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RESEARCH ARTICLE

COMPARATIVE MORPHOLOGY OF THE SNAKE SPECTACLE OF *PSAMMOPHIS SIBILANS* AND *SPALEROSOPHIS DIADEMA* BY USING ANATOMICAL AND HISTOLOGICAL STUDIES

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ABSTRACT

A morphological description of the spectacle of two snakes (*Psammophis sibilans* and *Spalerosophis diadema*) is provided in the current study. Five adult snakes of *P. sibilans* were captured from Qalyubia Governorate and five adult snakes of *S. diadema* were captured from Matrouh Governorate during April and May 2023. Periocular scales of both the snake species surround the eye opening. These scales are connected with the eye through the spectacle, and are formed of preocular, postocular, supraocular, and subocular scales. Morphologically, four types of periocular scales in the studied snakes exhibit variability in shape, color, and number. In *P. sibilans*, the pre-ocular scale has an irregular shape with black and little white color. The postocular scales consist of two small scales with rectangular shape and black color. The supraocular scale is one large scale with a plate-like shape and black color. The sub-ocular scales are two, and have a rectangular shape with white and little black color. In *S. diadema*, preocular scales are three with different sizes and brown color. The postocular scales are three small that have triangular shapes with brown color. The supraocular scale is one large, plate-like with brown color. The subocular scales are four, and have a triangular shape with brown and white color. Both snakes' spectacles have the same histological structure. The spectacle is comprised of triple-layers, and connects to the inner surface of the periocular scales through a transition zone, which enters into an integumental hinge region.

INTRODUCTION

One of the most significant clinical characteristics of the snake eye is existence of spectacle, which are essentially an extension of the skin that protects eyes of numerous squamates[1,2], including various species from the families Gymno-phthalmidae, Pygopodidae, Phyllodactylidae, Gekkonidae, Sphaerodactylidae, Scincidae, and Serpentes[3,4]. Typically, species that possess a spectacle lack the nictitating membrane[3-6]. The spectacle, a transparent, immobile vascular structure that covers the cornea[7], forms from the embryonic fusion of upper and lower eyelids[8,9]. The spectacle consists of hard keratin (the

stratum corneum, known as the spectacle scale) and soft tissues (epidermal epithelium and dermal stroma). The spectacle's dermal stroma is analogous to the cornea in that it features a lamellar structure composed of highly organized collagen fibers[9,10]. The spectacle allows the snake to maintain its eyes constantly open for spotting predators and prey[2], offers a protective physical layer for the eye where eyelids are lacking[8], and provides the eye with a significant refractive component that optically negates the cornea[9,11]. This layer of protection maintains the lubrication and moisture of the corneal surface[12]. Subspectacular space lies between the spectacle and the cornea[12,13], which can be regarded as similar to the conjunctival sac in vertebrate species that do not possess a spectacle[2] and allows free movement of the eye[9,14]. Subspectacular space is filled with Harderian gland secretions[13].

Maas *et al*.[12] studied the spectacle wound healing in *Python regius*. Da Silva *et al*.[9] reported the morphological description of spectacle of the *Python regius*. Guerra-Fuentes *et al*.[4] studied the spectacle in gymnophthalmid lizards *via* the looking glass. Van Doorn and Sivak[10] studied the spectral transmittance of the spectacle scale of geckos and snakes. Da Silva *et al*.[15,16] studied the comparative morphology of snake spectacles utilizing light and transmission electron microscopy and found that the morphology of the spectacle in the snake reflects its evolutionary adaptation and development. Snakes have adapted to thrive in different environments, which are reflected in their wide-ranging ecological roles and differing activity pattern. Due to this diversity, the visual and eye protection requirements differ among species[15]. This variation may influence the morphological characteristics of the spectacles in specific species. Thus, for this reason, the current study presents a description of anatomical and histological investigations of the spectacle of two snakes (*Psammophis sibilans* and *Spalerosophis diadema)*.

