The teeth and dentition of the filefish (Stephanolepis cirrhifer) revisited tomographically

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Abstract: The upper and lower tooth-bearing jaws of the filefish (Stephanolepis cirrhifer) were scanned using a micro-CT system in order to address the existing gaps between the traditional pictures of the morphology and histology. 2D tomograms, reconstructed 3D models and virtual dissection were employed to examine and evaluate the in situ geometry of tooth implantation and the mode of tooth attachment both separately and collectively. No distinct sockets comparable to those in mammals were evident, but shallow depressions were observed in the premaxillary and the dentary. The opening of the tooth pulp cavity was not simply oriented towards the apparent tooth base in a direction opposite to the tooth apex. The opening was distorted basoanteriorly or basoanterior edge of the opening appeared to closely match the contour of the shallow depression in the bone. These findings appear to suggest that micro-CT would be a useful modality concurrent with or in advance of histological investigations.

Keywords; alveolar sockets, ankylosis, fish teeth, 3D analysis, tooth attachment

Introduction

The thread-sail filefish, Stephanolepis cirrhifer (Japanese: “kawahagi”), is a well-known food fish widely served as “sashimi” in Japan. The filefish family (Monacanthidae) includes 102 species within 27 genera and has a close taxonomic relationship with the triggerfish family (Balistidae) [1]. These two sister families are therefore placed together within the suborder Balistoidei [2] in the Tetraodontiformes (Order#85; Nelson et al., Fishes of the World 5th ed., John Wiley & Sons, 2016).

In the mid-19th century, Sir Richard Owen published “Odontography” (Owen, A Treatise on the Comparative Anatomy of the Teeth, Hippolyte Bailliere Publisher, 1840-1845), and described in detail the teeth and dentition of the grey triggerfish (Balistes capriscus) [3]. Both Retzius and Owen made precise and valuable observations of the number, morphology and certain histologic details of the teeth and dentition of triggerfish. Among the observations of B. capriscus, the tooth attachment was notably interpreted as a double gomphosis. In fact, after examining Balistes bursa (synonym Sufflamen bursa) and two other balistids, Soule [3] reported that connective tissue comparable to the mammalian periodontal ligament connected the teeth and bone in shallow alveolar sockets. However, the presence of a periodontal ligament in triggerfish or other actinopterygians has not been commonly accepted (Berkovitz et al., The Teeth of Non-Mammalian Vertebrates, Elsevier, 2017).

With regard to filefish dentition in Japan, an early morphologic and histologic study of Monocanthus cirrhifer and Cantherines modestus (synonyms Stephanolepis cirrhifer and Thamnaconus modestus, respectively) was carried out by Sohiti Isokawa (Isokawa, Zool Mag 64, 194-197, 1955). Phylogenetic interrelationships in the balistoids were examined extensively by Matsuura [4], based on many anatomical characteristics including the tooth-bearing jaws, which were the premaxillary and the dentary. He showed that the balistoids and monacanthids were clearly separable by the number of teeth, and that a larger or smaller number of teeth in the balistoids was likely to represent a more primitive or an advanced systematic position, respectively; i.e. balistids for the former and monacanthids for the latter. A study of the upper jaw of S. cirrhifer at the ultrastructural level by Uehara & Miyoshi [5] made a significant contribution to histological knowledge of the tooth attachment. They demonstrated a fibrous attachment of the tooth to bone, but also stated: “The teeth appear to attach directly to the bone at the middle region of the labial and lingual components of the teeth”. These descriptions indicate that, despite accumulating observations, uncertainties still remain regarding the morphology and histology of the tooth-bone interface in filefish, partly arising from limitations in the morphological and histological methods employed, and also the historical, or conventional, definitions and usage of terminology such as gomphosis, periodontium, alveolar socket, fibrous attachment, etc.

