Original Research Article

A Study On Abnormal Structures Developed In Symbiont Bearing Benthic Foraminifera From Andaman Sea, India

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Abstract: The foraminifers are Protozoa with a shell (test) that consists of consecutive chambers that intercommunicate through oral cavity called foraminifera. The chambers are separated from each other by partitions called septa. The last chamber communicates with the exterior through one or several apertures. Cytoplasm that completely fills all the chambers emerges through these exterior apertures. Outside of the test the cytoplasm and emits as fine filamentous granular and reticulate pseudopodia. These pseudopodia often contain grains or fine particles of various kinds and play an important role in maintaining the living organism: movement, food supply, construction of new chambers, etc. Symbiotic algae (dinoflagellates) are often associated with the cytoplasm. The life cycle of the Foraminifera involves an alternation of generations. Foraminifera are broadly classified as planktonic and benthic. For the present study benthic foraminifera from the study area have harboured photosynthetic algae. The study has been carried out in the Andaman Sea, India. The samples were collected by Vanveen grab method during pre monsoon season at three different areas of different depth of South Andaman. The study has been carried out structural variation and symbiotic relationship algae. The study showed structural adaptations of the foraminiferans in the form of micro boring, pitted surface and tunnels in the shells of perforate and imperforate species. The remnant route system of the foraminiferans is adapted by symbiotic algae to uptake of CO₂ during the process of photosynthesis. Activities of symbiotic algae is light dependent and hence when light compensation is reached boring into the shell was stopped in the form of micro bores and diverted into the tunnels. These micro features in the foraminifer's shells were used for passage of CO for photosynthesis from the surrounding seawater. The results showed that the imperforate foraminiferan species have more adaptations than the perforate species.

Key Words: Benthic foraminifera, Micro boring, Tunnelling, Andaman sea

Introduction

The sea surface waters are supersaturated with respect to calcite; but Mg:Ca ratio in sea water is 5:1. Under these conditions how could the foraminiferans precipitate low Mg calcite? However, organisms like Scleractinian corals precipitates aragonite, whereas large benthic algal symbiont bearing foraminifers precipitate high Mg calcite shells. It was

thought that, prevalence of these two groups in geological periods is due to the successions in sea water chemistry. The periods with high Ca^{2+} concentration and low Mg^{2+} concentration the organisms preferred the precipitation of calcite.It is also known that the periods (Neogene) with low Mg/Ca ratios have chosen aragonite precipitation. (Nooijer

et al., 2009). However, the recent studies from culture experiments assessed the effects of ongoing ocean acidification on the calcification of symbiot bearing reef foraminifera using a high precision pCO_2 have shown different calcification mechanism between hyaline and porecelaneous taxa. The calcification of the foraminiferal hosts and photosynthesis by algal endosymbionts are known to be linked (Fujita et al., 2011).

The occurrence of magnesium in marine skeletal hard parts was documented by (Silliman, 1846). Three different compositional phases viz., low Mg aragonite, low Mg carbonate, low Mg (< 4% Mg CO₃) and high Mg calcite (> 4% Mg CO₂) were distinguished by (Boggild, 1930). The high magnesium calcite in skeletal was considered as mineral dolomite and later it was revealed that, calcium ions were replaced by magnesium ions in calcite lattices (Chave, 1952). Mg calcite - the isomorphs of calcite was substituted with much smaller Mg ions resulted shrinking of calcite lattice that caused variations in the mineralogical structure and positional disorder of anion and cation. Marine calcifiers depositing Mg calcite of varying compositions are ranging from a few mol % to as much as 30%. There are distinct differences between different species that have strong taxonomic control on the magnesium content of calcite skeletons (Chave, 1954). The mechanisms controlling the magnesium content of marine calcite skeletons are poorly understood and exert different influences on different organisms.

