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Understanding The Structural Tissues of Pectoral Finless Albino African Catfish *Clarias gariepinus* Originating from Pond Cultivation

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Abstract. *The loss of pectoral fin is an undesirable trait always found in almost all catfish breeding programmes in Indonesia, so further investigation is needed to clarify this problem. This study aims to describe albinism in fish with pectoral fin deformity from an immunohistochemical perspective by evaluating the correlation between two proteins (TBX5 protein and TYR protein) from eye and skin tissues. We used an individual albino fish (TL = 42 cm) found by local farmers in the Kebomas district, Gresik, to study the histological differences between albino fish without pectoral fins and black fish with regular pectoral fins using descriptive methods. The histotechniques using HE staining followed the Bioscience Laboratory, and the immunohistochemical staining was performed according to the Biomedical Laboratory procedure at Brawijaya University. The slides were scanned using Aperio Scanning CS2. Morphological structure differences determination used a t-test (α = 0.05). Spearman's rank correlation assessed the relationship between fin morphology and albinism. The results showed significant differences between the skin and retinal thickness of the two fish (p <0.05), and the total retinal thickness of albino fish without pectoral fins was significantly thinner than that of regular fish. Rank correlations showed a positive association between pectoral fin loss and reduced skin and eye histology. The histochemical studies indicated that TBX5 is an important factor in pigmentation and fin development, but additional factors may disrupt TYR regulation in albino fish. The results of this study provide further evidence for considering pectoral fin phenotype as one of the evaluation factors to make the success of a breeding program.*

Keywords: *albino, cultivation, histology, pectoral fins, TBX5*

Citation
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INTRODUCTION

Fish farming is one of the inducers of biological evolution in fish. For many generations, husbandry patterns in captivity can alter fish performances due to the loosening of natural selection pressure in the cultivation environment (Hutchings & Fraser, 2008). Consequently, fish exhibit different performances in body structures and functions from their origin (Ahti et al., 2020; Z. Zhang et al., 2023). Factors such as selection pressure, density, lack of essential nutrients, and contamination in the maintenance water can affect these changes. The skin colour and fin shape are standard features that change the cultivated species, affecting prices and market acceptance (Colihueque & Araneda, 2014).

The loss of pectoral fins, a clear example of biological evolution in farmed fish, is particularly evident in the African catfish *Clarias gariepinus*. However, this incident has only been reported in Indonesia, particularly in the Hybridization Program to obtain superior strains. The pectoral finless *Clarias gariepinus* is not significantly different from fish with regular pectoral fins regarding growth ability, survival rate, and feed conversion ratio. Still, they face more significant difficulties in surviving, growing, and utilizing energy (Farikhah et al., 2020). Farikhah et al. (2020) reported that pectoral-finless is a heritable trait, which is relevant to the Iswanto & Suprapto (2015) explanation that pectoral finsless always appear in the *Clarias gariepinus* Breeding Program to create new strains. Several strains, such as the Dumbo strain (lele Dumbo), Sangkuriang strain (lele Sangkuriang), Phyton (lele Phyton), Paiton (lele Paiton), Masamo (lele Masamo), and Mutiara (lele Mutiara), always contain fins deformity with a ratio ranging from 4-30% of the population. The deformity level in Masamo strain>Sukhoi>Burma>Pyton>Sangkuriang>Paiton>Dumbo>Mutiara. Although undesirable traits are generally detrimental, they can even provide effective guidance on avoiding the emergence of unwanted traits in the breeding program.

The formation of T-box5 protein bands using a Western Blotting assay showed the genetic factors behind the loss of pectoral fins in *Clarias gariepinus*, which originated from pond cultivation. The high expression of TBX5 protein in adult pectoral finless fish indicates an inappropriate expression pattern, as regular adult fish do not express or weakly express TBX5 protein (Farikhah et al., 2020). Inappropriate expression of TBX5 can lead to various developmental problems and diseases (Zheng et al., 2015). The inappropriate expression of TBX5 suggests that the Tbx5 gene not only plays an important role in the developmental stages of the body and limbs (S. Zhang et al., 2024) but is also crucial in supporting individual survival and growth. The finding of albino fish without pectoral fins in *Clarias gariepinus* from a local farmer's pond led to the hypothesis that there is a correlation between pectoral fin loss and skin colour in fish.

This study aims to explore the correlation between TBX5 protein and Tyrosinase (TYR) protein of pectoral finless albino fish, to understand more clearly the role of TBX5 in African catfish *Clarias gariepinus* or popularly known as ‘lele dumbo’ and to describe the histological structure of the pectoral finless albinian African catfish *Clarias gariepinus* using histological and immunohistological perspectives. TBX5 is a transcription factor key to inducing the growth of pectoral fin buds (Bayramov et al., 2024). Although there have been many scientific studies related to TBX5 downstream genes, its relationship with skin color is not yet known, so scientific exploration of both can provide helpful information for improving the productivity of farmed catfish and developing healthier fish.

The target organs were determined based on previous studies that highlighted significant histological differences between pectoral finless fish and regular fish in the retinal layer and total skin. This study will provide a theoretical and practical basis for utilizing pectoral fin phenotype and skin colour to determine fish selection in breeding programs. This study contributes to global African catfish aquaculture, which is currently facing serious challenges in terms of sustainability (Barasa & Ouma, 2024; Kemigabo et al., 2019).

MATERIALS AND METHODS

This research was conducted from March to August 2024 at the Aquaculture Laboratory, Faculty of Agriculture, Universitas Muhammadiyah Gresik. This study used a descriptive method with lele dumbo *Clarias gariepinus* with albino pectoral fin loss (n =1) as the subject of research. The albino status of lele dumbo *Clarias gariepinus* in this study was determined visually according to Francis et al.(2023) by observing the loss of black colour and showing white or yellowish colour. The fish originated from a lele dumbo collector in Kedanyang village. They were found by chance in December 2022 from a population of table-size fish in Dahanrejo village, Kebomas district, Gresik regency.

The fish were then brought to the laboratory and kept in a semi-indoor concrete pond with a volume of 1.6 m^3 (dimensions length x width x height 2 m x 1 m x 0.8m) using black netting as a shelter. During maintenance, the fish were fed fresh waste fish. Fish health and pond water stability were monitored daily. The pond water was changed weekly by removing 50% of the bottom water through the outlet hole and adding fresh water through the inlet pipe.

