Ontogeny of scleral ossicles of giant amazon river turtles *Podocnemis expansa* Schweigger, 1812 (Testudines, Podocnemididae)

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Abstract

Scleral ossicles are bony plates in the eye sockets of many vertebrates, whose morphology, development and position differ in distinct groups of vertebrates. To investigate the development, number, shape and arrangement of the scleral ossicles of *Podocnemis expansa*, 15 embryos were collected, starting from the 18th day of natural incubation and 16 adult specimens belonging to the LAPAS collection, whose eye sockets were removed. The embryos and eye sockets were subjected to bone diaphanization and staining techniques. The scleral ossicles in this species of Testudines were found to be quadrangular, varying in number from 10 to 13, with 11.25 ± 0.93 in the right eye and 11.43 ± 0.81 in the left eye, occupying a position close to the anterior edge of the sclera. The ossicles develop intramembranously and begin to present ossification centers at stage 21, with ten ossification centers in the right eye and eight in the left eye. In stage 23, all the ossicles of the sclerotic ring increase in length and approach the adjacent ossicles, forming a ring. In stage 26, the scleral ossicles are already arranged in their definitive form.

Keywords: ossification, embryo, eye, alizarin, reptiles.

1 Introduction

The sclera is the outermost covering of the eye, a strong structure that preserves the shape of the eye socket, preventing internal and external pressures from modifying it. Another type of protection is provided by the presence of a sclerotic ring, a series of bony plates in fixed positions (HILDEBRAND and GOSLOW, 2006; MAHECHA and OLIVEIRA, 1988).

Bony scleral plates or ossicles are common in many vertebrates, including Birds, Testudines and some Squamata, but are absent in Ophidians, Crocodilians, Amphibians and Mammals (ANDREWS, 1996; FRANZ-ODENDAAL, 2005).

According to Hall (1981) and Pinto and Hall (1991), scleral papillae induce the formation of scleral ossicles. Hall and Miyake (1992) state that intramembranous ossification occurs in reptiles and birds. An experimental study of chicken embryos proposed that the scleral ossicles emerge as condensations of mesenchymal cells originating from the neural crest (HALL, 1981; JOHNSON, 1973). There is always an exact correspondence between the number of ossicles and papillae, with the latter preceding the development of the scleral ossicles (FYFE, FERGUSON and CHIQUET-EHRISMANN, 1988; FRANZ-ODENDAAL, 2005; HALL and MIYAKE, 1992).

The removal of one or more papillae from the sclera in chicken embryos in a given phase of their development prevents the induction of the ossicle beneath this removed papilla, while other ossicles of the ring, without rupture of the papillae, develop normally (COULOMBRE, COULOMBRE and MEHTA, 1962).

Several authors have confirmed the presence of the papillae in different species of Testudines, such as *Chelydra serpentina* (FRANZ-ODENDAAL, 2005; YNTEMA, 1968), *Carettochelys insculpta* (BEGGS, YOUNG, GEORGES et al., 2000), *Apalone spinifera* (GREENBAUM and CARR, 2002); *Pelodiscus sinensis* (TOKITA and KURATANI, 2001); *Chrysemys picta* (MAHMOUD, KLICKA and HESS, 1973); *Dermochelys coriacea* (RENOUS, RIMBLOT-BALY, FRETEY et al., 1989); and *Trachemys scripta* (GREENBAUM, 2002).

According to Modesto and Anderson (2004), birds and reptiles are organized in the same group and their scleral ossicles are morphologically similar (WALLS, 1942). Considering the similarities in the development of the papillae of birds and reptiles, it is possible that the scleral ossicles of all Testudines are induced by the scleral papillae (FRANZ-ODENDAAL, 2005).

Knowledge of the development of these small bones is relevant in the clinical treatment of reptiles, since there has already been a case report of fracture of the scleral ossicles of a *Buteo jamaicensis* hawk (LINDLEY, HATHCOCK, MILLER et al., 1988). According to those authors, the ossicles of the sclera serve as the place of origin of the striated ciliary muscles and provide protection for the intraocular content.