Also, Mg content of marine calcifiers depositing Mg – calcite decrease with increasing latitude and co varies with environmental changes such as light, temperature, seawater carbonate saturation, energy availability and organism dependent on the phototrophic symbionts. Mg/Ca compositional variation of planktonic foraminifera tests were used as proxy to determine the for past seawater temperature (Anand *et al.*, 2003), (Elderfield, 2000), (Hallock, 1981), (Lea *et al.*, 1999), (Nooijer *et al.*, 2009), Nurnberg *et al.*, 1996). Still the experimental studies support that, Mg/Ca is more responsive to seawater temperature, pH, salinity and (CO₃⁻²) concentration, but mounting evidences indicate biological

control for strong influence on Mg/Ca composition of foraminiferal calcite (Erez, 2003). With augment of test size of the foraminiferal not only increase bulk test Mg/Ca composition and also symbionts activity with test.

Relation between foraminifera and ocean chemistry is less clear and cloaked owing to calcification strategies. Foraminifera precipitate CaCO₃ in the ocean and play in global carbon cycle and used as major tool for paleo oceanographic study. They serve as excellent model organisms for cellular calcification processes.

Photosynthesis and calcification

Mechanism to elevate the carbonate concentration at the site of calcification might have assisted by the process of light enhanced calcification in symbiont bearing foraminifera by removal of CO_2 by photosynthesis which enhances calcification. $Ca^{2+} + 2 \ HCO_3 \rightarrow CaCO_3 + H_2 \ O + CO_2 \rightarrow CaCO_3 + CH_2 \ O + O_2$ Calcification $Ca^{2+} + 2 \ HCO_3 \leftarrow CaCO_3 + H_2 \ O + CO_2 \leftarrow CaCO_3 + CH_2 \ O + O_2$ Dissolution $Ca^{2+} + 2 \ HCO_3 \leftarrow CaCO_3 + H_2 \ O + CO_2 \leftarrow CaCO_3 + CH_2 \ O + O_2$

In the symbiont —bearing benthic foraminiferans it was shown that, both calcification and photosynthesis could be inhibited, without affecting each other (Saraswati, 2007), (Ter Kuile *et al.*, 1989b) Observations on mechanism of calcification in foraminifera, are mediated by alkaline sea water. It was concluded that, light enhanced calcification in foraminifera and, CO₂ removal or photosynthesis are not directly involved. Two alternative mechanisms i) light enhanced calcification ii) photosynthetic activities combined with symbiotic algae exist. Exchange of Ca²⁺ for 2H⁺ and enhance calcification and at the same time photosynthesis are certainly possible in foraminifera (Erez, 1983).

Photosynthesis and algal symbiosis

The advantages of algal symbiosis to foraminifera include (i) energy from photosynthesis (ii) enhancement of calcification and (iii) removal of host metabolites. Hallock (1981) used mathematical model to demonstrate that algal symbiosis provides substantial energetic advantages to foraminifera over symbiont-free foraminifera in nutrient-deficient environments.

Increasing nutrient influx is therefore detrimental to the survival of symbiont-bearing foraminifera and as a result, this group of foraminifera is a potential indicator of anthropogenic changes. They are subjected to vulnerability to coastal nitrification and ultra violet radiation. These symbiont bearing foraminifera can also be used for monitoring pollution and health of the coral reefs. The other important aspects of symbiont-bearing benthic foraminifera are their relatively longer life span (few months to few years) and larger size of the tests. In India such foraminifera mostly occur in reef environments of Lakshadweep and Andaman (Saraswati, 2007) as presented in Table 1.

Table1. Types of symbionts in modern Foraminifera (Saraswati, 2007)

Family	Genus	Symbiont type		
Peneroplidae	Peneroplis	Unicellular red alga		
Archaiadae	Archaias, Cyclorbiculina, Androsina	Chlorophyte		
Soritidae	Sorites, Amphisorus, Marginopora	Dinoflagellate		
Alveolinidae	Alveolinella, Borelis	Diatom		
Amphisteginidae	Amphistegina	Diatom		
Calcarinidae	Calcarina	Diatoms		
Nummulitidae	Nummulites, Operculina, Heterostegina,	Diatoms		
	Cycloclypeus.			

