



RESEARCH PAPER

First characterization report of natural pearl of *Pinctada fucata* from Gulf of Mannar



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Abstract The present study is aimed to characterize the natural pearl of *Pinctada fucata* from Gulf of Mannar by Scanning Electron Microscope (SEM) and Energy Dispersive Studies (EDS). Pearl oysters (*P. fucata*) from Kayalpattinam, Gulf of Mannar, were landed as a by-catch in the bottom set gill net at a depth of 4 m and collected for tissue culture studies. During mantle tissue dissection, a good lustrous, round pearl of 1.5 mm size was found in the mantle fold of pearl oyster *P. fucata*. This evidenced the existence of natural pearl oyster beds and natural pearls in this region. It was analyzed by Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS) to find out the composition of nacre. Parallel orientation of crystals to form the lamellar formation of nacre is clearly visible in SEM. Pseudo-hexagonal aragonite crystals arranged in a uniform layer and joined together to form a lamella with inter-lamellar matrix. Two forms of calcium (CaO and CaCO₃) obtained in EDS analysis. Calcium content in the natural pearl is 66.05% which clearly reveals the aragonite form. Niobium (Nb) was attained at 6% in natural pearl which is the first report in its kind and it may also play a role in the formation of lustrous layer. There are so many characterization reports available about mother of pearl in shell of different species, but this is the first report of natural pearl from *P. fucata*.

Introduction

Formation of natural pearl in a mollusc such as *Pinctada fucata*, *Pinctada maxima*, *Pinctada margaritifera*, *Pteria penguin* and *Haliotis varia* is an accidental event influenced by the environment. The chances of a perfect natural pearl are one in a million. Natural pearls produced by the pearl

oysters from the Gulf of Mannar is considered to be the best "Orient Pearls" and highly valuable. As pearl oyster fishery is banned since 1961, natural pearls have not been recorded for decades. This is the first authenticated report of rare occurrence of natural pearl of *Pinctada fucata* from Gulf of Mannar after 1960.

Pinctada fucata is a marine bivalve mollusk in the family Pteriidae and native of this species is Indo-Pacific region. It is also distributed in Red Sea, the Persian Gulf, coastal waters of India, Korea, Japan, China and the Western Pacific Ocean. It was first observed in Venezuela coastal waters (FAO, 2014).

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Pearl culture technology was developed by Japan and Australia and they are the significant producers of cultured pearls. The mantle tissue of living pearl oysters such as *P. fucata*, *P. maxima*, *P. margaritifera* and *P. penguin* is playing a vital role in the formation of nacre layer. The pearl oyster *P. fucata* is one of the best studied species in the aspect of economic importance (Bouchet, 2013). It consists of inner aragonitic nacreous and outer calcitic prismatic layers (Liu et al., 2012). The formation of nacreous layer shows potential model for the development of *in vitro* pearls. It can lead to the novel thoughts for synthetic crystallization processes to materials science (Checa & Rodriguez-Navarro, 2005). Though, some nacre layer of shell characterized, still there is no authenticated evidence for the characterization of natural pearls.

Therefore, a standard reference is needed to compare the *in vitro* and *in vivo* pearl with natural pearl in the aspect of structures (lustrous and brick and mortar) and percentage of CaCO_3 and other important elements.

The present study is aimed to characterize the natural pearl of *P. fucata* from Gulf of Mannar by Scanning Electron Microscope (SEM) and Energy Dispersive Studies (EDS).

Materials and methods

Sample collection

The animal with a shell length of 7.5 cm and width of 4.2 cm was collected from Kayalpattinam landing centre, Gulf of Mannar with the Latitude of $8^\circ 34' \text{N}$ and Longitude of $78^\circ 10' \text{E}$ as a by-catch in the bottom set gill net at a depth of 4 m and collected for tissue culture studies (Fig. 1). During mantle tissue dissection, a good lustrous, round pearl of 1.5 mm size was found in the mantle fold of a pearl oyster (Fig. 2).

Characterization of nacre layer (aragonite) formation

SEM and EDS

A good lustrous, round natural pearl from *P. fucata* with the size of 1.5 mm size was taken for the analysis of SEM and EDS. The samples were air-dried and coated with gold by ion-sputtering (JEOL-JFC1200E), and observed by SEM (JEOL-JSM 5600LV, Tokyo, Japan) and analyzed by an energy dispersive X-ray microanalyser (EDS, New Jersey, USA).

