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Original article

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com



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Microstructure of scales in selected lizard species

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ARTICLE INFO

Article history: Received 4 January 2017 Revised 22 February 2017 Accepted 19 March 2017 Available online 21 March 2017

Keywords: Scales Microstructure Reptiles Squamates Lizards Skin

ABSTRACT

In the present study, it was hypothesized that micromorphology of the surface of many lizard scales appears to mimic the topography of the habitat in which they live. Many authors have suggested that the microstructure of the superficial surface of scales have undergone important adaptations and have functional value in lizards. In this study, we investigated the variation and adaptation of the micromorphology and microstructure of the superficial surface of the dorsal and ventral scales from the mid-body region of *Stellagama stellio* (Agamidae), *Stenodactylus petrii* (Gekkonidae), *Acanthodactylus boskianus* (Lacertidae), *Eumeces schneideri* (Scincidae), *Trachylepis quinquetaeniata* (Scincidae), *Scincus scincus* (Scincidae), *Varanus griseus* (Varanidae), *Chameleo chameleon* (Chamaeleonidae). Skin specimens were prepared and analyzed using scanning electron microscopy. The dorsal and ventral scale surfaces had microstructure in the studied species and they exhibited unique patterns that somewhat resembled the topography of the microhabitats in which they lived. Similarity was detected in the three most related species, those having a common family, Scincidae. Ecomorphological relationships were detected between the dorsal and ventral scale microstructures and microstructure scale microstructures and microhabitats. We conclude that environmental factors have observable influences on the microstructure of lizard scales.

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1. Introduction

Morphological features and characteristics of reptiles may reflect their phylogenetic information and their evolution. External morphological features of reptiles vary in complexity and are affected by environmental factors in their habitat. Environmental factors and evolutionary origins may be the source of variation in the macrostructure and microstructure of reptiles. In particular, the appearance of particular traits should be correlated with changes in certain characteristics. To test this hypothesis, we examined the fine structure of the external surface of the lizard's scales. Studies on the external morphology of reptilian's scales are very important to show the environmental impacts of the

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microhabitats of reptiles and to determine the relationship between ecology and morphology (i.e., ecomorphology) of the species. In addition, the environmental impacts may extend to account for some natural selection (Ribas et al., 2004; Ljungström et al., 2015).

The scales of squamates are characterized by special structures to enable individuals to adapt to their surrounding environment. Thus, squamate scales have special structures that consist of rigid outer epidermal β -layer (β -keratin), which is underlain by the meso and then the α -layers. The outer most structure of squamate scales, called the oberhautchen, covers the β -layer (Irish et al., 1988). The surface of the oberhautchen frequently exhibits a complex, microscopical, three-dimensional structure first noted by Leydig (1873). The oberhaütchen and underlying layers may all be rucked to produce ridges on the scale surface (Harvey and Gutberlet, 1995). The overall structure of features of the oberhautchen surface and epidermal folding is referred to here as microornamentation (Ruibal, 1968; Arnold, 2002) or microstructure (Allam and Abo-Eleneen, 2012), and these features are readily studied by scanning electron microscopy. Despite this common special structure of squamate scales, Allam and Abo-Eleneen (2012) reported that the snake's microhabitats have many impacts on the scales outer surface microstructure.

http://dx.doi.org/10.1016/j.sjbs.2017.03.012

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There have been several studies on microornamentation in various groups of squamates (Arnold, 2002). There has been much argument regarding the function of reptilian's scale microornamentation and microstructures, whether the taxonomic variations are associated with systematics or ecological variation. Many studies have been conducted to determine ecological or taxonomical functions are primary. Gower (2003) found strong correlation between microornamentation and general ecology, whereas Harvey and Gutberlet (1995) advocated some phylogenetic utility of the scales' outer surface microornamentation. The microstructure features of the scales' outer surface may be the result of direct adaptation pressures and could be reliable indicators of interspecific relationships (Crowe-Riddell et al., 2016). Price (1982) reported that microornamentation patterns reflected the phylogenetic relationship, rather than environmental or habitats impacts and there was no evidence for correlation between microornamentation and habitats or environment. Renous and Gasc (1989) mentioned that both history and function had effects on the taxonomic patterns. Arnold (2002) studied lacertid lizard microornamentation and found explicit and applied approach to explain and understand variations in morphology through the phylogenetic (integrated historical) and functional analysis.

