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## The distribution of benthic foraminiferal assemblages in the north–west coastal region of Malacca Straits, Malaysia

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### PEER REVIEW

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#### Comments

Benthic foraminiferal taxa and their temporal and spatial distribution are scarcely studied in Malaysia. This paper is proved to be valuable for relevant fields of studies, wherein results elaborate the research aims and discussion has clearly explained the hypotheses.

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### ABSTRACT

**Objective:** To investigate the spatial and temporal distribution of benthic foraminiferal assemblages in relation with environmental parameters in Penang Island (the northern part of Malacca Straits, west coast of Peninsula Malaysia).

**Methods:** Foraminifera samples were obtained from 144 sediment samples collected bimonthly throughout a one year sampling period using Ponar grab. These samples were then fixed with 4% buffered formalin stained with Rose Bengal. Temperature, salinity, dissolved oxygen and pH were detected *in-situ* at six sampling points within each transect approximately one metre above the seafloor. Sediment samples collected was also used to determine particle size.

**Results:** A total of fourteen benthic foraminiferal genera obtained from two major groups belonging to the calcareous and agglutinated groups have been identified at all four sampling locations throughout the sampling period. The abundance of 13 out of 14 species were significantly affected by different sampling sites and times ( $P < 0.05$ ). Physicochemical variables comprising temperature, salinity, dissolved oxygen and pH differed among sites and sampling months ( $P < 0.05$ ).

**Conclusions:** The distribution of foraminifera in terms of abundance and presence of species indicated dominance by calcareous genera of foraminifera contributed by significantly great abundances of *Ammonia* sp. and relatively low abundance of agglutinated taxa. This pattern of distribution could indicate a close association between foraminifera and physicochemical parameters.

### KEYWORDS

Foraminifera, Spatial analysis, Temporal analysis, Malaysia

## 1. Introduction

Foraminifera include calcareous or agglutinated, test-bearing, single-celled and amoeboid-like organisms, found in great abundance in various marine environments encompassing the deep sea, coastal waters and intertidal habitats such as lagoons, estuaries, salt marshes and mangroves[1–3]. Their diversity is the highest in tropical waters, gradually declining towards the North and South

poles[4]. The distribution and abundance of foraminifera are influenced by bathymetry, physicochemical characteristics of sediment and water quality. Foraminifera assemblages have been used to interpret palaeoenvironmental conditions[5], palaeomonsoons[6], palaeodepth[7], palaeotemperature[8], tsunami[9], sediment transport[10] and pollution profiles[11,12]. From this perspective, there is a potential for foraminifera to become useful in tropical mangrove settings[13] because foraminifera characterized by agglutinated and calcareous

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test walls are among the most abundant and dominant meiofauna in mangroves[14–17]. Previous studies in the tropical Malaysian peninsula by previous authors have provided some information of benthic foraminifera from mangroves and estuarine environments[14,17–23]. However, the identification of foraminiferal taxa and their spatial and temporal distribution in the west coast of the Malaysian peninsula is relatively unknown.

