**RESEARCH**

# Anatomy, 3D micro-CT and semiquantitative elemental microanalysis in common carp vertebrae (*Cyprinus carpio*; Linnaeus, 1758)

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## Abstract

The study describes the anatomy vertebrae of the Common Carp (*Cyprinus carpio*; Linnaeus, 1758) using 3D micro-CT and Semiquantitative Elemental Microanalysis (EDX), which helps to understand the ontogeny and factors that determine the biomechanics of bones in wild fish. The study of Common carp vertebrae revealed a complex and heterogeneous structure, with a variety of components contributing to their function and stability. The vertebral bodies were composed of lamellar trabeculae and internal hollow spaces, while the neural and hemal arches were composed of bony trabeculae and connected by a lace-like structure. The results of EDX analysis demonstrated the presence of oxygen, nitrogen, sodium, carbon, magnesium, and chlorine, among others. The calcium to phosphorus ratio was within the ranges described in teleost, suggesting good bone health. The presence of magnesium, sodium, potassium, chlorine, and nitrogen is due to their essential role in different biological processes. In addition, a difference was observed between the trabeculae present in the vertebrae of the abdominal and caudal segments, suggesting a relationship between the density of each bone and its function. These findings contribute to the understanding of the structure and function of vertebrae in wild Common carp and may have implications for understanding the evolution of the vertebral column in fish in general.

**Keywords** Common carp · *Cyprinus* · Teleost · Wildlife · Vertebrae · Anatomy · EDX

## Introduction

The vertebral column of teleosts, specifically cyprinids, serves as an example of adaptability and structural complexity, reflecting the wide diversity and specialization observed in this group of fishes. This structure is regionalized into three distinct areas, each with unique morphological characteristics, which lays the groundwork for understanding the evolutionary and functional development of these structures in teleosts (De Clercq et al. 2017). Moreover, the plasticity of the teleost skeleton, evidenced by changes in its anatomy, mechanical properties, and meristic traits, highlights the capacity for morphological adaptation and variation in response to epigenetic factors, emphasizing the dynamic and adaptive nature of the skeleton (Witten & Hall 2015).

As a model of an adaptive and cosmopolitan species, the Common carp (*Cyprinus carpio*), a teleost species in the family Cyprinidae, possesses characteristics that allow it to survive and thrive in a wide range of aquatic environments worldwide (GISD 2023). The Common carp exhibits remarkable adaptability, including tolerance to a wide range of temperatures, with survival in waters ranging from 4 to 30 °C (Liang et al. 2015; Sfakianakis et al. 2004; Vajargah & Vatandoust 2022). It can adapt to different oxygen levels, surviving even in stagnant and poorly oxygenated waters (Zhao et al. 2014; Bauer & Schlott 2006). Additionally, it tolerates varying salinity levels, thriving in both freshwater and saltwater environments, such as rivers, lakes, reservoirs, lagoons, and estuaries (Mubarik et al. 2019; Ahmed & Jaffar 2022; Malik et al. 2018). The carp's omnivorous diet, which includes aquatic plants, invertebrates, and small fish, allows it to exploit diverse food resources (Wei et al. 2021; Horváth 2019). Moreover, it can grow up to one meter in length and several kilograms in weight, with a lifespan extending to several decades, enabling it to withstand long-term environmental changes (Vilizzi & Copp 2017; Vajargah

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& Vatandoust 2022; Hailu 2013). Collectively, these traits contribute to the Common carp's high adaptability.

In the vertebral anatomy of cyprinids (Kardong 2013), precise use of anatomical terms is crucial for understanding their structure and function. The centrum, or vertebral body, serves as a central support that protects the spinal cord. The neural and hemal arches form protective channels for the spinal cord and blood vessels. The spinous and transverse processes emerge from these arches, providing insertion points for muscles and ligaments, important for locomotion and structural stability. In the dorsal region, the parapophysis is vital for articulation with the ribs, facilitating organ protection and respiratory mechanics. The spine is adapted to the aquatic environment and is divided into abdominal, transitional, and caudal regions, each featuring specialized structures. The abdominal region, less mobile than in other vertebrates, is crucial for supporting the central nervous system. The transitional and caudal regions support the main muscles, allowing movements essential for survival. Specifically, the caudal region includes adaptive structures such as the Weberian apparatus (Witten & Hall 2015; Criswell et al. 2017), crucial for propulsion and sensory perception. Additionally, key components of the spine, such as the praezygapophyses and post-zygapophyses, facilitate the connection between vertebrae and promote controlled mobility. The terminal plates and intervertebral ligaments distribute loads and provide structural support, while the bone marrow spaces, important for hematopoiesis and buoyancy regulation, reflect the cyprinids' unique adaptation to their aquatic environment. These aspects highlight the complexity and evolutionary sophistication of the vertebral column in these fish.

In teleosts, the vertebral structure demonstrates marked functional adaptability, evidenced by the regionalization of the spine and the presence of zygapophyses, which facilitate adaptations to diverse habitats and lifestyles (De Clercq et al. 2017). Terminal plates, positioned between the intervertebral disc and the vertebral body, are crucial for load distribution and disc nutrition. The intervertebral ligaments provide additional stability, complementing the functions of the zygapophyses, while the bone marrow spaces, essential for hematopoiesis, reflect the metabolic activity of the vertebrae. These components, along with the neural and hemal arches, contribute to the functional diversity and adaptability of the spine across different body regions (De Clercq et al. 2017). The formation of vertebral bodies in teleosts, involvs the mineralization of the notochordal sheath without cartilage and highlights the unique contribution of the sclerotome in early vertebral development (Inohaya et al. 2007; Nordvik et al. 2005). These details underscore the rich evolutionary history and specialized adaptations of vertebral anatomy in teleosts and cyprinids, reflecting their rich evolutionary history and specialized adaptations.

The Common carp exhibits unique characteristics in its spine that are crucial for detailed study. Recently, micro-computed tomography (micro-CT) was used to examine the morphology of the vertebral bodies (centrum) of grass carp (*Ctenopharyngodon idella*), highlighting their amphicoelous shape with concave terminal plates at both ends (Barak et al. 2024). The results showed that while the vertebral centrum presents bilateral symmetry in the sagittal plane. Detailed analysis revealed that the cranial terminal plate is smaller and less circular than the caudal plate, suggesting a tapering of the vertebral body from caudal to cranial. Additionally, the cranial terminal plate proved to be more superficial than the caudal, indicating that the caudal part of the vertebral centrum is more robust. This morphological feature may be related to the need to withstand greater muscular tensions during swimming, creating a tension gradient that strengthens the caudal end of the centrum. These findings highlight the importance of considering the morphological and biomechanical variability of the spine in anatomical studies and for understanding the functional adaptation of cyprinids to their aquatic environment (Barak et al. 2024).

The Common carp, originally introduced from Europe and Asia to southern Chile between 1895 and 1920, has been shown to exert beneficial effects in polyculture ponds by reincorporating nutrients from sediments, enhancing mineralization, and improving oxygen availability in the soil, thus supporting ecosystem functioning (Rahman 2015). This species, initially confined to artificial lagoons, adapted well to the local environment, dispersing into nearby rivers due to excessive rainfall and overflow of these lagoons (Prochelle & Campos 2008). Today, the Common carp is firmly established from the central to southern regions of Chile, thriving in various habitats, including national reserves (Habit et al. 2006; Crichigno et al. 2016). The significance of the Common carp extends beyond its ecological impact; it is also valued in aquaculture as a food source and for its ornamental Koi varieties (Rahman 2015; Xu et al. 2014). In this context, the National Reserve Lago Peñuelas, part of Chile's National System of Protected Wild Areas, serves as a crucial water resource for the central zone of Chile. However, its role as a water supply has been severely compromised by the extensive agricultural use of the Aconcagua River waters (CONAF 1999). As of April 2022, Lago Peñuelas' reservoir has been reduced to less than 0.2 MMm$^3$ from a total capacity of 95 MMm$^3$, a decline of over 99.98% due to prolonged drought conditions lasting nearly a decade and the absence of significant rainfall (DGA 2022). This severe reduction in water volume and consequent high temperatures have drastically impacted the aquatic life in the reservoir. Notably, several specimens of the Common carp, a species known for its resilience and adaptability, were found dead from dehydration, scattered around the drying lakebed. This distressing scenario highlights the vulnerability of even

adaptable species like the Common carp to extreme environmental changes and underscores the broader implications of habitat loss and climate change on aquatic ecosystems.

