Microstructures of Shark Teeth from Late Miocene Deposits of Baripada Beds, Orissa, India

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ABSTRACT

Numerous shark teeth were collected during the recent geological field work (2017-2018) conducted at the well known shark bearing deposits of Makurmatia section of Late Miocene Baripada Beds, Orissa. Scanning Electron Microscope (SEM) analysis of some of the selected shark teeth (Galeorhinus sp., Carcharhinus sp., Carcharodon sp.) were carried out to study the microcrostructural arrangement of the teeth enamel. The scanning electron micrographs of the shark teeth enamel crystals are highly ordered and arranged in different fashions as single crystallite enameloid (SCE), bundle crystallite enameloid (BCE) which can be subdivided into parallel bundle enameloid (PBE) and tangled bundle enameloid (TBE) as compared to the basal neoselachian which present only the single crystallite enameloid (SCE). The TBE of the Carcharodon sp. is more matured and more compacted than the other two Carcharhinus sp. and Galeorhinus sp. The microstructural arrangement of Galeorhinus sp., Carcharhinus sp. and Carcharodon sp. suggest that these sharks belong to the modern Neoselachian group of sharks.

Keywords: Microstructure, Enamel, Dentine, shark teeth, Miocene, Baripada Beds.

INTRODUCTION

The studies of a microstructures of the teeth enamels of vertebrate fauna are significantly useful for the reconstruction of phylogeny, evolution of the teeth and enamel, their compositions, mechanical properties, and identification of diets of the animals (Gillis and Donoghue, 2007; Enax et al., 2012; Mao et al., 2015). In case of selachian teeth, microstructural analysis of the crystallite enameloid structures are being used for the determination of the homology and the phylogeny of the Chondrichthyan teeth (Gillis and Donoghue, 2007) and differentiation of Hybodont (primitive) shark teeth and the neoselachian (modern) teeth (Enault et al., 2015). The teeth enamels of the modern neoselachians have different complex structures such as single crystallite enameloid (SCE), bundle crystallite enameloid (BCE) which can be subdivided into parallel bundle enameloid (PBE), tangled bundle enameloid (TBE) and radial bundle enameloid (RBE) as compared to the basal neoselachian which present only the single crystallite enameloid (SCE) (Gillis and Donoghue 2007; Andreev and Cunny 2012, etc.).
Previous workers from Baripada Beds reported a rich fossil assemblages of sharks, batoids, teleost, turtles, crabs, crocodilian and mammalian remains (Jena, 1942; Mohanty, 1966, 1980; Ghose, 1956, 1959; Modak, 1952; Mehrotra et al., 1973; Sahni and Mehrotra, 1981; Mondal et al., 2009; Milankumar and Patnaik, 2010, 2013a, b, 2014). This fossiliferous assemblage, correlatable with those of the Miocene deposits of Kutch, Mizoram (Mondal et al., 2009, Patnaik et al., 2014, Milankumar, 2013) is considered to be deposited as a consequence of marine transgression during Middle to Late Miocene (Bhalla and Dev, 1988; Milankumar, 2013, Milankumar and Patnaik, 2014). Beside the vertebrate remains, an extensive account of invertebrate fossils including Gastropods, Bivalves, Foraminiferas, etc. had also been described (Tipper, 1906; Eames, 1936; Jena, 1942; Sharma, 1956; Chatterji and Adyalkar, 1962; Mohanty, 1966, 1980; Bhaumik et al., 2017 and reference therein). Mishra (1991, pp. 31-32, pls. 1-3) described in brief and illustrated microstructure of shark teeth comprising of Isurus pagoda, Isurus spallanzanii, Negaprion brevirostris, Carcharhinus gangeticus and Carcharodon carcharias from Baripada Beds, Orissa. Probably no other reference is available on the microstructural analysis of Neogene shark teeth from India.

