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## **Evolution of the dentition in holocephalans (Chondrichthyes) through tissue disparity**

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**Synopsis** The Holocephali is a major group of chondrichthyan fishes, the sister taxon to the sharks and rays (Elasmobranchii). However, the dentition of extant holocephalans is very different from that of the elasmobranchs, lacking individual tooth renewal, but comprising dental plates made entirely of self-renewing dentine. This renewal of all tissues occurs at the postero-lingual plate surface, as a function of their statodont condition. The fossil record of the holocephalans illuminates multiple different trends in the dentition, including shark-like teeth through to those with dentitions completely lacking individual teeth. Different taxa illustrate developmental retention of teeth but with fusion in their serial development. Dentine of different varieties comprises these teeth and composite dental plates, whose histology includes vascularized tubes within coronal dentine, merging with basal trabecular dentine. In this coronal vascularized dentine extensive hypermineralization forms a wear resistant tissue transformed into a variety of morphologies. Through evolution, hypermineralized dentine becomes enclosed within the trabecular dentine, and specialized by reduction into specific zones within a composite dental plate, with these increasing in morphological disparity, all reflecting loss of defined teeth but retention of dentine production from the inherited developmental package.

## Introduction

The chondrichthyans are a major group of jawed vertebrates, with a fossil record extending back over 450 million years (Andreev et al. 2016, 2017) and a wide representation across all continents. Modern chondrichthyans comprise the Elasmobranchii (sharks, skates, rays) and Holocephali (chimaeroids), which together differ from all other jawed vertebrates in lacking true bone within their skeleton. Although being sister taxa, the elasmobranchs and modern holocephalans have very different dentitions. In elasmobranch dentitions, the shape, size and number of teeth is highly variable between taxa. Individual teeth develop ahead of use (Fig. 1A, B), from dentally competent tissues deep within the lingual surface of the jaw, forming tooth series that are temporally and spatially organized along the jaw (Underwood et al. 2015, 2016). Teeth form initially as an enameloid cap (as a cooperatively formed tissue between ameloblast and odontoblast cells), with coronal and basal root dentine forming subsequently (orthodentine, osteodentine, see recent papers by Jambura et al. 2019a, b). Developing teeth ascend from the lingual jaw surface, and mature teeth reach functional positions on the jaw margin, subsequently shed at the labial edge of the jaw. Each successional series of teeth forms as part of a developmentally linked spatiotemporal group referred to as a whorl (e.g., Maisey et al. 2014, 2019).

Modern holocephalans, by comparison, possess just six dental plates that are retained throughout life and made only of dentine (enameloid is lacking; Fig. 1E), including the supporting, trabecular dentine and wear resistant hypermineralized dentine (Fig. 1C–G). This dentine shows a very different biological mechanism for mineralization and also involves the mineral whitlockite. Whitlockite is a Mg-rich material present in small proportions in bone, and other vertebrate teeth, but only known to be present as a nearly pure biomineral in holocephalan hypermineralized tissue (Ishiyama et al. 1991; Smith et al. 2019). Rather than forming an extremely hard external tissue only once (elasmobranch enameloid), hypermineralized dentine develops internally, and is continuously renewed, as multiple, serial events within the dental plates. Also, this hypermineralized dentine infills pre-designated developmental spaces, determined by the trabecular dentine framework (Lund and Grogan 1997; Stahl 1999; Smith et al. 2019; fig. 1C–G; Supplementary Information Fig. S1A, ov.s). Growth in these composite dental plates is from their postero-lingual margin. Feeding-related differential loss of dental material due to wear exposes the

hypermineralized dentine as elevations on the functional surface. This regrowth and wear mode of dentitions (statodont, structures are retained) makes holocephalans very different, developmentally and morphologically, from shark and ray dentitions (Fig. 1A, B). These show continuous formation and loss of teeth by shedding (lyodont, structures continuously lost), Despite this extreme dissimilarity of holocephalan and elasmobranch dentitions, these taxa have a sister group relationship (Fig. 2), leading researchers to seek shared characters between the dentitions. Evidence for these shared characteristics comes from both developmental data and the fossil record, as some early fossil holocephalan dentitions have ‘shark-like’ characters (Owen 1867; Schauinsland 1903; Patterson 1965; Lund 1977, 1982; Kemp 1984; Didier et al. 1994; Didier 1995; Stahl 1999; Smith et al. 2019, 2020; Johanson et al. in press).