Generally, the morphology of animals is well matched to their habitats as stated by Aubret et al. (2004), probably because their gene expression is tailored at the population or individual level to suit habitats conditions. Teixeira-Filho et al. (2001) referred to certain associations between claw curvature of lizard species and their different terrestrial occupation microhabitats. Many squamates are similar in their morphological patterns, and it becomes difficult to distinguish adaptive divergences by the naked eye because these divergences are imperceptible.

Scales protect the body of squamates, aid in distinction between families, allow moisture to be retained, aid in locomotion, occasionally aid in prey capture, and alter the surface characteristics, such as roughness to aid in camouflage (Abo-Eleneen and Allam, 2011). However, another study reported that scales do not only play an important role in distinguishing between families, but are important at the generic and species levels (Rocha-Barbosa and Moraes e Silva, 2009). Electron microscopy and ultrastructure studies of the squamate scale surfaces are very important for the analysis of scale microstructures. Several studies have focused on scale microstructure and microornamentation to suggest their functional significance (Gower, 2003; Allam and Abo-Eleneen, 2012) or to simply describe microanatomy (Chiasson and Lowe, 1989; Velloso et al., 2005). In addition, microstructures and microornamentations have been used as tools in ontogenetic and/or evolutionary studies of squamates (Harvey and Gutberlet, 1995).

The present study aimed to show microstructural features of the dorsal and ventral scales of eight different lizard species using SEM, as well as to discover relationships between the microstructure of scales and the niches for lizard species from five different families and from different and similar habitats. This study tested the hypothesis that lizard scale microstructures are correlated with habitats and/or with lizard lineage (phylogeny).



Fig. 1. (A–D) Scanning electron photomicrographs show the microstructure of trunk skin scales of *Laudakia stellio*. (A \times 2000, B \times 5000) dorsal scales, (C \times 2000, D \times 10000) ventral scales.

2. Materials and methods

The present study did not involve protected or endangered reptilians species. The procedures were conducted in accordance with the standards set forth in the guidelines for the care and use of experimental animals by the Committee for the Purpose of Control and Supervision of Experiments on Animals by the National Institutes of Health (NIH). All the used chemicals were purchased from Sigma chemical Company (St Louis, MO, USA). All other chemicals used were of analytical grade.