This study's comparator (control, n=1) was a certified broodstock, Clarias gariepinus catfish Mutiara strain, with black skin and regular pectoral fins. The fish were purchased from Mojokerto Aquaculture Installation, Jl. Dlanggu Gondang, Mojokerto 61372. Both samples (albino fish and black fish) were analyzed for morphometric and meristic characteristics. The measurement of morphometric characters includes total length (TL), standard length (SL), height length (HL), orbital length (OL) and mouth width (MW), with the selection of these characters based on standard characters related to indicators of fish growth aspects. The calculation of the meristic characters includes the number of dorsal fin rays, the number of caudal fin rays, the number of anal fin rays, the number of left pectoral fin rays, the number of right pectoral fin rays, the number of left ventral fin rays and the number of right ventral fin rays.

The histological work started by anesthetizing the fish and removing the left eye organ. The skin sample was taken from the left anterior medial body linea lateralis (1 cm^2). After preservation in Bouin's solution for less than 24 hours, histological slides were prepared according to the standard procedures of the Bioscience Center Laboratory, Brawijaya University, Malang, Veteran Street 169 Malang, East Java. Skin and eye preparations (± 5μm) were scanned using a Leica microscope. Histological images of fish eyes and skin were scanned using Aperio Image Scope 12.4.6 software for Windows. For retinal histomorphometry, the peripheral area of the retina (bottom) was selected along ± 1.25 mm from the ciliary body or the edge of the retina. For total skin histomorphometry, the skin area was measured vertically to the right of the centre of the anterior body axis for

±1 cm.

Two types of rabbit polyclonal antibodies, TBX5 antibody (GeneTex, Cat. No. GTX113849) and tyrosine hydroxylase antibody (GeneTex, Cat. No. GTX113016), were used for immunohistochemistry. The technique was performed according to the procedure developed by the Biomedical Laboratory, Faculty of Medicine, University of Brawijaya.

Fish morphometrics and meristics were analyzed descriptively using Excel 2010. Histometric retinal tissue and total skin measurements were performed using Aperio Image Scope 12.4.6 for Windows software by determining five fields of view. The relationship between pectoral fin loss and retinal and skin thickness was examined using Spearman's rank correlation at a 0.05 error level. The difference in length or thickness of the retina and skin between albino finless fish and regular fish was determined by the p-value (α=0.05). If p<0.05, the histomorphometric subregion of the retina and skin of albino fish is significantly different from that of regular fish. Conversely, if p>0.05, it is concluded that albino fish are not significantly different from regular fish. The structure of the retina and skin is associated with organ function in albino and blackfish, which is related to albinism and pectoral fin loss. Immunohistochemical image reading was performed by analyzing 100 histological images of eye organs (retina, each image counted from 5 fields of view) and skin (epidermis, dermis, each image counted from 5 fields of view) using the Image-J program. TBX5 and TYR protein abundance were analyzed for correlation per fish type using Excel 2010. Differences in the abundance of Tbx5 and TYR in pectoral finless albino fish and regular pectoral finless blackfish were analyzed separately per protein using T-test (α=0.05)

RESULTS AND DISCUSSION

The subject of this study was the rare albino catfish *Clarias gariepinus* with fin abnormalities, precisely the absence of pectoral fins on both sides. The fish was initially pinkish dorsally and whitish ventrally (Figure 1-2). Its eyes were black, indicating that the albinism of this specimen was partial. Previously, Zhong et al. (2021) had successfully performed an albinism study on the channel catfish *Ictalurus punctatus* Rafinesque, 1818 (Siluriformes: Ictaluridae), but had not associated it with the paired fin anomaly, which was present with the loss of both pectoral fins. The loss of pectoral fins has also never been studied in conjunction with albinism in other farmed species, including channel catfish, the leading aquaculture species in the United States, and it is growing rapidly in China. As the albino catfish ages, its colour changes from bright pink to brownish yellow. At two years of age, the albino fish, which has no pectoral fins, changes to an intriguing yellow-brown colour that indicates the albino type of fish, which contains pheomelanin (Figure 3). Colour changes in albino fish with age have never been reported in *Clarias gariepinus* catfish. In this study, as the partial albino, the skin's melanin content is present but low; this pigment remains active throughout the fish's life. As a result, the colour of the fish, which was initially bright pink, gradually becomes darker. Increasing age means a change in status from juvenile to mature, which alters the fish's hormonal cycle and affects the colour change of albino individuals to hyperpigmentation. Autophagy is one of the factors that slowly causes aging, which affects changes in skin colour (Lee, 2021).

Apart from the pectoral fins, all other fins are in normal condition and have soft rays. The dorsal fin extends from the back towards the tail, approaching the caudal peduncle, while the caudal fin is rounded. The anal fin is on the ventral side and extends from the base of the anus to the caudal peduncle. The pelvic fins are the only paired fins in this albino fish and are symmetrical between the left and right sides. The protrusion of the genitalia is visible, tapered, and reddish, indicating that this fish is male.

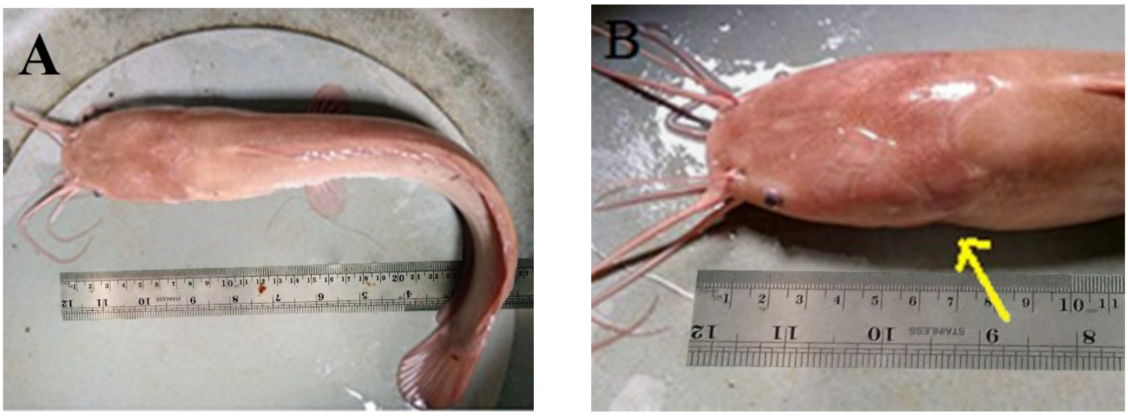

Figure 1. The first discovery of albino fish that does not have pectoral fins from fish farming in ponds. A. The dorsal morphology shows a bright pink, pale skin colour from anterior to posterior, including barbles and whole fins, but the head looks darker than other body parts (613x467 pixels). B. The yellow arrow shows that the pectoral fins are not present in the base of the operculum (612x461 pixels).

