

**BENTHIC FORAMINIFERA IN THE ARABIAN GULF: EFFECTS OF  
SEASONAL DYNAMICS, ENVIRONMENTAL PARAMETERS, AND  
MARINE POLLUTION ON THEIR DISTRIBUTION AND  
BEHAVIORS.**

BY

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This thesis is dedicated to my parents and wife for their endless love and encouragement.

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## LIST OF ABBREVIATIONS

<b>FD</b>	:	Foraminiferal Density
<b>Fc</b>	:	Foraminiferal Constancy
<b>D</b>	:	Dominance
<b>S</b>	:	Species Richness
<b>A/J</b>	:	Adults to Juveniles Ratio
<b>J</b>	:	Evenness
<b>E</b>	:	Equitability
<b>H'</b>	:	Shannon Diversity Index
<b>TOC</b>	:	Total Organic Carbon
<b>THC</b>	:	Total Hydrocarbons Contents

## **ABSTRACT**

Full Name : [Muhammad Arslan]  
Thesis Title : [Benthic Foraminifera in the Arabian Gulf: Effects of Seasonal Dynamics, Environmental Parameters, and Marine Pollution on their Distribution and Behaviours]  
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The Benthic Foraminifera are among the major carbonate producers in modern Arabian Gulf waters, and are found living in all marine habitats. The current study elucidates three areas of foraminiferal research, i.e., biology of foraminifera, effect of seasonality, and response to environmental pollution. In the first part, living behaviors of benthic foraminifera are observed in the laboratory conditions leading to their morphological and molecular characterization. The second part illustrates the effect of seasonality and environmental parameters on distribution of benthic foraminifera in unpolluted and polluted localities from eastern Bahrain and Saudi coastline. Lastly, the response of benthic foraminiferal assemblages is recorded along with their distribution patterns in polluted and unpolluted localities. This study is the first systematic baseline taxonomic as well as an environmental survey of benthic foraminifera carried out on the western side of Arabian Gulf.

## ملخص الرسالة

الاسم الكامل: محمد ارسلان

**عنوان الرسالة:** المنخربات القاعية في الخليج العربي: تأثير كل من الديناميكا الموسمية، العوامل البيئية، والتلوث البحري على توزيعها وسلوكياتها.

**التخصص:** علوم بيئية

**تاريخ الدرجة العلمي:** ٠٧ - ٠٩ - ٢٠١٥

تعتبر المنخربات القاعية من المنتجات الرئيسية للكربونات في المياه الحالية للخليج العربي وتتواجد في جميع البيئات البحرية. الدراسة الحالية تبين المجالات الثلاث لأبحاث المنخربات العلمية والتي هي: أحياء المنخربات، تأثيرات الموسمية على المنخربات، في الفصل الأول، تتم ملاحظة الأنماط السلوكية للمنخربات واستجابتها للتلوث البيئي. القاعية في المختبر تحت ظروف معينة وصولاً إلى توصيفها على الصعيدين البنيوي والجزئي. الفصل الثاني يوضح تأثير الموسمية والخواص البيئية على توزيع المنخربات القاعية في المواقع الملوثة وغير الملوثة بدءاً من شرقيّ البحرين وحتى الساحل أخيراً، تسجل استجابة تجمعات المنخربات القاعية بالإضافة إلى أنماط تركزها السعودي. في المواقع الملوثة والمواقع غير الملوثة. هذه الدراسة تعدّ أول تصنيف أساسي منظمّ فضلاً عن المسح البيئي للمنخربات القاعية الذي أُجري على الجانب الغربي من الخليج العربي.

# CHAPTER 1

## INTRODUCTION

### 1.1 What are Foraminifera?

Foraminifera are unicellular granuloreticulose eukaryotic micro-organisms which belong to the Kingdom Chromista [1]. Their etymological history begins in 1826 when d'Orbigny proposed a new order “Foraminiferes” for these organisms. Due to their similar shell coiling features analogous to gastropods and cephalopods, previously, foraminifera were considered as tiny molluscs [2]. However, the observation of the appearance of granuloreticulopods in living individuals convinced him to differentiate these organisms from molluscs. During his presentation to the Academie de Sciences on 7<sup>th</sup> of November 1825, he introduced them as a distinct order “foraminiferés” within the class “Cephalopodés” using two neo-Latin words, i.e., *foramin* (from *forare*) meaning an opening and *fer* (from *ferre*) meaning bearing. Afterwards, Von Eichwald (1830) Latinized the word foraminiferés by dropping diacritical mark, accenting the first “e”, and adding the terminal “-a” as “foraminiferea” [3]. Although, Brady (1884) acknowledged d'Orbigny for his name but he believed that a different name would have been better as some people might confuse the perforated surface features of calcareous test with aperture and older apertures which was the original characteristic behind his taxonomy [4]. Nevertheless, the name foraminifera was established by the 19<sup>th</sup> Century

British school of foraminiferologists, and is still in practice around the globe. In addition to this, earth scientists and biologists use the informal term “forams”, for simplicity in pronunciation during conversation [5].

Foraminifera have been seen in all marine environments, being planktic or benthic in mode of life. Currently, more than 5,000 species of modern foraminifera and 50,000 fossilized species have been identified so far [6]. Among them, the overwhelming majority are benthic whereas only 40 to 50 have been recognized as planktic [7, 8]. The benthic species dwell ocean’s bottom environments where they survive being free, sessile, epifaunal, epifaunal epiphyte, and/or infaunal depending on their nature of living. Epifaunal species are found to attach themselves onto the substrate, i.e., silt, sand, stones, rocks, and animal shells; epifaunal epiphytes attach to sea-grass or algae; and infaunal species live within the sediments [9, 10].

In addition to high diversity, benthic foraminifera also have a longer geological record compared to planktonic foraminifera. Stratigraphically, foraminifera appeared in latest Precambrian along with the first primitive metazoans [11]. The agglutinated Astorhizida were the earliest foraminifera that are preserved in the fossil record. Later on, miliolids appeared in early Carboniferous, followed by appearance of rotaliids in the Mesozoic and calcareous-cemented textulariids in the Jurassic. The earliest forms of foraminifera were all benthic in habitat, whereas planktic foraminifera appeared in mid Jurassic. Most probably, their behavior was meroplanktic (partially planktic) especially during later stages in their life cycle. Finally, during the Cretaceous period, development of greenhouse conditions and high sea levels resulted in the diversification of planktic foraminifera leading to a rapid evolutionary burst in the early Palaeogene along with the

appearance of larger benthic foraminifera. Thereafter, the population of larger foraminifera started dwindling again in the Miocene.

## **1.2 Classification of Foraminifera**

The history of foraminiferal classification starts at the beginning of 19<sup>th</sup> century when d'Orbigny developed first taxonomic system depending on their growth plans. Later on, a generalized classification was proposed by Schultze (1854) in which whole population of foraminifera was placed in two major groups, i.e., Monothalamia and Polythalamia, as per the presence of single and multi-chambered shells, respectively [12]. However, due to its limited scope and information, this primary division was replaced with another system of classification that focused on the presence or absence of pores in the walls of foraminifera [13]. The wall characterization gained tremendous popularity in 20<sup>th</sup> century and became one of the major criteria to differentiate higher level groups of foraminifera [14]. Consequently, differentiating them on the basis of ultrastructural and mineralogical features of the test wall, Loeblich and Tappan categorized foraminiferal population into 12 suborders [12, 15, 16]. The recent modifications in this classification system increased the number of orders (classes/subclasses) up to 16 without changing the foundation of the system [8, 17, 18].

Many studies have reported considerable variations in the morphology, dimension, and composition of the foraminiferal tests. These features are of great importance to taxonomists and this is why, up to now, foraminifera have only been classified on the basis of their wall composition and shell morphology. Three basic groups of foraminifera

have been recognized on the basis of their wall compositions. These are organic, agglutinated, and secreted [19].

The organic-walled forms possess a thin and non-rigid test of proteinaceous matter without further mineralization [20]. This is the reason that their fossil record is very poor. The order Allogromiida comes under the category of organic-walled forms [8].

The agglutinated forms construct their shells by cementing foreign particles of organic and/or mineral matter originally obtained from the sea floor [20]. This group of foraminifera is of great importance to geologists as the characterization of cementing material and bounded particles provide information about the environmental conditions and the type of sediment at the time of test construction [21]. However, some species have been found to be least selective in their behaviors as they use every type of material available in the sea floor [22].

The secreted forms are of two types, i.e. calcareous and siliceous, as they use calcium carbonate or more rarely silica in their test construction respectively. The calcareous forms have been divided in porcelaneous and hyalines (glassy) according to their light transmission characteristics. The porcelaneous forms are generally opaque in nature and reflect light, whereas the hyalines forms allow the complete passage of light and hence are translucent [22]. Furthermore, porcelaneous tests are imperforate with a milky appearance and their tests are constructed by the secretion of high-magnesium calcite needles from vesicles in the cytoplasm to the outer margin of the cell (characteristics of the Order Miliolida) [20]. However, in the hyaline forms, the test is perforated which is constructed by a bio-mineralization process exteriorly to the protoplasmic body by

adding a new lamella to the entire test every time a new chamber is formed [23]. The microstructure shows numerous pores and hence is termed calcareous perforate. Similar to the aperture, pores function as channels for the movement of cytoplasm, which carry food and/or waste products during metabolism [24, 25]. In contrast to calcareous forms, siliceous forms are extremely rare and their phylogenetic position has not been well investigated [26, 27].

### **1.3 Biology of Foraminifera**

Foraminifera are very successful group of amoeboid protists (Sen Gupta, 1998). Through the course of evolution, they have adopted themselves to perform fundamental functions of life similar to other living organisms. Foraminifera can eat, excrete, move, reproduce, grow, and respond to wide range of environmental stimuli [28].

The first biological study on foraminifera was conducted by Dujardin in 1835. His observations on the undifferentiated body and anastomosing granular pseudopodia (being able to split and rejoin) convinced him to place them under amoeboid instead of cephalopods while challenging the previous classification of d'Orbigny [8]. Later studies revealed the role of pseudopodia in motility, attachment, feeding, grazing, protection, structuring tests, and a few aspects of respiration as well as reproduction. In general, pseudopodia are the principle feature of foraminifera that provide them mechanisms to interact with the surroundings.

Generally, foraminifera can be distinguished from other protists in two ways.

1. Foraminifera possess granuloreticulopodia which are fine, thread-like pseudopodial structures having granular texture when examined under the light microscope [19]. These granuloreticulopodia emerge from one or numerous orifices present in their shells, and are used in feeding, predation, substrate fixation, physiological residue elimination, dislocation, and test construction [29]. Structurally, granuloreticulopodia are encased by a cell membrane and contain a core of cytoskeletal microtubules [28]. Their granular appearance is due to the presence of several organelles such as mitochondria, waste vacuoles (poop), phagosomes, and numerous other structures [8].
2. Foraminifera own a test or shell that covers the living individual and separates it from the surrounding environment. The shell could be organic, agglutinated, and/or secreted as explained earlier [19]. The growth of the shell is achieved by either increasing the overall size of chamber in unilocular and bilocular tubular forms or by adding a new chamber in multilocular forms [8].

These features have also been found to be closely related with other parameters such as physiological differences, ecological niche, habitat, and numerous types of reproductive strategies in their life cycles. The life cycle of foraminifera is characterized by heterophasic alternation of generations, i.e., gamogony or sexual reproduction, and schizogony or asexual reproduction [30]. Dimorphism is the fundamental characteristic of reproduced individuals in the form and size of the proloculus (first chamber). Briefly, individuals resulting from gamogony produce microspheric forms (small proloculus but larger test) whereas those resulting from schizogony produce megalospheric forms (large proloculus but smaller test) [22]. Gametes of foraminifera have been found to be biflagellated, triflagellated, or amoeboid.

During reproduction, certain foraminiferal species undergo through plastogamy for genetic recombinations. The plastogamy is a process in which two or more (but rarely) individuals come close to each other and join their apertures side by side with an animal cement or organic membrane in order to exchange gametes and/or protect the zygotes. Principally, internal septa and apertural sides dissolve giving rise to a single, broader chamber. Afterwards, the zygotes nurture to a 2 or 3-chambered stage and young individuals come out from the enclosing parent tests by dissolving the membrane. It is believed that the mechanism of foraminiferal plastogamy is an adaptation to turbulent waters in which there are less chance of gamete union and/or zygotes survival [31, 32].

Most benthic foraminifers are opportunistic omnivores [33]. They have adopted a wide range of feeding mechanisms including herbivory, bacterivory, suspensivory, detritivory, carnivory, mutualism and parasitism [34]. Some large calcareous forms can bear in the endoplasm naked photosymbionts, especially diatoms and dinoflagellates that aid in supplying energy [20] and some are able to sequester and house chloroplasts (chloroplast husbandry) but not the entire cell [19].

#### **1.4 New Insights in Foraminifera Classification**

There is very little information available on biology of foraminifera especially about their living behavior and molecular characterization. Furthermore, many of the foraminiferal species have never been studied alive. Classifying the hard-shelled foraminifera on the basis of shell morphology, and identifying living foraminiferal specimens using Rose Bengal staining has remained a common practice since decades. However, the effectiveness of the methods is quite unclear [35]. Furthermore, classification based on

morphology is questionable due to the influence of environmental factors, which makes distinction difficult between true species and ecophenotypes [36]. The situation gets even worse for the species with higher morphological variability worldwide [37]. For instance, more than 40 species and subspecies of *Ammonia* render its status uncertain and controversial [36, 37]. To overcome this issue, molecular characterization has been introduced recently which is bringing new insights into foraminiferal taxonomy. According to a study conducted by Pawlowski and colleagues, analysis of ribosomal RNA sequences can provide a strong foundation for the identification of the foraminiferal species without considering the test morphology. These molecular analyses also allow the evaluation of intraspecific morphological variability as well as estimation of the ecological significance of the different morphologic characteristics [38, 39].

Earlier studies, based on molecular analysis of ribosomal RNA (rRNA), have placed foraminifera near to the *Entamoeba* and *Dictyostelium* in the tree of life [38, 40]. However, recent investigation on multi-gene evidence of benthic foraminifera revealed that the phylum Foraminifera is a part of eukaryotic supergroup known as Rhizaria. Rhizaria comprises amoeboid and skeleton-building protists [41, 42]. More specifically, they form the monophyletic group Retaria together with the Polycystinea and Acantharea (i.e. Radiozoa) [42]. This fact further reveals the high level of uncertainty in the systematics of protists.

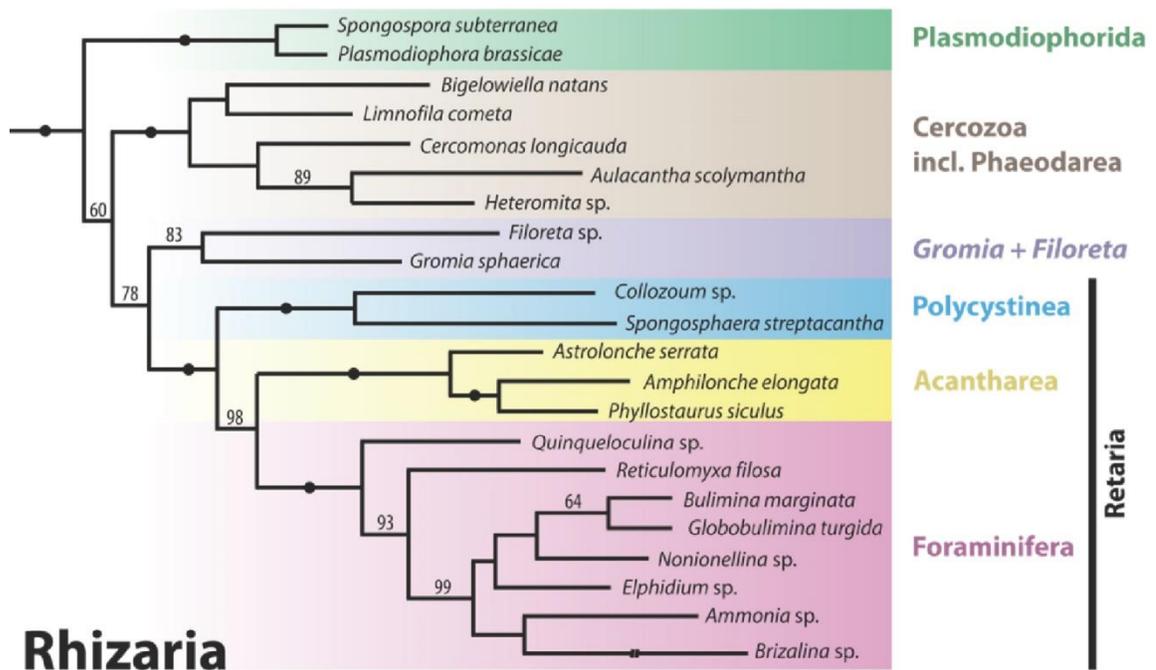


Figure 1: Position of phylum foraminifera in the tree of life (from xxx)

Most of the molecular phylogenies are based on the analyses of three rDNA regions, i.e. 3' fragment of the small subunit (SSU), internal transcribed region (ITS), and 5' fragment of the large subunit (LSU) [35, 43, 44]. Due to their unusual length (>3000 nucleotides), complete SSU sequences have been only obtained for a few species, generally for the order Rotaliida [45]. In addition to this, molecular phylogenies have also been inferred from actin, tubulin, and RNA polymerase [46, 47], but the number of analyzed species is very small. Recently, analysis of combined sequence data, inferred from single gene phylogenies, has also confirmed the process of evolution in Foraminifera [48]. Nevertheless, till today, no proper attempt has been made by including molecular data to construct a higher-level classification of the foraminifera.

In the near future, molecular biology will not only provide the specific definitions related to modern foraminifera along with their morphological classification, but will also establish database for the respective genetic information [38].

## **1.5 Justification of the Research**

In other parts of the world, benthic foraminifera have been widely used to study marginal marine, coastal, and marine shelf environments, and to assess environmental changes and the consequences of pollution [49, 50]. Recently, Al-Zamel and colleagues carried out a study of the benthic foraminifera from polluted areas of Sulaibikhat Bay (Kuwait), identifying assemblage changes relating to various pollution sources [51]. However, few studies have attempted to document the microfaunal communities of the Western Arabian Gulf. Furthermore, extensive development and human activities have already disturbed large areas of the coastal environment and many of Prof. John Murray's original localities (sampled in early 1960's) are already completely disturbed. Therefore, it is highly needed to conduct a survey of benthic foraminiferal populations to understand the environmental factors that may impact the marine biodiversity of the world's largest hypersaline sea before the natural habitat is lost forever. This study aims to address the above queries with a major focus on the seasonal distribution and pollution assessment covering the research gap for the microfaunal distributions in the western side of the Arabian Gulf. Furthermore, the section on living behaviors and molecular characterization is novel and have never done previously in the Arabian Gulf. The study is conducted along "Murray's pool transect", the "Corniche Al-Khobar", and Half-Moon Bay covering an assessment of

a range of environmental settings and pollution parameters from the foreshore to the offshore.

## **1.6 Significance of the Research**

This proposed study is timely because most of the areas are under threat due to infrastructural development. Coastal vegetation (mangroves) has already been threatened by human activities. Extensive development activities have disturbed large areas of the coastal environment along the Saudi Arabian and U.A.E. coastlines, and now many of Murray's original localities along the U.A.E. coast are located beneath parking lots (F. Fiorini, personal communication, 2011). In Bahrain, extension of the Corniche in Askar in 2013 poses a direct threat to Murray's Pool. There exists a need to conduct a survey of benthic foraminiferal populations, to document the biodiversity of the world's largest hypersaline sea. Furthermore, we need to understand the environmental factors that impact the marine biodiversity, and assess the role of human activities as a threat to these communities. Lastly, it is highly needed to build upon the early work of Murray to determine the relationship between the benthic communities and depositional sub-environments in those remaining areas that are still relatively undisturbed by development, before the natural habitat is lost forever. Therefore, this research would define a benchmark study that could be utilized for future environmental monitoring in the region.

## **1.7 Objectives of the Study**

The present study aims to quantify the environmental variability in the eastern coastline of the Arabian Gulf, concentrating on the benthic foraminiferal distribution patterns in modern sediments from the Arabian Gulf. The main purpose of this research is to provide baseline data for future environmental impact studies. The surveys focused on both disturbed and undisturbed areas by human activities. Specifically, the research addresses the following research objectives:

### **1. To investigate the living behaviors of benthic foraminifera leading to their molecular characterization**

To address this objective, living behaviors of benthic foraminifera are assessed during season of highest reproduction. Furthermore, the DNA of living specimens was sequenced for better classification. This objective of study illustrates the molecular biology of benthic foraminifera from the Arabian Gulf, the first ever documentation in the region.

### **2. To study the seasonal dynamics of living benthic foraminifera along with their environmental characterization in a relatively unpolluted area.**

In this objective, seasonal dynamics and standing crop is studied throughout the year. The objective further delineates the natural variability of the foraminiferal populations as a function of environmental parameters (water depth, sediment chemistry, and substrate parameters), to provide a basis for further comparisons to the disturbed areas. Overall, the

objective demonstrates effect of seasonal changes as well as environmental parameters on the distribution of living foraminiferal assemblages.

**3. To compare and assess the effects of environmental parameters and marine pollution on foraminiferal assemblages in unpolluted vs. polluted areas.**

To address this objective, different transects are selected with different pollution sources which are further compared with unpolluted zones. The objective describes the role of benthic foraminifera for detection of pollution as a bio-indicator.

-----

Protist Protest

Little protists of the sea

How do we treat thee?

As foraminifers, Oh wee beasties of the sea?

Or, shall it be, foraminifera,

for the plural or the singular?

Perhaps we can float the word “foraminiferan”

but then again,

it still would mean a single cell,

but how in hell

can foraminifera be for one and two,

when so many live in the ocean blue?

Please, please tell me Dr. Foram Man or M'am,

Is it -minifer, -minifera, or -miniferan? —S.E.W.

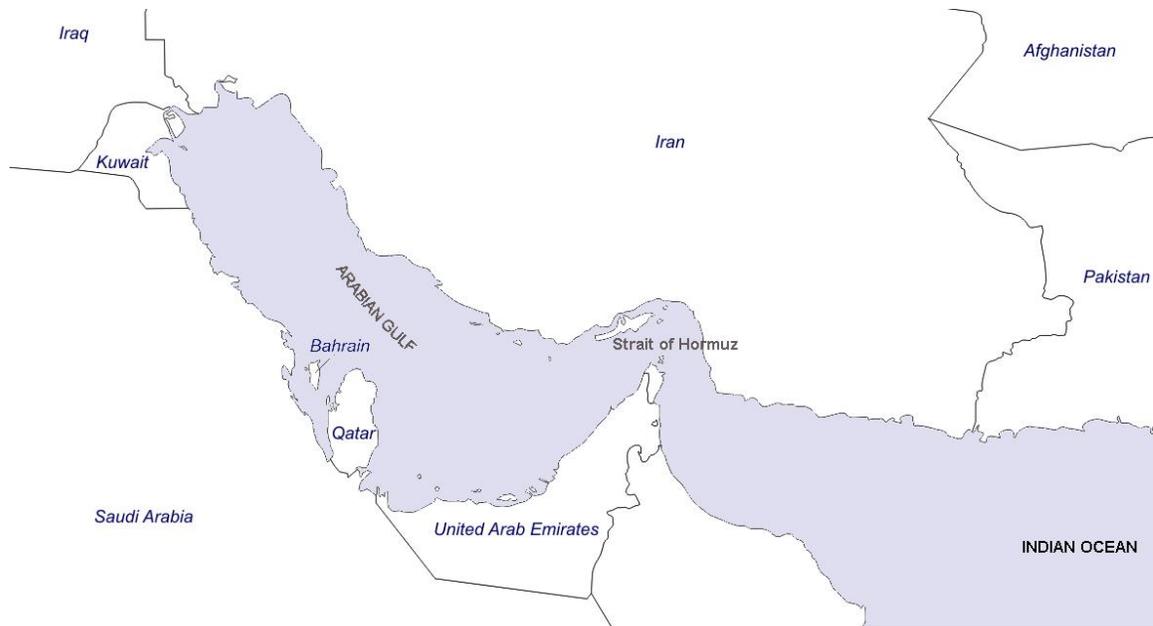
*Sally Walker*

## **CHAPTER 2**

### **LITERATURE REVIEW**

#### **2.1 The Arabian Gulf**

The Arabian Gulf is an almost completely landlocked, shallow, subtropical, and epicontinental basin with a single connection with the Indian Ocean via the Gulf of Hormuz. The basin is about 1000 km long and 200-300 km wide covering an area of 226000 km<sup>2</sup>. Furthermore, the Arabian shelf is bordered by lagoons, and slopes gently toward two deeper basins close to the Iranian shore. It is characterized by its shallowness with an average depth of 35 m, reaching its maximum depth of about 100 m in the 60 km wide passage of Strait of Hormuz, which connects it to the Indian Ocean (Figure 2). This simple topography is modified by the development of local rocky highs and islands sitting atop salt domes. The floor of Arabian Gulf is rich in biogenic carbonate sediments and evaporites, with aragonite muds common only in low-energy areas such as the lagoons and deeper basins. The oligotrophic waters are exceptionally clear, so that the whole Gulf lies within the photic zones [52].



**Figure 2: The Arabian Gulf**

Another characteristic feature of the Arabian Gulf is the abnormal hypersalinity, especially in the western side, which is due to its partial isolation from the Indian Ocean and excessive evaporation of approximately 144 cm/year [53]. This increase in salinity is mainly attributed to the surrounding arid land mass with temperatures up to 50°C and very low annual rainfall rate. The surface salinity in the central part of the Gulf is about 37-40‰ while the value reaches up to 40-50‰ towards the shallow parts of the Arabian Sea and 60-70‰ in remote lagoons and coastal embayments such as the Gulf of Salwah and Tarut Bay [52]. Sediments exhibit a longitudinal pattern with terrigenous sediments off the Iranian coast. Detailed information about the Arabian Gulf can be found in the studies of Emery (1956), Sugden (1963), Evans (1966) and a special volume on the Persian Gulf [54-56].

## 2.2 Earlier Foraminiferal Studies in the Region

The western part of the Arabian Gulf, with highest salinity, offers unique marine habitats which can be considered as a marine biodiversity hotspot. In the Arabian Gulf, the distribution of Foraminifera has attracted the attention of many scientists since the 18th century but, still, it is considered as a “terra incognita” meaning “unknown land” especially for Saudi coastline as far as its foraminiferal fauna is concerned [57]. This is, generally, because of the reason that still there is no proper catalogue of foraminifera available yet to date.

The first study on the Foraminifera in Arabian Gulf was carried out by Fichtel and Moll in 1798 [58]. The modern epoch commenced with Henson who briefly explained the presence of some living miliolids in the Gulf in his study on “Middle Eastern Tertiary Peneroplidae” [59]. Later on, Houbolt conducted a study on the Qatar offshore to investigate foraminiferal distribution along with the sedimentological problems of carbonate deposits [60]. Houbolt documented 20 genera and placed them into six groups based upon their particular depths, i.e., *Rotalia-Elphidium* (3-5 fathoms), *Textularia-Miliolidae* (6-14 fathoms), *Heterostegina* (below 14 fathoms), *Cibicides* (14-15 fathoms), *Rotalia-Cibicides* (12-43 fathoms), and *Rotalia-Elphidiella* (marls of the central part of the Gulf).

Soon after, Prof. John Murray conducted a series of studies in the western Arabian Gulf in the 1960's and 1970's, primarily dealing with the distribution of both living and dead foraminifera in the shallow-water environments of the United Arab Emirates (U.A.E.) shore [10, 34, 61]. The studies were based on sediment samples collected using a grab

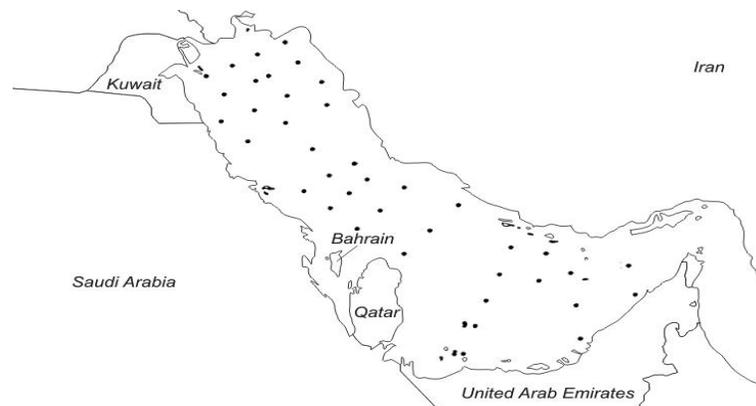
sampler by Imperial College in 1961 in an effort to map the sedimentary environments such as such as tidal drainage channel, shallow hypersaline lagoon, delta-shaped oolite band and nearshore shelf, off the U.A.E. coast. In most of the samples, populations of living foraminifera were found to be generally low, therefore, Murray mainly described the dead foraminiferal assemblages in the bottom sediments. However, in contrast to this, he reported clumped distribution of living foraminifera on hair-like epiphytic plants (e.g. sea weeds and sea grass) that suggest a worthy closer examination towards study of living fauna.

On the Iranian side, foraminifera have been studied by Lutze and other authors [62-64]. The publications of Lutze documented a catalogue of 52 species with a short discussion of the taxonomic problem and notes on the distribution of the foraminifera [63]. In the following year, Haake published a catalogue of 54 miliolid species [65]. His studies revealed that the species frequency increased with water depth along with increase in grain size and decrease in sedimentation rate. The maximum species frequency was found at water depths between 50 and 75 m. Another study conducted by Lutze and Wolf reported that the dominant distribution patterns were depending upon depth with a marked change at 35-40 m depth (shallow fauna with *Ammonia*, *Elphidium* etc.; and deep fauna with *Buliminacea*, *Cassidulina*, and *Cancris*) [66].

To date, the most extensive survey on foraminiferal population has been carried out in the Abu Dhabi region, where a variety of subenvironments were identified and sampled (i.e. inner lagoon, outer lagoon, coral banks, channels, algal-mangrove flats, frontal beach face, reef, back-reef lagoon, and nearshore shelf). In another study, three onshore–offshore transects were collected along the Trucial coast. Because of the high salinity and

temperatures, larger foraminifera are absent from the study area, and lagoonal assemblages are dominated by diverse miliolids, mainly *Peneroplis*, *Quinqueloculina*, and *Triloculina*. In the current-swept area, *Rosalina* is common, while the oolitic deltas contain miliolids with an admixture of calcareous benthics including *Ammonia*, *Elphidium*, *Parrina*, and *Eponides*. Seaweed growing in shallow areas also serves as a habitat for many epiphytic foraminiferans [67], especially *Miliolinella*, *Quinqueloculina*, *Rosalina*, and *Elphidium*. Murray reported that many foraminifera from the sediment samples are stained black in the samples examined, meaning that the grab samples had collected sub-Recent material. This interpretation corroborates the finding of few stained specimens. Agglutinated foraminifera belong mostly to the calcareous-cemented group, and are more common along the Iranian side of the Gulf [10].

Anber (1974) analyzed the foraminiferal content of 56 bottom samples from offshore of Kuwait, recognizing 120 species and subspecies [68]. Lately, Cherif et al., (1997) reported ninety-eight species from different parts of the Arabian Gulf. However, the coast of Bahrain, Qatar, and the Saudi coastline remained untouched during the whole study [69].



**Figure 3: Location map showing previously studied areas (Cherif et al. 1997)**

## **2.3 Response of Benthic Foraminifera to Environmental parameters**

Many studies have reported the relationships between environmental (ecological) parameters and foraminiferal populations [22, 70]. Among these parameters, temperature, dissolved oxygen, pH, water depth, salinity, substrate parameters, sediments grain size, nutrients, organic matter and solubility of calcium carbonate are of great importance. These parameters do not only affect the standing crop but are also responsible for morphological variations in foraminiferal assemblages [71]. The detailed description of these parameters along with the foraminiferal population is presented in the following sections.

### **2.3.1 Temperature**

Earlier researches have reported the significant effects of temperature on foraminiferal population and their distribution primarily by altering other physicochemical parameters. For example, relationship of temperature with dissolved oxygen is controlled by seasons and, therefore, high oxygen levels during winter and lower most during summer ultimately affect the benthic foraminiferal communities [72]. Generally, foraminifera can live between a wide range of temperature, i.e., 1 °C to 50 ° [73]; however, each species is adapted to a certain range of temperature for its successful reproduction [20]. Furthermore, human interventions in marine environment can significantly impair the water quality including temperature that ultimately affects the benthic foraminiferal communities.

### **2.3.2 Dissolved Oxygen (DO)**

As mentioned earlier, DO is an important environmental factor that affect the foraminiferal population. DO in the water has two main sources, i.e., (1) direct diffusion from the atmosphere and (2) photosynthesis by microbial and aquatic plants. Usually, oxygen availability is high at the sediment-water interface and, therefore, most aerobic benthic organisms dwell in this habitat. However, at a particular depth within the sediment, oxygen demand exceeds the supply and hence sediments become microxic (< 1 ml/l) or dysoxic/anoxic [8]. The top of the anoxic zone is typically within the upper decimeters of sediments, even when overlying waters are well-aerated [8]. On the contrary side, oxygen availability is not an obligatory factor of foraminiferal distribution as many of the benthic foraminifera inhabit oxygen-poor (microxic), anoxic and even sulfidic environments. However, oxygen depletion has been widely recognized as a stress factor, causing significant decrease in standing crops and species diversity. Furthermore, it may also result into dwarfism due to inefficient metabolism and allow proliferation of opportunistic species [70, 74].

### **2.3.3 pH**

The pH, an indicator of acidity in water and sediments, depends upon variations in temperature and dissolved oxygen. Generally, pH reflects the interface of seawater inputs and the quantity of organic matter present in particular environment. In principle, aerobic degradation of organic matter by microorganisms liberate CO<sub>2</sub> which produce carbonic acid, responsible in controlling the pH of medium. Many authors have reported a positive relationship between pH and dissolved oxygen in a diurnal cycle [75]. Another study illustrates the pronounced effects of change in pH on growth, survival and reproduction in

benthic foraminifera. It has also been observed that a pH below 7.5 can hamper the ability of living foraminifera to secrete calcite affecting their calcification rates [76, 77].

#### **2.3.4 Water Depth/Elevation Gradient**

Although, water depth is not found to have direct effects on distribution of benthic foraminifera; however, it can affect other parameters that may regulate their distribution in marine environment. As a matter of fact, elevation gradient is directly affected by the energy currents in terms of sediment particle size, in the offshore direction. Higher elevations are mainly comprised of silts and clay, however, lower elevations are generally sandy with significant ratio of bioclasts. In contrast to this, sediments are poorly sorted in the low-energy conditions typical of the deposition areas [78].

#### **2.3.5 Salinity**

Salinity, together with depth and temperature, has been found to be an important factor controlling foraminiferal abundance and distribution [79, 80]. Although, foraminifera can inhabit wide range of saline environments ranging between 0.5 - 57 PSU; however, more diverse assemblages have been seen in normal marine salinities, i.e., 35 PSU [20]. Armstrong and Brasier further reported that the lower salinities of lagoons and marshes favors population growth of certain hyaline forms, e.g., *Ammonia* and *Elphidium*, as well as the low-diversity assemblages of agglutinated foraminifera. Furthermore, it is reported that the littoral foraminifera are well adapted to strong salinity oscillations but their abundance tend to increase from low salinities (0.5) to typical sea water salinities (35-37). In waters with salinities higher than sea water, the number of species and standing crop decreases abruptly.

### **2.3.6 Substrate Parameters**

***Sediment Grain Size:*** The effects of substrate parameters on foraminiferal distribution patterns are still a matter of debate. According to Diz and colleagues, coarse grain substrate particles provide more favorable conditions to living benthic foraminifera for their settlement, whereas few other studies have reported more comprehensive results in the presence of fine particles [81]. Furthermore, some authors suggest that the shape of multilocular sedentary species depends on the substrate shape as the attached side of the test adopts the shape of the bottom [82-84]. In addition to this, Haake reported that some of the textularian species become broader in coarse sediments (e.g. *Textularia pseudogramen*).

***Organic Matter:*** Similar to the sediment grain size, effects of organic matter on foraminiferal population is also very complex. Some of the studies indicate that organic matter favors higher foraminiferal populations directly by providing food and indirectly by reducing predation and/or competition [27, 85-87]; however, a few studies reported a decrease in overall population of foraminifera with increase in organic matter content [88, 89]. In summary, the presence of organic matter seems to favor foraminiferal growth until the conditions turn into toxic, suboxic, or anoxic [90]. The detailed description on organically environment is presented in later sections (cf. section 2.4.1).

## **2.4 Response of Benthic Foraminifera to Marine Pollution**

The effects of marine pollution on benthic foraminifera have been well investigated over the last four decades. Although, the history on pollution effects of foraminifera is bit old, however, the first oriented study on benthic foraminifera as proxies of pollution was

conducted by Resig and Watkin in the early 1960s. Later on, several authors studied the effects of various types of pollution in a wide range of marginal marine polluted environments e.g. organically-enriched, human-induced eutrophy, hydrocarbons, and heavy metals [33, 49, 50, 91].

Benthic foraminifera generally respond to adverse ecological conditions mainly by undergoing (1) local extinctions, (2) modifications in the assemblages which include changes in standing crop, i.e., abundance and diversity, (3) size reduction/dwarfism, and (4) test abnormalities [33, 50, 90, 92, 93]. Both laboratory and field studies suggest that benthic foraminifera from unpolluted settings display less than 2% deformities [94], but this figure can rise to 50% in heavily contaminated areas [95]. The detailed description on each type of pollution source is presented in the following sections.

#### **2.4.1 Organic Matter Pollution**

Organic matter pollution could be due to two forms of organic matter, i.e. biodegradable and resistant. Effluents from domestic sewage, food industries, fertilizer plants, and agriculture are the primary source of biodegradable organic matter, however, resistant organic matter comes from paper and pulp mill effluents mainly comprising cellulose and lignin [90]. The presence of degradable organic matter affects the overall population of foraminiferal fauna [96, 97]. This is due the reason that organic matter benefits living individuals directly by providing food and indirectly by reducing competition [85, 98]. Therefore, the availability of dissolved organic material creates an artificially high nutrient environment that ultimately results in increased foraminiferal abundance [99-101]. However, organically rich environment, sometimes, result into the development of dysoxic conditions causing reduction in foraminiferal populations [102]; or during suboxic

conditions, lead to the appearance of opportunistic species [103]. This observation is further strengthened with the reports on increased foraminiferal diversity with increasing distance from a point source. Therefore, from these observations, it can be established that the flux of organic matter may cause alteration in the natural foraminiferal assemblages, compared to the background population [88, 104, 105].

***Eutrophication:*** As mentioned earlier, the degradation of organic matter leads to increased nutrient supply, which often causes eutrophication and stimulates the growth of opportunistic species. Recently, Minhat and colleagues assessed the effects of eutrophication pollutants on the distribution of benthic foraminifera in coastal waters, previously introduced in sea water due to fishing, ecotourism and floating cage cultures [106]. They attempted to determine the Pearson correlation for nitrates, nitrites, orthophosphates, and other physiochemical parameters with species abundance. Results illustrated a weak correlation with nitrates indicating a decrease in population with increasing pollutants level. Similarly, another study reports negative effects of eutrophication on porcelanous (miliolid) species, whereas no significant effects were found on hyaline taxa (Rotaliida), particularly nonionids, chilostomellids, buliminids uvigerinids, and bolivinids [107, 108].

#### **2.4.2 Hydrocarbons**

To date, very little work has been done to elucidate the effects of hydrocarbons on benthic foraminiferal communities. This is the reason that earlier studies are contradictory and have produced conflicting results. For instance, Mayer reported pronounced effects of hydrocarbon pollution on benthic foraminifera [109], whereas minor negative effects were reported by Lockin and Maddocks in the northern Caspian Sea and Yanko and Flexer in

Odessa Bay [50]. Furthermore, it is reported that the presence of hydrocarbons did not affect their relative abundance and diversity but resulted into morphological deformities [110]. Similarly, Witcomb and colleagues noted inhibited growth in *Ammonia beccarii* and *Allogromia laticollaris* leading to narcosis and death in laboratory experiments [8]. He further reported that the presence of hydrocarbons may cause a decrease in nutrient supply, primarily diatoms, which affected the standing crop. Likewise, other authors reported that the presence of hydrocarbons may also affect the respiratory functions and reproduction potential of benthic foraminiferal communities [109, 111]. However, a full understanding of the biological causes behind the inhibited growth and overall decrease in standing crop requires further research [8].

### **2.4.3 Heavy Metals/ Trace Elements**

Trace element geochemistry has been widely used to assess effects of pollution in terrestrial and marginal marine environments [33]. Trace elements and other contaminants are introduced to aquatic ecosystems and accumulate in sediments through disposal of liquid effluents, runoff and chemicals emanating from urban and industrial wastes, agricultural activities and atmospheric deposition [112]. In aquatic environments, sediments act both as sinks and sources of trace elements. Trace elements influence plant and animals in aquatic environments [113, 114]. As a result of their increased concentrations at successive trophic levels in the food web, trace elements are harmful to plant and animal life [115].

Trace element toxicity is complicated when compared to hydrocarbon pollution since both have the similar mode of toxicity i.e. mutagenesis. However, when studying elemental pollution, toxicity varies within individual elements, mainly depending on their

speciation parameters within the sample matrix. In particular, elevated concentrations of cadmium, lead, and mercury have been found to reduce the diversity of modern benthic foraminiferal assemblages and cause morphological deformities among the resilient species that remain [95]. For instance, Boltovskoy reported a stunted fauna in some lead-polluted areas of the northern Argentinean shelf [116]. Similarly, Setty and Nigam noted depauperate populations with high percentages of abnormalities near a titanium processing plant [103]. A similar observation was made by Naidu and colleagues in a harbor area where marine waters were polluted by variety of heavy metals (e.g. Cu, Fe, Pb, Zn, Ni, Cr, and As) coming from domestic sewage [117]. In general, it can be established that foraminiferal assemblages are drastically affected (both in abundance and deformities) due to elevated concentrations of trace elements compared to any other form of pollution.

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## CHAPTER 3

# Living Behaviors and Molecular Characterization of Benthic Foraminifera

### 3.1 Abstract

Since benthic foraminifera are recognized as proxies to assess environmental change, their biological behaviors in modern environments and in laboratory conditions needs to be well studied. The current study attempts to explain biology of benthic foraminifera in terms of their living behaviors and molecular characterization, from different regions of the eastern side of the Arabian Gulf. Accordingly, two major groups of benthic foraminifera, i.e. rotaliids and miliolids, are examined under laboratory conditions. Results illustrate that rotaliids extended their pseudopodia after 8 hours while staying in Petri plates, whereas miliolids took 24 hours, and even more time for some specimens, to show pseudopodial extensions. Furthermore, pseudopodia are extended out from the aperture toward the direction of movement with high rate of movement in rotaliids compared to miliolids. The high rate of movement in rotaliids is attributed to the extension of pseudopodia through all apertures, compared to miliolids in which pseudopodia come out from the primary aperture only, while the individual attaches these structures to the wall of hard substrate resulting in dragging of their bodies in the direction of pseudopodia. The study on molecular analysis reveals the presence of four groups, i.e. *Ammonia*, *Murrayinella*, *Glabratellina*, and *Elphidium*. Furthermore, BLAST analysis illustrates that none of the groups is previously identified at the species level. Overall, monophyletic clustering is observed among all major groups. Initially, *Ammonia*

showed two clusters reflecting the presence of two species, however, rovaliid alignment resolved the issue and placed all sequence of *Ammonia* in a single clade. Similarly, monophyletic clustering is observed for *Murrayinella*, *Glabratellina*, and *Elphidium*.

### **3.2 Introduction**

Benthic foraminifera are bottom-dwelling unicellular eukaryotes whose living behaviors are still a subject of debate. Amazingly, the majority of foraminiferal species have never been observed alive and among those that were studied, the authors do not provide sufficient information on their biology [35]. These few studies further present scattered information on their living behaviors in terms of survival, locomotion, and reproduction [32, 118]. It has been reported that the individuals of some species can live only for a few weeks, whereas some others can live for years. Similarly, some benthic foraminifera burrow actively, at burrowing rate of 82  $\mu\text{m}$  per minute, whereas others mostly attach or hide themselves to the surface of rocks or marine plants. Moreover, their average velocities are found to be varying among different species, with high movement rates in epifaunal species [118].