Five adult specimens of eight species of lizards were investigated. The lizard species selected in the present study included three species from one family (common lineage) and five species from five different families and inhabitants of different and similar habitats. These lizards were captured from the Egyptian regions and their evolutionary level differed. The first lizard, Stellagama (Agamidae), is a monotypic genus of agamid lizards containing the single species Stellagama stellio (Baig et al., 2012). Common names of this species include Stellion, Hardim, Hardun, Star Lizard, Painted Dragon, Starred Agama, Sling-Tailed Agama, and Roughtail Rock Agama. It is found in Greece, southwest Asia, and northeast Africa. It inhabits rocky and mountainous area of the desert. The second lizard, is the Frog-eved or Petrie's Gecko Stenodactvlus petrii (Gekkonidae), is desert-dwelling sand geckos found in sandy desert areas where it can be found among dunes and phytogenic mounds. It is strictly nocturnal and can often be found active during cold desert nights. It feeds nocturnally at the surface on active insects and other arthropods of the desert (Anderson, 1896). The third lizard, Bosk's fringe-toed lizard Acanthodactylus boskianus (Lacertidae), is a diurnal desert species preferring vegetated areas with gravel and stones, but less often sand (Pianka and Vitt, 2003). It is equally numerous in some of most extreme desert areas and at desert margins of cultivated lands of the Nile Valley and Delta. The fourth lizard, the Gold skink. Eumeces schneideri (Scincidae), is known as Berber or Skink Schneider's Skink, is a species of skink endemic to central and western Asia, and north Africa (Beolens et al., 2011). It inhabits sandy deserts with relatively dense vegetation. The fifth lizard is the five-lined Trachylepis guinguetaeniata (Scincidae), formerly Mabuva guinguetaeniata, sometimes called Rainbow Mabuya, the Bluetailed Skink (because of its the blue tail) or Rainbow Skink, which is a species of African skink in the subfamily Lygosominae (Carretero et al., 2016), is the most common lizard in the Nile Valley. It lives in gardens and other green area within towns and villages. It is also common among wild vegetation along the irrigation canals and the banks of the Nile. The sixth lizard is Sandfish Scincus scincus (Scincidae) which is a species of skink that burrows into the sand and swims through it. It is native to northern Africa and southwestern Asia, but is also kept as a pet elsewhere (Grandison, 1956). It inhabits sandy areas near the Mediterranean cost. The seventh lizard is Desert Monitor Varanus griseus (Varanidae) distributed in sandy areas of the desert and scrub country (Mertens, 1954); it inhabits sandy desert areas and is distributed in the northern oases of the western desert, including the western area of Faiyum and Cairo. The eighth lizard is Herbava Chameleo chameleon (Chamaeleonidae) found on trees and bushes in vegetated deserts, gardens, and cultivated areas with bushes or trees (Klaver and Böhme, 1986). It comes to ground only to move from one bush to another or may be to lay eggs. The distribution description of the studied lizards in Egypt is according to Saleh (1997).

Five adult female individuals of each species were anesthetized by light ether. Dorsal and ventral samples of skin, including scales,



Fig. 2. (A–D) Scanning electron photomicrographs show the microstructure of trunk skin scales of *Acanthodactylus boskianus*. (A \times 2000, B \times 5000) dorsal scales, (C \times 2000, D \times 10000) ventral scales.

were taken from the trunk region (mid-region) of the body and the taxa preserved alive for further laboratory demonstrations. The skin specimens of each lizard were collected from the mid-point of the sloughing cycle and after the sloughing phase. The collected skin samples were placed in labeled tubes and duly referred to every species. Distilled water and were added to the tubes for washing. The tubes were manually shaken for approximately 1 min to remove any impurities. The small samples were then removed, washed, and left to dry at room temperature for approximately 5 min. Samples were fixed in 4% glutaraldehyde. The skin samples were then washed in 0.1 M cacodylate buffer and post-fixed in a solution of 1% osmium tetroxide at 37 °C for 2 h. This procedure was followed by dehydration, critical point drying, and platinum-palladium ion-sputtering. The specimens were then examined under a scanning electron microscope (leol. ISM-5400LV). Scales were photographed and analyzed later under a scanning electron microscope using many magnifications.

3. Results

The microstructure of the outer surfaces of trunk scales of *S. stellio* lacked any definite structure. Fig. 1 showed that the micromorphology of both dorsal and ventral scales surface appeared as desert or mountain surface like-structures although the ventral scales appeared smoother than the dorsal.

The scales dorsal surface of *S. petrii* displayed unique forms of hairs, papilla, and microvillus-like structures (Fig. 2A and B), whereas the ventral scales show indefinite structures (Fig. 2C and D). Those structures, which appeared on the dorsal scales surface, may resemble grass.

In *A. boskianus*, the microstructure of the dorsal externalsurfaces of trunk scales showed specific structures, which appeared as aggregations of hay-like structures. In addition, a number of pits appeared at high magnifications (Fig. 3A and B). Conversely, the dorsal scales, the surface of ventral scales appeared smooth with zigzag grooves structures (Fig. 3C and D).