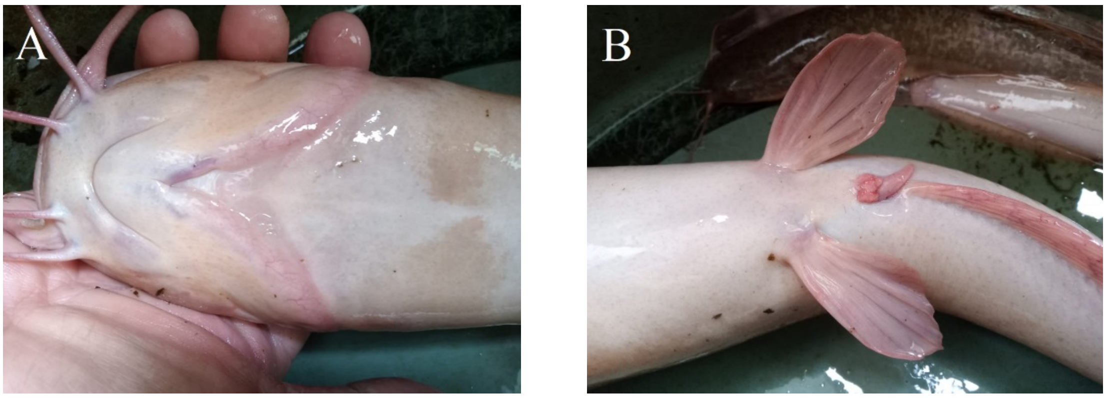

Figure 2. A. The ventrally anterior of an albino fish without pectoral fins shows a dominant pinky white skin with the tendency of the operculum margin (780x1040 pixel); B. The abdomen is predominantly white with genital papillae as the male secondary sexual char acteristics,and both the pelvic and anal fins show a pronounced pink colour (1040x780pixels).

Figure 3. The skin of an albino *Clarias gariepinus* catfish without pectoral fins turns yellowish-light brown after 2 years of age (771x316 pixels).

Table 1. The Morphometric characteristics of an albino African catfish, *C. gariepinus*, without pectoral fins, are different from those of blackfish with regular pectoral fins.

No.	Characteristic (mm)	Skin colour			
		Black	%relative to SL	Albino	% relative to SL
1.	Total Length (TL)	55.5	113.4	42	106.3
2.	Standard Length (SL)	48.5	100	39.5	100
3.	Head Length (HL)	10	20.5	6.6	16.7
4.	Mouth Width (MW)	6	12.4	4	10.1
5.	Body Depth (BD)	5	10.3	4.5	11.4
6.	Eye Diameter (ED)	1	2.1	0.8	2.0

Table 2. The meristic characteristic of an albino African catfish, *C. gariepinus*, without pectoral fins, compared to blackfish with regular pectoral fins

No.	Characteristic (unit)	Skin colour	
		Black	Albino
1.	Dorsal fin rays	61	68
2.	Left pectoral fin rays	10	0
3.	Right pectoral fin rays	11	0
4.	Left pelvic fin rays	6	5
5.	Right pelvic fin rays	5	5
6.	Anal fin rays	58	52
7.	Caudal fin rays	17	20

In this study, albino fish without pectoral fins were relatively shorter than blackfish with regular pectoral fins. The proportion of the head and mouth was more petite, both absolutely and relative to the SL, as measured by Head Length (HL) and Mouth Width (MW), which may indicate a possible difference in the ability of albino fish to catch food. The eye diameter of the albino fish was smaller in both absolute and relative terms, which may be related to the albino pigmentation affecting the development or function of the eyes. The Body Depth (BD) of the albino fish was slightly more significant than that of black fish with regular pectoral fins. Although the absolute body depth was smaller, in terms of percentage of SL, albino fish had a slightly deeper body and thus a more rounded shape than black fish with regular pectoral fins (Table 1). Albino fish have no left or right pectoral fins (0 fin rays). Therefore, the fish is different from the blackfish with pectoral fins. Without pectoral fins, the albino fish faces obstacles in balance, maneuverability when swimming, and the ability to withstand water currents. The increased dorsal and caudal fin rays might compensate for the loss of pectoral fins. The pelvic fins of albino fish are the same as those of blackfish with regular pectoral fins, and the left side is symmetrical to the right side of the fin. The anal fins of albino fish appear to be fewer, but the difference is slight (Table 2).

The histological structure of an albino pectoral-finless is smaller than a normal-finned blackfish (Figure 4, 4A, 4B). Generally, the eye comprises five essential parts, namely cornea, iris, lens, retina, and sclera, which are wrapped by extraocular muscles. Melanin pigment can be found in the iris, retina, and choroid, with unique characteristics in each location. The melanin in the iris is concentrated in the epithelium and stroma, totally different from its original development in the basement membrane, size of melanin granules, and packaging within the cells. The iris epithelium has a basement membrane with large melanin granules derived from the embryonic neuroectoderm, where tight junctions bind melanocytes.

In contrast, melanin in the stroma has no basement membrane; melanocytes are scattered and associated with fibroblasts, with small melanin granules derived from the neural crest (Moazed, 2020). The iris of albino fish is pale brown due to the lack of melanin pigment in the posterior and anterior parts. Meanwhile, the iris of blackfish seems thicker than albino-pectoral finless. It has long Iris Pigment Epithelial (IPE), where the spread melanin is found in the posterior and anterior stroma.

The retinal Pigment Epithelium (PE) is a single-layer cuboidal epithelium linked by tight junctions above Bruch's membrane. It contains the highest concentration of melanin in the eye, evident from the dark brown hue surrounding the retina, particularly in blackfish. In albino fish, PE is less developed due to the limited melanin production by melanocyte cells. The melanin protects the retina from excessive light that can harm photoreceptors and enhances visual acuity by reducing light scattering. In contrast, albino fish lack the ability for PE to protrude toward the ends of photoreceptor cells (Figure 3C), so albino fish appear only to have a thin, light brown PE. The choroid is located above the sclera and below the PE (Figure 4). Choroids produce melanin pigments in melanocytes that are concentrated in the suprachoroidal layer, consisting of loose connective tissue, and the tapetum lucidum layer, composed of dense choroidal tissue (Abd-elhafeez et al., 2024). The choroidal vascular network consists of three layers (from inside to outside): the choriocapillaris, stroma, and lamina fusca. The choriocapillaris supplies nutrients to the RPE and the outer third of the retina. The choroidal stroma contains dendritic melanocytes,

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fibroblasts, and mast cells and is provided by the long and short posterior ciliary arteries and the anterior ciliary arteries; drainage is via the whirlpool vessels. The whirlpool vessels and the optic nerve create additional attachment points between the uvea and the sclera. The lamina fusca is a thin, mesh-like attachment between the choroid and the sclera (Boote et al., 2020). The choroidal layer contains many blood vessels that carry oxygen and essential nutrients to the retina and function as a thermoregulator and light absorber (Kwame Ablordeppey et al., 2024).