The dental plates of modern holocephalans, all included within the Chimaeroidei, vary in shape and proportion, from robust plates with a largely crushing function in *Callorhinchus* (Fig. 1C–E) to gracile plates with a more cutting function in *Rhinochimaera* (e.g. Herman et al. 2001). In the genus *Callorhinchus* (Family Callorhinchidae), the dental plate was observed to develop from a single dental primordium (Schauinsland 1903; Kemp 1984; Didier 1995). Didier et al. (1994) studied this development in embryos of *Callorhinchus millii* and *Chimaera monstrosa* (Chimaeridae). In these taxa two distinct regions in the dental plates, the oral and aboral territories (particularly large in *Callorhinchus*), were observed to be separated by a thin layer of connective tissue. In some cases it was possible to observe a shallow crevice denoting the boundary between the two territories (Didier et al. 1994: fig. 2). This suggested dental plates were compound structures consisting of conjoined oral and aboral dental territories (e.g. Fig. 1E). Didier et al. (1994) also observed that each territory had a corresponding pulp cavity and a different orientation of the dentine tissues. Thus, this compound structure represented the morphological equivalent of separate teeth united to form a single unit. The distinction between the oral and aboral regions is reduced in the Chimaeridae and Rhinochimaeridae, but Smith et al. (2019: fig. 7A–E) identified disruptions of the tritoral tissues within the dental plate of *Harriotta* (Rhinochimaeridae) representing potential developmentally distinct timed units comparable to serial, separate teeth.

The fossil record, compared with extant forms, provides information as to how the holocephalan dentition has evolved, with predicted developmental change. Statodont holocephalan dentitions preserve important developmental growth information, even in fossils. This includes processes that mediated tooth fusion through the transition from separate, elasmobranch-style teeth, to the holocephalan condition lacking separate teeth (Owen 1867; Lund 1977, 1982; Didier 1995; Lund and Grogan 1997; Stahl 1999). Important in this regard are taxa resolved phylogenetically as stem-group holocephalans (Coates and Sequiera 2001; Coates et al. 2017, 2018; Frey et al. 2019; Fig. 2). The dentitions of these stem-group taxa show a succession of different morphologies, from those similar to the state in elasmobranchs (such as in *Cladoselache* and the Stethacanthidae; Fig. 3A) to ones closer to modern holocephalans (such as in *Myriacanthus*; Fig. 4). Furthermore, in some taxa, both continuously replaced teeth and continuous growth components are present within dentitions of the same genus or even individual. This combination of states is clearly present in *Helodus simplex* and other species of *Helodus* (Helodontiformes). *Helodus* combines a more shark-like dentition with replacement series of individual teeth, serially repeated along the jaw (Fig. 3B, C), but also shows fusion within each tooth whorl (e.g., Moy-Thomas 1936; Stahl 1999; Johanson et al. in press; Supplementary Information Fig. S1B). However, it is less clear whether, or how, multiple families in rows may have fused to form a dental plate and whether the dental plate represents a single tooth whorl or multiple tooth whorls fused together.

In order to improve our understanding of how modern holocephalan dentitions evolved, we review the morphology and histology of multiple representatives of the stem-group Holocephali, including taxa more closely related to the crown group holocephalans, with a particular focus on how the arrangement of mineralized tissues has changed over geological time (Fig.2).

## **Materials and Methods**

### *Specimens*

Both modern and fossil specimens were studied by X-ray micro-CT to reveal internal features of the dental plates. Modern specimens of *Callorhinchus* were obtained from commercial sources in South America; other modern chimaeroids were obtained from deep

water trawls in the North Atlantic by Marine Scotland. Specimens were skeletonized and dried. Fossil holocephalans are held in the Natural History Museum, London (NHM). Paleozoic stem-group holocephalans examined comprise the genera *Helodus*, *Edestus*, *Copodus*, *Poecilodus*, *Deltoptychius* and *Deltodus*. Taxa of the poorly defined ‘Edaphodontidae’, which is in an uncertain phylogenetic position either within or just outside the crown-group (extant) Holocephali, comprise the genera *Edaphodon*, *Ischyodus*, *Elasmodectes* and an unnamed *Ischyodus*-like form (E. Popov, personal communication 2020). In addition, an articulated specimen of *Myriacanthus* was studied but not scanned due to the size and fragility of the specimen. **Abbreviations:** **BMB**, Booth Museum of Natural History, Royal Pavilion & Museums, Brighton and Hove, Brighton, UK; **NHMUK** **PV P**, Natural History Museum, London, UK, fossil fish collections (Department of Earth Sciences).