Results

SEM

The SEM reveals the presence of lamellar formation of nacre layer and organic matrix. Numerous layers of nacre forms single pearl. The outer scanning of the pearl shows the presence of growing nacre layer. The organic matrix provides nucleation sites for crystal formation and influences the types of crystal produced. Addition of more crystals resulted in the formation of nacreous layer. Fig. 3 shows the complete view of the natural pearl under Scanning Electron Microscope with scattered crystals over a lamellar layer. Scattered orthorhombic and rhombohedral (hexagonal) shapes of crystals embedded over the mineral lamellae were seen in enlarged view in Fig. 4. Aragonite forms of crystals were arranged in parallel lines clearly visible in Fig. 5 which shows the growing stage of nacre layer. In another area of the same pearl, parallel growth of crystals were seen joining together to form a lamellar layer sandwiched in an organic matrix (Fig. 6). In another view, organic layers similar to the growth rings were observed in concentric parallel lines (Fig. 7). An over view of the lamellar nacre layer with

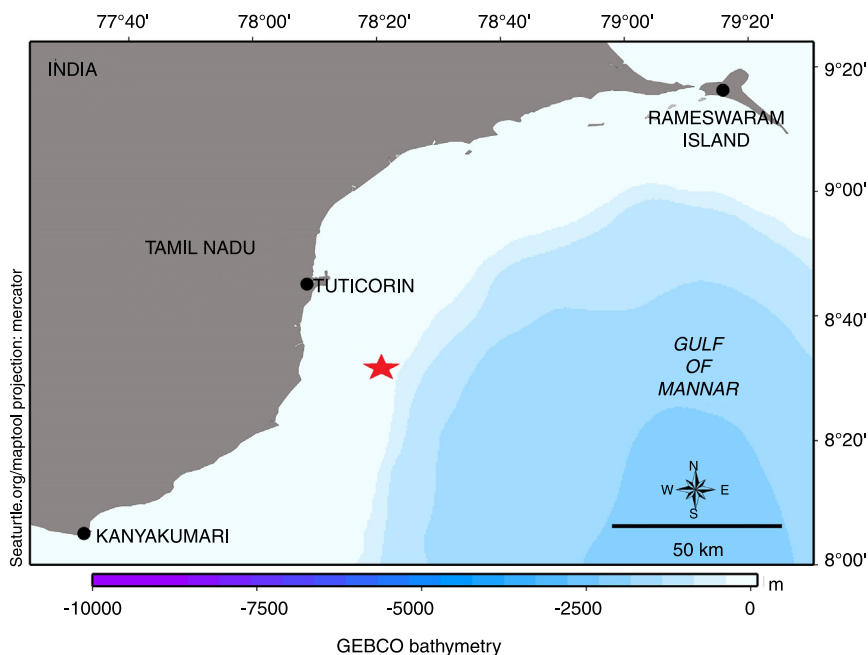


Figure 1 The study area.



Figure 2 Natural pearl embedded in mantle tissue of *P. fucata*.

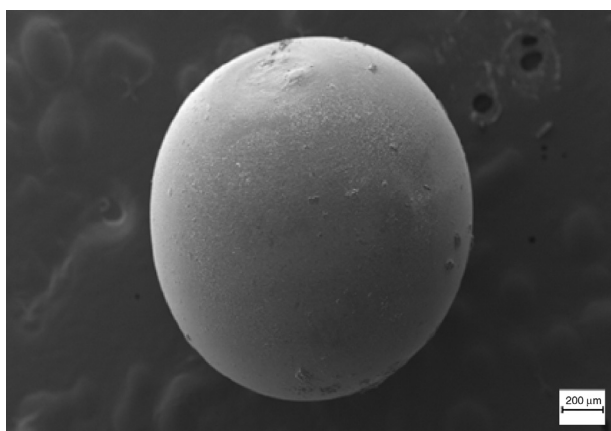


Figure 3 SEM image of complete view of natural pearl of *P. fucata*.

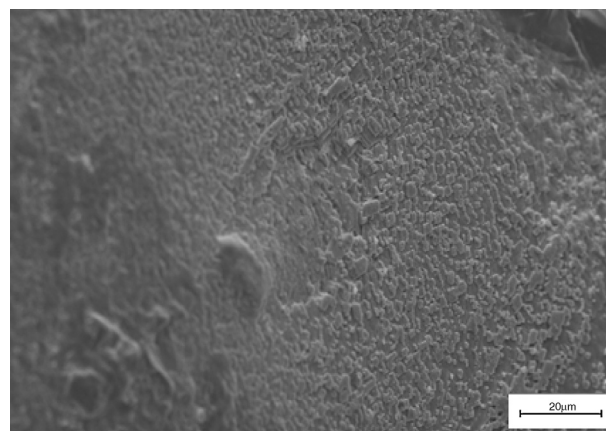


Figure 5 Parallel growth pattern of nacre crystals.

