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Understanding the mechanisms of polyphyodonty: insights gained from tooth replacement in fish

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‘…any tooth or dentition can provide the foundation for a deeper understanding of a variety of processes governing multi-generational tooth odontogenesis’.

Fraser, G. J. and THIERY, A. P. 2019. Evolution, Development and Regeneration of Fish Dentitions. *In* JOHANSON, Z., UNDERWOOD, C. J. and RICHTER, M. (eds.) *Evolution and Development of Fishes*, Cambridge University Press, 160–171 pp.

‘…the durability of the teeth renders them not less available to the palaeontologist in the determination of the nature or affinities of extinct species, of whose organisation are not unfrequently the sole remains’.

OWEN, R. 1845. *Odontography; or, A treatise on the comparative anatomy of the teeth; their physiological relations, mode of development, and microscopic structure, in the vertebrate animals.* v. 1. H. Baillière, London, 1–762 pp

Abstract

Most jawed vertebrates replace their teeth throughout life (polyphyodonty) and there is a great drive to understand the developmental basis of this mechanism. The extreme diversity of fish dentitions offers rich opportunities for investigation. Here, surface feature observations and X-ray micro-CT virtual sections are used to identify tooth replacement mechanisms in fossil and modern fish, which are evaluated in light of existing research. A consensus exists that tooth replacement requires a ‘dental lamina’; an epithelial connection between predecessor and replacement tooth, which provides the putative stems cells required for long-term tooth renewal. This single epithelial connection also enables only one tooth to be replaced by one successor, at any one time. The findings herein show this is not the case in the crushing dentitions of an extinct group of fishes, the pycnodonts. Instead, tooth positioning suggests an opportunistic, gap-filling addition, where teeth fill space arising from tooth damage, loss, and the geometry of neighbouring teeth. Contrastingly, in the modern fish specimens, the mechanisms by which teeth are regenerated are recognisable. However, the crushing dentitions of seabream show occasional unusual change in tooth size, shape, and positioning, over one tooth generation. These crushing dentitions, and those of two other modern specimens, exhibit a close-packed, near-tessellating ‘anamestic’ patterning. A range of research is drawn on to propose hypotheses for these observations. In pycnodonts, I propose that gap-filling was enabled by the oral epithelium retaining an odontogenic potential throughout life, possibly facilitated by stem cells that generate taste buds. I propose that tooth positioning and morphology in pycnodont, seabream and other crushing dentitions is an adaptive phenotypic response to mechanical strain at the crushing surface, a known phenomenon in cichlids. I suggest that alternative sources of stem

cells to predecessor teeth, and mechanoreception-mediated tooth morphology and patterning, are promising areas for future study.

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1 Introduction

1.1 Background

There is currently a surge in research interest in the mechanisms which enable tooth development and replacement in fish. This has emerged for a few key reasons. One is that most fish, as well as most other non-mammalian vertebrates, continually replace their teeth throughout life (polyphyodonty). Understanding the molecular and physiological basis of this impressive regenerative capacity could enable therapies for human tooth loss, a strong research driver, as well as further our understanding of epithelial regenerative biology (Bei 2009; Thesleff & Tummers 2009; Jussila & Thesleff 2012; Fraser & Thiery 2019). Another reason is that morphological diversity of the dentitions of jawed vertebrates (gnathostomes) is vast. To understand the evolutionary and developmental biology ('Evo-devo') responsible for this variety, more diverse research models of fish have been required. This is unsurprising considering more than half of extant vertebrates are teleost fish (more than 30,000 species), and when non-teleost, bony fish (osteichthyans), and cartilaginous fish species (chondrichthyans) are also considered, 'fish' are by far the most diverse and dominant group of vertebrates (Fraser & Thiery 2019). Serendipitously, the demand for more research models coincided with the emergence of advanced genomic, cellular and developmental technologies, meaning any organism is now a potential research model for EvoDevo studies (Tucker & Fraser 2014). The great diversity of vertebrate dentitions is particularly interesting in light of the relatively new understanding of how the genetic mechanisms underlying tooth development are highly conserved across the whole vertebrate clade (Bei 2009; Jernvall & Thesleff 2012). It is a process that has remained stable over a large proportion of evolutionary time (~400 million years), and the immense variety in tooth shape, rate of development, and number of tooth generations in vertebrates has been attributed to ‘developmental tinkering’ (Lieberman & Hall 2007; Tummers & Thesleff 2009; Jernvall & Thesleff 2012; Rasch *et al.* 2016; Fraser & Thiery 2019). In fishes, this adaptability has enabled feeding in, and therefore colonisation of, nearly all global aquatic habitats, and is likely a major reason for their success. Researchers are interested in this ‘paradox’ of how extreme morphological diversity in vertebrate dentitions has occurred with highly conserved underlying genetics; what exactly occurs in the process of ‘tinkering’? And how do some fish start life with a standard, conserved dentition, and during the process of multiple tooth replacements, transform their dentition into a dramatically different morphology, such as that of the pufferfish beak? (Fraser *et al.* 2012; Jernvall & Thesleff 2012; Thiery *et al.* 2017). Detailed understanding of fish tooth replacement mechanisms is required to investigate these questions, as it is the ability to produce multiple generations of teeth itself that is thought to be the key facilitator of the extreme diversity. It is thought that repeated replacement gives more opportunity for novelty to evolve in the dentition than in other parts of the anatomy, or other ‘developmental modules’ (Tucker & Fraser 2014). This has conferred such an advantage on fish species that they have been able to adapt to their immense range of environments to feed. In the wider context of understanding the evolution of novelty, and therefore evolution generally, the dentition has been identified as a unique system for study (Tucker & Fraser 2014; Thiery *et al.* 2017).

Fish are also important for tooth replacement studies because they occupy the earliest regions of the vertebrate phylogeny. As discussed, the vast majority of today’s fish species are teleosts, which are included in the ray-finned osteicthyans:

the actinopterygians. Although teleosts are highly derived, their dentition is similar in many respects to that of basal actinopterygians. They and other fish groups are therefore vital for interpreting teeth higher in the vertebrate phylogeny, shedding light on their evolutionary history. Furthermore, a significant advantage of researching fish is that they have the oldest fossil record in the vertebrate clade, with fossils of some of the earliest vertebrates available to study. Fortunately, teeth are the most mineralised component of the vertebrate anatomy, and therefore are the most common type of vertebrate fossil (Debiais-Thibaud *et al.* 2015). With all these factors combined, we can see that fish provide great potential in understanding tooth development and replacement, and why this has recently proved such an attractive research focus.

1.2 Project aims and objectives

Due to the conservation of the genetic mechanisms underlying tooth development and regeneration, Fraser & Thiery (2019) contend that '..any tooth or dentition can provide the foundation for a deeper understanding of the variety of processes governing multigenerational odontogenesis'. This project aims to:

- Evaluate the tooth replacement mechanisms in fossil and modern fish dentitions against knowledge of tooth replacement mechanisms arising from recent EvoDevo and historical research.
- Identify replacement mechanisms which are unexplained or little understood, and therefore do not correspond with current knowledge, and where appropriate, propose alternative developmental hypotheses. Such findings may open up new lines of enquiry for the EvoDevo research community.

- Identify replacement mechanisms which correspond to/are consistent with known mechanisms. These will further our understanding of established, known replacement mechanisms, either by confirming their presence in new individuals of a species, or by revealing their occurrence in other taxa.
- Create new, publicly accessible x-ray microcomputed tomography (XCT) data and images of fossil and modern fish dentition specimens. This data will be available for future investigation as understanding of the mechanisms of polyphyodonty develop over time.

The dentition specimens were investigated using traditional observation of surface features and also x-ray microcomputed tomography (XCT), to create virtual sections within the specimens. These data aimed to capture the tooth replacement mechanisms that were present *in vivo.*

1.3 Overview of the selection of study specimens

Dentition specimens were selected for study for various reasons, in order to address the study's aims. The decision for each selection is detailed in Chapter 3. Broadly, specimen selections were:

- dentitions of species which had previously been shown to exhibit unusual tooth replacement, apparently unexplained by current knowledge of replacement mechanisms. These were highlighted in the literature before the emergence of XCT techniques; XCT technology was used here to investigate their tooth replacement mechanisms in more depth than had previously been possible (fossil species *Pycnodus zeaformis*, *Pycnodus maliensis* and other species from the order Pycnodontiformes for comparison).

- dentitions of modern species with functional morphology that was analogous to that of the unusual fossil specimens, and therefore their replacement mechanisms were hypothesised to also be of interest (*Pogonias cromis*, *Labrus bergylta* and a representative of the family Sparidae). The sparid specimen exhibited an intergenerational tooth-shape change that was greater than that explained by the literature, therefore further sparid dentitions were also studied for comparison.

- dentitions of species that are known EvoDevo research model species, thereby testing research outcomes in new individuals and against new XCT data (*Salmo salar*, *Polypterus senegalus, Gasterosteus aculeatus*).

  Investigation of these species could also potentially contribute to a hypothesis for a tooth replacement mechanism in pycnodonts, due to their reported ‘lamina-less’ tooth replacement.

- dentitions of species whose tooth replacement mechanisms have yet to be investigated, and are of interest in the context of known tooth replacement mechanisms. As tooth replacement is generally understood to be orderly and controlled, specimens of *Amia calva* and *Gadus morhua w*ere selected, as their tooth patterning has been described as ‘random’ and ‘haphazard’ respectively (sections 3.9 and 3.11). *Amia calva* is also phylogenetically located within the Holostei, sister group to the Teleosts, therefore potentially providing some evolutionary context to the teleost dentitions studied. The pycnodonts are phylogenetically located between the Polypterids (*P.senegalus* is studied here) and the Amiids, therefore the selection of *Amia calva* also potentially provides some evolutionary context to the pycnodonts’ tooth replacement.

1.4 Outline of thesis

In order to effectively compare a specimen's tooth replacement mechanism with those revealed by existing research output, it is necessary to initially summarise this output, i.e. what we currently know about how teeth develop and are replaced. This knowledge has developed significantly in the last 30 years, owing to molecular and cellular research into how vertebrate teeth development initiates and then progresses to functionality, as well as how teeth are replaced, or continually replaced. Chapter one (the current chapter) presents the background as to why fish are particularly interesting in this field, describes the aims of this study, and gives a brief overview of how specimens were selected to achieve these aims.

Chapter two provides an overview of what we know about vertebrate tooth development and replacement, and what is still to be understood, drawing on a wide range of key research literature. Background information on the pycnodont order is included, and why they were identified as unusual and interesting in their tooth replacement, and therefore selected for study.

Chapter three presents the study's material and methods; a description of the sourcing and selection of the study specimens, and the techniques used to analyse their surface features and internal structure.

Chapter four, the results section, comprises descriptions of the specimens' surface features and virtual sections obtained via XCT, including any tooth replacement mechanism identified.

Chapter five comprises interpretation and discussion of the results; this includes identifying known and unexpected tooth replacement mechanisms or features, with hypotheses proposed to explain the latter, where possible.

Chapter six summarises key findings from the interpretation and discussion arising from the specimens' analyses and draws general conclusions. Proposals for further work are suggested.

2 Tooth development and replacement

2.1 Why are there teeth? The adaptive advantage of teeth, and their evolutionary origin

The importance of teeth in the evolution of vertebrates cannot be overestimated; teeth considerably increased the efficiency of the jaws in feeding, providing a significant survival advantage. Since teeth first appeared, they have undergone a great variety of morphological and structural adaptations which assist in a range of food-processing functions: cutting, slicing, grinding, crushing, scraping, grasping and piercing (Berkovitz & Shellis 2017), the latter two functions often enabling the efficient killing of prey. The existence of more than one type of tooth morphology in the same dentition (heterodonty) allows for combinations of functions, providing even greater efficiency in obtaining and processing food.

2.1.1 The developmental and evolutionary origin of teeth

The utility of teeth for acquiring and processing food could lead us to assume that teeth have only ever occurred in the mouth, oral jaws, or pharyngeal jaws of the pharynx (collectively the 'oropharyngeal cavity'), as observed in the majority of today's living vertebrates. However, tooth-like structures can develop in the oropharyngeal cavity as teeth, and in the skin as denticles, also known as placoid scales. As summarised by Donoghue & Rücklin (2016), Ørvig (1977) identified teeth and skin denticles (dermal denticles) as being derived from a homologous developmental unit, which he named the odontode, a term which has since been popularly used. All odontodes share the same basic structure; Fraser *et al.* (2010) summarise Ørvig's description (1977) of odontodes as all structures that comprise a mineralised hard tissue unit, consisting of attachment bone, dentine or similar dentinous tissue, sometimes with a superficial layer of enamel/enameloid, formed from a single papilla.

Odontodes are a type of epithelial appendage, originating from the ectoderm embryonic layer (although see section 2.1.2 for discussion concerning debated endodermal origin). Other ectodermal epithelial appendages include hair follicles, teeth, scales, feathers, and mammary, sweat, and salivary glands (Biggs & Mikkola 2014) .

All epithelial appendages share an origin as placodes: local thickenings of epithelium overlying condensed mesenchyme (Ørvig 1967). Their stages of development are strikingly similar, the first visible sign of development being the thickening of the epithelium to form the placode, with the condensing mesenchyme beneath it forming the papilla. Next the placode buds into (e.g. tooth, hair, mammary gland), or out of (e.g feather) the mesenchyme, comprising the bud stage. The epithelium has diverse patterns of growth that give rise to the varied types of epithelial appendage, with epithelial folding and branching determining the appendage or organ's final shape (for reviews see Pispa & Thesleff 2003; Biggs & Mikkola 2014; Dhouailly *et al.* 2019). However, molecular signalling instruction for this epithelial patterning and eventual shape appears to come from the mesenchyme (Pispa & Thesleff 2003). Across the diversity of epithelial appendage types, consistent is a molecular 'cross-talk', or reciprocal and sequential interaction between epithelium and mesenchyme, which regulates development, and is crucial for successful morphogenesis (Pispa & Thesleff 2003; Biggs & Mikkola 2014; Dhouailly *et al.* 2019). Section 2.2 describes the key stages of tooth morphogenesis. Specific to odontode development among the epithelial appendages is the production of mineralised tissues: dentine or dentine-like tissue and enamel or enameloid.

The shared developmental origins of the many and varied epithelial appendages has led them to be generally accepted as homologous structures, although they vary in mature form, function and regenerative capacity (Pispa & Thesleff 2003; Biggs & Mikkola 2014; Dhouailly *et al.* 2019; Hulsey *et al.* 2020). This homology hypothesis is supported by genetic data which show the same genes (or their orthologues) are involved in the development of diverse epithelial appendages, sometimes in species separated by several hundred millions of years of evolution (Hulsey *et al.* 2020). The example frequently given (e.g. Pispa & Thesleff 2003; Hulsey *et al.* 2020) is that of Ectodyplasin (Eda) and its receptor Edar, single mutations of which cause loss of both teeth and hair, among other symptoms, in mice, dogs and humans (Sadier *et al.* 2014). Remarkably, mutations of their orthologues in reptiles and fish also affect scale development (Kondo *et al.* 2001; Harris *et al.* 2008; Di-Poï & Milinkovitch 2016).

A popular model for how these homologous structures evolved is that of co-option; the gene regulatory network responsible for developing one structure such as a tooth or scale was later ‘repurposed’ elsewhere (Martin *et al.* 2016; Hulsey *et al.* 2020) e.g. the proposal that gene regulatory networks producing reptilian scales were co-opted in the development of feathers and mammalian hair (Chang *et al.* 2009; Hulsey *et al.* 2020).

It is the principle of co-option which is thought to have resulted in teeth and skin denticles. However, as summarised by Fraser & Thiery (2019) and Berkovitz & Shellis (2017), it has been a matter of contention for over two decades as to which evolved first. Specifically, whether denticles first evolved in the skin of jawless fishes and were co-opted into the mouth and then the pharynx of jawed fishes (the ‘outside-in’ hypothesis), or teeth first evolved independently from denticles in the oropharyngeal cavity, before the origin of jaws, and were later co-opted to the jaws (‘inside-out’ hypothesis). In addition, proponents of the ‘inside-out’ hypothesis proposed that teeth evolved more than once (see section 2.1.3). Recently, less evidence has been put forward for ‘inside-out’. Fraser & Thiery (2019) contend that at least a palaeontological consensus seems to be emerging in support for ‘outside-in’, citing Donoghue & Rücklin (2016), who present a summary of counter-evidence for each key piece of evidence presented for ‘inside-out’. This approach was also taken by Witten *et al.* (2014). Berkovitz & Shellis (2017) cite both these reviews of the evidence, as well as Benton (2015), to conclude: ‘the balance of opinion supports the classical view: teeth evolved from dermal odontodes in jawed vertebrates and they evolved only once’. The lack of counter-argument recently forthcoming appears to substantiate this claim. However, it is notable that some key conclusions drawn by Donoghue & Rücklin (2016) rest on phylogenetic evidence, and are therefore arguably vulnerable to future phylogenetic revisions. An overview of the debate is given here.

2.1.2 The ‘inside-out’ vs. ‘outside-in’ debate on the evolutionary origin of teeth

The ‘outside-in’ hypothesis, that denticles first evolved in the skin of jawless fishes and were co-opted to a jaw location and a tooth function (Reif 1982), is considered to be the traditional, classical hypothesis for the origin of teeth (Donoghue & Rücklin 2016). This is consistent with the favoured, general model for the developmental origin of epithelial appendages previously outlined; gene regulatory networks that produce one appendage (e.g. skin denticles) are co-opted at another anatomical site, resulting in another (e.g the mouth and pharynx) (Hulsey *et al.* 2020). Witten *et al.* (2014) explain the ‘outside-in’ hypothesis as: ‘teeth derive from odontogenically competent ectoderm [of the skin] that expanded into the mouth cavity and interacted with competent neural crest-derived mesenchyme’ (see section 2.2 for more detail regarding the ectodermal/mesenchymal reciprocal interactions of tooth development). The ‘outside-in’ hypothesis is generally considered to include the subsequent transference of the ectoderm’s odontogenic competence to the endoderm of the pharynx, enabling the development of pharyngeal teeth or denticles (Rücklin *et al.* 2012) .

The ‘outside-in’ hypothesis was safely the consensus view up until it was first challenged by Smith & Coates (1998). They proposed that teeth appeared before jaws, developing from the endoderm of the pharynx, rather than ectoderm of the skin external to the mouth (Smith & Coates 1998, 2000, 2001) . Furthermore, Smith & Johanson (2003), proposed that teeth evolved independently in placoderms, chondrichthyans and osteichthyans.

The previous, longstanding and undisputed consensus supporting the ‘outside-in’ hypothesis rested confidently on the similarity in both the structure and developmental process of teeth and skin denticles (see e.g. Ørvig 1977; Reif 1982; Huysseune & Sire 1998), combined with fossil evidence that skin denticles appear earlier in the vertebrate phylogeny than jaws, and therefore teeth (see e.g. Donoghue & Sansom 2002; Donoghue & Rücklin 2016). The significant similarity in structure and development is what unites skin denticles and teeth as the mineralised epithelial appendages that are odontodes, as described and named by (Ørvig 1977) (see above, section 2.1.1). Fig. 2.1, compiled by Donoghue & Rücklin (2016), details the distribution of skin denticles, oral/pharyngeal denticles and teeth/tooth-like structures across the vertebrate phylogenetic tree, drawing on key vertebrate phylogenies constructed since 2000. This clearly demonstrates how early in the vertebrate phylogeny skin denticles appeared, long before the evolution of jaws. The terminal groups on the cladogram that exhibit skin denticles, prior to the evolution of jaws in the placoderms, collectively comprise the 'ostracoderms' (literally 'shell-skins'). The term refers to their dermal armour, and ostracoderms are commonly known as the armoured jawless fish (Benton 2015) . Pertinent to the 'outside-in'/inside-out debate, contained within the ostracoderms are the thelodonts (see below).

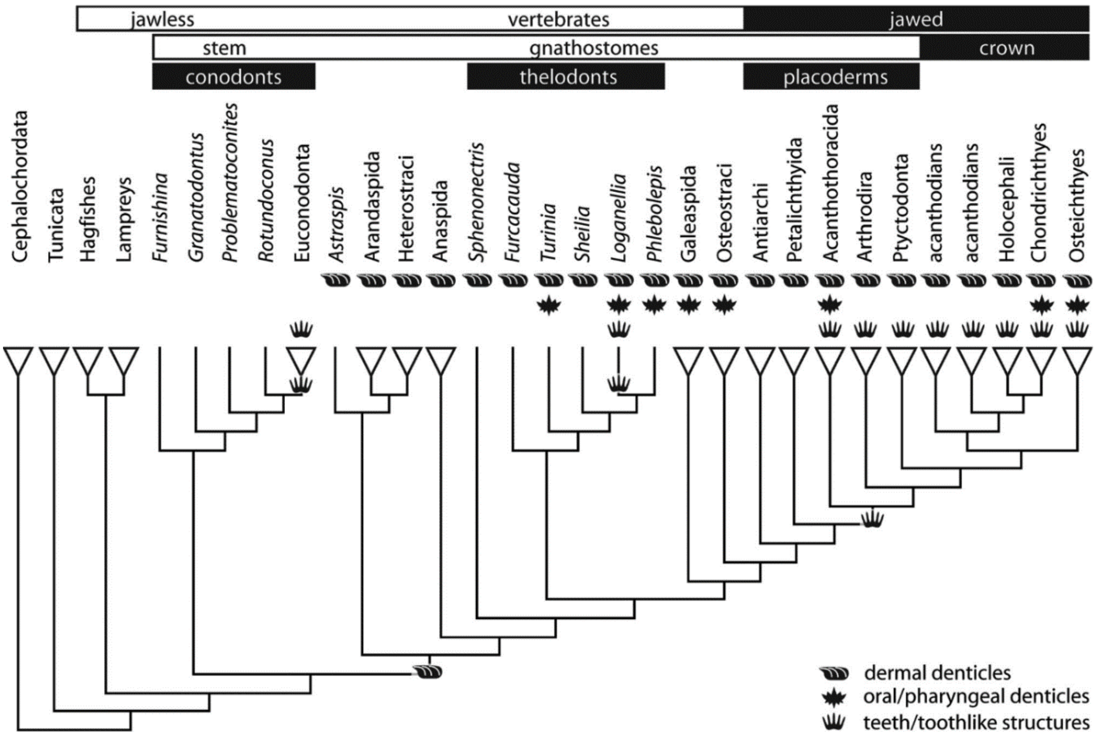


Fig. 2.1 Distribution of dermal denticles, oral/pharyngeal denticles and teeth/tooth-like structures, plotted on a phylogenetic tree of vertebrates. Taken from Donoghue & Rücklin (2016).

2.1.3 Fossil evidence challenging the classic 'outside-in' hypothesis

Three key pieces of fossil evidence significantly destabilised the 'outside-in' hypothesis. Foremost was the re-interpretation of a group of extinct eel-like animals, the conodonts, from invertebrates to jawless vertebrates (Donoghue *et al.* 2000). The conodonts exhibit tooth-like elements comprised of dentine- and enamel-like tissues, identified with certainty as dentine and enamel by Donoghue & Sansom (2002).

Importantly, conodonts appear tens of millions of years before the emergence of a dermal skeleton (Donoghue & Rücklin 2016), and therefore skin odontodes/denticles. As such, it was reasoned that the conodont fossils comprised evidence that tooth-like structures were present in the vertebrate oropharyngeal cavity long before skin denticles evolved. Tooth evolution from skin denticles (the ‘outside-in hypothesis) was therefore impossible, and the competence to develop teeth must have later been co-opted from the pharynx anteriorly to the oral jaw margins, in the gnathostomes (Smith & Coates 1998, 2000, 2001; Smith 2003).

However, evidence of how eucondonts (true conodonts) evolved from within the group the paraconodonts has broken the line of descent linking the enamel-bearing tooth like elements of the eucondonts to the jawed vertebrates (Murdock *et al.* 2013). The tooth elements of paraconodonts consist only of dentine, or a dentine-like substance. Therefore, the feature of the enamel cap is an innovation specific to the paraconodont/conodont phylogenetic branch, and so it cannot have been present in the most recent common ancestor of the eucondonts and the jawed vertebrates. Euconodont dentine (or a dentine-like substance) however could have been in inherited through paracondonts from such a shared ancestor with jawed vertebrates. However, evidence for the presence of dentine in paracondonts is thought to be unsafe, as this relies on identifying its associated development with enamel (or an enamel-like substance), which is absent. Therefore overall, the use of conodonts as evidence that teeth or toothlike elements first evolved in the vertebrate mouth, long before the evolution of skin denticles, is undermined by the severance of the ancestral link between their enamel- and dentine-like tissues and those of the jawed vertebrates; they cannot be homologous, and have evolved independently and convergently. With this, important support for the inside-out hypothesis is lost. In addition, Blieck *et al.* (2010) and (Turner *et al.* 2010) strongly contend that conodonts should not even be placed within the vertebrate phylogeny, due to an extremely low level of cephalisation and the lack of a dermal skeleton, among other problems with, or absences of key diagnostic vertebrate characters. Turner *et al.* (2010) also describe in detail many problems with the concept of conodont tooth elements being homologous with odontodes and teeth, including their comparative structure, fabric and topology.

The second source of fossil evidence presented in support of the 'inside-out' hypothesis drew on the discovery by Van der Brugghen & Janvier (1993) of oropharyngeal denticles in the thelodont *Loganellia scotica.* Thelodonts are members of the ostracoderms, the jawless, armoured fish. Therefore, the oropharyngeal denticles of *L. scotica* were suggested to be another example of evidence for tooth-like structures evolving before, and independently of jaws (Smith & Coates 1998, 2000). In addition, the arrangement and patterning of the pharyngeal denticles are also interpreted to be evolutionarily significant. Van der Brugghen & Janvier (1993) refer to the denticles as similar to tooth whorls that occur in palaeozoic sharks, and Donoghue & Rücklin (2016) describe how they are 'aggregated into compound scales through accretionary growth', and that those within the pharynx are aligned in parallel, orientated in the same direction. As such, thelodont 'tooth whorls' are interpreted as for foreshadowing the orderly replacement pattern manifest as files (including whorls) of replacement tooth families in chondrichthyans. The patterning and replacement in these phylogenetically distant groups, and the regulatory systems producing them, are viewed as homologous (Smith & Coates 2001; Smith 2003). It is proposed that like the teeth themselves, tooth patterning and successional replacement mechanisms present on the jaws of gnathostomes were co-opted from the pharyngeal endoderm of jawless fish, and not from the ectoderm of the skin (Smith 2003).

Although the evidence for the ‘inside-out’ hypothesis presented by thelodont fossils appears compelling, Donoghue & Rücklin (2016) state that more often than not, the pharyngeal denticles of *L. scotica* specimens are not arranged in an organised pattern, aligned in the same direction, but much more variable orientations. However, their main refutation of the fossil evidence lies again in phylogenetics. *L. scotica* and the jawed vertebrates do not share a most recent common ancestor that had oropharyngeal denticles, or tooth whorl-like structures in the pharynx. As was the case with the conodonts, thelodonts with these features are derived within their clade. Therefore the development of oropharyngeal denticles and successional tooth replacement in the tooth whorl-like structures of the thelodont pharynx, are not homologous with the teeth and replacement mechanisms of the jawed vertebrates (gnathosotomes), but both are a consequence of convergence (Rücklin *et al.* 2011; Donoghue & Rücklin 2016).

The third piece of fossil evidence to destabilise the ‘outside-in’ hypothesis came from the earliest known jawed vertebrates, the extinct placoderms. Historically, this group of armoured fish have most often been considered the monophyletic sister group to extant jawed vertebrates (the chondrichthyans and osteichthyans) (e.g. Janvier 1996; Goujet 2001). The placoderms have also generally been considered to be toothless – ‘edentate’- making do with bony elements of their jaws functioning like teeth, with bony fangs occluding into grooves. However, toothlike structures have been found and described on the oral jaws of the most derived placoderms, the arthrodires, exhibiting successional replacement and composed of dentine (Johanson & Smith 2003, 2005; Smith & Johanson 2003). A pulp cavity, vasculature and bone of attachment revealed by XCT has reinforced their identification as teeth (Rücklin *et al.* 2012). The teeth lack a hypermineralised enamel or enameloid layer or 'cap', but this is possibly due to its secondary loss in the most derived species of placoderms (Giles *et al.* 2013). Furthermore, new teeth were added to the jaws in an organised way in rows, indicating that the arthrodires had mechanisms for regulating and patterning teeth (Smith & Johanson 2003). Proponents of the inside-out hypothesis contended that as the jaws of the more basal placoderms are edentate, the teeth present on the oral jaws of derived placoderms are evidence that teeth evolved independently within the placoderm clade. They interpret that the oral teeth were co-opted from the endoderm of the pharynx and not the ectoderm of the skin. This was due to the finding that denticles patterned in rows were identified in part of the pharyngeal cavity of all the major placoderm taxa, differing significantly to denticles on the skin external to the mouth, in both morphology and arrangement. This co-option from pharynx to oral jaws was also proposed to have occurred in the crown gnathostomes, with the placoderm pharyngeal patterning compared to that of the pharyngeal tooth whorls of the thelodont *L. scotica.* These co-option events were proposed to have occurred separately in placoderms, acanthodians (extinct crown gnathostomes), osteichthyans and chondrichthyans. Therefore, teeth on the oral jaws in these groups had evolved independently and convergently, from edentate ancestors in each lineage.

As with the other two lines of significant fossil evidence, this proposal has also been contested and refuted. This was on the grounds that the pharyngeal denticles of the placoderms bear no structural similarity to teeth, lacking at least a dentine-like substance, and being composed only of bone (Rücklin *et al.* 2012). It was argued that they therefore cannot be considered as odontodes, thus cannot have been co-opted convergently as teeth to the jaws of the placoderms and key lineages of crown gnathostomes. In addition, the phylogeny of the placoderms has been revised as polyphyletic, placing the arthrodires, previously thought to be one of the most derived placoderms in a monophyletic clade, as more closely related to the crown gnathostomes than all other ‘placoderms’ (Donoghue & Rücklin 2016, and references therein). As such, and as the only placoderms to possess teeth on the jaws, the teeth of the arthrodires and the oral and pharyngeal denticles and teeth of the crown gnathostomes are interpreted to be homologous; they evolved as the odontogenic capacity of external skin extended into the jaws and then the endoderm of the pharynx (Rücklin *et al.* 2012)

Fraser & Thiery (2019) contend that overall, at least a palaeontological consensus appears to have emerged in favour of an external origin of odontodes before oropharyngeal teeth i.e. ‘outside-in’. Perhaps in line with this, Smith and Johanson, early and key proponents of ‘inside-out’, refer to the revised phylogenetic position of the arthrodires and suggest that teeth may have a single origin at the node connecting arthrodires and crown gnathostomes (Smith & Johanson 2012). However, they caution the reader to note that teeth may have evolved independently in chondrichthyans, due to their teeth evolving millions of years after an early history dominated by scales.

In summary, fossil evidence for the ‘inside-out’ hypothesis has not been universally accepted and the consensus appears to lean towards ‘outside-in’. However, evidence provided by evolutionary developmental studies has contributed a further important dimension to the debate, referred to as the ‘inside *and* out hypothesis’.

2.1.4 The ‘inside *and* out’ hypothesis for the origin of teeth

Fraser *et al.* (2010) presented a review of data demonstrating that odontodes develop from ectodermal and endodermal epithelia, and a combination of both, when combined with mesenchyme derived from migrated neural crest cells (ectomesenchyme). This therefore renders void the concept of either the ectoderm of the skin, or the endoderm of the pharynx, conferring by co-option odontogenic potential to the oral jaws (and in the case of ‘outside-in’, also to the endoderm of the pharynx): co-option being central to both ‘outside-in’ and ‘inside-out’ hypotheses. In addition, despite some differences, teeth mainly develop using a common set of genes, from whichever germ layer they derive (Fraser *et al.* 2009, 2010). Fraser and co-workers (Fraser *et al.* 2010) emphasise that rather than the germ layer or location, what is important when considering the origin of teeth is the signalling ‘interplay’ between the genes (or gene regulatory networks: GRNs) of the epithelium and underlying ectomesenchyme. Teeth develop wherever these GRNs are expressed and interact; their hypothesis was thus named ‘inside and out’. This was predicted to also be the case for all odontodes. Later Debiais-Thibaud *et al.* (2011) and Martin *et al.* (2016) provided compelling evidence for this. They showed that the skin denticles and teeth of an extant shark (the small-spotted catshark, *Scyliorhinus canicula*) have many of the same genes directing their development, comprising a core odontode GRN (oGRN). As such it was suggested that ‘all odontodes share a deep molecular homology’ (Martin *et al.* 2016). Concordant with this and relating to a wider evolutionary context, conserved epithelial and mesenchymal GRNs are suggested to result in a deep homology of all vertebrate epithelial appendages

(Cooper *et al.* 2017, 2023; Hulsey *et al.* 2020), in their many and varied forms (section 2.1.1), including the different odontode types. With the ‘inside and out’ hypothesis, Fraser *et al.* (2010) aimed to shift the debate away from which type of odontode evolved first and its location in the skin or oropharynx. Rather, they propose that both skin and oropharyngeal odontodes share a deep molecular homology, enabling both to develop wherever an epithelial GRN came into contact with a neural crest-derived ectomesenchymal GRN in the underlying mesenchyme, forming an oGRN. Skin odontodes met a need for a protective function, and orophyngeal odontodes food processing and predatory functions. It may not be possible to determine which of these evolved first (Fraser *et al.* 2010). Arguably, acceptance of a shared deep molecular homology circumvents the need to attribute one type of odontode as evolving from the other. In terms of odontode evolution, this ‘inside and out’ hypothesis focusses on the oGRN as the homologous unit, rather than focussing on epithelial cell types. The oGRN underpins the developmental module of the odontode, on which selection operates, resulting in oropharyngeal odontodes adapted for feeding (teeth) and skin odontodes adapted for protection and armour (denticles); see Stock (2001) for an overview of developmental modularity relating to the dentition.

2.1.5 Have taste buds enabled the successional replacement of teeth?

A key character that has long been viewed to set teeth apart from skin odontodes is the ability to successionally replace in an orderly manner, with teeth often organised into families, in readiness to replace functional teeth (Johanson & Smith 2003, Smith *et al.* 2009*a*; Fraser & Smith 2011; Tucker & Fraser 2014; Martin *et al.* 2016; Fraser *et al.* 2020). Contrastingly, skin odontodes do not have such a system of successional regeneration and are added to fill expanding areas during development and growth, or are replaced on demand when lost or damaged (Reif 1978, Smith *et al.* 2009*a*; Maisey & Denton 2016; Martin *et al.* 2016; Cooper *et al.* 2017, 2018). Although Martin *et al.* (2016) showed that most of the skin odontode and tooth oGNR genes studied in the catshark *S. canicula* were the same and so evolutionarily highly conserved, a conspicuous difference in this species (and also in the 'little skate' ray *Leucoraja ericacea*), was the strong expression of the gene *sox2* in developing tooth odontodes, but no expression in skin odontodes. They identify Sox2 as a known regulator (and therefore marker) of adult epithelial stem cells, which in turn are known to be essential for the lifelong generation of successional teeth (Martin *et al.* 2016, references therein, and see section 2.5.1). As successional regeneration does not occur in skin denticles, the presence of Sox2 is proposed as a marker to differentiate between teeth and skin odontodes.

Martin *et al.* (2016) ask the question of how *sox2* 'became incorporated' into an 'otherwise identical oGRN' of the skin denticle. In the context of the long-running inside-out/outside-in debate, this perhaps rather presumptuously implies an evolutionary sequence of teeth deriving from skin denticles. They suggest that the incorporation of *sox2* into the oGRN facilitated the presence of a sustained epithelial stem cell population in odontodes, which in turn enabled their successional regeneration, thus becoming teeth. Though arguably a bold suggestion, Martin *et al.* (2016) also propose a compelling hypothesis for the mechanism by which this occurred. They describe how in the catshark *S. canicula* a field of epithelial cells expressing *sox2* ('Sox2+' cells) gives rise to the development of both teeth *and* the most anterior rows of taste buds. As such the cells are termed 'Sox2+ progenitor cells', and their location/field 'the odontogustatory band' (OGB). This expands on the established term of 'the odontogenic band', as it delineates the epithelial region from which not only teeth will develop, but also the anterior taste buds. A description of tooth development and successional replacement in *S. canicula* is given in section 2.5.9.

Like teeth, taste buds are epithelial appendages that are renewed throughout life, and in mammals, studies have shown that taste buds are also continually renewed from populations of Sox2+ stem cells (Beidler & Smallman 1965; Okubo *et al.* 2006, 2009). Taste buds evolved long before teeth at the emergence of the earliest vertebrates, and today are present in extant gnathostomes and the extant jawless vertebrate, the lamprey (Kirino *et al.* 2013). Interestingly, taste buds originated in the endoderm, and this is the case in extant mice, in which taste bud progenitor cells arise from the pharyngeal endoderm (Okubo *et al.* 2006). Martin *et al.* (2016) propose that the common origin in *S. canicula* of *sox2*-expressing tooth and taste bud progenitors from the same epithelial field, the OGB, suggests that they are linked developmentally. They suggest that evolutionary precursors of the progenitors that enable odontodes to continually regenerate, could have been derived from precursors of those that enable taste buds to continually regenerate. They suggest that the oGRN was integrated into the sox2-modulated GRN of taste buds (tGRN), when odontodes extended their range into the oral cavity through evolution, an example of a heterotopic shift. This therefore provides the mechanism by which odontodes acquired *sox2* into their oGRN, enabling the presence of permanent epithelial stem cell populations and successional regeneration, engendering the developmental innovation of teeth.

Martin *et al.* (2016) maintain that their proposed mechanism does not resolve the problem of whether the first odontodes were skin odontodes (outside-in) or from within the pharynx (inside-out) before their heterotopic shift to the jaws. However, if the latter were true, the homology of skin denticles, oral teeth and taste buds does not appear to be accounted for. It implies that *sox2* was lost, rather than incorporated into the oGNR in the development of skin odontodes. This would in turn imply that skin denticles evolved after taste-linked evolution of oral teeth, which according to the summary of vertebrate phylogenies assembled by Donoghue & Rücklin (2016) is clearly not supported by the fossil record. Or alternatively, the extremely similar oGRNs of the oral teeth and skin denticles evolved independently, which in view of the level of molecular homology could be considered unlikely. Overall the work of Martin *et al.* (2016) linking taste buds to teeth appears to give weight to the 'outside-in' hypothesis, support for which had, as discussed previously, perhaps already achieved the balance of opinion (Witten *et al.* 2014; Benton 2015; Donoghue & Rücklin 2016; Berkovitz & Shellis 2017; Fraser & Thiery 2019). However, the fossil evidence presented for the inside-out hypothesis sparked over 20 years of research and vigorous debate, and arguably the counter-evidence outlined in this section draws heavily on phylogenetic evidence that could conceivably be revised in future.

2.1.6 Evidence from stem osteichthyans, stem gnathostomes and early sarcopterygians

Relatively recently, fascinating analyses of fossils of stem osteichthyans *Andreolepis* (Chen *et al.* 2016, 2017) and *Lophosteus* (Chen *et al.* 2020), as well the Devonian sarcoptergygians *Oncychodus* (Doeland *et al.* 2019) and *Powychthys* (King *et al.* 2021) have shown that in these very early fishes, the same dentigerous bone exhibited both teeth and oral denticles (in each report, denticles are more generally named odontodes). This was also observed in the Devonian sarcopterygian *Holodipterus gogoensis*, many years earlier (Campbell & Smith 1987). Teeth are distinguished from the other odontodes as those which show evidence for repeated resorption and shedding at that same tooth position (see section 2.1.5), indicating a recognisable one-for-one replacement, summarised diagrammatically across the study specimens in King *et al.* (2021). In each case, the other odontodes/denticles simply grew over one another, or exhibited tooth addition at the dentition margins. Very interestingly in terms of the much-debated origins of teeth, King *et al.* (2021) postulate that the progression from non-replacing odontodes to replacement revealed by resorption/shedding (therefore, to 'teeth') appears gradual, and is selected for in parts of the bone where space was restricted. This evolutionary sequence is supported by the study of even earlier species, the Early Devonian stem gnathostomes, the acanthothoracids. Vaškaninová *et al.* (2020) showed these exhibited odontode overgrowth and addition, but no evidence of replacement, and therefore teeth.

2.2 How teeth develop

The basic steps of tooth morphogenesis - the process by which tooth formation is controlled and tooth cells are spatially distributed and differentiated during development - are similar across all vertebrates (Luckett 1993; Huysseune & Sire 1997, 1998; Jernvall & Thesleff 2012) , and were first described well over 100 years ago (Owen 1845; Leche 1895). We now know that tooth morphogenesis is regulated by complex epithelial and mesenchymal signalling interactions, a two-way, molecular cross-talk between the two tissue types (Thesleff & Sharpe 1997; Jernvall & Thesleff 2000; Bei 2009; Tummers & Thesleff 2009). The mesenchyme comprises neural crest cells which have migrated from the margins of the neural tube into the jaws. It is therefore ectodermal in origin, and so is more accurately termed ‘ectomesenchyme’ to indicate this. The oral epithelium can be endodermal and/or ectodermal in origin, depending on the species (Smith 2003; Soukup *et al.* 2008; Fraser *et al.* 2009).

2.2.1 Where teeth develop

Teeth develop from either the oral or pharyngeal epithelium; therefore within what is known as the oropharyngeal cavity, or oropharynx. In osteichthyans, the plesiomorphic condition is for teeth to be distributed throughout the oropharyngeal cavity, with more derived taxa having only marginal, palatal and pharyngeal teeth (Huysseune & Sire 1998; Stock 2001; Huysseune 2006). There may be a combination of two or all three of these tooth-forming regions, or just one. For example, the zebrafish exhibits the highly derived state of pharyngeal teeth only (Huysseune 2006; Stock 2007). Whichever the location, oral and pharyngeal teeth develop via highly similar genetic mechanisms and are developmentally homologous (Fraser *et al.* 2009). As such, the following description of tooth morphogenesis can be generally applied to oral or pharyngeal teeth. The term 'oral' is most commonly used in the literature, as historically tooth development has been most extensively studied in the mouse, which only has an oral dentition e.g. (Jernvall & Thesleff 2000; Tucker & Sharpe 2004; Bei 2009; Tummers & Thesleff 2009; O'Connell *et al.* 2012; Lan *et al.* 2014). To take both the oral and pharyngeal locations of tooth development into account, the term 'dental' e.g. 'dental epithelium' will be used in this review.

2.2.2 Initiation of tooth development

Tooth development starts with the expression of genes in specific regions of the oropharyngeal epithelium, generating signalling molecules which initiate change of uniform epithelium towards a dental fate (Fraser & Thiery 2019). These regions of expression (in the now 'dental epithelium') are termed the 'odontogenic band' or ‘odontogustatory’ band (Jernvall & Thesleff 2012; Martin *et al.* 2016; Fraser & Thiery 2019), and key signalling molecules have been identified as sonic hedgehog (Shh) and pituitary homeobox 2 (Pitx2) in several taxa (Keränen *et al.* 1999; Fraser *et al.* 2006; Buchtová *et al.* 2008; Vonk *et al.* 2008; Smith *et al.* 2009; Rasch *et al.* 2016). The first morphogenic change is that the epithelium comprising the odontogenic band thickens, and in many taxa also invaginates, becoming what is termed the ‘dental lamina’ from which all initial teeth will develop (Berkovitz *et al.* 2002; Jernvall & Thesleff 2012; Fraser & Thiery 2019). The more accurate yet less frequently used term for this tissue is the ‘primary dental lamina’ e.g. (Luckett 1993; Thesleff 2014). Bony fish are unusual in that their thickened odontogenic band does not invaginate, and so they do not form a dental lamina as usually characterised i.e. an infolding of the epithelium (Huysseune & Sire 1998; Fraser *et al.* 2004, Smith *et al.* 2009*b*; Berkovitz & Shellis 2017). However teeth still develop from this thickened odontogenic band directly (Huysseune & Sire 1998; Berkovitz & Shellis 2017; Fraser & Thiery 2019).

To briefly clarify terminology: the term ‘dental lamina’ is sometimes also used to refer to the odontogenic epithelial tissue that later generates replacement teeth (see section 2.4.1), when this arguably should more accurately be termed the ‘successional dental lamina’. This takes a structurally diverse range of forms, but in all cases it is the specific epithelial region/structure, derived from the the primary dental lamina, responsible for the regeneration of teeth. For example, in bony fish, the successional dental lamina is an epithelial strand which develops from either the outer epithelium of the predecessor tooth, or the epithelium immediately surrounding it - see section 2.4.2), giving rise to its replacement successor (Huysseune & Thesleff 2004; Huysseune 2006; Jernvall & Thesleff 2012). Although structurally very different, in sharks the term ‘successional dental lamina’ (or simply ‘successional lamina’), also refers to a specific site of tooth generation regeneration (Martin *et al.* 2016; Fraser *et al.* 2020) – see section 2.4.1. However, here we are focussing on the formation of initial teeth, in a specific region of the dental epithelium known as the (primary) dental lamina (Thesleff 2014). Due to its prevalent use in the research literature, the term ‘dental lamina’, rather than ‘primary’ dental lamina will be used in this review.

In forming the oral, marginal dentition, the dental lamina usually takes the shape of an arch-shaped band, which follows the curved line of the primitive oral cavity margin e.g. (Fraser *et al.* 2008). This shape varies across the different types of tooth-bearing bone and taxa, most notably in the elasmobranchs, where the large area of the dental lamina has been described as ‘sheet-like’ (Berkovitz & Shellis 2017). In snakes, a single odontogenic band and therefore subsequently dental lamina spans both the palatine and pterygoid bones (Richman & Handrigan 2011). Whatever the shape, the dental lamina giving rise to the first teeth always comprises a thickening of the epithelium, corresponding to the preceding odontogenic band and invaginated into the underlying mesenchyme (Fig. 2.2). As noted previously, the same is true of bony fish, but without the dental epithelium having invaginated (therefore this epithelial region is not strictly known as a dental lamina, but simply the odontogenic band). In all taxa, from within this thickened epithelial band, cells start to proliferate and further invaginate at certain positions, creating local thickenings termed ‘placodes’, the beginnings of tooth germs (Bei 2009; Jernvall & Thesleff 2012). In mammals (and most likely other vertebrate groups), it is the restricted expression of the signalling molecules Shh and Pitx2 within the dental lamina that demarcates the placodes (Jernvall & Thesleff 2012). Further proliferation, invagination and convolution of the epithelium which makes up the placode gives rise to the tooth, through what are described as the bud, cap and bell stages of tooth morphogenesis (described below) (Luckett 1993; Bei 2009; Jernvall & Thesleff 2012). As previously mentioned, a constant cross-talk of reciprocal, reiterative, inductive signals between the mesenchyme and epithelium controls this process (Jernvall & Thesleff 2000; Bei 2009; Tummers & Thesleff 2009; Thesleff 2014). Four major signalling pathways and their inhibitors comprise these interactions; those of the BMP, FGF, Hh and Wnt ligands and receptors; Bei (2009) summarises key research which demonstrates the nature of the tight control these pathways exert on tooth formation. Any modifications in how the pathways interact with each other result in abnormal tooth number, shape, patterning, replacement, or combinations of these e.g. (Fraser *et al.* 2013). The four pathways are highly conserved in evolution, and remarkably, the extreme variation in tooth pattern, shape, size, number and rate of replacement that we see across the vertebrate clade is engendered by tinkering with them (Thesleff 2003; Bei 2009; Tummers & Thesleff 2009; Fraser *et al.* 2013).

The details of the stages of tooth morphogenesis vary across vertebrate taxa, however the general sequence of developmental events is similar (Luckett 1993; Huysseune & Sire 1997, 1998; McGeady *et al.* 2006; Jernvall & Thesleff 2012; Berkovitz & Shellis 2017) and described below (Fig. 2.2).

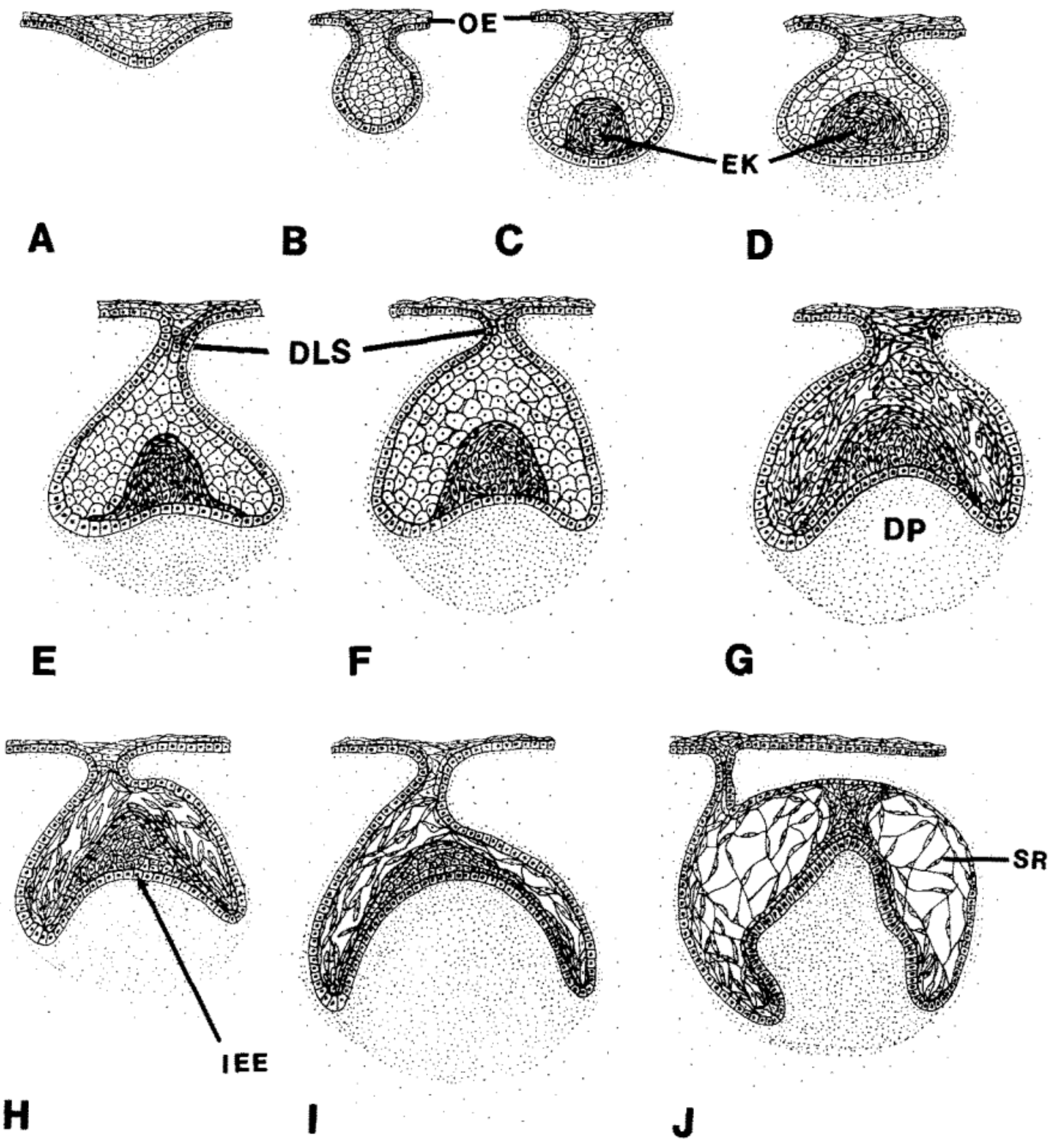


Fig. 2.2 Developmental stages of tooth development (therian mammalian dentition).

A, dental lamina; B-D, early, middle and late bud stages; E-G, early, middle and late cap stages; H-J early, middle and late bell stages. All stages shown in transverse section through centre of each tooth germ. *Abbreviations*: DLS, dental lamina stalk; DP, dental papilla; EK, enamel or epithelial knot; IEE, inner enamel epithelium or IDE, inner dental epithelium; OE, oral epithelium; SR, stellate reticulum. Taken from Luckett (1993).

2.2.3 The bud stage (Fig. 2.2B-D)

The oral epithelium comprising the placodes (at this stage now termed ‘dental epithelium’, or ‘dental lamina’) is drawn down, or invaginates, further into the mesenchyme. Cells of the epithelium signal to the underlying mesenchyme, causing mesenchymal cells to condense around the invagination, and a dental bud is formed (Luckett 1993; Huysseune & Sire 1997; Berkovitz *et al.* 2002; McGeady *et al.* 2006;

Jernvall & Thesleff 2012). The mesenchyme will continually condense around the developing tooth throughout each developmental stage until it is formed. In mammals, a group of epithelial cells at the base of the bud form a nodular, knot-like arrangement, due to their own aggregation as well as a slight thinning and loosening of the surrounding cells. This will become the 'enamel knot' in later cap and bell stages (Luckett 1993), which functions as a signalling control centre in tooth cusp formation (Jernvall *et al.* 1994). As reviewed by Thesleff (2014), enamel knots subsequently induce the formation of secondary enamel knots, determining the sites of tooth cusps in molars. Interplay of activator and inhibitor signalling molecules produced by the enamel knots pattern the shape of the cusps and the tooth crown. Fraser *et al.* (2013) suggest that fishes, and perhaps all vertebrates with complex cusp shapes, possess primitive enamel knot-like signalling centres that function to control cusp number, sharpness and size. This is supported by various studies in reptiles and fishes (Jernvall & Thesleff 2012, references therein).

At the late bud stage, the dental epithelium at the base of the tooth bud flattens, a necessary prerequisite for its invagination during the following cap stage (Luckett 1993).

2.2.4 The cap stage (Fig. 2.2E-G)

During the bud stage, the process of the dental epithelium being drawn down into the mesenchyme continues, forming the tooth bud, until its base is flattened, as described above. Throughout the process, mesenchyme continually condenses around the tooth bud. During the subsequent cap stage, this mesenchymal condensation continues. The flattened dental epithelium folds in on itself, or invaginates, wrapping itself round the central part of the condensing mesenchyme, creating a 'cap' shape (Luckett 1993; Jernvall & Thesleff 2012; Berkovitz & Shellis

2017). The mesenchyme within the concavity of the ‘cap’ is termed the ‘dental papilla’, the cells of which increase in number and size as the invagination progresses (Luckett 1993; Berkovitz & Shellis 2017). The dental papilla is the future source of the dental pulp and the odontoblasts, the specialised cells which produce the hard tissue dentine (Berkovitz *et al.* 2002; Berkovitz & Shellis 2017) (see below, bell stage). The mesenchyme surrounding the tooth germ forms the dental follicle, or sac (Berkovitz *et al.* 2002; McGeady *et al.* 2006; Berkovitz & Shellis 2017). In mammals, the dental follicle will later be responsible for forming the tooth attachment tissues (cementum, periodontal ligament and alveolar bone), and this is probably true in other vertebrates (Berkovitz & Shellis 2017, references therein). In most amphibians and bony fish however, a dental follicle/sac is either absent or restricted to the basal region of the tooth germ. This is probably related to their attachment to the bone later occurring here, and not at the sides of the tooth germ. Thecodont (see section 2.2.7) fish teeth however, do have a well-developed follicle around them as they develop (Berkovitz & Shellis 2017, references therein).

In mammals, cells in the centre of the enamel knot enlarge and elongate during the cap stage, forming a ridge-like epithelial cord (known as the enamel cord). The cells immediately surrounding the enamel cord gradually become more loosely associated, comprising the primordia of the stellate reticulum, named as such due to its star-shaped cells, and which becomes distinct during the bell stage (Luckett 1993) (see below, bell stage).

Sections of the dental epithelium are named according to location; the inner dental epithelium (IDE) comprises the epithelium forming the concavity of the cap, the remainder comprising the outer part of the tooth germ is termed the outer dental epithelium (ODE). These both become more distinct and are recognisable at this stage, due to the continued loosening of the central epithelial cells (Luckett 1993).

A note on terminology: in mammals e.g. (Luckett 1993; Jernvall & Thesleff 2012) and reptiles e.g. (Handrigan & Richman 2010; Richman & Handrigan 2011; Jernvall & Thesleff 2012) the dental epithelium is known as the enamel epithelium, and the IDE and ODE are therefore termed the inner and outer enamel epithelium respectively. Following Berkovitz & Shellis (2017), the terms dental epithelium, IDE and ODE will be used in this review, to encompass all vertebrates including those whose teeth possess enameloid rather than enamel. Similarly, the tooth germ is referred to as the enamel organ in mammals (Luckett 1993; Jernvall & Thesleff 2012), reptiles (Handrigan & Richman 2010; Richman & Handrigan 2011) and amphibians (Sire *et al.* 2002; Davit-Béal *et al.* 2006, 2007), but the term 'tooth germ' will be used here, to include all vertebrates.

Furthermore, with regard to terminology, transverse sections and diagrams representing the stages of tooth germ development commonly include a cord-like, epithelial connection between the tooth germ and the primitive oral cavity e.g. (Luckett 1993, Fig.1;Berkovitz & Shellis 2017, Fig. 9.15; McGeady *et al.* 2006, Fig. 19.9). This comprises the original infolding/invagination of the dental lamina, and its subsequent extension into the underlying mesenchyme, and so is usually simply referred to as the dental lamina e.g. (McGeady *et al.* 2006; Richman & Handrigan 2011; Jernvall & Thesleff 2012; Berkovitz & Shellis 2017). Luckett (1993) uses the term 'dental lamina stalk'.

Section 2.4.4 describes how some researchers refer to the region of cells located between the IDE and the ODE as the middle dental epithelium (MDE), and it is significant in the search for the mechanism responsible for polyphyodonty, as stem cells have been putatively identified here in some species (Huysseune & Witten 2008; Vandenplas *et al.* 2014, 2016*b*). The nature of this layer varies across vertebrate taxa; in mammals - and it seems in reptiles (Berkovitz & Shellis 2017, references therein) - it comprises the previously mentioned loosely associated epithelial cells at the late cap stage, (Luckett 1993), and the primordia of the stellate reticulum, which is not fully developed until the late bell stage. The main function traditionally ascribed to the stellate reticulum is a mechanical one, protecting underlying developing dental tissues and maintaining the tooth germ's shape. Its relatively widely spaced cells gradually result in the tooth germ's rounded shape, at this point consequently termed the 'bell stage'. The spaces between the cells of the stellate reticulum contain significant quantities of glycosaminoglycans, and connect to each other via cell processes (Berkovitz *et al.* 2002; Berkovitz & Shellis 2017).

2.2.5 The bell stage (Fig. 2.2H-J)

The folding in, or invagination of the inner dental epithelium is more extensive at this stage. The mesenchyme comprising the dental papilla increasingly condenses.

Species-specific cusp patterns emerge. A signalling centre known as the enamel knot forms at the tip of the inner dental epithelium (initially during the cap stage, see above, section). This will give rise to the tip of the crown, and therefore create a single-cusped tooth. In multi-cusped mammalian teeth, secondary enamel knots form at the locations of future cusps (Jernvall & Thesleff 2012). The stellate reticulum is fully developed by the late bell stage. Its intercellular spaces become fluid-filled, and this is thought to be due to the osmotic effect of high concentrations of glycosominoglycans (Berkovitz *et al.* 2002; Berkovitz & Shellis 2017).

Formation of the organic matrices of the tooth's hard tissues takes place in the late bell stage. Within the dental papilla of all vertebrates, the outer layer of mesenchymal cells nearest the IDE differentiate into cells called odontoblasts, which start to synthesise and secrete dentine. In mammals, reptiles and adult amphibians, the cells of the adjacent IDE also differentiate, into cells termed ameloblasts, which produce enamel (Berkovitz & Shellis 2017). In fish and larval urodeles - the amphibian order including salamanders and newts - enameloid is produced (and the term ameloblast is not used for the corresponding IDE cells as their role varies among these groups). Enamel or enameloid and dentine are therefore produced at the interface of the IDE and the dental papilla.

In mammals, reptiles and adult amphibians, the developing ameloblasts, known as pre-ameloblasts, initiate this process. They exert an inductive influence on the adjacent mesenchymal cells of the dental papilla, causing them to become more columnar and differentiate into odontoblasts. The odontoblasts start producing dentine, the presence of which induces the ameloblasts to secrete enamel. Only a thin layer of dentine is required for enamel secretion to be induced almost immediately (Berkovitz *et al.* 2002; Berkovitz & Shellis 2017). In fish and larval amphibians however, enameloid is laid down first and is fully formed *before* the deposition of dentine matrix begins (Berkovitz *et al.* 2002; Berkovitz & Shellis 2017).

The dental papilla gives rise to the pulp cavity of the tooth. As the odontoblasts and ameloblasts secrete dentine and enamel respectively, they move away from these hard tissues. Ameloblasts move towards the tooth surface and the oral cavity, while the odontoblasts move into the pulp cavity.

Unlike osteoblasts that form bone, odontoblasts and ameloblasts do not become trapped within the matrices they produce but remain on its surface. As the dentine and enamel are laid down, the stellate reticulum becomes thinner, and the crown of the tooth takes shape (McGeady *et al.* 2006).

Development of secondary, replacement teeth starts at the late bell stage in some taxa. In reptiles and mammals for example, this process starts with the cellular proliferation of, and therefore extension of, the ODE of the primary tooth germ on its lingual side. This region of cells is continuous with the cord of dental lamina cells connecting the tooth germ with the primitive oral cavity (Luckett 1993, Fig. 2; Jernvall & Thesleff 2012, Fig.4). The resulting extended cord of cells is known as the ‘successional dental lamina’, and it is from the tip of this that a replacement tooth starts to develop (see section 2.4.2). Timing of this event varies between different species.

Also in mammals, the initial epithelial downgrowth, or the cord of cells that connects the tooth germ with the oral epithelium above breaks down (McGeady *et al.* 2006). In most other taxa this does not happen and a connection with the oral epithelium is maintained.

2.2.6 Final growth

Once the enamel/enameloid and dentine formation is advanced, the more basal portion of the tooth begins to form. In elasmobranchs, attachment tissues form directly from the base of the tooth crown (Berkovitz & Shellis 2017), however in most other vertebrates the tooth elongates to form the shaft or root (the process of which is described here). The elongation begins where the IDE and ODE meet, at the edge or rim of the bell-shaped concavity. In some species, this apex is known as the ‘cervical loop’. From this point of contact, the cells of the IDE and ODE proliferate and extend into the underlying mesenchyme, forming a tube-like structure, referred to as the epithelial sheath, within which the ‘shaft’ or ‘root’ of the tooth forms, depending on the species. This sheath induces the mesenchymal cells of the dental papilla to differentiate into odontoblasts and form dentine, which is continuous with the dentine produced by the odontoblasts during the formation of the crown. Due to the absence of the stellate reticular layer, ameloblast differentiation does not occur, therefore the root or shaft is not covered in an enamel or enameloid layer. As the increase in dentine production continues, the pulp cavity is gradually reduced in size to a narrow ‘root canal’.

It is while the tooth elongates forming the shaft or root, that the tooth moves vertically towards the oral cavity to its functional position, where it attaches to the jaw skeleton. This process is termed tooth eruption (see section 2.2.10). In some groups of bony fish and reptiles, the teeth may have been developing in a sideways or ‘recumbent’ position, and so they have to rotate as they come into function (Berkovitz & Shellis 2017; Leuzinger *et al.* 2020).

2.2.7 Tooth attachment and implantation in vertebrates

The process of tooth attachment to the jaw skeleton varies across vertebrate taxa. In general, it has been shown that the epithelial sheath has the important role of controlling the deposition of tooth attachment tissues (Berkovitz & Shellis 2017). In reptiles (excepting crocodylians), amphibians and actinopterygians, outgrowths of dentine from the root or shaft meet the bone, and form a composite tissue in which bone and dentine are in intimate contact, the beginning of a fusion process called ankylosis (Davit-Béal *et al.* 2007; Zahradnicek *et al.* 2012). This term is derived from the Greek ‘ankulos’, meaning constricted, and refers to the little freedom of movement within the resulting joint; a joint being where a bone meets another, or in this case where a tooth meets bone (Bertin *et al.* 2018). The attachment created by the composite tissue is consolidated by deposition of bone, originating from the jaw bone. The exact location of this attachment, i.e. to which part of the root or shaft the bone connects, depends on the geometrical arrangement of the tooth in relation to the bone: the type of 'dental implantation'.

It has been claimed that confusion has arisen in the literature concerning the terminology of dental implantation and attachment, due to conflation of the two (Bertin *et al.* 2018; Haridy 2018). Bertin *et al*. contend that dental implantation strictly concerns the geometrical aspects of the tooth-bone interface, and that dental attachment mostly concerns the histological features occurring at this interface. In addition, Berkovitz and Shellis identify the incorrect use of the term 'cementum' by some authors, stating it should not be used outside the context of the thecodont tooth (Berkovitz & Shellis 2017). Here, the terminology used by Bertin *et al.* (2018) is used, as it aims to distinguish between attachment and implantation. Fig. 2.3 shows the different types of dental implantation exhibited in vertebrate teeth.

Where the tooth and tooth-bearing bone are positioned in a pleurodont arrangement, attachment is consolidated by bone deposited between the labial side of the tooth, and the lingual side of the bone (the Greek prefix 'pleuro' means 'to the side'). This is the main point of contact between the tooth and the jaw, although other tooth-bone contact may exist around the tooth, providing support. Acrodont implantation describes teeth positioned on the crest of the bone (Fig. 2.3). Pleurodont and/or acrodont implantation, with ankylosed attachment, are present in most fish, amphibians and reptiles, excluding crocodylians. Crocodylians and mammals exhibit a thecodont implantation only. This is where teeth are set deep in a symmetrical chamber in in the jaw, known as the alveolus, or often simply termed a socket. The depth of the alveolus is at least equal to the height of the crown. The four walls of the alveolus have comparable heights, although minor differences may exist. In crocodilians and mammals, the tooth is attached to these walls of the jaw bone by a fibrous, non-mineralised tissue, the ‘periodontal ligament’. This is instead of the mineralised attachment tissues arising from dentine and bone, described above, comprising ankylosis and exhibited by other taxa. The ligament confers a flexibility on the implantation that is lacking in ankylosis; it allows a slight degree of tooth movement, acting as a shock absorber during feeding (McGeady *et al.* 2006). This type of attachment is known as ‘gomphosis’, originating from the Latin ‘gomphus’, meaning peg, and refers to spatial relationship between the tooth and the bone in the joint. A periodontal ligament, and therefore gomphosis, is not always present in thecodont implantation however. Some fish species have thecodont teeth, but the attachment tissue is mineralised rather than fibrous, and therefore the teeth are ankylosed. Thecodont implantation in fishes also differs to that of crocodilians and mammals, as the socket does not continue through multiple tooth generations, each replacement tooth erupts through its own channel that then becomes its socket (Berkovitz & Shellis 2017).

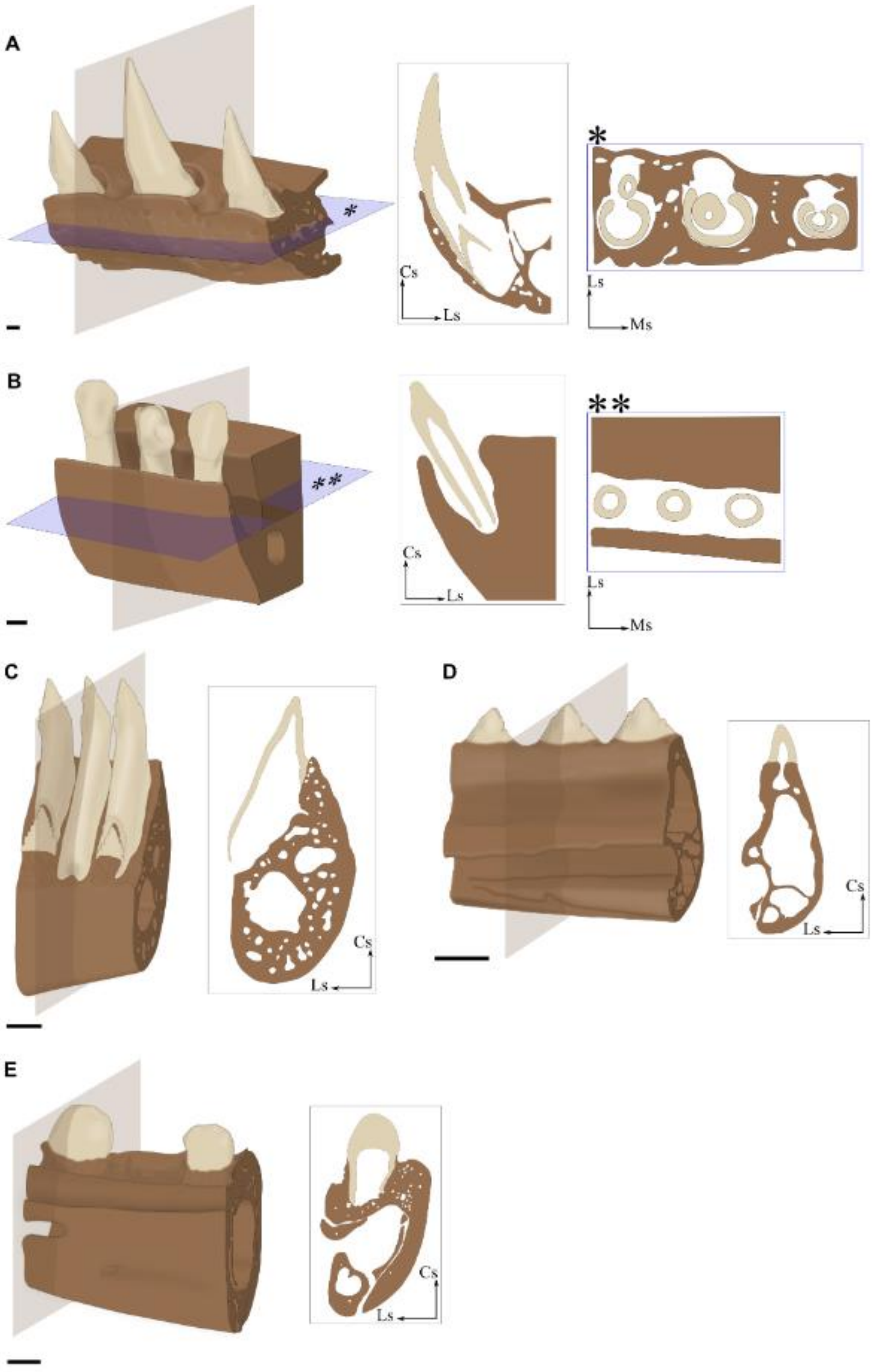


Fig. 2.3 Implantation geometry illustrated by 3D portions of maxilla (A,B) and mandible (C–E) associated with virtual sections.

A, Thecodont implantation in the Nile crocodile (*Crocodylus niloticus*); B, Aulacodont implantation in the Porpoise (*Phocoena* sp.); C, Pleurodont implantation in the Green iguana (*Iguana iguana*); D, Acrodont implantation in the Graceful chameleon (*Chamaeleo gracilis;* E, Subthecodont implantation in the Tegu (*Tupinambis teguxin*). *Abbreviations*: Cs, coronal side; Ls, lingual side; Ms, mesial side. Scale bar represents 1 mm. Taken from (Bertin *et al.* 2018).

2.2.8 Modes of tooth attachment in actinopterygians

Generally, actinopterygian teeth are either directly attached (ankylosed) to a bone of attachment (which is in turn fused to the jawbone), or are attached to it via an intervening fibrous, collagenous tissue. This tissue provides the tooth with flexibility in relation to the jawbone and absorbs shock during feeding (Fink 1981; Berkovitz & Shellis 2017; Rosa *et al.* 2021). Fink (1981) describes four different types of attachment in actinopterygians (Fig. 2.4), and explains that during a tooth's growth, each type allows varying amounts of dentine to develop. This variation arises from different levels of mineralisation of pre-dentine, or collagenous tissue, prior to its potential complete mineralisation to form dentine. Pre-dentine is the fibrous, collagenous tissue described above, conferring flexibility between tooth and bone. Berkovitz & Shellis (2017) refer to this pre-dentine/collagen as 'fibrous attachment', and this is the term which will be used herein. Hinged teeth occur when there is no fibrous attachment at either the anterior or posterior side of the tooth, but instead, direct attachment between the tooth (dentine) and the bone of attachment. This allows movement of the tooth around this direct point of connection between two hard tissues, which acts as a hinge. Hinged teeth allow prey to enter the mouth but prevent it from leaving (Berkovitz & Shellis 2017).

Berkovitz & Shellis (2017) add to Fink's scheme of attachment types, adding one in which the bone of attachment comprises a pedestal, termed a 'pedicel' (Berkovitz & Shellis 2017; Rosa *et al.* 2021). A pedicel is only present when fibrous attachment is also present. The pedicel comprises a 'collar' or cylinder of attachment bone, the inside of which is often concave, supporting the tooth in a cup-shaped surround. This creates what is known as 'pedicellate attachment', and is the most common type among teleosts (Berkovitz & Shellis 2017).

In fishes with intraosseous tooth development, teeth erupt from a bony crypt or trough, through an eruption channel (section 2.4.2, Trapani 2001), and usually then attach to the surface of the dentigerous bone at their bases e.g. in piranhas (Berkovitz & Shellis 1978). Significantly for the specimens studied herein, there is a different situation in the families Labridae and Sparidae, both of which exhibit intraosseous development. In these groups, the sides of the tooth, the lateral surfaces, become ankylosed to the bone surrounding the eruption channel. This is via a spongy bone of attachment, between the tooth and jaw surfaces. Attachment bone is also deposited at the base of the tooth. This comprises the type of thecodont implantation particular to fishes (see above), and the attachment bone is mineralised (unlike fibrous attachments in crocodylians and mammals) (Berkovitz & Shellis 2017; Rosa *et al.* 2021). Interestingly, in some species such as the gilthead seabream (*Sparus aurata*) and plaice, a pedicle is ankylosed within the eruption channel, meaning a fibrous attachment is also present.

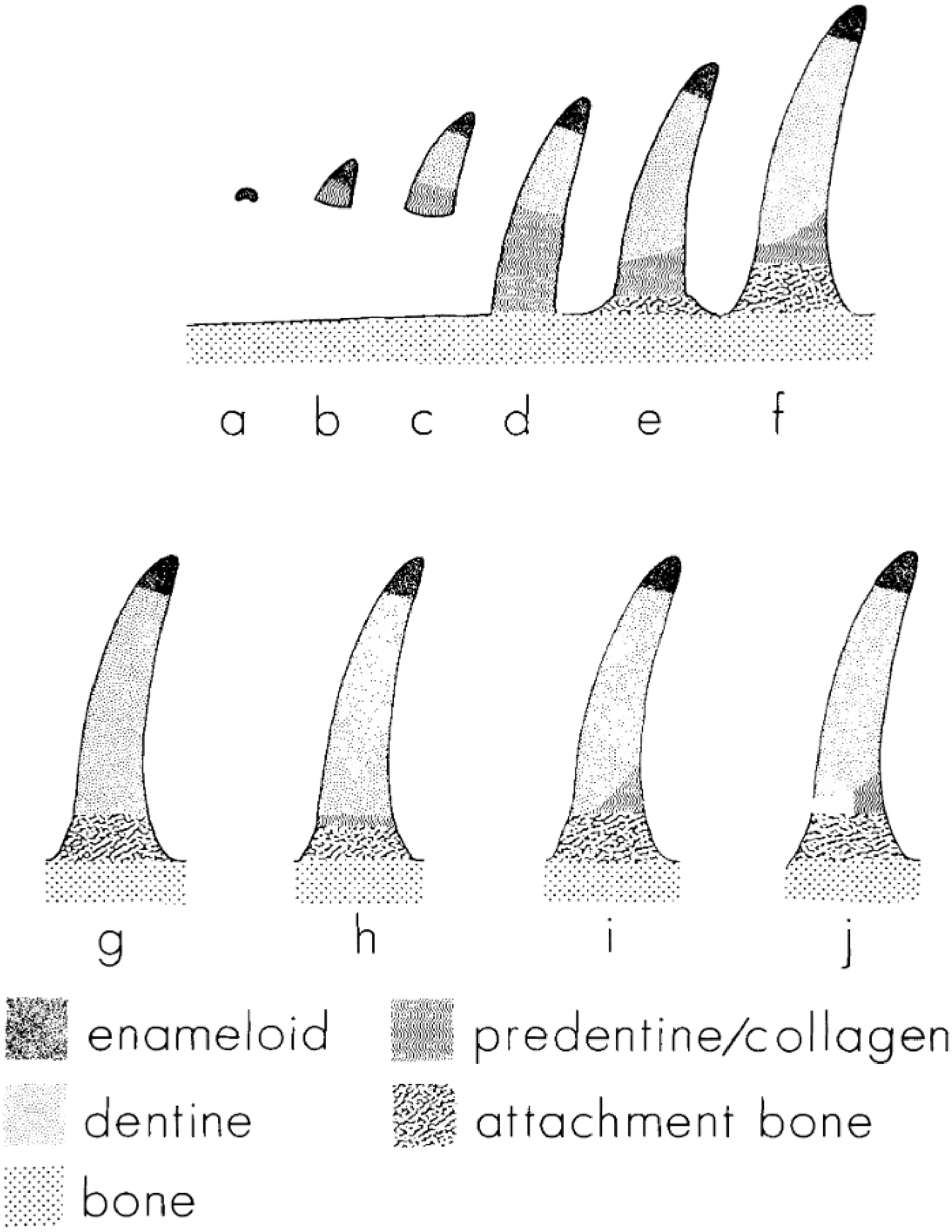


Fig. 2.4 Ontogeny of tooth attachment modes, as described by Fink (1981).

