Development and Replacement of Oral Teeth in Balistidae and Monacanthidae (Acanthopterygii: Tetraodontiformes)

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ABSTRACT

Tetraodontiformes includes approximately 350 living species of pufferfishes and allies. Oral teeth of tetraodontiforms vary widely in morphology, from several pointed individual teeth seen in boxfishes (Ostraciidae) to the single fused dental beak of ocean sunfishes (Molidae). Comparative dental morphology of tetraodontiforms has yet to be fully characterized using available technology such as Computerized Tomography. I present an in-depth morphological analysis of oral dentition within Balistoidea, a superfamily within Tetraodontiformes, to understand differences in dentition between its two living families, Balistidae (Triggerfishes) and Monacanthidae (Filefishes). I describe the morphology and replacement modes of the oral dentition of exemplar species from the two families using osteological materials, histological sections, and CT images, and place these observations in the context of dental structure in other lineages of tetraodontiforms. Balistoids are unique within tetraodontiforms, and other teleosts in general, in that they have a mammalian-like tooth formula in which tooth sizes but not numbers increase during life. Additionally, their teeth occlude like a chisel pounding against an anvil in a way that is similar to the incisor teeth of rabbits. This arrangement, combined with an elongate central cusp, maximizes crushing effectiveness in a pattern distinct from other tetraodontiforms.

INTRODUCTION

Dental morphology provides core insight into evolutionary relationships within the order Tetraodontiformes, and many of its subgroups are named on the basis of dental anatomy. Most teleosts replace teeth throughout life (Bemis et al., 2005; Huysseune and Witten, 2006; Bemis and Bemis, 2015; Bemis et al., 2019), and mechanisms of tooth replacement vary in parallel with different dentitions. An array of adult dentitions occur in Tetraodontiformes, as exemplified by the number of teeth in the upper jaw, which ranges from a single beak (e.g., Molidae and Diodontidae), to left and right beaks (e.g., Triodontidae and Tetraodontidae), to three or four individual teeth in the outside series (e.g., Balistoidea), to as many as six individual teeth (e.g., Ostraciidae). Many aspects of tetraodontiform tooth replacement as well as the mechanism for forming their characteristic beak-like structures, however, remain unknown. I elected to focus here on a group within Tetraodontiformes that have individual teeth. I was interested to compare similarities and differences between Tetraodontiformes and outgroup taxa as most teleosts have individual teeth.

Within Balistoidea, there are 42 living species of triggerfishes (Balistidae) and 107 living species of filefishes (Monacanthidae). They span habitats from shallow water reefs to pelagic marine environments, and are known for derived modes of locomotion using the dorsal and anal fins and unusual teeth that pluck and crush prey (Nelson et al., 2016). Together, these two extant families and fossils from the Eocene to Recent make up Balistoidea, one of several well supported clades within Tetraodontiformes. Evolutionary relationships within Tetraodontiformes have been controversial for many years (Fig. 1). For example, on the basis of muscle characters, Winterbottom (1974) postulated that the Ostracioidea (Boxfishes and Trunkfishes) were more closely related to Balistoidea (Triggerfishes and Filefishes) than to Tetraodontoidea (the Three-

Toothed Puffer, Ocean Sunfishes, Porcupinefishes, and Puffers). Subsequent morphological research on living and fossil Tetraodontiformes by Santini and Tyler (2003) placed ostracioids as the sister group of tetraodontoids. In a more recent molecular phylogenetic study, Santini et al. (2013) placed Triodontidae near the base of Tetraodontiformes in a clade with Triacanthoidea and Ostracioidea. Monophyly of Balistoidea remains undisputed across all of these studies and character sources. The strong morphological evidence for a close relationship between these two families includes features of the dentition, which offers many potentially informative phylogenetic characters at the gross anatomical and histological levels.

I studied two exemplar species of balistoids using micro-CT and histology: the Grey Triggerfish (Balistidae: *Balistes capriscus*) and Planehead Filefish (Monacanthidae: *Stephanolepis hispidus*). I also surveyed tooth morphology in skeletal preparations of 19 additional species of balistoids as well as comparative material from all ten extant families of tetraodontiforms.

MATERIALS AND METHODS

Anatomical Terminology. For general nomenclature of cranial bones, I follow Tyler (1980) and Grande and Bemis (1998). The term "mesial" refers to a tooth or portions of a tooth that are most proximal to the mandibular or premaxillary symphyses. Thus, the mesial-most tooth is directly adjacent to the symphysis. I follow Tyler (1980) in using the terms "outer" and "inner" to refer to teeth on the labial (outer) and lingual (inner) sides of each premaxilla. I use the term bone of attachment in the sense of Bemis et al. (2019) to mean the bony tissue that attaches the dentine of a tooth to its dentigerous bone. Ankylosis is achieved when the ligaments connecting the bone of attachment to the dentigerous bone are mineralized; such ankylosis corresponds to tooth attachment Type 1 of Fink (1981). I follow Bemis et al. (2005, 2019) in using the term locus to refer to a location on the jaw where a tooth is developing, is present, or was formerly present. I also use the four-part scoring system developed by Bemis et al. (2005) to characterize stages in tooth replacement at a particular locus: Absent, Incoming, Functional, or Eroding. The term replacement pore was defined by Bemis et al. (2005) to refer to an opening in the dentigerous bone through which a developing tooth germ can pass into the medullary cavity of the bone. Once a new tooth germ has reached the medullary cavity, it will continue to develop, grow, and erupt. In some cases, such as Bluefish (*Pomatomus saltatrix*), the tooth erupts directly into the overlying locus to replace the older tooth. In this pattern, replacement pores are only present at some stages of tooth replacement. In other cases, such as the Atlantic Cutlassfish (*Trichiurus lepturus*), a tooth germ migrates into the medullary cavity through its replacement pore, completes its development, and then erupts through its replacement pore (Bemis et al., 2019). In this pattern, replacement pores may be evident throughout all stages of tooth replacement. Further nuances of these two patterns remain to be fully documented and understood, but, in both cases, activity of osteoclasts is required to allow a new tooth to reach the medullary cavity and to erupt.

