Original article

Anatomy of the scleral ossicles in brazilian birds

Lima, FC.*, Vieira, LG., Santos, ALQ., De Simone, SBS., Hirano, LQL., Silva, JMM. and Romão, MF.

Wild Animal Research Laboratory, Faculty of Veterinary Medicine, Federal University of Uberlândia – UFU, Av. Dr. Luiz Antonio Waack 1365, Umuarama, CEP 38402-030, Uberlândia, MG, Brazil

*E-mail: fabianocl21@hotmail.com

Abstract

The sclera is the outermost layer of the eye. In some vertebrates, it consists of a scleral ring of osseous plates that afford protection against pressure and help maintain the shape of the eyeball. The morphology, number, development and position of the scleral ossicles differ in distinct groups of vertebrates. Therefore, the objective here was to examine the number, shape and arrangement of the scleral ossicles in Brazilian birds of different orders. The study involved 208 birds of 18 orders, which died from causes unrelated to eye infections. The birds' eyeballs were removed and subjected to diaphanization and staining of the ossicles with Alizarin red S. All the eyeballs analyzed presented scleral ossicles in a fixed position in the central portion of the eyeball, but which varied in size, shape and number. The number of bony plates in the various bird species varied from 11 to 16, with a modal number of 14, and their shape was predominantly quadrangular, showing a similar pattern in species of the same order. Some specimens presented differences in the number of ossicles in the right and left eyeball, as in *Spheniscus magellanicus*, *Elanus leucurus*, *Ramphastos toco* and others and *Leptodon cayanensis* also presented a scleral sesamoid bone. The distribution and morphology of the ossicles vary according to the taxonomic group, although species of the same order present a similar pattern.

Keywords: alizarin, eyeball, morphology, skeleton.

1 Introduction

The sclera is the outer covering of the eye, a tough structure that preserves the shape of the eyeball, preventing internal and external pressure from modifying its shape (HILDEBRAND and GOSLOW, 2006; MACHECHA and OLIVEIRA, 1998; FRANZ-ODENDAAL, 2008). Its skeleton is composed of scleral cartilage and ossicles, bony plates arranged jointly with the cartilage in a fixed position surrounding the sclera without articulating with any other skeletal elements. Some vertebrates such as fish, reptiles and birds possess both elements, while others, such as crocodilians and ophidians, have only scleral cartilage (FRANZ-ODENDAAL and HALL, 2006; WARHEIT, GOOD and QUEIROZ, 1989).

According to Hall (1981) and Pinto and Hall (1991), the scleral papillae induce the formation of the scleral ossicles, and there is always an exact correspondence between the number of ossicles and papillae (COULOMBRE, AJ. and COULOMBRE, JL., 1973). Hall and Miyake (1992) explain that the scleral papillae ossify intramembranously in reptiles and birds and endochondrally in fish. Experimental studies of *Gallus gallus* embryos demonstrated that the scleral bone skeleton emerges from the condensations of mesenchymal cells originating from the neural crest (HALL, 1981; JOHNSON, 1973; FRANZ-ODENDAAL, 2005; MIYAKE and HALL, 1992).

The individual role of each ossicle is still a subject of controversy, but two collective functions are well described. The first is to protect and support the eyeball during deformation while flying or diving (ROMER, 1956), and the second is to aid the function of the ciliary muscles, especially in the anterior portion of the cornea, suggesting a role of visual accommodation (KING and MCLELLAND, 1984; COULOMBRE, AJ. and COULOMBRE, JL., 1973; FRANZ-ODENDAAL and VICKARYOUS, 2006). It has also been suggested that these structures may help binocular vision, enabling the animal to adjust the shape of the cornea to modify its focusing power (LINDLEY, HATHCOCK, MILLER et al., 1988; COULOMBRE, AJ. and COULOMBRE, JL., 1973; CURTIS and MILLER, 1938), although any inference about its function should consider its generalized occurrence in several taxa (FRANZ-ODENDAAL, 2008).

The morphology, number and distribution of the scleral ossicles vary greatly among different groups of animals (VIEIRA, SANTOS and LIMA 2007). In most birds, the ossicles are rectangular in shape, while in others they are slightly elongated and concave (HALL, 1981; PINTO and HALL, 1991). However, their detailed pattern is still unknown in several animals, and this knowledge would be highly relevant in several clinical and therapeutic areas. Therefore, the objective of this work was to investigate the number, shape and arrangement of the scleral ossicles in birds of different orders.

