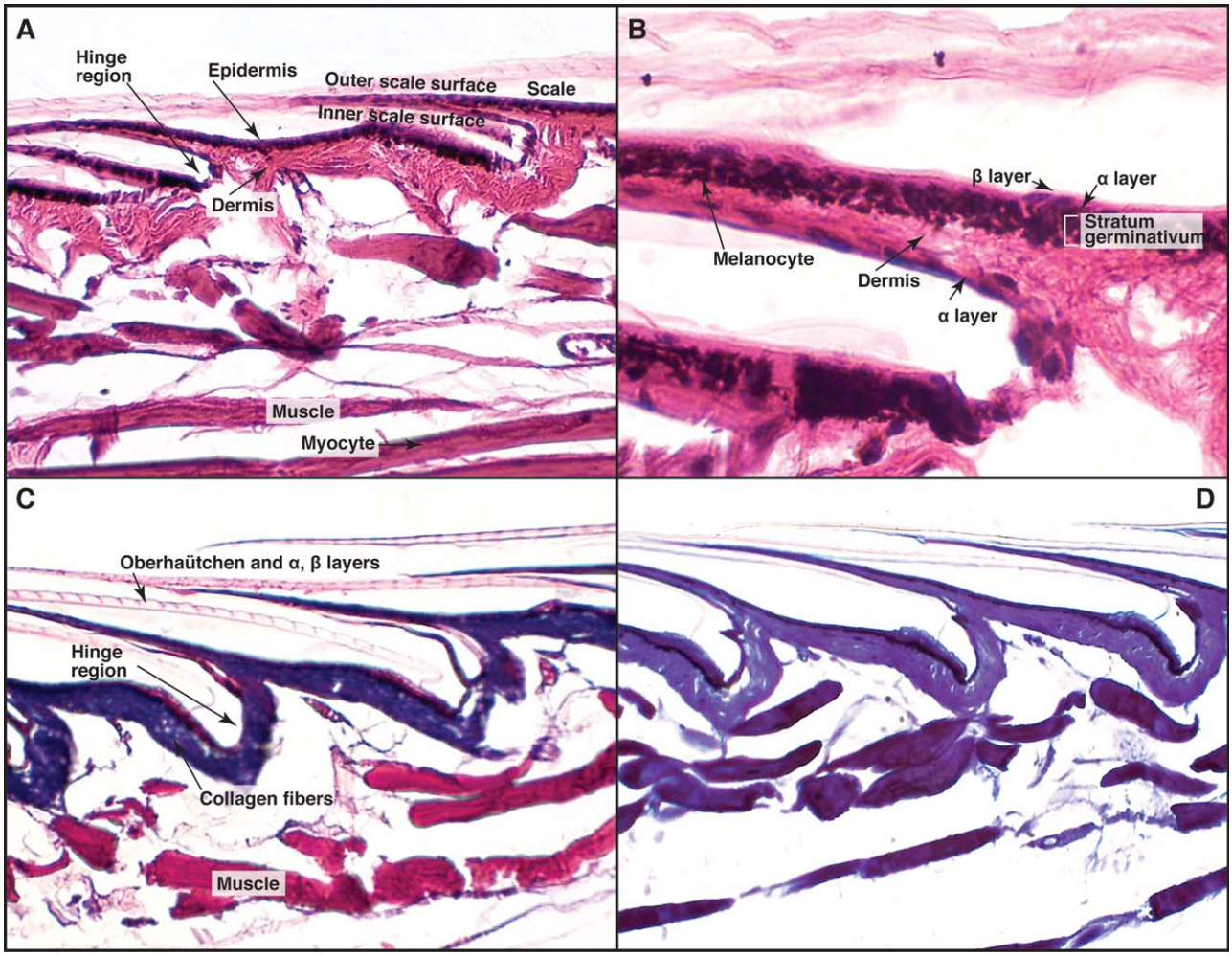

Fig. 5. The skin of *Indotyphlops braminus*. Photomicrographs of histological sections of the dorsal scales, **A.** H & E, ×100; **B.** Higher magnification showing details of the epidermis, dermis, and muscle, H & E, ×400; **C.** Preparation showing collagenous fibers distribution, Masson's trichrome, ×100; **D.** Protein concentration, Bromophenol blue, ×100.

