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Regeneration of the caudal fin of the evolutionary ancient tropical gar *Atractosteus tropicus*

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Abstract

Background The tropical gar (*Atractosteus tropicus*), a member of the Lepisosteidae family, is native to regions extending from southeastern Mexico to southern Costa Rica. This species serves as a unique bridge between tetrapods and teleosts due to its phylogenetic position, slow evolutionary rate, dense genetic map, gene similarities with humans, and ease of laboratory cultivation. As a taxonomic sister group to teleosts like the zebrafish (*Danio rerio*), known for its high regenerative capacity, it remains unclear whether the tropical gar shares a similar ability for regeneration.

Results This study aims to elucidate the caudal fin regeneration process in tropical gar through skeletal and histological staining methods. Juvenile specimens were observed over a two-month period, during which they were fed brine shrimp, and anesthetized with 1% eugenol for caudal fin amputation. Samples were collected at various days post-amputation (dpa). Alcian blue and alizarin red staining were used to highlight skeletal regeneration, particularly the formation of new cartilage, while histological staining with hematoxylin and eosin was performed to observe tissue regeneration at the amputation site.

Conclusions The findings reveal a remarkable ability for caudal fin regeneration in juvenile tropical gar. Given the Garfish evolutionary relationship with teleosts, this opens new avenues for research into tissue regeneration across different groups of Actinopterygii.

Keywords Garfish, Tropical gar, Caudal fin regeneration, EvoDevo

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Background

Atractosteus tropicus (*A. tropicus*) is a fish species belonging to the Lepisosteidae family. Fossil evidence from America, Europe, and India suggests that this species first appeared around 70 million years ago during the Cretaceous period [1]. The Lepisosteidae family is divided into two genera: *Lepisosteus* and *Atractosteus*.

The morphology of *A. tropicus* distinguishes it from most other freshwater or brackish fish. It has an elongated, cylindrical body, with a light olive-green coloration on the dorsum, accented by black spots, and a lighter, almost white belly. Its mouth is elongated, equipped with strong inward-curving canine teeth. The body is covered in diamond-shaped, hard scales. Both the dorsal and anal fins are positioned near the caudal fin [2].

The habitat of *Atractosteus tropicus* (Tropical Gar) spans from eastern to southern Mexico, particularly in the states of Veracruz, Tabasco, Campeche, and Chiapas [3]. This species holds cultural significance in Tabasco, Mexico, where it is an emblematic freshwater resource in local cuisine (Fig. 1A). The Tropical Gar is also found across four Central American countries: Guatemala,