Milankumar and Patnaik (2014) discussed a brief account of the shark fauna comprising of 13 genera and 37 species from Baripada Beds and their palaeoecological and palaeoclimatic significance. The present paper gives a brief preliminary data on the microstructural features of some selected shark teeth of the genus Galeorhinus, Carcharhinus and Carcharodon from the Late Miocene, Baripada Beds, Orissa, India.

**Fig. 1:** A. Palaeogeographic map of Indian-Subcontinent during the deposition of Middle and Late Miocene sediment (Modified after Sahni and Mitra, 1980; Adnet et al., 2007; Milankumar, 2013); B. Location map of study area; C. Lithosection of Mukurmatia (After Milankumar, 2013).
STRATIGRAPHY OF THE AREA

The Late Miocene Marine deposits of Baripada Beds are well exposed along the Burhabalang River showing lateral facies variation and also the variation in the thickness of each lithology at certain localities expose near Satpautia, Itamundia, Mukurmatia areas, etc. These deposits lie above the Precambrian metamorphic basement (Bose, 1904) and are again unconformably overlain by the conglomerate beds belonging to the Pleistocene age in some areas such as Kuliana, Itamundia, Mukurmatia (Fig. 1 A, B) which are further overlain by the Quaternary sediments (Milankumar, 2013). These marine sediments are rich in various vertebrates and invertebrate fossils of different taxa. The generalised stratigraphy of the marine Baripada Beds are comprising of the greenish shale at the lowermost unit in which the base is not exposed overlain by the limestone, sandstone, greenish, yellowish to greyish shale at the top. The shale, limestone, sandstone and quaternary laterite succession as exposed at locality Mukurmatia (Fig. 1C) shows progressively arenaceous nature of the uppermost shale bed towards the top. The sandstone unit is poorly sorted; immature contains numerous highly rounded pebbles and conglomerates of different composition (Mondal et al., 2007).

**Fig. 2**: Detail microstructure feature of the enameloid crystallite structures of neoselachii shark teeth (longitudinal view) (Modified after Enault et al., 2012).

MATERIAL AND METHOD

Shark teeth of the genera *Galeorhinus* (Family Triakidae), *Carcharhinus* (Family Carcharhinidae) and *Carcharodon* (Family Lamnidae) were chosen for microstructural analysis (Fig. 3). These teeth were collected from the maceration of the 500 kgs. of shale samples. The process of maceration follows those of Milankumar and Patnaik (2014). The collected fossils were photographed under Leica M205C microscope housed at Palaeontology and Biostratigraphy Laboratory, Department of Geography and Geology, Central University of Punjab, Bathinda. All the teeth samples were mounted using the M-seal adhesive before cutting and polishing. The mounted teeth were cut using Struers Discoplan-TS and then finely polished using the corundum powder. All the samples were...
first grounded using coarser powder and then to finer powder for a smooth surface. The polished surfaces were etched by using 16% of HCL for 60 seconds. The surfaces of the prepared samples were slightly washed with acetone and they were subjected to gold coating. The gold coated samples were studied under the Carl Zeiss Merlin Compact 6073 Scanning Electron Microscope (SEM) housed at Central Instrumentation Laboratory, Central University of Punjab, Bathinda. Then microstructural features of the enameloid crystallite structures of shark teeth as observed under the SEM (Fig. 2) were discussed. The collected specimens are catalogued under the catalogue numbers MKS/BJG/ and stored at the Palaeontology and Biostratigraphy Lab. Department of Geography and Geology, Central University of Punjab, Bathinda.

