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Research Article

A comparison of head scale microstructure between genera *Eristicophis* and *Pseudocerastes*

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Abstract

In the present study, it was hypothesized that micromorphology undergoes a specific evolutionary path at the time of species derivation. Many authors have suggested that the microstructure of the superficial surface of scales has undergone important adaptations and has functional value in snakes. For testing, this hypothesis, the skin micro-ornamentation of the snakes' genera *Pseudocerastes* and *Eristicophis* was studied using a dino-lite digital microscope and scanning electron microscopy. Skin samples from the dorsal part of the head in these related vipers were photographed and compared with each other in terms of divergence time. This study demonstrates that microstructures show a progression from an uneven honeycomb (a plesiomorphy) to a smoother and network-free (an autapomorphy) surface structure.

Keywords: *Eristicophis*, Micromorphology, Microornamentation, *Pseudocerastes*, Scale

Introduction

Many researchers claim that the morphology of organisms is generally well matched and related to the environments in which they live, apparently because gene expression is regulated at the population or individual levels according to local conditions (Aubret et al. 2004). In reptiles, the body surface is covered with scales that assist in successful terrestrial life. The snake's skin is made up of two principal layers, the dermis, and the epidermis. The epidermis of snakes consists of six main layers (Klein & Gorb, 2012). The innermost epidermis layer is called the stratum germinativum. The outer layer is made up of the clear layer, lacunar layer, α layer, Mesos layer, β layer, and Oberhautchen (Abdel-Aal et al. 2011; Berthé et al. 2009; Klein & Gorb, 2012). Oberhautchen layer is in direct contact with the environment. Oberhautchen has a delicate surface structure called microornamentation (Leydig, 1873; Ruibal, 1968). Scale shape, property, and arrangement of microornamentation can serve as important characteristics for evolutionary and systematic investigations (Leydig, 1873; Picado, 1931; Pough et al. 2003; R. M. Price, 1982). Many researchers have studied scale microornamentation in different taxa using electron microscopy (Bea & Fontarnau, 1986; Chiasson & Lowe, 1989; Hoge & Santos, 1953; R. Price, 1990; Price & Kelly, 1989; Price, 1982; Spinner et al. 2013; Stille, 1987). The microstructure of the scales can help us to the study various biological aspects of animals. For examples it is used for examined systematics, ecology, and conservation of Monitor lizards (Bucklitsch et al. 2016), the wettability of the Gaboon viper scales (Spinner et al. 2014) and the study of mechanical performance of scales in fishes (Allison et al., 2013). Family Viperidae includes about 378 species of venomous snakes (Uetz & Hosek, 2021), which are distributed all over the world except in Australia and Antarctica (Phelps, 2010). This family is divided into three subfamilies; Viperinae, Crotalinae, and Azemiopinae. The genera *Pseudocerastes* Boulenger, 1896 and *Eristicophis* Alcock & Finn, 1897 belong to subfamily Viperinae, comprises three and one species of vipers, respectively; *Pseudocerastes fieldi* Schmidt, 1930 (Field’s horned viper), *Pseudocerastes persicus* (Duméril et al. 1854) (Persian horned viper), *Pseudocerastes urarachnoides* Bostanchi et al. 2006 (Spider-tailed viper) and *Eristicophis macmahonii* Alcock & Finn, 1897 (Leaf-nosed viper) (Bostanchi et al. 2006) (de Pous et al., 2016; Fathinia & Rastegar-Pouyani, 2010; Fathinia et al. 2014). *P. persicus* and *P. fieldi* adapted to sandy and rocky areas, *P. urarachnoides* inhabits hill and high grounds mostly composed of gypsum, and *E. macmahonii* lives in fine loose sand of shifting dunes. The distribution of each species has been shown in figure 1. Based on the morphological characteristics, *P. urarachnoides* is significantly different from *P. fieldi* and *P. persicus* (Bostanchi et al., 2006; Fathinia & Rastegar-Pouyani, 2010; Phelps, 2010). However, there are slight morphological differences between *P. fieldi* and *P. persicus*. Unlike morphology, based on a molecular study, *P. urarachnoides* and *P. persicus* have a smaller genetic distance than each of which to *P. fieldi*, with equal genetic distance to both *P. persicus* and *P. urarachnoides*. *Eristicophis* is the closest living relative to *Pseudocerastes* (Fathinia et al. 2018).