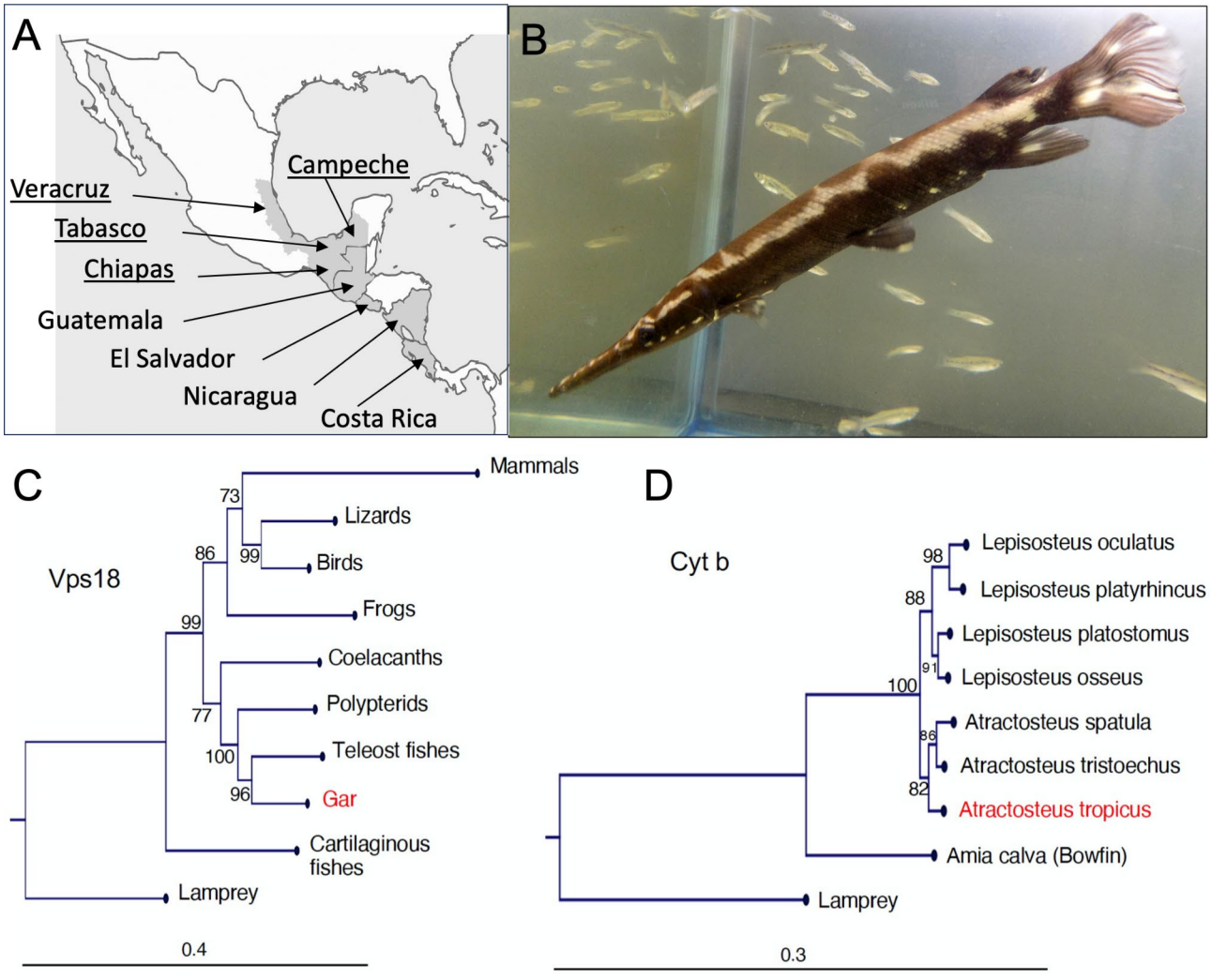

Fig. 1 The subject of study is the Garfish *Atractosteus tropicus*. (**A**) The geographical location of *Atractosteus tropicus*, it's in the East and the South of Mexico in four states (underlined) and four different Central American countries. (**B**) here is shown one of the juvenile individuals use it in this study. (**C**) Phylogenetic tree using Vps18 protein sequences (highly conserved intracellular traffic protein) to show the position of the Garfish among several animals. (**D**) Phylogenetic tree using cytochrome b protein sequences to show the phylogenetic position of the Garfish *Atractosteus tropicus* in their taxonomically family Lepisosteidae. The map in (**A**) was obtained from Wikimedia commons according to the CC BY-SA 4.0 Attribution-sharealike 4.0 international (https://creativecommons.org/licenses/by-sa/4.0/#ref-appropriate-credit), and slightly modified for our purposes with Adobe Photoshop 2021

El Salvador, Nicaragua, and Costa Rica [4]. In Nicaragua, populations thrive in the lakes and the San Juan River and its tributaries. In Guatemala, it inhabits the swampy wetlands of the Sierra de Lacandón, located in the municipality of La Libertad within the Petén Department. Along the Pacific slope, its range extends from southeastern Chiapas to the Gulf of Fonseca in Nicaragua [5], and in northern Costa Rica, it can be found in the wetlands of the canton of Los Chiles and its districts; Los Chiles, Caño Negro, El Amparo, and San Jorge [6]. Unfortunately, wild populations of *A. tropicus* are in decline due to fishing pressure and habitat alterations. Their conservation status is considered "deteriorated," with a sustained decrease in wild populations observed in several municipalities of Tabasco [7]. However, Tropical Garfish farming programs in Mexico, particularly in the states of Tabasco and Campeche, have seen considerable success [3].

Several teleost fish species have been extensively studied at the genetic level, with some having their entire genomes sequenced. For species such as Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), Nile tilapia (*Oreochromis niloticus*), and channel catfish (*Ictalurus punctatus*), genetic characterization and genomic programs are at least partly driven by economic interests [8]. Other fish species, notably the zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*), are widely used as "fish mice" in developmental biology. These species serve as well-established, complementary models for studying various aspects of vertebrate organogenesis [9–11].

Caudal fin regeneration in zebrafish has been extensively studied [12]. This process, which takes approximately 3 to 4 weeks, is epimorphic, meaning that new tissue arises from the proliferation of cells within the blastema formed at the amputation site [13]. Zebrafish caudal fin regeneration occurs in three distinct phases: wound healing, blastema formation, and regenerative outgrowth. During wound healing, neutrophils are the first to migrate to the injured tissue, followed by the migration of non-proliferative epithelial cells that cover the wound. The epidermis then becomes proliferative, forming multiple layers [14]. The blastema forms at approximately 12 h post-amputation (hpa) and contains progenitor cells. Within the blastema core, fibroblasts produce mesenchymal cells, while dedifferentiated osteoblasts remain beneath the epidermal cells on the lateral sides. Molecular pathways, including FGF, Shh, and RA, regulate the proliferation of blastemal cells [12]. In the outgrowth phase, osteoblasts differentiate to form the bone matrix, creating new fin rays. It is known that these cells respond to canonical Wnt signaling to maintain their proliferative state [15]. Additionally, it is believed that osteoblasts are not the sole source of new radial bones; a group of cells expressing matrix metalloproteinase 9 (mmp9) can also differentiate into osteoblasts, contributing to the formation of new bone in the regenerating zebrafish caudal fin [16].

The Spotted Gar (*Lepisosteus oculatus*), a member of the Lepisosteidae family, is noteworthy as the first ray-finned fish to have its genome sequenced [17] that has not undergone the teleost-specific whole-genome duplication. Due to the Lepisosteidae family's phylogenetic position, slow evolutionary rate, dense genetic map, and relative ease of laboratory cultivation, the Tropical Gar serves as a unique bridge between tetrapods and teleost biomedical models.

Genomic and phylogenetic analyses have shown that the Spotted Gar provides a critical link between teleosts and tetrapods, particularly in terms of genome organization. The identification of orthologous genes, which include ancient homologous genes lost in both teleosts and tetrapods, has significantly advanced our understanding of the evolution of vertebrate-specific traits, such as adaptive immunity, mineralized tissues, and gene expression regulation. Importantly, this connection between Lepisosteidae and tetrapods has enabled the identification of potential gene-regulatory elements shared by both teleosts and humans, which could not have been discovered through direct comparisons between tetrapods and teleosts alone [17].

