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1	Holocephalan (Chondrichthyes) dental plates with hypermineralized
2	dentine as a substitute for missing teeth through developmental
3	plasticity
4	
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26 Abstract

27 All extant holocephalans (Chimaeroidei) have lost the ability to make 28 individual teeth, as tooth germs are neither part of the embryonic development 29 of the dental plates, nor of their continuous growth. Instead, a 30 hypermineralized dentine with a unique mineral, whitlockin, is specifically 31 distributed within a dentine framework into structures that give the dental 32 plates their distinctive, species-specific morphology. Control of the regulation 33 of this distribution must be cellular, with a dental epithelium initiating the first 34 outer dentine, and via contact with ectomesenchymal tissue as the only 35 embryonic cell type that can make dentine.

36 Chimaeroids have three pairs of dental plates within their mouth, two in 37 the upper jaw and one in the lower. In the genera Chimaera, Hydrolagus and 38 Harriotta, the morphology and distribution of this whitlockin within each dental 39 plate differs both between different plates in the same species and between 40 species. Whitlockin structures include ovoids, rods and tritoral pads, with 41 substantial developmental changes between these. For example, rods appear 42 before the ovoids, and result from a change in the surrounding trabecular 43 dentine. In *Harriotta*, ovoids form separately from the tritoral pads, but also 44 contribute to tritor development, while in *Chimaera* and *Hydrolagus*, tritoral 45 pads develop from rods that later are perforated to accommodate the 46 vasculature. Nevertheless, the position of these structures, secreted by the 47 specialized odontoblasts (whitloblasts), appears highly regulated in all three 48 species. These distinct morphologies are established at the aboral margin of 49 the dental plate, with proposed involvement of the outer dentine. We observe 50 that this outer layer forms into serially added lingual ridges, occurring on the

anterior plate only. We propose that positional, structural specificity must be
contained within the ectomesenchymal populations, as stem cells below the
dental epithelium, and a coincidental occurrence of each lingual, serial ridge
with the whitlockin structures that contribute to the wear resistant oral surface.

56 Keywords Chimaeroidei, dentine, dentition, Holocephali, development,
57 whitlockin

58

59 **1 INTRODUCTION**

60 Among chondrichthyans, the extant holocephalan dentition is specialized and 61 unique, with three pairs of continuously growing tooth plates. Compared to 62 other chondrichthyans (sharks and rays), these extant holocephalans have 63 lost the ability to make individual teeth (tooth agenesis), with tooth germs not 64 being observed during embryonic development. However, teeth are present in 65 fossil, stem holocephalans such as *Helodus* and *Cladoselache* (e.g., Stahl, 66 1999; Coates et al., 2018; Frey et al., 2019), and are arranged in tooth files or 67 families, comparable to the shark and ray dentitions (Dean, 1894; Moy-68 Thomas, 1936; Johanson et al., in press). The phylogenetic position of these 69 stem taxa supports a loss of teeth in extant holocephalans. 70 Tooth plates in these extant groups are entirely composed of three types 71 of dentine, one of which is hypermineralized, forming into distinct patterns 72 among taxa, despite the lack of teeth in development and growth. The focus 73 of our paper is to describe the histogenesis and renewal of dentine in upper 74 jaw dental plates of Chimaera and Hydrolagus (Family Chimaeridae), 75 following on from a recent description in *Harriotta* (Family Rhinochimeridae;

76 Smith *et al.*, 2019). The arrangement of the dentine types, each of a different 77 hardness, accounts for the morphology of the worn oral surface and is specific 78 to each taxon, once the juvenile morphology has transformed into that of the 79 adult (Smith et al., 2019). Different rates of wear during feeding create raised 80 areas and fossae that combine to make a highly adapted and functional 81 dental surface (e.g., Figure 1c, d). All the tissues are renewed by aboral 82 growth, possibly with epigenetic input to the late timing of extra mineralization 83 (Smith et al., 2019), potentially controlled by the odontoblasts within the 84 trabecular dentine.

85 Previously, we described a new type of extra hard, hypermineralized 86 dentine (renamed whitlockin, from the generic term pleromin) in the dental 87 plates of *Harriotta* of both adult and juvenile forms (Smith *et al.*, 2019). This 88 whitlockin contains the magnesium-rich mineral whitlockite ((B-Ca3 $Mg_{X}(PO4)_{2}$) that, during dentine mineralization towards the oral surface of 89 90 the tooth plate, replaces the more typical hydroxyapatite, and becomes more 91 compact. This hypermineralized tissue was described as tritoral dentine 92 (reviewed in Didier, 1995; Stahl, 1999) forming the distinctive patterns of 93 specifically ordered ovoids (beads), rods, and tritoral pads, that after wear of 94 the surrounding softer trabecular dentine, become exposed at the oral surface 95 as projections, representing the morphological pattern of the wear-resistant 96 whitlockin. It was proposed that a pre-pattern of spaces in the trabecular 97 dentine, at the growing aboral surface, preceded formation of the ovoids, rods 98 and tritoral pads, with secretion of whitlockin into these spaces by specialized 99 odontoblasts (whitloblasts). In establishment of this pattern, whitlockin was confined within these spaces, where a single layer of the specialized 100