MATERIAL AND METHODS

Five adult snakes of *P. sibilans* were captured from Kafr Shukr (30°34′30″N, 31°17′26″E), Qalyubia Governorate, Egypt, and five adult snakes of *S. diadema* were captured from Dabaa city (31°02′00″N, 28°26′00″E), Matrouh Governorate, Egypt, during April and May 2023. The dissection of specimens was performed in accordance with the guidelines of the Aswan University Research Ethics Committee (approval number: ASWU/05/SC/ZO/24-01/06). An Olympus microscope and a Toup camp XCAM full HD camera (model SZ61, Olympus Europa SE & Co. KG, Hamburg, Germany) were used to take photos of the sample heads, which were preserved for a long period in 2% phenoxy ethanol after being fixed in 10% formalin for two weeks for anatomical study. According to previously published standards, the anatomical language for the orbital tissue was established[17].

For light microscopy examinations, the specimens' heads were sliced in half lengthwise. Following three days of storage in 10% neutral formalin, the samples were decalcified in EDTA for two weeks. The samples were dehydrated in ethyl alcohol, washed for three days in methyl benzoate, then embedded in paraffin wax and serially sectioned (7 µm). Sections were stained using Masson's trichromic and hematoxylin/eosin stains[18]. The photos were captured using an Olympus camera (model DP74) mounted on an Olympus microscope (type BX43).

RESULTS

Anatomical investigations of the spectacle of *P. sibilans* and *S. diadema*

The spectacle is an integumental structure fixed, transparence with curvature covering the cornea of *P. sibilans* and *S. diadema* (Figures 1 and 2). The length of the spectacle of the *P. sibilans* is ~3.88 mm vertical and ~4.72 mm horizontal. The thickness of the spectacle of *P. sibilans* is ~0.06 mm. The length of the spectacle of the *S. diadema* is ~4.32 mm vertical and ~4.81 mm horizontal.