snakes (Rocha-Barbosa and Moraes e Silva, 2009). The epidermis in *C. cerastes* is thicker than the epidermis of *I. braminus* and *D. zarudnyi* (Fig. 4C,D), and is highly keratinized. The light photomicrograph images with different histological preparations illustrate the well-defined *stratum germinativum* with cuboidal cells containing spherical nuclei. The α layer composed of two layers, a well-defined mesos layer, and a highly compact and chromophilic corneous β-protein layer (Figs. 4C,D and 6B). Furthermore, we find that collagenous fibers are distributed throughout the dermis. Bromophenol blue staining revealed a high concentration of proteins in the epidermis and a scattered distribution in the dermis (Fig. 6C,D).

DISCUSSION

The integument of the three species studied exhibited high morphological diversity (see also Radwan, 2004; Mostafa and Abo-Eleneen, 2006). Though the scales of these three squamates varied considerably in geometry, the stratification was generally conserved. The two snakes studied exhibit similar overall morphology and are consistent with previous observations on snakes such as *Orthriophis taeniurus* (Maderson, 1965) *Leptotyphlops dulcis*, *Tantilla gracilis*, *T. nigriceps*, *Sonora episcopa*, and *Virginia striatula* (Jackson and Reno, 1975), *Natrix tessellata* and *Cerastes vipera* (Abo-Eleneen and Allam, 2011).

In histological sections the epidermis of *C. cerastes* is thicker than that of *D. zarudnyi* and *I. braminus* and similar to that of an avian (Sawyer and Borg, 1979) and mammalian epidermis (Akiyama et al., 1999). The thickness of the epidermal layer does not restrict snake's locomotion or distensibility because skin flexibility is maintained due to hinge regions between the scales (Sawyer et al., 2000).

Amphisbaenians, being strictly burrowing reptilian squamates, exhibit a morphology and behavior that is very distinct from other squamates (Kearney, 2003). The skin of *D. zarudnyi* is very distinct from snakes, including surface active and fossorial snake species, by having tubercular rather than elongated and flattened scales. The organization of scales in these animals in annuli