Overview of specimens examined. I examined one exemplar species for the family Balistidae (*Balistes capriscus*) and one exemplar species for the family Monacanthidae (*Stephanolepis hispidus*). Additionally, I surveyed the dentition in seven other species of balistids (*Balistes carolinensis*, *B. polylepis*, *B. vetula*, *Canthidermis maculatus, C. sufflamen*, *Melichthys niger*, *M. vidua*; summarized in Table 1), and 13 other species of monacanthids (*Aluterus laevis*, *A. monoceros*, *A. schoepfi*, *A. scriptus*, *Cantherhines macrocerus*, *C. pullus*, *Meuschenia freycineti*, *M. scaber*, *Monacanthus pardalis*, *M. spilosoma*, *Stephanolepis auratus*, *S. setifer*, *Thamnaconus degeni*; summarized in Table 2). I also examined a variety of fishes from within

Tetraodontiformes. A complete list of specimens is provided in Materials Examined; institutional abbreviations follow Sabaj Pérez (2014).

To assess individual variation in jaw and tooth morphology as well as jaw morphology of smaller individuals, I prepared micro-CT datasets for 10 individuals of similar size from a large sample (125) of *Stephanolepis hispidus*. Within that sample of 10, I selected two individuals for higher resolution CT scanning and one individual for histology. Table 3 summarizes data specific to the 10 individuals randomly selected from the larger sample (CU 34396).

Osteology. I prepared and studied dry skeletons following the methods described in Bemis et al. (2004). Specimens examined summarized in Materials Examined.

CT Imaging. I prepared and studied several specimens (*Balistes capriscus*: MIR-006; *Stephanolepis hispidus*: CU 79394, CU 34396A-J).

I CT scanned dry skeletal specimens (MIR-006, CU 79394) and whole preserved specimens (CU 34396A-J) using the Xradia Versa XRM-500 nano-CT scanner in the Biotechnology Resource Center Multiscale Imaging Facility at Cornell University to prepare data sets for specimens MIR-006 (at 24.69 µm resolution), CU 79394 (at 21.89 µm), and CU 34396 (at 12.5 µm for I and J, and at 50 µm for A-J).

These CT datasets were used to make 3D volume reconstructions with OsiriX™ (version 7.0.2, 64-bit edition) DICOM imaging software (Rosset et al., 2004) and Horos™ (version 2.0.1, 64-bit edition) on Apple Macintosh computers running macOS Sierra 10.12.6. To view internal anatomy, I digitally dissected or sectioned reconstructions within OsiriX™.

Histology. I decalcified oral jaws of two specimens using Formic Acid A (Humason, 1972; 20% Formic Acid), washed and dehydrated specimens for paraffin embedding, cut sections at 6- 10 µm, and stained them with H&E (hematoxylin and eosin; Cornell Veterinary College Diagnostic

Laboratory). This method preserves cellular detail but removes minerals. Blocks were cut for cross-sections and frontal sections to examine multiple planes within the jaw.

Figure preparation. I used a Canon 5D Mark II digital camera to record color macrophotographs. Photographed histological sections were completed with an Olympus SZX12 microscope equipped with an Olympus DP70 digital camera. Adobe Photoshop CC (version 2017.1.1) was used to adjust images for color balance and contrast. Plates and line drawings were prepared in Adobe Illustrator CC (version 21.1.0).

RESULTS

Premaxilla. In balistids, each premaxilla contains four outer teeth that face directly downward from their loci, and three inner teeth that protrude slightly from their loci. The outer teeth extend slightly forward from the jaw over the dentary teeth. Outer teeth are smaller at more posterior loci (LP1-4, RP1-4; Fig. 2AB). In monacanthids, each premaxilla contains three outer teeth and two inner teeth. The third tooth is much wider and flatter than the other premaxillary teeth, and I refer to it as the “side tooth” because it appears to be ankylosed on its side rather than at its base (LP1-3, RP1-3; Fig. 2CD). Functional teeth in both groups are deeply socketed and firmly ankylosed to the premaxilla.

Dentary. In balistids, each dentary contains four lateral teeth that share a similar morphology to the outer teeth on the premaxillae directly above each tooth loci (LD1-4, RD1-2; Fig. 2AB). In monacanthids, each dentary contains three lateral teeth that do not share a similar morphology to the outer teeth of the premaxillae (LD1, RD1; Fig. 2CD). Dentary teeth of balistoids are smaller at more posterior loci. In both balistids and monacanthids, functional teeth are deeply socketed and firmly ankylosed to the dentary.

Outer teeth. The outer teeth of balistoids are ankylosed to the premaxillae and dentaries and extend slightly forward from the jaw. The teeth contact each other on their mesial and distal sides, only separating at the bases where they exit the loci and at the tips of the teeth. In balistids, all outer teeth are similarly shaped, but vary in size (Fig. 2B). In monacanthids, tooth shape differs along the jaw. The tooth in the first locus has a tall cusp similar to those of balistid teeth. The tooth in the second locus is angled such that the higher tip meets the more mesial adjacent tooth, and the lower tip meets the posterior tooth's height. The tooth in the third locus is similar to that of the second, but is characterized by a flatter, less-angled tip (Fig. 2D).

Replacement of outer teeth. The outer teeth develop in replacement pores on the labial sides of the premaxillae and dentaries. They develop intraosseously such that the tooth develops inside the bone, and the incoming tooth emerges through the locus of the existing tooth. As teeth develop at the locus, the tip of the incoming tooth protrudes into the pulp cavity of the existing tooth, thus maintaining tooth function throughout the replacement process (LPF1, LPI1; Fig. 3AB). Replacement pores remain present throughout all steps of tooth function and replacement. In balistids, there is some evidence for alternate tooth replacement where teeth at alternating loci match during stages of development. However, in monacanthids, all functional teeth examined had incoming replacements, and as such there is no evidence for alternate tooth replacement.

Inner teeth. Inner teeth are ankylosed to the lingual side of each premaxilla. Because of their attachment on the lingual side, the teeth protrude into the oral cavity from the loci. The loci of the inner teeth alternate with those of the outer teeth, but inner teeth still maintain direct contact with neighboring inner teeth as well as direct contact with the outer teeth (INLPF1-3; Figs. 3C, 4B).

Replacement of inner teeth. Replacement of the inner teeth matches those of the outer teeth but occurs on the lingual side of the premaxillae (Fig. 5AB). In monacanthids and balistids, tooth germs enter the bone of attachment through the replacement pore and develop intraosseously beneath the functional tooth locus (Fig. 6B).

Cusps. Functional outer teeth of balistids are flat with a long, narrow cusp in the middle of the tip (LP1-4, LD1-4; Fig. 2A). Functional outer teeth of monacanthids are flat with a short cusp at the tip on only the mesial-most premaxillary and dentary tooth (LP1, RD1; Fig. 2CD).