Anterior (or labial) border of each tooth to the left. Growth takes place in epithelium, not shown. A, Early tooth bud stage, enameloid cap present. B, Predentine develops proximal to enameloid and grows toward bone. C, Dentine is formed by mineralization of predentine. D, Predentine extends to bone; dentine formation continues. E, Mineralization nears tooth base; note that anterior tooth border mineralizes proximally further than posterior border; attachment bone forms at tooth base. F, Late-stage replacement tooth or mature, primitive Type 3 tooth. G, Mature Type1 tooth. H, Mature Type 2 tooth; note collagenous ring at base. I, Mature, derived Type 3 tooth; note that dentine extends to attachment bone on anterior border. J, Mature Type 4 tooth; note that collagen is lacking between anterior dentine border and attachment bone. Taken from (Fink 1981)

2.2.9 Tooth attachment and implantation in mammals and crocodylians

Tooth attachment and implantation in mammals and crocodylians arises from a different sequence of events to that of other vertebrate taxa. However, the formation of the epithelial root sheath, and root within it, takes place as described above. The sheath (known in mammals and crocodylians as Hertwig's epithelial root sheath, or HERS) adopts the same role, and induces the mesenchymal cells of the dental papilla to differentiate into odontoblasts and form dentine. Again, ameloblast differentiation does not occur, rendering the root free of any enamel layer. Consistent with other taxa, as the increase in dentine production continues, the pulp cavity is gradually reduced in size to a narrow root canal. The major differences arise due to the presence of the dental sac in mammals and crocodylians. During the bell stage of development, mesenchymal cells surrounding the tooth condense and form a vascular mesenchymal layer, the dental sac. This is also known as the dental follicle and is absent in other taxa. The layer of mesenchymal cells immediately adjacent to the developing root differentiate into cementoblasts. These produce cementum, a bone-like connective tissue, which covers the root of the tooth. It has been shown that Hertwig's epithelial root sheath begins to break down prior to this stage, which is necessary for mesenchymal dental sac cells to differentiate into cementoblasts (Berkovitz & Shellis 2017). Cementoblasts become trapped inside the cementum matrix they produce, and are termed cementocytes, comparable to osteoblasts and osteocytes in bone development. Mesenchyme at the outer layer of the dental sac gives rise to osteoblasts, which produce bone that forms the alveolus, the structure which anchors the tooth in the jaw. It is from within the intervening, middle layer of mesenchyme, between the cementum and alveolar bone, that the periodontal ligament develops, made of tough collagenous fibres. The periodontal ligament becomes attached to the bone of the alveolus and the cementum covering the root. As such the periodontal ligament confers the ability to move slightly on the tooth, and therefore a flexibility, within the alveolus.

2.2.10 Tooth eruption

As described previously, it is during the tooth's final stages of growth, as the structures of the shaft or root develop, that the tooth moves vertically towards, and breaks through the oral epithelium to reach its functional position. This constitutes the process of eruption, and once the tooth has reached its functional position it becomes attached to the jaw skeleton (Berkovitz & Shellis 2017). How the tooth is controlled so that it reaches its functional position is not fully understood, and a subject of much ongoing research interest (for overview of the field, see Berkovitz *et al.* 2002; Sarrafpour *et al.* 2013). In mammals and crocodylians there is some evidence to indicate that the eruptive force is generated by the periodontal ligament and its precursor the dental sac (Cho & Garant 2000; Berkovitz & Shellis 2017, see also section 2.6.7 for the proposed involvement of mechanical stress for eruption of permanent teeth). In taxa with extraosseous tooth replacement (section 2.4.2) - most bony fish and reptiles and all amphibians, an important factor for eruption is growth in the length of the tooth shaft. This growth brings the tooth tip closer to the epithelium. However once the tooth becomes fixed to the jawbone at the base and movement stops, exposure of the tooth tip may rely on remodelling of the epithelium (Huysseune *et al.* 1998; Van der heyden *et al.* 2000; Huysseune & Sire 2004; Vandervennet & Huysseune 2005; Berkovitz & Shellis 2017).

Eruption only occurs once the crown is fully formed. After eruption, the stellate reticulum, ODE and ameloblasts are lost due to wear - the first time the teeth are used, exposing the enamel layer. As the ameloblasts (or equivalent) are lost, no more enamel or enameloid can be made once the tooth is erupted, though odontoblasts continue to make dentine throughout the life of the tooth. If the enamel becomes damaged, it is replaced by dentine (McGeady *et al.* 2006; Jernvall & Thesleff 2012).

What has been outlined so far is the development of a primary tooth. In general, secondary teeth, and if the species is polyphyodont, subsequent successor teeth, develop in the same way. However, what differs is the mechanism by which the successor tooth's development is initiated, compared to that of the primary tooth. Also, the manner in which the successor tooth replaces its predecessor varies between vertebrate taxa. These processes will be reviewed in section 2.4.

2.3 Why do teeth replace? The adaptive advantage of tooth replacement

As discussed, most non-mammalian vertebrates replace their teeth throughout life (polyphyodonty). Some vertebrates however are unable to continually replace their teeth, and only do so once, resulting in two generations (diphyodonty); the case for most mammals, including humans. Some do not replace their teeth at all, resulting in just one generation of teeth throughout a lifetime (monophyodonty), as in many rodents and cetaceans. This raises the question of why there is such variety in numbers of tooth generations, and indeed, why are teeth replaced at all?

Perhaps the most obvious reason for tooth replacement is compensation for wear: as teeth wear down, or become damaged or lost, new replacements are needed. The rates of replacement in some species can be very high, depending on how they feed, and what on. For example, the rock goby fish scrapes rock surfaces for food using bristle-like teeth, meaning that even light use blunts them (Berkovitz & Shellis 2017). Tooth replacement rates are hard to quantify, however the numbers of successional teeth, at progressive stages of development, present in readiness to take up a functional tooth position can be a good indicator of rate. In some gobies there are about 45 developing successional teeth associated with each functional tooth. Sharks and rays are famous for their high numbers of successional teeth and often rapid tooth replacement. Teeth rows or 'files' are brought towards the functional position on the jaw margin via a 'conveyor belt' of underlying tissue (a 'continuous, permanent' dental lamina, discussed in section 2.4.1) (Underwood *et al.* 2015; Martin *et al.* 2016; Rasch *et al.* 2016; Fraser *et al.* 2020) .

Growth of the animal is a second reason for tooth replacement. Growing animals need progressively more nutrition, and the jaws and dentitions must enlarge to keep pace with feeding requirements (Berkovitz & Shellis 2017). Dentitions can enlarge in two ways: teeth are added at new loci, filling space provided by growth, and/or small teeth are gradually replaced by progressively larger teeth, taking up more space in the growing jaw. Osborn (1973) notes that in general small jaws accommodate small teeth, but these would become inadequate as a jaw grows larger. Dentitions usually enlarge via both processes, however there are some exceptions, such as piranhas which grow their dentitions by tooth replacement only (Berkovitz & Shellis 1978).

A third reason for tooth replacement is for the dentition to adapt to changing diet and feeding methods during a lifetime. For example, large prey that require skill and strength to predate may be impossible for a young animal to consume, but possible for the adult. Change in shape and size of teeth via multiple tooth replacements is considered key to enabling different food sources in the adult (Streelman *et al.* 2003; Fraser *et al.* 2013; Tucker & Fraser 2014). Most commonly teeth become more complex, although this can vary, including some first generation teeth resembling miniature complex adult teeth, as in diphyodont mammals (Sire *et al.* 2002; Jernvall & Thesleff 2012).

A further benefit and selective advantage of diet change over an individual's life time is that parents and offspring do not have to compete for the same food. It is thought that evolution favoured the development of larval stages for this reason. Larval stages are present in an immense range of species, including fish, amphibians and insects, and typically have a different diet to the parent (Shefferson 2010).

2.3.1 Why numbers of tooth generations vary across taxa

As previously mentioned, polyphyodonty is considered likely to be the plesiomorphic state for the vertebrates. Subsequently it appears that throughout vertebrate evolution there has been a general reduction in numbers of tooth sets/replacements, concurrent with an increase in the complexity of tooth morphologies. This complexity comprises the development of cusps and multi-cusp tooth shapes. Some cusps are simple individual bumps, some are long and blade-like, and some cusps combine to form complex crest patterns (Jernvall & Thesleff 2012). These correlated trends are often characterised as a 'trade-off' between the number of tooth generations and the complexity of tooth morphology (Osborn 1973; Tucker & Fraser 2014). The most reduced number of replacements occurs in mammals, which at most have a maximum of just one tooth replacement and therefore two sets of teeth (diphyodonty), e.g. in humans, or no replacements and just one set (monophyodonty), e.g. in mice, rats, the striped skunk (Nievelt & Smith 2005). Many mammal taxa have no teeth at all (see below). In keeping with the 'trade-off' relationship, mammals also exhibit the most complex tooth morphologies within the vertebrates.

What selective advantage does complex tooth morphology confer? Varied morphologies of tooth crowns between taxa allows adaptation to diet e.g. in herbivorous mammals, specialised cusp shapes and often fusion into ridges enable efficient breakdown of fibrous plant material (Evans *et al.* 2007; Jernvall & Thesleff 2012). Mammals have evolved a progressively more regionalized dentition in terms of tooth morphology, known as heterodonty (Luo 2007). The morphologies comprise four tooth types: incisors, canines, premolars and molars, with each performing a specific food-processing role, e.g. piercing, slicing and/or grinding. Molars exhibit the most complex morphology. Possessing the full range of tooth types in the same dentition is advantageous for animals with a varied, omnivorous diet, as in humans.

Complex tooth crowns have also enabled interlocking occlusion of opposing teeth and cusps in some mammals, known as ‘precise occlusion’ (Osborn 1973; Jernvall & Thesleff 2012). The upper and lower teeth shear across each other to grind food. The shearing planes on the upper and lower complex teeth mutually wear against each other to produce the perfect fit of precise occlusion, and the grinding process is thus made significantly more efficient. Such a successful strategy is however not compatible with repeated tooth replacement, the mechanism vertebrates generally employ to remedy tooth wear. Repeated new teeth would constantly disrupt the precisely matched shearing planes. Mammals with precise occlusion have offset this problem by teeth having a particularly thick layer of very hard enamel, and being very firmly rooted in the jaw (Osborn 1973). The benefits of precise occlusion therefore must have been selected for over polyphyodonty in such taxa.

As well as thick enamel and strong roots, many taxa including horses, cows and rodents have evolved a further solution to tooth wear and loss – the development of a high crown, or ‘tall teeth’, known as ‘hypsodonty’ (Jernvall & Thesleff 2012). A subset, or derived case of hypsodonty is hypselodonty, in which teeth continue to grow throughout the life of the animal to compensate for wear. Both hypsondonty and hypselodonty have evolved multiple times independently across the mammalian clade. Hypselodonty is found within mammalian orders as diverse as the diprotodonts (mainly herbivorous marsupials including kangaroos, koalas and wombats), lagomorphs (rabbits, hares and pikas), rodents (including mice, rats, guinea-pigs and beavers) and proboscids (elephants and their extinct relatives such as mammoths and mastdodons) (Renvoisé & Michon 2014). Such continual growth, or the condition of 'ever-growing teeth', is made possible by the presence of stem cells and progenitor cells, located in structures at the base of the tooth called the cervical loops (see sections 2.3.1 and 2.5.3). These cells continually renew the tip of the tooth as a counter to wear (Harada *et al.* 1999). This process and its molecular regulation has been studied most in mice and rats due to their frequent use as laboratory animal models, and the search for the mechanism of polyphyodonty has greatly drawn on this work (see section 2.5.3).

As well as the numbers of tooth generations, and numbers of teeth per generation generally reducing over evolutionary time (as complexity increases), the range in locations of teeth in the oropharynx also appear to reduce. The plesiomorphic condition for osteichthyans is assumed to be that of teeth located throughout the entire oropharynx, on small tooth plates (Nelson 1969; Stock 2001) . Though no fossil or extant examples show this, some groups show extensive coverage of the oropharynx surface, as seen in *Amia calva*, the bowfin (Miller & Radnor 1973; Grande & Bemis 1998; Stock 2001; Smith 2003). Tetrapods however only have teeth present on the oral jaws, and many mammals have a partial oral set, often with a gap or 'diastema' between tooth types e.g. between canines and slicing molars in cats, and between incisors and grinding molars in horses. Birds, turtles, and some mammals (e.g. baleen whales, pangolins, anteaters) have lost their dentitions completely, and are therefore termed ‘edentate’. Such edentate taxa are thought to have been able to survive tooth loss by the previous evolution of a secondary tool for food uptake e.g. beaks, baleen, long adhesive tongues (Davit-Béal *et al.* 2009).

2.4 How teeth replace

2.4.1 Development of replacement teeth from a permanent dental lamina

As with the initiation of most primary teeth, replacement teeth also arise from tooth-generating tissue termed the dental lamina. This takes different forms across the major gnathostome taxa. In chondrichthyans, as previously mentioned, it takes the form of a large ‘sheet’ of cells, created by a particularly deep invagination of the oral epithelium, which runs parallel with the surface of the jaw cartilage. This dental lamina remains throughout ontogeny, not only producing the initial generation of teeth, but also all successional teeth from within the deepest part of the invagination (Tucker & Fraser 2014; Martin *et al.* 2016; Berkovitz & Shellis 2017; Fraser & Thiery 2019; Fraser *et al.* 2020). This region of the lamina is often termed the ‘successional lamina’ due to successional teeth arising from it, but note its significant morphological difference to the structure of the same name in Osteichthyes (see below, section 2.4.2). The sheet of epithelial cells comprising the chondrichthyan dental lamina, of which the successional lamina is part, has been described as a continuous ‘unit’ (Fraser & Thiery 2019), which structurally and often temporally contrasts greatly with the epithelial strands or offshoots produced by an osteichthyan tooth to develop its successor, as described in section 2.4.2.

As the chondrichthyan dental lamina comprises the epithelial tissue from which teeth develop throughout ontogeny, it is referred to as ‘permanent’, and also ‘continuous’, as it provides a continuous, retained connection between each successional, developing tooth (Reif 1982). Teeth are generated in large tooth families or ‘files’ ahead of function, lined up ready to sequentially take the place of the functional tooth, once it is shed. The developing successional teeth move through the dental lamina rather like a conveyor belt, ensuring a constant supply to the functional position at the front of the jaw margin (Tucker & Fraser 2014; Berkovitz & Shellis 2017; Fraser *et al.* 2020). The foremost and most developed successional tooth only takes its position once the whole functional predecessor tooth, including its attachment tissues, is completely shed.

2.4.2 Development of replacement teeth from a successional dental lamina

In contrast, in osteichthyan tooth replacement, the successor develops by the side of (usually lingually), or underneath the primary tooth while the roots or shaft of the primary tooth are being resorbed, causing its attachment to loosen. The primary tooth is eventually shed, and the successor erupts into the functional position and attaches to the jaw bone. This process repeats, with all functional predecessors being replaced by successors in this way (Witten & Huysseune 2009; Chen *et al.* 2016; Berkovitz & Shellis 2017; Johanson 2017). Resorption of the attachment tissues during tooth replacement is a key feature of the osteichthyan dentition.

In most osteichthyans, successor teeth start to develop from a strand of epithelial cells off-shooting from the functional predecessor tooth. Specifically, in amphibians, reptiles and mammals it develops from the ODE of the functional predecessor, at various stages in the predecessor’s development (Richman & Handrigan 2011; Berkovitz & Shellis 2017). In bony fish, the strand most commonly offshoots from the epithelium immediately next to the predecessor tooth, once it has erupted. In zebrafish, this epithelium is described as the epithelial fold immediately surrounding the erupted, exposed part of the predecessor tooth (called the ‘epithelial crypt’, comprised of ‘crypt epithelium’) (Huysseune & Sire 2004; Huysseune & Thesleff 2004; Huysseune 2006). In cichlids, Fraser *et al.* (2013) do not describe crypt epithelium, but they describe the epithelial downgrowth as ‘initiated as an epithelial invagination, labial to the predecessor tooth’. In each case, and importantly across all osteichthyan taxa that replace their teeth, the epithelial strand connects the predecessor tooth (or its immediately adjacent oral epithelium) to its successor, at least initially. Although very different to the large sheet of cells comprising the chondrichthyan dental lamina, this epithelial strand is also tooth-generating and therefore also comprises a dental lamina, known as a ‘successional dental lamina’ (SDL), or sometimes simply a ‘successional lamina’ (SL), (SDL is used here). Development from this epithelial strand/SDL proceeds in two possible ways. The strand can be short, giving rise to a replacement tooth developing in the soft tissue above the jaw bone, to which it will eventually attach. This is known as extraosseous, or extramedullary replacement and is the predominant type of replacement in fish e.g. in cyprinids such as the zebrafish, carps and true minnows (Trapani 2001; Huysseune & Thesleff 2004), and is present in all reptiles, except crocodylians (Fraser *et al.* 2013; Berkovitz & Shellis 2017). Alternatively, the epithelial offshoot/SDL grows into a long epithelial strand. This penetrates into the jaw bone through a pore termed the ‘gubernaculum’ (Shellis & Berkovitz 1976) or ‘gubernacular canal’ (Fraser *et al.* 2013), reaching the medullary cavity of the bone in which the new tooth germ starts to develop at the tip of the strand (Huysseune & Thesleff 2004; Vandervennet & Huysseune 2005) (Fig. 2.5). The ‘penetration’ is enabled by the remodelling of the bone to form a canal around the SDL strand, and it ensures an epithelial connection between the developing successor tooth and the oral epithelium (Fraser *et al.* 2013). Inside, the bone also remodels to accommodate the developing successor, forming a range of cavity shapes as described by Trapani (2001) e.g. bony crypts for individual teeth as seen in cichlids (Fraser *et al.* 2013) or larger cavities such as the ‘trenches’ housing successors across numerous tooth positions seen in *Hepsetus odoe*, the African Pike Characin (Trapani 2001, Fig. 5). Subsequently, eruption initiates and the developing tooth moves through and the bone, facilitated by its resorption (Witten 1997; Witten *et al.* 1999). The tooth attaches to the bone surface, becoming a functional tooth. Overall, this is process is known as intraosseous, or intramedullary replacement (Trapani 2001; Huysseune & Thesleff 2004; Vandervennet & Huysseune 2005; Fraser *et al.* 2013).

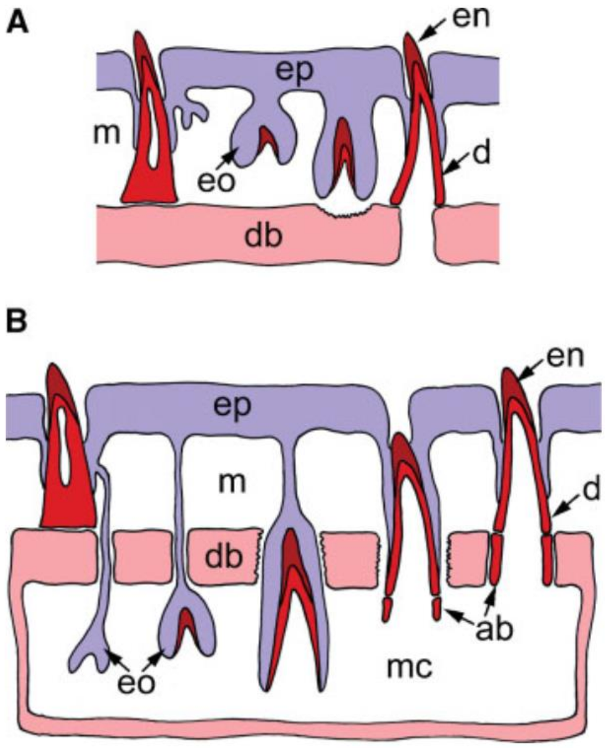


Fig. 2.5 Schematic representation of extraosseous (A) and intraosseous (B) replacement tooth formation in teleost fishes.

In both schemes, successive stages of tooth germ development are shown from left to right. The predecessor (functional tooth) is only represented once (far left of each figure) to show its relationship with the successor (replacement tooth). Bone, pink; oral epithelium, purple; tooth hard tissue, red. *Abbreviations*: ab, attachment bone; d, dentine; db, dentigerous bone; ep, oral or pharyngeal epithelium; en, enameloid; eo, enamel organ; m, mesenchyme; mc, medullary cavity. Taken from Huysseune & Thesleff (2004).

The plesiomorphic type of replacement in fish, and all osteichthyans, is considered to be extraosseous tooth replacement; it is consistently exhibited in basal fish groups, with intraosseous tooth replacement being the derived condition (Trapani 2001). As previously mentioned, extraosseous replacement is also the predominant type of replacement in fish, though intraosseous replacement has evolved in at least three clades within the teleosts (Trapani 2001; Huysseune & Witten 2006). Extraosseous replacement is present in all amphibians and reptiles except crocodylians. Mammals and crocodylians exhibit intraosseous replacement (Berkovitz & Shellis 2017).

Trapani (2001) suggests that two conditions were required for intraosseous replacement to evolve: developing replacement teeth must move beneath their functional predecessors, and the developing tooth must be encased in bone. This has generally become accepted as a definition of intraosseous replacement (e.g. Bemis & Bemis 2015 - but note ‘encased in bone’ is interpreted here as accounting for the gubernacular canal created by the bone’s penetration by the SDL). Whenever a tooth is replaced, either extraosseous or intraosseous replacement occurs, whether the tooth-bearing bone is oral or pharyngeal, and sometimes both types of replacement co-exist (Berkovitz & Shellis 1978; Huysseune & Thesleff 2004).

Frequently, e.g. in studied amphibians and most reptiles, the first replacement (or first successor) tooth also produces an epithelial offshoot whilst still developing, giving rise to a secondary successor existing concurrently. This can in turn give rise to a third, and so on. A ‘family’ of developing teeth results, which are destined to sequentially take up the original tooth’s position (Davit-Béal *et al.* 2006; Richman & Handrigan 2011; Whitlock & Richman 2013; Berkovitz & Shellis 2017). A few studied bony fish have been found to form tooth families by successor teeth developing directly from the outer dental epithelium of the predecessors, without the need of an offshoot epithelial strand (detailed in 2.4.4 below). Either way, a series of teeth at a tooth position develop ahead of function, as in the leopard gecko, *Eublepharis macularius* (Handrigan *et al.* 2010) , the Iberian ribbed newt *Pleurodeles waltl* (Davit-Béal *et al.* 2006), African bichir, *Polypterus senegalus* (Vandenplas *et al.* 2014) and Atlantic salmon, *Salmo salar* (Huysseune & Witten 2008).

Amphibians and reptiles develop an SDL from their initial teeth, producing a successor as described above. An SDL develops from the ODE of the primary successor, which extends and buds the next successor from its tip, and so the sequence continues (Davit-Béal *et al.* 2007; Richman & Handrigan 2011; Whitlock & Richman 2013). Importantly, the SDL of these groups differs significantly to that of bony fish, in that once the successor has sufficiently developed, the SDL is typically retained (Berkovitz & Shellis 2017). In bony fish, the SDL exists only temporarily until the successor tooth has sufficiently developed; a time limit varying across taxa, e.g. when the successor starts budding in the zebrafish (Huysseune 2006), and when it erupts in cichlids (Fraser *et al.* 2013). Therefore each replacement tooth forms its own, distinct SDL anew (Huysseune 2006; Jernvall & Thesleff 2012). In amphibians and reptiles, the frequent retention of the SDL in between successive teeth within a family means they are all connected by it (Davit-Béal *et al.* 2006; Handrigan & Richman 2010; Richman & Handrigan 2011; Jernvall & Thesleff 2012; Whitlock & Richman 2013), contrasting with the process in bony fish. As such, the SDL in most reptiles is described as ‘continuous’ and ‘permanent’, as opposed to the SDL’s description as ‘discontinuous’ and ‘non-permanent’ or transient’ in bony fish (Huysseune 2006; Jernvall & Thesleff 2012). From the descriptions of the studied amphibian specimens in the literature cited here, it can be deduced that the terms ‘continuous’ and ‘permanent’ also apply to the amphibian SDL. These distinctions between the different types of dental lamina in gnathostomes, based on structure and longevity, were identified and named in the significant work of Reif (1982).

Typically in amphibians and reptiles, the overall effect of successive tooth development via the SDL is that the tooth families, linked together by the retained SDL strand, somewhat resemble a chain of tomatoes on a vine, e.g. in the leopard gecko, *Eublepharis macularius* (Handrigan *et al.* 2010) and the ball python, *Python regius* (Handrigan & Richman 2010) (Fig. 2.8). As seen in both these studies, this sequential budding and retained connection occurs along and extends one side of the invagination (of the odontogenic band) forming the (primary) dental lamina, and therefore, as noted by Richman & Handrigan (2011): ‘the successional lamina is in effect the continuation of the dental lamina’ (Fig. 2.8). In many reptiles, the retained connection within tooth families is life-long (Handrigan *et al.* 2010; Whitlock & Richman 2013). Also, the chain can reach relatively high numbers; in fanged snakes approximately eight developing teeth are present - possibly as they are often lost in an attack, similar to numbers seen in some chondrichthyans (Zahradnicek *et al.* 2008; Tucker & Fraser 2014).

2.4.3 Regression of the successional lamina in mammals

Unlike all other tooth-bearing osteichthyans, including those within the tetrapods, mammals can only maximally develop two generations of teeth; they cannot develop a third. The initial and successor teeth develop very similarly to the process described in amphibians and reptiles, however the dental lamina connecting the initial, first tooth germ to the oral epithelium degrades during its development, as does the SDL connection to the replacement tooth (Buchtová *et al.* 2012; Jernvall & Thesleff 2012) (Fig. 2.6). It has been proposed that the lack of a third tooth is due to three possible reasons: the degradation of the dental lamina connecting the initial, primary tooth and the oral epithelium (Buchtová *et al.* 2012), the failure of an SDL to form from the replacement/permanent tooth bud, or that an SDL forms but then regresses before forming a tooth (Whitlock & Richman 2013). A rudimentary SDL developing from permanent teeth buds has been identified in some species, including humans (Ooe 1981), the straw-coloured fruit bat (Popa *et al.* 2016) and the bearded dragon (Richman & Handrigan 2011), implying the latter reason is most likely. Furthermore, mouse molars, which only have a single generation have also been found to have a small, rudimentary SDL, and intriguingly, it appears the proximity of the predecessor tooth to this SDL acts as an inhibitory influence on its development (Popa *et al.* 2019). When isolated from the molar in culture, the rudimentary SDL was ‘dramatically freed’ to develop into a cap stage-like tooth germ. Popa *et al.* (2019) speculate that the molar has an inhibitory effect on the Wnt signalling pathway required to initiate tooth development (see sections 2.5.1 and 2.6.1), and they note that Fjeld *et al.* (2005) found Wnt inhibitors to be strongly expressed in the mesenchyme around the rudimentary SDL at two particular stages in its development. Popa *et al.* (2019) suggest that the inhibition of a rudimentary SDL from the permanent tooth germ causes the restriction of mammal dentitions to (mostly) two generations. Any intervention to override this inhibition, to attempt to develop a third generation, could only happen during the short window of time between the formation of the second tooth germ and the loss of the rudimentary SDL.

Fraser *et al.* (2020) contend that the research endeavour to generate further tooth generations in mammals needs to broaden to include novel genetic markers of polyphyodonty. They point particularly to the rapid tooth regeneration of the successional lamina (SL) in the catshark (Martin *et al.* 2016) (section 2.5.9), which undergoes both initiation and pause phases in its regenerative dental cycle. Fraser *et al.* (2020) propose that understanding the genetic mechanisms controlling the pause phase may illuminate more generally how dental competence can be lost, and also regained.

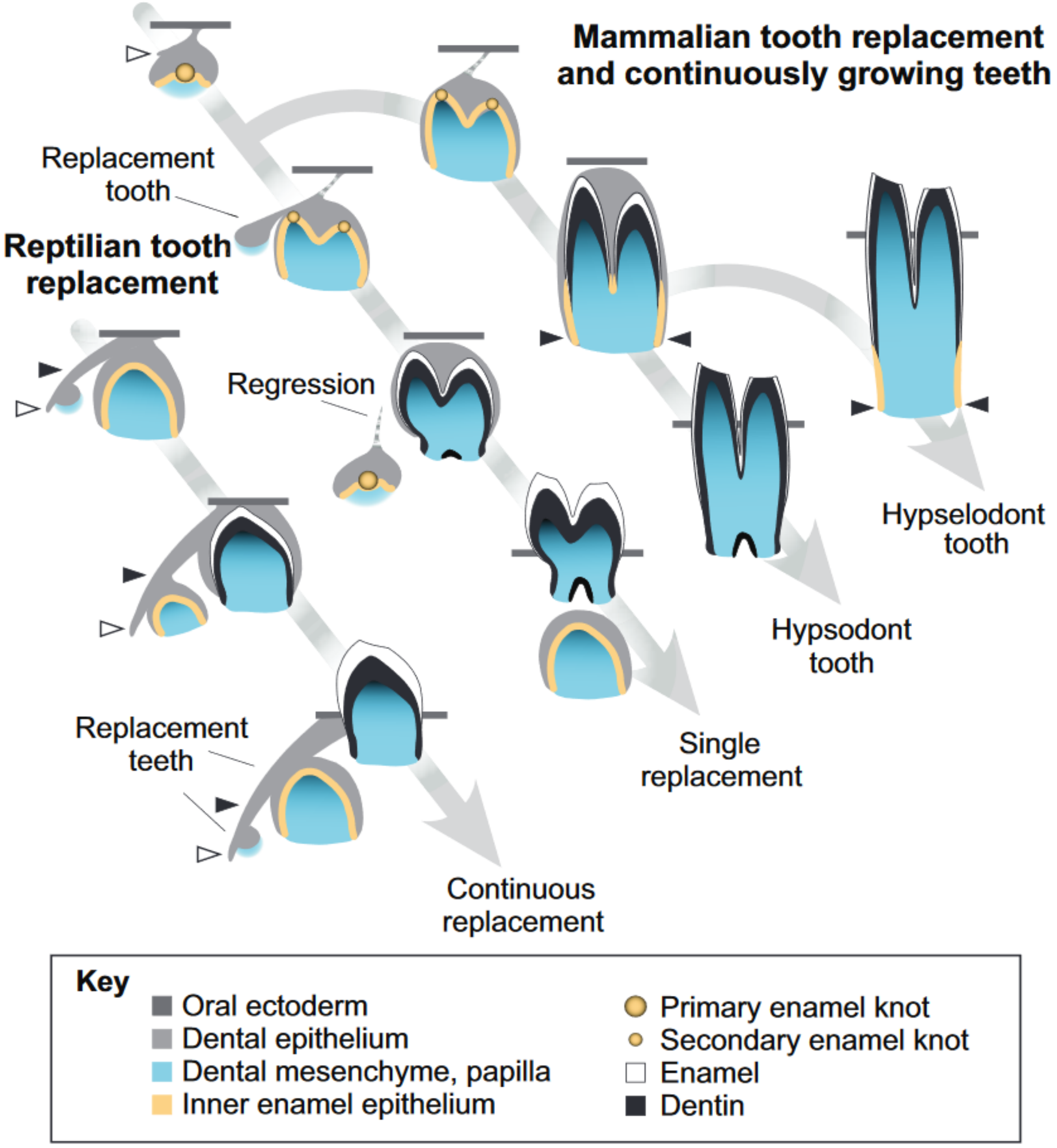


Fig. 2.6 Tooth renewal in different species

The reptilian mode of continuous tooth replacement (shown on the left) involves a dental lamina that extends to form a successional lamina (white arrowheads). A stem cell niche is retained in the dental lamina (black arrowheads). This form of replacement is also likely to be present in many fish. In mammals, the replacement tooth bud develops from the successional lamina as in reptiles, but the lamina regresses and continuous tooth replacement does not occur, beyong the single replacement. In many mammalian lineages teeth have become tall (hypsodont) by delaying root formation; hence, the teeth can wear more. The most-derived stage of tooth regeneration is hypselodonty, which is found, for example, in rodent incisors and vole molars, where the tooth retains stem cells at its base and continues to grow throughout the life of the individual. Taken from Jernvall & Thesleff (2012), with minor adaptation.

2.4.4 Development of replacement teeth without a dental lamina

Studies on the African bichir, *Polypterus senegalus* (Vandenplas *et al.* 2014), the wild Atlantic salmon, *Salmo salar* (Huysseune & Witten 2008) and the rainbow trout, *Oncorhynchus mykiss*, (Fraser *et al.* 2004, 2006*a*, *b*) reveal that in these species, the dental organ of the replacement tooth develops directly from the outer dental epithelium (ODE) of the predecessor tooth, without the need of an SDL. In each of these species, the ODE thickens locally on the posterior-lingual side of the predecessor tooth and invaginates around condensing mesenchymal cells, forming the IDE of the successor tooth. This crescent-shaped structure subsequently progresses through the recognised stages of tooth development (Fraser *et al.* 2006*b*; Huysseune & Witten 2008; Vandenplas *et al.* 2014). Huysseune & Witten (2008) show that in the wild Atlantic salmon, the ODE of the predecessor tooth is continuous with the IDE of its successor. Vandenplas *et al.* (2014) make the same observation in the African bichir, describing how the single cell layers comprising the ODE and IDE ‘merge with each other at the respective cervical loop of each tooth’ of a developing family. In between the ODE and the IDE, a population of cells exists which Huysseune & Witten (2008), term the ‘middle dental epithelium’ or MDE (Fig. 2.7). Subsequently, Vandenplas *et al.* (2014) identified the equivalent layer in African bichir and also used the term MDE. Importantly, Huysseune & Witten (2008) propose that the MDE functionally substitutes for an SDL, and that it supplies the ODE with a source of cells before its differentiation into a placode. They also speculate that the MDE provides a source of stem cells, whose descendants translocate to the forming ODE, similar to the stem cell-utilising mechanism for permanent growth in rodent incisors. They note however that their view contrasts with that of Fraser *et al.* (2006*b*), who considered the ODE itself to function as a transient dental lamina in rainbow trout.

Vandenplas *et al.* (2014) identify differences in the morphology of the cells comprising the MDE in the African bichir, compared to those of the wild Atlantic salmon. Unlike in the salmon, the cells are not homogeneous; cells nearest the IDE of the successor tooth have the phenotype of a stellate reticulum, with large intercellular spaces and a reticular aspect, whereas the remaining bulk of the cell population only has small intercellular spaces and are irregularly shaped. Despite the difference in morphological homogeneity of the MDE cells in the two species, Vandenplas *et al.* (2014) still name this cell population the MDE in the Africa bichir, indicating its equivalence to that of the wild Atlantic salmon (Huysseune & Witten 2008).

As hypothesised by Huysseune & Witten (2008) in wild Atlantic salmon, Vandenplas *et al.* (2014) also speculate that the MDE of the African bichir may contain stem cells that are supplied to the ODE, and whose descendants become the proliferating cells of the developing successor. However their investigation to determine the presence of stem cells was inconclusive (Vandenplas *et al.* 2014), as although no slow-cycling cells could be detected (generally considered to be the key diagnostic characteristic of stem cells), the authors discuss the uncertain reliability of the labelling method used in this case and generally, to detect stem cells, outlining the experimental and conceptual assumptions involved. A follow-up study aiming to detect stem cells in the MDE of wild Atlantic salmon (Vandenplas *et al.* 2016*b*) reached the same conclusion. It did however show the same cell proliferation patterns in both the wild Atlantic salmon and African bichir, when a new successor tooth is initiated: in the MDE, ODE and cervical loop, replenishing the IDE, as well as in the dental papilla before the odontoblasts become fully differentiated. A definitive mechanism for maintaining such localised cell proliferation and therefore tooth replacement over multiple generations however remains elusive in these species.

These searches for stem cells (Vandenplas *et al.* 2014, 2016*b*) form part of the recent, wider surge in research activity to answer the question of how polyphyodonty is made possible, discussed further in section 2.5.1 below. Fraser *et al.* (2020) state that there is ‘substantial evidence to suggest that polyphyodonty depends not only on the preservation of the DL [dental lamina, or presumably an epithelial structure functionally substituting as a transient dental lamina, such as the ODE as proposed by Fraser *et al.* (2006*b*)] but also the maintenance of an epithelial stem cell niche within the DL’. This wider research landscape contextualises the search for progenitor cells facilitating polyphyodonty in those species lacking an SDL, and also contextualises the hypothesis of the source of such cells being the MDE in the both the wild Atlantic salmon (Huysseune & Witten 2008) and the African bichir (Vandenplas *et al.* 2014), as well as the ODE in the rainbow trout (Fraser *et al.* 2006*b*).

It is interesting to note that in medaka and the three-spined stickleback, neither a discrete SDL can be identified, nor development of a successor tooth directly from the ODE of the predecessor, as in the African bichir, wild Atlantic salmon and rainbow trout (sections 2.5.7 and 2.6.3). It has therefore been suggested that a ‘Successional Dental Epithelium’ (SDE) is a more encompassing term (Square *et al.* 2021), to accommodate the varying forms of regenerative epithelia e.g a sheet of cells as in the chondrichthyan DL, a strand as in the SDL of most teleosts studied so far, a localised region as in three-spined stickleback and medaka (sections 2.6.3 and 2.6.4). This would arguably represent a significant departure from how tooth replacement is traditionally represented, but could present a useful means of description, as the number of examples which do not fit the DL/SDL histology, will likely increase over time.

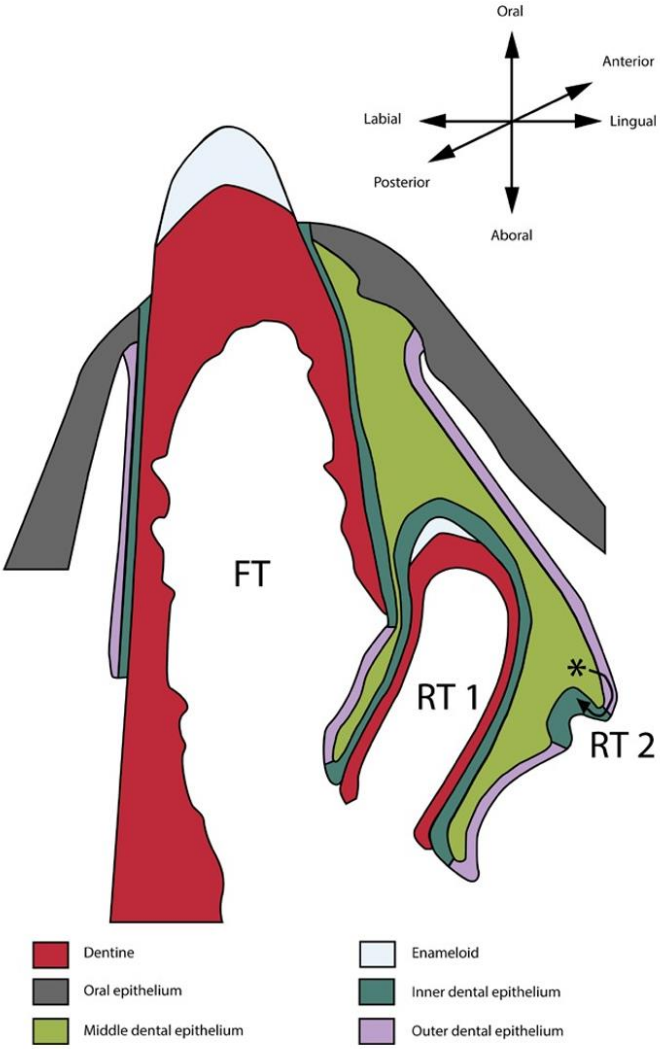


Fig. 2.7 Schematic representation of an African bichir, *Polypterus senegalus* tooth family with three members

Arrow indicates dynamic pattern of proliferating cells; asterisk indicates putative stem cell niche in middle dental epithelium. *Abbreviations*: FT, functional tooth; RT1, primary replacement tooth; RT2, secondary replacement tooth. Taken from Vandenplas *et al.* (2014).

2.4.5 ‘One-for-one’ replacement

In all the variations in the methods of replacement described herein, one aspect that unifies them all is that only one successor can replace one predecessor at any one time. This is facilitated by the epithelial connection between the two, in its varied forms. This is the case even if a family of teeth at a tooth position are present in advance of becoming the functional tooth; only one successor will succeed the functional predecessor at any one time. The term one-for-one replacement is commonly used, though ‘many for one’ is used to describe tooth families in waiting to replace one functional tooth. This has the potential to create confusion, as ‘many for one’ in the context of a tooth family would not suffice if attempting to describe the highly unusual occurrence of a non one-for-one replacement ratio at any one time (sections 2.6.3 and 2.7.2). Care must therefore be taken to describe context when using replacement ratios.

2.4.6 Tooth replacement without an SDL as plesiomorphic in actinopterygians

The African bichir *P.senegalus* belongs to the order Polypteriformes, which diverged early in the actinopterygian phylogeny, and the wild Atlantic salmon *S. salar*, and the rainbow trout *Oncorhynchus mykiss*, are both from the order Salmoniformes, which diverged relatively early in the teleost phylogeny (Near *et al.* 2012). Vandenplas *et al.* (2014) propose that these basal phylogenetic positions give support to Huysseune & Witten’s (2008) hypothesis that tooth replacement originating directly from the ODE of the predecessor tooth is a plesiomorphic character in the actinopterygians. This equates to tooth replacement without *any* type of dental lamina being the plesiomorphic condition for the actinopterygian clade, as not only is an SDL absent, but there exists no dental lamina (as classically defined) to utilise either. This is because in bony fish, first generation teeth develop from the odontogenetic band with no invagination to form a dental lamina (Berkovitz 1978; Huysseune & Sire 1997, Smith *et al.* 2009*a*; Berkovitz & Shellis 2017). Huysseune & Witten's (2008) proposal therefore challenges the historic, and arguably, superseded view that a dental lamina is a pre-requisite for any tooth to develop, and is a synapomorphy for all gnathostomes except placoderms (c.f. the conclusions of Reif 1982; Goujet 2001; Huysseune & Witten 2008; Smith *et al.* 2009*a*; Rücklin *et al.* 2012; Vandenplas *et al.* 2014; Berkovitz & Shellis 2017; Fraser & Thiery 2019). Authors who state a dental lamina is necessary for tooth development and regeneration may indicate from their conclusions elsewhere that certain epithelial structures i.e. the OB, ODE or MDE can 'functionally substitute' for a dental lamina, if a classical dental lamina does not exist e.g. (Fraser *et al.* 2006*b*, 2020; Fraser & Thiery 2019). See also section 2.6.4 for the recent proposal of a 'successional dental epithelium' as an all-encompassing regenerative tissue (Square *et al.* 2021).

2.5 Developmental mechanisms enabling polyphyodonty

As previously discussed (section 1.1), there is great research interest in determining the developmental mechanisms which enable polyphyodonty, with drivers including the potential to develop human therapies for tooth loss as well as understanding the evolutionary origins and history of teeth; for reviews see (Davit-Béal *et al.* 2007; Richman & Handrigan 2011; Jernvall & Thesleff 2012; Whitlock & Richman 2013; Tucker & Fraser 2014; Fraser & Thiery 2019; Fraser *et al.* 2020) and for key research examples see (Huysseune & Thesleff 2004; Davit-Béal *et al.* 2006, Fraser *et al.* 2006*b*; Huysseune 2006; Huysseune & Witten 2008, Smith *et al.* 2009*a*; Handrigan *et al.* 2010; Handrigan & Richman 2010; Jussila & Thesleff 2012; Juuri *et al.* 2012; Fraser *et al.* 2013; Juuri *et al.* 2013; Abduweli *et al.* 2014; Vandenplas *et al.* 2014; Martin *et al.* 2016; Rasch *et al.* 2016, Vandenplas *et al.* 2016*b*; Popa *et al.*

2019; Salomies *et al.* 2019; Fraser *et al.* 2020). Polyphyodonty and diphyodonty are of key interest for investigating the origins and evolutionary history of teeth, as successional regeneration via a dental lamina (or functional substitute), in a regulated pattern, with family-level organisation, are generally agreed to be the features that set teeth apart from skin odontodes, which replace on demand when space occurs (Reif 1982, Smith *et al.* 2009*a*; Fraser *et al.* 2010; Tucker & Fraser 2014; Donoghue & Rücklin 2016; Martin *et al.* 2016). A consensus to emerge from this work has been that in order for teeth to continually, successionally replace, a constant supply of progenitor cells is required, putatively identified as multipotent, odontogenic stem cells (Huysseune & Thesleff 2004; Richman & Handrigan 2011; Jernvall & Thesleff 2012; Fraser *et al.* 2013; Juuri *et al.* 2013; Abduweli *et al.* 2014; Tucker & Fraser 2014; Martin *et al.* 2016; Thiery *et al.* 2017; Fraser & Thiery 2019). In their research overview, Fraser & Thiery (2019) state that these progenitor/putative stem cells are born in the epithelium comprising the odontogenic band during its early development, and are subsequently located in the epithelium of the dental lamina in its varied forms (or functional substitutes for the dental lamina - see section 2.4.4). They enable initial tooth development followed by tooth regeneration, and this is the case for all vertebrates, whether polyphyodont, diphyodont or monophyodont. However, as previously discussed, if the dental lamina degenerates, further generations of teeth cannot continue (as in mono- and diphyodonts). This is generally considered to be because the connection is broken between the population of progenitor/stem cells and a potential new tooth (section 2.4.3 and 2.5.1). The population must always be maintained and be available via a physical connection when required, to create the new successor/replacement tooth, or in some cases, for continuous growth of teeth e.g. the rodent incisor (Harada *et al.* 1999, Smith *et al.* 2009*a*; Jernvall & Thesleff 2012; Tucker & Fraser 2014; Fraser & Thiery 2019).

This population of progenitor/stem cells has been putatively identified in species separated by vast evolutionary distance e.g. in mammals and sharks, as have the molecular mechanisms that regulate and initiate them (see section 2.6.1). Remarkably, this suggests the same key molecules have been conserved and relied upon for dental regeneration throughout the evolution of the vertebrates (Fraser & Thiery 2019).

2.5.1 The importance of stem cells

The question may reasonably arise, why are stem cells required for tooth initiation and replacement? Vandenplas *et al.* (2014) state 'in general, a stem cell can be defined as an undifferentiated cell that, upon division, produces one cell that remains undifferentiated, becoming the next stem cell, and one cell that undergoes proliferation and differentiation'. These proliferating, differentiated cells comprise the 'lineages' which become the various tissues that make up an organ e.g. the ameloblasts and odontoblasts in teeth. In the embryo, all tissues result from nearly 'totipotent' stem cells: those able to differentiate into any of the organism's tissues. At, or soon after birth, many tissues have a population of stem cells, and because as each stem cell divides one undifferentiated stem cell is produced (self-renewed), the capacity for renewal is retained, often throughout life. As such, stem cell-derived tissue regeneration can potentially occur throughout life, either in response to a specific environmental cue (e.g. the renewal of a lizard's tail if lost), or under the control of a programme that regulates renewal both temporally and spatially, as in teeth, hair, skin, scales and feathers (Fuchs & Segre 2000, Smith *et al.* 2009*a*; Fraser *et al.* 2013; Tucker & Fraser 2014).

As discussed previously, tooth replacement requires an epithelial connection between a predecessor tooth and its successor, which takes various forms, either a dental lamina (DL), SDL or a connection within the ODE of the predecessor tooth, from where the new tooth develops directly. This epithelial connection, renewed throughout life, is borne from putative epithelial stem cells, capable of differentiating into the tissues required to make a new tooth (Huysseune & Thesleff 2004; Fraser *et al.* 2013; Tucker & Fraser 2014). It is for this reason that a supply of stem cells is considered essential for tooth replacement, as well as the retention of the epithelial connection between predecessor and successor, without which regeneration fails, as in the case of a third tooth forming in diphyodont mammals. In addition, the role of the mesenchyme is yet to be ascertained in continual tooth replacement. Whitlock & Richman (2013) hypothesise that in the reptilian dentition, the clusters of putative stems cells located on the lingual surface of the dental lamina (first found by Handrigan *et al.* (2010) and described in section 2.5.4) are regulated by 'stationary niches' (the term 'niche' implying a population of stem cells) in the adjacent mesenchyme. In support of this hypothesis they cite Richman & Handrigan (2011), who propose that the canonical Wnt pathway in the putative stem cells of the dental lamina is activated by mesenchymal signals, causing them to divide and form rapidly dividing transit amplifying cells, leading to the development of the next tooth bud. Mesenchymal stem cells have been found in the dental pulp of continuously growing mouse incisors (Balic & Mina 2010; Feng *et al.* 2011; Zhao *et al.* 2014) , however Fraser *et al.* (2020) note that more study is required to understand the role of mesenchymal regulation of tooth regeneration, and the collaboration between epithelium and mesenchyme to maintain continual tooth replacement, given that we know that both are essential for any tooth to form (Jernvall & Thesleff 2012; Jussila & Thesleff 2012).

2.5.2 Locating stem cells using the BrdU pulse chase experiment

Given the general view that a population of progenitor/stem cells is crucial for sustaining successional tooth replacement, it is understandable that locating these populations provides an insight into the mechanism enabling polyphyodonty. Their location provides insight into their relationship with key structures such as the dental lamina and predecessor teeth. It also allows the tracking of the cell lineages the progenitor/stem cells give rise to, enabling the identification of signalling molecules and transcription factors which the cells express. This molecular identification is beginning to reveal the genetic mechanisms controlling tooth replacement (sections 2.5, 2.6.1 and 2.6.2).

The undifferentiated state of stem cells renders them difficult to identify and therefore locate. They are often characterised as having slow cell cycles (except during the initial stages of differentiation, when they rapidly proliferate) (Fuchs & Segre 2000), and this has frequently been used to as a means to identify them, using BrdU (5-Bromo-20-deoxyuridine) as a chemical label. BrdU is a thymidine analogue and is incorporated into the DNA of a cell in the S-phase of its cell cycle. Once this has occurred (a process referred to as the ‘pulse’), the cells’ BrdU label is searched for after a certain period of time (the ‘chase’ time), and only slow-cycling cells (putatively, stem cells) retain the label. Such cells are known as label-retaining cells (LRCs) and the technique is called ‘pulse-chase’ analysis. Vandenplas *et al.* (2014) provide many examples of this experimental approach across a range of species and organs, including the detection of LRCs in intestinal crypts, forebrain, kidney, eye, bone marrow and taste buds.

A BrdU pulse-chase experiment was used to find the first evidence for putative stem cells in a polyphyodont species, the leopard gecko (Handrigan *et al.* 2010), and subsequently in the American alligator (Wu *et al.* 2013), the corn snake (Gaete & Tucker 2013), and the medaka (Japanese ricefish) (Abduweli *et al.* 2014). In reptiles, successional teeth sequentially bud from and extend the labial side of the epithelial invagination comprising the primary dental lamina, enabling a retained connection between each tooth in the family (Fig. 2.8). The growing tip or 'free end' of the dental lamina becomes the successional dental lamina (SDL) when it extends off the newest tooth in a tooth family; as stated by Richman & Handrigan (2011), the successional (dental) lamina is in effect the continuation of the dental lamina. In the leopard gecko, Handrigan *et al.* (2010) found LRCs, and therefore a population of putative stem cells on the lingual side/face of the dental lamina. This was viewed to be a suitable quiescent environment for stem cells, with no teeth forming on this side of the lamina, but on the labial side. The putative stem cells appeared to be located in clusters in between the 'chains' of tooth families, in the interdental regions of the dental lamina, 'close to, but not at the tip' (Fig. 2.8). Gaete & Tucker (2013) also found putative stem cells in the same location in the corn snake. This location was later supported by the work of Juuri *et al.* (2013), who found that in the green iguana, leopard gecko, corn snake and ball python, expression of the stem cell marker Sox2 (see next section) 'overlapped the regions in the dental lamina where putative stem cells have been localized by Handrigan *et al.* (2010) in the leopard gecko'. Later, the location was further supported by studies on the bearded dragon by Salomies *et al.* (2019), who intriguingly also found a second stem cell niche location (see sections 2.5.5 and 2.6.1). Juuri *et al.* (2013) found the location of Sox2 expression in the American alligator to be different, and this agreed with the location of putative stem cells previously identified in this species by Wu *et al.* (2013) (see section 2.5.5).

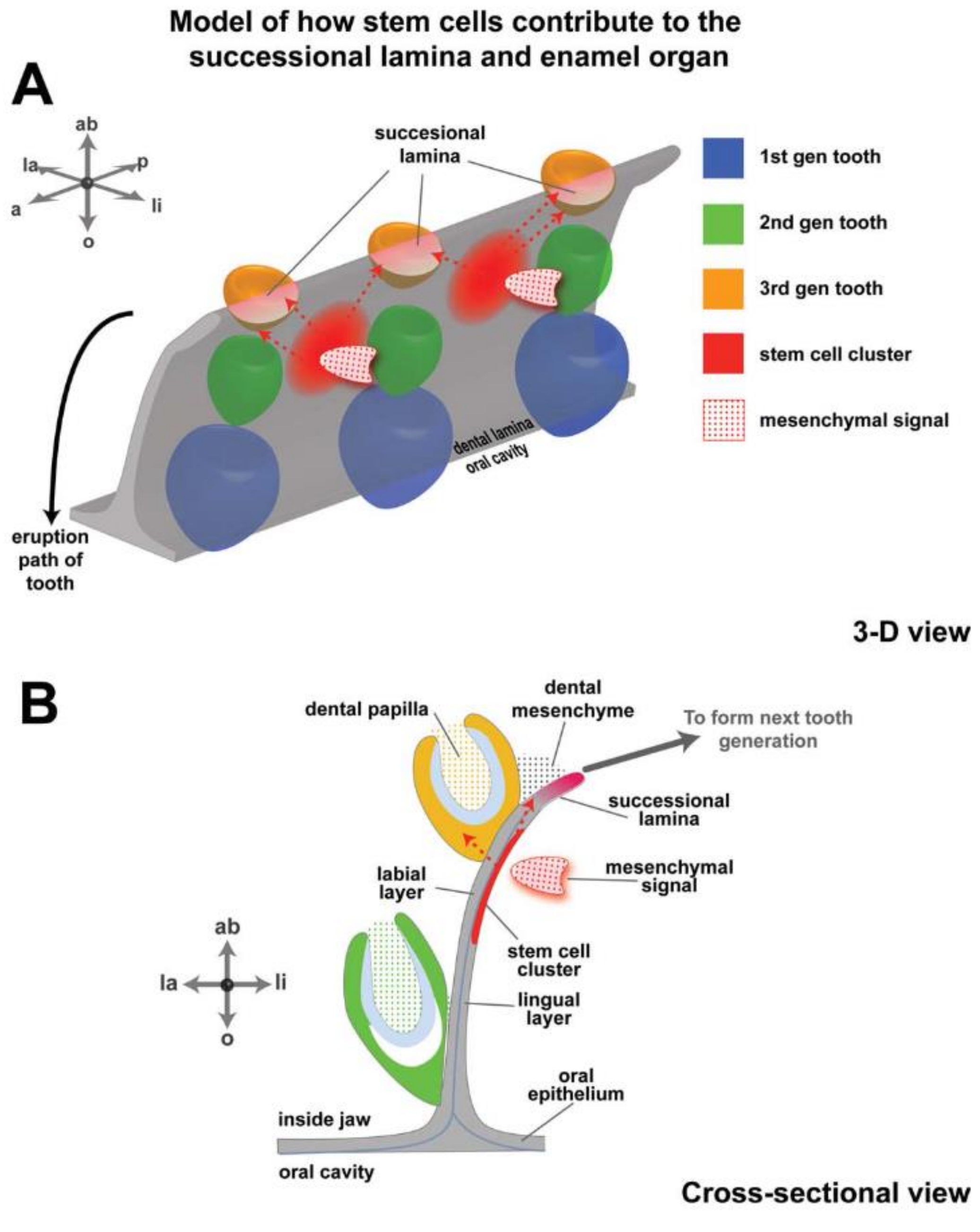


Fig. 2.8 Model proposed by Richman & Handrigan (2011) of how stem cells contribute to the successional lamina (or SDL), and enamel organs in polyphyodont reptiles.

A-B, Multiple tooth generations are arranged into tooth families and each family is connected to a ribbon-like dental lamina that spans the length of the tooth row. Between neighbouring tooth families label-retaining cells were found, referred to as ‘stem cell clusters’ based on their gene expression patterns and long-term retention of BrdU labelling in a pulse-chase experiment on juvenile leopard geckos. The clusters are invariably located on the lingual side of the dental lamina of marginal tooth rows. The simplified model suggests that the clusters are mainly found in the interdental regions and that they feed daughter cells into the successional laminae and newest forming enamel organs of the adjacent tooth families. It is proposed that mesenchymal signals may activate the Wnt pathway and this, in turn, induces asymmetric division of the stem cells where one cell remains a stem cell while the other becomes a rapidly dividing transit amplifying cell. Once amplified cells move into the successional lamina, further epithelial-mesenchymal interactions occur that lead to the formation of the next tooth bud. Taken from Richman & Handrigan (2011).

2.5.3 Locating stem cells using the stem cell marker Sox2

Since the first identification of putative dental stem cells in a species with continuous tooth replacement, the leopard gecko (Handrigan *et al.* 2010), methods other than BrdU pulse-chase analysis for identifying stem cells have been developed and commonly adopted. These rely on ‘marker molecules’ known to be expressed by stem cells. Juuri *et al.* (2012) showed that the epithelial stem cells responsible for the continual growth in the mouse incisor expressed the transcription factor sex-determining region Y-related box 2 (Sox2). Among other functions, Sox2 is known to maintain the undifferentiated stem state in embryonic and adult stem cells (Avilion *et al.* 2003). Harada *et al.* (1999) had previously identified and located the putative stem cells in the cervical loops of the mouse incisor. They had used BrdU pulse-chase analysis combined with a fluorescent label (DiI) technique, which followed the fate of the transit amplifying cells’ progeny as differentiating into ameloblasts. Once Juuri *et al.* (2012) had shown that these putative mouse incisor stem cells were reliably stem cells in this case, and that they expressed Sox2, Sox2 became the primary epithelial dental stem cell marker in subsequent research e.g. (Fraser *et al.* 2013; Gaete & Tucker 2013; Juuri *et al.* 2013; Abduweli *et al.* 2014; Martin *et al.* 2016, Vandenplas *et al.* 2016*a*; Popa *et al.* 2019).

As noted by Vandenplas *et al.* (2016*a*), although techniques relying on label retention and the expression of stem cell markers such as Sox2 do not unequivocally identify stem cells, they provide evidence in support of their presence and potential role in continual tooth replacement. Studies often use a combination of techniques to improve the robustness of findings e.g. (Gaete & Tucker 2013; Abduweli *et al.*

2014). Authors tend not to state the definitive presence of stem cells, but consistently use the term ‘putative’. However the general collective view appears to be that stem cells housed within the DL (or functional substitute) are necessary for tooth replacement, as summarised across a range of model species by Tucker & Fraser (2014).

The specific location of putative stem cell populations have been challenging to pin-point in bony fish e.g. the ‘indistinct bulge’ in medaka (Abduweli *et al.* 2014) (see section 2.5.7). Slow-cycling LRCs were not found at all when searched for in the Atlantic salmon and African bichir by Vandenplas *et al.* (2016*b*), following their hypothesis of stem cell presence in the MDE of both species (Vandenplas *et al.* 2014). In addition, Vandenplas *et al.* (2016*b*) seem the least willing to putatively ascribe stemness to cells expressing stem cell markers such as Sox2 in combination with BrdU label retention. They state ‘… co-localisation of Sox2 expression and LRCs is not sufficient evidence to conclude for dental stem cells being involved in tooth turnover, as they must be shown to produce progeny that shift in position as the replacement cycle progresses’. The latter has been demonstrated in the chondrichthyan model, the small spotted catshark (Martin *et al.* 2016; Fraser *et al.* 2020).

Encouragingly for tooth replacement research in bony fish, two cell populations expressing Sox2 have been identified in a range of cichlid species (Fraser *et al.* 2013). Due to their position, when compared to regenerating dental organs in other species, as well as the co-expression of certain genes, the authors were ‘tempted to speculate that each of these populations contributes to and/or regulates the stem niche for cichlid tooth replacement’. These cell populations were located at the labial oral epithelium, superficial to each invaginating SDL, and also in an intermediate layer between the IDE and ODE of the new, developing tooth. The authors consider the latter layer may be analogous to the stellate reticulum of mouse incisor cervical loops, known to house stem cells (Juuri *et al.* 2012). This location in cichlids is between the IDE and ODE of the same developing tooth, and so despite the similar description, different to the location of the MDE described by Huysseune & Witten (2008) and Vandenplas *et al.* (2014), between the ODE of the predecessor developing tooth and IDE of the successor.

As previously discussed, putative stem cell populations have been identified in the dental laminae of reptiles, via BrdU label retention and Sox2 expression, alongside other stem cell markers in some cases. This has been the case in key research models including the leopard gecko, corn snake, ball python, American alligator and bearded dragon (see section 2.5.2). In mammals, Sox2 has been used to identify putative stem cells in the dental lamina and/or the SDL, or rudimentary SDL, so far in the mouse e.g. (Juuri *et al.* 2012, 2013; Dosedělová *et al.* 2015; Popa *et al.* 2019; Kim *et al.* 2020), the ferret (Juuri *et al.* 2013; Jussila *et al.* 2014), pig (Popa *et al.* 2019) and human (Juuri *et al.* 2013; Fraser *et al.* 2019).

2.5.4 Proposed mechanism for polyphyodonty in reptiles involving stem cells

Richman & Handrigan (2011) proposed that in polyphyodont reptiles generally, the clusters of stem cells identified by Handrigan *et al.* (2010) in the leopard gecko dental lamina feed daughter cells into the successional laminae at the end of adjacent tooth families (possibly to both families either side of the cluster) (Fig. 2.8). This gives rise to the development of the newest tooth buds. This was supported by their previous work on tooth replacement in the ball python (Handrigan & Richman 2010), as well as the work of Gaete & Tucker (2013) on the corn snake. Richman & Handrigan (2011) also propose that this process is initiated by signals from mesenchymal cells, which may activate the Wnt signalling pathway in the dental lamina, causing the quiescent stem cells to divide, each producing one stem cell and one transit amplifying (TA) progenitor cell. As described previously, this is the cell which rapidly divides/proliferates to produce many TA cells, which after further divisions become fully differentiated cells (Fuchs & Segre 2000). The transit amplifying progenitor cells move into the successional lamina, leading to the formation of the next tooth bud. This model is remarkably similar to the system proposed in sharks (Martin *et al.* 2016; Fraser *et al.* 2020), where transit amplifying cells migrate from a slow-cycling stem cell niche to the shark successional lamina, facilitating the development of the newest teeth (see section 2.5.9).

2.5.5 The American alligator and the bearded dragon: reptile polyphyodonty model misfits?

Although the model proposed by Richman & Handrigan (2011) was a mechanism for polyphyodonty in reptiles generally, some very interesting exceptions have since been discovered. Wu *et al.* (2013) found that in the American alligator, dental laminae showed a more complex compartmentalization, with stem cells found only as an enlarged cell cluster, termed the dental lamina bulge, at the distal tip of the dental lamina. This contrasted with the stem cell clusters distributed more diffusely along the dental lamina (close to, but not in its tip) as first located in the leopard gecko (Handrigan *et al.* 2010), and proposed as a general distribution pattern in reptiles in Richman & Handrigan's (2011) model. Wu *et al.* (2013) hypothesised that 'the emergence of the distal bulge in the dental lamina may help to pattern clustered stem cells during alligator tooth cycling, that may maintain a niche for the persistence of tooth renewal for multiple generations'. Furthermore, unlike other studied reptiles, the dental lamina became disconnected from the oral epithelium, and adjacent tooth families were separated from one another by bone. However, tooth families were still interconnected by a thin, string-like dental lamina, equivalent to the interdental region of the dental lamina described by Richman & Handrigan (2011). A further significant feature of the study by Wu *et al.* (2013) which has not been addressed in other research reviewed here, was that experimental extraction of the functional tooth activated the putative stem cells in the dental lamina, and initiated a new tooth cycle. This could have important implications for understanding the control of the timing of tooth replacement and patterning.

Another research model which does not neatly fit the model proposed by Richman & Handrigan (2011) is the bearded dragon. Salomies *et al.* (2019) found a putative stem cell/progenitor niche in the bearded dragon in the same location as that found by Handrigan *et al.* (2010) in the leopard gecko, and therefore consistent with Richman and Handrigan's model thus far. The bearded dragon has polyphyodont and monophyodont tooth positions in the same jaw, and this stem cell niche was only found in the DL at polyphyodont tooth positions. However, at both monophyodont and polyphyodont tooth positions, surprisingly they also found a different population of putative stem/progenitor cells at the surface oral epithelium, in close proximity to both the DL invagination and a taste bud (Fig. 2.9). Intriguingly, the positioning of this population bears a strong resemblance to that of the stem cell/progenitor TTJ niche observed in the small-spotted catshark (Martin *et al.* 2016) (see section 2.5.9, below). Arguably its position also renders it similar to the putative stem cell population identified in the surface oral epithelium in cichlids (Fraser *et al.* 2013), adjacent to the invaginated epithelial downgrowth comprising the successional lamina (section 2.6.2, below). Furthermore, in the bearded dragon, Salomies *et al.* (2019) tracked the migration of oral epithelium putative stem cells' descendants (i.e.

TA cells, though not specifically described as these in the study) along the DL, to both the DL niche (present in polyphyodont positions only) and into the successional lamina (termed successional dental lamina by the authors – 'SDL'), where they contributed to the proliferative growth of both. This is also the case in the small-spotted catshark (Martin *et al.* 2016), though cell migration occurs to the SDL only, as no equivalent of the reptile DL putative stem cell population exists (section 2.5.9) (Fig. 2.9).

Importantly for the elucidation of the mechanisms that enable polyphyodonty in vertebrates generally, in the bearded dragon, this migration of oral epithelium putative stem cell descendants, and consequent growth in the SDL, occurs at both monophyodont and polyphyodont tooth positions of the same jaw. Salomies *et al.* (2019) therefore propose that SDL maintenance and growth is not enough in itself to enable successional tooth development.

In the search for factors that *do* enable successional tooth development, comparing the monophyodont and polyphyodont SDLs in the bearded dragon is of key interest, and important differences were observed between the two. Salomies *et al.* (2019) show that in the polyphyodont teeth, the direction of SDL proliferative growth was lingual, and in the monophyodont the growth projected downwards towards the jaw bone. They suggest that the growth in the lingual direction is likely to provide accommodation space for the replacement tooth. In the polyphyodont SDL, they also observed an asymmetric regional expression pattern of tooth replacement genes known to be conserved across vertebrate taxa e.g. Pitx1 and the Wnt readout gene Lef1. In the monophyodont SDL, the expression of these conserved genes was scattered, and not patterned into regional expression. Interestingly, consistent with the inverse relationship between Sox2 expression and and Wnt/β-catenin signalling observed in other polyphyodont models (e.g. the corn snake, see section 2.5.6 below), Sox2 expression was excluded from the SDL tip in the polyphyodont SDL, where Wnt/β-catenin signalling was located, as revealed by the presence of Lef1.

As such, Salomies *et al.* (2019) propose that as well as its direction of growth, regional patterning of gene expression levels (and gene expression dynamics) in the SDL is important for enabling vertebrate tooth replacement. Maintenance and growth of the SDL alone is not enough. As well as the regional patterning, there was generally a great difference in the levels of gene expression between monophyodont and polyphyodont SDLs, and also the mesenchymal tissues surrounding each SDL type.

Particularly significant in understanding the mechanism enabling polyphyodonty, Salomies *et al.* (2019) found a large number of new, as yet uncharacterised genes, likely involved in SDL proliferation/growth, and/or tooth initiation, as some genes were only expressed in the polyphyodont SDL.

It is important to recall here that there is no DL population of putative progenitor/stem cells in the bearded dragon monophyodont tooth positions (Fig. 2.9). Salomies *et al.* (2019) suggest that this population could play a key role in the organisation of the polyphyodont SDL, giving rise to its differences to the monophyodont SDL, which facilitate the repeated initiation of new teeth.

It is interesting to also recall at this point that Wu *et al.* (2013) observed a lack of connection between the oral epithelium and the DL in the American alligator. The lingual outer dental epithelium of a developing replacement tooth splits away from it to form a new dental lamina for the next new tooth (Wu *et al.* 2013, Fig. 3B). As such, there is no retained epithelial connection provided by the DL between developing teeth in a family, or to the oral epithelium, as in the leopard gecko and other studied lizards and snakes. Considering this, should an oral epithelium stem cell population ever be found in the American alligator, it does not appear possible that a TA cell migration, equivalent to that seen in the bearded dragon or small spotted catshark, could occur from the oral epithelium to the tooth forming region, due to the lack of retained epithelial connection. Consequently, the function this niche fulfils in these two species (suggested to be the facilitation of SDL growth in the bearded dragon) could not be fulfilled in the American alligator tooth-forming region. This appears also to be true of the leopard gecko and the other squamate species studied e.g. the ball python and corn snake, where no second putative stem cell population at the oral epithelium has been found. However, Salomies *et al.* (2019) imply, perhaps controversially, that the population could have been missed in these species, as they contend that the oral epithelium has ‘not been analysed in previous lizard and snake studies despite the strong Sox2 expression observed in this region’.

Overall, though the model proposed by Richman & Handrigan (2011) accounts for the tooth replacement mechanisms observed in most reptiles studied so far, the divergences from this model exhibited by the American alligator and the bearded dragon offer opportunities for new lines of enquiry. The bearded dragon offers a particularly rich source of new leads, given the observed similarities with the spotted catshark, contrasting regenerative ability within the same dentition, and the identification of new genes associated with replacing and non-replacing teeth.

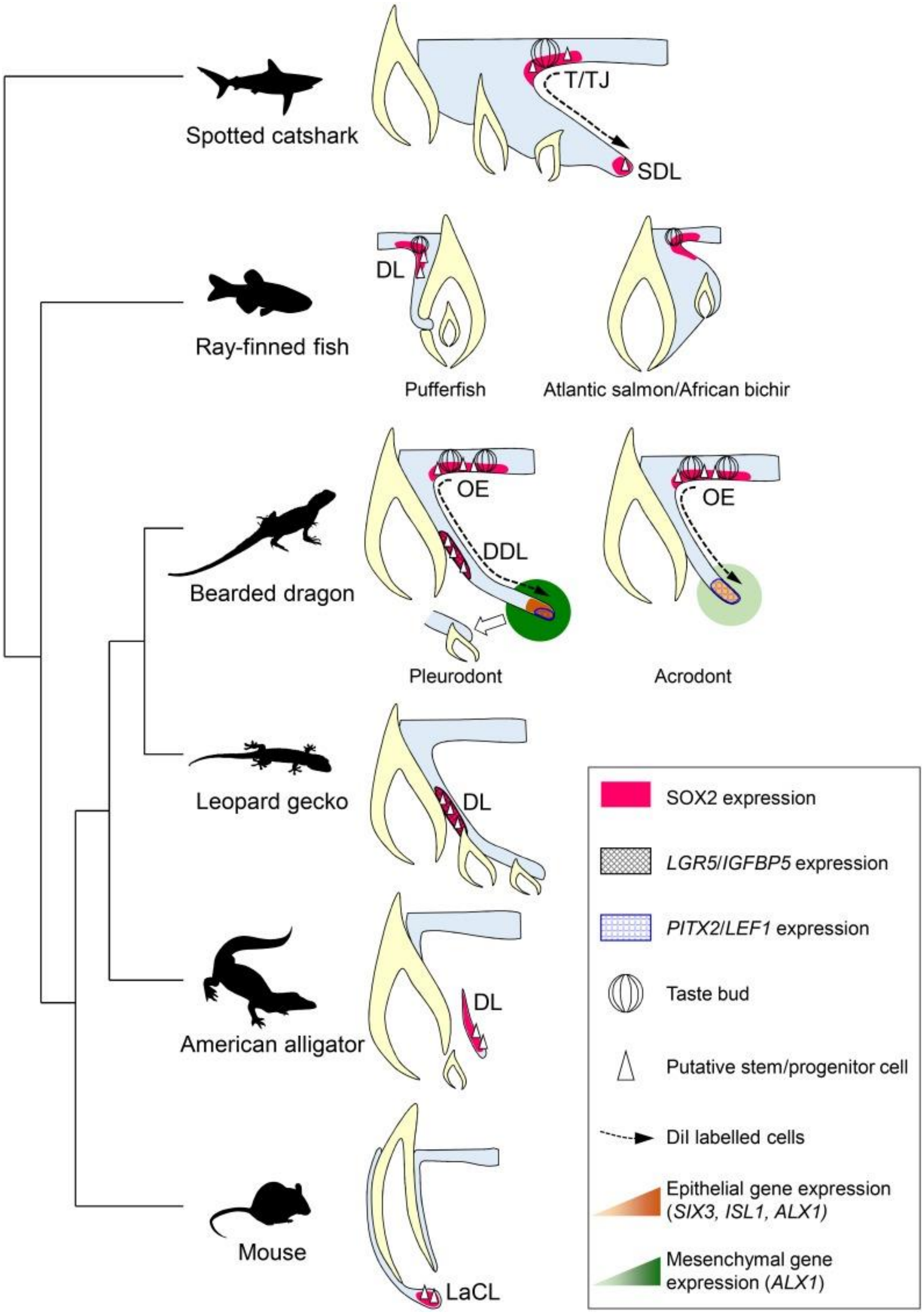


Fig. 2.9 Comparative models of cellular and molecular processes controlling oral tooth replacement in polyphyodont species

Schematic drawings of oral tooth replacement strategies in polyphyodont species with molecularly-characterized LRC populations: spotted catshark (Martin et al., 2016), pufferfish (Thiery *et al.* 2017), Atlantic salmon (Vandenplas *et al.* 2014, 2016*b*), African bichir (Vandenplas *et al.* 2014, 2016*b*), bearded dragon pleurodont (polyphyodont) and acrodont (monophyodont) teeth (Salomies *et al.* 2019), leopard gecko (Handrigan et al., 2010), and American alligator (Wu *et al.* 2013). The putative stem cell niche of monophyodont mouse incisor (Harada *et al.* 1999) is also shown for comparison, with SOX2-positive putative stem cells responsible for continual growth located in the labial cervical loop (LaCl) (Juuri *et al.* 2012). In pufferfish but also cichlid fish (Fraser *et al.* 2013), a SOX2-positive putative dental progenitor niche resides in the most superficial dental lamina (DL). In Atlantic salmon and African bichir, no epithelial LRCs have been identified despite positive SOX2 expression in the OE and ODE transition zone. In the leopard gecko, LRCs expressing adult stem cell markers such as IGFBP5 and LGR5 reside on the lingual side of the DL. In the American alligator, putative stem cells localize to the distal enlarged bulge of the DL. In spotted catshark, SOX2-positive putative dental progenitors migrate from the superficial taste/tooth junction (T/TJ) towards the successional dental lamina (SDL). In bearded dragon pleurodont teeth (polyphyodont), LRCs are located both in the SOX2-positive oral epithelium (OE; region similar to the T/TJ) and SOX2-positive DDL (region similar to the gecko DL). During regeneration, cells migrate from the superficial OE towards the SDL, the SDL shows focal expression of SDL marker genes (PITX2/LEF1), and both the SDL and surrounding mesenchyme exhibit relatively high expression of newly identified dental genes (ALX1/SIX3/ISL1), thus leading to the initiation of replacement tooth. In acrodont teeth (monophyodont), LRCs are also evident in the SOX2-positive OE and cell migration occurs from the superficial OE towards the SDL, thus contributing to SDL growth. However, the acrodont (monophyodont) SDL shows scattered expression of SDL markers and low expression of newly identified dental genes, most likely as a result of absence of DL stem/progenitor cells and SDL organization, and no replacement teeth are formed. Taken from (Salomies *et al.* 2019), with minor adaptation.

2.5.6 Compartmentalisation of gene expression in the SL and DL in the corn snake

Gaete & Tucker (2013) found evidence of compartmentalisation of gene expression corresponding to the structures of the successional lamina and the DL in the corn snake. Their findings provide support for the model proposed by Richman & Handrigan (2011), that stem cell clusters feed daughter cells into the successional laminae at the end of adjacent tooth families, to form the newest tooth buds. Note that the successional lamina is the equivalent to the structure termed the SDL by Salomies *et al.* (2019) in their research on the bearded dragon. Gaete & Tucker (2013) found that the DL and wider oral epithelium of the of the corn snake expressed Sox2, which as previously discussed, is a known to be a marker of stem cells (section 2.5.3). Furthermore, the expression of Sox2 in the lingual DL in the corn snake, at the (aboral) end closest to the SL, coincided with the localization of putative dental stem cells in the leopard gecko, which houses BrdU label-retaining cells and known hair stem cell markers (Handrigan *et al.* 2010) (section 2.5.2). This combination of evidence therefore indicates that the cells expressing Sox2 (Sox2+ cells) in the corn snake were stem cells. Sox2+ cells were absent in the SL, but activation of the canonical Wnt/β-catenin signalling pathway occurred in the SL, revealed by the expression of its target gene, transcription factor Lef1, in this region. These findings agreed with previous work that showed the Wnt/β-catenin signalling pathway inhibits Sox2 expression (Mansukhani *et al.* 2005). They are also consistent with the locations of Sox2 expression and Wnt/β-catenin signalling in the bearded dragon polyphyodont tooth positions (section 2.5.5). In keeping with Richman & Handrigan's (2011) proposed mechanism of reptile polyphyodonty, Gaete & Tucker (2013) hypothesised that Sox2+ putative stem cells in the lingual side of the aboral dental lamina may form progeny that end up in the successional lamina, which switch off the Sox2 gene.