Research on *Atractosteus tropicus* includes several key studies: the current state of knowledge in tropical Gar biology and cultivation [2], karyotype analysis and chromosomal variation in larvae and adults [18], population history and evolutionary relationships, and advances in feeding and nutrition [19]. However, no studies have yet explored the regenerative abilities of *A. tropicus*. In 2019, Darnet Sylvain et al. [20] conducted a study on the evolutionary origins of limb and fin regeneration across several species, including *Lepisosteus oculatus*. While they mentioned pectoral fin regeneration, caudal fin regeneration was not examined in their work. This study aims to establish the caudal fin regeneration capability of *A. tropicus* and highlight its potential as an emerging model of research.

Materials and methods

Source of organisms and maintenance

The juvenile garfish used in this study measured between 13 and 16 cm in length and were obtained from two different sources. For the initial experiments, garfish were purchased from authorized pet shop dealers. The juveniles used for Fig. 2 were reared in closed aquariums at the Laboratorio de Fisiología en Recursos Acuáticos (LAFIRA) of the División Académica de Ciencias Biológicas at the Universidad Juárez Autónoma de Tabasco. The fish were kept at low densities in 50×40×20 cm tanks filled with tap water pretreated with antichlorine and

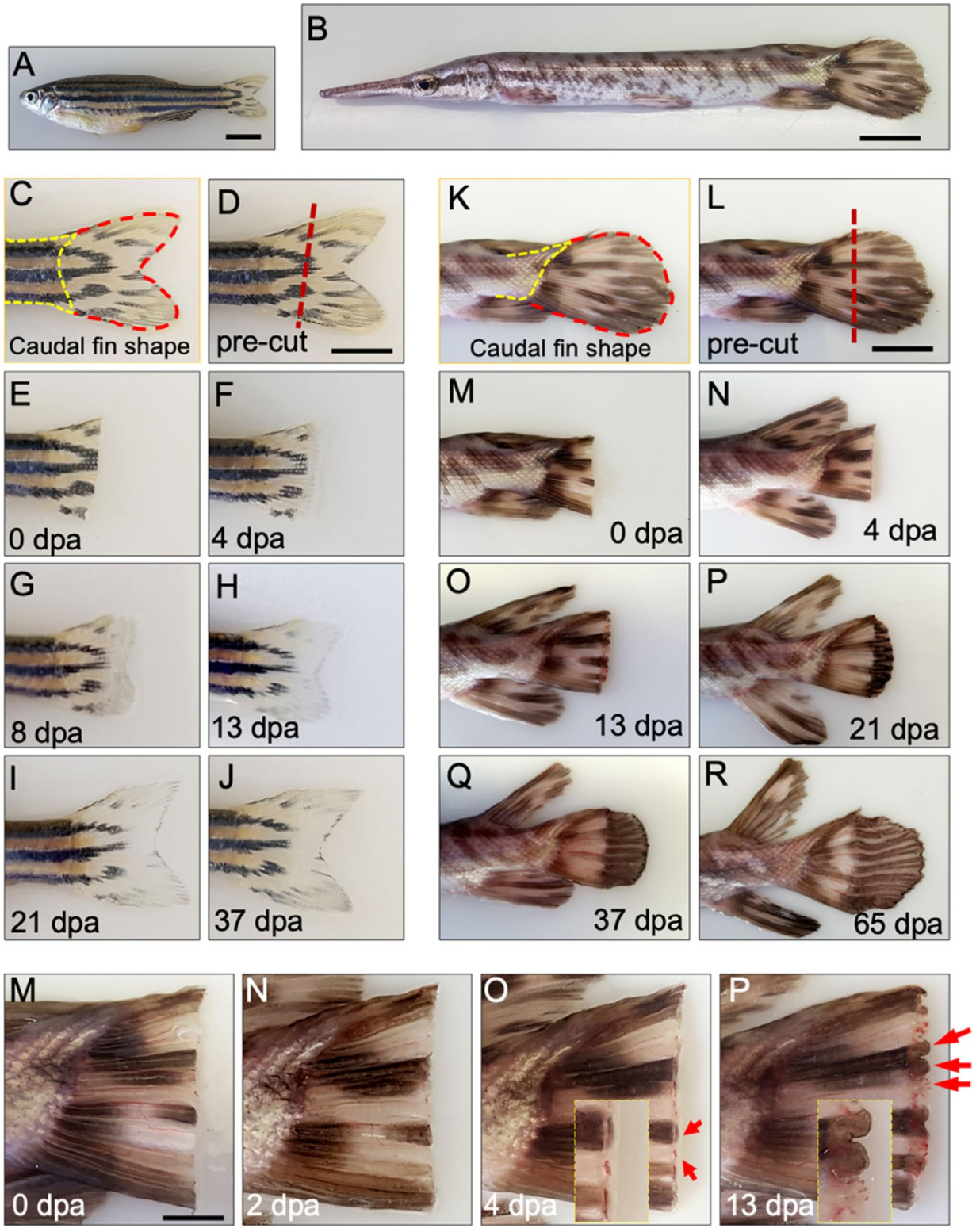

Fig. 2 Comparison of caudal fin regeneration between zebrafish (*Danio rerio*) and the Garfish (*Atractosteus tropicus*). (**A**) adult individual of zebrafish. (**B**) juvenile Garfish. (**C** – **K**) different time points pre- and post-amputation of the caudal fin. It takes about one month to regenerate a whole caudal fin in zebrafish. (**K** – **R**) Caudal regeneration in Garfish at different time points for about two months. (**M** – **N**) Initial steps of caudal regeneration in Garfish from 0 to 13 dpa (days post-amputation). At 4 dpa we detected the first visible signs of tissue regrowth that progress fast as could be seen at 13 dpa (see insets in white dotted boxes in **O** and **P**). Scale bars are (**A**) 5 mm (**B**) 20 mm (**D**) 5 mm (**L**) 10 mm (**M**) 2 mm. Figures **M** – **P** are shown twice enlarged at the bottom of the figure

