DIVERSITY AND DISTRIBUTION OF BENTHIC FORAMINIFERA IN MANGROVE FORESTS OF PENANG ISLAND

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DIVERSITY AND DISTRIBUTION OF BENTHIC FORAMINIFERA IN MANGROVE FORESTS OF PENANG ISLAND

by

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LIST OF SYMBOLS AND ABBREVIATIONS

ºC	degree Celsius
%	percentage
<	less than
>	more than
±	plus-minus
µm	micron or micrometer
2-D	two dimensional
aff.	closely related
Cu	copper
cf.	compare
cm	centimeter
df	degree of freedom
E	East
e.g.	for example
GPS	global positioning system
g	gram
g/L	gram per liter
ha	hectare
km^2	square kilometer
m	meter
mL	milliliter
mm^3	cubic millimeter
N	North
$(NaPO_3)_6$	sodium hexametaphosphate

Pb	lead
psu	practical salinity unit
S	South
SSU rRNA	small subunit ribosomal ribonucleic acid
s	second
sp.	species
sp. nov.	new species
spp.	several species
var.	variety
Zn	zinc

LIST OF APPENDICES

GLOSSARY

actin	cytoplasmic protein producing in its polymerized form short, contractile fibers
agglutinated	shell texture characterized by components gathered in the ambient environment and bound by organic or biomineralized cements produced by the cell
agamont	specimen grown from the zygote, producing either gamonts or schizonts in an asexual process
anthropogenic	referring to environmental change caused or influenced by people, either directly or indirectly
aperture	the primary opening of the foraminiferal shell cavity towards the ambient environment
apertural face	that surface of the chamber-wall that contains the main cameral aperture
apertural plate	a plate-like structure that restricts the base of an interiomarginal aperture
areal	positioned within the apertural face, neither at its base nor at the shell margin
biconvex	test having both sides convex (in coiled forms)
bifid tooth	divided into two branches, usually in milioline arrangement
biserial	trochospiral chamber arrangement with about 180° between consecutive chambers, producing two rows of chambers
boss	structure like a knob
bulla	a blister-like test element extending over the umbilicus of the ultimate whorl and covering primary, main or supplementary apertures
calcareous	test containing calcium carbonate
canaliculate	a structural pattern produced by repeated modes of canal disposition in the foraminiferal shell
chamber	the space and its enclosing biomineralized walls formed at one instar
chamber arrangement	the pattern of disposition of chambers in a shell

concave	having an outline or surface that curves inwards like the interior of a circle or sphere
cosmopolitan	occurring all over the world wherever there is a suitable habitat
cribrate	perforated by multiple holes, exclusively for numerous and small multiple apertures
crescentic	thin, curved shape that thicker in the middle and tapers to thin points at each end
discoidal	having a flat circular shape
dorsal	the side of a free, flattened organism turned away from its substrate
ecophenotypes	a modification of the phenotype of an organism, resulting from environmental influences, that is not heritable
ectoplasm	the microtubular outer zone of cytoplasm, that also forms the pseudopodia
elliptical	shaped like an ellipse
endemic	occurring in restricted geographic areas
endoplasm	the central part of protoplasm containing the nucleus or nuclei and in which the major metabolic processes take place
evolute	in spirally coiled foraminifera where due to chamber shape, the chamber lumina of a coil do not cover laterally those of the preceding coil
extraumbilical	an aperture in the primary chamber of coiled shells situated at the interior margin but not connected with the umbilicus
gamont	a specimen producing gametes in the process of reproduction irrespective of its involvement in meiosis (diploid gamonts) or not (haploid gamonts)
granular texture	appearance under crossed nicols of a fragment of the test wall showing minutely granulated crystals with irregularly shaped sutures
holotype	the single specimen showing all characteristics considered relevant to its identification and segregation at the time of its designation
hyaline	glassy and translucent in appearance
imperforate	lacking pores

infaunal	aquatic animals that live in the substrate of a body of water and which are especially common in soft sediments
inflate	increase in size or area
interiomarginal	aperture situated at suture between the distal wall and preceding coil
involute	in spirally coiled forms where due to chamber shape, the lumina of the chambers in one coil cover laterally those of the preceding coil
keel	a peripheral thickening of the shell
lateral	side view
limbate	a descriptive term for the thickened, more, or less prominent sutures on the test surface at the boundaries of chambers
lip	an everted, in lamellar forms imperforate extension of the chamber wall along an elongate cameral aperture
morphospecies	species distinguished from others only by its morphology
microgranular	appearance of walls composed of calcareous elements under the optical microscope, more stable and resistant to diagenetic recrystallization than porcelaneous walls
milioline	bilocular coils where all terminal apertures are positioned on one common axis (apertural axis), axis of coiling is normal to the apertural axis and is rotated so that several discrete angles exist between the median planes of consecutive chambers
non-indigenous	not produced, growing, living, or occurring naturally in a particular region or environment
ornaments, ornamental	the patterns formed at the shell surface that are generated by a regular modification of wall textures of the thickness portions of the wall that generates ribs, beads, papillae, pustules, or pseudospines
paralic	interfingered marine and continental deposits and environments found on the landward side of a coast, including alluvial, lagoonal, littoral, mangroves, and marshes
perforate	referring usually to walls possessing true pores
planispiral	chambers arranged in whorls where the net rate of movement along the growth axis to the net rate of movement away from the axis is zero, the spiral and umbilical sides of the test are identical and symmetrical

plugs	an expanding pile of thickened lamellae in axial position in an umbilicus or in an umbilical bowl
porcelaneous	composed of optically cryptocrystalline lathes and rods or needles of calcite produced in Golgi vesicles within the protoplast and transported through the cell wall by exocytosis to the site of wall construction
pores	a minute tubular perforation traversing a lamellar chamber wall, coated internally by an organic sheathe
proloculus	the initial chamber of a foraminiferal test without nepionic differentiation
pseudopodia	semipermanent or temporary extrathalamous ectoplasmic projections
pustules	a hemispherical to subconical inflational protuberance of the outer lamella
quinqueloculine	type of milioline coiling where discrete angles exist between the median planes of consecutive chambers is 72°
radiate	a single aperture, in terminal or margino-terminal position with radially directed, slit like, or pointed extensions
rim	the thickened margin of an aperture
spicules	a slender pointed usually hard body especially one of the minute calcareous or siliceous bodies that support the tissue of various invertebrate
spines	a calcite rod normal to the test surface, thin, round, triangular to triradiate in section, running through a hole in the outer lamella
spiral side	the side of the test in trochospiral forms which contains the proloculus
striae	a linear mark, slight ridge, or groove on a surface, often one of a number of similar parallel features
sutures	the line of adhesion of chamber wall to the previously formed test
symbionts	an organism living in symbiosis with another
taphonomic	the processes (such as burial, decay, and preservation) that affect animal and plant remains as they become fossilized
terminal	positioned at the distal end of a linear structure or of an elongate chamber

trochospiral	chamber arrangement in whorls or coils where the net rate of movement along the growth axis to the net rate of movement away from the axis is more than zero, spiral and umbilical sides are dissimilar, may be involute or evolute on either the spiral or the umbilical side
umbilical	a primary aperture of a chamber leading into an umbilicus
umbilical plugs	a pile of lamellae forming a solid, more, or less free-standing plug in the center of the umbilicus, often separated from foliar tips by a spiral fissure
umbilical side	in trochospiral tests, the side opposite to the spiral one
ventral side	the front, or lower side of an organism
whorl	coiling, the side of a flattened organism turned to its substrate