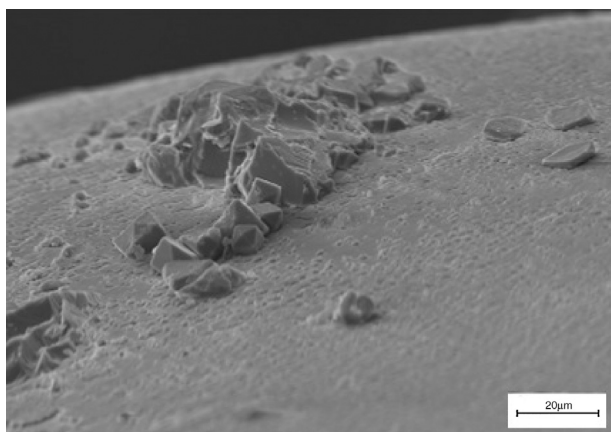


Figure 4 Scattered hexagonal shape of crystals embedded over the mineral lamellae in enlarged view.

thin layer of organic matrix and scattered crystals were seen in [Fig. 8](#).

EDS

The different surface of the natural pearl was analyzed by EDS which was composed of CaCO_3 and CaO . [Fig. 9](#) shows weight percentage of Ca and O with the element weight of

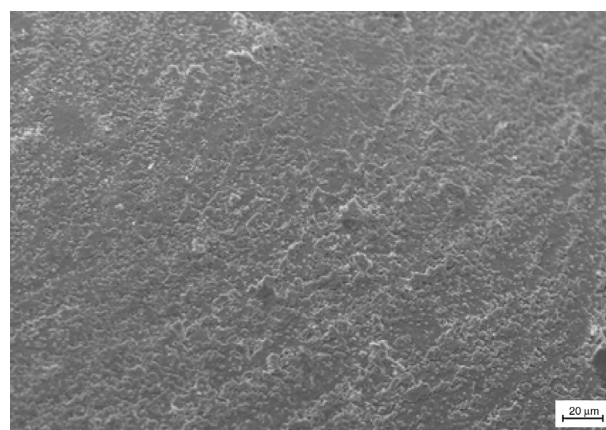


Figure 6 Parallel growth of crystals joined together to form a complete nacre layer.

66.05 and 57.82%, respectively ([Table 1](#)). [Fig. 10](#) shows EDS report of another surface of natural pearl which contains Ca, C and O additionally with Niobium (Nb) (6.41%) ([Table 2](#)).

Discussion

Many studies have shown that amorphous calcium carbonate plays a crucial role in the formation of mineralized tissues ([Miyazaki, Nishida, Aoki, & Samata, 2010](#)). Aragonite is the

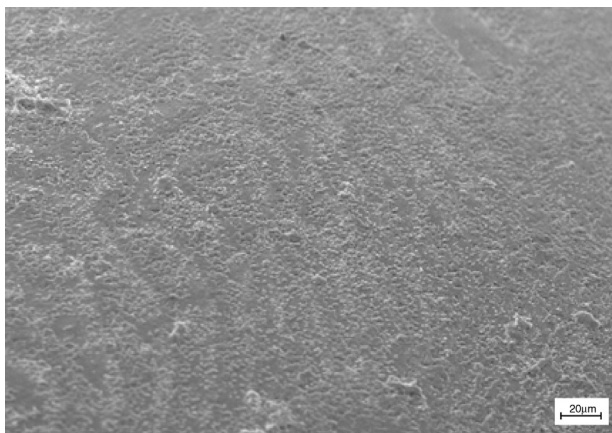


Figure 7 Lamellar growth of crystals sandwiched in organic matrix in parallel lines.

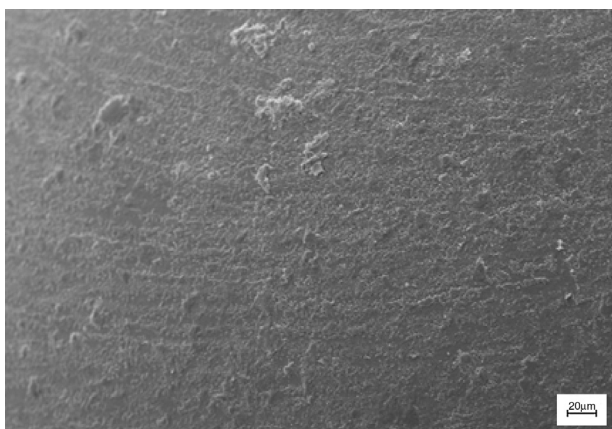


Figure 8 Over view of the lamellar nacre layer with thin layer of organic matrix and scattered crystals.