whitloblasts generate massive numbers of ramifying tubules that formed this
highly mineralized dentine (Smith et al., 2019: figs. 4, 6). However, details of
the formation of this dentine in development were confined to the lower dental
plates; the role of the dental epithelium surrounding the plates in forming any
tissues that shaped the structure of the dental plate, or the positioning and
timing of the whitlockin within the dental plate, is completely unknown.

107 Here we focus on tissues of the upper dental plates, from a variety of 108 chimaeroid taxa including different growth stages of Chimera monstrosa 109 Linnaeus, 1758 and Hydrolagus mirabilis Collett, 1904, as well as a juvenile 110 Chimaera sp. and a specimen of Harriotta. The study primarily used µCT-111 scanning and relative density differentiation (3µm-12µm voxels) to evaluate 112 the morphology and distribution of whitlockin relative to trabecular dentine, 113 and how these mineralized elements changed through growth. The tritoral 114 tissues of all three plates of each side of the upper and lower jaws have 115 specific intra-topographic patterns to the arrangement of the whitlockin. In 116 terms of potential mechanisms for patterning this tissue distribution, we have 117 observed uniquely iterative serial ridges of the outer dentine layer, only 118 present on the epithelially covered lingual surface of the anterior upper dental 119 plates. All trabecular dentine forms from odontoblasts at the aboral surface of 120 the plate, within the outer dentine (forming the ridges), these developmental 121 events are located in the cartilage furrow, nominally the location where the 122 dental epithelium also occurs (Smith et al., 2019). We also observed a co-123 incidence of new ridges with that of whitlockin formation and hypothesize a 124 developmental model in which the ridges on the outer dentine layer are 125 relevant to the pattern of rods and ovoids, whose formation is regulated at the aboral surface, the putative location of odontogenic stem cells of the pulp

127 cavity associated with the dental plate (Smith *et al.* 2019).

128

129 2 MATERIALS AND METHODS

- 130 All specimens from UK waters were obtained from scientific trawls by Marine
- 131 Scotland (Chimaera monstrosa, Hydrolagus mirabilis, Harriotta raleighana
- 132 Goode & Bean, 1895), with all individuals arriving on deck already dead as is
- 133 the case with deep water chimaeroids (Finlay Burns, Marine Scotland, *pers.*
- 134 *comm.*). The specimen of *Chimaera* sp. is from a commercial trawl off Taiwan
- 135 but further details are lacking. Callorhinchus milii Bory de Saint-Vincent,
- 136 1823 was obtained from Dr Catherine Boisvert (Monash University, Victoria,
- 137 Australia, Monash Animal Services (MAS) ethics permit MAS/ARMI/2010/01),
- 138 with animals euthanized via an overdose of tricaine in seawater (see
- 139 Johanson et al., 2015 for further details). No surgical procedures, nor
- 140 experiments, were performed.
- 141

142 **3 RESULTS**

- 143 **3.1** *Chimaera monstrosa* and *Chimaera* sp.
- 144 In *Chimaera*, the close fit of the anterior and posterior upper dental plates is
- apparent (Figures 1c, d, 2a, 3a, c, a.dpl, p.dpl, 2b, white arrows;
- 146 Supplementary Information File 1), such that when the density of whitlockin is
- segmented out (Figures 1d, arrow, 2c, 3c), the highly mineralized rods and
- 148 ovoids of the anterior plate are in close alignment with those of the posterior,
- presumed to result from tight morphological constraints. One feature on the
- 150 lingual surface of the anterior plate in all growth stages is the iterative,

sequentially developed ridges of the outer dentine layer. In the youngest
individual examined (14cm long; Figure 1c, rdg), 3–4 widely spaced ridges are
present, with small oval dentine units at the midline (*) of each dental plate.
Notably these are not hypermineralized tissues as are the rods found in these
plates (Figure 1d, ap.rd, pp.rd) but just elevations of the outer dentine layer.
Also at this growth stage, the hypermineralized rods are shorter, and do not
appear to extend through the dental plate.