#### *X-ray micro-CT and macrophotography*

Fossil and extant holocephalan specimens were imaged using X-ray micro-CT in the Image and Analysis Centre, NHM. Two instruments were used depending on their size and/or necessary resolution: a Nikon HMX ST 225 (Nikon Metrology, Leuven, Belgium) for larger specimens; a Zeiss 520 Versa (Carl Zeiss Microscopy GmbH, Jena, Germany) for smaller specimens. All acquisition parameters are listed in Table 1. Images and virtual sections from these scans were produced using Avizo software (<https://www.thermofisher.com/uk>). *Cladoselache* sp. (Fig. 3A) and *Myriacanthus paradoxa* (Fig. 4A) were photographed (Nikon D5600 camera); Helicon Focus software (<https://www.heliconsoft.com>) was used to improve depth of focus in Fig. 4A. Images in Fig. 4B, C were taken using a Zeiss Axio Zoom V16 (The Sackler Imaging Suite, NHM).

#### *Terminology*

Terms for dental tissue types are always controversial, and changing with new data (Ørvig 1985; Kemp 1994; Smith et al. 2019). So, we have clarified the basis of our terminology in the context of new observations on virtual sections from CT-scanning and mineralized sections, showing the range of variation in histology, as occurs in many durophagus dentitions (Supplementary Information Figs 1, 2). Principal patterns of the

internal morphology of holocephalan teeth have been well covered (Patterson 1965; Ørvig 1985; Zangerl et al. 1993; Stahl 1999), but there are differences in names given for the different types of dentine, particularly with respect to location relative to the blood vessels of the tritoral pad. For example, Zangerl et al. (1993: fig. 9) identified the hypermineralized dentine (whitlockin), forming around the blood vessels as ‘peritubular trabeculine’ and the dentine between (forming the bulk of the plate) as orthodentine. An objection to the latter is that Zangerl et al. (1993: 12) suggests that orthodentine does not infill preformed cavities, but this is not the case (Supplementary Information Fig. 2A, tri; Smith et al. 2019). With respect to ‘peritubular trabeculine’, Zangerl et al. (1993) use the term ‘peritubular’ to refer to the vascular canals oriented perpendicular to the biting surface; but the form of the word (‘tubular’) suggests that dentine is deposited within the canal, which conflicts with the part of the term, ‘peri’. Following on from Ørvig (1985), we reserve the term ‘peritubate’ for the less mineralized dentine surrounding the hypermineralized whitlockin immediately surrounding the blood vessel wall (Fig. SI 2 D, E). Formation of these two dentines may result from a transformation of the cells making whitlockin (whitloblasts) into regular odontoblasts. Details of these histologies are shown from transmitted light photomicrographs (Supplementary Information Fig. S2) and the overall plan as arranged in virtual sections (Supplementary Information Fig. S1A).

## **Anatomy of Tissue Structure**

### *Hypermineralized dentine*

In extant holocephalans, hypermineralized dentine (whitlockin, Smith et al. 2019; pleromin, Ørvig 1985) takes the form of tritoral pads, elongate rods and series of rounded structures called ovoids (see Didier 1995; Stahl 1999 for summary; Fig. 1C–G). These structures are held within a framework of less mineralized trabecular dentine (Fig. 1E; Supplementary Information Figure S1A), supported by the labial and lingual outer dentine of the dental plate. The trabecular dentine constitutes the tissue that provides preformed spaces for infill by hypermineralized dentine (now termed whitlockin, with the main mineral component being Mg-Whitlockite, relative to hydroxyapatite, Smith et al. 2019, figs. 14, 15). The whitlockin, as a specialized dentine tissue, surrounds parallel vascular canals normal to the biting surface of the compact tissue blocks (Fig. 1E, tritoral pads). The cells forming this

tissue (whitoblasts, also known as pleromoblasts, Ørvig 1985) encircle the vascular canal and are active in increasing the mineralization of whitlockin until late in formation, when these cells transform to deposit perivascular, less mineralized dentine. This restricts the vascular canals and seals them at the surface, resulting in wear differences that form circular depressions, termed ‘punctate surface’ (Fig. 1C, D). We call this ‘tubate dentine’ (equivalent to tubate vascular pleromin with peritubate dentine; Ørvig 1985), first appearing in members of the stem-group Holocephali (Fig. 2), histology as described below. The rods and ovoids are a non-tubate type of hypermineralized dentine (compact pleromin, Ørvig 1985: figs. 1–3), lacking the regular intrinsic arrangement of these vascular canals (Fig. 1F, G).