2 Material and methods

This study involved 208 birds of 18 orders supplied by the clinic of the Veterinary Hospital at the Federal University of Uberlândia, from January 2007 to May 2009. The birds had died of several causes, though none of them from eye infections. The eyeballs of each animal were removed and subjected to diaphanization and bone staining, following the method described by Davis and Gore (1936). The steps consisted of fixation in 10% formaldehyde, dehydration with ethyl alcohol, diaphanization with potassium hydroxide (KOH 2%), bone staining with Alizarin red S, and conservation of the material in glycerin.

3 Results and discussion

All the eyes presented scleral ossicles in a fixed position in the central portion of the eyeball (Figure 1). The ossicles varied in size, shape and number, and did not present a consistent pattern of distribution and dimensions in the different species (Figure 2). According to Modesto and Anderson (2004), birds and reptiles are organized in the same group and their scleral ossicles are morphologically similar, well developed (FRANZ-ODENDAAL and HALL, 2006), and contribute to preserve the shape of the eyeball (ROMER, 1956). In birds, the arrangement of the ossicles in the scleral cartilage forms a groove in the concavity of the eye, which plays an important role in accommodating the cornea, as well as in its protection (FRANZ-ODENDAAL and HALL, 2006).

These structures are delicate and have persisted throughout the evolution of several vertebrates (ROMER, 1956). They have been reported in *Gephyrostegus*, *Seymouria*, *Labidosaurus*, *Bolosaurus*, *Prolacerta*, *Sphenodon* and others, varying greatly in number and morphology (PAREDES, 1942), as well as in the birds evaluated. The ossicles of fishes and birds can be considered homologous, although they are not situated exactly in the same region and ossify differently in these two groups (EAMES, DE LA FUENT and HELMS, 2003; EAMES and HELMS, 2004).

The presence of ossicles forming incomplete rings has been reported in Saurischian birds, and complete rings with 13 to 15 plates in *Ornithischia* (OSTROM, 1961; GALTON, 1974). Heilmann (1926) found 14 ossicles in *Archaeopteryx*, although Wellnhofer (1974) reported that this bird had 'approximately 12' ossicles (QUEIROZ and GOOD, 1988). Among the groups of reptiles and teleost fish, the presence of the scleral skeleton is considered a synapomorphic trait that indicates homology among the gnathostomata (MAISEY, 1986; DONOGHUE, FOREY and ALDRIDGE, 2000; FRANZ-ODENDAAL and HALL, 2006), and its arrangement is considered a plesiomorphic trait.

The number of plates in the distinct birds varied from 11 to 16 (Table 1, Figure 2), and was similar in species of

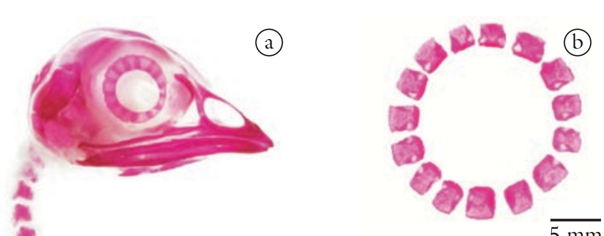

Figure 1. Photographs of the scleral ossicles of birds subjected to the protocol of diaphanization and bone staining with Alizarin red S. a) Ossicles of *Gallus gallus domesticus*; and b) Individual ossicles of *Caracara plancus.* Bar: 5 mm.

the same order. The modal number of ossicles was 14, with variations phylogenetically distributed among the different genera. In some taxa, the number of plates was uniform in the different specimens evaluated, as in *Ara nobilis*, *Coragyps atratus*, *Columba* sp. and *Tyto alba*. These may also represent the pattern for their order, given that in Strigiformes, for example, the mode was 15 ossicles in each eye, as well as in *T. alba*. According to Romer (1956), the number of plates that make up the ring varies from 2 to 32 among the different species. An average of 10 to 17 ossicles has been reported in the modern groups, and all the birds in this study lay within this interval.

