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ORIGINAL PAPER

Ultrastructure of enamel and dentine in extant dolphins (Cetacea: Delphinoidea and Inioidea)

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Abstract Longitudinal and cross sections of teeth from 17 species of the Recent dolphins (Delphinoidea and Inioidea) were examined under scanning electron microscope to study the arrangement and ultrastructure of dental tissues with reference to phylogenetic and functional constraints. For most species, enamel had a simple bi-layered structure of radial enamel and an outer layer of prismless enamel. The outer prismless layer varied from 5 to 30 % of enamel thickness. The enamel of Burmeister's porpoise (*Phocoena spinipinnis*) was entirely prismless. The prisms had an open sheath; tubules and tuft-like structures were common at the enamel-dentine junction. Cetacean dentine was characterized by irregularly distributed dentinal tubules in a relatively homogenous dentinal matrix. Radial enamel was observed in all Delphinoidea and in the franciscana (*Pontoporia blainvillei*), whereas the Amazon river dolphin (*Inia geoffrensis*) had prisms organized in Hunter–Schreger bands. HSB in enamel are regarded as a device for resisting propagation of cracks. These may occur due to increased functional demands, possibly related to the hardness of the species diet. Simplification in tooth shape and reduced biomechanical demands plausibly explain the primitive radial organization among delphinoids and *Pontoporia*. The HSB structure in the Amazon river dolphin, similar to those of extinct archaeocetes, seems to have secondary functional implications. However, the distribution of HSB in more-basal odontocetes is too poorly known to judge whether the HSB of *Inia* are a retained plesiomorphic feature or convergence.

Keywords Cetaceans · Dental tissues · Functional morphology · Phylogeny · Teeth

Abbreviations

EDJ	Enamel-dentine junction
HAP	Hydroxyapatite
HSB	Hunter-Schreger bands
IPM	Interprismatic matrix
OES	Outer enamel surface
PLEX	Prismless external enamel
SEM	Scanning electron microscope

Introduction

Enamel forms the outermost layer of reptilian and mammalian tooth crowns. It is a highly mineralized tissue formed by inorganic crystals of hydroxyapatite surrounded by a protein matrix (Lucas 2004). In most reptiles, the needle-like crystals have a simple organization and are oriented in parallel, extending toward the surface of the tooth. In mammalian enamel, however, hydroxyapatite crystals are organized into bundles of bounded crystals known as enamel prisms (Koenigswald and Clemens 1992).

Enamel prisms can vary in morphology, diameter, and density. Their patterns of organization and packaging are

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also variable, generating different types of complexity—the enamel types. At a higher level, the three-dimensional arrangement of enamel types (*Schmelzmuster*) may vary between different taxa. In addition, differences in the *Schmelzmuster* can be recognized along the tooth row in heterodont mammals, due to functional adaptation in different tooth types (Koenigswald and Clemens 1992).

The diversity of complex structures in mammalian enamel is the result of both biomechanical (or functional) adaptation and phylogeny. The relationship between stresses and complex structures reflects functional constraints, but phylogeny is also implicated in enamel differentiation and variability, as some specific enamel structures have close correlation with specific mammal taxa (Koenigswald 1997). Thus, investigations of enamel structure and organization among fossil and extant species may contribute to phylogenetic studies of cetaceans.

Due to the high mineral content and density of enamel and dentine compared to other skeletal elements, teeth are among the best-preserved structures in the fossil record. Teeth play a major role in studies of dietary adaptations and have the potential to elucidate behaviors including social activities, defense, and sexual signaling (Rensberger and Pfretzschner 1992). Numerous studies have sought to characterize and understand the enamel ultrastructure in fossil mammals, and in turn, to address issues of systematics and functional morphology/lifestyle (e.g., Carlson and Krause 1985; Rensberger and Pfretzschner 1992; Wood et al. 1999).

In cetaceans, the evolutionary transition from a terrestrial to an aquatic environment resulted in marked changes in the skeletal and body systems from Archaeoceti (extinct basal or archaic Cetacea) to Neoceti (crown or living Cetacea). These changes were also reflected in the masticatory apparatus and dental morphology, with the heterodont and molariform dentition of archaeocetes and early odontocetes and mysticetes becoming simplified into the conical and peg-like teeth of the most modern odontocetes (Sahni and Koenigswald 1997). These evolutionary/structural changes in cetacean teeth prompted several studies on the organization of dental tissues in cetaceans. Sahni (1981) and Maas and Thewissen (1995) studied the ultrastructural complexity of the enamel in Eocene basal archaeocetes. Sahni and Koenigswald (1997) characterized the enamel of archaeocetes from India, and included the extant species *Platanista gangetica* (Roxborough, 1801) for comparison. Ishiyama (1987) described the variability in the enamel structure in species of seven families of extant odontocetes, involving both prismatic and prismless organization.

This study describes the arrangement and ultrastructure of teeth in extant odontocete cetaceans, with its main focus on the enamel region and a brief account on the structure of dentine, to broaden our knowledge of living dolphins in a phylogenetic framework. Functional aspects and biomechanical implications of the enamel arrangement in cetaceans in comparison to other mammals are discussed.

Materials and methods

Material examined

Dental samples of modern odontocetes analyzed in this study (Table 1) were made available by Universidade Federal de Santa Catarina (UFSC, Brazil), Instituto Estadual de Pesquisas Científicas e Tecnológicas do Estado do Amapá (IEPA, Brazil), and Massey University (New Zealand). Specimens were obtained from the deceased stranded or incidentally caught animals. Where possible, teeth were extracted from the mid lower jaw. Otherwise, if the position was unknown, straight and unworn or mildly worn teeth were selected. One to two teeth of each specimen were analyzed.