Andrews (1996) and Franz-Odendaal (2005) analyzed several series of scleral ossicles in four species of Cryptodira: *Chelydra serpentina, Sternotherus odoratus*, *Trachemys scripta elegans* and *Chrysemys picta*. However, such a study has not yet been made of *P. expansa*.

Podocnemis expansa is a fluvial Testudine of the Podocnemididae family found in the Amazon River and most of its tributaries. It is a large species, with the largest specimens reaching more than 90 cm in length. Its meat and eggs are highly appreciated, constituting the basis for various dishes of Amazonian cuisine. This species is also locally called araú, jurará-açu and tartaruga-da-Amazônia (Amazonian turtle) (GASPAR, SILVA, SÃO-CLEMENTE, 2005; LUZ, STRINGHINI, BATAUS et al., 2003; MALVASIO, SOUZA, REIS et al., 2002).

The objective of this work is to examine the development, number, shape and arrangement of the ossicles of the sclera during the prenatal period and in adult specimens of *Podocnemis expansa*.

2 Material and methods

Fifteen embryos of *Podocnemis expansa* (Testudines, Podocnemididae) were collected during the spawning periods of 2005 and 2006 in the reproduction area protected by IBAMA/RAN (Reptile and Amphibian Conservation and Management Center) on the beaches of the Araguaia River in the state of Goiás, Brazil, in the region called Remansão (13º 20' 38.7" S and 50º 38' 05.7" W), under permit no. 117/2005-IBAMA/RAN. In addition, 16 adult specimens belonging to the collection of the Wild Animal Research Laboratory (LAPAS) were purchased and their eye sockets removed.

Egg specimens were collected randomly from an arbitrarily selected nest up to hatching. The stages of embryonic development were named according to the external morphological criteria described by Danni et al. (1990). All the embryos were removed from the eggs by cutting the shell with surgical scissors, isolating them from the vitellus and membranous sacs.

Embryos and eye sockets were subjected to diaphanization by potassium hydroxide (KOH) and bone staining with alizarin red S, and five embryos were also processed with the cartilage staining technique using alcian blue, following the methods described by Davis and Gore (1936) and Digenkus and Uhler (1977) modified at the Wild Animal Research Laboratory (LAPAS) of the Federal University of Uberlândia (UFU) Faculty of Veterinary Medicine.

The presence of ossification centers and the distinct stages of development of the bone elements that make up the scleral ossicles (Figure 1) were analyzed under a stereoscopic microscope (Olympus SZX-12) interfaced with an electronic camera (Olympus DP-10) and a digital camera (Olympus FE-100, 6.0). A schematic drawing was also made of the scleral bones (Figure 1), using six specimens of adult *P. expansa* fixed in formaldehyde from the LAPAS collection (Figure 2).

3 Results

The adult specimens of *Podocnemis expansa* displayed 11.25 ± 0.93 scleral ossicles in the right eye and 11.43 ± 0.81 in the left, varying from 10 to 13 ossicles. The ossicles are quadrangular and occupy fixed positions close to the anterior edge of the sclera (Figure 1).

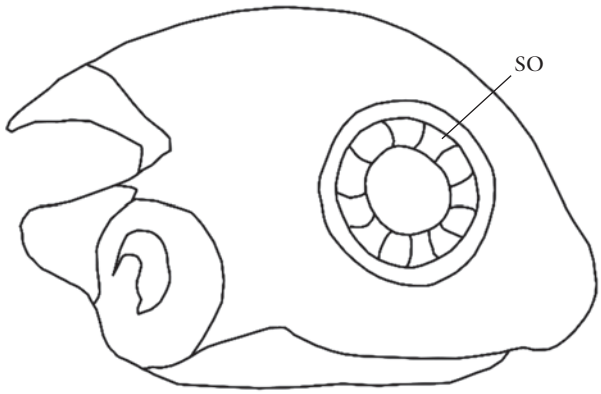

Figure 1. Schematic drawing of the scleral ossicles of adult *Podocnemis expansa*. SO, ossicles of the sclera.