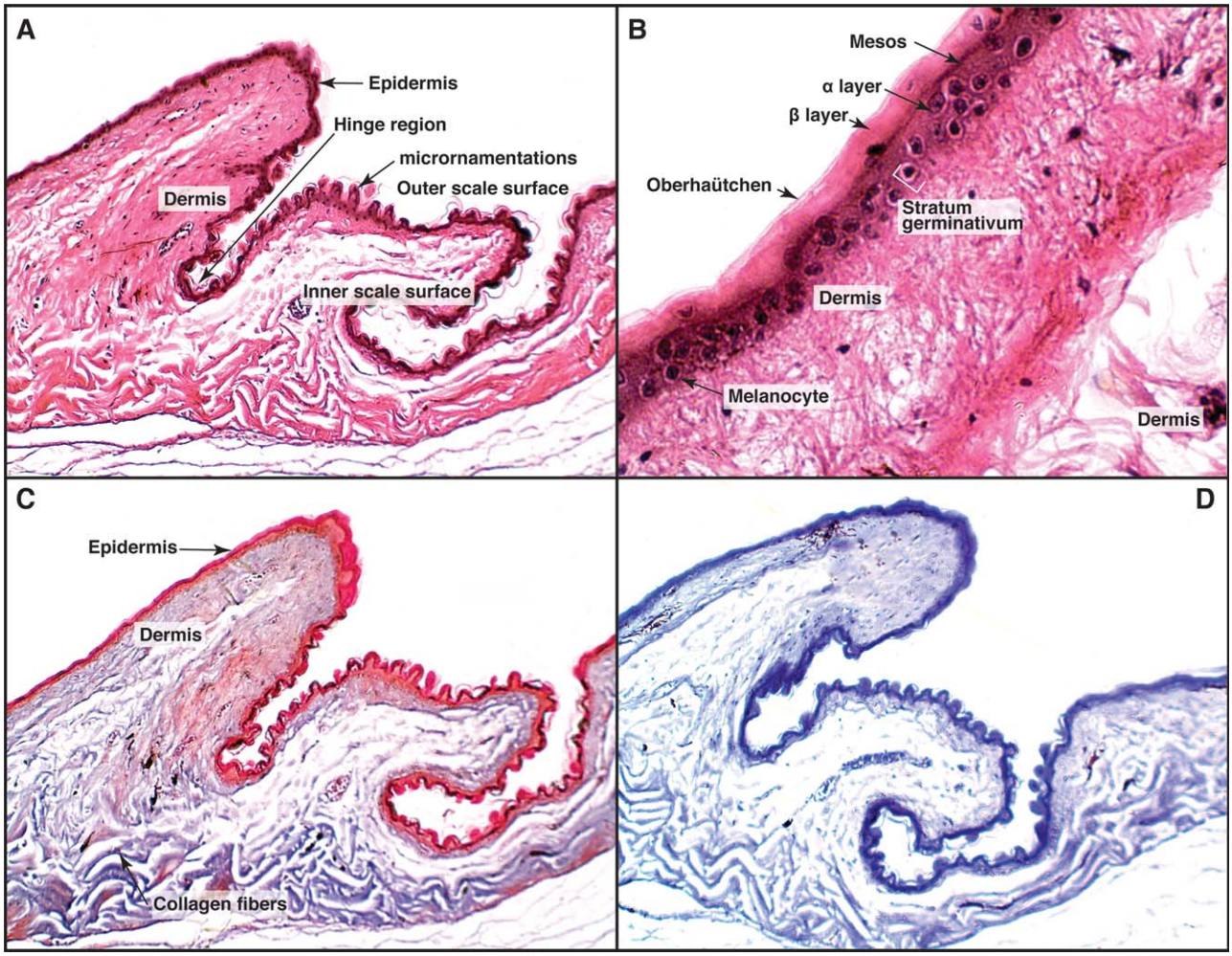

Fig. 6. The skin of *Cerastes cerastes*. Photomicrographs of histological sections of the dorsal scales, **A.** H & E, ×100; **B.** Higher magnification showing details of the epidermis and dermis, H & E, ×400; **C.** Preparation showing scattered distributed collagen fibers in the dermis, Masson's trichrome, ×100; **D.** High protein concentration in the epidermis, and less abundant in the dermis, Bromophenol blue, ×100.

might facilitate rectilinear locomotion independently used in conjunction with head movements for forward thrust to widen their burrows (Gans, 1978). Burrowing amphisbaenians might experience more friction than snakes performing similar locomotion, as snakes in general (and other lizards) have scales with a free outer margin (Jackson and Reno, 1975). These free margins are thin and flexible, reducing the extensive friction during locomotion through tube-shaped environments. The imbrication and posterior orientation of scales may also passively prevent debris and liquids from entering the hinge region between scales. The scales of *C. cerastes* are tough, which is consistent with the morphology (keeling and micro-ornamentation). These could be adaptations linked to the stress produced by desertic habitats (Rocha-Barbosa and Moraes e Silva, 2009).

Pigmentation differs among the species studied. Melanophores in *D. zarudnyi* and *C. cerastes* are distributed mainly in the dermis. In *D. zarudnyi* these cells are distributed in the row underlying the *stratum germinativum* and in *C. cerastes*, these cells are scattered in the dermis. In *I. braminus* melanosomes are restricted to the epidermis and are mixed with the *stratum germinativum* and other superior layers. In *D. zarudnyi* and *C. cerastes* melanosomes are concentrated in the corneous β-protein layer. Color change is a generalized squamate behavior and occurs to some extent in the species studied; whereas in the fossorial blindsnakes, *Leptotyphlops scutiform* and *Leptotyphlops dulcis*, it is known to occur as a defensive behavior (Visser, 1966; Gehlbach et al., 1968).