Therefore, it appears important to employ a new methodological approach for addressing the existing gaps in the current traditional picture of the morphology and histology. Fortunately, this can now be undertaken very efficiently and reliably using the recently introduced modality of micro-CT. The three-dimensional visualization made possible by this technique also allows consideration of the in situ geometry of tooth implantation and the mode of tooth attachment both separately and collectively, which is significant because these two aspects of the tooth are not necessarily dependent on each other for supporting the tooth [6]. The present study utilized a micro-CT system to examine the jaw teeth and dentition (excluding pharyngeal teeth) of S. cirrhifer in order to compare the findings of tomography with those of previous morphological and histological studies, focusing also on the development (and successional replacement) of teeth and the occlusal relationship of the upper and lower teeth.

Materials and Methods

Specimens

Four thread-sail filefish (Stephanolepis cirrhifer), all approximately 24 cm in total length, were fixed with 10% buffered formalin and labeled serially as Sc#1, 2, 3 and 4 for this study. These fish, from Goto, Nagasaki, were obtained through a local distributor via the Metropolitan Central Wholesale Market (Tsukiji, Tokyo) with the generous help of Professor Noriaki Koshikawa (Nihon University School of Dentistry; NUSD). Processing of the four specimens was carried out in accordance with the guidelines of the Animal Experimentation Committee of NUSD, which comply with the National Welfare and Management of Animals Act.

Some of the histological specimens of the teeth of S. cirrhifer, which had been examined in the early 1950s by Sohiti Isokawa, were still stored at the Department of Anatomy of NUSD. These specimens were re-examined using a modern microscope, and several images from those sections are incorporated into figures and cited in the Discussion.
CT scanning and observations

The upper and lower tooth-bearing jaws were scanned using a micro-CT system (R_mCT, Rigaku, Tokyo, Japan) with an X-ray exposure of 2 min, 90 kV, 100 μA, at magnifications of ×2, ×4, and ×6.7. Volume data were resliced with I-View software (J. Morita, Kyoto, Japan) and exported to Mango (version 4.6.9; Medixant, Poznan, Poland). Another DICOM viewer, Mango (version 4.1; Research Imaging Institute, University of Texas Health Science Center, San Antonio, TX, USA), was used to generate 3D-surface VR images, which were useful for examining the occlusal relationship of the upper and lower teeth.

The “Scalpel” tool in the RadiAnt was used preferentially to extract a tooth from the 3D-VR image, or to remove tissues other than teeth to a considerable extent. This work was aided greatly by controlling the window level (WL) and window width (WW) and also made it possible to measure reliably the maximum mesiodistal width of a functional tooth (predecessor) and its successional tooth, since in doing so both measurements was done on their surfaces of interest in an identical 3D image generated in the RadiAnt.

Symbols for teeth and their positions

The teeth and their positions within the jaws were expressed with symbols: any tooth in the outer and inner rows of the upper dentition in the maxillary was expressed as “o” and “i”, respectively, and any bowl-shaped tooth in the lower dentition in the dentary as “b”, followed by a number of the filefish dentition. The o1-3 teeth were subtrihedral in shape. At the apex, the tooth was a solid trihedron (typically in o1, Fig. 2D-F) and all three surfaces were covered with highly calcified tissue, which appeared to be enameloid. Further away from the apex, a hollow cavity appeared (Figs. 2G-I, 4A-D), and this changed to a groove which opened lingually, becoming wider towards the tooth base (Fig. 2H).

Observation of upper jaw tomograms (Fig. 2D-K) yielded further details of the filefish dentition. The o1-3 teeth were subtriangular in shape. At the apex, the tooth was a solid trihedron (typically in o1, Fig. 2D-F) and all three surfaces were covered with highly calcified tissue, which appeared to be enameloid. Further away from the apex, a hollow cavity appeared (Figs. 2G-I, 4A-D), and this changed to a groove which opened lingually, becoming wider towards the tooth base (Fig. 2H).

Results

Ten teeth in total were observed in the upper jaw: 3 each (o1-3) in the right and left sides in the outer row of dentition, and 2 each (i1-2) in the right and left sides in the inner row. In the lower jaw, 6 teeth – 3 each (b1-3) on the right and left – were aligned in a single row (Fig. 1).