Benthic and planktonic foraminifera are known as host algal symbionts. Symbiont- bearing planktonic foraminifera are wide spread calcifying protozoa has boring phototropic microalgae (Lee, 1969). Symbiont - bearing planktonic foraminifera can notify as hot spot of primary productivity in oligotrophic Seas. However the sources of inorganic carbon sustain symbiont photosynthesis has not been identified. Mechanism involved inorganic carbon uptake from the ambient seawater into foraminiferal cytoplasam and subsequent carbon fixation remains unknown. The current knowledge of CaCO₃ precipitation mechanism in symbiontbearing planktonic foraminifera is limited. The uptake and concentrating mechanism of calcium and carbonate is energy dependant and it has been suggested that the ions are stored in an inorganic carbon pool from the foraminiferal cytoplasm (Ter Kuile et al., 1989b).

Inorganic carbon is initially taken up in the form of bicarbonate (HCO $_3$) which is concentrated as carbonate (CO $_3$)

) in the special vesicle (Anderson, 2008). However, the significant of symbiotic algae in the regulation of calcification and the potential influence of other physiological and biochemical process await further studies. The physio-chemical microenvironment of symbiont bearing planktonic foraminifer's photosynthesis and respiration have caused significant changes in $\rm O_2$ and pH levels with rising levels of atmospheric $\rm CO_2$.

In consequent, the rise to oceanic inorganic carbon level, that has enhanced calcification in foraminifera by supplying them with extra bicarbonate. The accompanying decrease in seawater pH is estimated 0.3–0.4 units for the coming century may have counter effect to the extra supply of inorganic carbon and result in a net decreased growth rate. For coccolithophores it has been suggested that future ocean acidification will reduce net calcification, but for foraminifera micro- or mesocosm experimental data are inadequate.

Types of Symbioses

There are also significantly different ecological requirements of planktonic and benthic associations, both of which are considered here. Finally, one may distinguish between ectosymbioses (epibionts: external) and endosymbioses (endobionts: internal) and these imply fundamental differences in intimacy with the host.

Ectosymbioses

As a general rule, ectosymbioses, although sometimes highly specialized are usually less intimate and less modified associations than endosymbioses. Typically, they are separated into epiphytic (on plants) versus epizoic (on animals) associations. The term epibiotic, simply signifying that the organism occurs on a living surface is also useful. Marine protozoa may be either the active colonizers or the relatively passive hosts in such epibiotic associations.

Epibionts on the surface of larger Foraminifera

The surfaces of benthic foraminifers, particularly the soritids, are covered with colonies of bacteria, diatoms, and unicellular

green algae. Small species of the pinnate diatom *Amphora* spp.(Halamphora group) can fit in the crevices where the calcareous plates covering the chamberlets of *Amphisorus hemprichii* join together (Lee *et al.*,1983). The same foraminifers is often colonized by a unicellular green alga that creates pockets in the lateral walls of the test. Smaller foraminifers are heavily colonized by a species of *Fragilaria*.

Photobionts of Benthic Foraminifera

It appears that all the larger, shallow — water, benthic foraminifera, belonging to 11 families, can harbour photosynthetic cytobionts (Lee *et al.*, 1980) Lee *et al* (1983) Lee *et al.*, (1985a, Lee *et al.*, 1985b, Anderson, O. R. and Lee, 1991, Saraswati, P. K 2007.

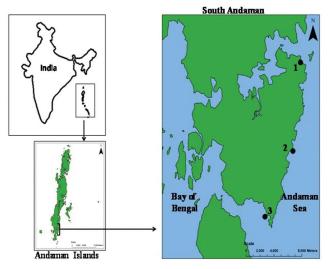


Fig .1. 1- Aberdeen Bay (25 m depth), 2- Burmanalla reef (12 m depth) and 3- Chidyatapu reef (2m depth)

Materials and methods

To study the symbiont bearing foraminifera the samples were collected through Van veen grab from the reefs in the Aberdeen Bay (25 m depth), Burmanalla reef (12 m depth) and Chidyatapu reef (2m depth) in Andaman Sea South Andaman, India. 25 grams of the samples were treated with Rose Bengal dye to distinguish living and deceased species. Hand picking of specimens after sieving and drying of the sediment samples are preserved in paleontological slides for further studies. All species are described based on the treatise of (Loeblich and Tappan 1988).