Pulp cavity. The pulp cavity in functional teeth of *Balistes capriscus* extends throughout the length of the tooth. Incoming teeth protrude into the pulp cavity as they emerge through the locus (Fig. 5A). All teeth examined in *Stephanolepis hispidus* also contained a large pulp cavity, but it is unclear if this extends as far distally as observed in *Balistes capriscus* (Fig. 6A).

Tooth shape. The teeth of balistoids are molded such that the outer premaxillary teeth fit together with each other and with the inner premaxillary teeth. The inner premaxillary teeth are cradled on the labial side by the outer teeth, allowing them to protect each other and likely optimize the biting force of the teeth by creating a wedge (Fig. 3C, 4B).

Symphyses. The right and left premaxillae and right and left dentaries attach by a zig-zag symphysial articulation reminiscent of a zipper. In balistids, this structure is not visible in frontal CT or transversal sections, but can be seen in sagittal and coronal sections using CT Multiplanar Reformation (MPR) reconstructions (Fig. 3D). In monacanthids, this zig-zag articulation is more apparent and can be visualized in 3D volume rendering of CT scans (Fig. 4C).

Jaw joint. In both balistids and monacanthids, a restrictive jaw joint between the quadrate and articular elements limits lateral movements of the dentary, yielding close occlusion of the teeth (Fig. 2AC).

DISCUSSION

The general tooth and jaw structure of balistids and monacanthids are similar to, yet markedly different from other tetraodontiforms. A suite of characters supports monophyly of Balistoidea. First, both families have a small number of individual teeth (when compared to other teleosts) and share a similar and unique arrangement of teeth in which the premaxilla has inner and outer series teeth and the dentary has only one series of teeth. Second, the individual teeth have a large pulp cavity and are closely associated with each other within the jaw. Third, balistoids share a tooth replacement mode in which teeth are replaced intraosseously whereby tooth germs enter the jaw through replacement pores, develop inside the bone of attachment, and erupt through existing tooth loci. However, certain aspects of tooth morphology differentiate the two families. First, balistids have four outer series teeth and three inner series teeth on each premaxilla and four teeth on each dentary, whereas monacanthids have three outer series teeth and two inner series teeth on each premaxilla and three teeth on each dentary. This is an intriguing distinction since specimens examined of both groups were similar in total length and, no ontogenetic variation in the total number of teeth was observed. This may be due to a smaller jaw length in monacanthids or to wider teeth more widely spaced along the jaw. Second, teeth of monacanthids were generally much flatter than those of balistids, which had a cusp tip on every tooth. These general differences in tooth morphology may relate to different feeding or defensive behaviors. In general, the similar morphology and replacement modes of Balistidae and Monacanthidae support the monophyly of the superfamily Balistoidea in Tetraodontiformes. Consistent differences in features of tooth shape and number support the interpretation that the two families diverged and possibly specialized for different diets early in their history.

Dentition. Because of the restrictive jaw joint, balistoid teeth occlude to form shearing and pounding surfaces much as in lepidosirenid lungfishes (Bemis, 1986). For example, the outer and inner series teeth in the upper jaw of triggerfishes and filefishes fit together precisely with the lingual side of each outer tooth touching the labial side of each inner tooth to form a larger, anvil-and-chisel-like structure against which the dentary teeth occlude. This arrangement of premaxillary teeth resembles that of the upper incisors of a rabbit. Unusually for teleosts, the number of functional teeth in the premaxillae and dentary bones is fixed in adult Balistoidea. Therefore it is possible to specify a mammalian-like tooth formula for Balistidae (4+3/4) and Monacanthidae (3+2/3). The tooth formula of monacanthids must be established early in ontogeny, for it is already apparent in the smallest *Stephanolepis hispidus* that I examined. As a balistoid ages, its replacement teeth only become larger and do not increase in number. It will be interesting to examine larval and juvenile balistoids to understand the early ontogeny of these mammalian-like tooth formulae.

Soft Tissues. Both *B. capriscus* and *S. hispidus* have extensive soft tissues surrounding the labial sides of the teeth. These padded lips likely protect the teeth and are probably especially important during early development of replacement teeth because the replacement pores are located on the labial sides of the premaxillae for outer series teeth and the labial side of the dentaries for their single series of teeth. The lips therefore function in a similar way to the rest of the skin to protect the fish. Bluefish and Atlantic Wolffish (*Anarhichas lupus*) also have extensive soft tissue to surround and protect tooth attachment sites (Bemis et al., 2005; Bemis and Bemis, 2015). The soft tissues of Atlantic Wolffish are associated with the replacement pores (Bemis and Bemis, 2015), as also seen in triggerfishes and filefishes, and it has been suggested that there is a similar pattern in Bluefish (Bemis et al., 2019). The presence of extensive soft tissues surrounding sites of tooth replacement appears to be common in teleost fishes. This hypothesis is further supported by the fact that teleosts that do not replace teeth through replacement pores on the surfaces of bones lack highly developed soft tissues surrounding and protecting the teeth. For example, Atlantic Cutlassfish replace their lateral teeth through a trench that lacks extensive soft tissue coverings (Bemis et al., 2019).

Dentition and Phylogeny of Tetraodontiformes. The pattern of individual teeth and their replacement in Balistoidea is most likely plesiomorphic for Tetraodontiformes as a group because similar patterns are observed in outgroups to the order. Other Tetraodontiformes, however, have very different arrangements including beaks formed of fused elements, crushing plates formed from individual teeth, and crushing plates that resemble stacks of plywood with alternating soft and hard layers. I expect further research on dentition, development, and replacement on all ten extant families of Tetraodontiformes will contribute to questions about how tooth evolution relates to diversity and relationships within the order.

Tooth Shape and Diet. Crofts and Summers (2014) demonstrate that the ideal tooth shape for organisms that consume hard-shelled prey is one that is flat with a long and narrow cusp at its tip. Such a cusp concentrates force on a specific point in the shell of the prey, requiring less force to break it. This tooth shape is seen in teeth of *Balistes capriscus* and in the mesial-most teeth of *Stephanolepis hispidus*. Even though the extended pulp cavities make the teeth essentially hollow, *B. capriscus* consume hard-shelled prey. Because most of the specimens I examined appeared not to have missing or broken teeth, the hollow pulp cavity does not affect tooth function when crushing prey. Therefore, it is likely that the tooth morphology described by Crofts and Summers (2014) prevents teeth from breaking by concentrating force without breaking the tooth shaft or attachment.