The ossification centers of the scleral ossicles in *Podocnemis expansa* do not begin at the same time, and are characterized by specific temporal patterns. The number of ossicles in each eye socket is also not uniform within this species. Variations were found between individual animals and between the left and right antimeres of the same animal.

Ten ossification centers were found at the beginning of stage 21 in the sclera of the right eye and eight ossification centers in the left eye. As can be seen in Figure 2a, the ossification centers are at the same level of development. In stage 22 (Figure 2b), 11 ossicles were observed in the right eye and 12 in the left eye socket. In this stage, the ossicles are still well separated from each other, but already have a clearly quadrangular shape.

In stage 23, all the ossicles of the sclerotic ring are close to the adjacent ossicles (Figure 2c), and in stage 24 the ossicles are joined to each other, forming a ring with discrete overlapping between the bony plates. In stage 26, the scleral ossicles are arranged in their definitive position (Figure 2d).

According to Franz-Odendaal (2008) and Warheit et al. (1989), the ossicles of the sclera are bony elements located in the sclerotic ring of the eye socket of many vertebrates, including *P. expansa*, differing in their morphology, development, number and position.

In studies of *C. serpentina*, Franz-Odendaal (2005) concluded that the process of ossification of the scleral ossicles is intramembranous, as it is in *P. expansa*. However and Andrews (1996) contradicts this information, claiming that the scleral ossicles of *C. serpentina* ossify endochondrally, as reported by Franz-Odendaal and Hall (2006) for teleost fishes.

According to Andrews (1996), *C. serpentina* and *C. picta* begin to ossify at stage 21, as occurs in *P. expansa*. The scleral ossicles in *C. serpentina* (FRANZ-ODENDAAL, 2005) start the process of ossification at the end of stage 19, and in stage 22 all the plates present ossification centers. After hatching, the scleral ossicles form a continuous ring, with the plates presenting discrete overlapping of their edges. Deraniyagala (1939) apud Franz-Odendaal (2005) observed a similar ossification pattern in *Dermochelys coriacea* and this

