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New insights into *Tibetodus gyrodoides* Young & Liu, 1954 (Actinopterygii, Pycnodontiformes) from the Qinghai-Xizang Plateau based on micro-CT data

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Abstract

The Pycnodontiformes is a distinctive group of fossil actinopterygian (ray-finned) fishes from the Late Triassic to Palaeogene. *Tibetodus gyrodoides*, reported in 1954, represents the only known fossil record of this group in China, holding significant scientific and historical importance. In this study, non-destructive techniques such as scanning electron microscopy (SEM) and micro-computed tomography (micro-CT) were utilised to reexamine the specimen, providing new insights into both the surface morphology and internal structures of the vomer and its teeth and investigating the process of tooth wear. A tooth covered by matrix was also discovered. Furthermore, an Elliptical Fourier Analysis (EFA) was conducted to quantitatively analyse the morphology of the palatal teeth in this specimen, adding new data for future research on pycnodontiforms and their feeding behavior.

Keywords: Pycnodontiformes, Qinghai-Xizang Plateau, micro-CT, tooth wear, EFA, morphospace

Introduction

The Pycnodontiformes is an extinct group of neopterygian fishes, characterised by specialized crushing dentition (Poyato-Ariza & Wenz, 2002; Kriwet, 2005; Poyato-Ariza, 2015). Given the ecological role of their prey items, they represent a major part of the marine actinopterygian (ray-finned fishes) diversity from the Late Triassic to Palaeogene (Cawley *et al*., 2021). Their fossil record extends back to the Late Triassic, with the peak diversity occurring during the Jurassic and Cretaceous periods, continuing into the middle Eocene (Cawley *et al*., 2021). More than 650 species of pycnodontiforms (pyconodont fishes) have been described to date, with fossils discovered worldwide, predominantly in shallow marine deposits, though they have also been found in freshwater sediments (Kriwet, 2005; Cavin, 2020). However, due to the reduced distribution of Jurassic and Cretaceous marine deposits in China, only one pycnodont specimen has been identified in China, *Tibetodus gyrodoides* Young & Liu, 1954 (Chang & Miao, 2004).

The specimen of *T. gyrodoides* was found in the Changdu area of the Xizang Autonomous Region, China (Fig. 1). It was reported in Chinese with English abstract by researchers from the Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences (IVPP, CAS) in 1954 (Young & Liu, 1954). This discovery fills a gap in the pycnodontiform fossil record in China and is the first vertebrate fossil from Xizang independently studied and published by the Chinese scientific community, holding significant scientific and historical importance.

In the original paper, the authors provided a line drawing of the specimen and described the morphology and arrangement of the vomerine dentition exposed on the surface of the matrix, along with preliminary morphometric measurements (Young & Liu, 1954). Seventy years later, fossil imaging, non-destructive testing, and data analysis techniques have advanced significantly, making it possible to extend the information provided in the original study with conventional methods. Therefore, this research revisited the specimen of *T. gyrodoides* by using scanning electron microscopy (SEM) and micro-CT to investigate its external morphology and internal structure. Additionally, geometric morphometric analyses were employed to conduct a more detailed quantitative analysis of the dentition morphology. Through these modern techniques, this research aims to uncover more