supplemented with vitamin B1. A waterfall filter system provided constant oxygenation. The water temperature was maintained at a steady 27°C, with a pH of 7.0 to 7.5, under a 12-hour light/12-hour dark photoperiod. The fish were fed daily with *Artemia* sp. nauplii (brine shrimp) and pellets. All procedures followed the Mexican Government's Official Norm (NOM-062-ZOO-1999) for laboratory animal welfare and were approved by the Institutional Committee of Bioethics in Research at Universidad Juárez Autónoma de Tabasco (UJAT-CIEI-2024-006).

Amputation procedure

The anesthesia method used was immersion [21], which involved submerging the fish in 1 L of a 1% eugenol solution in water, with no organic solvents. To minimize stress on the fish, it was removed from the anesthetic solution after a maximum of 30 s and placed on a predisinfected surface for amputation using a scalpel (No. 4 handle and No. 22 blade). A cross-sectional cut, approximately 1.8 to 2 cm long, was made on the caudal fin, after which the fish was returned to its tank. For sample collection for skeletal and histological staining, the fish was subjected to prolonged exposure to 1% eugenol for 20 to 30 min. A cross-section was made at the anterior part of the radials, and the caudal fin sample was then placed in absolute ethanol for skeletal staining or in 4% paraformaldehyde (PFA) for histological sectioning.

Cartilage and bone staining

Following the protocol by Hanken and Wassersug (1981) [22], as modified by Cuervo et al. (2012) [23], the sample was first fixed in absolute ethanol for up to 24 h. It was then permeated in acetone with constant agitation at 9.8 rpm using a CVP-9550 SCIENTIFIC® orbital shaker for 12 to 24 h. After permeation, the sample was stained with a solution of alcian blue and alizarin red, also with constant agitation at 9.8 rpm for 24 h. Following staining, the sample was washed three times with distilled water. Next, the sample was placed in a 10% KOH solution and 20% glycerin, prepared in distilled water, for a variable duration of 20 to 180 min, depending on the sample size. It was crucial to monitor this step carefully to prevent skeletal degradation. Finally, the sample was stored in a 50:50 glycerin/ethanol solution.

Histological preparations and staining

Following the protocol by Garciadiego-Cazares et al. (2004) [24], the sample was initially fixed in 4% paraformaldehyde (PFA) for 48 h. It was then placed in a decalcifying solution for 24 h, with the solution replaced for an additional two hours. After decalcification, the sample was transferred to phosphate-buffered saline (PBS) for 30 min, and subsequently dehydrated through a series of ethanol concentrations: 25%, 50%, 75%, and 100% with 30 min at each concentration. The sample could be left in absolute ethanol for up to one week if needed. Following dehydration, the sample was immersed in xylol 1 for up to one week, then transferred to xylol 1 for one hour and xylol 2 for one hour. It was then embedded in liquid paraffin at 56°C for one hour, with this embedding step repeated once more. The sample was placed in a histological embedding cassette filled with paraffin and left at room temperature until the paraffin solidified. Before sectioning, the sample was cooled to 4°C for 1 min, then cross-sections of 5 micrometers were made using a Leica RM212RT microtome. The sections were subsequently placed in a water bath at 48°C for 5 to 10 min. The samples were collected on TESPA-coated slides, which were then placed on a plate at 52°C for 5 to 15 min before being stored for histological staining.

Histological sections were rehydrated through a series of solutions: xylol 1 for 5 min, xylol 2 for 20 s, followed by ethanol (ETOH) at concentrations of 100%, 96%, 80%, 70%, 60%, 50%, double-distilled water 1 for 30 s, and double-distilled water 2 for 5 min. The samples were then immediately stained with Hematoxylin and Eosin (HE). For staining, the slides were immersed in Harris hematoxylin for 6 to 16 min, depending on the size and thickness of the sample. After staining, the samples were rinsed with tap water under constant flow for 5 min and examined under an Axio Imager A1 Zeiss® microscope. Next, the samples were briefly immersed in 1% acetic acid, then washed again with tap water under constant flow for 1 min. The staining of the nuclei was checked under the microscope. If necessary, the samples were treated with a saturated lithium carbonate solution and rewashed with tap water for 10 min. If the nuclei were still not properly stained, the lithium carbonate treatment was repeated. Finally, the samples were dehydrated by passing through 80% ethanol pre-eosin for 2 min, eosin for 1 to 2 min, and then immediately transferred to 96% ethanol. The dehydration process was completed using ethanol and xylol.