Replacement mode. In both extant families of Balistoidea, replacement teeth form directly beneath existing functional teeth. As a replacement tooth increases in size, it protrudes into the pulp cavity of the existing functional tooth. The attachment of the existing functional tooth begins to erode, and the new tooth pushes it out, eventually emerging from its locus. This pattern is possible because the pulp cavities extend nearly to the tooth tips, as in Bluefish (Bemis et al., 2005), in which a new tooth develops directly into the pulp cavity of the overlying functional tooth. Other fishes with large plucking teeth, such as the Sheepshead (*Archosargus probatocephalus*; Mook, 1977) do not replace into an existing pulp cavity, but instead through a new opening over the top of the existing tooth. In balistoids and Bluefish, then, the functional tooth must be fully eroded before the new tooth emerges from the bone, whereas in Sheepshead, a functional tooth can remain attached while the replacement tooth emerges.

Teleosts with intraosseous tooth replacement often display alternate tooth replacement patterns (e.g., Bluefish; Bemis et al., 2005). Common to many teleosts with alternate replacement is a relatively small number of teeth, but no teleosts that I have studied to date have as few individual teeth as Balistoidea. It is hard to assess whether there can be a truly alternate pattern when there are only three to four teeth in the jaw. It also may be important for fishes with such small numbers of teeth to always have functional teeth with replacements ready. Some teleosts, however, display a different mode of replacement. For example, the Tripletail (*Lobotes surinamensis*) replaces teeth in groups of four (Hilton and Bemis, 2005). This mode may link to the replacement mode in filefishes. The oral teeth of filefishes observed in this study appear to replace teeth on one side of the jaw all at once (in groups of three or less). One main difference, however, is that filefish teeth, while developing on a similar schedule to each other, enter the jaw and appropriate locus through replacement pores specific to its tooth locus. In Tripletails, this "grouped" replacement mode occurs in a single replacement pore (Hilton and Bemis, 2005). This comparison may provide further evidence of convergent evolution of tooth replacement modes in even distantly related teleosts.

Intraosseous tooth replacement and Tetraodontiformes. Although there is great diversity in tetraodontiform dental anatomy – including the anvil-and-chisel-like dentition of balistoids, fused beaks and crushing surfaces of tetraodontoids, and discrete molariform teeth in triacanthids and in a recently discovered Eocene fossil – all tetraodontiforms examined exhibit intraosseous tooth replacement (Tyler, 1980; Bemis et al., 2017; Trapani, 2001). I predict that this is the plesiomorphic condition for the group and will continue to test this by examining additional living and fossil Tetraodontiformes and closely related outgroups.

MATERIALS EXAMINED

Species	Catalogue # (TL)
Acanthostracion quadricornis	SB16-010 (243mm)
Aluterus heudelotti	SB16-009 (380mm)
Aluterus laevis	ANSP109144
Aluterus monoceros	216475SD; 094746SD
Aluterus schoepfi	088880SD; 21552SD; 90743SD; USNM283612; CU82134
Aluterus scriptus	88705SD; USNM111358
Anacanthus barbatus	ANSP109140
Aracana aurita	ANSP98627
Aracana flavigaster	ANSP33169; ANSP33169
Balistes capriscus	MIR-006; SB16-011 (359mm); 2163595SD; 94490SD; 211619SD; CU94612; CU91250; CU79397; CU95580;

	CU91251; CU90721; CU95761; CU79367; CU95582; CU93242; CU94612; CU91249; CU94614
Balistes carolinensis	210659SD
Balistes polylepis	ANSP109530
Balistes vetula	093252SD; ANSP16752; CU91253; CU95776; CU93425l; CU91254; CU91255
Cantherhines macroceros	21549SD
Cantherhines pullus	30819SD; 30818SD; 30816SD; 30830SD
Canthidermis maculatus	214487SD; 90916SD; CU94468
Canthidermis sufflamen	56735SD; CU93304; CU91284; CU91258; CU95775; CU95444; CU95774
Eubalichthys spilomelanurus	ANSP109810 (cleared and stained)
Laputa cingalensis	ANSP100831 (cleared and stained)
Melichthys niger	USNM273039; USNM111306; USNM273037
Melichthys vidua	ANSP109442 (cleared and stained)
Meuschenia freycineti	095466SD; 095467SD
Meuschenia scaber	92286SD; 92286SD; 92334SD
Monacanthidae	ANSP78224
Monacanthus hispidus	CU91259; CU34396; CU79394
Monacanthus pardalis	ANSP78218
Monacanthus setifer	CU91317 (cleared and stained)
Monacanthus sp.	CU83546 (cleared and stained)
Monacanthus spilosoma	ANSP84774

Navodon setosus	ANSP96426 (cleared and stained)
Ostracion quadricornis	ANSP109167
Ostracion triquetrum	ANSP16818
Pervagor spilosoma	CU79537 (cleared and stained)
Rhinecanthus aculeatus	CU95374
Sphoeroides spengleri	ANSP109531
Stephanolepis auratus	ANSP106265 (cleared and stained)
Stephanolepis hispidus	210670SD
Stephanolepis setifer	USNM273029
Stephanolepis sp.	USNM273026
Thamnaconus degeni	0986535SD; 098652SD
Triacanthus biaculeatus	ANSP109141
Triacanthus nieuhovii	ANSP109193

AUTHOR CONTRIBUTIONS

This work was submitted in partial fulfillment of the requirements for honors in Biology at Cornell University. The coauthors for the paper that will result from this research are William E. Bemis and Katherine E. Bemis. All three equally contributed to study design and data collection. Draft manuscript and figures were prepared by SB and edited by WEB and KEB.

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FIGURE LEGENDS

Figure 1. Comparison of interrelationships of families in the order Tetraodontiformes. (A) Phylogenetic interpretation from Winterbottom (1974) based on myological characters. Balistoidea and Ostracioidea are sister taxa colored in yellow, Triacanthoidea are an outgroup colored in green, and Tetraodontoidea are colored in blue. (B) Phylogenetic interpretation from Santini and Tyler (2003) based on osteological characters. Triacanthoidea are an outgroup colored in green, Balistoidea are colored in yellow, and Ostracioidea and Tetraodontoidea are sister groups colored in blue. (C) Phylogenetic interpretation from Santini et al. (2013) based on mitochondrial and nuclear characters. Triodontidae, Triacanthoidea, and Ostracioidea are all sister groups colored in green. Balistoidea is colored in yellow and a sister group to Tetraodontoidea colored in blue.