The study on living behaviors of benthic foraminifera was initiated by Dujardin who first reported the locomotion patterns of *Elphidium* sp., in natural settings. Afterwards, several authors reported detailed investigation of movements in different foraminiferal species [70, 118-120]. However, most of their observations were made on the glass surface of petri plates and, hence, their behaviors were subjected to laboratory environments [121, 122]. Later on, Severin and coworkers measured the vertical velocity of *Quinqueloculina impressa* in a natural environment (i.e. sand particles), in order to obtain the relationship between time of emergence and burial

depth [123]. Their principle objective was to measure the escape behavior of living individuals, attempting to avoid burial in the sediments. Besides locomotion and burying, other living behaviors were also studied by different authors. For instance, Lipps and Erskian reported plastogamy in *Glauvatella ornatissima* during sexual reproduction [32]. Similarly, Kitazato and coworkers described the breeding behaviors in four species of the genus *Glauvatella* and reclassified three morphogroups from the four morphospecies using morphological characters and interbreeding experiments [124].

The biology of foraminifera cannot be explained by studying living behaviors only but, indeed, it requires genetics study for their molecular characterization. In fact, conventional classification based on hard-shell morphology has been recently challenged by molecular biologists around the world [35]. Furthermore, it has been established that the morphology-based studies have largely underestimated the foraminiferal diversity. During the last decade, molecular techniques (i.e. analysis of rRNA sequences) offered new tools for the identification of foraminiferal species [125]. However, until now, very little data exists for the number of species for which DNA analysis have been performed.

The current study attempts to illustrate the overall biology of benthic foraminifera, including their living behaviors and molecular characterization, from different regions of the eastern coast of the Arabian Gulf, i.e., Eastern Bahrain, and the Saudi Coastline. For the molecular characterization, several species have been identified at genetic level in collaboration with the project foramBARCODING, a molecular

database of foraminifera, based at University of Geneva and by coordinating with Prof. Jan Pawlowski and Dr. Maria Holzmann (2014–2015).

### **3.3 Materials and Methods**

#### **3.3.1 Sample Collection**

Sediments containing living benthic foraminifera were collected from two areas, i.e. Eastern Bahrain and the Saudi Coastline. Water depths of the sampling stations range from 40 cm to 100 cm.

#### **3.3.2 Isolation of Foraminifera**

A significant number of living foraminifera were picked from a raw sample of 5 ml using a GENEX beta variable pipette (fixed at 200  $\mu$ l) under a reflected-light, binocular microscope. Isolated individuals were transferred to Petri plates containing sea water where their living behaviors were observed every 8 hours. Furthermore, the study of average life span was conducted for four species in glass jars (diameter of  $\frac{1}{4}$  inches) containing filtered sea water.

#### **3.3.3 Living Behaviors Study**

The laboratory observations on living behaviors particularly locomotion, attachment with substrates, and reproduction begun after 24 hours, as most of the living individuals migrate to their natural positions after a day (Kitazato, 1984). The observation were made on the glass surface of the Petri plates under a phase-contrast stereo inverted microscope. An automatic photographic system (Nikon) attached to the microscope was used to observe and record their behavior. Measurements on

velocity were made by photographing living individuals after 20 seconds intervals. Moreover, their movement rates were calculated using the following formula,

$$S = \frac{\Delta D}{\Delta t} = \frac{P_N - P_{N-1}}{t_N - t_{N-1}}$$

Where,  $P_N$  is the recent position at time  $t_N$  and  $P_{N-1}$  is previous position at time  $t_{N-1}$ .

### **3.3.4 Survival Response**

Survival response of 4 species was calculated using Kaplan-Meier procedure and Log rank test in order to study their average life span in glass jars having diameter of ¼ inches.

### **3.3.5 Identification and Classification**

The important species were photographed using scanning electron microscope (SEM), based at University of Geneva. The photographs were edited and compiled with Adobe Photoshop (Ps) CS7.

Several guides were used for identification, especially Loeblich and Tappan (1988), Boltovskoy (1980), Colom (1974), Jones (1994) and the Ellis and Messina (1942–2012) online catalogue. Most of the foraminifera were classified according to the generic classification proposed by Loeblich and Tappan (1988). For the higher levels of taxonomy, other than genus and species, the Worm's classification was followed (World Register of Marine Species – [www.marinespecies.org](http://www.marinespecies.org)).

### 3.3.6 Molecular Characterization

In collaboration with the project foramBARCODING based at the University of Geneva, several species were identified at genetic level. To accomplish this task, an extra field campaign was made in January 2015 with the aim of collecting live specimens. During this campaign, sites with high standing crop were revisited and sampled. Samples were collected with a spatula without disturbing the sediments floor and then stored in plastic boxes. In the laboratory, individuals with protoplasm were placed in sea water and allowed to settle for a couple of hours. The living foraminifera were isolated and dried at room temperature on Plummer Cell slides. At least 5 specimens of each species were separated and sent to the Department of Genetics and Evolution, University of Geneva, for molecular analysis.

In the University of Geneva, DNA was extracted from living specimens of each species belonging to *Ammonia*, *Elphidium* and *Glabratellina*, and *Murrayinella*. Afterwards, the extract was incubated at 60° C for 1 h, followed by a short centrifugation to remove insoluble material. A significant number of species were successfully analyzed using the methodology as explained earlier (Pawlowski 2000). Briefly, analyzed barcoding region, situated at the 3' end of the SSU rRNA gene, is amplified using the primer pairs (acgcamgtgtgaaacttg)-sB and (tgatccttctgcaggttcacctac). It is usually necessary to perform a nested PCR, replacing primer 14F3 with primer 14F1 (aagggcaccacaagaacgc). The barcoding region spans 6 foraminifera-specific hypervariable expansion segments, 37f, 41f, 43f, 45e, 47f and 49e, which were shown to be sufficiently variable to differentiate between closely related species. Most amplifications are done on single-cell DNA extractions.

Because of intra-individual polymorphism, the amplification products are cloned and 2-3 clones are sequenced.

### **3.3.7 Molecular Phylogeny**

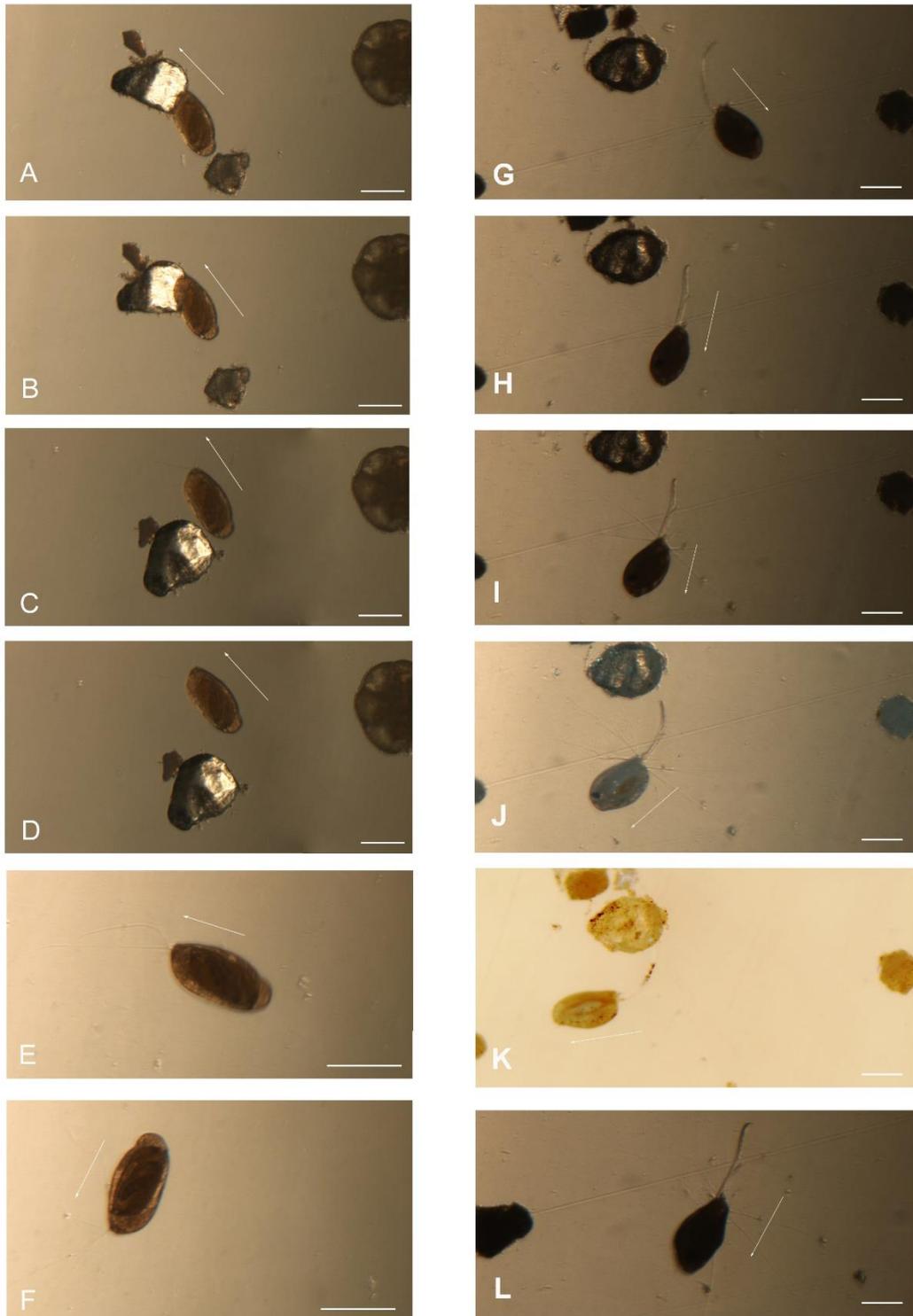
To build a phylogenetic tree, the results of sequencing were aligned in a software package SeaView 4.5.3 confirming the presence of several species. Furthermore, sequences were also aligned with other closely related sequences found in the GenBank database using software. The tree was calculated with BioNJ which is an improved neighbor-joining algorithm based on a simple model of sequence data.

## **3.4 Results**

### **3.4.1 Locomotion**

Locomotion in benthic foraminifera is principally driven by pseudopodial extensions. Emergence of these extensions are differently observed in different species. In miliolids, initially, the specimen was lying horizontally on the glass surface and then, after 24 hours, a single strand of pseudopodia came out from the primary aperture along with the cytoplasmic streaming. After some time, the extension becomes elongated leading to further branching, hence, resulting in locomotion. Initially, the individual was moving in a straight line with slow speed but later on it adopted a speedy curved path. In miliolids, the individuals are found to move in the direction of the apertural opening. The average speed in *Quinqueloculina seminula* and *Quinqueloculina poeyana* are recorded between 0.32 to 0.41 centimeters per hour. The movement patterns recorded for both species are presented in Figure 4.

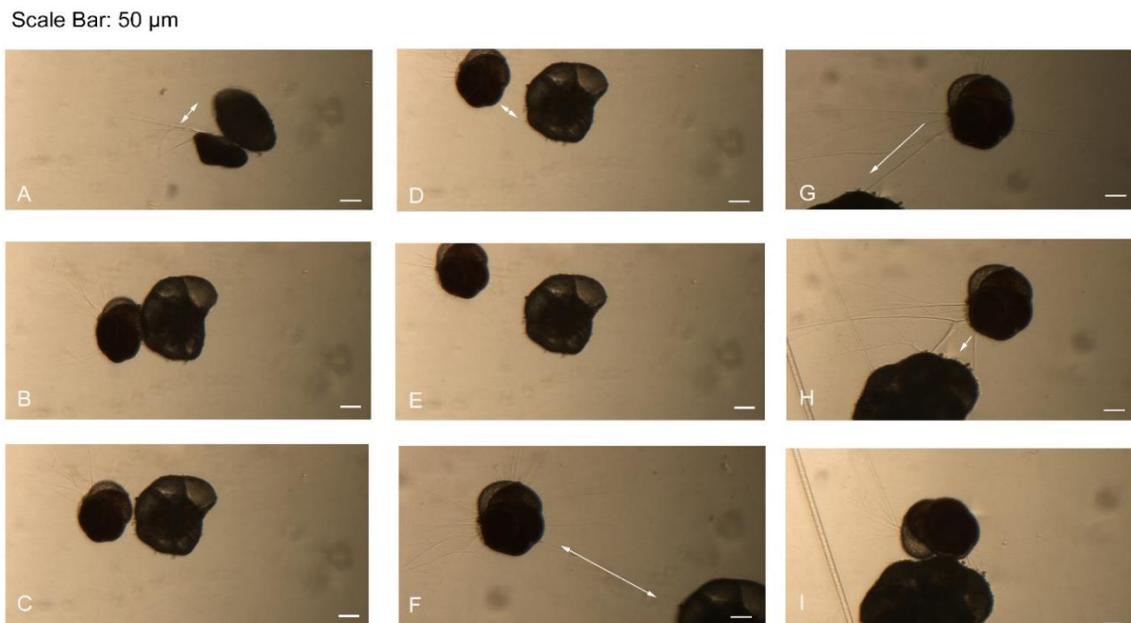
Scale Bar: 50  $\mu$ m



**Figure 4: Locomotion in two species of miliolids; (A-F) *Quinqueloculina seminula* (G-L) *Quinqueloculina poeyana***

Locomotion in species with supplementary apertures is relatively different from locomotion in miliolids. More specifically, in rotaliids, the individual extends its pseudopodia earlier than miliolids, i.e., after 8 hours staying in Petri plates. It attaches its pseudopodial extensions to the hard substrate and then the distally streaming protoplasm help drags the body forward. Furthermore, a clear cytoplasmic streaming of protoplasm showed bidirectional movements of viscous granules between the aperture and the tip of pseudopodia. However, the direction of movement was directly related to the apertural position and orientation of the foraminiferal test.

In rotaliids, individuals move in various directions depending upon the external stimuli. The average speed in *Glabrattellina* is recorded between 0.43 to 0.49 centimeters per hour. In the case of *Elphidium*, pseudopodia were observed in juveniles only with very little movement in hours. The photographic illustration is presented in Figure 5.



**Figure 5: (A-F) *Glabrattellina margaritaceus* pushing dead *Ammonia* away with its pseudopodial network (G-I) *G. margaritaceus* anchoring its pseudopodia with a hard biogenic substrate**

### 3.4.2 Relationship to Substrate

As it is mentioned earlier, extended pseudopodia in rotaliids anchor the living individual to the wall of hard substrate for locomotion; similarly, many species have shown a number of other living behaviors, such as hiding themselves beneath the substrate for protection and nutrition, in the presence of external/internal stimuli. For instance, miliolids move into the dark by hiding themselves under the sand particles due to light stimuli while examined under the light microscope (Figure 6).

Scale Bar: 50  $\mu\text{m}$

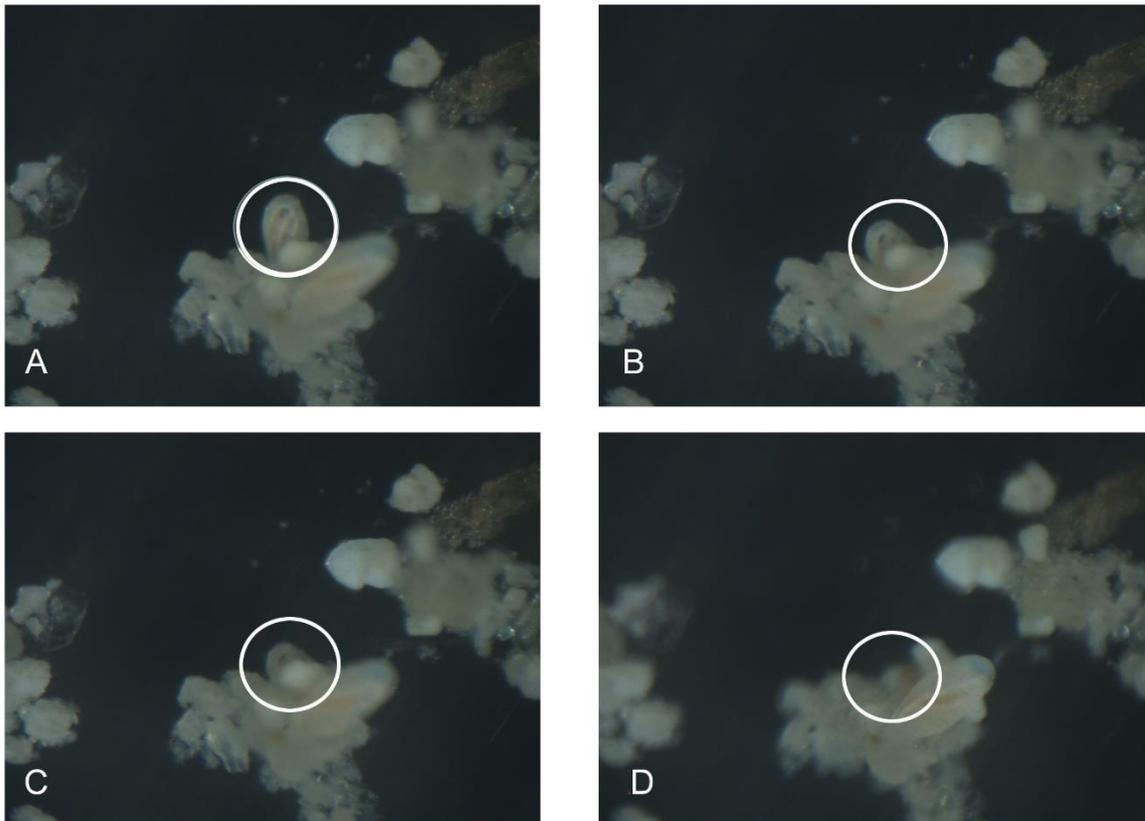
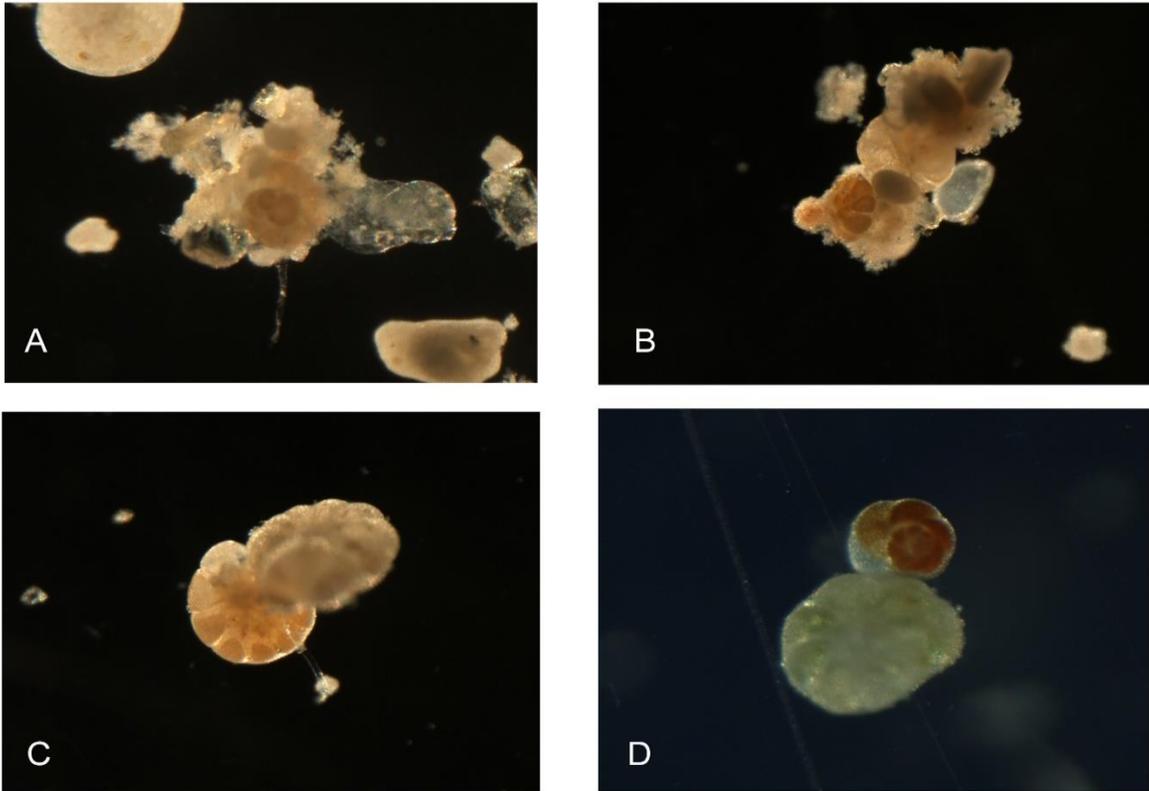


Figure 6: *Quinqueloculina seminula* hiding under the sediment substrate due to external light stimulus: (A) The individual is half hidden trying to move underneath the sediment clump (B-C) The individual is partially visible (D) The individual is completely hidden under the sediments.

Similarly, rotaliids love to attach their bodies onto biogenic hard substrates in order to get nutrition and protection. The observation was stronger for juveniles compared to adults who were able to survive independently. Furthermore, most of the living individuals were found to gather small sand particles around their bodies using their pseudopodia. The attachment was not limited to non-living substrate only as some of the living individuals were also stick to the other living foraminifera. The overall response of benthic foraminifera towards biogenic substrate is shown in Figure 7.

Scale Bar: 50  $\mu\text{m}$



**Figure 7: Living foraminifera adhering to hard biogenic substrates for protection and/or nutrition, (A) *Ammonia tepida* attracting sand particles (B) *Ammonia tepida* attaching to biogenic substrates and sand particles (C) *Ammonia* sp. adhering to a dead *Ammonia* (D) *Glabratella margaritaceus* attaching to a dead *Ammonia* with pseudopodial extensions**

### 3.4.3 Preservation of Pseudopodia

The visible pseudopodial extensions were preserved in an accidental observation in which live specimens were placed in the Petri plates containing seawater for more than a week. Resultantly, their pseudopodia were preserved in the cubic crystals of salt that precipitated from the seawater (Figure 8).

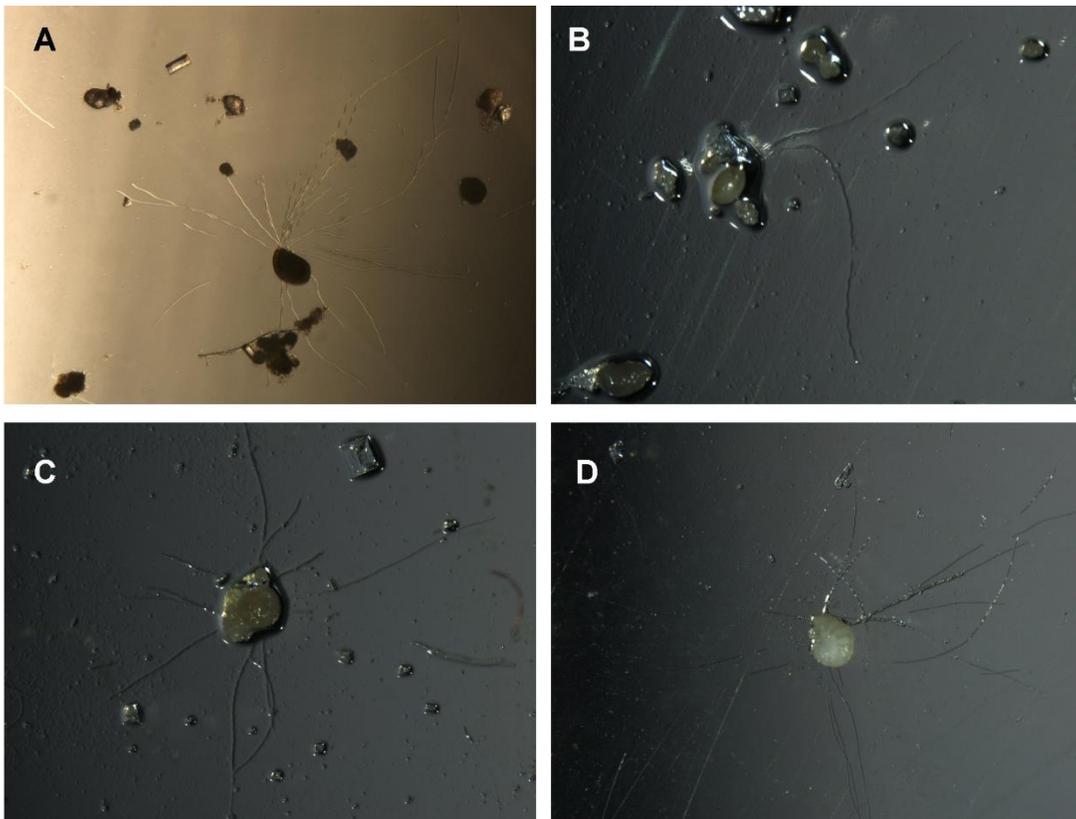


Figure 8: Preservation of pseudopodia in seawater salt crystals: (A) *Quinqueloculina* sp. 1; (B) *Quinqueloculina* sp. 2; (C) *Ammonia* sp. (D) *Elphidium advenum*

### 3.4.4 Symbionts-Bearing Foraminifera

Similar to the attachment of living individuals with other benthic fauna, some benthic foraminifera are found to host symbiotic algae in their protoplasm. The samples obtained from eastern Bahrain had good number of symbiont-bearing individuals, whereas no specimens were found in the samples obtained from the Saudi coastline. Furthermore,

there were more symbiont-bearing rotaliids than miliolids, i.e., 5-10 individuals vs. and 1-3 individuals per 5cc of sediments. Different specimens of symbiont-bearing foraminifera are shown in Figure 9.

Scale Bar: 50  $\mu$ m

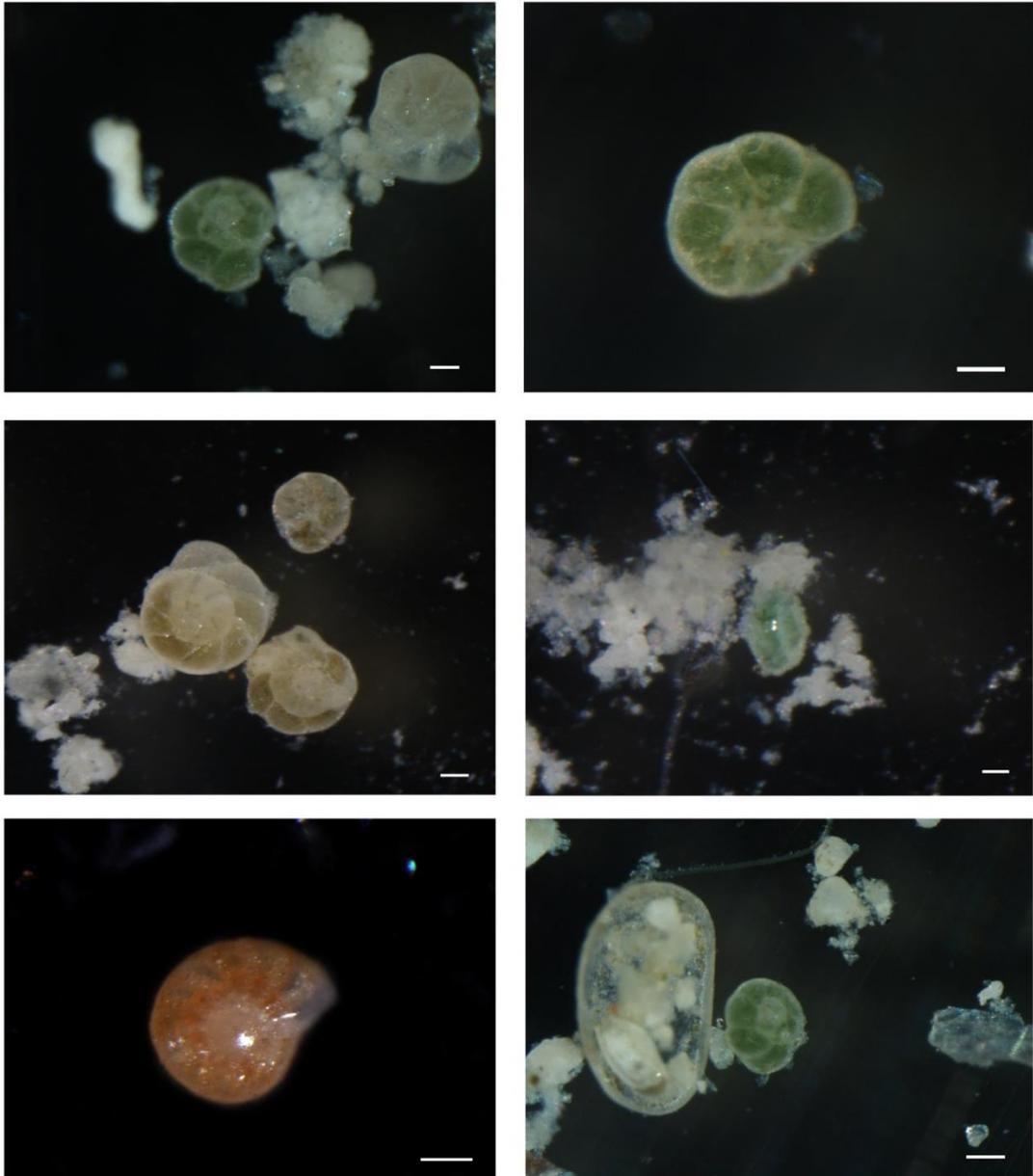


Figure 9: Light microscope photographs of symbiont-bearing foraminifera

### 3.4.5 Survival Response

This experiment was conducted for the project entitled “Forams in Space” under the Student Space Flight Experiment Program (SSEP). The ultimate objective was to study the bone loss in humans especially the calcium for the astronauts who spend a long time in space. Therefore, foraminifera were targeted because of their nature as calcium-carbonate producing organisms. Although, the project was designed by Hill Country Middle School, Austin, however, we conducted this experiment to answer the question that, how long forams can remain alive in a closed jar.

The results of Kaplan Meier analysis illustrated that the survivorship of *Quinqueloculina seminula* was higher than that of *Quinqueloculina poeyana*. Similarly, *Glabratellina* sp. survived longer than the *Ammonia* sp. Comparatively, the survivorship of rotaliids was higher than the miliolids as their pseudopodial network was observed even after the second day of staying in a glass jars. The graphical illustration of the analysis is shown in Figure 10.

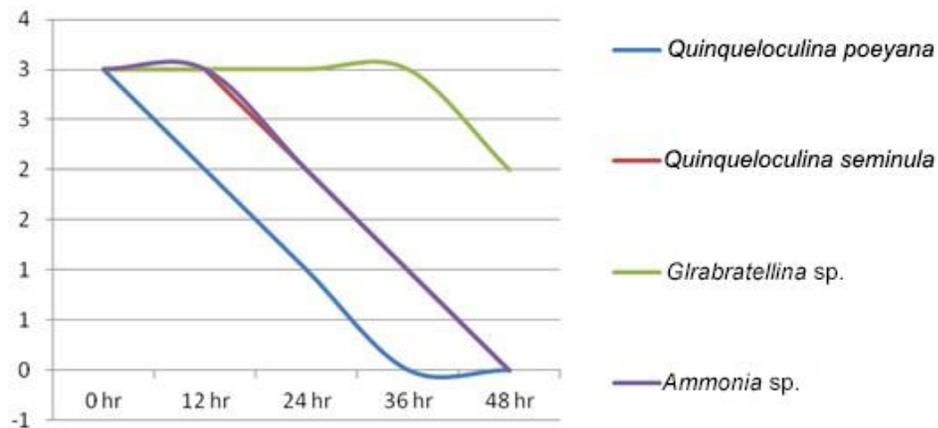


Figure 10: Kaplan-Meier analysis illustrating survival response for different species of rotaliids and miliolids.

### 3.4.6 Plastogamy: The Reproduction Phase

Among all living individuals, *Glauertella margaritaceus* is found to be involved in plastogamy during laboratory experiments. The observations revealed that 2 or more gamonts were joined together (not centered directly on one another) by their apertural sides to mutually exchange gametes. Initially, a cementing membrane bounded both sides to mutually exchange gametes. Initially, a cementing membrane bounded both individuals together resulting into formation of a single chamber. By separating the individuals with needle, opposing apertural sides and internal septa were found dissolved (Figure 11-C).

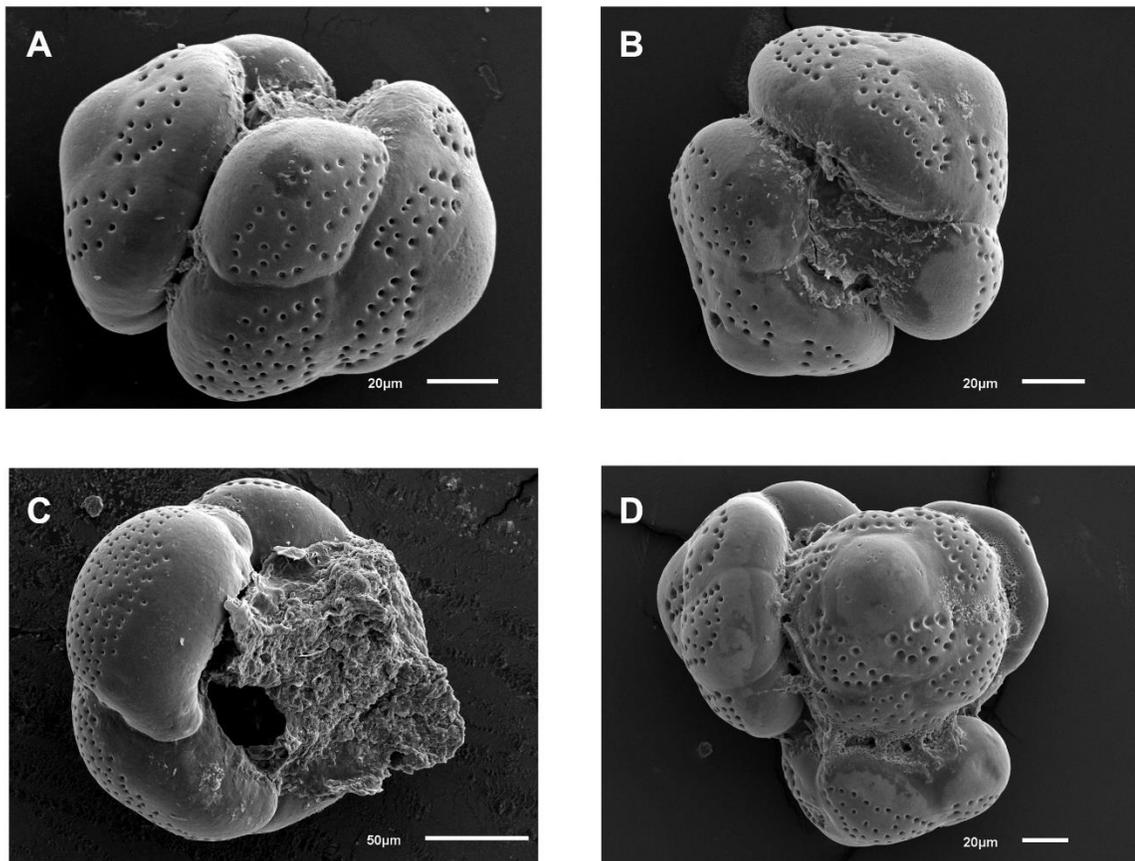


Figure 11: Plastogamy in *Glauertella margaritaceus*, (A-B) Plastogamous pair, attached by their apertural sides (C) Needle separated individual showing internal chamber and cementing material (D) Plastogamous complex of 5 individuals.

### 3.4.7 Systematics and Species Reports

Kingdom CHROMISTA

Subkingdom HAROSA

Infrakingdom RHIZARIA

Phylum FORAMINIFERA

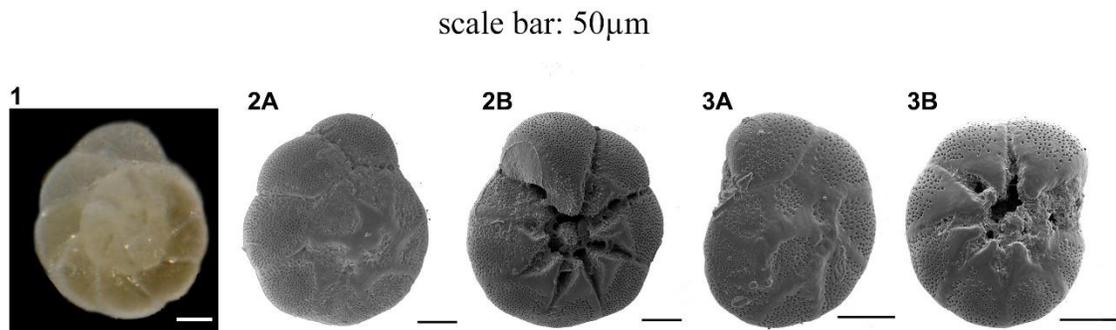


Figure 12: Different individuals of *Ammonia parkinsoniana*; (1) Light microscope photograph of a living specimen (2-3) SEM photographs: A) dorsal view and 2) ventral view.

**Original name:** *Rosalina parkinsoniana* (d'Orbigny, 1839)

#### Synonymised names

- *Ammonia beccarii* var. *parkinsoniana* (d'Orbigny, 1839)
- *Ammonia parkinsoniana* (d'Orbigny, 1839)
- *Rosalina parkinsoniana* d'Orbigny, 1839 (Synonym)
- *Streblus beccarii* var. *parkinsoniana* (d'Orbigny, 1839)

**Relevant Literature:** Boltovskoy, E., Giussani, G., Watanabe, S., & Wright, R. (1980). Atlas of benthic shelf foraminifera of the southwest Atlantic, W. Junk, 147 pp.

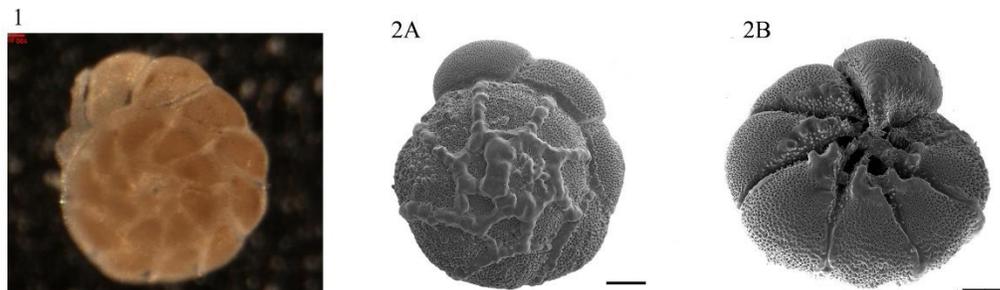
Sen Gupta, B. K., Smith, L. E., and Machain-Castillo. M. L. (2009). Foraminifera of the Gulf of Mexico, Pp. 87–129 in Felder, D.L. and D.K. Camp (eds.), Gulf of Mexico—Origins, Waters, and Biota. Biodiversity. Texas A&M Press, College Station, Texas.

**Morphological description:** Test outline circular, peripheral margin rounded;  $2\frac{1}{2}$  volutions, the last of which contains 8 chambers; wall yellow to yellow-brown, coarsely perforate; on the spiral side, early septal sutures limbate, spiral sutures depressed in the final whorl; on umbilical side, sutures depressed and leading into an umbilical cavity which may be filled with a knob; aperture a narrow slit at the base of the last chamber.

**Distribution in study areas:** This is an indigenous species, abundant in both areas, i.e. eastern Bahrain and the Saudi Coastline.

*Ammonia tepida* (Cushman, 1926)

scale bar: 50µm



**Figure 13: *Ammonia tepida* (1) Light microscope photograph (2) SEM photograph: A) dorsal view and 2) ventral view.**

### **Synonymised names**

- *Ammonia beccarii* subsp. *tepida* (Cushman, 1926)
- *Ammonia beccarii* var. *tepida* (Cushman, 1926)
- *Rotalia beccarii* var. *tepida* (Cushman, 1926)
- *Streblus beccarii* var. *tepida* (Cushman, 1926)

**Relevant Literature:** Hayward, B.W., Buzas, M.A., Buzas-Stephens, P., Holzmann, M., 2003. The lost types of *Rotalia beccarii* var *tepida* Cushman, 1926. *Journal of Foraminiferal Research*, 33, 352-354.

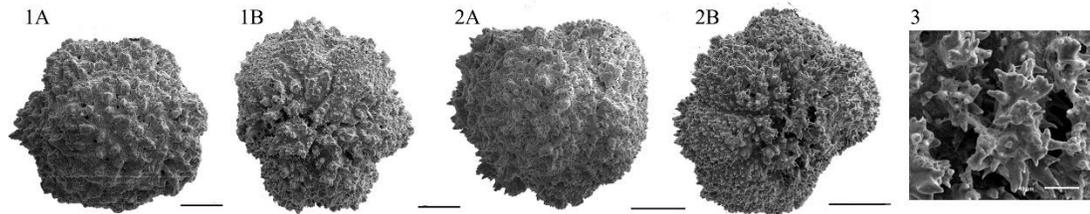
**Morphological description:** Test biconvex, with low trochospiral coil of 3 to 4 volutions, spiral side evolute, umbilical side involute, and may have large umbilical plug surrounded by umbilical fissure, final whorl with deeply incised umbilical, radial, and intraseptal spaces, sutural fissure straight or branching and appeared feathered on the umbilical side, umbilical and intraseptal spaces of earlier whorls filled with secondary lamellae, leaving only one vertical passage from each chamber of the penultimate whorl to the junction of the umbilical fissure and intraseptal spaces of the final whorl, early chambers closed toward umbilicus, no spiral canal present, periphery rounded to carinate; wall calcareous, optically radial, primarily bilamellar, moderately coarsely perforate, both surface may be ornamented by pillars and umbilical side may have transverse ridges, resulting from the feathered umbilical sutures when these are present; primary aperture an interiomarginal extraumbilical arch, bordered by a protruding lip and the umbilical end, space between the lip and the umbilical pillars may be filled by secondary lamellae and new chambers form.

**Distribution in study areas:** an indigenous species, commonly observed in both eastern Bahrain and Saudi Coastline, especially in dead assemblages.

Genus MURRAYINELLA Farias, 1977

*Murrayinella* sp. 1

scale bar: 50µm



**Figure 14: (1-2) SEM photographs OF Two different individuals of *Murrayinella* sp. 1 (A) dorsal view and (B) ventral view; (3) SEM photograph of Test wall**

**Relevant Literature:** Loeblich, A.E, and Tappan, H.N. 1987. Foraminiferal genera and their classification. Vol. 1. Van Nostrand Reinhold.

**Morphological description:** Test small, up to 0.2 mm in diameter, trochospirally enrolled, 4 to 6 rapidly enlarging, inflated to globular chambers forming about two and half whorl, sutures deeply depressed, umbilicus closed, periphery broadly rounded, peripheral outline lobulate; wall calcareous, hayaline, perforate, surface rugose to hispid; aperture a low interiomarginal slit, apparently extraumbilicall; sexual reproduction plastogamic.

**Distribution in study areas:** The species is recognized in organically enriched localities, i.e., eastern Bahrain (boat harbor transect) and Saudi Coastline (Sofitel hotel).

Superfamily GLABRATELLOIDEA

Family GLABRATELLIDAE

Genus GLABRATELLINA Seiglie & Bermúdez, 1965

*Glabratellina* sp. 1

scale bar: 50µm

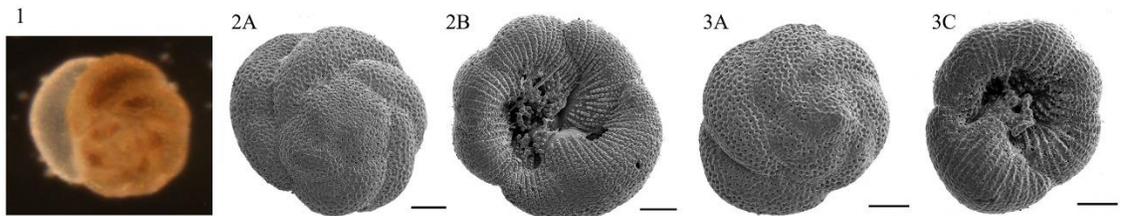


Figure 15: Different individuals of *Glabratellina* sp. 1; (1) Light microscope photograph of a living specimen (2-3) SEM photographs: A) dorsal view and 2) ventral view.

**Synonymised names**

- *Crumia* McCulloch, 1977 (subjective synonym)
- *Sabinia* McCulloch, 1977 (Junior homonym of *Saninia* Parona, 1909))
- *Sabinina* McCulloch, 1981 (subjective synonym)

**Relevant Literature:** Loeblich, A.E, and Tappan, H.N. 1987. Foraminiferal genera and their classification. Vol. 1. Van Nostrand Reinhold.