**Fig. 3:** Shark teeth from Miocene Baripada Beds. A-B. *Galeorhinus* sp. (MKS/BJG/01); C-H *Carcharhinus* sp. (C-D. MKS/BJG/03; E-F. MKS/BJG/07; G-H) MKS/BJG/04); I-J *Carcharodon* sp (MKS/BJG/10). Each scale bar is 2mm in length (A, C, E, G and I are in Labial views and B, D, F, H, J are in Lingual views).
RESULTS

Order CARCHARHINIFORMES, Compagno, 1977
Family TRIAKIDAE, J.E. Gray, 1851
*Genus Galeorhinus* Blainville, 1816
*Galeorhinus* sp.
(Fig. 3 A-B; Fig. 4 A-F)

**Material:** Isolated fragmented tooth under the specimen number MKS/BJG/01.

**Locality and Horizon:** Bluish grey to yellowish grey shale bed of Mukurmatia, Baripada Beds, Orissa.

**Comments:** The tooth of *Galeorhinus* sp. (MKS/BJG/01, Fig. 3 A-B), small in size, with a triangular high crown, inclined distally and serration on the distal edge. The *Galeorhinus* shark teeth were reported earlier from Baripada Beds (Mondal *et al.*, 2009; Milankumar, 2013; Milankumar and Patnaik, 2014) and also from Miocene of Mizoram (Ralte *et al.*, 2011). They are mainly found in coastal-pelagic of temperate continental, insular waters and mostly distribute around the subtropical region (Milankumar, 2013; www.fishbase.com).

**Descriptions on Microstructure:** The longitudinal surface of the SEM images of the sample (MKS/BJG/01) shows the well defined crystallite enamloid features of the tooth enamel which are demarcated from the Dentine by the Enamel Dentine Junction (EDJ). EDJ of the tooth is clearly visible (Fig. 4 A, B, C, D, E); the enamel of the teeth is very thin (Fig. 3 A) on the shoulder and thicker on the apex of the teeth. The thickness of the enamel is gradually decreased from the apex to shoulder and to near base of the teeth. Enameloid Crystallites are loosely packed and arranged in random orientation forming a sharp cutting surface to EDJ (Fig. 4 C, D). The unsystematic arrangement of loosely packed enamloid crystallate are slightly entangled with each other near the enamel-dentine junction. The parallel bundle enamloid (PBE) and a tangle bundle enamloid (TBE) structure of the enamloid crystallate are very faintly visible (Fig. 4 C, D, E). The parallel bundle enamloid (PBE) is not clearly shown or immature mineralization of crystallite on MKS/BJG/01, tangle bundle enamloid (TBE) is very loosely packed and the junction of the enamel and the dentine is poorly contacted indicating a low degree of biomineralization at the enamel portion. The Dentine tubules are clearly visible at the dentine part of the teeth (Fig. 4 A). The single Crystallite enamloid layer (SCE) are moderately shown (Fig. 4 F).

Family CARCHARHINIDAE, D.S. Jordan and Evermann, 1896
*Genus Carcharhinus* Blainville, 1816
*Carcharhinus* sp.
(Fig. 3 C-H; Fig. 5 A-F; 6 A-D; 7 A-D)

**Material:** Isolated fragmented teeth under the specimen number MKS/BJG/03, MKS/BJG/04, MKS/BJG/09.

**Locality and Horizon:** Bluish grey shale to yellowish grey shale beds of Mukurmatia, Baripada Beds, Orissa.

**Comments:** MKS/BJG/03(Fig. 3 C-D), MKS/BJG/04 (Fig. 3 E-F), MKS/BJG/09 (Fig. 3 G-H) are *Carcharhinus* teeth. The Genus *Carcharhinus* is the most dominant shark reported from the Miocene assemblages of India (Sahni and Mehrotra, 1981; Milankumar and Patnaik, 2014). Earlier reports on *Carcharhinus* shark teeth comes from the Miocene of Baripada Beds (Mehrotra, 1972; Mehrotra *et al.*, 1973; Sahni and Mehrotra, 1981; Mondal *et al.*, 2009, Milankumar and Patnaik 2014); Miocene of Kutch (Mehrotra *et al.*, 1973; Sahni and Mehrotra, 1981; Patnaik *et al.*, 2014) and Miocene of Mizoram (Ralte *et al.*, 2011), etc. The
Carcharhinus sharks are generally found in the warm temperate and tropical water (Roger et al., 2008; Milankumar and Patnaik, 2014).