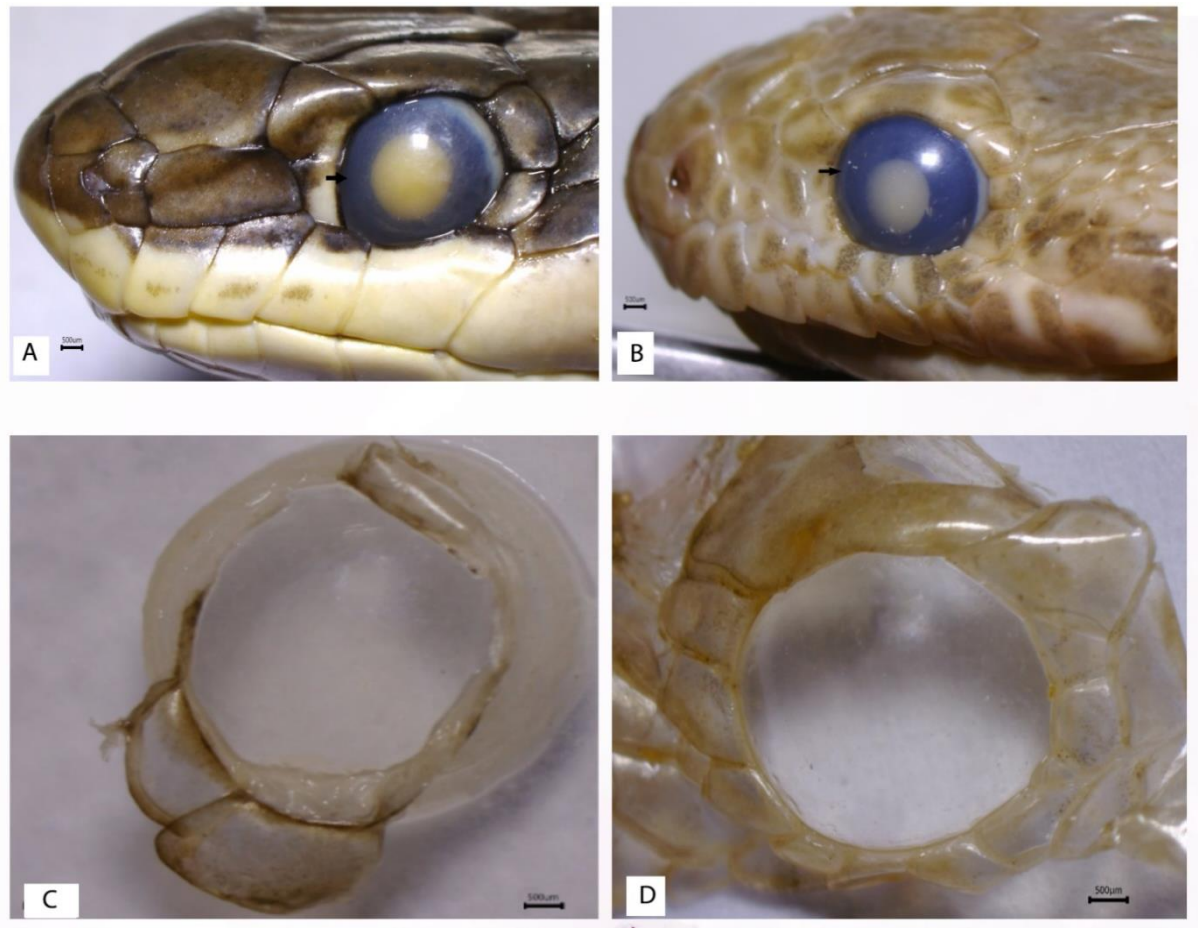

Figure 1: Photomacrograph of lateral view of head showing: **(A and B)** the spectacle (arrow), an integument structure fixed covering the cornea, **(C and D)** lateral view of spectacle showing transparence of spectacle. **(A and C)** *Psammophis sibilans*, **(B and D)** *Spalerosophis diadema*, (scale bar = 500 μm).

The thickness of the spectacle of the *S. diadema* is ~0.06 mm.

The periocular scales of both snake species surround the eye opening. The periocular scales are connected with the eye through the spectacle and create a little overhang around the spectacle at all 360 degrees. These scales are formed of preocular, postocular, supraocular, and subocular scales. Preocular scales are located immediately in front of the eye, while, postocular scales are located just behind of the eye. Supraocular scales are enlarged scales, and are located on the crown just above the eye, but the subocular scales lie just below. All scales in contact with the eye *via* spectacle (Figure 2C,D). The four types of periocular scales in both snake species exhibit variability in shape, color, and number.

In *P. sibilans*, the preocular scale is an irregular shape with black and little white color. Postocular scales consist of two small scales with rectangular shape and black color. The supraocular scale is one large scale with a plate-like shape and black color. Subocular scales are two (one large and one small) and rectangular shape with white and little black color (Figure 2C,D).

In *S. diadema*, preoculars scales are three with different size and brown color. Postocular scales are three small and triangular shapes with brown color. Supraocular scale is one large, plate-like with brown color. Subocular scales are four and triangular shapes with brown and white color (Figure 2C,D).