Gaete & Tucker (2013) elegantly demonstrated the function of the Wnt/β-catenin pathway in the corn snake SL. Experimental activation of the pathway throughout both the DL and SL caused abnormal patterning of tooth buds, with them appearing ectopically along the DL. Usually, with such activation occurring only the SL (revealed by the presence of Lef1), only the SL produces new teeth. Furthermore, when activated in the SL, the Wnt/β-catenin pathway facilitates the ordered and sequential emergence of new teeth, rather than the disordered arrangement of tooth buds observed in the DL, when it was activated there. This indicator of the role of the Wnt/β-catenin pathway is consistent with studies in other species on the ordered, sequential patterning of replacement teeth (Fraser *et al.* 2013). The bearded dragon study by Salomies *et al.* (2019) provides further insight, suggesting that the stem cell population in the DL influences regional gene expression patterning within the SL, therefore its complex organisation and orderly production of new teeth (however, see below for further discussion regarding this suggestion and the role of the Wnt/β-catenin pathway).

2.5.7 Locating stem cells in medaka

In the medaka fish *Oryzias latipes*, Abduweli *et al.* (2014) found BrdU LRCs in an ‘indistinct bulge’, forming at the end of tooth families forming in the pharyngeal jaw. They state ‘unlike in the dentition of toothed reptiles, the dental lamina in the pharyngeal dentition of medaka was not clearly discernible as discrete structures. Current data indicate that the dental lamina in medaka pharyngeal dentition corresponds to the indistinct bulge at the posterior end of each tooth family where the slow-cycling LRCs and Sox2-expressing cells are located’ (see section 2.5.3 regarding Sox2 use as a stem cell marker). This observation is consistent with our knowledge of bony fish lacking a permanent DL. Could the ‘indistinct bulge’ containing putative stem cells comprise the cells of an MDE, arising from the development of the newest tooth germ directly from the ODE of its predecessor, as in the Atlantic salmon, African bichir (see section 2.4.4)? It is unclear if this could be the case, though the ‘indistinct bulge’ is described as arising from odontogenic epithelium, and ‘next to the most posterior tooth germs in each tooth family’, suggesting it is possible. In medaka, there certainly is no epithelial downgrowth (comprising an SDL), as most commonly seen in bony fish. Also, the timing of replacement tooth initiation is different to that via an epithelial downgrowth/SDL, where the SDL develops from the surface oral epithelium once the predecessor tooth has erupted (Huysseune 2006; Fraser *et al.* 2013); in medaka the immediate predecessor tooth is not yet erupted.

As described previously, Fraser *et al.* (2006*b*) contended the ODE functionally substitutes as a transient DL in rainbow trout. Vandenplas *et al.* (2014) and Huysseune & Witten (2008) proposed the MDE, closely apposed to the ODE, to be the DL functional substitute in both the African bichir and Atlantic salmon respectively, hypothesising the specific location of stem cells to be the MDE. The discovery by Abduweli *et al.* (2014) of putative stem cells in what could be the equivalent general region in medaka, could be viewed as support for either of these proposals. However, their location in medaka is likely not well enough resolved.

2.5.8 Developmental link between teeth and taste buds

Using Sox2 identification to establish their location, Martin *et al.* (2016) found evidence for a link between the putative stem/progenitor cells which give rise to teeth in sharks, and those that give rise to taste buds. As previously discussed, they showed that the epithelia comprising the odontogenic band in the small-spotted cat shark, *Scyliorhinus canicula*, is bi-functional; it includes cells fated to develop putative stem/progenitor cells that give rise to both teeth and taste buds in close proximity. As such, they proposed the renaming of the odontogenic band (OB) the odontogustatory band (OGB), reflecting both the tooth and taste competence of this region of the oral epithelium. Bloomquist *et al.* (2015) also found the same phenomenon in the OBs of cichlids, leading Fraser & Thiery (2019) to suggest the term odontogustatory band for these and other teleosts. This relatively recent, significant development is now a key consideration in the debate on the evolutionary origin of teeth (section 2.1.5). This work has also shed light on the mechanism by which putative stem/progenitor cells facilitate tooth replacement in sharks, and possibly vertebrates more generally. A brief outline of this process is given here.

2.5.9 Putative stem/progenitor cells and the shark tooth replacement mechanism

As described previously, all teeth in both upper and lower shark jaws are interconnected within a continuous, sheet-like, jaw-length dental lamina, formed by a deep invagination of the ondontogenic band (OB). In the catshark, as outlined in the previous section, the discovery of the dual competence of this OB, with its inclusion of putative stem/progenitor cells that give rise to taste buds as well as teeth, has led to the suggested name the ‘odontogustatory band’, or OGB. The catshark DL forms as usual in sharks, as a deep invagination of the OGB. As the invagination occurs in the embryo, a population of Sox2+ putative stem/progenitor cells clusters at the edge of the invagination, known as the taste-tooth junction (TTJ) (Martin *et al.* 2016). The name is due to the outer surface oral epithelium’s potential to develop taste buds, with the term ‘surface’ used here to indicate the epithelium outside the deeply invaginated DL. These cells at the TTJ form a continuous band along the edge of the DL, following the approximately arched shape of the catshark jaw margin (Martin *et al.* 2016; Fig. 3F and J). From this population, Sox2+ putative stem/progenitor cells are either directed to contribute to development of taste buds nearby, at the oral surface, or migrate deep into the dental lamina, where at the terminal, distal end they form a second population from which successional teeth develop (Fig. 2.9). As such, this distal tip of the dental lamina comprises the catshark’s successional lamina (SL, although possibly equivalent to the successional dental lamina, SDL, in the bearded dragon). Martin *et al.* (2016) demonstrated this migration by labelling the Sox2+ putative stem/progenitor cells at the TTJ with lypophilic dye (DiI). This meant they could track the label, and therefore the movement of the cells, revealing their direct incorporation into either new teeth, or the supporting cells of taste buds adjacent to the TTJ. In addition, through tracking mitotic activity using the marker proliferating cell nuclear antigen (PCNA), they showed that by the generation of the third tooth, proliferation of cells in both populations of Sox2+ putative stem/progenitor cells dropped markedly, necessary for the establishment of slow-cycling, adult stem cell niches. The resulting niches at the

TTJ and SL enable the lifelong production of successional teeth, with putative stem/progenitor cell migration from the former to the latter occurring repeatedly. Drawing on the work of Martin *et al.* (2016) and Rasch *et al.* (2016), Fraser *et al.* (2020) describe how this repeated migration contributes the perpetual cycle of tooth replacement in the catshark, and how the overall cycle operates.

The ‘starting’ point of the perpetual cycle could be considered as the upregulation of Wnt/β-catenin signalling within the Sox2+ progenitor niche within the SL. This upregulation is associated with increased proliferation of the SL, and the onset of tooth initiation (Martin *et al.* 2016; Rasch *et al.* 2016; Fraser *et al.* 2020). Once this occurs, the developing teeth physically move through the DL in the ‘conveyor belt’-like manner (see section 2.4.1), towards to oral surface. During this journey, they become sufficiently developed to take up the functional position (Fraser *et al.* 2020) . It is thought that developing teeth in the catshark secrete signalling molecules that inhibit the development of new teeth in close proximity, creating a ‘zone of inhibition’ (ZOI) surrounding them (Fraser *et al.* 2020). Such a signal could counteract the initiatory action of Wnt/β-catenin signalling on the progenitor cells of the SL, and overwhelm it, causing new tooth production to stop. However, as the newly initiated tooth physically moves away from the SL in the ‘conveyor belt’, the effect of the inhibitory molecules secreted by the tooth is diluted with increased distance. The Wnt/β-catenin signalling in the SL can then overwhelm the reduced inhibitory signal, and initiation of a new tooth is activated. Therefore, the Wnt/β-catenin signalling and its consequent tooth production is paused temporarily, rather than stopped completely. Importantly, it is during this pause phase that new progenitor cells are pooled towards the SL (having started out their migratory journey from the TTJ), ensuring a constant supply for continual tooth development at the SL. This ‘start/stop’ process allows enough space to open up behind the preceding tooth, for a new tooth to initiate. This would otherwise be impossible in the physically restricted SL, the furthest tip of the DL invagination, which is surrounded by cartilage. The start/stop process also enables alternate patterning of teeth in the catshark DL. While one family is in a start phase, initiating a new tooth, the two adjacent families either side of it are in the pause phase. This ensures that the positions of teeth in adjacent families are offset from each other, enabling close packing and the maximum use of space in the DL (Fraser *et al.* 2020, Fig. 6). The ZOI of each tooth not only ensures consistent distance between developing teeth within a family, but also between teeth of adjacent families, creating the ‘interdental space’ (Fraser *et al.* 2020, Fig.6). In addition, the alternating timing across families of functional teeth emerging at the jaw margin results in there never being more than one empty space between any two teeth in the functional tooth row. Strong geometric patterning exists in developing chondrichthyan teeth more generally (Underwood *et al.* 2015, 2016; Smith *et al.* 2018), with alternate or regular spacing between adjacent families. Based on the cyclical mechanism of perpetual tooth development in the catshark described by Fraser *et al.* (2020), the same authors suggest that the variations in this geometric patterning across species arise from an interplay between two variables: the timing of the ‘start’ phase of tooth initiation in adjacent tooth families in relation to each other, and the strength of the inhibitory signal secreted by each tooth, dictating the size of the ZOI and therefore the amount of space between all teeth.

Fraser *et al.* (2020) propose that understanding the genetic trigger that forces daughter, TA cells away from the TTJ, or from niches of slow cycling stem/progenitor cells in polyphyodonts more generally, is invaluable in the quest to understand the mechanisms underpinning of lifelong, successional tooth replacement. They also propose that understanding the genetic mechanisms of the pause-phase in the chondrichthyan regenerative dental cycle may illuminate more generally how dental competence is lost in all non-polyphyodonts (including humans), and therefore how it could potentially be regained.

2.6 Control of tooth replacement

2.6.1 The molecular basis of replacement tooth initiation

As described in section 2.5.5, migration of Sox2+ stem/progenitor cells from a surface niche to the SDL, via the DL, occurs in the bearded dragon (Salomies *et al.* 2019). This is strikingly similar (and possibly synapomorphic) to the migration of Sox2+ stem/progenitor cells to the SL of the small-spotted catshark, *S. canicula* (Martin *et al.* 2016) (section 2.5.9). Following Salomies *et al.* (2019), the SL of the small-spotted cat shark is considered equivalent to the bearded dragon SDL, and so is termed SDL here, for easy comparison.

As described above, in both the bearded dragon and the small-spotted catshark, upregulation of Wnt/β-catenin signalling is associated with increased proliferation of the SDL. Martin *et al.* (2016) and Fraser *et al.* (2020) associate this with the initiation of tooth development. However, it is intriguing to note that the upregulation of Wnt/β-catenin signalling in the SDL of the monophyodont tooth positions of the bearded dragon appeared to cause proliferation of the SDL, but tooth initiation did not follow, whereas it did in the polyphyodont positions.   Salomies *et al.* (2019) therefore do not attribute the Wnt/β-catenin upregulation (and consequent SDL proliferation) as the sole cause of tooth initiation. They propose that as well as SDL proliferation, complex organisation of the SDL is key (see section 2.5.5). This organisation occurs at the levels of proliferative and directional growth, relative amounts of gene expression, and regionalisation and patterning of gene expression. Also, it involves a large number of as yet uncharacterized developmental genes and signalling pathways. This work certainly indicates there is much to be uncovered in terms of the molecular basis of the initiation of a replacement tooth. Indeed, citing Salomies *et al.* (2019), Fraser *et al.* (2020) urge a shift of research focus away from genetic markers for tooth replacement first discovered in the mouse model, in favour of new markers specific to the regulation of polyphyodonty. Salomies *et al.* (2019) also propose the putative stem/progenitor cell population in the DL, typical of reptile dentitions (Richman & Handrigan 2011), which is present in the bearded dragon polyphyodont tooth positions but absent at the monophyodont, is likely to have a key role in the complex organisation of the SDL. It is however important to note that no equivalent niche is present in the small-spotted catshark DL.

The findings of Salomies *et al.* (2019) are evidently an important consideration for understanding the process of successional tooth development in the small-spotted catshark, reptiles, and vertebrates in general, as they may suggest that SDL proliferation alone, associated with upregulation of Wnt/β-catenin signalling, is not enough to cause initiation of successional teeth. However, upregulated Wnt/β-catenin signalling has been associated with successional tooth initiation in a range of vertebrates, e.g the leopard gecko SDL (Handrigan *et al.* 2010), American alligator DL bulge (Wu *et al.* 2013), small-spotted catshark SDL (Martin *et al.* 2016; Rasch *et al.* 2016) and ferret SDL (Jussila *et al.* 2014). This calls into question the claim of Salomies *et al.* (2019) that upregulated Wnt/β-catenin signalling itself is not causative of tooth initiation. In this context, and in light of the work by Popa *et al.* (2019), Martin *et al.* (2016) and Fraser *et al.* (2020), could it be possible that the bearded dragon monophyodont tooth may have secreted an inhibitor which overrode the action of Wnt/β-catenin signalling, rather than it not having a causative effect? Further comparison of the monophyodont and polyphyodont teeth in the bearded dragon certainly holds promise for understanding the role of the Wnt/β-catenin signalling pathway in successive tooth initiation.

The findings of Gaete & Tucker's (2013) study on the corn snake dentition, outlined previously, are interesting to compare with those of Salomies *et al.* (2019). They found that only in the SDL could upregulation of Wnt/β-catenin signalling result in the sequential and ordered initiation of successional teeth. When Wnt/β-catenin signalling was experimentally upregulated in the DL, it increased cell proliferation and many additional teeth developed, but in disordered, ectopic positions along the DL. In keeping with the proposal of Salomies *et al.* (2019) , this implies that there is a level of organisation and genetic control present in the SDL which is absent in the DL. It seems likely that the complex organisation illuminated by Salomies *et al.* (2019) in the bearded dragon SDL is the key factor in the corn snake SDL, which, when interacting with the Wnt/β-catenin pathway, allows the ordered, sequential production of new teeth. However, in contrast to the conclusions drawn by Salomies *et al.* (2019), Gaete & Tucker's (2013) findings indicate the upregulation of the Wnt/β-catenin signalling as causative of tooth initiation.

2.6.2 Control of timing, positioning and shape in tooth replacement

As previously discussed, the immense diversity of vertebrate dentitions, in terms of tooth shape, size, patterning, rate of development and replacement, replacement mechanisms, and number of tooth generations is particularly interesting in light of the how the genetic mechanisms underlying tooth development are highly conserved across the whole vertebrate clade (Bei 2009; Jernvall & Thesleff 2012). Tooth development is a process that has remained stable over a large proportion of evolutionary time (~400 million years), and the observed great diversity has been attributed to ‘developmental tinkering’ (Lieberman & Hall 2007; Tummers & Thesleff 2009; Jernvall & Thesleff 2012; Rasch *et al.* 2016; Fraser & Thiery 2019). But what might this ‘tinkering’ comprise?

Fraser *et al.* (2013) used cichlid dentitions to explore how teeth develop complex shapes and patterning (in terms of positioning), while undergoing programmed replacement at the same time. They highlight how the co-ordination of these three processes is poorly understood in most systems but is often functionally crucial. For example, feathers for flight, scales for swimming and teeth for feeding must be of the appropriate, shape, size and structure (morphology), must be patterned/positioned correctly and must develop at the right time ensuring there are no gaps, in order to collectively achieve their function.

Fraser *et al.* (2013) investigated the role of five signalling pathways known to be important in controlling two other lifelong regenerative systems, hair and feathers, as well as the development of mouse molars and incisors: BMP, FGF, Hh, Notch, Wnt/β-catenin. Interference with these pathways in vivo using small molecules known to inhibit each, revealed the pathways’ effects on tooth morphogenesis, patterning and replacement. All five pathways affect shape and contain genes involved in putative signalling centres associated with cusp morphogenesis. Cusp number, sharpness and shape was manipulated via the pathway inhibitors. Many pathway molecules were expressed in the same locations in mammal tooth enamel knots (from which cusp development and therefore shape is directed, see section 2.2.5). This led the authors to suggest that fishes and all vertebrates with tooth cusps possess primitive enamel knot-like signalling centres which control size, sharpness and cusp number. The curvature of the tooth, and the amount and location of enameloid laid down was also manipulated using the pathway inhibitors. Interference with BMP, Notch and Wnt/β-catenin pathways affected the process of cichlid tooth replacement. Inhibition of BMP and Notch pathways caused a complete absence of tooth replacement at certain tooth positions. Wnt/β-catenin pathway inhibition caused tooth replacement to be delayed, but not completely lost at certain positions. These results, combined with the observed location of where the various pathway genes were expressed, led the authors to conclude that co- and sequentially expressed pathway molecules in specific locations and at specific times allowed 'spatio-temporal complementarity', i.e. enough control to fine-tune and schedule the processes of tooth morphogenesis, patterning/positioning and timing of replacement with respect to each other.

Interestingly, the strongest changes in morphology and tooth replacement (during the small molecule inhibition experiments) resulted from inhibition of gene expression which is localised to two cellular domains/regions. These domains exhibited most signalling pathway activity, including expression of sox2, the stem cell marker. One of these expression domains is a cell population of the labial oral epithelium, superficial and adjacent to each invaginating SDL. The second is located in the layer of cells between the ODE and the IDE, in the cervical loops of the replacement tooth (Fig. 2.9). Fraser *et al.* (2013) speculate these two domains may correspond to the locations of putative stem cell niches in other species, which enable perpetual tooth replacement. They note that the former domain has a similar location to the stem-like cell niches in the non-tooth forming regions of the reptile dental lamina (Handrigan *et al.* 2010). However, with reference to research conducted after the 2013 paper's publication, a far greater similarity could be argued for the putative surface stem cell niches in the oral epithelium of the small-spotted catshark (at the superficial taste/tooth junction) (Martin *et al.* 2016) and of the bearded dragon (Salomies *et al.* 2019) (Fig. 2.9). Fraser *et al.* (2013) compare the second population to the location housing a putative stem cell niche in the zebrafish (Huysseune & Thesleff 2004; Huysseune 2006). They also suggest it is analogous to stellate reticulum of the mouse incisor cervical loop, known (not putatively, but reliably) to house a stem cell niche (Harada *et al.* 1999, 2002; Wang *et al.* 2007; Juuri *et al.* 2012). Again, referring to work subsequent to that of Fraser *et al.* (2013) , this putative stem cell niche in cichlids could perhaps also be comparable to the 'middle dental epithelium' of the African bichir (Vandenplas *et al.* 2014, 2016*b*) as well as the Atlantic salmon (Huysseune & Witten 2008, Vandenplas *et al.* 2016*b*). However, as previously discussed, this 'middle' epithelium is between the ODE and IDE of the predecessor and successor tooth respectively, not of the same tooth. Although not the same location, perhaps the locations of these putative stem cell locations are linked in terms of their evolution.

These studies on putative stem cell niches in different species, as well as the presence of the stem cell marker Sox2 in both cichlid cellular domains highlighted by (Fraser *et al.* 2013), lend support to the speculative proposal by Fraser *et al.* (2013) that these two cellular domains/regions which they found to exhibit most signalling pathway activity are stem cell niches, necessary for lifelong tooth replacement.

Fraser *et al.* (2013) attribute the great dental diversity exhibited by closely related cichlids, all originating from Lake Malawi, East Africa to the variation in tooth morphology, pattern and tooth replacement enabled by the interaction between signalling networks. This complex interaction allows the fine-tuning and integration of tooth morphogenesis, patterning and replacement across space and time.

‘Tinkering’ with the interactions between signalling pathways enables the relatively fast evolution of different tooth phenotypes between cichlid species. Tummers & Thesleff (2009) presented a review of similar studies on mice. Four of the five above signalling pathways were focussed on (BMP, FGF, Hh, Wnt/β-catenin), and although development of both tooth shape and pattern were attributed to them, their influence on replacement could not be tested as mice do not replace their teeth. A key example given of how the key signalling pathways can affect shape and morphology was the asymmetrical development of the continuously growing mouse incisor. By modulating the complex networks of FGF, BMP and activin signals, proliferation and differentiation of epithelial stems cells can be regulated, causing asymmetric growth and therefore shape. The amount of enamel laid down is also affected, as this depends on the differentiation of stem cells into enamel-producing ameloblasts (Harada *et al.* 1999, 2002; Wang *et al.* 2007).

Tummers & Thesleff (2009) emphasise the complexity of the interactions of the signalling pathways, involving multiple activators, inhibitors and negative and positive feedback loops. They highlight how many inhibitors work across more than one pathway, and that co-operation between several inhibitors is common. As such, the intricacy of the interactions becomes apparent, befitting the precise control that Fraser *et al.* (2013) conclude is required to fine-tune and schedule the processes of tooth morphogenesis, patterning/positioning and timing of replacement, in relation to each other. Consonant with the conclusions of Fraser *et al.* (2013), Tummers & Thesleff (2009) hypothesise that the diversity of tooth shapes and dental patterns in vertebrates may have resulted from tinkering with the conserved signal pathways, organised into complex networks, during evolution.

A separate research team's findings independently provide support for this hypothesis. Pharyngeal tooth number in the three-spined stickleback *Gasterosteus aculeatus* is known to vary greatly by population, with those in freshwater localities possessing significantly more teeth than marine (Cleves *et al.* 2014, 2018; Miller *et al.* 2014; Ellis *et al.* 2015; Hart *et al.* 2018). Gain in tooth number is facilitated by shifts in tooth positioning and timing of replacement: an increase in the amount of space taken up by the tooth field, a decreased inter-tooth spacing, and an increased rate in replacement, all of which occur relatively late in ontogeny (Cleves *et al.* 2014; Ellis *et al.* 2015). The resulting increase in pharyngeal tooth number has evolved convergently in freshwater populations in separate North American lakes. Interestingly, a significant finding is that upregulation of the gene *Bmp6* appeared to increase tooth number in both freshwater populations, and the authors hypothesised that modulating different components of the BMP signalling pathway altered tooth replacement stem cell dynamics. This is consistent with the findings of Fraser *et al.* (2013) who found that inhibition of the BMP pathway in the cichlid *Pseudotropheus lombardoi* teeth in some tooth positions were not replaced.

Replacement rate has been observed to be controlled such that it varies in different parts of the dentition. Pacific lingcod, *Ophiodon elongatus* have increased rates of tooth replacement at the posterior of the lower pharyngeal jaw relative to the anterior, and to other tooth-bearing bones in the dentition (Carr *et al.* 2021). This is interpreted to be an evolutionary adaptation to a higher risk of tooth breakage in the posterior region, which is compensated for by the higher replacement rate to maintain function in the dental battery. Alternatively, or in addition, it is suggested the functional impact of tooth breakage or damage could be less at the anterior of the lower pharyngeal jaw and at other tooth-bearing bones, therefore requiring lower replacement rates. The authors initially hypothesised that tooth replacement rate could be increased in response to a particularly broken tooth or damage e.g. a hypothesis arising from the present work (section 5.2.5 , Collins & Underwood 2021), but found no evidence for this in the Pacific lingcod, as results were the same in feeding and non-feeding experimental groups. They attribute the regional differences in replacement rate across the dentition as likely due to a ‘spatially and temporally driven developmental network’, again consistent with the findings of Fraser *et al.* (2013) and Tummers & Thesleff (2009).

2.6.3 Variation from the one-for-one replacement ratio and patterning

Control of when a tooth replaces a predecessor, its position and its morphology all influence tooth patterning. However, recent research has uncovered another factor to consider. When a tooth is replaced, a one-for-one replacement ratio is consistent, enabled by the epithelial connection between a predecessor and successor, in all its varied forms: permanent DL, permanent or temporary SDL, direct development from the predecessor. Traditionally there has been no known mechanism for another replacement ratio to occur. An unusual finding in the three-spined stickleback, *Gasterosteus aculeatus* however has recently called this into question (Square *et al.* 2021), as have key findings of this present work (Collins & Underwood 2021) (section 5.2.8). Of 67 replacement tooth germs studied in the pharyngeal jaws of *G. aculeatus*, 17 (25%) were singularly ‘abutting’ two erupted teeth, both of which showed signs of dislodgement (interrupted mineralisation and osteoclast activity their bases) (Square *et al.* 2021, Fig. 3d). These were interpreted to be possible ‘one-for-two’ replacement events. As discussed in section 2.6.2, tooth number in sticklebacks is known to vary greatly by population, with those in freshwater localities possessing significantly more teeth than marine (Cleves *et al.* 2014, 2018;

Miller *et al.* 2014; Ellis *et al.* 2015; Hart *et al.* 2018). One-for-two replacement events could greatly increase the tooth-shedding rate, doubling it at many tooth locations where two neighbours are dislodged concurrently, therefore significantly reducing the number of teeth in the pharyngeal jaw. Square *et al.* (2021) hypothesise that up- or down- regulation of one-for-two replacement events could contribute to the tooth number differences in freshwater vs. marine populations of sticklebacks. Further investigation could explore the genetic and/or cellular basis for how such regulation would, or does, occur. From observations of tooth replacement histology in the pharyngeal tooth fields in sticklebacks, Square also suspects that their replacement patterns are stochastic (pers. comm., Dec. 2021); sometimes new tooth germs ‘run into’ an erupted tooth or two on their journey towards the tooth plate, but sometimes they do not. It is also possible that sometimes they might not run into their predecessor, Square refers to this type of event ‘one-for-another’. He also observed possible examples of ‘two-for-one’ replacement (two germs appeared to be dissociating the base of one erupted tooth). However, examples of this were far fewer in number, and it is possible that there was another, previously dissociated tooth (i.e. a predecessor absent at the time of sample preparation), meaning the ratio would have been one-for-one. Perhaps two teeth were replacing two adjacent predecessors concurrently, possibly even in a co-ordinated fashion. Overall, each of Square’s above hypotheses (pers. comm. 2021) represent a significant departure from what is understood about the mechanisms of tooth replacement, and thus strongly merit further investigation.

2.6.4 A ‘successional dental epithelium’ (SDE)

Square *et al.*'s (2021) proposal of a ‘successional dental epithelium’ (SDE), comprising a cellular domain, or region, rather than a DL or SDL as previously used in the literature, could conceivably make conceptual space for replacement ratios other than one-for-one. In fish, less emphasis on a singular connecting strand of epithelial cells (the SDL) engendering one new tooth per predecessor could possibly allow for the potential generation of more than one tooth. Square *et al.* (2021) propose that the term SDE also encompasses the growing number of examples of fish tooth replacement where a discrete, histologically distinct SDL is not present, e.g. African bichir (Vandenplas *et al.* 2014, 2016*a*), Atlantic salmon (Huysseune & Witten 2008, Vandenplas *et al.* 2016*b*), rainbow trout (Fraser *et al.* 2004, 2006*b*), medaka (Abduweli *et al.* 2014), sticklebacks (Square *et al.* 2021). In stickleback oral and pharyngeal tooth replacement, the replacement teeth are derived directly from a ring or ‘collar’ of epithelial cells surrounding the predecessor tooth’s site of eruption (Square *et al.* 2021, Fig. 2d and additional file 1: Fig S1). This therefore represents yet another variation on the mechanisms of replacement observed so far, as other ‘lamina-less’ mechanisms involve direct development from the ODE of the predecessor tooth itself (Atlantic salmon, rainbow trout, African bichir), excepting medaka, in which an epithelial ‘indistinct bulge’ at the end of a tooth replacement family corresponds functionally to an SDL (Abduweli *et al.* 2014) (section 2.5.7). As such, the proposed new term ‘SDE’ more accurately captures the range of replacement mechanisms studied so far.

Supporting evidence for the existence of an SDE is provided by the identification of nine genes expressed in both the naïve SDL of the zebrafish and the epithelium surrounding the predecessor tooth in the three-spined stickleback. These nine genes were from a selection of twelve chosen for investigation, based on previously published data indicating their involvement in tooth or hair regeneration, namely in the SDL or hair follicle stem. The nine were selected for study as they were found to be expressed in the three-spined stickleback SDE, and the remaining three were not.

Both the zebrafish and the three-spined stickleback also exhibit active Wnt signalling and *Lef1* expression (a Wnt read-out gene) during the early stages of tooth morphogenesis (Square *et al.* 2021). Therefore overall, despite the three-spined stickleback's lack of an SDL, and the two species diverging approximately 250 million years ago, the results presented by Square *et al.* (2021) indicate both species deploy a similar genetic programme during tooth replacement and development. The authors conclude that the SDE is highly conserved, and note that a set of genes orthologous to the identified nine shared genes is known to mark epithelial stem cells in the mouse hair follicle. They suggest that related progenitor cells may therefore be present in other regenerative epithelial appendages. The presence of gene orthologues in mice epithelial stem cells also supports the presence of stem cells in the three-spined stickleback SDE and the zebrafish SDL.

2.6.5 Phenotypic change through replacement

In their review of the evolution and development of vertebrate dentitions, Tucker & Fraser (2014) attribute the great phenotypic diversity of dentitions in part to the capacity for life-long regeneration (polyphyodonty). They contend that this offers a 'developmental window' through which selection and therefore evolution can operate (referred to as 'evolutionary tinkering'). This is because repeated replacement offers an opportunity for the phenotypic products of the complex and intricate interactions between conserved signalling pathways to be realised (Tummers & Thesleff 2009; Fraser *et al.* 2013). Diverse phenotypic adaptations to environment and ecological niches are made possible, often during ontogeny (see section 2.6.6, below). Through replacement, tooth morphology can shift in a lifetime from simple to complex, which confers a novel adaptive advantage within a community. Polyphyodont teeth in fish generally start out as simple unicuspid cone shapes in the primary dentition. An extreme example of just how complex fish tooth morphology can become is the highly modified pufferfish beak (Fraser *et al.* 2012). Non-polyphyodont groups such as mammals do not have the opportunity for such change during ontogeny. Polyphyodont groups such as the cichlids and three-spined sticklebacks have been particularly evolutionarily agile, with significant diversity occurring over a relatively short time in evolutionary terms, among closely related species (Illes & Fryer 1972; Streelman *et al.* 2003; Fraser *et al.* 2008, 2013; Ellis *et al.* 2015).

2.6.6 Environmentally induced adaptive phenotypic plasticity

The above discussion concerning phenotypic adaptation (section 2.6.5) is set within a conventional evolutionary framework; adaptation occurs over many generations via natural selection, and in vertebrate dentitions, greatly facilitated by the flexibility offered by interactions between conserved signalling pathways. This includes phenotypic adaptation programmed into ontogeny e.g. an increase in tooth number on the pharyngeal jaw of the three-spined stickleback, later in life, or an increased complexity of tooth shape or patterning via repeated, lifelong tooth replacement. However another strategy is increasingly being recognised for its contribution to evolution; the ability of an individual organism to change its phenotype in response to local environmental change, known as adaptive phenotypic plasticity. Karagic *et al.* (2020) review investigations of this phenomenon in vertebrate dentitions, the most extensive of which outside of mammals has again focussed on the pharyngeal dentitions of African cichlids. Cichlid study species have included *Cichlasoma citrinellum* (Meyer 1990), *Amphilophus citrinellus* (Muschick *et al.* 2011) and

Astatoreochromis alluaudi, the latter being a particular research focus over the last 60 years (e.g. Greenwood 1965; Hoogerhoud 1986; Huysseune 1995; Hulsey *et al.* 2008; Gunter & Meyer 2014). These species exhibit a well-known example of environmentally induced adaptive phenotypic plasticity. The pharyngeal jaws have a crushing function, and studies by Gunter *et al.* (2013), Gunter & Meyer (2014) and Schneider *et al.* (2014) show that changes in mechanical strain are sensed at the jaws' crushing surfaces. These changes are caused by variations in the fishes' diets: increases in either hard molluscs which require force to crack them open, or soft food such as insects and plant debris which do not. Switching these food sources in an individual's lifetime triggers a developmental response which changes tooth phenotype in their lower pharyngeal jaws; either to large 'molariform' crushing teeth (in dentitions exposed to the hard diet), or to small, fine slender teeth (in dentitions exposed to the soft diet) (Gunter & Meyer 2014, Fig. 1). Bone density of the lower pharyngeal jaw also increases in the 'hard diet dentitions', as does the size of the tooth field (Huysseune 1995, 2000; Gunter *et al.* 2013; Gunter & Meyer 2014; Schneider *et al.* 2014).

Importantly, in view of the above discussion regarding the control of tooth replacement (sections 2.6.1 and 2.6.2), the molecular basis for this environmentally induced response was investigated. Nineteen candidate plasticity genes, which encode a combination of transcription factors, signalling and structural proteins, were found to contain putative binding sites for mechanically responsive transcription factors in their promoter regions (Schneider *et al.* 2014). In summary, the mechanical strain of crushing hard food was observed to directly affect and regulate a complex gene network (Schneider *et al.* 2014, Fig.7), resulting in adaptive phenotypic change. The candidate genes investigated mainly controlled bone remodelling and muscle development, rather than the patterning and timing of tooth replacement. However, these aspects of tooth replacement were demonstrably affected by mechanical strain: in hard diet dentitions tooth size increased, and positioning changed due to a decrease in tooth number and increase in size of the tooth field. Although timing or rate change of tooth replacement was not a focus of the molecular studies, it was evident that the size and positioning changes were enabled over time through the replacement process (Gunter & Meyer 2014, Fig. 2; Schneider *et al.* 2014).

A. alluaudi exhibits conventional one-for-one tooth replacement, via a temporary epithelial downgrowth, the SDL (section 2.4.2). Huysseune (2000) discusses how the decrease in tooth number seen in the hard diet dentitions must be due to non-replacement of functional teeth (therefore a reduction in tooth replacement rate) and/or suppression of new tooth positions as the tooth field size grows. In both cases, Huysseune (2000) proposes that some mechanism must control the timing and position of the initiation of the epithelial downgrowth/SDL. She suggests that decisions concerning these are made in the epithelium. With reference to the ‘tinkering’ or signalling pathway modulation subsequently described by Tummers & Thesleff (2009), and Fraser *et al.*'s (2013) study on the control of timing, shape and positioning of cichlid teeth during replacement, it is arguably a safe assumption that the pathways highlighted in both pieces of research are at least partially responsible for control of tooth initiation in *A. alluaudi*. Huysseune (2000) also postulated that once mechanical strain change is registered at the crushing surface in *A. alluaudi* dentitions, decision-making is also made about tooth shape, in either the epithelium or the mesenchyme.

Interestingly, in relation to the findings of the current study, Huysseune (2000) contends there are limits to the level of change that can occur between successive tooth generations, and states ‘we do not yet know, in quantitative terms, how much one tooth generation can change with regard to the previous one in terms of tooth size, shape and position …however, it is likely that the changes in features observed here [the *A. alluaudi* dentition] in conditions of altered function are of a magnitude which can only be accomplished through successive tooth generations'. Also relevant to the findings of the current study, Huysseune notes that in the same example, there is a close correlation between how much wear a functional predecessor tooth exhibits and the developmental stage of its successor, and observes these events appear synchronized. She hypothesizes that ‘wear of a functional tooth and initiation of its successor are the result of a common, mechanical, factor (pressure on the teeth by hard food particles)’ (Huysseune 2000).

Fraser *et al.* (2013) showed that manipulation of certain signalling pathways caused absences/non-replacement at some tooth positions (inhibition of BMP and Notch pathways) and shape change (inhibition of BMP, FGF, Hh, Notch and Wnt/β-catenin pathways) (section 2.6.2). An important next step in understanding the effect of mechanical strain on the control of tooth replacement - and the potential involvement of environmentally induced adaptive phenotypic plasticity in tooth replacement mechanisms in general - would be to repeat the cichlid hard/soft diet experiments and test the effect on these known ‘tooth replacement’ signalling pathways.

Interestingly, another environmentally induced adaptive tooth phenotype has been identified in another fish species, the sheepshead fish, *Archosargus probatocephalus*, (Worcester 2012). An increase in enamel thickness was observed as a function of food hardness (enamel is referenced, rather than enameloid). In contrast, fish fed on a soft diet responded with an increase in dentine thickness. In the hard diet experimental group, there was also a significant increase in the percentage of functional jaw surface covered by teeth in upper and lower jaws, increasing opportunity for crushing food. This suggests a possible phenotypic response in terms of tooth size, shape, close-packed patterning, and possibly increased number of teeth in the sheepshead fish, which could be a focus of future study. The change in enamel thickness is intriguing, as enamel-producing ameloblast cells are lost through the process of eruption. The increase in enamel is therefore thought to develop in successional teeth during the replacement process, assumed to be gradual over numerous tooth generations (Karagic *et al.* 2020). It is perhaps useful here to again compare the experimental results of (Fraser *et al.* 2013), where manipulation of signalling pathways in cichlid dentitions caused variations in enameloid patterning (Hh) and mineralisation defects (Notch and Wnt/β-catenin).

In their review of research in the field of phenotypic plasticity in vertebrate dentitions, Karagic *et al.* (2020) list many examples of environmentally induced adaptive phenotypic responses in mammals. Although only able to come into effect in either one or no tooth replacements, many plastic responses are observed. They highlight how forces exerted though mastication when feeding can move incisor positions in rats and that this effect is termed ‘orthodontic movement’, exploited for decades in human dentistry to align teeth (Karagic *et al.* 2020, references therein). They contend that tooth movement as a plastic response to diet variation could be influential in terms of evolutionary success (e.g. by increasing efficiency at capturing and processing prey) and therefore should be studied more extensively. Importantly for understanding the control of the timing of tooth replacement initiation, human studies have shown delays to eruption in primary and/or replacement teeth due to malnutrition (e.g. Heinrich-Weltzien *et al.* 2013), premature birth, maternal age (Wu *et al.* 2019) and maternal smoking habit (Żądzińska *et al.* 2016) among other causes.

These many examples of phenotypic plasticity represent a departure from the traditional evolutionary frame of reference for research into the control of tooth replacement (e.g. Ellis *et al.* 2015). Karagic *et al.* (2020) conclude from their review of the field that environmentally induced phenotypic plasticity has been largely ignored across studies on vertebrate tooth diversity. It appears likely this is a significant oversight, particularly in view of the extremely successful radiation of cichlid species, possibly due to their agile environmentally induced phenotypic responses. In the context of the present study of tooth replacement mechanisms in fish, environmentally induced phenotypic plasticity is a key consideration.

More widely, the potentially missed significance of environmentally induced phenotypic plasticity to the process of selection and evolution is being addressed. The leading theory of a mechanism by which it contributes to evolution draws on the example of the explosive speciation of cichlids. A hypothetical ‘plastic’ ancestor is proposed, which colonised the lakes of East Africa, rapidly filling and phenotypically adapting to diverse trophic niches. Secondarily, the most successful of these initially plastic phenotypes are thought to have become ‘genetically fixed’, through the process of genetic assimilation (Gunter & Meyer 2014, references therein). Thus, a relatively new, and significant mechanism by which natural selection could operate is proposed.

2.6.7 Further insights concerning response to mechanical strain during tooth replacement

A mechanism for sensing strain in replacing human teeth has been investigated, with interesting findings in view of the above research on environmentally induced adaptive phenotypic plasticity in the pharyngeal jaws of cichlids. Sarrafpour *et al.* (2013) found evidence that bite-force in the human jaw is detected by soft tissues in the unerupted replacement (permanent) tooth follicle (or 'sac'). These follicular soft tissues then direct bone remodelling at the inner surface of the replacement tooth's bony crypt. This remodelling comprises an increase in osteoclast activity and therefore bone resorption above the tooth, facilitating its upward movement and eruption. This is thought to be due to a compressive force generated above the tooth crown by the bite, where bone resorption occurs, combined with a tensile force generated in the follicle beneath the root apices, where osteoblasts deposit bone (Sarrafpour *et al.* 2013, Figs. 5 and 7). The authors also suggest a key role for the periodontal ligament, as they identified it as also detecting strain, as effectively as the dental follicle. They note that related to this, the periodontal ligament is known to exhibit mechanosensor activity during orthodontic treatment (Nilforoushan & Manolson 2009; Kang *et al.* 2010; Wu *et al.* 2011).

Such a mechanism in human tooth replacement that utilises mechanical strain is likely to be interesting to researchers investigating the mechanisms of polyphyodonty. This is particularly so in view of the importance of mechanical strain in cichlid polyphyodonty, as discussed previously.

The role of mechanical stress and strain in mammal tooth replacement has recently been further illuminated. In the miniature pig, the SDL of the replacement (permanent) tooth initiated only when the predecessor (deciduous) tooth began to erupt (Wu *et al.* 2020; Wu & Wang 2020). Prior to this, accumulated mechanical stress inside the mandible had prevented the replacement occurring. Once eruption of the predecessor started (reconstructed experimentally in a cultured mandible slice, through a reduction in pressure), the consequent release/reduction in mechanical stress inside the mandible induced the downregulation of the integrin b1-RUNX2-Wnt pathway in mesenchyme located between the predecessor and successor. In addition, and significantly in view of its role in other species, the reduction in mechanical stress also induced upregulation of Wnt signalling in the epithelium of the resting SDL, triggering initiation of the replacement/permanent tooth development. This was also the case when the release of pressure was reconstructed in a human tooth germ. Wu *et al.* (2020) propose that biomechanical stress-associated Wnt modulation could shed light on the mechanisms of initiation in integumentary organ lifelong regeneration. In the context of the present study, it also is of great relevance in understanding the mechanisms of tooth initiation enabling polyphyodonty. It is interesting to compare the findings of this study with those of Wu *et al.* (2013), where extraction of functional teeth in the American alligator prompted activation of putative stem/progenitor cells, and the development of the successional/replacement teeth. In light of the effect of predecessor tooth eruption in the miniature pig, it seems possible that reduction in biomechanical stress caused by the American alligator tooth extraction, could have induced development of its replacement.

2.6.8 Further examples of unusual tooth replacement

The one-for-two tooth replacement in the three-spined stickleback described by Square *et al.* (2021) and the examples of environmentally induced phenotypic plasticity described here, such as that as observed in *A. alluaudi*, are challenging to accommodate within the framework of our current understanding of tooth replacement mechanisms. In addition, in the polyphyodont species discussed so far, any natural (non-experimental) change in tooth morphology or number has occurred gradually. This gradual change is either over multiple tooth generations during ontogeny e.g. cichlids' adaptive phenotypic responses to hard or soft diets, or over generations through natural selection e.g. evolved tooth gain in three-spined sticklebacks and pufferfish beak development.

However, studies on crushing molariform teeth in the adult Atlantic wolffish *Anarhichas lupus* (Bemis & Bemis 2015) show that their teeth can change shape significantly in just one tooth generation. Highly unusually, all crushing teeth present on the oral jaws (vomer, dermopalatines and posterior dentaries) are lost at the same time, and subsequently replaced at the same time. As replacement occurs simultaneously, all teeth are at a similar stage of development and no tooth takes up more space than another. Therefore, each individual tooth can accommodate the shape of its neighbour, growing to fit any space left by it. They appear to mutually tessellate with each other, meaning the replacement event overall is space-filling. A more efficient creation of a continuous crushing surface is formed than would be otherwise achieved, with no gaps. Bemis & Bemis (2015) term this space-filling pattern 'anamestic'. Replacement teeth are irregularly shaped, and not the same shape as their predecessors due to this process. Despite such simultaneous replacement being rare, the replacement of each individual tooth with respect to its successor is conventional, exhibiting a one-for-one replacement ratio, and is intraosseous. Interestingly, it is not known what controls the timing of the replacement event. Suggested controlling factors include endogenous rhythms, tooth wear, seasonal maturation, spawning, or environmental triggers. The latter is particularly interesting in view of environmentally induced adaptive phenotypic plasticity observed in cichlid tooth replacement (section 2.6.6). The replacement event appears to occur annually and is likely linked to breeding season, when feeding is reduced or the fish switch to softer prey, therefore the need for the dentition is reduced (Bemis & Bemis 2015, references therein).

Piranhas are also highly unusual in simultaneously replacing teeth in the oral jaws, though not all teeth are replaced at once. In the species *Serrasahmis rhombeus* and *Pygocentrus nattereri,* a quadrant of the oral jaws is replaced at any one time, with the upper and lower quadrants usually replacing simultaneously (therefore 50% of all teeth, and on one side of the mouth) (Shellis & Berkovitz 1976; Berkovitz & Shellis 1978).

2.7 Unusual tooth replacement in fossil pycnodonts

As teeth and jaws of vertebrates have a high preservation potential, tooth replacement in adult (or fully ossified) fishes can be documented among extinct forms where a good fossil record exists, as well as among modern species. Whilst many fossil fish have tooth replacement patterns comparable to modern species, this is not the case within the pycnodonts, an extinct clade within which all species possessed well-developed and distinctive crushing dentitions. As outlined previously, pycnodont dentitions have been selected for the present study as they show a tooth replacement pattern which is challenging to interpret, in view of the one-for-one tooth replacement observed across the vertebrate clade. These dentitions and their unusual replacement pattern were highlighted in the literature before the emergence of XCT techniques (Longbottom 1984). XCT technology is used here to investigate their tooth replacement mechanisms in more depth than had previously been possible, focussing on fossil species *Pycnodus zeaformis*, *Pycnodus maliensis* and other species from the order Pycnodontiformes for comparison (Chapter three outlines the process of selecting study specimens).

In order to review what is known about pycnodont tooth replacement, it is helpful to briefly discuss the group more generally.

2.7.1 The pycnodonts

The order Pycnodontiformes, or the ‘pycnodonts’ are an extinct group of neopterygian fishes; the actinopterygian (ray-finned) fishes which evolved a lighter skeleton and scales, and more powerful, efficient jaws. Pycnodonts lived from the late Triassic (Norian) to Eocene (Ypresian-Lutetian), spanning approximately 175 million years, and have no descendants today. Originally thought to be the sister group to the teleosts (Nursall 1996*a*), the most recent phylogenetic analysis places the pycnodonts as one of the most, if not the most, basal of the neopterygians (Poyato-Ariza 2015) (Fig. 2.10). They were primarily marine and often reef dwelling, with heterodont dentitions, including crushing teeth on the vomer and prearticular bones. Their distinctive, shiny, bead-like teeth and isolated dentitions are well known to many palaeontologists; these, as well as many complete specimens, have been found worldwide, generally correlating with the margins of the Tethys sea (Nursall 1996*b*; Martín-Abad & Poyato-Ariza 2013). *Pycnodus maliensis* and *Pycnodus zeaformis* are the two of the latest occurring species, from the Eocene of Tamaguélelt in the Republic of Mali (Longbottom 1984; O’Leary *et al.* 2019). As with many pycnodonts, the species are defined by the morphology and arrangement of the molariform ‘crushing’ teeth, borne on the vomer and prearticular bones. These bones and their teeth are referred to as the vomerine and prearticular dentitions (e.g. Longbottom 1984; Poyato-Ariza 2003; Kriwet 2005; O’Leary *et al.* 2019).

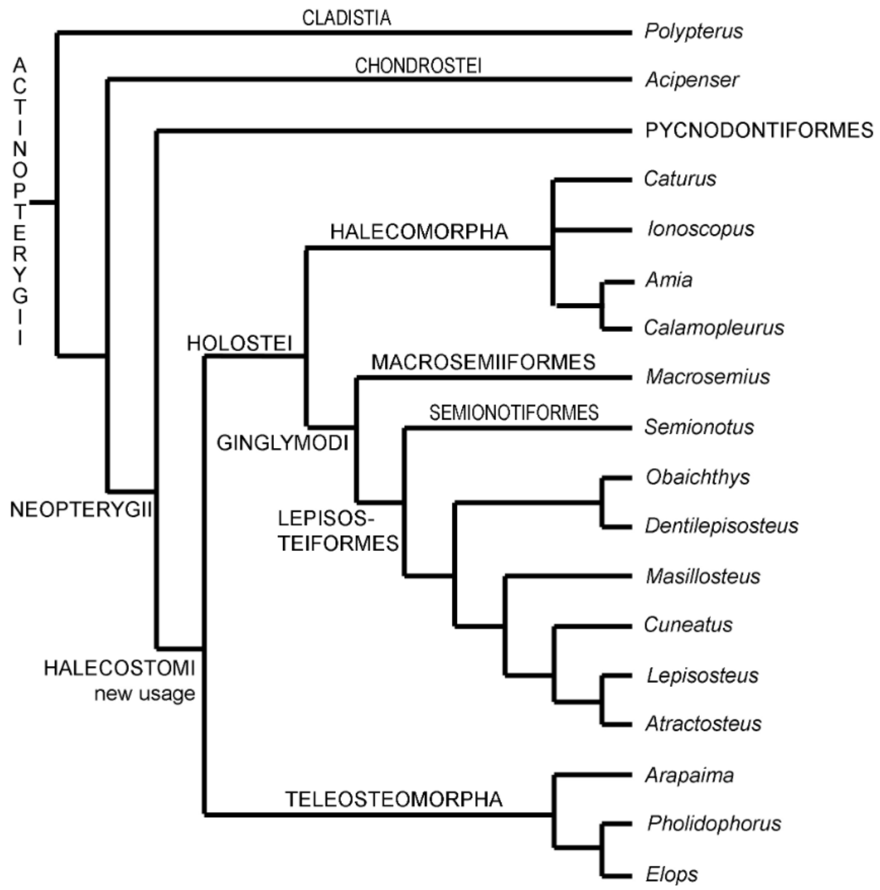


Fig. 2.10 Neopterygian phylogeny showing the basal position of pycnodonts among groups included in the phylogenetic analysis. Created by, and taken from Poyato-Ariza (2015).

2.7.2 Observations of unusual tooth replacement in pycnodonts

As described by Longbottom (1984), several *Pycnodus* species' crushing dentitions exhibit an unusual morphology, and prompt the question of how their teeth were replaced. Longbottom described how the small vomerine and prearticular dentitions from juvenile individuals differ from the corresponding large dentitions of mature individuals of the same species. The anterior region of the large dentitions is often covered in small, round, irregularly positioned teeth, whereas the small dentitions show proportionally larger, elliptical teeth arranged in orderly, regular rows throughout (Fig. 4.1). Previous authors have stated that pycnodont dentitions grew by the addition of new teeth at the posterior of the vomer and prearticular bones, and that there does not appear to be any replacement of individual teeth (Woodward

1895, p. 194; Thurmond 1974, p. 110). This would suggest that the small, round teeth at the anterior of the large dentitions are the oldest and should show the most wear. However, Longbottom observed that these small teeth show little or no wear. The teeth with most wear are the most anterior of the regularly arranged teeth, with the most posterior, regularly arranged teeth showing less wear than these. Interestingly in the largest specimens, Longbottom (1984) observed that the most anterior regularly arranged teeth are so worn that the pulp cavity is exposed within the ‘outline of the original ellipse’. Longbottom postulated that the dentitions grew by addition at the rear of the elliptical teeth in rows, but that the juvenile, regular pattern was progressively replaced from the front by the irregularly patterned, small round teeth. This could perhaps compensate for wear in the oldest teeth.

The small round teeth do not appear to have followed a one-for-one replacement pattern, as being much smaller they are more numerous per unit area than the large elliptical teeth that preceded them. Therefore, multiple teeth have taken the place of a single tooth. It is this which presents an interpretive challenge in the context of other tooth replacement studies, as only mechanisms for one tooth taking the place of one preceding tooth, at any one time, are known (although modern stickleback tooth replacement also presents an interpretive challenge in this regard – section 2.6.3). As reviewed in the current chapter, this ‘one-for-one’ replacement ratio is enabled by an epithelial connection between the predecessor tooth and its successional replacement. In bony fish, this connection mostly comprises either a strand of cells (an SDL,) or the replacement tooth germ off-shooting directly from the outer epithelium of a single predecessor tooth. Therefore Longbottom’s observations (1984) within *Pycnodus* dentitions of multiple small teeth positioned in the same location as fewer previous, large teeth, apparently cannot be explained by these replacement mechanisms.

Longbottom (1984) stated that such small, round irregular teeth are common in *Pycnodus* species but rare in other pycnodont genera, though she had observed them in the genera *Gyrodus*, *Coelodus*, *Eomesodon* and *Macromesodon*.

Subsequent to Longbottom's study, replacement of teeth in pycnodont dentitions has been observed but interpreted as an 'occasional and not a consistent feature and probably occurred mainly in the posterior part of the dentition' (Kriwet 2005). Similarly, Poyato-Ariza & Wenz (2005) observed a replacement tooth at the posterior of a pycnodont prearticular, and suggested this could be possible generally, but specified at the posterior of the bone.

Overall, these recent conclusions, and the long-held view that new teeth added on at the back of pycnodont vomer and prearticular dentitions as they grew, do not explain the small round teeth located at the anterior of dentitions with little or no wear, as observed by Longbottom.

Interestingly, over 100 years before Longbottom's study, Fricke (1875) observed small round teeth located between regularly arranged large teeth in the pycnodont *Mesodon laevior*, but did not discuss them further.

2.8 Overview of approach of the present study

In this study, I investigate how large teeth were replaced with multiple small teeth in *Pycnodus zeaformis* and *Pycnodont maliensis* using XCT to virtually dissect the specimens, combined with observation of surface features. I repeat these techniques with other pycnodont species, and modern fish specimens with analogous functional morphologies for comparison. I interpret the findings in light of known osteichthyan tooth replacement mechanisms, reviewed in this chapter. As previously outlined, I also use the same techniques to investigate tooth replacement mechanisms in other modern fish specimens, and evaluate them against research outcomes reviewed here i.e. tooth replacement mechanisms elucidated from historical and recent EvoDevo research.

3 Material and method: selection and sourcing of specimens, and methodology

Section 1.2 describes the aims of this study, which were inspired by the assertion that '..any tooth or dentition can provide the foundation for a deeper understanding of the variety of processes governing multigenerational odontogenesis' (Fraser & Thiery 2019).

A range of fossil and modern specimens were selected to address the present study's aims. The rationale for each specimen's selection and its relation to the study's aims are given in this chapter. Firstly, the sources of the specimens are described.

3.1 Sourcing of specimens

Seventeen fossil, and thirteen modern specimens were selected for study. All specimens except two modern specimens were sourced from the collection of the Natural History Museum, London, U.K. (NHMUK; BMNH), where they are currently stored. Details of the specimen identification, source, and for the fossils, locality are provided (Table 1; Table 2). For evolutionary context, Fig. 3.2. shows the locations of the modern species selected for study on a fossil-calibrated phylogeny by Hughes *et al.* (2018), of 300 crown actinopterygian taxa, representing all major actinopterygian lineages. Fig. 2.10 (section 2.7.1) shows the phylogenetic location of the pycnodonts.

3.2 Table 1. Fossil pycnodont identification and locality details

Specimen	NHMUK specimen identification number	Identification details from NHMUK	Fossil material locality	Fossil material formation	Notes and references
Pycnodus zeaformis, vomer, from mature individual	NHMUK PV P 60915	Labelled ‘PARATYPE Pycnodus zeaformis – Longbottom’. Collected, named and described by A. Longbottom.	Tamaguélelt, Taoudenit Basin, Republic of Mali	Tamaguélelt Formation, Phosphates, lower or middle Eocene	Longbottom (1984), p7, Fig 13. O’Leary *et al.* (2019) suggest an age for the Tamaguélelt Formation as ‘Eocene, possibly lower Eocene’.
Pycnodus zeaformis, vomer, from juvenile individual	NHMUK PV P 75895	Collected and identified by A. Longbottom (pers. comm. 2019)	Tamaguélelt, Taoudenit Basin, Republic of Mali	Tamaguélelt Formation, Phosphates, lower or middle Eocene	Collecting locality confirmed as per Longbottom (1984), (A. Longbottom, pers. comm. 2019) O’Leary *et al.* (2019) suggested an age for the Tamaguélelt Formation as ‘Eocene, possibly lower Eocene’.
Pycnodus maliensis, part of left prearticular	NHMUK PV P 75894	Collected and identified by A. Longbottom (pers. comm. 2019)	Tamaguélelt, Taoudenit Basin, Republic of Mali	Tamaguélelt Formation, Phosphates, lower or middle Eocene	Collecting locality confirmed as per Longbottom (1984), (A. Longbottom, pers. comm. 2019) O’Leary *et al.* (2019) suggested an age for the Tamaguélelt Formation as ‘Eocene, possibly lower Eocene’.

Pycnodont, *Pycnodus*, part of (right?) prearticular	NHMUK, unregistered	Genus identified by A. Longbottom (pers. comm. 2022)	Record not kept. Likely to be Tamaguélelt, Taoudenit Basin Tilemsi valley, Republic of Mali	Record not kept. Tamaguélelt formation? Likely to be from oolitic deposit at Tamaguélelt, Taoudenit Basin, Republic of Mali (Longbottom 1984), see notes and references.	Confirmed as likely to have been collected on 1981 Tilemsi Valley expedition as per Longbottom (1984), (A. Longbottom, pers. comm. 2022) It is unclear if the oolitic deposit referred to by Longbottom (1984) is part of the Tamaguélelt formation, which was defined by (O’Leary *et al.* 2019) and described as a ‘12~17m thick heterogeneous succession of carbonates, shales and phosphates…’
Mesodon nicoleti, part of left prearticular	NHMUK PV P 7437	Labelled *Mesodon nicoleti*,	Duingen, Germany	Formation not known. From NHMUK label: Lower Portlandian ‘Loc’n: Duingen, Harz’. In pencil: ‘Hanover’.	Portlandian is an obsolete term, equivalent to the late Tithonian stage, the latest stage of the Jurassic (Cope 2008) Duingen is 121 km from Harz, and 52 km from Hanover.
Pycnodont specimen, *Pycnodus*? part of right prearticular	NHMUK, unregistered	Purchased, unverified location and age given by vendor as Khouribga, Morocco, Early Eocene	Not known	Not known	
Coelodus mantelli, part of left prearticular	NHMUK PV P 13282	Labelled ‘Left splenial, *Coelodus mantelli* Agassiz’	Cowlease Chine, Atherfield, Isle of Wight, UK	Vectis Formation? See notes and references. From NHMUK label: Wealden (Sandstone overlying Hypsilophodon Bed)	No stratigraphical information is given in NHMUK collection data (however is likely the same as that given for NHMUK PV P 13288, see below). Label description of ‘sandstone overlying Hypsilophodon Bed’ indicates that fossil was likely from the ‘white rock’ of the Vectis Formation, lowermost Aptian stage, Early Cretaceous. This was a lagoonal

					environment arising from marine transgression (Coram *et al.* 2017). Consistent with NHMUK label, although the term ‘Wealden’ now refers to Wealden Group.
Coelodus mantelli, part of right prearticular	NHMUK PV P 13288	Labelled ‘Right splenial, *Coelodus mantelli* Agassiz’	Cowlease Chine, Atherfield, Isle of Wight, UK	From NHMUK collection data: ‘Vectis Formation, Barremian – Aptian stage, Early Cretaceous’. From NHMUK label: Wealden (Sandstone overlying Hypsilophodon Bed)	Label description of ‘sandstone overlying Hypsilophodon Bed’ indicates that fossils were likely from the ‘white rock’ of the Vectis Formation, lowermost Aptian stage, Early Cretaceous. This was a lagoonal environment arising from marine transgression (Coram *et al.* 2017). Consistent with NHMUK label, although the term ‘Wealden’ now refers to Wealden Group.
Coelodus mantelli, part of left prearticular	NHMUK PV P 13290	Labelled ‘Right splenial, *Coelodus mantelli* Agassiz’	Atherfield, Isle of Wight, UK	From NHMUK collection data: ‘Vectis Formation, Barremian – Aptian stage, Early Cretaceous’ From NHMUK label: Wealden Shale	Likely from the same depositional environment as NHMUK PV P 13282, NHMUK PV P 13290. ‘Wealden Shales’ is an obsolete term, equivalent to the Vectis Formation, within the Wealden Group (https://data.bgs.ac.uk/id/Lexicon/NamedRockUnit/VTIS)
Pycnodus pachyrhinus, vomer	NHMUK PV P 610	Labelled ‘HOLOTYPE *Pycnodus pachyrhinus* Egerton. Vomerine dentition’	Sheppey, Kent, UK	From NHMUK label: London Clay	The term London Clay Formation is in current use https://data.bgs.ac.uk/id/Lexicon/NamedRockUnit/LCLT Notes on label: ‘HOLOTYPE of *Pycnodus pachyrhinus* Egerton 1877: 54, pl.4, f1,2.

					Desc. & fig'd A. Longbottom 1984. Bull. B.M.N.H. (Geol) 38 (1). P21,22, figs 25c, 29’.
Pycnodus toliapicus, part of left prearticular	NHMUK PV OR 38826	Labelled *Pycnodus toliapicus* – Agassiz.	Sheppey, Kent, UK	From NHMUK label: London Clay Lower Eocene	The term London Clay Formation is in current use https://data.bgs.ac.uk/id/Lexicon/NamedRockUnit/LCLT Notes on label: ‘Bowerbank colln. Desc. Egerton. 1877, Geol. Mag. (2) vol iv. p.53. Desc. & fig'd A. Longbottom 1984. Bull. B.M.N.H. (Geol) 38 (1); p11,12 Fig 18. (38825)’.
Anomoeodus superbus, each specimen is part of a prearticular	NHMUK PV P 7237-1 NHMUK PV P 7237-4 NHMUK PV P 7237-6	Labelled *Anomoeodus superbus,* A.S Woodward NHMUK catalogue description: ‘5 portions of splenial’. Specimens are labelled 1-5; 1, 4 and 6 are studied here.	Cambridge, UK	From NHMUK collection data: Cambridge Greensand member, West Melbury Marly Chalk Formation Cenomanian stage, Late Cretaceous From NHMUK label: Cenomanian	The term West Melbury Marly Chalk Formation is in current use https://webapps.bgs.ac.uk/lexicon/lexicon.cfm?pub=WMCH
Pycnodus tattami, part of vomer	NHMUK PV P 18825	Labelled ‘HOLOTYPE *Pycnodus tattami* White. Vomerine dentition’	Wurno, Sokoto province, N. Nigeria	Formation not known. From NHMUK label: Landenian (Scree)	Landenian is an obsolete term, equivalent to Thanetian - Early Ypresian stages (Geyter *et al.* 2006). Notes on label: Descr. & fig’d. E. I. White, 1935. Geol Surv. Nigeria Bull. 14, p43, figs. 10, 10a. Desc.& fig'd. A. Longbottom 1984. Bull. B.M.N.H. (Geol) 38 (1): 19. Fig26c.

Polygyrodus cretaceous, vomer	NHMUK PV OR 39048	Labelled *Gyrodus cretaceus,* however it is identified as *Polygyrodus cretaceus* in the collection database, with the ‘identificaton qualifier’ listed as Vullo *et al.* (2017).	Lewes, E. Sussex, UK	Lewes Nodular Chalk Formation? See notes and references . Turonian (Vullo *et al.* 2017) From NHMUK collection data: Chalk group. Late Cretaceous, Cenomanian – Maastrichtian	Figured in Vullo *et al.* (2017), a report describing the Serrasalmimidae, a new family of pycnodonts. Dated by authors as Turonian. Its locality therefore suggests it is from the Lewes Nodular Chalk Formation of the Turonian (BGS Litho- and biostratigraphical correlation chart for the Chalk group of Southern England. P894998). Notes on label: ‘Bowerbank Colln Descr. + fig’d A. S. Woodward, 1909. Foss Fishes English Chalk Pal. Soc., P.167, Pl xxxv, fig. 5’
Polygyrodus cretaceous, left and right prearticulars (and dentaries?)	NHMUK PV P 11157	Two labels with different identifcations: *Gyrodus cretaceus* - Agassiz, 1844, and *Polygyrodus cretaceus* (Ag) Identified as *Polygyrodus cretaceus* by Vullo *et al.* (2017), and taken as the correct identification here.	Cuxton, Kent, UK	Lewes Nodular Chalk Formation? See notes and references. Turonian (Vullo *et al.* 2017) From NHMUK collection data: Chalk group. Late Cretaceous, Cenomanian – Maastrichtian From NHMUK label: Middle Chalk, Zone of *T. gracilis*	Figured in Vullo *et al.* (2017), see notes for NHMUK PV OR 39048. Dated by authors as Turonian. Zone of *T. gracilis* is a belemnite zone; *Terebratulina gracilis* equates to the *T. lata* Zone and basal *S. plana* Zone of current usage. These zones equate with the New Pit Chalk Formation and lower part of the Lewes Nodular Chalk Formation in southern England (Earthwise$^{\mathrm{TM}}$, British Geological Survey, https://earthwise.bgs.ac.uk/index.php/Rowe_(1900)_-_Terebratulina_gracilis_Zone) This therefore supports the dating of Turonian by Vullo *et al.* (2017) and the NHM label designation of Middle Chalk.

Considering the location of NHMUK PV OR 39048, Lewes Nodular Chalk Formation is inferred.

3.3 Table 2. Modern actinopterygian specimen identification details

Specimen	**NHMUK specimen identification number**	**Identification details from NHMUK**	**Notes and references**
Sparid specimen, upper oral jaw	BMNH 2016.9.23.1	Labelled ‘*Sparus* sp’. The genus *Sparus* contains only one species, *Sparus aurata*. However, the highest certain taxonomic level for this specimen is Family Sparidae*. Common name: the seabreams and porgies	Family identification agreed with NHMUK (J. Maclaine, pers. comm. 2020)
Pogonias cromis, lower pharyngeal jaw	BMNH 2019.11.8.1	Labelled ‘*Aplodinotus*?’. The genus *Aplodinotus* contains only one species, *Aplodinotus grunniens*, the freshwater drum. However, the specimen is identified here as *Pogonias cromis*, the black drum (see notes and references).	Species identified following Sasaki (1989) and Green (1941), supported by Grubich (2003), and agreed with NHMUK (J. Maclaine, pers. comm. 2020)
Labrus bergylta, lower pharyngeal jaw	BMNH 20175.22.1	Labelled ‘*Labrus bergylta Ascanius*, 1767’. Common name: ballan wrasse	Species identification supported by Berkovitz & Shellis (2017) and Evans *et al.* (2019)
Calamus leucosteus, lower oral jaw and left upper oral jaw	BMNH 2010.12.18.29	Labelled & in NHMUK collection data: *Calamus leucosteus* Jordan & Gilbert in Jordan, 1885 Family Sparidae Common name: whitebone porgy	Species identification support not found, except brief description of front teeth in ‘Species Identification Guide for Fishery Purposes’ by the Food and Agricultural Organization (FAO) of the UN (Carpenter 2002). Part of a key for species identification within the Sparidae.

Archosargus probatocephalus, left upper oral jaw	BMNH 2020.8.17.2	Labelled *Archosargus probatocephalus* Family Sparidae Common name: sheepshead	Species identification supported by Berkovitz & Shellis (2017) and (Deang *et al.* 2018)
Sparus aurata, lower oral jaw	BMNH 2020.11.2.2	Labelled & in NHMUK collection data: *Sparus aurata* Linnaeus, 1758 Family Sparidae Common name: gilthead seabream	Species identification supported by Azevedo *et al.* (2021)
Pagrus auratus (synonym *Chrysophrys auratus*), articulated left upper and lower oral jaws	BMNH 2020.11.2.1	Labelled & in NHMUK collection data: *Chrysophrys auratus* (Forster in Bloch & Schneider, 1801) Family Sparidae Common name: silver seabream or Australasian snapper	*Chrysophrys auratus* and *Pagrus major* were redescribed as one species, *Pagrus auratus* by Paulin (1990) See also FishBase: accepted 'Catalogue of Life' species status of *Chrysophrys auratus* is now *Pagrus auratus.* https://www.fishbase.se/summary/Pagrus-auratus https://www.catalogueoflife.org/data/taxon/75KB4 No full dentition description found, however, brief description of dentition in Paulin (1990) combined with photograph in informal resource of Australian Museum supports the identification of this specimen as *Pagrus auratus* https://australian.museum/learn/animals/fishes/snapper-pagrus-auratus-bloch-schneider-1801/
Salmo salar (male, in mating season), head, dry specimen	BMNH 2017.4.4.3	Labelled & in NHMUK collection data: *Salmo salar* Skull, dry Common name: wild Atlantic salmon (male)	Species identification supported by Berkovitz & Shellis (2017) and Konow & Sanford (2008)

Salmo salar (female), skull, dry specimen	BMNH 2017.4.4.2	Labelled & in NHMUK collection data: *Salmo salar* Skull, dry Common name: wild Atlantic salmon (female)	Species identification supported by Berkovitz & Shellis (2017), Konow & Sanford (2008) and Huysseune *et al.* (2007)
Polypterus senegalus head, wet specimen	Unregistered specimen from Kyle Martin, personal collection	Identified by Kyle Martin (visiting researcher to NHMUK) as *Polypterus senegalus* (pers. comm. 2020) Head including soft tissue, wet specimen Common name: Senegal bichir, African bichir	Species identification supported by Clemen *et al.* (1998), Berkovitz & Shellis (2017) and Giles *et al.* (2017)
Gastrosteus aculeatus wet specimen	BMNH 1912.7.10.7	Labelled & in NHMUK collection data: *Gasterosteus aculeatus* Linnaeus, 1758 Common name: three-spined stickleback	Species identification supported by Ellis *et al.* (2016)
Amia calva skull, dry specimen	NHMUK, unregistered	Identified by Dr Zerina Johanson (NHMUK) as *Amia calva* (pers. comm. 2022) Common name: bowfin	Species identification supported by Miller & Radnor (1973) and Berkovitz & Shellis (2017)
Gadus morhua skull, dry specimen	Unregistered specimen from teaching collection, Dept. of Earth and Planetary Sciences, Birkbeck, Univ. of London.	Identified by Dr Zerina Johanson (NHMUK) and Dr Charlie Underwood (Birkbeck, University of London) as *Gadus morhua* (pers. comm. 2022) Common name: Atlantic cod	Species identification supported by Berkovitz & Shellis (2017), Idaho Virtual Museum https://virtual.imnh.iri.isu.edu/Osteo/View/Atlantic_Cod/691 and Holmbakken & Fosse (1973)

*Specimen not identified to genus or species level as the geographic region from which the specimen was collected is uncertain, and the classification of sparids at sub-familial levels is primarily based on squamation patterns and meristic features, as well as dentition type (Santini *et al.* 2014). Furthermore, the current taxonomy, (e.g. Smith

& Smith 1986; Fiedler, K. 1991), is incongruent with more recent morphological and molecular phylogenetic analyses (Hanel & Sturmbauer 2000; Day 2002; Orrell & Carpenter 2004; Hanel & Tsigenopoulos, 2011; Santini *et al.* 2014), rendering *Sparidae* systematics in a state of flux.

3.4 Initial selection of fossil specimens for study

Pycnodus zeaformis and *Pycnodus maliensis* fossil vomers and prearticulars were firstly selected for study as Longbottom’s observations are challenging to interpret in view of current understanding of tooth replacement mechanisms. In contrast with outcomes of recent EvoDevo research, a one-for-one relationship does not appear to exist between replacement teeth and their predecessors.

Information on the provenance of these specimens is given below, providing geological context as well as insight into the identity of the many clasts of rock attached to the specimens’ surface.

3.4.1 Collection of *Pycnodus zeaformis* and *Pycnodus maliensis* fossils, and their geological context

Specimens of *Pycnodus zeaformis* and *Pycnodus maliensis* were collected from the phosphate deposit at Tamaguélelt, in the Tamaguélelt Formation of the Taoudenit Basin, Republic of Mali, during an expedition of the Natural History Museum (UK) and Kingston Polytechnic in 1981. The taxonomy of the expedition’s pycnodont finds was studied by Longbottom, with several species revised, and new species described including *Pycnodus zeaformis* and *Pycnodus maliensis* (Longbottom 1984). Fossils from the site have been the subject of a number of publications (e.g. Arambourg *et al.* 1951; Patterson & Longbottom 1989; O’Leary *et al.* 2006; Tapanila *et al.* 2008). NHMUK-registered specimens from the Mali expedition used in the present study were: a large *Pycnodus zeaformis* vomer from a mature individual, a small *Pycnodus zeaformis* vomer from a juvenile individual, and part of a *Pycnodus maliensis* prearticular (Table 1). The age of the Tamaguélelt Formation, and therefore the specimens, was estimated by Longbottom to be middle Eocene.

O’Leary *et al.* (2019) suggested an updated age of ‘Eocene, possibly lower Eocene’. For more details of the specimens’ geological setting, see Longbottom (1984) and O’Leary *et al.* (2019).

The three *Pycnodus zeaformis* and *Pycnodus maliensis* dentitions are very well preserved and there is little rounding. Although disarticulated and disassociated, both *Pycnodus zeaformis* vomers are relatively complete. The *Pycnodus maliensis* specimen is a small part of the prearticular bone. The dentitions have many small objects, often angular, attached to them. These are of similar density to the teeth and easily mistaken for them, but were identified by A. E. Longbottom as phosphatic grains from the surrounding conglomerate (pers. comm. 2018). With reference to the comprehensive taphonomic study of the Tamaguélelt Formation’s phosphate conglomerates by Tapanila *et al.* (2008), I suggest that the majority of these grains, or phosphatic clasts, are derived from fish bones and coprolites. These are either whole or fragmented, including whole water-worn coprolites which have lost their diagnostic features. The phosphatic clasts are distinguishable from the *Pycnodus* teeth by their predominantly yellow to light brown colour, rough texture and irregular, varied shapes, as opposed to the smooth, shiny, dark brown teeth, which are consistently rounded or elliptical.

3.5 Search of the NHMUK collection for *Eomesodon, Macromesodon, Gyrodus* and *Coelodus* study specimens

As previously described, Longbottom had not only observed the small, round irregularly patterned anterior teeth in the *Pycnodus* specimens collected from Mali, but also in the genera *Eomesodon, Macromesodon, Gyrodus* and *Coelodus* (Longbottom 1984). Therefore, the NHMUK collection was searched for specimens from these genera to study, which exhibited the same patterning of small teeth observed in *Pycnodus zeaformis* and *Pycnodus maliensis* at the dentition anterior.

3.5.1 Representation of *Eomesodon, Macromesodon, Gyrodus* and *Coelodus* dentitions in the NHMUK collection

A.S.Woodward assigned several species of the pycnodont genus *Mesodon* to *Eomesodon,* a new genus which he erected (1916). Most other species of *Mesodon* were later transferred to *Macromesodon*. There are currently 18 specimens registered as *Eomesodon* in the NHMUK collection, many of which are still physically labelled *Mesodon* (e.g. see NHM data portal https://data.nhm.ac.uk/). The collection has many more specimens designated as *Mesodon* than *Eomesodon*. Although Longbottom did not mention irregular anterior patterning of small teeth in *Mesodon*, the *Mesodon* specimens of the NHMUK collection were searched for this feature, for the current study. This was due to the similarity of *Eomesodon* and *Mesodon* vomer and prearticular dentitions, and because some *Mesodon* specimens could have been overlooked for attribution to *Eosomesodon.*

There are no *Macromesodon* specimens present in the NHMUK collection, nor is there online specimen data available. This may be explained by the genus's significant revision (Poyato-Ariza & Wenz 2002) during which it was restricted to just two species: *Macromesodon macropterus* and *Macromesodon bernissart*. Subsequently the genus was revised and renamed *Turbomesodon*, (Poyato-Ariza & Wenz 2004). However, no *Turbomesodon* species are represented in the NHMUK collection, and there is no specimen data online, therefore *Turbomesodon* was not readily available for study.

Gyrodus and *Coelodus* specimens are well represented in the NHMUK collection.

3.5.2 Presence and location of irregularly patterned small teeth in *Eomesodon, Mesodon, Gyrodus* and *Coelodus* dentitions of the NHMUK collection.

It was not possible to discern irregular patterning of small teeth located at the anterior of the *Eomesodon*, *Gyrodus* and *Coelodus* vomer and prearticular dentitions, in the NHMUK collection. This is because the anterior dentition is often missing, and of those few which are complete, the anterior irregular patterning of small teeth is not present. Some specimens are isolated, individual teeth, or small fragments of dentition bearing only a few teeth. *Eomesodon* and *Gyrodus* were therefore not selected for study (see below regarding *Coelodus*). All dentitions are under five cm in length, indicating that they may be from juveniles. If anterior irregular patterning of small teeth only occurred in mature individuals of these species, this could explain the absence of this feature in the collection. Some *Mesodon* dentitions in the collection were found to exhibit the feature, and one was selected for study.

Coelodus, Mesodon and Eomesodon showed a different type of irregular of patterning which was of interest. Small, teeth are positioned between larger teeth which are regularly patterned in rows, in varied, and seemingly unrelated locations across the dentitions. This is similar to patterning exhibited in the *Pycnodus zeaformis* and *Pycnodus maliensis* specimens from Mali, and patterning observed by Fricke (1875). This feature was not discussed by Longbottom (1984). I selected three *Coelodus* specimens exhibiting this feature.