158 At the next growth stage (Figure 2), there has been substantial wear on 159 both anterior and posterior dental plates, as indicated by the thickness of the 160 sclerotic dentine (sensu Smith et al., 2019; Figure 2b, g, h, sod), forming in 161 response to wear. The hypermineralized rods of the anterior plate are longer, 162 and extend through more of the anterior plate, while on the posterior dental 163 plate, more rods are present when compared to the 14cm individual (Figure 164 2c). The number of ridges on the lingual surface of the plate has increased as 165 well, to seven, with the most oral ridge showing wear (Figure 2a, b). These 166 are absent from the comparable surface on the posterior dental plate (Figure 167 2a). The posterior end of these ridges is expanded and bulbous, particularly 168 the most aboral ridges (Figure 2b, d, f, white asterisk), the histology of the 169 lower jaw plates of *Harriotta* showing that this outer dentine layer does 170 become even more mineralized (Smith et al., 2019). Just below these ridges, 171 the aboral margin of the plate is composed of an open network of trabecular 172 dentine (Figure 2f, h, tb). In section, the ridges surround trabecular dentine 173 (Figure 2g, h), with coincidence of early whitlockin formation of the rod and the ridge itself (Figure 2g, opposing white arrows). Sclerotic dentine fills the 174 175 ridges in the same manner as it fills the spaces within the trabecular dentine

176 closer to the oral surface (Figure 2g, h). Additionally, the ridges themselves

appear to be set off from the rest of the plate, with a shallow furrow

developing posteriorly (Figure 2f, arrow 1) and an elongate oral-aboral ridgeanteriorly (Figure 2f, arrow 2).

180 The next two growth stages are represented by a subadult *C. monstrosa* 181 and a juvenile Chimaera sp. (Figure 3), and in the upper dental plate of the 182 younger of these individuals (Figure 3a–e), the hypermineralized dentine 183 forms a series of ovoids in both the anterior and posterior dental plates 184 (Figure 3b, ov). Along with this is a small rod located posteriorly and 185 parasymphysially (Figure 3b). The posterior dental plate includes multiple 186 series of ovoids, along with a small number of rods, at the anterior end. As 187 well, there are two elongate rods, lingual and medial to the ovoids (Figure 3a, 188 b). There are 6–7 ridges on the lingual surface of the anterior plate, although 189 the bulbous ends are not as apparent (Figure 3c, e), and a shallow furrow and 190 elongate ridge mark the boundaries of this region within the plate (Figure 3e, 191 arrows 1 and 2). In section (Figure 3d), there again appears to be some 192 spatial relationship between the series of rods and the lingual ridges, including 193 those with developing rods that are less mineralized (grey (d) in Figure 3d; 194 see also Figure 8). In the older individual (Figure 3f–i), the number of lingual 195 ridges stays more or less constant. The anterior dental plate includes both a 196 series of ovoids and of rods, but in the posterior dental plate, the rods are 197 being modified to produce the tritoral pads, with spaces for the vasculature 198 appearing aborally within the whitlockin tissue (Figure 3g).

199

200 3.2 Hydrolagus mirabilis

201 Anterior and posterior dental plates of a young juvenile and adult of 202 Hydrolagus (Figures 4, 5) are comparable to those of *Chimaera*, with an ovoid 203 series of whitlockin in the anterior plate and a parasymphyseal rod in the adult 204 (Figure 5b), and a small number of extra rods in the ovoid series in the 205 juvenile (Figure 4b). With respect to the posterior plate, in the earlier growth 206 stage, both an ovoid series and rods are present along the labial margin of the 207 plate, with two rods present lingually and medially. As well, a tritoral pad is 208 developing posterior to these two rods, appearing to form via the incorporation 209 of individual ovoids (Figure 4b, black arrowheads on left side of image, 210 Supplementary Information files 1–3). In the adult, broad, well-developed 211 tritoral pads are present in this position, and with respect to the more medial 212 rods, the more posterior has become intensely vascularized to resemble the 213 more labial pad. However, the more anterior rod shows only a few openings 214 that represent incorporation of blood vessels within the mineralized dentine. 215 Along the labial margin, both series of ovoids and rods are present (Figure 216 5b).

217 In both growth stages, trabecular dentine below the oral surface has 218 been infilled with sclerotic dentine (Figures 4d, e, 5d, e), and becomes deeply 219 worn, exposing the ovoids and tritoral pads that resist deep wear (Figures 4c, 220 d, f, 5c, d, f). Serial ridges are present on the lingual surface of the anterior 221 dental plate, with five thick ridges in the juvenile, comparable in morphology to 222 those in *Chimaera* (Figure 4). However, in the adult, the ridges are more 223 numerous but less distinct (Figure 5). Bulbous expansions at the end of these 224 ridges are absent in both these growth stages (Figures 4c, f, 5c, f). As in 225 *Chimaera*, there may be correspondence between the ridges and the forming

ovoids (Figures 4e, 5e, double arrow). As well, trabecular dentine forms in
advance of these ridges forming in both growth stages (Figures 4f, 5f) but
always inside a shell of outer dentine. The posterior furrow and oral-aboral
ridge are present in both growth stages although the furrow appears to be
deeper in the adults (Figures 4f, 5f, arrows 1 and 2).