The aim of this study is to investigate scale structure in *Eristicophis* and *Pseudocerastes* snakes and compare the microstructure findings to molecular results to infer evolutionary outcomes. Therefore, using SEM the microstructure variation in the surface of head scales surface is compared to explore the evolutionary-taxonomic association in the concerned species.

Materials and methods

The materials used in the present study were obtained from snake specimens collected in fieldwork and stored in 70% ethanol in the reptile collection of the Razi University Zoological Museum (RUZM) and the Reza Babaei Savasari Zoological Collection (ZMRBS). 10 adult snake specimens from 4 species (Three specimens of *P. fieldi* (two female, one male); four of *P. persicus*(two female, two male); three of *P. urarachnoides* (one female, two male) and two of *E. macmahonii* (one female, one male)) were examined. No sexual dimorphism were observed in the structure and appearance of scales in the studied species.

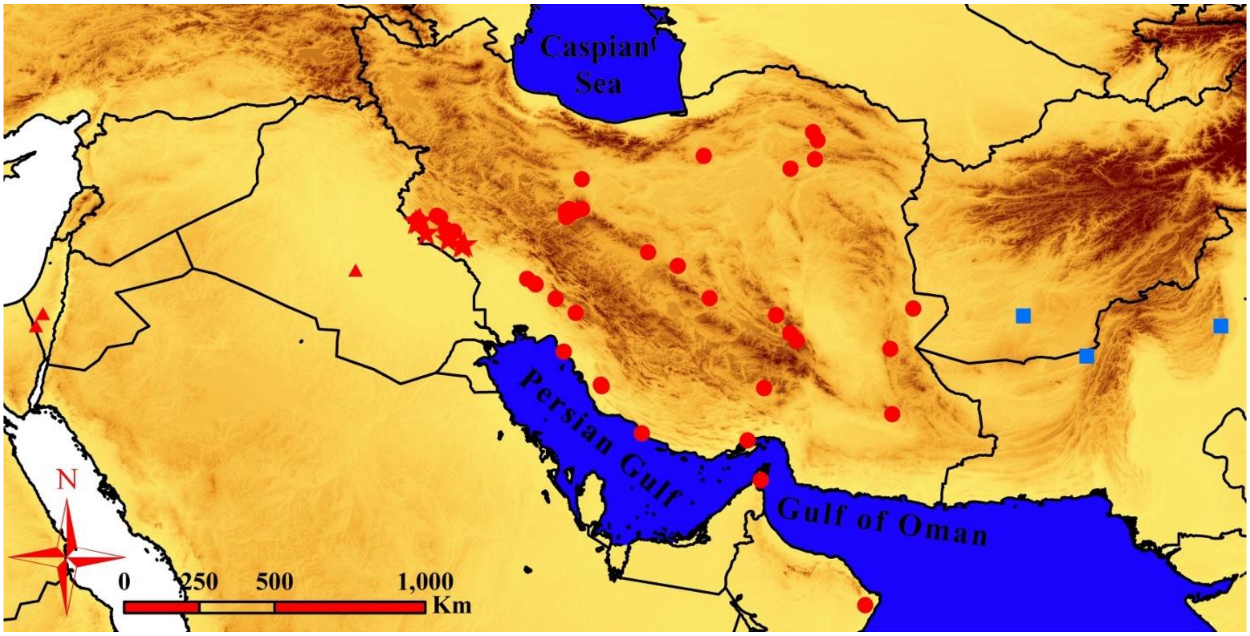

Figure 1. Distribution of *Pseudocerastes* and *Eristicophis*; *P. fieldi* (Red triangle), *P. persicus* (Red circle), *P. urarachnoides* (Red Star) and *E. macmahonii*(Blue square).

Initially, head scales in each sample were photographed by dino-lite digital microscope (AM4113ZT). At the next step, to investigate the microornamentation of scales, dorsal sections of skin scales were taken from the upper head. The skin sections were placed in tubes and filled with distilled water and neutral soap. The tubes were shaken manually for about one minute to remove any impurities, then washed with distilled water and dried at room temperature for about five minutes. Next, the scales were mounted on scanning electron microscope stubs with a thin veneer of Araldite glue. The Stubs were coated with gold and photographed using a scanning electron microscope (Hitachi S-2460 N SEM) at 25 kV under various magnifications.