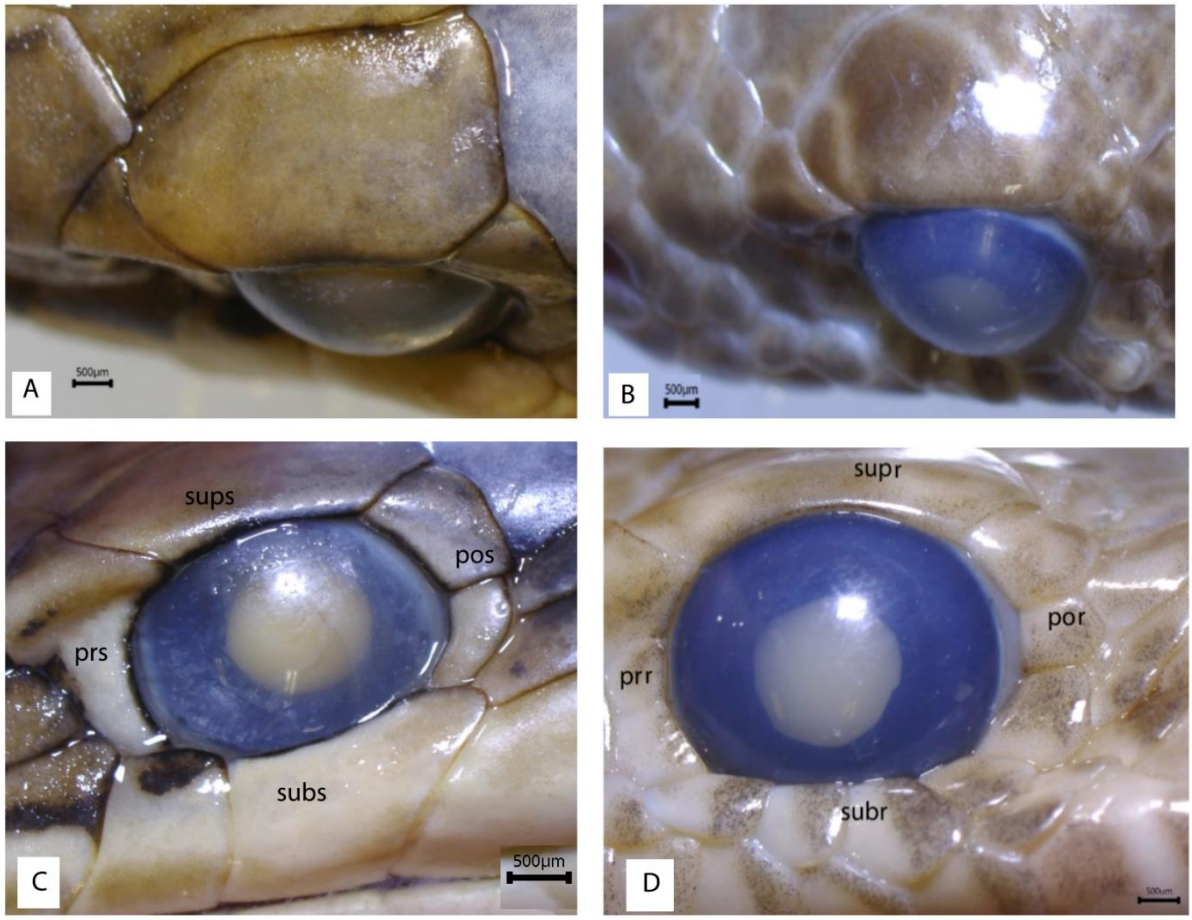

Figure 2: Photomacrograph of dorsal view of head showing: **(A)** curvature of *Psammophis sibilans* spectacle, **(B)** curvature of *Spalerosophis diadema* spectacle, **(C)** lateral view of eye of *P. sibilans* showing periocular scales of spectacle form of preocular scale (prs), postocular scales (pos), supraocular scale (sups), and subocular scales (subs), **(D)** lateral view of eye of *S. diadema* showing periocular scales of spectacle form of preocular scales (prr), postocular scales (por), supraocular scale (supr), and subocular scales (subr), (scale bar = 500 µm).