**Morphological description:** The specimens coarsely perforated, with globular chambers. Tests small, trochospiral, planoconvex to concavoconvex, few whorls of five to six chambers, that appears crescentic on the gently domed spiral side and subtriangular on

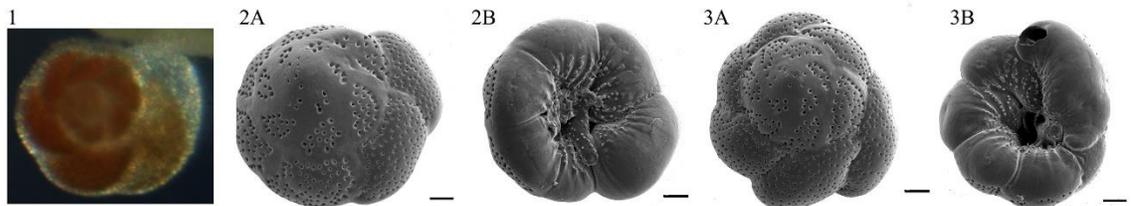
the centrally concave umbilical side, sutures flush, oblique and gently curved on the spiral side, nearly radial and sinuate on the umbilical side, periphery rounded: wall calcareous, finely perforate, smooth on spiral side, umbilical side with radiating granular or finely pustulose striae; aperture interiomarginal, probably just interior to umbilical flap.

**Distribution in study areas:** The *Glabrattella* sp. 1 is recognized in eastern Bahrain with high population in polluted locality. In contrast to this, the species is not found in Saudi Coastline sandy substrate but in carbonates sediments (Zabnah Lagoon).

Genus GLABRATELLA Doreen, 1948

*Glabrattella margaritaceus*

scale bar: 20 $\mu$ m



**Figure 16:** Different individuals of *Glabrattella margaritaceus*; (1) Light microscope photograph of a living specimen (2-3) SEM photographs: A) dorsal view and 2) ventral view.

**Relevant Literature:** Hayward, B.W., Grenfell, H.R., Reid, C.M., Hayward, K.A. 1999. Recent New Zealand shallow-water benthic Foraminifera: Taxonomy, ecologic distribution, biogeography, and use in paleoenvironmental assessment. Institute of Geological and Nuclear Sciences Monograph, 21, 258 p.

Loeblich, A.E, and Tappan, H.N. 1987. Foraminiferal genera and their classification. Vol. 1. Van Nostrand Reinhold.

**Morphological description:** Test enroll in low trochospiral coil, chambers inflated and globular, enlarging rapidly as added, four to five in the final whorl, sutures curved, depressed, periphery rounded; wall calcareous, finely perforated but may be more coarsely perforate on the spiral side, surface smooth, except for radial striaea and rows of pustules leading to the umbilicus; aperture a low interiomarginal slit; sexual reproduction plastogamic.

**Distribution in study areas:** The species is recognized in eastern Bahrain in both polluted and unpolluted localities. In contrast to this, no specimens are observed on the Saudi Coastline.

Family ELPHIDIIDAE

Genus ELPHIDIUM de Montfort, 1808

*Elphidium advenum* (Cushman, 1922)

scale bar: 50µm

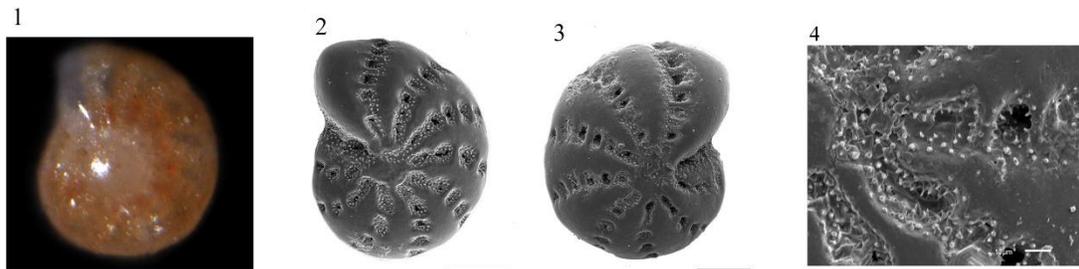


Figure 17: *Elphidium Advenum*; (1) Light microscope photograph of a living specimen (2-3) SEM photographs of each side of the individual (4) SEM photograph of test wall

**Synonymised names**

- *Cribrononion advenum* (Cushman, 1922) (Synonym)
- *Elphidium advenum* subsp. *advenum* (Cushman, 1922)
- *Polystomella advena* Cushman, 1922 (Synonym)

**Relevant Literature:** Gross, O. 2015. *Elphidium advenum* (Cushman, 1922). In: Hayward, B.W., Cedhagen, T., Kaminski, M., Gross, O. 2015. World Foraminifera Database. Accessed through: Hayward, B.W., Cedhagen, T., Kaminski, M., Gross, O. (2015) World Foraminifera Database.

**Morphological description:** Test planispiral, bilaterally symmetrical; sutural canal system opens into a single row of pores; septal bridges usually hollow and contain a retral process; aperture a series of large circular pores at base of aperture face.

**Distribution in study area:** The specie is rare in the modern (living) population; but most often in dead assemblages; highly abundant in sandy beaches on the Saudi Coastline more importantly near the Movenpick resort on Half-Moon Bay.

**Class TUBOTHALAMEA**

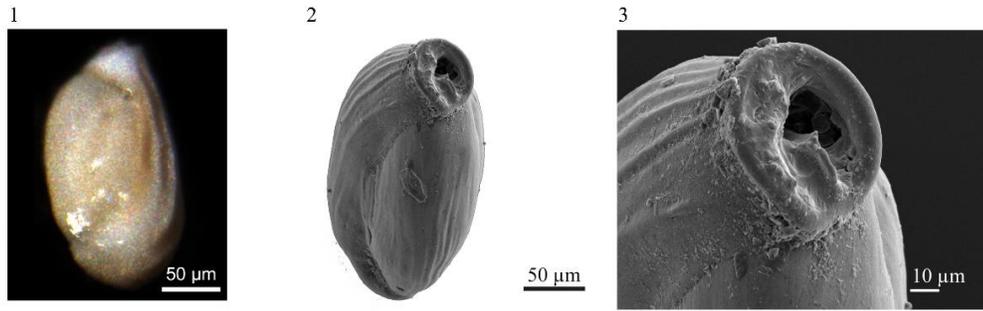
Order MILIOLIDA

Superfamily MILIOLOIDEA

Family HAUERINIDAE

Genus QUINQUELOCULINA d'Orbigny, 1826

*Quinqueloculina poeyana* d'Orbigny, 1839



**Figure 18:** *Quinqueloculina poeyana*; (1) Light microscope photograph of a living specimen (2-3) SEM photographs

**Relevant Literature:** Parker, J.H. 2009. Taxonomy of foraminifera from Ningaloo Reef, Western Australia. *Memoirs of the Association of Australasian Palaeontologists*, 36, 1-810.

B. K. Sen Gupta, L. E. Smith, and M. L. Machain-Castillo. 2009. Foraminifera of the Gulf of Mexico, Pp. 87–129 in Felder, D.L. and D.K. Camp (eds.), *Gulf of Mexico—Origins, Waters, and Biota. Biodiversity*. Texas A&M Press, College Station, Texas.

**Morphological description:** Test elongate, quinqueloculine, about three times higher than wide, roundly triangular in cross-section, oral end truncated and slightly flaring, aboral end rounded and slightly inflated, periphery rounded; chambers long and thin, widest at the aboral end, thinning and slightly flaring at the oral end; sutures indistinct, depressed and broadly curved, subparallel to test axis; aperture areal in terminal face of final chamber, not produced, low arch-shaped, with large flattened area between aperture and suture; apertural rim thickened and everted around top, with tooth; tooth low, bifurcate, trough shaped; wall smoothly finished, matte.

**Distribution in study areas:** The species is recognized in eastern Bahrain.

*Quinqueloculina seminula* (Linnaeus, 1758)

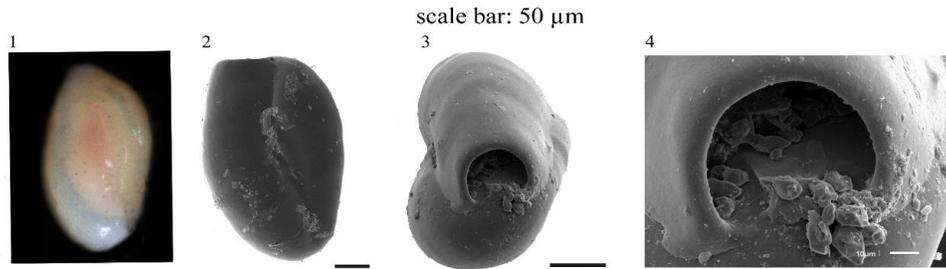


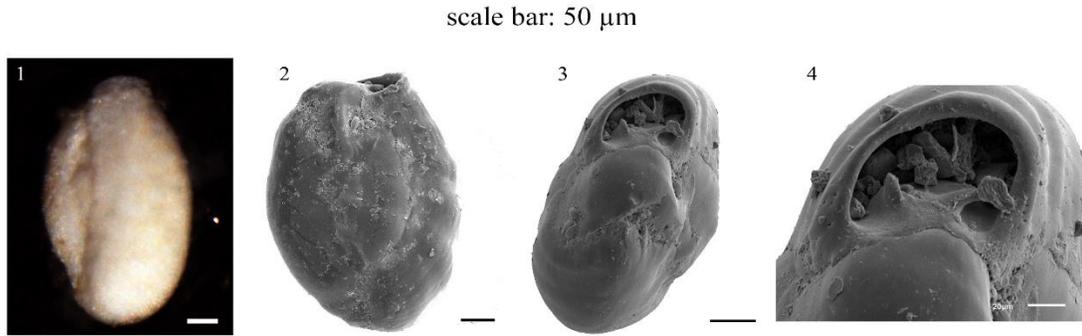
Figure 19: *Quinqueloculina seminula*; (1) Light microscope photograph of a living specimen (2-4) SEM photographs

**Relevant Literature:** Parker, J.H. 2009. Taxonomy of foraminifera from Ningaloo Reef, Western Australia. *Memoirs of the Association of Australasian Palaeontologists*, 36, 1-810.

**Morphological description:** Test elongate, ovate in lateral view, ovate in cross-section, oral end truncated, aboral end slightly produced, periphery rounded; coiling quincloculine throughout; chambers slightly inflated aborally; sutures depressed, slightly curved; aperture an arch-shaped opening, with tooth; tooth short, bifid, V-shaped, on short stem; wall opaque, smoothly finished, polished.

**Distribution in study areas:** The species is recognized in eastern Bahrain only in unpolluted locality. However, dead individuals were found in old assemblages.

*Quinqueloculina* sp. 1



**Figure 20: *Quinqueloculina* sp. 1; (1) Light microscope photograph of a living specimen (2-4) SEM photographs**

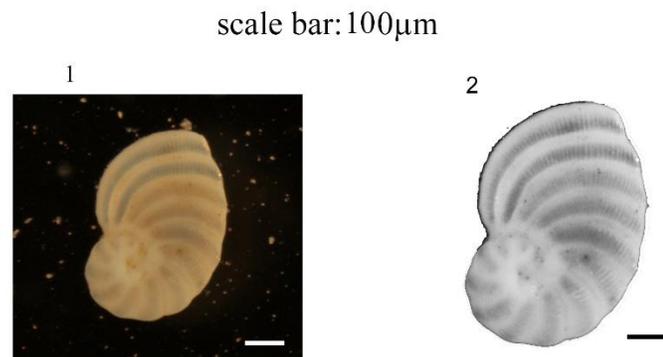
**Distribution in study areas:** The species is recognized in eastern Bahrain only in unpolluted locality. However, dead individuals were found in old assemblages.

Superfamily SORITOIDEA

Family PENEROPLIDAE

Genus PENEROPLIS de Montfort, 1808

*Peneroplis proteus* d'Orbigny, 1839



**Figure 21: *Peneroplis proteus* (1) Light microscope photograph of a living specimen (2) SEM photographs**

**Relevant Literature:** Loeblich, A.R., and Tappan, H.N. 1987. Foraminiferal genera and their classification. Vol. 1. Van Nostrand Reinhold.

**Morphological description:** Test compressed, early stage planispirally enrolled and involute, later chambers rapidly increasing in breadth and strongly arched but of nearly constant height resulting in a flaring test, interior of chambers not subdivided, sutures slightly depressed; wall calcareous, porcelaneous, perforate in juveniles stage, later imperforate, surface with numerous striae or grooves alternating with fine ribs aligned parallel to the test periphery, fine pseudopores commonly present in the grooves between the surface ribs, aperture in the adult consisting of a linear or alternating series of large, circular to oval or irregular pores, each bordered by a distinct elevated lip.

**Distribution in study areas:** The species is recognized in eastern Bahrain as well as in Saudi coastline especially in the area of Zabnah on Half-Moon Bay. The dead assemblages were more often in both localities.

Genus MONALYSIDIUM Chapman, 1900

*Monalysidium* sp. 1

scale bar: 100µm

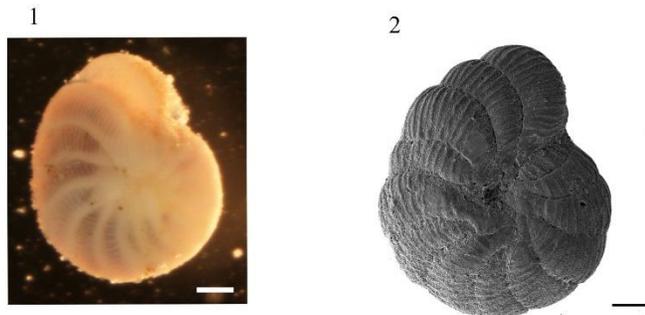


Figure 22: *Monalysidium* sp. 1; (1) Light microscope photograph of a living specimen (2) SEM photographs

### Synonymised names

- *Dendritina* (*Monalysidium*) Hofker, 1951 (Opinion of Loeblich & Tappan, 1987)
- *Peneroplis* (*Monalysidium*) Chapman, 1900
- *Spirolina* (*Monalysidium*) Chapman, 1900

**Relevant Literature:** Loeblich, A.R., and Tappan, H.N. 1987. Foraminiferal genera and their classification. Vol. 1. Van Nostrand Reinhold.

**Morphological description:** Test small, with relatively large planispiral coil, consisting of about nine ovoid chambers, later uncoiling and rectilinear, with short inflated chambers, sutures distinct and constricted, radial in the coiled stage, horizontal in the rectilinear stage; wall calcareous, porcelaneous, thin, surface smooth, distinctly punctuate; aperture terminal, simple, circular, bordered by and everted lip with a fimbriate or scalloped margin.

**Distribution in study areas:** The species is recognized in eastern Bahrain as well as in Saudi coastline especially in the area of Zabnah on Half-Moon Bay.

Genus COSCINOSPIRA Ehrenberg, 1839

*Coscinospira* sp. 1

scale bar: 100µm



**Figure 23:** *Coscinospira* sp. 1; (1) Light microscope photograph of a living specimen (2-3) SEM photograph

**Relevant Literature:** Loeblich, A.R., and Tappan, H.N. 1987. Foraminiferal genera and their classification. Vol. 1. Van Nostrand Reinhold.

**Morphological description:** Test free, spiroline, large, up to 2.2 mm in length, slightly flattened, early stage planispirally enrolled and evolute with numerous chambers per whorl, biumbilicate, later uncoiling with up to six inflated rectilinear chambers, sutures radial and straight to slightly arched in the coiled stage, horizontal in the uncoiled part; wall calcareous, porcelaneous, surface longitudinally finely striate; aperture terminal, cribrate, of numerous rounded pores centered in the terminal face.

**Distribution in study areas:** The species is recognized in eastern Bahrain as well as in Saudi coastline especially in the area of Zabnah in Half-Moon Bay. The dead assemblages were high in numbers in both localities.

### 3.4.8 Molecular Characterization

For sequence analysis, fragment of the rRNA subunits are amplified for the species of *Ammonia*, *Glabratellina*, *Peneroplis*, *Elphidium* and miliolids. Resultantly, only 13 amplifications are obtained against 26 specimens; and, unfortunately, no amplification was obtained for miliolids and *Peneroplis* species. For the successful amplifications, more than one clone was sequenced. Finally, circular phylogenetic tree was generated by the BioNJ method which revealed the presence of five monophyletic clades, comprising two distinct clades for *Ammonia*, one for *Glabratellina*, one for *Murrayinella*, and one for *Elphidium*. The relative groups are shown in Figure 24.

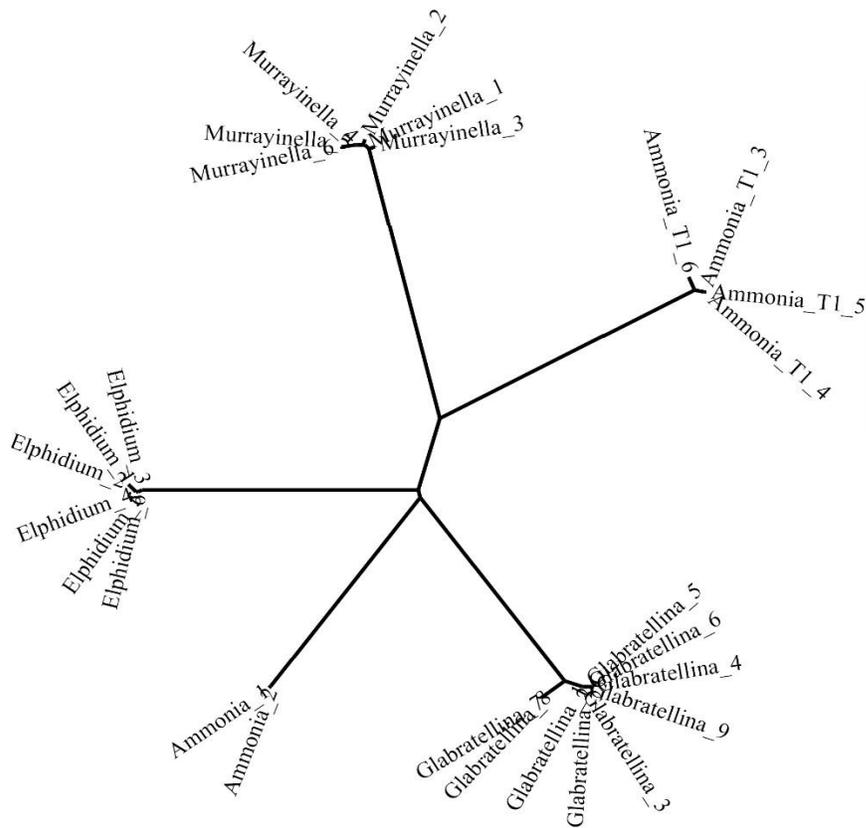


Figure 24: Circular (phylogenetic) tree illustrating clustering among different sequences

Hereby, two clades of *Ammonia* (i.e. **Ammonia\_T1** and **Ammonia**) apparently reflect the presence of two species, earlier reported as *Ammonia tepida* and *Ammonia parkinsoniana*. However, BLAST analysis of clad **Ammonia\_T1** with *A. tepida* (Accession Number: AF533835) revealed 88% similarity; while clad **Ammonia** did not show any significant similarity with *A. parkinsoniana* (Accession Number: X99824). Besides this, within the NCBI database, the clad **Ammonia** have shown 94% similarity with the 18S rRNA sequence of *Ammonia* sp. 124, while **Ammonia\_T1** reflects 98% similarity with *Ammonia* sp. 646, previously submitted to NCBI.

The sequence similarity of *Murrayinella* species are not significant for any of the previously submitted sequences, however, they are found most closely related to the *Murrayinella globosa*, *Schlumbergerella floresiana*, *Calcarina defrancii*, and *Haynesina germanica*. Similarly, *Glabratellina* sequence are found similar to the *Planoglabratella opercularis*, *Angulodiscorbis quadrangularis*, and *Glabratella opercularis*; and *Elphidium* sequence showed close association with the previously submitted sequences of *Elphidium aculeatum*, and *Elphidium macellum*. The detailed description of the sequences including their similarity with other sequences of NCBI is presented in Table 1.

After adding the **Ammonia** sequence to rotaliids alignment, instead of general alignment, it turned out that the clad **Ammonia** belongs to clad **Ammonia\_T1**. The phylogenetic trees for each group, inferred with Maximum Likelihood (ML) algorithm, reflect that the two clades of *Ammonia* are monophyletic as shown in Figure 25. Therefore, at certain extent, molecular study reveals that both groups of *Ammonia* belong to the same species including **Ammonia\_T1**, which had been erroneously referred to as *A. tepida* in earlier

publications. Similarly, trees for other groups showed monophyletic lineage as shown in Figure 26-28.

**Table 1: BLAST report and similarity of obtained sequences with NCBI database**

Species	DNA (bp)	Highest Similarity in NCBI Database	% Similarity	Accession Number
Ammonia_1	1,060	<i>Ammonia</i> sp. 124 partial 18S rRNA gene, isolate 124, clone 1	94	HE598562.1
Ammonia_2	1,060	<i>Ammonia</i> sp. 124 partial 18S rRNA gene, isolate 124, clone 1	94	HE598562.1
Ammonia_T1_3	1,069	<i>Ammonia</i> sp. 646 partial 18S rRNA gene, isolate 646, clone 1	98	HE598563.1
Ammonia_T1_4	1,069	<i>Ammonia</i> sp. 646 partial 18S rRNA gene, isolate 646, clone 1	98	HE598563.1
Ammonia_T1_5	1,076	<i>Ammonia</i> sp. 646 partial 18S rRNA gene, isolate 646, clone 1	96	HE598563.1
Ammonia_T1_6	1,070	<i>Ammonia</i> sp. 646 partial 18S rRNA gene, isolate 646, clone 1	94	HE598563.1
Murrayinella_1	948	<i>Murrayinella globosa</i> partial 18S rRNA gene, isolate 18070, clone 34	88	LN714808.1
Murrayinella_2	900	<i>Schlumbergerella floresiana</i> 18S rRNA gene, isolate 2482	85	FM877705.1
Murrayinella_3	941	<i>Calcarina defrancii</i> 18S rRNA gene, isolate 866	90	FM877704.1
Murrayinella_4	940	<i>Calcarina defrancii</i> 18S rRNA gene, isolate 866	90	FM877704.1
Murrayinella_5	916	<i>Haynesina germanica</i> isolate 2732.2 small subunit ribosomal RNA gene	83	KF042529.1
Murrayinella_6	916	<i>Haynesina germanica</i> isolate 2732.2 small subunit ribosomal RNA gene	83	KF042529.1
Glabratellina_1	1,023	<i>Planoglabratella opercularis</i> partial 18S rRNA gene, isolate 18053, clone 1	92	LN714815.1
Glabratellina_2	1,023	<i>Planoglabratella opercularis</i> partial 18S rRNA gene, isolate 18053, clone 1	92	LN714815.1
Glabratellina_3	1,023	<i>Planoglabratella opercularis</i> partial 18S rRNA gene, isolate 18053, clone 1	92	LN714815.1
Glabratellina_4	1,021	<i>Planoglabratella opercularis</i> partial 18S rRNA gene, isolate 18053, clone 1	92	LN714815.1
Glabratellina_5	1,023	<i>Planoglabratella opercularis</i> partial 18S rRNA gene, isolate 18053, clone 1	92	LN714815.1
Glabratellina_6	1,023	<i>Planoglabratella opercularis</i> partial 18S rRNA gene, isolate 18053, clone 1	92	LN714815.1
Glabratellina_7	1,025	<i>Angulodiscrobis quadrangularis</i> isolate AQ159 small subunit rRNA gene	94	AF194076.1
Glabratellina_8	1,019	<i>Angulodiscrobis quadrangularis</i> isolate AQ159 small subunit rRNA gene	93	AF194076.1
Glabratellina_9	1,024	<i>Glabratella opercularis</i> SSU rRNA gene (partial)	92	Z69614.1
Elphidium_1	808	<i>Elphidium aculeatum</i> SSU rRNA gene (partial)	81	Z69618.1
Elphidium_2	808	<i>Elphidium aculeatum</i> SSU rRNA gene (partial)	81	Z69618.1
Elphidium_3	815	<i>Elphidium macellum</i> isolate 6228.2 small subunit rRNA gene	85	JN655702.1
Elphidium_4	811	<i>Elphidium macellum</i> isolate 6228.2 small subunit rRNA gene	86	JN655702.1

PhyML ln(L)=-13622.6 2164 sites GTR 4 rate classes

0.2

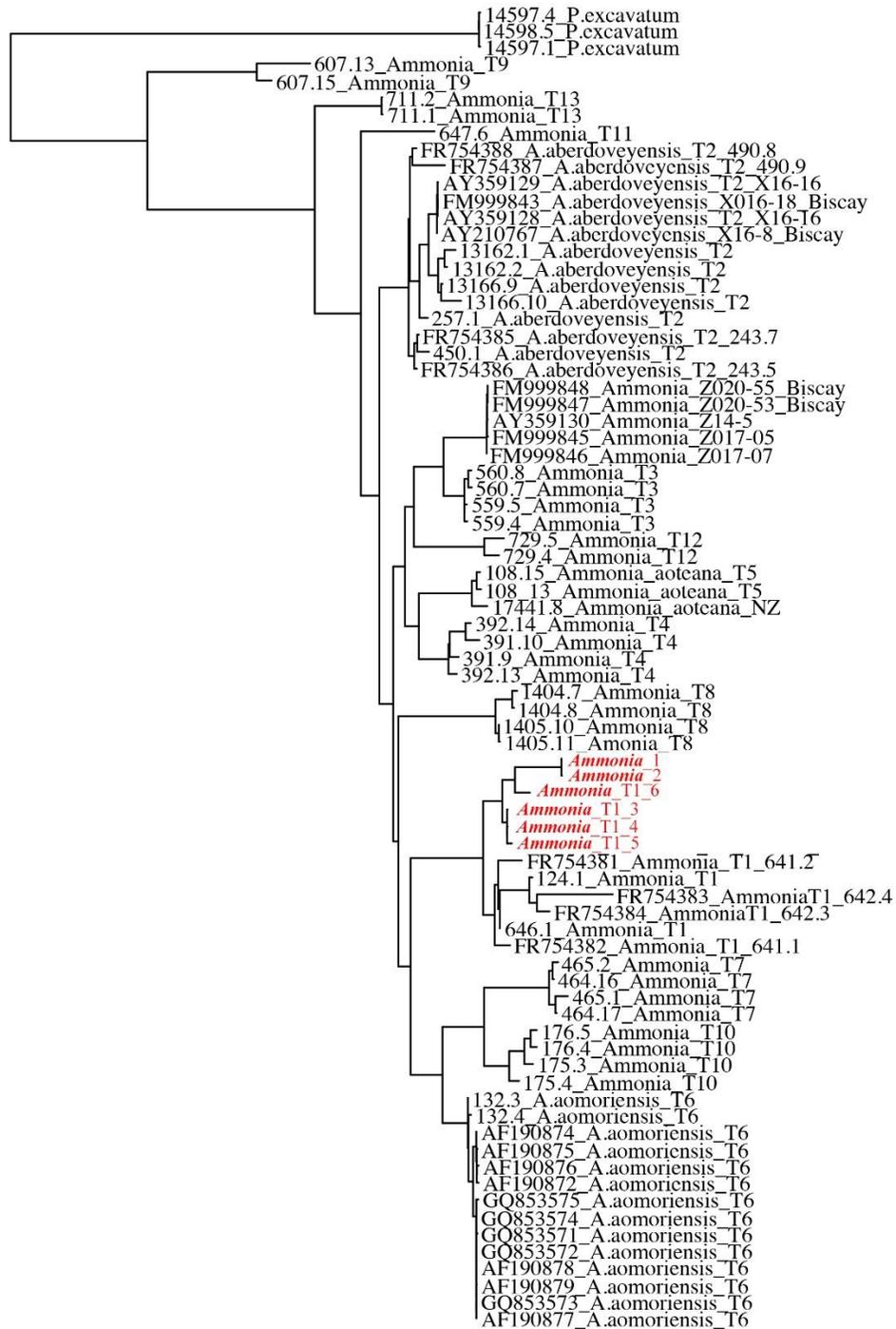
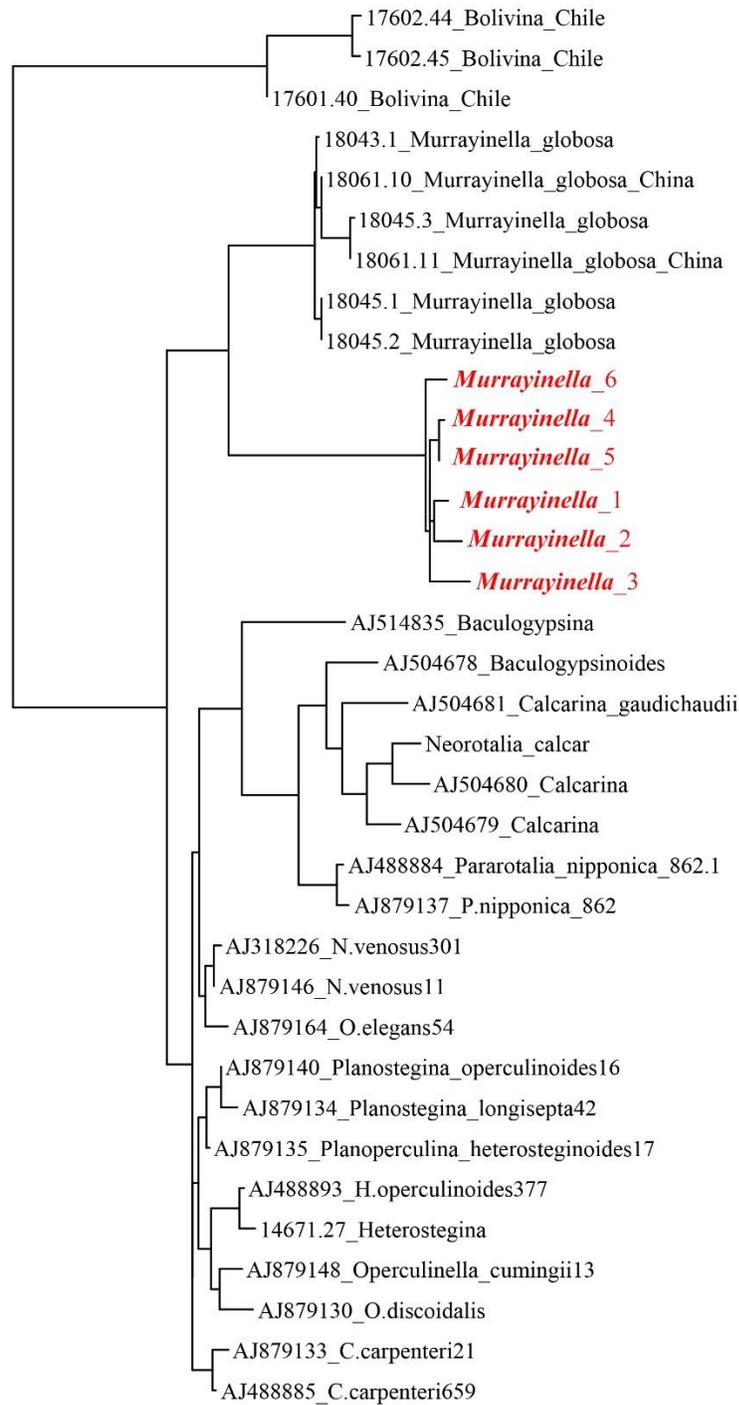


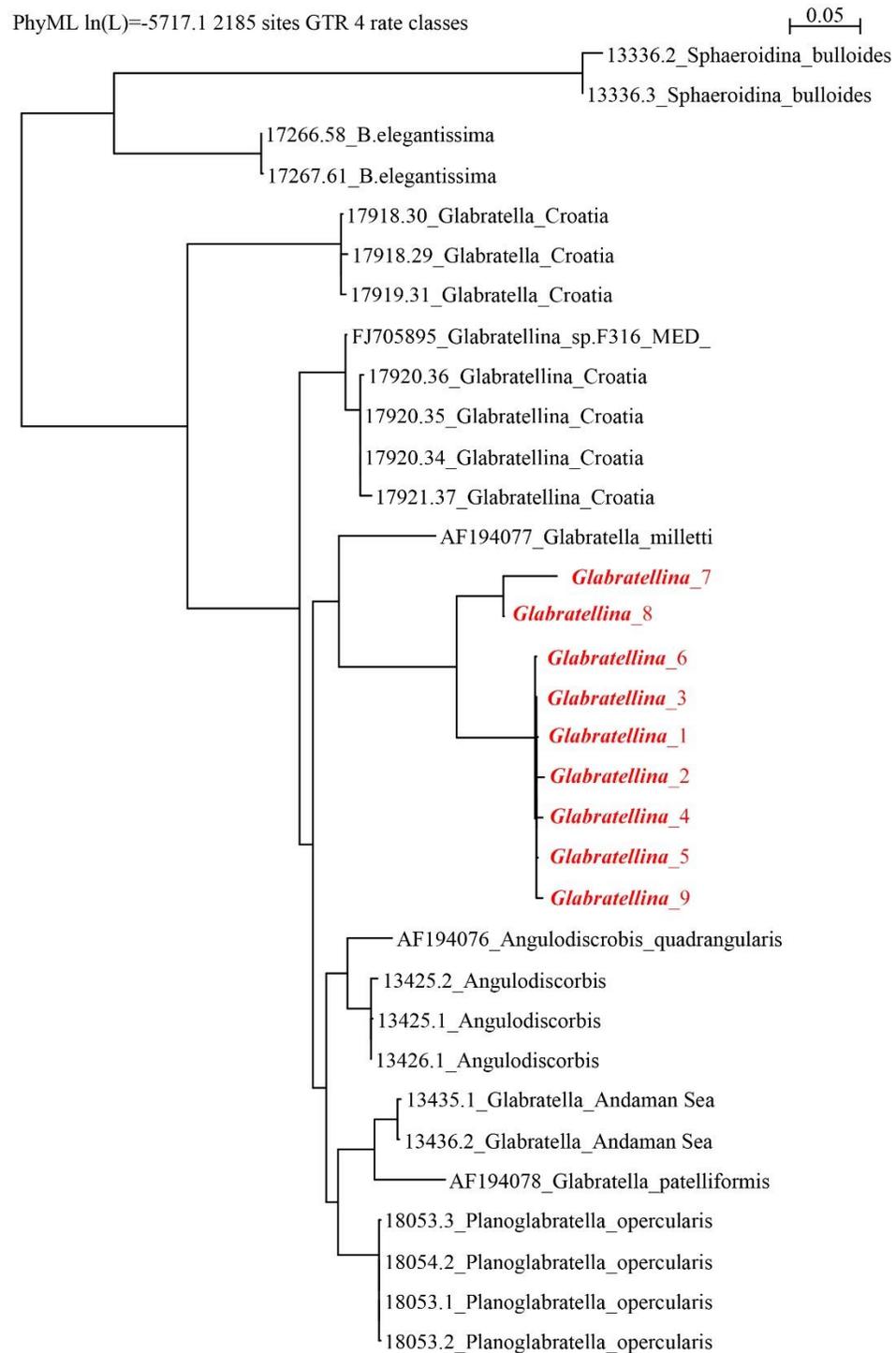
Figure 25: Phylogenetic analysis of 18S rDNA sequences of different species of *Ammonia* using the ML method. All genotypic groups are monophyletic and supported by high bootstrap values (97%-100%).

PhyML ln(L)=-6620.2 2185 sites GTR 4 rate classes

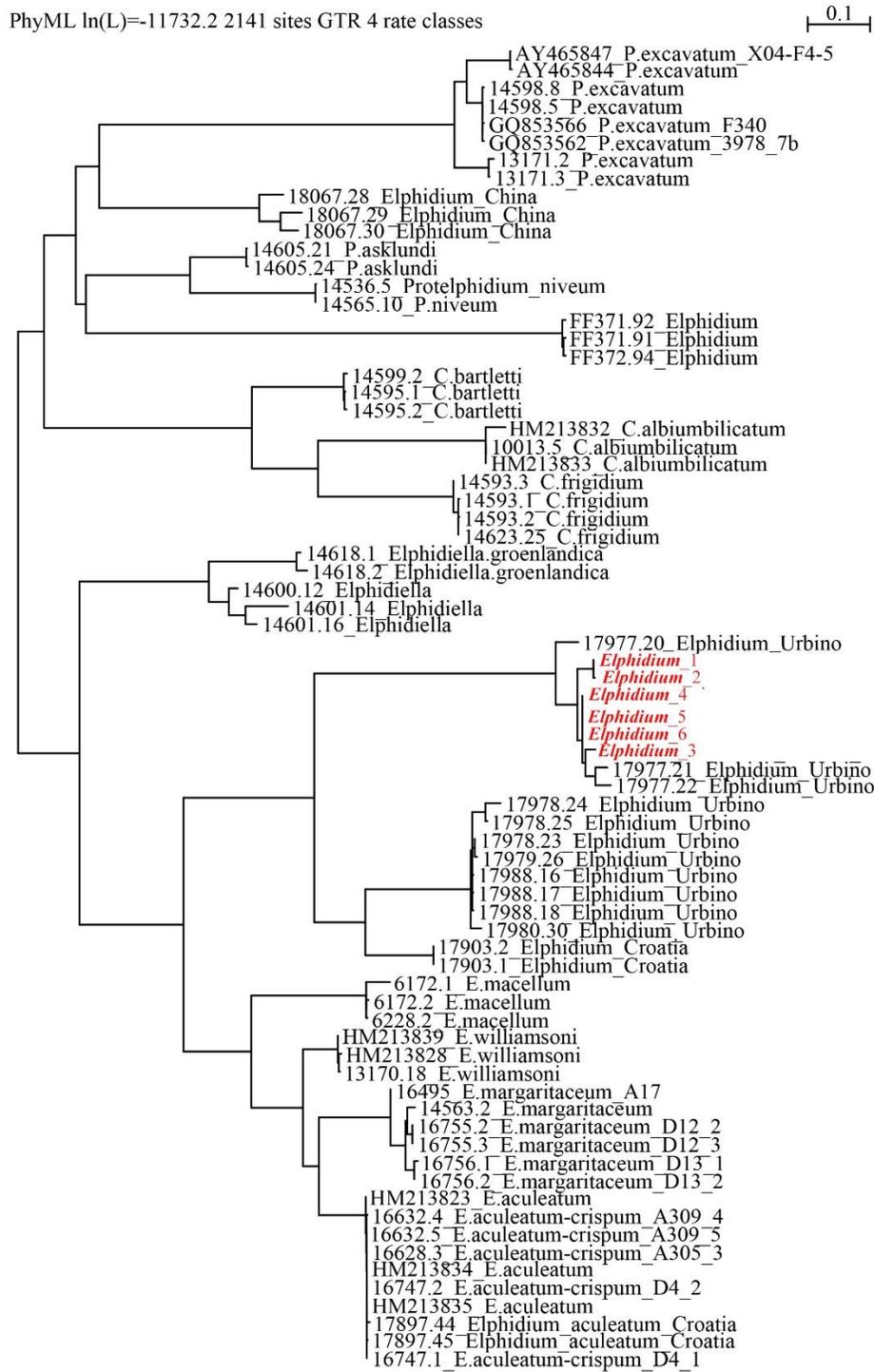
0.1



**Figure 26: Phylogenetic analysis of 18S rDNA sequences of different species of *Murrayinella* using the ML method. All genotypic groups are monophyletic and supported by high bootstrap values (97%-100%).**



**Figure 27: Phylogenetic analysis of 18S rDNA sequences of different species of *Glabratellina* using the ML method. All genotypic groups are monophyletic and supported by high bootstrap values (97%-100%).**



**Figure 28: Phylogenetic analysis of 18S rDNA sequences of different species of *Glabratellina* using the ML method. All genotypic groups are monophyletic and supported by high bootstrap values (97%-100%).**

### 3.5 Discussion

Sensitivity of benthic foraminifera towards different environs makes them a useful tool to understand the past conditions. Their use as proxies is subjected to certain limitations which can be achieved successfully by studying their biology in modern marine environment as well as in laboratory condition. In this study, the overall biology of benthic foraminifera from different localities of the Arabian Gulf is investigated in terms of living behaviors and molecular characterization.

Results on living behaviors illustrate that rotaliids, mainly *Ammonia* and *Glabratellina*, were able to develop the pseudopodial network easily, compared to the miliolids (i.e. 8 hours vs. 24 hours respectively). This could be due to the reason that rotaliids are more adoptable to the environmental changes than miliolids. Earlier research support this finding in terms of stressed environment where rotaliids were bearing unfavorable environs while miliolids started disappearing [107, 108]. Furthermore, high rate of movement was observed in rotaliids compared to miliolids as, in rotaliids, the pseudopodia were extended out through all apertures along with the bidirectional movement of cytoplasmic streaming; whereas in miliolids, extension was observed from the primary aperture only. This bidirectional movement of protoplasm has also been discussed by Bowser and coworkers [126, 127]. However, in either case, the individual was anchoring its pseudopodia to the wall of hard substrate resulting into dragging of their bodies in the direction of pseudopodia. The possible reason behind this attachment was to find the hard substrates in order to avoid inhospitable conditions. Gupta and coworkers reported the attachment of numerous foraminifera to vestimentiferan tube

worms in cold hydrocarbon seeps in order to avoid the anoxic conditions at the sediment-water interface [8]. Similarly, external light was also resulting into unfavorable conditions, for which the individual was hiding underneath the substrate clump. It is also reported that light is the main factor controlling the distribution and growth of many species especially the ones bearing symbionts.

Besides locomotion, the living individuals were found to gather small sand particles around their bodies using their pseudopodia, which shows their innate behavior of living in a benthic environment. Furthermore, the living individuals were adhering to the hard biogenic substrate which may be providing them nutrition in addition to protection [118]. The locomotion behavior is often observed for epifaunal species as they colonize the sediment surface or live attached to hard substrates that elevate them above the sediment surface [62]. In addition to this, during reproduction, organisms are recruited to a hard substrate when larvae settle onto the surface and survive metamorphosis to become adults [8]. The high juvenile population of *Glabratellina margaritaceus* is attributed to plastogamy, which is a most successful way of reproduction compared to asexual methods. Furthermore, it provides protection and ultimately high survival rate to gametes growing into zygotes. Myers also reported that plastogamous pair allows zygotes grow to a 2- or 3-chambered stage which later comes out as juvenile from the breeding pair.

The study on molecular analysis yielded amplification of specimens belonging to only four groups, i.e. *Ammonia*, *Murrayinella*, *Glabratellina*, and *Elphidium* which reflect their confirmed presence in the selected localities. These groups have been previously identified genetically from different parts of the world, however, BLAST analysis did not find any match at species level. Therefore, the identification of each group is primarily

considered under morphological observations. The DNA sequences of 26 individuals are assigned with the isolation numbers from 18137 to 18162 in the foramBARCODING project.