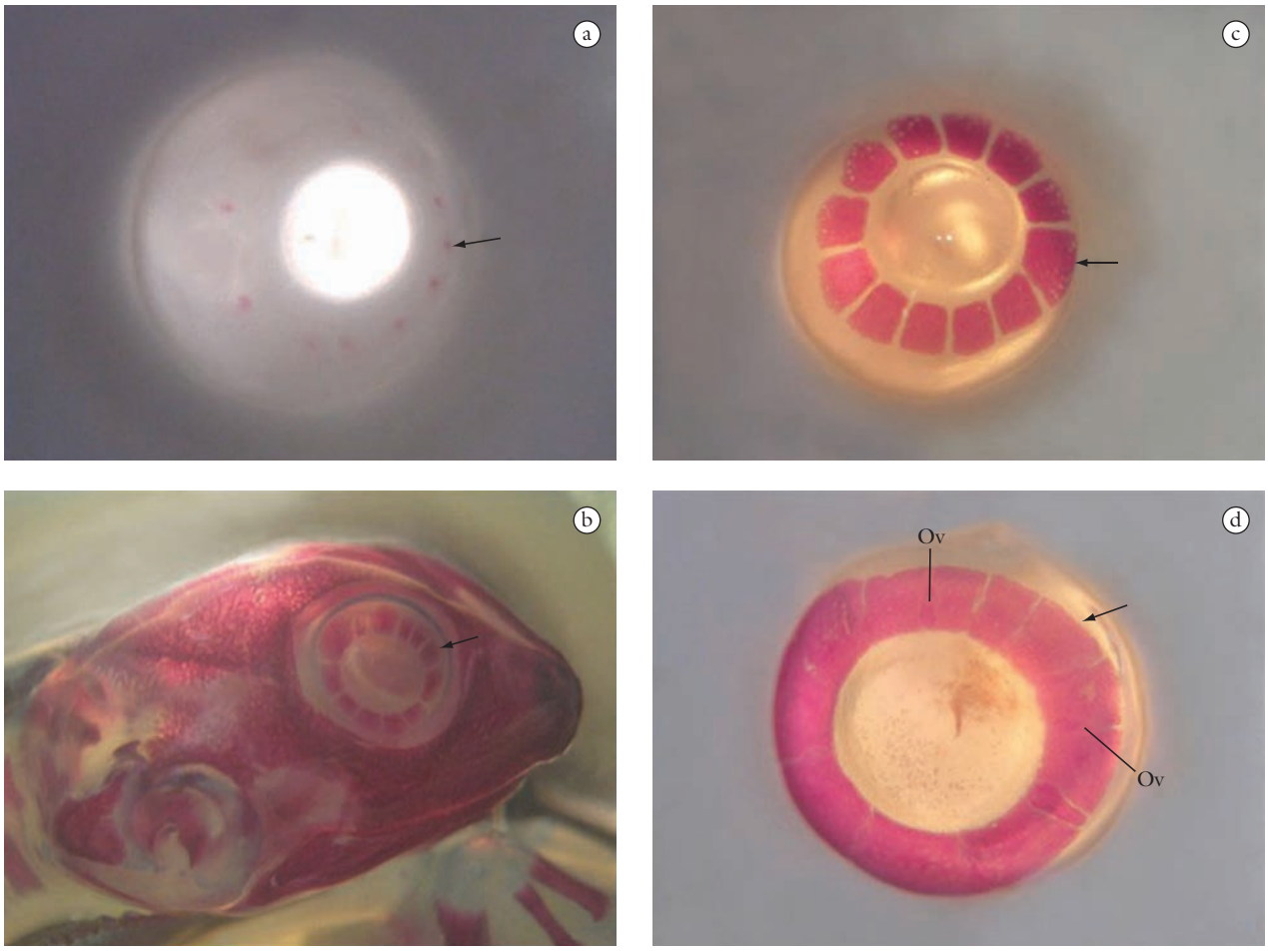

Figure 2. Photographs of *Podocnemis expansa* eyes. Right eye – corneal view. a) stage 21; b) stage 22; c) stage 23; d) stage 24. Arrow – ossicles of the sclera, Ov – overlapping. Diaphanization by KOH and bone staining with alizarin red S (a, c and d) and cartilage staining with alcian blue (b). (a, c and d) x 16, (b) x 7.

pattern is also seen in chicken embryos (COULOMBRE and COULOMBRE, 1973) as well as in *P. expansa*.

Warheit et al. (1989) stated that a sclerotic ring composed of overlapping bony plates enables the ring to grow during ontogeny. Hildebrand and Goslow (2006) suggest that the ring, with an arrangement of overlaps at the edges of the bony plates, gives the eye socket greater resistance against deformations and allows for better development of the bony plates.

In addition to the ossification pattern, the number of ossicles in the sclera also varies among taxa (FRANZ-ODENDAAL, 2005; WARHEIT, GOOD and QUEIROZ, 1989). Warheit et al. (1989) proposed that the difference in the number of ossicles among taxa can be attributed to the peculiar growth of each taxon.

In primitive fishes, the ring consisted of four plates, which were reduced to two elements. Most teleosts today have only two ossicles situated anteriorly and posteriorly in the eye socket (NAKAMURA and YAMAGUCHI, 1991). In the ancestral crossopterygians of the terrestrial vertebrates and in the most ancient fossils of amphibians, the number of plates increased substantially and a large number is still present in reptiles and birds (FRANZ-ODENDAAL, 2005; HILDEBRAND and GOSLOW, 2006; SANES, 1992). *Gallus gallus* has 13 to 14 scleral ossicles in each eye (PALMOSKI and GOETINCK, 2005). *Alectura lathami*, *Numida meleagris* and *Aburria aburri* have 14 scleral ossicles, while *Crotophaga ani* and *Tauraco hartlaubi* have 12 and 13 scleral ossicles, respectively (WARHEIT, GOOD and QUEIROZ, 1989).

Underwood (1970) reported that in Testudines, the number of ossicles varies from 6 to 13. *C. serpentina* has 10 scleral ossicles (FRANZ-ODENDAAL, 2005), and *P. expansa* has an average of 11.25 ± 0.93 ossicles in the right eye socket and 11.43 ± 0.81 in the left.