Based on comparisons among ratites, tinamids and other fossil birds (QUEIROZ and GOOD, 1988), the primitive number of ossicles is 14 to 15, but today the number varies according to the group evaluated. Pelicaniforms present 14 to 15 (CURTIS and MILLER, 1938), while *Sula* reportedly possess the smallest number of ossicles, 10 (LEMMRICH, 1931; CURTIS and MILLER, 1938), and the modal number in Phalacrocoracidae is 13. According to Underwood (1970), Lacertas invariably have 14 ossicles with a fixed superposition pattern in the scleral cartilage, unlike Amphisbaenia, in which they are variable and irregular. These variations may be associated with the niche these animals occupy, which can be inferred for the different birds, since these traits are variable among the different genera, but maintain a pattern if one compares similar ethologies.

Both domestic and wild Galliformes possess a modal number of 14 ossicles. Queiroz and Good (1988) reported the presence of 14 ossicles in *Penelope jacquacu* and *P. purpurascens*, *P. albipennis* and *Perdix perdix*, the same number observed in *Penelope ochrogaster*. In *Piaya cayana*, *Crotophaga ani* and *Cariama cristata*, the authors describe the presence of 12 ossicles. The number is similar in these related Cuculiformes, although in *C. cristata* the number of plates varied from 12 to 14. The aforementioned authors found 15 ossicles in *Struthio*, *Dromaius*, *Crypturellus cinnamomeus* and *Tinamotis pentlandii*, while Lammrich (1931) found 12 in *Catarrhactes chrysocome*, *Sula bassanus*, *S. capensis*, *S. serrator*, *S. abbotti*, and *Todus todus*, as well as in 11 other species of Psittacidae, which seems to be the standard number for this group, albeit with a slight variation in *Ara ararauna*, *Aratinga solstitialis* and *Amazona* sp.

There may be differences in the number of ossicles present in the right and left eye in some species, or even in individuals. Only one *Spheniscus magellanicus* analyzed here presented 13 ossicles in the right sclera and 14 in the left, and *Elanus leucurus* had 15 in the right eye and 16 in the left, while in *Ramphastos toco* the number varied from 12 to 14 in both antimeres. In the only specimen of *Leptodon cayanensis*, the left eye had a small peripheral one, which Franz-Odendaal and Vickaryous (2006) described in *Bubo bubo* as a scleral sesamoid bone.

The ossicles exhibit a predominantly quadrangular shape (Figure 2), as reported by Franz-Odendaal and Hall (2006), albeit without uniformity. In Falconiformes, they are square or rectangular, in Psittaciformes, Columbiformes and Gruiformes they are trapezoidal, and in birds of the order Piciformes they are irregular and have sinuous edges. In reptiles, the ossicles are normally flat, although they may sometimes be slightly curved. Their arrangement surrounding the sclera creates superpositioning of the ossicles