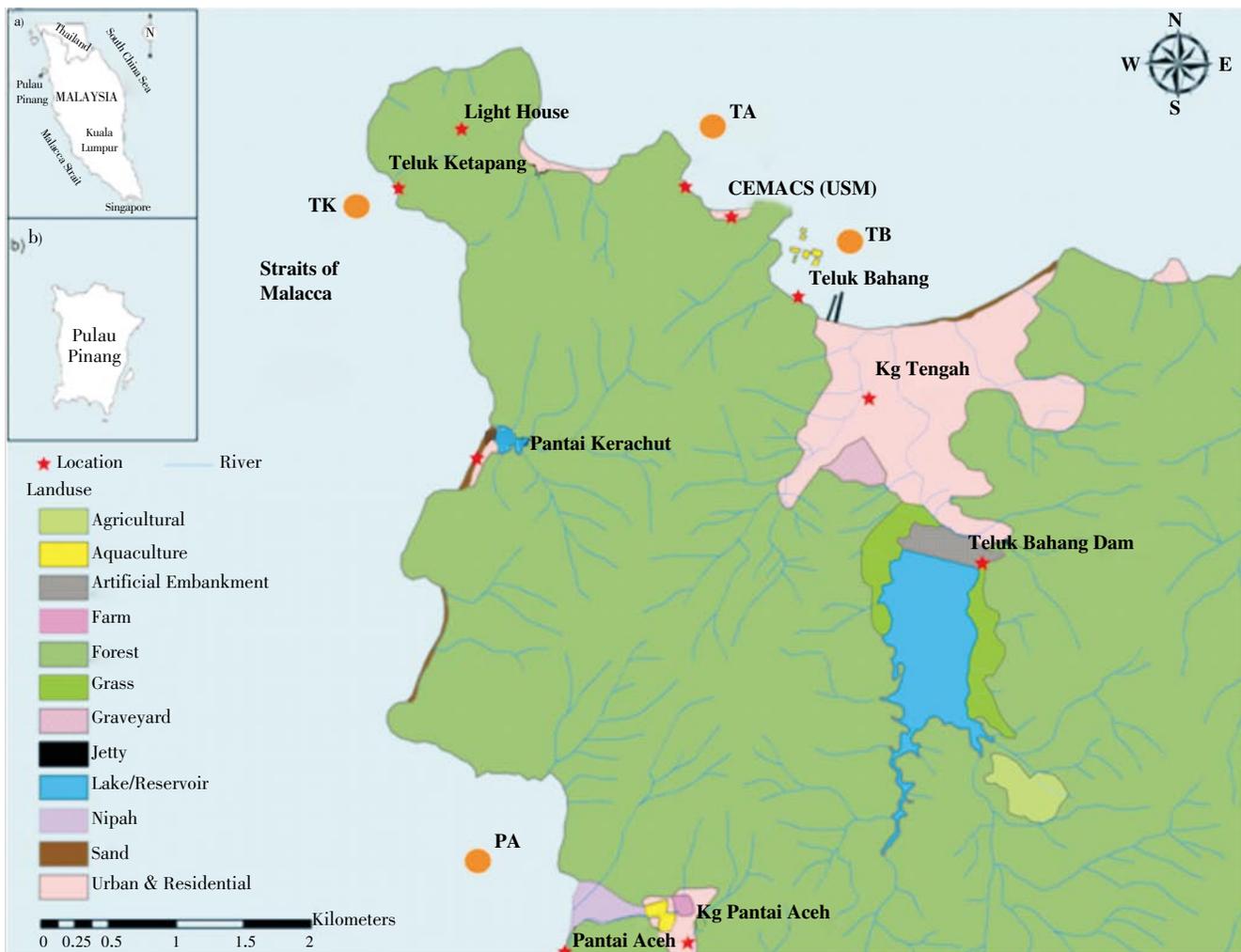
The objective of this study was, therefore, to identify the spatial and temporal distributions of foraminifera in Penang National Park, Malaysia. Because of the complex characteristics of the intertidal ecosystems in Malaysian coasts, this study was carried out concurrently with detailed characterization of the environment. This included measurements of water temperature, salinity, dissolved oxygen and pH and determination of sediment particle size. This concurrent sampling approach was done to investigate potential physicochemical factors that could explain patterns of foraminifera community structure at multiple spatial and temporal scales.

## 2. Materials and methods

### 2.1. Study area

This study was conducted along the coastal waters of Penang National Park, Penang Island (Figure 1). Sampling sites were chosen based on the degree of human activities within the area. Samples were collected from four study sites, which comprised of Teluk Bahang (5°47.1' N, 100°20.8' E), Teluk Aling (5°47.7' N, 100°19.8' E), Teluk Ketapang (5°39.6' N, 100°15.8' E) and Pantai Aceh (5°46.2' N, 100°16.6' E).

The Teluk Bahang coastal area was subjected to floating-cage culture activities and was used by locals for artisanal fishing. Teluk Aling is a sheltered, coastal area situated nearby a marine research centre. Teluk Ketapang is a sheltered bay situated 3 km away from the Teluk Bahang site, and is considered the least disturbed site, because there were no developments along its intertidal area. The Pantai Aceh site is situated further south, closer to mangroves, and was characterized by the occurrence of



**Figure 1.** Map showing location of study sites and sampling points along the coastal waters of Penang.

Locations used to sample foraminifera and physicochemical variables include (a) Peninsular Malaysia, (b) Penang Island (Pulau Pinang) and study sites at Penang National Park marked with orange dots. Map legend shows human activity, types of ecosystems and sediments in Penang National Park.

extensive mudflats. This area received freshwater input from the Pinang River. Major parts of the mangroves at Pantai Acheh had been cleared for aquaculture ponds and housing, contributing to the effluent (*i.e.* domestic, aquaculture and agriculture) discharge into the river<sup>[24]</sup>.

