

The enameloid microstructure of the teeth of synechodontiform sharks (Chondrichthyes: Neoselachii)G. Cuny[#] & S. Risnes*[#]Corresponding author

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Abstract

The so-called ‘triple-layered’ enameloid of neoselachian sharks is made of two main units: a superficial one and an internal one including the parallel-bundled enameloid and the tangled-bundled enameloid. The Triassic Synechodontiformes possess a parallel-bundled enameloid in which radial bundles are not very well-developed, contrary to what have been observed in more recent Synechodontiformes and other neoselachian sharks. The well-developed enameloid ridges that ornament the crown of many Synechodontiform sharks are superficial structures and show exactly the same organisation as in the cutting edges of more recent neoselachian sharks.

We propose that two different mechanisms lead to the formation of ridges at the surface of the crown in neoselachian shark teeth. Ridges may result from an early mineralisation process during tooth development, or may mineralise near the end of the tooth development. Finally, on the basis of both tooth morphology and enameloid microstructure, the species “*Hybodus*” *minor* is transferred into the genus *Rhomphaiodon*.

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Abbreviations

D	Dentine	RB	Radial Bundles
EDX	Energy Dispersive X-ray	RCEL	Ridge/Cutting Edge Layer
PBE	Parallel-Bundled Enameloid	SCE	Single Crystallite Enameloid
R1	External layer of the RCEL made of single crystallites	SEM	Scanning Electron Microscope
R2	Internal layer of the RCEL made of bundles of crystallites	SLE	Shiny-Layered Enameloid
		TBE	Tangled-Bundled Enameloid

1. Introduction

Because of their ‘conveyor-belt’ replacement system, shark teeth are among the most commonly found vertebrate fossils. These teeth are made of three main hard tissues. An external hypermineralised tissue called enameloid, which cover a regular layer of orthodontine surrounding a wide pulp cavity. The root of the teeth is made of osteodontine, a tissue that sometimes fills in the pulp cavity (Cappetta, 1987). Reif (1973) demonstrated convincingly that the teeth of neoselachian sharks are characterised by a triple-layered enameloid made of an internal tangled-bundled enameloid (TBE), a middle parallel-bundled enameloid (PBE) and an external shiny-layered enameloid (SLE) (See Cuny *et al.*, 2001 for details about the terminology used in this article). Radial bundles of crystallites are also present through the PBE and TBE. Among these layers, the PBE is considered a synapomorphy of the neoselachians (Gaudin, 1991; Maisey, 1984a, b, 1985; Reif, 1977; Thies, 1982; Thies & Reif, 1985), although this character is secondarily lost in the posterior teeth of *Heterodontus* (Maisey, 1985; Thies, 1982) as a mechanical adaptation for a durophagous diet (Preuschoft *et al.*, 1974).

The earliest neoselachian sharks found in the fossil record belong to the Synechodontiformes, an order of unclear phylogenetic affinities among the Neoselachii (see discussion). The earliest Synechodontiformes appeared in the Permian (Ivanov, 2000) and the last ones disappeared in the Palaeocene (Cappetta, 1987). The surface of the crown of synechodontiform teeth is often ornamented with prominent ridges, both on their lingual and their labial side. The aim of the present work is to study the enameloid microstructure of synechodontiform and other sharks in section and to compare the results with previous surface-etching studies in order to understand the formation process of the crown ornamentation in shark teeth.

2. Materials and methods

To complement earlier surface studies (Cuny, 1998; Cuny & Benton, 1999; Cuny *et al.*, 1998, 2000, 2001; Duffin, 1980, 1993; Godefroit *et al.*, 1998), the following teeth were sectioned (all housed in the department of Oral Biology at the University of Oslo):

- 11 teeth of “*Hybodus*” *minor* (Neoselachii: Synechodontiformes, FSA1–6, FSA8–12) from the Rhaetian (Upper Triassic) of Manor Farm Quarry (Gloucestershire, southwestern England)
- 3 teeth of “*Hybodus*” *minor* (FSA7, FSB6, FSB10) from the Rhaetian of Cribbs Causeway (Bristol, southwestern England)
- 2 teeth of *Rhomphaiodon nicolensis* (Neoselachii: Synechodontiformes, RNB4, RNB18) from the Norian–Rhaetian (Upper Triassic) of Saint–Nicolas–de–Port (Lorraine, France)
- 1 posterior tooth of *Synechodus rhaeticus* (Neoselachii: Synechodontiformes: Palaeospinacidae, SRB5) from the Rhaetian of Holwell Quarry (Somerset, southwestern England)
- 1 anterior tooth of *Synechodus rhaeticus* (SRB9) from the Rhaetian of Habay–la–Vieille (Belgium)
- 2 teeth of *Paraorthacodus eocaenus* (Neoselachii: Synechodontiformes: Palaeospinacidae, PEB16, PEB26) from the Palaeocene of Dormaal (Belgium)
- 1 tooth of *Striatolamia macrota* (Neoselachii: Galea: Lamniformes: Odontaspidae, SMB7) from the Ypresian (Eocene) of Muddy Creek (Virginia, USA)
- 1 tooth of *Lissodus minimus* (Hybodontiformes: Lonchidiidae, LMB13) from the Rhaetian of Cribbs Causeway (Bristol, southwestern England)

All the teeth were embedded in resin. The large teeth were sectioned using a diamond circular saw and polished first using fine sandpaper, and then polishing powder. Smaller teeth (5 mm or less) were ground using coarse sandpaper and then polished in the same way as for the larger samples. Most of the teeth were sectioned transversely, perpendicular to the apico–distal axis of the cusp, at different height of the crowns when several specimens were available, and whenever possible, both the main cusp and the lateral cusplets were sectioned. When enough specimens were available a longitudinal section was also made following the labio–lingual plane. The tooth of *Striatolamia macrota* was large enough to provide both transverse and longitudinal section. It was sectioned transversely near the base, and the main cusp was then sectioned longitudinally. Flat crushing teeth, like those of *Lissodus minimus* and the posterior ones of *Synechodus rhaeticus*, were sectioned perpendicular to the long (mesio–distal) axis of the crown.