### 3.6 Genetics Atlas and Sequence Report

#### Ammonia\_1

DNA: 1,060 bp

```
AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACGCGGGAAATCTTACCGGGTCC
GGACACACTGAGGATTGACAGATATATACACCGTCAATACTTGTTATTGCGGTGTTGAAAGATGCTAGTT
CTTTCATGATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTTCGTGGAGTGATCTGTCTGCTTAATTGCG
TATCATTAAAGAGACCTAGTATACGCGTAAGACTTCGTTTTACGGTTCGTGACCCCCCTCACGGGCGTGT
GTCGCACGTACGAGTCATACGCACAGGTCTCCGATAGCAACGAACGTGACCGTACTCTATTGTTGCACGC
AATGTATGCACCTTTTTGGTGTGTA ACTACCGCTGCTTAGCATATTTTCGTACCCTCGTGGTGC GTTGTAT
GCATTAAACTATAGAGACCGCTGTCTTTTCTTTAAACCAGAGGAAGGATACGGCAATAACAGGTCTGTGA
TGCCCTCAGATGTTCCGGGCTGCACACGTGCTACAATGATCATTGCACTGTGCATCTAACCCAATGTGCGT
GGACGCCACGGTGTATTGCGCTTCGGCGTATATGCATCAGTTGGTTCGACCACGCCGAACCTACTTCGAAA
GTAAAATTTTTAAGTGGGTAATCCATTAGAAGTAATGACTCGCATAGACCATGGTACACATTTATGTACG
CGCAGGTTCTACCCGGCCGGCCTTTTGTGTCGGTGCAGTGCAGTGTAGCTTGTGTTTCGTACGTACCACTCCG
TATTAATTCATACGTGGGGATAGATCATTGTTTAATTGTTGGTCTCGGTCTTA ACTAGGAATGCCTTGTAC
GGGTCTCTGGTTCAACATAACCACCCGGAATACGTCCCTGCCCTTTGTACACACCCGCCCGTCGCTCTTACCG
ATGGATTATACTATGAATCTATAGGACTGCCAAAGTTTGGGTCTCTCTCGAGAGACACGCTTAGTGGAAA
TATATATGAATAGTGTGATCTAAAGGAAAGAGAAGTCGTAACAAGGCATCGGTAGGTGAACCTGCAGAA
GGATCA
```

#### Ammonia\_2

DNA: 1,060 bp

```
AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACGCGGGAAATCTTACCGGGTCC
GGACACACTGAGGATTGACAGATATATACACCGTCAATACTTGTTATTGCGGTGTTGAAAGATGCTAGTT
CTTTCATGATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTTCGTGGAGTGATCTGTCTGCTTAATTGCG
TATCATTAAAGAGACCTAGTATACGCGTAAGACTTCGTTTTACGGTTCGTGACCCCCCTCACGGGCGTGT
GTCGCACGTACGAGTCATACGCACAGGTCTCCGATAGCAACGAACGTGACCGTACTCTATTGTTGCACGC
AATGTATGCACCTTTTTGGTGTGTA ACTACCGCTGCTTAGCATATTTTCGTACCCTCGTGGTGC GTTGTAT
GCATTAAACTATAGAGACCGCTGTCTTTTCTTTAAACCAGAGGAAGGATACGGCAATAACAGGTCTGTGA
TGCCCTCAGATGTTCCGGGCTGCACACGTGCTACAATGATCATTGCACTGTGCATCTAACCCAATGTGCGT
GGACGCCACGGTGTATTGCGCTTCGGCGTATATGCATCAGTTGGTTCGACCACGCCGAACCTACTTCGAAA
GTAAAATTTTTAAGTGGGTAATCCATTAGAAGTAATGACTCGCATAGACCATGGTACACATTTATGTACG
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TATTAATTCATACGTGGGGATAGATCATTGTTTAATTGTTGGTCTCGGTCTTA ACTAGGAATGCCTTGTAC
GGGTCTCTGGTTCAACATAACCACCCGGAATACGTCCCTGCCCTTTGTACACACCCGCCCGTCGCTCTTACCG
ATGGATTATACTATGAATCTATAGGACTGCCAAAGTTTGGGTCTCTCTCGAGAGACACGCTTAGTGGAAA
TATATATGAATAGTGTGATCTAAAGGAAAGAGAAGTCGTAACAAGGCATCGGTAGGTGAACCTGCAGAA
GGATCA
```

#### Ammonia\_T1\_3

**DNA: 1,069 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACGCGGGAAATCTTACCGGGTCC  
GGACACACTGAGGATTGACAGATATACGTCGTGCGTTGAGCTCTCTCGGGGGCCGAGCGCATGACTGAA  
AGATGCTAGTTCTTTCATGATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTCGTGGAGTGATCTGTCT  
GCTTAATTGCGTATCATTAAAAGAGACCTAGTATACGCGTAAGACTTCGTTTTACGGTTCGTGACCCCCCT  
CACGGGCGTGTGTCGCACGTACGAGTCATACGCACTGGTCTCCGATAGCAACGAACGTGACCGTACTCTA  
TTGTTGCAGCGAATGTATGCACCTTCCCAGGTGATCTACCGCTGCTTAGTGCGTATGCATACCTCGGTGCG  
TGTCGCACATTAAGTATAGAGACCGCTGTATTTCTTTAAACCAGAGGAAGGATACGGCAATAACAGGT  
CTGTGATGCCCTCAGATGTTCCGGGCTGCACACGTGCTACAATGATCATTGCACTGTGCATCTAACCCAAT  
GTGCGTGGACGCCACGGTATGTATTTATGCTTCGGCGTAGTATATATCAGTTGGTTCGACCGCGCCGAACC  
TACTTCGAAAGTAAAAATTTCTCAGTGGGTAATCCATTAGAAGTAATGACTCGCATAGACCATGGTACACA  
CTTATATATGTACGCGCAGGTTCTACCCGGCCGGCCTTTGTGTCCGGTGCAGTGCCTAGCTTGTGTTTCGT  
ACGTACCACTCAGTATTAATTCATACGTGGGGATAGATCATTGTTAAATTGTTGGTCTCGGTCTTAACTAG  
GAATGCCTTGTACGGGTCTCTGGTTCAACATACCACCCGGAATACGTCCCTGCCCTTTGTACACACCGCCC  
GTCGCTCTTACCGATGGATTATACTATGAATCTATAGGACTGCCAAAGTTTGTGTCTCTCGGGACACGCTT  
AGTGGAATATATATGAATAGTGTGATCTAAAGGAAAAGAGAAGTCGTAACAAGGCATCGGTAGGTGAAC  
CTGCAGAAGGATCA

**Ammonia\_T1\_4**

**DNA: 1,069 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACGCGGGAAATCTTACCGGGTCC  
GGACACACTGAGGATTGACAGATATACGTCGTGCGTTGAGCTCTCTCGGGGGCCGAGCGCATGACTGAA  
AGATGCTAGTTCTTTCATGATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTCGTGGAGTGATCTGTCT  
GCTTAATTGCGTATCATTAAAAGAGACCTAGTATACGCGTAAGACCTCGTTTTACGGTTCGTGACCCCCCT  
CACGGGCGTGTGTCGCACGTACGAGTCATACGCACTGGTCTCCGATAGCAACGAACGTGACCGTACTCTA  
TTGTTGCAGCGAATGTATGCACCTTCCCAGGTGATCTACCGCTGCTTAGTGCGTATGCGTACCTCGGTGCG  
TGTCGCACATTAAGTATAGAGACCGCTGTATTTCTTTAAACCAGAGGAAGGATACGGCAATAACAGGT  
CTGTGATGCCCTCAGATGTTCCGGGCTGCACACGTGCTACAATGATCATTGCACTGTGCATCTAACCCAAT  
GTGCGTGGACGCCACGGTATGTATTTATGCTTCGGCGTAGTATATATCAGTTGGTTCGACCGCGCCGAACC  
TACTTCGAAAGTAAAAATTTCTCAGTGGGTAATCCATTAGAAGTAATGACTCGCATAGACCATGGTACACA  
CTTATATATGTACGCGCAGGTTCTACCCGGCCGGCCTTTGTGTCCGGTGCAGTGCCTAGCTTGTGTTTCGT  
ACGTACCACTCAGTATTAATTCATACGTGGGGATAGATCATTGTTAAATTGTTGGTCTCGGTCTTAACTAG  
GAATGCCTTGTACGGGTCTCTGGTTCAACATACCACCCGGAATACGTCCCTGCCCTTTGTACACACCGCCC  
GTCGCTCTTACCGATGGATTATACTATGAATCTATAGGACTGCCAAAGTTTGTGTCTCTCGGGACACGCTT  
AGTGGAATATATATGAATAGTGTGATCTAAAGGAAAAGAGAAGTCGTAACAAGGCATCGGTAGGTGAAC  
CTGCAGAAGGATCA

**Ammonia\_T1\_5**

**DNA: 1,076 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACGCGGGAAATCTTACCGGGTCC  
GGACACACTGAGGATTGACAGATATACGTCGTGCGTTGAGCTCTCTCGGGGGCCGAGCGCATGACTGAA  
AGATGCTAGTTCTTTCATGATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTCGTGGAGTGATCTGTCT  
GCTTAATTGCGTATCATTAAAAGAGACCTAGTATACGCGTAAGACTTCGTTTTACGGTTCGTGACCCCCCT  
CACGGGCGTGTGTCGCACGTACGAGTCATACGCACTGGTCTCCGATAGCAACGAACGTGACCGTACTCTA  
TTGTTGCAGCGAATGTATGCACCTTCCCAGGTGATCTACCGCTGCTTAGTGCGTATGCGTACCTCGGTGCG  
TGTCGCACATTAAGTATAGAGACCGCTGTATTTCTTTAAACCAGAGGAAGGATACGGCAATAACAGG  
GTCTGTGATGCCCTCAGATGTTCCGGGCTGCACACGTGCTACAATGATCATTGCACTGTGCATCTAACCCA  
ATGTGCGTGGACGCCACGGTATGTATTTATGCTTCGGCGTAGTATATATCAGTTGGTTCGACCGCGCCGAA  
CCTACTTCGAAAGTAAAAATTTCTCAGTGGGTAATCCATTAGAAGTAATGACTCGCATAGACCATGGTACA  
CACTTATATATGTACGCGCAGGTTCTACCCGGCCGGCCTTTGTGTCCGGTGCAGTGCCTAGCTTGTGTTTC

GTACGTACCACTCAGTATTAATTCATACGTGGGGATAGATCATTGTTTAATTGTTGGTCTCGGTCTTAACT  
AGGAATGCCTTGTACGGGTCTTTGGTTCAACATACCACCCGAATACGCGAATCCTTCGGCCCTTTGTACA  
CACCGCCCGTCTGCTCTTAGCGATGGATCATACTATGAATCTATAGGACTGCCAAAGTTTGTGTCTCTCGGG  
ACACGCTTAGTGAAATATATATGAATAGTGTGATCTAAAGGAAAGAGAAGTCGTAACAAGGCATCGGT  
AGGTGAACCTGCAGAAGGATCA

**Ammonia\_T1\_6**

**DNA: 1,070 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACGCGGGAAATCTTACCGGGTCC  
GGACACACTGAGGATTGACAGATATACGTCGTGCGTTGAGCTCTCTCGGGGGCCGAGCGCATGACTGAA  
AGATGCTAGTTCTTTCATGATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTCGTGGAGTGATCTGTCT  
GCTTAATTGCGTATCATTAAAAGAGACCTAGTATACGCGTAAGACTTCGTTTTACGGTTCGTGACCCCCCT  
CACGGGCGTGTGTCGCACGTACGAGTCATACGCACAGGTCTCCGATAGCAACGAACGTGACCGTACTCTA  
TTGTTGCAGCGAATGCATACGCACTCTGTGCTGTATCTACCGCTGCTTAGCATAATTTACGCCTCTCAGA  
GACGTGTTGTATGCATTAAACTATAGAGACCGCTGTCTTTTCTTTAAACCAGAGGAAGGATACGGCAATA  
ACAGGTCTGTGATGCCCTCAGATGTTCCGGGCTGCACACGTGCTACAATGATCATTGCACTGTGCATCTA  
ACCCAATGTGCGTGGACGCCACGGTGTATTGCGCTTCGGCGTATATGCATCAGTTGGTCGACCACGCCGA  
ACCTACTTCGAAAGTAAAATTTCTCAGTGGTAATCCATTAGAAGTAATGACTCGCATAGACCATGGTAC  
ACTTTAATGTACGCGCAGGTTCTACCCGGCCGGCCTTTTGTGTCGGTGCAGTGCCTAGCTTGTGTTTCGT  
ACGTACCACTCAGTATTAATTCATACGTGGGGATAGATCATTGTTTAATTGTTGGTCTCGGTCTTAACTAG  
GAATGCCTTGTACGGGTCTCTGTTCAACATACCACCCGAATACGTCCCTGCCCTTTGTACACGCCGCC  
GTCGCTCTTACCGATGGATTATACTATGAATCTATAGGACTGCCAAAGTTTGTGTCTCTCTCGGGACACGC  
TTAGTGAAATATATATGAATAGCGTGATCTAAAGGAAAGAGAAGTCGTAACAAGGCATCGGTAGGTGA  
ACCTGCAGAAGGATCA

**Murrayinella\_1**

**DNA: 948 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACGCGGGAAATCTTACCGGGTCC  
GGACACACTGAGGATTGACAGGCAATATAATCGCACTCGATGCGACATCAAATATGCTAGTCCTTTCATG  
ATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTCGTGGAGTGATCTGTCTGCTTAATTGCGTTTCACTA  
AGGGCCTATATATTCATGCGTGTGTAGGGTTGCAGCGAATGCATTCATACTGCTACGCTCACCTCATACG  
CTGTTAGGTCTGAAAGCAACGAACGTGACCGCAACCTCTTGTGCTCAACACATACCAGTGCTTTTATT  
GCACTGTGAGGCTATTTTTAAAAGTAGAGGGACCGCTGCTACTTTCTTAAACCAGAGGAAGGTTGCGGGCAA  
TAACAGGTCTGTGATGCCCTTAGATGTTCCGGGCTGCACACGTGCTACAATGATTATTGCAGTGAGCATC  
TCATTTTATACACACCGCATGCGCGAGTCGTACACAATGTGTTTCGCTCTGCGCGCGGTAAGCCTGCTTC  
GAAAGTAAAGTGGGTAATCAATTAGAAGTAATGATTTTCTTTTTTATCAGCACACATATATACGGCGTCA  
TTACCCGGCTGTCTTGTGGCAGTTTTTGTGCGTATTGATGTTTCTTACCGTATGTGCGATTGTCAATTCA  
TGGTGGGGACAGACCATTGTTAATTGTTGGTCTCGGTCTTAACTAGGAATGCCTTGTACGGGTCTTTGGTT  
CAACAAACCACCCGGAATACGTCCCTGCCCTTTGTACACACCGCCCGTGCCTTACCGATGGACTTCTCT  
GTGAGTTTGTAGGGACCGCTCCATGGAACTTAAACGAACAGTGTGGTCTAAAGGAAAGAGAAGTCGTAA  
CAAGGCATCGGTAGGTGAACCTGCAGAAGGATCA

**Murrayinella\_2**

**DNA: 900 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACGCGGGAAATCTTACCGGGTCC  
GGACACACTGAGGATTGACAGGCAATATAATCGCACTCGATGCGACATCAAATATGCTAGTCCTTTCATG  
ATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTCGTGGAGTGATCTGTCTGCTTAATTGCGTTTCACTA  
AGGGCCTATATATTCATGCGTGTGTAGGGTTGCAGCGAATGCTCACACCTCACACAGCGTGTAGGTCTT  
GAAAGCAACGAACGTGACCGCAACCTCTTGTGCTCAACGTGACCGGAGGCTATTTTTAAAAGTAGAGG  
GACCGCTGTTACTTTCTTAAACCAGAGGAAGGTTGCGGCAATAACAGGTCTGTGATGCCCTTAGATGTTT

CGGGCTGCACACGTGCTACAATGATTATTGCAGTGAGCATCTCATTTTTATCTTACACACCGCGCGTGCGC  
GGGTCATGTAAGCAGGCCTGCTTCGAAAGTAAGTGGGTAATCAATTAGAAGTAATGATTCCTTTTTTTAT  
CAGCACACATATATACGGCGTCATTACCCGGCTGTCCTTGTGGCAGTTTTGTGCGTATTGATGTTTCTT  
ACCGTATGTGCGATTGTCAATTCATGGTGGGACAGACCATTGTTAATTGTTGGTCTCGGTCTTAACTAGG  
AATGCCTTGTACGGGTCTTTGGTTCAACAAACCACCGGAATACGTCCCTGCCCTTGTACACACCGCCC  
GTCGCTCTTACCGATGGACTTCTCTGTGAGTTTGAGGGACCGCTCCATGGAAACTTAAACGAACAGTGTG  
GTCTAAAGGAAAGAGAAGTCGTAACAAGGCATCGGTAGGTGAACCTGCAGAAGGATCA

**Murrayinella\_3**

**DNA: 941 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACGCGGGAAATCTTACCGGGTCC  
GGACACACTGAGGATTGACAGGCAATATAATCGCACTCGATGCGACATCAAATATGCTAGTCCTTTCATG  
ATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTTCGTGGAGTGATCTGTCTGCTTAATTGCGTTTCTACTA  
AGGGCCTATATATTCATGCGTGTGTAGGGTTGCAGCGAATGCTCACACCTCACACAGCGTGTAGGTCTT  
GAAAGCAACGAACGTGACCGCAACCTCTTGTTCCTCAACACATACCAGTGCTTTATTGCACTGTGAGGC  
TATTTTAAACTAGAGGGACCGCTGTTACTTTCTTAAACCAGAGGAAGGTTGCGGCAATAACAGGTCTGT  
GATGCCCTTAGATGTTCCGGGCTGCACACGTGCTACAATGATTATTGCAGTGAGCATCTCATTTTTATCTTA  
CACACCGCATGCGCGAGTCGTACACCATGTGTTTCGCTCTGCGCGCGGTAAAGCCTGCTTCGAAAAGTAAG  
TGGGTAATCAATTAGAAGTAATGATTTTCCCTTTCCGTATCAGCACACATATATACGGCGTCATTACCCGG  
CTGTCCTTGTGGCAGTTTTGTGCGTATTGATGTTTCTTACCGTATGTGCGATTGTCAATTCATGGTGGGG  
ACAGACCATTTGTTAATTGTTGGTCTCGGTCTTAACTAGGAATGCCCTGTACGGGTCTTTGGTTCAACAAAC  
CACCCCGAATACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCTTACCGATGGACTTCTCTGTGAGTTT  
GAGGGACCGCTCCATGGAAACTTAAACGAACAGTGTGGTCTAAAGGAAAGAGAAGTCGTAACAAGGCAT  
CGGTAGGTGAACCTGCAGAAGGATCA

**Murrayinella\_4**

**DNA: 940 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACGCGGGAAATCTTACCGGGTCC  
GGACACACTGAGGATTGACAGGCAATATAATCGCACTCGATGCGACATCAAATATGCTAGTCCTTTCATG  
ATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTTCGTGGAGTGATCTGTCTGCTTAATTGCGTTTCTACTA  
AGGGCCTATATATTCATGCGTGTGTAGGGTTGCAGCGAATGCTCACACCTCACACAGCGTGTAGGTCTT  
GAAAGCAACGAACGTGACCGCAACCTCTTGTTCCTCAACACATACCAGTGCTTTATTGCACTGTGAGGC  
TATTTTAAACTAGAGGGACCGCTGTTACTTTCTTAAACCAGAGGAAGGTTGCGGCAATAACAGGTCTGT  
GATGCCCTTAGATGTTCCGGGCTGCACACGTGCTACAATGATTATTGCAGTGAGCATCTCATTTTTATCTTA  
CACACCGCATGCGCGAGTCGTACACCATGTGTTTCGCTCTGCGCGCGGTAAAGCCTGCTTCGAAAAGTAAG  
TGGGTAATCAATTAGAAGTAATGATTTCCCTTTCCGTATCAGCACACATATATACGGCGTCATTACCCGGC  
TGTCCTTGTGGCAGTTTTGTGCGTATTGATGTTTCTTACCGTATGTGCGATTGTCAATTCATGGTGGGGA  
CAGACCATTTGTTAATTGTTGGTCTCGGTCTTAACTAGGAATGCCCTGTACGGGTCTTTGGTTCAACAAACC  
ACCCGGAATACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCTTACCGATGGACTTCTCTGTGAGTTT  
AGGGACCGCTCCATGGAAACTTAAACGAACAGTGTGGTCTAAAGGAAAGAGAAGTCGTAACAAGGCAT  
GGTAGGTGAACCTGCAGAAGGATCA

**Murrayinella\_5**

**DNA: 916 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACGCGGGAAATCATAACCGGGTCC  
GGACACACTGAGGATTGACAGGCAATATAATCGCACTCGATGCGACATCAAATATGCTAGTCCTTTCATG  
ATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTTCGTGGAGTGATCTGTCTGCTTAATTGCGTTTCTACTA  
AGGGCCTATATATTCATGCGTGTGTAGGGTTGCAGCGAATCCTCACACCTCACACAGCGTGTAGGTCTT  
GAAAGCAACGAACGTGACCGCAACCTCTTGTTCCTCAACACATACCAGTGCTTTATTGCACTGTGAGGC  
TATTTTAAACTAGAGGGACCGCTGTTACTTTCTTAAACCAGAGGAAGGTTGCGGCAATAACAGGTCTGT

GATGCCCTTAGATGTTCCGGGCTGCACACGTGCTACAATGATTATTGCAGTGAGCATCTCATTATCTTA  
CACACCGCATGCGGAGTCGTACACAATGTGTTTCGCTCTGCGCGGGTAAAGCCTGCTTCGAAAAGTAAG  
TGGGTAATCAATTAGAAGTAATGATTCCTTTTTTATCAGCACACATATATATGGTGGCATCAGTCGTAC  
GCACTGATGTCTCTTACCGTATGTGCGATTGTCAATTCATGGTGGGGACAGACCATTGTTAATTGTTGGTC  
TCGGTCTTAACTAGGAATGCCTTGTACGGGTCTTTGGTTCAACAAACCACCCGGAATACGTCCCTGCCCTT  
TGTACACACCGCCCGTCGCTCTTACCGATGGACTTCTCTGTGAGTTTGAGGGACCGCTCCATGGAAACTTA  
AACGAACAGTGTGGTCTAAAGGAAAGAGAAGTCGTAACAAGGCATCGGTAGGTGAACCTGCAGAAGGA  
TCA

**Murrayinella\_5**

**DNA: 916 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACGCGGGAAATCATAACCGGGTCC  
GGACACACTGAGGATTGACAGGCAATATAATCGCACTCGATGCGACATCAAATATGCTAGTCCTTTCATG  
ATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTTCGTGGAGTGATCTGTCTGCTTAATTGCGTTTCACTA  
AGGGCTATATATTCATGCGTGTGTAGGGTTGCAGCGAATCCTCACACCTCACACAGCGTGTAGGTCCCT  
GAAAGCAACGAACGTGACCGCAACCTCTTGTTCCTCAACACATACCAGTGCTTTATTGCACTGTGAGGC  
TATTTTAAACTAGAGGGACCGCTGTTACTTTCTTAAACCAGAGGAAGGTTGCGGCAATAACAGGTCTGT  
GATGCCCTTAGATGTTCCGGGCTGCACACGTGCTACAATGATTATTGCAGTGAGCATCTCATTATCTTA  
CACACCGCATGCGGAGTCGTACACAATGTGTTTCGCTCTGCGCGGGTAAAGCCTGCTTCGAAAAGTAAG  
TGGGTAATCAATTAGAAGTAATGATTCCTTTTTTATCAGCACACATATATATGGTGGCATCAGTCGTAC  
GCACTGATGTCTCTTACCGTATGTGCGATTGTCAATTCATGGTGGGGACAGACCATTGTTAATTGTTGGTC  
TCGGTCTTAACTAGGAATGCCTTGTACGGGTCTTTGGTTCAACAAACCACCCGGAATACGTCCCTGCCCTT  
TGTACACACCGCCCGTCGCTCTTACCGATGGACTTCTCTGTGAGTTTGAGGGACCGCTCCATGGAAACTTA  
AACGAACAGTGTGGTCTAAAGGAAAGAGAAGTCGTAACAAGGCATCGGTAGGTGAACCTGCAGAAGGA  
TCA

**Glabratellina\_1**

**DNA: 1,023 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACGCGGGAAATCTTACCGGGTCC  
GGACACACTGAGGATTGACAGGTTTTATCCAGTTTCTACTCGTAGAGCTGGTGTCAAAAATGCTAGTCCT  
TTCATGATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTTCGTGGAGTGATCTGTCTGCTTAATTGCGTT  
TCACTACGAATCTACATTAACGTAACGTTTTCGAGATGGACTGATCCCTCCCGCTCTCTGAGTGTGGAGT  
TGTGCGTCTTTCATCGTTACCGCATCGTACAACGTATGATTCTGAAAAGCAACGAACGTGACCGCAACCT  
CTTGTTCCTGTATTCCAAAACAGTTTGCCTCCGTGCTATTCTGTATAAACAGGCCTTATATTAATAAAC  
TAGAGGGACCGCTGTTACTTTCTTAAACCAGAGGAAGGTTGCGGCAATAACAGGTCTGTGATGCCCTTAG  
ATGTTCCGGGCTGCACACGTGCTACAATGATTATTGCAGTGAGCATCTCATATTCTCACATCTCAGCGTGA  
GCCGCTATCTTGCTTCGGCTTGATGGTGTGCTCTACGCGGGATCAAGCCTGCTTCGAAAAGTAAGTGGGT  
AATCAATTAGAAGTAATGATTCCTTTATATGCACGTCTATGTTTGGCGCTGATCCCTTGACTAACTCTT  
GTTAGCTTCTGTGTGCGTTCAGTGAAGCTCTTAGCTTTCTACCATTTCGTGCAACTGTCAATTCATGGTG  
GGGACAGACCATTGTTAATTGTTGGTCTCGGTCTTAACTAGGAATGCCTTGTACGGGTCTTTGGTTCAACA  
AACCACCCGGAATACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCTTACCGATGGATTTCTCTGTGAG  
TTTGAAGGACTGGCTTTTTTGCTATGGAAATTCAAACGAACAGTGTGATCTAAAGGAAAGAGAAGTCGTA  
ACAAGGCATCGGTAGGTGAACCTGCAGAAGGATCA

**Glabratellina\_2**

**DNA: 1,023 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACGCGGGAAATCTTACCGGGTCC  
GGACACACTGAGGATTGACAGGTTTTATCCAGTTTCTACTCGTAGAGCTGGTGTCAAAAATGCTAGTCCT  
TTCATGATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTTCGTGGAGTGATCTGTCTGCTTAATTGCGTT  
TCACTACGAATCTACATTA AACGTACGTTTTCGAGATGGACTGATCCCTCCCGCTCTCTGAGTGTGGAGT  
TGTGCGTCTTTCCATCGTTACCGCATCGTACAACGTATGATTCTGAAAAGCAACGAACGTGACCGCAACCT  
CTTGTTGCCTGTATTCCAAAACAGTTTGCCTCCGTGCTATTCTGTATAAACAGGCCTTATATTAATAAAC  
TAGAGGGACCGCTGTTACTTTCTTAAACCAGGGGAAGGTTGCGGCAATAACAGGTCTGTGATGCCCTTAG  
ATGTTCCGGGCTGCACACGTGCTACAATGATTATTGCAGTGAGCATTTTCATATTCTCACATCTCAGCGTGA  
GCCGCTATCTTGCTTCGGCTTGATGGTGTGCTCTACGCGGGATCAAGCCTGCTTCGAAAAGTAAGTGGGT  
AATCAATTAGAAGTAATGATTTCCTTTATATGCACATCTATGTTTGGCGCTGATCCCTTGACTAACTCTT  
GTTAGCTTCTGTGTGCGTTCAGTGAAGCTCTCTAGCTTTCTACCATTTCGTGCAACTGTCAATTCATGGTG  
GGGACAGACCATTGTTAATTGTTGGTCTCGGTCTTAACTAGGAATGCCTTGACGGGTCTTTGGTTCAACA  
AACCACCCGGAATACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCTTACCGATGGATTTCTCTGTGAG  
TTTGAAGGACTGGCTTTTTTGTATGGAAATTCAAAACGAACAGTGTGATCTAAAGGAAAAGAGAAGTCGTA  
ACAAGGCATCGGTAGGTGAACCTGCAGAAGGATCA

**Glabratellina\_3**

**DNA: 1,023 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACGCGGGAAATCTTACCGGGTCC  
GGACACACTGAGGATTGACAGGTTTTATCCAGTTTCTACTCGTAGAGCTGGTGTCAAAAATGCTAGTCCT  
TTCATGATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTTCGTGGAGTGATCTGTCTGCTTAATTGCGTT  
TCACTACGAATCTACATTA AACGTACGTTTTCGAGATGGACTGATCCCTCCCGCTCTCTGAGTGTGGAGT  
TGTGCGTCTTTCCATCGTTACCGCATCGTACAACGTATGATTCTGAAAAGCAACGAACGTGACCGCAACCT  
CTTGTTGCCTGTATTCCAAAACAGTTTGCCTCCGTGCTATTCTGTATAAACAGGCCTTATATTAATAAAC  
TAGAGGGACCGCTGTTACTTTCTTAAACCAGAGGAAGGTTGCGGCAATAACAGGTCTGTGATGCCCTTAG  
ATGTTCCGGGCTGCACACGTGCTACAATGATTATTGCAGTGAGCATCTCATATTCTCACATCTCAGCGTGA  
GCCGCTATCTTGCTTCGGCTTGATGGTGTGCTCTACGCGGGATCAAGCCTGCTTCGAAAAGTAAGTGGGT  
AATCAATTAGAAGTAATGATTTCCTTTATATGCACATCTATGTTTGGCGCTGATCCCTTGACTAACTCTT  
GTTAGCTTCTGTGTGCGTTCAGTGAAGCTCTCTAGCTTTCTACCATTTCGTGCAACTGTCAATTCATGGTG  
GGGACAGACCATTGTTAATTGTTGGTCTCGGTCTTAACTAGGAATGCCTTGACGGGTCTTTGGTTCAACA  
AACCACCCGGAATACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCTTACCGATGGATTTCTCTGTGAG  
TTTGAAGGACTGGCTTTTTTGTATGGAAATTCAAAACGAACAGTGTGATCTAAAGGAAAAGAGAAGTCGTA  
ACAAGGCATCGGTAGGTGAACCTGCAGAAGGATCA

**Glabratellina\_4**

**DNA: 1,021 bp**

AAGGGCACCACAACAACGCGTGGAGCATGTGGCTTAATTTGACTCAAGCAGCGGAAATCTTACCGGGTCC  
GGACACACTGAGGATTGACAGGTTTTATCCAGTTTCTACTCGTAGAGCTGGTGTCAAAAATGCTAGTCCTT  
TCATGATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTTCGGGAGTGATCTGTCTGCTTAATTGCGTTT  
ACTACGAATCTACATTA AACGTACGTTTTCGAGATGGACTGATCCCTCCCGCTCTCTGAGTGTGGAGTTG  
TGCGTCTTTCCATCGTTACCGCATCGTACAACGTATGATTCTGAAAAGCAACGAACGTGACCGCAACCTCTT  
GTTGCCTGTATTCCAAAACAGTTTGCCTCCGTGCTATTCTGTATAAACAGGCCTTATATTAATAAACTAG  
AGGGACCGCTGTTACTTTCTTAAACCAGAGGAAGGTTGCGGCAATAACAGGTCTGTGATGCCCTTAGATG  
TTCCGGGCTGCACACGTGCTACAATGATTATTGCAGTGAGCATCTCATATTCTCACATCTCAGCGTGAGCC  
GCTATCTTGCTTCGGCTTGATGGTGTGCTCTACGCGGGATCAAGCCTGCTTCGAAAAGTAAGTGGGTAAT  
CAATTAGAAGTAATGATTTCCTTTATATGCACATCTATGTTTGGCGCTGATCCCTTGACTAACTCTTGT  
AGCTTCTGTGTGCGTTCAGTGAAGCTCTCTAGCTTTCTACCATTTCGTGCAACTGTCAATTCATGGTGGG  
ACAGGCCATTGTTAATTGTTGGTCTCGGTCTTAACTAGGAATGCCTTGACGGGTCTTTGGTTCAACAAAC  
CACCCGGAATACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCTTACCGATGGATTTCTCTGTGAGTTT

GAAGGACTGGCTTTTTTGGCTATGGAAATTCAAACGAACAGTGTGATCTAAAGGAAAGAGAAGTCGTAAC  
AAGGCATCGGTAGGTGAACCTGCAGAAGGATCA

**Glabratellina\_5**

**DNA: 1,023 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACGCGGGAAATCTTACCGGGTCC  
GGACACACTGAGGATTGACAGGTTTTATCCAGTTTCTACTCGTAGAGCTGGTGTCAAAAATGCTAGTCCT  
TTCATGATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTTCGTGGAGTGATCTGTCTGCTTAATTGCGTT  
TCACTACGAATCTACATTAACAGTACGTTTGCAGATGGACTGATCCCTCCCGCTCTCTGAGTGTGGAGT  
TGTGCGTCTTTCCATCGTTACCGCATCGTACAACGTATGATTCTGAAAGCAACGAACGTGACCGCAACCT  
CTTGTTGCCTGTATTCCAAAACAGTTTGCACCTCCGTGCTATTCTGTATAAACAGGCCTTATATTAATAAAC  
TAGAGGGACCGCTGTTACTTTCTTAAACCAGAGGAAGGTTGCGGCAATAACAGGTCTGTGATGCCCTTAG  
ATGTTCCGGGCTGCACACGTGCTACAATGATTATTGCAGTGAGCATCTCATATTCTCACATCTCAGCGTGA  
GCCGCTATCTTGCTTCGGCTTGATGGTGTGGTCTACGCGGGATCAAGCCTGCCTCGAAAGTAAGTGGGT  
AATCAATTAGAAGTAATGATTTCCTTTATATGCACATCTATGTTTGGCGCTGATCCCTTGACTAACTCTT  
GTTAGCTTCTGTGTGCGTTCAGTGAAGCTCTCTAGCTTTCTACCATTTCGTGCAACTGTCAATTCATGGTG  
GGGACAGACCATTGTTAATTGTTGGTCTCGGTCTTAACTAGGAATGCCTTGTACGGGTCTTTGGTTCAACA  
AACCACCCGGAATACGTCCCTGCCCTTGTACACACCGCCCGTCGCTCTTACCGATGGATTTCTCTGTGAG  
TTTGAAGGACTGGCTTTTTTGGCTATGGAAATTCAAACGAACAGTGTGATCTAAAGGAAAGAGAAGTCGTA  
ACAAGGCATCGGTAGGTGAACCTGCAGAAGGATCA

**Glabratellina\_6**

**DNA: 1,023 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACGCGGGAAATCTTACCGGGTCC  
GGACACACTGAGGATTGACAGGTTTTATCCAGTTTCTACTCGTAGAGCTGGTGTCAAAAATGCTAGTCCT  
TTCATGATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTTCGTGGAGTGATCTGTCTGCTTAATTGCGTT  
TCACTACGAATCTACATTAACAGTACGTTTGCAGATGGACTGATCCCTCCCGCTCTCTGAGTGTGGAGT  
TGTGCGTCTTTCCATCGTTACCGCATCGTACAACGTATGATTCTGAAAGCAACGAACGTGACCGCAACCT  
CTTGTTGCCTGTATTCCAAAACAGTTTGCACCTCCGTGCTATTCTGTATAAACAGGCCTTATATTAATAAAC  
TAGAGGGACCGCTGTTACTTTCTTAAACCAGAGGAAGGTTGCGGCAATAACAGGTCTGTGATGCCCTTAG  
ATGTTCCGGGCTGCACACGTGCTACAATGATTATTGCAGTGAGCATCTCATATTCTCACATCTCAGCGTGA  
GCCGCTACCTTGCTTCGGCTTGATGGTGTGGTCTACGCGGGATCAAGCCTGCCTCGAAAGTAAGTGGGT  
AATCAATTAGAAGTAATGATTTCCTTTATATGCACATCTATGTTTGGCGCTGATCCCTTGACTAACTCTT  
GTTAGCTTCTGTGTGCGTTCAGTGAAGCTCTCTAGCTTTCTACCATTTCGTGCAACTGTCAATTCATGGTG  
GGGACAGACCATTGTTAATTGTTGGTCTCGGTCTTAACTAGGAATGCCTTGTACGGGTCTTTGGTTCAACA  
AACCACCCGGAATACGTCCCTGCCCTTGTACACACCGCCCGTCGCTCTTACCGATGGATTTCTCTGTGAG  
TTTGAAGGACTGGCTTTTTTGGCTATGGAAATTCAAACGAACAGTGTGATCTAAAGGAAAGAGAAGTCGTA  
ACAAGGCATCGGTAGGTGAACCTGCAGAAGGATCA

**Glabratellina\_7**

**DNA: 1,025 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACGCGGGAAATCTTACCGGGTCC  
GGACACACTGAGGATTGACAGGTTCTATCCATATGTTTTTAAACGTATGGTGTCAAAAATGCTAGTCCTT  
TCATGATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTTCGTGGAGTGATCTGTCTGCTTAATTGCGTTT  
CACTACGAATCTTCTTTAATAGTGTGTTTGTGAGATGGTCTGATCCCTCCCACGCTCTCTGAGTGTGGCG  
AGTTGTGCGTCTTTCCATCGTTACCACATCGCACAAACGTATGATTCTGAAAGCAACGAACGTGACCGCAA  
CCTCTTGTTGCCTGTATCTCCAAAACAGTCTGCACCTCTGTGCTCTCTGTATAAACAGGCCTTTTTTATTAATAA  
ACTAGAGGGACCGCTGTTACTTTCTTAAACCAGAGGAAGGTTGCGGCAATAACAGGTCTGTGATGCCCTT  
AGATGTTCCGGGCTGCACACGTGCTACAATGATTATTGCAGTGAGCATCTCATATTTTACACATCACTTGC  
GCGAGCTCCTTAACTTTGGGTTATGGTGTCTCTGTGCGTGATAAAGCCTGCCTCGAAAGGTCAGCGGGT

AATCAATTAGAAGTAATGATTTCCCTTTATATGCACATTTATGCTTGGCACTGTTCCCCATGGCTAGTCTTC  
GTGCTAGTTCCCTGTGCGTGTTTCAGTGGGCCCTTAGGGTCTCCTACCTTGCCTGCAATTGTCAATTCATGGTG  
GGGACAGACCATTGTTAATTGTTGGTCTCGGTCTTAAGTACAGGAATGCCTTGTACGGGTCTTTGGTTCAACA  
AACCACCCGGAATACGTCCCTGCCCTTTGTACACACCGCCGTCGCTCTTACCGATGGATTTCTCTGTGAG  
TTTGAAGGACTGGCCTTCTGTGCTATGGAAATTCAAACGAACAGTGTGATCTAAAGGAAAGAGAAGTCGT  
AACAAGGCATCGGTAGGTGAACCTGCAGAAGGATCA

**Glabratellina\_8**

**DNA: 1,019 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACGCGGGAAATCTTACCGGGTCC  
GGACACACTGAGGATTGACAGTTTTATCCAGTTTCTACTCGTAGAGCTGGTGTCAAAAATGCTAGTCCCT  
TTCATGATTATGTGATAGGTGGTGCATGGCCGTCTTAGTTTCGTGGAGTGATCTGTCTGCTTAATTGCGTTT  
CACTACGAATCTACATTAACGTACGTTTTCGAGATGGACTGATCCCTCCCGCTCTCTGAGTGTGGAGTT  
GTGCGTCTTTCCATCGTTACCGCATCGTACAACGTATGATTCTGAAAGCAACGAACGTGACCGCAACCTC  
TTGCTGCCTGTATTTCCAAAACAGTCTGCACTCTGTGCTCTCTGTATAAACAGGCCTTTTTATTAATAAACT  
AGAGGGACCGCTGTTACTTTCTTAAACCAGAGGAAGGTTGCGGCAATAACAGGTCTGTGATGCCCTTAGA  
TGTTCCGGGCTGCACACGTGCTACAATGATTATTGCAGTGAGCATCTCATATTTTACACATCACCTGCGCG  
AGTCCCTTAACCTTTGGGTTATGGTGTCTCTGTGCGTGATAAAGCCTGCTTCGAAAGTCAAGCGGTAATC  
AATTAGAAGTAATGATTTCCCTTTATATGCACATTTATGCTTGGCACTGTTCCCCATGGCTAGTCTTCGTGC  
TAGTTCCCTGTGCGTGTTTCAGTGGGCCCTTAGGGTCTCCTACCTTGCCTGCAATTGTCAATTCATGGTGGGA  
CAGACCATTGTTAATTGTTGGTCTCGGTCTTAACTAGGAATGCCCTGTACGGGTCTTTGGTTCAACAAACC  
ACCCGGAATACGTCCCTGCCCTTTGTACACACCGCCGTCGCTCTTACCGATGGATTTCTCTGTGAGTTG  
AAGGACTGGCTTTTTTGTATGGAAATTCAAACGAACAGTGTGATCTAAAGGAAAGAGAAGTCGTAACA  
AGGCATCGGTAGGTGAACCTGCAGAAGGATCA

**Glabratellina\_9**

**DNA: 1,024 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACGCGGGAAATCTTACCGGGTCC  
GGACACACTGAGGATTGACAGTTTTATCCAGTTTCTACTCGTAGAGCTGGTGTCAAAAATGCTAGTCCCT  
TTCATGATTATGTGATAGGTGGTGCATGGCCGTCTTAGTTTCGTGGAGTGATCTGTCTGCTTAATTGCGTT  
TCACTACGAATCTACATTAACGTACGTTTTCGAGATGGACTGATCCCTCCCGCTCTCTGAGTGTGGAGT  
TGTGCGTCTTTCCATCGTTACCGCATCGTACAACGTATGATTCTGAAAGCAACGAACGTGACCGCAACCT  
CTTGTTCCTGTATTCCAAAACAGTTTGCCTCCGTGCTATTCTGTATAAACAGGCCTTATATTAATAAAAC  
TAGAGGGACCGCTGTTACTTTCTTAAACCAGAGGAAGGTTGCGGCAATAACAGGTCTGTGATGCCCTTAG  
ATGTTCCGGGCTGCACACGTGCTACAATGATTATTGCAGTGAGCATCTCATATTTCTCACATCTCAGCGTGA  
GCCGCTATCTTGCTTCGGCTTGATGGTGTGCTCTACGCGGGATCAAGCCTGCTTCGAAAGTAAGTGGGT  
AATCAATTAGAAGTAATGATTTCCCTTTATATGCACATCTATGTTTGGCGCTGATCCCTTACTAACTCTT  
GTTAGCTTCTGTGTGCGTTCAGTGAAGCTCTTAGCTTTCTACCATTTCGTGCAACTGTCAATTCATGGTG  
GGGACAGACCATTGTTAATTGTTGGTCTCGGTCTTAACTAGGAATGCCTTGTACGGGTCTTTGGTTCAACA  
AACCACCCGGAATACGTCCCTGCCCTTTGTACACACTGCCCGTCGCTCTTACCGATGGATTTCTCTGTGAG  
TTTGAAGGACTGGCCTTCTGTGCTATGGAAATTCAAACGAACAGTGTGATCTAGAGGAAAGAGAAGTCGT  
AACAAGGCATCGGTAGGTGAACCTGCAGAAGGATCA

**Elphidium\_1**

**DNA: 808 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACACGGGAAATCTTACCGGGTCC  
GGACACATTGAGGATTGACAGACATGACCTTAATTAATAATTTTGGTCTTACAAAGATGCTAGTTC  
TTTCATGATTATGTGATAGGTGGTGCATGGCCGTCTTAGTTTCGTGGAGTGATCTGTCTGCTTAATTGCGT  
TTCATATTTAAATTTAATTACGCCTACCTCTGTGTAGTGTGTGATACTATGTTTGAAGGCAACGAACGTGA  
CCGTATTCTTATATTTAATTTAATTTTAAAGAGACCGCTGATTCTCTTTTTAAACCAGAGGAAGGTTTC

GGCAATAACAGGTCTGTGATGCCCTTAGATGTTCCGGGCTGCACACGTGCTACAATGATCATTTTCATTAA  
GTACTIONAAACCCTCCTTCGAAAAGTTGAGTGGGTAATCAATTA AAAAGTAATGACTCTATTCTATTATGACTC  
ACACTCACACTATCTTATTTTAATTTAATTTAATTTAATTTAATTTAGTAATTGTCTTATTGTTAATTCTTATTGTTG  
GGACAGTCCATTGTTAATTGTTGGTCTCGCTTTTAACTAGGAATGCCTTGTACTGGTCTTTGGTTCAACAA  
ACCACCAGGAATACGTCCCTGCCCTTTGTACACACCCGCCGTCGCTCTTACCGATGAACTCTGCTATGAGT  
TTGAAGGATGTATTAATTAATTTAATTTATGCGAATAGTTTGGTTTAAAGGAAAGAGAAGTCGTAACAAG  
GCATCAGTAGGTGAACCTGCAGAAGGATCA