Our research question focuses on elucidating how the different regions of the vertebral column of the Common carp (*Cyprinus carpio*; a representative of the Cyprinidae) reflect their specific biomechanical functions using advanced imaging techniques. The aim of this study is to describe the vertebral structure of this species by examining the morphological differences between the cervical, dorsal, and caudal regions of the spine, using methods such as micro-computed tomography (micro-CT) and digital radiography. This analysis seeks not only to identify unique structural features in the vertebrae of the different sections but also to interpret these features in the context of the biomechanical and evolutionary function of the Common carp's vertebral column. Here, we present morphological characteristics of the vertebrae and the caudal skeleton of the Common carp, including a biomechanical and morphofunctional interpretation, which would be useful as a reference for ecomorphological and biological research.

## Materials and methods

*Sample collection*. Skeletons of common carp (n = 6) were obtained from Lago Peñuelas National Reserve, Region de Valparaíso, Chile (341 masl; 33°09′08″S 71°31′50″W). These specimens were deceased and had been skeletonized prior to collection, ensuring no live animals were used. Once collected, each specimen was labeled and measured for precise identification at the Laboratory of Animal & Experimental Morphology of the Institute of Biology of the Pontificia Universidad Católica de Valparaíso. To document their initial condition, all specimens were photographed using a Canon Rebel T3i digital camera. The vertebral columns of these carps were then carefully dissected to extract three complete vertebrae from each specimen. The examination of the vertebral structure and the vertebral count previously mentioned were conducted through visual inspection of the dry bone preparations. These vertebrae were selected for their representation of typical vertebral morphology and were used for subsequent internal structure studies, elemental microanalysis, and histological examinations. This selection was made to ensure that the results reflected general vertebral characteristics, independent of anatomical variations such as those influenced by the shape of the head or tail. To identify and describe the vertebral segments, structural criteria related to the position of the fins and the specific characteristics of the vertebrae in each segment were adopted according to Barak et al. (2024). This approach facilitated a detailed exploration of the vertebral anatomy of the carp.

The vertebral column was divided into three main regions: the abdominal, transition and the caudal. The abdominal region included the vertebrae anterior to the first dorsal fin spine, and the transition vertebrae, where the parapophyses are fused to the vertebral body without articulation with the ribs. The caudal region comprised the vertebrae located before and after the first anal fin spine, (called preanal and postanal by Naseka 1996), respectively. This classification provided a solid foundation for further analysis and comparisons within the group of cyprinids.

*Micro-CT acquisition and image processing*. Three-dimensional (3D) reconstructions derived from datasets obtained from the vertebrae of the abdominal, caudal, and complex-caudal segments were analyzed using semi-automatic segmentation to create 3D models of all vertebrae. For this purpose, we utilized a Bruker SkyScan 1278 micro-CT, operating under specific parameters: a source voltage of 59 kV, source current of 692 µA, and an image voxel size of 51.489 µm. The exposure time for each image was 23 ms, with the total scan duration amounting to approximately 57 min and 53 s. Image analysis was conducted using Slicer software version 4.11.2021026 (Fedorov et al. 2012) with the Slicer Morph extension (Rolfe et al. 2021) for DICOM files that facilitated 3D reconstruction. The images illustrating this manuscript were processed with CTvox software (Version 3.3.1, 3D SUITE Software, Bruker MicroCT, Kontich, Belgium).

*Scanning Electron Microscopy and Semi-Quantitative Elemental Microanalysis*. For visualization, a chemical contrast detector (Backscatter, BSE Comp.) was used at a variable pressure of 20 Pa and a working distance of 7 mm, employing a Hitachi SU3500 scanning electron microscope (Tokyo, Japan). Images were captured under an energy of 20 kV and analyzed using the Hitachi software controller. To quantify chemical elements in vertebral bone tissue, an energy-dispersive X-ray spectroscopy (EDX) detector, the Bruker XFlash® 410 M with a resolution of 133 eV at the MnKα line, was coupled to the same microscope. This system was operated using the Quantax Esprit 1.8.1 software (Bruker, Belgium), and volumes of interest (VOIs) from the intervertebral joint region and the centrum were selected for analysis.

*Radiography*. To describe the characteristics of the vertebral column and caudal skeleton, we used a digital radiographic system that included a high-frequency JADE X-ray generator, DRGEM—4 kW, 100 kHz, and an external membrane console programmed at 41 25 kV and 1.0 mAs. The digital X-ray tablet used had dimensions of 2004 mm in width, 650 mm in depth, and 712 mm in height, with the distance from the X-ray cathode to the tablet being 98 cm. The

radiography time ranged from 0.01 to 10 s, with an average exposure time per sample of approximately 3 s.

*Histology.* Vertebrae sections from Common carp were harvested and fixed in 4% formaldehyde for 24 h at a controlled room temperature of 22 °C. The decalcification process for the vertebrae of the Common carp was conducted using the KOS system (Milestone Medical). Vertebral samples were initially submerged in a decalcifying solution of 10% formic acid and 10% hydrochloric acid in equal parts. The decalcification was performed at a constant temperature of 37 °C for 4 h. After this process, the samples were removed from the KOS system and allowed to cool for approximately 10 min before being rinsed under running water for another 10 min. The samples were then processed in a Leica tissue processor (TP1020, Leica Microsystems, Switzerland) for 12 h, followed by dehydration in a graduated ethanol series, clearing in xylene, and embedding in paraplast (Paraplast Plus embedding medium; melting point: 54 °C; Sigma-Aldrich Chemical Co., St Louis, MO, USA) to prepare for sectioning. Serial sections were cut at a thickness of 5 μm and routinely stained with hematoxylin and eosin to visualize basic structural details. For collagen analysis, we utilized the histochemical Picrosirius staining technique (Salinas et al. 2016). This method enhances the birefringence of collagen fibers under polarized light, allowing for detailed observation of collagen alignment and density. Different regions of the vertebral column were examined using a Leica®DM750 optical microscope equipped with polarized light capability (Leica Microsystems, Switzerland), focusing particularly on the intervertebral joint and on the longitudinal bony trabeculae of the vertebral body. Image analysis was conducted using ImageJ v1.49 software (National Institutes of Health, USA), which facilitated the quantification of aligned and non-aligned collagen fibers. The "color segmentation" plug-in was employed to distinguish and quantify the different types of collagens based on their color properties and alignment within the tissue (Salinas et al. 2018). This comprehensive approach allowed for a nuanced understanding of the structural and compositional characteristics of vertebral collagen in Common carp.