Figure 2. Overview of teeth and dentition of balistoids. (A) Left lateral view of jaw in *Balistes capriscus* (MIR-006). Left premaxilla and dentary each with four teeth are shown. (B) Anterior view of jaw in *Balistes capriscus* (MIR-006). Both halves of the premaxillae and dentaries are shown. Some replacement pores appear larger than others to indicate either an incoming tooth or a newly ankylosed tooth. Such enlarged replacement pores have been labeled. Tooth loci LP1 contains an incoming tooth and has been labeled as such. (C) Left lateral view of jaw in *Stephanolepis hispidus* (CU 91259). Left premaxilla and dentary are shown. Three teeth are visible in the premaxilla, but only one tooth is visible in the dentary due to close occlusion of the upper and lower jaws. (D) Anterior view of jaw in *Stephanolepis hispidus* (CU 91259). Both halves of the premaxillae and dentaries are shown and each tooth loci is labeled. All replacement pores of teeth are circled in red,

and all teeth are labeled for loci position. Note restrictive jaw joint. LP: Left Premaxilla; LD: Left Dentary; RP: Right Premaxilla; RD: Right Dentary. Scale bar = 5mm.

Figure 3. CT reconstructions of left premaxilla of *Balistes capriscus* (MIR-006). (A) Left lateral view. Replacement pores circled in red. Developing tooth LPI1 is replacing the LPF1 tooth by erupting through the locus. (B) Virtual CT section showing how the tooth grows directly into the pulp cavity of the overlying LPF1 functional tooth. Large pulp cavity of developing tooth labeled. (C) Lingual view of three inner teeth of the premaxilla. Replacement pores circled in red. (D) Coronal MPR section. Note zig zag symphysis connecting the left and right premaxillae. Scale bar = 5mm.

Figure 4. CT reconstructions of dentition in *Stephanolepis hispidus* (CU 34396 I). (A) Left lateral view. Note enlarged yet empty replacement pore. (B) Lingual view. (C) Anterior view. Note zig zag symphysis connecting the left and right dentaries. Incoming teeth labeled. Replacement pores circled in red. Enlarged replacement pores labeled. Scale bar = 10mm.

Figure 5. Histology of premaxilla in *Balistes capriscus*. (A) Cross section of premaxilla. Note large pulp cavity of functional outer tooth. (B) Frontal section of premaxilla. Anterior to left. Developing teeth and all outer and inner series teeth labeled.

Figure 6. Dentition of *Stephanolepis hispidus* (CU 34396 E). (A) Histology of cross sectioned jaws. Premaxillae and dentaries labeled. Outer series and inner series teeth labeled. (B) Closer image of area enclosed in red box of (A) to highlight osteological stages. (C) CT image to provide context for histology.

TABLES

Table 1. Balistidae examined.

Taxon	ID Number
Balistes capriscus	MIR-006
Balistes capriscus	SB16-011 (359mm)
Balistes capriscus	2163595SD
Balistes capriscus	94490SD
Balistes capriscus	211619SD
Balistes capriscus	CU94612
Balistes capriscus	CU91250
Balistes capriscus	CU79397
Balistes capriscus	CU95580
Balistes capriscus	CU91251
Balistes capriscus	CU90721
Balistes capriscus	CU95761
Balistes capriscus	CU79367
Balistes capriscus	CU95582
Balistes capriscus	CU93242
Balistes capriscus	CU94612
Balistes capriscus	CU91249
Balistes capriscus	CU94614
Balistes carolinensis	210659SD
Balistes polylepis	ANSP109530
Balistes vetula	093252SD
Balistes vetula	ANSP16752
Balistes vetula	CU91253
Balistes vetula	CU95776
Balistes vetula	CU93425
Balistes vetula	CU91254
Balistes vetula	CU91255
Canthidermis maculatus	214487SD
Canthidermis maculatus	90916SD
Canthidermis maculatus	CU94468
Canthidermis sufflamen	56735SD
Canthidermis sufflamen	CU93304
Canthidermis sufflamen	CU91284
Canthidermis sufflamen	CU91258
Canthidermis sufflamen	CU95775
Canthidermis sufflamen	CU95444
Canthidermis sufflamen	CU95774
Melichthys niger	USNM273039
Melichthys niger	USNM111306
Melichthys niger	USNM273037
Melichthys vidua	ANSP109442
Rhinecanthus aculeatus	CU95374

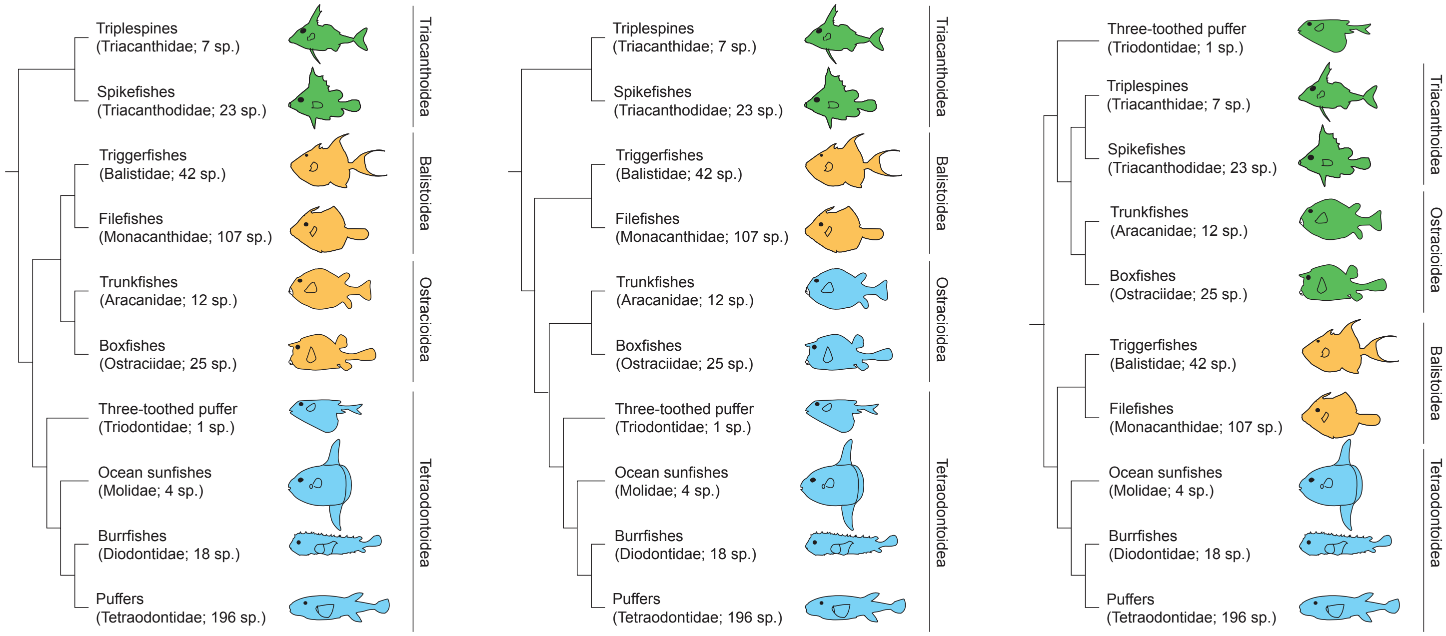
Table 2. Monacanthidae examined.