3.6 Selection of *Polygyrodus cretaceus*

Vullo *et al.* (2017) erected a new family of pycnodonts, Serrasalmimidae, which includes species with extraordinary, piranha-like dentitions, adapted for cutting and slicing. These dentitions are mostly adapted for a predatory, carnivorous lifestyle, contrasting greatly with the durophagous crushing dentitions for which pycnodonts are well known, and characterised by. This previously undiscovered lineage spanned from the early Late Cretaceous for 40 million years, and included three new genera and the known genus *Polygyrodus* White, 1927. *Polygyrodus cretaceus* is an early species in the family's evolutionary history. It has a crushing dentition and is of interest to the current study, due to its highly irregular tooth patterning, with barely discernible rows. This implies an orderly one-for-one tooth replacement mechanism may not be present. The specimens selected were the vomer and prearticular of *Polygyrodus cretaceus* figured in (Vullo *et al.* 2017, fig. 2), and housed in the NHMUK collection.

All other known pycnodonts of the Palaeogene, including genus *Pycnodus*, are of the family Pycnodontidae, from which the new family, Serrasalmimidae, is phylogenetically and ecologically distant (Fig. 3.1). Both families were the only Pycnodontiformes to survive the end-Cretaceous mass extinction. From the early durophagus form exemplified by *Polygyrodus cretaceus*, Serrasalmimids evolved a greatly reduced number of tooth rows in both vomers and prearticulars, and labiolingually compressed, sharp teeth, which became highly derived, slicing blades (Vullo *et al.* 2017, fig. 2). This transformation is another feature of interest to this study, as perhaps the Serrasalmimid pycnodonts could rapidly adapt their dentition to environmental pressures and change, as seen in modern three-spined sticklebacks and cichlids. Vullo *et al.* (2017) infer that the evolution seen in the Serrasalmimidae took advantage of the appearance of new prey types in changing marine ecosystems. Indeed, the authors contend that the new family provides evidence that pycnodonts basal to the superfamily Pycnodontoidea (Fig. 3.1) were more ecomorphologically diverse than previously thought, and that it provides evidence of pycnodonts' 'remarkable plasticity of shape and diet'.

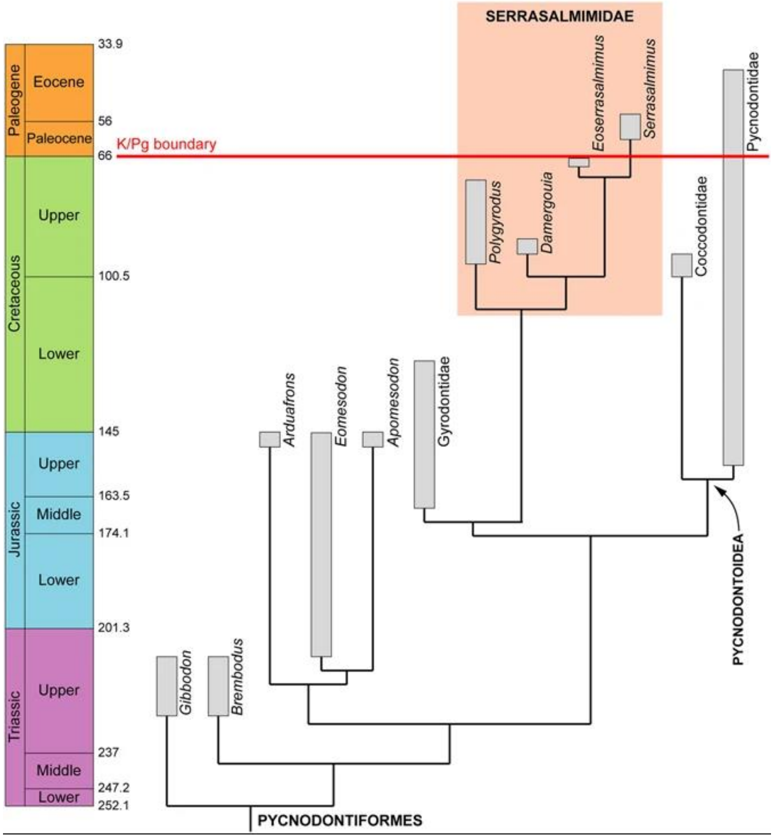


Fig. 3.1 Calibrated phylogenetic hypothesis of pycnodontiform interrelationships, from Vullo *et al.* (2017).

This cladogram shows the disparate origins of Paleogene pycnodont fishes, from the family Serrasalmimidae (erected by the authors) and the family Pycnodontidae.

3.7 Selection of further pycnodont dentitions for study

Two specimens were selected for study from NHMUK which are not from the registered collection. One, a pycnodont prearticular, was of interest because small teeth were located where parts of large teeth had broken off. Also, the dentition was made up of an unusually large proportion of irregularly patterned small round teeth, with only a few large teeth remaining. This specimen is identified by A. E. Longbottom (pers. comm 2022) as a prearticular of a pycnodont, genus *Pycnodus*, collected on the Tilemsi valley expedition of 1981, Republic of Mali (Table 1). The specimen is referred to here as '*Pycnodus* specimen, part of (right?) prearticular, NHMUK unregistered specimen'.

The second unregistered specimen sourced from NHMUK was donated to the museum by David J. Ward, scientific research associate of the museum, for the purpose of use in this study. The specimen was purchased in Khouribga, Morocco, and is of unverified provenance (Table 1). It is not identified to species level, however its similarity to other *Pycnodus* specimens indicates this as the likely genus; as such it is referred to here as *Pycnodus*?. The specimen is of interest due to the presence of irregularly patterned small round teeth at its anterior, in the location where large teeth are absent from regularly patterned tooth rows.

Other pycnodont specimens of the NHMUK collection were searched for any evidence of irregular patterning, and/or patterning that suggests a tooth replacement ratio other than one-for-one. *Pycnodus toliapicus*, *Pycnodus tattami* and *Pycnodus pachyrhinus* and *Anomoeodus superbus* met these requirements and so were all of interest. One specimen of each of these *Pycnodus* species was selected, and three specimens of *Anomoeodus superbus*.

Specimen details are provided in Table 1. *Pycnodus toliapicus* and *Pycnodus pachyrhinus* specimens were collected in Sheppey, Kent, UK, from the London Clay formation (Table 1) and are therfore likely contemporaneous to the *Pycnodus* species collected from Mali.

3.8 Initial selection of modern specimens for study

As with the fossil specimens, modern specimens were selected to address the present study's aims (section 1.2).

Generally, the irregularity of tooth patterning in the pycnodont specimens, be it to varying degrees, suggested that their tooth replacement mechanisms may not be explained by existing research, where orderly one-for-one replacement is accounted for, and not ‘many-for-one’ (in the sense described herein). Therefore, as a starting point, modern fish dentitions were selected for comparison, whose crushing morphologies appear to be functionally analogous to those of the pycnodonts. Virtual sections and surface features were analysed and insights into their tooth replacement mechanisms were compared with those of the pycnodonts. I chose a specimen from the family Sparidae (sp. indet.), the seabreams, as well as *Pogonias cromis* (the black drum) and *Labrus bergylta* (the ballan wrasse) (Table 2). All were available from NHMUK (specimens from the museum’s modern fish collection are registered with the prefix BMNH). As the pycnodonts have no descendants today, the functional analogy of the modern species dentitions and the pycnodont dentitions has evolved convergently.

There are many sparids in the NHMUK collection, and the sparid specimen selected was unusual for two reasons: it was particularly large, and it had a tooth developing directly under a much larger tooth, visible through a crack in the larger tooth. As previously discussed, as far as I can ascertain from the literature, a significant change in tooth size is considered most likely to be gradual over multiple tooth generations. As well as having a crushing morphology analogous to the pycnodonts, this specimen was therefore of further interest. The sparid collection was searched more widely for this feature, but not found. However, four more sparid dentition specimens were selected for study, in case the feature could be discerned through XCT. These were also interesting because each had a mixture of large and small teeth, some irregular patterning, and small teeth positioned in gaps between large teeth; some gaps so small they appeared squeezed between them. The four sparids were species *Calamus leucosteus*, *Archosargus probatocephalus, Sparus aurata* and *Pagrus auratus* (Table 2).

3.9 Selection of *Gastrosteus aculeastus* (three-spined stickleback) and *Gadus morhua* (Atlantic cod).

Square *et al.* (2021) offered the most promising lead from modern fish research in terms of offering an alternative mechanism to one-for-one replacement, and therefore a three-spined stickleback specimen was selected, to compare its features with the authors' outcomes, and to potentially provide insight into the irregular pycnodont tooth patterning.

Square *et al.* (2021) highlighted the 'haphazard' adult tooth arrangement of cod oral teeth, as described by Holmbakken & Fosse (1973), who contended that 'the epithelium covering the whole broad tooth bearing area in the Gadidae seems to have the capacity of forming new tooth germs at any site at any time'. Holmbakken & Fosse (1973) also observe that successors seemed to develop in the immediate vicinity of predecessors. As both these observations may bear relevance to the irregular, non-one-for-one replacement in both pycnodonts and sticklebacks, existing XCT data of a cod specimen, *Gadus morhua* (Atlantic cod) was selected and investigated. This is the same species studied by Holmbakken & Fosse (1973), though they referred to it by its synonym *Gadus callarius*.

3.10 Selection of *Salmo salar* (Atlantic salmon) and *Polypterus senegalus* (African bichir)

Research on *Salmo salar* (Atlantic salmon) (Huysseune & Witten 2008) and *Polypterus senegalus* (African bichir) (Vandenplas *et al.* 2014) also represented a step away from conventional understanding of tooth replacement as it was reported as ‘lamina-less’, i.e. without a successional dental lamina providing the single regenerative, epithelial link between predecessor and replacement tooth. Instead, the replacement develops directly from the outer dental epithelium (ODE) of the predecessor tooth, facilitated by a putatively identified stem cell niche. The replacement ratios do not diverge from one-for-one, as in the stickleback, however these species were selected for study nonetheless. As this is relatively under-researched mechanism, I postulated there may be potential for for more than one tooth to develop from a single predecessor, perhaps drawing on a single stem cell niche. While this would be a hypothesis to investigate on a molecular level, here the intention was to note evidence in the two species of more than one replacement tooth developing from a single successor (therefore a ‘many-for-one’ replacement ratio), and thus hypothetically relevant to the pycnodonts. The aim was also to investigate if the positioning of any replacement teeth in the study specimens is consistent with the findings of the previous research i.e. a single replacement tooth developing directly from a single predecessor.

3.11 Selection of *Amia calva* (bowfin)

Amia calva was selected for study due to its dentition appearing highly irregularly patterned, with numerous teeth seemingly crowded onto the tooth-bearing bones. The overall effect appears disorderly. I cannot find research on the species’ tooth replacement since that of Miller & Radnor (1973), who investigated tooth patterning in the context of the Zahnreihen theory (Edmund 1960). They concluded that their findings were not consistent with the theory. As such they were investigating patterns in development stages between tooth positions, rather than mechanisms for replacement at each tooth position. They noted many regions of the dentition lacking pattern, a ‘randomness’ to the timing of replacements between tooth positions, and that this randomness increased with the age of the fish. As this echoes Longbottom’s observation of irregular tooth patterning at the postulated oldest part of the pycnodont dentition, this finding was of interest to this study. The selection of *Amia calva*, one of only 15 living member species of the Holostei, also improved representation of the major clades of the Actinopterygian crown group (Fig. 3.2). A cladistian (*Polypterus senegalus)* and many teleosts were already selected for study. With the placement of Pycnodontiformes as a stem neopterygian clade, basal to the Holostei and Teleostei, but more recent than the cladistia (including the Polypterids) (Poyato-Ariza 2015) (Fig. 2.10), comparison of tooth replacement mechanisms of *Polypterus senegalus* and the Teleosts could set pycnodont tooth replacement in an evolutionary context.

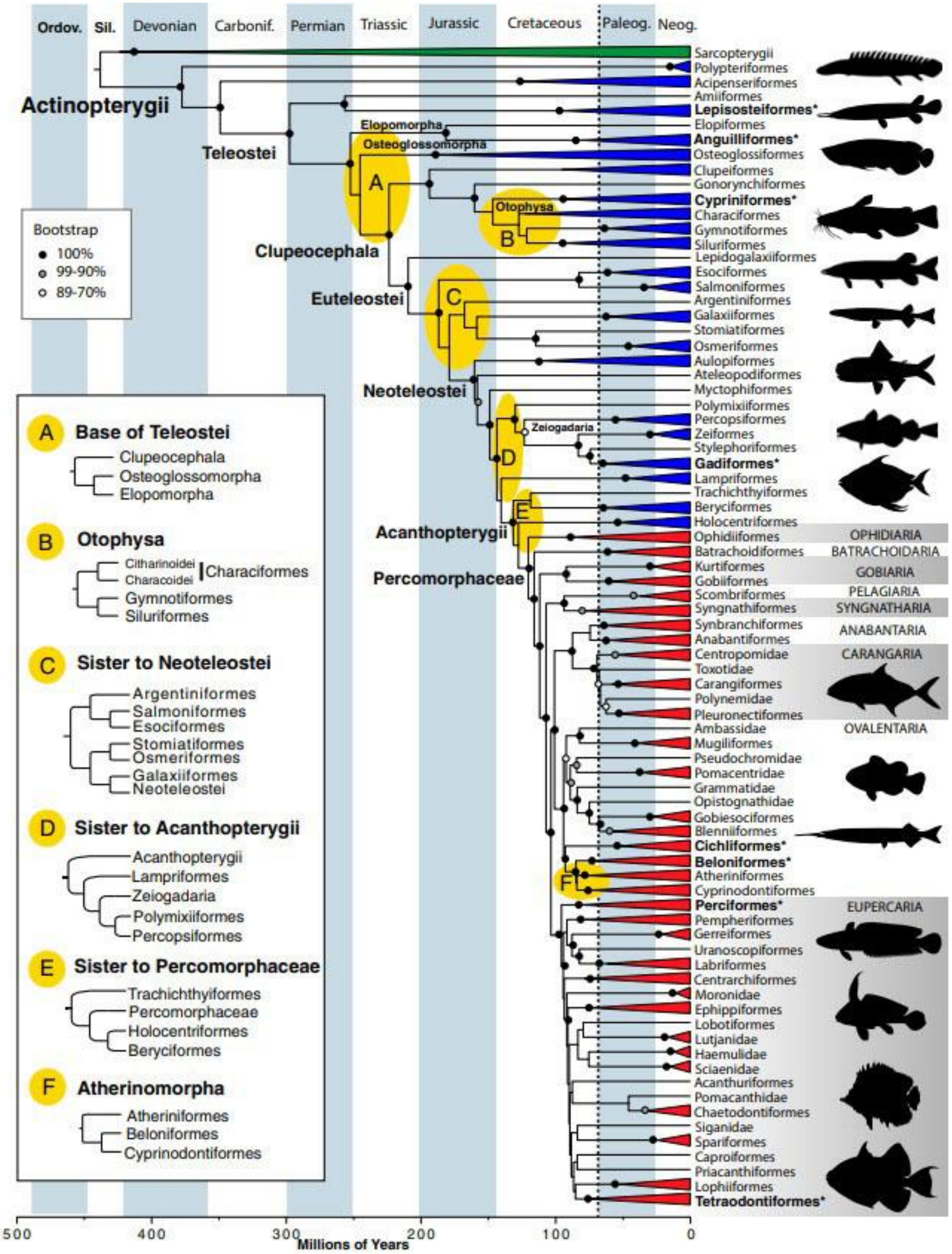


Fig. 3.2 Fossil-calibrated phylogeny of 300 taxa representing all major actinopterygian lineages (blue and red) and three sarcopterygian outgroups (green) compiled by Hughes *et al.* (2018).

Taxa marked with asterisks contained one of the eight model genomes used in the phylogenetic analysis. The areas highlighted in yellow ovals (A–F) indicate uncertain topologies, with alternatives given on the left, obtained via another test (GGI).The dotted vertical line denotes the Cretaceous–Paleogene boundary. Animal silhouettes courtesy of PhyloPic (http://www.phylopic.org). Taken from Hughes *et al.* (2018)

3.12 Methodology

The specimens were investigated using traditional observation of surface features, combined with the technique of X-ray microcomputed tomography (XCT). XCT produced virtual sections within the specimens, enabling visualisation of the specimens' internal structure. By combining data from the surface and inside the specimen, the aim was to gain an insight into tooth replacement mechanisms that were present *in vivo.*

XCT was performed with a Nikon Metrology HMX ST 225 (Nikon Metrology, Leuven, Belgium) at the Imaging and Analysis Centre at NHMUK. Given the variety of specimens, parameters used for XCT were adjusted for each. Tomographic reconstruction was performed using CT-agent software (Nikon metrology GmbH, Alzenau, Germany). Rendering and visualisation were achieved using the software application Avizo 9.3 (Thermo Fisher Scientific, Hillsborough, OR, USA). *Salmo salar*, *Gadus morhua* and *Amia calva* XCT data (Table 2) obtained by the same methodology was generously provided by Dr Zerina Johanson, Earth Sciences department, NHMUK.

To achieve the aim of creating new, publicly accessible XCT data and images of fossil and modern fish dentition specimens, for future investigation, XCT data produced in this study was uploaded to the MorphoSource digital repository (project C1154): https:// www.morphosource.org/projects/0000C1154

4 Results

This chapter comprises descriptions of each specimen; their surface features and XCT virtual sections. Statements are made as to the type of tooth replacement mechanism identified, or if it cannot be discerned.

4.1 Fossil pycnodont specimens

A note regarding specimen interpretation: anterior/posterior, lateral/medial orientiations are determined following Longbottom (1984, Fig.2).

4.1.1 *Pycnodus zeaformis*, vomer from mature individual, NHMUK PV P 60915, and vomer from juvenile individual, NHMUK PV P 75895

Surface features of* Pycnodus zeaformis, *vomer (mature individual)

This specimen is a vomer of *Pycnodus zeaformis*, its large size indicating it is from a mature individual (NHMUK PV P 60915; Fig. 4.1A (i, ii)). As seen in other large *Pycnodus* vomers obtained from the same region in Mali, the anterior region is covered in small round teeth, positioned in an irregular pattern (Longbottom 1984). These have fewer wear marks, with smoother, shinier surfaces, than the most anterior large teeth. Small round teeth are also positioned between large teeth at other varied locations on the vomer. In two locations, a small tooth has developed within an area exposed by part of a large tooth breaking off. The large teeth become progressively flatter towards the anterior of the vomer and this is supported by the XCT virtual sections (Fig. 4.1F). Some tooth crowns have rounded holes in their enameloid, likely due to wear. Pits remain where the crowns of small round teeth and whole teeth have been lost. Very small phosphatic clasts are also located between the teeth. These are yellow to light brown in colour, with a rough texture and have varied and irregular shapes. These features make the clasts distinguishable from the shiny, smooth, dark brown teeth, which are consistently rounded or elliptical in shape.

Surface features of* Pycnodus zeaformis*, vomer (juvenile individual)

This specimen is identified by A. E. Longbottom as the vomer of a juvenile individual of the species *Pycnodus zeaformis* (pers. comm. 2019) (NHMUK PV P 75895; Fig. 4.1B). The relatively large region of small round teeth seen in the large vomer of a mature individual is absent (compare Fig. 4.1A (i), (ii) with B). It is possible that a relatively small region of small round teeth may have been located on a section which has broken off, as the anterior of the specimen does not appear to be complete. As seen on the large vomer, small teeth are positioned between large teeth in varied locations. Small phosphatic clasts are also located between the teeth, as well as within areas exposed by parts of large teeth breaking off. The clasts are a yellow light brown colour, with varied texture and are irregularly shaped. Their shape is particularly varied in this specimen, and with reference to Tapanila *et al.* (2008), I suggest the clasts could include coprolites with a range of morphologies. The clasts are readily distinguishable from the shiny, smooth, dark brown teeth, which are consistently rounded or elliptical in shape.

XCT virtual sections of the* Pycnodus zeaformis*, vomer (mature individual)

XCT virtual sections of the mature *Pycnodus zeaformis* vomer (NHMUK PV P 60915; Fig. 4.1C–G) show that its dentigerous bone has been damaged or resorbed towards the anterior end; the near-horizontal structure of the upper bone layer appears to have broken down (Fig. 4.1C–D). There, small round teeth have developed. For some small round teeth, a zone of resorbed tissue is visible which surrounds the tooth and cross-cuts the pre-existing tissue of neighbouring teeth (Fig.

4.1D, E, G). In these 2D virtual sections, this zone appears as a circular ring, and is darker than the surrounding tissue due to its lesser density. Small round teeth have developed, or are in the process of developing, in gaps between both large, regularly arranged teeth (Fig. 4.1D, F, G) and other small teeth (Fig. 4.1E). Teeth have developed on the surface of the dentigerous bone, and not from a crypt within it, therefore the mode of replacement is recognizable as extraosseous. The large teeth become progressively flatter towards the anterior (Fig. 4.1F). A one-for-one relationship between predecessor and successor teeth cannot easily be discerned, nor can families of successional teeth be identified.

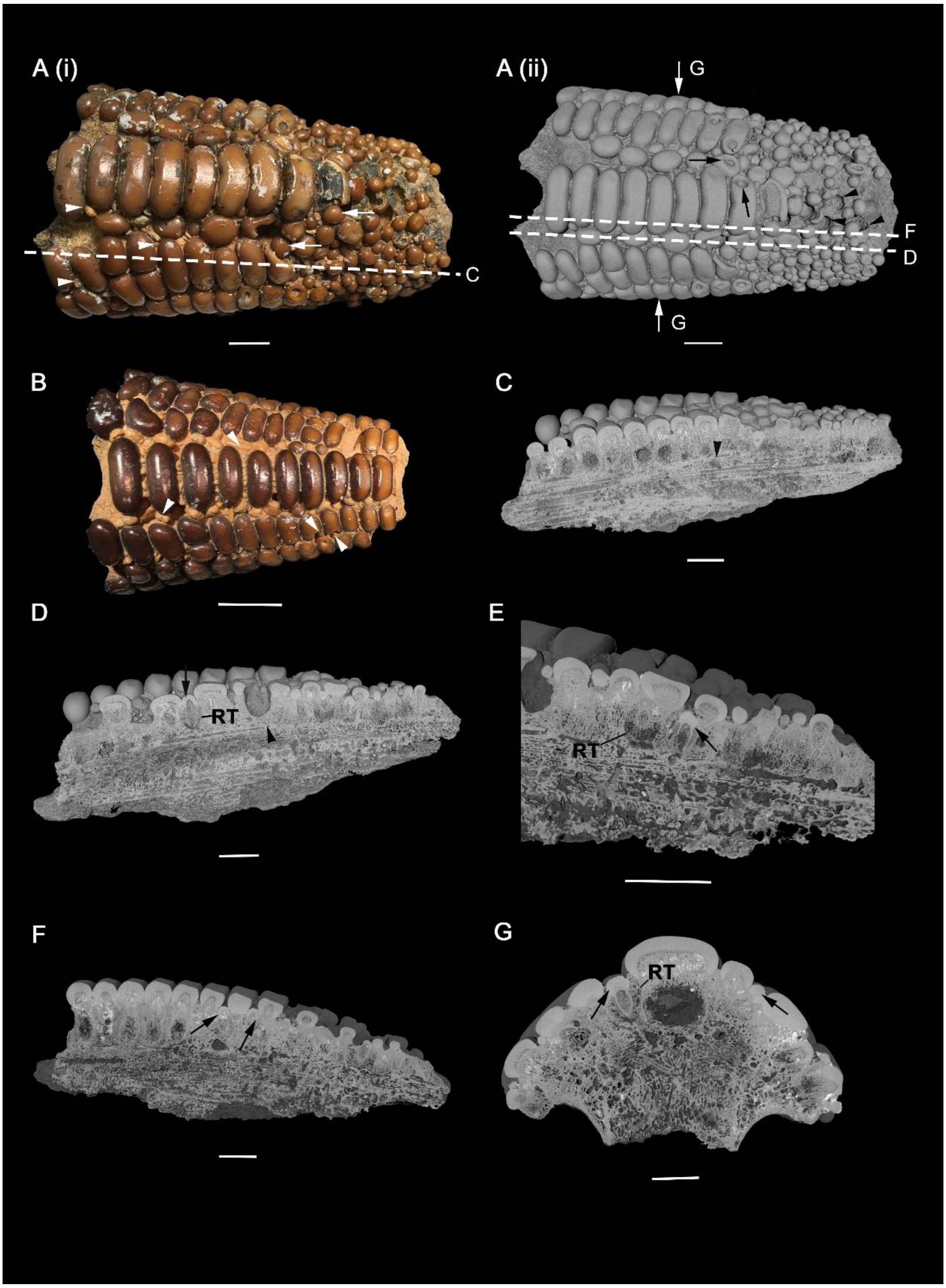


Fig. 4.1 Surface features and XCT virtual sections of *Pycnodus zeaformis*, vomer from mature individual, NHMUK PV P 60915, and vomer from juvenile individual, NHMUK PV P75895

Posterior to anterior direction is left to right (A-F). A (i), photograph of specimen from mature individual, showing small, round teeth covering the anterior region in an irregular pattern, and positioned between large teeth in varied locations; arrows indicate small teeth which have developed within areas exposed by parts of large teeth breaking off; distinct from teeth, lighter-coloured, irregularly and variously shaped, rough textured, very small phosphatic clasts are also located between teeth (arrowheads indicate examples); line shows the position of the virtual section in C. A (ii), XCT surface render of the same specimen from mature individual; some tooth crowns have rounded holes in their enameloid, likely due to wear (black arrows indicate examples); pits remain where the crowns of small round teeth and whole teeth have been lost (black arrowheads indicate examples); lines show the position of the virtual sections in D and F; white arrows show the lateral extent of XCT transverse virtual section in G. B, photograph of specimen from juvenile individual, showing a few small round teeth positioned between large teeth; note the absence of the relatively large anterior region of small round teeth seen in the mature vomer; distinct from teeth, lighter-coloured, irregularly and variously shaped, small phosphatic clasts of varied texture are also located between teeth, including two located within areas exposed by parts of large teeth breaking off (arrowheads indicate examples). C–F, XCT longitudinal virtual sections of the mature vomer NHMUK PV P 60915; the position of section C is shown by the line in A (i); the position of sections D and F are shown by the lines in A (ii); the position of section E is 1mm medial to D; arrowheads (C, D) indicate the point beyond which the upper layer of dentigerous bone has broken down, in the anterior direction; small teeth have developed, or are in the process of developing in gaps between both small and large teeth (arrows indicate examples); zones of resorbed tissue (RT) surround some teeth, which cross-cut the pre-existing tissue of neighbouring teeth; large teeth become progressively flatter towards the anterior of the specimen (F). G, XCT transverse virtual section of the mature vomer NHMUK PV P 60915, the position of section G is shown by the arrows in A (ii), indicating the lateral extent of the section; small teeth have developed, or are in the process of developing between large teeth (arrows indicate examples); a zone of resorbed tissue (RT) surrounds a small tooth which cross-cuts the pre-existing tissue of neighbouring teeth. All scale bars represent 1 cm.

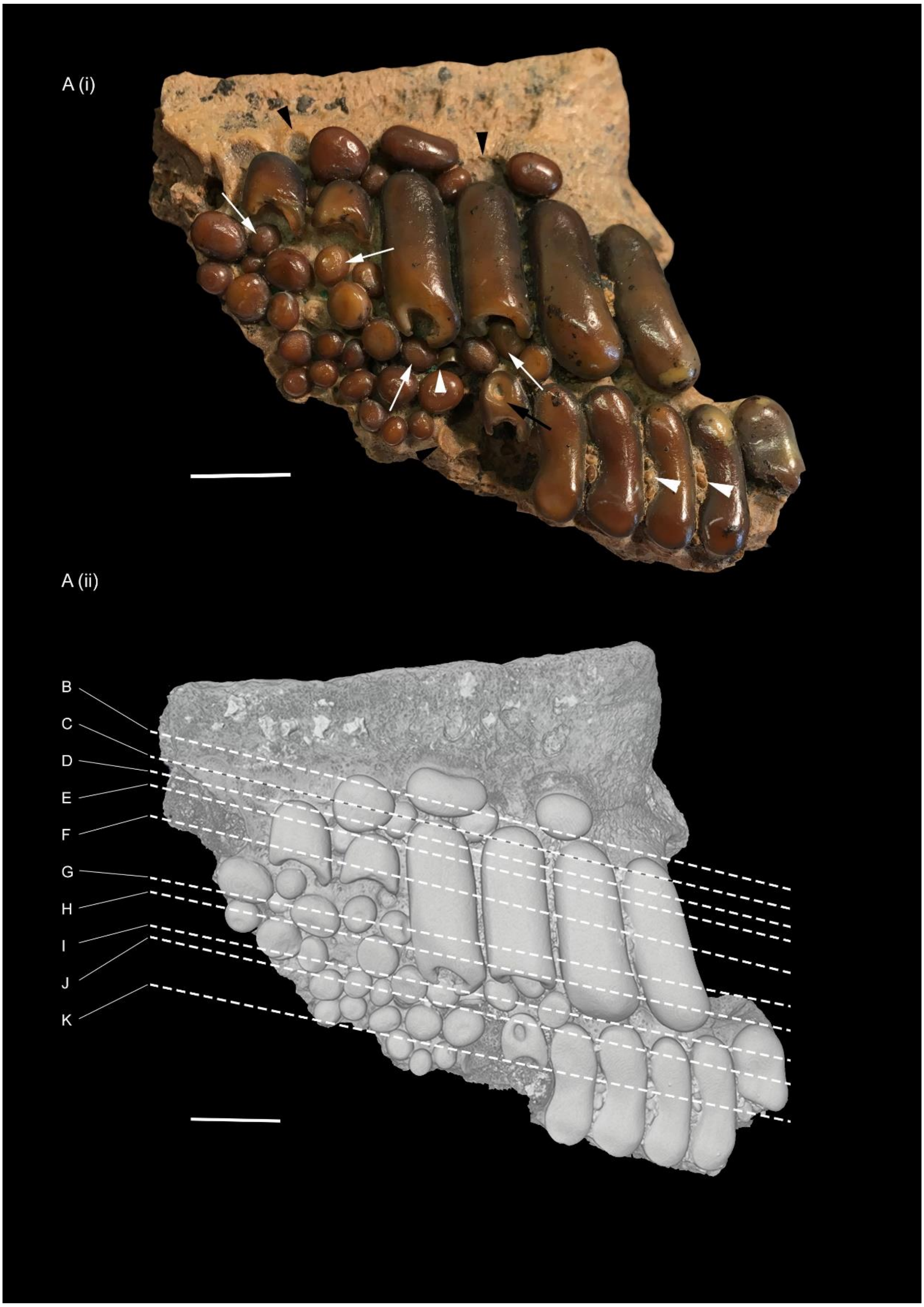
4.1.2 *Pycnodus maliensis,* part of left prearticular, NHMUK PV P 75894

Surface features of **Pycnodus maliensis,** *part of left prearticular*

This specimen is identified by A. E. Longbottom as part of a prearticular of the species *Pycnodus maliensis* (pers. comm. 2019) (NHMUK PV P 75894; Fig. 4.2A (i, ii)). Small teeth are located between large teeth, and in four locations occupy areas exposed by the breaking off of parts of large teeth. Small teeth are also located in a region where large teeth are absent. A tooth crown has a rounded hole in its enameloid, likely due to wear. Pits remain where the crowns of small round teeth and whole teeth have been lost. As with the *Pycnodus zeaformis* specimens, phosphatic clasts are located between the teeth. Unusually, one clast appears to be a fish vertebra, which is consistent with Tapanila *et al.*’s (2008) analysis of the Tamaguélelt phosphate conglomerate as comprising 27% bone clasts, of which 89% are fish elements. The other phosphatic clasts attached to this specimen are yellow to light brown in colour, very small, are of varied texture, and varied, irregular shape. As with the *Pycnodus zeaformis* specimens, the clasts are distinct from the shiny, smooth, dark brown teeth, which are consistently rounded or elliptical in shape.

XCT virtual sections of **Pycnodus maliensis,** *part of left prearticular*

As with the mature *Pycnodus zeaformis* vomer, the XCT virtual sections of the *Pycnodus maliensis* prearticular (NHMUK PV P 75894; Fig. 4.2B–K) show a zone of resorbed tissue surrounding some small round teeth, which cross-cuts the pre-existing tissue of neighbouring teeth. Small round teeth have developed, or are in the process of developing, in gaps between both large and small teeth (Fig. 4.2C, D, E, G), and within areas exposed by parts of large teeth breaking off (Fig. 4.2G-I). A detached tooth crown is located in a cavity left by a lost whole tooth, or possibly by the loss of its own dental papilla and attachment tissues, in life or post-mortem (Fig. Fig. 4.2J). A fish vertebra derived from phosphatic conglomerate is wedged between teeth (Fig. 4.2I). Cracks are present at the base of some tooth crowns, running to the dentigerous bone, potentially preceding their loss (Fig. 4.2H-J). Teeth have developed on the surface of the dentigerous bone, and not from a crypt within it, therefore the mode of replacement is recognizable as extraosseous. Again, a one-for-one relationship between predecessor and successor teeth cannot easily be discerned, nor can families of successional teeth be identified.


A (i)
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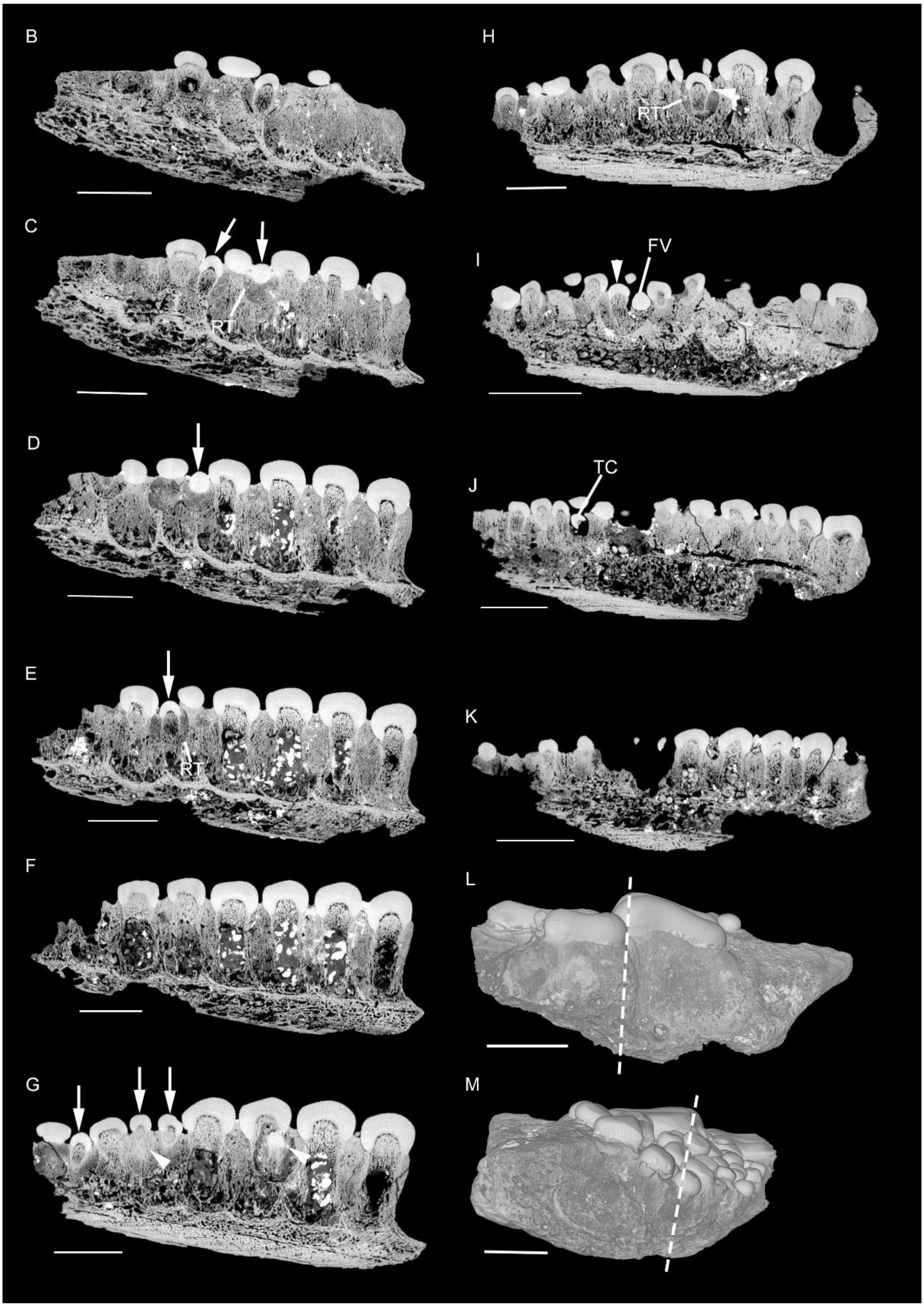


Fig. 4.2 Surface features and XCT virtual sections of *Pycnodus maliensis*, part of left prearticular, NHMUK PV P 7589

Posterior to anterior direction is right to left (A-K), medial to lateral direction is top to bottom (A (i-ii)). A(i), photograph of the specimen, showing small, round teeth located between large teeth, and in a region where large teeth are absent; white arrows indicate small teeth which have developed within areas exposed by parts of large teeth breaking off; distinct from teeth, lighter-coloured, irregularly shaped, very small clasts of varied texture, derived from phosphatic conglomerate are also located between teeth, including a fish vertebra (white arrowheads indicate examples); black arrow indicates tooth crown with a rounded hole in enameloid, likely due to wear; pits remain where the crowns of small round teeth and whole teeth have been lost (black arrowheads indicate examples). A (ii) XCT surface render of the specimen; lines show the position of the virtual sections in B-K. B–K, XCT virtual sections of the specimen, the position of sections B-K are shown by the lines in A (ii); arrowheads (G-I) indicate small round teeth, each located where part of a large tooth has broken off. The tooth indicated by the right arrowhead in G and the arrowhead in H, corresponds to tooth indicated by far right arrow in A (i); arrows (C, D, E, G) indicate small teeth which have developed, or are in the process of developing, in gaps between both large and small teeth; a detached tooth crown (TC) is located in a cavity left by the loss of its dental papilla and attachment tissues, or by another whole tooth, in life or post-mortem, in J; a fish vertebra derived from phosphatic conglomerate is wedged between teeth (FV) in I; cracks are present at the base of some tooth crowns, running to the dentigerous bone (H-J); zones of resorbed tissue surround some teeth, cross-cutting the pre-existing tissue of neighbouring teeth (RT indicates examples). L-M, XCT surface render of the specimen from the posterior (L) and anterior (M) view; line shows the position of the virtual section in H. All scale bars represent 1 cm.

4.1.3 Pycnodont specimen (order Pynodontiformes), *Pycnodus*? part of right prearticular, NHMUK unregistered specimen

Surface features of Pycnodont specimen,* Pycnodus?, *part of right prearticular

This specimen is part of a right prearticular from a pycnodont, putatively identified as genus *Pycnodus* (NHMUK unregistered specimen; Fig. 4.3A). Teeth are light brown to grey in colour with a smooth but non-shiny surface. Large teeth are regularly patterned in rows, with distinct, repeated tooth-shapes which are elliptical to bean-shaped. Small round teeth are located in the anterior-most region of the specimen in an irregular pattern, where large teeth are absent. Two small round teeth, and a pit left by a lost small round tooth, are located adjacent to the rows of large teeth, outside of the anterior region of small teeth. Pits remain where whole small round teeth, and the crowns of small round teeth have been lost. The two most anterior teeth in the most medial row of large teeth have rounded holes in their enameloid, likely due to wear, located at the pulp cavity. The most anterior tooth in the adjacent row of large teeth is the most worn of its row, with rounded parts broken off. This rounded shape could indicate a resorptive action of the small round teeth as replacements. However, the XCT virtual section does not support this (Fig. 4.3B), as there is no evidence of a replacement small tooth present beneath or immediately adjacent to the large tooth, causing its resorption. Clasts of significant size from the matrix rock are not present between teeth, as observed in the *P. zeaformis* and *P. maliensis* specimens.

XCT virtual section of Pycnodont specimen*, Pycnodus?, *part of right prearticular

An XCT virtual section of the *Pycnodus?* right prearticular (NHMUK unregistered specimen; Fig. 4.3B) shows each large tooth in the second-most medial row is worn at the base of the crown, causing rounded gaps between the teeth on the virtual section. This wear pattern could explain the rounded holes broken off the crown of the most worn, and most anterior tooth in the row (Fig. 4.3A). A very faint line or crack may be discerned between the crown and dentigerous bone, in the second-most posterior tooth, similar to lines representing fibrous attachment in modern fish (see section 2.2.8). As this is not a consistent feature among other teeth, it is most likely to be a crack. The surfaces of the large tooth crowns are progressively flatter towards the anterior. A small round tooth which has lost its crown is shown, located within the anterior region of irregularly-patterned small teeth (Fig. 4.3A). A shark tooth is embedded in the fossil matrix. Teeth have developed on the surface of the dentigerous bone, and not from a crypt within it, therefore the mode of replacement is recognizable as extraosseous. Again, a one-for-one relationship between predecessor and successor teeth cannot easily be discerned, nor can families of successional teeth be identified.

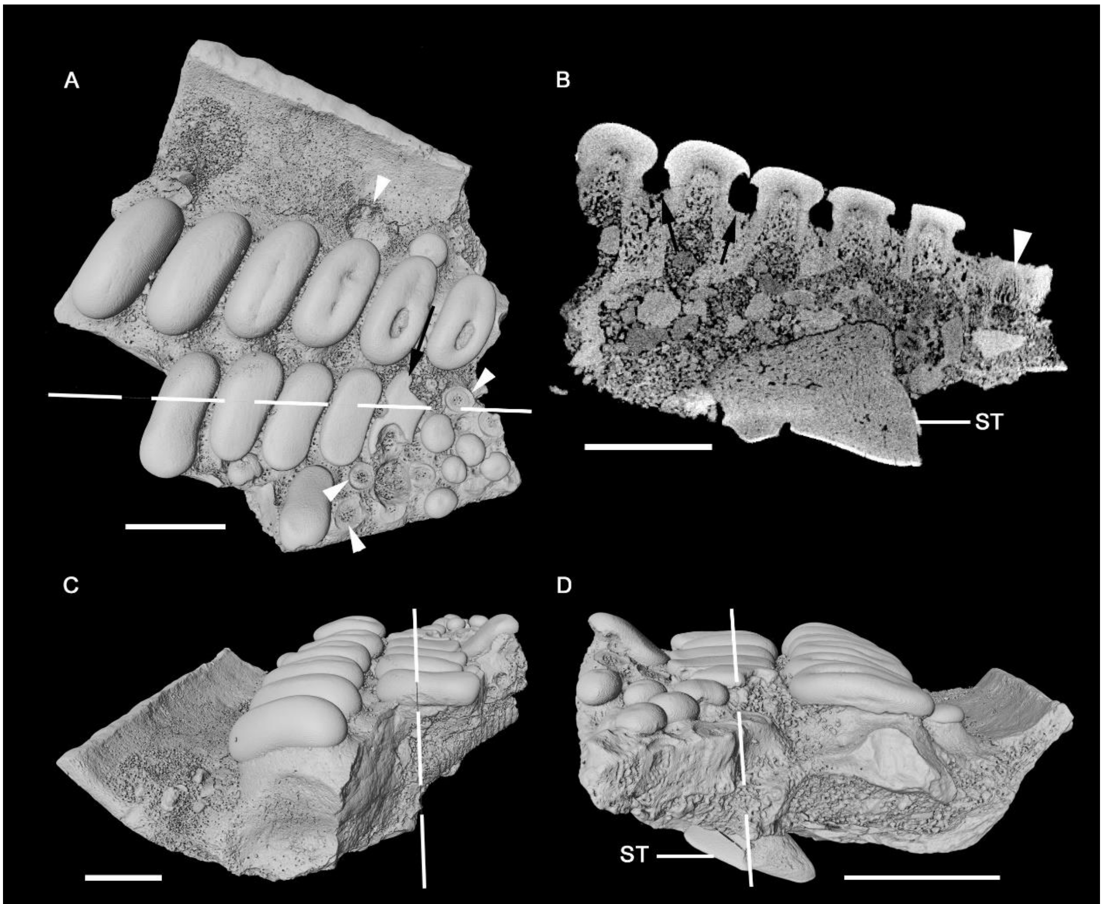


Fig. 4.3 Surface features and XCT virtual section of a specimen of the order Pycnodontiformes, *Pycnodus*?, part of right prearticular, NHMUK unregistered specimen.

Posterior to anterior direction is left to right (A-B), medial to lateral direction is from top to bottom (A). A, XCT surface render of the specimen showing small, round teeth located in the anterior-most region in an irregular pattern, where large teeth are absent; large teeth are regularly patterned in rows, with distinct, repeated tooth-shapes which are elliptical to bean-shaped; two small round teeth, and a pit left by a lost small round tooth, are located adjacent to the rows of large teeth, outside of the anterior region of small teeth; pits indicate where the crowns of small round teeth, and whole small round teeth have been lost (arrowheads indicate examples); the two most anterior teeth in the most medial row of large teeth have rounded holes in their enameloid, at the location of the pulp cavity, likely due to wear; arrow indicates tooth which is the most anterior and most worn of its row, parts broken off it are rounded, suggesting a possible resorptive action of the small round teeth as replacements (however, see B); clasts of significant size from the matrix rock are not present between teeth; line shows the position of the virtual section in B. B, XCT virtual section of the specimen, the position of the section B is shown by the line in A; no evidence of a replacement small tooth is present beneath or immediately adjacent to the anterior-most tooth, causing its resorption, corresponding to the tooth indicated by the arrow in A; each tooth in the row is worn at the base of the crown, causing rounded gaps between teeth on the virtual section (arrows indicate examples). This wear pattern could explain the rounded holes broken off the crown of the most anterior tooth; the surfaces of the crowns are progressively flatter towards the anterior; arrowhead indicates remains of a small round tooth which has lost its crown, corresponding to pit indicated by the far right arrowhead in A; a shark tooth (ST) is embedded in the fossil matrix. C-D, XCT surface render of the specimen from the posterior (C) and anterior (D) view; a shark tooth (ST) is embedded in the fossil matrix, corresponding to the shark tooth indicated in B; line shows the position of the virtual section in B. All scale bars represent 1 cm.

4.1.4 *Pycnodus* specimen, part of (right?) prearticular, NHMUK unregistered specimen

Surface features of **Pycnodus** ***specimen, part of (right?) prearticular***

This specimen is identified by A. E. Longbottom (pers. comm 2022) as a prearticular of a pycnodont, genus *Pycnodus,* collected in the Tilemsi valley, Republic of Mali (NHMUK unregistered specimen; Fig. 4.4A) (Longbottom 1984). There are not enough large teeth present on the specimen to identify the species, among the *Pycnodus* species collected (Longbottom 1984), as it is the large teeth which exhibit diagnostic features. The partial and whole remains of the crowns of only five large teeth are present, arranged in a row. These are elliptical to bean-shaped. Following Longbottom (1984), from the spatial orientation of this tooth row in relation to the rest of the specimen, it can be deduced this was the most medial row. The specimen’s designation as either a right or left prearticular is uncertain, due to the uncertainty of the posterior to anterior direction, which also requires a greater number of large teeth to ascertain. Assuming surface wear increases towards the anterior of the row, posterior to anterior is left to right, and the specimen is therefore likely a right prearticular (Fig. 4.4A).

Teeth are light brown to grey in colour with a smooth but mostly non-shiny surface. Some teeth appear to have retained a shiny substance on the surface which has mostly worn off. The two most complete large teeth, at the end of the row putatively identified as the posterior, have retained more of the shiny substance, and are a darker brown colour compared to all other teeth.

Most of the specimen is covered in small, round teeth, in an irregular pattern. This includes locations where large teeth are absent from their row, and in areas where parts of large teeth have broken off. Numerous pits remain where the crowns of small round teeth and whole small round teeth have been lost. Some small tooth crowns have a rounded hole in their enameloid, likely due to wear, and some parts of small teeth have broken off. Clasts of from the matrix rock are not present between teeth.

XCT virtual section of **Pycnodus** *specimen, part of (right?) prearticular*

An XCT virtual section of the *Pycnodus* specimen, part of (right?) prearticular (NHMUK unregistered specimen, Fig. 4.4B), with posterior to anterior direction putatively identified as right to left, and referred to as such in this description. The virtual section shows a small round tooth located where part of a large tooth has broken off. The remaining parts of the large tooth between which the small tooth is located, are seen on the section. A zone of resorbed tissue is putatively identified surrounding the small tooth. Teeth which have lost their crown are shown, the remainder of the tooth tissue present below the location of the lost crowns. The two most anterior teeth small teeth have particularly flattened surfaces. A small part of enameloid has broken, or ‘chipped’ off the most posterior tooth, and a crack runs across the base of its crown. There are not enough large teeth present to compare the shape and position of any similar cracks, to assess whether this could represent the location of fibrous attachment in life (see section 2.2.8). Teeth have developed on the surface of the dentigerous bone, and not from a crypt within it, therefore the mode of replacement is recognizable as extraosseous. Again, a one-for-one relationship between predecessor and successor teeth cannot easily be discerned, nor can families of successional teeth be identified.

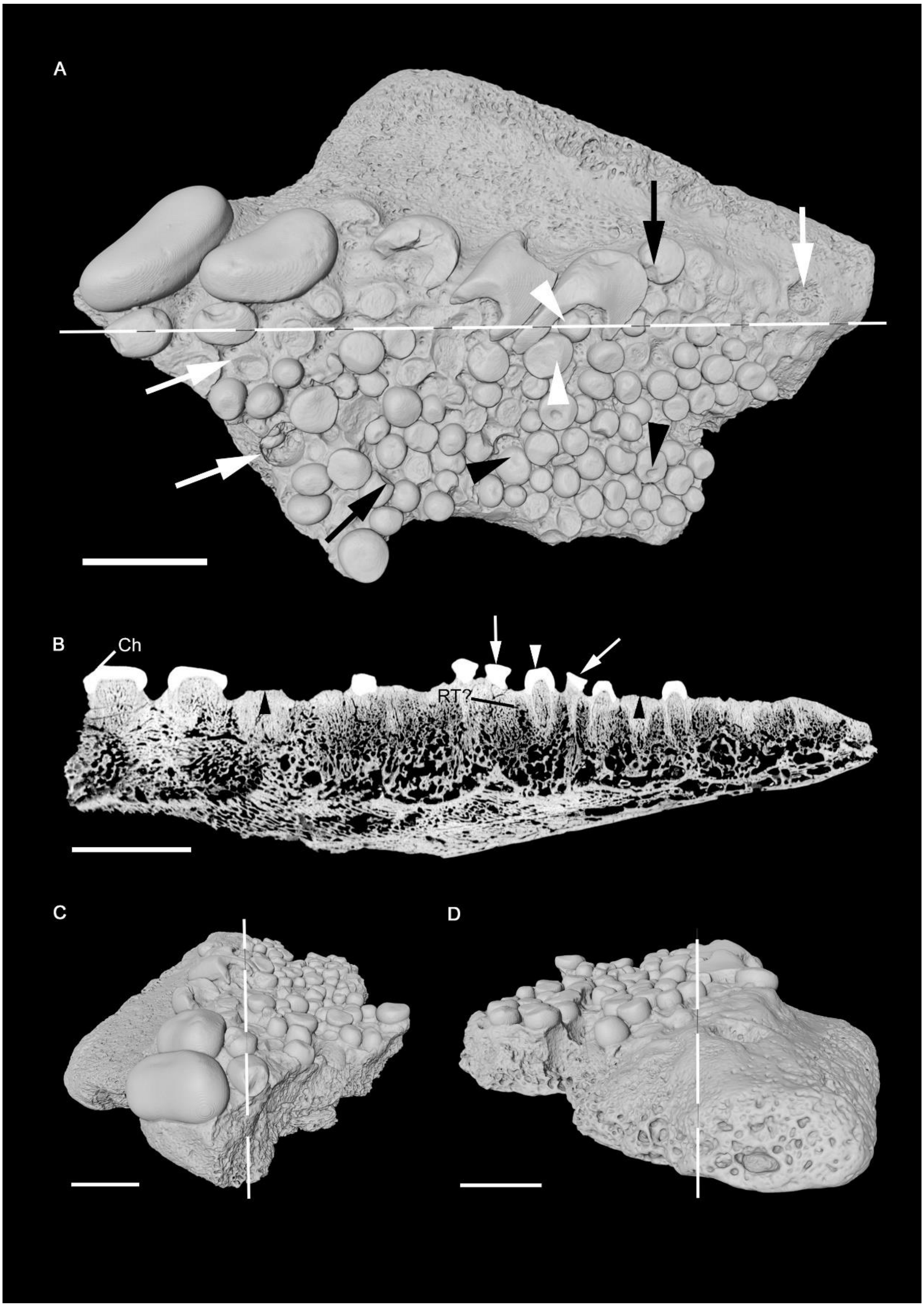


Fig. 4.4 Surface features and XCT virtual section of *Pycnodus* specimen, part of (right?) prearticular, NHMUK unregistered specimen.

Posterior to anterior direction is putatively left to right (A-B), medial to lateral direction is top to bottom (A). A, XCT surface render of the specimen, showing most of the specimen is covered in small, round teeth, in a region where large teeth are absent, and in an irregular pattern; the partial and whole remains of the crowns of only five large, teeth are present, arranged in a row; large teeth are elliptical to bean-shaped; white arrowheads indicate small teeth which have developed within an area exposed by part of a large tooth breaking off; numerous pits remain where the crowns of small round teeth and whole small round teeth have been lost (white arrows indicate examples); some small tooth crowns have a rounded hole in their enameloid, likely due to wear (black arrowheads indicate examples); some parts of small teeth have broken off (black arrows indicate examples); clasts of from the matrix rock are not present between teeth; line shows the position of the virtual section in B. B, XCT virtual section of the specimen, posterior to anterior direction is putatively left to right, and referred to as such in this description; the position of the section B is shown by the line in A; white arrowhead in B indicates a small round tooth located where part of a large tooth has broken off, corresponding to tooth indicated by the upper white arrowhead in A; white arrows indicate remaining parts of the large tooth between which the small round tooth is located; a zone of resorbed tissue is putatively identified surrounding the small tooth (RT?); some teeth have lost only their crown, retaining tissue below the crown (black arrowheads indicate examples); the two most anterior, small teeth have particularly flattened surfaces; a small part of enameloid has broken, or chipped off the most posterior tooth (Ch); a crack runs across the base of the crown of the most posterior tooth. C, XCT surface render of the specimen from the posterior view; line shows the position of the virtual section in B. D, XCT surface render of the specimen from the anterior view; line shows the position of the virtual section in B. All scale bars represent 1 cm.

4.1.5 *Mesodon nicoleti*, part of left prearticular, NHMUK PV P 7437

Surface features of Mesodon nicoleti, part of left prearticular

This specimen is part of a left prearticular of *Mesodon nicoleti* (NHMUK PV P 7437; Fig. 4.5A, C). Teeth are black with a smooth, very shiny surface. The most medial row is comprised of large teeth, regularly patterned, with distinct, repeated tooth-shapes which are elliptical to bean-shaped. Small, round teeth are located in the anterior-most region in an irregular pattern. Round teeth of an intermediate size are located posterior to these, in rows which are not clearly defined. The small, round anterior teeth have a more peg-like morphology compared to the flatter, larger rounded teeth located more posteriorly. Small round teeth are located in gaps between the rows of larger teeth, and also adjacent to the most medial and lateral rows, on the outer edges of the specimen. Pits remain where the crowns of small round teeth and whole teeth have been lost. A large elliptical tooth and an intermediate sized round tooth both have rounded holes in their enameloid. Except for the most posterior tooth, teeth of the most medial row share wear facets at similar positions and angles, on the lateral end of each tooth (Fig. 4.5A, C, E, F). This wear appears to increase towards the anterior. Clasts of significant size from the matrix rock are not present between teeth.

XCT virtual sections of* Mesodon nicoleti, *part of left prearticular

XCT virtual sections of the *Mesodon nicoleti* prearticular (NHMUK PV P 7437; Fig. 4.5B, D) show small round teeth which have developed, or are in the process of developing in gaps between larger round teeth. The trend of an increase in the number of small teeth and decrease in the number larger teeth towards the anterior is shown along the sections. Cracks run across the base of some tooth crowns. A rounded hole is shown in the enameloid of a medium-sized round tooth, with no resorption evident from below the enameloid to cause it (Fig. 4.5B). A pit is left where the crown and some of the tissues below it have been lost (Fig. 4.5D). The anterior-most tooth is attached unusually deeply in the bone, with a long dental papilla, compared to the teeth of most of the studied pycnodont specimens (Fig. 4.5B, D). This tooth morphology is similar to that observed in *Polygyrodus cretaceous* (Figs 4.15, 4.16). Teeth have developed on the surface of the dentigerous bone, and not from a crypt within it, therefore the mode of replacement is recognizable as extraosseous. Again, a one-for-one relationship between predecessor and successor teeth cannot easily be discerned, nor can families of successional teeth be identified.

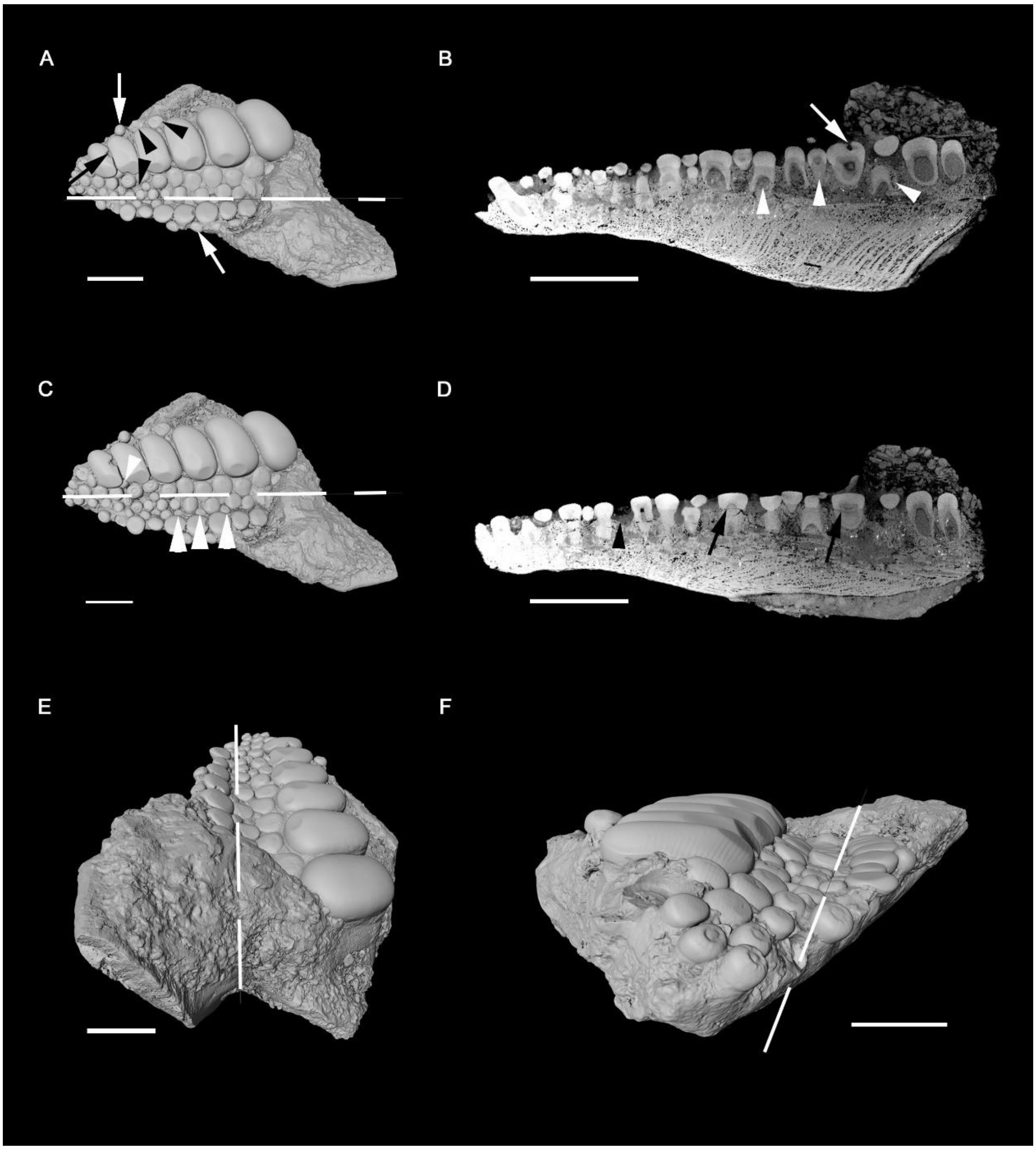


Fig. 4.5 Surface features and XCT virtual sections of *Mesodon nicoleti,* part of left prearticular, NHMUK PV P 7437.

Posterior to anterior direction is right to left (A-D), medial to lateral direction is top to bottom (A, C). A, XCT surface render of the specimen, showing small, round teeth located in the anterior-most region in an irregular pattern; the most medial row is comprised of large teeth, regularly patterned, with distinct, repeated tooth-shapes which are elliptical to bean-shaped; round teeth of an intermediate (‘medium’) size are located adjacent to this row, in rows which are not clearly defined; the medium-sized round teeth are flatter than the small round teeth, which have a more peg-like morphology; small round teeth are located and in gaps between the rows of medium-sized and large teeth (white arrowheads in C indicate examples, some teeth are obscured by section line in A); small round teeth are located adjacent to the most medial and lateral rows, on the outer edges of the specimen (white arrows indicate examples); pits remain where the crowns of small round teeth and whole teeth have been lost (black arrowheads indicate examples); some teeth have rounded holes in their enameloid (black arrow indicates example); except for the most posterior tooth, teeth of the most medial row share wear facets at similar positions and angles, on the lateral end of each tooth, most clearly seen in views E and F. This wear appears to increase towards the anterior; line shows the position of the virtual section in B. B, XCT virtual section of the specimen, the position of the section B is shown by the line in A; white arrowheads indicate small round teeth which have developed, or are in the process of developing in gaps between larger round teeth, corresponding to teeth indicated by three lower arrowheads in C; the trend towards an increase in smaller teeth and decrease in larger teeth, towards the anterior, can be see along this section and in D; white arrow indicates a rounded hole in the enameloid of a medium-sized round tooth, with no resorption evident from below the enameloid to cause it; the anterior-most tooth in the virtual sections shown in B and D and are attached unusually deeply in the bone, and have a long dental papilla compared to the teeth of most of the studied pycnodont specimens. C, XCT surface render of the specimen, as described in A; line shows the position of the virtual section in D. D, XCT virtual section of the specimen, the position of the section D is shown by the line in C; the trend towards an increase in smaller teeth and decrease in larger teeth, towards the anterior, can be see along this section and in B; a crack runs across the base of some tooth crowns (black arrows indicate examples). These may represent the location of fibrous attachment in life, as seen in modern fish (see section 2.2.8); black arrowhead indicates a pit where a whole tooth has been lost, corresponding to the tooth indicated by the lower black arrowhead in A. E-F, XCT surface render of the specimen from the posterior (E) and anterior (F) view; except for the most posterior tooth, teeth of the most medial row share wear facets at similar positions and angles, on the lateral end of each tooth; anterior teeth have a more rounded, peg-like morphology compared to the flatter, larger rounded teeth located more posteriorly; line shows the position of the virtual section in B. All scale bars represent 1 cm.

4.1.6 *Coelodus mantelli,* parts of left and right prearticulars, NHMUK PV P 13282, NHMUK PV P 13288, NHMUK PV P 13290

Surface features of three specimens of Coelodus mantelli, *parts of left and right prearticulars*

These three specimens of *Coelodus mantelli* comprise a part of: a left prearticular (NHMUK PV P 13282; Fig. 4.6A, C), a right prearticular (NHMUK PV P 13288; Fig. 4.7A, C), and a right prearticular (NHMUK PV P 13290; Fig. 4.8A, C). Teeth are dark grey to brown, with a smooth, shiny surface. Large teeth are regularly patterned in rows, with distinct, repeated elliptical shape. Small, rounded teeth are located in regions where large teeth are absent, towards the anterior of the specimens. They are irregularly patterned and mostly round, but also have irregular shapes. Unusually among the pycnodont specimens studied, some teeth in rows are comparable in size to the irregularly patterned rounded teeth. Some small rounded teeth have developed within areas exposed by parts of large teeth breaking off. Small rounded teeth are also located adjacent to the most medial row of large teeth, on the outer edge of one specimen (Fig. 4.7). Unusually, small round teeth are not located between rows of large teeth, with one exception (Fig. 4.8A, C). Pits remain where the crowns of small round teeth and whole teeth have been lost. Where the crowns of some teeth have broken off, a hole is exposed which comprised the pulp cavity in life (Fig. 4.6A; Fig. 4.8A). Some tooth crowns have rounded or elliptical holes in their enameloid, likely due to wear. One large tooth, part of which has broken off, has a large central hole (Fig. 4.7). Some rows of regularly patterned, large teeth show distinct, shared wear patterns, with holes and wear facets in their enameloid at similar positions and angles on each tooth (Fig. 4.6E-F; Fig. 4.7E-F). Unusually, there is a large hole running vertically and completely through the dentigerous bone of one specimen, highly likely due to pyritization of the fossil (Fig. 4.8A-C). This specimen also has cracks in its surface, causing a shallow pit in a region adjacent to the most medial teeth (Fig. 4.8A, E, F). In another specimen, dentigerous bone surrounding the base of the most medial tooth crowns (and lost crowns) appears to be worn away (Fig. 4.7E-F). Clasts from the matrix rock are not present between teeth.

XCT virtual sections of three specimens of **Coelodus mantelli***, parts of left and right prearticulars*

XCT virtual sections are visualised for part of: a left prearticular (NHMUK PV P 13282; Fig. 4.6B, D), a right prearticular (NHMUK PV P 13288; Fig. 4.7B, D), and a right prearticular (NHMUK PV P 13290; Fig. 4.8B, D). A repeated wear pattern within a row of large teeth is shown clearly on the anterior of each tooth in Fig. 4.7D. This pattern corresponds to the repeated holes in enameloid located at a similar position and angle in each tooth, described as a surface feature of this specimen (Fig. 4.7). A large tooth crown, the side of which has broken off, has a large, central hole. Here, the tooth has lost dentine and pulp cavity tissues, as well as enameloid (Fig. 4.7B). In another specimen, a trend of flattening towards the anterior, particularly among the small round teeth is evident (Fig. 4.6B, D). Pits remain where whole teeth have been lost (Fig. 4.6D). Rounded holes in enameloid described as a surface feature are observed in the virtual sections (Fig. 4.6B; Fig. 4.8B). The high density material represented by white on all virtual sections is likely iron pyrites, explaining the large hole running entirely through one of the specimens (Fig. 4.8A-C). In one specimen, cracks run from the base of tooth crowns through the dentigerous bone (Fig. 4.6B, D), and its surface has cracks at the medial edge. This specimen appears heavily pyritised, and cracks are not present in another specimen exhibiting virtually no pyritization (only flecks of white are present on its virtual section) (Fig. 4.7B, D). This suggests that the cracks are a result of poor fossil preservation and did not occur in life. As with the other pycnodonts studied, in each specimen teeth have developed on the surface of the dentigerous bone, and not from a crypt within it, therefore the mode of replacement is recognizable as extraosseous. Again, a one-for-one relationship between predecessor and successor teeth cannot easily be discerned, nor can families of successional teeth be identified.

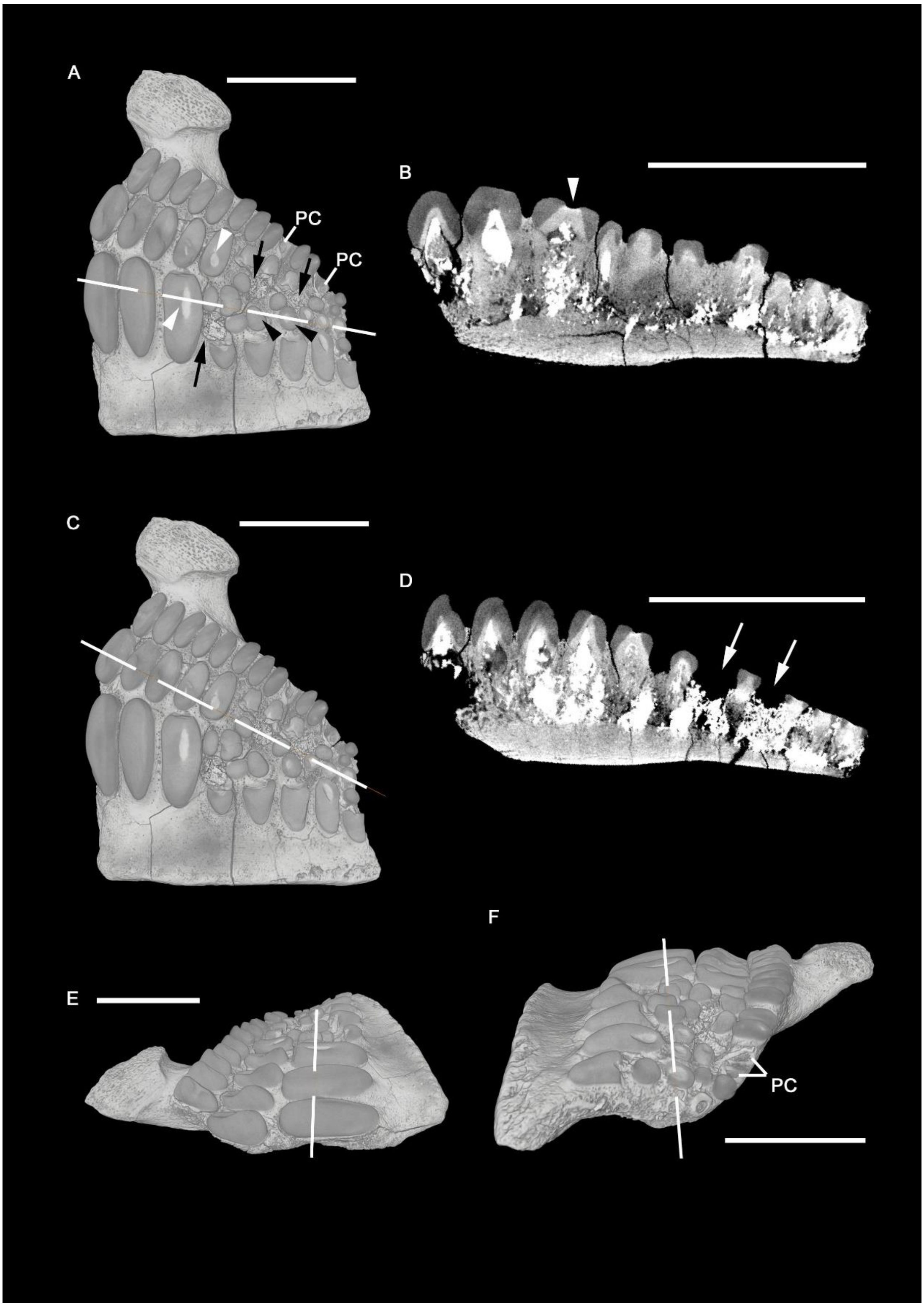


Fig. 4.6 Surface features and XCT virtual sections of *Coelodus mantelli*, part of left prearticular, NHMUK PV P 13282.

Posterior to anterior direction is right to left (A-D), medial to lateral direction is bottom to top (A, C). A, XCT surface render of the specimen, showing small teeth located in regions where large teeth are absent, towards the anterior; small teeth are irregularly patterned, mostly round but also have irregular shapes; large teeth are regularly patterned in rows with distinct, repeated elliptical shapes; some small teeth have developed within areas exposed by parts of large teeth breaking off (black arrowheads indicate examples); some teeth in rows are comparable in size to the irregularly patterned rounded teeth; pits are located where crowns of small round teeth have been lost (black arrows indicate examples); crowns of two large teeth have broken off, exposing hole that comprised the pulp cavity (PC) in life; some tooth crowns have a rounded or elliptical hole in their enameloid, likely due to wear (white arrowheads indicate examples); line shows the position of the virtual section in B. B, XCT virtual section of the specimen, the position of the section B is shown by the line in A; teeth become progressively less rounded and flatter towards the anterior of the specimen; white arrowhead indicates a hole in a tooth crown, corresponding to the tooth indicated by the lower white arrowhead in A; cracks run from the base of tooth crowns to the dentigerous bone, as well as within the dentigerous bone; white indicates a high density material, very likely iron pyrites. C, XCT surface render of the specimen; line shows the position of the virtual section in D. D, XCT virtual section of the specimen, the position of the section D is shown by the line in C; teeth become progressively less rounded and flatter towards the anterior of the specimen; arrows indicate two pits where crowns of small round teeth have been lost, corresponding to pits indicated by the middle and right arrows in A; cracks run from the base of tooth crowns to the dentigerous bone, as well as within the dentigerous bone; white indicates a high density material, very likely iron pyrites. E-F, XCT surface render of the specimen from the posterior (E) and anterior (F) view. These views show clearly a shared wear pattern among the teeth of two most lateral, regularly patterned rows. Enameloid holes and wear facets are at similar positions and angles on each tooth; tooth crowns have broken off, exposing a hole that comprised the pulp cavity in life (PC indicates examples); line shows the position of the virtual section in B. All scale bars represent 1 cm.

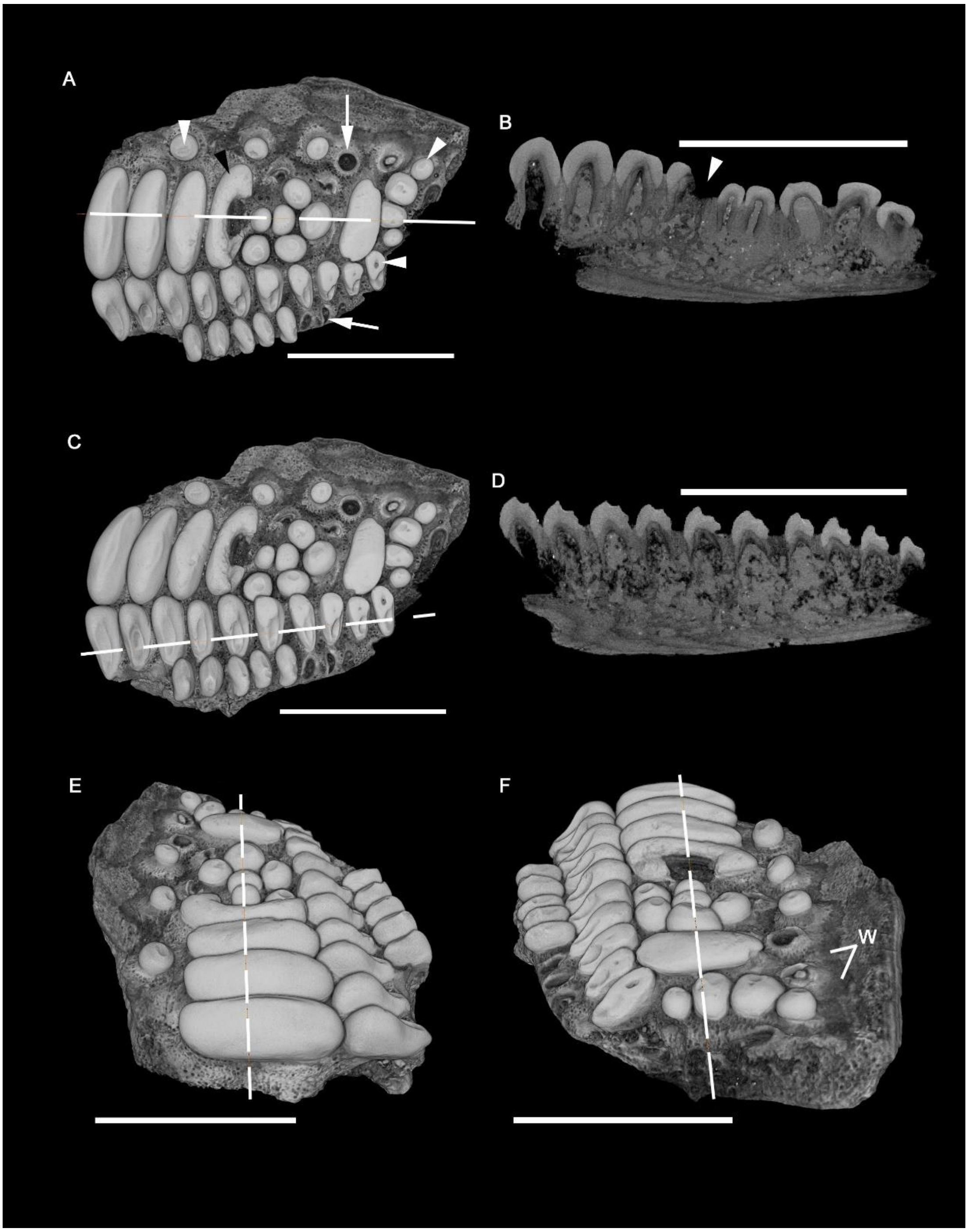


Fig. 4.7 Surface features and XCT virtual sections of *Coelodus mantelli*, part of right prearticular, NHMUK PV P 13288.

