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Gross histological review of the scleral cartilage found in catfish (Silurus triostegus)

[1] Nasser Akeel Nasser, [2] Oday Alawi Al-Juhaishi

1, 2 Department of Anatomy and Histology /College of Veterinary Medicine /Tikrit University/ Iraq.

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Corresponding Author:
Name:
Nasser Akeel Nasser
E-mail:
naser_a@st.tu.edu.iq
Tel: 0772 646 6199

ABSTRACT

Background and Objectives: Silurus triostegus is a native catfish of the Iraqi Tigris and Euphrates river basin. It has both ecological and economic importance. Although some studies have focused on its ecology and behavior, no extensive studies have shed light on its histology, especially the eye histology of Silurus triostegus. Scleral cartilage is such an element, found in the outer skeleton of many non-mammalian vertebrates. It provides structure and may serve another adaptive function in aquatic species. The aim of this study was to investigate the morphology and histology of the scleral cartilage in bottom-dwelling teleosts.

Methods: 20 histological eye samples were collected and processed using standard paraffin embedding and hematoxylin and eosin staining techniques.

Results: Histological examination showed a distinct layer of hyaline cartilage posterior to the choroid. The cartilage showed typical features, including lacunae containing single chondrocytes, a homogeneous ground substance, and collagen fiber bundles that continue into the sclera. The photoreceptor layer, pigmented choroid, and choroidal capillary were also well defined, representing a well-structured retina.

Conclusion: These findings confirm the presence of scleral cartilage that provides support and rigidity to the ocular structure of Silurus triostegus, suggesting great adaptability in response to the surrounding environment..

Introduction

The retina is a multiple layer neural tissue responsible for photoreception and visual signal processing it is histologically organized into a series of distinct layers that is responsible of phototransduction and synaptic function and signal transmission to the optic nerve. The inner retinal layers are involved in the neural signal conduction while the middle layers responsible of synaptic interactions between retinal neurons and the outer layers contain photoreceptor cell bodies and segments important for light detection. The retinal pigment epithelium forms the outermost retinal layer and supports photoreceptor maintenance and metabolic exchange and visual pigment recycling. this structure provides visual processing and functional integration with adjacent ocular tissues (1). The choroid is a thin highly vascular connective tissue layer located between the retina and the sclera and it supports the outer retinal layers and it is responsible of light absorption through its pigmented stroma. the choroid is formed of multiple vascular layers including large and medium vessels and an inner capillary layer adjacent to the retina. In some vertebrates a tapetal region may be present within the choroid enhancing visual sensitivity under dim light conditions by reflecting light back into the retina. The choroid is responsible of retinal nourishment and optical regulation and visual adaptation (2). The sclera forms the outer fibrous layer of the eye and it composed of dense connective tissue rich in collagen fibers and irregular collagen structure that provides mechanical strength and opacity for the internal ocular structures and preventing stray light penetration. the sclera is formed by many layers including the episcleral and stromal layer and inner pigmented zone and is continuous with cornea through the corneoscleral junction and finally the optic nerve head. the sclera responsible of maintaining the eye globe integrity and supports retinal positioning. and contributes to proper optical alignment in coordination with the cornea (3). *Silurus triostegus* is a native catfish to the Iraqi Tigris and Euphrates river basin. It is one of the most important economic and ecological fish species in Iraq. It inhabits rivers and marshes and reservoirs across the country specially in the southern marshlands. it was First described in Mosul in 1843, and is important to Iraq fishery (4).(5), accounting for up to 8.5% of total production. Taxonomic identification of this species debates persists regarding its classification, as it shows close resemblance to *Silurus glanis* and *Silurus asotus* (6). (7). *Silurus triostegus* is a bottom-dwelling nocturnal predator that feeds primarily on native fish and invertebrates making it a good example of a bioindicator species and Its population present is influenced by environmental changes such as damming, pollution, and predator decline (7). Despite changes in local population it remains a vital protein source and subject of continued biological and ecological study (8).One such overlooked structure is the scleral cartilage is a component of the ocular skeleton found in many non-mammalian vertebrates including birds, reptiles, and teleost fish (9). Scleral cartilage is typically embedded within the fibrous sclera, forming either a thin ring-like structure or a cup-shaped element at the posterior of the eyeball, depending on species (10). While its exact function remains yet to be studied, it is thought to maintain the ocular rigidity and eye shape during visual accommodation, which plays an important role in aquatic species facing increasing and decreasing levels of pressure (11). In teleosts, this cartilage is represented in a wide range of morphologies, from narrow rings in zebrafish (*Danio rerio*) to deep, posteriorly extending structures in cavefish (*Astyanax mexicanus*), often resembling the scleral cartilages seen in reptiles and birds. (10) Cartilage development, or chondrogenesis, it is a carefully controlled and evolutionarily conserved process, involving coordinated interactions between epithelial and mesenchymal cells, cell condensation, matrix formation, and the maturation of chondrocytes into their hypertrophic form (9).(10). Scleral cartilage in teleost is formed of chondrocytes embedded within a matrix resembling hyaline cartilage, with a dense fibrous outer layer and center rich in cells (12). The aim of this study is to give an in-depth investigation of the ocular structures of the *Silurus triostegus* and specifically note the presence of the scleral cartilage among its components, which is an adaptable component whose presence, Thickness, and function are affected by its surrounding environment, which may or may not be present in teleost and other types of fishes. Given the ecological and economic value of the Iraqi catfish, which is considered a genus specific to the Iraqi Euphrates and Tigris rivers. And the lack of studies regarding those species histologically. This study will provide valuable data for the histological components of the ocular structures, which will be beneficial to conduct future studies and broaden the knowledge to try to further understand how aquatic species respond to the different environmental niches.