Results

In photographing with Dino camera, the surface of the head scales in the *Pseudocerastes* species was slightly different. Head scales in all the three species are overlapped and keel of each scale is more prominent anteriorly, becoming flat toward the posterior end (Figs. 2A-5A). The keel undoubtedly does not extend to the posterior margin of scale in *P. fieldi*, while it does not or faintly reaches the posterior margin in both *P. persicus* and *P. urarachnoide* (Figs. 2A- 4A). In *E. macmahonii*, the scales of head are not overlapped, the keel is more prominent all along its length than *Pseudoceratstes* spp., wider in anterior and narrower gradually toward its posterior end (Fig. 5A).

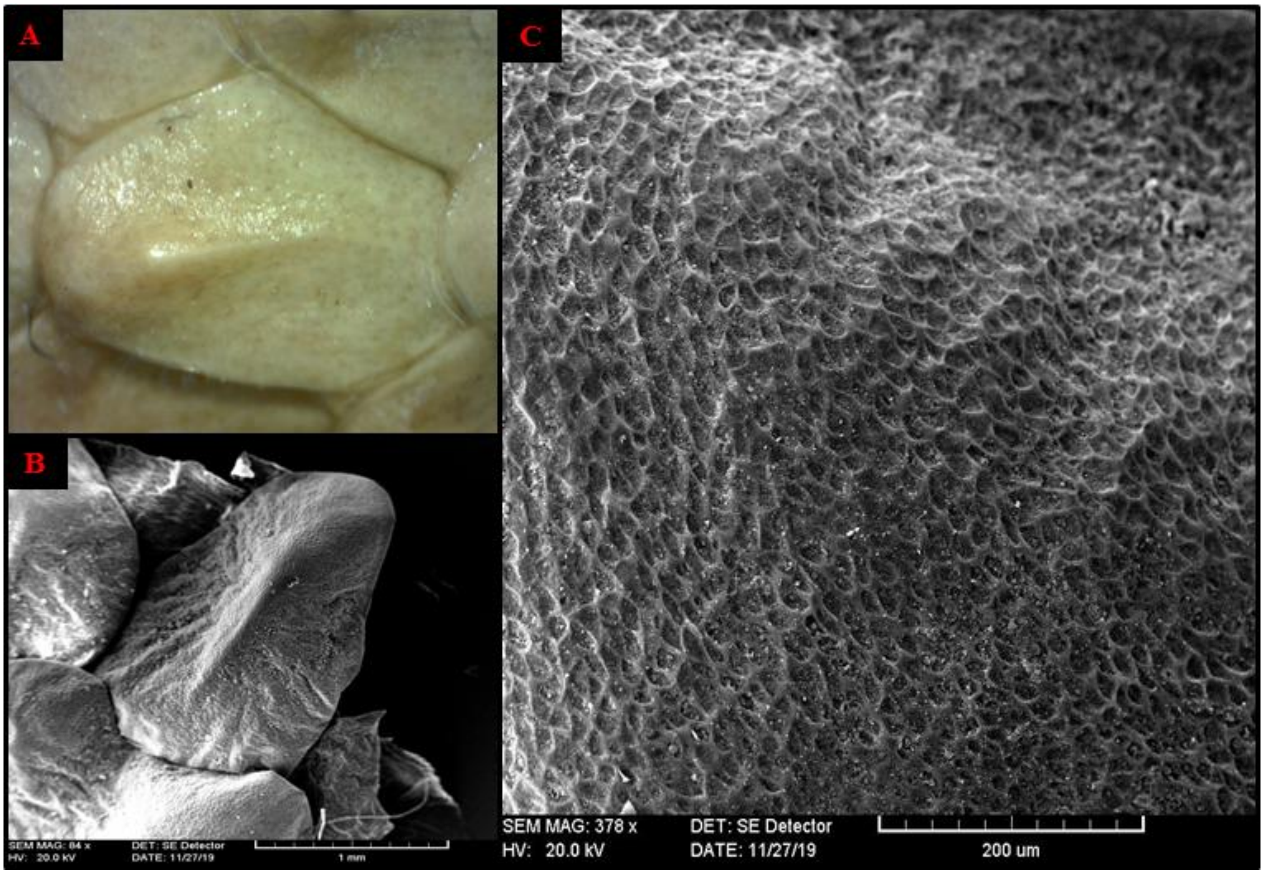

Figure 2. Dino-lite digital microscope show the topology of the head scales (A, 86X) and Scanning electron photomicrographs show the microstructure of head scales in *P. fieldi* (B, 84X and C, 378X).