231

232 **3.3 Harriotta raleighana**

233 In this juvenile specimen (Figure 6; Smith et al., 2019: fig.17a), with respect to 234 whitlockin, the anterior dental plate has both rods and ovoids, while in the 235 posterior plate, tritoral pads and ovoids are present (Figure 6C). In older 236 individuals, these rods are replaced within the dental plate by ovoids, and the 237 tritoral pads are better developed (Smith et al., 2019: figs. 1D, 17). On the 238 specimens examined, there is no indication of posterolingual rods on the 239 posterior dental plate (Smith et al., 2019: fig. 17), and instead, in the youngest 240 individual available (Figure 6C), two short series of ovoids are present. 241 However, these do not transform into tritoral pads (Smith et al., 2019: fig. 17). 242 One important difference between the Chimaeridae and Harriotta is the 243 absence of lingual ridges on the anterior dental plates of Harriotta (Figure 6a, 244 b, e). However, while these are not conspicuous on the lingual surface of the 245 outer dentine, the distinct region of the plate associated with the ridges in the 246 other taxa described here can be recognized and defined by the furrow and 247 oral-aboral ridge (Figure 6e, arrows 1, 2).

248

249 **3.4 Anterior versus posterior dental plates**

250 With respect to the whitlockin in the anterior dental plate, only rods or series 251 of ovoids are formed, with rods appearing dominant in early growth stages 252 (e.g., Figure 2c), and ovoids later, implying a developmental change in the 253 shape of the space, but not their location, within the trabecular dentine. Rods 254 are retained within the anterior dental plate, normally in a parasymphysial 255 position. By comparison, the posterior dental plate is more variable and 256 includes broad tritoral pads, which are blocks of highly vascularized whitlockin 257 (e.g., Figure 5b, 6a), more characteristic of adult dentitions. In earlier growth 258 stages the position of the tritors is occupied by whitlockin rods (Figures 3b, g, 259 4b) that subsequently become vascularized (Figures 3g, 5b). This 260 characterizes the more lingual, midline tritors, but in the case of labial tritors, 261 evidence from the earlier growth stage of *Hydrolagus* (Figure 4b; 262 Supplementary Information) indicates that the tritor is forming from the 263 incorporation of ovoids into the tritor. Thus, it seems that the tritors develop 264 differently in the posterior dental plates, by intrusion into whitlockin rods by 265 blood vessels lingually, and by addition of material to surround preexisting 266 blood vessels, labially. These dental plates show more morphological and 267 developmental diversity than previously appreciated.

268

269 **3.5 Synthesis of interpretation**

In all of the extant holocephalan species studied here the hypermineralised
tissue, whitlockin, whether ovoids, rods or tritoral pads, occurs in different
arrangements within the dental plate, all highly regulated. As part of this
regulation (Smith *et al.*, 2019), spaces for the whitlockin are preformed in the
trabecular dentine comprising much of the dental plate, at the aboral surface,

275 with whitlockin deposited within the spaces by specialized odontoblasts; our 276 new observations implicate superficial dentine ridges, also newly forming 277 lingually at the aboral surface of the anterior dental plate, in this patterning of 278 the rods and ovoids. These ridges are less mineralized than the whitlockin, 279 with locations that seem to concur with the position of the newly forming rods 280 and ovoids (Figures 2–4); a superimposed colour image (Figure 8) 281 summarizes our interpretation of the process by which dental epithelium could 282 induce the canonical ectomesenchymal tissue to make the whitlockin type of 283 dentine in the correct arrangement for each species, discussed further below. 284 Along with this, a clear ontogenetic pattern is present, with the whitlockin rods 285 dominating the lateral parts of the plates of the younger individuals; 286 positionally these rods are replaced by ovoid series in older individuals. As 287 well, rods appear to be located medially in younger individuals, to be replaced 288 in these positions by the tritoral pads.

289

290 4 Discussion

291 Previously Smith et al. (2019) showed the detailed arrangement of the 292 specialized hypermineralized dentine forming in set spaces within the 293 trabecular dentine, and that its mineral composition incorporated a novel 294 mineral form, called whitlockin, in preference to the more generic term 295 pleromin (Kemp, 1984; Ørvig, 1985; Didier et al., 1994; Stahl, 1999). Only the lower dentition of Harriotta (representing the Rhinochimaeridae) was studied 296 297 but it was concluded that this whitlockin determined the specific shape of the oral surface, as it was more resistant to wear than the supporting dentine, and 298 299 was regulated by renewal at the aboral surface by an unknown process.