The sections were etched for 45s in diluted HNO₃ at 0.1% (pH=1.73) and were coated with a gold–palladium alloy. The sections were studied with a Philips Environmental Scanning Electron Microscope (ESEM) using an acceleration voltage of 10 kV. In addition, another tooth of *Striatolamia macrota* from Muddy Creek was etched 35s in 10% HCl and its lingual surface was observed with a Cambridge Stereoscan 250MK3 SEM, using an acceleration voltage of 25 kV.

3. Results

3.1. “Hybodus” minor and *Rhomphaiodon nicolensis*

The 14 sections of “*Hybodus*” *minor* teeth (13 transverse and one longitudinal) and the two transverse sections of *Rhomphaiodon nicolensis* allow the recognition, both on the main cusp and lateral cusplets, of a very thin SCE layer, which is better seen on the longitudinal section than on the transverse ones, but the bundled layers (PBE + TBE) are very much intermingled and difficult to separate in transverse section. No differences in the microstructure of the enameloid were observed between the teeth of “*H.*” *minor* from Cribbs Causeway and from Manor Farm Quarry and those of *R. nicolensis* from Saint-Nicolas-de-Port. However, contrary to what have been claimed on the basis of surface-etching studies (Cuny, 1998; Cuny & Benton, 1999, Cuny *et al.*, 2000; Godefroit *et al.*, 1998), the ridges ornamenting the labial and lingual side of the crown are not made of an extension of the PBE showing a change of orientation of its bundles. The ridges are made of a superficial tissue, which is clearly separated from the PBE beneath (figure 1B–E). There is no difference in structure between the

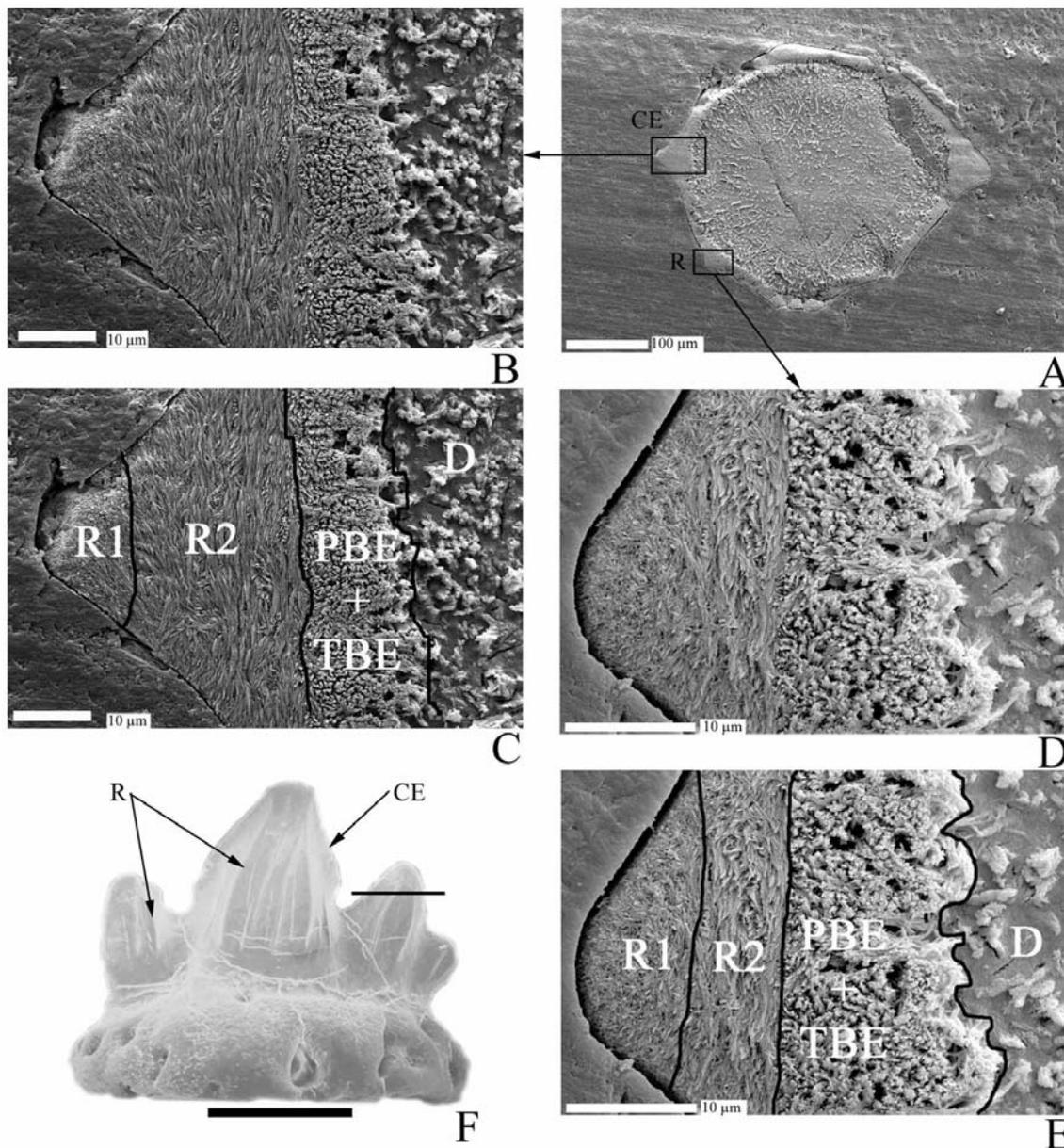


Figure 1. *Rhomphaiodon* (“*Hybodus*”) *minor*. A–E: Tooth FSA1; A: Transverse section of a lateral cusp; B: Detail of a cutting edge; C: Interpretation of B; D: Detail of a ridge; E: interpretation of D; F: Tooth of *Rhomphaiodon* (“*Hybodus*”) *minor* in lingual view showing where the transverse section of the lateral cusp was done. Scale bar F = 500 µm. Photograph by the authors.