Methods

Teeth were surface-cleaned with alcohol and embedded in epoxy resin (Epofix™ Cold-Setting Embedding Resin, Struers, Copenhagen, Denmark) using silicon molds. After setting for 24 h, specimens were sectioned using a MOD13 diamond wheel in an Accutom-50 high-speed saw (Struers, Copenhagen, Denmark) under water irrigation. Cross-sectional and longitudinal sections (Fig. 1) were polished in a TegraPol-21 polisher (Struers, Copenhagen, Denmark) with silicon carbide paper (1,200–4,000 grit) and sonicated in an ultrasonic cleaner for 3 min in between the polishing sessions. Final polishing was achieved with 1 µ diamond paste.

In order to reveal detailed enamel structure, polished sections were etched with 37 % phosphoric acid (Scotchbond™ Etchant, 3 M/ESPE, USA) for 10 s and again sonicated for 3 min. Samples were then coated with gold palladium for scanning electron microscope (SEM) observation. The secondary and backscatter electron microscopic images were obtained in a JEOL JSM-6700F Field Emission SEM (JEOL Ltd., Tokyo, Japan), operating at 5 kV and 10 µA. Magnifications ranged from 30× to 7,000×.

Previous studies of mammalian enamel ultrastructure have demonstrated that overall enamel organization is generally consistent among individuals of the same species, but may vary among species (Carlson and Krause 1985; Maas and Thewissen 1995). Most species in our study were represented by a single specimen because of the rarity of material for destructive sampling. Although we believe that

Table 1 Species analyzed names follow the "List of marine mammal species and subspecies of the Society for Marine Mammalogy" (Committee on Taxonomy 2011)

Species	Author and year	Common name
Delphinoidea		
Phocoena spinipinnis	Burmeister, 1865	Burmeister's porpoise
Steno bredanensis	(G. Cuvier in Lesson, 1828)	Rough-toothed dolphin
Stenella frontalis	(G. Cuvier, 1829)	Atlantic spotted dolphin
Stenella clymene	(Gray, 1850)	Clymene's dolphin
Stenella coeruleoalba	(Meyen, 1833)	Striped dolphin
Sotalia guianensis	(Van Bénedén, 1864)	Guiana dolphin
Delphinus capensis	Gray, 1828	Long-beaked common dolphin
Tursiops truncatus	(Montagu, 1821)	Bottlenose dolphin
Globicephala melas	(Traill, 1809)	Long-finned pilot whale
Cephalorhynchus hectori	(Van Bénedén, 1881)	Hector's dolphin
Lagenodelphis hosei	Fraser, 1956	Fraser's dolphin
Orcinus orca	(Linnaeus, 1758)	Killer whale
Pseudorca crassidens	(Owen, 1846)	False killer whale
Lagenorhynchus obscurus	(Gray, 1828)	Dusky dolphin
Lagenorhynchus cruciger	(Quoy and Gaimard, 1824)	Hourglass dolphin
Inioidea		
Pontoporia blainvillei	(Gervais and d'Orbigny, 1821)	Franciscana dolphin
Inia geoffrensis	(Blainville, 1817)	Amazon river dolphin

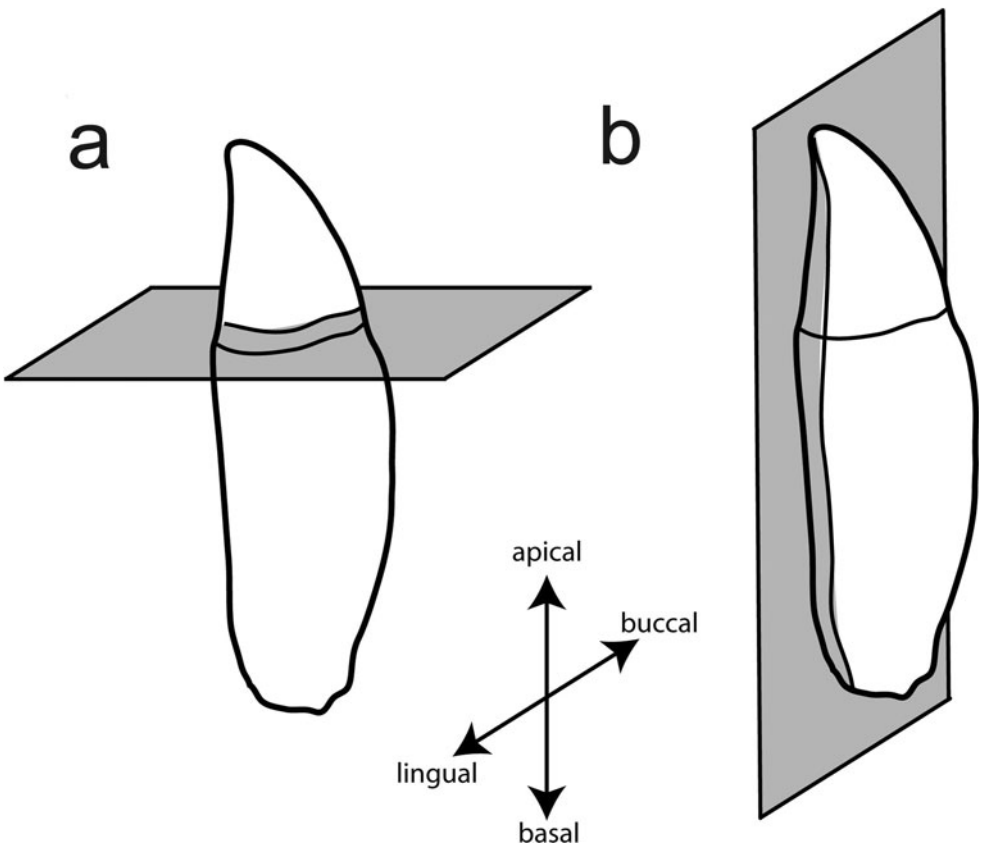


Fig. 1 Cross-sectional (**a**) and longitudinal (**b**) sectioning planes in cetacean teeth