**Elphidium\_2**

**DNA: 808 bp**

AAGGGCACCACAAGAACGCGTGGAGCACGTGGCTTAATTTGACTCAACACGGGAAATCTTACCGGGTCC  
GGACACATTGAGGATTGACAGACATGACCTTAATTA AAAATTTAATTTTGGTCTTACAAAGATGCTAGTTC  
TTTCATGATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTCGTTGGAGTGATCTGTCTGCTTAATTGCGT  
TTCATATTTAAATTTAATTACGCCTACCTCTGTGTAGTGTGTGATACTATGTTTGAAGGCAACGAACGTGA  
CCGTATTCTTATATTTAATTTAATTATTTAAGAGACCGCTGATTCTCTTTTAAACCAGAGGAAGGTTTC  
GGCAATAACAGGTCTGTGATGCCCTTAGATGTTCCGGGCTGCACACGTGCTACAATGATCATTTTCATTAA  
GTACTIONAAACCCTCCTTCGAAAAGTTGAGTGGGTAATCAATTA AAAAGTAATGACTCTATTCTATTATGACTC  
ACACTCACACTATCTTATTTTAATTTAATTTAATTTAATTTAATTTAGTAATTGTCTTATTGTTAATTCTTATTGTTG  
GGACAGTCCATTGTTAATTGTTGGTCTCGCTTTTAACTAGGAATGCCTTGTACTGGTCTTTGGTTCAACAA  
ACCACCAGGAATACGTCCCTGCCCTTTGTACACACCCGCCGTCGCTCTTACCGATGAACTCTGCTATGAGT  
TTGAAGGATGTATTAATTAATTTAATTTATGCGAATAGTTTGGTTTAAAGGAAAGAGAAGTCGTAACAAG  
GCATCAGTAGGTGAACCTGCAGAAGGATCA

**Elphidium\_3**

**DNA: 815 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACACGGGAAATCTTACCGGGTCC  
GGACACATTGAGGATTGACAGACATAACGACCTTAATTAATTTAATTTTGGTCTTAAATTA AAAAGACGCTA  
GTTCTTTTCATGATTATGTGATAGGTGGTGCATGGCCGTTCTTAGTTCGTTGGAGTGATCTGTCTGCTTAATT  
GCGTTTTTCATATTTAAATTTAATTACTTCTACTTTAAGTAGTTGTATTATACTGTTTGAAGGCAACGAACGT  
GACCGTATTCTTATACTTAATTTAATTATTTAAGAGACCGCTGATTCTCTTTTAAACCAGAGGAAGGTT  
TCGGCAATAACAGGTCTGTGATGCCCTTAGATGTTCCGGGCTGCACACGTGCTACAATGATCATTTTCATTA  
AGTAGCTAAAATCCTCCTTCGAAAAGTTGCCTTACGAGGTAATCAATTA AAAAGTAATGACTCTATACTTTAA  
ATGACTCACACTCACACTATCTTATTTTAATTTAATTTAATTTAATTTAATTTAGTAATTGTCTTATTGTTAATTCT  
TATTGTTGGGACAGTCCATTGTTAATTGTTGGTCTCGCTTTTAACTAGGAATGCCTTGTACTGGTCTTTGGT  
TCAACAAAACCACCAGGAATACGTCCCTGCCCTTTGTACACACCCGCCGTCGCTCTTACCGATGAACTCTG  
CTATGAGTTTGAAGGATGTATTAATTAATTTAATTTATGCGAATAGTTTGGTTTAAAGGAAAGAGAAGTC  
GTAACAAGGCATCAGTAGGTGAACCTGCAGAAGGATCA

**Elphidium\_4**

**DNA: 811 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACACGGGAAATCTTACCGGGTCC  
GGACACATTGAGGATTGACAGACATGACCTTAATTA AAAATTTAATTTTGGTCTTACAAAGATGCTAGTTC  
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TTCATATTTAAATTTAATTACTTCTACTTTAAGTAGTTGTATTATACTGTTTGAAGGCAACGAACGTGACC  
GTATTCTTATATTTAATTTAATTATTTAAGAGACCGCTGATTCTCTTTTAAACCAGAGGAAGGTTTCGG  
CAATAACAGGTCTGTGATGCCCTTAGATGTTCCGGGCTGCACACGTGCTACAATGATCATTTTCATTAAGT  
ACTTAAACCCTCCTTCGAAAAGTTGCCTTACGAGGTAATCAATTA AAAAGTAATGACTCTATACTTTAAATG  
ACTCACACTCACACTATCTTATTTTAATTTAATTTAATTTAATTTAATTTAGTAATTGTCTTATTGTTAATTCTTATT  
GTTGGGACAGTCCATTGTTAATTGTTGGTCTCGCTTTTAACTAGGAATGCCTTGTACTGGTCTTTGGTTCA  
ACAAACCACCAGGAATACGTCCCTGCCCTTTGTACACACCCGCCGTCGCTCTTACCGATGAACTCTGCTA

TGAGTTTGAAGGATGTATTAATTAATTTAATTTATGCGAATAGTTTGGTTTAAAGGAAAGAGAAGTCGTA  
ACAAGGCATCAGTAGGTGAACCTGCAGAAGGATCA

**Elphidium\_5**

**DNA: 811 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACACGGGAAATCTTACCGGGTCC  
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TTTCATGATTATGTGATAGGTGGTGCATGGCCGTCTTAGTTCGTGGAGTGATCTGTCTGCTTAATTGCGT  
TTCATATTTAAATTTAATTACTTCTACTTTAAGTAGTTGTATTATACTGTTTGAAGGCAACGAACGTGACC  
GTATTCTTATATTTAATTTAATTATTTAAGAGACCGCTGATTCTCTTTTAAACCAGAGGAAGGTTTCGG  
CAATAACAGGTCTGTGATGCCCTTAGATGTTCCGGGCTGCACACGTGCTACAATGATCATTTCATTAAGT  
ACTTAAACCCTCCTTCGAAAAGTTGCCTTACGAGGTAATCAATTAATAAGTAATGACTCTATACTTTAAATG  
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GTTGGGACAGTCCATTGTTAATTGTTGGTCTCGCTTTTAACTAGGAATGCCTTGACTGGTCTTTGGTTCA  
ACAAACCACCAGGAATACGTCCCTGCCCTTGTACACACCGCCCGTCGCTCTTACCGATGAACTCTGCTA  
TGAGTTTGAAGGATGTATTAATTAATTTAATTTATGCGAATAGTTTGGTTTAAAGGAAAGAGAAGTCGTA  
ACAAGGCATCAGTAGGTGAACCTGCAGAAGGATCA

**Elphidium\_6**

**DNA: 811 bp**

AAGGGCACCACAAGAACGCGTGGAGCATGTGGCTTAATTTGACTCAACACGGGAAATCTTACCGGGTCC  
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TTCATATTTAAATTTAATTACTTCTACTTTAAGTAGTTGTATTATACTGTTTGAAGGCAACGAACGTGACC  
GTATTCTTATATTTAATTTAATTATTTAAGAGACCGCTGATTCTCTTTTAAACCAGAGGAAGGTTTCGG  
CAATAACAGGTCTGTGATGCCCTTAGATGTTCCGGGCTGCACACGTGCTACAATGATCATTTCATTAAGT  
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ACTCACACTCACACTATCTTATTTAATTTAATTTAATTTAATTTAGTAATTGCTTATTGTTAATTCTTATT  
GTTGGGACAGTCCATTGTTAATTGTTGGTCTCGCTTTTAACTAGGAATGCCTTGACTGGTCTTTGGTTCA  
ACAAACCACCAGGAATACGTCCCTGCCCTTGTACACACCGCCCGTCGCTCTTACCGATGAACTCTGCTA  
TGAGTTTGAAGGATGTATTAATTAATTTAATTTATGCGAATAGTTTGGTTTAAAGGAAAGAGAAGTCGTA  
ACAAGGCATCAGTAGGTGAACCTGCAGAAGGATCA

## **CHAPTER 4**

# **Seasonal Variations, Environmental Parameters, and Standing Crop Assessment of Benthic Foraminifera in eastern Bahrain, Arabian Gulf**

### **4.1 Abstract**

Living benthic foraminifera in a relatively unpolluted site offshore Bahrain in the Arabian Gulf, were studied to determine the seasonal variability of their populations, as well as environmental parameters that may affect their distribution. The maximum foraminiferal density was observed during winter with the assemblages primarily dominated by rotaliids and secondarily by miliolids. The high population is attributed to an increased number of juveniles. A relationship between sediment grain size and the foraminiferal density reveals that juveniles were most abundant on coarse-grained sandy substrate and less abundant on fine-grained substrates. In spring, the foraminiferal density decreased, and the lowest values were observed during summer. The population increased again in autumn with highest juvenile/adult ratios. Moreover, results of relative abundance and species consistency show that *Ammonia* and *Glabratellina* are consistent from the shallowest to the deepest station, whereas miliolids occurred only at deeper stations. The numbers of peneroplidae and *Elphidium* also increased along the depth transect. Environmental characterization reveals that although the site is subject to eutrophication caused by nitrates and sulfates, pollution caused by hydrocarbons and heavy metals is not

significant. The assessment of 63 heavy metals showed that none of the metals had concentrations that exceed internationally accepted norms [the devised level of Effect Range-Low], but with high concentration of strontium. The lack of a significant environmental effect of heavy metals is confirmed by the Foraminiferal Abnormality Index of <2%. Likewise, no hydrocarbon contamination was detected in the water or sediment samples. We conclude that the site in Bahrain is not yet adversely affected by human development, and therefore can provide baseline information for future comparison and assessment of foraminiferal assemblages in contaminated zones of the Arabian Gulf.

**Keywords** — Arabian Gulf, Benthic Foraminifera, Standing Crop, Eastern Bahrain.

## **4.2 Introduction**

Benthic foraminifera represent a diverse group of marine protists that are ubiquitously distributed in marine and transitional marine habitats [10]. Their distributional patterns are generally dependent on both environmental conditions and seasonal variations [34, 78]. Their assemblages reflect environmental gradients such as water depth, physicochemical parameters of water, substrate parameters, availability of nutrients, and the effects of anthropogenic pollution, in addition to natural seasonality related to their reproductive cycle [34, 78]. Benthic foraminifera have been widely used to study environmental changes in marginal marine, coastal, and marine shelf environments (i.e. see review in Murray and Alve, 2002). For example, Sarita and coworkers illustrated environment specific spatial and seasonal distribution of living benthic foraminifera in the estuary of Guadiana (southeastern Portugal) [78]. In another example, Frontalini and coworkers reported low diversity benthic

foraminiferal assemblages in the lagoon of Santa Gilla (Italy) affected by industrial, agricultural, and domestic discharges [49]. Benthic foraminifera have been widely used as bioindicators and for assessing the health of marine ecosystem as consequences of pollution [33, 88, 95].

The western part of the Arabian Gulf is the world's largest hypersaline sea [128], and as such it offers unique marine habitats to foraminiferal assemblages. The history of foraminiferal study in the Arabian Gulf is not new and their distributional patterns, taxonomy, and ecology have been largely investigated [10, 69, 72, 129-134]. However, human activities are now posing major threat on the Arabian Gulf coastal environments, both onshore and offshore. Coastal vegetation (mangroves) in the area has been already affected by human activities [135]. Extensive human activities also disturbed large areas along the Saudi Arabian and U.A.E. coastlines, and now many of Murray's (1966a, b, c, d) original sample localities along the U.A.E. coast are located beneath parking lots (F. Fiorini, pers. comm., 2011). Furthermore, as the Arabian Gulf is exploited as one of the main oil producing regions of the world, more than half of the world's petroleum is transported through the Gulf [136]. As a result, hydrocarbon drilling activities combined with extensive urbanization are systematically disturbing coastal areas [137, 138].

The understanding of the foraminiferal distributional patterns requires consideration of a broad range of seasonal and environmental factors. Studies elucidating the effects of temporal variations on benthic foraminiferal assemblages are few and are mostly based on standing crop assessment without assessing the effects environmental parameters [72, 139]. Basson and Murray reported temporal variations of four intertidal foraminiferal species in a lagoon from western Bahrain, whereas Scott and coworkers presented

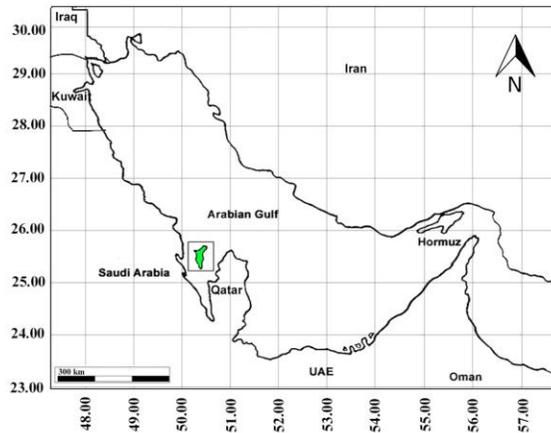
temporal variations of benthic foraminiferal assemblages under aquaculture operations. However, there is a still need to document and infer the role of environmental factors coupled with seasonal variations from an undisturbed area. Furthermore, it is essential to establish baseline studies of foraminiferal assemblages for future environmental assessment, and to provide controls for monitoring the effects of anthropogenic activities that threaten marine ecosystems.

The chapter summarizes (a) documentation of the seasonal variations of foraminiferal density (FD) and distribution of living benthic foraminifera in a coastal area of eastern Bahrain; and (b) environmental characterization of the study area by evaluating the pollution levels and the ecological quality of the area.

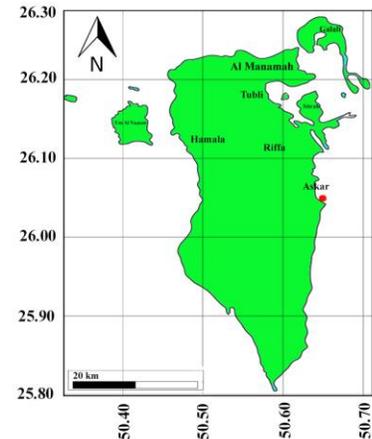
#### **4.2.1 Study Area**

The study was conducted south of the town of Askar, a fishing village on the eastern coast of Bahrain (Fig. 29A, B). The locality was selected because it is located in a protected cove next to the Bahrain Department of Fisheries research station, and therefore relatively undisturbed by human activities. The sample locality is just offshore from a small lagoon that was originally investigated for foraminifera by Basson and Murray [72]. This lagoon, which partially lies within the property of the research station represents the only site within the Kingdom of Bahrain that has been previously studied for foraminifera, and has become known to our research group as “Murray’s Pool” [140]. The foreshore to offshore transect off “Murray’s Pool”, located at 26°02'37.11" N, 50°37'32.77" E, was sampled for this study (Fig. 29C).

A)



B)



C)

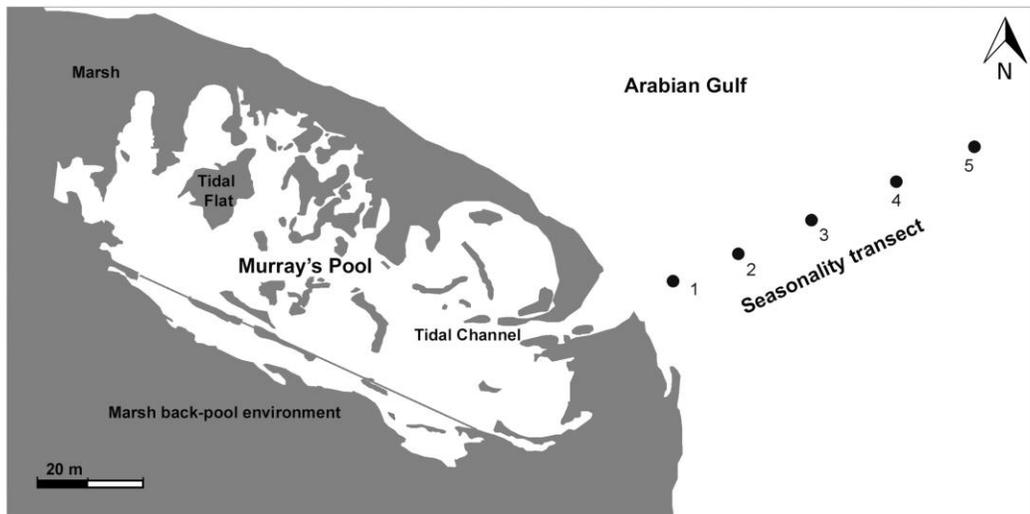


Figure 29: Geographical context of “Murray’s Pool” in the Arabian Gulf: (A) The Arabian Gulf (B) location map showing study area in eastern Bahrain (C) the depth transect with sampling locations.

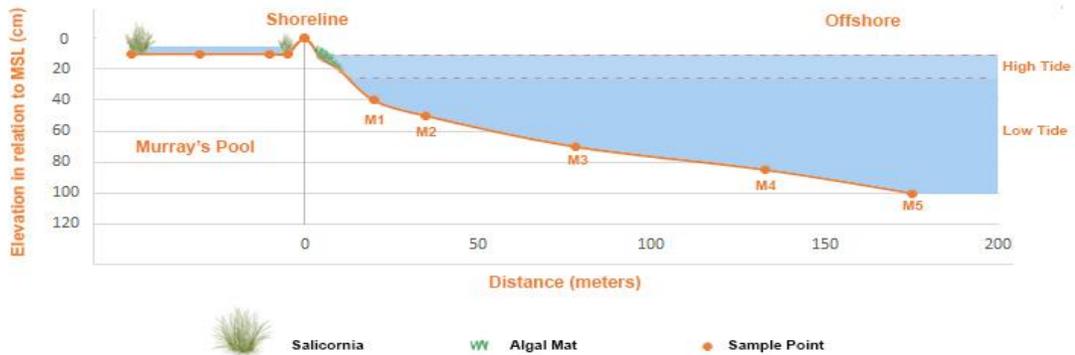
The coastal area of eastern Bahrain is microtidal (<1 m) with a diurnal rhythm [141]. The foreshore is wide, slopes very gently, and is characterized by a soft, silty, sandy carbonate sediment veneer discontinuously overlying lithified hard-ground [72]. Furthermore, the foreshore is occasionally covered with a bloom of an algal mat spreading over the sediments, particularly during summer, with isolated patches of sea grass beginning about

50 m from the shoreline. On the eastern side of Bahrain, water temperature varies between 17.5°C in winter to 36.6°C in summer, whereas salinity remains mostly constant throughout the year, i.e. 45-46‰ [72, 141].

## 4.3 Materials and Methods

### 4.3.1 Sampling Strategy

In order to assess the seasonal effects, five samples were collected during four seasons (i.e., winter, spring, summer, and autumn), from the foreshore area along the depth transect offshore from “Murray’s Pool” (Figure 29C). Sampling sites were placed at 17, 50, 125, 200 and 250 m from the shore and locations were determined by GPS. The winter sampling was carried out in late December 2013, followed by a spring survey in early March 2014, a summer one at the end of May 2014, and an autumn one in early October 2014. The whole study comprised 20 intertidal bottom water and sediments from water depths of 35 cm to 1.0 m (Figure 30).



**Figure 30: The seasonality transect profile with respect mean sea level.**

Bottom waters were sampled by dipping well-rinsed glass jars at each station prior to the sediment sampling to avoid any alteration of physicochemical parameters. Sediment

samples with a depth of ~1.2 cm (volume ~57.6 cm<sup>3</sup>) were collected with a spatula taking care not to disturb the sediment floor, and placed into plastic storage boxes fitted with a lid that was secured under water. A layer of aluminum foil was placed over the jar mouth to avoid sediment contact with the plastic cap. Both water jars and sediments boxes were immediately transported to the laboratory for analysis. Sample processing was carried out at the Research Institute and Environmental Sciences labs at King Fahd University of Petroleum and Minerals (Saudi Arabia). Sediment and water samples used for the characterization of the environmental quality (eutrophication indicators, heavy metals and hydrocarbons) were only analyzed during the winter season.

#### **4.3.2 Benthic Foraminifera Analysis**

In the laboratory, 5 cm<sup>3</sup> of sediment was taken from each box. Each sample was carefully washed with seawater through a 63 µm mesh sieve. Finally, the entire residue was microscopically analyzed and the total numbers of living foraminifera (both adults and juveniles) were wet-picked under a reflected-light binocular microscope based on the presence of protoplasm. We visually distinguished "living" (protoplasm-filled tests except in the last chamber) from "dead" (protoplasm-empty or degraded) as described previously [142]. Foraminiferal assemblage parameters of the standing crop were calculated, including the adult/juvenile (A/J) ratio (individuals with diameter less than 150 µm were considered as juveniles), foraminiferal density (FD, number of living individuals per 5 cm<sup>3</sup>), generic diversity ("richness", S), foraminiferal dominance (D), and faunal constancy ( $Fc = \frac{n}{N} \times 100$ , where n is the number of samples where the species occurs and N is the total of samples collected). Foraminifera were taxonomically identified at genus level for juveniles and at species level for adults with the aim to

understand the total standing crop and species representation during each season. Taxonomical identification was carried largely following the monographs of Hottinger, Loeblich and Tappan and Hayward and workers [1, 15, 22, 143, 144]. Because much of the assemblages consisted of juveniles, we did not attempt to resolve the species taxonomy. Group diversity was further assessed by the Fischer  $\alpha$  index and the Shannon diversity index ( $H' = -\sum p_i \times \ln p_i$ ) (Shannon, 1948; Shannon and Weaver, 1963) as well as evenness (J), and equitability (E). The above mentioned diversity indices were calculated using the PAST – PAleontological STatistics data analysis package (version 1.68). The diversity indices were derived to compare between samples in this study and are not comparable to studies that have reported species level diversity indices. Therefore, the diversity indexes must be considered with care being calculated at the group level. Lastly, Foraminiferal Abnormality Index (FAI) was calculated to possibly document the effect of pollution [92]. The most important foraminiferal species were photographed using a scanning electron microscope (SEM).

### **4.3.3 Physicochemical Parameters of Water**

Salinity, temperature, and pH were measured in situ using YSI multi-probe during each sampling period. However, conductivity, bicarbonate alkalinity, and turbidity were evaluated in the laboratory using PC-BODTM Stand Alone System (MANTECH-YSI probes) by running samples in duplicates.

### **4.3.4 Grain Size Analysis**

In order to determine the grain-size distribution of the sediments along the transect, samples were treated initially with an H<sub>2</sub>O<sub>2</sub> solution to remove the organic matter. Afterwards, standard analysis was performed by taking 50 grams of each sample

followed by manual sieving and drying at 60°C. The grain size distributions were statistically and graphically summarized to understand the porosity and permeability for later analysis [145].

#### **4.3.5 Total Organic Carbon (TOC) Analysis**

For TOC analysis, approximately 200 mg of the dried and ground sample was weighed and placed in ceramic boats. Afterwards, sample was suspended in a diluted hydrochloric acid solution thrice a day to break down all the carbonates present in the sample resulting into removal of total inorganic carbon (TIC). Lastly, the suspensions were injected and analyzed in Shimadzu TOC-Vcsh Total Organic Carbon Analyzer for TOC analysis. Standards and samples were weighed in duplicates and five calibration points were taken for drawing a calibration curve.

#### **4.3.6 Eutrophication Pollution Analysis**

The eutrophication indicators ( $\text{SO}_4^{2-}$ ,  $\text{PO}_4^{2-}$ ,  $\text{NO}^{-3}$ , and  $\text{NO}^{-2}$ ) were detected by using Ion chromatography (IC- Metrohm 850 Professional system, Switzerland). The seawater samples were prepared by performing 1000-fold dilution in ultra-pure water. Prior to analysis, the standard solutions of 10 ppm concentration were prepared for each ion and then injected into the system to assess the performance and calibration of the instrument [146].

#### **4.3.7 Heavy Metals Analysis**

In order to determine the heavy metal contents in the sediments, 5 g of each sample was dried under the light bulb at low temperature to prevent the evaporation of heavy-metals, then reduced to fine powder. Thereafter, the heavy metal content was investigated in all

the sediments by Activation Laboratories Ltd. (Ontario, Canada, <http://www.actlabs.com>) that analyzed a fraction of 0.5 g of a sample for 63 elements using inductively coupled plasma mass spectrometry (ICP-MS), which is a multi-element technique capable of measuring concentrations at very low detection limits (mg/kg-1 to µg/kg). The sample material was digested in aqua regia (0.5 ml H<sub>2</sub>O, 0.6 ml concentrated HNO<sub>3</sub> and 1.8 ml concentrated HCl) at 90°C in a microprocessor controlled digestion block for 2 hours. The analyses were performed under standard quality control protocols.

#### **4.3.8 Hydrocarbons Analysis**

Hydrocarbon extraction from the sediments was performed using the ASE 200 accelerated solvent extraction system, a procedure to extract organic solvents at high temperature and pressure above the boiling point as described as Method 3545 in U.S. EPA SW-846 Methods. In order to perform this analysis, representative samples of 5 g of sediments from each station was taken and homogenized equally with commercially available hydrant for removal of moisture content. The mixture was directly enclosed into the sample cells which were subsequently installed on the system to statically extract the hydrocarbons under 100°C temperature and 500 psi pressure for 20 min. Finally, compressed gas allowed extraction of hydrocarbon from the sample cell to the collection vessel using n-hexane. For quality control, samples were run in duplicates and surrogate spiking was performed to assess the extraction efficiency.

Analyses of the extracts were performed using gas chromatography flame ionization detector (GC/FID) Agilent technology 7890A GC system. Separations were performed using a 30 m × 0.32 mm internal diameter Varian capillary column. The carrier gas supply was helium with column flow rate of 25 mL/min and the pressure was regulated

by hydrogen and air flowing at rate of 30 mL/min and 300 mL/min, respectively. The column temperature during transfer was 60°C. It was maintained for 1 min, and then programmed at 10°C/min to 150°C for 12 min. The temperature of the flame ionization detector (FID) was 200°C. Peaks were integrated using a Chrom Card system (CE Instruments). Finally, quantification of the total hydrocarbon content (THC) was calculated using a hydrocarbon window of C10 to C36 calibration standards.

#### **4.3.9 Statistical Analysis**

In order to determine the assemblages' relationship with environmental parameters, multivariate techniques principal component analysis (PCA) and cluster analysis (CA) were performed using Statistica v6.0. Prior to statistical analysis, the data was normalized and an additive logarithmic transformation  $\log(1 + X)$  was performed to eliminate the effects of orders of magnitude differences between different environmental variables. The CA was applied to identify the similarities between sampled stations. The analysis was based on the Euclidean distance and the Ward's linkage method that produced dendrograms with exceptionally well-defined clusters [147] where each cluster includes stations with a similar spatial distribution pattern [148]. The PCA attempts to recognize the responsible factors explaining pattern of correlation within a set of observed variables. In a PCA, it is also possible to compute additional variables (biotic data) which do not contribute to the results.

## 4.4 Results

### 4.4.1 Environmental Characterization of the Study Area

The spatial variability of environmental parameters, i.e. physicochemical parameters of water and geochemical parameters of sediments, were analyzed along the depth transect during each season. Furthermore, the current level of pollution is evaluated in terms of eutrophication indicators, heavy metals and hydrocarbons during the foraminiferal peak season (i.e. winter). Pollution parameters were compared with the benthic foraminifera in order to assess their effects on living assemblages. The salinity, temperature, pH, conductivity, turbidity and bicarbonate alkalinity of seawater results are presented in Appendix 1 (a, b). All physicochemical parameters showed minor variation between the sampling stations i.e. salinity  $45.6 \pm 0.6$  PSU, temperature  $24.3 \pm 3.2^\circ\text{C}$ , pH  $8.23 \pm 0.04$ , conductivity  $54656 \pm 1777$ , turbidity  $0.73 \pm 0.01$ , and bicarbonate alkalinity  $103.7 \pm 1.1$  (Appendix 1a,b). Results of grain size analysis documented a gradual decrease of fine sand contents seaward for each season (Appendix 1). The TOC ranged from 3379 mg/kg to 10035 mg/kg with the highest value at shallowest stations (Appendix 1a,b).

The environmental quality of both water and sediment was assessed during the season of highest reproduction i.e. winter in order to relate to benthic foraminiferal assemblages. The level of nitrates and sulfates was high in all samples but their concentration decreased along the transect (Appendix 2). Compared to the ER-L (Effect Range – Low) and ER-M (Effect Range – Median) values reported for the United States Environmental Protection Agency's (USEPA) sediment guidelines (Long et al., 1995; Ligero et al., 2002), none of heavy metals were beyond the permitted standards; however, strontium

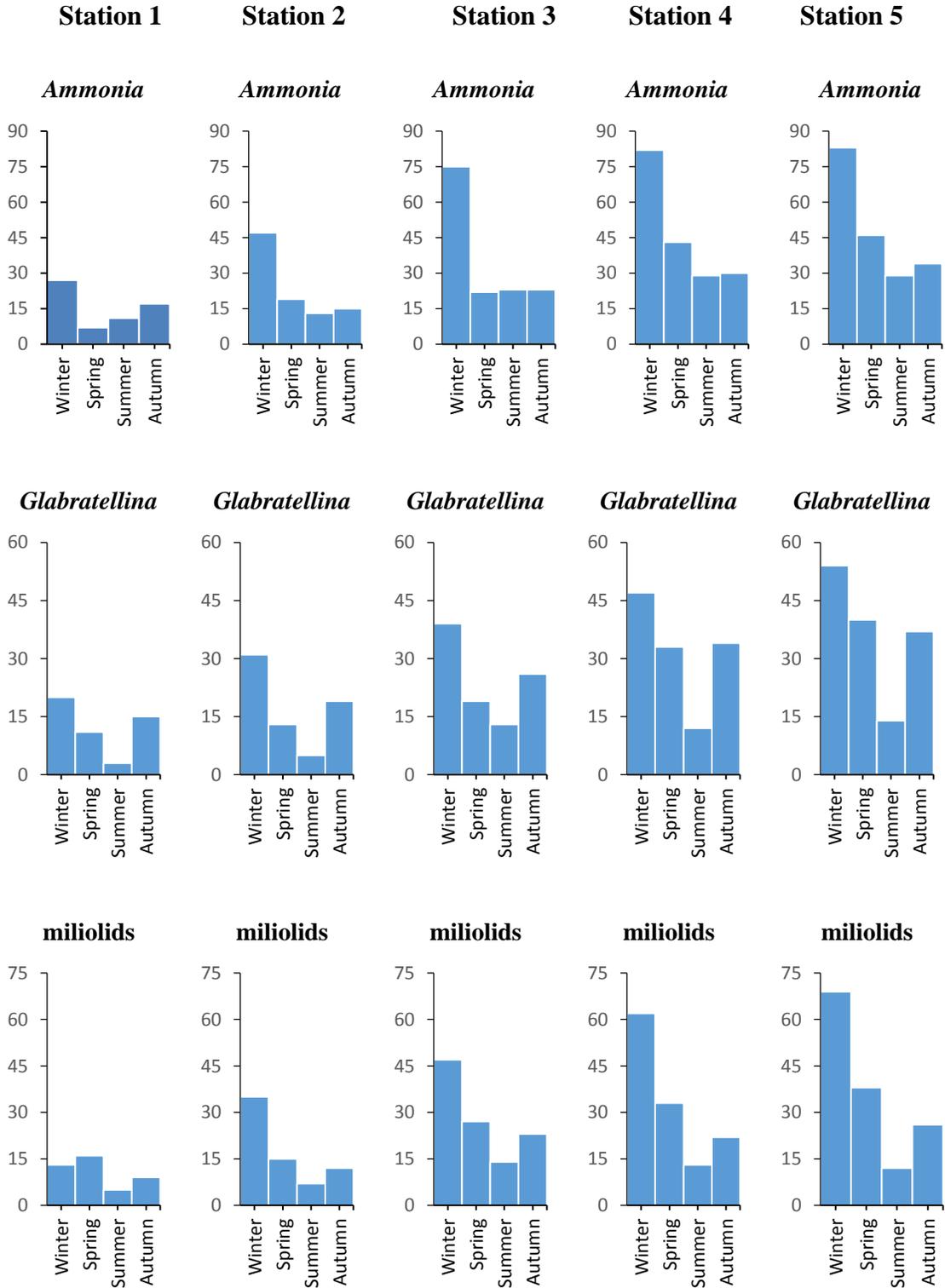
exhibited higher values. The highest value of hydrocarbons (9.18 ppm), as THC, was found in the first station and the THC content reduced seaward (Appendix 2). No hydrocarbons were detected in stations 3, 4 and 5 (Appendix 2).

#### **4.4.2 Benthic Foraminifera Analysis**

All the studied samples contained abundant and well-preserved living benthic foraminifera. The foraminiferal density varied between 19 and 215 with a mean of 86.4 individuals per 5 cm<sup>3</sup>. The foraminiferal density increased along the transect and the highest numbers were found in the station 5 during all the seasons (Appendix 3). There was a marked increase in foraminiferal density from stations 1 to 4 and then it foraminiferal density did not vary considerably between stations 4 and 5. The highest foraminiferal density values were found in the winter samples and the lowest in the summer sample (Appendix 3). The higher value of foraminiferal density was mainly due to the increased number of juveniles along depth transect in autumn, winter and spring whereas, in summer, the juvenile's population remained approximately constant in all the stations (Appendix 3). More specifically, in the depth transect, the juveniles' population increased from 58% to 71% during autumn, 24% to 49% during winter, and 21% to 37% during spring. Overall, the absolute relative abundance of juveniles was at the highest (65%, on average) during autumn and then reduced to 39% in winter, 28% in spring, and 27% in summer (Appendix 3).

Only six taxonomical groups were found to be living at the moment of collection. These were *Ammonia*, *Glabratellina*, *Elphidium*, *Brizalina*, miliolids (*Cycloforina* and *Quinqueloculina*) and peneroplidae (*Monalysidium*, *Coscinospira*, and *Peneroplis*) (Plate

1). Their absolute abundances along the depth transect and during different seasons are presented in Figure 31, 32, and Appendix 3.



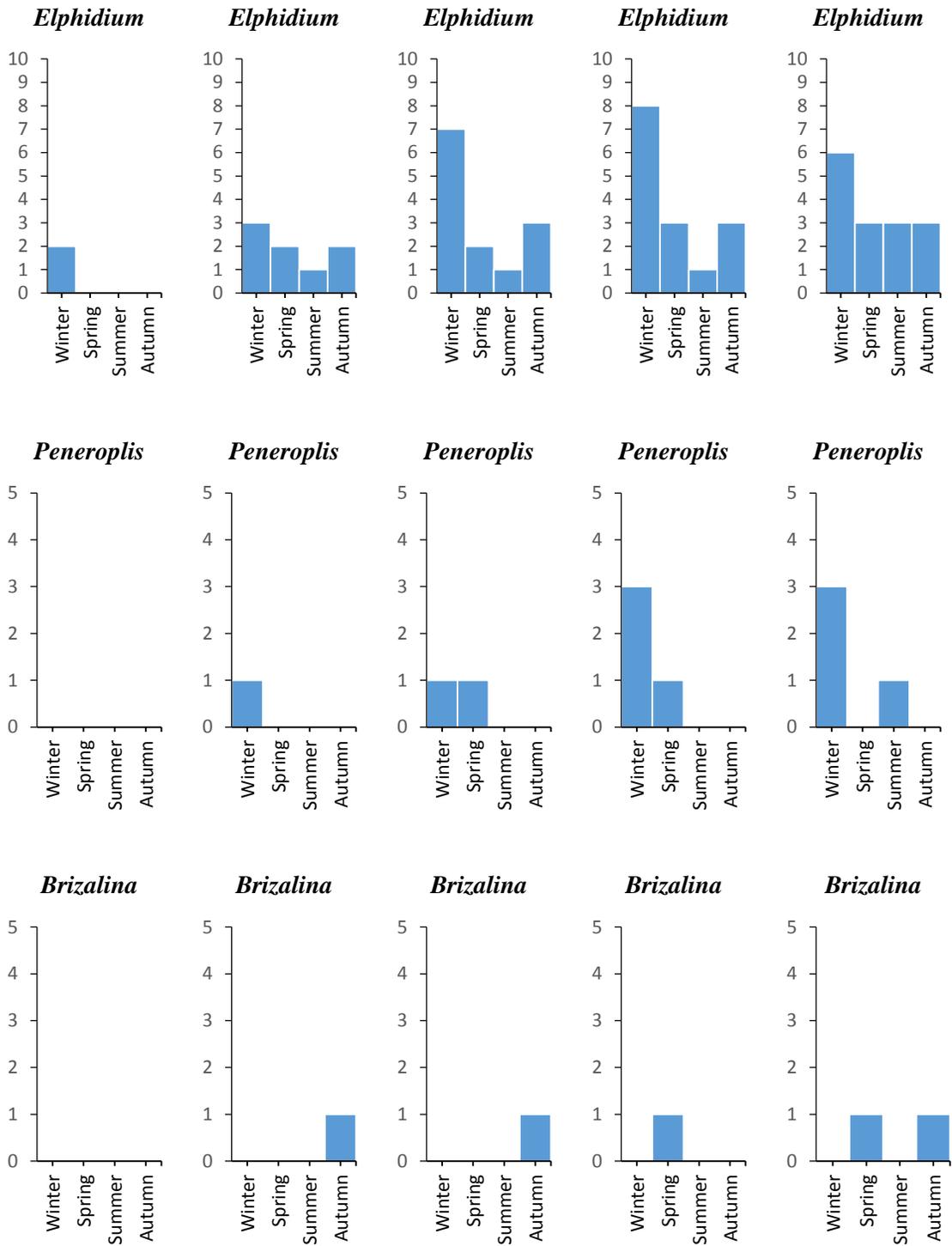


Figure 31: Seasonal variations in the relative abundance of six benthic foraminiferal groups.

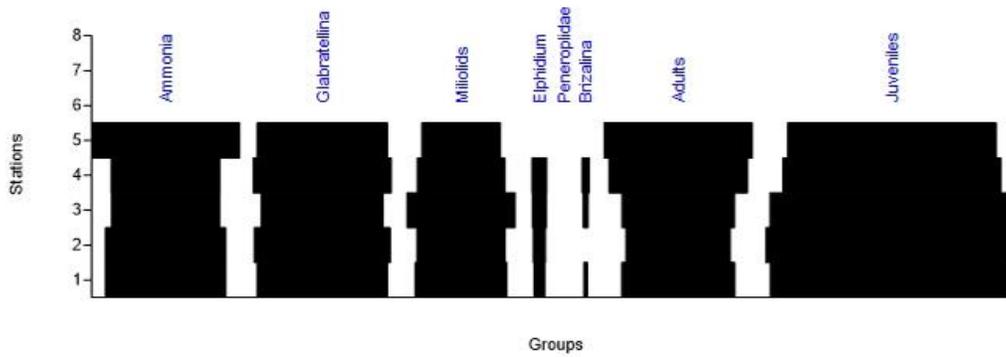
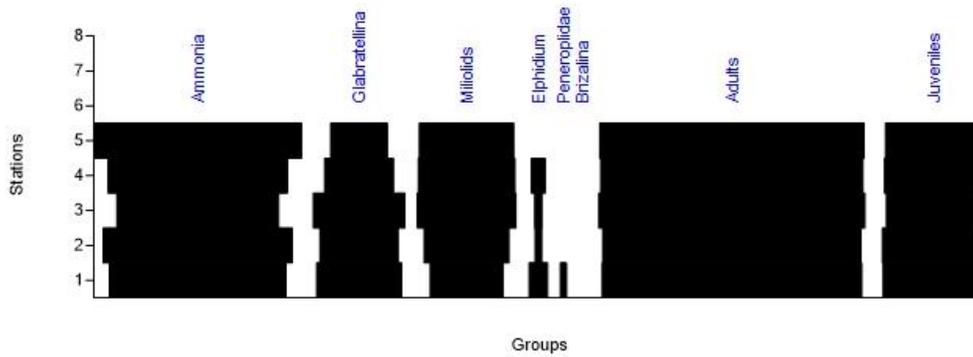
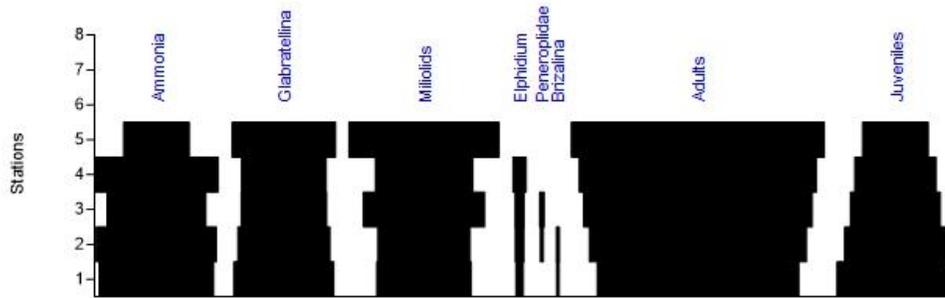
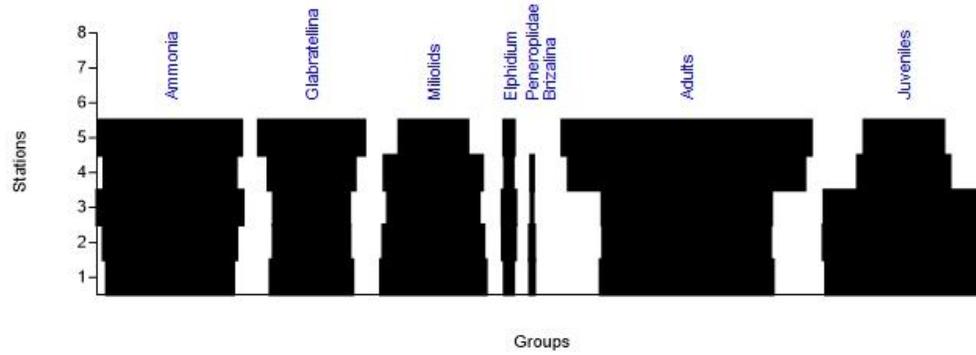


Figure 32: Spindle diagrams and the relative abundance of benthic foraminiferal groups.

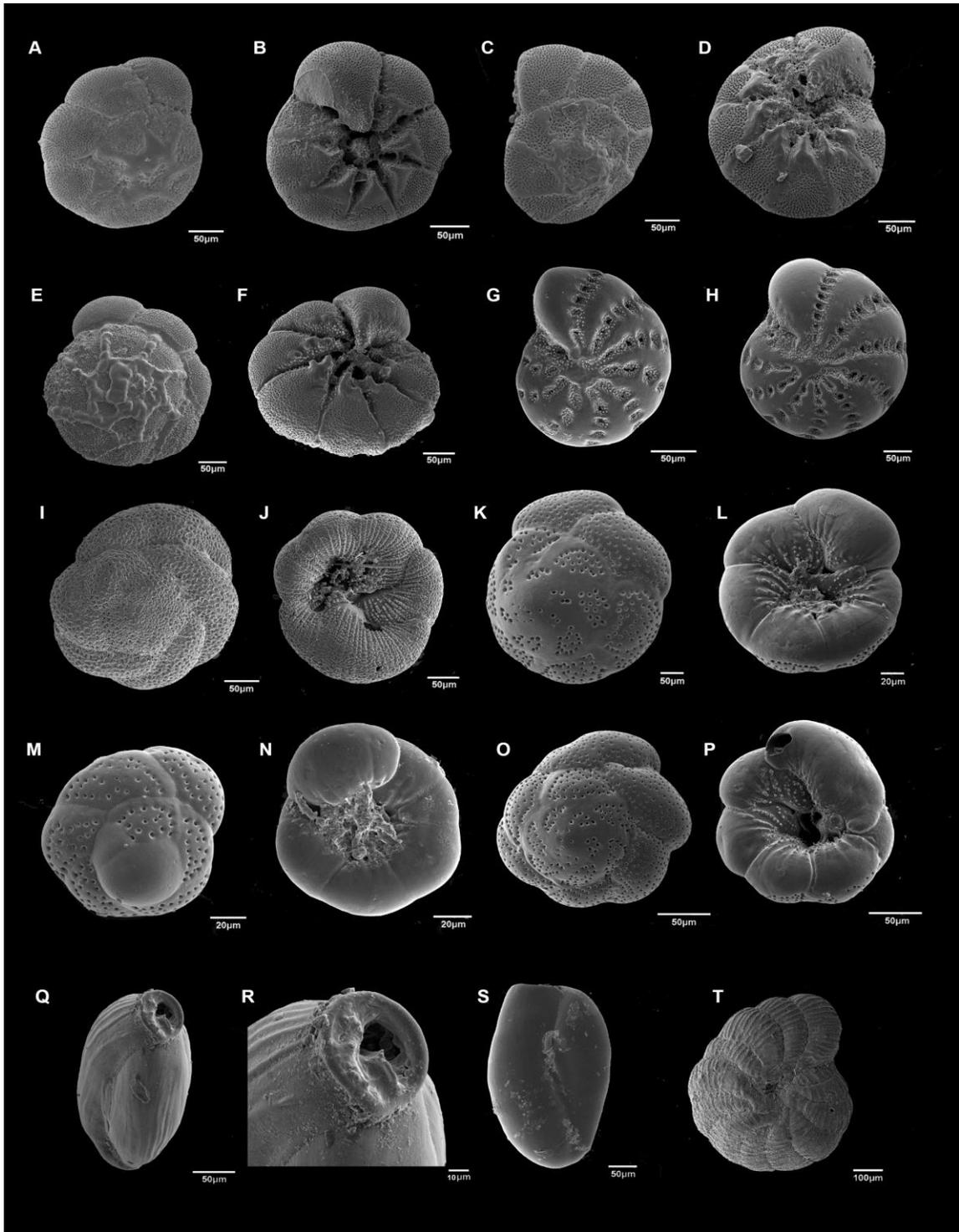


Plate 1: Scanning electron micrographs of the selected foraminiferal specimens. A. *Ammonia* cf. *A. parkinsoniana* (dorsal view) B. *Ammonia* cf. *A. parkinsoniana* (ventral view). C. *Ammonia* cf. *A. parkinsoniana* (dorsal view) D. *Ammonia* cf. *A. parkinsoniana* (ventral view); E. *Ammonia tepida* (dorsal view), F. *Ammonia tepida* (ventral view); G. *Elphidium excavatum*; H. *Elphidium advenum*; I. *Glabratellina* sp. 1 (dorsal view); J. *Glabratellina* sp. 1 (ventral view); K, M, O. *Glabratellina* sp. 2 (dorsal views); L, N, P. *Glabratellina* sp. 2 (ventral views); Q, R. *Quinqueloculina poeyana* (lateral view); S. *Quinqueloculina seminula* (front view); T. *Monalysidium* sp. (dorsal view).