As noted, one important feature of the hypermineralized dentine in extant holocephalans is that deposition occurs within preformed spaces made by the trabecular dentine, and surrounding organized vascular canals (Smith et al. 2019). In separate teeth, by comparison, dentine is deposited centripetally and incrementally by odontoblast cells within a pulp cavity. Within the tritoral pads and ovoids, enlarged cell bodies are joined as a membrane that lines the forming whitlockin next to the vascular tissue (specialized odontoblasts; pleromoblasts [Kemp 1984] or whitoblasts [Smith et al. 2019]). From this organized layer of cells, a massive number of tubules ramify and extend into the center of the dentine that is becoming hypermineralized. Amongst and between the tubules, mineral crystals are formed without an organic matrix, as deposition occurs within disorganized membranous saccules located among these tubules (Smith et al. 2019: figs 3, 6). By contrast, within the trabecular dentine, and inner and outer layers of the dental plate, a disorganized network of dentine tubules extends from separate randomly spaced odontoblasts (Smith et al. 2019: figs. 4, 9).

Another characteristic of hypermineralized dentine, demonstrated for *Harriotta* and *Chimaera*, is that this mineral is a magnesium-rich form of whitlockite, rather than hydroxyapatite (Families Rhinochimaeridae, Chimaeridae; Ishiyama et al. 1991; Smith et al. 2019: fig. 9g). Given this distribution, a higher percentage of whitlockite than hydroxyapatite may potentially be a feature of extant holocephalans, although the Callorhynchidae have not yet been examined, nor have closely related fossil taxa (Fig. 2). In the fossil holocephalans described below, sections through the teeth or dental plates show

that increased mineralization is similar in position to extant forms but restricted coronally (Johanson et al. in press: fig. 3E, F). However, in the living groups, hypermineralization extends deeper into the trabecular dentine, where tritoral pads, ovoids and rods are forming as developing tissue, less mineralized (Smith et al. 2019: figs. 1, 2, 12, 13. 16; Supplementary Information, Fig. S1A, compare highly mineralized tissue at the biting surface, mineralization decreasing to the base of the plate).

### **Distribution of Characters among the Holocephali**

The phylogenetically most basal members now considered to be part of the Holocephali (Coates and Sequiera 2001; Coates et al. 2017, 2018; Frey et al. 2019; Fig. 2) have both a body form and a dentition superficially more similar to modern sharks than holocephalans. The teeth have multiple, high and pointed cusps, not joined at the level of the crown (e.g., *Cladoselache*, Fig. 3A) but fused at the root. These teeth are retained in a spiral whorl on the labial side of the jaw and not shed (e.g. Williams 2001). This pattern of cusps teeth fused basally is also present within even more basal taxa such as *Doliodus* (Maisey et al. 2014, 2019), along with *Ptomacanthus* (Brazeau 2012) and other taxa previously identified as acanthodians but now resolved phylogenetically as stem-group chondrichthyans (e.g., ischnacanthiforms [Burrow et al. 2018]; Zhu et al. 2013). This phylogenetic distribution (Fig.2) suggests that the partial fusion within tooth whorls is primitive to chondrichthyans, with the separate teeth seen in elasmobranchs being a derived character (Brazeau and Friedman 2004).

Despite the fused teeth of basal chondrichthyans, some early stem-group Holocephali do have separate teeth as in elasmobranchs (“individualized tooth bases”, Brazeau and Friedman 2004: 805). This suggests that separation of tooth bases to form a succession of separate teeth has evolved on multiple occasions. Separate teeth are present in the petalodonts (Stahl 1999), as well as in genera such as *Debeerius* (Grogan and Lund 2000). In addition, some holocephalans display both separate and joined teeth. In the Eugeneodontida, genera such as *Agassizodus* have small lateral teeth which appear to be separate and continuously replaced (e.g. Tapanila et al. 2018). However, the symphyseal tooth whorl of *Agassizodus*, as in other eugeneodontids, is at least partially fused. In the closely related *Edestus*, the individual symphyseal teeth are more shark-like in appearance

and are arranged so that each tooth base is overlapped by the next tooth crown, as also occurs within the sharks (Fig. 3D; also Taylor and Ademec 1977). This suggests that the tooth bases are not fused into a whorl and the teeth are at least partially separate (Fig. 3E), whilst the retention of teeth long enough for the tooth whorl to form an anteriorly-directed structure (Tapanila et al. 2018) suggests that partial fusion or interlocking of the roots (as in some batoids) is present. Whilst individual teeth of *Helicoprion* display a very similar shape to those of *Edestus*, they are clearly fused basally as the entire tooth whorl is retained, with a spiral structure forming during ontogeny (Tapanila et al. 2013). Similar basal fusion, or partial fusion, of rather shark-like teeth is seen in Iniopterygiformes such as *Iniopteryx* (Fig. 2; Zangerl and Case 1973: fig. 3A).