KEPELBAGAIAN DAN TABURAN FORAMINIFERA BENTIK DI HUTAN PAYA BAKAU PULAU PINANG

ABSTRAK

Foraminifera adalah salah satu daripada pelbagai kumpulan organisma yang menghuni persekitaran bakau, namun kajian tempatan mengenai foraminifera bentik adalah terhad. Kajian ini telah dijalankan bagi mengenal pasti kepelbagaian dan corak taburan spesies foraminifera di tiga hutan bakau di barat daya Pulau Pinang iaitu Teluk Tempoyak, Pulau Betong, dan Kuala Sungai Pinang. Sejumlah 648 sampel (N = 18 stesen × 3 kawasan bakau × 12 bulan) di permukaan sedimen dengan kedalaman 1 cm telah diambil secara rawak menggunakan penyodok semasa air pasang terendah. Pensampelan telah dijalankan secara bulanan dari bulan Mac 2017 hingga Februari 2018. Hasil kajian telah menunjukkan kepelbagaian species adalah rendah hingga sederhana (Index *H'*: 0-1.4), sesuatu yang biasanya bagi persekitaran bakau. Sebanyak 29 spesies foraminifera bentik telah dikenal pasti, yang didominasi oleh jenis cengkerang aglutinat dengan gabungan jenis hialin dan porselin. Foraminifera bentik di hutan bakau Pulau Betong telah mencatatkan kepelbagaian spesies tertinggi (29 spesies), diikuti bakau Teluk Tempoyak (25 spesies) dan bakau Kuala Sungai Pinang (19 spesies). Species yang telah ditemui merupakan species yang lazimnya ditemui di kawasan paya bakau dan juga di persekitaran persisir laut. Taburan species telah menunjukkan variasi yang signifikan antara faktor lokasi, zon, dan musim yang berlainan di tiga hutan bakau ($p < 0.05$). Analisis komponen utama telah menunjukkan kandungan bahan organik dan ukuran partikel zarah sebagai parameter utama yang mempengaruhi taburan spesies foraminifera bentik. Analisis kesepadanan kanonikal telah menunjukkan tindak balas positif zarah berpasir terhadap kelimpahan tinggi genus jenis hialin berkapur, *Ammonia* dan *Elphidium* di zon antara pasang surut rendah hingga pertengahan. Manakala, genus jenis aglutinat, *Ammotium*, *Trochammina*, dan *Miliammina* banyak terdapat dalam sedimen dengan kandungan bahan organik yang tinggi serta substrat berlumpur di zon antara pasang surut atas. Analisis kluster hierarki telah mengelaskan spesies penunjuk kepada tiga kumpulan yang merujuk kepada tahap kurungan yang berbeza. Indeks kurungan (I_C) telah dikira berdasarkan spesies penunjuk terpilih yang mencatatkan nilai antara 0 hingga 0.4 di hutan bakau Teuk Tempoyak dan Pulau Betong, sementara hutan bakau Kuala Sungai Pinang merekodkan nilai antara 0 hingga 1. Interpolasi spasial nilai $\mathrm{I_C}$ telah menunjukkan aliran yang meningkat dari zon antara pasang surut yang rendah ke zon antara pasang surut atas. Julat nilai I_C telah menunjukkan bahawa hutan bakau Kuala Sungai Pinang adalah persekitaran yang paling tinggi tekanan persekitaran. Jumlah bilangan individu foraminifera, dominasi cengkerang aglutinat dan assosiasi spesies tertentu menunjukkan keadaan tekanan persekitaran yang disebabkan oleh faktor setempat yang boleh dijadikan sebagai data asas untuk aktiviti pemantauan dan pengurusan biodiversiti hutan bakau di masa hadapan, khususnya di Pulau Pinang.

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ABSTRACT

Foraminifera is one of the diverse groups of organisms inhabiting mangrove environment, but local studies regarding benthic foraminifera are limited. Therefore, this study was conducted to examine the diversity and distribution patterns of the foraminifera species in mangrove forests around Penang Island, which were Teluk Tempoyak, Pulau Betong, and Kuala Sungai Pinang. A total of 648 surface sediment samples (N=18 points × 3 mangrove forests × 12 months) of 1 cm thick were randomly collected using scoops during the lowest spring tide. The sampling was conducted monthly from March 2017 to February 2018. The results showed low to moderate species diversity (H' index: 0-1.4), typical of a mangrove environment. Overall, 29 benthic foraminifera species were identified, predominantly agglutinated with some combination of hyaline and porcelaneous tests. Benthic foraminifera in Pulau Betong mangrove recorded the highest species diversity (29 species), followed by Teluk Tempoyak (25 species) and Kuala Sungai Pinang (19 species). The species idetified are commonly found in mangrove swamps and in the coastal environment. Species distribution demonstrated significant variation among the different locations, zones, and seasonal factors in the three mangrove forests ($p < 0.05$). Principal components analysis showed that organic matter content and particle size type were the main parameters that influenced the distribution of benthic foraminifera species. Canonical correspondence analysis showed positive respond of sandy particles to the high abundance of calcareous hyaline test genera, *Ammonia* and *Elphidium* at the lower to middle intertidal zones. Meanwhile, the agglutinated test genera, *Ammotium*, *Trochammina*, and *Miliammina* were highly abundant in sediments of high organic matter content with silty-muddy substrate in the upper intertidal zone. Hierarchical cluster analysis grouped the indicator species into three types of assemblages which demonstrated different levels of confinement. Index of confinement (I_C) calculated based on selected indicator species recorded values between 0. to 0.4 in both Teluk Tempoyak and Pulau Betong mangroves, while Kuala Sungai Pinang mangrove recorded I_C ranged from 0 to 1. Spatial interpolation of I_C value showed an increasing trend from lower intertidal to the upper intertidal zones. The range of I_C values indicated that Kuala Sungai Pinang mangrove was the most stressful environment followed by Teluk Tempoyak and Pulau Betong mangroves. Foraminifera total number of individuals, the dominance of agglutinated tests and the specific association of species reflect the stress condition caused by local factors that could be served as baseline data for future biodiversity monitoring and management of mangrove forests, particularly in Penang Island.

CHAPTER 1

INTRODUCTION

Foraminifera are among widely studied single-celled (protists) organisms with widely distribution in all marine environments, including marginal (e.g. mangrove, lagoons, salt marshes and estuaries), coastal as well as deep-sea environments (Murray, 2006). They existed since the beginning of Cambrian era and evolved until now, making them as good proxy for tracing evolutionary history of life on earth (Beck Eichler & Barker, 2020). Foraminifera typically produces shell, which commonly refers to as ‘test’ because protoplasm covered the exterior of the test (Debenay, 2012). This unique test preserved well in the sediments making them as suitable index fossil used by geologist for oil exploration (Bhargava Sharma et al., 2018). Foraminifera are relatively high in abundance, and they quickly responds to environmental changes, making them as an excellent tool to reconstruct past environmental conditions (Gooday, 2003).

Foraminifera are divided into two types based on their lifestyle, benthic and planktic. Benthic foraminifera occupy most of benthic habitat while planktic foraminifera restricted to open ocean. This organisms have been used as bioindicator to monitor environmental changes in various ecosystems because they are easy to collect, and only small volume are needed for statistically significant analyses.