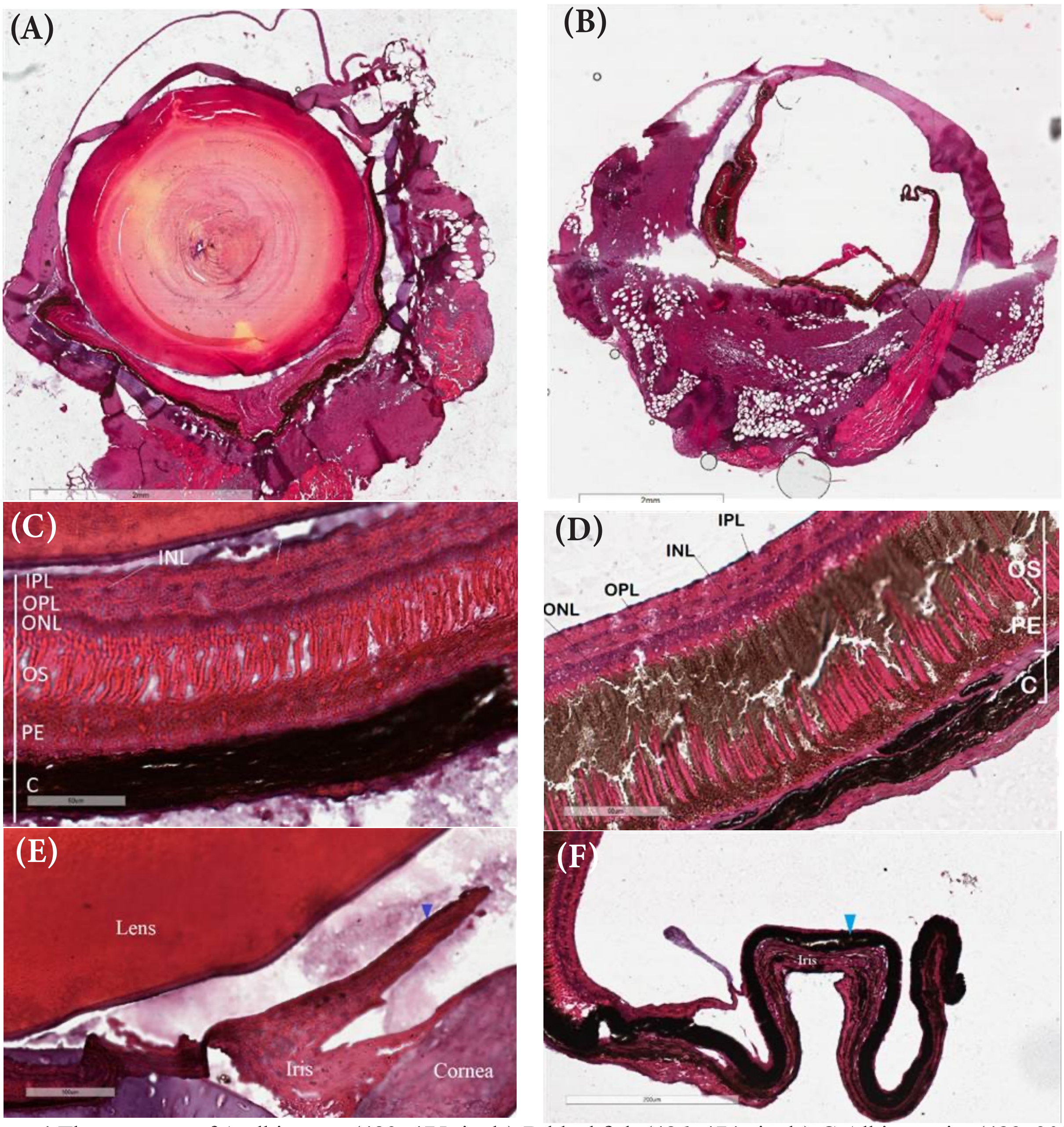

Figure 4.The structure of A.albino eye (489x475pixels);B.blackfish (486x474 pixels);C.Albino retina(489x309 pixels); D. Blackfish retina (490x309 pixels); E. Albino iris (476x275 pixels); F. Blackfish iris (489x278 pixels); (annotation: C: Choroid; PE: Pigment Epithel; OS: Outer Segment; ONL: Outer Nuclear Layer; OPL: Outer Plexiform Layer; PNL: Inner Nuclear Layer; IPL: Inner Plexiform Layer; blue head arrow: Iris Pigment Epithelium (IPE).

Table 3. The retinal histomorphometric between albino African Catfish without pectoral fins and the blackfish with regular pectoral fins

No.	Layer	The fish skin (Average±SD)		Statistical value
		Albino	Blackfish	
1	Choroid	31.71±18.38 µm	13.91±5.40 µm	*p* 0.00<0.05
2	Total retina	69.99 ±28.61 µm	110.10±20.11 µm	*p* 0.00<0.05

The choroid of albino fish is thicker and darker than that of blackfish (Figure 3C-D), possibly due to the higher activity of the choroidal blood vessels in transporting blood. Increasing the rate and volume of blood in the choroid can reduce the risk of retinal damage from UV rays and help regulate eye temperature to protect nerve cells. This adaptation may also improve the relatively weak vision of albino fish by providing enough nutrients and oxygen to the retina. This finding contradicts the study of Venkatesh et al. (2022), which shows that the choroid of albino humans is thinner than that of normal individuals, possibly due to differences in visual mechanisms between aquatic and terrestrial organisms. Albino rats also showed thinner and paler choroid than wild-type ones (Sarkar et al., 2024).

Albino fish showed thinner retinas than blackfish ($p<0.05$, Table 3). However, both have the same seven main layers, namely: pigment epithelium (PE), outer segment (OS), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), and inner plexiform layer (IPL). After the IPL, there is the Inner Limiting Membrane (ILM) and ganglion cell layer (GCL) (not shown in Figure 3), which are thin layers near the lens membrane, indicating that the retina of albino fish is thinner than that of blackfish. This finding not only provides new insights into the unique characteristics of albino fish but also aligns with previous studies of retinal thickness in mice, where the albino retina was found to be reduced by 50% compared to black mice, reinforcing the validity of our research (Ubels et al., 2022).

A striking difference is seen in the PE, which is less and lighter in albino fish. It only covers part of the photoreceptor nerves. In contrast, the PE of blackfish is thicker, with a dark brownish colour that extends to the ends of the receptor nerves. The receptor cells in the OS, both rods and cones, in albino fish are shorter than those in normal fish. The PE migration of albino fish was significantly different from that of blackfish ($p<0.05$, Table 3), characterized by a brown layer above the choroid that did not reach the photoreceptor nerves (Figure 4C). Meanwhile, blackfish showed extreme migration to touch the tip of the OS layer, away from the PE at the base of the retina (Figure 4D). The choroid of albino fish was very thick, as seen from the deep black colour below the PE (Figure 4C, Table 3), indicating more blood flow through the choroid's blood vessels than typical fish. Blood vessels transport blood cells that carry oxygen and nutrients to support the work of receptor cells in the retina.