The lingual surface of o1-3 and b1-3 was basically a smooth rectangle with an obtuse apex (Fig. 1D-F). There was evidence of apparent wear at the apex of some mesial teeth. Teeth in a row were closely aligned with each other, and the mesial pair (o1 or b1) was larger than the others except for o3, which appeared wider than o1 when examined without mucosa (Figs. 1F, 2A).

The lingual surface of i1-2 was exposed more widely over the oral mucosa (Fig. 1B). The surface consisted of a rectangular part with a rounded edge at the base and a triangular part with an apex bent slightly towards the labial side (Fig. 2B, C). The rectangular part had a series of perikymata-like characteristic linear grooves. The apex of the triangular part in i1 and i2 projected towards gaps in o1-2 and o2-3, respectively, and thus the apical parts of the outer and inner rows of the upper teeth were intercalated, forming a continuous dental arch (Figs. 1B, 2A-C).

In the lower dentition, there was a lingual cingulum in each of b1-3, making the lingual surface of the teeth curved. The size of this shallow bowl-like concavity was largely proportional to the size of the “bowl” teeth themselves (Figs. 1C, 3B, C). An array of these bowl-like concavities immediately behind the labial apical edge appeared to aid durophagy in this fish, since the “bowl” was exposed entirely over the oral mucosa (Figs. 1C, 3C).

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Teeth showed a highly calcified plate-like morphology with an apical ridge (Figs. 2F-H, 4C, D), the latter giving rise to one central and two peripheral ridges facing the bone; the radiopacity of these ridges was lower than the plate-like part (Figs. 2I-K, 4D-F).

Tomograms of the lower jaw showed that the b1-3 teeth and their successors were trihedral. A solid apex and transition of the pulp cavity from tubular to groove-like were also observed in b1-3, and the enameloid covering (Fig. 3D-K) was basically similar to that in o1-3. In addition, the surfaces of the lingual bowl-like concavities in b1-3 and in their successors were covered with a radiopaque layer of enameloid (Figs. 3L-Q, 4H-J).

Tomograms at higher magnification revealed the link between functioning teeth and their supporting bone. The bone did not come close to the apices in any of the three types (o, i, b) of functional teeth. In the upper jaw, the o1-3 teeth and their successors were ankylosed to the supporting bone at the tooth base and/or at the basal one third in the ridges facing the bone (Fig. 4A, F). In the lower jaw, the b1-3 teeth were again slightly ankylosed to the bone at the tooth base (Fig. 4H-J). In contrast, no bony ankylosis was found in the developing successional teeth.

The successional teeth appeared to develop beneath the functioning predecessors. The erupted teeth and their successors were aligned almost coaxially, but the axis of o3-o3s was tilted considerably distally compared to those of o1-o1s and o2-o2s (Fig. 5A, B). The width of successional teeth was expressed as a ratio of the maximum mesiodistal width of the successor to that of its predecessor (Table 1).
measured in this study was approximately 10% larger than the corresponding predecessor teeth (Table 1), although the former might have developed further before their eruption.

The development of successional teeth was asynchronous. They varied in both size and tomographic signal intensity, and therefore close examination of multiple tomograms was necessary in order to trace the developing successors. In addition, some of the developing teeth detectable in tomosgrams could not be visualized in 3D-VR images (e.g. right i2s of Sc#3 in Figs. 5A, 6). In this series of four samples (Sc#1-4), only one tooth (left o1 in Sc#3) had been exfoliated among 64 functional teeth examined, and the number of successors evaluated as “calcified”, “detectable” and “undetectable (radiographically)” was 41, 7 and 16, respectively. The distribution of successors is illustrated in a recording chart in Fig. 6.