## 2.2. Sampling of foraminifera assemblages and physicochemical variables

The sample of sediment for the collection of foraminifera was obtained from a total of 144 sediment samples collected bimonthly between October 2010 and September 2011. Six sampling points were laid out at each of the four study site, giving a total of 24 sampling points. These sampling points were determined and recorded using the Global Positioning System. The selection criteria for these sampling locations was based on a systematic sampling design, in which random points were chosen at 200 m intervals starting from 200 m away from shore to 1200 m in the offshore area. Subsequent sampling throughout the entire duration of the study was also done consistently at these determined sampling points. At each sampling point, three samples were taken at these 200 m intervals to determine the distribution of foraminifera at specific scales.

The three samples obtained from each sampling point within each sampling point consisted of three bulk sediment samples collected using a 6 inch×6 inch×6 inch small Ponar grab. Three subsamples were taken from the Ponar grab using a hand corer, with each subsample consisting of 5 cm inner diameter sediment cores which were then transferred into 250 mL pre-labelled containers. These samples were then fixed with 4% buffered formalin, to minimize the degradation of foraminiferan tests, and stained with Rose Bengal, to detect live foraminifera<sup>[25]</sup>. Although there is the possibility of Rose Bengal adhering to dead as well as live foraminiferan tissue, thus causing overestimation of abundance, precautions were taken to avoid bias derived from Rose Bengal staining. Firstly, the process and time frame of staining all the samples were standardized, and secondly the amount of Rose Bengal used was increased so live foraminifera would absorb the stain in greater amounts than dead foraminifera, then a more accurate count of foraminiferal abundance could be achieved.

Due to the great densities of foraminifera in marine sediments, two 8 cm<sup>3</sup> sub-samples were taken from each 5 cm diameter sediment core previously subsampled from the Ponar grab for a more efficient and accurate quantitative analysis of the foraminifera. The subsamples were wet-sieved using 1000 µm and 63 µm sieves<sup>[26]</sup>. The residue on the 63 µm sieve was then carefully inspected under a dissecting microscope. Total counts of foraminifera,

identified to the genus level, were obtained from each of the 8 cm<sup>3</sup> samples, and used to describe foraminiferal assemblages. Taxonomic identifications were made based on taxonomic keys obtained from morphological descriptions of foraminifera species by previous authors<sup>[1,27–29]</sup> to the smallest taxonomic levels possible. Subsequently, identified genera were categorized into functional groups comprising taxa which could tolerate extreme environmental stressors stress tolerant, and groups based on the morphology of foraminifera which include agglutinated and other small miliolids. Selected foraminiferal samples were cleaned using a fine brush, dried and sent for imaging by scanning electron microscopy (Carl–Zeiss SMT, Oberkochen, Germany).

Physicochemical parameters were measured *in-situ* comprising of temperature, salinity, dissolved oxygen and pH using a conductivity, temperature and depth meter. Temperature and salinity were measured by YSI 30 meter and pH by a portable EcoTestr™ pH 2 meter. Dissolved oxygen was measured using YSI 52 dissolved oxygen meter. Sediment samples collected were also analysed to determine particle size based on methods of Eleftheriou<sup>[30]</sup>.