the structural features we describe in these specimens were typical of each species, we drew our conclusions with some caution. The principal characteristics analyzed were the prisms and prism sheaths (shape and size), the spatial organization of prisms and interprismatic matrix, and the overall organization of the enamel in each tooth (Carlson and Krause 1985; Maas and Thewissen 1995). Anatomical terminology followed Koenigswald and Sander (1997).

Results

Thickness and overall organization

The thickness of enamel varied among the taxa studied. For small delphinids (Hector's dolphin, long-beaked common dolphin, Fraser's dolphin, Guiana dolphin, dusky dolphin, and 3 species of the genus *Stenella*), the enamel was 100–160 μm thick. Interestingly, the hourglass dolphin, also a small delphinid, had the thinnest enamel cover of only 45–90 μm in thickness. In species with larger body sizes, such as the bottlenose and rough-toothed dolphins, the enamel was 200–350 μm thick, with a maximum reached in the long-finned pilot whale and in the false killer whale of 350–450 μm. In contrast, however, thinner enamel was registered in Burmeister's porpoise (Delphinoidea) and the franciscana (Inioidea), two species that are not closely related but that share small body and tooth sizes. For both species, enamel was 75–100 μm thick. Interestingly, the killer whale, a large species with bigger tooth size, presented with only moderately thick (160–230 μm) enamel. Thicker enamel was found in the Amazon river dolphin, 315–415 μm thick in the conical anterior teeth and 475–515 μm thick in the molariform-like posterior teeth. In general, thickness of buccal and lingual enamel was similar in longitudinal sections, but sometimes the buccal enamel was slightly thicker. No correlation was found between enamel thickness and irregular enamel surface. From the two species with wrinkled enamel, only the Amazon river dolphin had the thickest enamel, while the rough-toothed dolphin had an enamel layer comparable in thickness to other species of similar body size.

In most dolphins, the overall organization of enamel (also known as *Schmelzmuster*) was a simple, double-layered structure (Fig. 2a, e), common to all cetaceans with exception of the Burmeister's porpoise (*Phocoena spinipinnis*) (Fig. 2b). The latter species had prismless enamel from the enamel-dentine junction (EDJ) to the outer enamel surface (OES). For all the other species, prisms arise near the EDJ and extend close to the OES, where they disappear in a layer of prismless enamel. In longitudinal sections, prisms were seen with inclinations parallel to almost perpendicular in relation to the EDJ. The organization and position of prism characterizes the enamel type in dolphins as radial. No decussation was observed in the prismatic layer in most species, except the Amazon river dolphin (*Inia geoffrensis*). This was the only species where Hunter-Schreger bands were observed both in longitudinal and cross-sectional specimens (Fig. 2c, d). Adjacent bands showed inclination angles to up to 90 % between each other, characterizing a strong decussation. Thickness of bands varied, but an average thickness of 10–15 prisms was estimated from SEM longitudinal sections.

Prisms and interprismatic matrix (IPM)

Cross sections of the prisms (seen in cross-sectional and longitudinal sections) revealed an incomplete prism sheath. Prisms were open basally and arranged in rows that were not aligned, but in alternating positions. This means that the tail of one prism was located in between the top parts of two prisms in the row below (Fig. 2f). This fits the Pattern 3 of Boyde's (1965) classification of enamel prisms. Maximum diameter of prisms varied from 3 to 5 μm in species with prismatic enamel. A few prisms with complete prism boundaries (Pattern 1 of Boyde) were also recorded in some species, mostly near the tooth tip or in the most external layers of prismatic enamel (Fig. 3a). However, the bulk of prismatic enamel was composed of prisms with an incomplete sheath. Interprismatic matrix (IPM) surrounded the prisms and IPM crystallites were parallel to one another, but ran at a slight angle to the prism long axes.

Outer enamel surface (OES) and Enamel-dentinal junction (EDJ)

The outer surface of enamel consisted of prismless enamel in all species evaluated. Here, HAP crystallites were organized parallel to each other and with their long axis directed toward the OES, but lacked any other higher level of structural organization. Prismless enamel appeared to be more acid-resistant than prismatic enamel in etched longitudinal and cross sections.

The outermost layer of prismless enamel (PLEX) varied in thickness among species. It represented 30 % or more of the enamel thickness in long-beaked common dolphin, franciscana, and Clymene's dolphin (Fig. 3b). On the other hand, PLEX represented no more than 5 % of enamel thickness for the false killer whale and the striped and rough-toothed dolphins. In the latter species, transition from PLEX to prismatic enamel was not as gradual as seen for the other dolphin species. PLEX interlocked with the prismatic layer in an unusual manner (Fig. 3c). Thinner PLEX was also recorded in the Amazon river dolphin, where molariform-like teeth showed a prismless layer