## Results

The skeletons of the Common carp exhibited an average total length of 36 cm ($\pm 2.5$ cm). The vertebral column of these specimens consisted of an average of 34 amphicoelous vertebrae, ranging from 32 to 38 vertebrae, each generally characterized by the presence of neural and hemal arches emerging from the upper and lower parts of the vertebral bodies (Fig. 1). The vertebral column was subdivided into three main segments: the anterior segment, consisting of the abdominal or trunk vertebrae (V1 to V11), the middle segment composed of transitional vertebrae (V12 to V17), and the posterior segment, consisting of the caudal vertebrae (V18 to V34). The distinction between the abdominal and caudal regions was typically identified at the level of vertebra 15 or 17 (approximate dorsal fin), marking a clear functional and structural change in the vertebral column (Fig. 2A). The first vertebra was distinguished by its specialized morphology, adapted to articulate with the skull, while the last caudal vertebra was integrated into the caudal skeletal complex, known as the urostyle complex. Each vertebra



**Fig. 1** **A** Vertebrae of Common carp (*Cyprinus carpio*; Linnaeus, 1758), **B** Volume-rendered micro-computed tomography of a typical vertebra, **C** Illustration of structures present in a typical vertebra. *Ns*: neural spine; *Na*: neural arch; *Vb*: vertebral body; *Ha*: hemal arch; *Hs*: hemal spine, *Cc*: canal cord
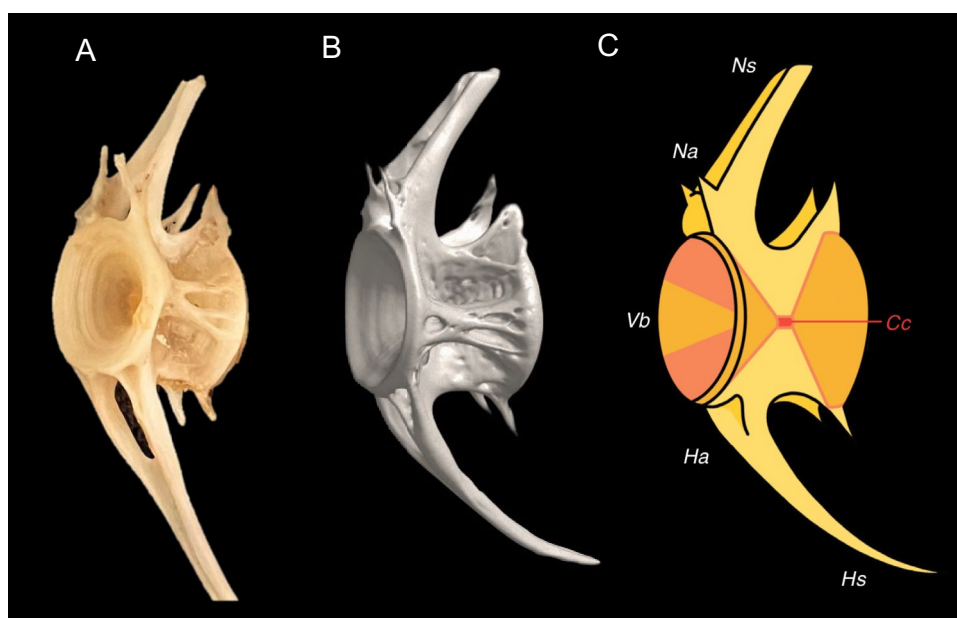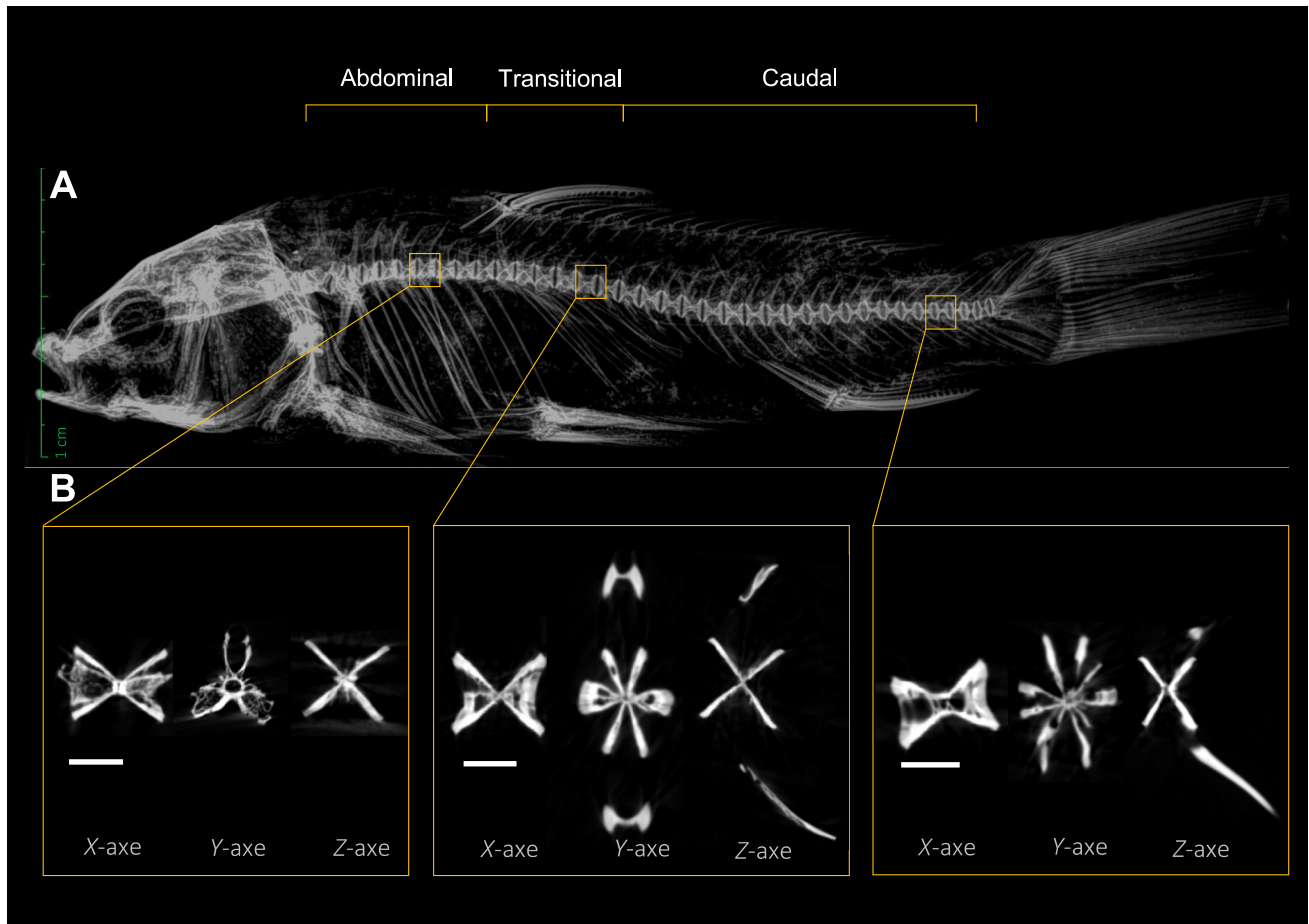
**Fig. 2** **A** Common carp skeleton (*Cyprinus carpio*; Linnaeus, 1758). **B** Digitals X-ray image displays the volume of interest (VOI). Each vertebral body was formed by two hollow cones fused at their vertices. Vertebrae are oriented so that anterior is to the left and posterior to the right. **C** Volume-rendered computed tomography images showing lateral (X-axis), transverse (Y-axis), and horizontal (Z-axis) sections of vertebral bodies from abdominal, transitional and caudal. Scale bars: 5 mm

displayed a "diabolo" shape with the exception of the first and the last (prior to the caudal complex). The vertebral centrum had an amphicoelous shape, similar to a biconcave hourglass, surrounded by a complex three-dimensional network of trabeculae, both longitudinal and transverse. The spinal cord cavities in these vertebrae were not directly connected due to the ossification of the neural canal, which could imply additional protection for the spinal cord in an aquatic environment.

The vertebrae individually exhibited specific anatomical characteristics according to the studied segments, with similarities and differences among them. Each vertebra presented an amphicoelous vertebral body with an hourglass shape, consisting of two robust and smooth cones, typical of the vertebral structure that allows for fluid movements underwater. The vertebrae from the abdominal segment were more robust than those from the caudal segment. However, in the abdominal vertebra, a complex network of longitudinal and transverse trabeculae was observed, forming an intricately

perforated mesh with holes, grooves, and ridges. In contrast, the transitional vertebra shared the amphicoelous shape but was distinguished by having branched longitudinal trabeculae and one or two transverse trabeculae at the midpoint, also reinforced with ridges of compact bone tissue. The neural and hemal arches of the transitional vertebra were prominent and robust, giving rise to curved spinous processes oriented caudally. The caudal vertebra similarly maintained the amphicoelous shape and the two robust cones, but its trabeculae were predominantly longitudinal, some branched, and equally reinforced. The neural and hemal arches were small in the caudal vertebrae, and the spinous processes were straight and longer than in the transitional vertebrae. Additionally, both transitional and caudal vertebrae exhibited pre-zygapophyses and post-zygapophyses, although in the caudal ones these were smaller, sharper, and oriented almost at 90 degrees, facilitating greater articulated stability.