The stem-group holocephalan *Helodus* gives a particularly good insight into the mechanism of tooth fusion. *Helodus simplex* (Helodontiformes) is well known to show fusion of both the tooth base and the tooth itself (Moy-Thomas 1936; Patterson 1965; Stahl 1999; Coates et al. in press; Johanson et al. in press; Supplementary Information Figure S1B). In this species, some tooth whorls comprise separate teeth with unfused, if somewhat interlocking, roots (Fig. 3B, C). Other parts of the dentition show fusion of the tooth but with clear separation of the cusps, whilst tooth whorls from the most posterior parts of the jaw show fusion of both the root and crown, leading to a dental morphology originally considered to belong to a separate genus, *Pleuroplax* (Supplementary Information Figure S1B).

In addition to the change in tooth fusion within *Helodus*, it is notable that the tooth crown is entirely formed of tubate dentine (Fig. 3C, Supplementary Information Figure S2A, B), with the vascular canals joining the oral surface of the tooth at an angle but wearing to give a punctate appearance. This type of vascular dentine is also seen within the Eugeneodontida, for example in *Edestus heinrichi* (Fig. 3E). Therefore, the presence of teeth is a phylogenetically basal characteristic of the Holocephali, as has long been recognized (Owen 1867; Moy-Thomas 1936; Patterson 1965; Lund 1977; Didier 1995; Stahl 1999). Additionally, having a dentition comprised of distinct tubate dentine within the crown is a basal character.

As noted above, this dentine also characterizes a range of fossil holocephalans, with dental plates dominated by tubate dentine and the characteristic punctate surface, but

forming a range of plate morphologies; these include the groups Psammodontiformes, Copodontiformes, Menaspidiformes and Cochliodontiformes (Fig. 3F–M; Patterson 1965; Stahl 1999). The dental plates are of varying shapes, for example the flatter *Copodus* versus the curving *Deltodus* and the wavy oral surface of *Poecilodus* (Fig. 3F, H, J, L). However, in CT-data this dentine is consistently tubate across the dental plate, supported by a thin base of trabecular dentine (Figure 3G, I, K, M; Supplementary Information Figure S2C, D). Also relevant is that in these groups, the number of dental units has become reduced (i.e., relative to taxa such as *Helodus*, the Eugeneodontida and the Iniopterygiformes), but a larger number of dental plates are still present in the dentition compared to extant taxa, for example, in *Psammodus* (Psammodontiformes; Stahl 1999). Thus, this tubate dentine is homologous in separate teeth in taxa such as *Helodus*, and in these forms where teeth have been lost (Supplementary Information Figure S2).

Another characteristic of the extant holocephalans is the framework of less mineralized trabecular dentine that surrounds the distinct and separate tritoral pads, ovoids and rods (Smith et al. 2019; Fig. 1C–G). As noted, the tritoral pads are composed of tubate dentine, morphologically similar to that seen in many Paleozoic holocephalans (Fig. 3, Supplementary Information Figure S2), including those with separate teeth. A multiple stage process can be envisaged: A transition from stem-group holocephalans with teeth composed entirely of tubate dentine (*Helodus*, *Edestus*), through to dentitions where hypermineralized dentine has become constricted into discrete structures (tritoral pads), to the non-tubate dentine structures with some vascularization around the periphery (rods, ovoids). This would involve a compositional change from largely apatite to whitlockite, the change from tubate tissue forming a surface layer, to one being partially enclosed into the dentine to form tritoral pads, and the incorporation of rods and rows of ovoids into the preformed trabecular dentine of the plate (Fig. 2).

These blocks of tubate dentine incorporated into the dental plate as tritoral pads are first observed in fossil taxa such as *Ischyodus* (Fig. 4A, B) and *Edaphodon* (Family ‘Edaphodontidae’; Johanson et al. in press; Fig. 4C, D; Supplementary Information Figure S2E, F). In both genera, these tritoral pads dominate, and histologically they are surrounded by trabecular dentine that forms the framework of the entire dental plate (Fig. 4B, D). A thinner and more surficial tritoral pad is present in ‘*Ischyodus*’ *curvidens* (Fig. 4H–J) and

may mark a morphological intermediate between the state in stem-group and extant holocephalans.

The rods and ovoid series have a restricted distribution within the extant Holocephali, being found only in the families Rhinochimaeridae (Fig. 1G, H) and Chimaeridae (Stahl 1999; Smith et al. 2019). In these taxa, the ovoids and rods are well-organized within the dental plate. For example in *Hydrolagus* (Fig. 1G, H), a pair of elongate rods is present on either side of the jaw symphysis in the anterior upper dental plate, with multiple series of closely spaced ovoids extending along the labial plate margin. Because these ovoids and rods form in pre-existing dentine spaces, this suggests some patterning mechanism is present, regulated by developmental processes at the forming surface of the dental plate (Smith et al., 2019; Supplementary Information Figure S1A, *Harriotta*). With respect to fossil taxa, in the family ‘Edaphodontidae’, ‘*Ischyodus*’ *curvidens* (Fig. 4H, I) shows a series of ovoids symphysially, in combination with the thin tritoral pad (Fig. 4J).