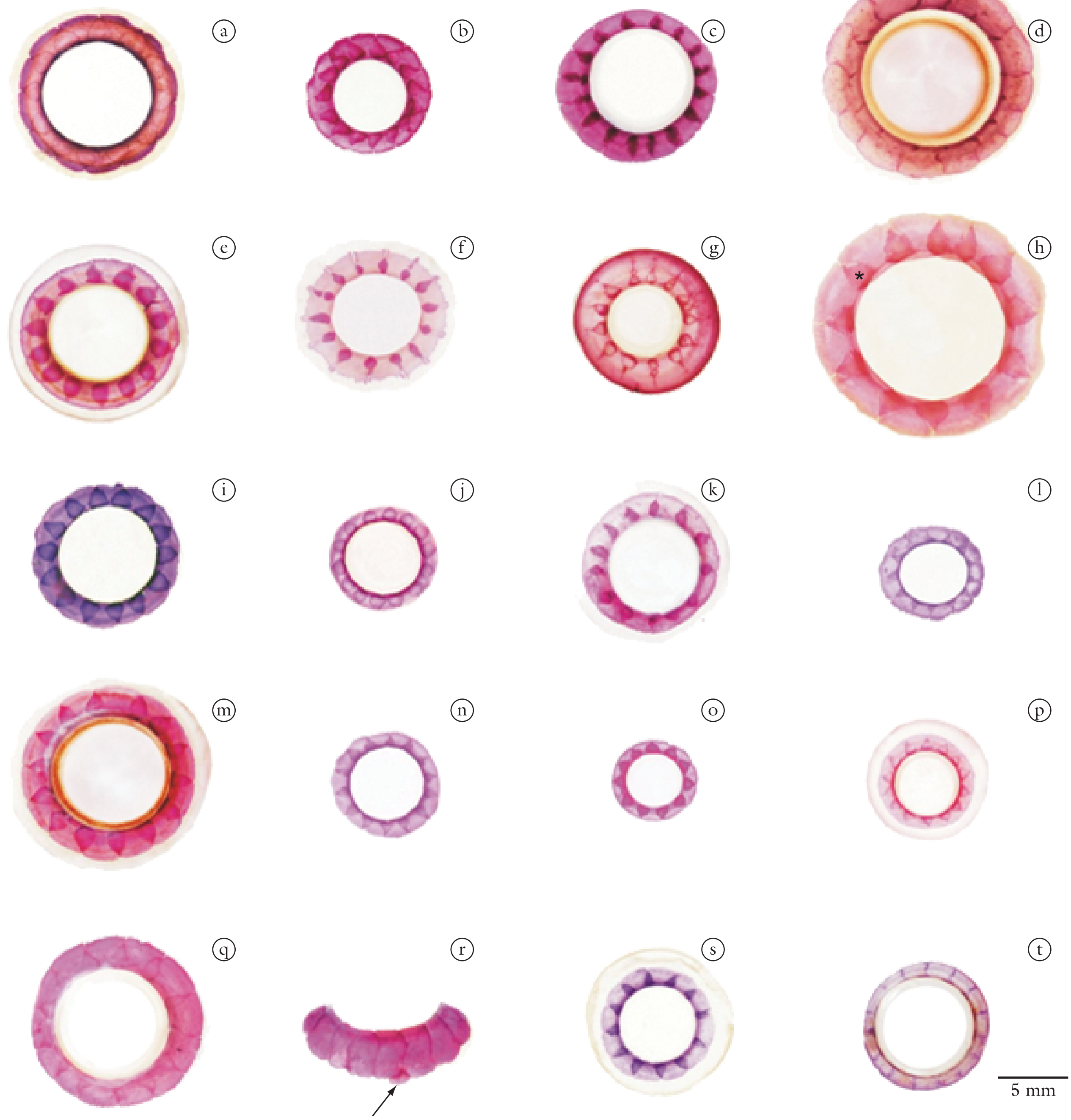

Figure 2. Photographs of the scleral ossicles of several birds. a) *Piaya cayana* RE (right eye); b) *Nothura minor* RE; c) *Theristicus caudatus* RE; d) *Elanus leucurus* LE (left eye); e) *Penelope ochrogaster* RE; f) *Piculus* sp. LE; g) *Melanerpes candidus* LE; h) *Ramphastos toco* RE (*superposition); i) *Aramides calopterus* LE; j) *Megarhynchus pitangua* RE; k) *Ara ararauna* LE; l) *Chloroceryle americana* LE; m) *Crax fasciolata* RE; n) *Vanellus chilensis* RE; o) *Columbina talpacoti* LE; p) *Turdus rufiventris* RE; q) *Cariama cristata* RE; r) *Leptodon cayanensis* LE (sesamoid bone (arrow); view of temporal face); s) *Aratinga leucophthalmus* RE; and t) *Nyctibius griseus* LE. View of anterior pole. Bar: 5 mm.

in most species. In some cases these plates touch each other at the edges, forming a kind of suture, but in most cases they imbricate in a differentiated pattern. This superposition offers an advantage to animals, since it allows for the expansion and contraction of this structure to accommodate the eyeball (ROMER, 1956). The superposition pattern provides evidence of the homology of the ossicles, based on the evaluation of events in which the same number of ossicles derived from the loss in different episodes (QUEIROZ and GOOD, 1988).

In Falconiformes, the ossicles were imbricated in a straight line throughout their extent, while in Psittaciformes,

Table 1. Number of ossicles in birds of different species.