## 2.3. Statistical analysis

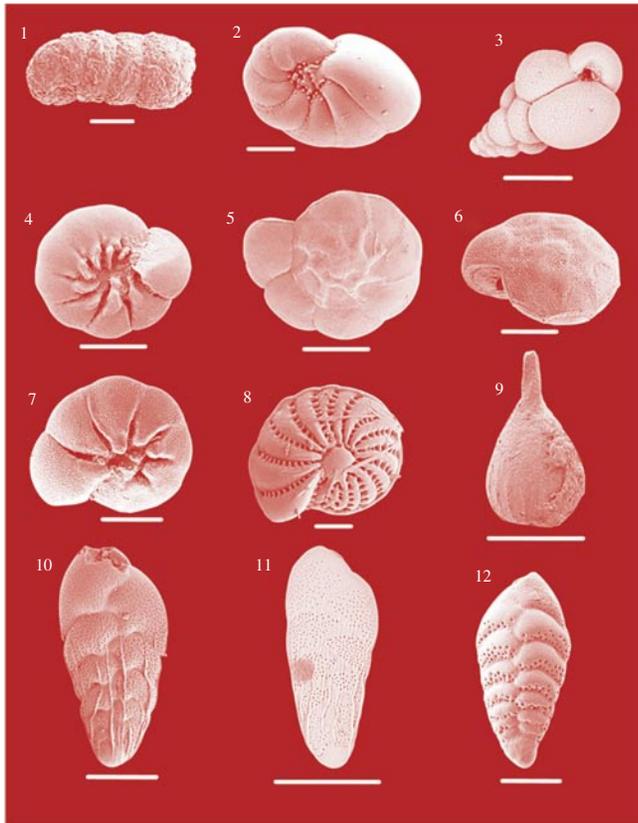
Foraminiferal abundance and environmental variables were analysed using a general linear model in STATISTICA (Statsoft Pty Ltd) incorporating fixed factors comprising sampling times ( $n=6$ ) and sites ( $n=4$ ), with transects ( $n=6$ ) as replicates. Assumptions for homogeneity of variances were checked using the Cochran test. If variances were insufficiently homogeneous, transformations were applied to the data and if the homogeneity was still not achieved, a more conservative  $P$  value was used (namely,  $P<0.01$ ). Transformations of the data were obtained using square root, four root or Log ( $x+1$ ) transforms based on the results of the Cochran test, and examination of scatterplots of means *v.s.* standard deviations was established. If there were significant effects of time and site, a *post hoc* test using Tukey's honest significant difference was done for comparison of means for the significant variables.

## 3. Results

### 3.1. Distribution of foraminiferal assemblages

A total of 14 benthic foraminiferal genera and their abundance in percentage was identified at all four study sites throughout the sampling period (Table 1). The occurrences of foraminifera species across all sites and sampling points are shown in Table 2 and Figure 2.

The benthic foraminiferal assemblages were dominated by calcareous foraminifera, which thrive in hypohaline conditions. *Ammonia* sp. was distributed at all stations with an average density of 63 individuals/cm<sup>3</sup>. By comparison, only 1 individual/cm<sup>3</sup> of *Asterorotalia* sp. was found at selected stations. The agglutinated group was dominated by *Ammobaculites* sp. at all stations except Pantai Aceh with an average density of ~2 individuals /cm<sup>3</sup>.



**Figure 2.** Scanning electron microscope images of live foraminifera (scale bar: 100 µm). Species name according to plate number: 1. *Ammobaculites agglutinans* (d’Orbigny, 1846); 2. *Nonion* sp.; 3. *Eggerella advena* (Cushman, 1922); 4, 5, 6, 7: *Ammonia tepida* (Chushman, 1926); 8: *Elphidium advenum* (Cushman, 1922); 9: *Lagena* sp.; 10, 11: *Bolivina striatula* (Cushman, 1922); 12: *Bolivina spathulata* (Williamson, 1858).

**Table 1**

The mean density of foraminiferal genera (individuals/8 cm<sup>3</sup>) at four sites.

Foraminiferal genera	Teluk Bahang	Teluk Aling	Teluk Ketapang	Pantai Aceh	Relative abundance (%)
<i>Ammonia</i> (ST)	830.64±212.71	761.22±239.42	51.00±14.83	358.08±116.38	79.00
<i>Elphidium</i> (ST)	15.78±5.66	25.81±12.98	0.64±0.21	10.06±4.51	3.00
<i>Quinqueloculina</i> (OSM)	–	5.58±3.25	0.25±0.14	1.28±0.83	0.44
<i>Textularia</i> (AG)	1.61±0.46	1.19±0.45	0.31±0.10	1.17±0.33	2.00
<i>Ammobaculites</i> (ST)	26.00±7.76	12.00±4.02	0.64±0.18	1.39±0.60	5.00
<i>Reophax</i> (AG)	0.58±0.18	1.78±0.49	1.42±0.24	1.08±0.24	6.00
<i>Nonionoides</i> (ST)	2.00±0.98	1.11±0.55	0.78±0.54	3.08±1.11	1.00
<i>Astacolus</i> (OSM)	8.97±3.05	2.00±0.92	0.14±0.08	1.39±0.89	2.00
<i>Lagena</i> (OSM)	–	0.03±0.03	0.08±0.06	1.36±0.35	0.14
<i>Fissurina</i> (OSM)	0.06±0.06	0.61±0.32	0.28±0.15	1.39±0.37	0.49
<i>Bolivina</i> (ST)	0.22±0.16	1.25±0.81	1.19±0.71	5.14±1.58	1.00
<i>Asterorotalia</i> (ST)	–	0.03±0.03	0.03±0.03	0.22±0.14	0.05
<i>Eggerella</i> (AG)	0.06±0.06	0.03±0.03	0.19±0.11	1.06±0.30	0.33

Data are expressed as mean±SE. Functional groups in parenthesis based on morphology of foraminiferan tests comprising other small miliolids (OSM), stress tolerant (ST) and agglutinated (AG). Relative abundance is the mean abundance averaged from the four sites.