Identification of Ultra structures

FEI Quanta 200 Environmental Scanning Electron Microscope (ESEM) was used to identify the ultra structures such as micro boring by endolithic micro-organisms that bored borehole less than 100µm the wide that correspond exactly with the outline of the boring organisms in the benthic foraminifers: Spirolina arietinus, Quinqueloculina pulchella, Peneroplis cylindraceus, Elphidium crispum, Bolivina subspinescens, Siphogenerina striata, Calcarina spengleri and Peneroplis planatus; Micro borings are straight, curved and formed dense networks with dendritic appearance. The endolithic micro- organisms colonize the calcareous, siliceous and phosphatic substrate. Excavation of microborer towards the interior of the host is light dependent. Once the light compensation depth for photosynthesis is reached, boring either stopped and formed as pit or proceeds parallel to surface and produced as tunnel (Garcia-Pichel, 2006).

The uptake of CO_2 from the surrounding seawater has taken takes place through the thin lateral walls of symbiont bearing foraminifera, since CO_2 can parameter through the very thin pitted walls. Thinned walls with pits, furrows, microboring and tunnels facilitate the passage of CO_2 for the symbiotic algae in their photosynthetic process.

Results

Types of micro boring, pits and tunnels observed

The tunnelling and boring features are short, curved in *Spirolina arietinus* and densely distributed. The length of boring ranges from 64µm to 112µm and the width of the boring is 20 µm (Fig 2.1). In *Peneroplis cylindraceus* the tunnelling and boring features are straight, curved and randomly distributed. The boring length of is varies from 25.77µm to 46.24µm and the width of the boring is 3.1 µm (Fig 2.2). In *Quinqueloculina pulchella* the tunnelling and boring features are long, curved and they are randomly distributed. The length of boring range from 29µm to 105µm and the width of the boring is 2.8 µm (Fig 2.4). In *Quinqueloculina intricate* the tunnelling and boring features are straight, curved and distributed randomly. The length of boring is 32µm and the width of the boring is 5 µm (Fig 4.2). In *Peneroplis planatus* the tunnelling and boring features are long, coiled and they are thickly distributed. The

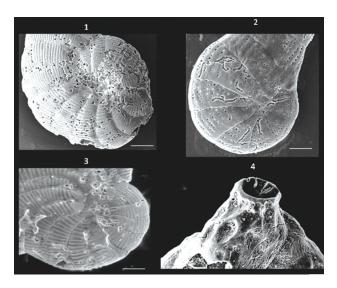


Fig. 2. 1. Shows the Environmental Scanning Electron photomicrographs of micro boring, tunnelling and pitted surface of *Spirolina arietinus*, 2. Shows the Environmental Scanning Electron photomicrographs of microboring, tunnelling and pitted surface of *Peneroplis cylindraceu*, 3. Shows the Environmental Scanning Electron photomicrographs of microboring and pitted surface of *Peneroplis planatus*, 4. Shows the Environmental Scanning Electron photomicrographs of microboring, tunnelling and pitted surface of *Quinqueloculina pulchella*.