Phylogenetic tree building

The phylogenetic trees in Fig. 1C and D were made from multiple sequence alignments using ClustalW with the BLOSUM matrix, and the rooted phylogenetic trees were made by the neighbor joining method with the Bootstrap mode using 1,000 replications. These multiple alignments and phylogenetic trees were made using the MacVector software (18.6.4), the percentage of best replicated trees that were clustered together is shown next to each of the tree branches. The Vps18 phylogenetic animal tree (Fig. 1C) was made using the Vps18 protein sequences of: *Mus musculus* (for mammals) ID: AK029109.1, *Chelonia mydas* (for lizards) ID: XM_027818182.3, *Gallus gallus* (for birds) ID: NM_001199655.2, *Xenopus tropicalis*

(for frogs) ID: NM_001127982.1, *Latimeria chalumnae* (For Coelachants) ID: XM_064565099.1, *Polypterus senegalus* (For Polypterus) ID: XM_039741060.1, *Larimichthys crocea* (For Teleosts) ID: XM_010744363.3, *Lepisosteus oculatus* (For Garfish) ID: XM_006632431.2, *Amblyraja radiata* (For Cartilaginous fishes) ID: XM_033026715.1, and *Petromyzon marinus* (For Lamprey) ID: XM_032964217.1. For the phylogenetic tree in Fig. 1D we used the protein sequences of Cytochrome b of: *Lepisosteus oculatus* ID: JF912051.1, *Lepisosteus platyrhincus* ID: JF912048.1, *Lepisosteus platostomus* ID: JF912054.1, *Lepisosteus osseus* ID: JF912058.1, *Atractosteus spatula* ID: JF912045.1, *Atractosteus tristoechus* ID: PP331196.1, *Atractosteus tropicus* ID: JF912046.1, the Bowfin *Amia calva* ID: PP331004.1, and the Lamprey *Petromyzon marinus* ID: KJ684768.1. In both trees the Lamprey sequences, either Vps18 or Cytochrome b were used to root the trees.

Photographs

Photographs were taken in the Discovery V20 Carl Zeiss® vertical microscope with a 1.0X FWO 81 mm lens, using an AxioCam HRc Zeiss® camera, and in the Axio imager A1 Zeiss® microscope using 5X, 10X, and 20X lenses.

Results

The tropical gar (*A. tropicus*), a member of the Lepisosteidae family, is distributed across eastern and southern Mexico and Central America (Fig. 1A). This fish is strictly freshwater and brackish, inhabiting rivers, marshlands, and shallow lagoons with abundant vegetation. Morphologically, it features an elongated body, prominent jaws, and ethmoids; a premaxilla with two complete rows of conical teeth, resembling an alligator's; laterally compressed branchial arches with 57 to 62 gill rakers on the first branchial arch; and a series of fulcrum scales along the middle fins (Fig. 1B). As a phylogenetic sister group to teleost fishes, the garfish's evolutionary position has been illustrated using sequences from the highly conserved trafficking protein Vps18, which helped construct a phylogenetic tree depicting its relationships with other animals (Fig. 1C). Additionally, we utilized cytochrome b sequences to build a phylogenetic tree demonstrating the position of *A. tropicus* within the Lepisosteidae family (Fig. 1D).

Regeneration is a complex biological process that allows animals to restore the shape, structure, and function of lost or damaged body parts. Although many vertebrates exhibit significant regenerative abilities during embryonic and neonatal stages, few retain this capacity into their juvenile and adult stages. In this study, we compared caudal fin regeneration in zebrafish (*Danio rerio*) and juvenile garfish (*A. tropicus*) under identical conditions. Zebrafish typically regenerate their caudal fin within about one month (Fig. 2A–J), while garfish require slightly over two months for complete regeneration (compare Fig. 2E vs. 2 M and Fig. 2J vs. 2R). Initial regeneration in Garfish can be observed as early as 4 days post-amputation (dpa) (Fig. 2N and inset), with more advanced regeneration evident by 13 dpa (Fig. 2O and inset). Although comparative studies ideally use organisms of similar age, zebrafish and Garfish differ greatly in size. Zebrafish as adults reach up to 4 cm, while Garfish can grow up to 1.25 m as adults. Therefore, juvenile garfish, measuring between 13 and 16 cm, were used instead in this study to ensure they could be easily maintained under controlled laboratory conditions.

Given the small sample size in our initial experiment (shown in Fig. 2), we decided to repeat the study with a larger cohort of 15 juvenile garfish, each measuring approximately 13–16 cm in length (Fig. 3). We assessed the gradual increase in caudal fin size by plotting the mean length measurements before amputation (Pre-amputation), at 0 days post-amputation (dpa), and at subsequent intervals of 14, 28, and 42 dpa. Error bars represent standard deviations. As anticipated, the caudal fins exhibited continuous and consistent regrowth across the different juvenile *A. tropicus* specimens, reaching their original size by 42 dpa (Fig. 3).

To investigate cartilage and bone regrowth, we employed specific staining techniques for cartilage and bone (see Materials and Methods section) (Fig. 4A–G), as well as hematoxylin and eosin (HE) staining (Fig. 4H–K). From day 0 post-amputation (dpa) (Fig. 4B) to 4 dpa (Fig. 4C), we observed the initial extension of rays cartilages, which began to show signs of early branching by 6 dpa (Fig. 4D).

Our original staining protocol clearly highlighted skeletal structures but did not reveal the tissue between the rays. To address this, we modified the protocol by increasing the KOH clearing solution concentration from 10 to 20%, allowing us to visualize the tissue regrowth between the cartilage, including pigment cells (close-up at 7 dpa in Fig. 4E). Reverting to the standard protocol, we observed more frequent branching of the rays at 9 and 12 dpa, with branches increasing from 2 to 4 (Fig. 4F–G).