*Ammonia* was consistently present in all the stations during each season and dominated (39.8%, on average) the benthic foraminiferal assemblages (Appendix 3). The second most abundant group was the miliolids (28.4%, on average) followed by *Glabratellina* (28.3%, on average). Near the foreshore (stations 1 and 2), *Ammonia* and *Glabratellina* were the most abundant taxa, but the relative percentage of miliolids increased in the seaward stations (stations 3, 4 and 5) (cf. Appendix 3). In contrast, *Brizalina* sp. was rare and found only during the spring and autumn seasons. Furthermore, a large number of *Ammonia* specimens were found during each season.

On the basis of Shannon's  $H'$ , the lowest values of diversity were documented in station 1 for all seasons, and lower values were found in summer. The highest diversity values were found at stations 4 and 5 during winter; stations 3 and 4 during spring; and stations 2 and 3 during autumn. During summer, the Shannon's  $H'$  values are nearly constant in stations 2 and 3 (Fig. 33; Appendix 3). The dominance ranged from 0.31 (3a) to 0.43 (1s), with the highest values found in summer and close to shore, particularly at station 1 in all seasons. Results of foraminiferal constancy reveal that 100% *Ammonia*, *Glabratellina* and miliolids were found during all seasons. Constancy for *Elphidium* was 100% in winter only and reduced to 80% in spring, summer and autumn (Table 2).

**Table 2: Fc in different stations along transect during each season.**

Parameters	Ammonia	Glabratellina	Miliolids	Elphidium	Peneroplidae	Brizalina
Winter	100%	100%	100%	100%	80%	0%
Spring	100%	100%	100%	80%	40%	40%
Summer	100%	100%	100%	80%	20%	0%
Autumn	100%	100%	100%	80%	0%	60%

Peneroplidae constancy was found as 80% during winter, 40% during spring, 20% during summer, and 0% during autumn. In contrast to these results, no living *Brizalina* specimens were found during winter and summer seasons, however, constancy increased to 40% in spring and 60% in autumn (Table 2). Relatively low values (<2%) of foraminiferal alteration index were documented.

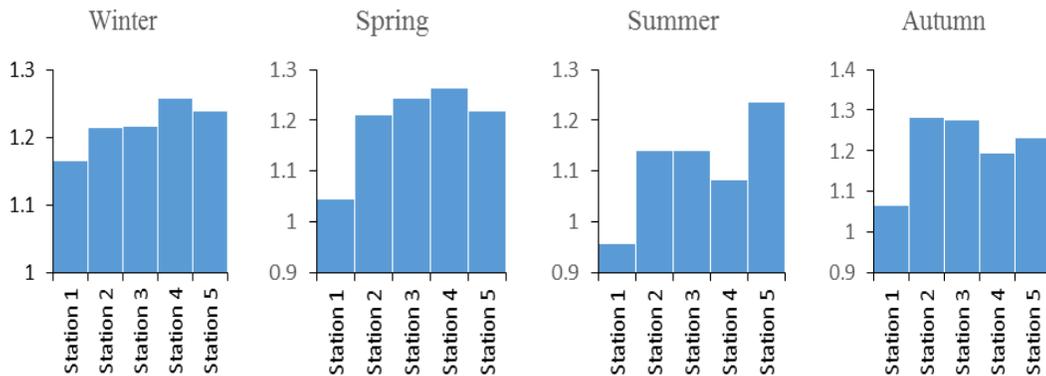
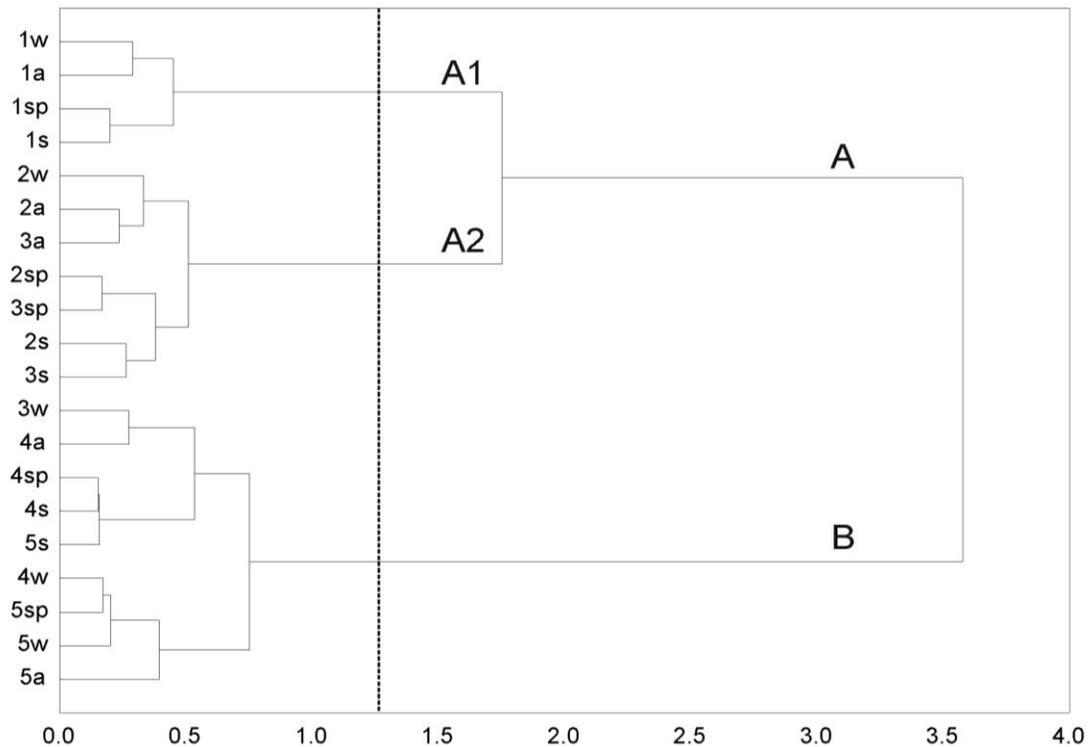


Fig. 33: Shannons H' showing the changes in diversity along the depth transect during each season.

#### 4.4.3 Statistical Analysis

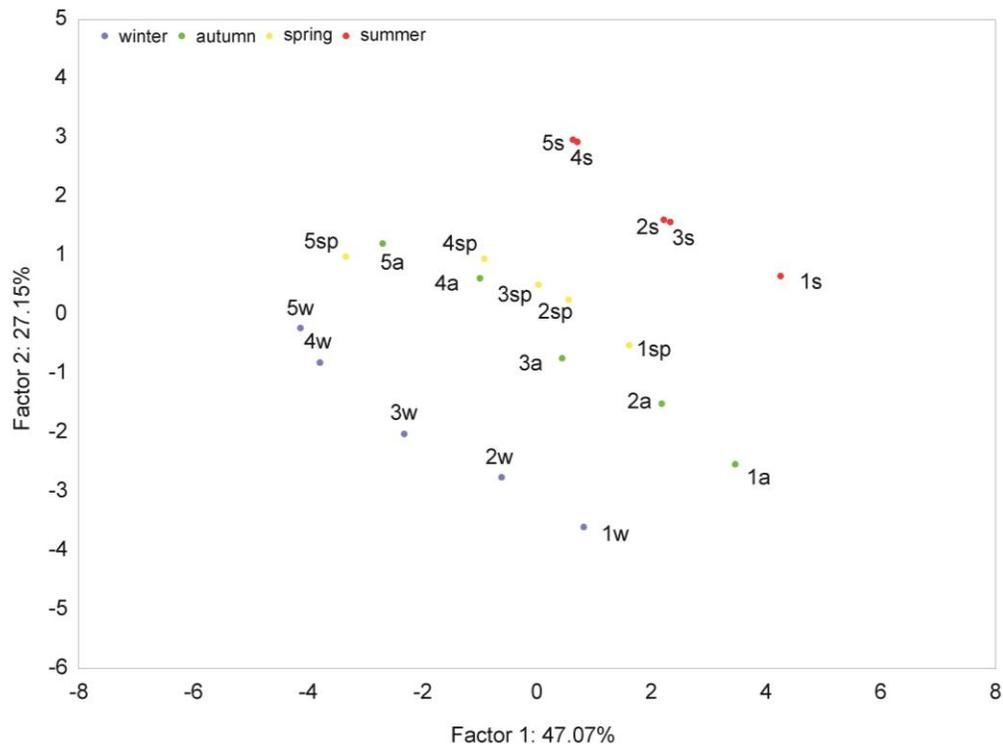
The cluster analysis resulted in the grouping of samples into two main clusters (A and B) and two subclusters (A1 and A2) (Fig. 34). Cluster A represents the nearest stations to the shore for all seasons; and Cluster B groups all the offshore stations. Cluster A has been further subdivided: cluster A1, which includes station 1 samples from all seasons, and cluster A2, which groups together mostly stations 2 and 3 stations of each season. Cluster A1 samples are characterized by the shallowest water depth, and highest values of silt, clay and TOC content. It shows the lowest level of foraminiferal density and diversity, and the highest level of dominance, and includes *Ammonia*, *Glabratellina*, miliolids and *Elphidium*. Cluster A2 includes stations 2 and 3 characterized by relatively lower values of TOC compared to cluster A1 and intermediate sand content. This cluster is also

characterized by relatively higher foraminiferal density and significant higher values of diversity compared with cluster A1. It also has more abundant *Elphidium* and relatively less *Ammonia* compared with cluster A1, the assemblages of this cluster also contains very low percentages of peneroplidae and *Brizalina*. Cluster B groups stations 4 and 5 from all seasons that are deeper and are dominated by the lowest values of fine fraction and TOC. In terms of benthic foraminiferal assemblages, this cluster shows the highest values of Foraminiferal density, and diversity and the taxa representing it are similar to subcluster A2 in terms of relative abundance. Furthermore, cluster A shows the lowest foraminiferal population particularly due the low number of juveniles whereas Cluster B exhibits the highest foraminiferal density and number of juveniles.



**Figure 33: Dendrogram classification of the stations produced by a Q-mode cluster analysis using the Euclidean distance.**

The Q-mode PCA further confirms the recognition of these groups of stations (clusters and subclusters) (Fig. 35). The PCA shows that ~74.0% of the data variance can be explained by the first two principal components (factors). On the basis of Q-mode PCA plan, the first component can be interpreted as the depth transect (foreshore-offshore gradient), whereas the second component might be related to the seasonality (Fig. 35).



**Figure 34: Q-mode PCA ordination diagram plotting samples. The first component can be interpreted as the depth transect (foreshore-offshore gradient), whereas the second component might be related to the seasonality.**

More precisely, physicochemical parameters mainly grain size and salinity are the predominant elements in the first component, while the contribution to the second component was mainly due to seasonality and TOC (Fig. 6). In order to better understand the relationships of biotic and abiotic data, secondary variables (biotic) were plotted on the factor-planes (Fig. 7). It is clear that foraminiferal density and assemblage indexes

(H', S, J and E) are linked to the first component, whereas *Ammonia* and *Glabratellina* are related though weakly to the second one.

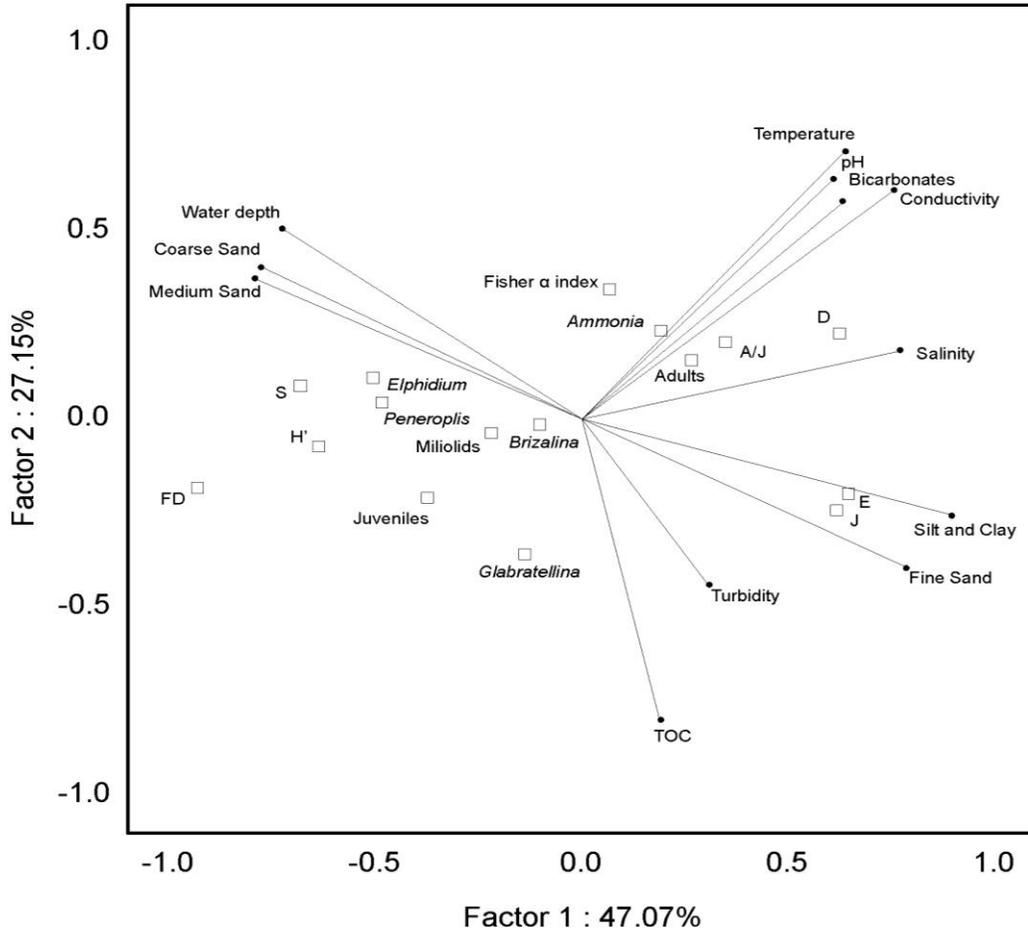


Figure 35: R-mode PCA ordination diagram projecting variables on the factor-planes (1x2). The secondary variables are marked with a square.

#### 4.5 Discussion

In this study, several factors influencing the distribution of living foraminiferal assemblages in the western Arabian Gulf (eastern Bahrain) have been described. These factors include seasonal variations of physicochemical parameters, sediment grain sizes, total organic carbon, and pollution due to nitrates, sulfates, heavy metals and

hydrocarbons. The locality was initially investigated by Basson and Murray, who reported temporal variations in four intertidal foraminiferal species (*Ammonia beccarii*, *Elphidium advenum*, *Brizalina pacifica*, and *Nonion* sp.) and mainly focused only on the standing crop assessment of the pool [72]. The present study extends their findings in terms of environmental characterization as well as seasonality in a seaward transect. Our results show that the highest foraminiferal density is found in winter, which is similar to the findings of Basson and Murray. The highest foraminiferal density might be due to the reproduction of species of rotaliids and miliolids in early autumn, which is indicated by the presence of high juvenile numbers. The effect of seasonality on standing crop has also been reported by other authors; for example, the highest population during winter was observed by Basson and Murray (1995) and Korsun and Hald (2000), during spring by Heinz and Hemleben (2003), during spring and summer by Ellison (1984), and during spring and autumn by Fontanier et al. (2003) [72, 149-152].

Grain size is known to influence the benthic foraminiferal assemblage in terms of diversity, density, and species composition [81, 153], which is further controlled by the hydrodynamic regime of the environment [154]. The coarser sediments are transported and deposited by faster-flowing currents than finer sediments, which instead tend to be deposited in quieter waters [155]. On the Arabian side of the gulf, the seafloor slopes more gently towards its center than on the Iranian side and the average grain size increases as energy increases [156]. In the depth transect, high percentage of coarse grains particles indicate, most probably, the presence of faster flowing currents though a possible production of biogenic grains as in carbonate environments cannot be excluded. However, presence of fine grain sediments in the stations close to shoreline could be due

to low energy conditions in the shallow water environment. Living foraminifera, particularly juveniles, were more commonly found in samples with higher coarse sand content. The positive relationship between juveniles' population and sediment grain size might suggest that coarse-grained sediments may better support the reproduction of gametes and the survival of juveniles when compared with fine-grained sediments. Coarse-grained sediments also offer favorable conditions to benthic foraminifera in terms of providing habitat to the flora (e.g., microalgae and bacterial films) that ultimately provides food/nutrients to the living population [81, 157-159]. Lastly, high inertia of the first principle component (i.e. particle grain size) agrees with Murray's niche theory, which states that the distributions patterns of benthic foraminifera are controlled by environmental factors (reaching critical thresholds alone or in combination) [160].

Another reason for the lower population of juveniles near the foreshore seems to be due to the presence of algal mat. Our analyses suggest that the site might be influenced by eutrophication particularly by elevated nitrates and sulphates, and the algal mat spreading along the beach at shallow water depth might support it. The presence of algal mat may hinder adults to reproduce and result in a decrease in foraminiferal density near the foreshore [161]. In the offshore direction, algal growth diminishes as water depth increases, which results in more favorable conditions for foraminifera. Increasing foraminiferal density in an offshore direction during an eutrophication event has also been reported in earlier studies [161, 162]. Higher TOC content in nearshore stations 1 and 2 could be due to the high primary productivity of algae. However, the overall TOC content decreased along the transect as coarser sediments allow less TOC to accumulate compared to finer sediments [163]. The highest levels of TOC were found in winter and

autumn, double the content of the other two seasons. The highest foraminiferal density occurred in winter. Slight variations among physicochemical parameters were recorded for the depth transect during each season. These minor variations could be due to the mixing between bottom and surface water in the shallow coastal areas. However, this integrated perspective may provide an understanding of the factors influencing population dynamics as a whole rather a decreasing or increasing profile along the transect in each season [164].

In addition to the foraminiferal density, diversity in the depth transect varied due to changes in environmental parameters. Of the six groups of living foraminifera found in the depth transect, *Ammonia* and *Glabratellina* were found to be dominant in each season, miliolids were dominant only in winter and spring, and *Elphidium*, peneroplidae and *Brizalina* were never dominant (Dominant: refers to >20% in relative abundance). More specifically, the species structure for each group was as follows: one species of *Ammonia* (*Ammonia* sp. 1 cf. *A. parkinsoniana*), two species of *Glabratellina* (*Glabratellina* sp. 1 and sp. 2), one species of *Elphidium* (*Elphidium advenum*), one species of *Brizalina* (*Brizalina pacifica*), three species of miliolids (*Quinqueloculina seminula*, *Quinqueloculina poeyana*, and *Quinqueloculina* sp. 1), and three species of peneroplidae (*Monalysidium* sp. 1, *Coscinospira* sp. 1, and *Peneroplis pertusus*). Previously, in the same locality but from the pool, Basson and Murray reported the temporal variation of four species, i.e. *Ammonia beccarii*, *Elphidium advenum*, *Nonion* sp., and *Brizalina pacifica*; however, no *Glabratellina*, miliolids or peneroplidae were found. In contrast, *Nonion* sp. was not found in the present study. Earlier studies have also reported different species of *Ammonia*, *Elphidium*, and miliolids in the shallow water

environment of the Arabian Gulf, however, *Glauvatellina* has not been recorded [69, 165, 166].

Foraminiferal constancy revealed that both *Ammonia* were consistently present along the transect, irrespective of sediment grain size and seasonal variations. This supports the finding that some rotaliids might be capable of reproducing rapidly in many different environments [88]. For instance, *Ammonia tepida* has been reported as an opportunistic species along the Mediterranean coast in the vicinity of a sewage sludge disposal site and other sources of pollution [49, 167, 168]. In contrast, miliolids were less abundant near the foreshore (1<sup>st</sup> and 2<sup>nd</sup> stations), but their relative abundance increased in the offshore direction. This could be due to the fact that miliolids were affected by eutrophy, however, their relative percentage increased with decrease in the pollutants concentration along the depth transect (Appendix 1; Figs. 3, 7). Similarly, the relative abundance of *Elphidium* increased along the depth transect which could be due to their high affinity with coarse sand particles. On the contrary, *Glauvatellina* were consistently present along the transect irrespective of the seasonal variations and grain size; however, no earlier reports are available on their distributional patterns in shallow water environments. Comparatively, both *Elphidium* and *Glauvatellina* showed higher abundance in winter and lowest in summer but their overall increased in the seaward direction. Lastly, *Brizalina* are found only twice, in spring and autumn. They are found in the fine to medium grain substrate compared to very fine or coarse grain environments. Debenay et al. (2001) correlated the presence of this genus with fine-grained sediments [153].

Among sediment grain size, it can be established from that coarse and medium sand particles allow the majority of the juvenile population to survive and reproduce

successfully compared with the clay and fine sand. Clay and fine sand better supported the adult population. Similarly, the higher concentration of nitrates and sulfates near the foreshore can be seen to affect the population at these stations. The elemental analysis and their comparison with devised levels indicated that the area is not affected by heavy metal pollution except strontium when compared with other parts of the world [169-171]. The elevated strontium could be due its affinity with gypsum and other carbonates which are abundant in the Arabian Gulf [172, 173]. The overall foraminiferal alteration index was <2%, which supports the finding that the area is unpolluted. Similarly, the absence of THC pollution further confirms that the site is not affected by hydrocarbons, and can be considered as reference station for future studies.

#### **4.6 Conclusions**

This chapter summarizes the abundance, diversity, and assemblage composition of benthic foraminifera along a depth transect in eastern Bahrain. We observed pronounced seasonality in the benthic foraminiferal populations. The highest standing crop was observed in winter, while the highest proportion of juveniles was found in autumn. The proportion of juveniles along the transect increased in the offshore direction. Analysis of heavy metals, hydrocarbons, and nutrients indicates that the studied site is not polluted, and therefore provides baseline information for future studies related to pollution.

## CHAPTER 5

### **Benthic Foraminifera in Sandy (Siliciclastic) Coastal Sediments of the Arabian Gulf (Saudi Arabia)**

#### **5.1 Abstract**

The chapter presents the finding of benthic foraminiferal assemblages in a unique siliciclastic sediment substrate of the Saudi coastline. In the locality, only two genera, namely *Elphidium* and *Ammonia* were observed in the depth transect. The genus *Elphidium* dominated the foraminiferal assemblages that support the fact of its resistant nature in siliciclastic environment; where mechanical action of water waves and the minor accumulation of organic matter do not support abundant and highly diversified foraminiferal assemblages. Environmental analysis suggests the site as completely unpolluted and hence can be considered as a benchmark for future studies along the Saudi coastline. To best of our knowledge, this is the first report presenting information on foraminiferal assemblages from a siliciclastic environment of the Arabian Gulf as previous studies were reported from carbonate sand and muddy environment in the Arabian Gulf.

**Keywords:** Benthic Foraminifera, *Elphidium*, Siliciclastic, Saudi Coastline

## 5.2 Introduction

The nature of sediment mineralogy in the sea floor is attributed to the water temperature and salinity. For instance, the content of  $\text{CaCO}_3$  increases with increasing water/temperature. Resultantly, distribution and dynamics of organisms in the benthic environment is directly or indirectly affected by the sediment nature. Greiner reported that the arenaceous species occupied low salinity areas due to the lack of availability of  $\text{CaCO}_3$  for the secretion of calcareous tests [174]. He further argued that the organisms with hyaline tests need  $\text{CaCO}_3$  less environment compared to the porcelaneous organisms mainly due to the construction of their wall types. This finding lead him to envisioned that a gradient from low availability of  $\text{CaCO}_3$  (arenaceous), intermediate availability (hyaline) to high availability (porcelaneous). In addition to this, thick walls and large size of benthic foraminifera in the shallow waters is also been observed as a function of  $\text{CaCO}_3$  availability [70].

The current report presents the finding of benthic foraminiferal assemblages in a unique sediment substrate of the Saudi coastline (50°09.620' E, 26°06.222' N). The coastal area covers sandy beaches that are comprised of siliciclastic sediments, dominated by quartz sand. The foreshore is wide with a gentle slope. The water temperature varies between 17° and 31°C, and salinity is approximately 45-46‰ throughout the year. The sea floor consists of loose sand with no vegetation coverage [141].

### 5.3 Materials and Methods

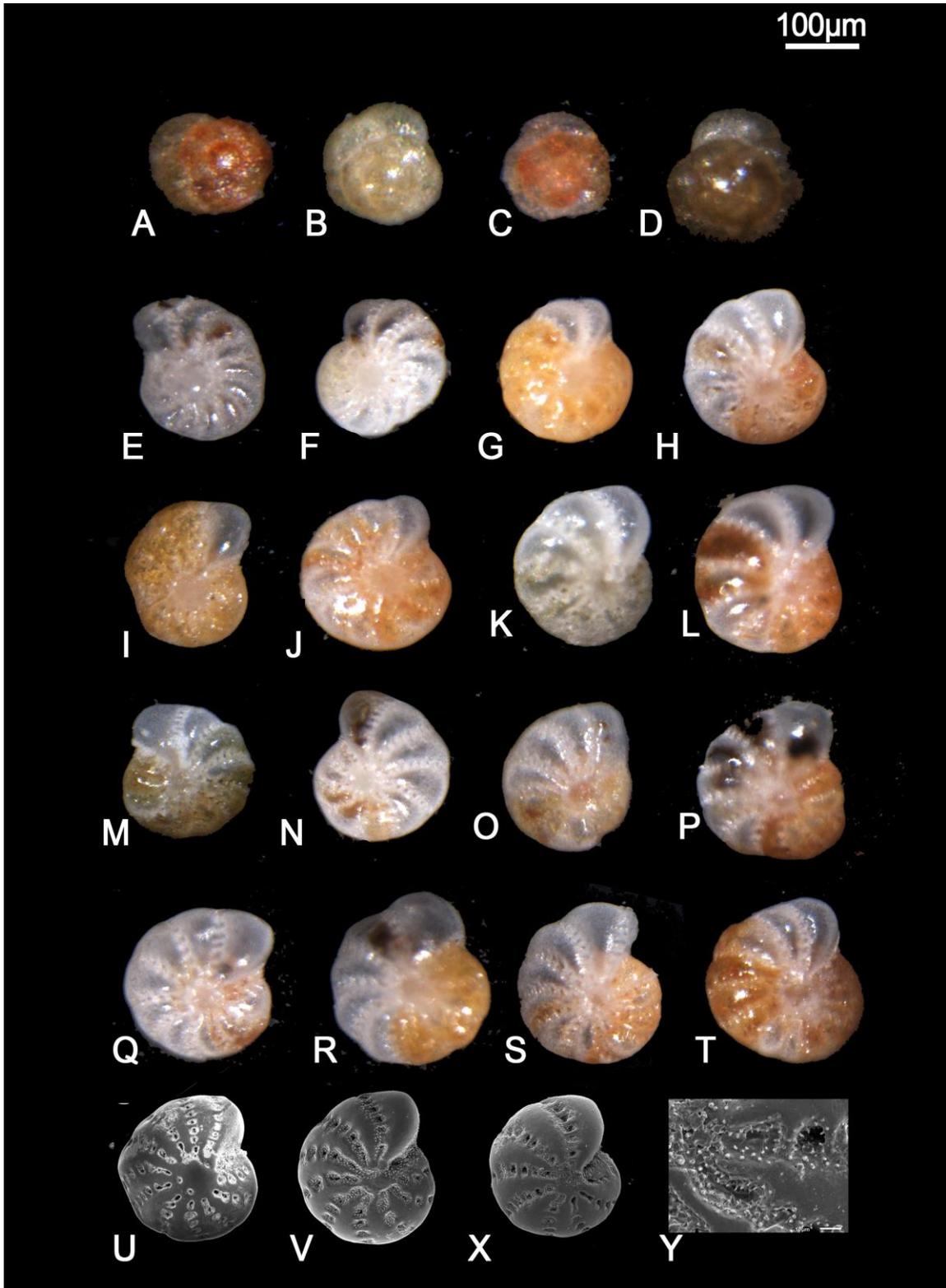
Five samples were collected from a shallow depth transect during the season of highest foraminiferal density, namely winter January 2015, as previously reported [175, 176]. The transect profile data are presented in Appendices 4-6. Water was sampled by dipping well-rinsed glass jars at each station prior to the sediment sampling to avoid any alteration of physicochemical parameters. Sediment samples with a depth of 1.0 cm (volume  $\sim 57.6 \text{ cm}^3$ ) were collected with a spatula taking care not to disturb the sediment floor, and placed into plastic storage boxes fitted with a lid that was secured under water. Both water jars and sediments boxes were immediately transported to the laboratory for analysis. Sample processing was carried out at the Research Institute and Environmental Sciences labs at King Fahd University of Petroleum and Minerals (Saudi Arabia). Sediment and water samples were used for the characterization of the environmental quality (eutrophication indicators, heavy metals and hydrocarbons).

In the laboratory, benthic foraminifera, grain size, dissolved oxygen (DO), eutrophication level, total organic carbon (TOC), total hydrocarbon content (THC), and heavy metals concentration were analysed. Moreover, spatial variability of environmental parameters (i.e., physicochemical parameters of water and geochemical parameters of sediments) was analysed and the current level of pollution was evaluated in terms of eutrophication indicators, heavy metals, and THC. Siliciclastic nature of sediment was further confirmed by digesting the sediments in 10% hydrochloric acid (HCl) as suggested earlier [177]. The pollution levels were compared with the benthic foraminifera to assess their effects on living assemblages.

## 5.4 Results

The results of physicochemical parameters of water and geochemical parameters of sediments reflected minor variations between the sampling stations, i.e., salinity  $45.5 \pm 0.1\%$ , temperature  $19.5 \pm 0.1^\circ\text{C}$ , pH  $8.0 \pm 0.2$ , and DO as  $8.7 \pm 0.3$  mg/l. Grain size analysis documented the prevalence of medium- and coarse-grained sand (i.e., average of 48.4% for medium grain and 40.2% coarse grain sand) (Appendix 4). The acid test showed that the quartz content ranged between 83% and 87% in the depth transect (85.2%, on average) (Appendix 4).

Analysis of environmental quality assessment for both water and sediments reflected presence of very low concentrations of nitrates and sulphates in all the samples (mostly  $< 1.0$  mg/l and 8.58 mg/l, respectively). TOC ranged from 144 mg/kg to 562 mg/kg with the highest values in the seaward stations (Appendix 5). Similarly, THC ranged between 25.35 and 29.56  $\mu\text{g/l}$ . Compared to the ER-L (Effect Range – Low) and ER-M (Effect Range – Median) values reported for the United States Environmental Protection Agency's (USEPA) sediment guidelines (Long et al., 1995; Ligerio et al., 2002), out of 63 heavy metals, none were in excess of the permitted standards (Appendix 5).



**Plate 1:** A-T. Light microscope images, U-Y.SEM microscope images. A-D. Specimens belonging to genus *Ammonia*. E- X. Specimen belonging to genus *Elphidium* (mainly *Elphidium advenum*); Y. *Elphidium advenum* detail of septal bridges of specimen X.

The abundances of living benthic foraminifera ranged between 32 and 131 individuals/10 cm<sup>3</sup>. The foraminiferal assemblages comprised only two genera, i.e., *Elphidium* and *Ammonia*; the former being dominant (85.5%). The nearshore stations (1<sup>st</sup> and 2<sup>nd</sup> stations) were devoid of living specimens. The living specimens occurred from the 3<sup>rd</sup> station seaward, and remarkably increased in the offshore direction (Appendix 6). The overall foraminiferal assemblages varied significantly in the offshore stations and most of the population consisted of adult specimens. Very few abnormalities were observed within the living assemblages, which would reflect the stable, undisturbed and unpolluted nature of this locality.

## 5.5 Discussion

The siliciclastic nature of the sediments does not provide ideal conditions for benthic foraminifera. However, the common occurrence of *Elphidium advenum* suggests that their resistant nature in siliciclastic sediments that are commonly well oxygenated and prevent the accumulation of organic matter [178]. This type of substrate is also influenced by the mechanical action of water waves that, in turn, strongly shapes the benthic foraminiferal assemblages. This normally leads to the destruction of small and more fragile specimens. Moreover, the TOC content was lower in near foreshore stations as mentioned previously. It is most likely for these reasons that no living specimens were found at the first two stations that instead occurred below a water depth of 50 cm. The presence of a few rotaliids, namely *Ammonia*, with broken tests further confirm the adverse effects of hydrodynamic action. Earlier studies in the Tyrrhenian Sea between Sardinia and Corsica reported the dominance of *Elphidium crispum*, *Eponides*

*concameratus*, and *Textularia agglutinans* in area influenced by strong currents [179]. Species with a keel are quite resistant to breakage and hence, are abundant at shallow water depth and in sandy substrates. Nevertheless, the results are contradictory to Buzas who reported that the mineralogy of the sediment is not important in regulating foraminiferal densities [180]. The absence of pollution also supported by the fact that the foraminiferal population displays no abnormalities. In general, the locality is an unpolluted site in the Saudi offshore, with a unique sediment nature along with its foraminiferal assemblages.

## **5.6 Conclusions**

The study concludes that the substrate nature of the sediments directly affect the foraminiferal population in addition to the seasonality and environmental parameters. In the locality of siliclastic Saudi transect, only two genera (*Elphidium* and *Ammonia*) were observed in the depth transect with *Elphidium* being dominated the foraminiferal assemblages. This support the fact that the group has resistant nature to quartz environment; where mechanical action of water waves and the minor accumulation of organic matter do not support abundant and highly diversified foraminiferal assemblages. Environmental analysis revealed that site as completely unpolluted and hence the distribution of species is primarily based on sediment nature. Lastly, the locality can be considered as a benchmark for future studies along the Saudi coastline with siliclastic benthic environment.

## CHAPTER 6

### **Benthic Foraminifera in Eastern Bahrain and the Saudi Coastline: Relationship to Local Pollution Sources**

#### **6.1 Abstract**

This chapter aims to document the response of benthic foraminiferal assemblages along with their distribution patterns in four different transects from eastern Bahrain and Saudi coastline. Results illustrate that the transect from eastern Bahrain is subjected to pollution by nutrients, organic matter, and hydrocarbons. In the transect, seven taxonomical groups were recognized including *Ammonia*, *Glauvatellina*, *Murrayinella*, *Elphidium*, *Brizalina*, miliolids, and peneroplids. By comparing the findings with unpolluted transect, it is found that *Murrayinella* is appeared as an opportunistic taxon due to the presence of high organic matter pollution. However, pollution due to heavy metals was not significant, and hence the deformities index is not high. In the second transect from the Saudi coastline, environmental analysis reflects that the site is polluted with high organic matter and hydrocarbons which resulted in a significant reduction of the benthic foraminiferal population. Although the locality was not polluted with heavy metals, however, minor deformities were observed due to the THC pollution. However, in total, three groups were recognized, i.e., *Ammonia*, *Elphidium*, and *Murrayinella*, with *Ammonia* being the dominant one. Lastly, the environmental quality assessment of the third transect from Saudi coastline in a lagoon indicated presence of both natural and anthropogenic stresses due to high salinities, warm waters, and higher levels of chromium. Resultantly, foraminiferal fauna bears an abnormality index of 8.9% to 11.3% at different stations of the transect. Furthermore,

due to the unique lagoon environment, the peneroplids was the dominant group along with *Ammonia*, *Murrayinella*, *Elphidium*, and miliolids. Finally, the study compares the findings of different pollution sources with unpolluted transects in the region.

**Keywords:** Benthic Foraminifera, Organic Matter, Saudi Coastline, Deformities,

## 6.2 Introduction

Benthic foraminifera have been widely exploited as bio-indicators for the environmental quality assessment of marine ecosystems [33, 88, 181, 182]. Their distributional patterns are influenced by natural marine environmental conditions and by the possible presence of different sources of pollution [86]. Benthic foraminifera might respond to adverse environmental conditions in terms of abundance and diversity, appearance of opportunistic taxa, changes in foraminiferal assemblages' composition and morphological abnormalities [49, 92, 95].

Many studies have documented increased numbers of foraminiferal tests in organically-rich areas [183, 184]. It has been established that foraminifera might benefit by the presence of organic matter that directly represents a source of nutrients and might indirectly reduce predation and/or competition. The availability of organic material and its quality promote the increase of the overall foraminiferal density. However, excess of organic matter may lead to the oxygen deficiency with the consequence disappearance of the most sensitive taxa, the increase of opportunistic groups, decrease in diversity, and a change in the microhabitat succession [88, 185-187]. As a consequence, flux of organic matter may cause alteration of natural foraminiferal assemblages [88, 104, 188]. In addition to the foraminiferal abundance, oxygen-deficient environments can also limit the foraminiferal

diversity [95]. Several species have been found to be tolerant or opportunistic to various pollution sources including organic matter, heavy metals, and chemicals. On the basis of these observations, a distinction has been developed to differentiate pollution-tolerant taxa from pollution-sensitive taxa [88].

In addition to the foraminiferal abundance, dysoxic environments can also regulate the foraminiferal diversity [50]. Several species have been found to be tolerant or opportunistic to various pollution sources including organic matter, heavy metals, and chemicals [98, 103]. On the basis of these observations, a distinction has been developed to differentiate pollution-tolerant taxa from pollution-sensitive taxa [90].

Besides changes in abundance and diversity, the foraminiferal response has also been recorded in morphological deformities. It has been observed that the frequency of morphological deformities depends on a number of factors including abnormal salinity [93], low nutrient levels [189], rapidly changing environment, and pollution due to heavy metals [190]. However, morphological deformities due to hydrocarbons have not been proved to be significant compared to the heavy metals [33, 94].

This chapter summarizes the response of benthic foraminiferal assemblages along with their distribution patterns in four different transects from eastern Bahrain and the Saudi coastline. The study further aims to compare the results with our earlier findings of the relatively unpolluted Murray's pool seasonality transect. To best of our knowledge, this is the first report on this subject from the area.

## Study Area

The study was conducted in four depth transects, one transect from eastern Bahrain and three transects from the Saudi Coastline. The GPS coordinates of the localities (transects) are presented in Table 3.

**Table 3: Transects locations along with their coordinates**

<b>Transect</b>	<b>Location</b>	<b>Station</b>	<b>Longitude</b>	<b>Latitude</b>
Transect # 1	Eastern Bahrain	Askar village	50°36'59.3" N	26°4'32.69" E
Transect # 2	Saudi Coastline	Sofitel Hotel	50°13'15.5" N	26°16'37.56" E
Transect # 3		Zabnah Beach	50°12'34.4" N	25°39'40.81" E

The coastal area offshore to Bahrain transect is microtidal (<1m) with a diurnal rhythm. The foreshore is wide and slopes very gently, and is characterized by a silty, sandy carbonate sediments. The water temperature varies between 17° and 31°C, and salinity is approximately 45-46 PSU throughout the year. Recently, boat traffic, construction along the corniche, and domestic sewerage discharge has resulted in a deterioration of the environmental health of the area. The transects along the Saudi coastline comprise sandy beaches. The foreshore is wide with a gentle slope, and is characterized by sandy siliciclastic sediments.

## 6.3 Materials and Methods

### 6.3.1 Sampling Strategy

In order to assess the pollution effects, five samples were collected from each transect during the season of highest reproduction i.e., winter January 2015 (earlier reported). The

whole study comprised 20 bottom sediments along with the sediment surface water collected from the coastline to 250 m offshore, from water depths of 25 cm to 70 cm.

Water was sampled by dipping well-rinsed glass jars at each station prior to the sediment sampling to avoid any alteration of physicochemical parameters. Sediment samples with a depth of 1.0 cm (volume ~ 57.6 cm<sup>3</sup>) were collected with a spatula taking care not to disturb the sediment floor, and placed into plastic storage boxes fitted with a lid that was secured under water. A layer of aluminum foil was placed over the jar mouth to avoid sediment contact with the plastic cap. Both water jars and sediments boxes were immediately transported to the laboratory for analysis. Sample processing was carried out at the Research Institute and Environmental Sciences labs at King Fahd University of Petroleum and Minerals (Saudi Arabia). Sediment and water samples used for the characterization of the environmental quality (eutrophication indicators, heavy metals and hydrocarbons) were only analyzed during the winter season.

### **6.3.2 Laboratory Analysis**

In the laboratory, both biotic and abiotic factors such as benthic foraminiferal analysis, grain size analysis, eutrophication pollution, total organic carbon (TOC), total hydrocarbon content (THC), and heavy metals concentration were analyzed as described previously [175]. Moreover, the spatial variability of environmental parameters (i.e., physicochemical parameters of water and geochemical parameters of sediments) were analyzed, and the current level of pollution evaluated in terms of eutrophication indicators, heavy metals, and hydrocarbons. Finally, the pollution parameters were compared with the benthic foraminifera in order to assess their effects on living assemblages.

### 6.3.3 Statistical Analysis

Prior to statistical analyses, all the available biotic and abiotic data of the two transects were logarithmically transformed  $\log(1+X)$  and test for normality through the Kolmogorov-Smirnov test, as most of the variables fail for normality, nonparametric statistics were applied. The Mann-Whitney U test, a nonparametric test, was used to check the significant difference between the two transects for any parameters ( $p < 0.01$ ). In order to evaluate the relationships among variables a correlation matrix (Spearman's rho) was calculated for all the biotic and abiotic data. These two analyses were performed in Statistica v6.0. Non-metric multi-dimensional scaling (nMDS) ordinations derived from Bray-Curtis similarity matrices were used to document the differences among the two transects in the abiotic parameters and in the benthic foraminiferal assemblages (Clarke and Gorley 2001, Clarke and Warwick 2001). Furthermore, the formal significance of the differences in either the benthic foraminiferal assemblages and abiotic parameters was tested by means of the analysis of similarity (one way ANOSIM). In order to define the contribution of each biotic and abiotic parameters to the observed is similarity between the two transects a SIMPER (Similarity Percentages) analysis. For this analysis, a fourth-root transformation of the data was applied. The nMDS, ANOSIM and SIMPER analyses were carried out in PRIMER v.5.2.9. Descriptive analysis were performed using SPSS software package and comparisons between treatments were carried out by one-way analysis of variance (ANOVA). Duncan's test was applied for ANOVA after testing homogeneity of variance. Furthermore, spindle diagrams, and diversity indices were calculated using PAST software.

## **6.4 Results**

### **6.4.1 Eastern Bahrain – Boat Harbor, Transect # 1**

#### **Environmental characterization of the study area**

Physicochemical parameters of water showed minor variations between the sampling stations and the two transects. Accordingly, salinity ranged between 43.9 and 45.9 ( $45.4 \pm 0.7$ ), and temperature varied between 20.1 and 20.8 ( $20.9 \pm 0.8$ ) (Appendix 7). Results of grain size analysis revealed the prevalence of medium-grained sand followed by fine sand (i.e., 43.5% and 40.8%, respectively). The coarser sand fraction increased in the seaward direction, whereas the fine fraction (silt and clay) diminished (Appendix 7). Further examination of coarser particles under a stereomicroscope revealed that the fraction size  $>63 \mu\text{m}$  was mainly constituted by reworked bioclasts.