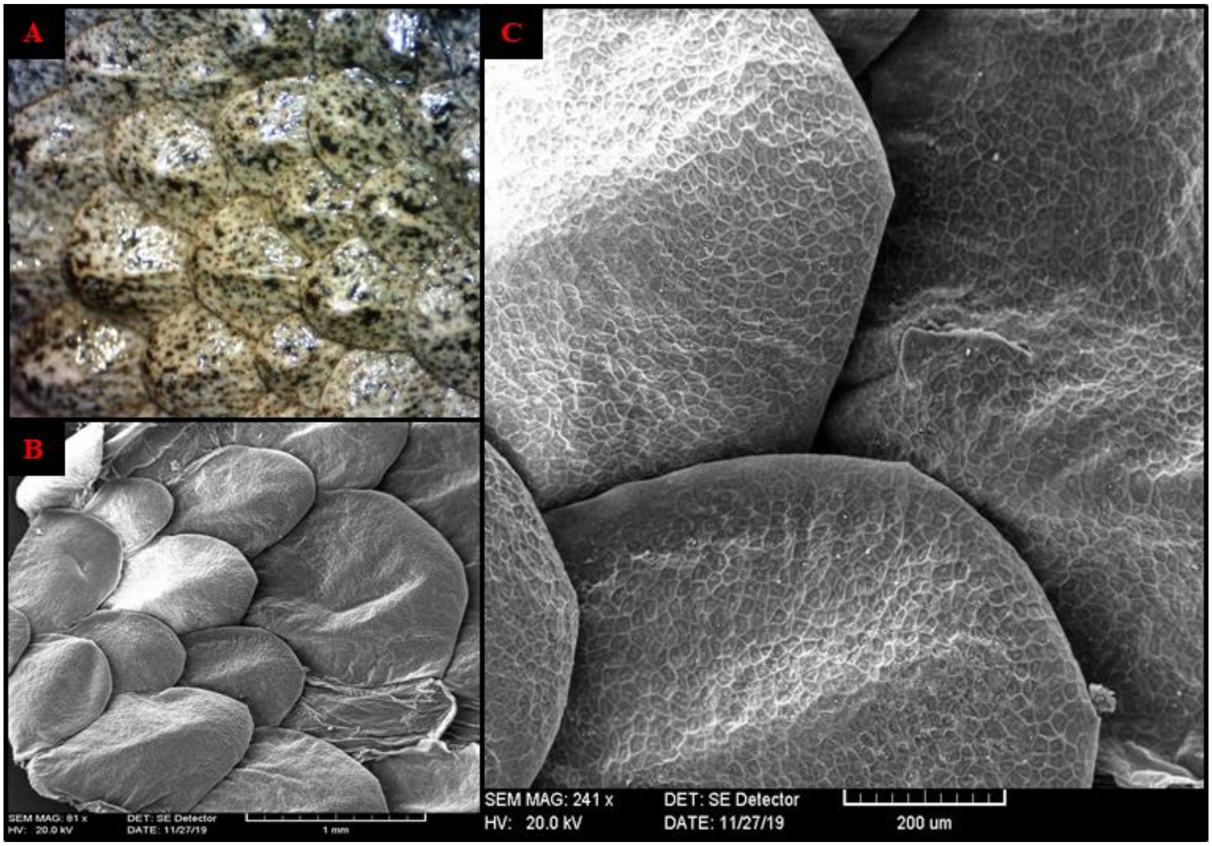

Figure 3. Dino-lite digital microscope show the topology of the head scales (A, 86X) and Scanning electron photomicrographs show the microstructure of head scales in *P. persicus* (B, 81X and C, 241X).