4 Conclusion

The species *P. expansa* has a scleral ring with 11.25 ± 0.93 ossicles in the right and 11.43 ± 0.81 in the left eye socket, varying from 10 to 13 ossicles.

The ossicles are quadrangular in shape and develop intramembranously, beginning to present ossification centers at stage 21 and reaching their definitive arrangement in stage 26.

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References

ANDREWS, KD. An endochondral rather than a dermal origin for scleral ossicles in cryptodiran turtles. *J. herpetol.* 1996, vol. 30, p. 651-662.

BEGGS, K., YOUNG, J., GEORGES, A. et al. Ageing the eggs and embryos of the pig-nosed turtle, Carettochelys insculpta (Chelonia: Carettochelydidae), from northern Australia. *Can. J. Zool.* 2000, vol. 78, no. 1, p. 373-392.

COULOMBRE, AJ. and COULOMBRE, JL. The skeleton of the eye: II overlap of the scleral ossicles of the domestic fowl. *Dev. Biol.* 1973, vol. 33, no. 2, p. 257-267.

COULOMBRE, AJ., COULOMBRE, JL. and MEHTA, H. The skeleton of the eye. I. Conjunctival papillae and scleral ossicles. *Dev. Biol.* 1962, vol. 5, p. 382-401.

DANNI, TMS., DARDENNE, MAR. and NASCIMENTO, SM. Estudo morfológico do desenvolvimento embrionário da tartaruga-da-amazônia, *Podocnemis expansa*, pelomedusidae. *Rev. Brasil. Biol.* 1990, vol. 50, p. 619-625

DAVIS, DD. and GORE, UR. Clearing and staining skeleton of small vertebrates. *F. Mus. Natl History.* 1936, vol. 4, p. 3-15.

DERANIYAGALA, PEP. *The Tetrapod Reptiles of Ceylon*. Ceylon: Colombo Museum, 1939.

DIGENKUS, G. and UHLER, L. Differential staining of bone and cartilage in cleared and stained fish using alcian blue to stain cartilage and enzymes for clearing fish. *Stain Technol.* 1977, vol. 52, p. 229-232.

FRANZ-ODENDAAL, TA. and HALL, BK. Skeletal elements within teleost eyes and a discussion of their homology. *J. Morphol.* 2006, vol. 267, no. 11, p. 1326-1337.

FRANZ-ODENDAAL, TA. Intramembranous ossification of scleral ossicles in Chelydra serpentina. *Zool.* 2005, vol. 109, no. 1, p. 75-81.

FRANZ-ODENDAAL, TA. Scleral Ossicles of Teleostei: Evolutionary and Developmental Trends. *Anat. Rec.* 2008, vol. 291, no. 2, p. 161-168.

FYFE, DM., FERGUSON, MWJ. and CHIQUET-EHRISMANN, R. Immunocytochemical localisation of tenascin during the development of scleral papillae and scleral ossicles in the embryonic chick. *J. Anat.* 1988, vol. 159, p. 117-127.

GASPAR, A., SILVA, TJP. and SÃO-CLEMENTE, SC. Insensibilização e Rendimento de Carcaça de Tartaruga-da-Amazônia (*Podocnemis expansa*). *Braz. J. Food Technol.* 2005, vol. 8, no. 1, p. 57-61.

GREENBAUM, E. A standardized series of embryonic stages for the emydid turtle Trachemys scripta. *Can. J. Zool.* 2002, vol. 80, no. 8, p. 1350-1370.

GREENBAUM, E. and CARR, JL. Staging criteria for embryos of the spiny softshell turtle, Apalone spinifera (Testudines: Trionychidae). *J. Morphol.* 2002, vol. 254, no. 3, p. 272-291.

HALL, BK. and MIYAKE, T. The membranous skeleton: the role of cell condensations in vertebrate skeletogenesis. *Anat. embryol.* 1992, vol. 186, no. 2, p. 107-124.