Studies on benthic foraminiferal assemblages from low-latitude intertidal mangrove wetlands have been conducted to gain information on the past ecological conditions and on the impact of human-induced changes towards marine ecosystem (Vilela et al., 2011; Moriaki et al., 2012; Culver et al., 2013). Relative abundance of benthic foraminifera assemblages forms vertical zones along the salt marsh gradient have been reported to correlate with tidal levels (Edwards & Horton, 2000; Edwards et al., 2004; Kemp et al., 2011; Strachan et al., 2015). The benthic foraminiferal assemblages have also been studied over the surface of salt marshes to relate the faunal occurrences with ecological parameters (Scott et al., 2004; Murray, 2006; Strachan et al., 2015). Benthic foraminifera have been developed as a tool for environmental monitoring in contemporary contaminated and polluted environments (Frontalini et al., 2009; Frontalini & Coccioni, 2011; Foster et al., 2012; Debenay et al., 2015). The effect of riverine influx-dominated ecological parameters has been investigated using living assemblages of benthic foraminifera which reflected the influence of dissolve oxygen and organic carbon control on the distribution (Suokhrie et al., 2021). Dubois et al. (2021) and Bouchet et al. (2020) have applied benthic foraminifera as bioindicator to monitor the ecological impact of aquaculture farms which revealed that opportunistic and tolerant species predominated near the farms, while sensitive species were found further away.

Since the early 2000s, there has been a lot of interest in study on benthic foraminifera studies in Malaysia, with most of the research focusing on coastal waters (Faiz et al., 2017; Culver et al., 2019; Minhat et al., 2020, 2021; A’ziz et al., 2021). Despite their significant advantages as bioindicators, some environment such as mangrove are less studied. Identifying the abundance and distribution of foraminifera is essential to perceive current condition of mangrove ecosystem. Therefore, the occurrence and knowledge of foraminifera in mangrove areas and their interaction with the surrounding environment is required.

Globally, the mangrove distribution majorly populated between the latitudes of 30° N and S (Tomlinson, 1986). The extent of mangrove forests in Peninsular Malaysia as of 2017 was estimated to be 1.09×10^5 ha (Omar et al., 2018). Mangrove forests in Penang Island have rapidly declined from 1.7×10^3 ha in 2012 to only 680 ha in 2015, mainly due to construction developments and reclamation projects beginning in 1960 (Chee et al., 2017; Satyanarayana et al., 2018). Some of the mangroves were invaded with aquaculture ponds, especially in Pulau Betong and Kuala Sungai Pinang while mangrove patches in Batu Maung were cleared and transformed into a dumping site (World Rainforest Movement, 2002; NST, 2019, 2018). The rest of the island's mangrove areas grew in small patches, such as in Teluk Tempoyak, except mangroves in Balik Pulau.

1.1 Problem Statements

Rapid urban and infrastructure development, such as beach resorts, industry, and aquaculture ponds, resulted in the destruction of mangrove ecosystems (Jusoff & Taha, 2008). The destruction of mangrove ecosystem increases potential serious threats to human safety along the shoreline, including erosion, flooding, storm waves, and surges. Mangrove loss will also cause a decline in coastal water quality, reduce biodiversity, disintegrate fish nursery habitat, and fish catches, adversely affect adjacent coastal habitats (Mumby et al., 2004) and eliminate a significant resource for human communities that usually rely on mangroves for numerous products and services (Gilman et al., 2006). Despite these addressed issues on Penang Island's mangrove forests, less attention was given to the effect on biodiversity of organisms in this area, such as benthic foraminifera. Therefore, a baseline record of population diversity were essential to monitor ecological changes.

Since previous studies have identified most common benthic foraminifera from coastal waters, limited studies were carried out in mangroves. Since the fact that benthic foraminifera consist of numerous ecophenotyes, it is important to study the ecological characteristics of their surroundings. Several studies on ecological characteristics of mangroves foraminifera have been carried out worldwide, however only limited investigations have been conducted in Malaysia, especially in Penang Island. There were only two studies performed on mangrove benthic foraminifera assemblages in Penang Island (Hawkes et al., 2007; Shah Rijal, 2017). Both studies have been carried out in a short period of time and the taxonomic identification of the species recovered were not described in detail, thus, making some of the species reported have invalid scientific name. For example, the genera *Ammonia* and *Elphidium* exhibit high morphological variation due to environmental variability (usually temperature and salinity) of their niches (ecophenotypes), thus the tendency of misidentification resulted in giving an invalid species identity occurred (Hayward & Hollis, 1994; Melis & Violanti, 2006; Mina et al., 2013; Camacho et al., 2015). The problem has persisted across the globe in various types of environments (Hayward et al., 1994; Barbosa & Suguio, 1999; Pawlowski & Holzmann, 2014).

1.2 Objectives

The main aim of the present study was to determine the distribution of benthic foraminifera in three mangrove forests in Penang Island. The specific objectives were:

1. To identify benthic foraminifera species in mangrove sediment based on their morphology and to describe the population diversity.

2. To determine the environmental parameters that influence the distribution patterns of benthic foraminifera in the mangrove ecosystem.

3. To determine indicator species of benthic foraminifera in mangrove forest and their implication for environmental stress.

CHAPTER 2

LITERATURE REVIEW

2.1 Biology of Foraminifera

2.1.1 Structure of cell

Foraminifera are widely distributed single-celled protists in marine environments, from intertidal areas to deep seas and from polar to tropical climate. Foraminifera cell consists of soft tissue (cytoplasm) that primarily enclosed in a test (shell). The test is the main characteristics used by many taxonomists to identify foraminifera species. The test made up of secreted organic matter (tectin), secreted minerals (e.g. calcite, aragonite, or silica) or arenaceous (agglutinated) particles (Murray, 2006).

Benthic foraminifera are grouped based on their size. The larger foraminifera commonly exceed 3 mm^3 in volume, have intricate internal morphologies which requires observation through thin sections. Smaller type (<3 mm^3) benthic foraminifera is identified based on their simple external structures (BouDagher-Fadel, 2008).

Two types of cytoplasm are present in foraminifera cell, endoplasm, and ectoplasm (Figure 2.1A). In multi-chambered planktic foraminifera, the ectoplasm forms bubble capsule (Figure 2.1B). The ectoplasm is also known as the network of pseudopodia, known as reticulopodia (Murray, 2006). The structure is fine, thread-like and has a granular texture when viewed under a light microscope, which is distinct from other protozoan groups (Lee, 1990; Tendal, 1990; Bowser, 2002). Other protozoa does not have the granular texture and the reticulopodia disintegrated readily due to ocean currents. Consequently, some species of foraminifera utilized spines or elongated particles consists of sponge spicules to support the structure (Murray, 2006).