Here we have demonstrated the different morphologies of the oral 300 301 surface in the upper four plates, compared to the two lower jaw plates, 302 sculpted by wear resistant dentine (Figure 7a-c). These differences between 303 the plates, as well as different morphologies among the taxa, are determined 304 by intraspecific modulation of each dental plate. These surface morphologies 305 are dependent on the properties and arrangement of the tissues, as modified 306 in occlusion. For example, in *Hydrolagus* upper dental plates, a marginal 307 antero-labial ridge with hard, translucent whitlockin ovoids, set in less hard 308 sclerotic osteodentine, surrounds a deep, extensive fossa of the less 309 mineralized sclerotic osteodentine (double-headed arrow, Figure 7a, see also 310 µCT scans Figure 5a–f). Notably, the occlusion of the four upper plates can 311 be appreciated in lateral and frontal views (Figure 1a, b), where the close 312 alignment of the anterior with the posterior plate is shown, also in occlusal 313 views (Figures 2b, 3e, f, 4a, f). They act as one unit of the upper jaw to meet 314 with the anterior edges of the two lower jaw plates. Also, the close alignment 315 of the hypermineralized rods and ovoids between anterior and posterior plates 316 adds to this functional link, as a feature suggesting common morphological 317 control to form a morphology against which the lower plates bite (Figures 1d, 318 2c, 3g, 4b, 5b, 6c, lower jaw ovoid stack, 7b).

We discuss the likely control of this patterning by a dental epithelium surrounding the plate margins, of the continuous growth plates, modeled on new observations of distinctive ridged, lingual surfaces on the anterior upper dental plate (Figures 1a, 2a–h, 3a–l, 4a–f, 5a–f, 7a, 8b). During dental plate development, an outer shell forms, composed of dentine formed by odontoblasts creating two layers, surrounding the whitlockin and trabecular 325 dentine. The outer layer forms by apposition to the inner, demonstrated by the 326 odontoblast tubules directed inwards from cells located on the outermost surface of the dental plate (Smith et al., 2019: figs. 3A, 4B), while the inner 327 328 layer forms from odontoblasts associated with the trabecular dentine. The 329 outer layer, in particular, is heavily mineralized and hence translucent, 330 revealing the structures beneath (Figure 7b, double arrow; Smith et al., 2019: 331 figs 1A, 2A, 3A, 7C). We have assumed these odontoblasts are 332 ectomesenchymal cells that deposit the dentine layer from beneath a layer of 333 epithelium, but currently have no supporting soft tissue histology (see Figure 334 7e). Nevertheless, if this is accepted as a cellular model, then control of 335 growth at the aboral surface can be by epithelial-mesenchymal interactions 336 that regulate the timing and positioning of new tissues.

337 Observations from the anterior dental plates of chimaeroids 338 (Hydrolagus, Figure 7a, c, with plates in situ) provide a model for proposed 339 epithelial control for the serial production of ridges on the lingual surface of 340 the plate but also dentine produced by ectomesenchymal derivatives (see 341 below). Notably, these ridges are not heavily mineralized (low density, missing when whitlockin structures are segmented out, e.g., Figure 2b), and form 342 343 aborally in advance of the trabecular dentine spaces where rods, ovoids and 344 tritors will develop (Figures 2g, 4e). The ridges coincide with these developing 345 whitlockin elements on the labial surface, contributing to the antero-labial 346 ridge (Figures 2g, 3d, 4e, 5e, 8b, nos. 0–4), as well, sclerotic dentine forms 347 within the ridges, spreading through the plate to form the fossa of the wear 348 surface (Figures 2g, 7d).

349 We also compared the distribution of the hypermineralized dentine in 350 embryos of *Callorhinchus milii* (red, Figure 7e, ple; Kemp 1984: figs. 3B, 5) 351 and that of the adult (Figure 7f, false colored red), with the whitlockin of rhinochimaeroids and chimaerids, forming as separate ovoids within 352 353 trabecular dentine (Figure 7d, false colored red, rings denoting capsular 354 dentine). In Callorhinchus (Callorhinchidae, sister group to Rhinochimaeridae 355 + Chimaeridae) the whitlockin comprises continuous antero-posterior ridges, 356 surrounded by trabecular dentine, continuous growth of each is aboral to form 357 low contoured wear surfaces (Figure 7f). Control of the specific arrangement 358 of this whitlockin must be generated in the embryo by pattern regulation of the 359 cell layers, dental epithelium and ectomesenchyme, as described in the 360 analysis of embryos of Callorhinchus (Kemp, 1984). The ectomesenchyme is 361 canonically accepted to be necessary to produce dentine, also is derived from 362 cranial neural crest, as demonstrated in teeth of an osteichthyan fish (Kundrat 363 et al., 2008).