Micro-CT allowed for a detailed visualization of the mineralized portions in the abdominal and caudal segments

of the vertebral column. Fully mineralized vertebral arches and spines were distinguished, along with vertebral bodies that exhibited distinctive terminal plates, showing an "X" shape representative of complete mineralization and mature vertebral structure. Axial (X), sagittal (Y), and coronal (Z) views revealed the internal structures of the abdominal, anal, and post-anal vertebrae with exceptional clarity. In the axial view, the abdominal vertebrae displayed a typical amphicoelous shape, with vertebral centers resembling an hourglass, characterized by the presence of two opposing cones. The internal texture of these vertebrae showed bone marrow spaces surrounded by a well-organized trabecular pattern. In the sagittal view, the neural and hemal arches were highlighted, extending from the dorsal and ventral parts of the vertebral bodies, respectively, clearly outlining the channels through which the spinal cord and blood vessels pass. The bone marrow spaces appeared as lighter areas within the denser bone tissue, suggesting the presence of active marrow tissue in the hematopoietic process. The coronal view offered a transverse perspective, displaying the symmetry and arrangement of the vertebral structures. Details of the trabecular bone tissue extending along the vertebral bodies could be observed. The anal and post-anal vertebrae maintained the amphicoelous pattern but showed differences in the size and shape of the bone marrow spaces and in the arrangement of the trabecular bone tissue, reflecting the functional transition along the spine. Additionally, a pattern of flattening in the terminal plates of some vertebrae was noted, specifically in their conical morphology, accompanied by a reduction in the intervertebral spaces, suggesting a decrease in disc height that could be the result of natural variations or degenerative processes. Despite these observations, no signs of abnormal fusion of the vertebral bodies nor evidence of ectopic chondrogenesis in the terminal plates were detected, indicating the absence of active bone pathology in these areas (Fig. 2B).

The caudal complex (urostyle complex) consisted of a set of four preural vertebrae (Pu), characterized by having less robust vertebral bodies with a concave amphicoelous shape similar to an hourglass. This complex included the preural vertebrae from Pu4 to Pu1, forming the structure of the most caudal segment of the axial axis (Fig. 3C). These vertebrae lacked neural and hemal arches, but two long and sharp spinous processes (dorsal and ventral) were present. Unlike previous segments, these vertebral bodies, while maintaining an amphicoelous hourglass shape with two robust and smooth cones, were shorter. Additionally, each side of the vertebral body displayed a thick and double longitudinal bone structure composed of compact bone tissue fused in the center. The dorsal surface of the first preural (Pu1 + U) displayed a rudimentary neural arch (Rna), which articulated with the epural (Epu). A pleurostyle (Pls) was also observed on the neural arch, articulating with the hypurals

(Hpu 2—6). The ventral surface of the first preural articulated with the parhypural (Pah) and hypural 1 (Hpu1; Fig. 4), highlighting the complexity of the skeletal connections in this terminal region of the vertebral column.

The detailed histological and microstructural analysis of the vertebrae in the intervertebral joint region and along the longitudinal trabeculae revealed a differentiated microarchitecture between the abdominal and caudal vertebrae. The abdominal vertebrae were distinguished by an internal architecture composed of radially oriented bone trabeculae around the central core, creating a series of cavities that gave the vertebral tissue a spongy texture. A high density of these trabeculae was observed in the abdominal vertebrae, particularly in the intervertebral joint region, where compact bone tissue predominated along with oval-shaped canaliculi. A distinctive pattern of predominantly aligned collagen was noted, colocalized at $79.28 \pm 5.68\%$ with non-aligned collagen fibers. On the other hand, the caudal vertebrae exhibited a network of thicker and less dense bone trabeculae compared to the abdominal ones. The intervertebral joint region was characterized by a lower density of canaliculi and more compact bone tissue, with a predominance of aligned collagen ($69.33 \pm 3.71\%$) over non-aligned collagen (Fig. 5). Regarding the longitudinal bone trabeculae of the vertebral body, the presence of compact bone tissue with a high density of canaliculi and a predominance of aligned collagen ($62.1 \pm 4.22\%$) was notable. Additionally, elemental analysis of both vertebral regions identified the presence of calcium and phosphorus in the vertebral cone and in the longitudinal bone trabeculae of the vertebral body (Fig. 6; Table 1).

## Discussion

In the field of fish structural biology, the present research contributes to the characterization of the vertebrae of the Common carp. To date, to our knowledge, there has been no study that integrates radiography, micro-CT, differential collagen staining, and semi-quantitative elemental analysis to describe these structures. This study provides valuable knowledge in terms of the macroscopic and microscopic anatomy of the vertebrae, as well as offering insights into the factors that affect bone biomechanics, such as the composition of collagen and the elements constituting the extracellular matrix and bone structure. Although variability in the number of vertebrae among the specimens of Common carp examined was observed, it is noteworthy that such variation did not necessarily correlate with differences in body length, challenging the notion that vertebration has a direct impact on the size or morphology of the body. Conversely, in taxonomically unrelated species such as the seabass, it has been reported that certain nutritional factors, including vitamin C deficiency, can
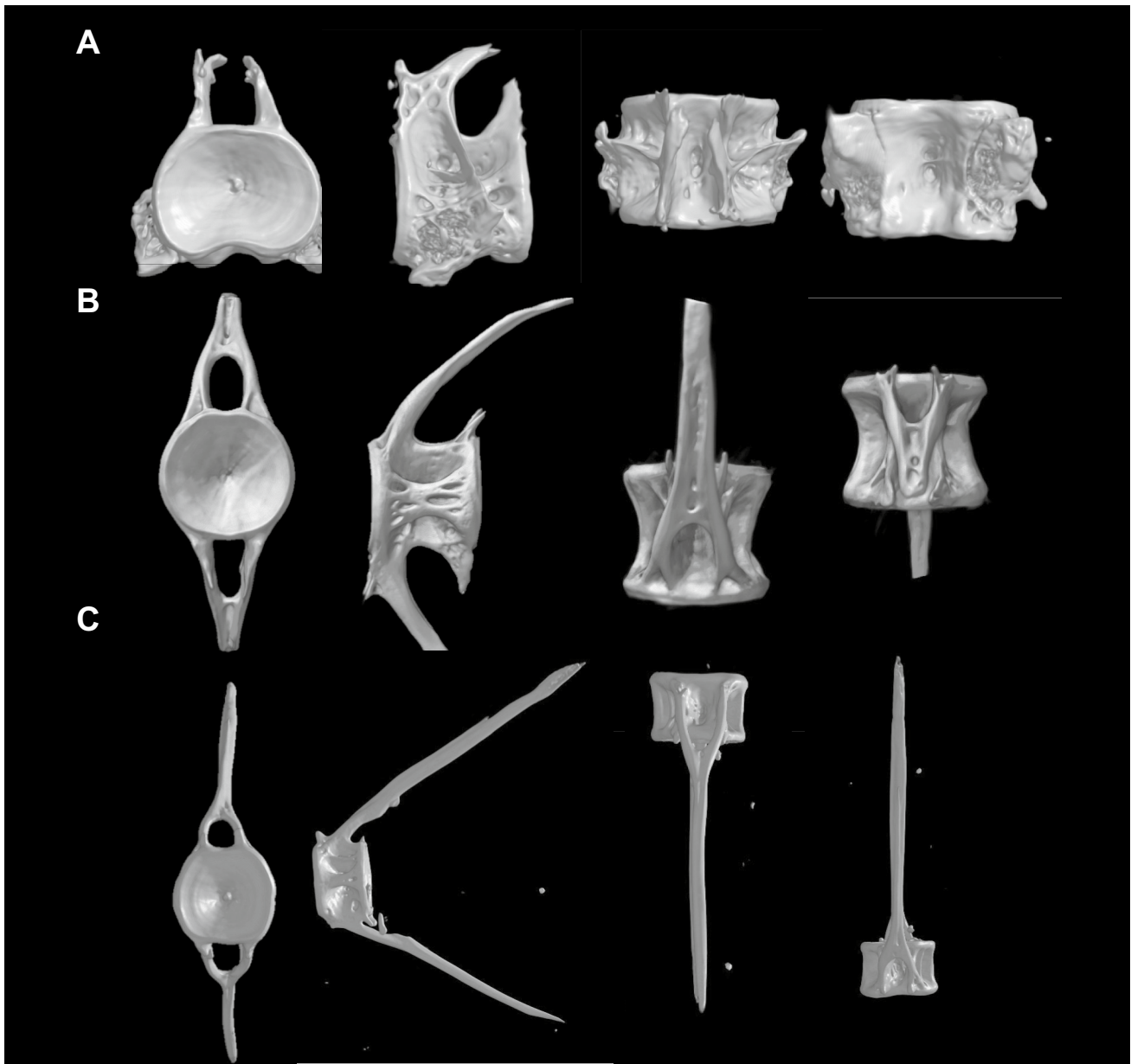
**Fig. 3** Volume-rendered micro-computed tomography (micro-CT) images of vertebral bodies. Anterior, lateral, dorsal and ventral views of the **A** abdominal, **B** transitional and **C** caudal vertebra of Common carp (*Cyprinus carpio*; Linnaeus, 1758)