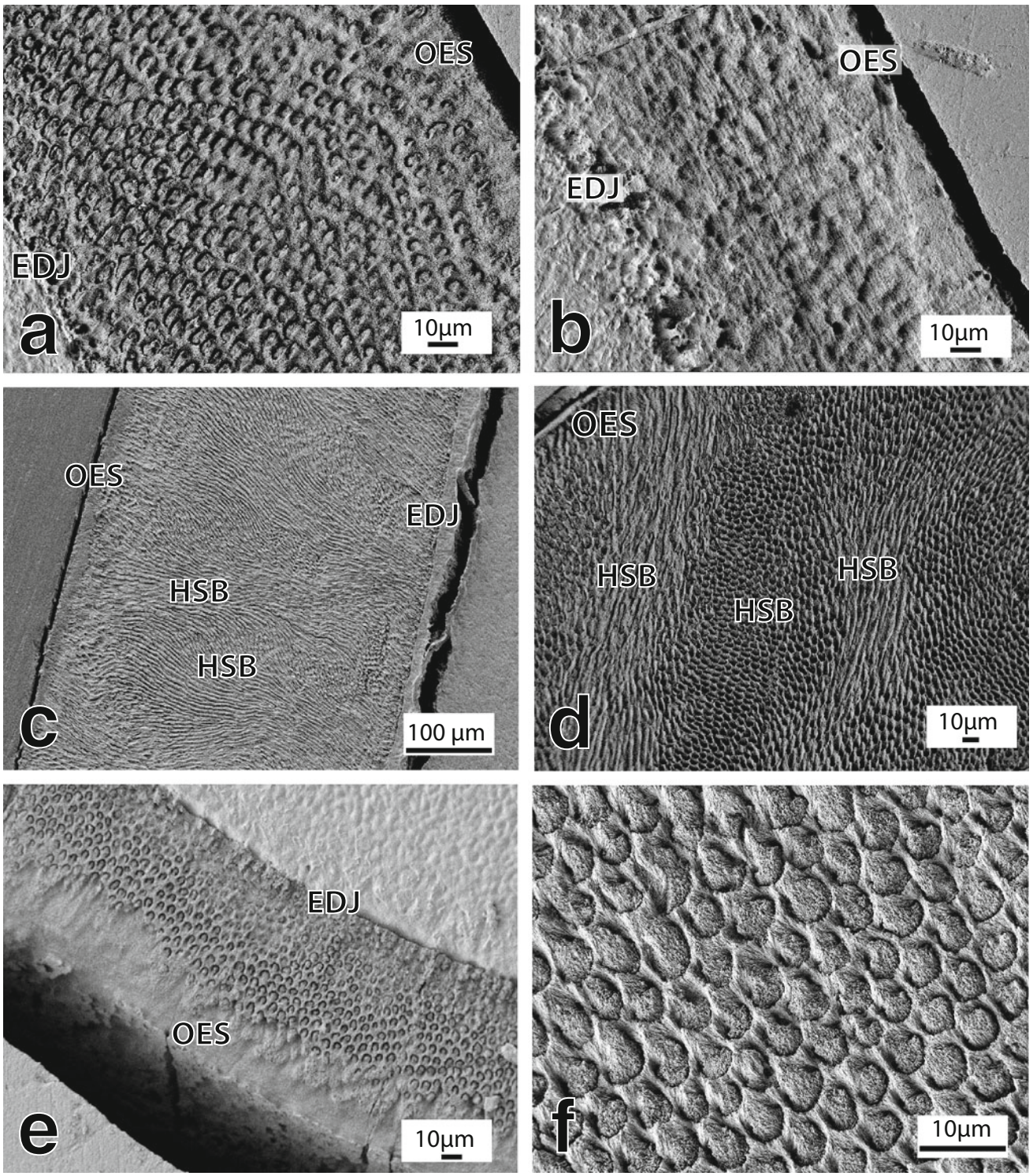
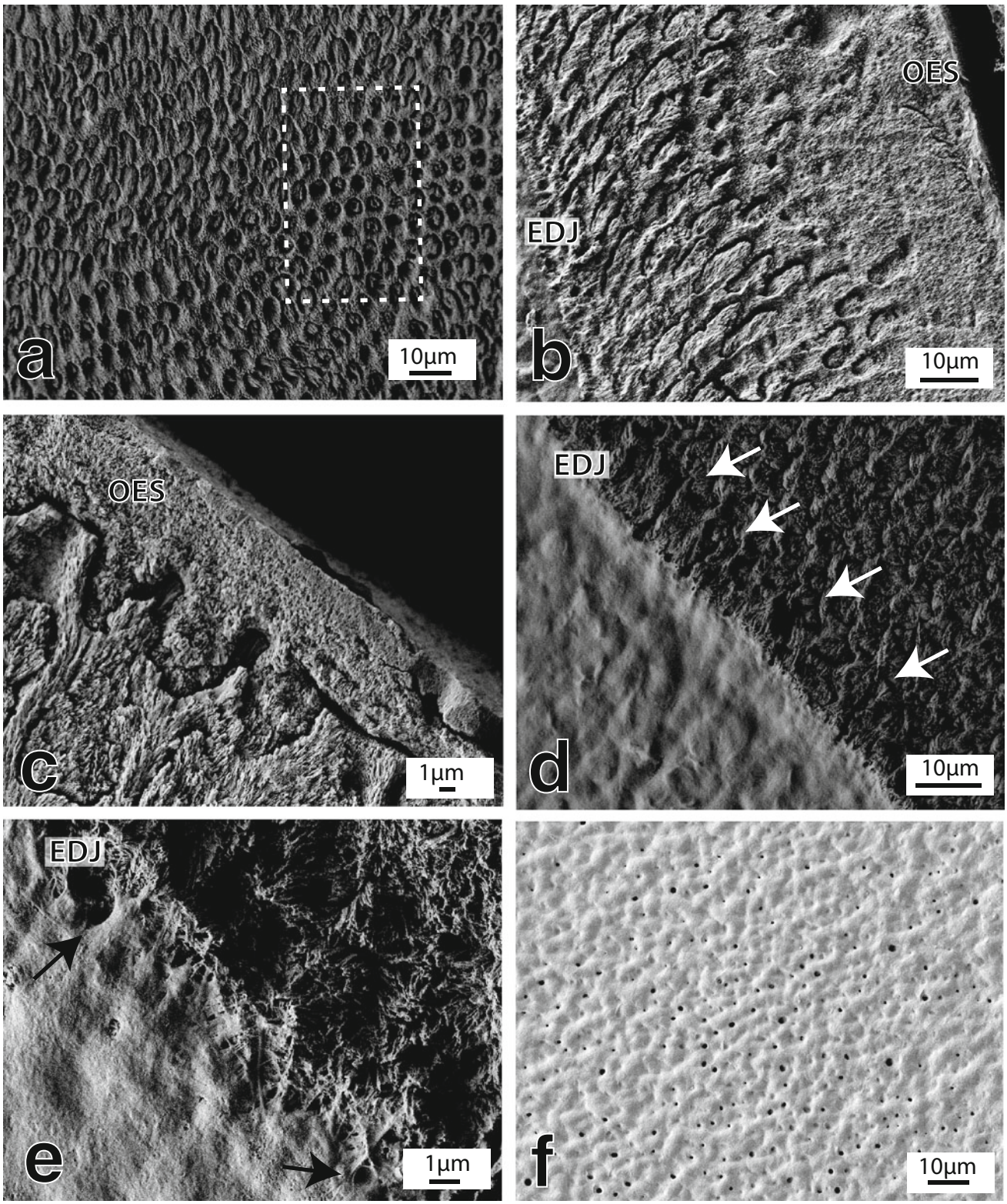