*Elasmodectes* has an unusual upper dental plate shape that shows an anteroposterior line of ovoids along the lingual part of the oral surface of the plate (Fig. 4E). However, internally, there is an extensive layer of trabecular dentine, but then several series of randomly packed ovoids (Fig. 4F, G). Lower dentitions of *Elasmodectes* (Fig. 4K–O) likewise have ovoids, but no tritoral pads. In section (Fig. 4L–O), a more basal region of trabecular dentine can be seen, with irregular ovoids within this dentine (the ovoids are seen as black spaces, where mineral content is low, indicating early formation) (Fig. 4L, N).

The presence of non-patterned ovoids in the family ‘Edaphodontidae’ differs from the ovoids in the crown-group Holocephali. As noted, in *Harriotta*, the framework trabecular dentine forms as spaces into which the dentine (whitlockin) of the discrete tritoral pads and ovoids is secreted (Smith et al. 2019; Supplementary Information Figure S2). We suggest that the evolution of the capacity to form these discrete spaces, through developmental plasticity, is regulated at the base of the dental plate, possibly by stem cells for making dentine. This originated with taxa such as *Ischyodus* within the Jurassic, including ‘*Ischyodus*’ *curvidens*, possessing a tritoral pad and ovoids. However, ovoids, but not tritoral pads, were present far earlier in the Carboniferous *Chondrenchelys*. In *Chondrenchelys*, these ovoids appear radially arranged at the oral surface (Finarelli and Coates 2012), suggesting a patterning to the ovoid series, with patterning also

characterizing extant holocephalans. Either this patterning is lost in taxa such as *Ischyodus* and *Elasmodectes*, then evolved convergently, or perhaps the surficial patterning in *Chondrenchelys* is not indicative of internal patterning, as seen in '*Ischyodus*' *curvidens* (Fig. 4E–G).

### **Retention of Teeth in Crownward Stem Holocephalans**

As previously discussed, shark-like teeth (e.g. with cusps) are found in taxa such as *Helodus* (Helodontiformes) and *Edestus* (Eugeneodontida; Fig. 3B–E). In these the teeth are separate or show varying degrees of fusion within the tooth series, to resemble a dental plate (Moy-Thomas 1936; Stahl 1999; Johanson et al. in press; (Supplementary Information Figure S1B). The general trend, as described above, is to lose teeth completely, however, in another stem holocephalan, *Myriacanthus* (Figs. 2, 5), teeth may be retained within the body of the dental plate. The overall dentition of *Myriacanthus* is very unusual (Patterson 1965). However, in the anterior upper dental plates, several round, worn structures are visible, showing some degree of organization (Fig. 5B, white arrowheads). These were described remnants of tooth crowns by Lund and Grogan (1997: 93), and they do show what appears to be mineralized tubate dentine, with individual openings of the incorporated vascular canals visible at the worn oral surface (Fig. 5B, C, white arrows). This is reminiscent of dentine in the crowns of the individual teeth of *Helodus* and *Edestus*, as described above.

### **Discussion**

Although the dentitions of stem-group holocephalans such as *Cladoselache* have elasmobranch-type dentitions with teeth organized into tooth families, more crownward taxa including *Helodus* have separate teeth, but also fusion of teeth (Moy-Thomas 1936; Stahl 1999; Coates et al. in press; Johanson et al. in press). The presence of separate teeth is clearly a plesiomorphic character for the Holocephali, while in *Helodus* and *Edestus* (Fig. 2) the tooth crown is comprised of tubate dentine that characterizes the dental plates of other fossil holocephalans (Figs. 3, Supplementary Information Fig. S1B–E, S2). This tissue, proposed as homologous across these taxa (Supplementary Information Figure S2), has been modified in terms of shape and distribution within the dental plate, through the

history of the group. As well, during the evolution of the holocephalan dentition, the trabecular dentine that forms the framework tissue of these fossil holocephalan dental plates expands from its restricted location at the base of the dental plate (Supplementary Information Fig. S2C, D), and acquires the developmental capability (from tissues at the postero-lingual margin of the plate) to form spaces for the restricted deposition of whitlockin, that includes dentine forming the tritors, rods and ovoids (e.g., Supplementary Information Figs S1A, S2C, F).