Observations of individual teeth extracted digitally from the 3D-VR images were photographed during re-examination of the specimens that had been prepared by Sohiti Isokawa in the early 1950s. In a section through the mesiodistal plane of the lower dentition (A), interdental bundles of collagen fibers (asterisks) and the site of ankylosis (boxed) between b2 and its supporting bone (bo) are shown. In a parasagittal section (B, labial side is oriented right, mu; labial mucosa), the bundles of collagen fibers connecting b1 and bo are shown. Boxed areas in A and B are enlarged in C and D, respectively. The arrowhead indicates the site of ankylosis between the dentin of b2 (d.b2) and bo, both of which are located directly next to predentin (pd). In d.b2, cells are entrapped in dentin, implying an odontodentin nature. The arrow indicates the transition of the odontoblastic cell layer (od) to osteoblasts in the surface of the tuberculated bo. Diamonds indicate dentin or its equivalents in the outer surfaces of the dentin. Sections are stained with hematoxylin-eosin (A-D). The image of a specimen stained by silver impregnation (E) is histologically comparable to D.

The occlusion of the upper and lower teeth was well delineated by 3D-surface VR images (Fig. 7). When the jaws were closed, the apex and the distal nipping edge of b1 were in close contact with the lingual surface of i1, but not with o1. Similarly, b2 was in contact with both i1 and i2, and b3 was in contact with i2. Thus, an arch-shaped area of contact was seen between the i1-2 and b1-3 dentitions. Just behind this arch-shaped contact, the mucosal membrane of the oral cavity was protected with hard tissue armor, i.e. a roof provided by four inner teeth in the upper, and a basal terrace formed from an array of bowl-like concavities in the lower dentition.

**Discussion**

The present study proved that it was possible to investigate and describe the highly elaborate morphologies of the tooth and dentition of *S. cirrhifer* comparing it with the apex of the unerupted successor (compare Fig. 5C and D); in addition, a worn apex appeared indented at its tip, indicating a difference in hardness between the outer enamolid and the inner dentin (apical aspect in Fig. 5C). Differences in the basal aspects evident in Fig. 5C and D indicated that the thickness of the dentin was still increasing in the successor (Fig. 5D). Moreover, perikymata-like linear grooves had been developing on the lingual surface of the unerupted successor (i1s in Fig. 5F), where the bone-side morphology was still incomplete compared to that of the erupted tooth (compare Fig. 5E and F).

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in greater detail using a modern micro-CT system. Some of the features described had already been reported by Sohiti Isokawa in 1955. Matsura [4] and Uehara and Miyoshi [5] through perceptive observations based on morphological and histological approaches. However, the present approach employing 2D tomograms and reconstructed 3D models was undoubtedly advantageous, even though histological investigations at the light and electron microscopy levels are still indispensable for studies of tooth morphology.

Since analysis of datasets generated by micro-CT allows non-destructive observations, the *in vivo* geometry of teeth in the specimens can be interrogated serially back and forth as 3D models from various directions, allowing identification of overall functional and successional teeth *in situ* (Figs. 1-4). In addition, "virtual dissection", described as "digital extraction from 3D-VR" was possible using a scalpel tool (Fig. 5). This approach yielded a number of valuable findings, which were beneficial for in-depth understanding of the tomographic images. The smallest voxel size in the original images was 30 μm, which was feasible for evaluating the link between the teeth and bone (Fig. 4) and for measuring the width of developing teeth *in situ* in the 3D models (Table 1). Kazzazi and Kranioti [7] reported that measurements on 3D-VR images were more accurate and reliable than traditional odontometrics carried out on real teeth with digital calipers.