In *E. schneideri*, the microstructure of dorsal scales appeared rough, graded and serrated broad layers (Fig. 4A and B) whereas the ventral scale surface appeared corrugated at low scale (Fig. 4C) and may be like the surface of sandy dunes at high scale (Fig. 4D). In T. quinquetaeniata, the microstructure of dorsal and ventral scale surfaces were similar to some extent to the scales of E. schneideri, but the serrated layers of dorsal scales in T. quinquetaeniata appeared small, compact and accumulated (Fig. 5A and B). Additionally, the ventral scale surfaces in T. auin*auetaeniata* was more corrugated and resembles the sand surface more than did E. schneideri (Fig. 5C and D). In Scincus scincus, the microstructures of ventral and dorsal scales appeared similar. At the low scale, the surface of both ventral and dorsal scales appeared as broad graded layers and resembled the costal surface (Fig. 6A and C). The surface of dorsal scales appeared darker than the surface of ventral scales. At the current high magnification, many bits have been observed in the surface of dorsal and ventral scales (Fig. 6B and D). Although E. schneideri, T. quinquetaeniata, and S. scincus, have the same lineage, significant similarity in scale surface micromorphology has been detect only between E. schneideri and T. quinquetaeniata.

The microstructure of the dorsal and ventral scales of the *Varanus* lizard showed unspecific structures and the surface appeared rough and looked like to the desert soil surface where the *V. griseus* lives (Fig. 7). In *Chameleon* lizard, the micromorphology of external



Fig. 3. (A–D) Scanning electron photomicrographs show the microstructure of trunk skin scales of *Sphenops sepsoides*. (A ×2000, B ×5000) dorsal scales, (C ×2000, D ×10000) ventral scales.



Fig. 4. (A–D) Scanning electron photomicrographs show the microstructure of trunk skin scales of *Eumeces schneideri*. (A ×2000, B ×5000) dorsal scales, (C ×2000, D ×10000) ventral scales.



Fig. 5. (A–D) Scanning electron photomicrographs show the microstructure of trunk skin scales of *Mabuya quinquetaeniata*. (A \times 2000, B \times 5000) dorsal scales, (C \times 2000, D \times 10000) ventral scales.



Fig. 6. (A–D) Scanning electron photomicrographs show the microstructure of trunk skin scales of *Scincus scincus*. (A ×2000, B ×5000) dorsal scales, (C ×2000, D ×10000) ventral scales.



Fig. 7. (A-D) Scanning electron photomicrographs show the microstructure of trunk skin scales of Varanus griseus. (A \times 2000, B \times 5000) dorsal scales, (C \times 2000, D \times 10000) ventral scales.

dorsal and ventral scales surface displayed in indefinite structures (Fig. 8), and perhaps mimics the morphology of the external surface of some old tree trunks.

4. Discussion

Previous studies of microstructures and microornamentation in lizards and snakes found intraspecific and interspecies variations associated with the ontogeny, body region, scales size, lineage, and habitats (Gower, 2003; Rocha-Barbosa and Moraes e Silva, 2009: Allam and Abo-Eleneen, 2012). These variations and modifications arose not only in visible aspects, but also in microscopic structures. Such alterations may have been caused by the optimal adaptation of the reptilians to their environmental niche (Velloso et al., 2005). The differences in microornamentations and microstructures may allow a functional interpretation (Stewart and Daniel, 1973). The investigations of valuable adaptation in cases of morphological divergences may provide ideas of new evolutionary adaptation complex (Williams and Peterson, 1982). Gower (2003) mentioned the idea that variations in scales outer surface microstructures may correspond to the different environmental impacts to which the reptilians were exposed during the acquisition of the microornamentation. The external microscopical variations of the scale surfaces may be related to the effects of selective pressures brought about by the features of reptilian's habitats (Allam and Abo-Eleneen, 2012).

The present study used investigated eight species of lizards from different families upgraded in the evolution scale, close and far in lineage from each other, and inhabiting similar and different habitats. It is likely that small differences in microstructures may bring great benefit to the occupation of distinct microhabitat, and during squamate evolution synapomorphies may have arisen giving the same solution to different adaptive issues. Even when the microstructural forms were similar, and the distribution and the lineage origin differed, gives us a characteristic microstructure to each lizard (Arroyo and Cerdas, 1985).