Posterior to anterior direction is left to right (A-D), medial to lateral direction is top to bottom (A, C). A, XCT surface render of the specimen, showing small teeth located in regions where large teeth are absent, including adjacent to the most medial row of large teeth on the outer edge of the specimen; small teeth are irregularly patterned and mostly round, but also have irregular shapes; large teeth are regularly patterned in rows with distinct, repeated elliptical shapes; some teeth in rows are comparable in size to the irregularly patterned, rounded teeth (teeth in the most lateral row and anterior of adjacent row); pits are located where crowns of teeth (white arrows indicate examples) and possibly whole teeth have been lost; black arrowhead indicates the crown of a large tooth, part of which has broken off, which has a large central hole; some tooth crowns have rounded holes in their enameloid, likely due to wear (white arrowheads indicate examples); a repeated, distinct, wear pattern is consistent among teeth of the second-most lateral row, with the anterior and lateral sides of each tooth exhibiting a hole in a similar shape and position; line shows the position of the virtual section in B. B, XCT virtual section of the specimen, the position of the section B is shown by the line in A; white arrowhead indicates large central hole in tooth crown, part of which has broken off, corresponding to tooth indicated by black arrowhead in A. The hole represents the loss of dentine and pulp cavity tissues, as well as enameloid; very small white flecks indicate a high density material, very likely iron pyrites. C, XCT surface render of the specimen; line shows the position of the virtual section in D. D, XCT virtual section of the specimen, the position of the section D is shown by the line in C; a distinct, repeated wear pattern is present anteriorly on each tooth, corresponding to holes described in A, E and F; very small white flecks indicate a high density material, very likely iron pyrites. E-F, XCT surface render of the specimen from the posterior (E) and anterior (F) view, clearly showing a shared wear pattern among the teeth of two most lateral, regularly patterned rows. Enameloid holes and wear facets are at similar positions and angles on each tooth, with deeper and more defined holes on the second-most lateral row; dentigerous bone surrounding the base of the most medial tooth crowns (and lost crowns) appears to be worn away (W indicates examples); line shows the position of the virtual section in B. All scale bars represent 1 cm.

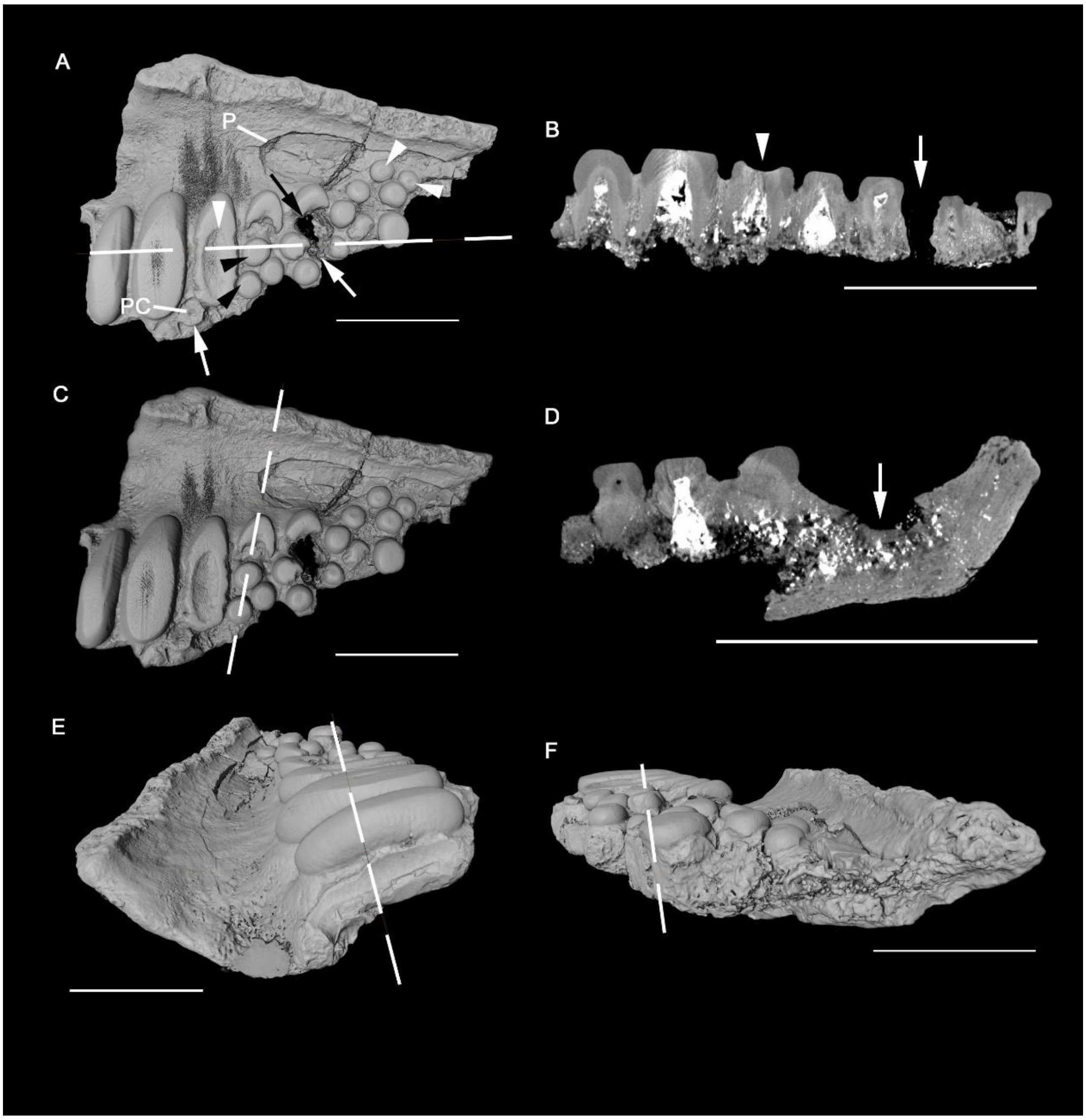


Fig. 4.8 Surface features and XCT virtual sections of *Coelodus mantelli*, part of right prearticular, NHMUK PV P 13290.

Posterior to anterior direction is right to left (A-C), medial to lateral direction is top to bottom (A, C) and right to left (D). A, XCT surface render of the specimen, showing small teeth located in regions where large teeth are absent; small teeth are irregularly patterned and mostly round but also have irregular shapes; large teeth are regularly patterned in one incomplete row, with distinct, repeated elliptical shapes; some small teeth have developed within areas exposed by parts of large teeth breaking off (black arrowheads indicate examples); some tooth crowns have a rounded or elliptical hole in their enameloid, likely due to wear (white arrowheads indicate examples); pits are located where crowns of small round teeth have been lost (white arrows indicate examples); loss of a tooth crown has exposed the hole that comprised the pulp cavity in life (PC). This is also the only singular small tooth located between two relatively complete large teeth, of the three studied prearticulars of *C. mantelli*; a region at medial edge of dentigerous bone has cracks on the surface, with some cracks causing a large shallow pit (P); black arrow indicates a large hole running vertically and completely through the dentigerous bone. This is located at a region of possible previous weakness, as the edge of the hole includes the edge of a broken large tooth and a pit (far right white arrow) where a small tooth has been lost; line shows the position of the virtual section in B. B, XCT virtual section of the specimen, the position of the section B is shown by the line in A; most posterior tooth crown is rounded, its neighbour has a flat wear facet on the top of the tooth; white arrowhead indicates large hole in large tooth crown, corresponding to tooth indicated by far left white arrowhead in A; the remainder of teeth (all small and rounded) are flat; arrow indicates hole through dentigerous bone, corresponding to hole indicated by black arrow in A; white indicates a high density material, very likely iron pyrites. C, XCT surface render of the specimen; line shows the position of the virtual transverse section in D. D, XCT virtual transverse section of the specimen, the position of the section D is shown by the line in C; two most posterior teeth correspond to the teeth indicated by black arrowheads in A. These have developed in an area exposed by part of a large tooth breaking off; arrow indicates large shallow pit in the surface of the dentigerous bone, corresponding to region indicated by (P) in A; white indicates a high density material, very likely iron pyrites. E-F, XCT surface render of the specimen from the posterior (E) and anterior (F) view, showing clearly the region at the medial edge of the dentigerous bone with cracks on the surface. Some cracks have caused a large shallow pit, indicated by (P) in A; line shows the position of the virtual section in B. All scale bars represent 1 cm.

4.1.7 *Pycnodus pachyrhinus*, vomer, NHMUK PV P 610, holotype

Surface features of* Pycnodus pachyrhinus, *vomer

This specimen is an almost complete vomer of *Pycnodus pachyrhinus* (NHMUK PV P 610, holotype; Fig. 4.9A, C). Teeth are light brown with a smooth, shiny surface. Large teeth are regularly patterned in rows, with distinct, repeated shapes. Smaller, round-to-oval teeth are located in regions where large teeth are absent, and in an irregular pattern. In comparison with other studied pycnodont specimens, there are fewer small, rounded, irregularly patterned teeth, and these are larger. Their larger size means there is less of a size difference between the large teeth regularly patterned in rows, and the irregularly patterned rounded teeth, in comparison with other pycnodont specimens studied. A small rounded tooth has developed within an area exposed by part of a large tooth breaking off. The anterior-most large tooth at the tip of the specimen is present in the median row of large teeth, despite a gap in the same row immediately posterior to it. Two small rounded teeth are located in this gap. Approximately half of the crown of the anterior-most large tooth has broken off from its anterior side. The tooth’s surface is more worn than all others in its row, with the outline of the pulp cavity visible. Unusually among the pycnodont specimens studied, the rows of large teeth appear to be almost tessellated with each other, though not completely so as there are gaps between all teeth. This is seen most clearly when viewed from the anterior and posterior (Fig. 4.9E), where the rows appear completely tessellated. Pits remain where whole small and large teeth have been lost. Some tooth crowns have rounded holes in their enameloid, likely due to wear. These are mostly located at the lateral ends of large teeth, and centrally at the location of the pulp cavity, most clearly seen in the posterior view (Fig. 4.9E). Three holes run vertically and completely through the dentigerous bone, highly likely due to pyritization of the fossil (Fig. 4.9A). Clasts from the matrix rock are not present between teeth.

XCT virtual sections of **Pycnodus pachyrhinus,** *vomer*

XCT virtual sections of the *Pycnodus pachyrhinus* vomer (NHMUK PV P 610, holotype; Fig. 4.9B, D) show that large, regularly patterned teeth have been lost towards the anterior of the specimen, and retained towards the posterior. Large cracks run across the base of the crowns of the two small rounded teeth located within the median row of regularly patterned large teeth. The cracks are so significant that the teeth appear to be detached from the dentigerous bone, apart from very small region of contact on the posterior side of the most posterior small rounded tooth. The high density material represented by white on both virtual sections is highly likely to be iron pyrites; the fossil therefore appears highly pyritized, with many regions and lines of white. The cracks at the base of the two small rounded teeth are likely due to this, particularly as there is a dense region of white between the two teeth. Pyritization is also likely responsible for: three holes present within the second-most posterior large tooth in section B (Fig. 4.9B), the holes throughout the specimen, represented by black in section D (Fig. 4.9D), and the three holes running completely through the specimen, best seen in the surface view A (Fig. 4.9A). The pulp cavity of the most posterior large tooth in section B appears to be completely infilled with iron pyrites. The two most anterior teeth of the four large, regularly patterned, whole teeth in sections B and D (Fig. 4.9B, D) have thin, pyritized lines (or cracks), across their crown bases. It is possible this is associated with the tooth's attachment in life, either representing fossilised bone of attachment or a narrow dividing zone of unmineralised fibrous tissue (see section 2.2.8, Berkovitz & Shellis (2017) and c.f. modern sparids, Fig. 4.20, Fig. 4.21). It seems plausible that whichever the mode of attachment used in life, the connecting tissue may have comprised a line of weakness, in terms of fossil preservation. Interestingly, the two most posterior large teeth in B and the posterior-most tooth in D do not have this line, and there is only a short part of a line present in the second-most posterior tooth in D. A hypothesis as to the cause may be that these teeth are younger/newer - following the hypothesis of Longbottom (1984) (section 2.7.2), in which new large teeth are added to the back of the row as the fish grows. The older, anterior, large teeth may have had a looser attachment due to wear, therefore a weaker connection between the crown and bone of attachment in life, affecting fossil preservation. As with the other pycnodonts studied, teeth have developed on the surface of the dentigerous bone, and not from a crypt within it, therefore the mode of replacement is recognizable as extraosseous. Again, a one-for-one relationship between predecessor and successor teeth cannot easily be discerned, nor can families of successional teeth be identified.

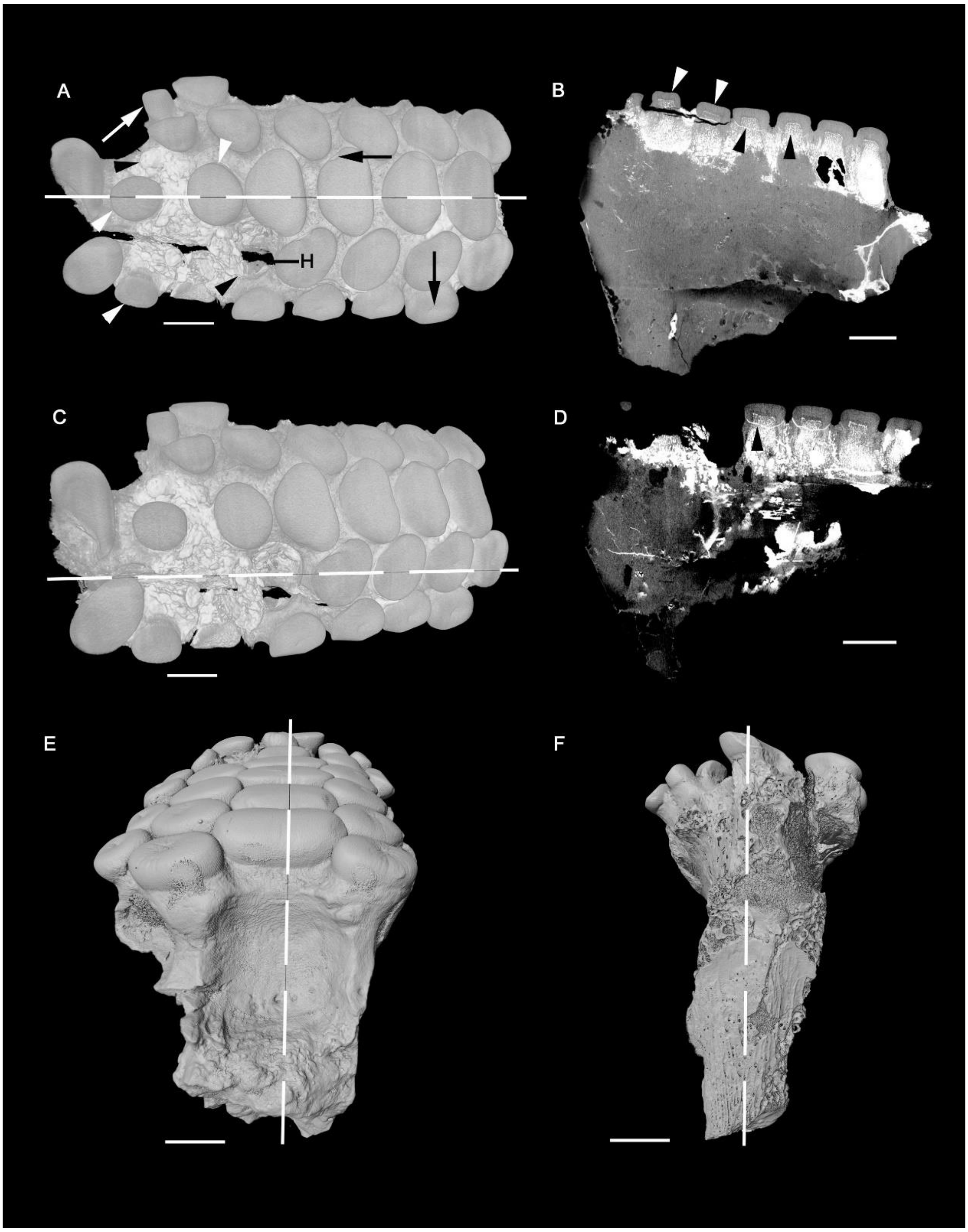


Fig. 4.9 Surface features and XCT virtual sections of *Pycnodus pachyrhinus*, an almost complete vomer, NHMUK PV P 610, holotype.

Posterior to anterior direction is right to left (Longbottom 1984) (A-D). A, XCT surface render of the specimen; large teeth have distinct, repeated shapes and are arranged in regularly patterned rows; white arrowheads indicate smaller rounded-to-oval teeth located in regions where large teeth are absent, and in an irregular pattern; the anterior-most tooth large tooth in the median row is present but with approximately half of the crown broken off from its anterior side. Its surface is more worn than all other large teeth in its row, with the outline of the pulp cavity visible; white arrow indicates a small round tooth which has developed within an area exposed by part of a large tooth breaking off; the rows of large teeth appear to be almost tessellated with each other, incompletely due to gaps between all teeth; pits remain where whole small and large teeth have been lost (black arrowheads indicate examples); some tooth crowns have rounded holes in their enameloid, likely due to wear (black arrows indicate examples). These are mostly located at the lateral ends of large teeth and centrally at the location of the pulp cavity, most clearly seen in E; three large holes run vertically and completely through the dentigerous bone (H indicates example); line shows the position of the virtual section in B. B, XCT virtual section of the specimen, the position of the section B is shown by the line in A; arrowheads indicate two teeth with cracks at the base of the crown (likely due to pyritization), corresponding to the rounded teeth indicated by the two upper white arrowheads in A; white indicates a high density material, very likely iron pyrites; black arrowheads indicate thin pyritized lines or cracks, running across the base of two large tooth crowns. These may represent the location of attachment tissue in life, as seen in modern fish (Berkovitz & Shellis (2017), section 2.2.8 herein); three holes located within the pulp cavity of the second-most posterior large tooth are likely due to pyritization; the pulp cavity of the most posterior large tooth appears completely pyritized. C, XCT surface render of the specimen; line shows the position of the virtual section in D. D, XCT virtual section of the specimen, the position of the section D is shown by the line in C; white indicates a high density material, very likely iron pyrites; thin pyritized lines, or cracks, run across the base of two large tooth crowns, and in a very short line on the anterior side of the second-most posterior tooth (black arrowhead indicates example). These lines may represent the location of attachment tissues in life (see B); whole teeth have been lost from the anterior tooth positions in the row; many holes are present in the dentigerous bone, represented by regions of black. These are likely due to pyritization. E-F, XCT surface render of the specimen from the posterior (E) and anterior (F) view, showing clearly in E that the rows of large teeth appear to be tessellated with each other from this perspective; line shows the position of the virtual section in B. All scale bars represent 1 cm.

4.1.8 *Pycnodus tattami*, part of vomer, NHMUK PV P 18825, holotype

Surface features of* Pycnodus tattami*, part of vomer

This specimen is part of a vomer of *Pycnodus tattami* (NHMUK PV P 18825, holotype; Fig. 4.10A, C). Teeth are dark brown to light brown in colour with a smooth surface. Large teeth are regularly patterned in rows, with distinct, repeated shapes. Unusually among the pycnodont specimens studied, only one small rounded tooth is present. This is located in in a gap left by the loss of the second-most anterior large tooth in a row. The whole surface of the vomer can be viewed as an outline drawing in Longbottom (1984), p19. The vomer's most lateral rows are near-square or 'lozenge'-shaped. This shape, and the shape of the vomer itself, are features unique to this species, among the *Pycnodus* species described by Longbottom (1984). The vomer shape is unusal in that the anterior-posterior axis of the most lateral row is about 45° to that of the median row (Longbottom, 1984). This creates a steeper-sided, more cylinder-like volume around the central anterior-posterior axis. XCT virtual sections are therefore more likely to include more than one tooth row. The lateral and second-most lateral rows of large teeth appear to be positioned in a near-interlocking pattern, though the geometry of their shapes does not allow them to interlock completely. This is seen most clearly when viewed from the anterior and posterior (Fig. 4.10E-F). Pits remain where the crowns of large teeth have been lost. Some tooth crowns have rounded holes in their enameloid, likely due to wear. One such hole in the specimen's most anterior large tooth comprised part of the pulp cavity in life. Clasts from the matrix rock are not present between teeth.

XCT virtual sections of **Pycnodus tattami***, part of vomer*

XCT virtual sections of the *Pycnodus tattami* vomer (NHMUK PV P 18825, holotype; Fig. 4.10B, D) show a crack running across the base of a large, anterior tooth crown, possibly representing the location of attachment tissue in life (section 2.2.8). A crack also runs from the base of most posterior tooth crown shown, through the dentigerous bone. The only small rounded tooth on the specimen is located in a gap left by the loss of the second most anterior large tooth, in a regularly patterned row (Fig. 4.10D). Tooth crowns become progressively flatter towards the anterior. A pit in the dentigerous bone resulting from loss of a large tooth crown corresponds to a pit observed on the surface (Fig. 4.10A-B). The high density material represented by white on both virtual sections is highly likely to be iron pyrites; the fossil therefore appears highly pyritized, with many regions of white. Pulp cavities have become infilled or partially infilled with iron pyrites within the fossil. Pyritization is also likely responsible for the large holes in some pulp cavities, represented by black in virtual sections B and D (Fig. 4.10B, D). A second row of pulp cavities appears to be present underneath those of the teeth. This is likely due to the virtual section cutting through the adjacent row's pulp cavities, due to the shape of the vomer (see above). As with the other pycnodonts studied, teeth have developed on the surface of the dentigerous bone, and not from a crypt within it, therefore the mode of replacement is recognizable as extraosseous. Again, a one-for-one relationship between predecessor and successor teeth cannot easily be discerned, nor can families of successional teeth be identified.

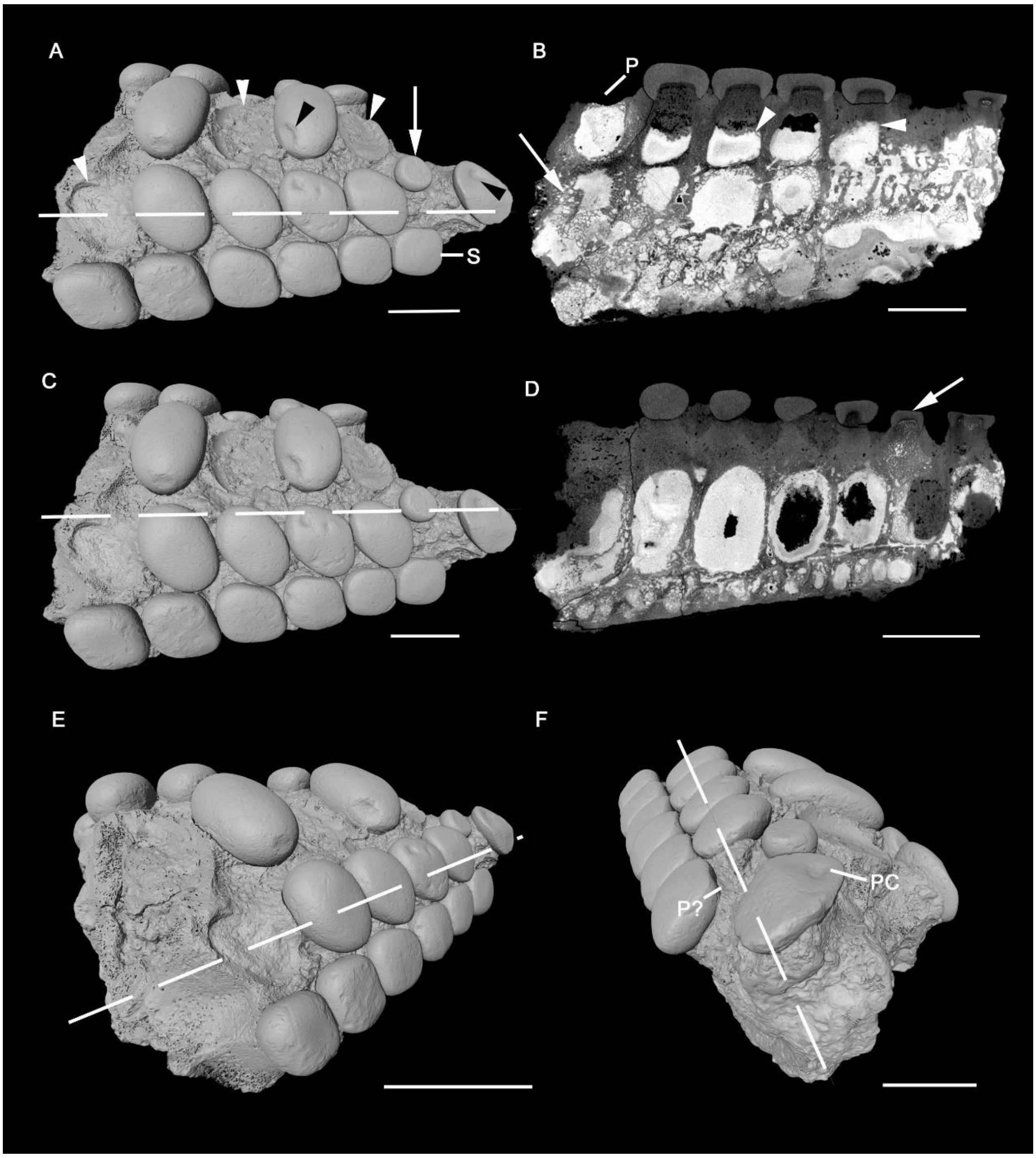


Fig. 4.10 Surface features and XCT virtual sections of *Pycnodus tattami*, part of vomer, NHMUK PV P 18825, holotype.

Posterior to anterior direction is left to right (Longbottom, 1984) (A-D). A, XCT surface render of the specimen showing large teeth with distinct, repeated shapes, arranged in regularly patterned rows; arrow indicates the only small rounded tooth on the specimen, located in a region where a large tooth is absent from a regularly patterned tooth row. The side of a pit left behind by the loss of the large tooth is putatively identified in F; pits indicate where large tooth crowns have been lost from rows of regularly patterned teeth (white arrowheads indicate examples); the near-square or ‘lozenge’ tooth shape (S) of the most lateral rows is unique among the *Pycnodus* species described in Longbottom (1984); the shape of vomer is unique among the *Pycnodus* species described in Longbottom (1984) in that the anterior-posterior axis of the most lateral row is about 45° to that of the median row (Longbottom, 1984), forming a steeper-sided, more cylinder-like volume around the central anterior-posterior axis (see E, F). XCT virtual sections are therefore more likely to include more than one tooth row (see B, D); the two most lateral rows of large teeth are positioned in a near-interlocking pattern, though the geometry of their shapes does not allow them to interlock completely; some tooth crowns have rounded holes in their enameloid, likely due to wear, and most clearly seen in views E and F (black arrowheads indicate examples). One hole located on the specimen’s most anterior large tooth comprised part of the pulp cavity in life (right black arrowhead); line shows the position of the virtual section in B.

B, XCT virtual section of the specimen, the position of the section B is shown by the line in A; the second-most anterior tooth has a crack at the across base of the crown, possibly representing the location of attachment tissues in life (section 2.2.8); a crack runs from the base of the most posterior tooth crown through the dentigerous bone; tooth crowns become progressively flatter towards the anterior; a pit (P) on the surface of the dentigerous bone resulting from loss of a large tooth crown corresponds to the pit indicated by the far left arrowhead in A; white indicates a high density material, very likely iron pyrites; pulp cavities have become infilled or partially infilled with iron pyrites within the fossil (arrowheads indicate examples); a large hole is present in the pulp cavity of the middle large tooth in the row. Smaller holes are present in the pulp cavities of the two most posterior large teeth (which retain crowns). The holes are represented by regions of black and are likely due to pyritization; arrow indicates apparent second row of pulp cavities present underneath those of the teeth. This is likely due to the virtual section cutting through the adjacent row’s pulp cavities, as a result of the shape of the vomer (see A).

C, XCT surface render of the specimen; line shows the position of the virtual section in D.

D, XCT virtual section of the specimen, the position of the section D is shown by the line in C; a crack is present at the base of the third-most anterior tooth crown, possibly representing the location of attachment tissue in life; a crack runs from the base of the most posterior tooth crown through the dentigerous bone; arrow indicates small rounded tooth located where a large tooth is absent from a regularly patterned row, corresponding to tooth indicated by arrow in A; tooth crowns become progressively flatter towards the anterior; pulp cavities have become infilled or partially infilled with iron pyrites within the fossil (as described in B); large and small holes are present in the pulp cavities of the teeth. The holes are represented by regions of black and are likely due to pyritization

E-F, XCT surface render of the specimen from the posterior (E) and anterior (F) view, showing clearly in F that the two most lateral rows of large teeth are positioned in a near-interlocking pattern; putative side of a pit (P?) caused by loss of a large tooth is shown in F. The pit contains the small rounded tooth indicated by the arrows in A and D; PC indicates hole comprising part of the pulp cavity in life, corresponding to the hole indicated by right black arrowhead in A; line shows the position of the virtual section in B. All scale bars represent 1 cm.

4.1.9 *Pycnodus toliapicus*, part of left prearticular, NHMUK PV OR 38826

Surface features of **Pycnodus tolipicus**, *part of left prearticular*

This specimen is part of a left prearticular of *Pycnodus toliapicus* (NHMUK PV OR 38826; Fig. 4.11A, C). Teeth are dark brown to light brown in colour with a smooth surface. Large teeth are regularly patterned in rows, with distinct, repeated shapes. The teeth of the two most lateral rows are round-to-oval. The partial and whole remains of the crowns of only three large teeth are present in the medial row. These have a long elliptical shape. The most anterior large tooth in the medial row has a large central hole, comprised of what was the pulp cavity in life, and worn away dentine and enameloid immediately surrounding it. The adjacent large tooth in the row is almost completely worn away, with only its most lateral third remaining. The adjacent, most posterior tooth of the three is whole. Small rounded teeth are located in the region where large teeth are absent from the most anterior part of the medial row, and in an irregular pattern. The two most lateral rows of large teeth appear to be positioned in a near-interlocking pattern, though the relatively large gap between the rows, and the geometry of their shapes does not allow them to interlock completely. This is seen most clearly when viewed from the anterior and posterior (Fig. 4.11E-F). Some tooth crowns have rounded holes in their enameloid, mostly at the location of the pulp cavity. Unusually among the Pycnodont specimens studied, some tooth crowns have a ridged pattern on their enameloid surface; both features are likely due to wear. Clasts from the matrix rock are not present between teeth.

XCT virtual sections of* Pycnodus toliapicus*, part of left prearticular

XCT virtual sections of the *Pycnodus toliapicus* specimen, part of a left prearticular (NHMUK PV OR 38826; Fig. 4.11B, D) show remaining parts of large teeth in the medial row which are worn down, including parts of the tooth crown either side of the most anterior large tooth's large central hole (described above). The two most anterior teeth are small and rounded, located where large teeth are absent at the anterior of the medial tooth row. In a virtual section through the second-most lateral row of large teeth, all teeth are whole, yet worn. Either faint black lines, or larger black lines which appear to be cracks, are located at the base of most tooth crowns in both virtual sections. These run completely across the crown base in most cases. The lines/cracks possibly represent the location of attachment tissue in life (section 2.2.8). A crack running across the base of the most posterior large tooth in the medial row continues down through the dentigerous bone. Regions of white – very likely iron pyrites - are located within some pulp cavities and in the dentigerous bone below the teeth, in both virtual sections. White lines run through the deepest part of the specimen, this is most likely within the matrix rock surrounding the fossil, as the specimen density is different to the bone here. In the medial row, a particularly dense white is located beneath the large central hole in the most anterior large tooth. Holes in the bone are represented by black; large holes are likely due to pyritization. Tooth crowns in the second-most lateral row become progressively slightly flatter towards the anterior. As with the other pycnodonts studied, teeth have developed on the surface of the dentigerous bone, and not from a crypt within it, therefore the mode of replacement is recognizable as extraosseous. Again, a one-for-one relationship between predecessor and successor teeth cannot easily be discerned, nor can families of successional teeth be identified.

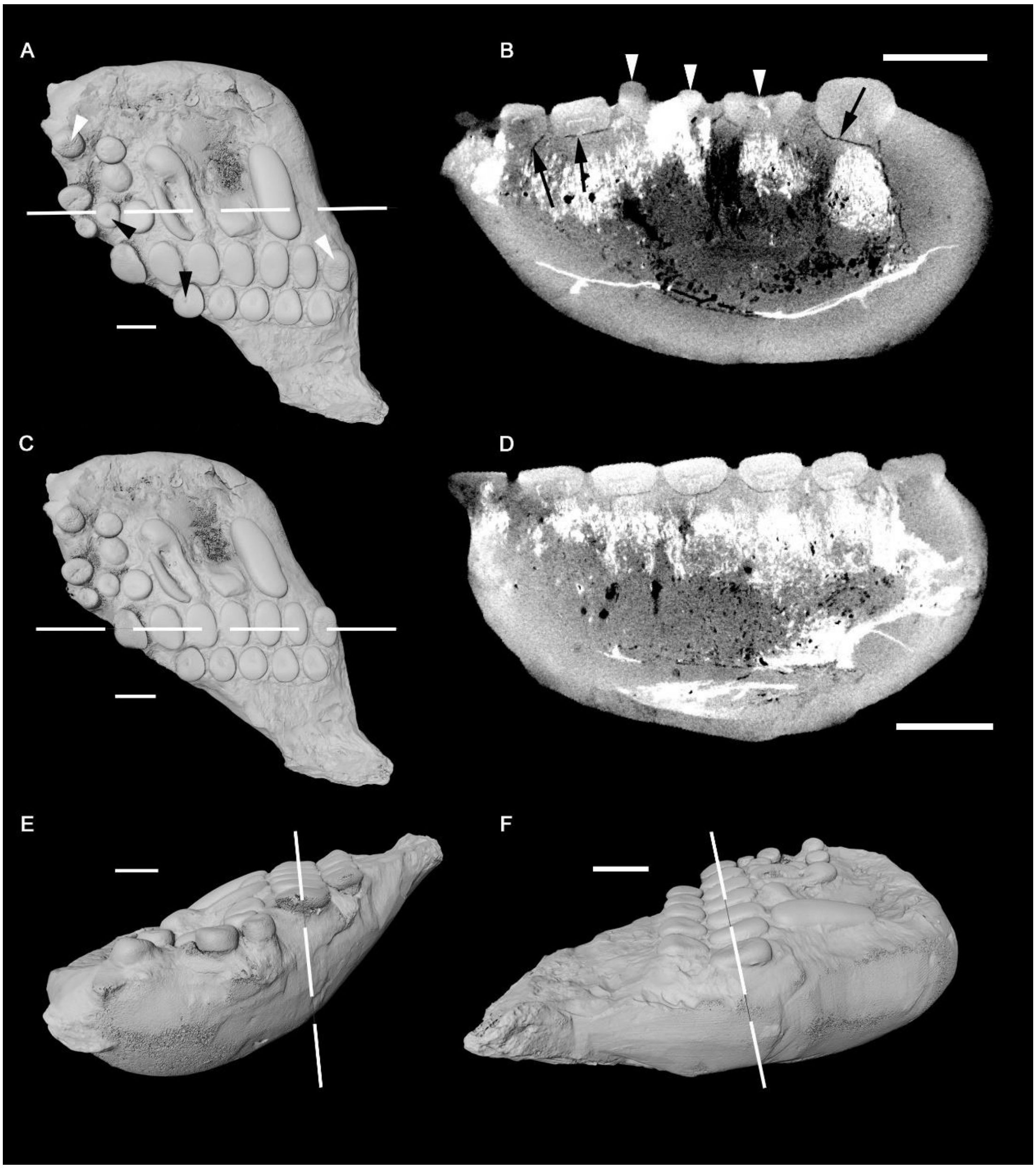


Fig. 4.11 Surface features and XCT virtual sections of *Pycnodus toliapicus*, part of left prearticular, NHMUK PV OR 38826.

Posterior to anterior direction is right to left (A-D), medial to lateral direction is top to bottom (A, C). A, XCT surface render of the specimen, showing large teeth with distinct, repeated shapes, arranged in regularly patterned rows; large teeth in the two lateral rows are round-to-oval shaped, large teeth in the medial row are long and elliptical; the most anterior large tooth in the medial row has a large central hole, comprised of what was the pulp cavity in life, and worn away dentine and enameloid immediately surrounding it; the middle large tooth in the medial row is almost completely worn away, with only its most lateral third remaining; the most posterior tooth in the medial row is whole; small rounded teeth are located in the region where large teeth are absent from the anterior end of the medial row, and in an irregular pattern; the two most lateral rows of large teeth are positioned in a near-interlocking pattern, though the relatively large gap between the rows and the geometry of their shapes does not allow them to interlock completely; some tooth crowns have rounded holes in their enameloid, mostly at the location of the pulp cavity, likely due to wear (black arrowheads indicate examples); some tooth crowns have a ridged pattern on their enameloid surface, likely due to wear (white arrowheads indicate examples); line shows the position of the virtual section in B.

B, XCT virtual section of the specimen, the position of the section B is shown by the line in A; white arrowheads indicate remaining parts of large teeth which are worn down, corresponding to the two most anterior large teeth in A. The two left-hand white arrowheads are located either side of the most anterior large tooth's large central hole; black arrows indicate cracks at the base of large and small tooth crowns, possibly representing the location of attachment tissues in life (section 2.2.8); a crack runs across the base of the most posterior tooth crown (far right black arrow), and continues down through the dentigerous bone; the two most anterior teeth are small and rounded, located where large teeth are absent at the anterior of the tooth row; white indicates a high density material, very likely iron pyrites; regions of white are located within some pulp cavities and in the dentigerous bone below the teeth. A particularly dense white is located directly beneath the large central hole in the most anterior large tooth (in between the remains of the tooth indicated by the two left-hand white arrowheads); lines of white run across the deepest part of the specimen (towards the bottom of the image). As the density of the specimen material is different to the dentigerous bone here, the white lines are likely within in the matrix rock of the fossil; holes in the bone are represented by black, with large holes likely due to pyritization.

C, XCT surface render of the specimen, line shows the position of the virtual section in D.

D, XCT virtual section of the specimen, the position of the section D is shown by the line in C; a faint black line, or crack, is present at the base of the five middle teeth, possibly representing the location of attachment tissues in life; regions of white – highly likely to be iron pyrites - are located within the pulp cavities, in the dentigerous bone below the teeth and in lines running through the deeper parts of the bone and possibly the fossil matrix rock (as described in B); tooth crowns become progressively slightly flatter towards the anterior.

E-F, XCT surface render of the specimen from the anterior (E) and posterior (F) view, showing clearly in F that the two most lateral rows of large teeth are positioned in a near-interlocking pattern; line shows the position of the virtual section in D. All scale bars represent 1 cm.

4.1.10 *Anomoeodus superbus*, parts of prearticulars, NHMUK PV P 7237-1, NHMUK PV P 7237-4, NHMUK PV P 7237-6

Surface features of three specimens of **Anomoeodus superbus**, *parts of prearticulars*

These three specimens of *Anomoeodus superbus* comprise a part of: a (left?) prearticular (NHMUK PV P 7237-1; Fig. 4.12A), a (right?) prearticular (NHMUK PV P 7237-4; Fig. 4.13A), and a (right?) prearticular (NHMUK PV P 7237-6; Fig. 4.14A). The designation of the specimens as left or right prearticulars is uncertain, as in the latter two specimens neither of the outer rows are comprised of the largest teeth. In prearticular specimens of *Pycnodus* species, the outer row of largest teeth, tilted at an upward angle towards the dentigerous bone's adjacent long edge, is identified as the medial row, following the method of Longbottom (1984). Once this medial row is identified, the designation of a left/right prearticular can be made.

However, in two of the *Anomoeodus superbus* specimens, the row of largest teeth is the middle row, meaning this method is not useful. Assuming the width of the combined rows decreases towards the specimen anterior, and surface wear increases towards the anterior, the posterior-to-anterior direction is left to right in all three specimens. Although specimen NHMUK PV P 7237-4 (Fig. 4.13A) is too small to identify these trends, comparison of its tooth shapes to those of the two larger specimens enables its posterior-to-anterior direction to also be postulated as left to right. For the three specimens overall: by determining the posterior-anterior direction, the tilt of the rows in relation to the outer long edge of the dentigerous bone, and from this, identifying the medial and lateral rows (Longbottom 1984), the specimens are postulated to be left, right and right prearticulars respectively. However, this assumes that the method used for *Pycnodus* holds for *Anomoeodus*, and as such left/right and medial/lateral designations are putatively assigned here.

Teeth are dark to light brown, with a smooth, shiny surface. Large teeth are regularly patterned in rows, with distinct, repeated shapes. The largest teeth are elliptical to bean-shaped, however in some cases the curvature creating the bean-shape occurs only at one end of the tooth, resulting in a slight l-shape (Fig. 4.12A, Fig. 4.14A). In each specimen, these largest teeth comprise one row. Either side of this row there is a row of regularly patterned rounded-to-oval teeth, of intermediate size (however one such row is lost in NHMUK PV P 7237-1, 4.12A). Some teeth of these rows have varying, irregular shapes e.g rounded-to-triangular (Fig. 4.14A). There are a few, irregularly patterned small rounded teeth located in between teeth of these rows (Fig. 4.12A, Fig. 4.14A). These are located on the outer edge of the specimen (whether a medial or lateral row). Pits remain where teeth of a similar size, shape and location to these have been lost, as whole teeth. Pits also remain where whole small rounded teeth have been lost from in between rows of teeth (Fig. 4.14A). Some tooth crowns have rounded holes in their enameloid, likely due to wear (Fig. 4.12E). Small parts of enameloid have broken, or ‘chipped’ off in various locations (Fig. 4.14C). Part of an anterior-most large tooth crown has broken off, forming a central hole at the location of the pulp cavity in life (Fig. 4.14C). Unusually, there are two holes running vertically and completely through the dentigerous bone of one specimen (Fig. 4.14). The teeth of specimen NHMUK PV P 7237-6 (Fig. 4.14) become progressively flatter and more worn towards the anterior of the specimen. This is supported by its XCT virtual sections (Fig. 4.14B, D). The virtual sections of NHMUK PV P 7237-1 reveal this also to be the case for the row of largest teeth in this specimen (Fig. 4.12B). Clasts from the matrix rock are not present between teeth in any of the three specimens.

XCT virtual sections of three specimens of* Anomoeodus superbus*, parts of prearticulars

XCT virtual sections are visualised for part of: a (left?) prearticular (NHMUK PV P 7237-1; Fig. 4.12B, D), a (right?) prearticular (NHMUK PV P 7237-4; Fig. 4.13B, D, F), and a (right?) prearticular (NHMUK PV P 7237-6; Fig. 4.14B, D). A crack runs across the base of a large tooth in specimen NHMUK PV P 7237-6 (Fig. 4.14B, D), connecting to a crack that runs vertically through the whole of the dentigerous bone. As there are no lines or cracks in the same position in other teeth on the virtual sections of this specimen, it is unlikely that the crack across the base of the crown represents the location of attachment tissue in life (section 2.2.8). It is however close to holes that run completely through the specimen, and so is likely to be associated with this damage. A faint line/crack is present across the base of the most-anterior of the three largest teeth in NHMUK PV P 7237-4 (Fig. 4.13B, D). However, this is also unlikely to represent the location of attachment tissue in life, as no other teeth in the specimen’s virtual sections have lines across the crown base. Teeth become progressively less rounded and flatter towards the anterior of the two largest specimens. In NHMUK PV P 7237-1 (Fig. 4.12D), the dentigerous bone between the three anterior-most teeth in the virtual section appears more worn down than in between the posterior teeth. The large central hole at the location of the pulp cavity in the anterior-most tooth of NHMUK PV P 7237-6 (Fig. 4.14B, D), is shown to have not reached the dentine at the position of the two sections. Also in this specimen, another part of the tooth enameloid is shown to be broken/‘chipped off’, corresponding to chipped enameloid observed on the surface (Fig. 4.14B). In specimen NHMUK PV P 7237-4, a virtual section shows a rounded hole observed in the surface enameloid to be significant, reaching through to the underlying dentine (Fig. 4.13E-F). As with the other pycnodonts studied, teeth have developed on the surface of the dentigerous bone, and not from a crypt within it, therefore the mode of replacement is recognizable as extraosseous. Again, a one-for-one relationship between predecessor and successor teeth cannot easily be discerned, nor can families of successional teeth be identified.

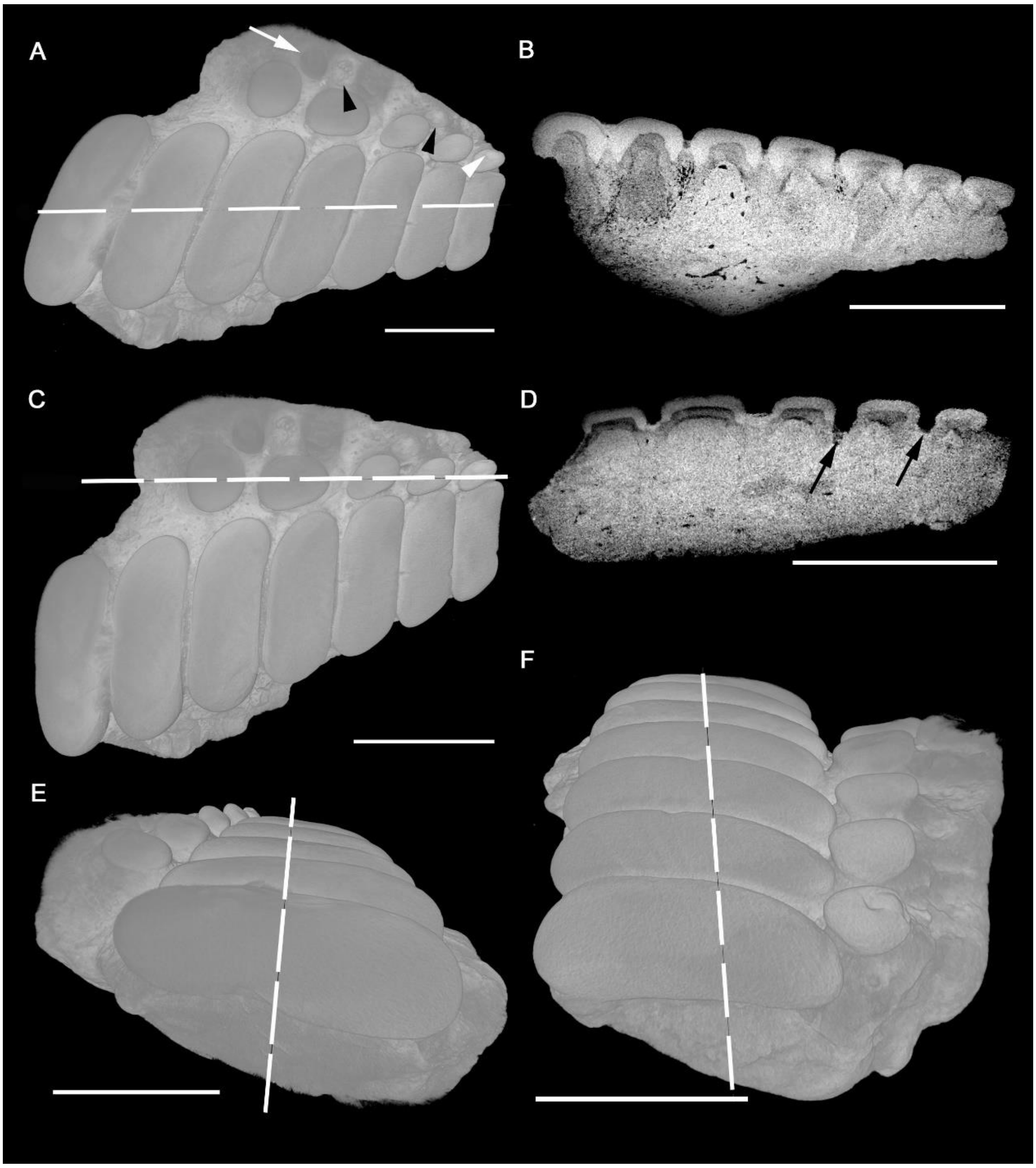


Fig. 4.12 Surface features and XCT virtual sections of *Anomoeodus superbus*, part of a (left?) prearticular, NHMUK PV P 7237-1.

Posterior to anterior direction is left to right (A-D), medial to lateral direction is putatively bottom to top (A, C). A, XCT surface render of the specimen showing large teeth with distinct, repeated shapes, arranged in regularly patterned rows. The largest teeth comprise one row and are elliptical-to-bean-shaped, with the lateral end curving anteriorly, resulting in a slight l-shape; adjacent and lateral to the row of largest teeth is a row of regularly patterned, medium sized, rounded-to-oval teeth which become more elliptical towards the anterior; white arrow indicates a small rounded tooth, located on the outer edge of the lateral row, and therefore on the outer edge of the specimen; black arrowheads indicate pits remaining where whole, small round teeth have been lost; white arrowhead indicates rounded hole in enameloid of the most anterior, medium-sized, regularly patterned tooth, likely due to wear; line shows the position of the virtual section in B. B, XCT virtual section of the specimen, the position of the section B is shown by the line in A; teeth become progressively less rounded and flatter towards the anterior of the specimen. C, XCT surface render of the specimen; line shows the position of the virtual section in D. D, XCT virtual section of the specimen, the position of the section D is shown by the line in C; black arrows indicate two regions of dentigerous bone between the three most anterior teeth which appear more worn down in comparison to the posterior inter-tooth regions.

E-F, XCT surface render of the specimen from the posterior (E) and anterior (F) view; line shows the position of the virtual section in B. All scale bars represent 1 cm.

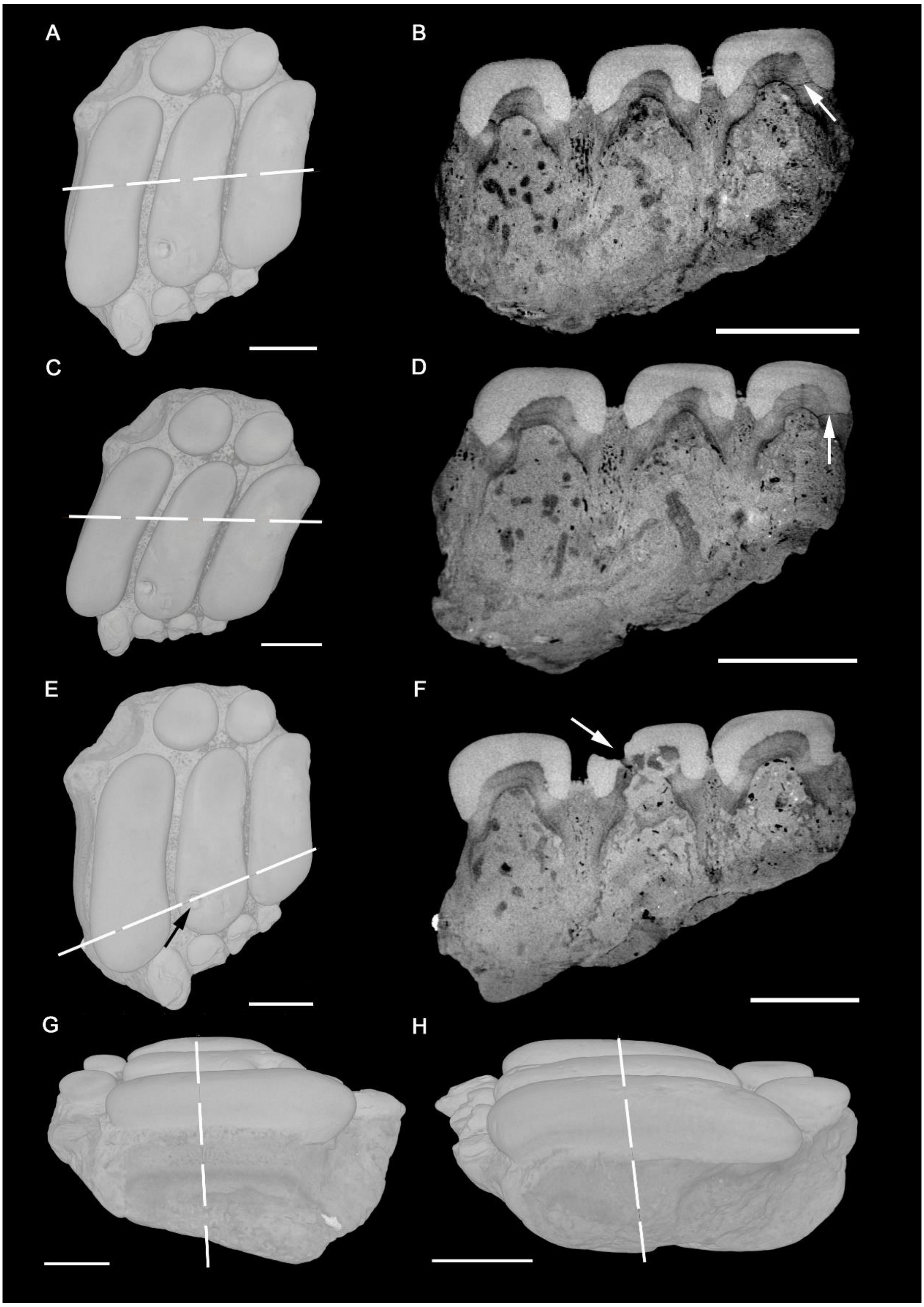


Fig. 4.13 Surface features and XCT virtual sections of *Anomoeodus superbus*, part of a (right?) prearticular, NHMUK PV P 7237-4.

Posterior to anterior direction is left to right (A-F), medial to lateral direction is putatively top to bottom (A, C, E). A, XCT surface render of the specimen showing three large teeth with distinct, repeated shapes, part of a regularly patterned row. These are elliptical-to-bean shaped, with the medial end curving anteriorly, resulting in a slight l-shape; parts of a row of regularly patterned, rounded-to-oval teeth are located either side of the row of three largest teeth; the row on the medial side of the largest teeth comprises only two remaining teeth, and a pit where a whole tooth has been lost; the row on the lateral side of the largest teeth comprises teeth which are partially broken off, with only the medial ends remaining in all but the most posterior tooth; the teeth in both rows either side of the the row of largest teeth are of intermediate size; line shows the position of the virtual section in B. B, XCT virtual section of the specimen, the position of the section B is shown by the line in A; arrow indicates a faint black line or crack across the base of the crown of the most anterior tooth. C, XCT surface render of the specimen; line shows the position of the virtual section in D. D, XCT virtual section of the specimen, the position of the section D is shown by the line in C; arrow indicates a faint black line or crack across the base of the crown of the most anterior tooth. This is very likely a continuation of the crack in B. E, XCT surface render of the specimen; arrow indicates hole in tooth surface; line shows the position of the virtual section in F. F, XCT virtual section of the specimen, the position of the section F is shown by the line in E; arrow indicates hole in tooth enameloid, reaching to the underlying dentine, corresponding to hole indicated by arrow in E. G-H, XCT surface render of the specimen from the posterior (G) view and anterior (H) view; line shows the position of the virtual section in B. All scale bars represent 0.5 cm.

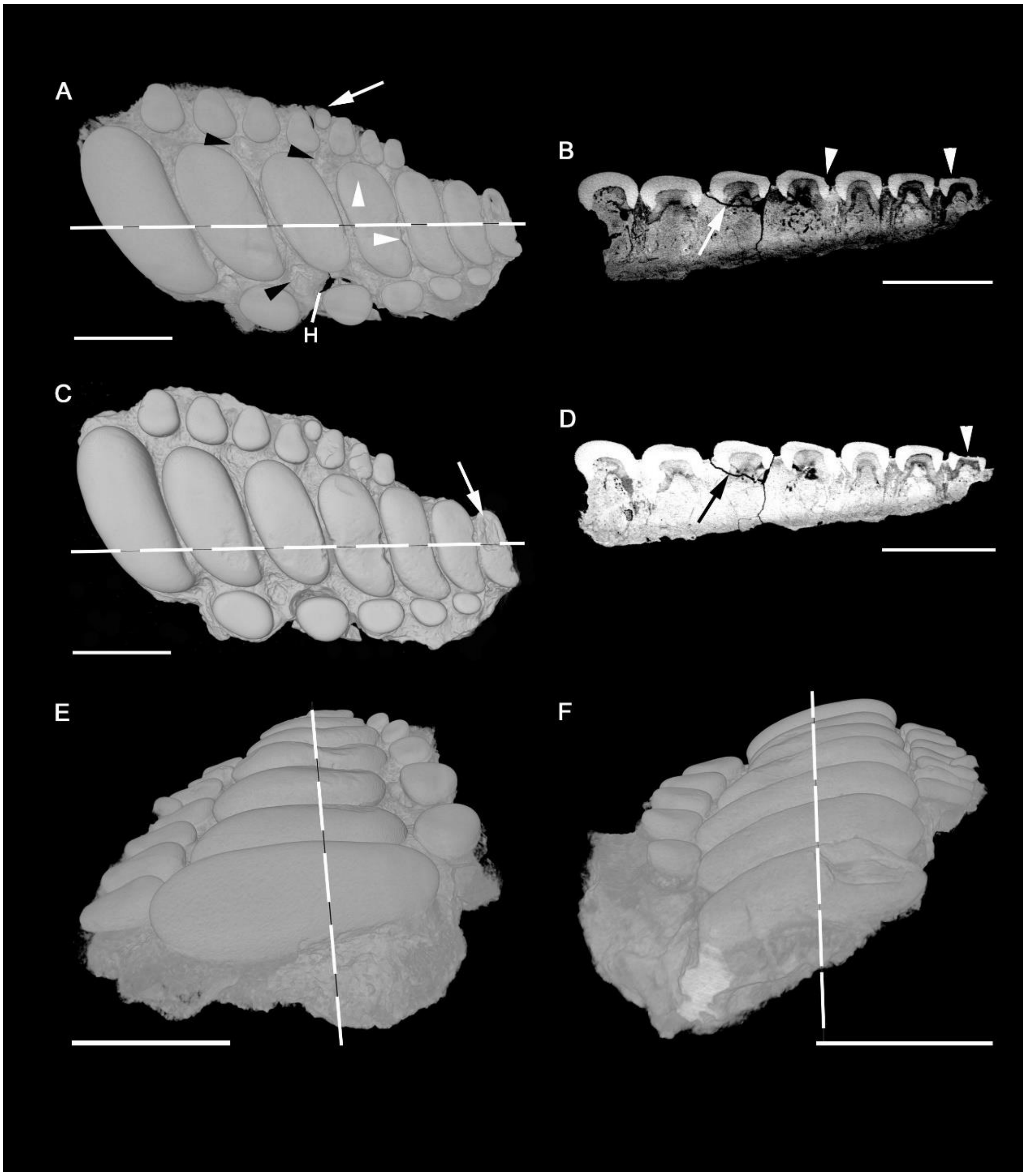


Fig. 4.14 Surface features and XCT virtual sections of *Anomoeodus superbus*, part of a prearticular, NHMUK PV P 7237-6.

Posterior to anterior direction is left to right (A-D), medial to lateral direction is putatively top to bottom (A, C). A, XCT surface render of the specimen showing large teeth with distinct, repeated shapes, arranged in regularly patterned rows. The largest teeth comprise one row and are elliptical-to-bean shaped, with the lateral end curving anteriorly, resulting in a slight l-shape; rows of regularly patterned, medium-sized, rounded-to-oval teeth are located either side of the row of largest teeth. Teeth towards the posterior of the most medial row have an irregular, rounded-to-triangular shape; white arrow indicates a small rounded tooth, located on the outer edge of the medial row, and therefore on the outer edge of the specimen; black arrowheads indicate pits remaining where whole, small round teeth have been lost from in between rows of larger teeth; white arrowheads indicate where parts of enameloid have broken, or chipped off; two holes run vertically and completely through the dentigerous bone (H indicates example); large teeth become progressively flatter and more worn towards the anterior of the specimen, this is supported by the XCT virtual sections (B, D); line shows the position of the virtual section in B. B, XCT virtual section of the specimen, the position of the section B is shown by the line in A; arrow indicates crack across the base of a tooth crown which connects to a crack running vertically through the whole of the dentigerous bone; teeth become progressively less rounded and flatter towards the anterior of the specimen; arrowheads indicate where parts of tooth enameloid have broken off - left arrowhead corresponds to 'chipped off' enameloid, the right arrowhead corresponds to large hole in tooth surface indicated by arrow in C. C, XCT surface render of the specimen; arrow indicates large hole in tooth surface; line shows the position of the virtual section in D, slightly medial to that in B. D, XCT virtual section of the specimen, the position of the section D is shown by the line in C; arrow indicates crack across the base of a tooth crown which connects to a crack running vertically through the whole of the dentigerous bone; teeth become progressively less rounded and flatter towards the anterior of the specimen; arrowhead indicates hole in tooth enameloid, corresponding to large hole in the tooth surface indicated by arrow in C. E-F, XCT surface render of the specimen from the posterior (E) and anterior (F) view; line shows the position of the virtual section in B. All scale bars represent 1 cm.

4.1.11 *Polygyrodus cretaceus*, vomer, NHMUK PV OR 39048

Surface features of **Polygyrodus cretaceus,** *vomer*

This specimen is a vomer of *Polygyrodus cretaceus* (NHMUK PV OR 39048; Fig. 4.15A, C). Teeth are medium brown in colour with a smooth, slightly shiny surface (Vullo *et al.* 2017; Fig. 2A). The specimen is embedded in plaster of Paris. Teeth of various sizes are irregularly positioned in no clear pattern, though an anterior-posterior central row of the large teeth is discernible. Following Vullo *et al.* (2017), the large, most lateral teeth were likely arranged in rows. Some small teeth are located in small gaps between larger teeth. Crown morphology is near-conical but most crowns are flat on the top surface, likely due to wear. A shelf near the base marks a point under which the sides slope inwards creating a peg-like shape, seen most clearly in Fig. 4.15E-F. In some crowns the shelf demarcates a doughnut-like ring (cingulum) comprising the base. All crown bases are near-circle or near-oval shapes. Crowns are compressed anterior-posteriorly towards the apex, facilitating a cutting function in this derived dentition of a likely predatory species of pycnodont (Vullo *et al.* 2017). Many crowns have small cusp-like bumps on their surface, seen most clearly in Fig. 4.15E-F. Some tooth crowns have central holes in their enameloid due to wear and some crowns have broken off completely; both reveal the hole that comprised the pulp cavity in life. Teeth of the right-most lateral row share a wear facet of the same angle, facing medially. Teeth of the left-most lateral row also share a wear facet of the same angle, facing medially (mirroring that of the right). However, the presence of the shared facet and angle is less clearly defined in the left-most lateral row, than in the right (Fig. 4.15E-F). Clasts of significant size from the matrix rock are not present between teeth.

XCT virtual sections of **Polygyrodus cretaceus,** *vomer*

XCT virtual sections of the *Polygyrodus cretaceus* vomer (NHMUK PV OR 39048; Fig. 4.15B, D) show small teeth located in small gaps between larger teeth, one in a particularly small gap. The pointed shape of the of the largest crowns shows their compressed morphology, with each having a flattened top surface to varying extents. One crown has a flat surface due to the virtual section cutting through the doughnut-like cingulum at its base, rather than higher up the crown (Fig. 4.15D). The structure of the enameloid appears folded (Fig. 4.15B), or with holes or channels within it. Teeth are tall with pulp cavities which are long dorsal-ventrally. Fine black lines or cracks are present at the base of some tooth crowns, one of which crosses a whole crown base (Fig. 4.15D). These lines/cracks possibly represent the location of attachment tissue in life (section 2.2.8). As with the other pycnodonts studied, teeth have developed on the surface of the dentigerous bone, and not from a crypt within it, therefore the mode of replacement is recognizable as extraosseous. Again, a one-for-one relationship between predecessor and successor teeth cannot easily be discerned, nor can families of successional teeth be identified.

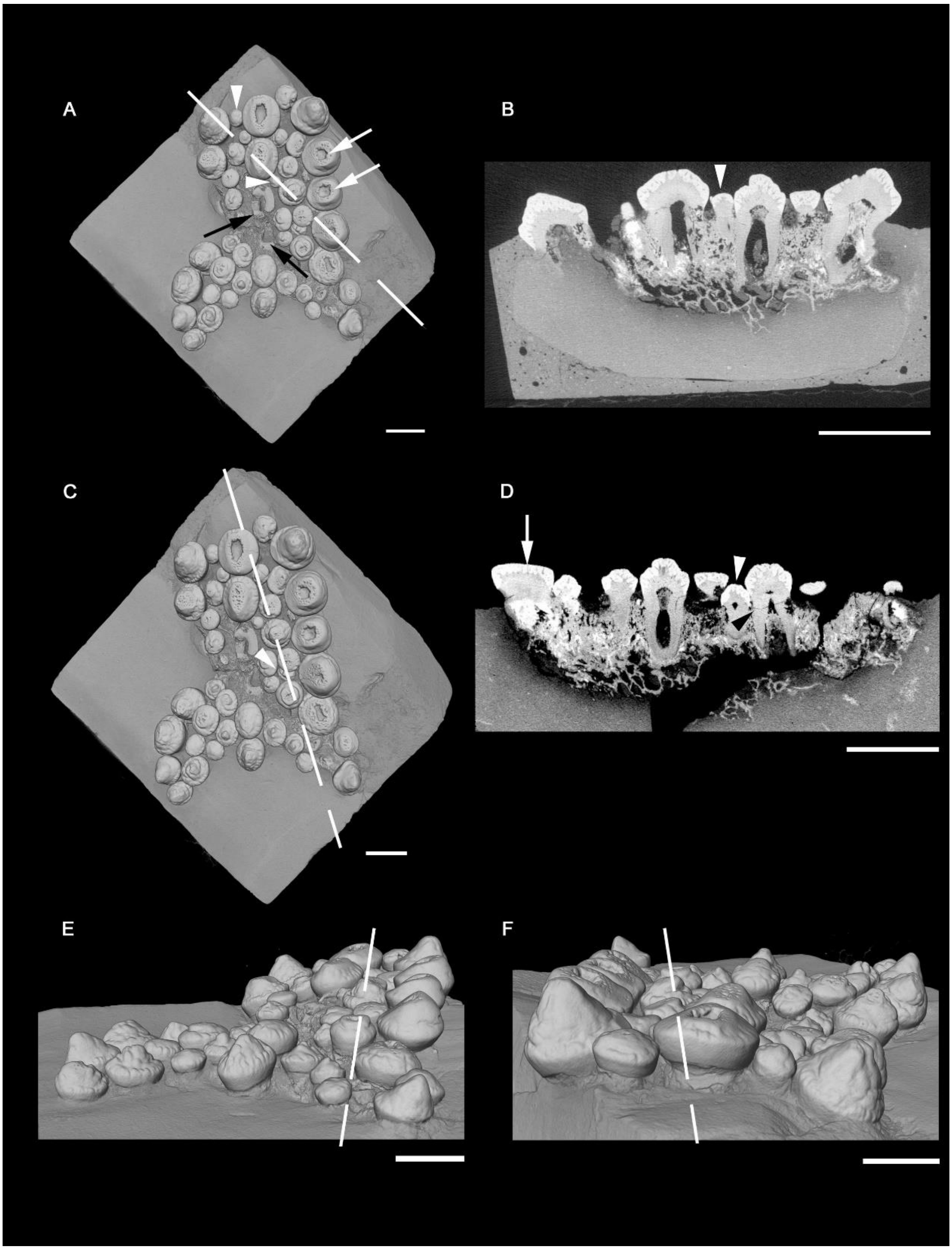


Fig. 4.15 Surface features and XCT virtual sections of *Polygyrodus cretaceus*, vomer, NHMUK PV OR 39048.

Specimen is embedded in plaster of Paris. Posterior to anterior direction is bottom to top (A, C) and right to left (B, D). A, XCT surface render of the specimen showing teeth of various sizes irregularly positioned in no clear pattern, though an anterior-posterior central row of the large teeth is discernible. Following Vullo et al. (2017), the large, most lateral teeth were likely arranged in rows; some small teeth are located in small gaps between larger teeth (arrowheads indicate examples); crown morphology is near-conical but most crowns are flat on the top surface, likely due to wear. A shelf near the base marks a point under which the sides slope inwards creating a peg-like shape, most clearly seen in views E and F. In some crowns the shelf demarcates a doughnut-like ring (cingulum) comprising the base. All crown bases are near-circle or near-oval shapes; crowns are compressed anterior-posteriorly towards the apex, facilitating a cutting function in this derived dentition of a likely predatory species of pycnodont (Vullo et al. 2017); many crowns have small cusp-like bumps on surface; some tooth crowns have central holes in their enameloid due to wear (white arrows indicate examples) and some crowns have broken off completely (black arrows indicate examples), both reveal the hole that comprised the pulp cavity in life; line shows the position of the virtual section in B.

B, XCT virtual section of the specimen, the position of the section B is shown by the line in A; arrowhead indicates small tooth located in small gap between two larger teeth, corresponding to tooth indicated by lower arrowhead in A; pointed shape of the of the largest crowns shows their compressed morphology, with each having a flattened top surface to varying extents; structure of enameloid appears folded, or with holes or channels within it; teeth are tall with long pulp cavities dorsal-ventrally.

C, XCT surface render of the specimen; arrowhead indicates small tooth in a particularly small gap between two larger teeth; line shows the position of the virtual section in C.

D, XCT virtual section of the specimen, the position of the section D is shown by the line in C; white arrowhead indicates small tooth in a particularly small gap between two larger teeth, corresponding to tooth indicated by arrowhead in C; fine black lines or cracks are present at the base of some tooth crowns, black arrowhead indicates one which crosses a whole crown base; crown morphology is as described in B; white arrow indicates crown which has a flat surface due to the virtual section cutting through the doughnut-like cingulum at its base, rather than higher up the crown. This crown corresponds to the anterior-most tooth of the central row of large teeth in C.

E-F, XCT surface render of the specimen from the posterior (E) and anterior (F) view; crown morphology described in A is more clearly observed from these views; teeth of the right-most lateral row share a wear facet of the same angle, facing medially; teeth of the left-most lateral row share a wear facet of the same angle, facing medially, mirroring that of the right. This is less clearly defined than in the right-most lateral row; line shows the position of the virtual section in D. All scale bars represent 1 cm.

4.1.12 *Polygyrodus cretaceus*, left and right prearticulars and putative dentaries, NHMUK PV P 11157

Surface features of **Polygyrodus cretaceus***, left and right prearticulars and putative dentaries*

This specimen is a near-complete lower dentition of *Polygyrodus cretaceus* (NHMUK PV P 11157; Fig. 4.16A, C), comprising left and right prearticulars and putative dentaries (Woodward 1912; Vullo *et al.* 2017). Teeth are medium brown in colour with a smooth, slightly shiny surface (Vullo *et al.* 2017; Fig.2B). The specimen is embedded in plaster of Paris. One tooth has broken off and is stuck to the plaster of Paris mount, therefore its location does not represent its life position (Fig. 4.16C). Following Vullo *et al.* (2017), anterior-posterior rows are discernible, particularly within the largest teeth; examples are two rows of mostly large teeth through which virtual sections are visualised here. Some small teeth are located in small gaps between larger teeth. A small tooth appears to ‘fit’ or tesselate with the curve of an incomplete adjacent tooth crown (Fig. 4.16A). Another tooth has a large part of its crown broken off, the point of breakage forming a curved line, possibly ‘fitting’ the curve of an adjacent tooth crown (Fig. 4.16A). Crown morphology is near conical but some crowns are flat on the top surface, likely due to wear. A shelf near the base marks a point under which the sides slope inwards creating a peg-like shape, most clearly seen in Fig. 4.16E-F. In some crowns the shelf demarcates a doughnut-like ring (cingulum) comprising the base. All crown bases are near-circle or near-oval shapes. Crowns are compressed anterior-posteriorly towards the apex, facilitating a cutting function in this derived dentition of a likely predatory species of pycnodont (Vullo *et al.* 2017). Some crown apices are bent slightly anteriorly (not apparent in the vomer), seen most clearly in Fig. 4.16E-F. Some crowns have small cusp-like bumps on surface, seen most clearly in Fig. 4.16F. These are far fewer and less pronounced than those on the crowns of the vomer. Some tooth crowns have central holes in their enameloid due to wear and some crowns have broken off completely, both reveal the hole that comprised the pulp cavity in life. Clasts of significant size from the matrix rock are not present between teeth.

XCT virtual sections of* Polygyrodus cretaceus*, left and right prearticulars and putative dentaries

XCT virtual sections of the *Polygyrodus cretaceus* left and right prearticulars and putative dentaries (NHMUK PV P 11157; Fig. 4.16B, D) show cracks are present at the base of some tooth crowns, mostly running across the whole crown base. These cracks possibly represent the location of attachment tissue in life (section 2.2.8). A small part of enameloid has broken, or chipped off a large tooth crown (Fig. 4.16B). There is a central hole in the enameloid of another large crown, due to wear (Fig. 4.16D). The pointed shape of some tooth crowns shows their compressed morphology. Some have a flat surface, likely due to wear, and due to the virtual section cutting through the doughnut-like cingulum at the base, rather than higher up the crown. Teeth are tall with pulp cavities which are long dorsal-ventrally. White indicates a high density material; white within the dentigerous bone, at the base of the central teeth in the section, is very likely iron pyrites. Uniquely among the pycnodont specimens studied, a possible successional, replacement tooth is identified (Fig. 4.16D). This is located beneath an anterior tooth, suggesting a one-for-one replacement mode at this location. It is unclear whether it would have replaced the tooth immediately above it, or the most anterior tooth, which is putatively identified as located on the left dentary (Fig. 4.16C). If the dentary tooth were replaced, this could explain why successional, one-for-one replacement may be present in this specimen and no others studied, as they do not have dentaries. Elsewhere on the specimen, teeth have developed on the surface of the dentigerous bone, and not from a crypt within it. Here, the mode of replacement is recognizable as extraosseous and a one-for-one relationship between predecessor and successor teeth cannot easily be discerned, nor can families of successional teeth be identified.

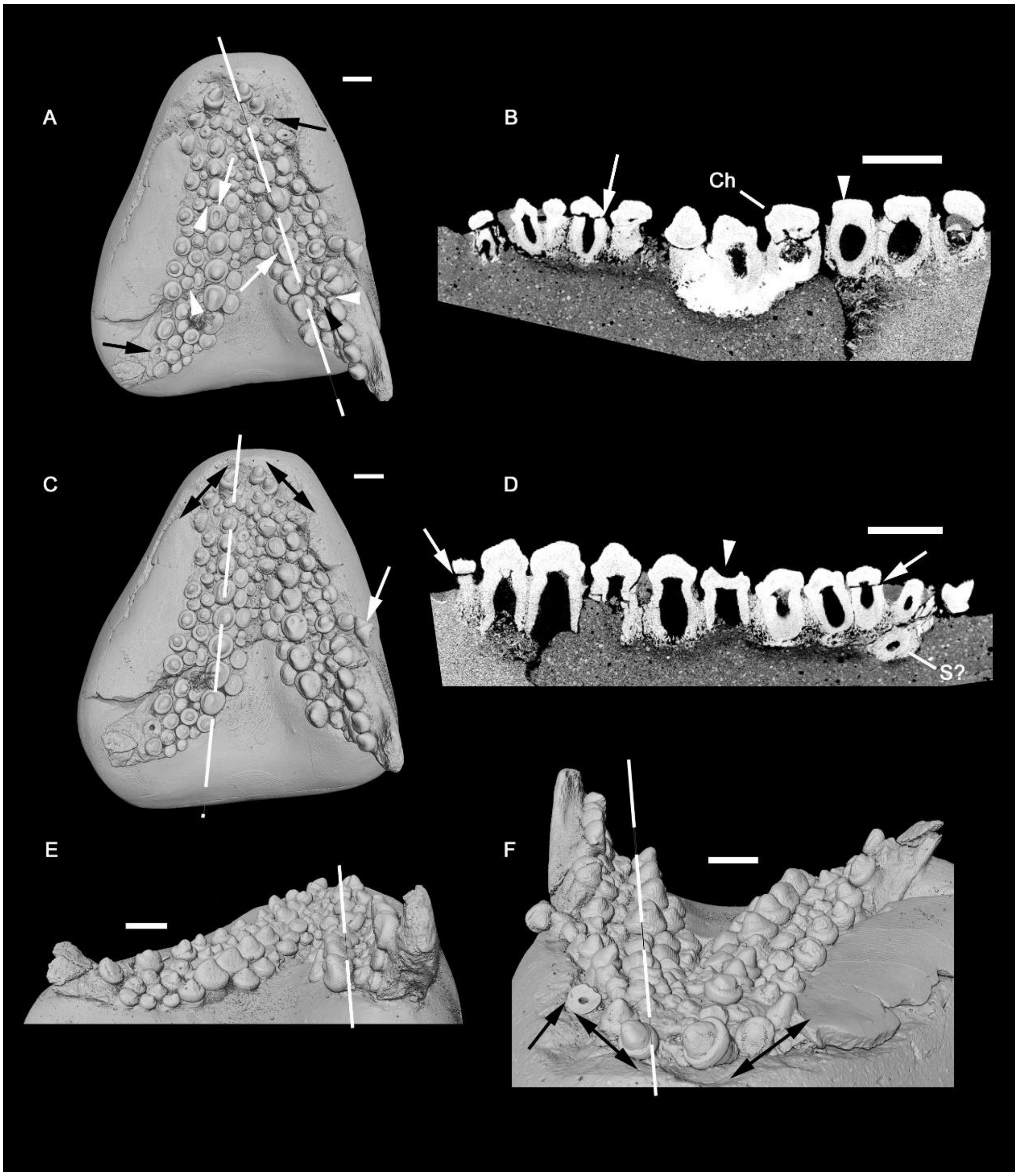


Fig. 4.16 Surface features and XCT virtual sections of *Polygyrodus cretaceus*, left and right prearticulars and putative dentaries (Woodward 1912; Vullo et al. 2017), near-complete lower dentition, NHMUK PV P 11157.