Taxon	ID Number
Aluterus heudelotti	SB16-009 (380mm)
Aluterus laevis	ANSP109144
Aluterus monoceros	216475SD
Aluterus monoceros	094746SD
Aluterus schoepfi	088880SD
Aluterus schoepfi	21552SD
Aluterus schoepfi	90743SD
Aluterus schoepfi	USNM283612
Aluterus schoepfi	CU82134
Aluterus scriptus	88705SD
Aluterus scriptus	USNM111358
Anacanthus barbatus	ANSP109140
Cantherhines macroceros	21549SD
Cantherhines pullus	30819SD
Cantherhines pullus	30818SD
Cantherhines pullus	30816SD
Cantherhines pullus	30830SD
Eubalichthys spilomelanurus	ANSP109810
Laputa cingalensis	ANSP100831
Meuschenia freycineti	095466SD
Meuschenia freycineti	095467SD
Meuschenia scaber	92286SD
Meuschenia scaber	92286SD
Meuschenia scaber	92334SD
Monacanthidae	ANSP78224
Monacanthus hispidus	CU91259
Monacanthus hispidus	CU34396
Monacanthus hispidus	CU79394
Monacanthus setifer	CU91317
Monacanthus sp.	CU83546
Monacanthus spilosoma	ANSP84774
Monacanthus pardalis	ANSP78218
Navodon setosus	ANSP96426
Pervagor spilosoma	CU79537
Stephanolepis auratus	ANSP106265
Stephanolepis hispidus	210670SD
Stephanolepis setifer	USNM273029
Stephanolepis sp.	USNM273026
Thamnaconus degeni	0986535SD
Thamnaconus degeni	098652SD

Table 3. Measurements of ten individuals examined from CU 34396 (A-J). TL: total length; FL: forked length; Pre-D: pre-dorsal length; Pre-A: pre-anal length; HL: head length; PL (I): number of outer and inner (I) left premaxillary teeth; PR (I): number of outer and inner (I) right premaxillary teeth; DL: number of left dentary teeth; DR: number of right dentary teeth. All measurements in millimeters. * indicates that it was difficult to make an accurate count due to jaw occlusion.

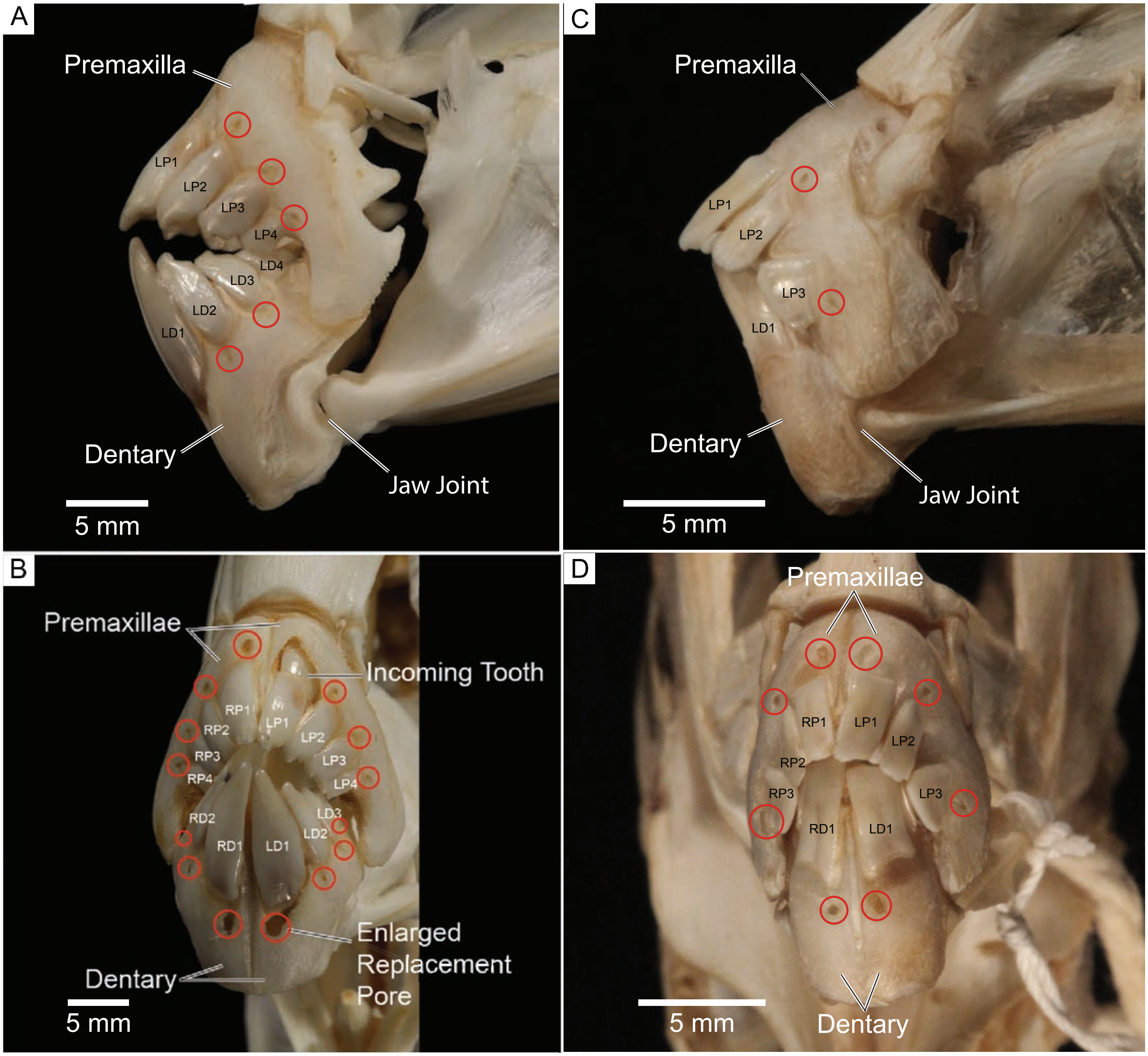
Num	TL	FL	Pre-D	Pre-A	HL	PL (I)	PR (I)	DL	DR
A	75.5	70.8	33.3	36.2	18.6	3(2)	3(2)	2*	2*
B	74.1	64.5	31.0	34.8	20.3	3(2)	3(2)	3	3
C	75.2	71.9	32.7	35.0	19.7	3(2)	3(2)	3	2*
D	81.2	73.4	37.3	35.6	20.2	3(2)	3(2)	2	2
E	90.8	90.3	41.5	43.5	24.1	3(2)	3(2)	3*	3*
F	78.6	74.6	35.4	35.0	20.8	3(2)	3(2)	2*	2*
G	73.8	66.8	30.7	33.1	18.8	3(2)	3(2)	2*	3*
H	69.0	66.8	30.9	33.8	18.3	3(2)	3(2)	2*	2*
I	82.4	78.7	37.8	40.2	22.0	3(2)	3(2)	3	3
J	90.2	85.0	39.4	42.4	23.5	3(2)	3(2)	3	3