S. stellio, is a monotypic genus of agamid lizards containing a single species, lives in rocky and mountainous area of the desert and described as a primitive lizard (Baig et al., 2012). The microstructure of the dorsal and ventral scales surface of *S. stellio* displayed coarse as desert soil surface. The rough surface of the scales in *S. stellio* may be correlated to its inhabited coarse environment. Ribas et al. (2004) accounted for the relationship between ecology and morphology.

The hairs, papilla, and microvilli-like structure that appeared on the outer dorsal scale surface of *S. petrii* may be specific and adapted structures. This type of gecko is nocturnal and feeds on surface active insects. As such, the detected scale structures may be adapted sense structures to help in the mode of feeding and nocturnal life. El-Shershaby et al. (2008) detected sense hairs in the skin of some lizards. In addition, this gecko lives in phytogenic mounds and duns; therefore, the surface structures may be resemble grass shape, as a type ecological adaptation. This result may be in agreement with Gower (2003) who proposed that smoother and more regular microornamentations may confer advantages to smooth niche.

A. boskianus is a desert species preferring vegetated and cultivated areas with gravel and stones, but less often sand. We believe that the microstructure of dorsal scales of this lizard resemble that of hay because it always lives under the hay, which is densely distributed in vegetated deserts. The microstructure of ventral scale surfaces appeared similar to the soil surface that may be caused



Fig. 8. (A–D) Scanning electron photomicrographs show the microstructure of trunk skin scales of *Chameleon chameleon*. (A \times 2000, B \times 5000) dorsal scales, (C \times 2000, D \times 10000) ventral scales.

by the fractional affects in-between ventral scales and soil, as reported by Berthé et al. (2009).

The three lizards; *E. schneideri*, *T. quinquetaeniata*, and *S. scincus* belong to the Scincidae family, have intermediated position in the evolution scale (Yan et al., 2008) and inhabit sandy habitats where S. scincus lives always beside the cost, E. schneideri, and T. quinquetaeniata prefer vegetated land (Saleh, 1997). S. scincus dorsal and ventral scales showed unique microstructures differing from the others, in ways that may be related to its specific niche. The scale surfaces in S. scincus affected by its Mediterranean coastal habitat that may be due to the frictional effect as reported by Berthé et al. (2009). There is a high similarity degree between dorsal and ventral scales microstructures of E. schneideri and T. quinquetaeniata, this resulted from the common habitats and lineage (Pough et al., 2003; Berthé et al., 2009). Despite the common lineage of those three lizards, the high similarity in scales microstructures was detected only between *E. schneideri* and *T.* quinquetaeniata, which live in similar habitats. This result is in agreement with our previous results (Allam and Abo-Eleneen, 2012).

V. griseus (Varanidae) is a desert lizard with an advanced evolutionary position (Kelly et al., 2008). The results of the present study showed that microstructures of dorsal and ventral scales are rough and similar; however, it needs to be clarified why this roughness is caused by the coarse habitat and frictional affects. The appearance of trunk scale microstructure may have resulted from the adaptation with the environment (Ribas et al., 2004). Also, Price (1982) observed morphological similarities in the microstructures of the scales of aquatic, arboreal, and fossorial squamates.

C. chameleon (Chamaeleonidae) is entirely arboreal, and in the most advanced and highly specialized clade of lizards (Townsend and Larson, 2002). The detected ventral and dorsal microstructures of the scales surface were unique in that it mimicked to a certain extent based on the topography of its inhabited habitats (tree trunks). Ribas et al. (2004) previously reported on the relationship between ecology and morphology. The ventral scales surfaces appeared more crimped because of the fractional affects (Berthé et al., 2009). In conclusion, the dorsal and ventral scaled surfaces of the trunk region of each of the eight lizards were correlated with microhabitat even though the lizards have a common lineage. Thus, this study detected a correlated relation between the lizard habitats and scale microstructure.

Acknowledgement

The authors would like to thank the Deanship of Scientific Research at Princess Nourah bint Abdulrahman University for funding this work through the Research Groups Program Grant no.(RG-1438-002).

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