HALL, BK. Specificity in the differentiation and morphogenesis of neural crest-derived scleral ossicles and of epithelial scleral papillae in the eye of the embryonic chick. *J Embryol Exp Morphol.* 1981, vol. 66, p. 175-190.

HILDEBRAND, M. and GOSLOW, GE. *Análise da estrutura dos vertebrados.* São Paulo: Atheneu, 2006.

JOHNSON, LG. Development of chick embryo conjuntival papillae and scleral after hydrocortisone treatment. *Dev. Biol.* 1973, vol. 30, p. 223-227.

LINDLEY, DM., HATHCOCK, JT., MILLER, WW. et al. Fractured scleral ossicles in a red tail hawk. *Vet. radiol. ultrasound.* 1988, vol. 29, p. 209-212.

LUZ, VLF., STRINGHINI, JH., BATAUS, YSL. et al. Rendimento e composição química de carcaça da tartaruga-da-Amazônia (*Podocnemis expansa*) em sistema comercial. *Rev. bras. zootec.* 2003, vol. 32, no. 1, p. 1-9.

MAHECHA, GAB. and OLIVEIRA, CA. An Additional Bone in the Sclera of the Eyes of Owls and the Common Potoo *(Nictibius griseus)* and its Role in the Contraction of the Nictitating Membrane. *Acta anat.* 1988, vol. 163, no. 4, p. 201-211.

MAHMOUD, IY., KLICKA, J. and HESS, GL. Normal embryonic stages of the western painted turtle (Chrysemys picta belli). *J. Morphol.* 1973, vol. 141, p. 269-279.

MALVASIO, A., SOUZA, AM., REIS, ES. et al. Morfologia dos órgãos reprodutores de recém-eclodidos de *Podocnemis expansa* (Schweigger, 1812) e *P. unifilis* (Troschel, 1848) (Testudines, Pelomedusidae). Rev. *Inst. Pau Bras.* 2002, vol. 5, p. 27-37.

MODESTO, SP. and ANDERSON, JS. The phylogenetic definition of Reptilia. *Syst Biol.* 2004, vol. 53, no. 5, p. 815-821.

NAKAMURA, K. and YAMAGUCHI, H. Distribution of scleral ossicles in teleost fishes. *Mem. Fac. Fish. Kagoshima Univ.* 1991, vol. 40, p. 1-20.

PALMOSKI, MJ. and GOETINCK, PF. An analysis of the development of conjunctival papillae and scleral ossicles in the eye of the scaleless mutant. *J. Exp. Zool.* 2005, vol. 174, no. 2, p. 157-164.

PINTO, CB. and HALL, BK. Toward an understanding of the epithelial requirement for osteogenesis in scleral mesenchyme of the embryonic chick. *J. Exp. Zool.* 1991, vol. 259, no. 1, p. 92-108.

RENOUS, S., RIMBLOT-BALY, F., FRETEY, J. et al. Caracteristique du développement embryonnaire de la Tortue Luth, Dermochelys corciacea (Vandelli, 1761). *Ann. Sci. Nat. Zool.* 1989, vol. 13, p. 197-229.

SANES, JR. On the republication of the Hamburger-Hamilton stage series. *Dev. Dyn.* 1992, vol. 195, no. 4, p. 229-230.

TOKITA, M. and KURATANI, S. Normal embryonic stages of the Chinese soft-shelled turtle Pelodiscus sinensis (Trionychidae). *Zool. Sci.* 2001, vol. 18, no. 5, p. 705-715.

UNDERWOOD, G. The eye in: GANS, C. *Biology of the Reptilia.* London: Academic Press, 1970.

WALLS, GL. The Vertebrate Eye and its Adaptive Radiation. *Inst. Sci. Bull.* 1942, vol. 19, p. 467-472.

WARHEIT, KI., GOOD, DA. and QUEIROZ, K. Variation in numbers of scleral ossicles and their phylogenetic transformations within the pelecaniformes. *Auk.* 1989, vol. 160, no. 3, p. 383-388.

YNTEMA, CL. A series of stages in the embryonic development of Chelydra serpentina. *J. Morphol.* 1968, vol. 125, no. 2, p. 219-252.

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