Fig. 2 **a** Radial enamel with thin prismless outer surface in Hector's dolphin (*C. hectori*) in longitudinal section, magnification ×700. **b** Prismless enamel in the Burmeister's porpoise (*P. spinipinnis*) in longitudinal section, magnification ×1,000. **c** HSB in the Amazon river dolphin (*I. geoffrensis*) in longitudinal section, magnification ×270. **d** Enamel of the Amazon river dolphin (*I. geoffrensis*) in cross section evidencing HSB, magnification ×330. **e** Radial enamel with prismless outer surface in the franciscana (*P. blainvillei*) in cross section, magnification ×500. **f** Detail of open sheath prisms and surrounding IPM of the bottlenose dolphin (*T. truncatus*) seen in cross section, magnification ×2,000. *EDJ* enamel-dentine junction, *HSB* Hunter-Schreger bands, *OES* outer enamel surface



corresponding to 8 % or less of the enamel thickness, while in conical teeth it represented less than 3 %. For the other species analyzed, PLEX represented between 10 and 25 % of the enamel thickness.

The enamel-dentine junction (EDJ) was well defined in most of the species analyzed, showing a sharp boundary between the two zones (Fig. 3d, e). Prisms originated at the boundary or immediately after a row of densely compacted

◀ **Fig. 3** **a** Closed prisms (*dashed lines*) together with open sheath prisms (*top left*) in the long-finned pilot whale (*G. melas*), magnification ×1,000. **b** Thick layer of prismless enamel in the Clymene dolphin (*S. clymene*) seen in longitudinal section and showing smooth transition from prismatic radial to prismless enamel, magnification ×1,400. **c** Thin prismless layer interlocking with prismatic enamel in the rough-toothed dolphin (*S. bredanensis*) seen in cross section, magnification ×4,000. **d** Tuft-like structures (*arrows*) in the EDJ of the Atlantic spotted dolphin (*S. frontalis*) seen in cross section, magnification ×1,600. **e** Detail of the enamel-dentine junction (EDJ) in the long-beaked common dolphin (*D. capensis*), showing tubules (*arrows*) and collagen fibers extending toward the enamel, magnification ×7,000. **f** Dentinal surface of the franciscana (*P. blainvillei*) seen in cross section, magnification ×1,000. *EDJ* enamel-dentine junction, *OES* outer enamel surface

crystallites that was few μm thick. The Burmeister's porpoise was the only species where the EDJ was not so evident and the transition between zones was smoother (Fig. 2b).

Special features

Tubules and tuft-like structures were often observed at the EDJ of dolphins, both in cross-sectional and longitudinal sections (Fig. 3d, e). Tubules were observed flush with the EDJ or in the basal enamel adjacent to the EDJ and measured roughly 0.5 μm or less in diameter. Tuft-like structures were seen at the EDJ boundary projecting to the basal area of enamel (Fig. 3d). Tufts were ribbon-like in shape and ran longitudinally toward the OES. Sometimes tufts also projected to the underlying dentine, where they showed a more complex structure. Bridge-like projections of dentine were observed between the two walls of the tufts, below the EDJ area. From the SEM images, the darker coloration of the tuft-like structures in contrast to the underlying enamel suggests that they may represent less-mineralized areas.

Dentine

Dentinal surfaces exposed in longitudinal and cross sections were simple and relatively featureless. In cross section, the surface was uneven and revealed irregularly distributed dentinal tubules in a relatively homogenous dentinal matrix (Fig. 3f). The diameter of dentinal tubules on average measured 1 μm or slightly more. No vascularization was apparent in the dentinal tissue.