These structures and their histology have been observed in extant taxa, and can be extrapolated to fossil forms. Fossils assigned to the Family ‘Edaphodontidae’ include both ovoid series and tritoral pads (*Ischyodus curvidens*) with the loss of these ovoids characterizing the Family Callorhinchidae, where only tritoral pads are present. As well, the tritoral pad increases in size in the Callorhinchidae. The ovoid series in taxa such as *Elasmodectes* lack the organization that is so characteristic of the extant forms. This suggests that precise patterning of the trabecular dentine to form spaces for this dentine only evolved over time. Although fossils, these taxa as described herein can provide substantial evidence, including developmental information as data relevant to interpretation of the evolution of the diversified, toothless holocephalan dentition within the Chondrichthyes.

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## FIGURE AND TABLE CAPTIONS

**Fig. 1** Comparison of chondrichthyan dentitions, digital 3D rendering of X-ray CT data. **(A, B)** Elasmobranchii, including **(A)** the shark *Lamna nasus* (Selachii; porbeagle shark) and **(B)** the ray *Aetobatus* sp (Batoidea: eagle ray), showing individual teeth arranged into tooth series. Holocephali, including **(C–E)** *Callorhinchus milii* (Callorhinchidae: elephant shark) and **(F, G)** *Hydrolagus* sp (Chimaeridae: rabbitfish). **(C)** dental plates in the upper and lower jaws (oral view); **(D)** tritoral pads of hypermineralized dentine, trabecular dentine removed, virtually; **(E)** antero-posterior section through the upper and lower jaws, showing dental plates including framework trabecular dentine and hypermineralized tritoral pads. **(F)** anterior upper dental plates (oral view), showing hypermineralized tritoral pads and surrounding framework trabecular dentine; **(G)** tritoral pads, ovoids and rods of hypermineralized dentine, alone. **Abbreviations:** **1–3**, hypermineralized tritoral pad dentine of the anterior upper, posterior upper and lower dental plates, respectively; **ab**, aboral territory; **a.dpl**, anterior upper dental plate; **l. jaw**, lower jaw; **l. jaw cart**, lower jaw cartilage; **l.dpl**, lower dental plate; **or**, oral territory; **ov**, hypermineralized ovoid dentine; **p.dpl**, posterior upper dental plate; **rd**, hypermineralized dentine rod; **t**, teeth; **trab**, trabecular dentine forming framework on the dental plate; **tri.pd**, hypermineralized tritoral pad; **u. jaw cart**, upper jaw cartilage; **vc**, vascular canal. **(F, G)**, from Smith et al. 2020. *J Fish Biol* <https://doi.org/10.1111/jfb.14302> [Figs 5a, b].

**Fig. 2.** Simplified and composite phylogeny of the chondrichthyans, showing the relative positions of stem group holocephalan clades. Adapted from Stahl (1999), Coates et al. (2017, 2018), Frey et al. (2019). Dental characters mapped above the phylogeny.

**Fig. 3.** Holocephali, digital 3D rendering of X-ray CT data of Paleozoic stem-group taxa. **(A)** NHMUK PV P.9264, *Cladoselache* sp, jaw with multiple series of teeth; **(B, C)** NHMUK PV P.8216, *Helodus simplex*, **(B)** partial anterior tooth whorl of showing separate teeth, **(C)**, virtual section showing separate tooth crown formed of tubate dentine and separate, if strongly interlocking, roots. **(D, E)** NHMUK PV P.75723 *Edestus heinrichi*, **(D)** part of the symphyseal dentition; **(E)** virtual section of **(D)** showing separate tooth crowns and closely packed elongate roots (white arrows). **(F–M)** dental plates dominated by vascularized tubate dentine and thin layer of trabecular dentine, white lines show lines of

virtual section. (**F, G**) NHMUK PV P.2601 *Copodus auriculatus*; (**H, I**) NHMUK PV P.2437 *Deltoptychius acutus*; (**J, K**) NHMUK PV P.2444 *Deltodus sublaevis*; (**J, K**) NHMUK PV P.2462 *Poecilodus jonesii*. (**F, H, J, L**) occlusal view of the single plate; (**G, I, K, M**) virtual section showing internal structure. **Abbreviations:** as in previous figures. Scale bars= (**A**) 2 cm; (**C**) 0.2 cm; (**D, E**) 1 cm; (**H**) 4 mm; (**I**) 3 mm; (**J**) 3 mm; (**K**) 1 mm; (**L**) 3 mm; (**M**) 2 mm. (**B, C**) from Johanson et al. in press. Ichthyological Explorations of Freshwaters. Verlag Pfeil, Munich [Figs 1B, D].