induce skeletal malformations and affect vertebral count (Berillis 2015). Additional studies in teleosts suggest that environmental conditions—including temperature, salinity, and oxygen—impact vertebral configuration (Fjelldal et al. 2013). Understanding the determinants of the number of vertebrae in Common carp remains a challenge that requires further research. Future studies, focused on specific populations and genetic and environmental variables, are imperative to elucidate the underlying causes of the observed variability and their implications on the physiology and evolution of these fish. These findings underscore the need to continue exploring the biological complexity that defines vertebral morphology in different fish species.

## Structural diversity of the vertebra in the common carp

The vertebral centrum of the Common carp aligns with the general morphological pattern observed in teleosts (Sakashita et al. 2019; Schultze & Arratia 2013; Barak et al. 2024). It is crucial to recognize that the macroscopic structure of the vertebrae is not homogeneous and, as confirmed by
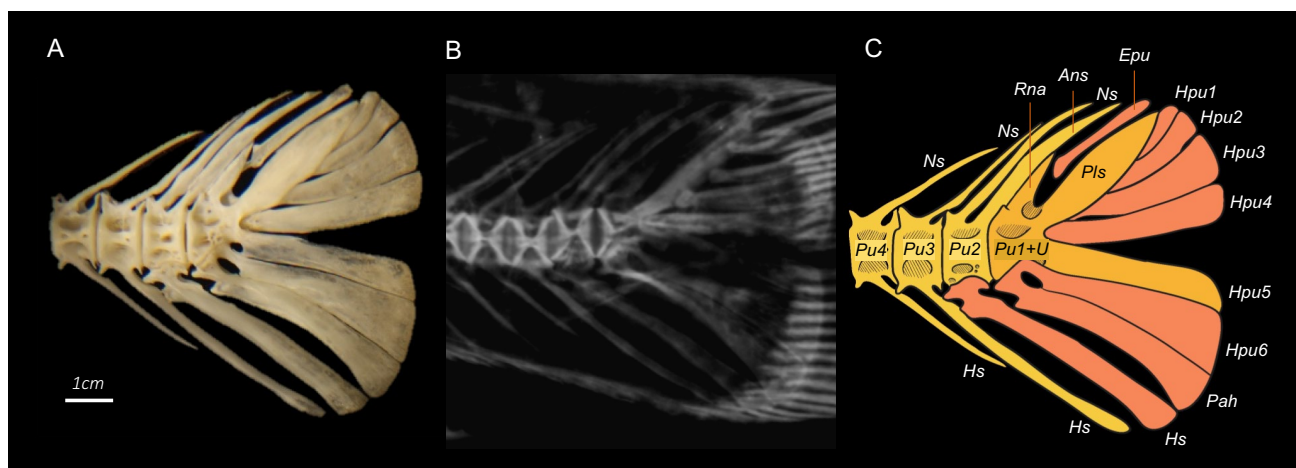
**Fig. 4** Photograph, radiograph, and illustration of the caudal skeleton complex of the common carp (*Cyprinus carpio*; Linnaeus, 1758). *Ns*: neural spine; *Hs*: hemal spine; *Pu*: pre-ural vertebrae, *Pu1 + U*: first preural; *Rna*: rudimentary neural arch; *Ans*: Accesory neural spine; *Epu*: epural vertebra; *Hpu*: hypural vertebra; *Pah*: parhypural vertebra

histological studies, neither are the microscopic characteristics. The shape variation between abdominal and caudal vertebrae, particularly in the hemal and neural arches, is undoubtedly linked to their respective functions — the articulation with the head for abdominal vertebrae and participation in the caudal peduncle for caudal vertebrae. Although all vertebrates share endoskeletal elements that constitute the vertebral axis, the vertebrae themselves are not structurally homologous across different classes of vertebrates and evolve through diverse patterning mechanisms (Arratia et al. 2001). The neural and hemal arches of the transitional vertebrae in the Common carp exhibit notable structural robustness, indicating their role in protecting the spinal cord and supporting caudal movement. Micro-CT and scanning electron microscopy have revealed that these arches are composed of intricate bony trabeculae connected by a cord-like structure, which enhances both their strength and flexibility (Eastman et al. 2014). These morphological characteristics are adapted to the biomechanical demands placed on the vertebrae. The robust and prominent neural and hemal arches maintain the stability and integrity of the spinal column during various movements and stresses encountered in the aquatic environment (Naseka 1996; Baxter et al. 2022). These structural features underscore the importance of the vertebral column in the Common carp, providing essential protection for the spinal cord and the mechanical support necessary for efficient locomotion, thereby highlighting the critical role of the vertebral column in the species' survival and functionality (Fjelldal et al. 2013; Baxter et al. 2022). The vertebral bodies exhibit an amphicoelous hourglass shape common among vertebral segments, which is typical in fish (Fig. 2B; Nordvik et al. 2005). Nonetheless, the pattern of the longitudinal bony trabeculae in the vertebral body varies between segments, as has been observed in other species (Laerm 1976; Arratia et al. 2001; Eastman et al. 2014; Sakashita et al. 2019). These traits reflect an evolutionary adaptation aimed at optimizing the function and biomechanical efficiency of the spine in Common carp. From a radiological perspective, the integrity of vertebral mineralization is critical for biomechanical function and structural stability, and these images provide evidence of the overall health and robustness of the skeleton in Common carp.