The generic model based on Callorhinchus milii shows both an 364 365 enclosing layer of epithelium of the blastema for the entire dental plate 366 including a 'diffuse mesenchyme' at the formative, aboral side, with a 'dense 367 'mesenchyme' at the postero-lingual, aboral surface (ide, md, Figure 7e; from 368 Kemp, 1984: fig. 5B). We propose a similar tissue arrangement in Chimaera 369 and *Hydrolagus*, creating the serial pattern of ridges on the anterior dental 370 plate (Figure 8b, nos. 0–4). In the youngest specimen of *Chimaera*, one 371 symphysial and two marginal rods are present, whereas the older juvenile has four marginal plus a symphysial rod (Figures 1c, d, 2a-c), coinciding with 372 373 serial addition to the lingual ridges (Figure 2g). This suggests an increase in

the number of rods forming the anterior margin with regulated growth, as
apparent in the growth series of *Chimera monstrosa* (Figure 8a, nos. 0–2, 8b,
nos. 0–4).

377 Also, there appears to be a correlation of lingual ridges with new 378 formation of ovoids (replacing the rods) in the oldest Chimaera dental plate 379 (arrows, Figures 2g, 3d, 8d) with regulation by the epithelium in close contact 380 with this surface. A coloured schematic superimposed on one half on the 381 anterior plate (Figure 8b, nos. 0-4) presents an explanation of the 382 relationships of ridges to internal plate structure. Although the lingual ridges 383 appear to show some positional relationship to the developing rods and 384 ovoids of the antero-labial margin of the plate, a distinct region forms at the 385 symphysial margin (Figure 8a, b, rod no. 0), being separated by an oral-aboral 386 ridge, as well, the posterior part of the plate is defined by a distinct furrow 387 (Figures 4f, 5f, arrows 1 and 2).

388 It is important to note that although there is a potential developmental 389 relationship between the lingual ridges and both the developing trabecular 390 dentine and hypermineralized whitlockin (lime green, orange, Figure 8b), the ridges have formed in advance of the mineralized ovoids, as well as the 391 392 patterned spaces within the trabecular dentine (Figure 4d–f). And, the ridges 393 are absent from the posterior dental plate in the three taxa examined here, 394 despite possessing substantially organized whitlockin, while the ridges appear 395 to become less distinct with growth although whitlockin deposition is ongoing 396 (Figure 5d, e). Moreover, these ridges are absent from *Harriotta*, whose anterior upper dental plate is also characterized by a series of patterned rods 397

and ovoids, while the lingual face where these ridges occur in *Chimaera* and *Hydrolagus* can also be identified in *Harriotta* (Figure 6e, arrows 1 and 2).

401 **5 Conclusions**

402 Individual teeth are present in stem group holocephalans such as *Helodus* 403 (e.g., Stahl, 1999), arranged in tooth families comparable to other 404 chondrichthyans such as sharks and rays. The dentition of *Helodus* also 405 presents evidence for tooth fusion (Moy-Thomas, 1936; Patterson, 1964; 406 Stahl, 1999; Johanson et al. in press), making it an ideal intermediate in the evolutionary transition from stem- to crown group holocephalans, with 407 408 complete tooth fusion in the latter (e.g., Ørvig, 1957). Previously, researchers 409 have attempted to identify evidence for this tooth fusion in extant taxa, such 410 as the recognition of discrete and stacked oral and aboral territories in 411 *Callorhinchus*, said to be equivalent to the separate teeth of shark and ray 412 tooth families (Didier et al., 1994). Separate growth phases were also described in Harriotta (Smith et al. 2019: fig. 7A, C), although this was not 413 414 thought to represent the development of individual teeth. Developmentally, the 415 tooth plates in crown group holocephalans have been demonstrated to be 416 417 et al. 2016), with an infolding of epithelial tissue and corresponding aggregation of mesenchymal cells (Didier et al., 1994). The tooth plate formed 418 from this single primordium (Schauinsland, 1903; Kemp, 1984; Didier, 1995), 419 420 but separate tooth germs were not observed as part of the embryonic development of the dental plates, nor of their continuous growth. Despite this 421 422 loss, significant patterning of dental elements, related to highly functional

- 423 dentitions, occurs in all extant taxa. How this patterning is regulated is
- 424 unknown, but may be correlated with development of outer and inner dentine
- tissue, controlled by a dental epithelium and dentally committed
- 426 ectomesenchyme.
- 427

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- 435

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posterior dental plate, rdg, ridges on lingual surface of the anterior dental
plate. White arrows in (d) indicate position of close contact between the
anterior and posterior dental plates, scale bar=1mm. White asterisk in (c)
indicates small oval dentine units at the midline of each dental plate.