Histological sections combined with HE staining provided additional insights into caudal fin regeneration. At 2 dpa (Fig. 4H), 5 dpa (Fig. 4I), 8 dpa (Fig. 4J), and 9 dpa (close-up in Fig. 4K), we observed the formation of numerous new chondrocytes and epidermal cells at the tips of each radial, indicated by blue arrows in Fig. 4I and J, and the close-up in Fig. 4K. In a live *A. tropicus* specimen at 16 dpa, we could clearly distinguish the new tissue and the branching of newly regenerated rays, which appeared red during the regeneration process (Fig. 4L).

We also present the phylogenetic distribution of the garfish relative to other animals (Fig. 5). The garfish is an

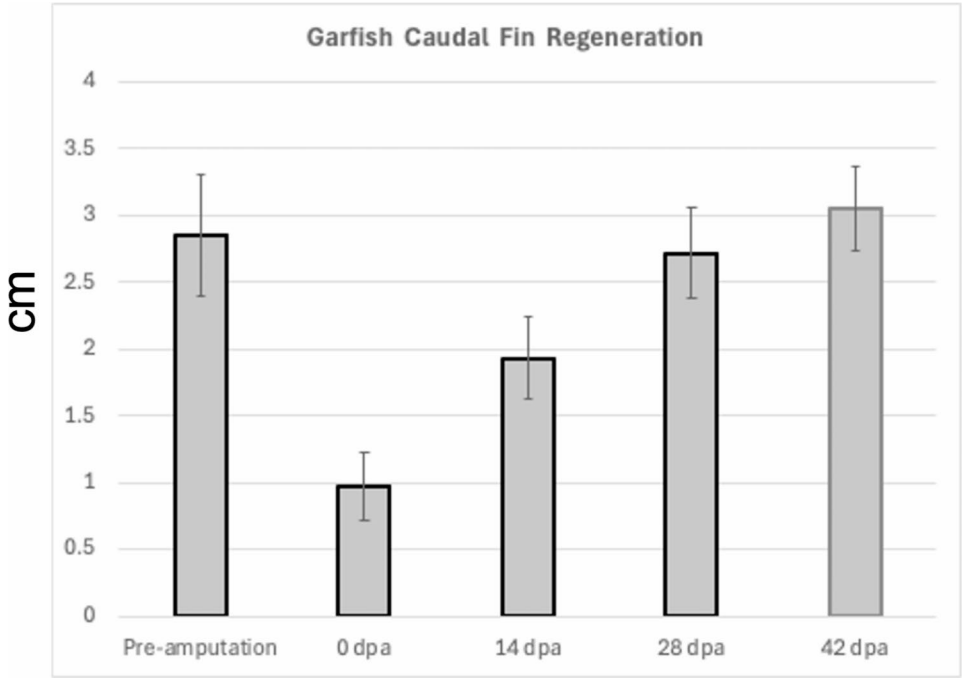

Fig. 3 Caudal fin regeneration in 15 Garfishes. In this graph means values are shown for the Pre-amputation caudal fin and the regrowth velocity for the caudal fin after amputation as measured at 0, 14, 28 and 42 dpa. Error bars show Standard Deviation

ancestral lineage to modern animals and represents the sister group to teleost fishes, which are the most diverse group of fishes known today.

Discussion

Gars belong to an ancient group of fish known as the Lepisosteidae [1]. This family comprises seven species across two genera: *Lepisosteus* and *Atractosteus*. The *Lepisosteus* genus includes four species: *Lepisosteus osseus*, *Lepisosteus platyrhincus*, *Lepisosteus platostomus*, and *Lepisosteus oculatus*. The *Atractosteus* genus includes three species: *Atractosteus spatula*, *Atractosteus tropicus*, and *Atractosteus tristoechus*.

Recent genomic analysis of *L. oculatus* has revealed sequences that are unique to teleosts or tetrapods, often shared by ray-finned fish, lobe-finned vertebrates, and mammals, including humans [17]. Fish possess a remarkable ability to regenerate various body parts, although this regenerative capacity varies significantly among different species [12]. Generally, there is an inverse correlation between a species' evolutionary development and its regenerative ability; more primitive animals typically exhibit greater regenerative capabilities compared to more advanced ones [24].

Typically, studies on fin regeneration focus on teleostean actinopterygians, such as the zebrafish, whose regenerative abilities are confined to the dermal rays of their fins [25, 26]. The regenerative capacity of zebrafish caudal fins has been extensively studied [12]. Our study investigates the regeneration of the caudal fin in *Atractosteus tropicus* from 0 to 65 days post-amputation, focusing on the regrowth of the amputated rays. Following amputation, the wound heals and forms a blastema, which proliferates and generates new ray growth. This process shows similarities to that observed in zebrafish.

Analysis of data from 15 garfishes revealed that the caudal fin reached its original length after 42 days post-amputation (dpa). Throughout this period, the *Atractosteus tropicus* specimens were fed daily and maintained in optimal freshwater conditions. These controlled conditions may differ significantly from those in their natural habitat. External factors such as nutrition, stress, water quality, and temperature could potentially influence the regeneration of the caudal fin.