Histological investigations of the spectacle of *P. sibilans* and *S. diadema*

Histological investigation of the spectacle of the *P. sibilans* and *S. diadema* showed that the spectacle comprises of triple-layers: the outer epithelium, stroma, and inner epithelium (Figure 3). The outer epithelium of the spectacle of both snake species is made up of one or two basal germinal cells containing oval and rounded nuclei, and covered with thick keratin layer. The overall thickness of the outer epithelium measures approximately 32.62 µm in *P. sibilans* and 45.65 µm in *S. diadema* (Figure 3A,B). The stroma of the spectacle of both snake species is the largest layer at the spectacle, locates underneath the outer epithelium's basement membrane, and contains layers of organized collagen fibrils interlaced with blood vessels. Stroma of the spectacle of the *P. sibilans* measures approximately 27.79 µm, and the stroma of the spectacle of the *S. diadema* measures approximately 29.64 µm (Figure 3). The inner epithelium of the spectacle of both snake species is very thin and consists of a single layer of simple squamous epithelium cells that covers the posterior surface of the spectacle, thereby it forms the border to the subspectacular space. This cell layer measures approximately 2.30 µm in *P. sibilans* and approximately 2.73 µm in *S. diadema* (Figure 3A,B).

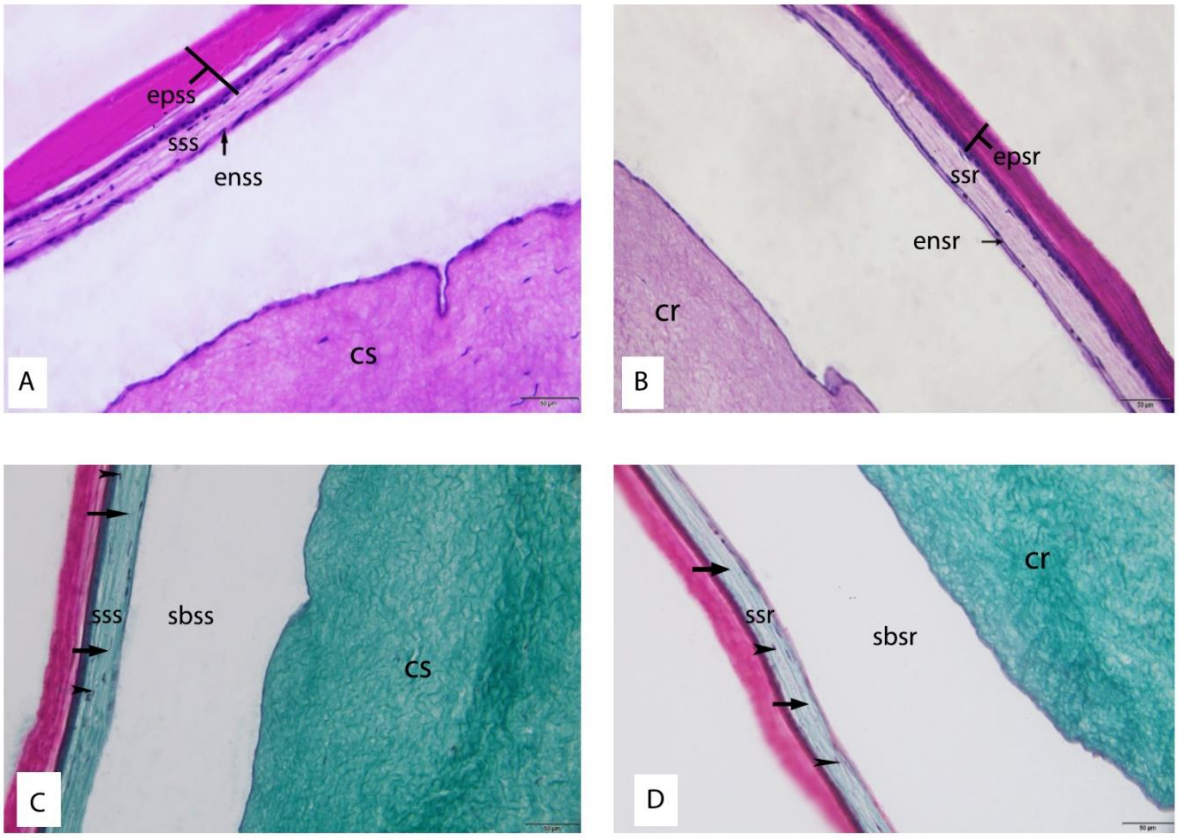

Figure 3: Photomicrograph of a transverse section of eye showing: **(A)** *Psammophis sibilans* spectacle comprises of triple-layers; outer epithelium (epss), stroma (sss), and inner epithelium (enss), cornea (cs), **(B)** *Spalerosophis diadema* spectacle comprises of triple-layers; outer epithelium (epsr), stroma (ssr), and inner epithelium (ensr), cornea (cr), **(C)** *P. sibilans* stroma (sss) contains layers of organized collagen fibrils (arrow) interlaced with blood vessels (arrowhead), cornea (cs) and subspectacular space (sbss), **(D)** *S. diadema* stroma (ssr) contains layers of organized collagen fibrils (arrow) interlaced with blood vessels (arrowhead), cornea (cr) and subspectacular space (sbsr) (**A and B:** hematoxylin and eosin stain, **C and D:** Masson's trichromic stain, magnification: 400×, scale bar = 50 μm).

The spectacle connects to the inner surface of the periocular scales through a transition zone, which enters into an integumental hinge region. At the edge of the spectacle (a transition zone), thickness of spectacle of the *P. sibilans* and *S. diadema* increases approximately 129.92 μm in *P. sibilans* and approximately 131.06 μm in *S. diadema*, while becoming thinner in the spectacle proper, approximately 60.78 μm in *P. sibilans* and approximately 69.19 μm in *S. diadema* (Figure 4). At edge of the spectacle (a transition zone) of *P. sibilans* and *S. diadema*, the basal cells of the outer epithelium enlarged and became taller compared to flattened basal cells of the spectacle proper. The rigid outer keratin layer of the outer epithelium diminished in thickness toward the transition zone, giving spectacle surface a wavy look typical of an integumental hinge region. Stroma's thickness increased toward the hinge area. The structure of the inner epithelium stayed the same across the transition zone (Figure 4). The spectacle of the *P. sibilans* and *S. diadema* is distanced from the cornea by subspectacular space. Subspectacular space lies between the outer surface of cornea and the inner epithelium of spectacle is filled with secretions that producing by the Harderian gland of both snake species (Figure 4).