Materials and methods

Ethical Approval:

On April 28, 2025, the Habwan Ethics Commit tee at Tikrit University's College of Veterinary Me dicine approved all animal experimentation proce dures in accordance with global standards for the care of lab animals.
(Approval number: Veterinary Medicine, Tuesday , 104).

Sample Collection and Fixation

Twenty eyeball samples were taken from Silurus triostegus (catfish) in the area. The catfishes were acquired from a licensed local fish market in Salah Al-Din Governorate, Iraq, and were delivered right away to the lab without being stored prior to eye extraction. The catfish employed in this investigation weighed between 900 and 1000 kg and were around 6 months old. Since the fish's sex had no bearing on the study's goals, it was disregarded. The entire eyeballs of the gathered catfish were meticulously enucleated at the University of Tikrit's Animal House Facility. The eyes were immediately fixed by submerging them in 10% neutral buffered formalin. After that, the fixed samples were moved to the Histology Laboratory for standard tissue processing and microscopic analysis.

Biological Sample

In this study, twenty eyeball samples from Silurus triostegus (catfish) were used. The fish were about six months old and weighed between 900 and 1000 kg. Only healthy catfish with no visible eye abnormalities were included. During the regular histological examination of the eye utilizing the obtained samples, special attention was paid to the scleral layer and other ocular tissues. Samples were collected in April 2025 (spring season).

Chemicals and Reagents

Fixative for tissue preservation using 10% Neutral Buffered Formalin (NBF) for 24 hours and then the fixative was changed to ensure proper fixation for another 48 hours (days in total). Ethanol series dehydration agents (70%, 80%, 90%, 95%, and 100%) for tissue dehydration in two hours to ensure that all the water had been evaporated. Before embedding, the samples were cleared in xylene (30 minutes in two consecutive changes) till they were completely transparent. during the de-alcoholification process. For sectioning techniques, the tissues were then infiltrated and embedded in molten paraffin wax at 58°C over four hours and then blocked with pure paraffin to produce solid blocks of tissue which could be subjected to microtomy.