## Radiographic analysis of vertebral morphology

Radiography of the abdominal, transitional, and caudal vertebrae of the Common carp provided evidence of both shared characteristics and distinctive variations in their bone structure (Fig. 2). The common amphicoelous morphology, representative of the hourglass shape across all vertebrae, highlights an adaptation for flexibility and force dissipation during swimming, essential for the fish's undulatory movements. Studies suggest that the internal shape of vertebrae can predict swimming modes and ecological adaptations, emphasizing the significance of vertebral shape in swimming biomechanics (Donatelli et al. 2021). The trabecular bone tissue patterns present in each vertebral segment of the Common carp suggest structural optimization for load management and mechanical stress distribution. Detailed studies using advanced imaging techniques, such as micro-CT, have shown that the trabecular bone in vertebrae is highly porous and anisotropic, enhancing its ability to withstand mechanical loads and resist fractures. The trabecular architecture in vertebral bodies is particularly adapted to handle compressive forces, with variations in density and orientation reflecting regional biomechanical demands (Baxter et al.
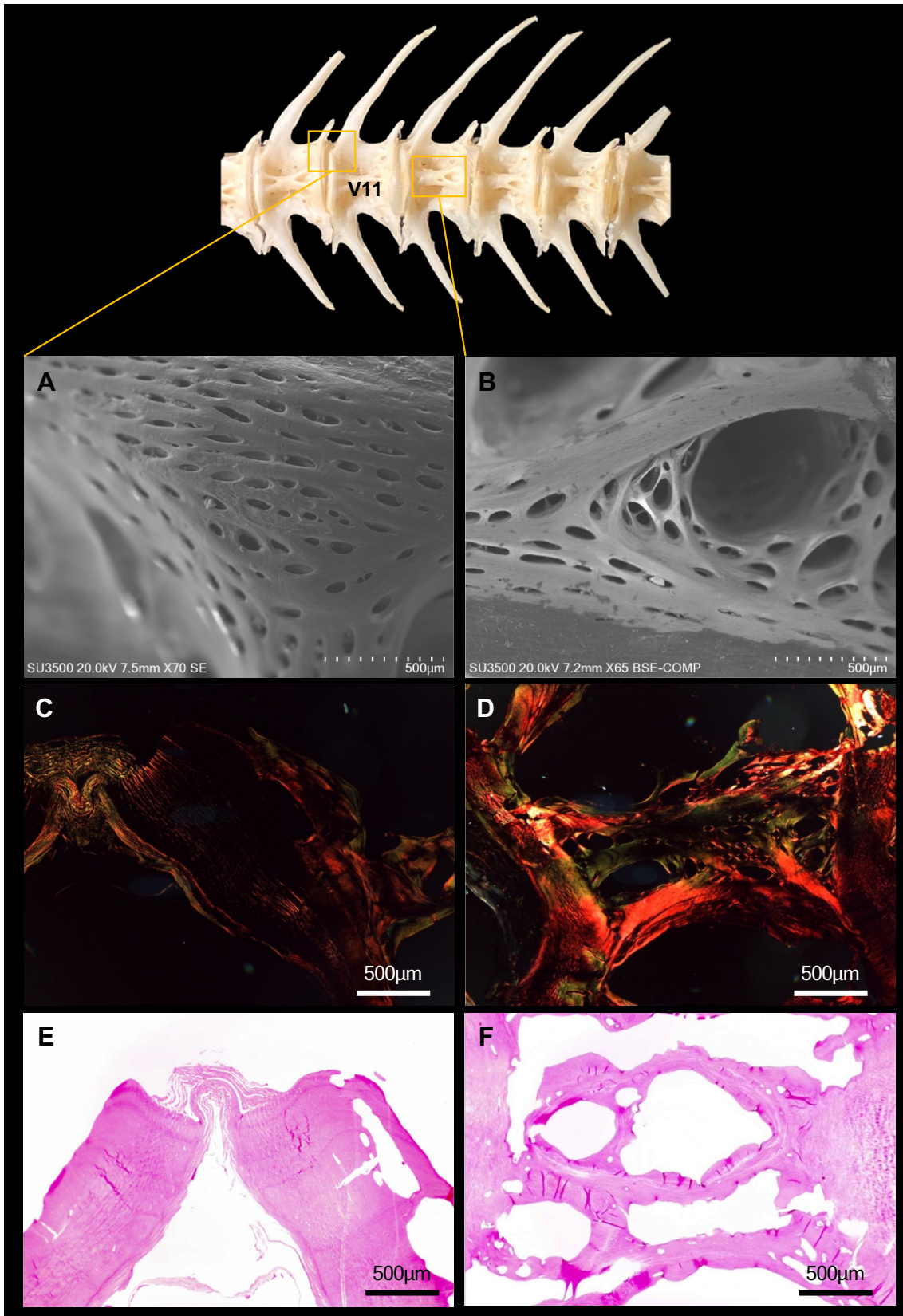
**Fig. 5** Scanning electron microscopy, hematoxylin eosine and picrosirius red staining in Common carp (*Cyprinus carpio*; Linnaeus, 1758). (**A-C**) vertebral cone region and (**B-D**) longitudinal trabecula
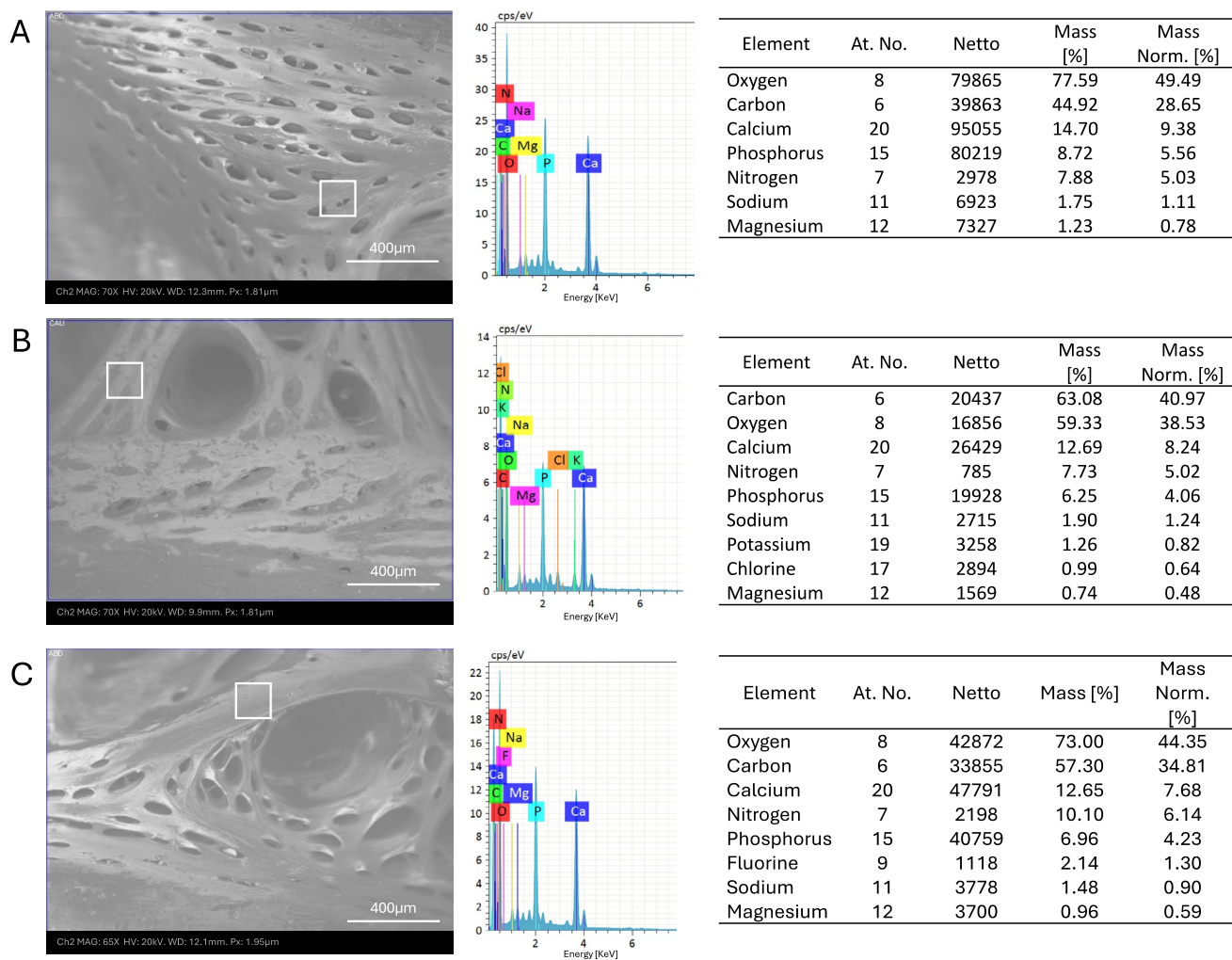
**Fig. 6** SEM and Elemental Microanalysis EDX in **A** abdominal, **B** transicional and **C** caudal vertebra of Common carp (*Cyprinus carpio*; Linnaeus, 1758)