**Table 2**

Occurrence of foraminifera genera at six sampling points within a transect from each of the four sites.

Genus	Teluk Bahang					Teluk Aling					Teluk Ketapang					Pantai Aceh								
	S1	S2	S3	S4	S5	S6	S1	S2	S3	S4	S5	S6	S1	S2	S3	S4	S5	S6	S1	S2	S3	S4	S5	S6
<i>Ammonia</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Asterorotalia</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Hopkinsina</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Nonion</i>	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
<i>Elphidium</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
<i>Quinqueloculina</i>	-	-	-	-	-	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+
<i>Bolivina</i>	-	+	-	-	+	+	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+
<i>Reophax</i>	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Ammobaculites</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
<i>Eggerella</i>	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
<i>Textularia</i>	+	+	+	+	+	+	+	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+
<i>Astacolus</i>	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+
<i>Lagena</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
<i>Fissurina</i>	-	-	-	-	+	+	+	+	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+

S: Sampling points in the sampled transect. +: present, -: absent.

Overall, 13 out of 14 foraminiferal species were significantly affected by different sites and sampling occasions, however the effects of main and interacting factors were different for each species ( $P < 0.05$ ; Table 3). The density of dominant species *Ammonia* spp., varied significantly spatially and temporally ( $P < 0.05$ ; Table 3) while the density of *Asterorotalia* sp. was not significantly different among the sampling sites and occasions ( $P > 0.05$ ; Table 3).

**Table 3**

ANOVA detailing the effect of time, site and sampling occasions on the abundance of 14 foraminifera genera.

Foraminifera genera	Time (df=5)		Site (df=3)		Time×site (df=15)		Error (df=120)
	MS	F	MS	F	MS	F	
<i>Textularia</i> sp. (0.5√x)	2.10	3.01*	1.34	1.92	0.97	1.39	0.70
<i>Lagena</i> sp.	1.72	1.64	15.82	15.09*	1.57	1.50	1.05
<i>Ammobaculites</i> sp. (0.25√x)	2.42	3.23*	10.33	13.79*	0.47	0.63	0.75
<i>Ammonia</i> sp. [Log (x+1)]	11.68	3.31*	46.65	13.23*	5.43	1.54	3.53
<i>Astacolus</i> sp. (0.5√x)	11.91	7.30*	25.34	15.53*	1.86	1.14	1.63
<i>Fissurina</i> sp.	7.65	3.54*	12.26	5.67*	2.29	1.06	2.16
<i>Nonion</i> sp. (0.5√x)	6.90	5.95*	5.87	5.06*	0.72	0.62	1.16
<i>Reophax</i> sp. (0.5√x)	1.71	2.86*	1.94	3.24*	0.93	1.55	0.60
<i>Eggerella</i> sp.	1.67	2.04	8.54	10.45*	1.87	2.29*	0.82
<i>Bolivina</i> sp. (0.5√x)	7.46	6.70*	8.71	7.82*	2.80	2.51*	1.11
<i>Elphidium</i> sp. (0.25√x)	1.87	2.19	3.78	4.43*	2.24	2.63*	0.85
<i>Hopkinsina</i> sp.	0.83	2.73*	1.80	5.93*	0.66	2.17*	0.30
<i>Quinqueloculina</i> sp. (0.5√x)	2.30	1.59	4.25	2.93*	2.65	1.83*	1.45
<i>Asterorotalia</i> sp.	0.13	0.61	0.38	1.82	0.17	0.81	0.21

\*  $P < 0.05$ .

For foraminifera categorized under functional groups, only the abundance of two functional groups were significantly affected by sampling sites and occasions, comprising other small miliolids (time×site;  $F_{15, 120}$ : 2.08,  $P < 0.05$ ) and stress tolerant (time×site;  $F_{15, 120}$ : 2.04,  $P < 0.05$ ). The abundance of agglutinated groups were not different among sites and sampling occasions ( $P > 0.05$ ).