Staining Reagents:

For a general histological description use the stain hematoxylin and eosin (H&E). A popular histological staining technique used to examine paraffin embedded sections in order to see the tissue structure and cell morphology is the Hematoxylin and Eosin (H&E) staining protocol. Using a rotary microtome slices with a thickness of 4-6 µm are first cut and then floated in a bath of warm water. (40–45 °C), flattening them then mounting them on spotless glass slides and drying them for 30–60 minutes or overnight at 37–40 °C. Deparaffinization starts the staining process by immersing slides in two or three changes of xylene for three to five minutes each to dissolve the paraffin wax. Next, the slides are rehydrated using descending alcohol (100 percent, 95 percent, and 70 percent), and finally they are rinsed in distilled water. The slides are submerged in hematoxylin solutions (Harris staining method) for five to eight minutes before being rinsed under running tap water for three to five minutes in order to stain nuclei. To differentiate the sections, they are briefly dipped for 5 seconds in 1% acid alcohol to eliminate unnecessary nuclear stain and then they are rinsed under running water for one to two minutes followed by Bluing. In order to cure the nuclear stain, stain-free slides are blued by immersing them in a 0.2 percent ammonia water or Scott tap water equivalent for 30 seconds to 1 minute, followed by another rinse in neutral stainless water for two to three minutes. After that, sections are stained with 0.51 percent eosin Y solution for 30 to 2 minutes to give the cytoplasm and extracellular components a pink to red hue. Sections are mounted in a synthetic resin (DPX) and covered with a glass coverslip after being dehydrated using consecutive mixes of ethanol (95 and 100 percent) and cleaned with two to three changes of xylene, lasting two to three minutes each. Sharp, blue to purple nuclei, pink to red cytoplasm, pink to orange collagen fibers, and bright red or orange red blood cells should all be visible under a microscope. To ensure equal staining, the amount of staining or differentiation time would be changed to rectify light nuclei, excessive background staining, or light cytoplasm staining. Proper timing, handling of reagents, and removal of all superfluous wax were crucial(13).

Microscopic Examination

The retinal layers including choroid, sclera, and corneal ocular structures were all examined under X10 and X40 magnification using light

microscope. As part of the recording and analysis pictures of the examination were taken using a microscope camera.

Results and discussion

The retina was sharply visible along with the inner limiting membrane, and several layers of nerve cells and fibers were present. The rods and cones were observed in direct contact with the choroid, which contained abundant melanin pigment. The choroid exhibited a dense staining pattern, and its stroma consisted of loosely arranged connective tissue and bar of hyaline cartilage was noted along the course of the choroid, which was posteriorly surrounded by collagen bundles of sclera Figure (1) H&E stain 10x.

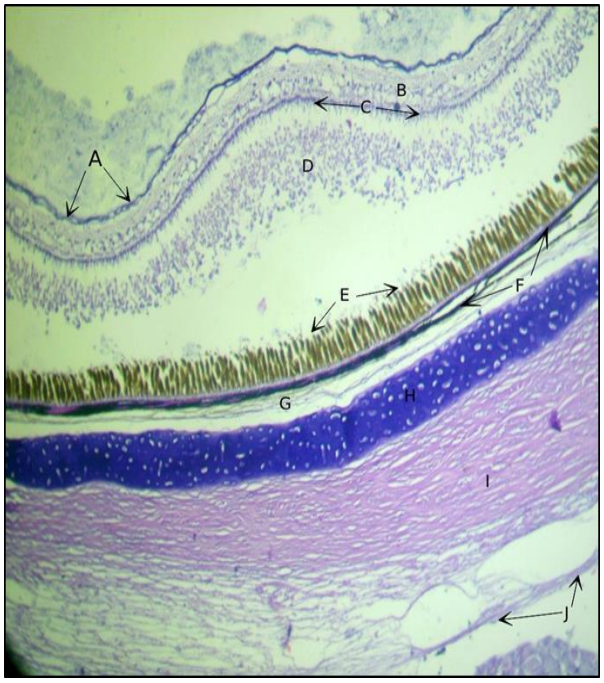

Figure (1) Retina, Choroid, and Sclera inner limiting membrane (A) inner nuclear layer (B) External plexiform layer (C) outer nuclear layer (D) Photoreceptors (E) Retinal pigmented epithelium (F) Choroid (G) Hyaline cartilage (H) bundle of collagen of sclera (I) nerve bundle (J) H&E stain 10x.