The level of nitrates was higher in the polluted transect than in the unpolluted, without any significant trend along the transects, whereas sulphate showed an opposite pattern (Appendix 8). The TOC averaged 10448 mg/kg (=1.05%) in the polluted transect that is higher than the unpolluted one (7296 mg/kg) (Appendix 8). Similarly, THC was also found to be higher in the polluted transect (average of  $67.37 \mu\text{g/g}$ ) than in the unpolluted transect ( $2.24 \mu\text{g/g}$ ). Compared to the ER-L (Effect Range – Low) and ER-M (Effect Range – Median) values reported for the United States Environmental Protection Agency's (USEPA) sediment guidelines, none of the considered heavy metals were beyond the permitted standards in both transects; however, but strontium (Appendix 8). The Concentration Factor (CF) of selected heavy metals (Cr, Ni, Cu, Zn, As, Ag, Cd,

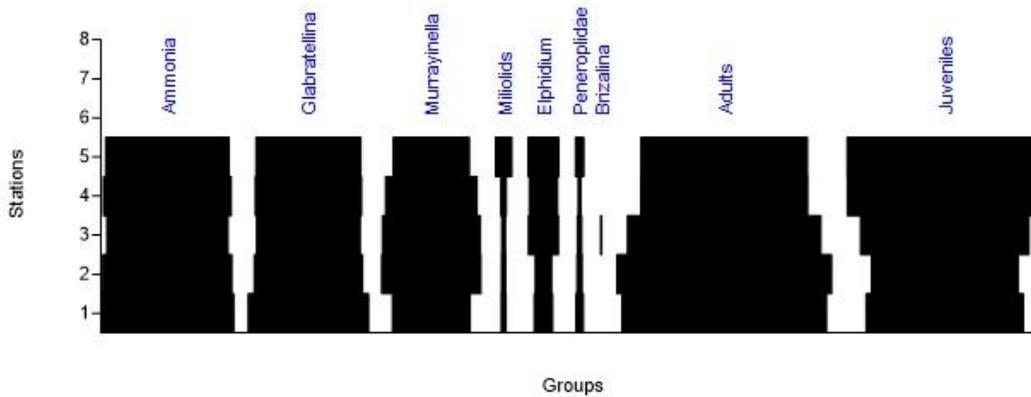
Hg, V, Mn, Fe, Co and Pb) and the Pollution Load Index (PLI) were calculated following Martins et al. (2013).

### **Benthic foraminifera**

All the studied samples from both transects contained abundant and well-preserved living benthic foraminifera. The overall FD was higher in the polluted transect than in the unpolluted transect. More specifically, FD varied between 176 to 309 individuals (average of 254) in the polluted transect, whereas in the unpolluted transect, FD ranged between 62 to 215 individuals (average of 153). Furthermore, the polluted transect showed an increase of FD up to the third station and then decreasing values whereas the unpolluted exhibited a clear increasing trend seaward (Appendix 9). In addition, an opposite trend of juveniles was observed where a gradual decrease was found for the polluted transect and a steady increase for the unpolluted one (Appendix 9). Seven groups (i.e., *Ammonia*, *Glauvatellina*, *Murrayinella*, *Elphidium*, *Brizalina*, miliolids and peneroplids) were identified in the polluted transect, with the addition of genus *Murrayinella* that was absent in the unpolluted transect (Appendix 9). Considering the relative abundance in all samples, the most abundant groups in the polluted transect were *Ammonia*, *Glauvatellina*, and *Murrayinella*; whereas, the unpolluted transect was mainly characterized by *Ammonia*, *Glauvatellina*, and miliolids. *Ammonia* was consistently present in both transects and dominant, it represents 35.1%, on average in the foraminiferal assemblages of the polluted transect that is approximately similar but relatively lower than that in the unpolluted transect (41.5%, on average). The second most abundant group was the *Glauvatellina*, which represented 30.7% of the living assemblages in the polluted transect and slightly less abundant in the unpolluted transect

(26.0%). Miliolids population was significantly lower in the polluted transect compared to the unpolluted transect representing 1.8% and 28.3% of the living assemblages, respectively (Appendix 9). The dominance of *Ammonia* and *Glabratellina* was nearly constant in both polluted and unpolluted transects with no specific trend. *Murrayinella* was observed only in the unpolluted transect whose relative percentage was increased up to 3<sup>rd</sup> station and then decreased in later stations. By contrast, miliolids were abundant in the unpolluted transect with increasing population in the depth transect, whereas their numbers were significantly reduced in the polluted transect but relatively high proportion in the station 1 (Appendix 9). However, due to their less numbers in polluted transect, it is difficult to compare and correlate the transect behaviors with unpolluted transect. *Elphidium* represent a minor component of the living assemblages in both transects, its abundance is relatively higher in the nearshore stations than commonly decreased in the offshore direction (Fig. 37).

The Shannon's  $H'$  in both transects showed opposite behavior with respect to each other. The highest  $H'$  values was observed at station 1 for polluted transect and a gradual decrease was observed along the transect length. On the other hand, in unpolluted transect, lowest  $H'$  values was observed in the station 1 which was increased gradually in the later stations. By contrast, higher values of Fisher  $\alpha$  were observed at in the near shore stations for both polluted and unpolluted transects; however, the value was highest in the 3<sup>rd</sup> station for polluted transect and 2<sup>nd</sup> station for unpolluted transect. The results of richness illustrate no significant variations in each transect whereas high evenness values are observed in nearshore stations whose values were decreased horizontally for both transects (Appendix 9).



**Figure 37: Spindle diagrams and the relative abundance of benthic foraminiferal groups in the boat harbor transect.**

### **Statistical Analysis**

Results of Mann-Whitney U Test shows substantial differences between the two transects. More specifically, the parameters temperature, pH, fine sand, medium sand, nitrates, sulphates, THC, Cr, Cu, As, Pb, CF, PLI, *Ammonia*, *Murrayinella*, miliolids, *Elphidium*, S, H', and Fisher  $\alpha$  index are significantly different between the polluted and the unpolluted transects ( $p < 0.01$ ) (Appendix 10). The Spearman's rho correlation analysis showed significant correlation among major abiotic and biotic variables ( $P < 0.05$ ). Regarding abiotic factors, TOC, THC, and certain trace elements such as Cr, Cu, As, V, Mn, Fe, and Pb were strong positively correlated with the fine grain substrate (i.e. silt and clay, fine sand), whereas strong negative correlation was observed with the medium grain substrate (medium sand). It is important to mention here that majority of the abiotic factors did not show strong correlation with coarse grain substrate except a few trace elements (V, Mn, Fe, and Co) who depicted strong negative correlation with coarser particles.

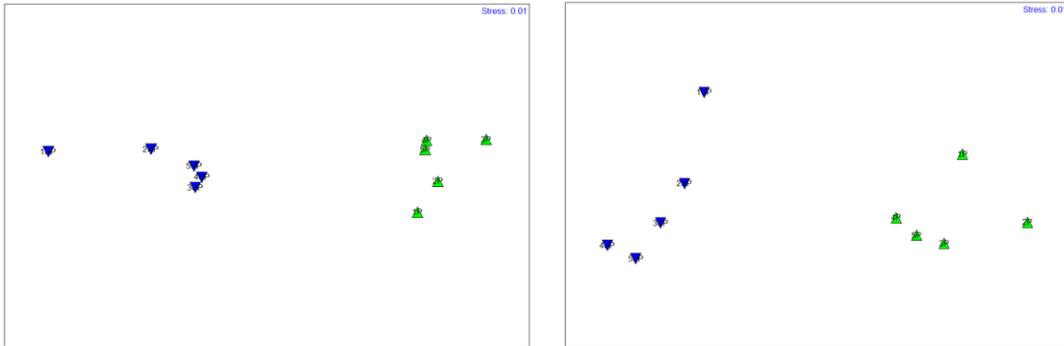
For biotic factors, *Ammonia* and miliolids are found to be strong negatively correlated

with the fine grain substrate, strong positively correlated with the medium grain substrate, and weak positively correlated with the coarse grain substrate. In contrast to this, *Murrayinella* and *Elphidium* showed strong positive correlation with the fine grain substrate, strong negative correlation with the medium grain substrate, and weak negative correlation with the coarse grain substrate. *Glabratellina* and *Brizalina* did not show any strong correlation with the substrate parameters but a moderate positive correlation was found with the fine grain substrate, a moderate negative correlation was found with the medium grain substrate, and a moderate to weak negative correlation was found with the coarse grain substrate. Besides, peneroplids showed weak negative correlation with silt and clay, weak positive correlation with the fine sand, weak negative correlation with the medium sand, and moderate positive correlation with the coarse sand.

In addition, correlation analysis with nitrates, sulfates, TOC and THC were also performed. The strong negative correlation was observed for *Ammonia* and miliolids with nitrates and THC whereas strong positive correlation were found for *Glabratellina*, *Murrayinella*, and *Elphidium*. For sulphates, *Murrayinella*, *Elphidium*, and peneroplids showed strong negative correlation and *Ammonia* showed strong positive correlation. Lastly, TOC had strong positive correlation with *Murrayinella* but strong negative correlation with miliolids.

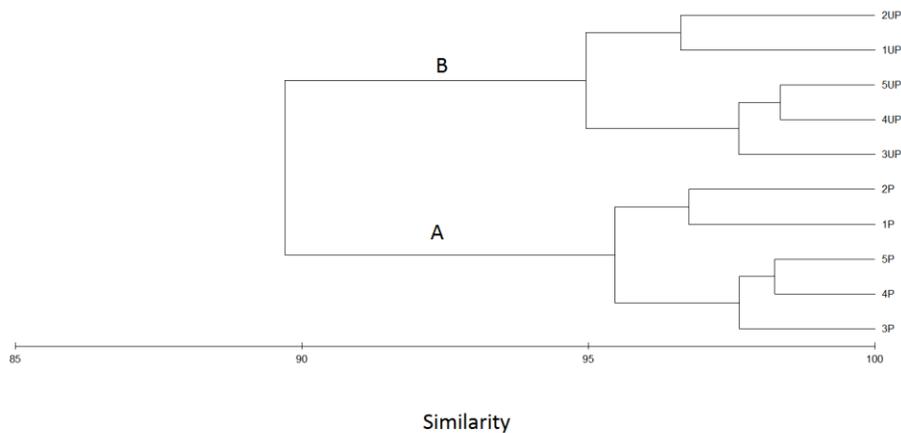
The nMDS, which simultaneously considers all the variables, separated the samples in two distinct groups (stress 0.01) that reflect the transect either when all variables or foraminiferal ones are considered (Fig. 38). The separation of the two groups were significant as revealed by ANOSIM ( $p < 0.001$ ,  $R=0.98$ ) for either all variables or foraminiferal ones. The SIMPER analysis applied to abiotic parameters reveals 13.1% of

dissimilarity between the two transects and identifies CF, THC, sulphates, Pb, PLI and TOC as the parameters most responsible for this dissimilarity (Appendix 11). On the other hand, the average dissimilarity of foraminiferal variables is 10.9% and is mainly due to *Murrayinella*, miliolids and FD (Appendix 12).



**Figure 38: The non-metric multidimensional scaling (nMDS) ordination for polluted and unpolluted transects: (a). nMDS considering all variables, (b). nMDS considering foraminifera.**

The nMDS ordination for the samples from both polluted and unpolluted transects shows marked separation among samples. The Q-mode CA further confirms the recognition of these groups in two clusters (clusters and subclusters) (Fig. 39).



**Figure 39: Dendrogram classification of the stations from polluted and unpolluted transects produced by a Q-mode cluster analysis. Cluster A represents the stations from polluted transect**

while and Cluster B groups all unpolluted stations (seasonality transect).

## **6.4.2 Saudi Coastline – Sofitel Hotel, Transect # 2**

### **Environmental characterization of the area**

The sea floor was covered with patches of brown algae spreading over the sediments surviving polychaetes population in the nearshore samples. The direct discharge of hotel waste has resulted the area to be foul-smelling, consequently leading to the deterioration of environmental health of the area. The studied samples contained dirty and less well-preserved living benthic foraminifera.

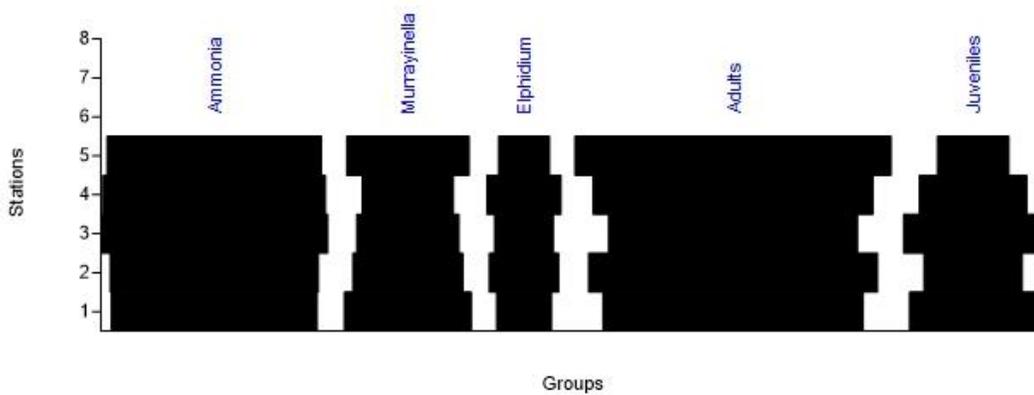
The physicochemical parameters of water and geochemical parameters of sediments showed minor variations between sampling stations. In the depth transect, average salinity was recorded as  $45.6 \pm 0.0$  PSU, temperature as  $22.3 \pm 0.1$ °C, pH as  $8.8 \pm 0.2$ , and DO as  $3.3 \pm 0.2$  (Appendix 13). Regarding geochemical parameters, grain size analysis illustrated prevalence of medium and fine grained sand (i.e., average of 35.5% for medium grain and 34.2% for fine-grain sand). However, in general, the content of coarser particles increased in the seaward direction (Appendix 13). The detailed description on water depth (transect profile), salinity, temperature, and pH is presented in Appendix 14.

Analysis of environmental quality assessment for both water and sediments reflected presence of nitrates and sulfates in all the samples. Furthermore, there average concentration increased along the depth transect (Appendix 14). TOC ranged from 7770 mg/kg to 21500 mg/kg with the highest values in the last stations (Appendix 14). Overall, TOC also increased along the length of transect. Furthermore, THC ranged between 114.61 mg/L to 548.96 mg/L with increasing concentration in the seaward direction

similar to the nutrients and TOC (Appendix 14). Compared to the ER-L (Effect Range – Low) and ER-M (Effect Range – Median) values reported for the United States Environmental Protection Agency’s (USEPA) sediment guidelines (Long et al., 1995; Ligeró et al., 2002), none of heavy metals were beyond the permitted standards; however, strontium exhibited higher values (Appendix 14).

**Benthic foraminifera**

In this transect, a total of three groups were recognized, i.e., *Ammonia*, *Elphidium*, and *Murrayinella*. The foraminiferal density ranged between 38 and 67 with the mean of 53.8 individuals per 5 cm<sup>3</sup>. However, the overall standing crop increased along the transect and the highest numbers were observed at station 4 (Appendix 15). Results on adults to juveniles’ population reflected that most of the population was adult, ranging from 64.2% to 81.6% (Appendix 15). This is the second locality where a significant number of individuals from the group *Murrayinella* were observed throughout the transect (Appendix 15, Figure 41).



**Figure 41: Spindle diagrams and the relative abundance of benthic foraminiferal groups in the Sofitel hotel transect (Al-Khobar).**

Considering the total absolute abundance in all samples, the most abundant groups were *Ammonia* (149 specimens), followed by *Murrayinella* (77 specimens) and *Elphidium* (43 specimens). *Ammonia* was consistently present at all the stations and dominated (55.3%, on average) the benthic foraminiferal assemblages. The second most abundant group was the *Murrayinella*, (28.6%, on average) and lastly *Elpidium* (15.9 %, on average). No living miliolids or peneropliids were observed in the transect; however, dead assemblages reflected the presence of miliolids alongside the hyaline forms and a few peneropliid species. The relative distribution of dead foraminifera in a representative sample was found as follows; rotalids 45%, miliolids 31%, peneropliids 24%. Lastly, the FAI was observed to be about 2.6% and deformities were mainly found in the species of *Ammonia*.

### **6.4.3 Saudi Coastline – Zabnah Lagoon, Transect # 3**

#### **Environmental characterization of the area**

The physicochemical parameters of water and geochemical parameters of sediments at Zabnah lagoon reflected unique parameters. Results reflected high deviations from the other transects, but slight variations within the sampling stations. In the Zabnah lagoon, salinity was observed  $47.2 \pm 0.0$  PSU, temperature  $31.5 \pm 0.0^\circ\text{C}$ , pH  $8.3 \pm 0.0$ , and DO as  $7.1 \pm 0.0$  (Appendix 16).

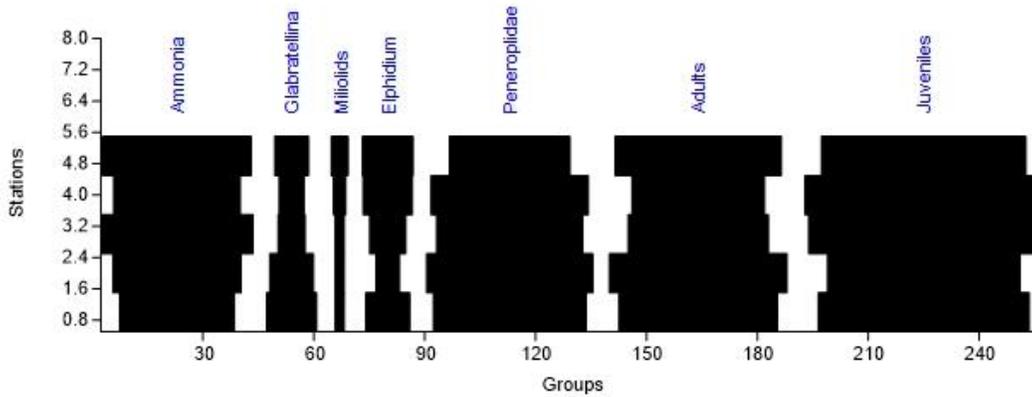
Grain size analysis documented the prevalence of medium-grained sand followed by coarse grained sand and fine grained sand, i.e., average of 47.8% for medium grained sand, 26.6% for coarse grained sand and 19.8% for fine grained sand. Moreover, no significant trend on grain size distribution was observed along the length of transect

(Appendix 16). The water depth was almost same at all the stations and their values are presented in Appendix 16.

Analysis of nutrients level in the water samples suggest the presence of low concentrations of nitrates and sulfates in the samples (ranging between 13.59 to 23.71 mg/L for nitrates and 2132 to 2673 mg/L for sulfates). However, no significant trend or spatial distribution of nutrients was observed (Appendix 17). TOC ranged from 423.5 mg/kg to 609 mg/kg without any distribution pattern (Appendix 17). Similarly, THC ranged between 39.74 to 66.96 mg/L (Appendix 13). Heavy metals analysis reflects that the level of chromium was higher than the ER-L (Effect Range – Low), i.e., 122 ppm in the 4<sup>th</sup> station. Besides, strontium also exhibited higher concentration (i.e. >5000 ppm), however, its significance cannot be understood due to unavailability of USEPA sediment guidelines for this element.

### **Benthic foraminifera**

In the Zabnah lagoon transect, the abundance of benthic foraminifera ranged between 120 to 132 individuals/5 cm<sup>3</sup>. The overall foraminiferal density remained nearly constant at all the stations (Appendix 18). Similarly, the juveniles' population varied from 43% to 61.4% without having any specific distribution patterns (Appendix 18). In general, the foraminiferal fauna was dominated by two groups, i.e., peneropliids and *Ammonia*, however, a total of five taxonomical groups and were recognized in the analyzed samples. These were *Ammonia*, *Murrayinella*, *Elphidium*, miliolids (*Quinqueloculina* and *Triloculina*), and peneropliids (*Monalysidium*, *Coscinospira*, and *Peneroplis*). Their absolute abundances along the depth transect are presented in Figure 42 and Appendix 18.



**Figure 42: Spindle diagrams and the relative abundance of benthic foraminiferal groups in the Zabnah lagoon transect (Half Moon Bay).**

Considering the total absolute abundance in all samples, the most abundant groups were the peneroplids (252 specimens), and *Ammonia* (228 specimens). The peneroplid individuals were consistently present at all the stations and dominated (40.2%, on average) the benthic foraminiferal assemblages. The second most abundant group was the *Ammonia* (36.2%, on average) (Appendix 18). The *Elphidium* population varied between 6.3% and 13.6%, with relatively a higher population near the foreshore. In contrast, very few miliolids were observed throughout the transect, whose populations varied between 2.4% and 4.6%. The foraminifera abnormality index (FAI) varied from 8.9% to 11.3% in the offshore direction and abnormal specimens were mainly belonging to the peneroplid group.

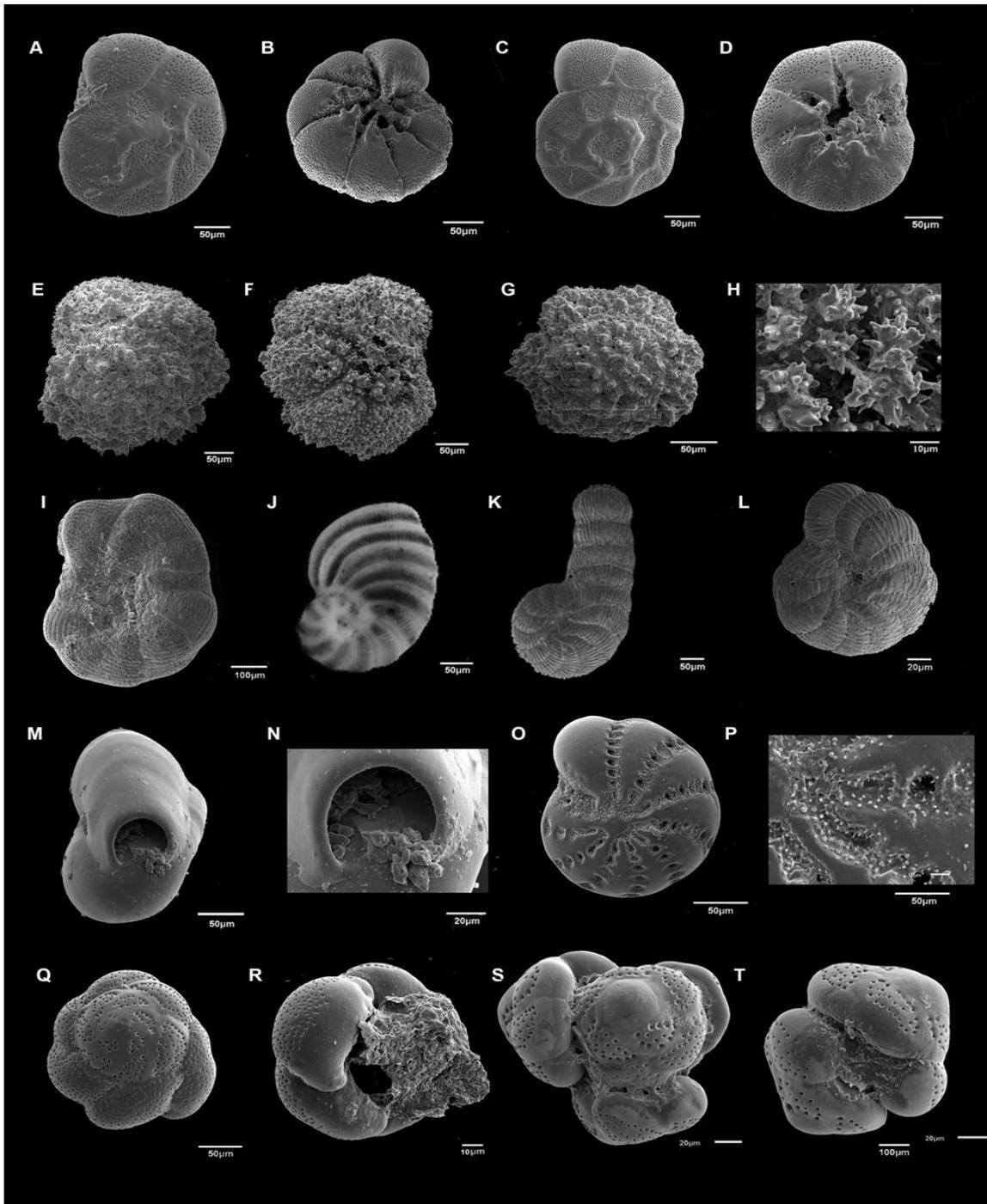


Plate 2: Scanning electron micrographs of selected foraminiferal specimens. A. *Ammonia* cf. *A. parkinsoniana* (dorsal view) B. *Ammonia tepida* (dorsal view) C. *Ammonia tepida* (ventral view) D. *Ammonia* cf. *A. tepida* (ventral view); E. *Murrayinella* sp. 1 (dorsal view), F. *Murrayinella* sp. 1 (ventral view); G. *Murrayinella* sp. 1; H. *Murrayinella* sp. 1 (high magnification); I. *Conscinospira* sp. 1 (dorsal view); J. *Peneroplis proteus* (dorsal view); K, L. *Monalysidium* sp. 1 (dorsal view), M, N. *Quinqueloculina poeyana* (front view with high magnification); O, P. *Elphidium advenum* (ventral view with high magnification); Q. *Glabratellina* sp. (ventral view); R, S, T. Plastogamy in *Glabratellina* sp.

## 6.5 Discussion

This chapter attempts to explain the relationship of local pollution sources on benthic foraminiferal communities at different localities in eastern Bahrain and the Saudi coastline. These factors includes nutrients pollution, organic carbon pollution, elemental pollution, and hydrocarbons pollution. Furthermore, physicochemical parameters of the water and geochemical parameters of sediments (i.e., sediment grain size) is also discussed. To date, no study on benthic foraminifera for these localities has been performed. This study further presents the comparison of polluted localities with relatively unpolluted localities, which may help micropaleontologists to compare the findings with future pollution episodes.

### **Transect # 1: Eastern Bahrain (Boat Harbor)**

The results of the “Boat Harbor” transect reflect the assessment of local pollution sources on benthic foraminiferal assemblages in a polluted locality of the eastern Bahrain along with its comparison with an unpolluted locality. Both of the localities reflects same environmental and geochemical conditions and the distance between both of the localities is less than 1 km. The unpolluted locality was initially investigated by Arslan and coworkers, in which six foraminiferal groups (*Ammonia*, *Glabratellina*, *Elphidium*, miliolids, peneroplids, and *Brizalina*) were observed along with no background pollution. Moreover, *Brizalina* was found to be a seasonal genus as it was only present during spring and autumn [175].

In the transect, the overall concentrations of pollutants particularly organic carbon, hydrocarbons and trace elements in the sediment samples were found to be significantly

higher when compared with the unpolluted transect. The relative high concentrations of pollutants could be attributed to the recent ship traffic, domestic sewage discharge, and waste coming from the mariculture. In addition, the biological decomposition of sewage waste produces biological nutrients especially nitrates along with liberation of organic carbon under aerobic conditions [191, 192].

It has been well-established that the distribution of foraminifera in the coastal environments is a function of nutrients, organic matter and hydrocarbons [88, 107]. The high standing crop in the polluted transect could be due to the higher availability of biodegradable organic matter. Hence, the presence of free organic carbon may help foraminifera to increase their population. Moreover, some of the studies indicate that the organic matter favors higher foraminiferal populations directly by providing food and indirectly by reducing predation and/or competition [85-87, 153]. The presence of plastogamic clusters of living foraminifera in the sampled stations confirms the significant role of organic carbon as well as “winter” as season of reproduction (cf. Figure 3) [175]. The highest FD values in the third station with gradual decrease in both directions may be attributed to the TOC, i.e. strong positive correlation of 0.79 between FD and TOC.

The presence of seven foraminiferal groups with the addition of genus *Murrayinella* in the polluted transect suggest its appearance as an opportunistic group in the organically polluted environment. The strong positive correlation of *Murrayinella* with nitrates, TOC, and THC further confirm that the group was favored by polluted environmental conditions. Previously, *Murrayinella* has been observed in the coastal waters at 0–2 m water depths; however, no study reports the effect of organic carbon on their presence

and abundance [193]. Besides, *Ammonia* and *Glabratellina* were abundant in all the stations of both polluted and unpolluted transect. This reflects their resistant nature towards high organic matter and supports the finding that some of the rotaliids are capable of surviving and reproducing rapidly in every environment [88]. For instance, genus *Ammonia* has been reported as an opportunistic species along the Mediterranean coast in the vicinity of a sewerage sludge disposal site and other sources of pollution [49, 167, 168]. By contrast, very few miliolids were found in the polluted transect which suggest that the group were adversely affected by organic pollution when compared with unpolluted transect [108]. *Elphidium*, peneroplids and *Brizalina* represent a minor component of the living assemblages in both transects, which support the earlier finding of lower FD in the unpolluted transect [175].

Slight variations among physicochemical parameters were recorded along the depth transect. These minor variations could be due to the mixing between bottom and surface water in the shallow coastal areas. However, this integrated perspective may provide an understanding of the factors influencing population dynamics as a whole rather a decreasing or increasing profile along the transect [164].

### **Transect # 2: Saudi Coastline: Sofitel Hotel**

Analysis of environmental quality assessment from the “Sofitel Hotel” transect document the presence of high organic pollution. Higher contents of TOC, THC, and nutrients are primarily due to the recent discharges of hotel waste, which results in an increase in the concentration of organic carbon. This is the reason that the sea floor is covered with patches of brown algae spreading over the sediments, and a living polychaete population in the

nearshore samples. Furthermore, hotel waste has resulted the area to be foul-smelling, consequently leading to the worsening of environmental health of the area. This is the reason that dirty and less well-preserved living benthic foraminifera were observed in all the samples. In the Sofitel hotel transect, the population was very low with maximum of 67 individuals in 4<sup>th</sup> station. This could be due to the reason that the presence of high THC results in reduced reproduction, decreased nutrient supply, inhibited growth rate, and necrosis of living individuals [111]. Morvan and coworkers also reported a reduction of the reproduction rate in the presence of high THC [194].

Similar to the “Boat Harbor” transect, no living miliolids or peneroplids were observed in all the samples due to fact that the locality was intensively affected by eutrophy and algal mat over the sea floor [175]. However, their presence in dead assemblages support the fact that the site is only recently influenced by anthropogenic pollution [107]. Moreover, the overall standing crop constituted an adult population with only a few juveniles, which illustrate that the environmental conditions were not favorable for adult individuals to reproduce and juveniles to survive [111]. However, the presence of *Murrayinella* further confirms its ability to resist the polluted environments (explained previously).

Although, physicochemical parameters of water showed minor variations between sampling stations, however, the average temperature was higher than at the other localities, with very low dissolved oxygen, i.e.,  $3.3 \pm 0.2$ . This may also hinder the living individuals to reproduce and survive due to less metabolism and resource competition. Regarding grain size analysis, no specific spatial trend was observed in the transect except that the content of coarser particles increased in seaward direction which may supported adults to reproduce and, hence, the juveniles to survive [175]. Regarding the

FAI, similar to the boat harbor transect, none of the heavy metals were beyond the permitted standards. Even for strontium, a few stations exhibited values >5000 ppm (Appendix 15). However, as the sediment guidelines for strontium has not been reported by USEPA, therefore, the FAI cannot be related with high level of strontium. Nevertheless, the role of THC for FAI cannot be ignored (described earlier).

### **Transect # 3: Saudi Coastline: Zabnah Lagoon**

Environmental quality assessment of “Zabnah Lagoon” transect illustrate the presence of moderate levels of nutrients, organic matter, and hydrocarbons. However, the locality has its own unique feature due to its restricted contact with the open sea. Although, most of physicochemical parameters of water showed minor variations between sampling stations and other localities, however, the natural salinity of the lagoon water was observed highest as well 47.2 PSU. Similarly, the average temperature was highest compared to all other localities (i.e., 31.5°C), In August, the water temperature in this shallow lagoon approaches 36°C. Moreover, the sediments nature of the locality is carbonates which makes it different from other localities of the Saudi coastline. Mostly, the benthic floor is rocky with patches of algal mat spreading over the sediments, supporting an abundant gastropos population in the lagoon. The locality contains well-preserved living benthic foraminifera in all the samples.

The foraminiferal fauna consists of two dominating groups, i.e., peneroplids, and *Ammonia*. Probably, the peneroplids are leading due to the unique nature of the lagoon (as described earlier) and presence of rotaliids seems to be due to their survival in a stressed environment, i.e., temperature, salinity, high organic carbon [88]. Regarding grain size,

no specific spatial trend was observed in the transect, which does not build any correlation between the substrate parameters and benthic foraminiferal fauna. Furthermore, presence of five groups of benthic foraminifera could be due to the fact that the locality is not harshly polluted with nutrients, organic matter, and/or THC [175]. The study of dead assemblages also suggests no important differences between the dead and living fauna.

Regarding FAI, two major factors could have been responsible for deformities. These are higher concentration of chromium (greater than ER-L) and higher salinity [93, 190], or even the high summer temperatures. The deformities in peneroplids species due to the high concentration of chromium have been reported in other studies as well [195, 196]. For example, Youssef reported elevated concentrations of Cr in the tests of living *Peneroplis planatus* with deformed chambers [196].

## **6.6 Conclusions**

This chapter elucidates the response of benthic foraminiferal assemblages along with their distribution patterns in four different transects from eastern Bahrain and the Saudi coastline. We observed pronounced effects of different kinds of pollution/stresses in the benthic foraminiferal populations. The locality in eastern Bahrain is organically polluted in which an opportunistic fauna appeared with a high overall standing crop. On the Saudi coastline, one unpolluted and two polluted transects were identified. In the unpolluted transect, *Elphidium advenum* was the only species to survive successfully due to a sandy substrate nature. The main reason behind this observation was the mechanical action of waves which strongly influenced the benthic foraminiferal assemblages and resulted in the destruction of small and more fragile specimens. This is the reason that only a few rotalliids were observed in the transect. The third transect reports presence of THC and TOC pollution which

caused significant reduction of benthic foraminiferal population. Finally, effect of natural stress and elemental pollution was observed in the last transect which resulted into high deformities mainly in peneroplid individuals.

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## Appendices

**Appendix 1(a): Water depths, physicochemical parameters of the bottom water, grain-size and Total Organic Carbon (TOC) of the seasonality transect.**

		1w	2w	3w	4w	5w	1sp	2sp	3sp	4sp	5sp	1s	2s	3s	4s	5s	1a	2a	3a	4a	5a
<b>Water depth</b>	cm	40.0	52.0	71.0	96.0	102	40.0	50.0	70.0	85.0	100	35.0	50.0	75.0	92.0	102.0	45.0	58.0	71.0	85.0	100.0
<b>Salinity</b>	PSU	45.8	45.7	45.4	44.1	43.9	45.8	45.8	45.8	45.8	44.7	46.1	46.1	46.1	46.1	46.1	46.0	45.9	45.9	45.9	45.9
<b>Temperature</b>	°C	20.2	20.1	20.1	20.1	20.1	23.4	23.4	23.4	23.3	23.3	28.8	28.8	28.8	28.8	28.8	25.1	25.1	25.0	25.1	25.1
<b>pH</b>		8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.3	8.3	8.3	8.3	8.3	8.2	8.2	8.2	8.2	8.2
<b>Conductivity</b>		52325	52224	52120	52122	52132	54937	54574	54476	54215	54101	57581	57136	56694	56592	56574	55947	55353	54991	54855	54165
<b>Turbidity</b>		0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.8	0.8	0.8	0.7	0.7
<b>Bicarbonates</b>		102.1	102.3	101.9	102.1	102.0	104.3	104.9	104.5	104.3	102.5	104.6	104.2	103.9	105.1	104.8	104.2	104.3	104.1	104.3	103.8
<b>TOC</b>	mg/kg	9321	10035	8642	5105	3379	4522	4757	4623	4486	4329	5191	4988	4445	4131	3946	8746	8310	7133	6470	5935
<b>Silt and Clay</b>	%	10.6	6.9	4.4	3.0	2.8	12.1	7.6	7.6	5.4	3.5	14.1	9.9	13.0	4.4	6.5	13.6	14.0	10.5	6.6	1.6
<b>Fine Sand</b>	%	40.7	37.4	32.5	29.2	29.3	34.6	34.0	33.0	28.8	21.9	44.8	35.4	39.7	33.7	31.5	47.4	41.5	31.3	23.2	20.0
<b>Medium Sand</b>	%	48.3	50.4	52.1	55.6	54.8	50.0	52.8	52.3	57.4	63.2	39.9	51.3	42.5	54.5	53.7	37.7	39.8	52.8	59.0	68.6
<b>Coarse Sand</b>	%	0.4	5.3	11.0	12.2	13.1	1.3	5.8	7.2	8.4	11.5	1.2	3.4	4.9	7.4	8.3	1.3	4.8	5.5	11.2	9.9

**Appendix 1(b): Descriptive analysis of water depths, physicochemical parameters of the bottom water, grain-size and Total Organic Carbon (TOC) of the seasonality transect.**

		min	max	mean	s.d.	w	sp	s	a	1	2	3	4	5	A1	A2	B
<b>Water depth</b>	cm	35.0	102.0	71.0	23.4	72.2	69.0	70.8	71.8	40.0	52.5	71.8	89.5	101.0	40.0	60.9	92.6
<b>Salinity</b>	PSU	43.9	46.1	45.6	0.6	45.0	45.6	46.1	45.9	45.9	45.9	45.8	45.5	45.2	45.9	45.9	45.3
<b>Temperature</b>	°C	20.1	28.8	24.3	3.2	20.1	23.4	28.8	25.1	24.4	24.4	24.3	24.3	24.3	24.4	24.9	23.9
<b>pH</b>		8.2	8.3	8.2	0.0	8.2	8.2	8.3	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2
<b>Conductivity</b>		52120	57581	54655	1777	52184	54460	56915	55062	55197	54822	54570	54446	54243	55197	55064	54097
<b>Turbidity</b>		0.7	0.8	0.7	0.0	0.7	0.7	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
<b>Bicarbonates</b>		101.9	105.1	103.7	1.1	102.1	104.1	104.5	104.1	103.8	103.9	103.6	103.9	103.3	103.8	104.0	103.4
<b>TOC</b>	mg/kg	3379	10035	5929	2036	7296	4543	4540	7319	6945	7023	6211	5048	4397	6945	6327	5158
<b>Silt and Clay</b>	%	1.6	14.1	7.9	4.1	5.5	7.2	9.6	9.2	12.6	9.6	8.9	4.8	3.6	12.6	9.9	4.2
<b>Fine Sand</b>	%	20.0	47.4	33.5	7.2	33.8	30.5	37.0	32.7	41.9	37.1	34.1	28.7	25.7	41.9	36.0	27.8
<b>Medium Sand</b>	%	37.7	68.6	51.8	7.7	52.2	55.1	48.4	51.6	44.0	48.6	49.9	56.6	60.1	44.0	48.8	57.7
<b>Coarse Sand</b>	%	0.4	13.1	6.7	4.0	8.4	6.9	5.0	6.5	1.0	4.8	7.1	9.8	10.7	1.0	5.3	10.3

**Appendix 2: Concentrations of nitrates, sulphates, chlorides and bromides on bottom water, total hydrocarbon content (THC) in sediments, and heavy metals for winter sampling from seasonality transect.**

Parameters		Station 1	Station 2	Station 3	Station 4	Station 5	ER-L	ER-M
Nitrates	ppm	142.4	140.9	138.9	139.1	138.4		
Sulphates	ppm	4450	4365	4273	4124	3944		
Chlorides	ppm	2981	2862	2763	2745	2653		
Bromides	ppm	664	667	663	680	680		
THC	µg/g	9.18	1.7	< 0.1	< 0.1	< 0.1		
Cr	ppm	7	5	4	< 1	< 1	81	370
Ni	ppm	13.5	13	11.1	11.4	10.8	20.9	51.6
Cu	ppm	3.85	4.89	2.88	1.7	3.92	34	270
Zn	ppm	8.5	11.4	8.1	6.6	18.9	150	410
As	ppm	1.8	1.9	1.7	1.4	1.6	8.2	70
Ag	ppm	0.036	0.028	0.028	0.016	0.084	1	3.7
Cd	ppm	0.06	0.09	0.08	0.05	0.09	1.2	9.6
Au	ppb	< 0.5	< 0.5	< 0.5	< 0.5	4.1	46.7	218
Hg	ppb	< 10	< 10	20	< 10	< 10	150	710
Ti	%	0.016	0.007	0.008	0.003	0.004		
S	%	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05		
P	%	0.011	0.009	0.007	0.005	0.016		
Li	ppm	1.7	1.2	1	0.8	1.1		
Be	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
B	ppm	23	21	16	17	16		
Na	%	0.547	0.736	0.554	0.51	0.571		
Mg	%	1.97	1.32	1.08	0.39	0.69		
Al	%	0.24	0.14	0.13	0.08	0.11		
K	%	0.06	0.06	0.05	0.04	0.04		
Bi	ppm	< 0.02	< 0.02	< 0.02	< 0.02	0.02		
Ca	%	14.3	16.2	12.5	14.7	11.8		
Sc	ppm	0.5	0.3	0.3	0.2	0.2		
V	ppm	7	4	3	2	3		
Mn	ppm	58	27	26	11	16		
Fe	%	0.28	0.2	0.18	0.13	0.15		
Co	ppm	1.3	1	0.9	0.7	0.7		
Ga	ppm	0.72	0.42	0.34	0.14	0.12		
Ge	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
Rb	ppm	2.7	1.9	1.7	1.2	1.5		

Parameters		Station 1	Station 2	Station 3	Station 4	Station 5	ER-L	ER-M
Sr	ppm	3780	3720	3350	> 5000	3070		
Y	ppm	2.74	1.67	1.66	0.94	1.14		
Zr	ppm	1	0.8	0.9	0.8	0.5		
Nb	ppm	0.4	0.2	0.3	0.1	0.2		
Mo	ppm	0.44	0.78	0.44	0.3	0.41		
In	ppm	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02		
Sn	ppm	0.22	0.24	0.17	0.08	0.43		
Sb	ppm	0.08	0.1	0.09	0.09	0.15		
Te	ppm	0.12	0.24	0.23	0.37	0.22		
Cs	ppm	0.15	0.09	0.11	0.07	0.1		
Ba	ppm	37.9	33.3	25	23.7	40.3		
La	ppm	3.1	1.9	2	1	1.4		
Ce	ppm	6.39	4.35	4.13	2.88	3.22		
Pr	ppm	0.7	0.4	0.4	0.2	0.3		
Nd	ppm	2.51	1.44	1.55	0.92	1.19		
Sm	ppm	0.5	0.3	0.3	0.2	0.2		
Se	ppm	0.7	1.1	0.8	0.8	0.6		
Eu	ppm	0.1	< 0.1	< 0.1	< 0.1	< 0.1		
Gd	ppm	0.5	0.3	0.3	0.2	0.2		
Tb	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
Dy	ppm	0.4	0.2	0.2	0.1	0.2		
Ho	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
Er	ppm	0.2	0.1	0.1	< 0.1	< 0.1		
Tm	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
Yb	ppm	0.2	< 0.1	< 0.1	< 0.1	< 0.1		
Lu	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
Hf	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
Ta	ppm	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05		
W	ppm	< 0.1	< 0.1	< 0.1	0.1	< 0.1		
Re	ppm	0.001	0.001	0.001	0.001	0.003		
Tl	ppm	0.07	0.07	0.07	0.02	0.05		
Pb	ppm	6.34	9.19	7.25	1.45	4.19		
Th	ppm	0.5	0.3	0.3	0.2	0.2		
U	ppm	2.2	2.6	2.4	3.3	2.1		

**Appendix 3 (a). Benthic foraminiferal assemblages' parameters and relative abundances of the recognized taxa for any recognized cluster and subclusters from seasonality transect.**