This study takes a novel approach to understanding the correlation between pectoral fin deformation and albinism in African catfish *Clarias gariepinus*. By focusing on the eyes and skin, the essential organs that demonstrate the performance of genes that cause individuals with albinism, we have discovered significant differences in the structure of the albino retina compared to that of normal-finned blackfish. The albino retina exhibits a thinner retina and little melanin pigment content due to the cells not running the melanogenesis normally. The melanin in the apical process of the retinal Pigment Epithelium could not reach the tip of the Outer Segment (OS) receptor cells. Our findings also align with previous studies, showing that fish without pectoral fins have a thinner histological retinal structure than those with regular pectoral fins. The migration of melanin pigment in the apical process of the Pigment Epithelium (PE) is also lower in pectoral finless fish than in normal pectoral finned fish (Farikhah et al., 2019). Albino fish, we found, have the lowest thickness and very little melanin content at the base of the stem of the Outer Segment (OS) photoreceptor cells. Based on these findings, the shape of the pectoral fins and the skin colour of a dumbo catfish can reflect the thickness of the retina and the levels of melanin in the eye.

Pectoral fins and fish eyes have similar organ development in the embryonic phase. Fish fin buds grow during gastrulation around 16 hours post fertilization (h-PF), and the cleithrum appears on the sixth day after fertilization (d-PF). Mesenchyme is formed at 8-9 d-PF, followed by the growth of scapulacoracoid at 13 d-PF. Gradually, lepidotrichia I-III and fin rays IV-VII, then VIII-XII will appear. Skeletal development is accompanied by the development of the pectoral fins' muscles, vascular tissue, and nerve tissue (Shkil et al., 2022). At the same time in the embryogenesis period, melanin pigment is synthesized in the eye organ. However, it is thought that the genes involved in the eye are different from the formation of melanin in the skin (Wang et al., 2021). This process occurs in a limited period at the beginning of the life phase during embryogenesis, before the embryo hatches into a larva. After that, melanosomes will maintain melanin throughout the fish's life (Kreis et al., 2020).

The first stage of fish eye development is the same as that of vertebrates in general, starting from the specifications of the retina. Retinal development begins in the initial neurulation stage by expressing transcription factors that determine the eye plane in the anterior neural plate. The first morphogenetics is to form a Vesicle Optic (VO). VO is divided in two: the distal/ventral portion expresses retina-specific genes, marking the prospective retinal region, while the dorsal/outer region expresses the transcription factor Otx2, marking the prospective Retinal Pigmented Epithelial region (RPE) (Zilova et al., 2021). This finding is essential to obtaining information regarding pectoral fin anomalies that cannot be directly traced during the complete embryogenesis. Individuals carrying paired fin anomalies are always found after passing through the juvenile or table size phase. Skin pigmentation in farmed fish is not just a surface-level characteristic but is deeply rooted in their genetics (Liu et al., 2024). Fish skin pigments are markers of biological and biochemical differences between individuals in a population, aiding in selection; for example, colour is used as a selection criterion, which has an impact on the economic value and tendency of farmers to cultivate Qingtian PF (*Cyprinus carpio*) carp in a co-culture system with paddy fields (He et al., 2023). For instance, research on albino fish has revealed that genes related to melanin synthesis, including the tyrosinase enzyme, function weakly. The tyrosinase enzyme, crucial in melanogenesis, is present in minimal amounts in fish that have lost their pectoral fins. A recessive gene could not produce functional proteins, affecting melanogenesis and pectoral fin formation.

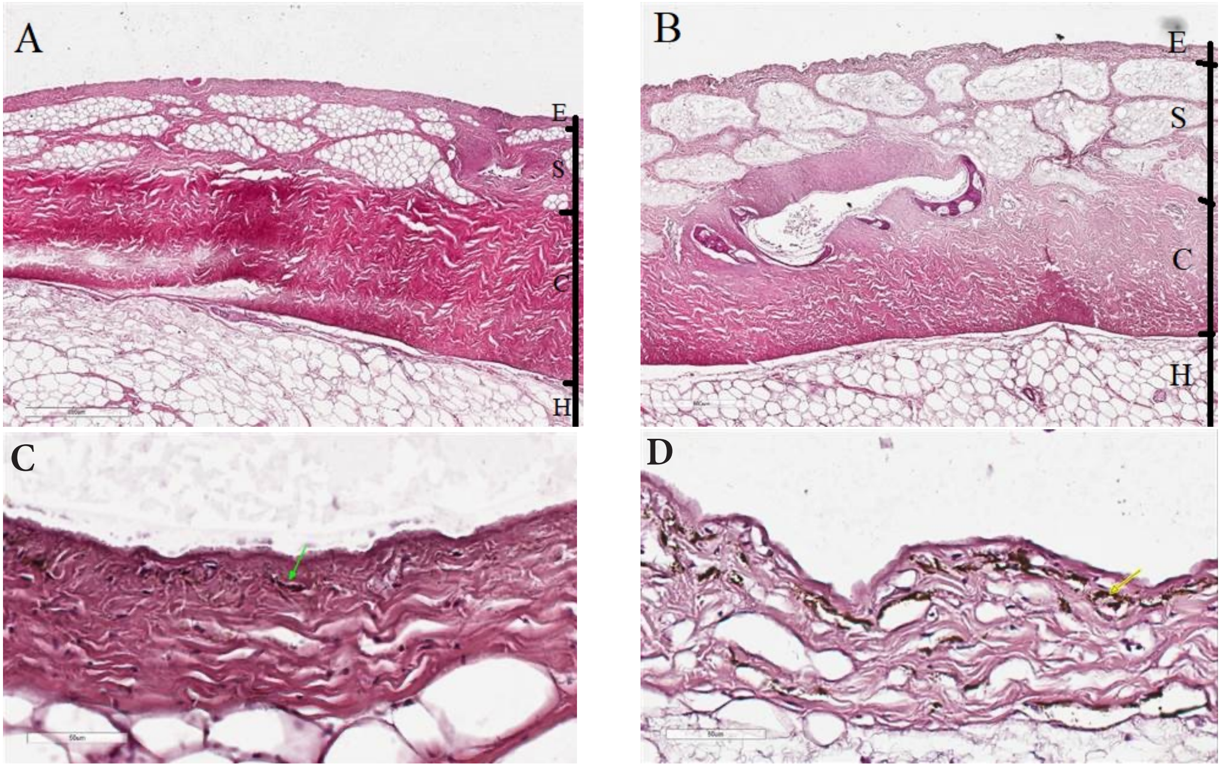

Figure 5. Total skin tissue structure of A. An albino African catfish *Clarias gariepinus*, without pectoral fins (474x346 pixels) and B. Blackfish with regular pectoral fins (490x360 pixels); C. The distribution of melanin in the epidermis of albino fish without pectoral fins (471x272 pixels) compared to D. black fish with regular pectoral fins (481x278 pixels) marked by the coloured arrow lines.