Enamel ultrastructure and phylogenetic pattern

All species with prismatic enamel showed a combination of Pattern 1 (closed) and Pattern 3 (open) prisms. Open sheath prisms were the predominant prism pattern throughout the enamel, while closed prisms could be seen in reduced numbers, most commonly close to the tooth surface. Burmeister's porpoise (*P. spinipinnis*) was the only species where the enamel layer had no prisms.

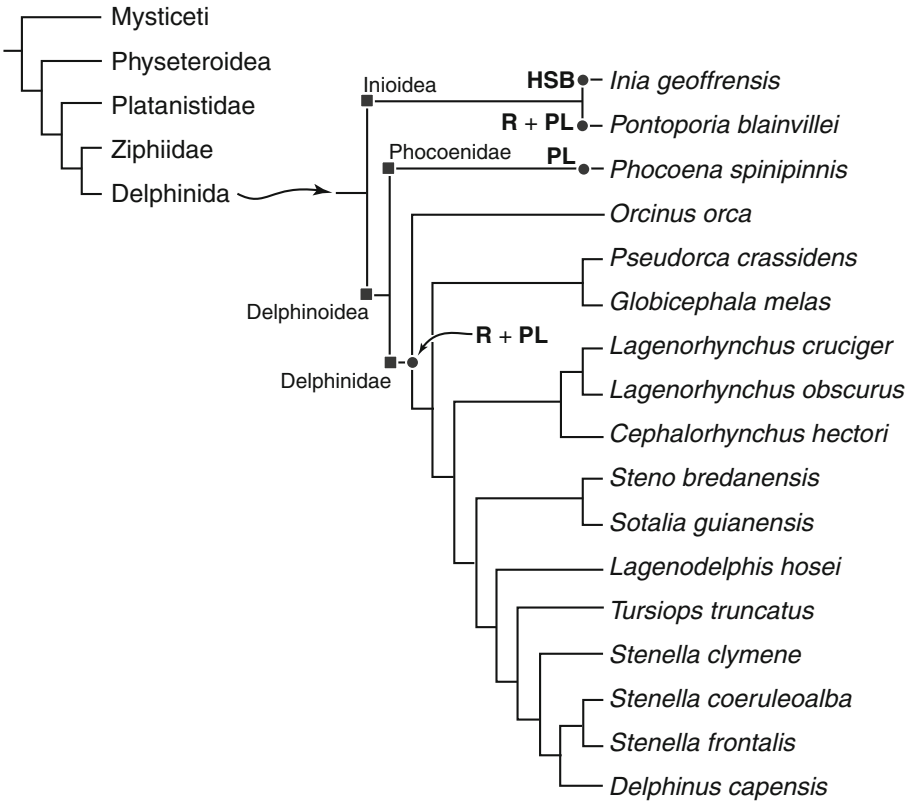


Fig. 4 Generalized cetacean phylogeny (*top left*), phylogenetic relationships of the species analyzed in this study based on Steeman et al. (2009) (*right*) and the *Schmelzmuster* of cetacean enamel. *HSB* Hunter–Schreger bands, *PL* prismless, *R + PL* radial + prismless

On the other hand, more diverse structures were observed at the *Schmelzmuster* level when a phylogenetic framework is taken into account (Fig. 4). The combination of an inner layer of radial enamel and an outer layer of prismless enamel was the common trend, and it was observed in all Delphinidae species as well as in the inioid *Pontoporia blainvillei* (franciscana). Interestingly, the Amazon river dolphin (*I. geoffrensis*), the other inioid species sampled, revealed a completely different and more elaborate *Schmelzmuster* organized in Hunter-Schreger bands.

Discussion

The enamel structure of the recent cetaceans was diverse, revealing several levels of structural complexity. At the prism level, the dominant pattern was an open enamel sheath. The prism cross section of cetaceans fitted the so-called Pattern 3 of Boyde's classification. In summary, the Boyde classification of prism patterns comprises prisms with closed boundaries and horizontal arrangement (Pattern 1) and prisms with incomplete prism boundaries (Pattern 2 and 3). Pattern 2 prisms are smaller and arranged in longitudinal columns, while Pattern 3 prisms are bigger and horizontally arranged in alternating position (Boyde 1965). However, this classification is not always applicable, and it has been argued that a simpler classification (open vs. closed prisms) would be preferable, due to the high variability of prism cross section from the EDJ to the outer surface and that this system does not cover the diversity of structures in mammalian enamel (Koenigswald and Sander 1997; Maas and Dumont 1999).

The previous generalized idea of odontocete enamel being characterized by closed prisms (Boyde 1971) is not correct, as already implied by previous studies (Ishiyama 1987). In our study, closed prisms were sometimes found close to the outer enamel surface (notably in *G. melas* and in the species of *Stenella*), but always less frequent than open sheath prisms. An arrangement with open sheath prisms predominant and closed prisms scattered close to the tooth surface is also a common trend recorded in many terrestrial mammals and archaeocetes (Maas and Thewissen 1995; Maas and Dumont 1999; Hillson 2005). In contrast to Ishiyama (1987), who reported closed prisms (Pattern 1) as prominent in *P. blainvillei*, we observed open prisms (Pattern 3) in this species as well as in the other odontocetes. The average prism size (maximum diameter) of roughly 5 µm was similar among all species, independent of differences in body and tooth size, and was consistent with what was reported for humans and other land mammals (Ten Cate 1998).