cutting edges of the cusps (one mesial and one distal) and the ridges ornamenting the rest of the crown (figure 1). The cutting edges are simply better developed than the ridges (figure 1A). The ridge/cutting edge layer (RCEL) is divided into an external layer of a thick single crystallite enameloid in which the crystallites are randomly oriented and an internal layer made of bundles of crystallites oriented perpendicular to the axis of the ridges and parallel to its surface (figure 1C, E). However, the basal layer of bundles is lacking in the basal part of the ridges and cutting edges, which are only made of a so-called ‘single crystallite enameloid’ (SCE, Reif, 1979, figure 2A–B). The PBE and TBE are also very poorly defined at the base of the crown. The bundled layer appears only higher on the crown. Also, the superficial part of the external layer may show, near the surface, crystallites with a preferential orientation, perpendicular to the surface (figure 2F). Laterally, the RCEL appears to be in continuity with the SLE covering the rest of the crown. However, the SLE may be so thin between the ridges that

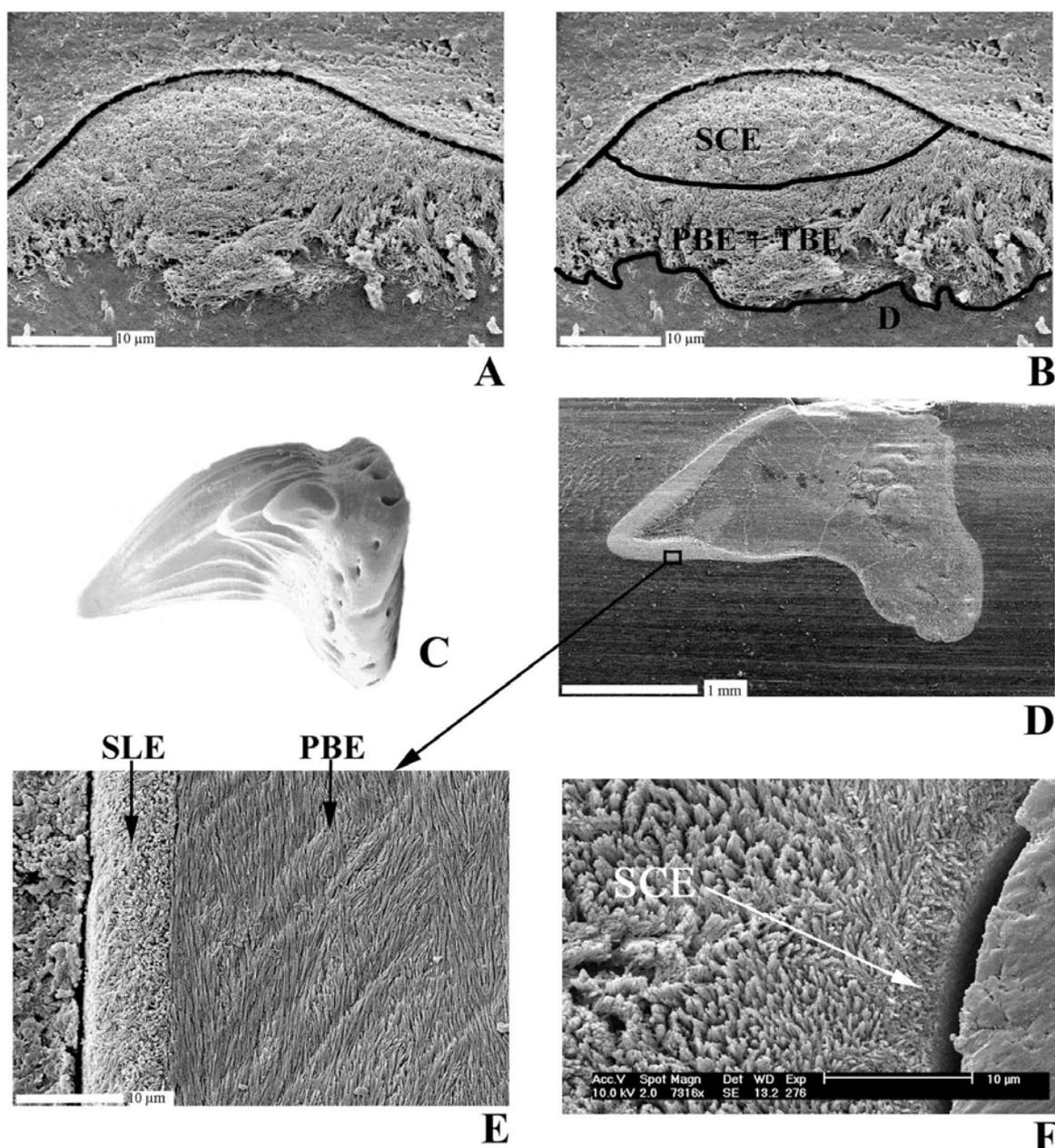


Figure 2. Rhomphaiodon (“Hybodus”) minor. A: Tooth FSA6, transverse section of a ridge near the base of the crown made only of SCE; B: Interpretation of A; C: Position of a tooth of Rhomphaiodon (“Hybodus”) minor before the longitudinal section was made, parallel to the surface of the page; D: Tooth FSB6 in longitudinal section; E: External part of the enameloid at mid-height of the crown showing the shiny-layered enameloid (SLE, left) on the surface and the parallel bundled enameloid (PBE, right), image rotated 90° clockwise; F: Transverse section of tooth FSA9, detail of a ridge showing a superficial layer (arrow) made of crystallites perpendicular to the surface. Photograph by the authors.

it does not appear in many of the transverse sections we have studied. When visible, it always shows a sharp boundary with the PBE.