The mode of tooth attachment in balistoids has long been a controversial topic. Owen described this as a "double gomphosis" in *B. capriscus*, but appeared to imply that the teeth were implanted in alveolar sockets, while bony struts projecting from alveolar bone were implanted in the narrow spaces between the teeth. Isozawa (1955) reported that the teeth in *S. cirrhifer* were barely ankylosed on the bone margin via predentin (Fig. 8). Soule [3] reported that in *S. bursa* and two other balistids the tooth attachment comprised a shallow alveolar socket, a periodontal ligament and acellular cementum. Berkovitz and Shellis (as stated in "The Teeth of Non-Mammalian Vertebrates", 2017) appeared to be unconvinced by the latter results and was more inclined to favor an attachment by ankylosis to shallow bony depressions. Uehara and Miyoshi [5] showed that the upper teeth of *S. cirrhifer* were tightly fixed to the jawbone by bundles of fibrils and also that the teeth appeared to attach directly to the bone at the middle portion of the labial and lingual components of the teeth. The present study examined and visualized three-dimensionally the tooth shape and related geometry in the jaws of *S. cirrhifer*, and this revealed unequivocally that there were no distinct sockets comparable to those in mammals, whereas instead shallow bone depressions were observed (Fig. 5G, H). The edge of the pulp cavity opening was being barely ankylosed in these depressions (arrowheads in Figs. 4, 5 and 8).

The present 3D-VR resolved the uncertainty of how the filefish teeth were ankylosed to shallow depressions in the bone. The opening of the tooth pulp cavity was not simply oriented towards the apparent tooth base in a direction opposite to that of the tooth apex. The opening was distorted basoposteriorly in o1-3 and b1-3, and basoanteriorly in i1-2, and therefore part of the basoposterior or basoanterior edge of the opening was ankylosed to the bone (Figs. 4, 5C, E). This meant that the tooth was pleurodont, and not ankylosed to the bone by the actual labial or lingual walls. Pleurodonty is a mode whereby the labial (or lingual) surface of a tooth is set against the labial (or lingual) side of the tooth-bearing bone. The distribution of such pleurodont ankylosis along the edge of the pulp cavity opening appeared to match well with the contour of a shallow depression in the supporting bone (Fig. 5G, H).

The presence of a periodontal ligament and cementum as an attachment apparatus for the teeth of balistids has been reported [3], but these two tissues are considered to be peculiar to the Crocodileida and Mammalia, being non-existent in almost all Osteichthyes. Indeed, Isokawa reported in 1955 that nothing that could be regarded as cementum was present, but that "predentin" was evident in the boundary area of the teeth and bone of *S. cirrhifer*. This was confirmed by re-examination of the histological specimens he had prepared in the early 1950s (Fig. 8C). It was also recognized that predentin or its equivalents extended to the outer surface of the edge of pulp cavity opening, thus providing an anchorage for fibers connecting tooth to bone and tooth to tooth. These two types of fibers were also confirmed in the histological specimens (Fig. 8) and also reported in an electron microscopy study by Uehara and Miyoshi [5]. The tooth-to-tooth fibers should not be described as "periodontal", and the tooth-to-bone fibers were considered to be part of the "fibrous attachment" relatively common in Osteichthyes [8], indicating that the fibrous attachment coexists with ankylosis in the tooth of this species, *S. cirrhifer*, reinforcing the tooth attachment cooperatively.

The surface rendering in this study showed how the upper and lower teeth of *S. cirrhifer* meet together (Fig. 7), i.e. forming a continuous arch consisting of the apex and its distal nipping edge of b1-3 coming completely into contact with the lingual surface of i1-2. Since the dentary is articulated with the quadrate, and the maxillary and premaxillary are articulated with the palatine, the upper and lower jaws are both able to rotate in species of this family [4,9]. Thus, the flat and wide lingual surfaces of teeth i1-2 (Fig. 7) would serve as a cutting or crushing board after pecking and capturig prey. The feeding habits of the filefish may vary from herbivorous to carnivorous, with many being omnivorous [2,4,10]. The stomach contents of *S. cirrhifer* caught in the southern Shikoku, Japan, were reported to include amphipods, barnacles and sea urchins [11], most of which would have hard shells and/or calcified structures. Thus, the four inner teeth as well as an array of bowl-like concavities in the lower dentition, both being covered with enameloid tissue, might be functionally relevant as an armor for protecting the mucosal membrane during biting.

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Conflict of interest
None.

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