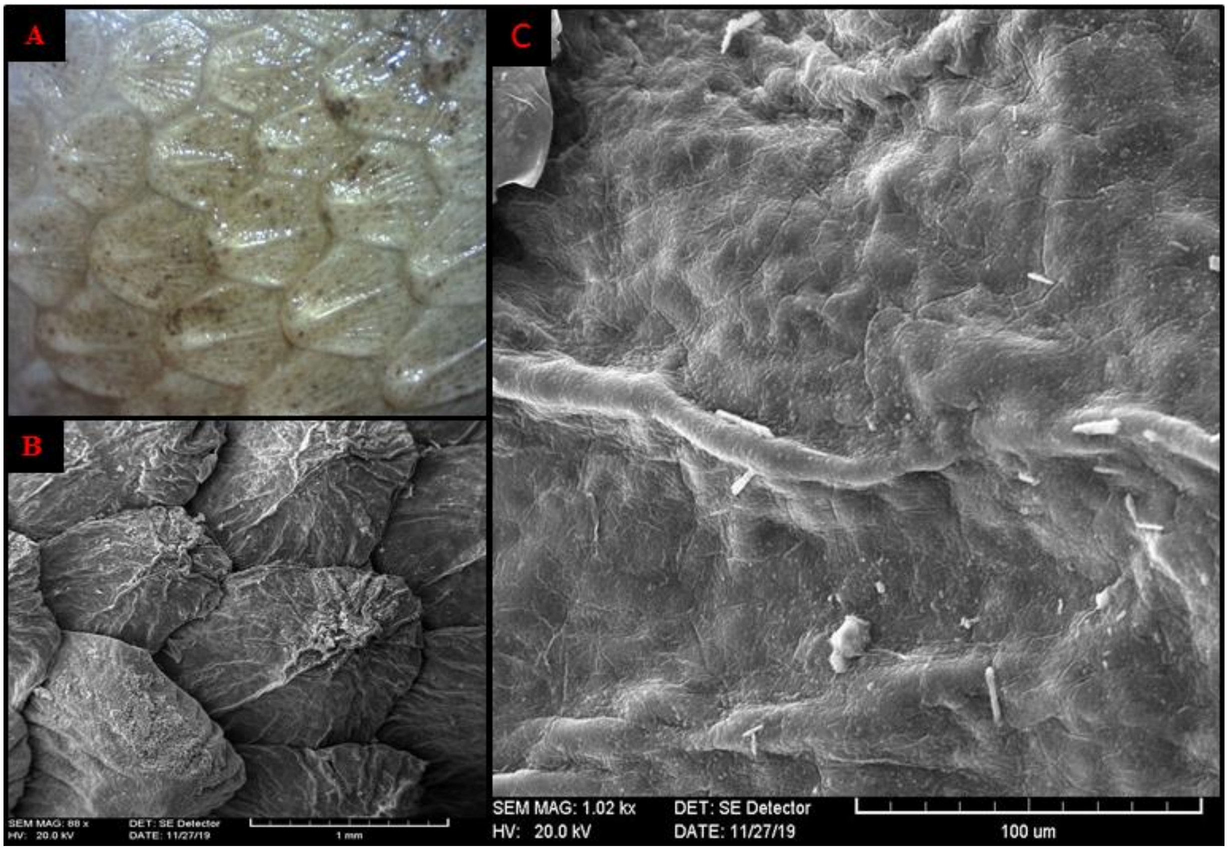

Figure 4. Dino-lite digital microscope show the topology of the head scales (A, 46X) and Scanning electron photomicrographs show the microstructure of head scales in *P. urarachnoides* (B, 88X and C, 1000X).

In SEM photographing, the microstructure of the external surfaces of head scales resemble irregular honeycomb structure in *P. fieldi* (Fig. 2B and C), lattice-like structure in *P. persicus* (Fig. 3B and C), and it is smooth and granular in *P. urarachnoides* (Fig. 4B and C). Head scale microornamentation of the *E. macmahonii* is very similar to *P. fieldi* but the honeycomb or lattice structure was found to be more irregular than that of *P. fieldi* (Fig. 5C). In *P. persicus*, these honeycomb structures have lost their depth and turned into spongy lattice structures.