It is important to note that our study compared caudal fin regeneration between adult zebrafish and juvenile *A. tropicus* (around 5 months old). It is possible that caudal fin regeneration in *A. tropicus* may only occur during its juvenile stage and not in adults. To date, there are no reports on fin regeneration in adult individuals of any of the eight Holostei species. Additionally, the large size of adult *A. tropicus* makes such experiments challenging. While juvenile *A. tropicus* takes approximately twice as long to regenerate the caudal fin compared to adult zebrafish, however studying the Gar juvenile fin regeneration remains to be an intriguing model for evolutionary research.

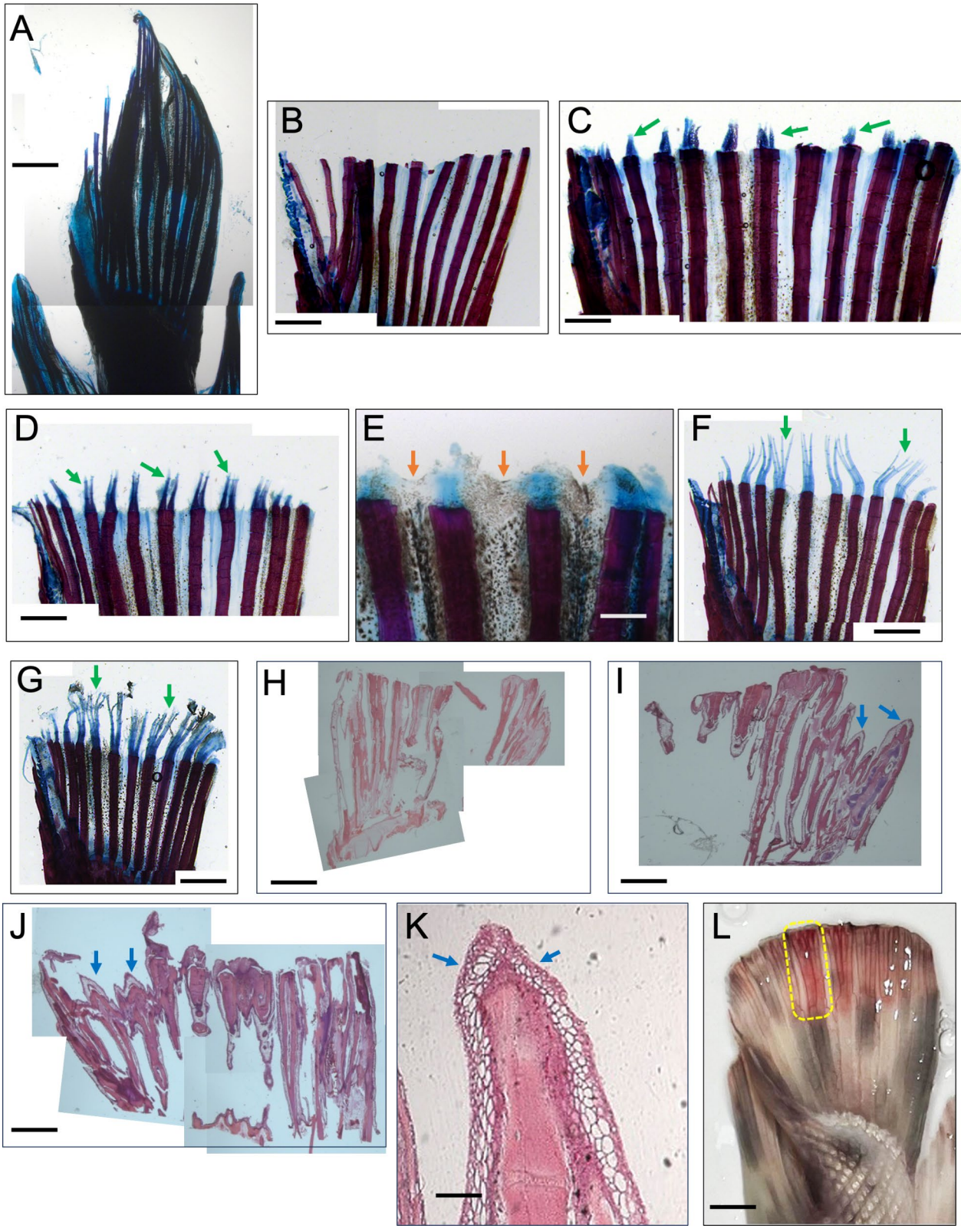

Fig. 4 (See legend on next page.)

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Fig. 4 Garfish skeletal bone regeneration. Cartilage and Bone staining's in control fish (**A**) and at different post-amputation times (**B** – **G**). (**A** and **B**) control and 0 dpa respectively. (**C**) At 4 dpa the regrowth of the cartilage is readily apparent (see green arrows). (**D**) At 6 dpa the rays cartilage is growing steadily and showing signs of branching (see green arrows). (**E**) In the close-up at 7 dpa we could observe that the tissue between growing between the rays (see orange arrows), pigment cells are clearly visible. (**F** and **G**) At 9 and 12 dpa respectively, the rays cartilage has grown considerable, and rays show several branches (see green arrows). We also used HE staining at (**H**) 2 dpa. (**I**) 5 dpa. (**J**) 8 dpa, and (**K**) close up at 9 dpa where new chondrocytes can be observed at the tip of the new formed rays. (**L**) At simple view on the stereomicroscope, at 16 dpa, a regenerated tail shows branched rays (See yellow dotted box). Scales bars are (**A**) 3 mm, (**B**, **D**, **F**, **G** - **J**) 3 mm, (**C**) 2 mm, (**E**) 0.5 mm, (**K**) 0,2 mm, and (**L**) 3 mm