Burke Figure 1. Comparisons of interrelationships of families in the order Tetraodontiformes.

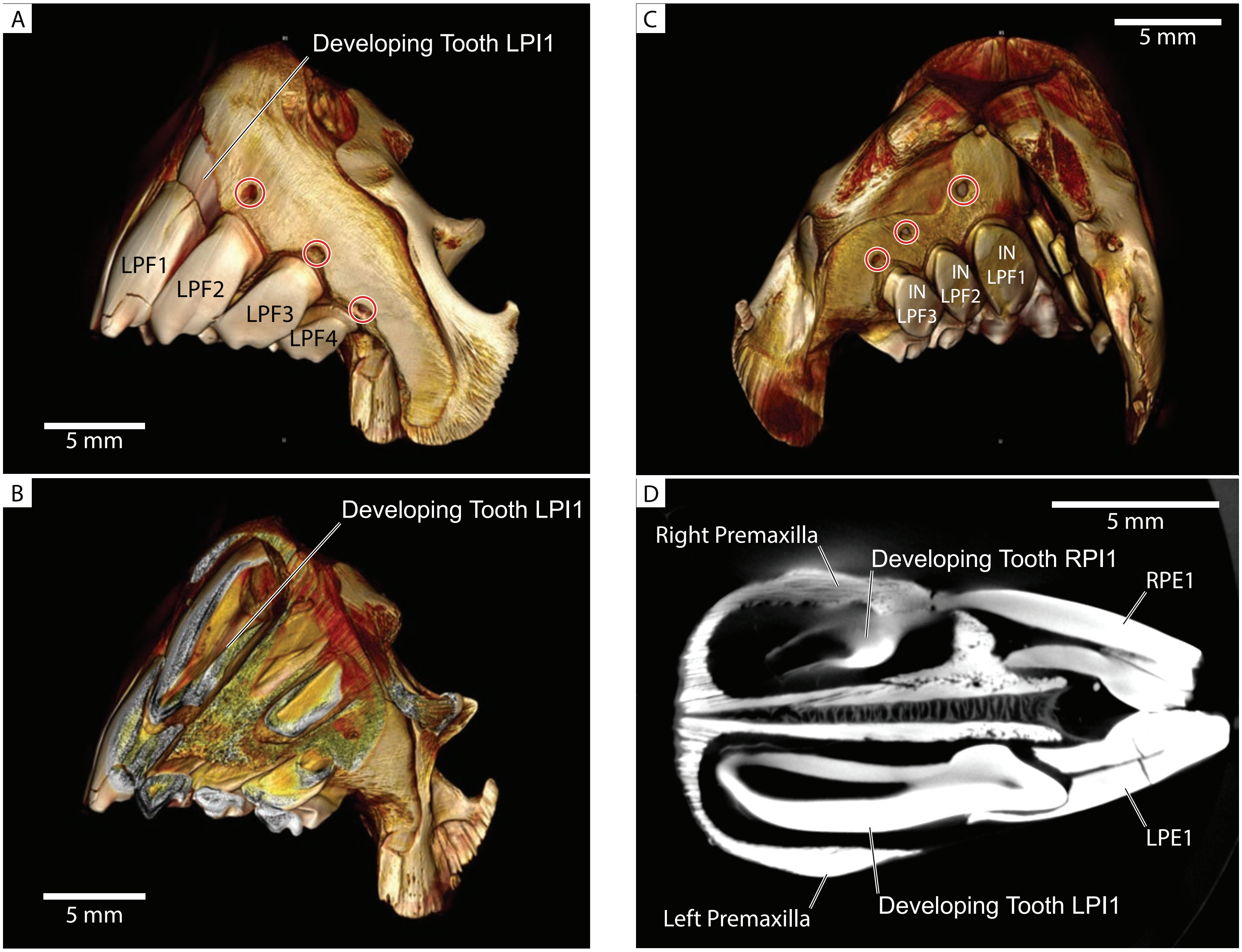


A. Based on Winterbottom (1974): morphology B. Based on Santini and Tyler (2003): morphology C. Based on Santini et al. (2013): molecules