The longitudinal section shows a SLE with a maximum thickness of 10 μm at mid-height of the crown, on the lingual side (the cusp has a diameter of 1.2 mm at its base, figure 2C–E). It is made of a single crystallites enameloid mainly oriented perpendicular to the surface (figure 2E). The addition of the TBE results in an increase of the thickness of the enameloid in the upper part of the crown (figure 2D). At mid-height, on the lingual face, the enameloid is 83 μm thick while it reaches 166 μm at the apex. Transverse sections made at different heights of the crowns confirm that the TBE is well-developed only in the upper third of the crown, both in the main cusp and the lateral cusplets. The TBE is intermingled outward with the PBE and inward with the dentine (figure 3A). Therefore, it never shows clear boundaries. Near the apex of the crown, the radial bundles of

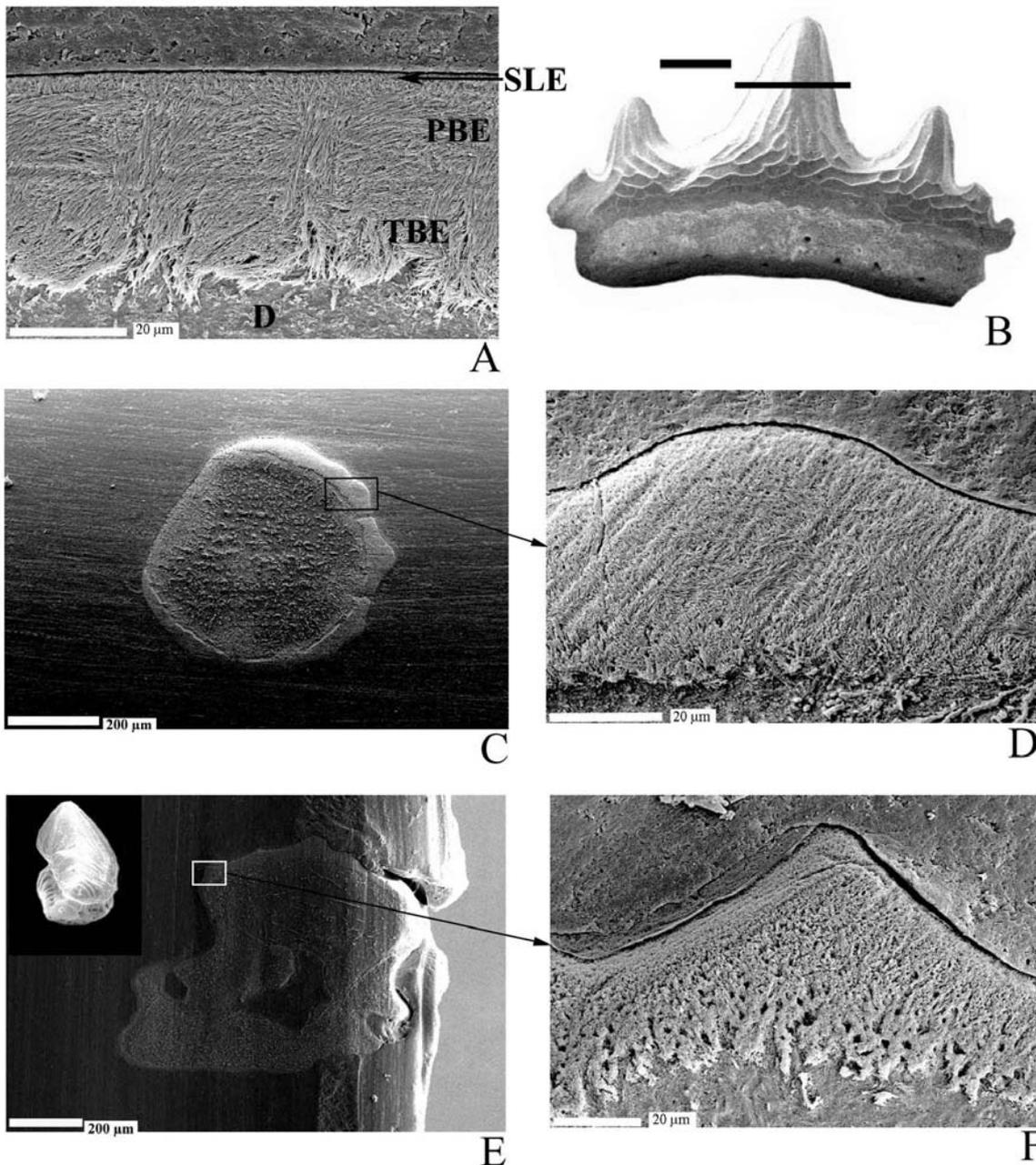


Figure A: Rhomphaiodon (“Hybodus”) minor, tooth FSB6, longitudinal section of the enameloid near the base of the crown; B: Tooth of Synechodus rhaeticus in labial view showing the location of the transverse section of the main cusp shown in C, scale bar = 500 μm ; C: Transverse section of the main cusp of the anterior tooth SRB5; D: Transverse section of a ridge of the anterior tooth SRB5; E: Section of the posterior tooth SRB9 of Synechodus rhaeticus perpendicular to its mesio-distal axis, upper left corner showing the tooth before sectioning; F: Section of a ridge of the posterior tooth SRB9. Photograph by the authors.

the PBE appear to originate from the TBE, while near the base, where the TBE is reduced or absent, they cross the whole thickness of the enameloid. At the TBE/dentine boundary the organisation of the crystallites into bundles disappears and crystallites, randomly oriented, are observed.

3.2. *Synechodus rhaeticus*

The transverse section of the main cusp of the anterior tooth shows an enameloid made of poorly defined bundles of crystallites. The external layer is similar to a PBE, showing transverse section of longitudinal bundles, while the internal one is more reminiscent of a TBE, showing a more wavy aspect (figure 3B–D). Neither a SLE, nor a RCEL were observed and both the cutting edges and ridges appear to be made of a PBE-like tissue. The PBE appears to be devoid of radial bundles. The contact area between the enameloid and the dentine is more distinct than in “*Hybodus*” *minor* and *Rhomphaiodon nicolensis*, the two tissues showing almost no intermingling. However, crystallites randomly oriented were observed at the base of the enameloid, similar to “*H.*” *minor* and *R. nicolensis*.

A posterior tooth of *Synechodus rhaeticus* was sectioned longitudinally according to its labio–lingual axis. The enameloid shows the same structure as in the anterior tooth except that it is more intermingled basally with the dentine (figure 2E–F). The enameloid of *S. rhaeticus* shows therefore a lesser degree of organisation than in “*Hybodus*” *minor* and *Rhomphaiodon nicolensis*.