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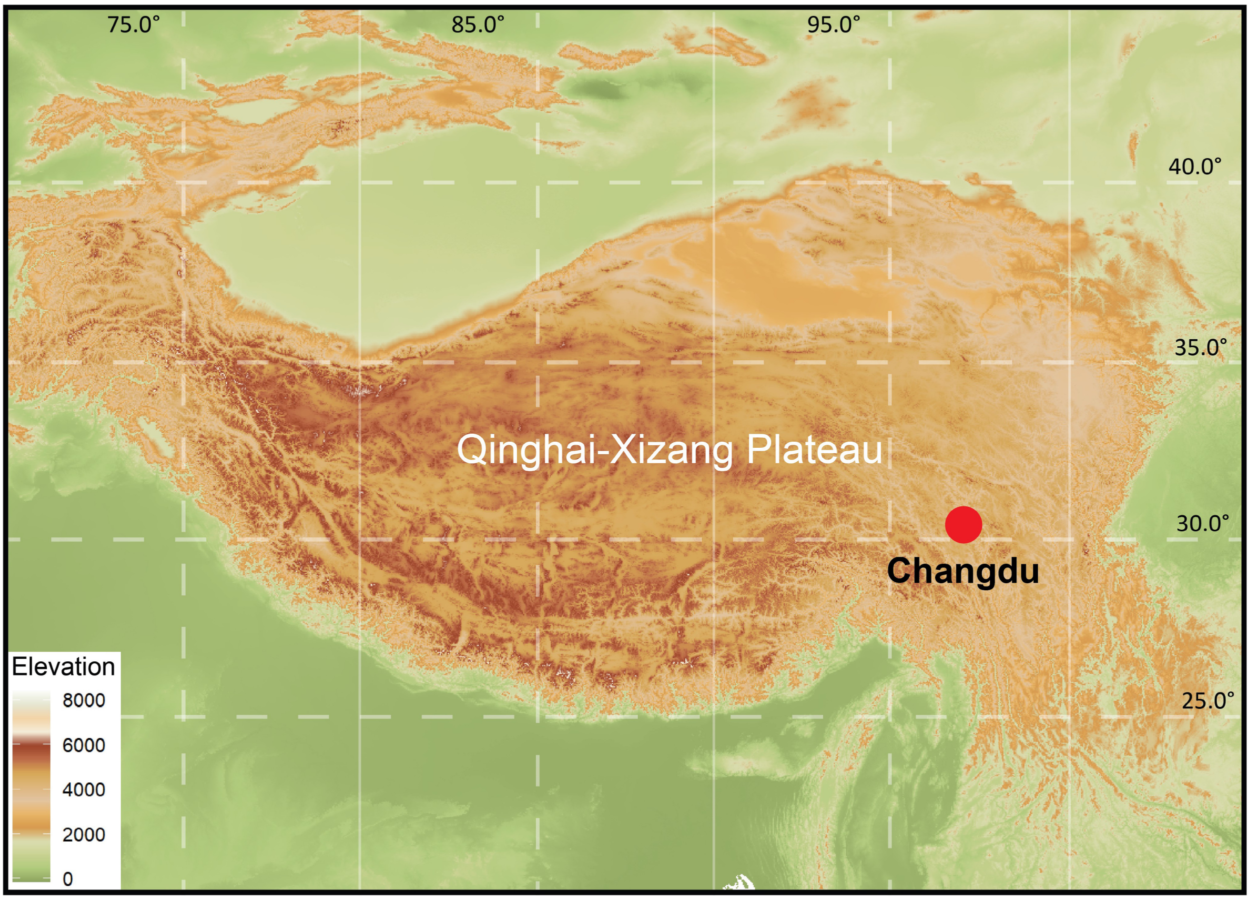

FIGURE 1. A Digital Elevation Model (DEM) map of the Qinghai-Xizang Plateau. The red dot indicates Changdu City, Xizang Autonomous Region, China, where the fossil of *Tibetodus gyrodoides* was found.

information about *T. gyrodoides* and contribute new data for future, more comprehensive studies on pycnodontiform fishes.

Material and methods

The only specimen of *T. gyrodoides* was discovered in Changdu area of the Xizang Autonomous Region, China. It was collected by a local resident near a temple, though the exact location and the stratigraphic horizon are unclear. However, based on the lithology of the matrix and surrounding geological data, the specimen is inferred to originate from the Upper Jurassic to Lower Cretaceous strata (Young & Liu, 1954; Li, 1955). In 1952, the specimen was donated to Dr Pu Li of the Chinese Academy of Sciences, the leader of the comprehensive scientific expedition to Xizang (Tibet) launched in 1951, and later, in 1954, it was studied and published by Dr Chung-Chien Young and Hsien-Ting Liu of IVPP. The specimen is deposited in the specimen collection of IVPP under the catalogue number IVPP V718 (Young & Liu, 1954).

The specimen was examined and photographed at IVPP with a Nikon D850 and Nikkor 105 mm macro lens, a Nikon SMZ1500 binocular and a Hitachi S-3700N Scanning Electron Microscope. The images were processed using Adobe Photoshop 2024 for focus stacking and stitching.

Micro-CT scanning was used to image the specimen by the GE v|tome|x m300&180 at IVPP. Scan parameters: 160 kV, 140 µA, with a voxel size of 16.18 µm. Segmentation, virtual sections and rendering of the tomography slices were performed using VGSTUDIO MAX 2022.2 at the University of Bristol.