The eyes of fish are similar to vertebrates, with the exception of nearly all teleost whose pupil diameter is fixed. Vertebrates retinas are multiple-layer neural tissues that process the output of light detecting photoreceptors. Depending on the kind and species, the retina may contain over 100 different types of neurons. (14). The posterior uvea is made up of vascular tissue called the choroid which is thin and varies in color. It is located between the retina and sclera in the back and links the ciliary body in front. The inner surface of the choroid has a single layer of capillaries that nourish the outer layers of the retina, making it extremely vascular. Numerous melanocytes in the choroidal stroma provide the retina with a dark optical background. (2). The sclera is made up of a fibrous and thick layer that is continuous with the cornea (12). Our findings revealed that the photoreceptors of the retina were arranged into tall rod and shorter cone cells, and the choroid contained a well-developed choroidal capillary plexus with distinct melanin pigmentation within its stroma. The hyaline cartilage was clearly visible, showing lacunae, each containing a single chondrocyte and surrounded by ground substance. Collagen bundles surrounding the cartilage continued posteriorly into the sclera Figure (2) H&E stain x40.

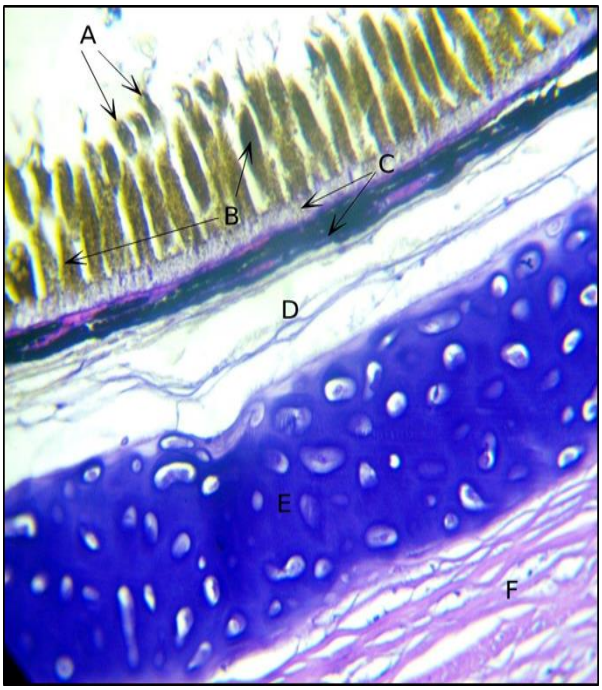

Figure (2) Photoreceptors of retina Rods (A) cons (B) Retinal pigmented epithelium (C) cherio capillary plexus (D) Hyaline cartilage (E) Collagen bundle of sclera (F) H&E stain x40.

Because there are differences in diversity of habitat, the change from marine to terrestrial habitats has a significant impact on fish species diversity (15). Teleost fishes inhabit many different environments. Over the course of this evolution, several of these visual adaptations have developed. This is demonstrated in marine fish by variations in spectral sensitivity between species that live at different depths (eg, shallow versus deep) and between seasons. The size and quantity,

and distribution of retinal cell types may all change as a result of these cellular adaptations (16). Rhod opsin molecular level like opsins or other components of the process of phototransduction (17) Our findings revealed that the photoreceptors were arranged in a single layer of sensory cells consisting of tall rods and shortercones, all enclosed by the retinal pigment epithelium. The choroid contained a fine choriocapillary plexus with melanin pigmentation and delicate stromal connective tissue and A bar of hyaline cartilage was observed traversing the choroid and sclera, containing prominent chondrocytes within lacunae of the matrix. Collagen bundles were seen posterior to the cartilage Figure (3) H&E stain x40.