Table 4. The skin histomorphometry between albino-pectoral finless and blackfish-normal pectoral fins African Catfish *Clarias gariepinus* in five observational fields

No	Layer	Layer thickness (Average±SD)		Statistical value
		Albino	Blackfish	
1	Epidermis (E)	113.89±16.45 µm	139.8417±39,67 µm	*p* 0.18>0.05
2	Stratum adiposum (SA)	356.34 ±73.40 µm	814±58.18 µm	*p* 0.00<0.05
3	Stratum Compactum (C)	873.96 ±115.20 µm	780.95±123.22 µm	*p* 0.19>0.05
4	Total skin	1,344.19 mm± 183.55µm	1,735 mm ±95.84 µm	*p* 0.00<0.05

The overall structure of pectoral finless *Clarias gariepinus* (Figure 5A) was thinner (*p* 0.00<0.05, Table 4). The difference between the two is visible in the stratum adipose layer, where the albino fish was thinner than the blackfish. In contrast, the other two layers (epidermis, stratum compactum) showed equivalent thickness (Figure 5B).The outermost surface of the skin of albino fish without pectoral fins is relatively smoother than that of blackfish with regular pectoral fins, illustrating the different life histories of fish in their environment (Madkour et al., 2023). The epidermis contains several layers of cells, including mucous cells, club cells, pigment cells, and stratified squamous epithelial cells (Dhamayanti et al., 2024), which play a role in self-protection against both environmental changes that can cause stress and the invasion of harmful pathogens. The secretory role dominates the performance of the cells that make up the epidermis (Awad & Mohamedien, 2023). Mucus cells produce mucus containing antimicrobial compounds (Díaz-Puertas et al., 2023). Club cells act as a natural alarm system, transmitting danger signals by secreting pheromones. Pigment cells produce melanin, a dark brown substance that plays a role in communication, attack, and defence (Aldermn & Gillis, 2024). Squamous epithelial cells are alive as the respiratory layer of fish (Dhamayanti et al., 2024). The dermis is divided into the stratum adiposum and stratum compactum. Albino fish have little melanin pigment in their epidermis, as indicated by a few dark brown pigment spots scattered throughout the epidermal layer (Figure 5C). In contrast, black-skinned fish contain more pigment spots (Figure 5D). The melanin in the epidermis of albino fish appears to be small spots, while the pigment cells in blackfish appear to be more realistically sized with an amorphous shape.

The African catfish *Clarias gariepinus* is known to have a thick skin (Domiszewski et al., 2020). We found that the skin thickness of the black fish was 1,735 mm ± 95.84 µm, while that of the albino fish was 1,344.19 mm ± 183.55 µm. The significant difference in skin thickness has implications for the physiological function of the skin as the first line of defense, which is different between albino fish without pectoral fins and black fish with complete pectoral fins. Scaleless fish use mucus for self-defense against external threats. The skin is the primary body barrier, with the first layer protecting the fish from pathogen attack. The stratum adiposum (SA) of albino fish is smaller ($p<0.05$) with a sharp-angled curve shape, while blackfish have a larger stratum adiposum with a more blunt-angled curve shape. The number of SA units in albino fish is relatively lower and fills the dermal layer (Figure 5). The Stratum Compactum of albino fish is the same as that of blackfish ($p>0.05$, Table 4) but tends to be thicker in albino fish. The correlation between pectoral fin morphology and skin colour with eye and skin, calculated using Spearman's rank, is positive for total retina (rho = 0.65; Z = 3.82) and total skin (rho = 0.88; Z = 3.39). The results have practical implications for the study of fish health and genetics.

Albinism is a disorder of body colour caused by changes in the body's melanin production patterns due to individual genetic changes. Albinism is one of the body colour disorders caused by changes in the body's melanin production patterns due to individual genetic changes. This study highlights the relationship between melanin levels in the body and pectoral fin morphological abnormalities, which may be related to similarities in dynamics, signaling pathways, and gene regulation. Melanin pigment is formed in melanocytes by biochemical reactions that convert tyrosine to eumelanin or pheomelanin. Mutations in genes involved in the melanogenesis pathway can inhibit melanin production. The Tyr gene plays an important role in melanogenesis; its mutations cause albinism in many vertebrates (Bian et al., 2021; Utzeri et al., 2021); for example, goldfish *Carassius auratus* has albinism due to the knockout of the Tyr gene (Li et al., 2024). The novel exploration of albinism in the Welsh catfish *Silurus glanis* was reported by Ozerov et al. (2024). He found 1355 skin genes and 614 fin genes directly affecting albinism, of which 149 genes overlap, are particularly intriguing. Furthermore, discovering 189 differential genes associated with albinism that function in the eye, some of which also overlap with genes in the skin and fins, provides a fresh perspective on the work of genetic factors in skeletal abnormality in farmed fish.

In addition to mutational factors, the cause of albinism is increased homozygosity in the population, especially in the context of oculocutaneous albinism (OCA), a recessive genetic status. The recessive genes concentrated in captivity reveal why albino fish are more commonly found in aquaculture environments and even at relatively higher frequencies in the population than in wild environments. The small, adequate population size favors inbreeding in farmed fish. Therefore, the offspring are likelier to be homozygous albino recessive (Zhong et al., 2021). Zhong et al. also reported an increase in albinism in fish. The albinism in this study is thought to be due to individuals' high homozygosity, so the fish cannot produce sufficient melanin pigment. The popularity of catfish as a farmed species can be a driver of increased homozygosity locus but also can be found in frequency, which can also result from increased homozygosity of individuals in the population—farmed fish. Albinism is also found with paired fin anomalies, suggesting the possibility of phenotypic integration of the same developmental process.