Geometric morphometric analyses were employed to compare the morphological differences in the vomerine dentition quantitatively. The occlusal views of 36 complete vomerine teeth from the specimen were rendered using VGSTUDIO MAX 2022.2. The shapes of occlusal surfaces were extracted using Adobe Illustrator 2024 and converted into binary images. The outlines were extracted and analysed using the Momocs package in R, and then the results were visualised (Ihaka & Gentleman, 1996; Bonhomme *et al*., 2014). All outlines were aligned for size and centroid using Generalized Procrustes

Analysis (GPA), but rotational alignment was omitted to preserve the directional information of the dentition. Elliptic Fourier Analysis (EFA) was then performed to compare all dentition shapes quantitatively. A total of 26 harmonics were considered, capturing over 99% of the cumulative harmonic power, which measures the shape information and allows for accurate reconstruction of actual morphologies. A virtual morphospace was generated by Principal Component Analysis (PCA) on the preordination data to visualise the main shape variations.

Systematic palaeontology

Order Pycnodontiformes
Family Cyrodontidae
Genus *Tibetodus* Young & Liu, 1954

***Tibetodus gyrodoides* Young & Liu, 1954**

Material. IVPP V718 (Holotype), a nearly complete vomer, with dentition exposed on the matrix surface.

Remarks. The vomer of *T. gyrodoides* bears five longitudinal rows of teeth, with the central axes of each row aligned in a relatively straight line, exhibiting an organized arrangement (Fig. 2). The main tooth row is developed along the midline of the vomer, symmetrically flanked by two lateral tooth rows on each side. No accessory tooth develops in between tooth rows. The number of teeth preserved in each row is as follows: 8, 9, 7, 9, 8. The vomerine dentition forms a pavement-like structure. Most teeth are not in close contact with each other, and the teeth in adjacent rows are alternatively arranged to maximise surface coverage within a limited area. The lateral rows incline outward from anterior to posterior, forming an angle of approximately 22° between the two outermost lateral tooth rows. In cross-section, the tooth arrangement forms a curved arc (Fig. 3A). The main tooth row is the most prominent, projecting highest above the vomer surface, while the lateral rows gradually incline outward. In addition to the five exposed rows of teeth on the surface, micro-CT scans reveal a tooth buried within the matrix (AD in Fig. 2C, D). This tooth is located between the anterior end of the main tooth row and the left lateral row 1, smaller than the other preserved vomerine teeth and irregular in shape.

The virtual sections indicate that parts of the vomer are preserved within the matrix, but due to the effects of recrystallisation, the morphology of the vomer is difficult to distinguish (Fig. 3A, B). The cross-section of the tooth crowns in the vomerine dentition is nearly an elongated oval, slightly expanded on lateral sides. The tooth roots extend deep into the vomer, with thin walls surrounding a large undivided pulp cavity. No replacement teeth were observed at the base of the pulp cavity (Fig. 3A), as seen in another pycnodont fish (Matsui & Kimura, 2022).

The occlusal surfaces of the vomerine teeth exhibit ornamentation, with the lateral row showing clearer ornamentation than the main row in this specimen and the posterior vomerine teeth displaying more distinct ornamentation than the anterior ones. The last vomerine teeth in the lateral rows exhibit the most distinct ornamentation, featuring an oval central pit encircled by irregular, radiating canals (Fig. 3F).

Within the same tooth row, a gradual change in ornamentation can be observed; when comparing from posterior to anterior (Fig. 3C–F), it becomes evident that the radiating grooves are gradually shallow first (Fig. 3E), leaving only the central pit on the occlusal surface, which also progressively decreases in size (Fig. 3D). Eventually, the pit disappears altogether, leaving the occlusal surface completely smooth (Fig. 3C), with shallow depression occasionally appearing (Fig. 2B, white arrows). Virtual sections of the same row also reveal that the posterior teeth have slightly raised teeth crowns, while the anterior crowns gradually flatten (Fig. 3B). This phenomenon is related to the tooth replacement mode and the usage pattern in pycnodonts (Kriwet, 2005; Poyato-Ariza & Wenz, 2005). During the developmental process, new vomerine teeth are added posteriorly to replace older teeth, meaning that the anterior teeth are older and have undergone longer periods of abrasion (Poyato-Ariza & Wenz, 2005). Interestingly, the external ornamentation of the lateral row teeth tends to disappear first, which may be linked to the occlusal relationship with the prearticular dentition (Kriwet, 2005). As only four teeth were preserved in the main tooth row, it is unclear whether the weaker ornamentation of the central teeth is inherent or the result of more extensive abrasion.