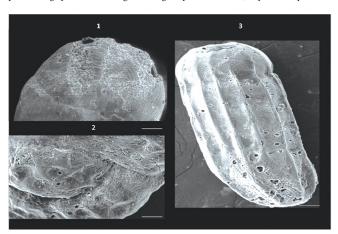


Fig. 3. 1. Shows the Environmental Scanning Electron photomicrographs of microboring, tunnelling and pitted surface curved out by symbiotic algae in *Spirolina arietinus*, 2. Shows the Environmental Scanning Electron photomicrographs of Microboring, Tunnelling and pitted surface *Quinqueloculina intricata*, 3. Shows the Environmental Scanning Electron photomicrographs of microboring tunnelling and pitted surface *Siphogenerina striata*.

boring length varies from 62.5 to 188 μ m μ m and the width of the boring is ranges from 4 μ m to 6.25 μ m (Fig 2.3). In *Spirolina arietinus* the tunnelling and boring features are found as straight, curved and distributed unevenly. The length and width of the bores counted in the species varies from 132 μ m to 288 μ m and 17 μ m to 35 μ m (Fig 3.1). In *Siphogenerina striata* the tunnelling and boring features are observed as straight, curved and distributed they are randomly. The length of

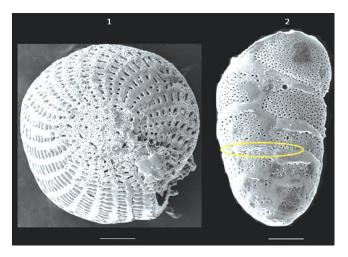


Fig. 4. 1. Shows the Environmental Scanning Electron photomicrographs of pitted surface in *Elphidium crispum*, 2. Shows the Environmental Scanning Electron Microphotographs of Tunnelling in *Bolivina subspinescens*

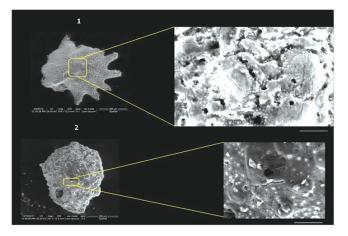


Fig. 5. 1. Shows the Environmental Scanning Electron photomicrographs of microboring, tunnelling and pitted surface *Calcarina spengleri*, 2. Shows the Environmental Scanning Electron photomicrographs of microboring, tunnelling and pitted surface *Calcarina spengleri*.

boring measured as 52 μ m and width 3 μ m (Fig 3.3). The tunnelling and boring features in *Bolivina subspinescens* are found as straight curved and they are randomly distributed. The length and width of the bores measured as 124 μ m to 240 μ m width of the boring is 8 μ m (Fig 4.2). In *Calcarina spengleri* boring features observed small, curved with dense distribution pattern. While the length of bores exhibit variation from 22 μ m to 107 μ m and the width maintain same as 1.8 μ m (Fig 5.1).

Surface pits are observed in the abnormal features were occurred in *Elphidium crispum, Spirolina arietinus, Peneroplis planatus, Quinqueloculina intricate, Siphogenerina striata Quinqueloculina intricate Calcarina spengleri* and

Peneroplis cylindraceus. Some pits measured as small as 25 im and found as circular in shape with ragged edges. Others looked like sink holes on the surface of the shells, and when they coalesced into large pockmarks up to the size of 100µm and appeared as crumbly. Appearance was evident. Pitting is

systems adapted by symbiotic algae to uptake of ${\rm CO}_{_2}$ during the process of photosynthesis.

Microbores, tunnels and pits in the perforate and imperforate symbiont —foraminifera serve the purpose of inward passage of CO₂ from the surrounding seawater with

Table 2. List of ultra structure such as microboring, tunnels and pitted surfaces observed through Environmental Scanning Electron Microscope (ESEM) in Miliolida and Hyaline (Rotalida and Buliminida)

Miliolid				Hyaline					
Species	Spirolina arietinus	Peneroplis planatus	Peneroplis cylindraceu	Quinqueloculina pulchella	Quinqueloculina intricata	Calcarina spengleri	Elphidium crispum	Siphogenerina striata	Bolivina subspinescens
Micro boring	✓	✓	✓	✓	✓	✓	✓	✓	✓
Tunnelling	✓	\checkmark	✓	✓	\checkmark	\checkmark	×	\checkmark	\checkmark
Pitted surface	✓	✓	✓	\checkmark	✓	✓	✓	✓	✓

^{✓-} Present × - Absent

Table 3. Percentage of the surface areas of miliolida and hyaline (Rotalida and Buliminida) affected by tunnels, microboring and pitted nature and un affected surface areas were measured and through Environmental Scanning Electron Microscope (ESEM) photographs of the respective species.