3.3. *Paraorthacodus eocaenus*

Two transverse sections of teeth of *Paraorthacodus eocaenus* show an enameloid with a similar organization as in “*Hybodus*” *minor* and *Rhomphaiodon nicolensis*. The main difference is seen at the level of the PBE where the radial bundles appear to be more numerous and more distinct than in the two Triassic taxa (figure 4). When attaining the boundary with the SLE and RCEL, the radial bundles usually bend themselves to become parallel to it, a feature already observed in modern Lamniformes by one of us (S.R., unpublished data). This makes the boundary between the two tissues even sharper than in the Triassic Synechodontiformes. Sometimes, at the level of the RCEL, they cross the boundary and enter the RCEL over a short distance (figure

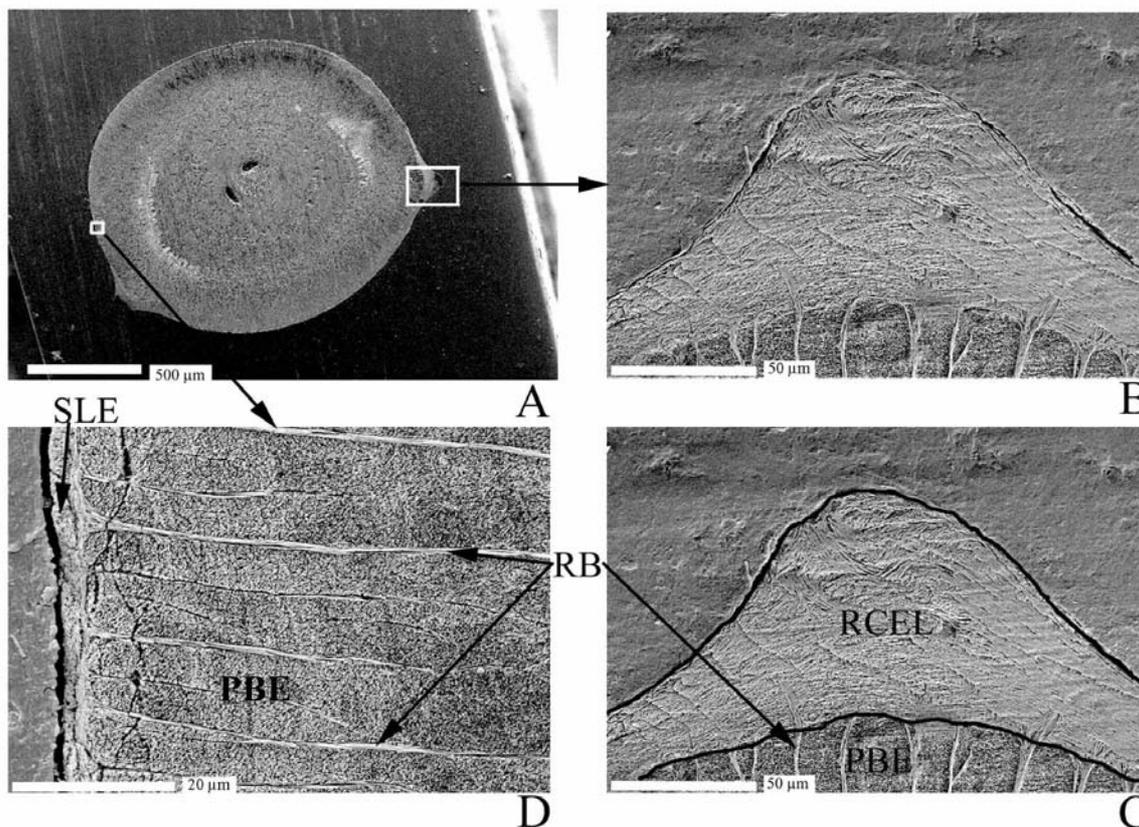


Figure 4. A–D: *Paraorthacodus eocaenus*, tooth PEB26; A: transverse section of the main cusp; B: Transverse section of a cutting-edge; C: Interpretation of B; D: Transverse section of the external part of the enameloid showing the SLE (left) and the PBE with its radial bundles (right).

4B). The contact area between the dentine and the TBE is poorly defined, the two tissues being intermingled (figure 4B–C). The layer made of randomly oriented crystallites partly embedded within the matrix of the dentine is less developed than in "*H*" minor, *R. nicolensis* and *Synechodus rhaeticus*. It is whole bundles of crystallites that are intermingled with the dentine in *P. eoacaenus*. At the level of the cutting edges and ridges, the pattern is similar to that of older Synechodontiformes but the layer of single crystallite enameloid is here much more reduced than in the latter. Ridges and cutting edges are thus made mainly of bundles of crystallites, but showing a more oblique orientation than in the Triassic taxa hitherto studied (figure 4B–C).

3.4. *Striatolamia macrota*

The longitudinal and transverse sections of the tooth of *Striatolamia macrota* reveal an enameloid which is very similar to that of *Paraorthacodus eoacaenus*: numerous radial bundles through the PBE and cutting edges mainly made of oblique bundles of crystallites. The longitudinal section shows an increase of the thickness of the TBE towards the apex of the crown. The main difference between the two taxa appear in the transverse section of the ridges ornamenting the lingual face of the crown of *S. macrota* where there is no special structure except a slight thickening of the SLE on top of each ridge. The pattern observed in synechodontiform ridges is indeed absent and the ridges appear to be the result of a simple folding of the surface of the crown (figure 5A). Comparing with those of *P. eoacaenus*, the ridges of *S. macrota* are less developed, and obviously their formation follows a different pattern than in the Synechodontiformes. There are no ridges on the labial face of the crown.