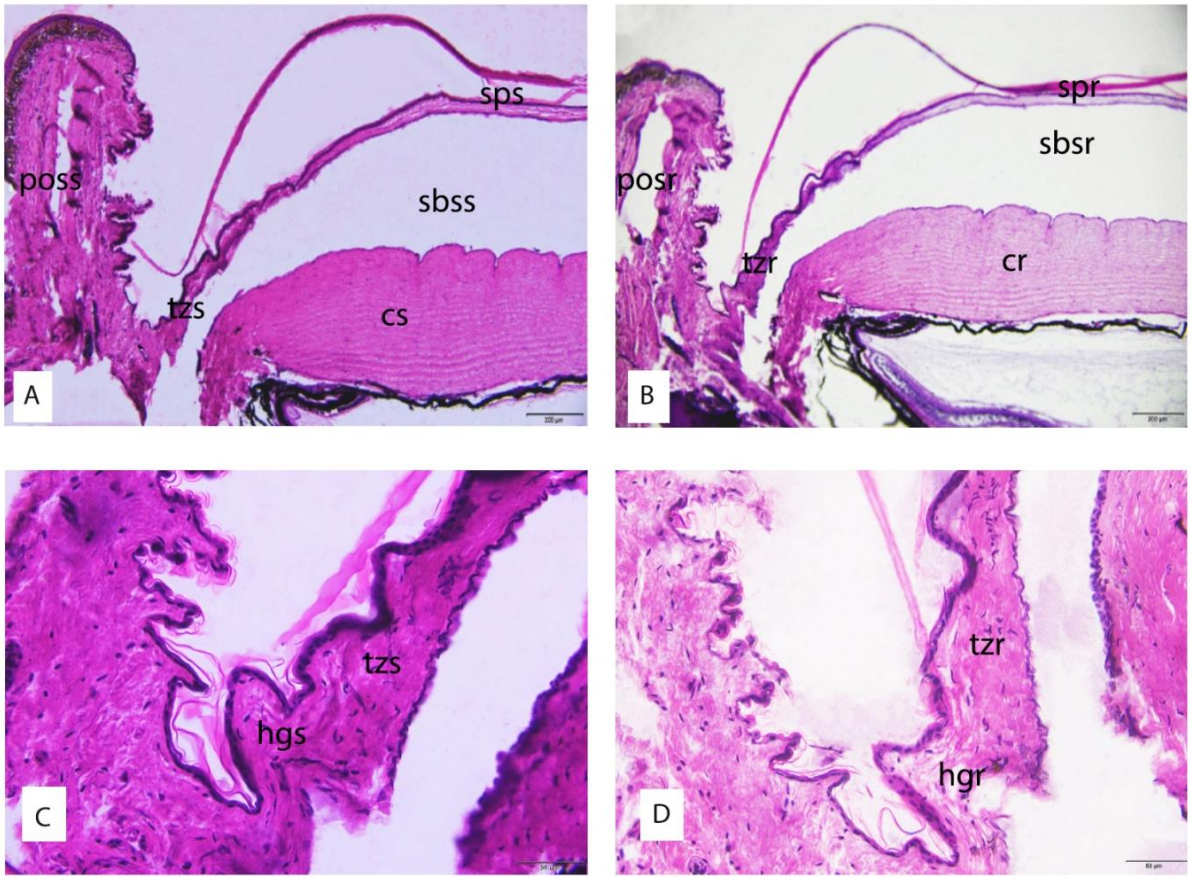

Figure 4: Photomicrograph of a transverse section of eye showing: **(A)** *Psammophis sibilans* spectacle (sps) connects to the inner surface of the periocular scales (poss) through a transition zone (tzs), and is distanced from the cornea (cs) by subspectacular space (sbss), **(B)** *Spalerosophis diadema* spectacle (spr) connects to the inner surface of the periocular scales (posr) by a transition zone (tzr), and is separated from the cornea (cr) by subspectacular space (sbsr) (**A and B**: hematoxylin and eosin stain, magnification: 200×, scale bar = 100 μm), **(C)** at edge of the *P. sibilans* spectacle (a transition zone) (tzs) and hinge region (hgs), **(D)** at edge of the *S. diadema* spectacle (a transition zone) (tzr) and hinge region (hgr) (**C and D:** hematoxylin and eosin stain, magnification: 400×, scale bar = 50 μm).

DISCUSSION

In snakes, the spectacle is formed by fusion of the eyelids during development[9,12]. A secondarily derived spectacle found in certain species of geckos and skinks. Wyneken[19] referred that these species have transparent lower eyelids made of clear scales that allow for limited vision even when the lids are closed. This modification reaches its extreme form in certain geckos and skink, *Ablepharus* sp.[20]. The present study revealed that a spectacle connects the periocular scales of *P. sibilans* and *S. diadema*, which encircle an eye opening, and form of preocular, postocular, supraocular, and subocular scales. The four types of periocular scales of two studied snakes are different in shape, color, and number. Van Doorn and Sivak[10] indicated that spectacle scales may differ between snakes and geckos and between snake families.

Despite that the spectacle of snakes varies in thickness between species[2], it is nearly identical in both snakes in the present study. Da Silva *et al*.[16] indicated that thinner spectacles are observed in arboreal and terrestrial species, while thicker spectacles are present in burrowing and aquatic species, Thus, snakes that live underground or are