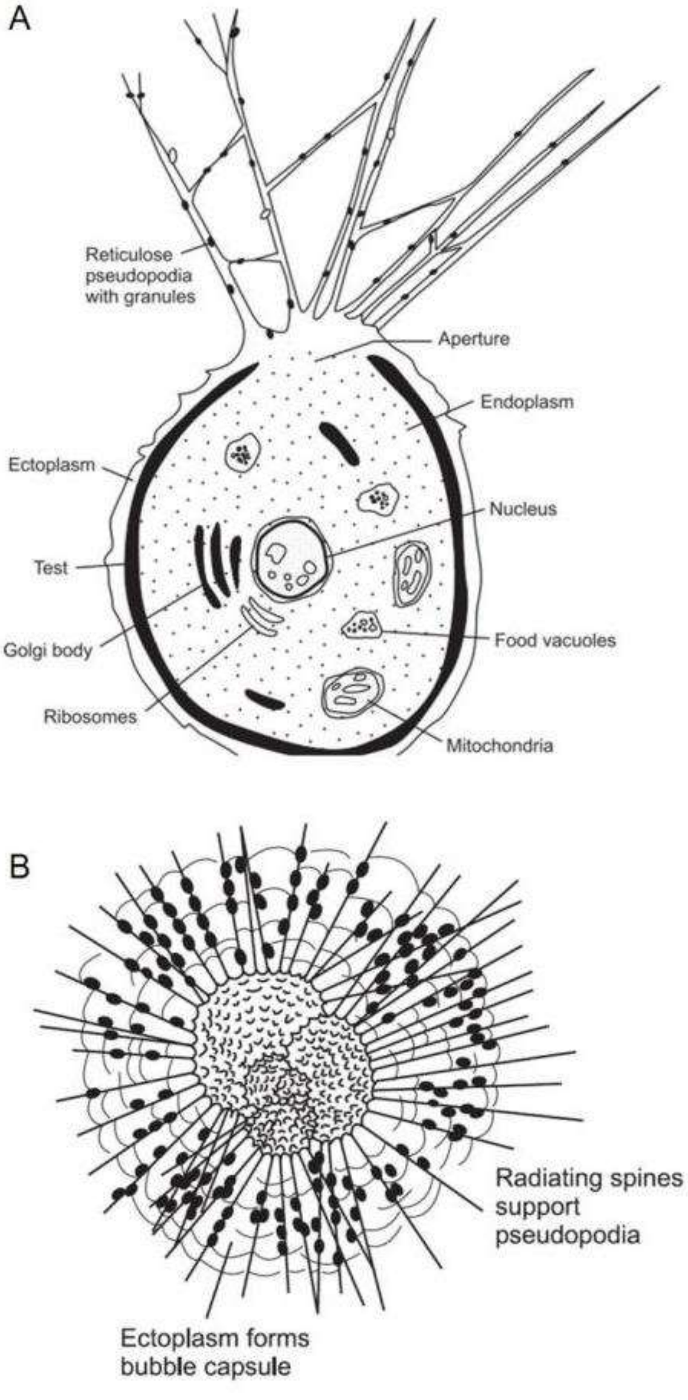

Figure 2.1: (A) Cross-section of a living single-chambered foraminifera as seen with transmitted light. (B) Multi-chambered planktic foraminifera showing radiating spines and pseudopodia. Source: Armstrong & Brasier (2004)

The branches of reticulopodia are utilised for movement, anchoring to the substrate and to capture food, such as diatoms or bacteria (Hemleben et al., 1989; Armstrong & Brasier, 2004) (Figure 2.1). The reticulopodia movement consists of extension, bending and withdrawal, which respond to environmental stimulation such as chemical substance (xenobiotics), adhesivity of the substrate and tonicity (Martin, 2000; Travis et al., 2002; Murray, 2006). Reticulopodia have shown to flow inward when introduced to a hypertonic seawater (Martin, 2000). Researchers have utilized fluorescent imaging techniques to monitor real time movements of reticulopodia and their cytological alteration under stressful conditions (Frontalini et al., 2019; Golen et al., 2019; Tyszka et al., 2019). The shell (test) formation through controlling morphogenesis and biomineralization of benthic foraminifera are controlled by dedicated F-actin-driven pseudopodial structures that associated with microtubular structures (Frontalini et al., 2019; Tyszka et al., 2019).

2.1.2 Habitat

Foraminifera are divided into two types based on their mode of life; planktic and benthic. Benthic foraminifera are distributed from shallow intertidal areas to the deep seas, while planktic forms are abundant in the water column of open seas at various depths. A ‘microhabitat’ is referred to as smaller part of the corresponding habitat, constitute of an integration of physical, chemical, and biological properties. This microhabitat is found at the interface between sediment and water. Infaunal foraminifera are benthic foraminifera that live at the bottom of the water column and epifaunal, lives at the upper layer of the sediment (Murray, 2006).

Corliss (1991) divided benthic foraminifera from Atlantic Ocean into epifaunal, shallow epifaunal, intermediate infaunal and deep infaunal based on their vertical distribution. The composition of species in each distribution are indicated in Table 2.1.

Table 2.1: Vertical distribution of benthic foraminifera

	Description	**Species**
Epifaunal	Taxa at the top centimeter of the substrate	*Cibicides lobatulus*, *Cibicides* sp., *Bulimina* spp., *Cibicidoides kullenbergi, Cibicidoides* sp., *Discorbis williamsoni*, *Hoeglundina elegans*, *Planulina* sp., *Planulina wuellerstorfi*, Miliolid
Shallow epifaunal	Taxa that is confined to the 0-2 cm interval	*Brizalina* sp., *Lenticulina* sp., *Pseudonodosaria* sp., *Pullenia bulloides, Robertina bradyi*, *Trifarina* sp., *Uvigerina peregrina*
Intermediate infaunal	Taxa that is found between 1-4 cm	*Cibicidoides bradyi*, *Melonis barleeanum, Pullenia quinqueloba, Pullenia simplex*
Deep infaunal	Taxa that lives within subsurface population maximum below 4 cm	*Chilostomella oolina*, *Florilus labradorica, Fursenkoina* sp., *Globobulimina affinis*, *Nonion grateloupi*, *Nonionella turgida*

Source: Corliss (1991)

Sen Gupta (2003) stated that benthic foraminifera vertical distribution by Corliss (1991) was arbitrary as the species was arranged according to tests morphology. Epifaunal taxa are characterised with (a) plano-convex, biconvex, and rounded trochospiral, lacking large pores or only present on one side and (b) miliolids, while infaunal taxa have rounded planispiral, triserial, spherical, flattened biserial with pores all over the test (Corlis & Chen, 1988; Corliss, 1991). Moreover, the infaunal species are known to inhabit various depth in the sediments and may be subjected to short-term variations of their movements (Barmawidjaja et al., 1992; Linke & Lutze, 1993).

Although the morphology of the test may explain the vertical distribution of foraminifera, Buzas et al. (1993) pointed that vertical distribution based on test morphology only elucidate about 75%, with most of the studies were based on the assemblages in the deep sea. Several studies suggested that all species in mangrove sediments should be considered as infaunal as the sediments are soft particularly the muddy content (Buzas et al., 1993; Jorrisen et al., 1995; Berkeley et al., 2007; Culver et al., 2013).

Studies on foraminifera microhabitat indicated the most vital controlling factors for benthic foraminifera vertical distribution pattern are bottom-water oxygenation, food availability, competition and predation, and bioturbation (Barmawidjaja et al., 1992; Linke & Lutze, 1992; Buzas et al., 1993; Goldstein et al., 1995; Jorissen et al., 1995; Licari et al., 2003; Sen Gupta, 2003; Duchemin et al., 2005; Fontanier et al., 2006; Mojtahid et al., 2010; Dessandier et al., 2016; Koho et al., 2017). Jorrisen et al. (1995) utilised these controlling factors to construct the TROX model, which defines foraminiferal microhabitat depth because of the negative interplay between oxygen and food availability in deep-water environments (Figure 2.2).