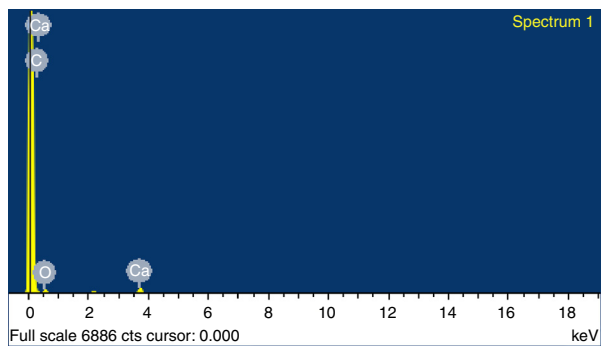


Figure 9 EDS report surface of natural pearl of *P. fucata* which contains Ca and O.

Table 1 Weight percentage of Ca and O of natural pearl of *P. fucata*.

Element	Weight%	Atomic%
OK	57.82	110.37
Ca K	66.05	50.33

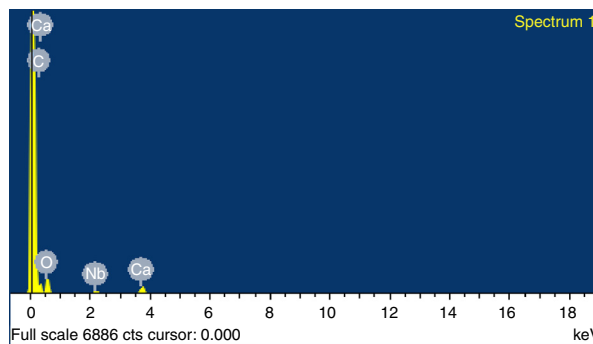


Figure 10 EDS report of the surface of natural pearl of *P. fucata* which contains Ca, C, O and Nb.

Table 2 Weight percentage of Ca, C, O and Nb of natural pearl of *P. fucata*.

Element	Weight%	Atomic%
C K	18.42	28.46
OK	50.84	58.98
Ca K	24.34	11.27
Nb K	6.41	1.28

purest form of calcium carbonate which is the same as that of calcite (Addadi, Joester, Nudelman, & Weiner, 2006). In the present study, EDS reports revealed that the natural pearl containing Ca and O which is in calcium oxide form. But the Ca weight percentage of natural pearl is 66%. It may be due to the calcination reaction in which calcium carbonate reduced to calcium oxide due to the high temperature (John Daintith, 2008). The formula is, $\text{CaCO}_3 \rightarrow \text{CaO} + \text{CO}_2(\text{g})$

In pearl formation an alternation of crystalline and matrix layers also occurs; and from the known age of the pearl it can be calculated that two to eight double layers form each day (Watabe, 1952). The mantle tissue is responsible for shell formation and secretes a matrix complex that includes proteins, polysaccharides and lipids. It has been suggested that the insoluble proteins provide the framework and mechanical properties of the shell, whereas the soluble proteins are involved in crystal nucleation and growth (Belcher et al., 1996).

The typical stepped mode growth of nacre demonstrated by Checca, Okamoto, and Ramirez (2006) is clearly visible in the natural pearl. Growth of nacre between lamellae may vary between shells and within the same shell which justifies the different growth of nacre in different surface of the same pearl (Checca et al., 2006). Wada (1961) reported the crystalline layers of naces in *Haliotis discus* and *Turbo cornutus* and also reported nacre consisted of mosaic arrangement of microcrystals. The present study reveals the presence of vertical growth of rhombohedral calcite over the lamellar layer of aragonite crystals.

The outer calcite prismatic layer is always related to the proteins secreted from the outer epithelial cells of the edge of the mantle, whereas the inner aragonite nacreous layer is related to the proteins of the pallial region (Xiang et al., 2014).

Though, calcium is playing a major role in the formation of nacre layer, but Nb also observed in this study with 6%

occurrence. Niobium is a lustrous, grey, ductile, paramagnetic metal in Group 5 of the periodic table (Gupta & Suri, 1994).

This study is the first authenticated characterization report of natural pearl of *P. fucata*. The earlier reports of nacre layer formation focused on CaCO₃, but this report revealed the role of CaO by calcination and the presence of Nb which also may be the reason for the formation of lustrous layer. Therefore further study needs to be focused on Nb and CaO, and its role in nacre layer formation.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgement

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