Dermal collagen fibers vary in distribution among the species studied and are associated with the gross morphology of the skin. Studies on the arrangement of dermal collagen fibers in lizards (Mohammed, 1989; Radwan, 2004) and snakes (Jayne, 1988) indicated that these fibers might facilitate lateral undulation and body distension during the swallowing of large prey. Subcutaneous adipose tissue is prominent in the hypodermis of *D. zarudnyi*. Adipose tissue has also been reported in the geckos *Underwoodisaurus milii* (Elkan, 1976) and *Cyrtopodion scabrum* (Mohammed, 1989). In desert reptiles fat metabolism of adipose tissue produces water as an end product

(Cloudsley-Thompson, 1971). This layer might also play a role in thermoregulation as an insulator or can serve as cushioning for the internal organs, especially considering the external pressure consequence from burrowing through the soil.

The two snakes studied differed from the amphisbaenian in the large amount of carbohydrates in the epidermis and dermis. The epidermis of *D. zarudnyi* contained only a moderate amount of polysaccharides and in the dermis just a small amount of PAS positive material was observed. This observation is consistent with previous reports in other amphisbaenians (Abo-Eleneen, 2008).

Polysaccharides were found distributed throughout the layers of the epidermis, except in the *stratum germinativum*, as well as in the dermis; thus, this observation is consistent with previous observations in lizards and snakes (Mohammed, 1992). Polysaccharides are found in the epidermis of metazoans and have been reported in the integument of cephalopods (Srinivasan et al., 1969), echinoderms (Katzman and Jeanloz, 1969), fish (Seno et al., 1972; Banerjee 1980; Mittal and Banerjee, 1980), and snakes (*Natrix tessellata* and *Cerastes vipera*; Abo-Eleneen and Allam, 2011), these molecules have been hypothesized to work as intercellular cement (Henkart et al., 1973). In some tetrapods, such as amphibians, reptiles, and birds, epithelial polysaccharides serve to define integumentary features (Sengel, 1976; Mohammed, 1984; Abdeen et al., 2008).

The bromophenol blue stain revealed different patterns of distribution in the skin of species studied, and *I. braminus* and *C. cerastes* resemble the distribution reported in *Natrix tessellata* (Abo-Eleneen and Allam, 2011). As it is expected proteins are abundant in the scales; particularly where these molecules are needed to increase mechanical strength, hardness, and durability, which seems to be advantageous especially in harsh environmental conditions (Abo-Eleneen and Allam, 2011).

The scales of *D. zarudnyi* are attached differently from the scales of snakes, which have an imbrication and a free edge. The integument plays an important role in locomotion in limbless forms, especially in the amphisbaenian body, which is entirely in contact with the substrate. The integument is associated with muscles, and movement of the skin is determined by two sets of muscles in snakes and three sets in amphisbaenians (Wiedemann, 1932; Gans, 1960), which explains in part their distinct fossorial behavior. *D. zarudnyi* also has a regular arrangement of collagenous fibers and a dense layer of subcutaneous adipose tissue, which can function as a source of energy and a water reservoir, and can also play a role in temperature insulation and as a cushion for the internal organs. The role of water balance of the skin in these animals cannot be addressed directly in this study. Considering the habitat selection of these three species we can expect that *D. zarudnyi* is more susceptible to water loss, especially in compacted sand dunes. *I. braminus* is also fossorial, but it dwells in more mesic habitats or termite mounds where water conditions might be more favorable. Although it can be found in sandy dry environments, *C. cerastes* is also surface active and can cover larger areas where it can find a balance between warm temperatures to bask, and humidity to prevent dehydration (Johann, 1973; Mermod, 1970; Masood and Asiry, 2012).

In conclusion, the current study confirms that the morphological and histological configuration of the skin of *D. zarudnyi*, *I. braminus* and *C. cerastes* is adapted to their habitat and lifestyle. The ultrastructure of the skin of more squamates dwelling in similar habitats needs to be evaluated in order to confirm the observations of this study.

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