Specimen is embedded in plaster of Paris. Posterior to anterior direction is bottom to top (A, C), right to left (B) and left to right (D). A, XCT surface render of the specimen showing teeth of various sizes irregularly positioned in no clear pattern. Following Vullo et al. (2017), anterior-posterior rows are discernible, particularly within the largest teeth, examples are two rows of mostly large teeth through which virtual sections are visualised (B, D); some small teeth are located in small gaps between larger teeth (white arrowheads indicate examples); small tooth indicated by far left white arrowhead appears to ‘fit’ or tesselate with the curve of an incomplete adjacent tooth crown; black arrowhead indicates a tooth with a large part of its crown broken off, the point of breakage forming a curved line, possibly ‘fitting’ the curve of an adjacent tooth crown; crown morphology is near conical but some crowns are flat on the top surface, likely due to wear. A shelf near the base marks a point under which the sides slope inwards creating a peg-like shape, most clearly seen in views E and F. In some crowns the shelf demarcates a doughnut-like ring (cingulum) comprising the base. All crown bases are near-circle or near-oval shapes; crowns are compressed anterior-posteriorly towards the apex, facilitating a cutting function in this derived dentition of a likely predatory species of pycnodont (Vullo et al. 2017); some crown apices are bent slightly anteriorly (not apparent in the vomer), seen most clearly in views E and F; some crowns have small cusp-like bumps on surface, seen most clearly in view F. These are far fewer and less pronounced than those on the crowns of the vomer; some tooth crowns have central holes in their enameloid due to wear (white arrows indicate examples) and some crowns have broken off completely (black arrows indicate examples), both reveal the hole that comprised the pulp cavity in life; line shows the position of the virtual section in B.

B, XCT virtual section of the specimen, the position of the section B is shown by the line in A, posterior to anterior direction is right to left; cracks are present at the base of some tooth crowns (arrow indicates example), mostly running across the whole crown base; a small part of enameloid has broken, or chipped off a large tooth crown (Ch), corresponding to the crown indicated by the lower white arrow in A; pointed shape of some tooth crowns shows their compressed morphology; some tooth crowns have a flat surface, likely due to wear, and due to the virtual section cutting through the doughnut-like cingulum at the base, rather than higher up the crown (white arrowhead indicates example); teeth are tall with pulp cavities which are long dorsal-ventrally; white indicates a high density material, white within the dentigerous bone, at the base of the central teeth in the section, is very likely iron pyrites.

C, XCT surface render of the specimen; putative dentary teeth are adjacent to double-headed black arrows (Woodward 1912; Vullo et al. 2017); arrow indicates tooth which has broken off and stuck to the plaster of Paris mount, therefore its location does not represent its life position; line shows the position of the virtual section in D.

D, XCT virtual section of the specimen, the position of the section D is shown by the line in C, posterior to anterior direction is left to right; arrows indicate cracks at the base of some tooth crowns; crown morphology is as described in B; arrowhead indicates central hole in the enameloid of the crown due to wear, corresponding to tooth indicated by upper white arrow in A; possible successional, replacement tooth (S?) is located beneath an anterior tooth, suggesting a one-for-one replacement mode at this location. It is unclear whether it would have replaced the tooth immediately above it, or the most anterior tooth, which is putatively identified as located on the left dentary (see C).

E-F, XCT surface render of the specimen from the posterior (E) and anterior (F) view; crown morphology described in A is more clearly observed from these views; putative dentary teeth are adjacent to double-headed black arrows, corresponding to teeth indicated by double-headed black arrows in C; arrow indicates where tooth crown has broken off, exposing hole that comprised pulp cavity in life; line shows the position of the virtual section in B. All scale bars represent 1 cm.

4.2 Overview of surface features of fossil pycnodont specimens

4.2.1 Positioning of large teeth on the dentigerous bone

In all the studied pycnodont specimens, large teeth are consistently arranged in rows, with distinct repeated shapes. The shapes are mostly elliptical to bean-shaped, with *Anomoeodus superbus* having a slight l-shaped variation, and *Pycnodus tattami* a near-square or lozenge-shaped variation. The individual teeth become gradually smaller, and the tooth rows become closer together, as the shape of the vomer or prearticular tapers towards the anterior. This is more marked in the prearticulars, where the change in width between the posterior of the dentition and the anterior is greater than in the vomers. Only in *Polygyrodus cretaceus* is the identification of rows of large teeth unclear, though some are discernible.

4.2.2 Positioning of small teeth on the dentigerous bone

Longbottom (1984) stated that:

> ‘the phenomenon of small round irregularly-arranged teeth is common in *Pycnodus* species but rare in other genera. However, I have observed it in the vomers and spenials [prearticulars] of the genera *Gyrodus*, *Coelodus*, *Eomesodon* and *Macromesodon*’.

In the present study, small teeth are observed as not always ‘round’, in the sense of an implied circular shape, but sometimes elliptical/oval or irregularly shaped, with rounded rather than sharp contours. Excepting this detail, and with respect to small, irregularly-patterned teeth, Longbottom’s (1984) above statement is true of all pycnodont specimens studied here. These are species of the genera *Pycnodus* (five species, plus one unidentified, and one possible *Pycnodus* species), *Mesodon, Coelodus, Anomoeodus* and *Polygyrodus* (one species of each). They were selected from the NHM collection because they exhibit irregular patterning of small teeth, so that XCT analysis could be used to investigate tooth replacement where such patterning occurs. The search at NHMUK for vomers and prearticulars of pycnodont species with similar irregularly patterned small teeth was not exhaustive, and others likely exist in this and other collections. As the aim here was not to ascertain the range and extent of this phenomenon across pycnodont taxa, but to investigate mechanisms underpinning tooth replacement where irregularly patterned small teeth occurred, a sample of species was selected.

A consistent feature among all the pycnodont specimens studied here is that small, irregularly patterned teeth are positioned between large teeth in varied, seemingly unrelated locations across the specimen. These vary in number and are often located between large teeth, in gaps created by the geometry of their close alignment in rows. Their varied locations include the outer edge of prearticular bones, often with the end part of the tooth edging into a gap between teeth in the most-lateral or most-medial large tooth rows e.g. *Anomoedus superbus*, *Pycnodus tattami*. In some specimens, small teeth are located where part of a large tooth has broken off, within what would have been the outline of the original tooth. Of the specimens studied, this was observed in the vomers of *Pycnodus zeaformis* and *Pycnodus pachyrhinus,* and the prearticulars of *Pycnodus maliensis*, *Pycnodus pachyrhinus*, *Polygyrodus cretaceus*, *Coelodus mantelli* and the unidentified *Pycnodus* specimen.

Overall, it should be noted that of the pycnodont specimens studied, only the *Pycnodus zeaformis* mature vomer and the unidentified *Pycnodus* specimen are complete enough to indicate with certainty that irregular patterning of small teeth is a feature occurring at varied locations across the whole of the dentigerous bone.

In most specimens, pits are present in the dentigerous bone where whole small teeth or small tooth crowns have been lost. These present further evidence of irregular patterning of small teeth in varied locations on the dentigerous bone.

In many of the specimens studied, multiple small irregularly arranged teeth cover ‘patches’ of the dentigerous bone, located where large teeth are absent from single or multiple regularly patterned rows. This feature is present in the *Pycnodus zeaformis* mature vomer, and the prearticular specimens of *Pycnodus maliensis, Pycnodus toliapicus*, *Coelodus mantelli, Mesodon nicoleti*, an unidentified pycnodont species

(*Pycnodus?*) and an unidentified *Pycnodus* species. Such patches range from small areas relative to the specimen size in the *Mesodon nicoleti* and *Coelodus mantelli* specimens, to relatively large areas in *Pycnodus zeaformis* and *Pycnodus maliensis* specimens. In all cases, the patches are located towards the anterior of the specimen – significant for Longbottom's interpretation of tooth replacement. In all of the study specimens except *Pycnodus toliapicus*, this feature co-exists with that of small teeth positioned between large teeth in varied locations across the specimen, as described above.

In *Polygyrodus cretaceus*, large teeth, as well as small teeth are positioned in no clear pattern.

4.3 Modern fish specimens

4.3.1 Large specimen from the family Sparidae, the seabreams, upper oral jaw, BMNH 2016.9.23.1

Surface features of large sparid specimen, upper oral jaw

This specimen is an upper oral jaw of an unusually large sparid specimen (BMNH 2016.9.23.1; Fig. 4.17A (i, ii)). Teeth are a white to light brown colour, with a smooth shiny surface. The anterior teeth are long, pointed and incisiform, the remainder are rounded and molariform, providing a crushing function. The molariform teeth range in size, the largest tending to be more oval than the smaller, rounded teeth. Large teeth are arranged in rows lining the inner and outer margins of the bone, and are frequently worn flat. Smaller are teeth are positioned in between the rows of large teeth, in an irregular pattern, suggestive of close-packing. The largest teeth are located at the posterior of the inner jaw margins. One of these largest teeth is cracked with small pieces broken off its crown. Underneath it, two small teeth are developing, or have erupted, visible within the large tooth crown. One is more centrally located under the large, broken tooth than the other, which is located at the posterior edge.

XCT virtual sections of large sparid specimen, upper oral jaw

XCT virtual sections of the large sparid specimen, upper oral jaw (BMNH 2016.9.23.1; Fig. 4.17B-D) show that teeth appear to be following an orderly replacement process, with successor teeth positioned beneath functional predecessors. The replacement relationship is one-for-one, although not all functional teeth have successors in waiting. The two most posterior teeth on the right side of the jaw, which are also the largest, are pointing towards the anterior, unlike all other teeth (Fig. 4.17C). A large tooth has a large hole in its surface, and a smaller tooth is developing, or appears to have erupted, within its crown (Fig. 4.17). This small tooth corresponds to the tooth indicated by the arrow in Fig. 4.17A (i). The successor teeth are each developing in a crypt within the dentigerous bone, therefore the mode of replacement is recognizable as intraosseous. Tooth implantation is thecodont, and attachment type appears pedicellate, due to a slightly raised bone of attachment (the pedicel) and a very thin gap between tooth crown and bone of attachment, which would have accommodated fibrous tissue.

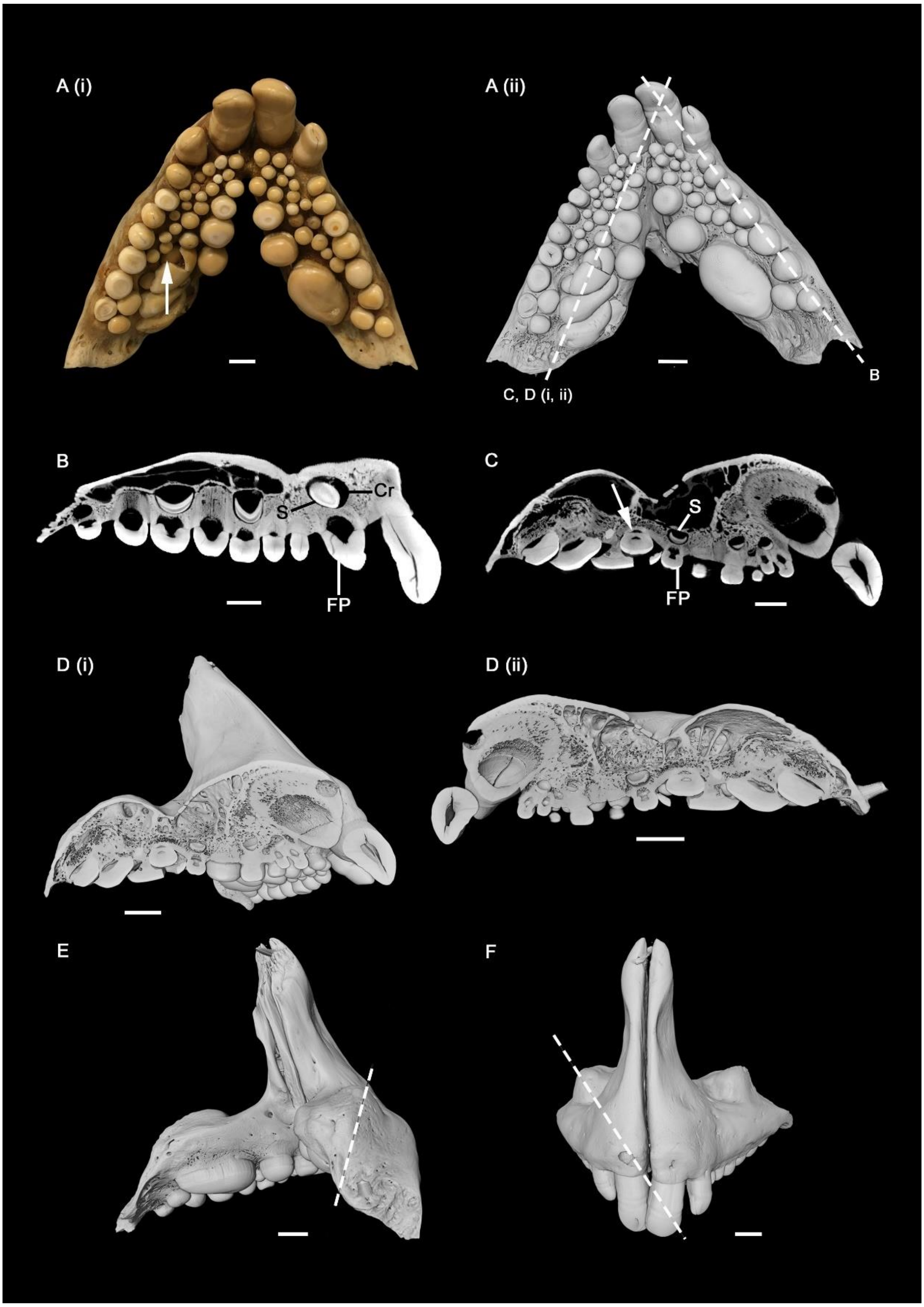


Fig. 4.17 Surface features and XCT virtual sections of sparid upper oral jaw, BMNH 2016.9.23.1.

A (i), photograph of the specimen, posterior to anterior direction is bottom to top; arrow indicates a small tooth developing within a larger broken tooth. A second small tooth is just visible posteriorly, also positioned underneath the same large tooth. A (ii), XCT surface render of the specimen, posterior to anterior direction is bottom to top; lines show the position of the virtual sections in B-D. B, XCT virtual section along the corresponding line shown in A (ii), posterior to anterior direction is left to right; tooth replacement process appears orderly and one-for-one, though not all functional teeth have successors in waiting; successional teeth are positioned beneath functional predecessors. C, XCT virtual section along the corresponding line shown in A (ii), posterior to anterior direction is left to right; arrow indicates a small tooth has erupted within the crown of a larger broken tooth, corresponding to tooth indicated by arrow in A (i); tooth replacement process appears orderly and one-for-one, though not all functional teeth have successors in waiting; successional teeth are positioned beneath functional predecessors. D, XCT virtual clipped section views along the corresponding line shown in A (ii), view from medial side (i), posterior to anterior direction is left to right, and lateral side (ii), posterior to anterior direction is right to left; 3D structure of pulp cavities and crypts containing developing successional teeth is shown; 3D structure of broken tooth crown containing a smaller tooth is shown. E-F, XCT surface render of the specimen from the posterior (E) and anterior (F) view; line shows the position of the virtual sections in C, D. *Abbreviations*: Cr, crypt; FP, functional predecessor tooth; S, successional tooth. All scale bars represent 1 cm.

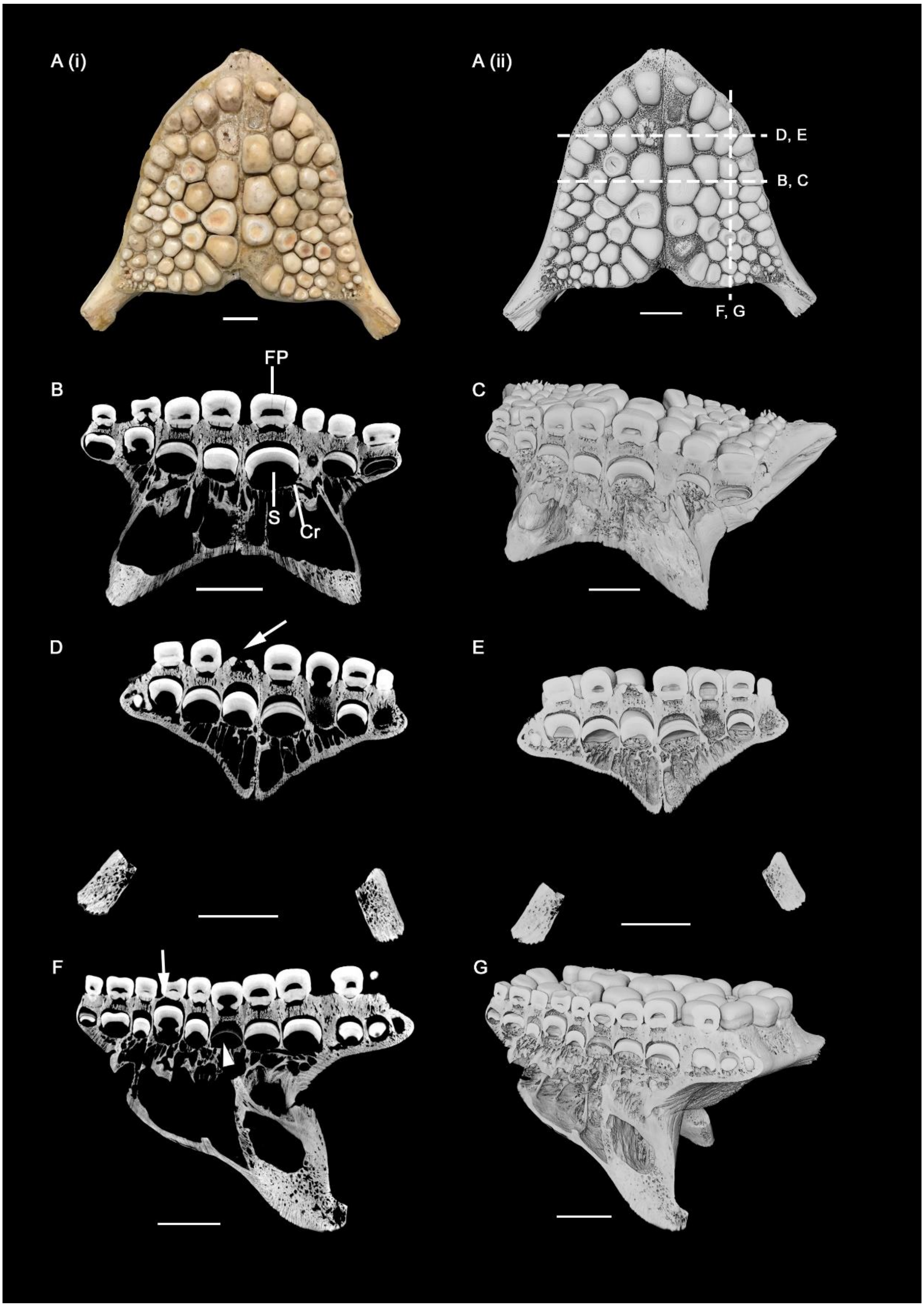
4.3.2 *Pogonias cromis*, black drum, lower pharyngeal jaw, BMNH 2019.11.8.1

Surface features of **Pogonias cromis***, black drum, lower pharyngeal jaw*

This specimen is a lower pharyngeal jaw of *Pogonias cromis*, black drum (BMNH 2019.11.8.1; Fig. 4.18A (i, ii)). Teeth are a white to light brown colour, with shiny surface. Their surface is slightly textured so that is not completely smooth, likely bevelled by the action of crushing mollusc shells. Teeth are molariform, but rather than being rounded, they are polygonal in shape. They are irregulary patterned in an arrangement that suggests close-packing. The polygonal tooth shapes are tessellated with each other, often very closely and almost touching, and occasionally touching. Shapes tend towards 5- and 6-sided shapes, and teeth become larger, towards the centre of the jaw. Teeth grade towards being particularly small and more rounded towards the two corners where the lateral and posterior edges of the crushing surface meet. Here, teeth are extremely small and some have been lost. Three of the largest teeth are lost, adjacent to the anterior-posterior midline. A relatively small, rounded tooth is lost from both left and right posterior regions, both close to the edge of the crushing surface.

XCT virtual sections of **Pogonias cromis***, black drum, lower pharyngeal jaw*

XCT virtual sections of the *Pogonias cromis*, lower pharyngeal jaw (BMNH 2019.11.8.; Fig. 4.18B–G) show that teeth appear to be following an orderly replacement process, with successor teeth positioned beneath functional predecessors. The replacement relationship is one-for-one, although not all functional teeth have successors in waiting. Successor teeth teeth are at varying stages of development in relation to each other, revealed by the varying thicknesses of enmaloid and dentine, and the absence of some successor and functional teeth. This indicates the timings of the replacement cycles vary between tooth positions, possibly ensuring an ongoing close-packed patterning of teeth. The successor teeth are each developing in a crypt within the dentigerous bone, therefore the mode of replacement is recognizable as intraosseous. A functional tooth crown has been lost, beneath which remodelling of the crypt walls has created a relatively large space for upward movement of the successional tooth (Fig. 4.18D-E). The bone above a successional tooth has remodelled and mostly broken down, directly beneath a functional tooth. This allows space for upward movement of the successional tooth as it erupts and replaces its predecessor (Fig. 4.18F-G). Tooth implantation is thecodont, and attachment appears to be ankylosed, as crowns appear to be in direct contact with the bone of attachment.


A (i)
A (ii)
D, E
B, C
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FP
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S
Cr
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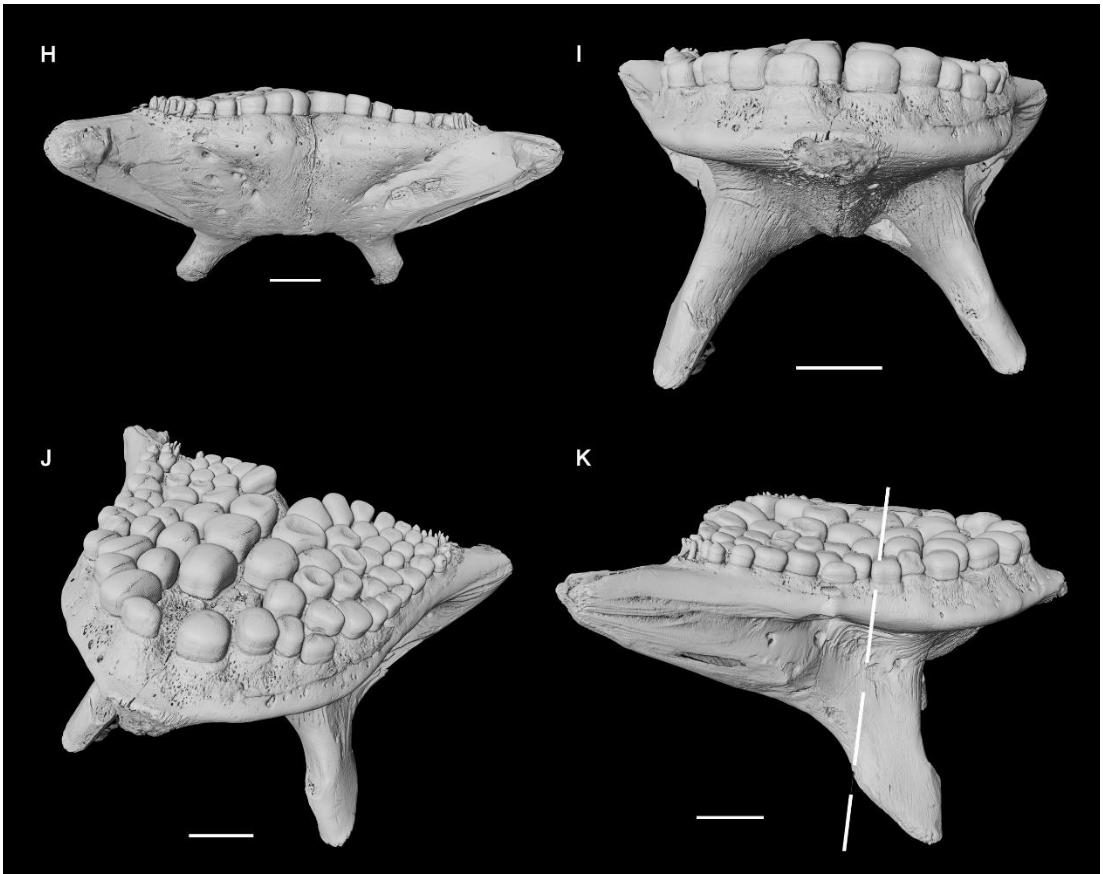


Fig. 4.18 Surface features and XCT virtual sections of *Pogonias cromis*, black drum, lower pharyngeal jaw, BMNH 2019.11.8.1.

A (i), photograph of the specimen, posterior to anterior direction is bottom to top (Sasaki 1989*b*; Grubich 2003). A (ii), XCT surface render of the specimen, posterior to anterior direction is bottom to top; lines show the position of the virtual sections in B-G. B, XCT virtual section along the corresponding line shown in A (ii), tooth replacement process appears orderly and one-for-one; successional teeth are positioned beneath functional predecessors. C, XCT virtual clipped section along the corresponding line shown in A (ii), view from anterior side (image has been flipped horizontally); 3D structure of pulp cavities and crypts containing developing successional teeth is shown. D, XCT virtual section along the corresponding line shown in A (ii), tooth replacement process appears orderly and one-for-one, though not all functional teeth have successors in waiting; arrow indicates location where a functional tooth crown has been lost, beneath which remodelling of the crypt walls has created a relatively large space for upward movement of the successional tooth. E, XCT virtual clipped section along the corresponding line shown in A (ii), view from posterior side; 3D structure of pulp cavities and crypts containing developing successional teeth is shown. F, XCT longitudinal virtual section along the corresponding line shown in A (ii), view from lateral side, posterior to anterior direction is left to right; tooth replacement process appears orderly and one-for-one; successional teeth are positioned beneath functional predecessors; arrow indicates point at which the wall of a crypt containing a successional tooth meets the functional tooth, the wall having remodelled to allow the successional tooth to move upwards; arrowhead indicates successional tooth in early stages of development with little enamel and dentine formed. G, XCT longitudinal virtual clipped section along the corresponding line shown in A (ii), view from lateral side, posterior to anterior direction is left to right; 3D structure of pulp cavities and crypts containing developing successional teeth is shown. H-I, XCT surface render of the specimen from the posterior (H) and anterior (I) view. J, XCT surface render of the specimen from the anterior and left view. K, XCT surface render of the specimen, view from right lateral side, posterior to anterior direction is left to right; line shows the position of the virtual sections in B, C. *Abbreviations*: Cr, crypt; FP, functional predecessor tooth; S, successional tooth. All scale bars represent 1 cm.

4.3.3 *Labrus bergylta*, ballan wrasse, lower pharyngeal jaw, BMNH 2017.5.22.1

Surface features of **Labrus bergylta***, ballan wrasse, lower pharyngeal jaw*

This specimen is a lower pharyngeal jaw of *Labrus bergylta*, ballan wrasse (BMNH 2017.5.22.1; Fig. 4.19A (i, ii)). Teeth are white with a smooth, shiny surface, and are positioned in an irregular pattern suggestive of close-packing. Teeth are rounded to near-circular in shape and gradually become larger from the outer edges towards the centre of the jaw. The combination of tooth shape and patterning likely facilitate a crushing function. Pits remain where whole teeth/and or tooth crowns have been lost, all at outermost tooth positions. Erupting teeth are visible below or at the surface of the bone (Fig. 4.19A (i, ii), G), each in the process of leaving the crypt through its eruption channel.

XCT virtual sections of **Labrus bergylta***, ballan wrasse, lower pharyngeal jaw*

XCT virtual sections of the *Labrus bergylta*, ballan wrasse, lower pharyngeal jaw (BMNH 2017.5.22.1; Fig. 4.19 B-E) show that many successor teeth appear to not be positioned directly under predecessor teeth, but below and adjacent to, therefore it is not clear which successor relates to which predecessor. This indicates the replacement cycle is at varying stages at different tooth positions. Functional predecessor teeth have likely already been shed directly above some successional teeth, which are in the process of moving into the resulting space. Successional teeth are absent below some functional teeth, though possibly not yet sufficiently developed to be visualised by XCT. A one-for-one replacement relationship is therefore less clearly discerned than in the Sparid and *Pogonias cromis* study specimens. However, the replacement process appears orderly and a one-for-one replacement relationship is likely. Some teeth are shown to be in the final stages of eruption with no bone above them (Fig. 4.19 B-C), corresponding to teeth visible below the bone surface, in the surface views (Fig. 4.19 A (i, ii) and G).

A virtual section plane has clipped the edge of a successional tooth, located adjacent to a functional tooth and its successor (Fig. 4.19 D-E). Its intermediary location vertically between them indicates a different tooth cycle timing between the two adjacent tooth positions. A space on the jawbone is visible from the surface view, in the location where this developing tooth (clipped by the virtual section) will erupt. This may represent a complementary, optimal replacement timing between adjacent tooth positions, which facilitates the ongoing close-packed patterning of teeth.

As the successor teeth are each developing in a crypt within the dentigerous bone, the mode of replacement is recognizable as intraosseous. Tooth implantation is thecodont, and attachment appears to be ankylosed, as crowns appear to be in direct contact with the bone of attachment.

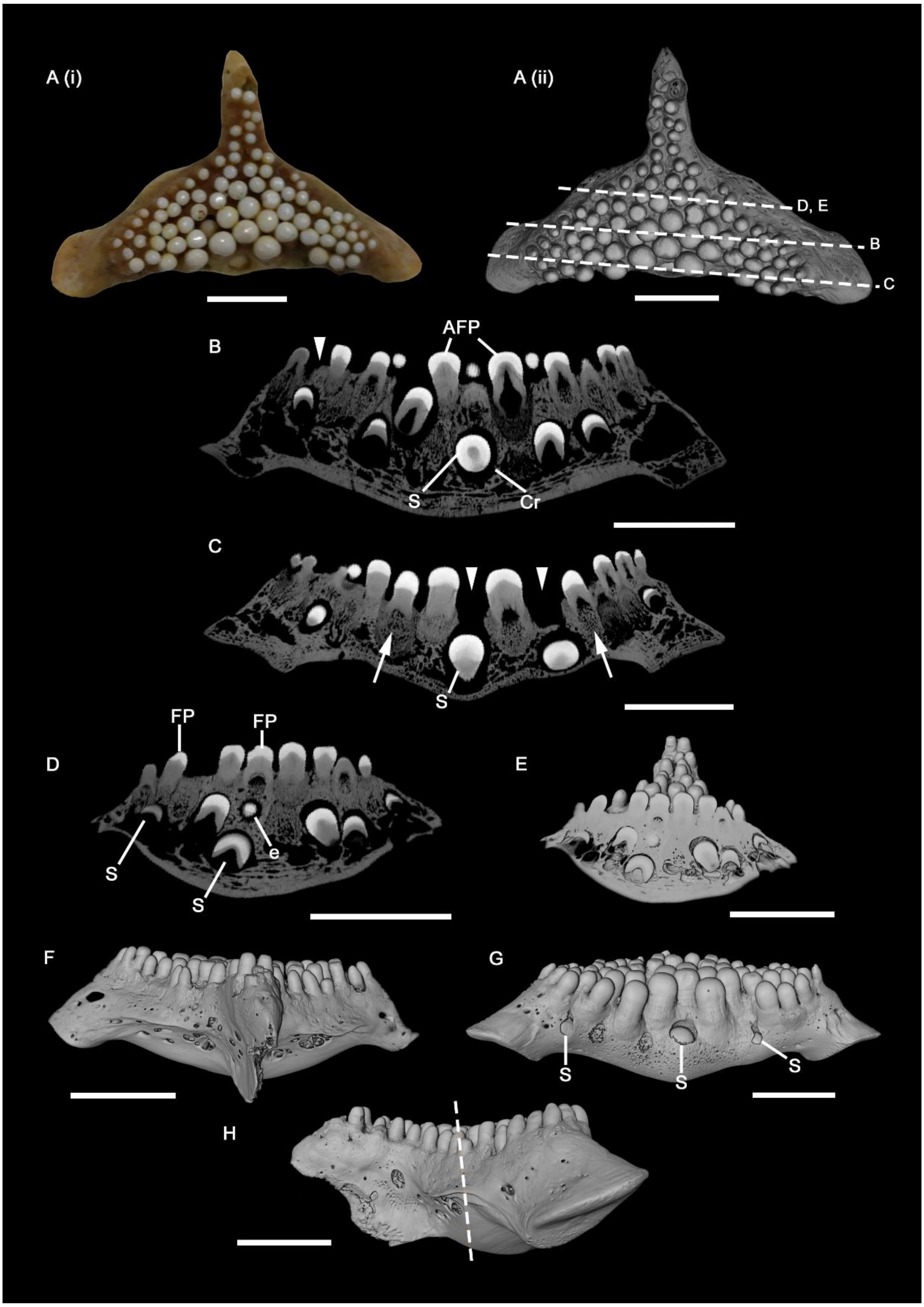


Fig. 4.19 Surface features and XCT virtual sections of *Labrus bergylta*, ballan wrasse, lower pharyngeal jaw, BMNH 2017.5.22.1.

A, photograph (i) and XCT surface render (ii) of the specimen, posterior to anterior direction is bottom to top (Evans *et al.* 2019); lines in A (ii) show the position of the virtual sections in B-E. B-D, XCT virtual sections along the corresponding lines shown in A (ii), view from posterior side; tooth replacement process appears orderly, a one-for-one replacement relationship is likely but less clear than in the Sparid and *Pogonias cromis* specimens, with the replacement cycle at varying stages at different tooth positions; functional predecessor teeth may have already been shed directly above some successional teeth, which are in the process of moving into the resulting space (arrowheads indicate example locations); successional teeth are absent below some functional teeth, though possibly not yet sufficiently developed to be visualised by XCT (arrows indicate example locations); the section plane has clipped the edge (e) of a successional tooth located in an adjacent tooth position to a functional tooth and its successor, appearing between them on the section. E, XCT virtual clipped section along the corresponding line shown in A (ii), view from posterior side; 3D structure of pulp cavities and crypts containing developing successional teeth is shown. F-G, XCT surface render of the specimen from the anterior (F) and posterior (G) view; successional teeth are visible from the surface in G, corresponding to successional teeth in C. Middle tooth labelled S in G corresponds to tooth labelled S in C. H, XCT surface render of the specimen from the from the left lateral view, posterior to anterior direction is right to left; line shows the position of the virtual section in D, E. *Abbreviations*: AFP, adjacent functional predecessor tooth; Cr, crypt; FP, functional predecessor tooth; S, successional tooth. All scale bars represent 1 cm.

4.3.4 *Archosargus probatocephalus* (family Sparidae), sheepshead fish, left upper oral jaw, BMNH2020.8.17.2.

Surface features of **Archosargus probatocephalus***, sheepshead fish, left upper oral jaw*

This specimen is a left upper oral jaw bone of a sparid, *Archosargus probatocephalus*, sheepshead fish (BMNH2020.8.17.2; Fig. 4.20A). Teeth are a yellowish white to light grey colour, with a smooth, shiny surface. Teeth on the anterior-most margin are long, chisel-shaped and incisiform. These have short, rounded, pointed incisiform teeth located immediately behind them. The remainder of the teeth on the bone are molariform, providing a crushing function. They range in size and shape, from smaller rounded teeth to larger, slightly elongated ovoid teeth. Although rounded/ovoid, each molariform tooth crown is slightly pointed/conical, with the tip of the point located centrally. Two molariform teeth located on the inner margin of the bone have lost this pointed tip, as the top of the crown has been worn flat. Larger teeth are arranged in rows lining the inner and outer margins of the bone, and become gradually larger towards the posterior. There is one exception however as one small round tooth is located at the most posterior region of the outer margin, not quite in line with the large tooth row. Three small round teeth, also smaller than other teeth in their row, are positioned in the equivalent location on the opposite, right side the jaw (bone is not figured here). Teeth generally smaller than the large teeth lining both left and right margins of the bone in rows, are postioned between these rows, in an irregular pattern suggestive of close-packing. Two teeth are located in very small gaps between neighbouring teeth, appearing ‘squeezed’ between them. This may represent a gapfilling tooth positioning.

XCT virtual sections of **Archosargus probatocephalus*****, sheepshead fish, left upper oral jaw***

XCT virtual sections of *Archosargus probatocephalus*, sheepshead fish, left upper oral jaw (BMNH2020.8.17.2; Fig. 4.20B-D) show that teeth appear to be following an orderly replacement process, with successor teeth positioned beneath functional predecessors. The replacement relationship is one-for-one, although not all functional teeth have successors in waiting. This is more frequently the case than a successor in waiting being present, possibly reflecting a long replacement cycle, and high durability of the functional teeth. A large successional tooth at the most posterior position is pointing towards the anterior, unlike all other teeth (Fig. 4.20B), as seen in the large sparid specimen. Small teeth are identified which are visible in the surface view as located in very small gaps between their neighbours (Fig. 4.20B). The successor teeth are each developing in a crypt within the dentigerous bone, therefore the mode of replacement is recognizable as intraosseous. Tooth implantation is thecodont, and attachment type is pedicellate, due to a slightly raised bone of attachment (the pedicel) and a very thin gap between tooth crown and bone of attachment, which would have accommodated fibrous tissue. The posterior-most functional teeth in both XCT virtual sections appear to not have a fully formed pedicel (as described in section 2.2.8). This could be due to eruption and attachment not yet being completed. Alternatively, it is possible that a different type of attachment will form at these positions.

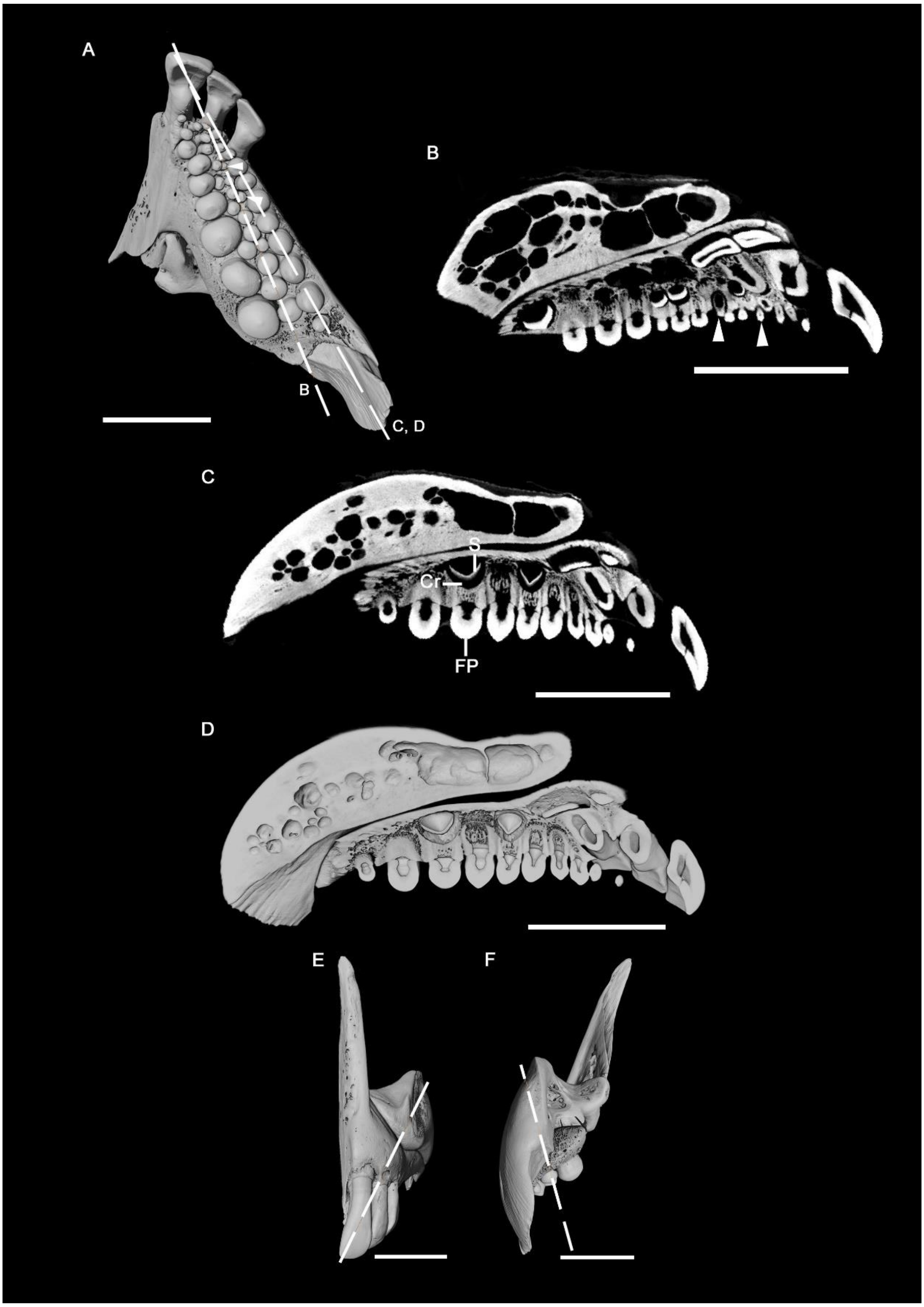


Fig. 4.20 Surface features and XCT virtual sections of *Archosargus probatocephalus* (family Sparidae), sheepshead fish, left upper oral jaw, BMNH2020.8.17.2.

A, XCT surface render of the specimen, posterior to anterior direction is bottom to top; arrowheads indicate small teeth which appear squeezed between two neighbouring teeth, possibly representing gap-filling; lines show the position of the virtual sections in B-D. B, XCT virtual section along the corresponding line shown in A, posterior to anterior direction is left to right; tooth replacement process appears generally orderly and one-for-one, though not all functional teeth have successors in waiting; successional teeth are positioned beneath functional predecessors, except at the most posterior tooth position where no functional tooth is present; arrowheads indicate small, possible gap-filling teeth corresponding to teeth indicated by arrowheads in A. C, XCT virtual section along the corresponding line shown in A, posterior to anterior direction is left to right; tooth replacement process appears orderly and one-for-one, though not all functional teeth have successors in waiting; successional teeth are positioned beneath functional predecessors. D, XCT virtual clipped section along the corresponding line shown in A, posterior to anterior direction is left to right, view from medial side; 3D structure of pulp cavities and of crypts containing developing successional teeth is shown. E-F, XCT surface render of the specimen from the (E) anterior and (F) posterior view; line shows the position of the virtual section in B. *Abbreviations*: FP, functional predecessor tooth; S, successional tooth; Cr, crypt. All scale bars represent 1 cm.

4.3.5 Specimens of three species from the family Sparidae, the seabreams (Fig. 4.21).

Fig. 4.21A-B: *Pagrus auratus* (synonym *Chrysophrys auratus*), silver seabream or Australasian snapper, articulated left upper and lower oral jaws, BMNH 2020.11.2.1

Surface features of* Pagrus auratus*, silver seabream, articulated left upper and lower oral jaws (left upper jaw only described).

Teeth are a grey/white to light brown colour, with a smooth, shiny surface. There are three, large pointed grasping teeth at the anterior of the jaw. The remainder of the teeth on the bone are molariform, providing a crushing function. There is one very large molariform tooth at the posterior-most position, on the inner margin of the bone. All other molariform teeth range from approximately 1/8th of the size of this tooth, and smaller. Among these, the larger teeth line the outer and inner margins of the bone, and positions immediately adjacent to, and surrounding the very large molariform tooth. One tooth in the row lining the inner margin appears to have its successor exposed where the two halves of the jaw were joined. In between the large teeth, smaller teeth are irregularly positioned in a pattern suggestive of close-packing. These generally get smaller towards the anterior of the bone. All teeth are rounded to ovoid, with the crowns of larger teeth on the outermost margin slightly pointed/conical, with the tip of the point located centrally.

XCT virtual section of **Pagrus auratus***, silver seabream, articulated left upper and lower oral jaws*

XCT virtual sections of the specimen show that teeth appear to be following an orderly replacement process, with successor teeth positioned beneath functional predecessors. The replacement relationship is one-for-one, although not all functional teeth have successors in waiting. As seen in the *Archosargus probatocephalus*, the absence of a successor visible beneath a functional tooth is more frequent that the presence of one**,** possibly reflecting a long replacement cycle, and high durability of the functional teeth. In the lower jaw, a functional predecessor tooth has been shed above a successional tooth, which is in the process of moving into the resulting space. The successor teeth are each developing in a crypt within the dentigerous bone, therefore the mode of replacement is recognizable as intraosseous. Tooth implantation is thecodont. Attachment type is pedicellate, which is particularly identifiable here, as the very thin gap between tooth crown and bone of attachment is clearly visualised. This contains fibrous tissue in life. The slightly raised bone of attachment (the pedicel) is less pronounced in the anterior incisors, suggesting direct fibrous attachment may be present at these locations, rather than pedicellate (see section 2.2.8).

Fig. 4.21C-F: *Calamus leucosteus,* whitebone porgy, lower oral jaw and left upper oral jaw, BMNH 2010.12.18.29:

Fig. 4.21C: *Surface features of* Calamus leucosteus*, whitebone porgy, lower oral jaw*

Teeth are a white to light yellow colour, with a shiny surface. Their surface is slightly textured so that is not completely smooth, likely bevelled by the action of crushing mollusc shells. Several small, round, pointed, grasping teeth are present at the anterior-most region of the right half of the jaw. These include two teeth approximately three times the height and volume of the others. All but one tooth position bears a functional tooth. Contrastingly, at the equivalent region on the left half of the jaw, all but one tooth position is empty. It is not known how many teeth were lost in life or post-mortem. At the empty anterior tooth positions, the pedicels and/or the bone separating the eruption channels between teeth, tessellate with each other, forming polygonal shapes. The remainder of the teeth on both the left and right bones are molariform, providing a crushing function. They form two rows adjacent to each other, running the length of each jaw bone. Unusually among the sparid study specimens presented herein, no small teeth are located between these rows. The teeth are round to ovoid-shape, crowns on the outer margin of both left and right bones are slightly pointed/conical, with the tip of the point located centrally. Teeth become gradually larger towards the posterior of both left and right bones, except for the presence of some small molariform, empty tooth positions, located in the lateral/posterior-most corners of each. Nearly half the molariform tooth positions are empty. At these empty positions, the pedicels seem to loosely accommodate each other's shapes, but not quite tesselate. The overall impression is that the molariform teeth are positioned in a manner that is optimally using space on the bone, and closely-packed.

Fig. 4.21D: ***XCT virtual section of*** **Calamus leucosteus,** ***whitebone porgy, left lower oral jaw***

An XCT virtual section of the specimen shows that teeth appear to be following an orderly replacement process, with successor teeth positioned beneath functional predecessors. The replacement relationship is one-for-one although two functional teeth do not have successors in waiting, two developing teeth do not have a functional predecessor above them and three tooth positions have neither successional or functional predecessor teeth. This represents variation in the tooth replacement cycles between different tooth positions. The most anterior successional molariform tooth is at the point of eruption, with no bone above it. Pedicels are visible with and without teeth attached, the latter correspond to the pedicels indicated by the two far right arrows in Fig. 4.21C.The narrow gaps between the tooth crowns and pedicels are visible, which contain fibrous tissue in life.

Fig. 4.21E: ***Surface features of*** **Calamus leucosteus,** ***whitebone porgy, left upper oral jaw***

As with the lower jaw, teeth are a white to light yellow colour, with a shiny surface. Their surface is slightly textured so that is not completely smooth, likely bevelled by the action of crushing mollusc shells. Several round, pointed, grasping teeth are present at the anterior-most region of the jaw. The outer row are approximately three times the height and volume of the others. Several of the tooth positions are empty among the pointed, grasping teeth. As observed in the lower jaw, at these empty positions, the pedicels and/or the bone separating the eruption channels between teeth tessellate with each other, forming polygonal shapes.

The remainder of the teeth on the bone are molariform. A row of these line in inner and outer margins of the jaw, and exhibit the same range of tooth shapes as the equivalent rows of the lower oral jaw. Unlike the lower jaw, small molariform teeth are located between these rows, in an irregular pattern suggestive of close-packing. As on the lower jaw, molariform teeth are missing, revealing the shape of their pedicels. Most of these empty pedicels are tessellating, whereas on the lower jaw they appear to loosely accommodate each other’s shapes, but not quite tesselate. This difference is likely due to the closer-packed arrangement of teeth on the upper jaw. As with the lower jaw, the overall impression is that the molariform teeth are positioned in a manner that is optimally using space on the bone, and closely-packed.

Fig. 4.21F: ***XCT virtual section of*** **Calamus leucosteus,** ***whitebone porgy, left upper oral jaw***

XCT virtual section of the specimen shows that the orderliness of tooth replacement process is difficult to discern, as many functional teeth are lost, and many functional teeth do not have successors in waiting. Unusally, two crypts appear to have fused, each containing developing a successional tooth. Overall for the upper and lower jaws of this specimen, the XCT virtual sections have shown that successor teeth are each developing in a crypt within the dentigerous bone, therefore the mode of replacement is recognizable as intraosseous. Tooth implantation is thecodont, and the attachment type is pedicellate.

Fig. 4.21G-J: ***Sparus aurata*****, gilthead seabream, lower oral jaw, BMNH 2020.11.2.2**

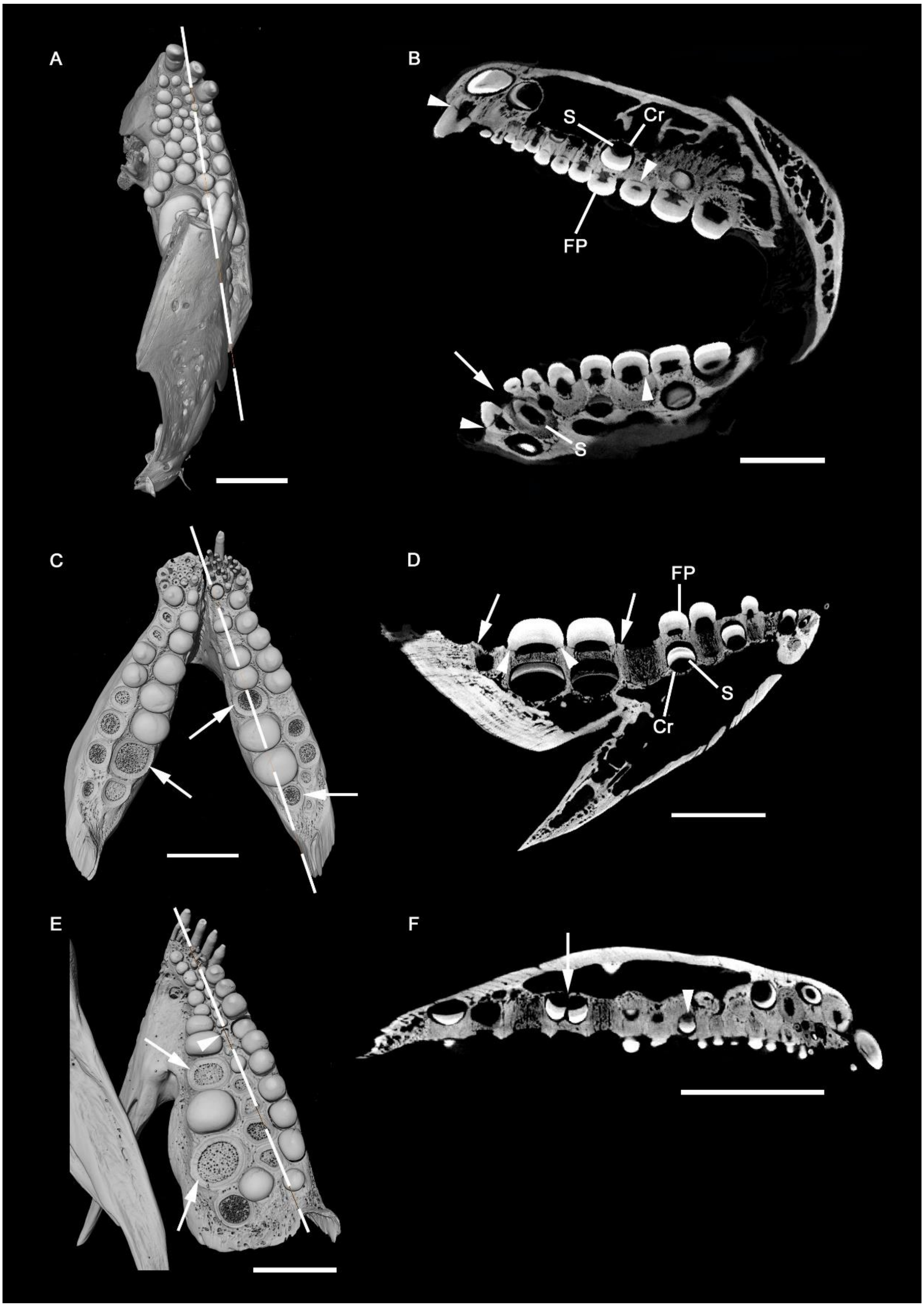
Fig. 4.21G: ***Surface features of*** **Sparus aurata,** ***gilthead seabream, lower oral*** **jaw**

Teeth are a white to light yellow colour, with a shiny, smooth surface. The anterior-most teeth on the outer margin are a rounded but with a pointed, chisel-like tip. Immediately behind these are small, pointed grasping teeth. The remainder of the teeth are rounded and molariform, providing a crushing function. The molariform teeth range in size, the largest tending to be more oval than the smaller, rounded teeth. The largest molariform teeth are at the posterior of the jaw and have an irregular, slightly curved kidney shape. Medium-sized molariform teeth line the outer margins. The smallest molariform teeth are located at the anterior, these are also the most irregularly patterned. Molariform teeth larger than these are generally positioned in discernible rows. Unusually, a tooth seems to be in the process of replacing a predecessor tooth which is comparatively much smaller, as revealed by the small empty pedicel above it (Fig. 4.21G).

Fig. 4.21H-J: ***XCT virtual sections of*** **Sparus aurata,** ***gilthead seabream, lower oral*** **jaw**

XCT virtual section of the specimen shows that a one-for-one replacement is likely, as two successional teeth are aligned under functional predecessors. However, three functional teeth do not have successors in waiting. Unusally, two successional teeth are comparatively much larger than their functional predecessors (Fig. 4.21H), with the views in Fig. 4.21 I-J supporting this. Also unusally, a serial addition of large teeth at the posterior-most region, rather than tooth replacement, is suggested by three teeth positioned in decreasing stages of development towards the bone surface (three teeth indicated by double-headed arrow in Fig. 4.21H). The most developed of these is also one of the two teeth that appears to be a much larger successor compared to its apparent functional predecessor (Fig. 4.21 H-J). It is therefore possible that it may not have a direct successional relationship with the functional tooth aligned above it, but rather be part of a mechanism of serial addition.

As the successor teeth are each developing in a crypt within the dentigerous bone, the mode of replacement is recognizable as intraosseous. Tooth implantation is thecodont, and attachment type is pedicellate.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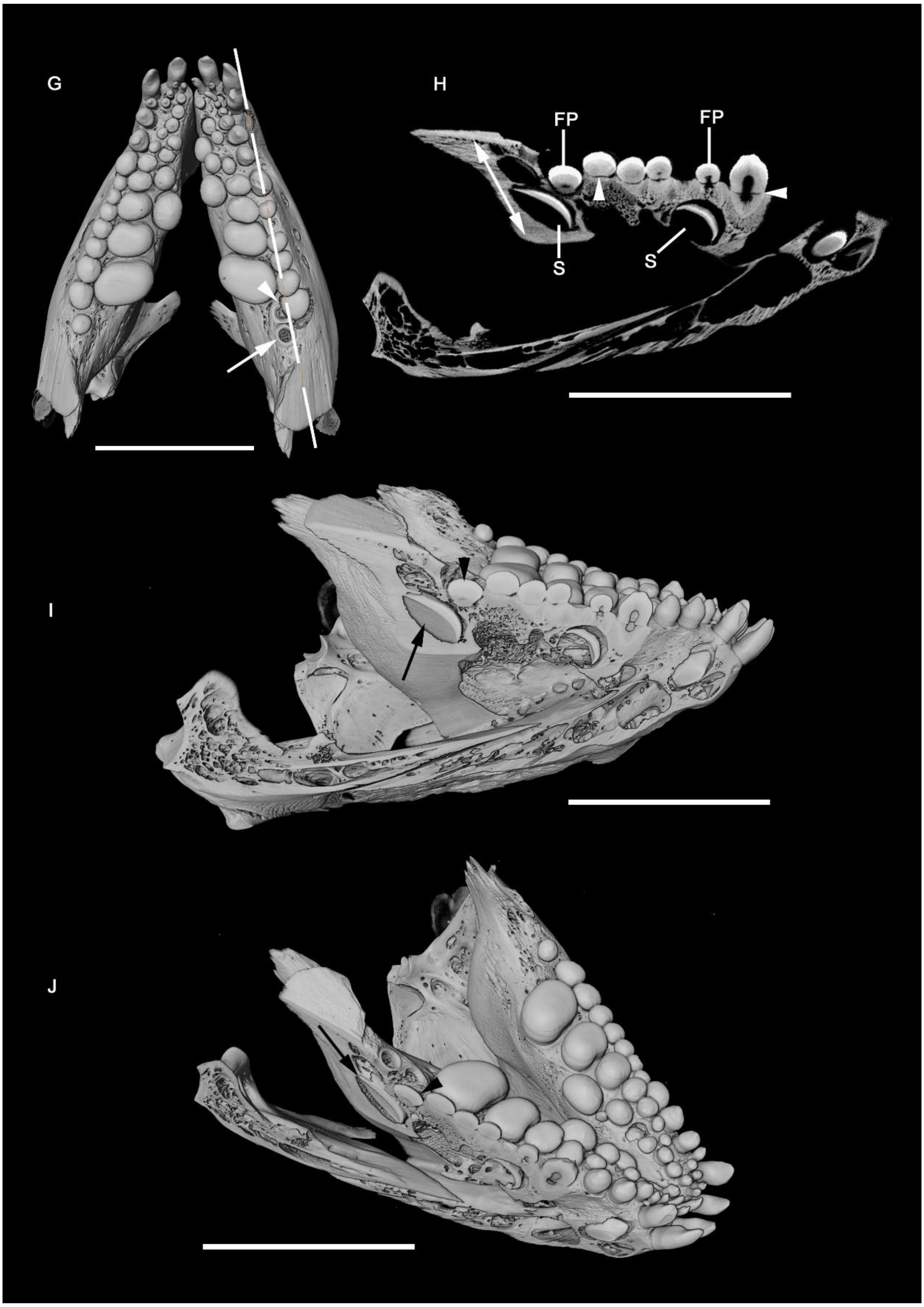


Fig. 4.21 Specimen surface features and XCT virtual sections of three species of sparid, the seabreams.

A-B, *Pagrus auratus* (synonym *Chrysophrys auratus*), silver seabream or Australasian snapper, articulated left upper and lower oral jaws, BMNH 2020.11.2.1:

A, XCT surface render of the specimen, posterior to anterior direction is bottom to top, left upper jaw partly visible with left lower jaw in foreground, ventral view; line shows the position of the virtual section in B;

B, XCT virtual section along the corresponding line shown in A, posterior to anterior direction is right to left; tooth replacement process appears generally orderly and one-for-one, though many functional teeth do not have successors in waiting, possibly these are yet to develop; successional teeth are positioned beneath functional predecessors; a functional predecessor tooth has been shed above a successional tooth, which is in the process of moving into the resulting space (white arrow indicates location); the pedicellate attachment is clearly visible in this specimen, as a narrow gap between the base of the tooth crown (arrowheads indicate examples), and the bone of attachment, slightly raised from the jawbone surface – the ‘pedicel’. The narrow gap contains fibrous tissue in life. This is distinguishable from a crack due to damage, as it occurs at a consistent location at the base of tooth crowns.

C-F, *Calamus leucosteus*, whitebone porgy, lower oral jaw and left upper oral jaw, BMNH 2010.12.18.29:

C, XCT surface render of the lower oral jaw, posterior to anterior direction is bottom to top; many tooth positions are empty, it is unclear how many teeth were lost in life or post-mortem; empty pedicels are clearly visible, on which the lost tooth had been supported and attached (arrows indicate examples, see D); nearly all small, pointed teeth are lost from the left anterior-most region of the jaw, in contrast to the right where most tooth positions are filled; pedicels and bone between the empty small tooth eruption channels are tessellated with each other; pedicels of adjacent molariform tooth positions do not quite touch each other, but seem to accommodate each other’s shapes; line shows the position of the virtual section in D;

D, XCT virtual section along the corresponding line shown in C, posterior to anterior direction is left to right; tooth replacement process appears generally orderly and one-for-one, although two functional teeth do not have successors in waiting; two developing teeth do not have a functional predecessor above them; three tooth positions have neither successional or functional predecessor teeth; the pedicellate attachment is visible as a narrow gap between the base of the tooth crowns and the pedicels (arrowheads indicate examples). The narrow gaps contain fibrous tissue in life. The pedicel comprises a cylindrical bone of attachment, on which the tooth is supported and attached via the fibrous tissue (arrows indicate examples, both of which correspond to pedicels indicated by middle and far right arrows in C).

E, XCT surface render of the left upper oral jaw, posterior to anterior direction is bottom to top; many pedicels are empty (arrows indicate examples); molariform tooth pedicels are close-packed and tessellating; arrowhead indicates a relatively small tooth erupting through the surface of the bone;

F, XCT virtual section along the corresponding line shown in E, posterior to anterior direction is left to right; orderliness of tooth replacement process is difficult to discern at the posterior half of the section as all functional teeth are lost; most small functional teeth at the anterior half of the section do not have successors in waiting; arrowhead indicates relatively small tooth erupting through the surface of the bone, corresponding to tooth indicated by arrowhead in E; arrow indicates two crypts which appear to have fused, each containing developing a successional tooth.

G-J, *Sparus aurata*, gilthead seabream, lower oral jaw, BMNH 2020.11.2.2:

G, XCT surface render of the specimen, posterior to anterior direction is bottom to top; arrowhead indicates a tooth which appears to be replacing a predecessor tooth which is comparatively much smaller, as revealed by small empty pedicels (arrow indicates example); line shows the position of the virtual section in H-J;

H, XCT virtual section along the corresponding line shown in G, posterior to anterior direction is left to right; orderly one-for-one tooth replacement is likely, but not clearly evident in this section due to a relative lack of successors; two successional teeth (labelled) are comparatively much larger than their functional predecessors (labelled); addition of large teeth at the posterior-most region, rather than tooth replacement is suggested by three teeth positioned in decreasing stages of development towards the bone surface (three teeth indicated by double-headed arrow); pedicellate attachment is revealed by narrow gaps between tooth crown and pedicel (arrowheads indicate examples), which contain fibrous tissue in life.

I-J, XCT virtual clipped section along the corresponding line shown in G, posterior to anterior direction is left to right, two views from lateral side; 3D structure of pulp cavities and of crypts containing developing successional teeth is shown; black arrow indicates large successional tooth, corresponding to the left successional tooth in H. Both view angles show its alignment with the smaller tooth above it (black arrowhead). The alignment appears to suggest that despite the size difference between the two teeth, there is a successor/functional predecessor relationship, possibly facilitated by the gap in the neighbouring functional tooth position. Abbreviations: Cr, crypt; FP, functional predecessor tooth; S, successional tooth. All scale bars represent 1 cm.

4.3.6 *Polypterus senegalus*, African bichir, head including soft tissue, wet specimen, unregistered from Kyle Martin, personal collection

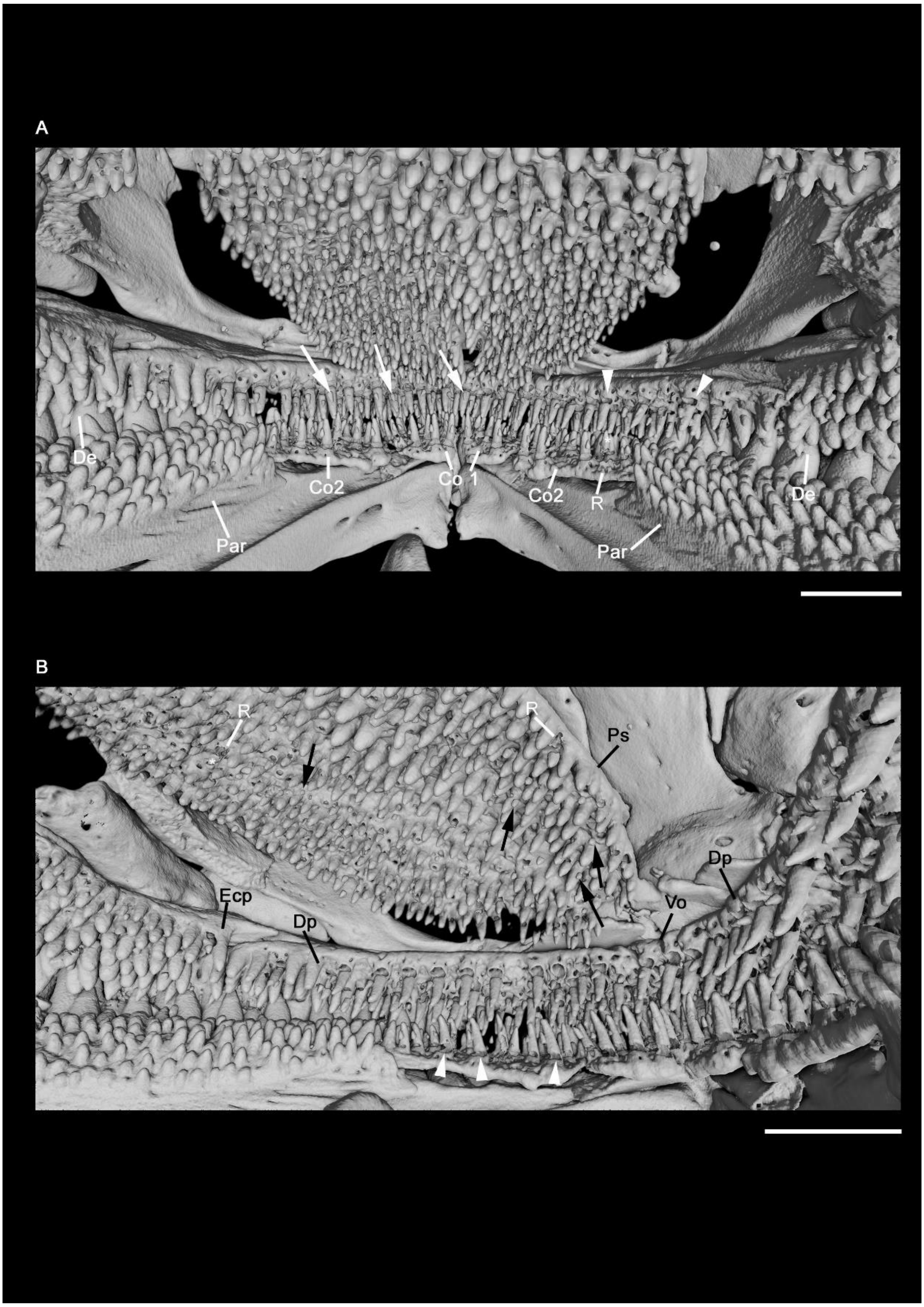
Fig. 4.22: Surface *features and XCT virtual sections of* Polypterus senegalus, *African bichir, head including soft tissue*

XCT surface renders of the specimen inside the oral cavity, viewed from posterior to anterior, show multiple, irregularly patterned conical teeth, which become more pointed and curved towards the marginal bones. A single row of much larger, curved conical teeth is located on both dentaries of the lower jaw, partially visible behind multiple smaller teeth located on the prearticular and coronoids 1 and 2. On the upper jaw, a single row of large, curved, conical teeth is located teeth on the maxillae (and premaxillae – not visible) of the upper jaw, partially visible behind multiple smaller teeth located on the ectopterygoid, dermopalatines and vomer.

Small replacement teeth are developing directly from the posterior-lingual side of functional predecessor teeth, this is more common on the bones located towards the margins of the oral cavity. Small replacement teeth are also developing from more varied positions in relation to functional teeth on the parasphenoid, trending towards the right lateral side. Many functional teeth towards the dentition margin have a hinged attachment, type 3 *sensu* Fink (1981), in which the tooth’s hard tissue (dentine) is attached to the bone of attachment only on the labial side (see section 2.2.8).

Some functional teeth can be seen to have nearly, or fully detached, following resorption of their bone of attachment and tooth base. This resorption is revealed by scalloped edging of both the bone of attachment and the detached tooth.

Teeth have developed on the surface of the dentigerous bone, and not from a crypt within it, therefore the mode of replacement is recognizable as extraosseous. XCT virtual sections indicate that those teeth which are not hinged have a straightforward ankylosed attachment. This is because teeth are directly joined to their bone of attachment with no gap, and therefore with no fibrous tissue in between.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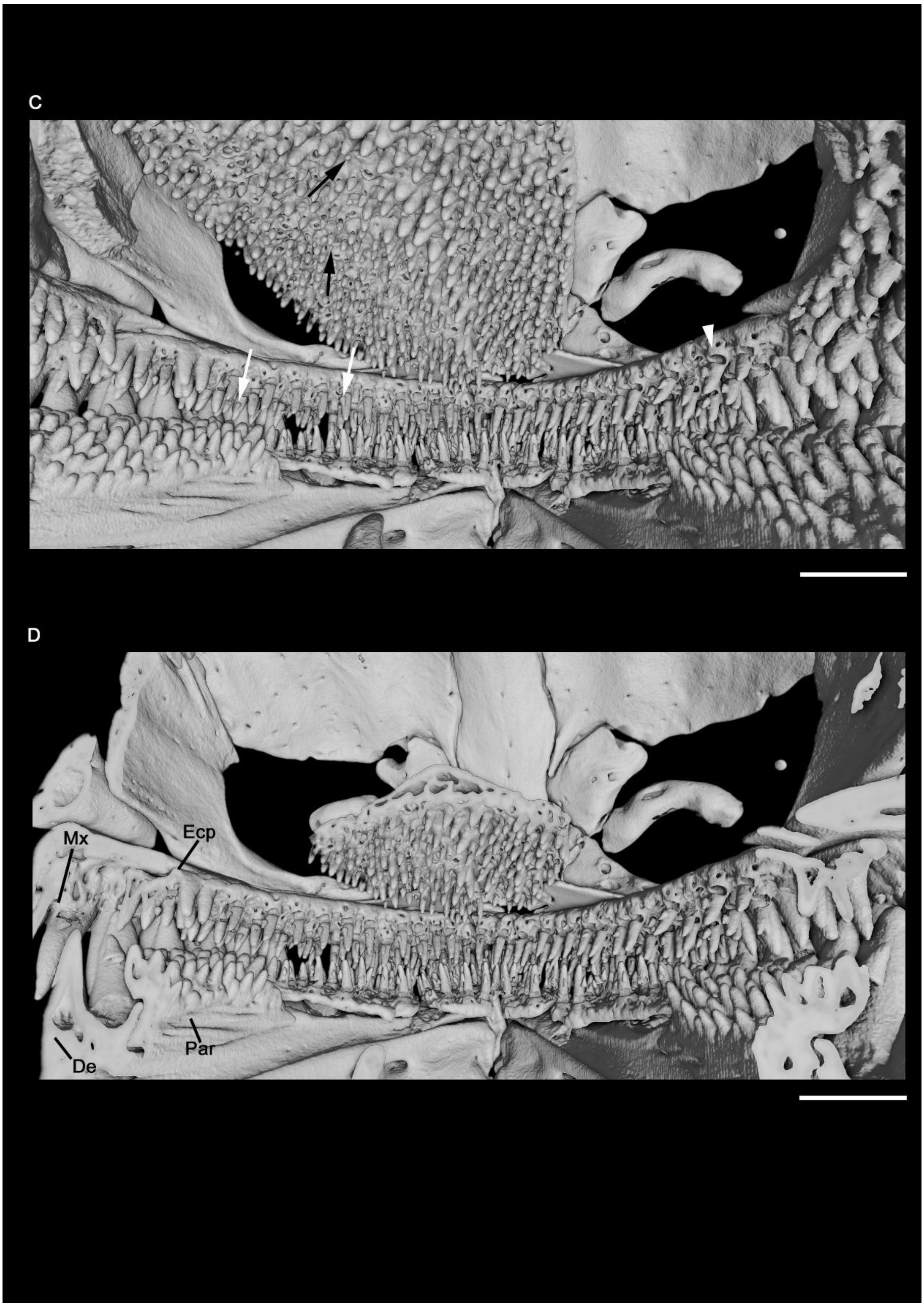

Fig. 4.22 Surface features and XCT virtual section within the oral cavity of *Polypterus senegalus*, unregistered specimen.

A-C, XCT surface renders of the specimen inside the oral cavity, viewed from posterior to anterior. Small replacement teeth are developing from the posterior-lingual side of functional predecessor teeth (white arrows indicate examples); small replacement teeth are also developing from more varied positions in relation to functional teeth on the parasphenoid, trending towards the right lateral side (black arrows indicate examples); many functional teeth towards the dentition margin have a hinged attachment type, type 3 *sensu* Fink (1981), the tooth's hard tissue (dentine) is attached to the bone of attachment only on the labial side (white arrowheads indicate examples); some functional teeth have nearly, or fully detached (white *), following resorption of their bone of attachment and tooth base. This resorption (R) is revealed by scalloped edging of both the bone of attachment and detached tooth, caused by the action of clastic cells in circular patterns; a single row of large, curved, conical teeth is located on both dentaries of the lower jaw (De), partially visible behind multiple smaller teeth located on the prearticular and coronoids 1 and 2; a single row of large, curved, conical teeth is located teeth on the maxillae (and premaxillae – not visible) of the upper jaw, partially visible behind multiple smaller teeth located on the ectopterygoid, dermopalatines and vomer. D, XCT virtual clipped section cutting through dentaries, prearticulars, maxillae, ectopterygoids and parasphenoid, 3D structure of pulp cavities and cavities within bones are shown. *Abbreviations*: Co1, Co2, coronoids 1 and 2; De, dentary; Dp, dermopalatine; Ecp, ectopterygoid; Mx, maxilla; Par, prearticular; Ps, parasphenoid; Vo, vomer. Anatomical labelling follows Clemen et al. (1998) and Giles et al. (2017). All scale bars represent 1 cm.

4.3.7 *Salmo salar*, wild Atlantic salmon (male, in mating season), head, dry specimen, BMNH 2017.4.4.3.

Fig. 4.23: ***Surface features and XCT virtual sections of*** **Salmo salar*****, wild Atlantic salmon (male, in mating season), head, dry specimen***

A single row of large, curved, conical teeth is located on upper jaw (on the maxillae, premaxillae and dermopalatines) as well as the lower jaw, on right dentary. In this specimen, the left dentary is broken off, and the tongue-bite apparatus is absent. Two teeth are attached to the vomer, visible in Fig. 4.23C. The anterior of the upper and lower jaws are hook-shaped (forming the 'kype'), due to secondary sexual adaption during the mating/spawning season (Huysseune *et al.* 2007; Berkovitz & Shellis 2017). The largest and most curved teeth are located on the most anterior, hook-shaped part of the premaxilla and dentary. Replacement teeth are developing directly from the functional predecessor tooth, orientated to its posterior-lingual side. Teeth are at varying stages of development at different tooth positions, consistent with Huysseune *et al.* (2007), and also consistent families of successional, replacement teeth being located at each marginal tooth position (Huysseune *et al.* 2007; Huysseune & Witten 2008; Vandenplas *et al.* 2014). Some functional teeth have detached, leaving gaps in a row. The scalloped edge on their remaining bone of attachment shows where it was resorbed. Teeth have developed on the surface of the dentigerous bone, and not from a crypt within it, therefore the mode of replacement is recognizable as extraosseous. XCT virtual sections indicate that teeth have an ankylosed attachment type, as teeth are directly joined to their bone of attachment with no gap, and therefore with no fibrous tissue in between.