FIGURE 2 Chimaera monstrosa Linnaeus, 1758, 49 cm juvenile upper

532 dentition, µCT-scans. In (a) oral view, including anterior dental plate with 533 ridges on the lingual surface, (b) upper dentition in oral view, with posterior 534 dental plate cut away to show internal structure, and ridges on the anterior 535 dental plate, asterisk indicates region shown in (d), white arrows indicate 536 close correspondence between anterior and posterior dental plates, (c) upper 537 dental plates rendered (Avizo) showing rods of hypermineralized dentine 538 (whitlockin) in both, (d) closeup of aboral anterior dental plate, showing 539 newest, bulbous postero-lingual end of the ridge, asterisk indicates newly 540 developing trabecular dentine below the most aboral ridge, (e) anterior dental 541 plate, with posterior plate almost completely cut away to show lingual ridges 542 relative to cartilage of the upper jaw, (f) symphyseal view of anterior dental 543 plate, with opposing plate almost completely cut away to show the ridges, 544 asterisk indicates bulbous aboral new ridge tissue, also three separate growth 545 regions (white arrows indicate direction of growth), anterior ridge, or column, 546 and posterior furrow on each side of the ridged zone (arrows labelled 1 and 547 2), (g, h) anterior dental plate, virtual section through developing rod, 548 framework trabecular dentine, ridges in section with forming sclerotic dentine 549 inside, (g) arrows indicate correspondence between the ridge and developing 550 rod. Abbreviations as in Figure 1, also brc, cartilage of the braincase; f.td, 551 forming trabecular dentine; rd, whitlockin rods; sod, sclerotic osteodentine, 552 uic, upper jaw cartilage. Scale bars, (a)=1.5cm, (e, f)= 1mm. 553





FIGURE 3 *Chimaera* spp., upper dentition, µCT-scans, (a-e) subadult

556 Chimaera monstrosa Linnaeus, 1758, (f-i) juvenile (20cm) Chimaera sp.

557 (unidentified), Taiwan. (a) *C. monstrosa*, (f) *Chimaera* sp., upper dentition in

- oral view, including anterior dental plate with ridges on the lingual surface of
- the dental plate, (b) *C. monstrosa*, (g) *Chimaera* sp., upper dentition rendered
- 560 (Avizo) showing rods and ovoids of hypermineralized dentine (whitlockin) in
- the both dental plates, and tritoral pads in the posterior plate (g), (c) C.

562 monstrosa, upper dentition in oral view, with posterior dental plate cut away to 563 show internal structure, including ridges on the anterior dental plate, (d) C. 564 monstrosa, (i) Chimaera sp., anterior dental plate, cut away to show section 565 through developing ovoids (hypermineralized whitlockin), surrounding 566 trabecular dentine, sclerotic (hypermineralized) trabecular dentine and the 567 ridges, in (d) the less mineralized ovoids can be seen at the aboral surface, (e) *C. monstrosa*, anterior and posterior dental plates, with opposing plates 568 569 cut away to show symphyseal face of the anterior plate and the ridges, 570 anterior ridge, or column, and posterior furrow on each side of the ridged zone 571 (arrows labelled 1 and 2, as in Figure 2f), (h) Chimaera sp., anterior plate in 572 oral view, with posterior dental plate cut away to show ridges on the anterior 573 dental plate. Abbreviations as in previous Figures, also d, less mineralized 574 dentine; tri, tritoral pad.



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576 FIGURE 4 Hydrolagus mirabilis Collet, 1904, µCT-scans. (a) upper dentition 577 in oral view, including anterior dental plate with distinct ridges on the lingual 578 surface of the dental plate, (b) upper dentition rendered (Avizo) showing 579 ovoids and rods of hypermineralized dentine (whitlockin) in both dental plates, 580 black arrowheads on developing tritoral pad on the left side of the image 581 showing ovoids being added to form the pad, (c) upper dentition in oral view, 582 with posterior dental plate cut away to show ridges on the anterior dental plate, (d, e) anterior dental plate, cut away to show section through 583 584 developing ovoids (hypermineralized), surrounding trabecular dentine, 585 sclerotic (hypermineralized) trabecular dentine and the ridges, (f) anterior and 586 posterior dental plates in symphyseal view, with opposing plates cut away to

587 show symphyseal face of the anterior plate and the ridges, numbered arrows 588 different regions of the plate (as in Figures 2f, 3e). Abbreviations as in

- 589 previous Figures.
- 590





FIGURE 5 Hydrolagus mirabilis Collet, 1904, µCT-scans, (a) upper dentition 593 in oral view, including anterior dental plate with ridges on the lingual surface of 594 the dental plate, (b) upper dentition rendered (Avizo) showing ovoids and rods 595 of hypermineralized dentine in the both dental plates, and tritoral pads on the 596 posterior plate, (c) in oral view, with posterior dental plate cut away to show 597 posterior face of the anterior dental plate and lingual ridges, (d, e) anterior 598 dental plate, cut away to show section through developing ovoids 599 (hypermineralized), surrounding trabecular dentine, sclerotic

(hypermineralized) trabecular dentine and the ridges, double-headed arrow
(e) indicating correspondence of developing ovoids and lingual ridges, (f)
anterior and posterior dental plates in symphyseal view, with opposing plates
cut away to show symphyseal face of the anterior plate and the ridges,
numbered arrows mark different regions of the plate as in Figures 2f, 3e, 4f.
Abbreviations as in previous Figures.