At the *Schmelzmuster* level, cetaceans revealed considerable diversity of enamel type combinations. An inner layer of radial enamel and an outer layer of prismless enamel was common for all Delphinidae analyzed, as well as for *P. blainvillei*; however, *Inia*, which is related closely to *Pontoporia*, showed a different structure. The pattern of inner radial and outer prismless enamel is the plesiomorphic *Schmelzmuster* among mammals, and the only type of prismatic enamel found in the earliest mammals as well as in many living marsupials and small-bodied eutherians (Maas and Dumont 1999; Koenigswald 2000). Very few large-bodied mammals have radial enamel as the dominant enamel type (Koenigswald 2000). In addition to being considered the most primitive enamel type, radial enamel is regarded as biomechanically more resistant to wear and thus has been retained in several mammalian groups, especially in those in which opposing tooth surfaces slide over each other (Koenigswald 1997, 2000). An even simpler *Schmelzmuster* was observed in the Burmeister's porpoise (*P. spinipinnis*), with prismless enamel extending from the EDJ to the outer surface. Hence, at least for some delphinoids, the enamel ultrastructure seems to have been simplified from the standard mammalian prismatic structure (Ishiyama 1987). However, a complex organization resulting in Hunter-Schreger bands was observed in the Amazon river dolphin (*I. geoffrensis*). HSB evolved multiple times in mammal groups during the Paleocene and Eocene (Koenigswald et al. 2010). HSB have been related to increases in body size and weight during eutherian evolution and diversification, and are considered to be a biomechanical reinforcement against enamel cracking (Koenigswald and Pfretzschner 1991; Maas and Dumont 1999; Koenigswald et al. 2010).

A noteworthy feature of cetacean enamel was the conspicuous prismless external layer, representing from 5 to about 30 % of the enamel thickness in the species analyzed here. Ishiyama (1987) also noted a significant prismless layer in delphinids, and mentioned prismless layers corresponding from 20 to 50 % of the whole enamel thickness. The formation of this prismless layer is related to enamel development, particularly the activity of ameloblasts. At the beginning of enamel deposition, ameloblasts lack Tomes' processes, resulting in a thin layer of prismless enamel near the EDJ. At the end of enamel deposition, after prismatic enamel has been laid down, ameloblasts lose their Tomes' processes and a thicker layer of prismless enamel is formed at the outer surface (Maas and Dumont 1999). The prismless external layer in cetaceans could mean that in this group the ameloblasts have a shorter time to secrete the enamel matrix proteins where the hydroxyapatite crystals grow, losing their Tomes' processes relatively early. Ishiyama (1987) suggested that this represents a sort of degenerative phenomenon or a less active enamel formation in cetaceans. The same idea was corroborated by molecular studies of the Enamelin gene, which produces

one of the structural proteins related to the formation of hydroxyapatite crystals secreted by ameloblasts (Meredith et al. 2009). The decay of Enamelin mirrors and the morphological degeneration of enamel in several edentulous and enamelless taxa, including cetaceans, suggesting that the shortened activity of ameloblasts is directly related to the enamel development and to the formation of a conspicuous prismless layer.

Most of the Recent delphinoids and inioids sampled here are raptorial predators that also rely on suction feeding to some extent, with the exception of the long-finned pilot whale (*G. melas*) and Burmeister's porpoise (*P. spinipinnis*), where suction feeding seems to be predominant (Werth 2006). For most of the raptorial feeders, prey is seized and grasped using the elongated jaws that bear many slender pointed teeth (Werth 2000). Mastication is negligible, except for the Amazon river dolphin (*I. geoffrensis*) that is capable of some mechanical reduction and processing of its prey. The occlusion of upper and lower teeth is also modified in cetaceans. Teeth do not come into contact through occlusal surfaces, but rather interdigitate with their mesial/distal surfaces sliding over each other (Ungar 2010). The repeated tooth-to-tooth contact between the margins of teeth (attrition) is considered the main cause of mesio-distal wear facets, while apical facets are caused by abrasion during food apprehension and processing (Loch and Simões-Lopes 2012). In cetaceans, however, abrasional wear should be less prominent than in many other mammals. For extant dolphins, simultaneous mild to moderate wear facets in the apex and lateral faces of teeth are relatively common, and the same condition was observed in the specimens used in this study.

It is known that crystallites oriented perpendicular to the surface are more resistant to wear, and that prismless enamel is more resistant to attrition and abrasion than prismatic enamel, due to the dense packing of crystallites (Maas and Dumont 1999). The presumed effective wear resistance could explain why a prominent prismless layer is well developed in some cetaceans. Although previous studies have implied that human prismless enamel is more radiodense than prismatic enamel (Gwinnett 1967), little is known about the mineral versus protein content in the prismless layer and its relationship with the ultrastructural arrangement and biomechanical response.

In relation to dentine and the EDJ, the ultrastructure of the dentinal tissue fitted the regular mammalian orthodentine standard: subparallel tubules embedded in a matrix of intertubular dentine rich in collagen fibers (Boyde 1980; Koenigswald et al. 2010). These fibers, oriented perpendicular to the long axis of the tubules, are responsible for the uneven and irregular surface observed in cross sections. At the EDJ, dentine collagen fibers often extended toward the enamel, where tubules and enamel tufts were often identified. Tubules were also recognized in archaeocetes (Sahni and Koenigswald 1997) and the Recent delphinoids (Ishiyama 1987), possibly related to a low degree of enamel mineralization. Tufts, which are also regarded as hypomineralized areas of enamel, have been considered as intrinsic crack-like structures that play a major role in damage tolerance and mechanical response. This is achieved by reducing the tensile strain within the enamel at the EDJ by minimizing crack initiation from the EDJ toward the surface, as a consequence of heavy localized contact loading (Lawn and Lee 2009).