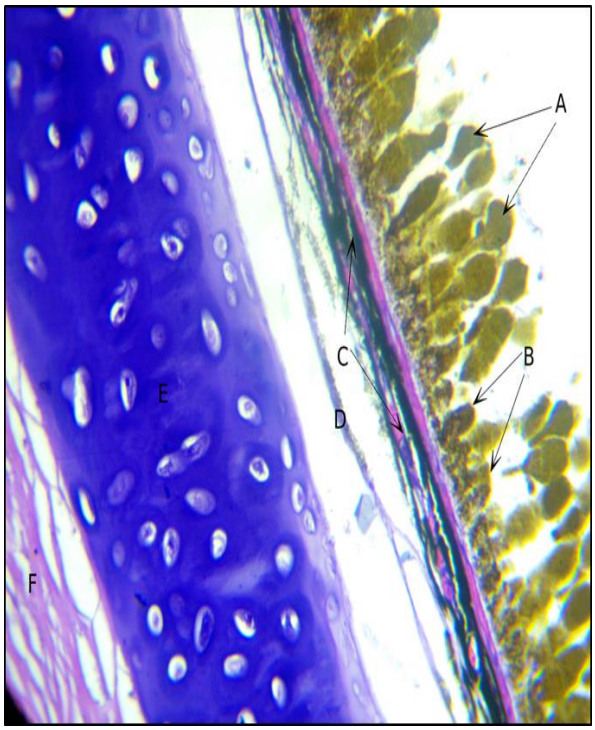

Figure (3) Photoreceptors of retina Rods (A) cons (B) Retinal pigmented epithelium (C) choroid capillary plexus (D) Hyaline cartilage (E) Collagen bundle (F) H&E stain x40.

Fish like Liza abu (Heckel) and Acanthobrama marmid (Heckel), Leuciscus vorax (Heckel), and Mesopotamichthys sharpeyi (Günther) are the primary food source for the Silurus triostegus, which also occasionally consumes aquatic insects and frogs, shrimp, and crabs and Detritus and aquatic plants (5). Cartilages form inside the scleral tissue in several animals. Fibrous connective tissue makes up the sclera of every vertebrate. The histological makeup of the fibers and cells in this layer of the eye might vary. Certain cells inside this layer of connective tissue develop into chondrocytes in many species, including fish. These cells release a large number of collagen type II fibers, which are then cross-linked to form cartilaginous elements. (18) (19) (20) (21). In our findings the photoreceptors (rods and cones) were arranged in a single row resting upon the outer limiting membrane. The choroid was rich in choriocapillaris plexus and showed prominent melanin pigmentation. Scattered elastic fibers were observed within the choroidal stroma. The hyaline cartilage was well defined containing chondrocytes within lacunae surrounded by ground substance followed by the collagen bundles of the sclera and skeletal muscle fibers Figure (4) H&E stain x10.

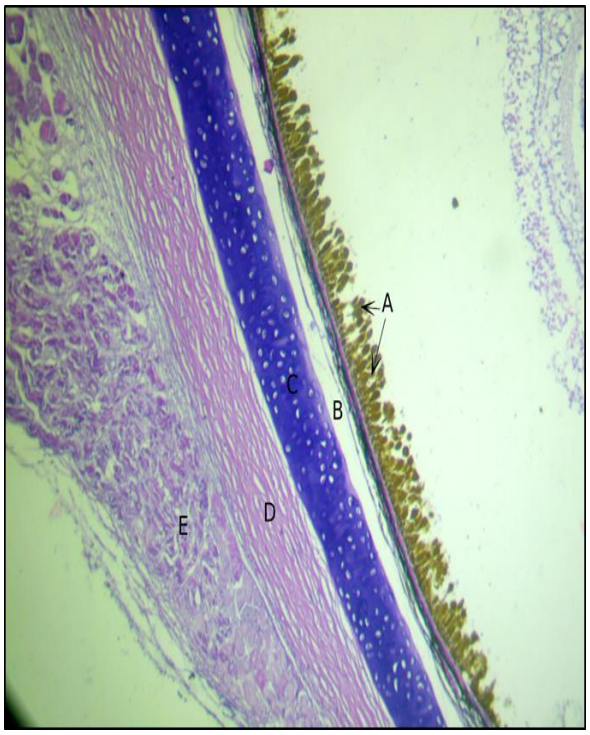

Figure (4) Photoreceptor of rods and cons (A) choroid (B) Hyaline cartilage (C) collagen bundle of sclera (D) Skeletal muscle fibers (E) H&E stain x10.

the histological components of the choroid are the suprachoroidal, large-vessel layer, intermediate-vessel layer and choriocapillaris. The cellular tapetum of carnivores is made up of reflecting crystals (tapetum cellulosum). An animal eyes shine in the dark because of the tapetum's reflecting qualities, which also give each animal's fundi their unique color. The breed and age and species and tapetum thickness all affect this color. The animal capacity to function in low light is enhanced by reflecting light through the retina twice. (2).