FIGURE 6 Harriotta raleaghana µCT-scans, (a-e) upper dentition in oral view,
(a) anterior and posterior dental plate showing close fit between them and
tritoral pad on posterior plate, (b) anterior dental plate with posterior dental
plate cut away to show posterior and lingual faces of the anterior plate,

612 lacking prominent ridges on the lingual surface of the dental plate, but with 613 some bulbous expansion that was associated with ridges in Chimaera (Figure 614 2), indicated by white arrow, (c) four upper dental plates rendered (Avizo) 615 showing ovoids and rods (whitlockin) as extensive below the worn surface, 616 and developing tritoral pads on the posterior plate, ovoids in symphyseal row 617 with antero-labial set of rods in anterior plate, (d) anterior dental plate, virtual section through developing rod (whitlockin), surrounding trabecular dentine, 618 619 sclerotic dentine near the wear surface, trabecular dentine only at forming 620 aboral surface, (e) symphyseal surface with opposing left plates cut away, 621 showing anterior dental plate (arrow 2 marks antero-symphyseal portion) and 622 posterior in close alignment at oral surface (arrow 1). Abbreviations as in 623 previous Figures.





FIGURE 7 Hydrolagus mirabilis, (a-c) photomicrographs in incident light of

627 upper and lower dental plates, (a) anterior upper plate shows ridges on lingual 628 surface (arrows), extent of worn tissue fossa (double arrow), translucent grey 629 rods, (b) lower dental plate tissue, transparency of most mineralized tissues 630 as in outer dentine (white double arrow) surrounding trabecular dentine, ovoid 631 stack not fully mineralized, (c) upper dentition *in situ* with soft tissue, plates 632 lined by epithelium, rostral snout present, (d) close up of virtual section 633 through anterior upper plate as in Figure 4d, e, colored false red to show 634 whitlockin as mineralized ovoids, and red rings the pre-formed capsular 635 spaces in the trabecular dentine, newest ridge forming aborally, coincidence 636 of ridges with new whitlockin forming (double arrows), (e) Callorhinchus milii 637 Bory de Saint-Vincent, 1823, schematic drawing of section through lower jaw 638 embryonic plate (Kemp 1984), false coloured red indicates whitlockin 639 formation (ple = pleromin) under an epithelium, with modified trabecular 640 dentine (mt), condensed mesenchyme (md), cartilage (c), scale bar=1mm, (f) 641 Callorhinchus milii, adult lower jaw, µCT rendered with whitlockin segmented. 642 false coloured red (Drishti), showing low ridges on the surface that form deep 643 into the trabecular dentine, above a cartilage furrow. Abbreviations as in 644 previous Figures, also car, cartilage furrow, ep, epithelium; nrdg, newest 645 ridge; ov st, ovoid stack; ros, rostral snout.



FIGURE 8 Chimaera monstrosa, µCT-scans, sub-adult upper dentitions, (a) 648 649 from Figure 1, (b) from Figure 3a-e, with the rod series explained as whitlockin 650 formed to an timed order at the aboral surface in the trabecular dentine, 651 determined by a dental epithelium, where on the lingual side, ridges of the 652 outer dentine layer form (Figure 8b, also Figures 1a, 2b, 3 d-e). (a) partially 653 segmented upper dentition (Avizo) showing three rods of the anterior plate, 654 no. 2 aligns with the rods of the posterior plate), no. 0 forms in the 655 symphyseal segment of the plate, 1, 2 along the labial ridge, (b) anterior dental plate with five rods and a coloured overlay to explain the co-incidence 656 657 of ridges with rods (0-4), tissues seen in virtual section relative to the surface 658 anatomy of the oral and lingual surfaces, whitlockin, orange; lime green, 659 trabecular dentine; olive green, sclerotic osteodentine dentine; moss green, 660 wear surface of the sclerotic dentine, arrowed in figure 7(a). Abbreviations as 661 in previous figures.

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663 Supplementary information

- 664 Hydrolagus mirabilis upper dental plates. Supplementary Information File 1,
- 665 movie of upper dental plates, Supplementary Information Files 2, 3 rotatable
- 666 .stl files of mineralized whitlockite components within the posterior dental
- 667 plates. Available on request from the Zerina Johanson (NHM,
- 668 z.johanson@nhm.ac.uk).

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