In this study, we investigated the expression of T-box 5 protein (TBX5) by TBX5 antibody and tyrosinase protein (TYR) by TYR antibodies. TBX5 was a key gene in forming pectoral fin buds, which is involved in the lack of melanin production in albino fish. We used eye and skin tissues. The detection results are represented by the brown colour (DAB) (Figure 6-7). Albino fish without pectoral fins express weaker immunohistochemical staining in the retina, especially in the retinal pigment epithelium (RPE) and inner nuclear layers. Other areas of the retina appear to have a more diffuse distribution of TBX5. Pectoral finned fish express more TBX5 immunoreactivity, particularly in the RPE and inner retina. Pigmentation in the outer retina is more pronounced, possibly because TBX5 plays a role in retinal differentiation and melanocyte pigmentation. The distribution of TBX5 is more localized than in albino fish. As with TBX5 expression in albino fish without pectoral fins, TYR expression remains detectable in eye and retinal tissues even though the fish have an albino phenotype. TYR in albino fish is usually inactive or has a loss-of-function mutation that disrupts melanin production. The presence of significant amounts of TYR protein in albino fish may indicate that TYR is still expressed but not functioning correctly, which means there may be a disruption in the post-translational regulation of TYR. Although melanin pigment is not produced, TYR expression may be a compensatory mechanism. The absence of pectoral fins may be related to the expression of TBX5, which plays a role in limb development. TYR expression is very strong in black fish with regular pectoral fins, consistent with the black-pigmented phenotype. Active TYR enables normal melanin biosynthesis to make pigment visible in the retina and eye tissue. Well-developed pectoral fins may reflect undisturbed TBX5 expression, supporting the link between TBX5 and limb development and pigmentation.

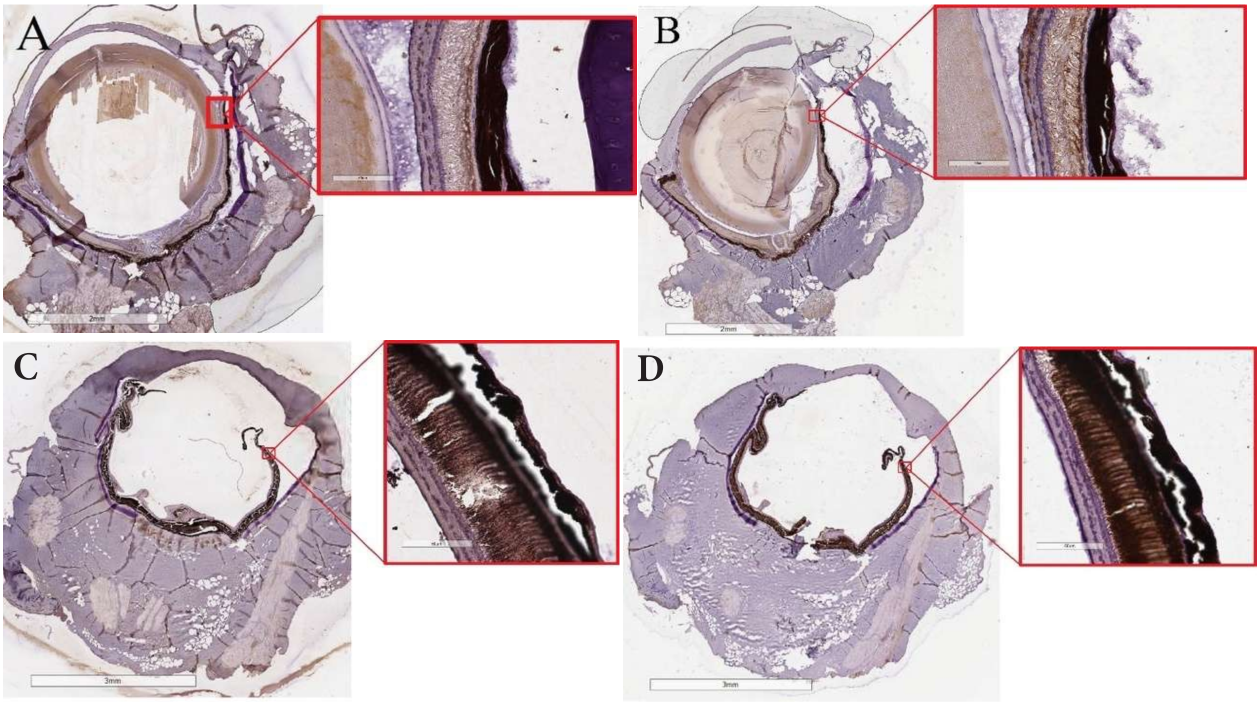

Figure 6. Histochemical activities of A. T-box 5 (TBX5) (5000x2688 pixels) and B. Tyrosinase (TYR) (5000x2869 pixels) in the African catfish *Clarias gariepinus* eye tissue of albino without pectoral fins; C. TBX5 (5000x3031 pixels) and D. TYR in blackfish with regular pectoral fins (5000x2883 pixels)

Table 5. The Antibody percentage (%) between albino-pectoral finless and blackfish-normal pectoral fins of eye and skin organs of African Catfish *Clarias gariepinus* in five observation

No.	Organ	%Antibody/observation field (%)				TBX5-TYR Correlation		T-test Albino-Blackfish	
		Albino		Blackfish		Albino	Blackfish		
		TBX5	TYR	TBX5	TYR			TBX5	TYR
1	Eye	17.116	13.438	20.623	20.051	(0.17)	0.94	p 0.53>0.05	p 0.38>0.05
2	Skin	25.643	31.196	9.613	6.241	0.29	0.54	p 0.00<0.05	p 0.00<0.05

TYR expression is retained in albino fish but is probably non-functional due to mutations or other regulatory mechanisms. Blackfish have active TYR expression, which supports normal melanin production. TBX5 may regulate TYR expression or pigment and limb development, which may explain the phenotypic differences between the two fish.

Correlation results (Table 5) to assess the relationship between TBX5 and TYR in pectoral fin abnormalities and pigmentation in fish; Normal Pectoral Fin Blackfish has a robust positive correlation (r = 0.94), indicating that TBX5 and TYR are expressed synchronously under normal conditions. The strong correlation between TBX and TYR in blackfish with regular pectoral fins suggests that TBX5 may regulate TYR expression or the downstream pathway of melanogenesis. TBX5 may play an active role in developing healthy and functional melanocytes, allowing TYR to fulfill its role in melanin production. In contrast, albino fish without pectoral fins show a weak negative correlation (r = -0.17), indicating that TBX5 and TYR expression are no longer coordinated. Presumably, TBX5 no longer supports melanogenesis, or there is a different regulation in albino fish that inhibits TYR function, although it is still expressed. TBX5 does not induce the downstream pathway required for functional melanocytes, leading to pigment failure even though TYR is still detected. The TYR activity in albino fish is consistent with the hypothesis that albinism is not just a problem of TYR but also of other factors that inhibit melanin production, such as impaired melanocyte differentiation, mutations in TYR downstream genes such as TYRP1, DCT or OCA2, or disruption of TYR post-translational modification. The TYR protein product catalyzes melanin synthesis by converting tyrosine to DOPA and then catalyzing dopaquinone to melanin, involving the transcription factor mitf and the genes pmel and slc24a5 (Bian et al., 2021). These findings could be significant in understanding the role of TBX5 in pigmentation and its possible relationship to the albino phenotype and pectoral fin development.