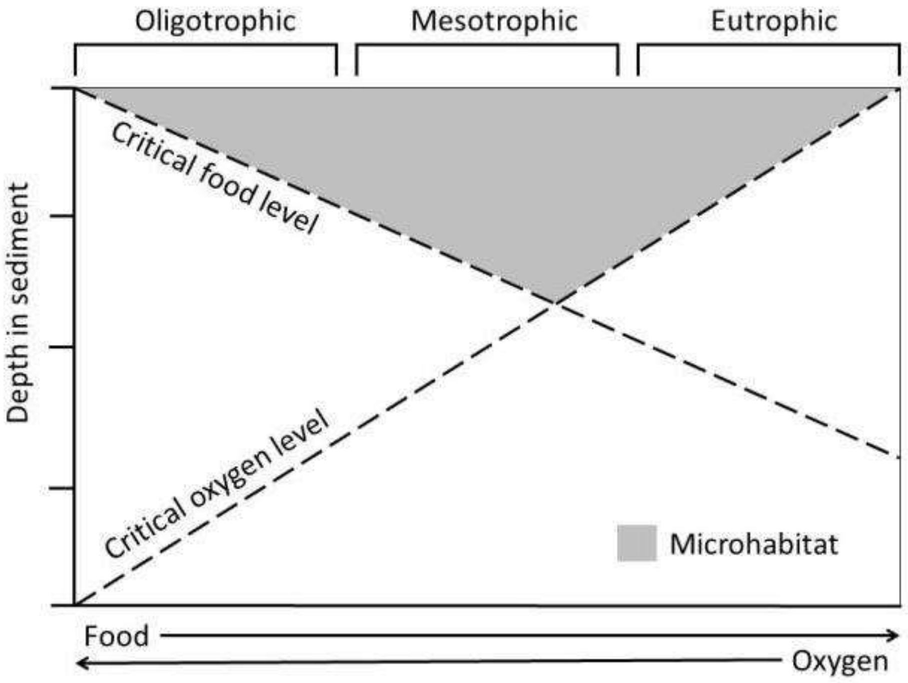

Figure 2.2: TROX model of benthic foraminifera microhabitats. Source: Berkeley et al. (2007)

The model suggests that the depth of benthic foraminifera species within the sediment can be determined by the availability of oxygen and food, especially in oligotrophic (low nutrient, high oxygen level) conditions. Thus, food availability become the limiting factors in deeper layer of sediments. As organic matter accumulates (mesotrophic), some of the materials are transported into deeper layers through bioturbation, supplying appropriate nourishment for infaunal population. Nevertheless, with an increase in organic flux (eutrophic), oxygen continues to be absorbed efficiently than bioturbation and diffusion. Thus, this condition contribute to oxygen as limiting factor. In extreme situation where all oxygen rapidly absorbed at the surface layer, the living foraminifera confined at the uppermost surficial layers (Jorissen et al., 1995).

A study by Dejardin et al. (2018) found that the epifaunal taxa calcify at the sediment-seawater interface with organic carbon accessibility. In contrast, the infaunal taxa can only calcify when there is a large concentration of organic carbon at the bottom. This condition might initiate the migration of the taxa to the sediment-seawater interface for feeding activities.

2.2 Classification of Foraminifera

The main method of foraminifera classification is based on morphological observation particularly test types. Main morphological characteristics that are used for classification are chambers arrangement, position of apertures and ornaments. Since the beginning of foraminifera classification system, species were initially assigned based on the plan of growth which are the chambers arrangements. Later, wall texture became another character to distinguish species of foraminifera. When new species are described, the species are named based on the name-bearing type specimen. Using the main morphological characteristics, it is highly possible to correctly classify foraminifera to genus level (WoRMS, 2019).

Growing interest of foraminiferal research worldwide has resulted in an increasing number of foraminiferal taxonomic system. However, the large number of species names listed may interpret as highly confusing. Murray (2007) estimated about 10 to 25% of modern benthic foraminiferal species names to be synonyms. Nowadays, in addition to morphology, molecular methods have been applied using specific markers to classify foraminifera. Molecular method is required mainly to identify species at higher taxonomic level particularly subspecies or varieties, for example the genera *Ammonia* and *Elphidium* (Hayward et al., 2004; Schweizer, 2011). These two species have been subject of long-standing controversies of species' naming (Schweizer, 2011). More than 40 morphospecies of *Ammonia* and 60 for *Elphidium* were reported however only a low number of species have been sequenced.

2.2.1 Classification based on morphology

2.2.1(a) Past classification

Foraminifera was first described as cephalopod by Linnaeus (1766) until taxonomic work by d'Orbigny (1826) that classified this organism as a distinct group from cephalopod and established the order Foraminifera (as described by Cifelli, 1990). According to Cifelli (1990), the taxonomic work by d'Orbigny (1826) was based on single character which was plan of growth as it was the most visible features. d'Orbigny (1826) classified foraminifera into five families, Stichostègues, Enallostègues, Helicostègues, Agathistègues and Entomostègues. The classification was later changed from families to orders and another two orders were added, Monostègues and Cyclostègues (as described by Cifelli, 1990).

During earlier study of foraminifera, most scientists regarded foraminifera diversity as representation of variation (Cifelli, 1990). After the discovery of foraminifera as protozoa by Dujardin in 1835, studies of foraminifera especially their classification has gained attention during 19th to 20th century (Cifelli, 1990; Sen Gupta, 2003). As stated by Cifelli (1990) and Sen Gupta (2003), most of the important foraminiferal systematics and classification have been studied by researchers in Europe and America but the commonly accepted classification was by Brady (1884) and Cushman (1928).

According to Cifelli (1990), foraminiferal classification by Brady (1884) was based on the collection of foraminifera taken from H. M. S. *Challenger* scientific expedition (1872-1876). The classification contained ten families with 29 subfamilies that were arranged in numerical order, from the most primitive (Gromidae) to the most advanced form (Numulinidae). Brady’s classification focused on dimorphism phenomenon and morphological comparison of each taxon, particularly between fossil and modern specimens. Brady’s classification was thought to be two-dimensional as the classification did not consider the stratigraphic disjunction between genera. Therefore, the classification was irrelevant according to evolutionary perspective.

Based on a review by Cifelli (1990), evolutionary regulation in foraminiferal classification was proposed by Cushman (1905) which divided foraminifera into stages of development; embryonic, nepionic, neanic and ephebic. Evolutionary regulation was previously applied to metazoans of which phylogenetic relationships between genera were done by comparing early and adult stages. However, the classification system was not able to provide evidence between stratigraphic and phylogenetic relationships. Thus, the classification was disputed by many foraminiferal researchers (Cifelli, 1990). Nevertheless, Cushman’s idea has recognized the important aspect of early ontogenesis stage (development from time of fertilization) for species classification.

The classification of Cushman was later revised in 1928 and documented 404 genera of foraminifera, grouped into 45 families. The test material was suggested as an essential character by Cushman and for classification, the test material must be given priority. The classification also takes into account the type specimens and type materials, as well as complying to the Rules of Zoological Nomenclature (Cifelli, 1990). Cushman’s classification was applied to classify foraminifera in subsurface stratigraphy during the period of oil exploration. Previous higher classification of foraminifera based on morphology is listed in Table 2.2.