**Table 1** Semi-quantitative elemental microanalysis in Common carp vertebrae (*Cyprinus carpio*; Linnaeus, 1758) by SEM–EDX

| Mass per cent (% W) | Abdominal | | | | Transition | | | | Caudal | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | DE | min | max | Mean | DE | min | max | Mean | DE | min | max |
| Oxygen | 46.3 | 2.8 | 44.4 | 49.5 | 36.5 | 1.8 | 35.2 | 38.5 | 35.0 | 1.2 | 34.18 | 35.86 |
| Carbon | 32.0 | 3.1 | 28.7 | 34.8 | 42.2 | 3.6 | 39.3 | 46.2 | 41.2 | 2.6 | 39.31 | 43.03 |
| Calcium | 6.1 | 4.3 | 1.3 | 9.4 | 7.7 | 2.7 | 4.8 | 10.1 | 9.5 | 0.9 | 8.87 | 10.08 |
| Phosforus | 3.6 | 2.4 | 0.9 | 5.6 | 3.5 | 1.1 | 2.3 | 4.3 | 4.3 | 0.1 | 4.2 | 4.33 |
| Nitrogen | 8.7 | 5.0 | 5.0 | 14.3 | 7.0 | 1.7 | 5.0 | 8.0 | 7.2 | 1.1 | 6.4 | 7.98 |
| Sodium | 0.9 | 0.2 | 0.7 | 1.1 | 1.1 | 0.2 | 0.9 | 1.2 | 1.2 | 0.2 | 1.11 | 1.34 |
| Magnesium | 0.7 | 0.1 | 0.6 | 0.8 | 0.3 | 0.1 | 0.2 | 0.5 | | | | |
| Silicon | 0.8 | | 0.8 | 0.8 | | | | | | | | |
| Aluminium | 0.5 | | 0.5 | 0.5 | | | | | | | | |
| Fluor | 1.3 | | 1.3 | 1.3 | 0.5 | 0.3 | 0.2 | 0.7 | | | | |
| Potasium | | | | | 0.8 | 0.1 | 0.7 | 0.8 | 0.9 | 0.3 | 0.73 | 1.12 |
| Chlorine | | | | | 0.6 | | 0.6 | 0.6 | 0.6 | 0.4 | 0.25 | 0.86 |
| Sulfur | | | | | 0.5 | | 0.5 | 0.5 | | | | |

2022; Barak et al. 2024). In Common carp vertebrae, the trabecular bone exhibits a complex network of interconnected rods and plates that contribute to the overall mechanical strength and flexibility of the spine (Fig. 2). This structural arrangement allows for efficient load transfer and energy dissipation during movements, thereby reducing the risk of structural damage (Fiedler et al. 2019, 2021). The bone marrow spaces, consistent throughout the spine, vary in size and shape, potentially reflecting functional differences in hematopoiesis. The trabecular architecture of the vertebrae in Common carp reflects a specific adaptation of its skeleton for biomechanical function and structural integrity, as demonstrated by the lacunarity analysis in trabecular bone tissue (Dougherty and Henebry 2002). Furthermore, the close relationship between form and function of trabecular bone underscores the relevance of this structure in resistance and load management (Smit et al. 1997). Previous studies have elucidated how mechanical forces are essential for adapting and maintaining the structure of trabecular bone, potentially reflecting the tissue's ability to adjust to varying levels of stress and preserve its functionality (Huiskes et al. 2000). The variability in bone marrow spaces along the spine, indicating differences in hematopoiesis, adds to the complex functionality of the vertebrae, maintaining both structural strength and the metabolic and hematopoietic capacities necessary for the organism.

However, the robustness of the vertebral bodies and the prominence of the neural and hemal arches differ among segments (Fig. 3). The abdominal vertebrae are notable for their greater robustness and more pronounced arches compared to the transitional and caudal vertebrae. Additionally, there is significant variability in the length and angulation of the spinous processes, particularly elongated in the caudal vertebrae. This pattern may be related to their role in supporting the fins and participating in caudal propulsion. Barak et al. (2024) analyzed the morphology of the vertebrae of the grass carp, providing data that can be extrapolated to understand the vertebral structure in the Common carp. They report that the vertebral bodies exhibit an hourglass shape and asymmetry between the cranial and caudal ends, with the caudal end being more robust, suggesting a specific adaptation to better withstand the forces generated during active swimming and caudal propulsion. In the context of the Common carp, this pattern of increased robustness in the caudal vertebrae may be related to their support function for the caudal fins and their critical role in propulsion during swimming. The more robust vertebrae, with elongated and angled spinous processes in the caudal region, would help manage mechanical stress during rapid tail movements, facilitating efficient and powerful swimming movements. The caudal vertebrae, while sharing the amphicoelous morphology, are distinguished by their greater articular complexity and the prominence of the spinous processes,

suggesting an evolutionary specialization linked to the biomechanical functions of the anal fins. Together, these observations reflect how the overall structural coherence of the vertebral column is modulated by adaptive variations in each segment, reaffirming the divergent evolution of bone structures in response to specific mechanical demands within the axial skeleton (Moran et al. 2016). Radiographic findings also indicated the presence of vertebral compressions and fusions in mild degrees, implying alterations in the intervertebral space rather than damage to the vertebral bodies per se. These anomalies suggest that altered mechanical load could cause the replacement of intervertebral tissue by cartilaginous tissues, resulting in vertebral compression and fusion. It remains unclear whether intervertebral tissue damage in wild conditions could be due to elevated biomechanical demands or excessive mechanical stress (Witten et al. 2005).

## Micro-CT analysis of trabecular architecture and bone resorption

In this study, we analyzed the intricate 3D internal structure of vertebral bodies from specimens using micro-CT scans (Figs. 2 and 3), identifying two common structural units: lamellar trabeculae and internal hollow spaces, which have been previously described in teleosts (Barak et al. 2024). Nordvik et al. (2005) observed that in Atlantic salmon, trabeculae in the lateral portion of each vertebral body radiate and branch from the center, with densely assembled osteoblasts covering the tips. They hypothesized that vertebral bodies grow outward by selectively adding bone at the trabeculae ends. Our findings confirm that such trabecular structures are typical in Common carp vertebrae. While some fish species exhibit internal hollow spaces, these do not depend on the arrangement of laminar trabeculae; rather, their presence varies among species and even among closely related species (Atkins et al. 2015). The determinants of these hollow spaces remain unclear and warrant further investigation. However, their presence in Common carp vertebral bodies can be a useful parameter for species identification. Notably, the walls of these hollow spaces often display an alveolar pattern due to multiple Howship lacunae, indicating osteoclastic activity (Francillon-Vieillot et al. 1990). The prevalence of trabeculae in the abdominal vertebrae suggests bone erosion at the vertebral center, as depicted in volume-rendered computed tomography images (Fig. 2C). This erosion uncovers a radiated bone architecture around the spinal canal, highlighting its mechanical properties. Our study implies ongoing vertebral bone turnover in the Common carp, with resorption predominating, a phenomenon that further studies should confirm. This dominance of bone resorption might result from accelerated

osteoclastic processes or a reduction in basic bone formation, as described in adult eels (López & Martelly-Bagot 1971; López 1973). Additionally, Sakashita et al. (2019) suggest that bone tissue adapted to withstand compression and tension is preferable, particularly in bones with a higher trabeculae proportion, like those in abdominal vertebrae. The higher surface-volume ratio of trabecular bone allows for extensive interaction with surrounding tissues, enhancing biomechanical responses such as increased bone remodeling and strength, observed in our histological analysis. The substantial presence of trabeculae also enhances the bone's capacity to distribute forces evenly, reducing the likelihood of fractures (Baxter et al. 2022; Sakashita et al. 2019). This trabecular architecture functions effectively under mechanical stress, distributing forces throughout the bone structure and providing a superior adaptation to diverse environmental conditions.