3.5. *Lissodus minimus*

The enameloid is a single crystallite enameloid (SCE) in which the crystallites show no preferential orientation. The enameloid is very compact except near the base where large canals, perpendicular to the enameloid/dentine boundary, are visible through the enameloid. Like in neoselachian sharks, the contact area between the enameloid and the dentine is unclear, the two tissue being intermingled (figure 5B–D). No special

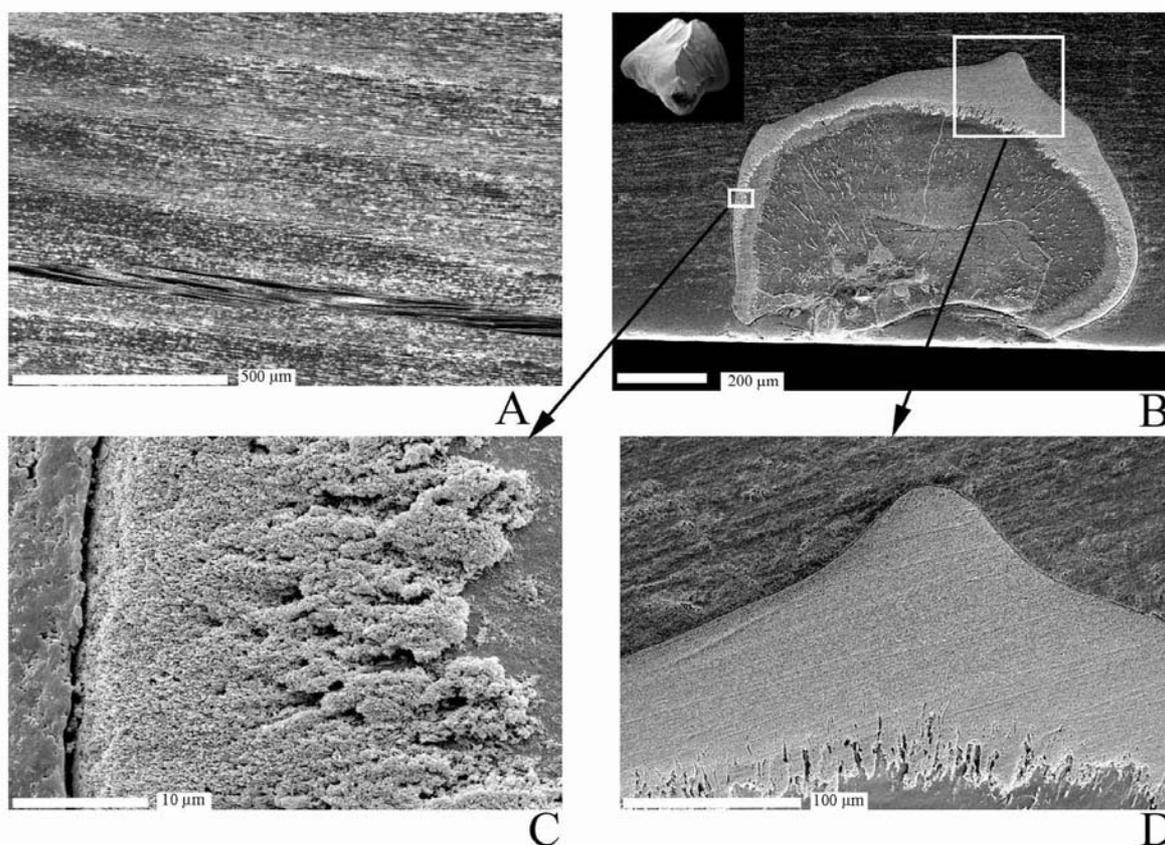


Figure 5. A: Lingual surface of the crown of a tooth of *Striatolamia macrota* etched 10s in 10% HCl showing the ridges as the result of a simple folding of the surface; B–D: *Lissodus minimus*, tooth LMB13; B: Section of the tooth perpendicular to its mesio-distal axis, upper left corner showing the tooth before sectioning; C: Section of the longitudinal crest; D: Section of the enameloid at the base of the crown, on the lingual side. Photograph by the authors.

structure can be observed at the level of the longitudinal crest (figure 5D) or at the level of the ridges. Both are made of a SCE similar to that observed in the rest of the crown. There is no distinct superficial tissue and the enameloid is made of a single layer.

4. Discussion

On the basis of tooth morphology, it has recently been claimed that Synechodontiformes are squalan sharks, closely related to the Hexanchiformes (Duffin & Ward, 1993). This view appears to be supported by the morphology of the neurocranium and the dermal denticles of the genus *Sphenodus* (Böttcher & Duffin 2000, but see Maisey *et al.* 2004 for a different opinion) although cranial characters of the genus *Synechodus* seem to favour the classification of the Synechodontiformes as galean sharks (Maisey 1985; Maisey *et al.* 2004). Cappetta (1987) indeed considered *Sphenodus* as a squalan shark and *Synechodus* as a galean shark, but the Synechodontiformes would not, in that case, be monophyletic. According to De Carvalho (1996), they may also represent the sister-group of the Galea + Squala (see also Maisey, 1985). A reassessment of the taxonomic position of this order, taking into account dental and non-dental characters, is therefore desirable but beyond the scope of this paper.

4.1. Remarks on the enameloid microstructure among neoselachian sharks

Previous surface-etching studies have demonstrated that the enameloid of the teeth of neoselachian sharks is triple-layered (Cuny, 1998; Cuny & Benton, 1999, Cuny *et al.*, 1998; Cuny *et al.*, 2000; Cuny *et al.*, 2001; Duffin, 1980, 1993; Godefroit *et al.*, 1998; Reif, 1973). This kind of study permits a clear recognition of three separate layers: Shiny-Layered Enameloid (SLE), Parallel-Bundled Enameloid (PBE), and Tangled-Bundled Enameloid (TBE). However, sections provide a somewhat different interpretation. The PBE and TBE are difficult to separate from each other, except in the upper part of longitudinal section (figure 3A), and are therefore better interpreted as two different arrangement of the same unit made of bundles of crystallites. On the contrary, there is a clear boundary between the PBE+TBE and the SLE+RCEL. The enameloid of neoselachian shark teeth appears therefore to be divided into two main units: a superficial unit and an inner bundled layer. In the inner unit, the orientation of the bundles of crystallites gradually changes from the external highly organised pattern of the PBE to the internal haphazard pattern of the TBE. At the enameloid/dentine boundary, the TBE loses its organisation into bundles, and crystallites haphazardly oriented have been observed, although this phenomenon is better developed in Triassic neoselachians than in younger taxa like *Paraorthacodus* and *Striatolamia*. This feature probably explains why a basal haphazard single crystallite layer was mentioned in *Rhomphaiodon nicolensis* and *Reifia minuta* by Duffin (1980, 1993). Duffin (1980, 1993) did not specify where on the crown he observed this haphazard single crystallite enameloid but it is likely to be in the lower part, where the TBE is very thin or absent. Subsequent observation of a true TBE in the teeth of *R. nicolensis* by Cuny (1998) in the upper part of the crown supports this interpretation. Current data thus indicate that the presence of such a layer is not a peculiarity of *Rhomphaiodon* and *Reifia*, but characterises all neoselachian teeth at the dentine/enameloid boundary, although it is better developed in Triassic taxa. Its observation through surface-etching analysis is only a matter of chance as this layer is always very thin, never exceeding 1 µm in thickness. In "*Hybodus*" *minor* and *R. nicolensis*, the PBE is very similar to that seen in other neoselachians even though there are less numerous radial bundles than in modern representatives of this clade.