surrounded by water require a greater protective layer than those that live in arboreal and terrestrial snakes. The spectacle of arboreal and terrestrial snakes may have become thinner in order to enhance their visual acuity[16]. Therefore, thickness relates to the necessity of safeguarding the eye from the environment[2]. Both snake species live in the same terrestrial habitat, so the present study agrees with other previous studies, which mentioned the thickness of the spectacle correlated to habitat. On the other hand, the spectacle diameters in both snakes exhibit great variability. The spectacle diameter of the *S. diadema* is larger than the diameter of the *P. sibilans*. Snakes that were used in this study are quite diversified in terms of activity patterns. Da Silva *et al*.[16] indicated that spectacle diameter was correlated to activity pattern. Liu *et al*.[21] pointed out the nocturnal species possess larger eyes compared to diurnal species in the same habitat. Vertebrates that rely on vision and forage at night possess larger eyes compared to their diurnal counterparts, like gecko[22], shorebirds[23], and primates[24]. The differences in spectacle diameter between both snakes are influenced by their activity patterns.

The histological evaluation showed that the spectacle resembles the skin composed of a stratum corneum, often known as the spectacle scale, a complex epidermis and a dermis[13]. Unlike most eyelids or the skin, the spectacle is transparence, perfectly adapted for vision. However, it shares a trait with the rest of the integument, which is its vascularity. The blood vessels of the spectacle of both snakes are located in the center layers of the stroma, similar to boas and pythons, whereas colubrids blood vessels are found closer to the inner epithelium in colubrids[15,25]. Da Silva *et al*.[15] mentioned that the position of the blood vessels of the spectacle in the center or inner layers of the stroma may influence the visual acuity of the snake. In conclusion, the anatomical and histological features of the spectacle of the eye in *P. sibilans* and *S. diadema*, highlighted how the thickness of the spectacle correlates with habitat, as well as the diameter of the spectacle of the both snakes influenced by their differing activity patterns.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interests.

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مقارنة مورفولوجية لنظارة ثعباني "*Psammophis sibilans* و *Spalerosophis diadema*" باستخدام الدراسات التشريحية والنسيجية

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تم تقديم وصف مورفولوجي لنظارة الثعبانين (*Psammophis sibilans* و *Spalerosophis diadema*) في الدراسة الحالية. تم اصطياد خمسة ثعابين بالغة من النوع "*P. sibilans*" من محافظة القليوبية، وخمسة ثعابين بالغة من النوع "*S. diadema*" من محافظة مطروح، خلال شهري أبريل ومايو 2023. تحيط حراشف محيط العين في كلا النوعين من الثعابين بفتحة العين. ترتبط هذه الحراشف بالعين من خلال النظارة وهي تتكون من حراشف أمام العين، وخلف العين، وفوق العين، وتحت العين. من الناحية المورفولوجية، تُظهر أربعة أنواع من الحراشف حول العين في الثعابين المدروسة تباينًا في الشكل واللون والعدد. في الثعبان "*P. sibilans*"، تُكون الحراشف الأمامية للعين شكلًا غير منتظم باللون الأسود ولون أبيض خفيف. وتتكون الحراشف الخلفية العين من حرشفتين صغيرتين ذات شكل مستطيل ولون أسود. والحراشف فوق العين عبارة عن حرشفة كبيرة واحدة، تشبه الصفيحة، سوداء اللون. الحراشف تحت العين ثنائية الشكل، مستطيلة الشكل، بيضاء اللون مع قليل من اللون الأسود. وفي ثعبان "*S. diadema*"، تتكون الحراشف الأمامية للعين من ثلاثة أحجام مختلفة بلون بني. أما الحراشف الخلفية للعين فهي ثلاثة أشكال صغيرة مثلثة الشكل بنية اللون. والحراشف فوق العين هي واحدة كبيرة تشبه الصفيحة بلون بني. أما الحراشف تحت العين فهي أربعة أشكال مثلثة، بنية وبيضاء اللون. تتشابه نظارة كلا الثعبانين في التركيب النسيجي. وتتكون النظارة من ثلاث طبقات، تتصل بالسطح الداخلي للحراشف المحيطة بالعين عبر منطقة انتقالية، تدخل في منطقة مفصلية لغشاء الجلد.