The morphology of vomerine teeth in pycnodont fishes is an important diagnostic feature (Poyato-Ariza, 2020). The five rows of vomerine teeth in *T. gyrodoides* exhibit varying morphologies, displaying heterodonty. The vomerine teeth in the main row, located along the midline, are the largest and relatively uniform in shape, displaying a flattened oval form. The teeth in the lateral rows are smaller than those in the main row, with the outermost two rows being smaller than the inner two, and their shapes are more irregular. Upon examining the line drawings in the original publication and specimen photographs, the outer edges of the lateral vomerine teeth appear to be straight. However, this impression is an artefact of projection effects and the fact that the outer edges remain partially embedded in the matrix. In this study, all vomerine teeth were adjusted to the occlusal view, revealing that the outer edges of the lateral rows still protrude outward to varying degrees (Fig. 2D).

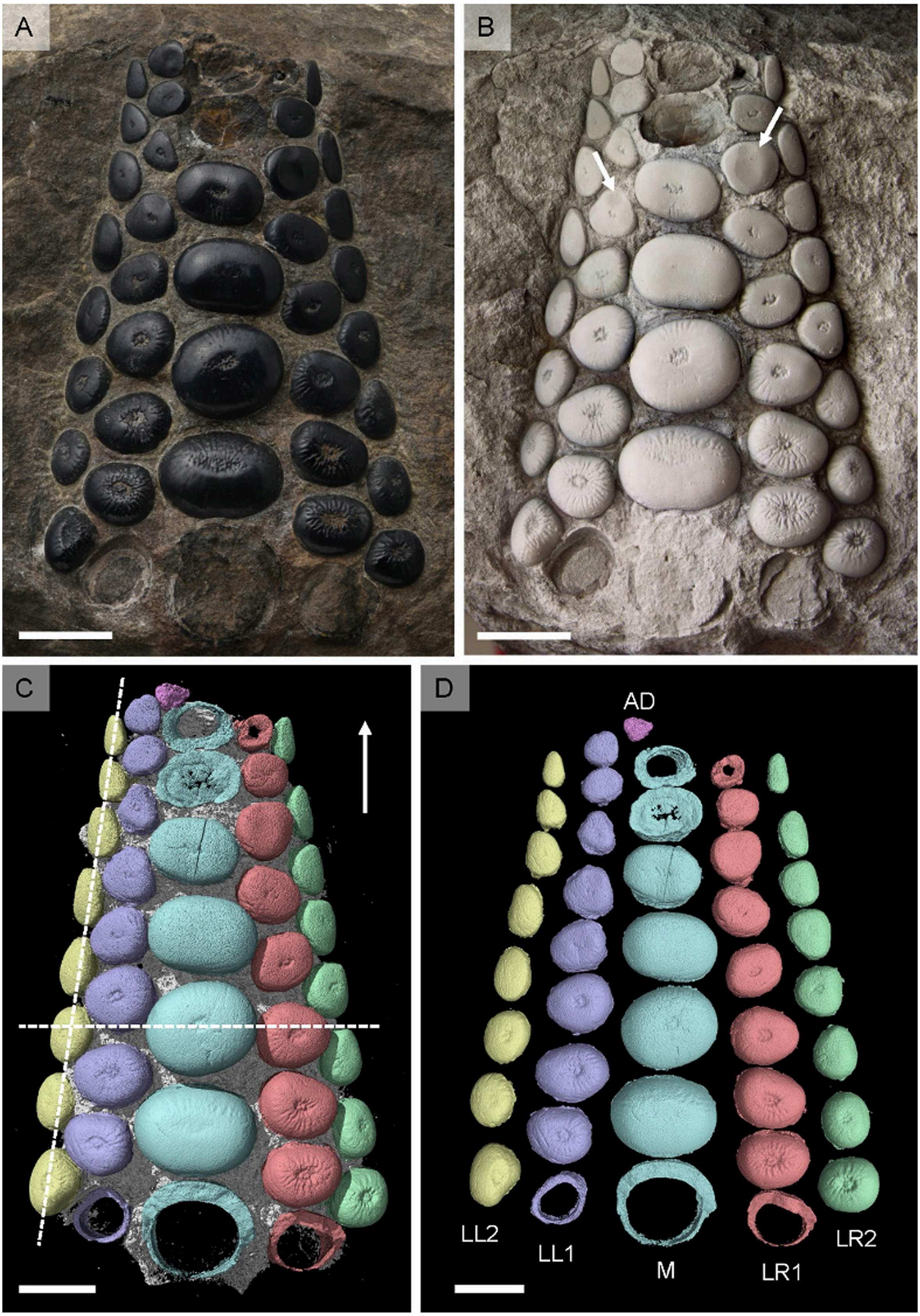

FIGURE 2. *Tibetodus gyrodoides* Young & Liu, 1954 (IVPP V 718). **A**, Vomerine teeth exposed on the surface of the matrix. **B**, Specimen dusted with ammonium chloride. **C**, Reconstruction of the specimen's micro-CT scan data, with the white areas representing the remains of the vomer. **D**, Occlusal surface views of all vomerine teeth. The white arrow in **C** points anteriorly. The white arrows in **B** indicate the smooth depression on the occlusal surface. White dashes lines in **C** indicate the virtual sections in Fig. 3. Abbreviations: AD, anterior dentition; M, main row; LL, left lateral row; LR, right lateral row. Scale bars = 5 mm.