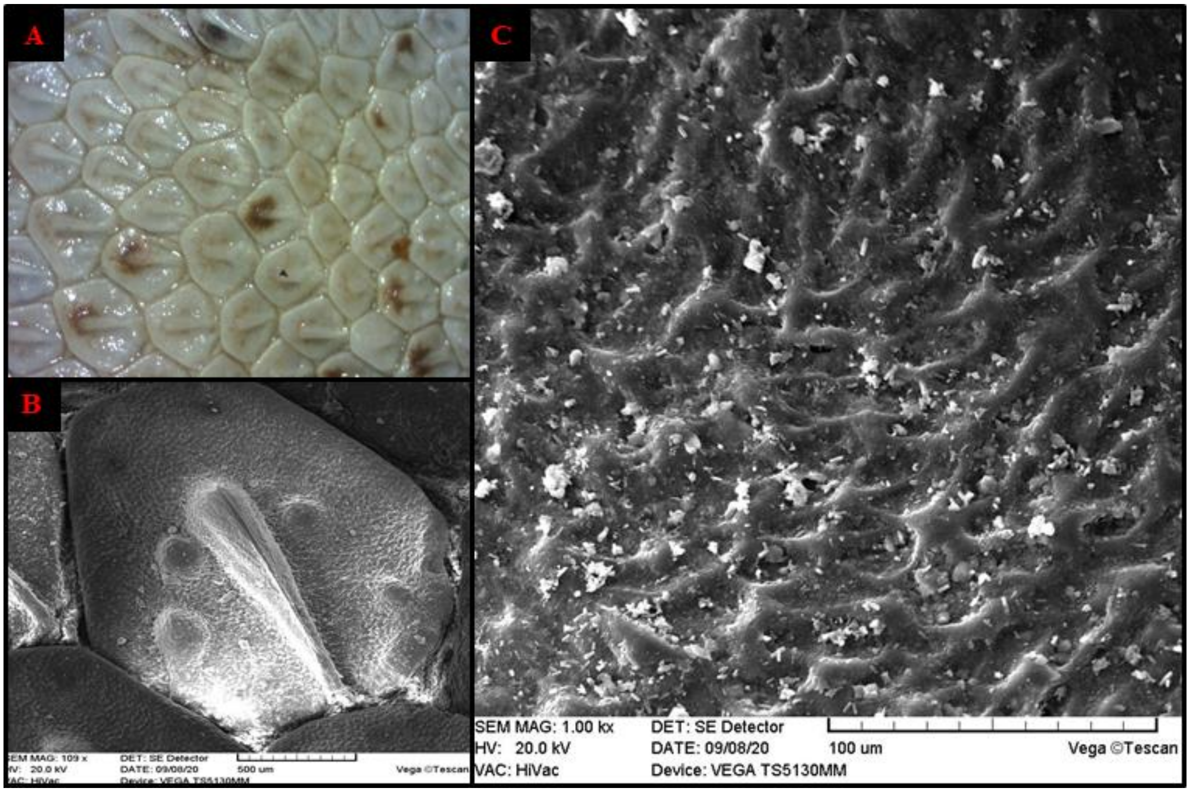

Figure 5. Dino-lite digital microscope show the topology of the head scales (A, 58X) and Scanning electron photomicrographs show the sensory pits (B, red arrow) and the microstructure of head scales in *E. macmahonii* (B, 100X and C, 1000X).