When comparing the results of studies from surface etching and sections, one of the main discrepancies appears in the distribution of the radial bundles of the PBE. Surface-etching studies indicate that these bundles are more numerous in the inner part of the PBE than in the outer part (Duffin, 1993; Cuny *et al.*, 1998) while sections show an even distribution of them through the whole thickness of the PBE. However, there is no clear boundary between the PBE and the TBE. From surface-etching studies, it may be very difficult to differentiate inner PBE from outer TBE and what are interpreted as radial bundles may be bundles belonging to the TBE, *i.e.* with an orientation different from those of the PBE. However, it is more difficult to explain the absence of radial bundles in the section of the PBE of the teeth of *Synechodus rhaeticus* while these bundles were observed using surface-etching techniques (Cuny *et al.*, 2000). All Triassic taxa studied so far show radial bundles less distinct and less numerous than in younger neoselachians, Synechodontiformes (*Paraorthacodus*) or not (*Striatolamia*). It seems therefore, that there is a trend in the evolution of these radial bundles, starting with poorly defined and rare ones in Triassic taxa and ending with numerous and highly distinct ones in Cenozoic taxa. This would suggest that these radial bundles evolved after the appearance of a triple-layered enameloid among neoselachian sharks. *Synechodus rhaeticus* would thus represent a very primitive stage where the radial bundles would be either rare or absent, with maybe some variation according to the tooth position, which would account for the discrepancies observed. More studies are needed to fully understand this problem. The recognition of a PBE and the absence of a SLE in section appear to contradict previous surface-etching studies of anterior teeth of *S.*

rhaeticus (Cuny *et al.*, 2000). However, as the bundles of the PBE are much less distinct than in the sections of the teeth of "*Hybodus*" *minor* and *Rhomphaiodon nicolensis*, the true nature of this tissue may be difficult to recognise using surface-etching technique, explaining the discrepancies in the interpretations. The absence of a SLE in section, whereas it was observed by surface etching, probably reflects the thinness of this layer, as it is often the case in the teeth of the Triassic taxa.

Both studies from surface etching and section indicate that the enameloid has a more complex organisation near the apex than at the base of the crown. The TBE is well-defined in the upper part of the crown only (figure 2D). Near the base, the bundles of crystallites forming the enameloid are mainly perpendicular to the contact area with the dentine but are not interwoven, hence the absence of a recognisable TBE. This explains why at the base of the crown the enameloid of the teeth of *Mucrovenator minimus* and *Synechodus rhaeticus* appears to be made only of bundles of crystallites perpendicular to the surface (Cuny *et al.*, 2000, 2001). The PBE itself is not very well-developed here. Similarly, the RCEL lacks a bundled layer at the base of the crown in the teeth studied so far. This lack of organisation of the enameloid at the base of the crown appears to be a very general feature of most, if not all, neoselachian sharks.

4.2. Formation of the ridges

The common pattern of the RCEL in the cutting edges and ornamentation ridges in many neoselachian sharks (*Hueneichthys*, *Grozonodon*, most *Synechodontiformes*, *Parasymbolus*, *Cretodus*) suggests that their formation results from the same process. However, other patterns of formation were observed. Teeth of *Striatolamia macrotia* show that ridges may also be made of a PBE, thus resulting of a simple folding of the surface of the enameloid. The same pattern was also observed in both the ridges and the cutting edges of the teeth of *Synechodus rhaeticus*.

According to Fossé *et al.* (1974), the mineralisation of the enameloid during the tooth development starts with the cutting edges of the crown. If ridges share the same structure as cutting edges, it is likely that they are also formed very early in the tooth development, an early mineralisation of the enameloid occurring in more than two points of the crown (the two cutting edges) but in as many as there are ridges. The formation of the RCEL would therefore be the result of an early mineralisation during tooth development, although its exact mechanism remains unknown. On the other hand, if ridges are the results of a simple folding of the surface of the enameloid, this is likely to happen later during the tooth development. Fossé *et al.* (1974), and Risnes (1990) demonstrated that the mineralisation of the main part of the enameloid starts at the PBE/TBE junction. The external part of the PBE will thus mineralise near the end of the process, allowing a folding of the surface until very late in the mineralisation process. However, this would imply that the SLE covering the ridges also mineralises near the end of the tooth development, contrary to the RCEL. The mechanism leading to the formation of that kind of ridge remains thus largely unknown. Anyway, this latter process would be applicable not only to some neoselachians, but also to hybodont sharks. Contrary to what happens in some reptiles (Sander, 1999), ridges cannot be pre-formed by the dentine in sharks as it mineralises after the enameloid. The microstructure of the ridges seems thus to indicate that the ornamentation of the crown of a shark tooth may result from two different mechanisms occurring at different time during its development.