Fig. 4: MKS/BJG/01; Scanning Electron Microscope Image of Galeorhinus sp. A) Showing Enamel and Dentine B &C) Magnified Image of Enamel and Dentine, C, D & F) Showing the SCE layer composed of loosely packed enameloid Crystallite structure, E & F) Magnified images of PBE+TBE and the SCE.

Descriptions on Microstructure: The teeth of Carcharhinus sp. (MKS/BJG/03) is small with pointed apex and high crown. The ultra-structure of the teeth enameloid comprises of two prominent layers namely SCE and well developed TBE layer (Fig. 5 C). SCE layer is present at the marginal sides of the teeth but not at the apex or middle part the enamel (Fig. 5 B). The SCE layer is composed of loosely arranged enameloid Crystallite which are structureless and random in nature. PBE layer is lacking in between the SCE and TBE layer of the enamel Crystallite. The TBE layer is visible as a compact, interwoven bundles of
enameloid Crystallite. The TBE layers extended up to EDJ from the SCE layers. However, at the middle of the teeth, TBE layers are extending from the cutting surface to the EDJ (Fig. 5 C, D). The thickness of the TBE layers are decreasing towards the lateral side of the teeth (Fig. 5 C). Dentin tubules are present at the dentin portion of the teeth having a tubular diameter of 2-6 μm. The core of the dentin is comprised of orthodentine (Fig. 5 E, F). The microstructure of the enameloid crystallite structure of MKS/BJG/04 is strongly developed from cutting edge of the tooth enamel to EDJ (Fig. 6 A, B, C, D). The teeth of *Carcharhinus* sp. (MKS/BJG/04) are showing a different enameloid crystallite structure from EDJ to cutting surface. Enameloid crystallite structure layers are well developed and crystallite structures are distinguishable as PBE layer and TBE layer. The PBE layer comprises of microstructure enameloid crystallites arranges in uniform bundles. This crystallite bundles are parallel to each other having a thickness of 30 μm from cutting surface to the PBE-TBE boundary (Fig. 6 B, C). The PBE layers are more compacted toward the EDJ, their crystallite structures are changing in orientation and interwoven with each other (Fig. 6 B; Fig. 7 C). The TBE layer is
around 60 μm thick from the EDJ. MKS/BJG/09 shows the presence of PBE and TBE layers but SCE layer is lacking (Fig. 7 A). The loosely formed bundles PBE layer enameloid Crystallites (Fig. 7 B) is 200 μm thick. This layer extends from cutting surface to the boundary of the TBE layer. The dentine portion of the teeth is showing prominent the dentin tubules (Fig. 7 D).

![Fig. 6: MKS/BJG/04; Scanning Electron Microscope Image of Carcharhinus sp., A. Showing Enamel and Dentine, B. Showing PBE and TBE layer, C. PBE layer, D. TBE layer.](image)

Order LAMNIFORMES, L.S. Berg, 1958
Family LAMNIDAE, J.P. Müller and Henle, 1838
Genus Carcharodon Linnaeus, 1758
Carcharodon sp.
(Fig. 3 I-J; Fig. 8 A-D)

**Material**: Isolated tooth under the specimen number MKS/BJG/10.

**Locality and Horizon**: Bluish grey shale to yellowish grey shale beds of Mukurmatia, Baripada Beds, Orissa.

**Comments**: The genus of *Carcharodon* was reported from the Miocene of Baripada Beds, Kutch, Piram Island and Bhavnagar (Mehrotra, 1972; Mehrotra *et al.*, 1973; Sahni and Mehrotra, 1981; Mondal *et al.*, 2009; Milankumar and Patnaik, 2014) and also forms Miocene of Mizoram (Ralte *et al.*, 2011). The genus *Carcharodon* is well known for its adaptation at marine, pelagic-oceanic and brackish environment and warm to temperate climates generally in subtropical regions.