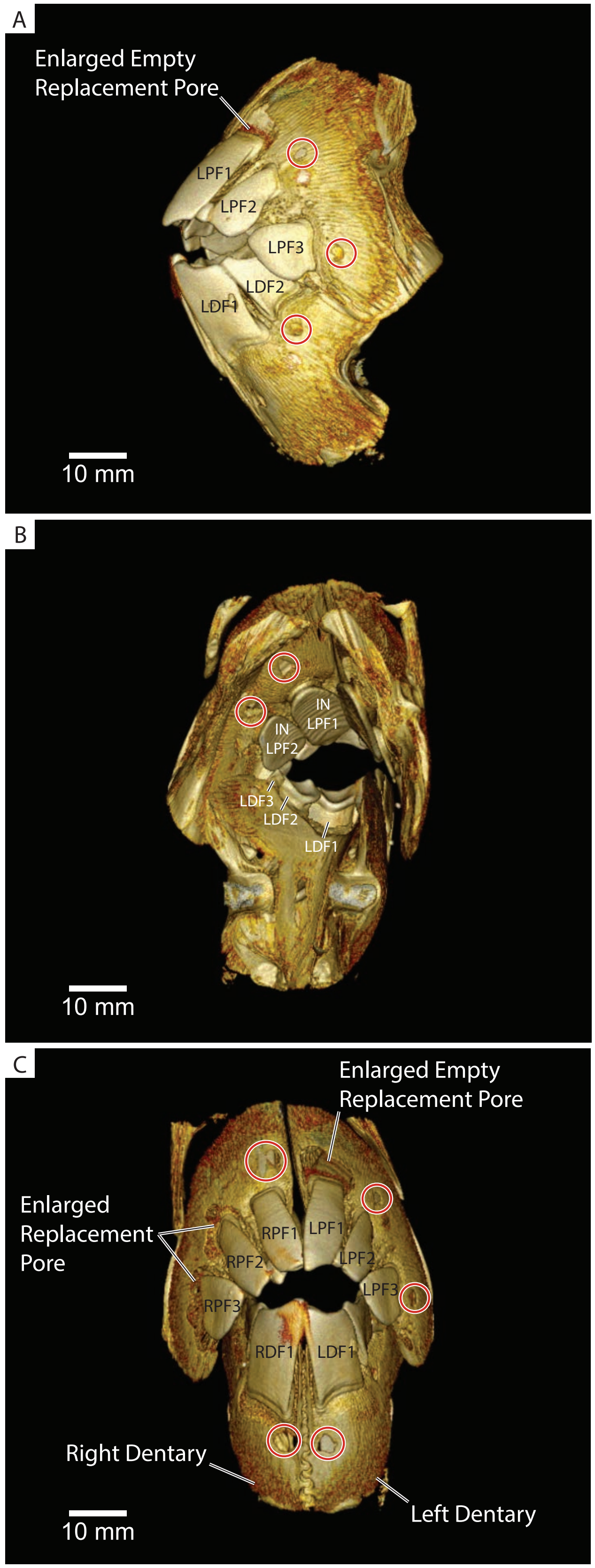
Burke Figure 2. Overview of teeth and dentition of balistoids.



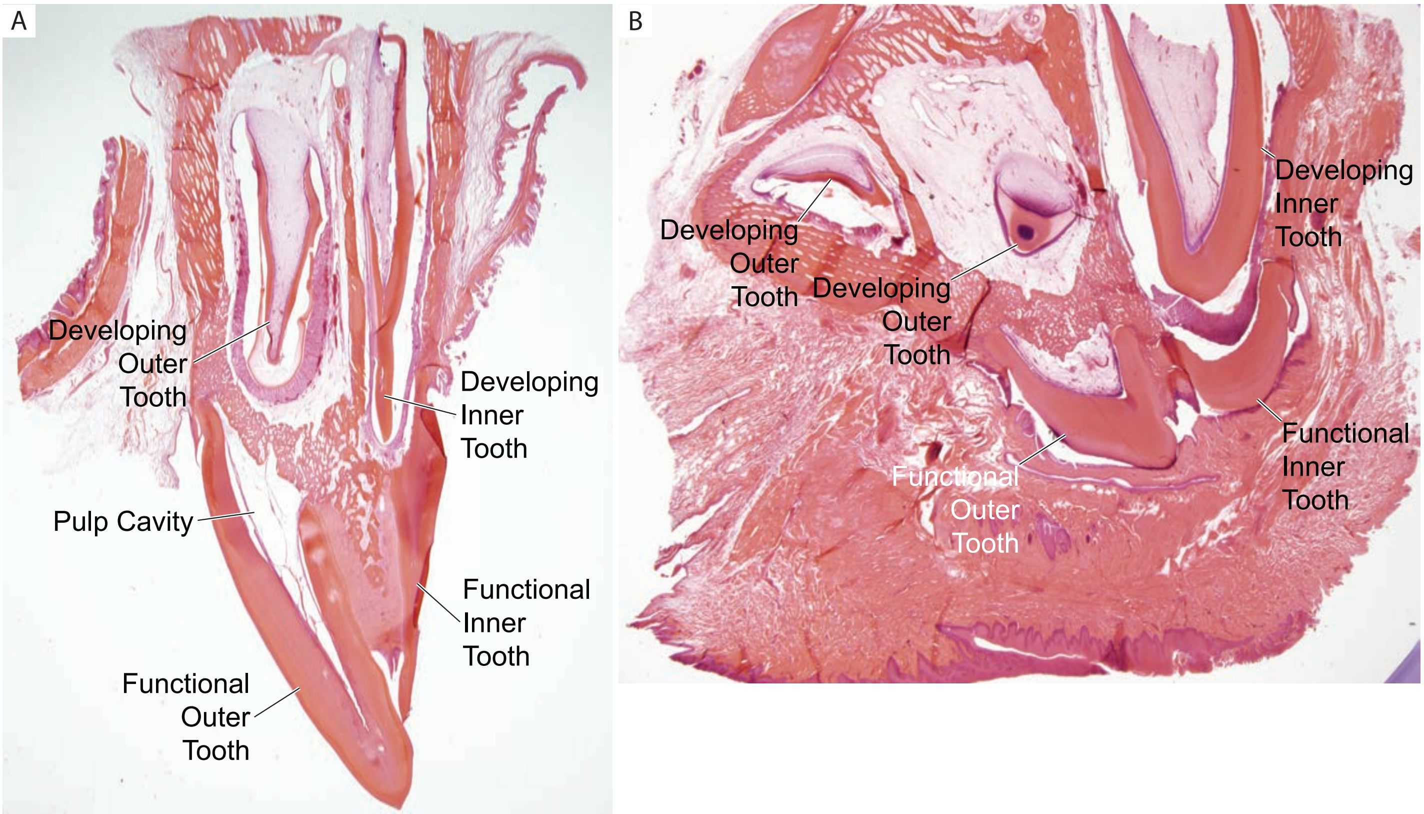
Burke Figure 3. CT reconstructions of left premaxilla of Grey Triggerfish, *Balistes capriscus* (MIR-006).



Burke Figure 4. CT reconstructions of dentition in Planehead Filefish, *Stephanolepis hispidus* (CU 34396 I).



Burke Figure 5. Histology of premaxilla in Grey Triggerfish, *Balistes capriscus*.



Burke Figure 6. Dentition of Planehead Filefish, *Stephanolepis hispidus* (CU 34396 E).