Táxon	Nº of specimes	Nº of ossicles RE	Nº of ossicles LE
STRUTHIONIFORMES			
Rhea americana	12	15-16	15-16
TINAMIFORMES			
Nothura minor	1	15	15
Nothura maculosa	1	15	15
ANSERIFORMES			
Amazonetta brasiliensis	1	15	15
Anas sp.	10	14	14
GALLIFORMES			
Colinus cristatus	1	13	13
Penelope ochrogaster	1	14	14
Crax fasciolata	1	14	14
SPHENISCIFORMES			
Spheniscus magellanicus	1	13	14
CICONIIFORMES			
Egretta thula	1	14	14
Tigrisoma sp.	1	14	14
Theristicus caudatus	4	14-16	15
CATHARTIFORMES			
Coragyps atratus	8	15	15
FALCONIFORMES			
Elanus leucurus	1	15	16
Buteo magnirostris	5	15	15
Buteo albicaudatus	1	15	15
Micrastur semitorquatus	1	14	14
Micrastur ruficollis	1	16	16
Caracara plancus	13	14-15	14-15
Gampsonyx swainsoni	1	15	15
Herpetotheres cachinna ns	1	14	14
Falco sparverius	1	14	14
Leptodon cayanensis	1	14	14+1
CUCULIFORMES			
Crotophaga ani	2	12	12
Piaya cayana	1	12	12
GRUIFORMES			
Rallus maculatus	1	15	13
Porphyrula martinica	2	14	14
Cariama cristata	8	12-13	12-14
Aramides calopterus	5	14	14-15
CHARADRIIFORMES			
Vanellus chilensis	4	15	15
COLUMBIFORMES			
Columba sp.	7	11	11
Columbina talpacoti	5	11	11
PSITTACIFORMES			
Ara nobilis	9	12	12
Amazona sp.	12	11-12	11-12
Aratinga solstitialis	7	12-13	12-13
Pyrrhura sp.	1	12	12
Ara ararauna	2	12-13	13
Aratinga leucophthalmus	7	12	12

Legend: RE - right eye; and LE - left eye.

Table 1. Continued...

Táxon	Nº of specimes	Nº of ossicles RE	Nº of ossicles LE
STRIGIFORMES			
Athene cunicularia	17	15-16	15-16
Tyto alba	4	15	15
Rhinoptynx clamator	2	15	15
Asio stygius	2	14-16	14-16
CAPRIMULGIFORMES			
Nyctibus griseus	4	15	15
CORACIIFORMES			
Chloroceryle americana	1	13	13
Ceryle torquata	1	14	13
PICIFORMES			
Ramphastos toco	27	13-14	12-13
Ramphastos dicolorus	1	13	13
Colaptes campestris	2	14-13	14
Piculus sp.	1	14	14
Colaptes punctigula	1	13	13
Melanerpes candidus	1	13	14
PASSERIFORMES			
Gnorimopsar chopi	2	13-14	14
Megarhynchus pitangua	1	14	14
Turdus rufiventris	1	14	14
Cyanocorax caeruleus	1	12	12

Legend: RE - right eye; and LE - left eye.

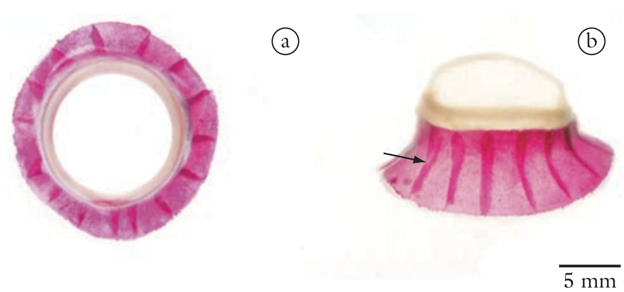

Figure 3. Photographs of the scleral ossicles of *Athene cunicularia* LE; a) view of anterior pole; and b) view of temporal face. Superposition (arrow). Bar: 5 mm.

Columbiformes, Coraciiformes, Passeriformes, Charadriiformes and Gruiformes, due to their morphology, the ossicles were in contact with each other mainly at the external edge of the sclera, giving the overlapping region a triangular aspect.

In fast-flying birds such as Falconiformes and Strigiformes (Figure 3) and aquatic birds such as *S. magellanicus*, the elliptical shape of the eyeball influences the morphology of the ossicles, which are elongated and, especially in Strigiformes, concave, giving the eyeballs of these birds a tubular shape. This apparatus provides passive help for the eyeball's resistance against deformation by water or during flight (ROMER, 1956), conferring visual acuity, support and protection (FRANZ-ODENDAAL and HALL, 2006). This extra support represents an advantage when the eyes are subjected to strong water pressures or during flight. According to Curtis and Miller (1932), the scleral ossicles and cartilage also aid in the adjustment of the pupil and the ciliary muscles involved in visual accommodation (FRANZ-ODENDAAL and HALL, 2006).

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