Table 2.2: Previous higher classification of foraminifera

d'Orbigny (1852)	Brady (1884)	Cushman (1928)
Orders	Families	Families
Monostègues	Gromidae	Allogromiidae
Cyclostègues	Miliolidae	Astrorhizidae
Stichostègues	Astrorhizidae	Rhizaminidae
Helicostègues	Lituolidae	Saccamminidae
Entomostègues	Textularidae	Hypermminidae
Enallostègues	Chilostomellidae	Reophacidae
Agathistègues	Lagenidae	Ammodiscidae
Suborder	Globigerinidae	Lituolidae
Aequilateralidae	Rotalidae	Textulariidae
Inaequilateralidae	Nummulinidae	Verneuilinidae
Nautiloidae	Subfamilies	Valvulinidae
Turbinoidae	Nubecularia	Fusulinidae
Asteriginidae	Miliolininae	Loftusiidae
Polymorphinidae	Hauerininae	Neusinidae
Textularidae	Peneroplidinae	Silicinidae
Miliolidae	Alveolininae	Miliolidae
Multiloculidae	Keramosphaerinae	Opthalmidiidae
	Astrorhizinae	Fischerinidae
	Pilulininae	Trochamminidae
	Saccammininae	Placopsilinidae
	Rhabdammininae	Orbitolinidae
	Lituolinae	Lagenidae
	Trochamminae	Polymorphinidae
	Endothryrinae	Nonionidae
	Loftusinae	Camerinidae
	Textularinae	Peneroplidae
	Bulimininae	Alveolinellidae
	Cassidulininae	Keramosphaeridae
	Lageninae	Heterohelicidae
	Nodosarinae	Hantkeninidae
	Polymorphininae	Buliminidae
	Ramulininae	Ellipsoidinidae
	Spirillininae	Rotaliidae
	Rotalinae	Amphisteginidae
	Tinoporinae	Calcarinidae
	Fusulininae	Cymbaloporettidae
	Polystomellinae	Cassidulinidae
	Nummulitinae	Chilostomeliidae
	Cycloclypeinae	Globigerinidae
	Eozooninae	Globorotaliidae
		Anomalinidae
		Planorbulinidae
		Rupertiidae
		Homotremidae
		Orbitoididae

Source: Compiled from Cifelli (1990)

2.2.1(b) Modern classification

By the late 20th century, another major classification of foraminifera was introduced by Loeblich & Tappan (1988) and remained as the foundation in most taxonomic studies of foraminifera until today. The classification divided Foraminifera into 12 suborders, which differed mainly in their test wall mineralogy and ultrastructure (Figure 2.3). A total of 3620 valid proposed taxa and 2455 genera were identified in this classification. Although new classifications were created after the introduction of the classification by Loeblich & Tappan (1988), the foundation remained unchanged.

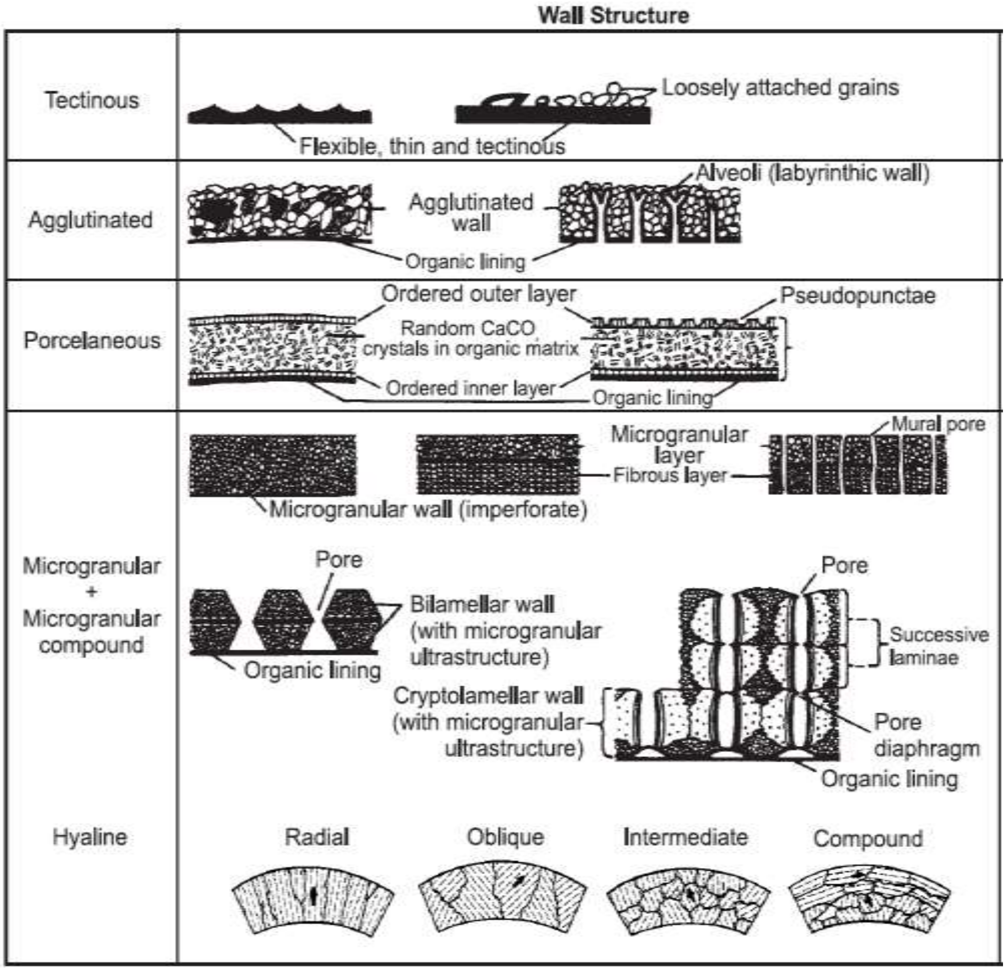

Figure 2.3: Summary of foraminifera wall structures based on scanning electron microscopy analyses. Source: Armstrong & Brasier (2004)

Sen Gupta (1999) and Mikhalevich (2004) made some modifications to the classification system by Loeblich & Tappan (1988), mainly on the ranking level of orders and classes. Kaminski (2004) modified the classification, specifically for the agglutinated group of foraminifera. The agglutinated classification was defined as a subclass composed of four orders which are determined by general morphology, wall architecture, and cement component (Kaminski, 2004). The cement used to hold the test together could be organic (Astrorhizida), canaliculate and calcareous (Textulariida), or organically cemented calcareous and microgranular types combined (Lituolida and Loftusiida). The modern classification of foraminifera based on morphology, categorized according to Orders/Suborders and Class is listed in Table 2.3.

Table 2.3: Modern classifications of foraminifera based on morphology

Loeblich & Tappan (1988)	Sen Gupta (1999)	Mikhalevich (2004)	Kaminski (2004)
Order	Orders	Order	Class
Foraminiferida	Allogromiida	Foraminifera	Foraminifera
Suborders	Astrorhizida	Classes	Subclass
Allogromiina	Lituolida	Astrorhizata	(agglutinated)
Textulariina	Trochamminida	Lagynana	Textulariia
Fusulinina	Textulariida	Astrorhizana	Orders
Involutinina	Fusulinida	Spirillinnata	Astrorhizida
Spirillinina	Miliolida	Ammodiscana	Lituolida
Carterinina	Carterinida	Spirillinana	Loftusiida
Miliolina	Spirillinida	Miliolata	Textulariida
Silicoloculinina	Lagenida	Miliamminana	
Lagenina	Rotaliida	Miliolana	
Robertinina	Buliminida	Nodosariata	
Globigerinina	Globigerinida	Hormosinana	
Rotaliina	Involutinida	Nodosariana	
	Robertinida	Rotaliata	
	Silicoloculinida	Textulariana	
		Rotaliana	
		Globigerinana	

Source: Modified after Pawlowski et al. (2013)

Generally, classification based on morphological description has been long accepted for species identification until now. Two main characteristics that are used for classification of foraminifera were plan of growth (chamber arrangement) and test structure. These characteristics were alternately given priority to classify foraminifera. Later, the foraminifera classification system was arranged according to phylogenetic relationships to accommodate evolutionary trends.