**Fig. 4.** Holocephali, digital 3D rendering of X-ray CT data of dentitions of the ‘Edaphodontidae’. White lines indicate virtual sections through the dental plate. (**A, B**) NHMUK PV P.324, *Ischyodus dolloi*, upper dental plate in occlusal view (**A**) and virtual section showing thick tritoral pad (**B**). (**C, D**) NHMUK PV OR 328871, *Edaphodon* sp., (**C**) upper dental plate in occlusal view, and virtual section (**D**). (**E–G**) NHMUK PV P.3107 *Elasmodectes falcatus*, (**E**) upper dental plate in occlusal view, and virtual section (**F, G**) showing disorganized packing of hypermineralized ovoids. (**H–J**) NHMUK PV P.5152 ‘*Ischyodus*’ *curvidens*, (**H**) lower dental plate in occlusal view, and virtual sections showing disorganized packing of hypermineralized ovoids (**I**) and shallow tritoral pad (**J**). (**K–O**) BMB 007295 *Elasmodectes willetti*, (**K**) lower dental plate in occlusal view, and virtual sections (**L–O**) showing trabecular dentine disorganized packing of hypermineralized ovoids. **Abbreviations:** as in previous figures. Scale bar- **E**= 5 mm; **F**= 4 mm; **K**= 5 mm. (**C, D**) from Johanson et al. in press. Ichthyological Explorations of Freshwaters. Verlag Pfeil, Munich [Figs 7D, E].

**Fig. 5.** Holocephali, macrophotography of NHMUK PV P. 4664, *Myriacanthus paradoxus*, (**A**) articulated skull, jaws and dentition. (**B**) closeup of anterior dental plate and part of the posterior dental plate. White arrowheads indicate organized teeth within the dental plate. (**C**), closeup of posterior dental plate, showing teeth within the dental plate, including tubate dentine. **Abbreviations:** as in previous figures, also **symph.t**, symphyseal tooth. Scale bar= 1 cm.

**Table 1** List of parameters used to characterize the specimens presented here. Note that for the Zeiss 520 Versa, the composition and thickness of filters are not disclosed by the manufacturer and only described as high energy (HE) or low energy (LE) filter; the number following the acronym give an indication relative to the thickness (the higher the number, the thicker the filter).

**Supplementary Information Figure S1.** (A) *Harriotta raleighiana*, virtual section through upper posterior dental plate showing more highly mineralized ovoid and tritoral pad dentine at the oral surface (white in this image). At the developing surface, the framework trabecular dentine is preforming spaces for the ovoids and tritoral pad. From this point, to the oral surface, these space are filled in with dentine that becomes hypermineralized. (A) from Smith et al. (2019 Zoology Letters.

<https://doi.org/10.1186/s40851-019-0125-3>; (B) *Helodus* sp. (NHMUK PV P8320), Johanson et al. in press. Ichthyological Explorations of Freshwaters. Verlag Pfeil, Munich. (C-F) serial sections through the teeth of *Helodus appendiculatus* (NHMUK PV P75938); (C) near crown surface; (D) trabecular dentine; (E) vascular canals surrounded by whitlockin hypermineralized dentine; (F) lamellar dentine. Abbreviations: **lam**, lamellar dentine; **od**, outer dentine layer of dental plate; **ov**, ovoid; **peri**, peritubate dentine; **tub**, tubate dentine; **tr**, trabecular dentine; **tri**, tritoral pad; **vc**, vascular canal, **whit**, whitlockin.

**Supplementary Information Figure S2.** (A, B) NHMUK PV P.8216, *Helodus simplex*, (A) partial anterior tooth whorl of showing separate teeth, occlusal view; (B), virtual section showing separate tooth crown formed of tubate dentine (pink) and trabecular dentine (blue); (C, D) NHMUK PV P.2437 *Deltoptychius acutus* (D) dental plate in occlusal view; (S), virtual section through the dental plate showing distribution of tubate dentine (pink), trabecular dentine (blue) and lamellar dentine. (E, F) NHMUK PV OR 328871, *Edaphodon* sp, (E) upper dental plate in occlusal view; (D) virtual section through the dental plate showing distribution of tubate dentine (pink) and trabecular dentine (blue). White lines in A, C, E, indicate sections seen in B, D, F. **Abbreviations:** **lam**, lamellar dentine; **tub**, tubate dentine; **trab**, trabecular dentine. (A, B, E, F) from Johanson et al. in

press. Ichthyological Explorations of Freshwaters. Verlag Pfeil, Munich [Figs 1B, D, 7D, E].