		1w	2w	3w	4w	5w	1sp	2sp	3sp	4sp	5sp	1s	2s	3s	4s	5s	1a	2a	3a	4a	5a
<i>Ammonia</i>	%	43.5	40.2	44.4	40.6	38.6	20.6	38.8	31.0	37.7	35.9	57.9	50.0	45.1	52.7	49.2	41.5	30.6	30.3	33.7	33.7
<i>Glabratellina</i>	%	32.3	26.5	23.1	23.3	25.1	32.4	26.5	26.8	28.9	31.3	15.8	19.2	25.5	21.8	23.7	36.6	38.8	34.2	38.2	36.6
Miliolids	%	21.0	29.9	27.8	30.7	32.1	47.1	30.6	38.0	28.9	29.7	26.3	26.9	27.5	23.6	20.3	22.0	24.5	30.3	24.7	25.7
<i>Elphidium</i>	%	3.2	2.6	4.1	4.0	2.8	0.0	4.1	2.8	2.6	2.3	0.0	3.8	2.0	1.8	5.1	0.0	4.1	3.9	3.4	3.0
Peneroplidae	%	0.0	0.9	0.6	1.5	1.4	0.0	0.0	1.4	0.9	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0
<i>Brizalina</i>	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.8	0.0	0.0	0.0	0.0	0.0	0.0	2.0	1.3	0.0	1.0
Adults	%	75.8	71.9	51.5	51.0	52.6	79.4	74.5	71.8	68.1	63.3	73.7	73.1	74.2	72.0	72.4	41.5	38.8	31.6	29.2	31.7
Juveniles	%	24.2	28.1	48.5	49.0	47.4	20.6	25.5	28.2	31.9	36.7	26.3	26.9	25.8	28.0	27.6	58.5	61.2	68.4	70.8	68.3
Ratio (A/J)		3.1	2.6	1.1	1.0	1.1	3.9	2.9	2.5	2.1	1.7	2.8	2.7	2.9	2.6	2.6	0.7	0.6	0.5	0.4	0.5
S		4.0	5.0	5.0	5.0	5.0	3.0	4.0	5.0	6.0	5.0	3.0	4.0	4.0	4.0	5.0	3.0	5.0	5.0	4.0	5.0
FD		62.0	117.0	169.0	202.0	215.0	34.0	49.0	71.0	114.0	128.0	19.0	26.0	51.0	55.0	59.0	41.0	49.0	76.0	89.0	101.0
D		0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.4	0.4	0.3	0.4	0.3	0.4	0.3	0.3	0.3	0.3
H'		1.2	1.2	1.2	1.3	1.2	1.0	1.2	1.2	1.3	1.2	1.0	1.1	1.1	1.1	1.2	1.1	1.3	1.3	1.2	1.2
E		0.8	0.7	0.7	0.7	0.7	0.9	0.8	0.7	0.6	0.7	0.9	0.8	0.8	0.7	0.7	1.0	0.7	0.7	0.8	0.7
J		0.8	0.8	0.8	0.8	0.8	1.0	0.9	0.8	0.7	0.8	0.9	0.8	0.8	0.8	0.8	1.0	0.8	0.8	0.9	0.8
Fisher $\alpha$ index		1.0	1.1	1.0	0.9	0.9	0.8	1.0	1.2	1.3	1.0	1.0	1.3	1.0	1.0	1.3	0.7	1.4	1.2	0.9	1.1

**Appendix 3 (b). Descriptive analysis on benthic foraminiferal assemblages' parameters and relative abundances of the recognized taxa with minimum, maximum and mean values and calculated mean values for any recognized cluster and subclusters.**

		min	max	mean	s.d.	w	sp	s	a	1	2	3	4	5	A1	A2	B
<i>Ammonia</i>	%	20.6	57.9	39.8	8.8	41.5	32.8	51	33.9	40.9	39.9	37.7	41.2	39.3	40.9	38	40.7
<i>Glabratellina</i>	%	15.8	38.8	28.3	6.5	26	29.2	21.2	36.9	29.2	27.8	27.4	28.1	29.2	29.2	28.2	28
<b>Miliolids</b>	%	20.3	47.1	28.4	6.1	28.3	34.9	24.9	25.4	29.1	28	30.9	27	27	29.1	29.7	27.1
<i>Elphidium</i>	%	0	5.1	2.8	1.5	3.3	2.4	2.5	2.9	0.8	3.6	3.2	2.9	3.3	0.8	3.3	3.2
<b>Peneroplidae</b>	%	0	1.7	0.4	0.6	0.9	0.5	0.3	0	0	0.2	0.5	0.6	0.8	0	0.3	0.7
<i>Brizalina</i>	%	0	2	0.3	0.6	0	0.3	0	0.9	0	0.5	0.3	0.2	0.4	0	0.5	0.3
<b>Adults</b>	%	29.2	79.4	59.9	17.2	60.6	71.4	73.1	34.6	67.6	64.6	57.3	55.1	55	67.6	62.3	54.6
<b>Juveniles</b>	%	20.6	70.8	40.1	17.2	39.4	28.6	26.9	65.4	32.4	35.4	42.7	44.9	45	32.4	37.7	45.4
<b>Ratio (A/J)</b>		0.4	3.9	1.9	1.1	1.8	2.6	2.7	0.5	2.6	2.2	1.7	1.5	1.5	2.6	2.1	1.5
<b>S</b>		3	6	4.5	0.8	4.8	4.6	4	4.4	3.3	4.5	4.8	4.8	5	3.3	4.6	4.9
<b>FD</b>		19	215	86.4	56.2	153	79.2	42	71.2	39	60.3	91.8	115	125.8	39	62.7	125.8
<b>D</b>		0.3	0.4	0.3	0	0.3	0.3	0.4	0.3	0.4	0.3	0.3	0.3	0.3	0.4	0.3	0.3
<b>H'</b>		1	1.3	1.2	0.1	1.2	1.2	1.1	1.2	1.1	1.2	1.2	1.2	1.2	1.1	1.2	1.2
<b>E</b>		0.6	1	0.8	0.1	0.7	0.7	0.8	0.8	0.9	0.8	0.7	0.7	0.7	0.9	0.7	0.7
<b>J</b>		0.7	1	0.8	0.1	0.8	0.8	0.8	0.8	0.9	0.8	0.8	0.8	0.8	0.9	0.8	0.8
<b>Fisher <math>\alpha</math> index</b>		0.7	1.4	1.1	0.2	1	1.1	1.1	1.1	0.9	1.2	1.1	1	1.1	0.9	1.2	1.1

**Appendix 4: Physicochemical parameters of the water, and grain-size parameters and quartz content of the sediments in the sampling stations.**

		<b>Station 1</b>	<b>Station 2</b>	<b>Station 3</b>	<b>Station 4</b>	<b>Station 5</b>	<b>min</b>	<b>max</b>	<b>mean</b>	<b>s.d.</b>
<b>Water depth</b>	cm	26	47	71	85	89	26	89	63.6	26.7
<b>Salinity</b>	‰	45.4	45.5	45.5	45.6	45.5	45.4	45.6	45.5	0.1
<b>Temperature</b>	°C	19.5	19.4	19.5	19.6	19.6	19.4	19.6	19.5	0.1
<b>pH</b>		7.9	7.9	7.9	8.2	8.2	7.9	8.2	8	0.2
<b>DO</b>	mg/l	8.9	8.9	8.9	8.3	8.3	8.3	8.9	8.7	0.3
<b>Silt and Clay</b>	%	1.3	1.4	0.9	0.9	0.9	0.9	1.4	1.1	0.2
<b>Fine Sand</b>	%	11.3	11.4	9.4	10.1	9.7	9.4	11.4	10.4	0.9
<b>Medium Sand</b>	%	46.3	48.1	49.6	49.6	48.3	46.3	49.6	48.4	1.4
<b>Coarse Sand</b>	%	41.1	39.1	40.1	39.4	41.2	39.1	41.2	40.2	1
<b>Quartz content</b>	%	87.3	87.5	84.9	83.5	83	83	87.3	85.2	2.1

**Appendix 5: Nitrates, sulphates, Total Organic Carbon (TOC), Total Hydrocarbon Content (THC), and Heavy Metals contents in siliciclastic transect.**

Parameters		Station 1	Station 2	Station 3	Station 4	Station 5	ER-L	ER-M
<b>Nitrates</b>	mg/l	1.4	>0.1	>0.1	>0.1	>0.1		
<b>Sulphates</b>	mg/l	9.1	8.3	8.9	8.2	8.4		
<b>TOC</b>	mg/Kg	152.5	144	388	496	562		
<b>THC</b>	µg/g	29.56	27.94	29.24	25.35	26.31		
<b>Cr</b>	ppm	11	8	461	11	11	81	370
<b>Ni</b>	ppm	10	4.1	16	8.8	5.2	20.9	51.6
<b>Cu</b>	ppm	15.2	3.99	9.88	22	5.31	34	270
<b>Zn</b>	ppm	6.8	4.2	3.6	4.3	5.9	150	410
<b>As</b>	ppm	1.8	0.9	1.8	1.3	1.1	8.2	70
<b>Ag</b>	ppm	0.011	0.01	0.09	0.046	0.017	1	3.7
<b>Cd</b>	ppm	< 0.01	0.04	0.01	< 0.01	0.02	1.2	9.6
<b>Au</b>	ppb	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	46.7	218
<b>Hg</b>	ppb	10	20	< 10	< 10	< 10	150	710
<b>Ti</b>	%	0.014	0.006	0.003	0.007	0.007		
<b>S</b>	%	< 1	< 1	< 1	< 1	< 1		
<b>P</b>	%	0.006	0.006	0.004	0.003	0.003		
<b>Li</b>	ppm	2.1	1.1	1.6	1.2	1.2		
<b>Be</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>B</b>	ppm	11	6	8	7	6		
<b>Na</b>	%	0.545	0.304	0.495	0.322	0.326		
<b>Mg</b>	%	0.42	0.29	0.29	0.28	0.27		
<b>Al</b>	%	0.2	0.1	0.13	0.13	0.12		
<b>K</b>	%	0.06	0.04	0.06	0.05	0.05		
<b>Bi</b>	ppm	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02		
<b>Ca</b>	%	2.76	2.98	2.13	2.35	2.44		
<b>Sc</b>	ppm	0.4	0.2	0.3	0.2	0.1		
<b>V</b>	ppm	9	3	5	6	5		
<b>Mn</b>	ppm	93	99	89	94	118		
<b>Fe</b>	%	0.73	0.75	0.94	0.9	1.01		
<b>Co</b>	ppm	1.8	1.2	1.5	1.5	1.5		
<b>Ga</b>	ppm	0.99	0.43	0.46	0.59	0.62		
<b>Ge</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Rb</b>	ppm	1.4	0.5	1.6	0.8	1.1		
<b>Sr</b>		132	143	135	109	116		
<b>Y</b>	ppm	1.68	1.04	1.11	1.1	0.92		
<b>Zr</b>	ppm	1.1	0.8	2.4	1.2	1.3		
<b>Nb</b>	ppm	0.2	0.2	0.2	0.2	0.2		
<b>Mo</b>	ppm	1.02	0.74	2.87	1.53	0.98		

Parameters		Station 1	Station 2	Station 3	Station 4	Station 5	ER-L	ER-M
<b>In</b>	ppm	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02		
<b>Sn</b>	ppm	0.64	0.34	0.6	1.02	0.48		
<b>Sb</b>	ppm	0.19	0.04	0.14	0.15	0.07		
<b>Te</b>	ppm	0.18	0.06	0.05	0.06	0.1		
<b>Cs</b>	ppm	0.07	0.05	0.05	0.04	0.04		
<b>Ba</b>	ppm	39.6	26.7	31	29.6	28.8		
<b>La</b>	ppm	2.3	1.7	1.8	1.8	1.7		
<b>Ce</b>	ppm	4.42	3.37	3.72	3.75	3.63		
<b>Pr</b>	ppm	0.5	0.4	0.5	0.4	0.4		
<b>Nd</b>	ppm	2.27	1.48	1.71	1.6	1.68		
<b>Sm</b>	ppm	0.3	0.3	0.3	0.4	0.3		
<b>Se</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Eu</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Gd</b>	ppm	0.4	0.2	0.4	0.3	0.3		
<b>Tb</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Dy</b>	ppm	0.2	0.2	0.2	0.2	0.2		
<b>Ho</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Er</b>	ppm	0.1	0.1	< 0.1	< 0.1	0.1		
<b>Tm</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Yb</b>	ppm	0.1	< 0.1	0.1	< 0.1	< 0.1		
<b>Lu</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Hf</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Ta</b>	ppm	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05		
<b>W</b>	ppm	0.1	0.2	< 0.1	0.1	0.2		
<b>Re</b>	ppm	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001		
<b>Pb</b>	ppm	2.16	1.42	2.35	2.32	1.7		
<b>Tl</b>	ppm	0.02	< 0.02	0.18	0.09	0.05		
<b>Th</b>	ppm	0.3	0.4	0.4	0.4	0.4		
<b>U</b>	ppm	0.4	0.5	0.4	0.4	0.4		

**Appendix 6: Nitrates, sulphates, Total Organic Carbon (TOC), Total Hydrocarbon Content (THC), and Heavy Metals contents in siliciclastic transect.**

		<b>Station 1</b>	<b>Station 2</b>	<b>Station 3</b>	<b>Station 4</b>	<b>Station 5</b>	<b>min</b>	<b>max</b>	<b>mean</b>	<b>s.d.</b>
<i>Ammonia</i>	n	0	0	5	19	17	0	19	8.2	9.2
<i>Elphidium</i>	n	0	0	27	109	114	0	114	50	57.2
<i>Ammonia</i>	%	0	0	15.6	14.8	13	0	15.6	8.7	8
<i>Elphidium</i>	%	0	0	84.4	85.2	87	0	87	51.3	46.9
<b>Adults</b>	%	0	0	15.6	16.4	17.6	0	17.6	9.9	9.1
<b>Juveniles</b>	%	0	0	84.4	83.6	82.4	0	84.4	50.1	45.7
<b>Ratio (A/J)</b>		0	0	0.2	0.2	0.2	0	0.2	0.1	0.1

**Appendix 7: Water depths, physicochemical parameters of the bottom water, grain-size and Total Organic Carbon (TOC) of the boat harbor transect.**

		Station 1	Station 2	Station 3	Station 4	Station 5	min	max	mean	s.d.
Water depth	cm	25.0	29.0	42.0	55.0	70.0	25.0	70.0	44.2	18.6
Salinity	PSU	45.8	45.8	45.9	45.6	45.5	45.5	45.9	45.7	0.2
Temperature	°C	21.6	21.6	21.8	21.7	21.4	21.4	21.8	21.6	0.1
pH		9.1	9.3	9.8	9.5	9.4	9.1	9.8	9.4	0.3
Silt and Clay	%	10.6	11.2	11.7	10.7	8.9	8.9	11.7	10.6	1.0
Fine Sand	%	48.5	51.0	50.8	45.3	43.6	43.6	51.0	47.8	3.3
Medium Sand	%	38.2	35.3	30.5	34.6	35.6	30.5	38.2	34.8	2.8
Coarse Sand	%	2.7	2.7	7.0	9.4	11.9	2.7	11.9	6.7	4.1

**Appendix 8: Concentrations of nitrates, sulphates on bottom water, total hydrocarbon content (THC) in sediments, and heavy metals for winter sampling from boat harbor transect.**

Parameters		Station 1	Station 2	Station 3	Station 4	Station 5	ER-L	ER-M
Nitrates	ppm	197.8	189.5	203.3	201.8	189.9		
Sulphates	ppm	14611	14871	14951	14189	11456		
TOC	mg/Kg	8540	9143	12525	11300.0	10730		
THC	µg/g	9.18	1.7	< 0.1	< 0.1	< 0.1		
Cr	ppm	14	30	39	9	10	81	370
Ni	ppm	10.1	25.6	5.6	6.1	5.1	20.9	51.6
Cu	ppm	117	52.9	18.2	8.41	13	34	270
Zn	ppm	64.9	115	20.4	17.8	27.2	150	410
As	ppm	3.1	6.9	3.4	2.4	2.7	8.2	70
Ag	ppm	0.161	0.361	0.056	0.052	0.035	1	3.7
Cd	ppm	< 0.01	0.09	0.05	0.01	0.07	1.2	9.6
Au	ppb	8.2	10	< 0.5	1.6	< 0.5	46.7	218
Hg	ppb	110	90	20	20	30	150	710
Ti	%	0.01	0.027	0.006	0.005	0.003		
S	%	< 1	< 1	< 1	< 1	< 1		
P	%	0.025	0.078	0.018	0.031	0.021		
Li	ppm	3.1	11.4	2	2.2	2.1		
Be	ppm	< 0.1	0.2	< 0.1	< 0.1	< 0.1		
B	ppm	45	96	32	34	29		
Na	%	2.14	4.56	0.934	0.878	1.14		
Mg	%	1.13	2.58	1.73	0.87	1.75		
Al	%	0.25	0.65	0.22	0.17	0.17		
K	%	0.13	0.29	0.08	0.07	0.07		
Bi	ppm	0.03	0.09	< 0.02	< 0.02	< 0.02		
Ca	%	26.6	47.2	30.4	31	25.6		
Sc	ppm	0.3	0.9	0.5	0.3	0.1		
V	ppm	10	29	8	7	8		
Mn	ppm	63	177	51	50	47		
Fe	%	0.42	0.97	0.23	0.29	0.25		
Co	ppm	1.3	3.3	0.7	0.9	0.7		
Ga	ppm	0.49	1.11	0.52	0.1	0.48		
Ge	ppm	0.1	< 0.1	< 0.1	< 0.1	< 0.1		
Rb	ppm	2.5	3.9	0.8	1	1.1		
Sr		4300	> 5000	> 5000	> 5000	3650		
Y	ppm	1.34	2.96	1.27	1.92	1.04		

<b>Zr</b>	ppm	1	1.1	0.8	0.7	0.4		
<b>Nb</b>	ppm	0.2	0.8	0.1	0.1	< 0.1		
<b>Mo</b>	ppm	0.59	2	0.53	0.3	0.37		
<b>In</b>	ppm	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02		
<b>Sn</b>	ppm	0.94	2.75	0.45	0.28	0.38		
<b>Sb</b>	ppm	0.16	0.36	0.12	0.08	0.1		
<b>Te</b>	ppm	0.61	1.07	0.72	0.8	0.64		
<b>Cs</b>	ppm	0.17	0.5	0.11	0.09	0.09		
<b>Ba</b>	ppm	54	179	38.4	44.8	30.5		
<b>La</b>	ppm	1.8	4.7	2.4	1.5	1.7		
<b>Ce</b>	ppm	3.81	10.5	4.2	3.61	3.53		
<b>Cd</b>	ppm	< 0.01	0.09	0.05	0.01	0.07		
<b>Pr</b>	ppm	0.4	1.2	0.4	0.5	0.3		
<b>Nd</b>	ppm	1.55	4.38	1.48	1.6	1.13		
<b>Sm</b>	ppm	0.3	0.8	0.3	0.3	0.3		
<b>Se</b>	ppm	0.7	0.8	0.4	< 0.1	< 0.1		
<b>Eu</b>	ppm	< 0.1	0.2	< 0.1	0.1	< 0.1		
<b>Gd</b>	ppm	0.4	1	0.3	0.4	0.2		
<b>Tb</b>	ppm	< 0.1	0.1	< 0.1	< 0.1	< 0.1		
<b>Dy</b>	ppm	0.2	0.6	0.2	0.3	0.2		
<b>Ho</b>	ppm	< 0.1	0.2	< 0.1	< 0.1	< 0.1		
<b>Er</b>	ppm	< 0.1	0.4	0.1	0.1	< 0.1		
<b>Tm</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Yb</b>	ppm	0.1	0.3	0.1	0.1	< 0.1		
<b>Lu</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Hf</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Ta</b>	ppm	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05		
<b>W</b>	ppm	0.1	0.3	< 0.1	< 0.1	0.1		
<b>Re</b>	ppm	0.002	0.003	0.001	< 0.001	0.004		
<b>Pb</b>	ppm	4.83	18.5	9.72	3.79	14.5		
<b>Tl</b>	ppm	0.23	0.47	0.12	0.09	0.07		
<b>Th</b>	ppm	0.3	0.7	0.3	0.3	0.2		
<b>U</b>	ppm	3.1	6.3	2.9	3.4	2.6		

**Appendix 9. Benthic foraminiferal assemblages' parameters and relative abundances of the recognized taxa for any recognized cluster and subclusters from the boat harbor transect.**

		<b>Station 1</b>	<b>Station 2</b>	<b>Station 3</b>	<b>Station 4</b>	<b>Station 5</b>	<b>min</b>	<b>max</b>	<b>mean</b>	<b>s.d.</b>	<b>W</b>
<i>Ammonia</i>	%	34.1	35.3	33.5	35.7	36.7	33.5	36.7	35.1	1.3	35.1
<i>Glabratellina</i>	%	29	29.4	28.6	29.9	33.3	28.6	33.3	30.3	2.1	30.3
<i>Murrayinella</i>	%	21.0	25.3	27.1	27.4	21.5	21.0	27.4	24.5	3.0	24.5
<b>Miliolids</b>	%	4.5	1.5	1.0	1.1	1.3	1.0	4.5	1.9	1.5	1.9
<i>Elphidium</i>	%	8.5	7.8	8.3	4.5	5.1	4.5	8.5	6.8	1.9	6.8
<b>Peneroplidae</b>	%	2.3	0.7	1.2	1.4	2.1	0.7	2.3	1.5	0.7	1.5
<i>Brizalina</i>	%	0.0	0.0	0.3	0.0	0.0	0.0	0.3	0.1	0.2	0.1
<b>Adults</b>	%	46.0	46.1	53.4	59.4	56.5	46.0	59.4	52.3	6.1	52.3
<b>Juveniles</b>	%	54.0	53.9	46.6	40.6	43.5	40.6	54.0	47.7	6.1	47.7
<b>Ratio (A/J)</b>		0.9	0.9	1.1	1.5	1.3	0.9	1.5	1.1	0.3	1.1

**Appendix 10. Mann-Whitney U Test illustrating substantial differences between the two transects**

Mann-Whitney U Test By variable Factor Marked tests are significant at p <.01000										
	Rank Sum - Group 1	Rank Sum - Group 2	U	Z	p-level	Z - adjusted	p-level	Valid N - Group 1	Valid N - Group 2	2*1sided - exact p
Salinity	35	20	5	1.5667	0.117186	1.58604	0.112731	5	5	0.150794
Temperature	40	15	0	2.61116	<b>0.009024</b>	<b>2.70281</b>	<b>0.006876</b>	5	5	<b>0.007937</b>
pH	40	15	0	2.61116	<b>0.009024</b>	<b>2.62714</b>	<b>0.008611</b>	5	5	<b>0.007937</b>
Silt and Clay	39	16	1	2.40227	0.016294	2.40227	0.016294	5	5	0.015873
Fine Sand	40	15	0	2.61116	<b>0.009024</b>	<b>2.61116</b>	<b>0.009024</b>	5	5	<b>0.007937</b>
Medium Sand	15	40	0	-2.61116	<b>0.009024</b>	<b>-2.61116</b>	<b>0.009024</b>	5	5	<b>0.007937</b>
Coarse Sand	24	31	9	-0.73113	0.464703	-0.73113	0.464703	5	5	0.547619
Nitrates	40	15	0	2.61116	<b>0.009024</b>	<b>2.61116</b>	<b>0.009024</b>	5	5	<b>0.007937</b>
Sulphates	15	40	0	-2.61116	<b>0.009024</b>	<b>-2.61116</b>	<b>0.009024</b>	5	5	<b>0.007937</b>
TOC	35	20	5	1.5667	0.117186	1.5667	0.117186	5	5	0.150794
THC	40	15	0	2.61116	<b>0.009024</b>	<b>2.6434</b>	<b>0.008208</b>	5	5	<b>0.007937</b>
Cr	40	15	0	2.61116	<b>0.009024</b>	<b>2.61911</b>	<b>0.008816</b>	5	5	<b>0.007937</b>
Ni	20	35	5	-1.5667	0.117186	-1.5667	0.117186	5	5	0.150794
Cu	40	15	0	2.61116	<b>0.009024</b>	<b>2.61116</b>	<b>0.009024</b>	5	5	<b>0.007937</b>
Zn	39	16	1	2.40227	0.016294	2.40227	0.016294	5	5	0.015873
As	40	15	0	2.61116	<b>0.009024</b>	<b>2.61116</b>	<b>0.009024</b>	5	5	<b>0.007937</b>
Ag	36	19	4	1.77559	0.075801	1.781	0.074914	5	5	0.095238
Cd	21.5	33.5	6.5	-1.25336	0.210076	-1.27679	0.201678	5	5	0.222222
Hg	39	16	1	2.40227	0.016294	2.51117	0.012034	5	5	0.015873
V	39.5	15.5	0.5	2.50672	0.012186	2.52982	0.011413	5	5	0.007937
Mn	37	18	3	1.98449	0.047203	1.98449	0.047203	5	5	0.055556
Fe	38	17	2	2.19338	0.028281	2.19338	0.028281	5	5	0.031746
Co	29	26	11	0.31334	0.754023	0.3254	0.744882	5	5	0.84127
Pb	40	15	0	2.61116	<b>0.009024</b>	<b>2.6434</b>	<b>0.008208</b>	5	5	<b>0.007937</b>
CF	40	15	0	2.61116	<b>0.009024</b>	<b>2.61116</b>	<b>0.009024</b>	5	5	<b>0.007937</b>
PLI	40	15	0	2.61116	<b>0.009024</b>	<b>2.61116</b>	<b>0.009024</b>	5	5	<b>0.007937</b>
<i>Ammonia</i>	15	40	0	-2.61116	<b>0.009024</b>	<b>-2.61116</b>	<b>0.009024</b>	5	5	<b>0.007937</b>

<i>Glbratellina</i>	37	18	3	1.98449	0.047203	1.98449	0.047203	5	5	0.055556
<i>Murrayinella</i>	40	15	0	2.61116	<b>0.009024</b>	<b>2.78543</b>	<b>0.005346</b>	5	5	<b>0.007937</b>
Miliolids	15	40	0	-2.61116	<b>0.009024</b>	<b>-2.61116</b>	<b>0.009024</b>	5	5	<b>0.007937</b>
<i>Elphidium</i>	40	15	0	2.61116	<b>0.009024</b>	<b>2.61116</b>	<b>0.009024</b>	5	5	<b>0.007937</b>
Peneroplidae	34	21	6	1.35781	0.174526	1.35781	0.174526	5	5	0.222222
<i>Brizalina</i>	30	25	10	0.52223	0.601509	1	0.317311	5	5	0.690476
Adults	24	31	9	-0.73113	0.464703	-0.73113	0.464703	5	5	0.547619
Juveniles	31	24	9	0.73113	0.464703	0.73113	0.464703	5	5	0.547619
Ratio (A/J)	24	31	9	-0.73113	0.464703	-0.73113	0.464703	5	5	0.547619
FD	38	17	2	2.193378	0.028281	2.193378	0.028281	5	5	0.031746
S	40	15	0	2.61116	<b>0.009024</b>	<b>2.78543</b>	<b>0.005346</b>	5	5	<b>0.007937</b>
H'	40	15	0	2.61116	<b>0.009024</b>	<b>2.61116</b>	<b>0.009024</b>	5	5	<b>0.007937</b>
E	19	36	4	-1.77559	0.075801	-1.77559	0.075801	5	5	0.095238
J	22	33	7	-1.14891	0.250593	-1.14891	0.250593	5	5	0.309524
Fisher $\alpha$	40	15	0	2.61116	<b>0.009024</b>	<b>2.61116</b>	<b>0.009024</b>	5	5	<b>0.007937</b>

**Appendix 11. The SIMPER analysis elucidating dissimilarity between the two transects and identifying CF, THC, sulphates, Pb, PLI and TOC as the parameters most responsible for this dissimilarity**

*Groups p & u*

Average dissimilarity = 13.08

Species	Group p	Group u	Av.Diss	Diss/SD	Contrib%	Cum.%
	Av.Abund	Av.Abund				
CF	607.44	31.76	1.88	3.81	14.37	14.37
THC	67.37	2.24	1.48	3.60	11.33	25.70
Sulphates	1477.60	4231.20	1.42	23.61	10.85	36.55
Pb	10.27	0.06	0.95	4.90	7.29	43.84
PLI	309.75	72.69	0.95	4.29	7.27	51.11
TOC	10447.60	7296.40	0.81	1.12	6.23	57.34
Cu	41.90	3.45	0.74	1.62	5.69	63.03
Cr	20.40	3.60	0.57	1.82	4.37	67.39
Hg	54.00	12.00	0.55	1.44	4.23	71.62
Zn	49.06	10.70	0.55	1.49	4.22	75.84
Mn	77.60	27.60	0.52	1.48	3.97	79.81
V	12.40	3.80	0.34	1.53	2.57	82.39
Water depth	44.20	72.20	0.31	1.48	2.38	84.76
Coarse Sand	6.72	8.40	0.29	1.32	2.23	86.99
Silt and Clay	10.61	5.54	0.24	1.62	1.84	88.83
Nitrates	196.46	139.94	0.23	8.48	1.77	90.61

**Appendix 12: The average dissimilarity of foraminiferal variables**

*Groups p & u*

Average dissimilarity = 10.89

Species	Group p	Group u	Av.Diss	Diss/SD	Contrib%	Cum.%
	Av.Abund	Av.Abund				
Murrayinella	24.14	0.00	4.31	27.88	39.62	39.62
Miliolids	1.75	28.30	2.31	7.00	21.18	60.80
FD	254.40	153.00	1.07	1.35	9.85	70.65
Peneroplidae	1.57	0.87	0.66	0.81	6.07	76.71
Elphidium	6.71	3.34	0.49	2.16	4.54	81.25
Juveniles	47.72	39.44	0.37	1.17	3.40	84.66
Brizalina	0.06	0.00	0.29	0.49	2.66	87.32
Ratio (A/J)	1.12	1.78	0.28	1.17	2.54	89.86
Adults	52.28	60.56	0.27	1.31	2.48	92.34

**Appendix 13: Water depths, physicochemical parameters of the bottom water, grain-size and Total Organic Carbon (TOC) of the Sofitel hotel transect (Al-Khobar).**

		<b>Station 1</b>	<b>Station 2</b>	<b>Station 3</b>	<b>Station 4</b>	<b>Station 5</b>	<b>min</b>	<b>max</b>	<b>mean</b>	<b>s.d.</b>
<b>Water depth</b>	cm	31.0	54.0	68.0	76.0	82.0	31.0	82.0	62.2	20.4
<b>Salinity</b>	PSU	45.6	45.6	45.6	45.7	45.6	45.6	45.7	45.6	0.0
<b>Temperature</b>	°C	22.4	22.4	22.3	22.3	22.3	22.3	22.4	22.3	0.1
<b>pH</b>		8.7	8.7	8.9	8.9	8.8	8.7	8.9	8.8	0.1
<b>DO</b>	mg/L	4.4	3.9	3.7	2.4	2.1	2.1	4.4	3.3	1.0
<b>Silt and Clay</b>	%	14.5	14.9	13.1	13.5	11.8	11.8	14.9	13.6	1.2
<b>Fine Sand</b>	%	32.5	33.4	37.5	34.3	33.1	32.5	37.5	34.2	2.0
<b>Medium Sand</b>	%	41.4	35.3	30.5	34.6	35.6	30.5	41.4	35.5	3.9
<b>Coarse Sand</b>	%	11.6	16.4	18.9	17.6	19.5	11.6	19.5	16.8	3.1

**Appendix 14: Concentrations of nitrates, sulphates on bottom water, total hydrocarbon content (THC) in sediments, and heavy metals for winter sampling from Sofitel hotel transect (Al-Khobar).**

Parameters		Station 1	Station 2	Station 3	Station 4	Station 5	ER-L	ER-M
Nitrates	ppm	73.3	78.5	71.9	85.3	86.2		
Sulphates	ppm	11582	13442	13521	14467	17624		
TOC	mg/Kg	7770	9214	11705	12955	21500		
THC	µg/g	114.613	164.40	338.34	359.514	548.960		
Cr	ppm	38	44	9	9	23	81	370
Ni	ppm	12.4	6.2	6	4.4	4.4	20.9	51.6
Cu	ppm	17.4	5.57	17.3	11.1	6.5	34	270
Zn	ppm	53.3	10.4	74.6	21.1	15.8	150	410
As	ppm	3.7	1.9	3.9	1.5	2.4	8.2	70
Ag	ppm	0.115	0.051	0.031	0.016	< 0.002	1	3.7
Cd	ppm	0.08	< 0.01	0.14	0.05	0.07	1.2	9.6
Au	ppb	7.6	0.6	1	0.6	< 0.5	46.7	218
Hg	ppb	70	20	20	< 10	< 10	150	710
Ti	%	0.008	0.003	0.005	0.005	0.004		
S	%	< 1	< 1	< 1	< 1	< 1		
P	%	0.043	0.02	0.021	0.018	0.015		
Li	ppm	3.7	1.9	2.2	2.1	1.7		
Be	ppm	0.1	< 0.1	< 0.1	< 0.1	< 0.1		
B	ppm	52	29	29	29	32		
Na	%	2.38	0.716	0.924	0.852	0.933		
Mg	%	1.67	0.54	1.65	2.32	1.56		
Al	%	0.26	0.16	0.18	0.2	0.15		
K	%	0.16	0.07	0.08	0.06	0.06		
Bi	ppm	0.03	< 0.02	< 0.02	< 0.02	< 0.02		
Ca	%	25.7	25.6	25.7	30.7	30.7		
Sc	ppm	0.3	0.2	0.3	0.3	0.2		
V	ppm	12	5	7	7	6		
Mn	ppm	66	38	62	55	36		
Fe	%	0.4	0.25	0.34	0.2	0.17		
Co	ppm	1.4	0.6	0.9	0.6	0.5		
Ga	ppm	0.52	0.25	0.82	0.28	0.37		
Ge	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
Rb	ppm	2.7	1	1.4	1.1	0.9		
Sr		3650	4620	3910	4660	> 5000		
Y	ppm	1.54	0.87	1.25	1.22	0.88		

Parameters		Station 1	Station 2	Station 3	Station 4	Station 5	ER-L	ER-M
<b>Zr</b>	ppm	0.7	0.5	0.4	0.6	0.5		
<b>Nb</b>	ppm	0.1	< 0.1	0.1	< 0.1	< 0.1		
<b>Mo</b>	ppm	0.69	0.44	0.4	0.59	0.27		
<b>In</b>	ppm	0.02	< 0.02	< 0.02	< 0.02	< 0.02		
<b>Sn</b>	ppm	1.08	0.23	0.64	0.35	0.22		
<b>Sb</b>	ppm	0.26	0.14	0.05	0.09	0.06		
<b>Te</b>	ppm	0.56	0.77	0.65	0.73	0.91		
<b>Cs</b>	ppm	0.19	0.08	0.09	0.1	0.09		
<b>Ba</b>	ppm	60.8	44.2	38	34.2	26.5		
<b>La</b>	ppm	1.9	1.2	2.1	2.1	1.7		
<b>Ce</b>	ppm	4.15	3.43	3.51	3.68	2.82		
<b>Pr</b>	ppm	0.5	0.3	0.4	0.4	0.3		
<b>Nd</b>	ppm	1.69	1.08	1.55	1.69	1.17		
<b>Sm</b>	ppm	0.3	0.3	0.3	0.3	0.2		
<b>Se</b>	ppm	0.2	< 0.1	0.5	0.4	< 0.1		
<b>Eu</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Gd</b>	ppm	0.3	0.2	0.3	0.3	0.2		
<b>Tb</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Dy</b>	ppm	0.2	0.1	0.2	0.2	0.2		
<b>Ho</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Er</b>	ppm	0.1	< 0.1	0.1	0.1	< 0.1		
<b>Tm</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Yb</b>	ppm	0.1	0.1	< 0.1	< 0.1	< 0.1		
<b>Lu</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Hf</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Ta</b>	ppm	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05		
<b>W</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Re</b>	ppm	< 0.001	0.003	0.001	0.001	0.002		
<b>Pb</b>	ppm	8.06	2.59	8.97	11.6	9.24		
<b>Tl</b>	ppm	0.08	0.06	0.05	0.04	0.04		
<b>Th</b>	ppm	0.2	0.2	0.2	0.2	0.2		
<b>U</b>	ppm	3.1	2.9	2.5	3.1	3		

**Appendix 15. Benthic foraminiferal assemblages' parameters and relative abundances of the recognized taxa for any recognized cluster and subclusters from the Sofitel hotel transect (Al-Khobar).**

		<b>Station 1</b>	<b>Station 2</b>	<b>Station 3</b>	<b>Station 4</b>	<b>Station 5</b>	<b>min</b>	<b>max</b>	<b>mean</b>	<b>s.d.</b>	<b>w</b>
<i>Ammonia</i>	%	55.3	57.4	58.5	53.7	53.1	53.1	58.5	55.6	2.3	55.6
<i>Murrayinella</i>	%	31.6	23.5	26.4	28.4	32.8	23.5	32.8	28.5	3.8	28.5
<i>Elphidium</i>	%	13.1	19.1	15.1	17.9	14.1	13.1	19.1	15.9	2.5	15.9
<b>Adults</b>	%	81.6	72.3	64.2	74.6	67.2	64.2	81.6	72.0	6.8	72.0
<b>Juveniles</b>	%	18.4	27.7	35.8	25.4	32.8	18.4	35.8	28.0	6.8	28.0
<b>Ratio (A/J)</b>		4.4	2.6	1.8	2.9	2.0	1.8	4.4	2.8	1.0	2.8

**Appendix 16: Water depths, physicochemical parameters of the bottom water, grain-size and Total Organic Carbon (TOC) of the Zabnah Lagoon transect (Half Moon Bay).**

		<b>Station 1</b>	<b>Station 2</b>	<b>Station 3</b>	<b>Station 4</b>	<b>Station 5</b>	<b>min</b>	<b>max</b>	<b>mean</b>	<b>s.d.</b>
<b>Water depth</b>	cm	26.0	29.0	34.0	32.0	35.0	26.0	35.0	31.2	3.7
<b>Salinity</b>	PSU	47.3	47.2	47.2	47.2	47.2	47.2	47.3	47.2	0.0
<b>Temperature</b>	°C	31.4	31.5	31.5	31.5	31.5	31.4	31.5	31.5	0.0
<b>pH</b>		8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	0.0
<b>DO</b>	mg/L	7.1	7.2	7.1	7.1	7.1	7.1	7.2	7.1	0.0
<b>Silt and Clay</b>	%	5.2	6.1	5.7	5.8	6.2	5.2	6.2	5.8	0.4
<b>Fine Sand</b>	%	21.4	21.3	19.7	20.4	16.3	16.3	21.4	19.8	2.1
<b>Medium Sand</b>	%	49.1	46.3	47.4	48.5	47.8	46.3	49.1	47.8	1.1
<b>Coarse Sand</b>	%	24.3	26.4	27.2	25.4	29.6	24.3	29.6	26.6	2.0

**Appendix 17: Concentrations of nitrates, sulphates on bottom water, total hydrocarbon content (THC) in sediments, and heavy metals for winter sampling from Zabnah Lagoon transect (Half Moon Bay).**

Parameters		Station 1	Station 2	Station 3	Station 4	Station 5	ER-L	ER-M
Nitrates	ppm	16.46	23.71	19.34	14.83	13.59		
Sulphates	ppm	2394	2453	2132	2234	2673		
TOC	mg/Kg	443.5	609	423.5	437.5	427.5		
THC	µg/g	39.074	51.45	49.35	41.56	66.96		
Cr	ppm	49	8	9	122	10	81	370
Ni	ppm	7.5	8.1	5.8	8.6	11	20.9	51.6
Cu	ppm	2.82	11.6	3.26	4.72	7.12	34	270
Zn	ppm	14.8	10.1	9.3	6.1	12.5	150	410
As	ppm	2.5	2.1	3.1	1.3	2.6	8.2	70
Ag	ppm	0.019	0.018	0.009	< 0.002	< 0.002	1	3.7
Cd	ppm	< 0.01	< 0.01	0.01	0.01	< 0.01	1.2	9.6
Au	ppb	0.6	< 0.5	0.9	0.6	0.8	46.7	218
Hg	ppb	20	< 10	< 10	10	< 10	150	710
Ti	%	0.006	0.006	0.009	0.007	0.013		
S	%	< 1	< 1	< 1	< 1	< 1		
P	%	0.007	0.005	0.003	0.005	0.01		
Li	ppm	2.2	2.1	1.9	1.6	2.6		
Be	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
B	ppm	95	64	62	56	72		
Na	%	2.05	1.25	1.48	1.18	1.46		
Mg	%	0.73	0.5	0.55	0.48	0.7		
Al	%	0.16	0.19	0.17	0.14	0.24		
K	%	0.12	0.09	0.12	0.08	0.11		
Bi	ppm	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02		
Ca	%	31.8	21.8	23.8	20.6	24.7		
Sc	ppm	0.1	0.2	0.2	0.2	0.6		
V	ppm	6	6	6	5	10		
Mn	ppm	47	65	69	47	68		
Fe	%	0.3	0.57	0.53	0.42	0.44		
Co	ppm	0.7	1.2	1	0.8	1.4		
Ga	ppm	0.16	0.72	0.6	0.47	0.58		
Ge	ppm	0.1	0.1	0.1	0.1	0.2		
Rb	ppm	1.5	1.4	1.3	1.2	1.5		
Sr		> 5000	4340	4580	3920	4790		

Parameters		Station 1	Station 2	Station 3	Station 4	Station 5	ER-L	ER-M
<b>Y</b>	ppm	1.57	1.52	1.41	1.33	1.95		
<b>Zr</b>	ppm	1.9	1.2	1.5	1.5	1.7		
<b>Nb</b>	ppm	0.1	0.2	0.2	0.1	0.2		
<b>Mo</b>	ppm	0.48	0.79	0.48	1.17	0.35		
<b>In</b>	ppm	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02		
<b>Sn</b>	ppm	0.33	0.63	0.28	0.38	0.37		
<b>Sb</b>	ppm	0.1	0.1	0.1	0.09	0.14		
<b>Te</b>	ppm	0.93	0.68	0.72	0.69	0.9		
<b>Cs</b>	ppm	0.07	0.09	0.04	0.13	0.17		
<b>Ba</b>	ppm	34	32.1	32.1	28.6	37		
<b>La</b>	ppm	2.3	1.9	2.1	2.1	2.6		
<b>Ce</b>	ppm	5.61	5.34	4.88	4.8	5.86		
<b>Pr</b>	ppm	0.6	0.5	0.6	0.5	0.6		
<b>Nd</b>	ppm	2.58	1.87	2.2	1.83	2.71		
<b>Sm</b>	ppm	0.5	0.4	0.5	0.3	0.6		
<b>Se</b>	ppm	< 0.1	< 0.1	0.2	< 0.1	< 0.1		
<b>Eu</b>	ppm	0.2	0.1	< 0.1	< 0.1	0.1		
<b>Gd</b>	ppm	0.5	0.3	0.4	0.4	0.5		
<b>Tb</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Dy</b>	ppm	0.3	0.3	0.2	0.2	0.3		
<b>Ho</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Er</b>	ppm	0.1	0.1	0.1	0.1	0.2		
<b>Tm</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Yb</b>	ppm	0.2	< 0.1	0.1	0.1	0.2		
<b>Lu</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Hf</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Ta</b>	ppm	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05		
<b>W</b>	ppm	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1		
<b>Re</b>	ppm	< 0.001	< 0.001	< 0.001	0.002	< 0.001		
<b>Pb</b>	ppm	2.93	2.36	2.14	1.71	2.49		
<b>Tl</b>	ppm	0.05	0.04	0.02	< 0.02	< 0.02		
<b>Th</b>	ppm	0.4	0.3	0.4	0.4	0.4		
<b>U</b>	ppm	2.7	2	2.1	2.1	2.2		

**Appendix 18. Benthic foraminiferal assemblages' parameters and relative abundances of the recognized taxa for any recognized cluster and subclusters from the Zabnah lagoon transect (Half Moon Bay).**

		<b>Station 1</b>	<b>Station 2</b>	<b>Station 3</b>	<b>Station 4</b>	<b>Station 5</b>	<b>min</b>	<b>max</b>	<b>mean</b>	<b>s.d.</b>	<b>w</b>
<i>Ammonia</i>	%	40.2	34.2	41.1	34.6	31.0	31.0	41.1	36.2	4.3	36.2
<i>Glabratellina</i>	%	9.1	6.7	7.3	11.8	13.5	6.7	13.5	9.7	2.9	9.7
<b>Miliolids</b>	%	4.5	3.3	2.4	2.4	2.4	2.4	4.5	3.0	0.9	3.0
<i>Elphidium</i>	%	13.6	13.3	9.7	6.3	11.9	6.3	13.6	11.0	3.0	11.0
<b>Peneroplidae</b>	%	32.6	42.5	39.5	44.9	41.3	32.6	44.9	40.2	4.7	40.2
<b>Adults</b>	%	44.9	35.8	37.9	48.0	42.9	35.8	48.0	41.9	5.0	41.9
<b>Juveniles</b>	%	55.3	64.2	62.1	52.0	57.1	52.0	64.2	58.1	5.0	58.1
<b>Ratio (A/J)</b>		0.8	0.6	0.6	0.9	0.8	0.6	0.9	0.7	0.1	0.7

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**Arslan, M.,** Kaminski, M.A., Tawabini, B.S., Ilyas, M., babalola, L.O., Frontalini, F.,  
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