Modern classifications (Table 2.3) are often used for identification of foraminiferal species and have become the basis for foraminifera species deposited and described in several databases. One of the databases is World Foraminifera Database (WFD), developed as component of the World Register of Marine Species (WoRMS) to provide information on accepted foraminifera species identified based on morphological characters (Hayward et al., 2019). Another alternative database known as ‘foraminifera.eu Project’ was developed by Hesemann (2015) which is based on the classification by Mikhalevich (2004).

2.2.2 Classification using molecular approach

Previous classification of foraminifera based on morphological features has now changed due to the advancement of molecular studies. Researchers working on foraminiferal have used molecular approach, based on amplification of partial small subunit and large subunit ribosomal deoxyribonucleic acid (rDNA) sequences (Holzmann & Pawlowski, 2000; Bowser et al., 2006). This region is situated at the 3’ end of the SSU rRNA gene and the primer pairs, s14F3 (acgcamgtgtgaaacttg)-sB (tgatccttctgcaggttcacctac) are regarded as barcoding region of foraminifera (Pawlowski & Holzmann, 2014).

As a result, the latest classification of foraminifera was proposed on the basis of molecular data. Although some of the evolutionary relationships between major groups are confirmed, not all species were sequenced and a substantially larger taxonomic sample is necessary, particularly from the orders Textulariida, Miliolida and Robertinida (Pawlowski et al., 2013). Based on the molecular data, Pawlowski et al. (2013) suggested evolution of foraminifera is a shift from single-chambered to multi-chambered tests.

A new proposed classification of foraminifera was also erected to phylum status based on molecular data (Hayward et al., 2019; WoRMS, 2019). The phylum is then divided into three classes based on the criteria below:

(i) Monothalamea - single-chambered species with organic and agglutinated walls
(ii) Globothalamea - multi-chambered species with mostly globular chambers
(iii) Tubothalamea - multi-chambered species with mostly tubular chambers

The three classes were separated into 14 orders, Allogromiida, Astrorhizida, Xenophyophoroidea, Miliolida, Spirillinida, Rotaliida, Robertinida, Textulariida, Carterinida, Lagenida, Fusulinida, Involutinida, Lituolida, and Loftusiida. Only two classes, Miliolida and Textulariida remained unchanged from the initial classification of d'Orbigny (1852) to the latest classification. The present classification of foraminifera is as follows (Hayward et al., 2019):

Kingdom Chromista Cavalier-Smith, 1981
Subkingdom Harosa Cavalier-Smith, 2010
Infrakingdom Rhizaria Cavalier-Smith, 2002
Phylum Foraminifera d'Orbigny, 1826

Class Monothalamea Pawlowski et al., 2013

Order Allogromiida Fursenko, 1958

Order Astrorhizida Lankester, 1885

Order Xenophyophoroidea Tendal, 1972

Class Tubothalamea Pawlowski et al., 2013

Order Miliolida Delage & Herouard, 1896

Order Spirillinida Hohenegger & Piller, 1975

Class Globothalamea Pawlowski et al., 2013

Order Rotaliida Lankester, 1885

Order Robertinida Loeblich & Tappan, 1984

Order Textulariida Delage & Herouard, 1896

Order Carterinida Loeblich & Tappan, 1981

Order Lagenida Delage & Herouard, 1896

Order Fusulinida Wedekind, 1937

Order Involutinida Hohenegger & Piller, 1977

Order Lituolida Lankester, 1885

Order Loftusiida Kaminski & Mikhalevich, 2004

Based on DNA fragments at the 3' end of the Small Subunit Unit (SSU) rRNA gene to identify species of foraminifera, 'foramBarcoding Project' was created as a complementary identification system. This database is coordinated by Prof. Dr. Jan Pawlowski and Prof. Dr. Maria Holzmann from the Department of Genetics and Evolution, University of Geneva. A total of 125 species of foraminifera has been sequenced and deposited in the database (Pawlowski & Holzmann, 2014). Recently, metabarcoding technique was applied to study the interaction of species-specific feeding preferences and potential niche and resource partitioning among foraminiferal population (Chronopoulou et al., 2019; Cordier et al., 2019; Frontalini et al., 2020; He et al., 2020). The V9 hypervariable region of the 18S rRNA gene within benthic foraminiferal cells elucidate the in situ feeding behaviour of living foraminifera in intertidal environment. The result have concluded that *Ammonia* sp. can predate on metazoan classes, whereas *Elphidium* sp. and *Haynesina* sp. are more likely to ingest diatoms (Chronopoulou et al., 2019).

2.3 Ecology of benthic foraminifera

2.3.1 General distribution

In marginal marine environments (salt marshes, mangroves, lagoons, estuaries, deltas, and fjords), the environmental parameters are usually sensitive to changes. Due to these conditions, species diversity and abundance of benthic foraminifera are lower than in continental shelf and deep seas (Murray, 2006). Common genera found in marginal marine environments are usually smaller benthic type such as *Ammoastuta*, *Ammotium*, *Arenoparrella*, *Haplophragmoides*, *Entzia*, *Balticammina*, *Trochammina*, *Miliammina*, *Elphidium*, *Haynesina*, *Ammonia*, *Quinqueloculina*, *Spirillina*, *Cibicides*, *Cassidulina*, *Discorbis* and *Bulimina*.

Meanwhile, larger foraminifera are highly abundant in carbonate environments (coral reefs). This type of foraminifera occupies coral reefs with a unique adaptation to algal symbionts (Murray, 2006). Common genera are *Calcarina*, *Amphisorus*, *Amphistegina*, *Baculogypsina*, *Heterostegina*, *Neorotalia* and *Operculina*.

The continental shelves to deep-sea environments are generally characterised with a stable salinity, temperature, and oxygen flux (Murray, 2006). Common genera found in continental shelves are *Adercotryma*, *Bigenerina*, *Cribrostomoides*, *Eggerelloides*, *Reophax*, *Saccammina*, *Textularia*, *Trochammina*, *Cornuspira*, *Miliolinella*, *Quinqueloculina*, *Cancris*, *Buccella*, *Brizalina*, *Cibicides*, *Elphidium*, *Globobulimina*, *Fursenkoina*, and *Cassidulina*. Deep seas foraminifera including *Cyclammina*, *Karreriella*, *Sigmoilopsis*, *Cibicidoides*, *Epistominella*, *Melonis*, *Planulina*, *Uvigerina*, *Hanzawaia* and from Class Monothalamea (*Syringammina*, *Ocultammina*, *Edgertonia*) lives in abyssal plains and trenches. The general distribution of foraminifera according to their test types are summarised in Table 2.4.