**Descriptions on Microstructure**: Microstructure of the teeth shows well developed enameloid crystallite structures (Fig. 8 A, B, C, D). Enameloid crystallite structure of PBE
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Layer comprises of a crystallite bundle arranged in parallel to each other and perpendicular to the cutting surface of the teeth (Fig. 8B, C). The TBE layer is showing the random orientation of the entangled enameloid crystallites. The TBE layers are more matured than the PBE layer enameloid structure and they are more compacted, crystallites are strongly interwoven as compare to those of the enameloid crystallite of PBE layer. SCE is feebly developed at the side edge of the specimen.

Fig. 7: MKS/BJG/9; Scanning Electron Microscope Image of Carcharhinus sp., A. Showing Enamel and Dentine, B. PBE layer, C. TBE layer, D. Dentine Tubule.

DISCUSSIONS AND CONCLUSION

Vertebrate tooth enamel are composed of hypermineralized tissues in the forms of Crystallite Enameloid matrix Structure (Reif, 1973; Francillon-vieillot et al., 1990). The teeth of the modern neoselachian shark teeth have triple layer of different microstructural feature (Reif, 1973; Duffin, 1980; Mishra, 1991; Cuny et al., 2001). The triple layer enameloid microstructures of sharks have been developed from the hypermineralization of SCE to PBE and again PBE to TBE (Reif, 1970; Andreev and Cunny, 2012; Enault et al., 2017) and this triple layers enameloid microstructure suggests the characteristic feature of modern neoselachian (Reif, 1973).

The teeth microstructural feature of the genus Galeorhinus shows the presence of Single Crystallite Enameloid (SCE) and Bundle Crystallite Enameloid (BCE). Similar ultrastructures of Galeorhinus sharks were studied by Gillis and Donoghue (2007), Botella et al. (2009). The Bundle Crystallite Enameloid, Parallel Crystallite Enameloid and Tangle Crystallite Enameloid are loosely packed and BCE layer is not well developed. Thus, Galeorhinus sharks are showing the similar characteristic features of modern neoselachian which are having triple crystallite layers (Reif, 1973; Cuny and Risnes, 2005; Moyer et al., 2015). In case of the Genus Carcharhinus, the teeth enameloid shows the presence of
Bundled Crystallite Enameloid (BCE) which are consisting of Parallel Bundled Enameloid (PBE) and Tangled Bundled Enameloid (TBE) and similar features are available in teeth microstructures of the genus *Carcharodon*. The teeth of *Carcharhinus* sp. and *Carcharodon* sp. also shows similar microstructures to those of modern neoselechian shark which are having SCE, Bundle Crystallite Enameloid layers, namely the PBE and TBE on the teeth enamel (Reif, 1973; Cuny and Risnes, 2005). Microstructures of Neoselechian shark such as *Sphenodus* sp., *Paraorthacodus* sp., *Synechodus* sp., *Welcommia bodeuri* and *Pachyhexanchus pockrandti*, *Prionace glauca* (Carcharhiniformes: Carcharhinidae) and the Great White Shark, *Carcharodon carcharias* (Lamniformes: Lamnidae) (Mishra, 1991; Botella et al., 2009; Moyer et al., 2015) are comparably similar to those the Miocene shark teeth from the Baripada Beds.

![Fig. 8](image_url)

**Fig. 8**: Scanning Electron Microscope Image of *Carcharodon* sp. (MKS/BJG/10), A. Showing Enamel and Dentine, B. Showing TBE layer, C. PBE layer, D. Magnified image PBE layer.

Thus, based on the arrangement of crystallite microstructure of the Miocene shark teeth (*Galeorhinus* sp., *Carcharhinus* sp. and *Carcharodon* sp.) from the Baripada Bed, it is concluded that *Galeorhinus*, *Carcharhinus* and *Carcharodon* sharks come under the modern Neoselachian group of sharks. The TBE of the *Carcharodon* sp. is more mature and more compacted as compare to those of *Carcharhinus* sp. and *Galeorhinus* sp. In *Galeorhinus* sp. crystallite enameloid is very slack and immature which may due to the lack of further mineralization or hypermineralization enameloid Crystallite layer.

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