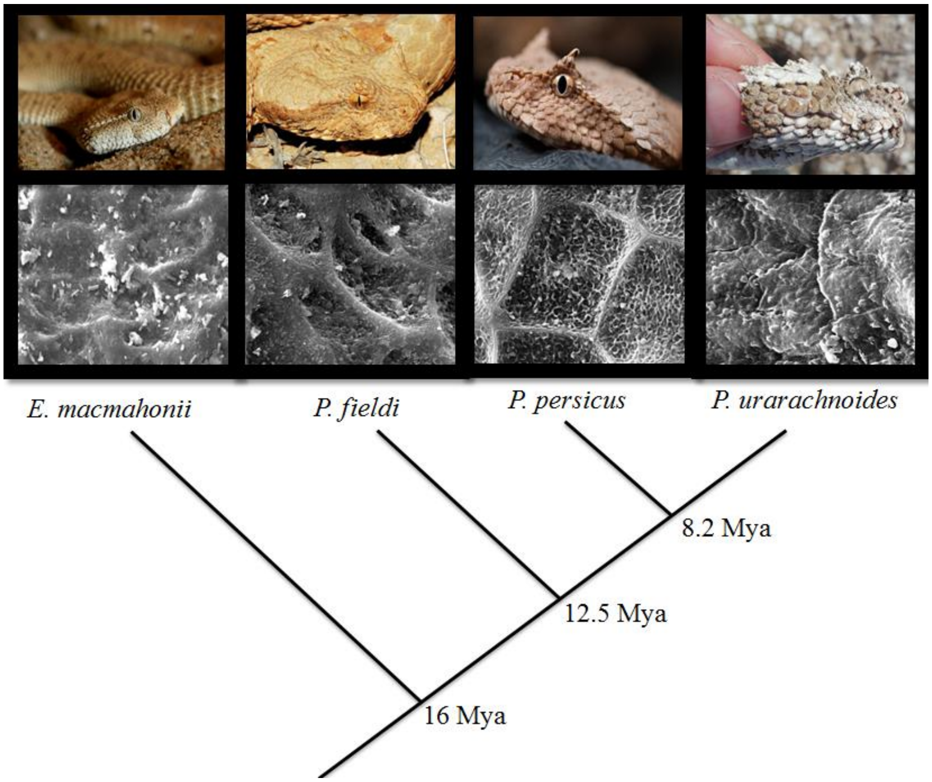

Figure 6. Cladogram depicting phelogenetic relationships, derivation time, the shape of the head and the microstructure of its scales (5000X) among the *Eristicophis* and *Pseudocerastes* genera.

Discussion

The morphological characteristics of reptiles are directly related to their ecology and may reflect phylogenetic and evolutionary trends, nutritional potential, ability to survive in specific habitats, and therefore they can be used to predict their lifestyle (Gower, 2003; Rocha-Barbosa & Moraes e Silva, 2009). Microstructural patterns allow to conclude that, over the course of evolution, modifications have arisen not only in macro but also in microstructures, and that such alterations allow optimal to adapt to their environmental niche (Velloso et al. 2005). The study of such significant adaptations in cases of morphological divergences may provide insights into new evolutionary adaptive complexes (Williams & Peterson, 1982). Therefore, the variation of scales microstructure in snakes can indicate the evolutionary path taking place in these reptiles. To have a better understanding of the evolutionary processes first, it is suggested to examine the phylogenetic relationships between species and their divergence time. According to a recent study, *P. fieldi* takes a basal position at the phylogenetic tree of *Pseudocerastes* as sister taxon to the clade comprising *P. persicus* and *P. urarachnoides* (Fthinia 2018). The divergence between *Pseudocerastes* and its sister taxon, *Eristicophis* have occurred around 16 Mya (Fathinia et al., 2018; Wüster et al. 2008). The divergence within the genus *Pseudocerastes* has started with the split of *P. fieldi* from its sister clade at ~12.5 Mya and continued with the split of *P. persicus* from *P. urarachnoides* at ~8.2 Mya (Fathinia et al., 2018) (Fig.6). Due to the fact that *E. macmahonii* is the closest living taxon of the genus *Pseudocerastes*, it is expected that they have more or less similar scale microstructure. In this study, almost similar honeycomb structures were observed in both *E. macmahonii* and *P. fieldi*, although the structures in *P. fieldi* were arranged and formed more regularly than in *E. macmahonii*. In *P. persicus*, these honeycomb structures have lost their depth and turned into spongy lattice microstructures, while in *P. urarachnoides* the lattice-like microstructures completely disappear, replaced by faint, irregular granules appearing on the surface of head scales. Thus, there is a progression from the uneven honycomb surface (a plesiomorphic state) to a smoother meshwork and finally to a smooth granulated surface (an autapomorphy) in these related vipers. Given the fact that *E. macmahoni* and *P. fieldi* are older taxa than *P. persicus* and *P. urarachnoides*, one might infer that the honey-comb microstructure is a plesiomorphic characteristic which has retaned in these taxa, but underwent some alterations in both younger sister taxa, *P. persicus* and *P. urarachnoides*, with a much more profound transformation in the latter than the former.

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