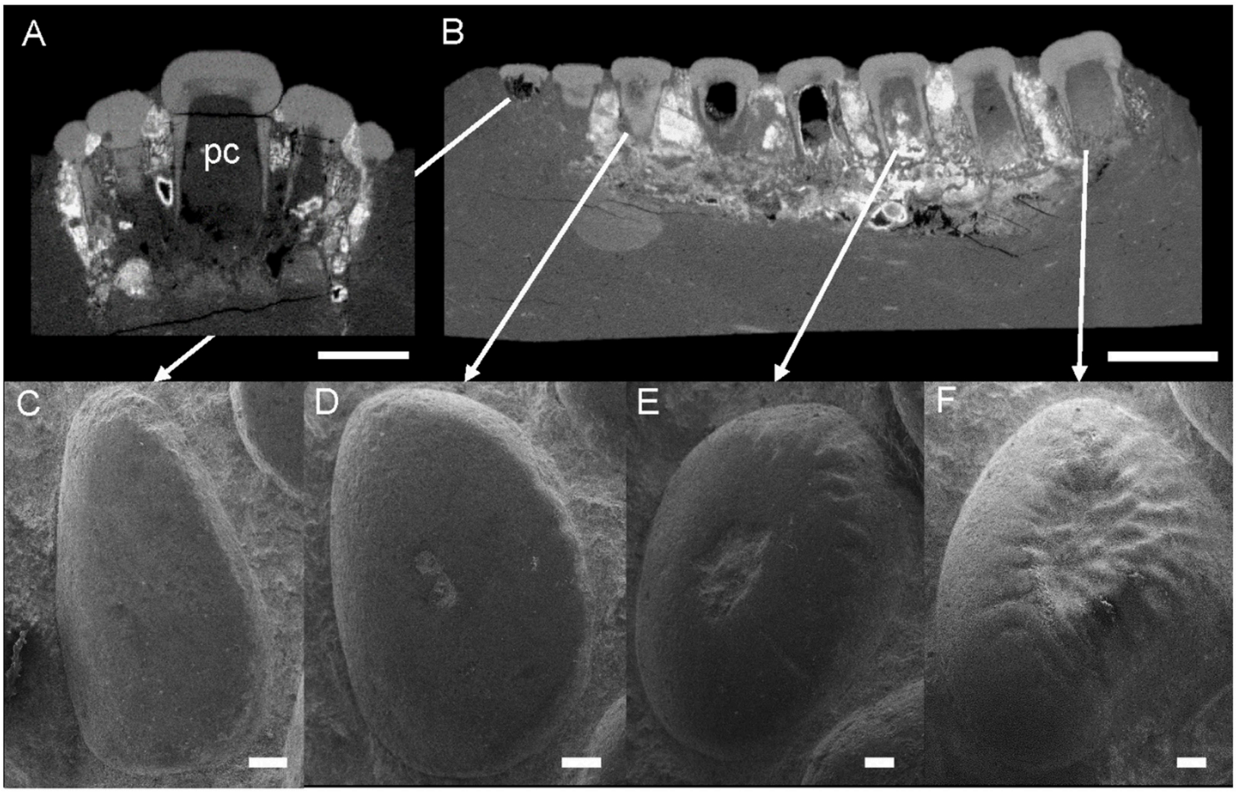

FIGURE 3. Virtual sections and SEM images of *Tibetodus gyrodoides* Young & Liu, 1954 (IVPP V 718). **A**, Cross direction. **B**, Longitudinal section of left lateral row 1. **C**–**F**, SEM images of vomerine dentition in the left lateral row 1, exhibit increasing abrasion of tooth ornamentation. White arrows indicate the position of the single teeth. Abbreviation: pc, pulp cavity. Scale bars = 5 mm in **A**, **B**, 200 µm in **C**–**F**.

In prior research on pycnodonts, the quantitative study of vomerine tooth shapes typically involves measuring the width and length axes and calculating their ratios (Kriwet, 2005; Poyato-Ariza, 2020). However, this method can introduce subjective bias for irregularly shaped teeth and may capture only a limited portion of the overall shape variation. To address this limitation, the present study applied Elliptical Fourier Analysis (EFA), allowing for a more comprehensive and objective tooth shape analysis. EFA decomposes the two-dimensional outline of a shape into a series of elliptical harmonics, each defined by four parameters that describe the major and minor axes of the ellipse and its orientation (Deakin *et al*., 2022). Combining EFA with statistical techniques like Generalized Procrustes Analysis (GPA) enables quantitative comparisons of tooth shapes, the construction of a morphospace, and the visualisation of results.

The shape analysis of the vomerine teeth in *T. gyrodoides* revealed that teeth from different rows occupy distinct regions in the morphospace (Fig. 4). The primary variation is in the length-to-width ratio, followed by the orientation of the outlines. The main tooth row shows minimal overlap with other rows, while the two pairs of lateral rows are symmetrically distributed along the PC1 axis. Theoretical morphology (grey outlines in the background) reveals that their shapes exhibit symmetry along the midline axis.

Discussion

The results of the micro-CT scan revealed more information that is not visible on the surface of the specimen. It is more evident that both the anterior and posterior ends of the vomer are incomplete. The newly discovered smaller tooth at the anterior end does not fit into any of the established rows, for which there are two possible explanations: The first is that during the early developmental stages of the vomer in pycnodont fishes, the teeth were arranged irregularly (Poyato-Ariza, 2020). The second possibility is the unique tooth replacement mode in certain pycnodonts, where multiple smaller teeth replace a single damaged or worn old tooth (Longbottom, 1984; Nursall & Maisey 1991; Collins & Underwood, 2021). However, due to the damage observed in the anteriormost part of the vomer, it is difficult to determine which scenario is applicable.