Miliolid				Hyaline					
Species	Spirolina arietinus	Peneroplis planatus	Peneroplis cylindraceu	Quinqueloculina pulchella	Quinqueloculina intricata	Calcarina spengleri	Elphidium crispum	Siphogenerina striata	Bolivina subspinescens
Non affected area	28%	64%	35%	58%	65%	73%	72%	56%	89%
Micro boring Tunnelling	16%	32%	19%	29%	8.5%	22%	0	20%	6%
Pitted surface									
	56%	4%	46%	13%	26.5%	5%	28%	24%	5%

often associated with the in combination of dissolution and giving a smooth polished look to the pits as abnormal features. Table 2 and 3 showed the surface areas excavated in the forms of pits, microboring and tunnels by symbiotic algae in the imperforate (Miliolida) and perforate (Hyaline) species. The percentage of surface area imperforate 46% unaffected, 54% affected by boring, pitted and tunnels. In perforate 74% of unaffected and 26% affected by boring, pitted and tunnels. From the study of ultra structures observed in the imperforate species the highest percentage of 54% of microboring, tunnels and pitted surface. Whereas the perforate species exhibit only26% affected surface area.

Discussion

Benthic foraminifers from the study area have harboured photosynthetic algae. Structural adaptations in the form of micro boring, pitted surface and tunnels in the shells of perforate and imperforate species are remnant route advent of symbiotic algae, with when the symbionts are active. It has been reported that imperforate foraminifera get additional supply of CO₂ for their symbiotic algae through aperture and direct uptake of CO₂ through the lateral test.

Carbonate microborer are found among filamentous or pseudo filamentous forms of both euhoutes (fungi, green and red algae). Micro boring towards the interior of the host is light dependent. Once the light compensation depth for photosynthesis is reached, boring either stopped and formed as pit or proceeds parallel to surface and produced as tunnel (Garcia-Pichel, 2006).

Benthic foraminifera, belonging to 11 families, can harbour photosynthetic cytobionts (Lee *et al.*, 1980), (Lee *et al.*, 1983) (Lee *et al.*, 1985a), (Lee *et al.*, 1985b), (Anderson, O. R. and Lee, 1991), (Saraswati, P. K. 2007). Mg/Ca compositional variation of planktonic foraminifera tests were used as proxy to determine the for past seawater temperature

(Anand *et al.*, 2003) , (Elderfield, 2000), (Hallock, 1981), (Lea *et al.*, 1999) , Nurnberg *et al.*, 1996). Still the experimental studies support that, Mg/Ca is more responsive to seawater temperature, pH, salinity and (CO_3^{-2}) concentration, but mounting evidences indicate biological control for strong influence on Mg/Ca composition of foraminiferal calcite (Erez, 2003).

The uptake of CO_2 from the surrounding seawater takes place through the thin lateral walls of symbiont bearing foraminifera, because CO_2 can pass through the very thin pitted walls. The lateral walls of symbiont bearing imperforate porcellaneous foraminifera are thinned with pits to facilitate CO_2 uptake from surrounding seawater for the symbiotic algae in their photosynthesis.

Activities of symbiotic algae is light dependent and hence, when light compensation is reached boring into the shell was stopped in the form of micro bores and diverted into the tunnels. These micro features in the foraminifer's were used for passage of CO₂ for photosynthesis from the surrounding seawater. From the study it is seen that higher percentage of pits micro bores and tunnels are found in imperforate than perforate species. Evaluation of surface areas of bores, pits and tunnels within shells of the host foraminifera of miliolids -imperforate and perforatehyaline group of species in the foraminifera by the intercellular symbiotic algae specify that 54%imperforate species are and only 26% are affected by micro bores, pitted and tunnels. The highest percentage 74% of surface area of perforate (hyaline) is unaffected where as the 46% of the surface area of the imperforate are unaffected.

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