## Collagen in vertebra

Type I collagen, predominantly found in the extracellular matrix of the vertebral bodies studied, plays a crucial role in maintaining spinal rigidity in wild fish, while its colocalization with non-aligned type II collagen adds viscoelasticity, essential for preserving flexibility and range of motion during swimming (Fig. 5). This collagen not only reinforces the spinal column by distributing compressive forces and resisting tension but also facilitates bone mineralization by serving as a substrate for calcium salt deposition, thereby increasing spinal rigidity (Yamada et al. 2021). The composition and proportion of collagen types in Common carp vertebrae have significant implications for both fish ecology and their nutritional value as a supplementary food source for humans and other animals. The presence of a higher proportion of aligned collagen in the vertebral centrum suggests that this area is well-suited to withstand bending and torsional forces commonly experienced during swimming and propulsion, enhancing the stability and damage resistance of the Common carp's vertebral column, thereby maintaining its swimming performance (Donatelli et al. 2021). Furthermore, since aligned collagen is involved in regulating bone mineralization (Yamada et al. 2021), it may indicate that the centrum has enhanced mineralization capacity, potentially leading to a more rigid and mineralized bone structure. However, the precise morphofunctional implications of aligned collagen concentration and proportion in the centrum of Common carp remain unclear, as they cannot be directly extrapolated from other species due to potential variations among species and environmental conditions. Thus, further research is necessary to fully understand these effects.

## Elemental composition and bone health in Common carp via EDX

Our study utilized, for the first time, an EDX detector to assess elemental composition in the bones of a wild fish, showcasing its significant potential in investigating bone mineralization and chemical makeup. The EDX facilitated a precise and non-destructive evaluation of elemental distribution within the vertebral body tissue, including essential elements such as calcium, phosphorus, magnesium, and trace elements. The most prevalent elements identified were oxygen, carbon, and calcium, crucial for the structural and functional integrity of bones. Oxygen and carbon are primary components of organic tissue, whereas calcium plays a vital role in bone mineralization. Variations in the concentrations of these elements across different vertebral segments might indicate local adaptations to the specific biomechanical demands of each region (Lall & Kaushik 2021). Additionally, phosphorus, nitrogen, and magnesium are essential for key biological processes. Phosphorus is a critical component of bone tissue and central to bone matrix construction and calcium regulation. Nitrogen suggests the presence of organic compounds such as proteins and nucleic acids, essential for tissue growth and repair. While present in smaller quantities, magnesium is crucial for numerous enzymatic reactions and the metabolism of calcium and phosphorus (Kim & Jung 2007). The analysis also detected traces of other elements like sodium, potassium, and chlorine, important for osmotic regulation and neuromuscular function. The presence of aluminum and silicon, albeit in minor amounts, could relate to the ingestion of sediments or particles from the fish's natural habitat, as these elements are common in aquatic and terrestrial environments (Wetzel & Likens 2000).

These findings are particularly crucial as they establish a baseline for future research aimed at understanding the relationship between nutrition, bone mineralization, and the health of the Common carp, as well as identifying patterns and factors influencing bone mineralization and health. Calcium, detected in the vertebrae of Common carp, is primarily sourced from its environment, such as the water and food it consumes. The presence of calcium and phosphorus was identified in the Common carp vertebrae, with a relative ratio of approximately 2:1, indicative of typical mineralization of mature and functional bone in areas critical for the structural integrity and biomechanics of the vertebrae. This is consistent with findings by Drábiková et al. (2021), where phosphorus deficiency in the diet was linked to spinal deformities in Atlantic salmon, including areas of unmineralized bone in the vertebrae, highlighting the importance of phosphorus in proper bone mineralization. Nordvik et al. (2005) describe how preformed bone tissues in salmonids mineralize through specific processes, resulting in a complex

structural organization that supports specific biomechanical functionalities. This includes the formation of different layers in the vertebral structure, where the inner layers have an orderly laminar collagen matrix similar to that observed in this study, crucial for structural integrity, while the outer layers are made of spongy bone with a more woven matrix, also playing a role in impact absorption and structural support. A deficiency in calcium or phosphorus can lead to bone weakness, osteoporosis, and bone deformities (Cotti et al. 2020), which can impact the fish's ability to swim, feed, and reproduce. Based on our results, we did not observe skeletal alterations in any of the specimens studied. Therefore, we can infer that while the appropriate (or normal) proportion of calcium and phosphorus is unknown in the Common carp, our data could be considered a baseline for further studies. We found no trace elements such as iron and zinc in the vertebral bone tissue.

EDX adds a practical dimension crucial for understanding bone health in widespread and wild fish species like the Common carp. Since bone growth and mineralization are inherently linked to the availability and absorption of essential microelements such as calcium and phosphorus, this analysis provides a solid foundation for assessing environmental impacts on the skeletal integrity of these fish. By identifying specific concentrations of these elements in the vertebrae, it is possible to correlate the quality of aquatic habitats with the bone health of carp populations, offering an invaluable tool for conservation efforts and fisheries management. This approach not only enhances our understanding of the biological processes affecting the Common carp but also sets a precedent for similar studies in other species of wild fish.

## Limitations of the study

Research efforts have often been impeded by the destruction of anatomical samples from dissections of wild species, such as the Common carp. To address this, micro-CT has been adopted as a non-invasive, non-destructive method to study vertebral anatomy. The 3D reconstruction from micro-CT scan proved invaluable for visualizing the spatial configuration of vertebral trabecular tissue and has enhanced the sharing of species information in digital libraries and museums. However, a notable limitation of 3D reconstruction is the poor visibility of certain structures, complicating the delineation of some vertebrae and the identification of specific arches, spines, and trabeculae due to image shading. This study confirms that the morphology of Common Carp vertebrae aligns with the species' ecology and phylogeny, allowing comparisons with similarly habituated and closely related animals. The use of radiographic and tomographic imaging for observing complete skeletons was found to be complementary,

suggesting that the findings could significantly support further research and serve as a valuable reference for identifying wild fish. A further limitation of our study revolves around the lack of detailed information regarding the size and number of fish used, an inherent challenge when dealing with wild specimens such as the Common carp. We addressed this limitation by utilizing measures of approximate length (around 36 cm), which provides a reliable indicator of the developmental stage and maturity of the species, as evidenced by similar methodologies in ecological and fish biology research (Johnson et al., 2005). Although the sample size was limited to six fish, this number aligns with the standards for preliminary and exploratory studies in vertebrate biology, where larger sample sizes may not be feasible due to conservation concerns and ethical considerations (Smith & Brown, 2010). To mitigate the impact of this limitation on our findings, we employed rigorous statistical methods to ensure that our results are robust and reflective of broader patterns within the population, thus providing valuable insights despite the sample size constraints. Additionally, future studies could leverage collaborative efforts with fisheries and wildlife agencies to access a broader range of specimens, thereby enhancing the generalizability of the research outcomes.

In *conclusion*, the morphological study of the vertebrae of the Common carp using micro-CT and EDX has revealed a complex and heterogeneous vertebral structure, characterized by a series of lamellar trabeculae and internal hollow spaces. These structural features are consistent across the studied vertebral regions, with each vertebra displaying a distinctive amphicoelous body that reflects its primary structural function. Histologically, an internal architecture was observed, which includes radially oriented bone trabeculae around a central core, providing a spongy texture crucial for the biomechanical functionality of the spine. Furthermore, a predominant pattern of aligned type I collagen was identified, essential for vertebral rigidity and bone mineralization, thereby facilitating the deposition of calcium salts that increase vertebral stiffness. Analysis using SEM and EDX allowed for a precise and non-destructive evaluation of the elemental composition in the bone tissues of the vertebrae, revealing the presence of essential elements such as calcium, phosphorus, magnesium, as well as other trace elements. These elements play a vital role in the structural and functional integrity of the bones, indicating adequate mineralization without signs of active bone pathologies. This in-depth analysis of vertebral morphology and elemental composition provides a detailed understanding of the bone structure in the Common carp, highlighting the sophistication of its vertebral anatomy.

## Declarations

**Conflict of interest** The authors declare no competing interests.

**Ethical approval** Approval by research ethics committees was not required to achieve the objectives. Skeleton care and handling were carried out in accordance with Chilean Law 20.380.

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