The relationship between TBX5 and TYR gene expression in two different groups of fish has been assessed. Albino fish without pectoral fins (r = 0.29) showed a weak positive correlation between TBX5 and TYR expression. Although there is a slight relationship between TBX5 and TYR expression, the relationship between the two is not strong, which may indicate that the regulation of TYR in albino fish without pectoral fins is not entirely dependent on TBX5. Other factors are more dominant in inhibiting or disrupting TYR expression, causing the fish to remain albino even though TYR is still expressed at some level. Blackfish with regular pectoral fins showed a moderate positive correlation (r = 0.54) between TBX5 and TYR expression. When TBX5 expression increases, TYR expression also tends to increase, although the relationship is not linear. These findings support the hypothesis that TBX5 regulates TYR expression, especially in fish with normal pigmentation. The lower correlation in albino fish may indicate that TBX5 does not function optimally in regulating TYR, possibly due to mutations or other disrupted regulatory factors. The higher correlation in black fish suggests that TBX5 plays a role in controlling TYR, which supports normal pigmentation.

Although a robust positive correlation was found between TBX5 and TYR, the limitation of this study, which used only one specimen as a sample, is a limiting factor for a broader generalization. Therefore, this study needs to be validated by examining more samples. The results of this study have at least provided a basis for including the pectoral fins phenotype as one of the important considerations in a selection and hybridization programme for *Clarias gariepinus* catfish to obtain superior fish.

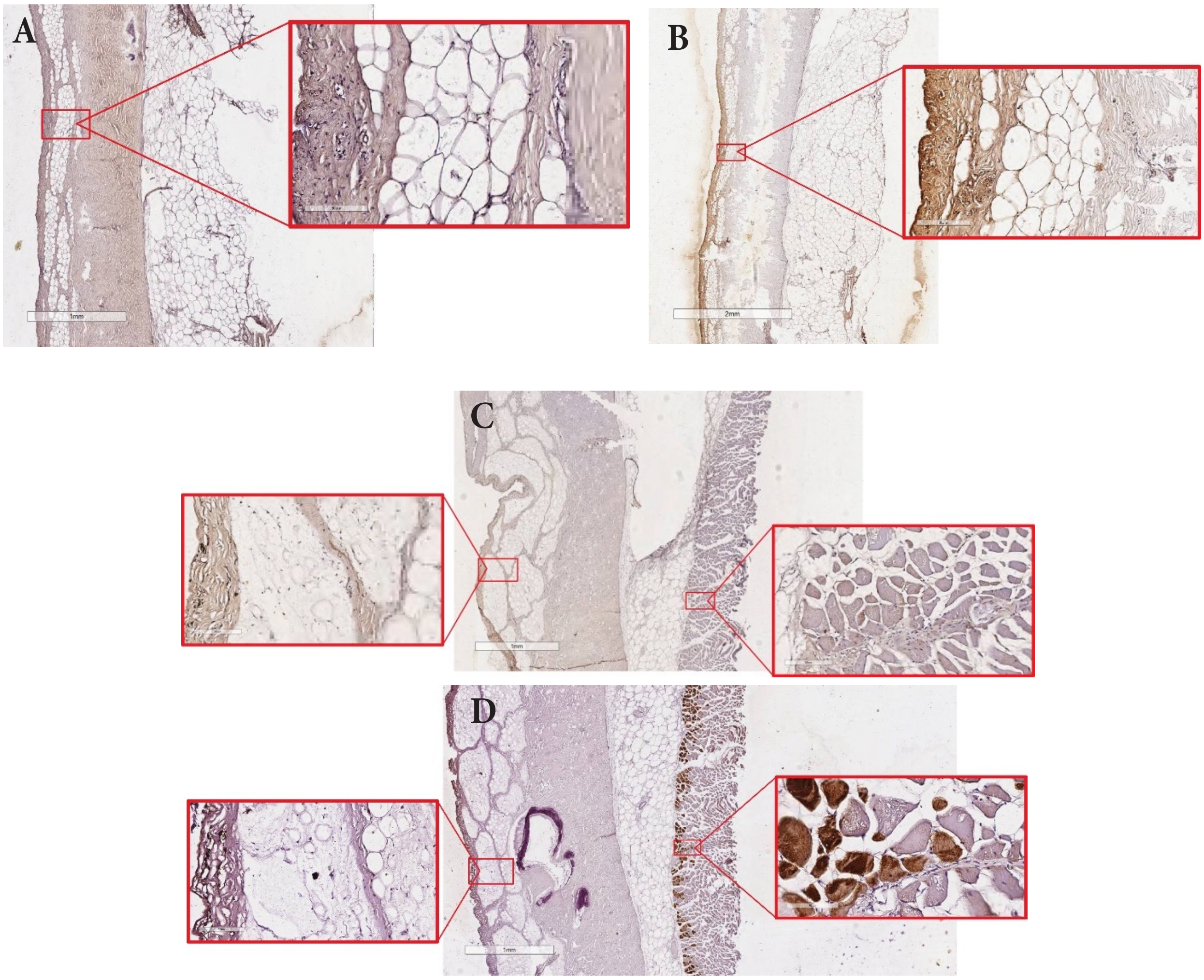

Figure 7. Distribution of A. TBX5 (5000x2624 pixels) and B. TYR in the African catfish *Clarias gariepinus* from skin tissue of albino without pectoral fins (5000x3030 pixels); C. TBX5 (5000x1680 pixels) and D. TYR in blackfish with normal pectoral fins (5000x1764 pixels)

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CONCLUSION

Our research has uncovered a significant difference in the total retinal and skin thickness of albino pectoral-finless fish compared to blackfish with regular fins, revealing important physiological differences. The positive correlation between albinism and pectoral fin anomalies with the total retinal and skin thickness directly impacts the fish's fitness. Our study of melanin, a key biochemical, has practical implications and provides insight into the relationship between pectoral fin absence and albinism in the African catfish *Clarias gariepinus*. Reactivity tests of two key proteins related to the pectoral fin pathway (TBX5) and melanin formation (TYR) showed a close correlation between TBX5 and TYR. However, they were poorly coordinated in individuals with pectoral fin abnormalities and melanin hypopigmentation. TBX5 is an important factor in pigmentation and fin development, but additional factors may disrupt TYR regulation in albino fish.

AUTHOR CONTRIBUTION

F: acted as principal investigator, **A:** analyzed data, and **D.T.R:** collected documentation of albino fish. **M.A:** established the immunohistochemical staining in pectoral finless-albino African catfish *Clarias gariepinus*.

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CONFLICT OF INTEREST

The authors do not have any conflict of interest regarding the research or the research funding.

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