Table 2.4: General distribution of foraminifera based on the test types

Type	Distribution
Proteinaceous	Found in freshwater as well as marine environments Known Order: Allogromiida
Agglutinated	Found in maritime habitats from shallow to deep water. Resistant to hypo-salinity and normal marine salinity (hypoxia or dysoxia). Known Orders: Astrorhizida and Lituolida
Calcareous	Found in marginal to deep water, and tolerant to hypo-salinity or hyper-salinity as well as normal marine salinity. Mostly characteristic of carbonate environments (back-reef lagoonal sub-environment). Known Orders: Miliolida, Nodosariida, Buliminida, Robertinida and Rotaliida
Planktic	Live in the water column and once dead, the tests sink to the seafloor. Highly tolerant to hyposalinity or hypersalinity as well as normal marine salinity. Occupy a range of depth environments within the water column. Also, harbour photosynthetic algal symbionts. Known Order: Globigerinida

Source: Modified after Jones (2013)

2.3.2 Environmental factors affecting benthic foraminifera distribution

Foraminifera dispersal patterns are regulated by environmental factors surrounding them. Consequently, the environmental factors will affect tolerances, preferences, abundance, and diversity of foraminifera. The main factors of foraminifera ecology are temperature, salinity level, dissolve oxygen content, substrate, and nutrients (Scott et al., 2004; Woodroffe et al., 2005; Murray, 2006; Horton & Culver, 2008; Kemp et al., 2011; Jones, 2013). Other important abiotic factors include calcium carbonate availability and pH for calcareous benthic and planktic types. Low carbonate condition have known to increase morphological abnormalities and inclusion of heavy metals in carbonate tests are possible evidence of environmental stress (Dejardin et al., 2018; Bergamina, et al., 2019; Guaman-Guevara et a; 2019; Humphreys et al, 2019). In contrast, larger foraminifera are influenced by harbour photo-symbionts, light availability, turbulence, and turbidity (Jones, 2013). Essential biotic or biological controls include food accessibility, predation, competitive interaction, and exclusion (Murray, 2006; Jones, 2013).

Salinity is recognized as one of the main factors in distribution of foraminifera. Foraminifera have been classified based on their tolerances and preferences to a range of salinity. Species of benthic foraminifera with narrow tolerances and preferences to salinity are known as stenohaline. Species of foraminifera that are known to live in this range of salinity including *Buliminella elegantissima*, *Bulimina elongata*, *Nonion depressulus*, *Fissurina lucida*, *Hanzawaia nipponica*, *Ammonia ketienziensis*, *Ammonia compressiuscula,* and *Elphidium advenum* (Alve & Murray, 2001; Li et al., 2015).

Species of foraminifera that tolerated a broad range of salinity are known as euryhaline (33-37 psu) (Murray, 2003; Jones, 2013). Some known euryhaline species are *Cribrononion subincertum*, *Elphidium hughesi*, *Helenina anderseni*, *Entzia macrescens*, *Miliammina fusca*, *Ammonia beccarii*, and *Ammonia tepida* (Alve & Murray, 2001; Cann et al., 2002; Melis & Violanti, 2006; Li et al., 2015). Foraminifera have also been found in extreme condition such as hydrothermal vent where species were dominated by agglutinated with organic cement tests and contained only rare hyaline taxa (Jonasson & Adams, 1996; Murray, 2003, Panieri 2006). Species that have been reported living in hydrothermal vent were *Fursenkoina rotundata*, *Fursenkoina cornuta*, and *Oridorsalis umbonatus* (Murray, 2003) from the Gulf of California and *Lepidodeuterammina ochracea* and *Trochammina nitida* from Panarea Island, Italy (Panieri, 2006). These species known as pioneer recolonizers in harsh hydrothermal vents because their tests were able to withstand dissolution after venting occurs (Panieri 2006).

Distribution of common benthic genera of foraminifera according to their salinity tolerance was documented by Scott et al. (2004), of which the distribution can be divided into five sub-environments: 1- salt marshes and mangrove, 2- brackish lagoons and estuaries, 3- inner shelf, 4- lagoons and carbonate platforms, 5- hypersaline lagoon (Figure 2.4).

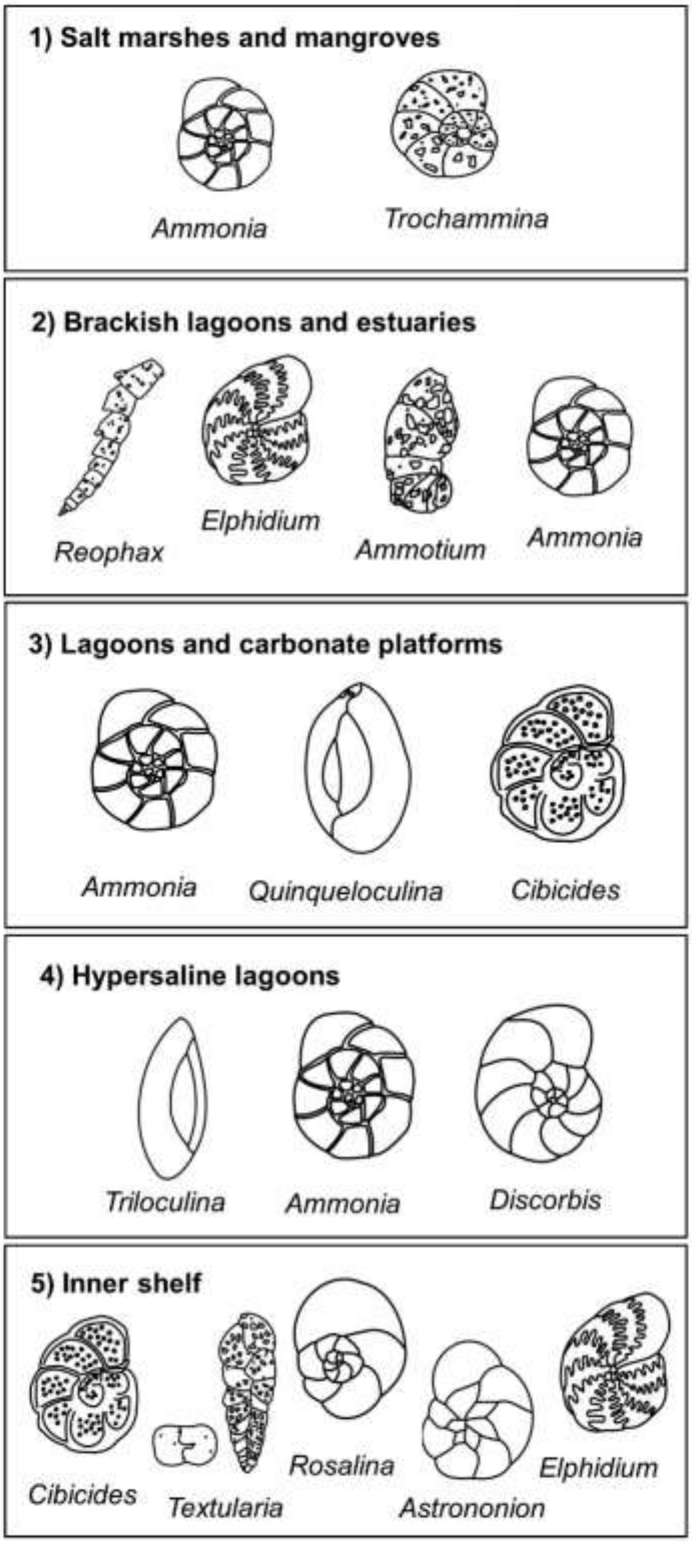

Figure 2.4: Typical benthic foraminifera genera distribution from brackish to normal marine environments. Source: Modified after Scott et al. (2004)