It is believed that the unusual and distinctive tooth form of dolphins reflects the shift to an aquatic lifestyle, which brought major changes in feeding methods and feeding apparatus from the basic mammalian condition (Werth 2000). Concurrent with significant alterations in the morphology of skull and jaws, teeth were simplified to become mainly subconical in shape, with an increase in number. This simplification in tooth form is also reflected in the thickness of the enamel cover. For the species reported here, the thickest enamel was observed in the Amazon river dolphin (around 500 μm), and thickness ranged from 100 to 200 μm on average for most of the other species. In archaeocetes, enamel thickness was reported as ranging from 400 to 500 μm (Sahni and Koenigswald 1997). For most mammals, the presence of a thick enamel layer would enhance resistance to contact-induced fracture, and would prolong tooth lifetime in case of progressive wear (Lucas et al. 2008). Thus, the relatively thin layer of enamel in cetaceans suggests limited utility in feeding and relaxed selection for tooth microstructure in comparison with other eutherians (Werth 2000).

Major changes in tooth morphology normally result in simplification of enamel microstructure in response to changed biomechanical demands (Koenigswald 1997). The cetacean clade is a good example of this. Most of the cetacean species studied have radial enamel, considered as the most primitive enamel type for mammals. Some species reduced the prismatic to prismless enamel, as is the case in the Burmeister's porpoise. The thick prismless layer in species with prismatic enamel suggests that ameloblasts lose their Tomes' processes very early, which could mean a relaxed selective pressure in producing a prismatic structure.

The only species to show a complex enamel structure was the Amazon river dolphin, where HSB were observed. This structure is regarded as a split-and-deviating device to resist and limit crack propagation in enamel and a biomechanical response to increased occlusal loads. It has evolved many times in parallel in several mammal groups (Koenigswald and Pfretzschner 1991). HSB were also present in the dentition of extinct archeocetes (Sahni 1981; Maas and Thewissen 1995; Sahni and Koenigswald 1997)

and the extant Ganges river dolphin (*P. gangetica*). Most archaeocetes had a moderately thick enamel layer ranging from 400 to 500 μm, with well-developed HSB and wide variation of prism cross section (Maas and Thewissen 1995; Sahni and Koenigswald 1997). Sahni and Koenigswald (1997) suggested that *Platanista* could have retained the precursor HSB enamel organization of archaeocetes, as this species was considered a basal odontocete in early studies. Recent molecular and morphological studies have shown that the Physeteroidea (*Physeter* and *Kogia* species), not *Platanista*, is the most basal odontocete group, and that *P. gangetica* is not closely related to inioids (*I. geoffrensis*, *P. blainvillei* and *Lipotes vexillifer* Miller, 1918) (McGowen et al. 2009; Steeman et al. 2009). The Physeteroidea, however, have a specialized feeding system when compared to other odontocetes, with a diet based mostly on squids (McAlpine 2002; Whitehead 2002). While sperm whales have a small cap of prismless enamel confined to the tooth tip (Ishiyama 1987), *Kogia* is regarded as either having no enamel or having a thin enamel cover in very few young specimens, which is worn away quite quickly (Plön 2004), and it is not known if HSB are present. The beaked whales (Ziphidae), another group of squid-eating basal odontocetes, also have either a very thin layer of prismless enamel or only dentine and cementum exposed at the crown (Ishiyama 1987).

HSB in the Amazon river dolphin (*I. geoffrensis*) could be interpreted as a plesiomorphic structure that was retained from more archaic cetaceans, being lost in other taxa due to enamel reduction and simplification. However, the distribution of HSB in more-basal odontocetes such as Physeteridae and Ziphiidae is too poorly known to judge whether the HSB of *Inia* and *Platanista* are a retained plesiomorphic feature or convergence. The study of fossil specimens would help to establish the patterns of HSB, particularly in modern groups of low species diversity and specialized feeding habits in which the species might have gone through some morphofunctional bottleneck or if the extant species is not representive of the earlier members of the clade. This is the case of Physeteridae, now represented by a single species, and formerly diverse both in form and species number (e.g., Bianucci and Landini 2006).

Apart from *Inia boliviensis* d'Orbigny, 1834, the franciscana (*P. blainvillei*) is the closest extant relative to *I. geoffrensis*; however, it is known that the two species are separated by several extinct forms (Geisler et al. 2012). *P. blainvillei* showed a radial and prismless organization without HSB, and similar to most delphinids. While *P. blainvillei* and most delphinoids feed on small fish and squid, *I. geoffrensis* can feed on freshwater turtles, crustaceans, bivalves, and armored catfish (Werth 2000). For this species, HSB seem to have the secondary functional significance, providing a protective function related to the hardness of the diet. In fact, *I. geoffrensis* is the only living cetacean to have molariform-like posterior teeth ornamented with a rugose layer of enamel (Flower 1867); suggesting that teeth may play a role in the mechanical reduction of hard prey (Werth 2000). It is difficult to interpret the enamel patterns seen in modern odontocete families which contain few living species, and the study of teeth from fossil odontocetes would provide wider taxon sampling, helping to resolve phylogenetic patterns of enamel.

The evolution of mammalian enamel has been driven by a combination of developmental constraints, functional influences, and phylogenetic history. Nonetheless, the wider distribution and variation of functionally important features suggest they are plastic and have been acquired independently many times, carrying limited phylogenetic information. However, to some extent, enamel ultrastructure could be useful to understand some specific evolutionary aspects of clades that share common functional adaptations (Maas and Dumont 1999). In addition, future understanding of the mechanical properties of cetacean enamel may help us elucidate broader aspects of dental functional biomechanics in terms of enamel complexity. Further studies considering a wider range of living and fossil odontocetes are necessary to elucidate the role of phylogenetic and functional constraints in the ultrastructure of cetacean enamel.

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