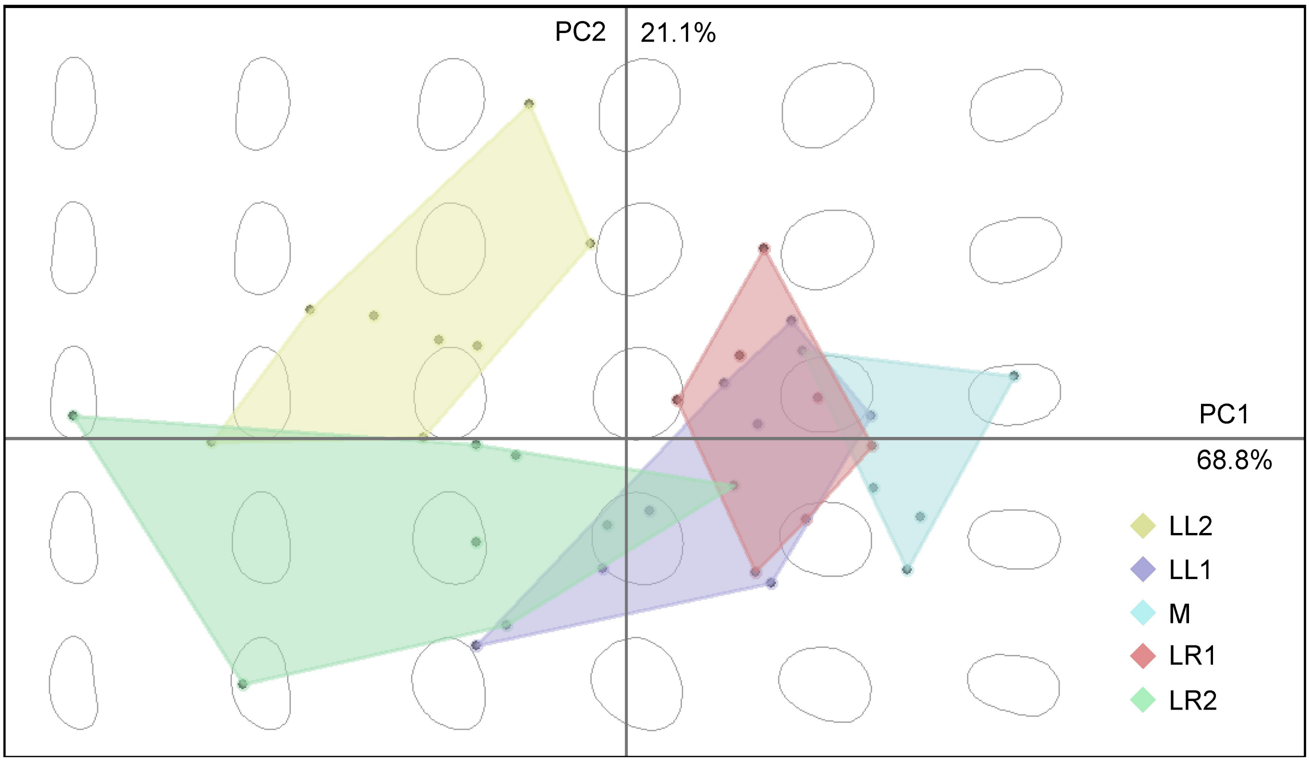

FIGURE 4. Empirical and theoretical morphospace of the vomerine dentition. Theoretical morphospace (grey outlines) with overlain empirical data of the shape of the vomerine dentition grouped in the different rows (coloured symbols and convex hulls).

The EFA method used in this study does not rely on landmarks, making it suitable for analysing tooth outlines that lack identifiable homologous anatomical markers, such as the teeth of pycnodonts. However, it is important to note that before performing EFA, Generalized Procrustes Analysis (GPA) is usually required to eliminate differences in size, position, and rotation. In the process of eliminating rotational differences, some spatial orientation information is lost, which is meaningful when comparing the shapes of teeth developed on the same bone. Therefore, no rotational alignment was performed in this study.

Among the more than 650 pycnodontiform species reported to date, over 70% only consist of teeth (Kriwet, 2005). Therefore, the study of the vast amount of dental morphological data is of great significance for understanding the ecology and evolution of pycnodont fishes. Employing EFA can effectively compress shape data, representing a complex shape using a limited set of parameters, allowing for quantitative comparisons, and can be used to assess the validity of prior biometric characteristics (Kriwet, 2005; Poyato-Ariza, 2020). This study represents an initial attempt at such quantitative analysis. Furthermore, EFA only captures two-dimensional morphological differences, but the three-dimensional morphology of pycnodonts' dentition also has crucial ecological significance. However, the micro-CT and scanning electron microscope (SEM) results in this study showed that the tooth surfaces have undergone significant wear, which will introduce substantial confounding factors in the quantitative comparison of three-dimensional morphology (Fischer *et al*., 2022).

The greatest limitation of the *T. gyrodoides* study is the unclear provenance and stratigraphic data of the fossil. Seventy years have passed, and all the participants in the original research have since passed away. Verifying the fossil locality mentioned in the original paper is also difficult. The uncertainty about the geological age limits the significance of *T. gyrodoides* in studying the classification and evolution of pycnodonts. Nevertheless, the discovery of *T. gyrodoides* fossils highlights the potential of the Mesozoic strata in the Changdu region of Xizang, China, to preserve more fish fossils. This provides valuable clues and directions for future field excavation work.

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