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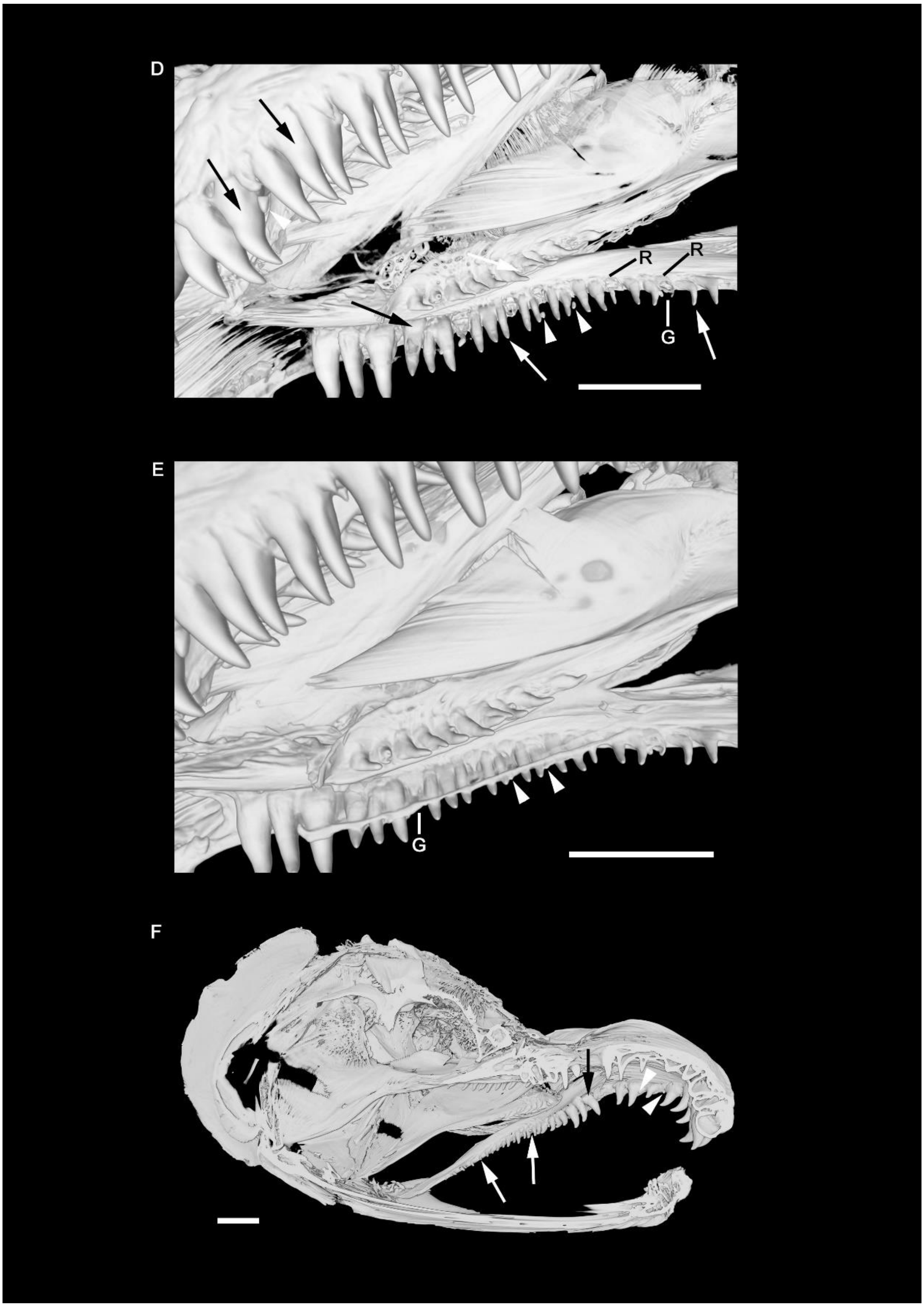
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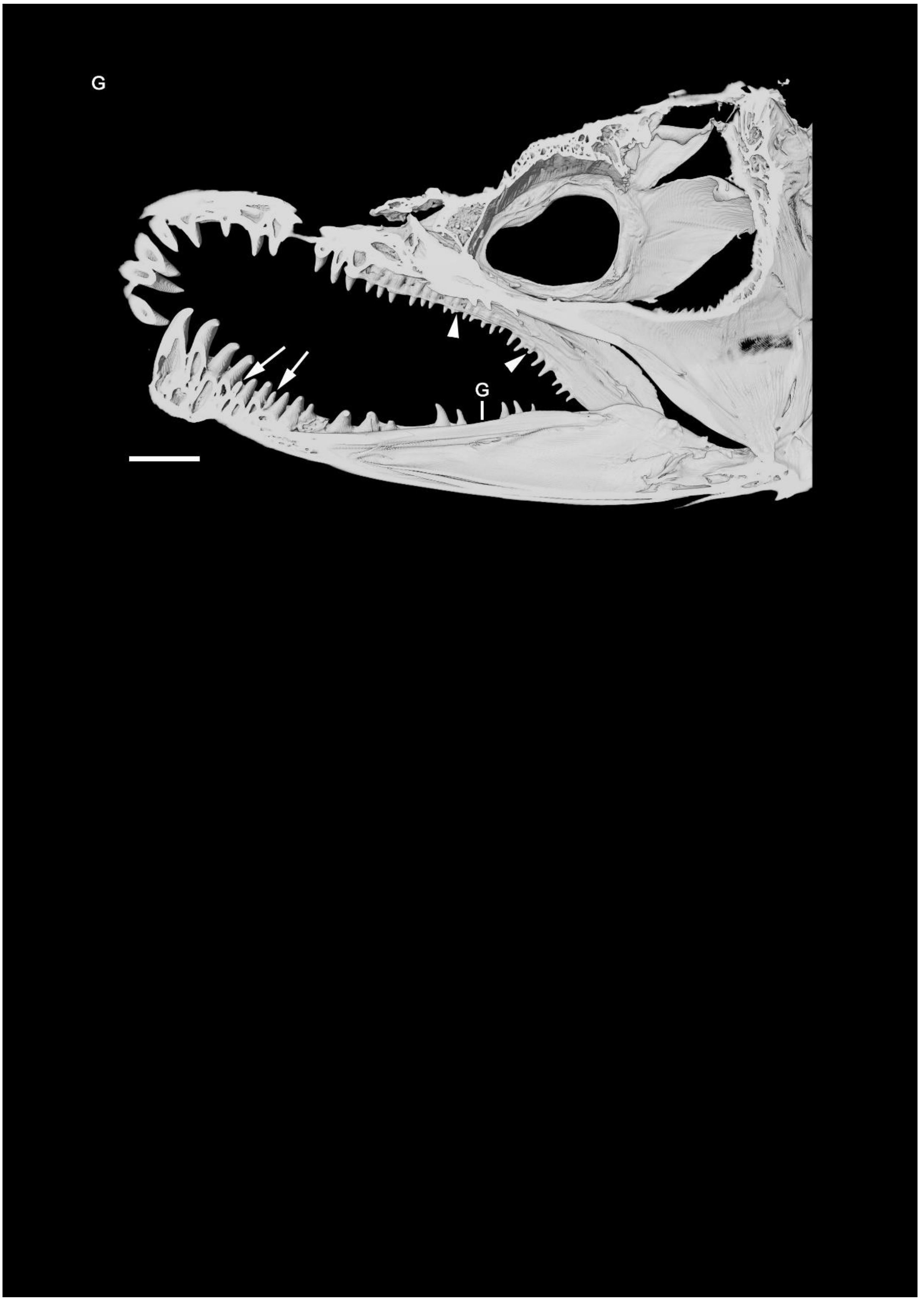


Fig. 4.23 Surface features and XCT virtual sections of *Salmo salar*, wild Atlantic salmon (male, in mating season), head, dry specimen, BMNH 2017.4.4.3.

A-B, XCT surface renders of the specimen from the anterior (A) and left lateral (B) view; a single row of large, curved, conical teeth is located on the maxillae, premaxillae and dermopalatines of the upper jaw; a single row of large, curved, conical teeth is located on the right dentary of the lower jaw, the left dentary is broken off, and tongue-bite apparatus is absent in this specimen; the anterior of the upper and lower jaws are hook-shaped (forming the 'kype'), due to secondary sexual adaption during the mating/spawning season (Witten & Hall 2003; Berkovitz & Shellis 2017); the largest and most curved teeth are located on the most anterior, hook-shaped part of the premaxilla and dentary.

C-E, XCT surface renders of the specimen inside the oral cavity: C, posterior to anterior view; D, close-up of right maxilla and right dermopalatine; E, view in D with more soft tissue visualised. Teeth are at varying stages of development at different tooth positions, consistent with Huysseune *et al.* (2007), and also consistent families of successional, replacement teeth being located at each marginal tooth position (Huysseune *et al.* 2007; Huysseune & Witten 2008; Vandenplas *et al.* 2014). Following Huysseune *et al.* (2007), the three types of arrow indicate examples of functional teeth (black arrows), advanced replacement teeth (white arrows), and young replacement teeth (white arrowheads); both young and advanced replacement teeth are orientated to the posterior-lingual side of the functional predecessor; tooth on right maxilla indicated by black arrow in D has not yet fully attached to the bone; surface oral epithelium is visualised in E, showing young replacement teeth developing at the epithelial surface, before attachment to the bone, as indicated by white arrowheads. These teeth correspond to teeth indicated by white arrowheads on right maxilla in C-D; some functional teeth have detached, leaving a scalloped edge on their bone of attachment due to resorption (R); some detached teeth have left a gap (G) in a tooth row; more functional teeth are present than in the female specimen. This may be due to the male requiring an optimally functioning dentition during spawning, as the kype likely contributes to a dominance hierarchy among males at the spawning ground (Huysseune *et al.* 2007; Berkovitz & Shellis 2017); the vomer is visible behind the premaxillae in C, with only two teeth attached; anterior-most teeth of the distal dentary (‘kype’) are not attached to the bone, as seen in C. Teeth may be in the process of attaching, or attachment to soft tissue may be advantageous for the modification of head shape during the lifecycle.

F, XCT virtual clipped section cutting through right premaxilla, maxilla and dentary, lateral view, with medial view of left side of upper jaw; 3D structure of pulp cavities and cavities within bones are shown; stages of tooth development are indicated using the same labels as in C-E.

G, XCT virtual clipped section cutting through the right premaxilla, maxilla, and the distal right dentary (part of the kype), medial view; 3D structure of pulp cavities and cavities within bones are shown; stages of tooth development are indicated using the same labels as in C-E; anterior-most teeth on the dentary are attached, seemingly in contrast to the corresponding teeth in C. These teeth are likely attached via a softer tissue than is visualised in C. *Abbreviations*: De, dentary; Mx, maxilla; Pmx, premaxilla; Dp, dermopalatine; Vo, vomer; VoT, vomerine tooth. Anatomical labelling of bones is derived from Konow & Sanford (2008) and Berkovitz & Shellis (2017). All scale bars represent 1 cm.

4.3.8 *Salmo salar*, wild Atlantic salmon (female) skull, dry specimen, BMNH 2017.4.4.2

Fig. 4.24: Surface *features and XCT virtual sections of* Salmo salar, *wild Atlantic salmon (female) skull, dry specimen*

A single row of large, curved, conical teeth is located on both dentaries of the lower jaw. Teeth are also located on the tongue, developing from the basihyal tooth plate, on the basihyal bone, both of which form part of the tongue-bite apparatus. A single row of large, curved, conical teeth is located on the maxillae, premaxillae and dermopalatines of the upper jaw. Teeth are at varying stages of the tooth cycle at different tooth positions (i.e. young or mature functional teeth, in resorption or shed), consistent with Huysseune *et al.* (2007), and also consistent with families of successional, replacement teeth being located at each marginal tooth position (Huysseune *et al.* 2007; Huysseune & Witten 2008; Vandenplas *et al.* 2014). However, direct evidence of replacement teeth prior to attachment to the bone, and therefore evidence of tooth families, is absent. This is because they would have been located in the soft tissue removed from this skeletal specimen. It is therefore also not possible to determine the positioning/orientation of developing replacement teeth in relation their functional predecessor. Teeth are in the process of being resorbed at the base, and some have been shed, leaving a gap in the tooth row. Teeth have developed on the surface of the dentigerous bone, and not from a crypt within it, therefore the mode of replacement is recognizable as extraosseous. XCT virtual sections indicate that teeth have an ankylosed attachment type, as teeth are directly joined to their bone of attachment with no gap, and therefore with no fibrous tissue in between.

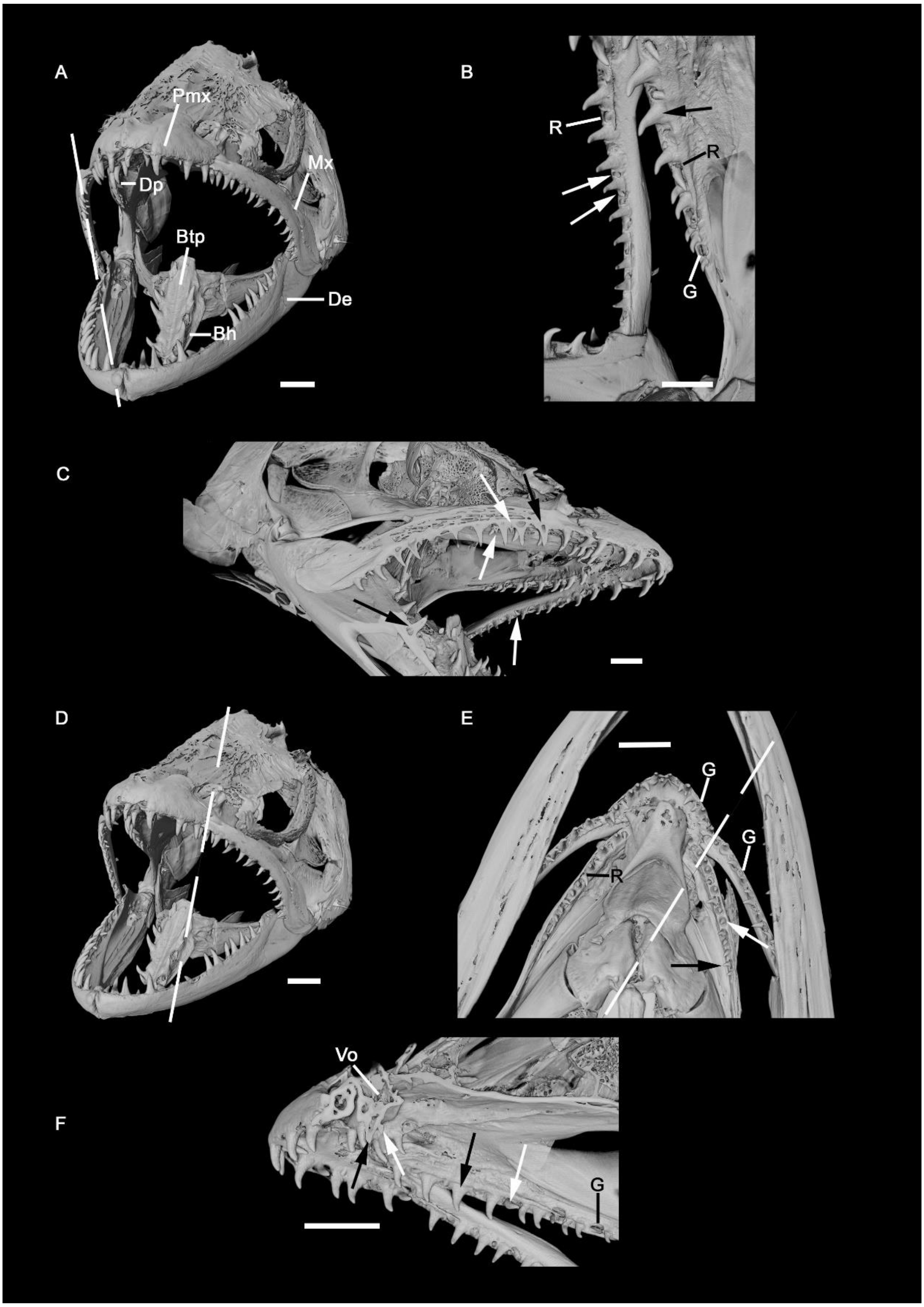


Fig. 4.24 Surface features and XCT virtual sections of *Salmo salar* (female) skull, dry specimen, BMNH 2017.4.4.2.

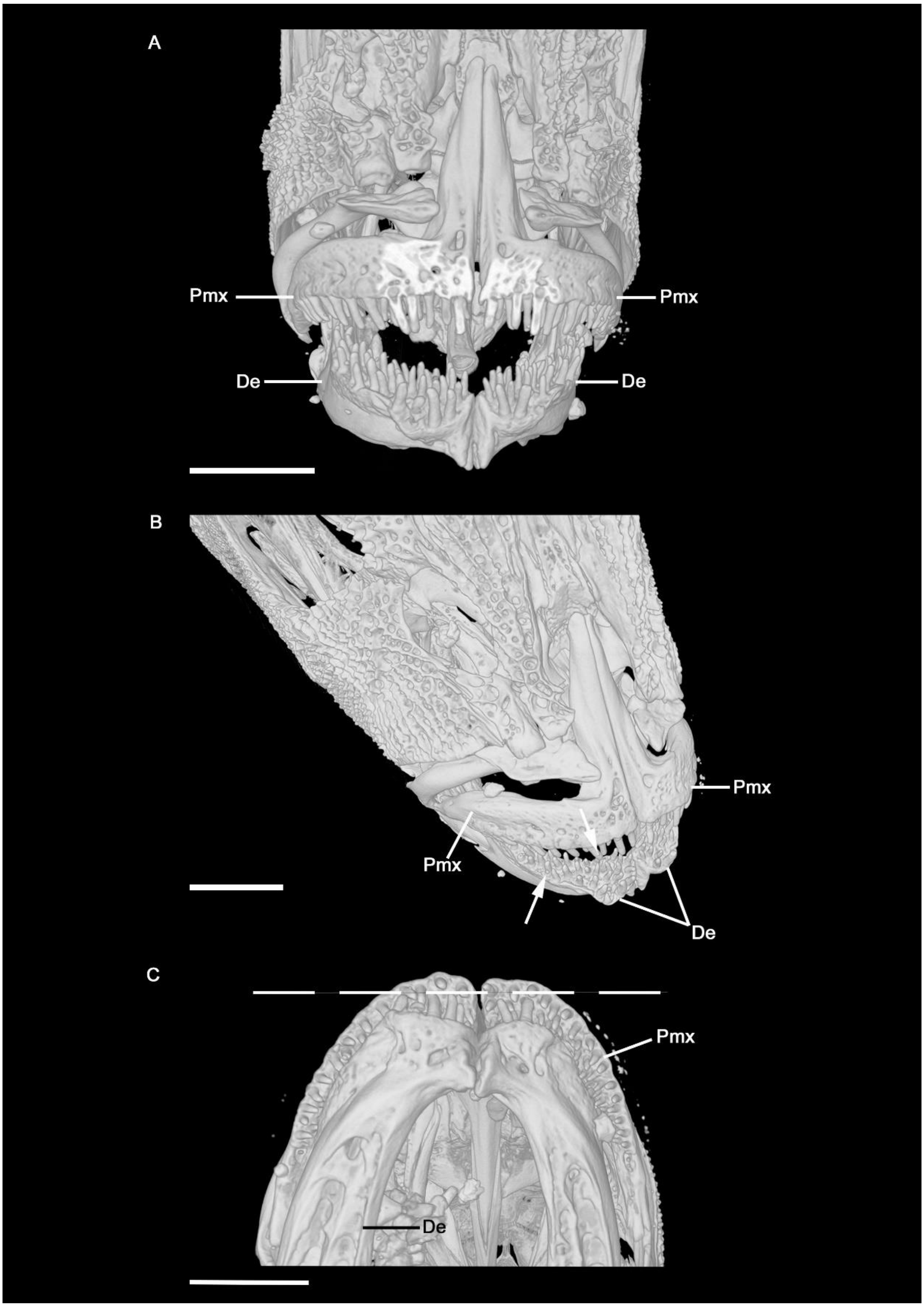
A, XCT surface render of the specimen from the anterior view; a single row of large, curved, conical teeth are located on both dentaries of the lower jaw; teeth are located on the tongue, developing from the basihyal tooth plate, on the basihyal bone, both of which form part of the tongue-bite apparatus; a single row of large, curved, conical teeth is located on the maxillae, premaxillae and dermopalatines of the upper jaw; line shows the position of the XCT virtual clipped section in C. B, XCT surface render of right maxilla and right dermopalatine, medial view. Teeth are at varying stages of development at different tooth positions, consistent with Huysseune et al. (2007), and also consistent with families of successional, replacement teeth being located at each marginal tooth position (Huysseune et al. 2007; Huysseune & Witten 2008; Vandenplas et al. 2014). Black arrows indicate mature functional teeth, white arrows indicate young functional teeth. Evidence of developing replacement teeth (either advanced or young, *sensu* Huysseune et al. (2007)) is not present, as these would not be attached to the bone, but located in the soft tissue, which is removed from this skeletal specimen; some teeth are in the process of being resorbed at the base (R); some teeth have been shed, leaving a gap (G) in the tooth row (labelling used in B is also used in C-F). C, XCT virtual clipped section along the corresponding line shown in A, cutting through the right maxilla and part of the right dentary; 3D structure of pulp cavities and cavities within bones are shown; young functional teeth indicated by white arrows on the right maxilla in C correspond to teeth indicated by white arrows in B. D, XCT surface render of the specimen from the anterior view (see A for description of anatomy); line shows the position of the XCT virtual clipped section in F. E, XCT surface render of the specimen from the ventral view; line shows the position of the XCT virtual clipped section in F. F, XCT virtual clipped section along the corresponding line shown in D, E, cutting through the premaxilla and behind it a small tooth-bearing bone, the vomer; 3D structure of pulp cavities and cavities within bones are shown. *Abbreviations*: De, dentary; Btp, basihyal tooth plate; Bh, basihyal bone; Mx, maxilla; Pmx, premaxilla; Dp, dermopalatine; Vo, vomer. Anatomical labelling of bones is derived from Konow & Sanford (2008) and Berkovitz & Shellis (2017). All scale bars represent 1 cm.

4.3.9 *Gastrosteus aculeastus*, three-spined stickleback, wet specimen, BMNH 1912.7.10.7

Fig. 4.25: ***Surface features and XCT virtual section of*** **Gastrosteus aculeastus,** ***three-spined stickleback, wet specimen***

XCT surface renders of the specimen from exterior views and from inside the oral cavity show multiple thin, papilliform teeth located on the premaxillae and dentaries, in an irregular pattern. Putative replacement teeth are identified on the dentaries, located on the labial side, and close to the functional predecessors, consistent with observations made by Square *et al.* (2021). Inside the oral cavity, viewing premaxillae from ventral perspective; one-for-one replacement ratios and other patterns are difficult to discern, possibly reflecting an irregular, stochastic nature of replacement, suggested by Square *et al.* (2021) to occur in the pharyngeal jaws of three-spined sticklebacks . Young teeth are not positioned closely, and next to a particular tooth, indicating no obvious predecessor. Some teeth are located close to, and equidistant between their two nearest neighbours. They could therefore, in view of the findings of Square *et al.* (2021), possibly be abutting two functional predecessor teeth, representing a one-for-two replacement event (Fig. 4.25D-E). The tooth would be developing from a region (SDE) of epithelium adjacent to both the functional predecessors, as observed by Square *et al.* (2021) in the pharyngeal jaw of the three-spined stickleback.

XCT virtual clipped section shows functional teeth attached to the surface of the bone with no replacement teeth developing in crypts beneath. No crypts containing replacements were found elsewhere in the dentition, indicating teeth are replaced extraosseously. This is contrary to the findings of Ellis *et al.* (2016), but consistent with those of Square *et al.* (2021), in which replacement teeth develop from an SDE (section 2.4.2). The XCT virtual clipped section indicates that teeth have an ankylosed attachment type, as teeth are directly joined to their bone of attachment with no gap, and therefore with no fibrous tissue in between.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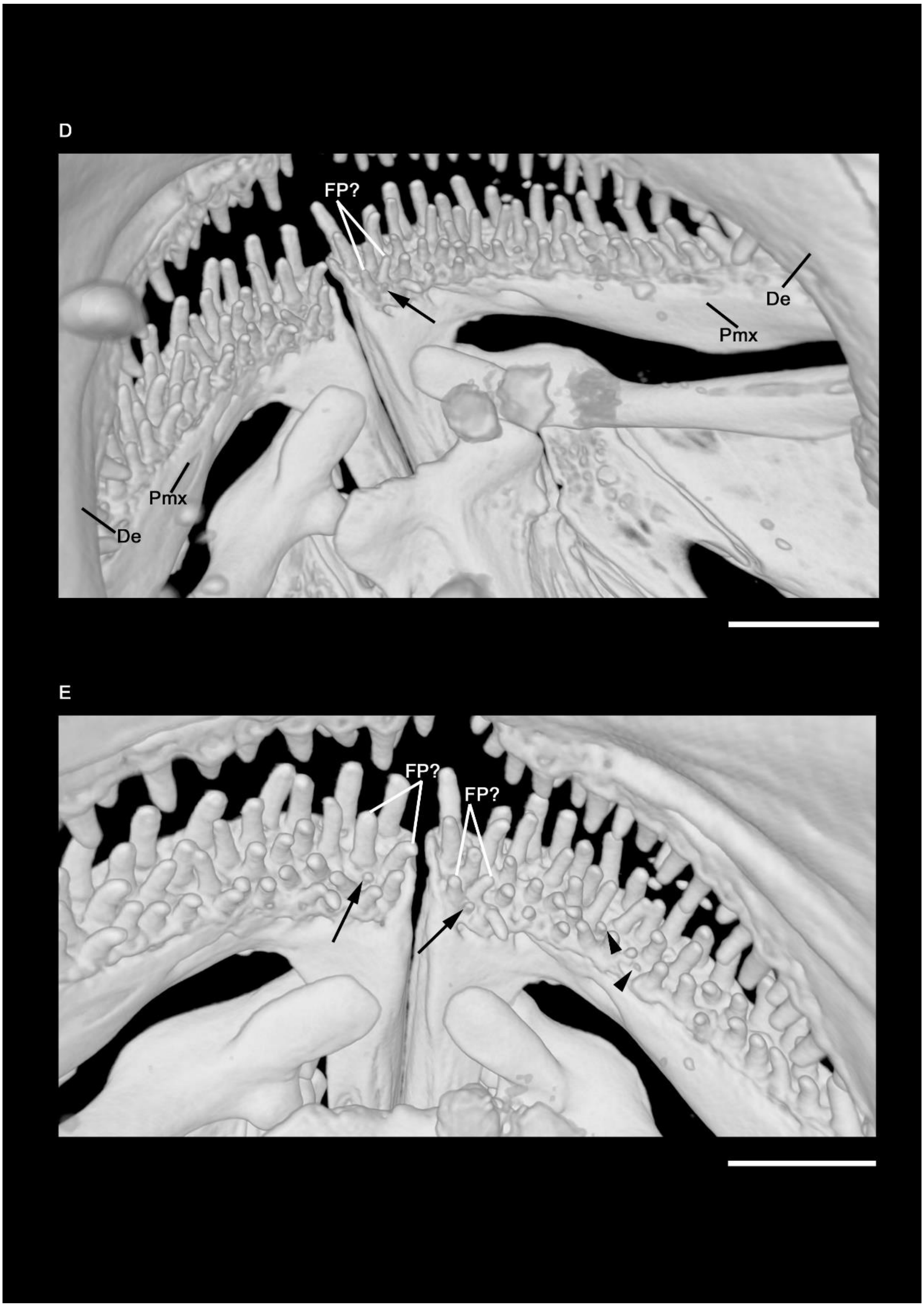


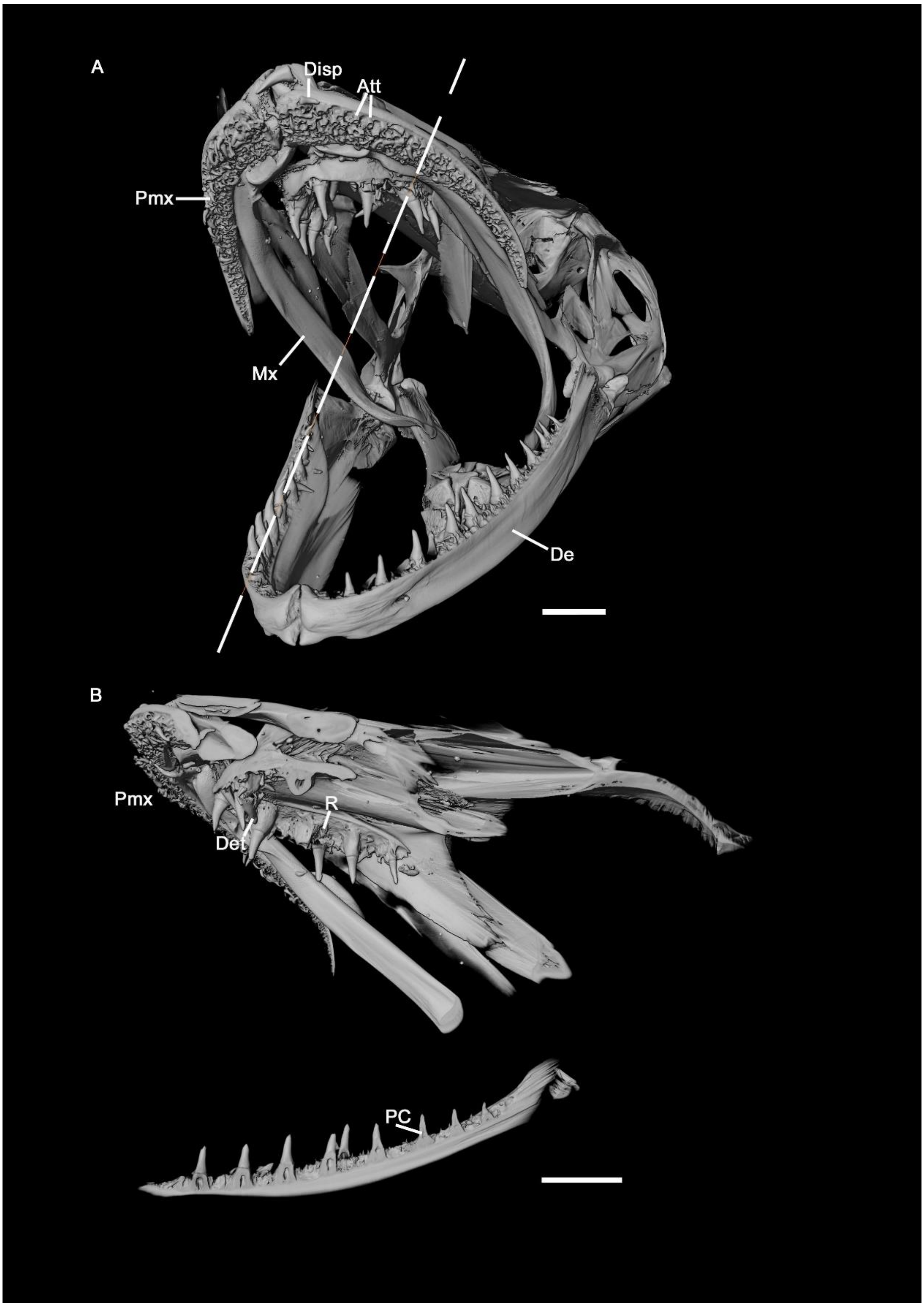
Fig. 4.25 Surface features and XCT virtual section of the oral dentition of *Gastrosteus aculeastus*, three-spined stickleback, wet specimen, BMNH 1912.7.10.7.

A, XCT surface renders of the specimen from anterior view, including a virtual clipped section of premaxillae along the corresponding line shown in C; multiple thin, papilliform teeth are located on the premaxillae and dentaries, in an irregular pattern; XCT virtual clipped section shows functional teeth attached to the surface of the bone, and no crypts within the bone containing developing teeth, indicating teeth are replaced extraosseously. B, XCT surface render of the specimen from anterior-dorsal view; the irregular pattern of multiple thin, papilliform teeth located on the dentaries is shown; arrows indicate putative replacement teeth developing from the labial side of functional predecessors, consistent with observations made by Square et al. (2021). C, XCT surface render of the specimen from the ventral view; line shows the position of the XCT virtual clipped section through the maxillae in A. D-E, XCT surface renders of specimen inside the oral cavity, viewing premaxillae from ventral perspective; one-for-one replacement ratios and other patterns are difficult to discern, possibly reflecting a stochastic nature of replacement, *sensu* Square *et al.* (2021); arrowheads indicate young teeth, with positioning that indicates no obvious predecessor; arrows indicate putative replacement teeth possibly abutting two functional predecessor teeth (FP?). Anatomical labelling follows Ellis *et al.* (2016). *Abbreviations*: De, dentary; Pmx, premaxilla. Scale bars represent: 2mm (A, B, C); 1mm (D); 0.5mm (E).

4.3.10 *Gadus morhua***, Atlantic cod, skull, from teaching collection of the Department Earth and Planetary Sciences, Birkbeck, University of London.**

Fig. 4.26: Surface ***features and XCT virtual section of*** **Gadus morhua,** ***Atlantic cod, skull***

XCT surface renders of the specimen from exterior views and from inside the oral cavity show curved, conical teeth are located on the premaxillae and the vomer of the upper dentition, and both dentaries of the lower jaw. While all teeth share a similar shape, they vary greatly in size. The variation is not gradual, with small and large tooth sizes generally contrasting greatly. Most teeth on the premaxillae are detached, it is not known the proportion which were lost post-mortem, leaving empty attachment bases (bone of attachment, or ‘attachment bone’ *sensu* Fink (1981)). Only one large tooth remains on the labial margin of the premaxillae, where usually a single row of large teeth is located on both bones (Berkovitz & Shellis 2017). This tooth appears it may have been slightly displaced post-mortem (see below). Large teeth are located on the dentaries and vomer. No regular patterning of teeth, or general association between functional teeth and their replacements can be discerned on the premaxillae, consistent with ‘haphazard’ tooth patterning described by Holmbakken & Fosse (1973). This is also the case on the vomer. Patterning is more regular on the dentaries. A repeating pattern along the left dentary of three replacement teeth in increasingly advanced stages of development, followed by a functional tooth, indicates tooth families may be present (and therefore one-for-one replacement), at each tooth position in this region (Fig. 4.26C, F). This was likely mirrored along the right dentary in life, but not evident here as developing teeth have probably been lost post-mortem. Some functional teeth have detached (Det) following resorption of their tooth base and attachment base. This resorption (R) is revealed by scalloped edging of both the detached tooth and attachment base, caused by the action of clastic cells in circular patterns. Some teeth are located on regions which are not usually dentigerous, a likely artefact where teeth are displaced post-mortem e.g due to capture damage (‘Disp’ indicates example). XCT virtual clipped section clearly shows that teeth have an ankylosed attachment type, as teeth are directly joined to their bone of attachment with no gap, and therefore with no fibrous tissue in between. Teeth have developed on the surface of the dentigerous bone, and not from a crypt within it, therefore the mode of replacement is recognizable as extraosseous.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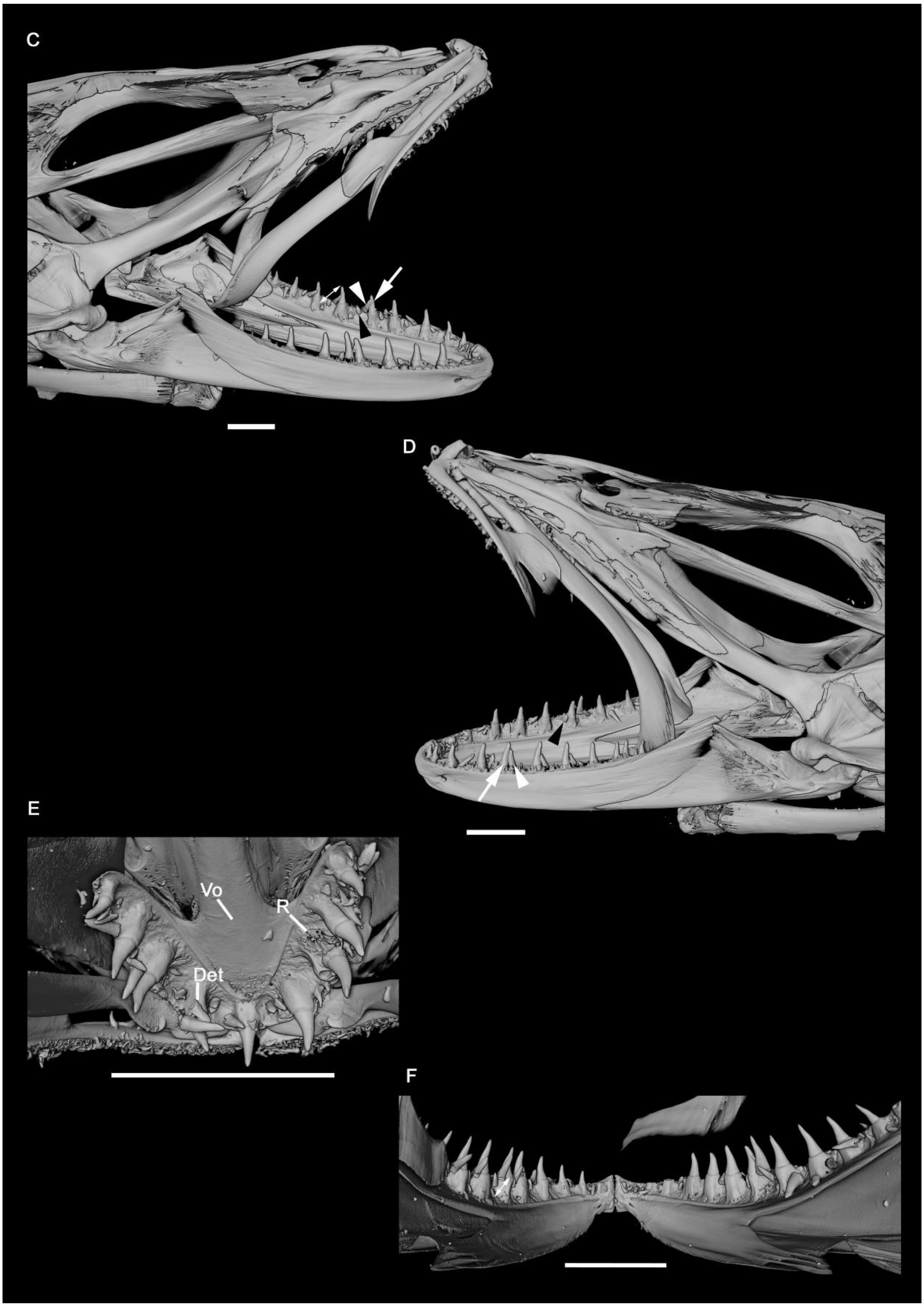


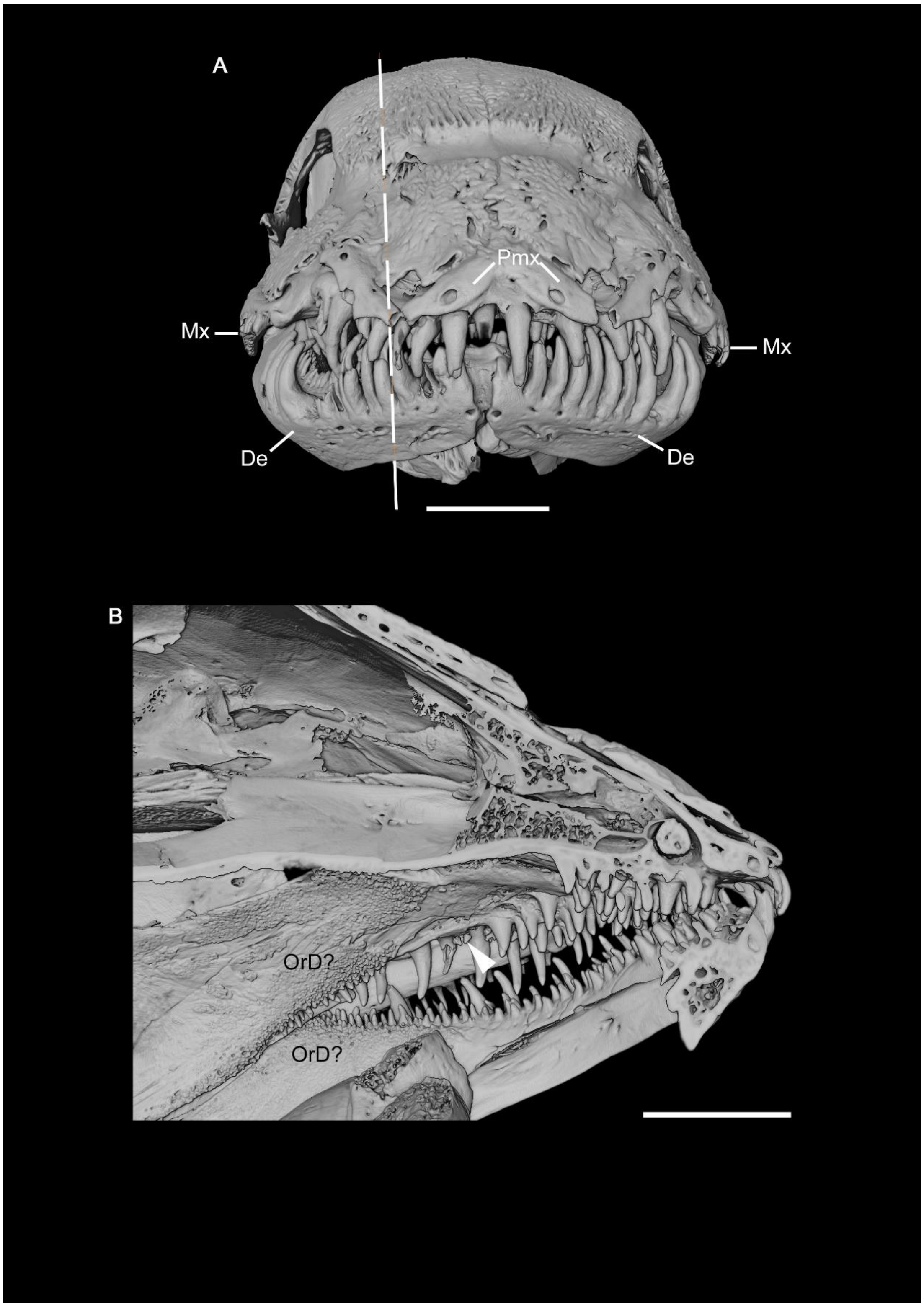
Fig. 4.26 Surface features and XCT virtual section of *Gadus morhua*, Atlantic cod, skull, from the Department of Earth and Planetary Sciences' teaching collection, Birkbeck, University of London.

A-D, XCT surface renders of the specimen from the anterior (A), right lateral (C) and left lateral (D) view, and virtual clipped section along the corresponding line shown in A, cutting through part of the vomer and right dentary, view from medial side (B). Attachment is by ankylosis, not pedicellate, therefore the bone of attachment is referred to as an 'attachment base' in this figure. Curved, conical teeth which vary greatly in size are located on both premaxillae of the upper jaw, both dentaries of the lower jaw, and the vomer; very few teeth on the premaxillae are attached, with only one large tooth remaining. It is not known the proportion which were lost post-mortem, leaving empty attachment bases (Att); the three types of arrow indicate examples of functional teeth (white arrows), advanced replacement teeth (white arrowheads), and young replacement teeth (black arrowheads). Some functional teeth have detached (Det) following resorption of their tooth base and attachment base. This resorption (R) is revealed by scalloped edging of both the detached tooth and attachment base. No regular patterning of teeth, or general association between functional teeth and their replacements is discernible on the premaxillae or vomer; patterning is more regular on the dentaries (see C, D); the largest teeth are present on the dentaries and vomer; some teeth are located on regions which are not usually dentigerous, a likely artefact where teeth are displaced post-mortem e.g due to capture damage (‘Disp’ indicates example); 3D structure of tooth pulp cavities (PC) and cavities within attachment bases are shown in B. A repeating pattern along left dentary in C of three replacement teeth in increasingly advanced stages of development, followed by a functional tooth, indicates tooth families may be present at each tooth position in this region (double-headed arrow indicates example of putative tooth family). E-F, XCT surface renders of the specimen inside the oral cavity, viewed from posterior to anterior; labelling as in A-D. *Abbreviations*: De, dentary; Mx, maxilla; Pmx, premaxilla; Vo, vomer. Anatomical labelling of bones is derived from Holmbakken & Fosse (1973) and Idaho Virtual Museum https://virtual.imnh.iri.isu.edu/Osteo/View/Atlantic_Cod/691. All scale bars represent 1 cm.

4.3.11 *Amia calva*, bowfin, skull, unregistered specimen, NHMUK

Fig. 4.27: ***Surface features and XCT virtual section of*** **Amia calva*****, bowfin, skull***

An XCT surface render of the specimen from the anterior view shows numerous large, curved, conical teeth located on the premaxillae and maxillae of the upper jaw and the dentaries of the lower jaw. A virtual clipped section and surface renders of the specimen inside the oral cavity, viewed from posterior to anterior, show extensive coverage of bones in ‘bumps’, putatively identified as oral denticles. These grade to a larger size and appear to become more tooth-like towards the dentition margin, until recognisable as teeth. This trend culminates in the largest teeth being those observed from the exterior. At which point in the dentition the transition to true teeth occurs is uncertain, however teeth are recognisable on the dentaries, coronoids, prearticulars, premaxillae, maxillae, dermopalatines, vomer and possibly the anterior-most region of the ectopterygoids. The teeth shapes are conical, to conical with a curve, with the tips of the curve pointing posteriorly. Generally, tooth patterning appears irregular. Most soft tissue is absent, meaning unattached developing teeth are unlikely to be present. A tooth replacement ratio therefore cannot be discerned. There is an increase in tooth size at tooth positions approaching the labial margin of the prearticulars, resembling developing tooth families. However, this is likely impossible without soft tissue present, to enable an epithelial connection between teeth. It could perhaps represent a type of serial tooth addition. There are a very few young teeth located close to functional teeth, possibly developing replacements, retained in soft tissue remnant, or perhaps just mature enough to attach to the bone. Resorption is identified where bone of attachment has scalloped margins, and where tooth bases are being broken down prior to detachment. The XCT virtual clipped section indicates that teeth have an ankylosed attachment type, as teeth are directly joined to their bone of attachment with no gap, and therefore with no fibrous tissue in between. Teeth have developed on the surface of the dentigerous bone, and not from a crypt within it, therefore the mode of replacement is recognizable as extraosseous.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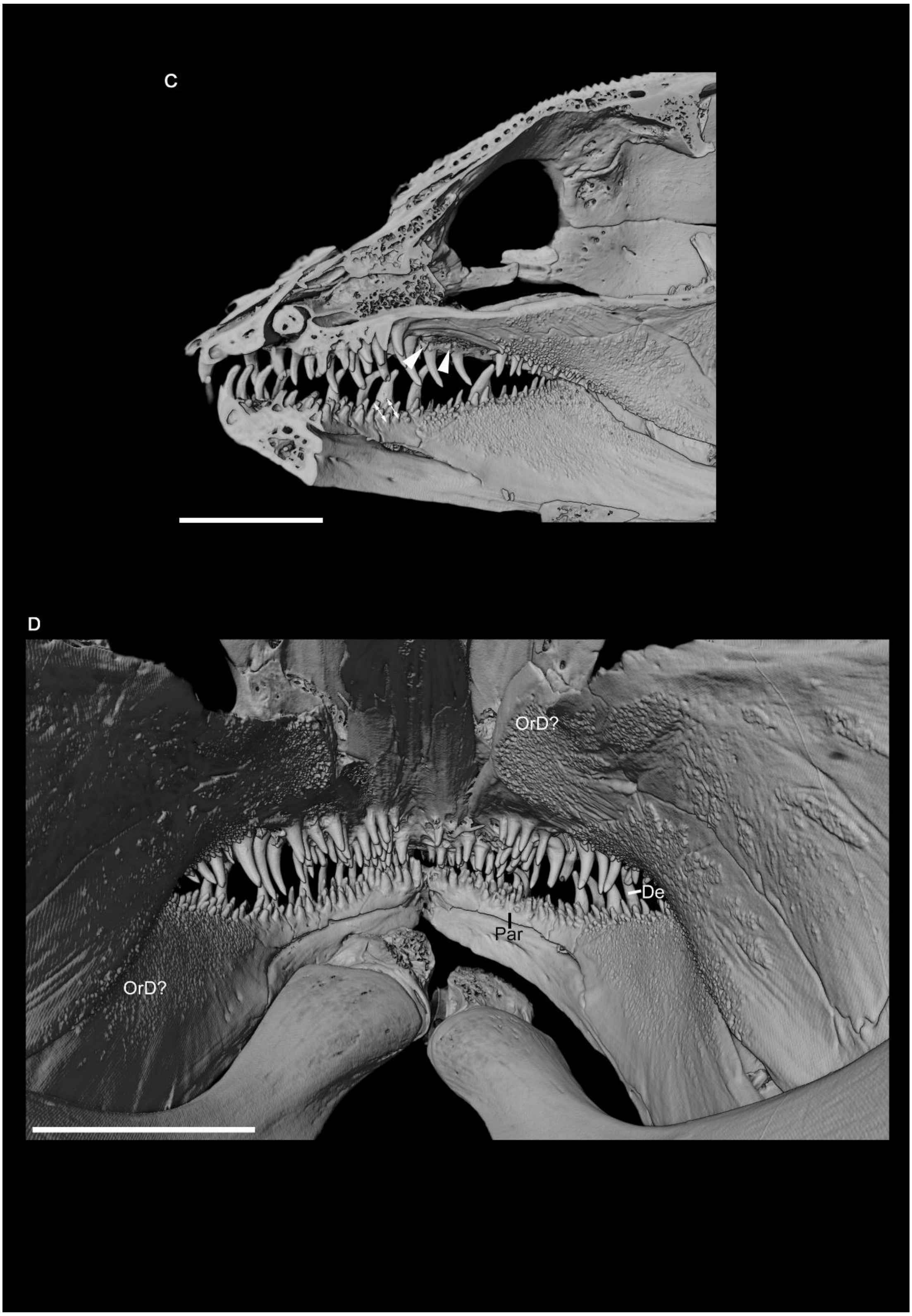
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Fig. 4.27 Surface features and XCT virtual section of the oral dentition of *Amia calva*, bowfin, skull, unregistered specimen, NHMUK.

A, XCT surface render of the specimen from the anterior view showing numerous large, curved, conical teeth are located on the premaxillae and maxillae of the upper jaw, and the dentaries of the lower jaw; line shows the position of the virtual clipped section in B-C; B-C, virtual clipped section cutting through the right premaxilla, dermopalatine, ectopterygoid, dentary and coronoid bones, from the lateral (B) and medial (C) view; functional teeth are attached to the surface of the bone with no replacement teeth developing in crypts beneath, indicating teeth are replaced extraosseously; numerous teeth are located within the oral cavity on the dermopalatines, vomer, and possibly anterior ecopterygoids of the upper dentition, and the coronoids and prearticulars of the lower dentition (seen more clearly in D-F); 3D structure of pulp cavities and cavities within bones is shown; teeth grade to a smaller size from the anterior to posterior of the oral cavity, until they are small rounded bumps, possibly oral denticles (OrD?); teeth increasing in size towards the labial margin of the prearticular resemble tooth families (examples indicated by white double-headed arrows in C); some young, putative replacement teeth have yet to attach to the bone (examples indicated by white single arrowheads). D-F, XCT surface renders of the specimen inside the oral cavity, viewed from posterior to anterior; putative oral denticles (OrD?) are present on the parasphenoid, ento- and ectopterygoids of the upper oral cavity (F) as well as the prearticulars of the lower jaw, appearing to grade to become true teeth towards the jaw margins; resorption (R) is indicated where bone of attachment has scalloped margins, and where tooth bases are being broken down prior to detachment; a young, putative replacement tooth has yet to attach to the bone (white arrowhead in F); *Abbreviations*: Cor, coronoid; De, dentary; Dp, Dermopalatine; Ecp, ectopterygoid; Ent, entopterygoid; Mx, maxilla; Par, prearticular; Pmx, premaxilla; Ps, parasphenoid; Vo, vomer. Anatomical labelling of bones is derived from Miller & Radnor (1973), Clemen *et al.* (1998), Berkovitz & Shellis (2017) and Giles *et al.* (2017). All scale bars represent 1 cm.

5 Discussion

5.1 Structure of discussion

Interpretation of the present study’s results is with reference to its aims, and I will initially address the first two:

- Evaluate the tooth replacement mechanisms in fossil and modern fish dentitions against knowledge of tooth replacement mechanisms arising from recent EvoDevo and historical research.
- Identify replacement mechanisms which are unexplained or little understood, therefore do not correspond with current knowledge, and where appropriate, propose alternative developmental hypotheses. Such findings may open up new lines of enquiry for the EvoDevo research community.

I will address both aims by evaluating two tooth replacement mechanisms unexplained by current knowledge: those observed in certain species of pycnodonts and modern sparids.

Next, I will address the first and third aims:

- Evaluate the tooth replacement mechanisms in fossil and modern fish dentitions against knowledge of tooth replacement mechanisms arising from recent EvoDevo and historical research.
- Identify replacement mechanisms which correspond to/are consistent with known mechanisms. These will further our understanding of established, known replacement mechanisms, either by confirming their presence in new individuals of a species, or by revealing their occurrence in other taxa.

I will address both aims by evaluating tooth replacement mechanisms identified in the study specimens, which are explained by and consistent with current knowledge.

5.2 An unexplained tooth replacement mechanism in pycnodont fishes

5.2.1 Small tooth irregular patterning in fossil pycnodont dentitions in relation to current understanding of tooth replacement

Irregular patterning of small teeth in the vomer and prearticular dentitions of certain pycnodont species is described here from surface feature observations (see section 4.2.2). This patterning presents a challenge to interpret in terms of how the teeth developed, based on our current understanding of tooth development and replacement, discussed in chapter two. ‘Patches’ or regions of irregularly patterned, multiple small, rounded teeth are located at the anterior of the dentitions, where large teeth are absent from regularly patterned rows. They appear not to have followed a one-for-one replacement pattern. e.g. on the anterior of the *Pycnodus zeaformis* mature vomer (Fig. 4.1). Being much smaller, the rounded irregularly patterned teeth are more numerous per unit area than the large teeth, which they have somehow taken the place of on the dentigerous bone. If direct tooth replacement had occurred, it appears that multiple small teeth have replaced a single large tooth, at one locus. As far as I can ascertain, there is no known developmental mechanism for this scenario in osteichthyans, or more widely in the gnathostomes; only the replacement of one tooth by one other, at any one time. As described previously, this is facilitated by an epithelial connection between the predecessor tooth and its replacement, in its varied forms. The only example found here in the literature that varies from a one-for-one predecessor/replacement ratio is that of *Gasterosteus aculeatus,* the three-spined stickleback. In this study, Square *et al.* (2021) describe examples of multiple teeth being replaced by one tooth; singular tooth germs were observed to be 'abutting' two erupted teeth, both of which showed signs of dislodgement. These were interpreted to be possible 'one-for-two' replacement events. Square suspected that other variations to the one-for-one ratio were present, including two-for-one, however reliable evidence of this was not obtained (pers. comm. 2021). A hypothesis of how Square *et al.*'s (2021) findings could relate to tooth replacement in pycnodonts is discussed below.

As well as multiple small teeth forming patches at the anterior of some study specimens, every study specimen exhibited small teeth located in varied, seemingly unrelated locations across the dentitions, in varying numbers. They appeared to have developed in even the smallest of geometric gaps between the large, regularly arranged teeth, and less frequently, where parts of large teeth have broken off. This gives a strong impression that the small teeth opportunistically developed in gaps on the dentigerous bone. More simply, the positioning of the small round teeth appears to be 'gap-filling'. It certainly appears unlikely, if not impossible, that small teeth developed before the large teeth, given the latter's arrangement in orderly rows. How would the small teeth have developed, if they filled gaps between large teeth on the dentigerous bone? From the known replacement mechanisms reviewed (chapter two), there seems to be no solution offered, as each new, replacement tooth is always associated with a predecessor, which appears to offer little freedom to gap-fill across the dentition (see below for a discussion re. gap-filling addition). Alongside this question, there also appears to be no solution as to how multiple teeth have apparently replaced singular large teeth.

5.2.2 XCT virtual sections of fossil pycnodont specimens in relation to current understanding of tooth replacement

The XCT virtual sections obtained in the present study aimed to visualise the specimens' internal structure and gain insight into tooth replacement mechanisms present *in vivo*. Can these offer answers to the above questions, arising from surface observations of the pycnodont vomer and prearticular dentitions?

The pycnodont XCT virtual sections all show teeth exhibiting extraosseous development (not replacement) on the dentigerous bone surface (Trapani 2001; Berkovitz & Shellis 2017). They do not show a discernible one-for-one relationship between any of the functional teeth, and a successor. Consistent with the surface feature observations, in many of the specimens, small teeth are seen to have developed, or are in the process of developing in gaps between both small and large teeth, as well as in areas exposed by parts of large teeth breaking off. The gaps between teeth are often very small, which gives the impression of developing teeth 'squeezing into', or growing to fit, whatever sized gap is available. Interestingly, around some small teeth, a zone of resorbed tissue is visible which cross-cuts the pre-existing tissue of neighbouring teeth. These cross-cutting patterns show that the small teeth are indeed younger than the larger teeth regularly patterned in rows, as suggested by the surface observation of how they seem to ‘fit around’ the existing rows.

Though there is evidence of neighbouring tissue having been resorbed by the developing teeth, there is no evidence of any direct one-for-one tooth replacement, where a predecessor tooth is in the process of being resorbed at the base and shed in favour of a successor, as is usual for osteichthyans (Witten & Huysseune 2009; Chen *et al.* 2016; Johanson 2017). Furthermore, the development of the small teeth does not appear organised, orderly and sequential, as is usual even more widely, in gnathostomes (Tucker & Fraser 2014; Berkovitz & Shellis 2017).

The only apparent, possible organisation is that small teeth ‘find’ and fill available gaps in the dentition. This patterning would have required an organising, genetic programming to instruct tooth positioning i.e. ‘if a gap exists - fill it’. Also, the data obtained in the present study relating to tooth wear supports the traditional view that large teeth were added onto the posterior of the dentition, extending the rows of large teeth, as the fish grew. This therefore also represents an organised, gap-filling addition of teeth, in this case in space created by ontogenetic growth, and patterned in orderly rows.

5.2.3 Comparison of tooth replacement in pycnodont and modern specimens

Although the morphologies of the pycnodont vomer and prearticulars seem to be functionally analogous to the crushing dentitions of the modern fish studied here, there appears to be no similarity in terms of how their teeth are replaced. Therefore, the modern specimens do not offer an insight into how pycnodonts developed new teeth without a one-for-one replacement ratio, or how developing teeth could have filled gaps.

The sparid, *Pogonias cromis*, and *Labrus bergylta* specimens all exhibit intraosseous replacement (Trapani 2001; Berkovitz & Shellis 2017), whereas the pycnodont specimens exhibit extraosseous tooth development or - assuming multiple anterior small teeth have somehow replaced singular large teeth - extraosseous replacement. Tooth replacement in the modern fish specimens is orderly, with successor teeth lined up to replace functional teeth; they are readily recognisable as typical of crown gnathostomes, in which the timing and spatial arrangement of teeth generally appear tightly programmed and controlled (Tucker & Fraser 2014; Berkovitz & Shellis 2017). In contrast, the tooth replacement observed in the pycnodont species appears disorderly, and a one-for-one replacement pattern cannot be discerned. Though in the sparids there is some flexibility of replacement tooth positioning indicated, and a change in tooth size, discussed below (section 5.3), a developmental mechanism to account for the great difference in the pycnodont observations is not discernible in the modern specimens.

5.2.4 Pycnodont tooth wear and its relationship with tooth replacement

A possible mechanism for tooth replacement in pycnodonts emerges when key observations from this study are considered alongside those of Longbottom, and her hypothesis concerning the positioning of oldest and youngest teeth on pycnodont vomer and prearticular bones. In this section, all references to this hypothesis come from Longbottom (1984).

Longbottom noted the presence of small round teeth located between large teeth in varied locations across the pycnodont vomers and prearticular bones she studied, but did not hypothesise as to why and how they developed. However, she did form a hypothesis on the origins of the patches of numerous irregularly patterned small, round teeth towards the anterior of the larger specimens. As previously discussed, Longbottom observed that on the larger, mature, *Pycnodus* vomer and prearticular bones from Mali, the most anterior regularly patterned, large teeth in rows show more wear than the posterior ones. This finding supported the long-held theory that pycnodonts 'never exhibited successional teeth' (Woodward 1893, p.433) but teeth were added to the posterior of the vomers and prearticulars, as these bones grew (Woodward 1895, p.194; Thurmond 1974. p.110). In this scenario, the most posterior of the large, regularly patterned teeth should be the least worn because they would be the most recently added, that is, the newest teeth on the bone. However, Longbottom (1984) observed that the anterior patches of small, round, irregularly patterned teeth showed 'little or no wear', implying they were in fact the newest teeth. Woodward (1893, p. 433) had even attributed these small anterior teeth to being a juvenile feature. Longbottom noted that small (juvenile) specimens from Mali had very few small round teeth. This is the case in the present study, when comparing the small *Pycnodonus zeaformis* vomer with the larger, mature vomer from the same species. In the small, juvenile vomer, large teeth in rows extend to the anterior tip, with no small, irregularly patterned round teeth present. Longbottom contended that in the mature vomers and prearticulars, if the anterior irregular patterning of small teeth were a juvenile feature they should have been the most worn on the bone, not the least. In fact, Longbottom observed that the most worn teeth were the most anterior of the large teeth in rows, sometimes with part of the tooth having 'disappeared entirely'. Due to the location of the small, round irregularly patterned teeth being immediately in front of these most worn, large teeth in rows, at the most anterior end of the bone, Longbottom reasoned that the small teeth had replaced the oldest large teeth in the region where most wear had occurred over the lifetime of the individual.

She suggested that the small round teeth progressively replaced the large, from the anterior towards the posterior.

Longbottom is uncertain about how replacement occurred, suggesting it was indirect, and ‘interstitial, with resorption of part of the pre-existing worn tooth’, concluding ‘the whole question of tooth replacement in pycnodonts merits further study’.

In the present study, consistent with Longbottom’s observations, the *Pycnodus zeaformis* mature vomer is also observed to exhibit a ‘patch’ or region of multiple, irregularly patterned small teeth, which completely covers its anterior. Other study specimens also exhibit this patterning. However, the *Pycnodus zeaformis* mature vomer is the most complete study specimen, and therefore shows most clearly how comprehensively the irregular patterning could cover the anterior. Interestingly, the XCT data show a deterioration of this vomer at its most anterior, with the structural integrity of the upper part of the dentigerous bone broken down. The location of this deterioration directly corresponds to the region above it where only the small, round, irregularly patterned teeth are present. Consistent with Longbottom’s hypothesis, the location of this deterioration could be due to it being the oldest part of the bone. The damage appears significant, suggesting that weakened, worn, old teeth could not provide this region with adequate protection during durophagous feeding. Therefore, given the apparent gap-filling patterning observed herein of small teeth elsewhere on the bone, in this and in other study specimens (section 4.2.2), I propose that the region of anterior, small, round, irregularly patterned teeth developed in the space created when the oldest large, regularly-arranged teeth were lost due to wear and /or jaw damage.

Counter to this suggestion, it is possible that the damage to, or deterioration of the dentigerous bone may represent its resorption by the small, round teeth. However, the deterioration appears too extensive to represent basal resorption of predecessor teeth and their immediate surrounding bone, and there is no known mechanism by which multiple small teeth could have replaced a single large tooth at one locus. Given the size difference of the teeth, this must have occurred. In the absence of conventional one-for-one tooth replacement, I propose that rather than direct replacement, gap-filling tooth addition has occurred, after tooth loss due to damage. In sections 5.2.8 to 5.2.11 below, I hypothesise as to how the new teeth could have been positioned to fill gaps.

In support of this hypothesis, as well as that of Longbottom, the XCT virtual sections clearly show the large teeth of the *Pycnodus zeaformis* mature vomer become progressively flatter, and therefore more worn, towards the anterior of the bone, indicating the anterior as the oldest part of the dentition. This trend is also evident in other pycnodont dentition virtual sections. Surface feature observations also show increased wear towards the anterior of the study specimens. This wear comprises increases in tooth flatness, numbers of broken teeth, lost teeth, cracks, holes, and exposed pulp cavities. Overall this increased trend in wear towards the anterior is observed in the mature *Pycnodus zeaformis* vomer, the *Pycnodus tattami* vomer, the unidentified pycnodont prearticular (*Pycnodus*?) and the prearticular specimens of *Pycnodus toliapicus*, *Anomoeodus superbus,* and *Coelodus mantelli*.

A useful next step would be a quantitative analysis of the wear marks on the anterior small teeth, compared to those on the large teeth at the back of the jaw. The anterior small teeth on the *Pycnodus zeaformis* specimen appear shinier and smoother than the most anterior, regularly patterned large teeth. Also, a comparative analysis of wear marks on small teeth and neighbouring large teeth at varied locations on the large vomer, could help test my suggestion that small teeth filled geometric gaps after large teeth were established in rows. Such an analysis would need to be interpreted with caution however, as these small teeth are generally located below the crushing surface, and therefore would likely be less worn than the large teeth, regardless of age.

Overall, the findings of this study generally support Longbottom's hypothesis, using data obtained from traditional observation of surface features, as well as XCT, a technique which was not available to Longbottom at the time of the Mali study. However, there is a significant difference in the hypotheses arising from the two studies. I propose that Longbottom's suggestion that the anterior small round teeth 'progressively replaced' the large teeth 'from the front' is incorrect. This implies a co-ordinated approach, which does not take into account the small round teeth located at varying locations across the dentition, including the posterior. Also, the 'resorption' and tooth loss which Longbottom observes comprises the absence of whole and partial teeth. It seems unlikely that a new tooth would resorb and replace only part of a predecessor, with the rest of the crown intact. Longbottom notes that no small teeth have been found 'within the pulp cavity of intact teeth', perhaps implying there was no direct replacement from underneath functional teeth, which is also absent on the XCT virtual sections. Given the correlation of the location of 'patches' of multiple small teeth with the oldest part of the dentition (as revealed by wear), as well as small teeth being located in geometric gaps between large teeth across the dentition, I suggest that the 'resorption' Longbottom observed is actually where small teeth have opportunistically filled gaps where parts of large teeth have broken off due to damage, or where teeth are lost completely.

5.2.5 Gap-filling as a wound-healing, protective function?

I suggest that the gap-filling by the small round teeth at the anterior of the *Pycnodus zeaformis* specimen, and in other study specimens with this feature, may have had a protective, healing function, post-damage and loss of the anterior large teeth. As discussed, it appears that gap-filling after tooth damage and loss was possible elsewhere on the *Pycnodus zeaformis* mature vomer, with small round teeth developing within the outline of two large teeth, where parts have broken off. This has also occurred in other study specimens e.g. the *Pycnodus maliensis* prearticular. Interestingly, in view of the debate surrounding the evolutionary relationship between teeth and skin denticles, the irregular, possibly gap-filling anterior tooth patterning observed here is reminiscent of wound-healing in some shark species. Reif (1978) showed experimentally in a leopard and nurse shark that if the skin was damaged by injury, irregularly patterned denticles filled the gap at the wound site as it healed. Prior to injury, this wound site had been filled with regularly arranged denticles in rows. This raises an intriguing question: could the pycnodonts have used an ancestral healing mechanism, common to both tooth and skin odontodes, to repair damage at the anterior of the vomers and prearticulars? Curiously, similar healing may have been observed in one of the earliest known gnathostome fossils. A broken odontode in the dentition of the early Devonian, stem gnathostome *Radotina* had been repaired with a new crown ‘inserted into the break’ (Vaškaninová *et al.* 2020).This was prior to the evolution of odontode shedding and replacement, and therefore before the evolution of ‘teeth’, as classically defined. As this has occurred in the dentition of *Radotina,* the observation supports the possibility that the pycnodonts retained an ancestral, odontode gap-filling healing mechanism, perhaps homologous to that seen in shark denticles. However, this feature would have been retained through a vast swathe of evolutionary time, from the early Devonian through to the Eocene. Given the conserved nature of the genetics underpinning tooth development however (Bei 2009; Jernvall & Thesleff 2012), this seems plausible.

5.2.6 Gap-filling tooth addition rather than direct tooth replacement in pycnodonts

Although the hypothesis proposed herein predicts that large teeth at the anterior of the *Pycnodus zeaformis* vomer, and other study specimens, were replaced by irregularly patterned small teeth, the proposed process by which this occurred is not that which is referred to as ‘tooth replacement’ in the literature. I suggest the process was, more accurately, indirect replacement, facilitated by gap-filling tooth addition. This is because small round teeth developed at, or were ‘added’ to, the anterior dentition, in space created by the damage and loss of the oldest teeth on the bone. There was no co-ordinated one-for-one replacement, as is usual in osteichthyans. I propose that the specimens exhibit gap-filling addition not only at their anterior, but across the whole dentigerous bone, in gaps caused by damage (whole and partial tooth loss), and the geometry of closely-aligned, large elliptical teeth in rows.

As such it is useful to briefly address the difference between the terms ‘tooth addition’ and ‘tooth replacement’, as used in the literature, in order to use current understanding of both processes to best inform a hypothesis as to how the irregularly patterned pycnodont teeth developed.

‘Tooth addition’ is the development of new teeth when space is provided by the growth of the underlying jaw (Chen *et al.* 2016). Examples include sequential addition of teeth in rows in lungfish toothplates (Smith & Krupina 2001) and within the placoderms, arthrodire gnathal plates (Rücklin *et al.* 2012). I suggest that the initiation of the first generation of all vertebrate teeth can be regarded as ‘tooth addition’, as no replacement has yet taken place. ‘Tooth replacement’ is when new teeth are added at faster rate than the jaw grows, and space is created for them by moving old teeth out of the way (Chen *et al.* 2016). As previously described, in osteichthyans, including ourselves, the basal tissues of the predecessor are resorbed by the new tooth before the crowns are subsequently shed (Witten & Huysseune 2009; Berkovitz & Shellis 2017). Evidence for this trait has emerged in some of the earliest osteichthyans: *Andreolepis hedei* (Chen *et al.* 2016, 2017) and *Lophosteus superbus* (Chen *et al.* 2020).

I suggest that unusually, and perhaps uniquely, the small round pycnodont teeth of this study exhibit tooth addition that was not just filling space created by ontogenetic growth (though I contend it would have should the opportunity have arisen). Instead, I propose that they were filling space created by damage, and filling the geometric gaps between established teeth. I do however suggest that the large teeth added to the back of the regularly patterned tooth rows, were filling space created by ontogenetic growth. The decreased wear towards the posterior of these rows supports this suggestion. This trend also supports that traditional view (with respect to the large teeth only), that pycnodont teeth were added on at the posterior, as the fish and therefore the dentigerous bone grew.

5.2.7 Towards a hypothesis for the developmental mechanism responsible for gap-filling tooth addition in pycnodonts

Given what is known about how new teeth are generated in vertebrate dentitions, key questions arise in terms of a developmental mechanism which could facilitate gap-filling tooth addition in pycnodonts. As Juuri *et al.* (2013) state: tooth renewal is initiated from epithelium associated with existing teeth. We have seen that in gnathostomes, generation of a new tooth requires an epithelial connection to a source of stem/progenitor cells. In osteichthyans, this comprises a direct epithelial connection with the predecessor tooth, or its immediately adjacent epithelium .This connection takes the form of an SDL, a direct connection with the predecessor ODE, or perhaps a region (a ‘collar’) of surrounding epithelium, the SDE (Square *et al.* 2021). If, in the pycnodont dentitions, new teeth had been positioned to fill gaps, which teeth could to fulfil the role of predecessor, and provide this essential epithelial connection?

The only two options appear to be either teeth which have been lost through damage, or existing, neighbouring teeth. Considering the former, some retained or remnant dental epithelia from the lost teeth may have existed for continued tooth competence in that region. This perhaps could have been either a successional dental lamina, or cells of the lost tooth’s outer dental epithelium. However, I am not aware of any such retention of regenerative tissue in modern fish. Access to regenerative epithelia is consistently reported as via a predecessor tooth present in the dentition, albeit often on the path to eruption and being shed (Huysseune & Thesleff 2004; Huysseune 2006; Fraser *et al.* 2013; Tucker & Fraser 2014). Also, this mechanism would not explain the development of new teeth in the geometric gaps between large teeth in rows, where no tooth loss appears to have occurred.

Considering the second option; when a new tooth develops, could a neighbouring tooth provide the epithelial connection required to provide access to stem/progenitor cells, fulfilling the role of the predecessor?

The proposal by Square *et al.* (2021) of regenerative epithelium comprising an SDE, rather than the discrete structure of an SDL, potentially ‘frees’ new teeth from the requirement to develop from a single specific predecessor. Rather, they develop from a region of epithelium, or in the case of the three-spined stickleback, a ‘collar’ surrounding a predecessor. Square considered it likely that there were ‘two-for-one’ replacement events happening in the oral dentitions of three-spined sticklebacks (pers. comm. 2021). There may be therefore be potential for new pycnodont teeth to have generated from SDE regions surrounding established teeth. If this were the case, new pycnodont teeth could only develop in proximity to the predecessor, as the SDE immediately surrounds the erupted tooth, in a ‘shallow collar of epithelial cells’ (Square *et al.* 2021). However, small, round pycnodont teeth sometimes occur relatively far away from a neighbouring tooth e.g. at the margins of the *Coelodus mantelli* prearticular (Fig. 4.7). Also, development from an SDE may not afford small, round pycnodont teeth the spatial freedom to gap-fill at any position across the dentition. It is difficult to assess if this could have occurred. In three-spined sticklebacks, development from an SDE precedes the loss of the tooth around which the SDE forms the collar. With pycnodont small round teeth seemingly frequently ‘squeezed’ in between large teeth, without the large teeth having been shed, it does not seem likely that this is the underlying mechanism for the small tooth development.

The next logical step is to identify developmental mechanisms which are known to result in tooth addition, rather than replacement. One mechanism is that of ‘serial addition’ in mammals (Juuri *et al.* 2013; Tucker & Fraser 2014). This is where permanent molars are sequentially added along the rear of the dentition, e.g. in almost all placental mammals including humans (Tucker & Fraser 2014). The new molars develop from an SDL budding from its neighbour, in a very similar way to how a replacement mammal tooth develops. However the successional tooth’s final position is adjacent to the predecessor, rather than in its place, and both function concurrently in the jaw (Juuri *et al.* 2013; Tucker & Fraser 2014). Tucker & Fraser (2014) describe this as horizontal, rather than vertical development. Interestingly, in order to compensate for wear and tooth loss in the most anterior molars, this process continues throughout ontogeny in five of the 5500 known mammal species: the nabarlek, or the little rock wallaby, *Petrogale concinna*, (Sanson *et al.* 1985), three manatee species (Domning 1982; Domning & Hayek 1984) and the silvery mole-rat, *Heliophobius argenteocinereus*, (Rodrigues *et al.* 2011). As this is an orderly, sequential process, it is possible this is similar to how large pycnodont teeth were added on to the posterior of regularly patterned tooth rows, as space was created by ontogenetic growth. In fish, the SDL is transient, unlike in mammals and reptiles, but it can give rise to an adjacent tooth, rather than a replacement in the same location (i.e. ‘horizontal’ development, as described above for mammal serial addition). Indeed, an orderly mechanism of this type is thought to facilitate tooth addition in lungfish (Smith & Krupina 2001) and arthrodires (Rücklin *et al.* 2012), as teeth add sequentially as the tooth plates underlying them grow. These examples are described as statodont dentitions, where teeth are not resorbed, shed and replaced, but all are retained to function together (Smith & Krupina 2001; Rücklin *et al.* 2012; Berkovitz & Shellis 2017).

It is challenging to envisage how this addition mechanism would work to produce new teeth in the gap-filling, irregular tooth patterning of the small teeth in pycnodonts, as opposed to the orderly patterning of the large teeth in rows. It would require an epithelial connection with a tooth, to give rise to each new small tooth formed. The XCT virtual sections did not seem to suggest such a mechanism governed small tooth positioning, as small round teeth were not orientated in such a way that they were associated with one particular neighbour (though it is not possible to be certain of this). There was certainly no sign of orderly patterning. Rather, small teeth appeared to be generated at just the right position to fill a gap between larger neighbouring teeth.

In general, as there is no identifiable one-for-one relationship, indicating an odontogenic epithelial connection between a predecessor and a successor in the pycnodonts dentitions, it is not possible to attribute any known regenerative mechanism to their small, irregularly patterned teeth.

5.2.8 A hypothesised tooth competence throughout the oral epithelium

Overall, the nature of the gap-filling pattern of teeth in pycnodonts seems to indicate that space is the deciding factor as to whether a new tooth develops, not whether there is a predecessor able to give rise to a new tooth in a particular gap. The simplest solution would be for the entire oral epithelium on the dentigerous bone to be able to generate new teeth, and fill gaps, throughout ontogeny. I suggest that this could have been possible, and hypothesise that the oral epithelium retained its initiatory potential for tooth addition (Fraser & Thiery 2019) throughout life; the same dental competence that forms the first teeth/primary dentition.

As defined by Tucker & Fraser (2014), dental regeneration is a ‘repeated and cyclical induction of tooth germs, from a set of well-maintained dental progenitor cells at each tooth position in the jaw, primed to replace each functional tooth… ’. Aside from the situation here comprising *additional* tooth germs as opposed to replacements at a particular site, the need for access to stem/progenitor cells is evidently necessary, if the pycnodont oral epithelium is to retain lifelong dental competence. I propose that if pycnodonts were able to develop teeth in gaps anywhere on this oral epithelium, the stem/progenitor cells would need to reside in, and extend throughout, the oral epithelium. From the review herein of developmental mechanisms in modern fish, is there a means by which this could occur?