Members of the infraclass Holostei are considered sister groups to the Teleosts (infraclass Teleosteomorpha). While Holostei emerged during the Permian period (299–251 mya), Teleosts appeared later and diversified during the Triassic period (250–201 mya), both within the Mesozoic era (see Fig. 5). *A. tropicus*, a member of Holostei, is one of the eight extant species and represents the closest ray-finned bony fish relative to Teleosts. Alongside the chondrostean Paddlefish and Polypterids, Holostei constitutes one of the three non-teleost taxonomic groups of living Actinopterygians (Fig. 5). These groups are valuable evolutionary models for understanding the innovations that contributed to the success of Teleosts, which include nearly 30,000 known species. Prior to this study, fin regeneration had been explored in non-teleost ray-finned bony fish such as Polypterus, Paddlefish, and the Spotted Gar [20, 23]. These ancient fish demonstrated the ability to partially regenerate pelvic fins when amputated at the proximal endoskeleton, differing from our study where amputations were performed at the distal dermal skeleton. Comparative transcriptomics data on limb regeneration between Polypterus and the salamander Axolotl reveal significant evolutionary similarities in gene regulatory networks for tissue regeneration [20], even between Actinopterygians and Sarcopterygians. The sequenced genome of the Spotted Gar [17] revealed that Garfishes do not share the teleost genome duplication, highlighting their unique evolutionary position. Thus, we propose that juvenile *A. tropicus* serves as an excellent model for tissue regeneration studies due to its pre-teleostean lineage.

The molecular signals regulating caudal fin regeneration in Garfishes remain unidentified. However, Fibroblast Growth Factors (FGFs) are potential candidates involved in cell proliferation and the subsequent differentiation of bone tissue.

Exploring the molecular signals that govern caudal fin regeneration in *A. tropicus* could be valuable. Specifically, genes such as *fgf20* and *fgf3* are worth investigating. In zebrafish, these genes are upregulated following caudal fin amputation, *fgf20* is expressed earlier, followed by *fgf3*, and these are crucial for fin regeneration. Both genes have been observed in the wounded epidermis and in blastema cells during the regeneration process [27].

Based on our observations, we can conclude that juvenile *A. tropicus* is a valuable model for studying caudal fin regeneration. Although the regeneration process in *A. tropicus* takes twice as long as it does in zebrafish, the significant phylogenetic distance between these two groups suggests that the shared genetic pathways for fin regeneration are ancient and evolutionarily conserved. This makes *A. tropicus* a promising model for exploring fundamental aspects of regenerative biology.

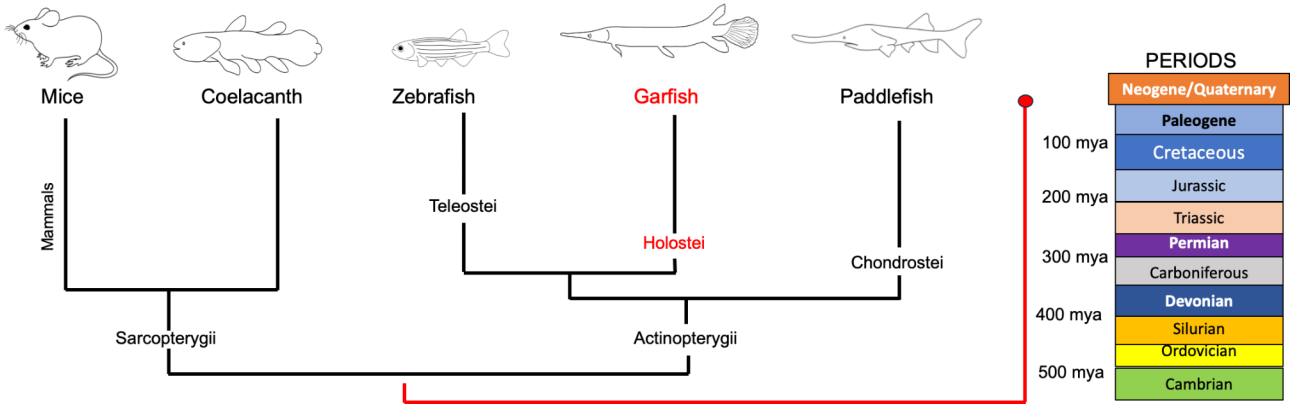

Fig. 5 Phylogenetic model showing the evolutionary position of Garfish between some other related animals. The Geological periods are represented by colored boxes at one side of the diagram from the Cambrian to the Quaternary period

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Author contributions
AJR-F and EM designed the experiments and wrote the manuscript. AJRF, SLF, JAMM, KYFR, RM-G, and ER-S carried out the experiments or help preparing figures for the manuscript. AJR-F, GGA-A, CAS-Q, RM-G, and CAA-G contributed with equipment, materials, reagents and discussions.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All experiments were in accordance to the protocol approved by the Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (NOM-062-ZOO-1999, 2001) [22], considering that the Garfish *A. tropicus* is listed in the IUCN (International Union for Conservation of Nature) as LC (Least Concerned), which mean is not considered an endangered species. *A. tropicus* it is also not part of the list of endangered organisms in the NOM-059-SEMARNAT-2010 from the Mexican government. Experimental protocols were approved by the Institutional Committee of Bioethics in Research from the "Universidad Juarez Autonoma de Tabasco" UJAT (UJAT-CIEI-2024-006).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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