4.3. Origin of elasmobranch enameloid

Whether the enameloid of shark teeth is of ectodermal or mesodermal origin has been subject of controversy over a century. The point of view expressed by Moss (1968) that both enamel and enameloid are of ectodermal origin and homologous in all vertebrate is now considered unlikely (see Kemp, 1999). According to Kemp (1999), shark enameloid differs from tetrapod enamel in two main respects: (1) in sharks the enameloid layer begins to mineralise before the start of dentine mineralisation, whereas the chronology of enamel versus dentine mineralisation is reversed in tetrapods, and (2) odontoblastic processes extend into the enameloid layer in shark teeth but are absent in tetrapod enamel.¹ Enameloid is therefore likely to be a product of mesodermal cells, but are they the only cells involved, or is the enameloid a joint product of both mesodermal and ectodermal cells? Based on SEM and EDX analysis of shark enameloid, Risnes (1990) concluded that it has a mesenchymal background, but a role played by the inner dental epithelium cannot be excluded. Both collagen-like proteins (indicating a mesodermal origin, Sasagawa, 1991) and amelogenins (indicating an ectodermal origin, Slavkin *et al.*, 1983; Samuel *et al.*, 1983) have been found in shark enameloid.

The two most closely related clades to the Neoselachii are the Hybodontiformes and the Ctenacanthiformes (Janvier, 1996), whose teeth possess only a single crystallite enameloid (SCE, Reif, 1973).

¹ Note that the third difference given by Kemp (1999), the presence of an aprismatic structure, is not valid as many tetrapods show an aprismatic enamel, see Sander (1999) for details.

The question is: to which neoselachian unit (SLE + RCEL or PBE + TBE) this SCE is homologous with? The sections of the teeth of *Lissodus minimus* that we have studied show the same intermingling between the base of the enameloid and the dentine as seen in neoselachian sharks. This suggests that the SCE of hybodont shark teeth is homologous with the neoselachian PBE+TBE unit. This hypothesis is further supported by the observation in the teeth of hybodonts from the Middle Triassic of Nevada of bundles of crystallites at the base of the SCE (Cuny *et al.*, 2001). As no triple-layered enameloid has been observed so far outside the Neoselachii, this would suggest that the SLE+RCEL unit, possibly of ectodermal origin, was a late addition to the enameloid during the evolution of the Elasmobranchii. Primitively, the enameloid would then have been a pure mesodermal product. This would also explain the absence of a RCEL in *Synechodus rhaeticus* which would have retained a primitive enameloid microstructure, which is in accordance with its stratigraphic age.

4.4. Affinities of “*Hybodus*” *minor*

“*Hybodus*” *minor* was originally described on the basis of a dorsal fin-spine from the British ‘Rhaetic’ by Agassiz (1836: 48, pl. VIIIb) who later in the same work ascribed teeth collected from the Rhaetic Bone Bed at Axmouth, Devon to the same species (Agassiz, 1836: 183, pl. XXIII; Duffin, 1985). Unfortunately, fin spines of *Hybodus* and *Lissodus* are indistinguishable and it is likely that the fin-spines referred to “*H.*” *minor* belong indeed to *Lissodus minimus* (Woodward, 1891; Duffin, 1985). Thus the classification of “*H.*” *minor* has centred on isolated teeth (Duffin, 1985).

When Duffin (1993) described *Rhomphaiodon nicolensis*, he noted that this species possesses teeth that are extremely similar morphologically to those of “*Hybodus*” *minor*, both at the level of the crown and the root. However, *R. nicolensis* displayed a triple-layered enameloid while at that time the teeth of “*H.*” *minor* were still believed to belong to a hybodont. The two taxa were therefore not considered to be related to each other. It was later demonstrated that the teeth of “*H.*” *minor* also possess a triple-layered enameloid and that they belong in fact to a neoselachian shark, not an hybodont one (Cuny *et al.*, 1998). However, the presence of a haphazard single crystallite enameloid in *R. nicolensis*, thought to be absent in the teeth of “*H.*” *minor*, precluded the attribution of the teeth of “*H.*” *minor* to the genus *Rhomphaiodon* (Cuny, 1998). The present study demonstrates that this layer is present in both and the teeth of “*H.*” *minor* can therefore be attributed safely to the genus *Rhomphaiodon*, which includes two species: *R. nicolensis*, the type species, and *R. minor*, nov. comb.

Indirect evidence sustaining the inclusion of these two species into the same genus include the fact that both the teeth of “*Hybodus*” *minor* and *Rhomphaiodon nicolensis* are almost always found in association with *Nemacanthus monilifer* fin-spines (Cuny, 1998). This would suggest that the teeth of *Rhomphaiodon* and the fin-spines of *Nemacanthus* belong to the same genus, the fin-spines being not diagnostic at the species level.

5. Conclusion

The so-called ‘triple-layered’ enameloid of neoselachian sharks is made of two main units: an external SLE+RCEL and an internal PBE+TBE. The Triassic *Synechodontiformes* possess a PBE in which radial bundles are not very well-developed, contrary to what have been observed in more recent taxa, *Synechodontiformes* (*Paraorthacodus*) or not (*Striatolamia*). Also, a general feature of the neoselachian enameloid is that it is more complex near the apex of the crown than at the base. However, it is always clearly recognisable and very different from the single crystallite enameloid seen in hybodont sharks and remains therefore a good indicator of the phylogenetic relationships of isolated shark teeth. On the basis of both tooth morphology and enameloid microstructure, the species “*Hybodus*” *minor* is transferred into the genus *Rhomphaiodon* as a thin layer of crystallites with a haphazard arrangement was demonstrated to be present at the enameloid/dentine boundary in most, if not all, neoselachian sharks.

The well-developed ridges that ornament the crown of many *Synechodontiform* sharks are superficial structures and show exactly the same organisation as in the cutting edges of more recent neoselachian sharks. On the contrary, the ridges ornamenting the lingual side of the crown of the lamniform *Striatolamia* show no special structure. It seems therefore likely that among neoselachian sharks, two different mechanisms may lead to the formation of ridges at the surface of the crown. One is the appearance of many early mineralisation sites at the surface of the crown, the other a mineralisation of the ridges occurring late during the tooth development. The well-developed ornamentation of hybodont shark teeth appears to be the result of a late mineralisation.

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