Conclusion

The study documents the presence of scleral hyaline cartilage in *Silurus triostegus* a native catfish to the Iraqi Euphrates and Tigris rivers. The histological analysis showed there is a distinct cartilaginous structure located along the posterior course of the choroid, characterized by chondrocytes within lacunae and a homogeneous ground substance with surrounding collagen bundles continuous with the sclera. The results confirm that S. triostegus possesses a true scleral cartilaginous element, which is an anatomical feature that could be or could not be present among teleost fishes. Although the sclera does not directly participate in vision, it plays an essential mechanical role in maintaining the shape of the globe, sustaining intraocular pressure and providing attachment for ocular muscles. In teleost that depend on lenticular accommodation, these functions differ from those of terrestrial species. the presence of cartilage in S. triostegus suggests that it may serve supporting roles, such as reinforcing the posterior globe or stabilizing the retina choroid interface or optimizing muscle attachment in aquatic environments. this study finding support the concept that scleral cartilage in teleost forms part of a developmental and morphological continuum shaped by ecological and phylogenetic, and functional effects in which this discovery contributes to more understanding of ocular skeletal adaptations and highlights the evolutionary flexibility of the vertebrate sclera. Further comparative studies among Siluridae and related families are recommended to clarify the structural variability and evolutionary significance of this often-overlooked structure.

Conflicts of interest

There are no conflicts of interest.

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none

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مراجعة نسيجية عامة للغضروف الصلبي في سمك السلور (Silurus triostegus)

ناصر عقيل ناصر [1], عدي علاوي الجحيشي [2]

1,2 فرع التشريح والانسجة, كلية الطب البيطري, جامعة تكريت, العراق.

الملخص

المقدمة والهدف : يُعد سمك السلور (Silurus triostegus) نوعًا من الأسماك المحلية في حوض نهري دجلة والفرات في العراق، ويُمثل قيمة بيئية واقتصادية مهمة. على الرغم من أن بعض الدراسات ركزت على بيئته وسلوكه، إلا أنه لم تُجرَ دراسات موسعة تسلط الضوء على نسيجه، وخاصة النسيج العيني لهذا النوع. يُعد الغضروف الصلبي أحد هذه العناصر، إذ يوجد في الهيكل الخارجي للعديد من الفقاريات غير الثديية، وهو مسؤول عن توفير الدعامة وقد يؤدي وظيفة تكيفية إضافية في الكائنات المائية. يهدف هذا البحث إلى دراسة الشكل الظاهري والتركيب النسيجي للغضروف الصلبي الموجود في هذا النوع من الأسماك القاعية.

طرائق العمل : تم جمع 20 عينة نسيجية للعين ومعالجتها باستخدام تقنية التضمين بالبارافين والصبغة الروتينية بالهيماتوكسيلين والأيوزين، وقد أظهرت وجود طبقة واضحة من الغضروف الزجاجي خلف المشيمية.

النتائج : أظهر الغضروف صفات نسيجية نموذجية، بما في ذلك الفجوات المحتوية على خلايا غضروفية منفردة، ومادة أساسية متجانسة، وحزم ألياف كولاجينية مستمرة مع الصلبة. كما كانت طبقة المستقبلات الضوئية والمشيمية المصطبغة والشعيرات الدموية المشيمية محددة بوضوح، مما يعكس شبكية ذات بنية متكاملة.

الاستنتاج : تؤكد هذه النتائج وجود الغضروف الصلبي الذي يوفر الدعم والصلابة للتركيب العيني في سمك السلور (Silurus triostegus)، مما يشير إلى قدرة تكيفية عالية استجابةً للبيئة المحيطة.

الكلمات المفتاحية: Silurus triostegus , سمك السلور، الغضروف الصلبي، نسيجيات الأسماك العظمية، التكيف المورفولوجي.