5.2.9 Further insight from research on taste buds

We have seen that putative stem cell niches located at the oral epithelium have been identified in cichlids (Fraser *et al.* 2013), as well as the bearded dragon (Salomies *et al.* 2019) and the small-spotted catshark (Martin *et al.* 2016; Fraser *et al.* 2020) . We have seen from this same research in the small-spotted catshark and the bearded dragon the proximity of the oral epithelium putative stem cell/dental progenitor niche with nearby taste-buds. In the small-spotted catshark, the region of the early oral epithelium called the odontogustatory band (OGB) is bi-functional; it contains putative stem/progenitor cells that give rise to both teeth and taste buds in close proximity (Martin *et al.* 2016).

It is reasonable to infer that the oral epithelium of pycnodont vomer and prearticular dentition contained taste buds. The chemical senses of olfaction and gustation are the most ancient of the sensory systems, evolving 500 million years ago (Hara 1994). As discussed previously, this was before the evolution of odontodes. Fish are more sensitive than mammals to water-soluble chemicals compounds, and taste is vital for the acceptance or rejection of food items (Hara 1994). In modern fishes, the taste buds are distributed widely, with variations between species, including in the lips, gill-rakers, pharynx, oral cavity, and on the body surface (Ishimaru *et al.* 2005).

Further to the work of Martin *et al.* (2016), showing the dual fate of putative stem/progenitor cells in the small-spotted catshark as taste buds or teeth, Bloomquist *et al.* (2019) have shown that in cichlids and mice, the oral epithelium retains a ‘plasticity’ to form either tooth and taste-like cell types. They found that the BMP signalling pathway is key in deciding either tooth or taste bud fate in stem/progenitor cells (ascertained by exposing cichlids to BMP inhibitors). Donati & Watt (2015) review several examples of ‘inter-organ plasticity’, to which Bloomquist *et al.* (2019) add teeth and taste buds. These include cells fated to develop scales which can be experimentally switched to form feathers (Wu *et al.* 2018) , and those fated to develop teeth can be switched to form hair in mice (Yoshizaki *et al.* 2014). Donati & Watt (2015) discuss how within each type of epithelium of the human body, many different compartmentalised stem cell populations exist, however, they are able to exhibit significant plasticity ‘when perturbed’. Intriguingly, in view of the present hypothesis for how gap-filling pycnodont teeth develop, this includes response to tissue damage (as well as transplantation, or tumour development). Examples are stem cells which would usually contribute to structures called the bulge or isthmus in hair follicles changing their cell type to those which repair the follicle epithelium more widely, with similar transformations happening in the intestine after damage. It is conceivable that stem/progenitor cells present throughout the pycnodont oral epithelium, replenishing taste buds throughout ontogeny, were able to switch to the development of new teeth, perhaps in response to damage, or another environmental trigger (see next section). Damage of the anterior dentigerous bone on the *Pycnodus zeaformis* vomer is evident, as well as broken teeth elsewhere on the bone. A response from the oral epithelium to develop new teeth would prevent further injury to the soft tissue. A similar response drawing on epithelial stem cells (though probably not taste-associated) is likely used in the previously mentioned example of wound healing in shark skin, producing scales (denticles) at the site of injury. It is known that unlike teeth, new skin denticles arise ‘on demand’ and not continually, only with growth, as space allows, or after damage or wounding (Martin *et al.* 2016).

Overall, though the small, round pycnodont teeth do not seem to have a predecessor tooth from which to receive stem/progenitor cells (as is deemed necessary for tooth production), it seems plausible that alternative mechanisms may exist which the pycnodont oral epithelium could have drawn on, to protect itself and gap-fill. It may have utilised an ancient, gap-filling odontode mechanism shared with skin denticles in sharks to develop new teeth, as a protective or healing measure. Or, as cells generating other epithelial appendages such feathers and hair can ‘plastically’ switch developmental pathways, it seems possible that cells fated to develop taste buds in the pycnodont oral epithelium could have switched to develop teeth. Or, quite simply, the genetic interactions which enable the development of the first dentition within the oral epithelium, creating ‘tooth competent’ regions, could be retained throughout life, which perhaps involve the at least some of the same genetic machinery governing skin wound-healing and ‘inter-organ plasticity’. It seems that a future area of research could explore whether a new tooth necessarily has to be associated with a predecessor tooth, and whether they could derive the necessary stem/progenitor cells from elsewhere. We have seen in the account of research herein that examples are emerging which don’t ‘fit’ the long-established model of ‘a dental lamina is required for tooth regeneration’ e.g. three-spined sticklebacks, African bichir, Atlantic salmon. However, as discussed, epithelial regions strongly associated with the predecessor tooth provide the regenerative function of an SDL in these examples. An alternative, non-dental source of stem cells could help explain the arrangement of new teeth in the Atlantic cod premaxillae, about which Holmbakken & Fosse (1973) state: ‘…the epithelium covering the whole broad tooth-bearing area seems to have the capacity of forming new tooth germs at any site at any time', and they describe the patterning as 'haphazard'. Although such 'free', irregular tooth positioning sounds very similar to that of small teeth in the pycnodont dentitions presented here, Holmbakken & Fosse (1973) somewhat 'backtrack', going on to say that 'each germ certainly originates from the epithelium close to the functional tooth to be replaced'. Therefore, it seems more likely that the Atlantic cod is in fact producing new, successional new teeth from an SDL, or perhaps an SDE of a predecessor. The latter perhaps is more likely, as it may allow a more irregular, 'haphazard' tooth patterning (see discussion below).

It would be interesting to assess the distribution of taste buds on modern fish crushing dentitions to gain an indication of whether there might have been extensive taste buds on an analogous crushing dentition of the pycnodonts. A specimen of the sparid Karanteen seabream, *Crenidens crenidens*, had 3460 taste buds in the main tooth-bearing regions of the oral and pharyngeal jaws combined (Fishelson *et al.* 2014), this species having a standard length of 206mm. This could imply a relatively strong presence of taste bud progenitor cells within this modern crushing dentition, and could be a useful potential area for further study.

Although it has not been possible to confidently identify a mechanism in extant fish which the pycnodont epithelium could have utilised to create gap-filling tooth addition, I contend that from the extant examples discussed, there is potential for it to have occurred. It appears there is still much to discover, with Bloomquist *et al.* (2019) even proposing that the 'under-appreciated stem cell populations' which give rise to either taste buds or teeth, offer potential bioengineering solutions to human tooth loss.

5.2.10 An adaptive phenotypic response to mechanical strain?

A gap-filling tooth addition in pycnodonts as a response to damage or injury seems plausible, given the deterioration of the anterior dentigerous bone observed in the XCT virtual section of the *Pycnodus zeaformis* vomer. However, would injury induce the observed gap-filling in the often extremely small geometric gaps between the large, elliptical teeth arranged in rows? Assuming there was a way for teeth to freely develop at a any position to fill any gap, what would the mechanism have been to initiate development? Would injury or damage always be a pre-requisite?

I hypothesise that in the pycnodont vomers and prearticulars, gaps caused by tooth damage and loss, as well as gaps caused by the geometry of the large elliptical teeth arranged in rows were all registered as an absence of pressure or ‘mechanical strain’ at the crushing surface during feeding, and that this absence of pressure initiated new tooth development. This gap-filling strategy could have been selected for as the crushing and grinding of molluscs on dentigerous bone surface was a risk to all exposed epithelium, even in the smallest of geometric gaps, especially over a lifetime. A general genetic programming of ‘if a gap exists – fill it’ would not only heal damaged areas, but provide a protective function against future damage. If this were the case, how could such a pressure-sensing mechanism function?

Though impossible to test the existence of a pressure sensitive mechanism in the extinct pycnodont species, a precedent is provided in nature in some extant species. We have seen that mechanical strain is detected in the dentition of some African cichlids (section 2.6.5), the miniature pig, and in humans. Research has shown that in humans, bite-force induced the eruption of a permanent replacement tooth (Sarrafpour *et al.* 2013). In the miniature pig and humans, release of pressure in the mandible (caused by eruption of the predecessor) triggered development of a permanent replacement tooth, from the resting SDL (Wu *et al.* 2020; Wu & Wang 2020).

Perhaps the pressure-sensitive mechanism which is most relevant to the current hypothesised gap-filling tooth addition in pycnodonts is the adaptive response of African cichlids to mechanical strain. As discussed, studies of the cichlid *Astatoreochromis alluaudi* (e.g. Gunter *et al.* 2013; Gunter & Meyer 2014; Schneider *et al.* 2014) show that changes in mechanical strain are sensed at the crushing surface of its pharyngeal jaws, and that these changes are caused by variations in hardness of the fishes' diets. The developmental response – speculated herein to involve the signalling pathways identified by Fraser *et al.* (2013), crucial to the shape, size and production rate of replacement teeth in cichlids - produces two tooth phenotypes. These are either large 'molariform' crushing teeth (in dentitions exposed to the hard diet), or small round teeth (in dentitions exposed to the soft diet) (Huysseune 1995, 2000; Gunter *et al.* 2013; Gunter & Meyer 2014; Schneider *et al.* 2014). In support of these studies' findings, Hulsey *et al.* (2008) showed that in the cichlid *Herichthys minckley*, the large, molariform teeth developed in the region of the lower pharyngeal jaw under the greatest mechanical strain during food intake. As discussed, Huysseune (2000) postulated that in *Astatoreochromis alluaudi*, once the mechanical strain change is registered at the crushing surface, decision-making about tooth shape occurs either in the oral epithelium or the underlying mesenchyme. This suggestion was subsequently supported by Schneider *et al.* (2014), who started to uncover pathways of a regulatory gene network responsive to mechanical strain, underlying the phenotypic plasticity. This network includes transcription factors which are directly responsive to mechanical strain, e.g. AP1, which takes a central role (see Schneider *et al.* 2014, fig. 7).

The large and small tooth phenotypes in *Astatoreochromis alluaudi* are strikingly similar to those of the pycnodont specimens, with the large, crushing molariform teeth having an analogous function, and both also having contrastingly much smaller teeth. Unlike pycnodonts, cichlids replace teeth via intraosseous, one-for-one replacement (Huysseune 1995, 2000; Fraser *et al.* 2013), and as described, pycnodonts do not have any form of successional replacement. However, I propose that the registering of mechanical strain in *Astatoreochromis alluaudi* and other cichlid species at the oral epithelium, inducing a significant change in tooth morphology, is important and relevant to the findings of the present study. I suggest it provides a means whereby a gap may have been registered as a lack of pressure in the pycnodont oral epithelium, subsequently resulting in the development of a new tooth of a significantly different size and morphology to others existing in the dentition.

5.2.11 How did small pycnodont teeth fit gaps so effectively?

The question of how the small pycnodont teeth appear to have developed to exactly the right size and shape to fill even the smallest of gaps in intriguing. The study by Bemis & Bemis (2015), on tooth replacement in the Atlantic wolffish, *Anarhichas lupus* appears relevant here, as the molariform oral teeth fit perfectly together, tessellating to form a continuous gap-free crushing surface. This is achieved despite teeth individually varying in shape and size. Bemis & Bemis (2015) describe this as a space-filling pattern, which they term ‘anamestic’. They suggest the pattern is likely facilitated by the teeth all growing simultaneously, meaning that at any one time, teeth are all at the same stage of development. This is not the case in pycnodonts, and so their development of teeth which are ‘just the right size’ for gaps cannot be via this process. However, the space-filling growth of individual teeth in the Atlantic wolffish does suggest a mechanism for each tooth to sense its own boundary, as well those of its immediate neighbours, which perhaps has an analogue in the pycnodonts.

There are a number of modern fish which have closely packed molariform, crushing dentitions which appear functionally analogous to those of the pycnodonts. While their teeth are not perfectly tessellated, like in the Atlantic wolffish, many have near continuous crushing surfaces, like *Pogonias cromis*, the black drum studied herein. This, the various sparid species, and *Labrus bergylta* (ballan wrasse) specimens of the present study all have crushing dentitions which have closely-packed tooth patterning to varying degrees. Tessellation of attachment tissues is observed in the the whitebone porgy sparid study specimens (see below discussion), and an 'inter-tooth' mechanosensory mechanism postulated. Despite their very different replacement systems, it is possible that pycnodonts used whichever mechanisms to gap-fill that these modern species use to closely pack teeth together.

To summarise, the existence of phenotypic plasticity in cichlids, resulting in significant change in tooth size and morphology, suggests that a mechanism exists in nature by which pycnodonts could have mechanically sensed, and filled gaps in their crushing dentitions with small, round teeth. How those teeth grew to fill gaps seemingly perfectly is uncertain, but there may be potential in exploring how close-packing and tessellation is achieved in modern fish (discussed below).

5.2.12 New evidence of putative mechanoreception in pycnodont dentitions

Cooper *et al.* (2022) recently reported the presence of the pycnodont *Phacodus punctatus*, as regurgitalites, in the Late Cretaceous, (Maastrichtian) phosphate beds of the Moroccan Oulad Abdoun Basin. In doing so, they also unexpectedly uncovered some highly unusual structures in the vomer and prearticulars of this species, which the authors speculate may have a mechanosensory function. The species has long been known as unique among pycnodonts for exhibiting dental ‘pitting’; fine punctations which cover the surface of the teeth (Cooper *et al.* 2022, references therein). The pits are so distinctive that the authors were able to reliably identify the species even from isolated teeth. Although a long-recognised feature of *Phacodus punctatus* (Dixon 1850), the function of the punctuations had never been understood or investigated. Cooper *et al.* (2022) made petrological thin sections of the specimens, and used scanning electron microscopy (SEM) to examine the punctuations’ form and structure.

Vertical petrological thin sections of a medial tooth of *Phacodus punctatus* (from either a vomer or prearticular) show the punctuations or pits are the openings of ‘canal-like dental tubules’ that run continually from the surface, through the enameloid and dentine layers and into the pulp cavity (see Cooper *et al.* 2022, fig. 6). The authors note they appear morphologically similar to dentine tubules (Mjör & Nordahl 1996), however such tubules have never been observed to run through the enamel/enameloid layer, as well as the dentinous layer. As such, Cooper *et al.* (2022) contend that proposing a function for the tubules is challenging. They initially observe how the tubules resemble the small channels (canaliculi) that contain osteocytes in vertebrate bone, however these have never been found in enamel/enamloid. The authors propose that alternatively, a sensory function could be inferred, due to the tubules running all the way through the enameloid to the tooth surface, combined with their high concentration on the surface. They speculate that the tubules could have contained nerves for mechanoreception, as their high concentration would confer a high sensitivity to mechanical pressure/strain during feeding. This could have served a protective function for the tooth, enabling detection of food hardness, and therefore which food items are safe to crush. Such a mechanism has been observed within mammal teeth, but only in the dentine and periodontal ligament, not the enamel (e.g. Chudler *et al.* 1985; Dong *et al.* 1993). However, it is interesting to note the instances of mechanoreception linked to mammal permanent tooth eruption (Sarrafpour *et al.* 2013; Wu *et al.* 2020; Wu & Wang 2020) and cichlid phenotypic plasticity (e.g. Schneider *et al.* 2014) described herein, although there is no involvement of tubules such as those seen in *Phacodus punctatus*, or to my knowledge, the nervous system.

Overall, the putative pressure-detecting mechanism identified in the pycnodont *Phacodus punctatus* differs from that which underpins the proposed hypothesis herein, as the former detects pressure created by feeding at the enameloid surface of the tooth, inducing nervous transmission of messages to the brain. The latter mechanism is located in the oral epithelium, detecting gaps between teeth as a lack of pressure, inducing a response to develop new teeth and fill the gaps. The findings of Cooper *et al.* (2022) are nevertheless significant for the proposed hypothesis of the current study. The punctuations and tubules in the vomers and prearticulars of *Phacodus punctatus* show that mechanoreception could have been present in this pycnodont species. I suggest this increases the plausibility that mechanoreception could also have been present in the oral epithelium of vomers and prearticulars in other pycnodont species. This would provide a means of positioning new teeth, developed as a phenotypically plastic response to gaps between teeth, whether created by tooth loss, damage, or the geometry of the large tooth rows.

5.2.13 An alternative interpretation

An alternative interpretation to the gap-filling hypothesis proposed herein could be that the small round teeth are denticles, and not teeth. As discussed previously, denticles and teeth are very similar structures (‘odontodes’). They share a deep homology in terms of tissues (e.g. dentine and enamel-like hard tissues) and developmental processes; as with all epithelial appendages (e.g. feathers, hair, mammary glands), they both develop from an epithelial placode. Indeed, Cooper *et al.* (2017) provided evidence for genetic homology between the placode development in shark denticles and that of the integumentary organs in amniotes, including mammals, conserved over 450 million years of evolution.

As previously discussed, skin denticles have been central to the debate on the origin of teeth, with Fraser *et al.* (2010) contending that odontodes could have developed ‘inside *and* out’ and that whichever occurred first is not important. Arguably, this view offers a convincing explanation of odontode homology, and so is helpful to briefly revisit here when considering the identity of the small, irregularly patterned pycnodont teeth. Fraser *et al.* (2010) propose that wherever and whenever epithelial and neural crest-derived ectomesenchymal gene networks came into contact and signalled to each other during development, odontodes would have evolved. They propose that the resulting odontode gene regulatory network (oGRN) was common to all odontodes regardless of location on the body, but only the oGRN located in the oropharynx became the tooth (dental) GRN. This is speculated to have arisen from the co-option of the GRN of taste buds in the oropharynx, conferring taste buds’ valuable capacity for continual lifelong replacement to teeth (but not to skin odontodes) (Martin *et al.* 2016). This key difference is often used as a defining feature of teeth; the ability to generate connected/associated successional replacements in an organised and controlled way, as well as in a patterned distribution (e.g. Fraser *et al.* 2010; Martin *et al.* 2016) . Contrastingly, denticles have unrelated replacements, and are often in a non-patterned, random distribution;

the opposite of what is considered usual for teeth (Fraser *et al.* 2010). A key point to note here, which can be overlooked when considering the origins of teeth, is that denticles can occur in the oropharyngeal cavity too in some osteichthyans. These oral denticles are sometimes simply referred to as ‘odontodes’, as opposed to teeth (Fraser *et al.* 2010; Chen *et al.* 2016; Doeland *et al.* 2019). As such, the small round teeth in pycnodonts could be interpreted to be oral denticles, especially when their irregular patterning is considered; as previously mentioned, when the skin of some shark species is damaged by injury, the resulting gap is filled by denticles in an irregular pattern (Reif 1978).

Despite the gap-filling pattern observed in the pycnodont specimens, I consider it highly unlikely that the small round odontodes are oral denticles. To my knowledge, no actinopterygian has exhibited the co-existence of teeth and denticles in the oropharyngeal cavity, and from the XCT data, they exhibit the tissues of true teeth. Also, oral denticles, unlike skin denticles, have not shown a patterning of gap-filling between teeth. As discussed, recent analyses of fossils of stem osteichthyans, and early sarcopterygians, summarised by King *et al.* (2021), have shown that in these very early fishes, the same dentigerous bone could exhibit both teeth and oral denticles/odontodes. However, in none of these early species did the denticles appear to develop opportunistically in gaps between teeth, in the manner observed in the pycnodonts. Denticles ‘overgrew’ previous ones, with layers of bone sometimes deposited first, on which denticles developed (Chen *et al.* 2016). Denticles are ‘graded’ spatially with teeth in terms of their position on the bone. King *et al.* (2021) give the example from the early sarcopterygian *Powichthys*, which exhibits a ‘continuum of odontode replacement mechanisms in the prearticular, with progressively more resorption [therefore tooth-like character], occurring towards the bone margin'. In the stem osteichthyan *Andreolepis*, such a gradation can also be seen between the inside and outside of the jaw (Chen *et al.* 2016). The jaw margin bears 'true' teeth with evidence of basal resorption and shedding at those positions, and denticles/odontodes are present on the labial side of the jaw, on the facial surface. These denticles show evidence of some resorption and organised patterning, therefore Johanson (2017) suggests that these represent a transitional zone between external odontodes and teeth. Therefore, although these early dentitions include both denticles and teeth, none show odontodes gap-filling between teeth, as in the pycnodonts. In pycnodonts, I consider the morphology of the large elliptical odontodes and their orderly arrangement in the rows qualify them as true teeth, despite these teeth being non-replacing. As discussed previously, their strict patterning indicates an orderly developmental process, similar to that of serial addition seen in mammal molars.

Overall, I consider the small round, hypothetically gap-filling odontodes of pycnodont vomers and prearticulars to have more similarities with teeth than with oral denticles.

5.3 Unexplained tooth replacement features in sparid specimens

Previously, I speculated that the sheepshead fish may utilise a mechanosensory mechanism during hard diet feeding, plastically adapting its tooth size and shape, enabling a close-packed pattern, and achieving the increased percentage of functional jaw surface covered by teeth, observed by Worcester (2012). If such a mechanism exists in the sheepshead fish, I hypothesise it may be used more generally in species which exhibit close-packed tooth patterning in crushing dentitions e.g. *Pogonias* *cromis*, the black drum. It could also offer a mechanism by which the small round teeth of pycnodonts filled gaps so effectively.

As previously described, for the present study, modern fish dentitions were selected for investigation of their tooth replacement mechanisms. The specimens initially selected were those with crushing morphologies which appear to be functionally analogous to those of the pycnodonts. These were a large specimen from the family Sparidae (sp. indet.), the seabreams, and a specimen each of *Pogonias cromis* (the black drum) and *Labrus bergylta* (the ballan wrasse).

As described, the four other sparid specimens studied were one from each of the species *Calamus leucosteus* (whitebone porgy), *Archosargus probatocephalus* (sheepshead fish), *Sparus aurata* (gilthead seabream) and *Pagrus* auratus (silver seabream). Across the different species of sparid studied herein, their tooth patterning is reminiscent of that in the pycnodont vomer and prearticulars. Each specimen has a mixture of large and small teeth, and the range in tooth size between large and small is more gradual than in the pycnodont dentitions. The large teeth are ‘loosely’ arranged in rows. Smaller teeth are irregularly positioned in between the rows of large teeth, and their patterning is suggestive of close-packing. As in the pycnodonts, some teeth are positioned in gaps so small that they appear ‘squeezed’ between teeth. The tooth patterning in sparids generally suggests the positioning of the small teeth is gap-filling, though the gaps are not filled so efficiently and tightly as in the pycnodont dentitions.

In the black drum and ballan wrasse specimens, there was no similarity with pycnodonts in terms of how their teeth are replaced. This initially also appeared to be the case with all the sparid specimens. The XCT virtual sections of the black drum, ballan wrasse, and sparid specimens all revealed that they exhibited orderly, one-for-one, intraosseous replacement.

There was, however, an interesting, feature observed from the surface in the large sparid. Two relatively small teeth had erupted within the crown of a much larger broken tooth (Fig. 4.17A (i, ii)). This intriguing observation is puzzling for two reasons. This appears to be an example of direct two-for-one replacement, for which there is no known mechanism in osteichthyans (as was problematic in interpreting observations in the pycnodonts). Secondly, this appears to comprise a significant change in tooth size over one tooth replacement generation. As discussed previously, Huysseune (2000) contends that significant change in tooth size, shape and position is likely only achieved through successive tooth generations. This was in reference to the magnitude of change seen in cichlids through adaptive plasticity. However, I propose that the change in tooth size observed here in one tooth generation, in the large sparid, is relatively large, and that this has unusually occurred in one tooth generation.

For both these reasons - the apparent two-for-one replacement, and significant size difference between tooth generations - this observed feature is not explained by existing knowledge of tooth replacement mechanisms in osteichthyans.

5.3.1 Towards hypotheses to explain two-for-one replacement in the large sparid, and the significant size change in one tooth generation

By observation of surface features only, the NHMUK collection was searched for sparids with two-for-one tooth replacement, or replacement teeth of significantly different size to their predecessor. No other examples were found through this method. However, the XCT virtual sections of the gilthead seabream specimen, *Sparus aurata*, revealed large teeth developing underneath, and therefore replacing, much smaller, functional teeth, at two locations (Fig. 4.21). Although the example observed in the large sparid comprises small teeth replacing a large tooth (rather than large replacing small), this is still interesting as it represents a relatively large size change, over one tooth generation. In the gilthead seabream, one of the small functional teeth destined for replacement has an empty tooth position next to it, possibly to accommodate the size of the new large tooth. It is also interesting that NHMUK label of the large sparid stated an uncertain identification of *Sparus aurata*, suggesting the feature of a large change in tooth size over one tooth generation might be found in other specimens of this species. This finding supports my hypothesis that the sheepshead fish, also a sparid, may have plastically adapted to a hard food diet with a change in tooth size and shape, enabling a close-packed patterning and the observed increased percentage of functional jaw surface covered by teeth (Worcester 2012). The XCT virtual section of the sheepshead fish conducted in the present study does not however capture evidence of this, as all replacement teeth seems to be of a similar size to their predecessors. Yet, interestingly, there are small teeth which have the appearance of being ‘squeezed’ into very small gaps, implying a gap-filling patterning.

The observation in the large sparid that two small teeth appear to be replacing one large tooth is challenging to explain, particularly in light of the intraosseous replacement evident in this specimen. To my knowledge, there is no known mechanism by which this could occur. All descriptions of intraosseous replacement comprise one tooth developing in one bony crypt. It is understood that the singular tooth develops from the tip of a single epithelial strand, the SDL, which penetrates the medullary cavity, originating from one predecessor tooth.

The XCT virtual section of the sparid *Calamus leucosteus* specimen, the whitebone porgy, reveals two bony crypts fused together, containing two developing teeth. Is this a mechanism by which developing, replacement small teeth can be positioned in close enough proximity to each other to replace one large tooth? Bone remodelling to accommodate such fusion of the crypts seems possible. Another question arising is: could just one SDL have penetrated the bone to give rise to both teeth? This seems very unlikely, as this has not been seen before, in the numerous tooth replacement studies carried out across a wide range of vertebrate taxa.

The only tooth replacement mechanism I am aware of which could be partially aligned to this observation of two teeth replacing one, is that which occurs in three-spined sticklebacks, utilising an SDE (Square *et al.* 2021). If an SDE, a collar-like region of oral epithelium existed around the large predecessor tooth, functioning as the required regenerative epithelium to produce a replacement, it is plausible that two teeth could develop rather than one. This has been suggested in the three-spined stickleback (T. Square, pers. comm 2021). However, a crucial difference is that the three-spined sticklebacks exhibit extraosseous replacement, where teeth generate directly from the oral epithelium, on surface of the dentigerous bone. The sparid exhibits intraosseous replacement, where the regenerative epithelium is required to penetrate into the medullary cavity, only ever observed in the form of a strand (the SDL) (Trapani 2001; Huysseune & Thesleff 2004). Therefore, unless two SDLs could derive from one predecessor tooth's SDE, this mechanism would not be feasible. Though the notion of two SDLs arising from one predecessor tooth's SDE is highly unlikely as this has not been observed elsewhere, it may be possible.

In view of the fused bony crypts observed in the whitebone porgy, it seems much more likely that a functional tooth has been lost, and its replacement has moved its positioning to be close to a neighbouring, developing tooth, meaning that together they erupt underneath the same large tooth. Such a strategy used across the same dentition could facilitate a rapid change in tooth number, as suggested in the three-spined sticklebacks (Square *et al.* 2021) . Interestingly, a change in number, size, shape and positioning would logically also facilitate a change in tooth patterning.

5.3.2 Sparid tooth damage registered as a change in mechanical strain?

One significant feature in this example is the large crack in the crown of the predecessor tooth (Fig. 4.17A (i, ii)). It is not certain if this occurred post mortem. If it occurred *in vivo*, the examples of phenotypic plasticity in cichlids and the sheepshead fish, which also exhibit intraosseous replacement, could inform a hypothesis as to how the change in tooth size has occurred. It is possible the damage to the sparid tooth crown was registered in the dentition as a change in mechanical strain, prompting its replacement. It is only possible to speculate whether the force which caused the crack, or subsequent structural weakness in the tooth after it occurred, would provide the signal to replace it. We know that in cichlids, significant change in tooth morphology occurs through the registering of mechanical strain at the crushing surface. I have hypothesised herein, from observations made in the sparid specimens, combined with the findings of Worcester (2012), that adaptive morphological change can occur over just one tooth generation. I therefore suggest that the small teeth have developed over one generation, as a result of the damage incurred to large, predecessor tooth.

It is interesting to speculate that if the damage was registered as a lack of pressure in the weakened sparid tooth, perhaps the hypothesised programming in pycnodonts, of ‘if a gap exists, fill it’, was operating here in the large sparid. As suggested for the anterior of the *Pycnodus zeaformis* specimen, where the dentigerous bone appears damaged, such a gap-filling strategy would remedy damage, by way of registering the decrease in pressure, or a gap. In both cases, small teeth have developed at the site of damage/injury, and so it could be inferred that the generation of multiple small teeth offer more geometric flexibility than large teeth, to adequately cover and protect the oral epithelium.

5.3.3 Examples of tessellation among modern study specimens

Bemis & Bemis (2015) propose that the ‘anamestic’ patterning of oral teeth in the Atlantic wolffish, which produces a continuous crushing surface, is made possible by the highly unusual simultaneous growth of all the teeth. They describe how the successional, replacement teeth develop intraosseously within the dentigerous bone. When all overlying functional teeth are shed at the same time, the bone separating the developing teeth erodes away, allowing them to come into contact with each other. As the teeth are all still growing and at the same developmental stage, each tooth can accommodate to the shapes of adjacent teeth, resulting in no gaps in the crushing surface i.e. a tessellation of tooth shapes. Bemis & Bemis (2015) acknowledge that simultaneous replacement is very uncommon in fish, with the best known example being that of piranhas, which simultaneously replace teeth in a jaw quadrant (Shellis & Berkovitz 1976; Berkovitz & Shellis 1978). However, they also highlight other anamestic crushing dentitions, where the teeth shapes are ‘rigorously geometric’, including the pharyngeal jaws of the black drum, *Pogonias cromis.* The surface observations of the black drum lower pharyngeal jaw presented herein, are consistent with this description. XCT virtual sections of the jaw also presented here show that, as in the Atlantic wolffish, tooth replacement is intraosseous, orderly and one-for-one. However, unlike the Atlantic wolffish, tooth replacement does not occur simultaneously. In the black drum, successional teeth are concurrently at various stages of development. For example, in one XCT virtual section, one tooth has little enamel or dentine formed yet, whereas another is nearly fully formed prior to eruption. Not all functional teeth have successors in waiting, and at one position a functional tooth has been lost, beneath which a successional tooth is located, due to move into its place. Despite this range of stages of the replacement process in the lower pharyngeal jaw, the black drum maintains a near-tessellated tooth pattern of varying geometric shapes, forming a continuous crushing surface. A variation of stages in the replacement cycle is also evident in the XCT virtual sections of the lower pharyngeal jaw of the ballan wrasse, *Labrus bergylta*, presented herein. Although this species does not produce a tessellated patterning of teeth, they are efficiently close-packed. Therefore, there must be a co-ordinating system of patterning to enable close-packing and near-tesselation of teeth, which does not require their simultaneous development within the jaw. Interestingly, Bemis & Bemis (2015) suggest intraosseous replacement is necessary for such patterning. They propose that development within bone is required, so that individual teeth can accommodate shapes of adjacent teeth. The reasoning for this is not explained further. It is assumed that the previous description of erosion of the bone separating developing teeth is significant, enabling contact between adjacent teeth during growth.

An interesting, possibly novel observation is made here in the lower oral jaw of the whitebone porgy, *Calamus leucosteus* (family Sparidae). All but one of the small, pointed, non-crushing teeth are absent from the left anterior-most region, in contrast to the right where most tooth positions are filled. It is not known if this happened post-mortem, but it is intriguing that the loss is differentiated by the left and right sides of the jaw. This is resembles tooth cycling in piranhas, where the teeth in jaw quadrants are lost simultaneously (Shellis & Berkovitz 1976; Berkovitz & Shellis 1978). From the remaining attachment tissues at the empty tooth positions, the tooth attachment mode for the anterior small pointed teeth appears to be pedicellate, as does that of the large molariform teeth (Hughes *et al.* 1994; Berkovitz & Shellis 2017; Rosa *et al.* 2021). Also of interest is that the pedicels and attachment tissues between the empty tooth positions appear to have geometrically tessellated with each other. This is the case for the small pointed, anterior teeth and to a lesser extent (as they are not tessellated as well), the attachment tissues of the molariform crushing teeth on this bone. Intriguingly, the closer-packed molariform teeth on the specimen's upper jaw appear very well tessellated (middle row, Fig. 4.21E).

This observation raises the question of whether this is the case for all teeth which are closely-packed; they may not achieve perfect tessellation, but do their individual bones of attachment/attachment tissues? Is a system similar to that of the Atlantic wolffish teeth operating, whereby each attachment bone makes contact with its adjacent neighbours, accommodating to their shapes? If this is the case in the whitebone porgy, simultaneous tooth replacement in the large teeth is not required, as the XCT virtual sections reveal different stages of the tooth cycle at different tooth positions. However, is the near-perfect tessellation of tooth attachments of the anterior teeth related to the fact that all teeth are absent in that same region? Could near-perfect tessellation be achieved by simultaneous replacement, proposed as necessary in the Atlantic wolffish, in that region of the jaw only? This is an interesting idea and simultaneous tooth replacement could possibly be a strategy used where optimal tessellation of teeth or tooth attachment tissues is an advantage.

It is interesting to note that some of the smaller teeth on the upper oral jaw of the same specimen (Fig. 4.21E) are located in very small gaps between large teeth, resembling the pycnodont gap-filling patterning.

5.3.4 Anamestic patterning via mechanoreception in attachment tissues?

Berkovitz & Shellis (2017) state that Sparidae species have thecodont attachment, or implantation *sensu* Bertin *et al.* (2018). Whether all sparids have a pedicellate, fibrous attachment is uncertain from the literature. It seems likely to be common, as all sparids referred to in studies on attachment by Fink (1981), Hughes *et al.* (1994) and Berkovitz & Shellis (2017) include pedicellate attachment (or simply a fibrous attachment is identified in Fink's (1981) study; his 'Type 2' attachment mode). Following Berkovitz & Shellis (2017), all four sparid species studied herein, as well as the unidentified large sparid, appear to have pedicellate attachment. Interestingly, in the silver seabream, *Pagrus auratus*, different teeth seem to exhibit pedicellate or direct fibrous attachment, on the same dentigerous bone.

In all the sparid specimens, as well as the black drum and ballan wrasse specimens studied here, tooth patterning appears to be anamestic (space-filling) *sensu* Bemis & Bemis (2015). All exhibit either a close-packed (sparids and ballan wrasse) or near-tessellating (black drum) tooth patterning, with regions of possible opportunistic gap-filling. I hypothesise that the anamestic patterning may arise from tessellation of their tooth attachment tissues. There is evidence of such tessellation in surface observations of the specimens presented herein. Where teeth have been lost, the outline of the attachment tissues is revealed. A potentially promising area of future study would be whether the attachment tissues, either bone of attachment or fibrous tissue, have a mechanosensory property. If so, it could provide a means by which adjacent teeth interact and accommodate the shape of each other's growth. Such spatial accommodation is proposed between tessellating teeth in the Atlantic wolffish (Bemis & Bemis 2015), but the mechanism by which it is achieved is not explored in detail. In any crushing dentitions which show anamestic patterning, it is plausible that as teeth erupt and attach to the jawbone, the attachment tissues could exert mutual pressure on each other. Furthermore, thecodont attachments in fish resemble those of mammals, where periodontal ligaments are known to be mechanosensory, and therefore responsive to pressure (Sarrafpour *et al.* 2013). While the sparids have attachment bone between the lateral surface of the tooth and the jaw bone, where the periodontal ligament is present in mammals, could this too be mechanosensory? Or could the fibrous tissue present in the tooth attachment of most fish species serve this function?

The bone vs. dentinous nature of the bone of attachment in fishes has been greatly debated, as summarised by Rosa *et al.* (2021) (references therein). Rosa *et al.* (2021) view bone and dentine as a being at opposite ends of a continuum, with the bone of attachment in different fish species falling on this continuum. Hughes *et al.* (1994) describe the pedicel of sparids to comprise mainly of a dentine-like tissue, called ‘canalar dentine’, as it contains canals which in turn contain osteocytes (or in this situation, they are called ‘osteodentocytes’). Significantly, osteocytes are known to be mechanosensitive (Robling & Bonewald 2020). Also, in humans, mechanoreception involving nerve stimulation has been shown to occur within the tooth, as well as in the periodontal ligament (Levy & Dong 2022). Therefore overall, I hypothesise that either dentinous or fibrous attachment tissues of teeth within anamestic crushing dentitions are used as mechanosensors, to detect the presence and shape of adjacent teeth, and tesselate with them during growth. It is tempting to compare the canals described by Hughes *et al.* (1994) of the sparid pedicel canalar dentine to the tubules observed by Cooper *et al.* (2022), in the crushing dentition of the pycnodont *Phacodus punctatus.* Cooper *et al.* (2022) note the pycnodont tubules' similarity to tubules that contain osteocytes in mammal teeth - although these tubules run through enameloid as well as dentine in pycnodonts, which has never been observed in mammals. The pycnodont and sparid tubules are nevertheless an intriguing comparison.

Overall, mechanoreception in the teeth of actinopterygian crushing dentitions merits further study. Although mechanoreception in cichlids has been a focus for much research, I propose two new aspects to investigate. Hypotheses presented herein are that mechanoreception facilitates adaptive responses in crushing dentitions that create anamestic, space-saving patterning e.g. in the Atlantic wolffish and sparids, and also to locate gaps in a dentition and develop new teeth to fill them, as in the pycnodont vomers and prearticulars.

5.4 Tooth replacement mechanisms consistent with current knowledge

5.4.1 Intraosseous and extraosseous tooth replacement, and tooth attachment types

In all study specimens except the pycnodonts, intraosseous or extraosseous replacement was identified, with each identification in line with literature for each species. Pycnodont specimens are hypothesised herein to exhibit tooth addition, rather than replacement. Attachment types of all specimens were observed to be the same as those predicted by the literature, although the hinged attachment (type 3, *sensu* Fink (1981)) in the prearticulars of *Polypterus* genera identified by Fink (1981) and quoted by Berkovitz & Shellis (2017) was not observed on the prearticulars studied here. It was instead observed on coronoids 1 and 2, the dentaries, vomer, ectopterygoids, and possibly the anterior parasphenoid. The research references used, and the observed condition of each species are summarised in the table below.

5.4.2 Table 3. Tooth replacement and attachment modes of study specimens

Species and common name	Specimen dentition type	References used	Extraosseous (E) or intraosseous (I) replacement	Observed attachment type, according to Fink (1981) / Berkovitz & Shellis (2017)
***Labrus bergylta*, ballan wrasse**	LPJ	(Berkovitz & Shellis 2017)	I	1/ thecodont, ankylosed
***Pogonias cromis*, black drum**	LPJ	(Berkovitz & Shellis 2017)	I	1/ thecodont, ankylosed
***Pagrus auratus*, silver seabream or Australasian snapper**	oral	(Hughes *et al.* 1994; Trapani 2001)	I	2/ thecodont, pedicellate
***Archosargus probatocephalus*, sheepshead fish**	oral	(Hughes *et al.* 1994; Berkovitz & Shellis 2017)	I	2/ thecodont, pedicellate
***Calamus leucosteus*, whitebone porgy**	oral	(Hughes *et al.* 1994; Berkovitz & Shellis 2017)	I	2/ thecodont, pedicellate
***Sparus aurata*, gilthead seabream**	oral	(Hughes *et al.* 1994; Trapani 2001; Berkovitz & Shellis 2017)	I	2/ thecodont, pedicellate
***Gastrosteus aculeastus*, three-spined stickleback,**	oral	(Ellis *et al.* 2016; Square *et al.* 2021)	E	1/ ankylosed
***Gadus morhua*, Atlantic cod**	oral	(Trapani 2001; Berkovitz & Shellis 2017)	E	1/ ankylosed
***Polypterus senegalus*, African bichir**	oral	(Fink 1981; Vandenplas *et al.* 2014; Berkovitz & Shellis 2017)	E	1/ ankylosed and 3 anterior hinged

***Salmo salar*, wild Atlantic salmon**	oral	(Fink 1981; Huysseune & Witten 2008)	E	1/ ankylosed
***Amia calva*, bowfin**	oral	(Fink 1981; Trapani 2001)	E	1/ ankylosed
All pycnodont study specimens	Vomer and/or prearticular	(Kriwet 2005)	E (development, rather than replacement)	1/ ankylosed

5.4.3 Putative serial addition of sparid molariform teeth

The lower oral jaw of the sparid the gilthead seabream, *Sparus aurata*, appears to exhibit a putative serial addition of its most posterior molariform teeth, rather than replacement. The three, non-erupted, developing molariform teeth are at decreasing stages of development towards the bone surface, and angled towards the anterior. This is unusual as it suggests the teeth closest to the surface will erupt last, and in fact the larger, deeper, but more anterior teeth will erupt first. There would be no advantage to this arrangement if the teeth were replacing as usual, in the same position, suggesting serial addition. This is interesting as it is reminiscent of molar serial addition at the posterior of mammal dentitions (Juuri *et al.* 2013; Tucker & Fraser 2014) as well as the hypthothesised serial addition of large, molariform teeth to the posterior of pycnodont crushing dentitions. In both cases, the large posterior teeth are added as ontogenic growth, and therefore space allows. This perhaps also occurs in the gilthead seabream, *Sparus aurata*, and sparids more generally. Perhaps this feature has been conserved to be deployed when needed, across the vertebrates. Alternatively, it may have evolved convergently in different vertebrate groups.

5.4.4 Irregular tooth patterning in the three-spined stickleback and the Atlantic cod

In agreement with Square *et al.* (2021), tooth replacement in the oral dentition of the three-spined stickleback (*Gastrosteus aculeastus*) specimen studied herein is extraosseous. Also in agreement with their study, there appears to be no discernible regular tooth patterning. The replacement ratio is difficult to discern as there is no consistent orientation for the replacement tooth relating to a predecessor, and so relies on identifying the 'nearest' predecessor tooth. This was also observed by Square *et al.* (2021), but in the pharyngeal jaws; in the oral dentition they observed replacements developing only labially to their predecessor. In the oral dentition here, two marginal teeth have replacements developing labially, the rest are in varied orientations, as described above. However, the oral replacement tooth sample size in the study by Square *et al.* (2021) is relatively low (n=8 between two fish), therefore it is possible their observed labial positioning of replacements is not general. In the present study, two young replacement oral teeth each appear to be approximately equidistant from the two nearest functional teeth, therefore it is possible these represent a one-for-two replacement ratio. This ratio was observed by Square *et al.* (2021) in 25% of replacement events (but in the pharyngeal jaws).

In general, the patterning observed herein supports the finding of Square *et al.* (2021) of an SDE enabling tooth replacement in the three-spined stickleback, rather than an SDL. The collar-like region surrounding the predecessor comprising the SDE, allows for more spatial flexibility in terms of where the replacement tooth originates from. This could explain the observation herein of varied orientation between young replacement teeth and a predecessor. It is interesting to note that the absence of an SDL is suggested by Square *et al.* (2021) to be the reason that successive generations of replacement teeth in the stem osteichthyan *Andreolepis* are in slightly varied orientations relating to each predecessor (Chen *et al.* 2016). They also suggest that tooth replacement without an SDL, and via an SDE, might therefore be plesiomorphic among osteichthyans; an interesting potential area for future study.

The present study generally supports the findings of Holmbakken & Fosse (1973), that tooth pattering in Atlantic cod (*Gadus morhua*) is ‘haphazard’ (however, see exception below re. the dentaries). As discussed previously, Holmbakken & Fosse (1973) state that ‘each germ certainly originates from the epithelium close to the functional tooth to be replaced’, but perhaps somewhat counter to this, that developing teeth are ‘scattered in a seemingly haphazard manner among the ankylosed teeth’. This suggests that patterning could be due to tooth replacement via an SDE, rather than an SDL, as in the three-spined stickleback (see above, this section). Comparison with further Atlantic cod specimens is needed, as the specimen used herein is dry, and many teeth have likely been lost post-mortem. Also, interestingly, this specimen has a repeating sequence along the dentaries, of three replacement teeth in increasingly advanced stages of development, culminating in a functional marginal tooth. This indicates organisation into tooth families – the opposite of ‘haphazard’ patterning. This represents greatly contrasting patterning in the same dentition. Speculatively, this contrast could indicate an ability for the SDE to generate replacements in similar orientations between generations where needed, or perhaps the presence of an SDL-based tooth replacement mechanism in the dentaries only.

5.4.5 Tooth replacement in the African bichir and Atlantic salmon

In section 2.4.4, research was reviewed on the African bichir, *Polypterus senegalus* (Vandenplas *et al.* 2014), the wild Atlantic salmon, *Salmo salar* (Huysseune &

Witten 2008) and the rainbow trout, *Oncorhynchus mykiss*, (Fraser *et al.* 2004, 2006*a*, *b*), describing how in each of these species, tooth replacement occurs without an SDL. Instead, the dental organ of the replacement tooth develops directly from the outer dental epithelium (ODE) of the predecessor tooth, at the posterior-lingual side. Progression through the recognised stages of tooth development follows, with the predecessor and developing replacement tooth still connected (Fraser *et al.* 2006*b*; Huysseune & Witten 2008; Vandenplas *et al.* 2014). Unusually for bony fish, the successional tooth can start to develop another, secondary successional tooth from its own ODE, whilst still developing. This is results in two successional and one functional tooth forming a three-member family, at one functional tooth position.

The absence of an SDL prompted a search for a stem cells in the African bichir and Atlantic salmon, as the maintenance of an epithelial stem cell niche within the DL or SDL, as well as preservation of the DL or SDL itself, has generally been viewed as the premise on which polyphyodonty depends (e.g. Fraser *et al.* 2020). Investigations of 'lamina-less' tooth replacement mechanisms such as these have made an important contribution to the ongoing goal of understanding the mechanisms of polyphyodonty. They also include the previously discussed studies on tooth replacement in the three-spined stickleback (Square *et al.* 2021) and medaka (Abduweli *et al.* 2014). However, perhaps the most extensive of these investigations to date have been the aforementioned studies on the African bichir and Atlantic salmon. Identification of a putative stem cell niche was made in each species, in an equivalent epithelial location called the MDE (Huysseune & Witten 2008; Vandenplas *et al.* 2014).

In line with these studies, the surface feature observations and XCT virtual sections of the African bichir and Atlantic salmon presented here show replacement teeth in both species developing from the posterior-lingual side of the predecessor tooth. In the African bichir, if a functional tooth is undergoing replacement, only one successor is visualised in the XCT data herein, not two. This may be because a third tooth in the family has not yet developed, or never forms enameloid before the functional tooth is shed. Because only hard tissue was visualised here, if the latter is the case, the three teeth would not be visible. Interestingly, tooth families with three members can be seen in some XCT renders of the male salmon specimen, where particularly soft tissue was visualised (Fig. 4.23D, E).

Generally it was difficult to discern regular patterns in tooth development stages along the rows of adjacent teeth, in the lower jaw dentition of the Atlantic salmon, as described by Huysseune *et al.* (2007). This may be because generally the tissue visualised was not soft enough to reveal the youngest teeth. Also, it is possible the male specimen had a higher number of mature, functional, teeth than is usual in the life-cycle. This could be due to needing an optimally functioning dentition during spawning, as the hook at the tip of the lower jaw (the ‘kype’) is a secondary sexual characteristic that likely contributes to a dominance hierarchy among males at the spawning ground (Huysseune *et al.* 2007, references therein). Although identifying a pattern in developmental stages between adjacent teeth was difficult, it was however possible to discern that teeth were at various developmental stages at different tooth positions.

While generally the data herein supports previous research on tooth replacement in the African bichir and Atlantic salmon, some possible additions to current knowledge were found. In the African bichir, on the parasphenoid bone, there appeared to be some variation in where the replacement teeth were positioned with respect to their predecessors. Within this variation, there appeared to be a trend towards the right lateral side of the predecessor tooth. One potential explanation for this positional variation could be that the teeth on this bone use an SDE during tooth replacement, rather than an SDL (as was suggested for the Atlantic cod and three-spined stickleback). Elsewhere in the dentition, all replacement teeth were located on the posterior-lingual side. I also noted in the African bichir, that the hinged teeth described by Fink (1981) as attachment type 3, were present on the following bones: coronoids 1 and 2, the dentaries, vomer, ectopterygoids, and possibly a few at the anterior parasphenoid - though I am uncertain if the latter represented partial resorption of ankylosed teeth at the base. Clemen *et al.* (1998) were followed for anatomical identification of bones. Surprisingly, I could see none present on the prearticulars. However, Fink (1981) and Berkovitz & Shellis (2017) only mention the prearticulars (or splenials) as the location of the type 3 hinged tooth attachment in *Polypterus*.

5.4.6 Tooth replacement in the bowfin, *Amia calva*

The extensive coverage by teeth of the inside of the oral cavity in the bowfin study specimen greatly resembles that of the African bichir. This could relate to their proximity to each other at the base of the actinopterygian phylogeny. Miller & Radnor (1973) attempted to discern regular tooth patterning across the bowfin dentition, but with difficulty. They noted many regions of the dentition lacked pattern, a ‘randomness’ to the timing of replacements between tooth positions, and interestingly, that this randomness increased with the age of the fish. I cannot find research specifically on the mechanism of tooth replacement in the bowfin, suggesting this as a useful area for future research.

The tooth replacement mechanisms represented in the bowfin and African bichir specimens initially appear very different, but this is difficult to compare as the bowfin is a dry specimen, with developing teeth likely lost with the soft tissue. The few putatively identified replacement teeth in the bowfin did not share the same orientation in relation to their predecessor.

The seemingly irregular tooth patterning of the bowfin also resembles that of the cod premaxillae (Holmbakken & Fosse 1973) and three-spined stickleback oral cavity (Square *et al.* 2021). As the SDE has been described in the three-spined stickleback, with a demonstrated equivalent function of an SDL (Square *et al.* 2021), it is tempting to attribute the irregular patterning seen in the bowfin to the same replacement mechanism. As discussed in relation to Atlantic cod, the SDE - a region, or 'collar' of regenerative epithelial tissue located around a predecessor tooth - is likely to confer a greater flexibility of positioning to the replacement, and therefore engender a more irregular patterning of teeth overall.

As discussed, Square *et al.* (2021) suggest tooth replacement via an SDE might be plesiomorphic in osteichthyans, as the irregular tooth patterning and replacement in the three-spined stickleback resembles that observed by Chen *et al.* (2016) in the stem osteichthyan *Andreolepis hedei*. The fact that an irregular pattern is also observed in the bowfin lends support to this proposal, considering its basal phylogenetic positioning among the actinopterygians. However, as the African bichir is phylogenetically basal to the bowfin, and develops replacements directly from the ODE (a very orderly regenerative system), this hypothetical evolutionary link between irregular tooth patterning of the bowfin with the that of the stem ostichthyans appears to be broken.

However, I propose that there may be a flexibility conserved in the actinopterygian phylogeny where a range of types of tooth replacement mechanism can be deployed in the same dentition, be it via an SDL, SDE, from the ODE, or any other mechanism yet to be discovered. The XCT visualisations herein of the African bichir oral dentition show that it does not seem to employ the same tooth replacement mechanisms on all its oral bones. For example, no teeth on the prearticulars appear to have any small replacements attached to them (resembling teeth in three-spined sticklebacks), unlike where this frequently occurs on the dermopalatines and coronoids.

In the bowfin, despite the highly irregular patterning in the upper oral cavity, surprisingly, orderly patterning is evident on the prearticulars.

It could be that different replacement mechanisms are relatively easily selected for, perhaps with the underlying GRN able to switch between either. The discovery by Square *et al.* (2021) that the SDL and SDE regenerative systems share a conserved battery of genes could support this.

One distinct feature of the bowfin oral dentition which is not evident in the other study specimens is the prevalence of ‘bumps’ that grade into teeth, approaching the jaw margins. This is greatly reminiscent of the gradual spatial transition from oral denticles to teeth in stem osteichthyans (Chen *et al.* 2016, 2017) and early sarcopterygians (Doeland *et al.* 2019; King *et al.* 2021). King *et al.* (2021) suggest this is dictated by reduced, restricted space towards the jaw margin, where replacement at a specific position needs to be guaranteed at the more functional locations. This is achieved through organised successional replacement, fulfilling the required characteristic of odontodes that are named ‘teeth’. It is possible this was also the case in the bowfin, considering it shows the same spatial trend. It is interesting to note that the ‘from the ODE’ tooth replacement mechanism observed herein, in the Atlantic salmon, African bichir, and possibly the Atlantic cod and bowfin, is located on, or near the jaw margin. This could therefore represent a strategy conserved from the stem osteichthyans, where the mechanism which allows the tightest, most organised specificity in terms of positioning the replacement tooth (ODE and perhaps SDL), at these functionally crucial, marginal positions, is selected for.

6 Conclusion

6.1 Introduction

The current drive surrounding research into the developmental mechanisms of polyphyodonty has motivations which are compelling to both the medical and Evo-Devo communities. The potential to devise a therapy for human tooth loss by ‘switching back on’ an aspect of our polyphyodont ancestors’ tooth replacement mechanism is a captivating possibility, and will likely become increasingly attainable as genomic, cellular and developmental technologies improve. Recent advancement in these technologies has also provided the opportunity to better understand the evolutionary history of teeth, and how the vast range of morphologically diverse vertebrate dentitions has evolved (Tucker & Fraser 2014). Their immense variety qualifies teeth as an excellent model organ for investigating the developmental basis of diversity, especially as seemingly paradoxically they show remarkable conservation of genetic mechanisms underlying tooth development and replacement. Fishes occupy the basal nodes of the vertebrate phylogeny (Johanson *et al.* 2019), and fortuitously, teeth are the hardest and most easily preserved part of an animal, meaning teeth have an excellent fossil record. Studying replacement of fish teeth is particularly valuable, as it is thought that polyphyodonty gives more opportunity for novelty to evolve in the dentition than other anatomical developmental modules (Tucker & Fraser 2014). In fish, this has meant they could adapt to a great range of environments to feed, and they have colonised nearly all aquatic environments globally. The polyphyodont dentition is a unique system for understanding the evolution of novelty, and therefore evolution generally (Tucker & Fraser 2014; Thiery *et al.* 2017).

This study has aimed to contribute to this effort to understand the developmental mechanisms underlying tooth replacement and polyphyodonty by evaluating tooth replacement mechanisms in light of outcomes from recent Evo-Devo and historical research. The study drew on the great morphological and taxonomic diversity of fossil and modern fishes represented in the collection of NHMUK. Unexplained tooth replacement mechanisms were identified in the specimens, with hypotheses for their development proposed. It is hoped that these findings and hypotheses might open up new opportunities for the Evo-Devo community to research. Mechanisms which are consistent with, and therefore explained by, previous research were also identified, confirming their presence in new individuals of a species. Some observations made herein may add to the conclusions of previous research.

Photographs and XCT data produced in this study were uploaded to the MorphoSource digital repository (project C1154): https://www.morphosource.org/projects/0000C1154. This was to achieve the study aim of creating and make available new XCT data and images of fossil and modern fish dentition specimens, for use in future investigations as understanding of the mechanisms of polyphyodonty develop over time, particularly as this is a fast-paced field of research.

Two key, overarching hypotheses are proposed herein to account for observations from the study specimens which are not explained by existing research. These involve mechanoreception, and an alternative source of stem cells to the predecessor tooth, to engender tooth replacement. For each of the study's unexplained observations, the below conclusion summarises hypothesised interpretations.

6.2 Hypothesised gap-filling tooth addition in pycnodonts

The first unexplained observation is that small, round teeth on the pycnodont vomers and prearticulars had developed in gaps that had been putatively been caused by tooth damage and loss (at the anterior dentition), or reliably caused by tooth damage and loss (where parts of teeth had broken off), or in gaps in varied locations caused by the geometry of the large elliptical teeth arranged in rows.

This observation was unexplained because traditionally pycnodont teeth are understood to have added to the posterior end of the vomers and prearticulars (Woodward 1893, 1895; Thurmond 1974). While Longbottom (1984) disagreed with this with respect to small round teeth at the anterior-most end of the dentitions, she suggested the small teeth progressively replaced the large from the front, towards the back. My observations of small teeth located in gaps where damage had likely or certainly occurred, or in geometric gaps between large teeth in varied locations on the dentition, and not restricted to the anterior, were unexplained by Longbottom's hypothesis of progressive replacement from the anterior.

Supporting evidence presented herein for damage having occurred at the dentition anterior comprised increased tooth wear towards the anterior (excluding the small round teeth which showed little or no wear), indicating this was the oldest part of the dentition, and deterioration of the dentigerous bone in this region. The small teeth appear to very effectively, and often near-perfectly, fit and fill gaps wherever they were located on the dentition. There was no evidence in the XCT data for one-for-one tooth replacement mechanism.

The patterning suggests a mechanism existed for gaps to be located and for new teeth to develop in them. In view of the reviewed research herein on modern cichlid crushin dentitions, I hypothesise that the gaps were registered as an absence of pressure or ‘mechanical strain’ in the oral epithelium, at the crushing surface during feeding, and that this absence of pressure initiated new tooth development. I propose this constituted a regenerative mechanism of gap-filling tooth addition, governed by a genetic programming of ‘if a gap exists – fill it’. This strategy could have been selected for as all exposed oral epithelium would likely be at risk from damage caused by the crushing and grinding of mollusc shells. Feeding could conceivably form sharp splinters of shell, that could access any gap. Development of small teeth may not only heal damaged areas but protect the oral epithelium in even the smallest of geometric gaps. Evidence of phenotypic plasticity in cichlids provides an example from nature of a very similar, pressure sensitive response mechanism affecting tooth replacement, also responding to feeding on molluscs in a crushing dentition (e.g. Huysseune 2000; Gunter *et al.* 2013; Gunter & Meyer 2014; Schneider *et al.* 2014). Other examples are drawn on in the above discussion of mechanoreception affecting tooth replacement, to support the hypothesis. In the miniature pig and humans, pressure change galvanises replacement of the primary tooth by the permanent tooth (Sarrafpour *et al.* 2013; Wu *et al.* 2020; Wu & Wang 2020).

The discovery by Cooper *et al.* (2022) of tubules running from the tooth surface to the pulp cavity, in the pycnodont *Phacodus punctatus,* led the authors to speculate they had a role in mechanoreception, serving a protective function to the tooth by enabling detection of food hardness. This postulated presence of mechanoreception in a pycnodont specimen gives support to the proposed hypothesis herein.

If this hypothesis is correct, I have raised the question of how the small gap-filling teeth were born from the oral epithelium, when in the development of non-primary teeth in vertebrates, access to a source of stem/progenitor cells is required, associated with predecessor tooth (Tucker & Fraser 2014). I hypothesise that overall, the simplest solution is that space is the deciding factor dictating where a new, small tooth develops, not proximity to a predecessor. I also hypothesise that the oral epithelium of the pycnodont vomer and prearticular dentitions retained an ability to generate teeth throughout life, without the need for a new tooth to be associated with a predecessor.

6.3 A hypothesised retained ondontogenic competence in the pycnodont oral epithelium throughout ontogeny

The question immediately arises as to how a retained odontogenic capacity could be achieved in the oral epithelium, if this hypothesis were correct. Could the same dental competence that forms the first teeth/primary dentition in most vertebrates (Fraser & Thiery 2019), be retained? If the generally accepted premise that access to a source of stem/progenitor cells is required for tooth generation after the primary dentition, from epithelium associated with predecessor tooth (Tucker & Fraser 2014), there appears to be no possibility this hypothesis could be correct. However, I propose that the stem/progenitor cells may be accessed from elsewhere – the stem cells which give rise to the taste buds. Bloomquist *et al.* (2019) showed experimentally that the in cichlids and the mouse, oral epithelium retains a ‘plasticity’ to form either tooth and taste-like cell types. Also, Martin *et al.* (2016)

revealed the dual fate of putative stem/progenitor cells in the small-spotted catshark as taste buds or teeth. Significantly for the medical research community, Bloomquist *et al.* (2019) even referred to the ‘underappreciated stem cell populations’ which give rise to either taste buds or teeth, as offering potential bioengineering solutions to human tooth loss. To assess the plausibility of such stem cell populations enabling gap-filling tooth addition in pycnodonts, it would be interesting to investigate the distribution of taste buds in modern fish with crushing dentitions. A specimen of the sparid Karanteen seabream, *Crenidens crenidens*, contained 3460 taste buds within its oral cavity and pharyngeal dentition combined (Fishelson *et al.* 2014), in a fish of 206 mm standard length, implying a relatively strong presence of taste bud progenitor cells within this modern crushing dentition. Overall, though this is a bold hypothesis, it may offer a mechanism for tooth generation in fish with highly irregularly patterned dentitions, and offer another mechanism of maintaining polyphyodonty.

6.4 How small teeth in pycnodonts filled gaps so effectively

Hypotheses have been proposed herein for how small teeth were positioned to fill gaps on the oral epithelium, and how oral competence was retained throughout ontogeny, facilitating gap-filling tooth addition in pycnodonts. An interesting question remains however as to how the number, size, shape and positioning of the small round teeth were such that they filled, or ‘fitted’ gaps so effectively, arguably perfectly in some cases. Suggestions are made herein, arising from a combination of the pycnodont gap-filling observation, and the next unexplained observation of the present study; the changes across one tooth generation observed in the sparid specimens.

6.5 Hypothesis to explain sparid ‘two-for-one’ replacement and large change in size in one tooth generation

The second unexplained observation was that in the large sparid, two relatively small, rounded teeth had erupted within the crown of a much larger, ovoid, broken tooth (Fig. 4.17). This appeared to represent two-for-one replacement, and a significant change in tooth size over one tooth generation. The latter is thought to likely only to take place over successive tooth generations (Huysseune 2000). XCT data of the gilthead seabream, *Sparus aurata*, also revealed a large size change over one generation, in two locations (Fig. 4.21). In these cases, the opposite change of a large tooth replacing two small teeth was observed. Interestingly, in the *Sparus aurata* specimen, one of the small functional teeth destined for replacement has an empty tooth position next to it, possibly to accommodate the size of the new large replacement tooth. This suggests a possible co-ordination of tooth positioning in relation to a single replacement and two predecessors.

The XCT virtual section of the sparid specimen the whitebone porgy, *Calamus leucosteus*, reveals two bony crypts fused together, containing two developing teeth (Fig. 4.21). The functional tooth space above these are empty. It suggests bone remodelling to accommodate such fusion of the crypts is possible, with the small teeth within them coming together during development, to be positioned in close enough proximity to each other to replace one large tooth (which has already been shed). Again, this appears to represent a positional co-ordination between replacements and predecessor. Returning to the initial puzzling observation of two small teeth replacing one large tooth in the large sparid specimen, I hypothesise that by altering the relative timing of the tooth replacement cycles in adjacent tooth positions, and by lateral movement of developing teeth, the apparent two-for-one replacement could have taken place. I suggest a functional tooth has been shed, and its developing replacement has moved laterally towards the developing tooth in the adjacent tooth position, close enough to eventually both take the place of the adjacent tooth's large functional predecessor located above it. There is a space immediately next to the large, cracked tooth which supports this idea. Such a strategy could engender a relatively rapid change in tooth number across the dentition. I suggest that this tweaking of replacement cycle timing, and movement of a developing tooth, in combination with a change in tooth size and shape over one generation could also effect a rapid change in tooth patterning in sparids.

It is evident from the data presented here, that unlike in cichlids, which also exhibit intrassous replacement, a large change in tooth size is possible in one tooth generation in *Sparus aurata*, and the large sparid specimen (sp. indet.). The tooth shape also can change from rounded to/from ovoid.

It is not possible to know if the crack in the large tooth of the large sparid occurred post-mortem. If *in vivo*, I speculate that the change described here with two small teeth taking its place may have been prompted by a sensed change in pressure, as the tooth was weakened. It is plausible that multiple small, rounded teeth are better able to fill the shape of any gap caused by damage - resembling the proposed gap-filling in pycnodonts.

6.6 'Anamestic' tooth patterning (close-packing and tessellation) via mechanoreception

Worcester (2012) showed experimentally that the sparid sheephead fish *Archosargus probatocephalus* exhibits a phenotypically plastic response to hardness of diet. As well as changes to thickness of dentine and enamel, there was change in percentage of jaw surface covered by teeth. This is likely facilitated by mechanoreception, as shown to be the case in cichlids, engendering a change in tooth shape and size in response to change in hardness of diet.

I hypothesise that a similar response to that observed by Worcester (2012) in the sheepshead fish, has occurred in the large sparid and the gilthead seabream, *Sparus aurata* specimens, presented herein. In both these study specimens, a large change in tooth size has occurred over one tooth generation. In the large sparid, I have suggested that movement of teeth as they develop has positioned two small teeth underneath one large tooth, to eventually take the place of this large tooth. I have suggested this is a means of changing tooth patterning in this region. Both these changes in tooth shape and positioning/patterning could contribute to a change in percentage of jaw surface covered by teeth, as described by Worcester (2012) in the sheepshead fish. The sheepshead fish, the gilthead seabream *Sparus aurata*, and the large sparid specimen of the present study, are all closely related in the family Sparidae.

Overall, I hypothesise that the sparids presented herein are able to undergo a change in tooth patterning as an adaptive, phenotypically plastic response. This response would be facilitated by mechanoreception, sensing changes to hardness of food, and I suggest, also gaps in the dentition. This would enable a close-packed patterning of small teeth in gaps, as seen in the sparid dentitions presented herein (particularly in the sheepshead fish, Fig. 4.20A). I suggest close-packed patterning in gaps also occurs as a phenotypically plastic response in the pycnodonts.

I suggest that close-packed patterning of small teeth in gaps as a phenotypically plastic response may also be present in other fish species with crushing dentitions outside of the Sparidae e.g. the ballan wrasse, *Labrus bergylta*, and the black drum, *Pogonias cromis* specimens presented here in. A future study in other species with crushing dentitions, similar to that undertaken by Worcester (2012) in the sheepshead would be a useful next step.

6.6.1 Perfecting the continuous crushing surface via tessellation

The close-packed tooth patterning in some species of fish with crushing dentitions is such that they exhibit remarkable tessellation of polygonal-shaped teeth, e.g. the Atlantic wolffish, *Anarhichas lupus* (Bemis & Bemis 2015) and the black drum, *Pogonias cromis* (specimen presented herein). Interestingly, I observed similar polygonal tessellation of attachment tissues in the small, anterior teeth of the lower oral jaw of the sparid, the whitebone porgy, *Calamus leucosteus*. I have previously speculated that this may have been possible due to simultaneous replacement of teeth in this region of the jaw (one half of the anterior-most region, bearing small, pointed, non-crushing teeth), as strangely, all teeth are absent there. Bemis & Bemis (2015) contend that simultaneous replacement is needed in the Atlantic wolffish, to achieve such near-perfect tessellation. Simultaneous replacement is unusual however, and not present in the larger, molariform crushing teeth on the same dentigerous bone in the whitebone porgy. Interestingly, the attachment tissues of these molariform teeth also tessellate, but only where they happen to meet, as they are not as closely packed as the small, pointed teeth. However, on the upper oral jaw of the same specimen, the middle row of molariform attachment tissues are tessellated well (Fig. 4.21E). I therefore propose that simultaneous replacement of teeth, is not required for tessellated tooth patterning in crushing dentitions, as suggested by Bemis & Bemis (2015), as it is not evident in the whitebone porgy molariform teeth.

Bemis & Bemis (2015) propose that tooth tessellation in the Atlantic wolffish is made possible by replacement teeth accommodating to the shapes of adjacent teeth, resulting in no gaps in the crushing surface. This implies that that each tooth somehow senses the presence and positioning of its neighbour. I hypothesise that this could be achieved by pressure-sensing, i.e. mechanoreception between the teeth. I support this proposal with the observation that attachment tissues appear to have tessellated in the whitebone porgy. Sparids have a thecodont implantation (Berkovitz & Shellis 2017) and pedicellate attachment (Hughes *et al.* 1994), which I contend may offer opportunities for the existence of mechanoreceptors within this mode of implantation and attachment type. Hughes *et al.* (1994) describe the pedicel of sparids as comprising a dentine-like tissue, called ‘canalar dentine’, which contains osteocytes. Significantly, osteocytes are known to be mechanosensitive (Robling & Bonewald 2020). It is therefore plausible that sparid pedicels enable tessellated tooth patterning. Another opportunity for pressure-sensing could be the bone of attachment, located between the lateral surface of the tooth and the jaw bone (Rosa *et al.* 2021), within the thecodont implantation. This location is comparable with that of the periodontal ligament in the mammal thecodont tooth, which is well known for its mechanosensory function. Bone of attachment is known to very likely contain a dentine component (Rosa *et al.* 2021), which may lend itself to this function. There is also a further opportunity for a mechanosensory tissue in sparids; the fibrous/ligamentous tissue connecting the crown to the pedicel, especially when considering the mechanosensory property of the mammalian periodontal ligament.

Overall, I hypothesise that close-packed tooth patterning within fish crushing dentitions is a phenotypically plastic response to pressure change at the crushing surface, facilitated by mechanoreception. I also hypothesise that perfecting this close-packing to form near-tessellated teeth occurs via mechanoreception between adjacent teeth, either in the tooth itself, but more likely in the attachment tissues. I suggest these mechanisms are likely to have been responsible for the spatially very effective gap-filling in pycnodonts by small round teeth.

6.7 Replacement mechanisms consistent with previous research

Excluding the pycnodonts, all tooth replacement mechanisms identified within the study specimens as either intraosseous or extraosseous replacement were consistent with previous research on each species, as were all identified tooth attachment types. Pycnodonts are hypothesised herein to exhibit tooth addition, rather than replacement. They exhibit extraosseous development on the on the dentigerous bone, rather than intraosseous development from within it.

There were some observations which broadened those made in previous research. The hinged tooth attachment (type 3, *sensu* Fink (1981)) was not present on the prearticulars as of the African bichir study specimen, representing and inconsistency with Fink (1981) and Berkovitz & Shellis (2017). However, they were instead observed on coronoids 1 and 2, the dentaries, vomer, ectopterygoids, and possibly the anterior parasphenoid. Interestingly, serial tooth addition was also putatively identified at the posterior of the gilthead seabream, *Sparus aurata* study specimen, as well as the intraosseous successional replacement indicated by Hughes *et al.* (1994) and Trapani (2001).

6.7.1 Three-spined stickleback and Atlantic cod

In general, the patterning observed in the three-spined stickleback study specimen supports the finding of Square *et al.* (2021), as there was no consistent positional relationship between a successor and predecessor tooth, and occasionally positioning of a small/developing tooth implied a one-for-two replacement ratio. However, this ratio was observed by Square *et al.* (2021) in the pharyngeal jaws, not the oral dentition, and only the oral dentition was studied herein. The overall irregular patterning in the oral dentition of the study specimen appeared consistent with the less restricted positioning enabled by development from an SDE, rather than an SDL.

This was also the case generally in the Atlantic cod study specimen. Holmbakken & Fosse (1973) described patterning of developing teeth in the upper and lower oral jaws of the Atlantic cod as haphazard, and I had difficulty discerning any pattern or orderly replacement on all bones apart from the lower jaw. Square *et al.* (2021) suggest tooth replacement via SDE in the Atlantic cod, which the irregular patterning observed herein supports. However, I observed a great contrast on the lower oral jaws, with putative regular, repeated organisation of teeth into families. This regular positioning suggested tooth replacement via an SDL or directly from the ODE of the predecessor tooth.

6.7.2 African bichir and Atlantic salmon

Replacement teeth in both the African bichir and Atlantic salmon study specimens developed from the posterior-lingual side of the predecessor tooth, as predicted by previous research (Huysseune & Witten 2008; Vandenplas *et al.* 2014, 2016*a*). The replacements developed directly from the predecessor, implying development directly from the ODE, which is also consistent with research. Only in the male salmon could the predicted third member of the tooth families be discerned (Fig. 4.23D, E). However, this is likely due to an artefact arising from the tissue visualisation.

In the African bichir, I noted some variation in where the replacement teeth were positioned with respect to their predecessors, on the parasphenoid bone. This variation included a tendency towards the right lateral side of the predecessor tooth. I speculate that teeth there developed from an SDE rather than an SDL, the region of regenerative tissue offering more positional flexibility than an SDL (as suggested above for the Atlantic cod and three-spined stickleback). Elsewhere in the dentition, all replacement teeth were consistently located on the posterior-lingual side.

The variation in tooth attachment type to previous research, in the African bichir, is noted above.

6.7.3 The bowfin, *Amia calva*

Consistent with the ‘random’ patterning noted by Miller & Radnor (1973), the tooth patterning in the oral cavity of the bowfin study specimen appeared highly irregular. There were only a few putatively identified replacement teeth in the bowfin, however this was a dry study specimen likely hindering the observation of developing young teeth. As with the three-spined stickleback, Atlantic cod, and putatively the parasphenoid bone of the African bichir, the general irregular patterning in the bowfin is suggestive of tooth replacement via an SDE.

As in the Atlantic cod, there was a surprising, incongruous regular patterning, on the bowfin lower jaw, this time on the prearticulars. As this is a dry specimen, as mentioned above, it is uncertain if this could represent tooth families. Other study specimens described herein exhibit more than one type of tooth replacement mechanism in the same dentition. Square *et al.* (2021) hypothesise that tooth replacement via an SDE is plesiomorphic in the osteichthyans, possibly accounting for the irregular patterning observed by Chen *et al.* (2016) in *Andreolepis*. While this seems likely, I hypothesise that there may be a flexibility conserved across the actinopterygians where a range of different types of tooth replacement mechanisms can be deployed in the same dentition, be it via an SDE, ODE or SDL, or any yet to be uncovered. Square *et al.* (2021) showed the SDE of the three-spined stickleback and the SDL of the zebrafish, *Danio rerio* share a battery of genes, supporting this suggestion. I speculate that the different replacement mechanisms are relatively easily selected for, as they share an underlying GRN able to switch between either.

I hypothesise that as observed in the stem osteichthyans and early sarcopterygians, and summarised by King *et al.* (2021), there is a trend in the study specimens, and speculatively, the actinopterygians generally, for those replacement mechanisms which guarantee replacement tooth positioning at a specific, tight location to be selected for on the functionally crucial, space-restricted jaw margins. Square *et al.* (2021) speculate that the SDE was used in *Andreolepis,* as successive generations of replacement teeth are in slightly varied orientations relating to each predecessor. Perhaps the ODE and SDL were evolved improvements. Among the study specimens herein, the trend is seen most obviously in the bowfin where ‘bumps’, which I postulate are oral denticles, grade into being more tooth-like as they approach the jaw margins. This observation in the bowfin is also interesting as it is a basal actinopterygian, therefore supportive of the summary given by King *et al.* (2021). It is also interesting to note that regeneration via an ODE is however known to be operating at a more basal phylogenetic location than the bowfin, in the African bichir.

7 Areas for future research

In the context of the drive to better understand the mechanisms underlying polyphyodonty, this study has aimed to use fossil and modern fish specimens to either support what has already been discovered, or provide new findings which are not explained by previous research.

There are many findings presented herein which are consistent with previous research. I suggest there are two overarching areas with the most potential for us to learn more. The first is the role of mechanoreception in determining shape, size, patterning/positioning of replacement teeth. How these factors are affected in the crushing dentitions of extant fish would likely be the most promising and illuminating place to start. We know that a hard diet affects tooth size and shape in cichlid pharyngeal jaws, but how is their tooth positioning and patterning determined? How do teeth interact with each other in crushing dentitions like those of the Atlantic wolffish and the black drum so that they near-perfectly tessellate? How does gap-filling by small teeth in crushing dentitions, like those in sparids occur? Another related line of enquiry, arising from observation of the study specimens, but also from research on stem osteichthyans, is how can space restriction on marginal dentitions affect the type of tooth replacement mechanism that occurs there?

The second area where I think there is most potential to learn more is in the regenerative origins of teeth which develop after the primary dentition. The current paradigm is that a developing tooth must be in some way ‘tied’ to its predecessor, via an epithelial connection; until recently the DL, SDL or develop directly from the predecessor ODE. This is because it needs to be supplied with the necessary stem/progenitor cells for it to develop. In actinopterygians, the discovery of the SDE by Square *et al.* (2021) has ‘loosened’ the connection and enabled us to understand how a replacement tooth can occur in slightly varied positions, in relation to its predecessor. This can account for irregular patterning to some extent, and a release from the one-for-one replacement ratio, but the physical distance between replacement and predecessor is still very close.

The example of pycnodont gap-filling could arguably not be accounted for by the SDE regenerative system, with some teeth relatively remote from others on the dentition edge, and new teeth located adjacent to teeth which have not been shed e.g. between large teeth in rows. I speculate that more examples might come to light where tooth positioning cannot easily be linked to a predecessor. The Atlantic cod and bowfin could even be such examples. In this study, I have hypothesised that ‘underappreciated’ populations of stem cells that supply progenitors for taste bud development, present throughout ontogeny, could be being used in some species to position and develop new teeth. The homology of teeth and taste buds, relevant in the debate on the evolution of teeth, has been shown (Martin *et al.* 2016), as has their ‘inter-organ plasticity’ (Bloomquist *et al.* 2019). Another suggested mechanism is that the dental competence that gave rise to the primary dentition is retained throughout life. This however does not seem possible, without a source of stem cells from which to draw on.

Fish dentitions, often irregularly patterned, and immensely diverse will continue to be a rich source for us to gain more insights into how teeth are generated throughout ontogeny. Though their genetics are lost in time, the use of fossil fishes such as the pycnodonts opens up another vast range of diversity for study, where tooth positioning can prompt us to ask new questions about how tooth replacement works.

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