### 3.2. Physicochemical variables

Univariate analyses revealed physicochemical variables differed significantly across sampling occasions and sites ( $P < 0.05$ ). Salinity was the only variable affected by main factors sites (Site;  $F_{3, 120}$ : 6.39,  $P < 0.01$ ) and sampling occasions (Time;  $F_{5, 120}$ : 16.59,  $P < 0.01$ ). Overall, average salinity was (29.52±0.08)‰. Salinity in the month of April and June 2011 was significantly greater compared to other months (Tukey HSD,  $P < 0.05$ ). Salinity was significantly greater at Teluk Bahang at 29.79‰ ( $P < 0.05$ ) compared with salinity at Teluk Aling (29.61‰), Teluk Ketapang (29.58‰) and Pantai Acheh (29.11‰).

The other environmental variables, comprising dissolved oxygen, pH and temperature were affected by interactions among sampling sites and occasions (time×site,  $P < 0.01$ ). The mean values of these physicochemical parameters recorded were dissolved oxygen at (5.21±0.08), pH at (8.43±0.01), and temperature at (29.97±0.05) °C.

All sediment grain sizes differed significantly across Sites ( $P < 0.05$ ). Sediment samples consisted of different ratios of mud and sand. The sediment composition at Pantai Acheh was 99% silt and clay, Teluk Ketapang 98% silt and clay+2% sand, Teluk Aling 92% silt and clay+8% sand, and Teluk Bahang 82 % silt and clay+18% sand.

## 4. Discussion

Benthic foraminiferal taxa and their temporal and spatial distribution are scarcely studied in Malaysia. Our results indicated that foraminiferal assemblages were mainly dominated by calcareous forms throughout the sampling period. The most abundant genus was *Ammonia*, which dominated in most of the sampling points. Other taxa, typical of inner-shelf environments, included *Elphidium*, *Textularia*, *Ammobaculites*, *Reophax*, *Nonion*, *Quinqueloculina* and *Astacolus*. The same taxa were found at several localities in the Indian Ocean in the northern part of Malaysia, including Kuala Teriang, Langkawi and Sungai Burong, and Penang<sup>[19]</sup>. Benthic foraminiferal assemblage compositions differed among multiple spatial and temporal scales, reflecting the variability of the sites sampled. The taxonomic composition in this study suggested that

foraminifera were typical of marine groups, generally dominated by calcareous groups and rarely agglutinated forms. For example, the high dominance of *Ammonia* in a majority of the samples indicated that this genus preferred shallow inner shelf habitats with warm, slightly hyposaline waters<sup>[31,32]</sup>.

Based on the water quality analysis, there are no signs of pollution in the coastal water. Previous studies done around this area also indicated similar water quality conditions<sup>[22,24]</sup>. However, the sediment qualities and foraminifera assemblage indicated, otherwise, as what inferred from prior published reports of the relationship of foraminifera species abundance in different environmental conditions<sup>[32]</sup>. The high abundance of *Ammonia* and low occurrences of the other taxa could be associated with relatively low oxygen level (<5 mg/L), and the presence of higher sand components in the bottom sediment<sup>[33,34]</sup>. Hallock<sup>[32]</sup> explained that in normal marine salinities, abundant food source with intermittent hypoxia can create a favourable environment for *Ammonia*. Similar distribution of *Ammonia* was reported by Jayaraju *et al.* in the Andaman Sea<sup>[35]</sup>. This suggests that *Ammonia* is a stress-tolerant taxon and could dominate highly stressed environments in the coastal waters of the Malacca Straits<sup>[34–36]</sup>. Likewise, this could explain why *Elphidium* was not highly abundant, as *Elphidium* spp. were reported to dominate tropical estuarine-lagoonal environments characterized by higher oxygen concentrations in less hypoxic sediment<sup>[33,34]</sup>.

In comparison with calcareous genera (*Ammonia* and *Elphidium*), the presence of agglutinated taxa could be associated with smaller sediment grain size. This could be linked to high silt clay content in the sediment, as recorded throughout all four sampling sites which were predominated by these muddy sediments. In addition to sediment grain size, changes in the abundance of agglutinated to calcareous taxa have also been linked to fluctuations in salinity, as agglutinated foraminifera usually dominate lower salinities<sup>[21,36]</sup>. This could explain why agglutinated genera were most abundant in Pantai Acheh, which was characterized by low salinity compared to other sampled sites. Therefore, it is possible that the distribution patterns of agglutinated foraminiferal assemblages in this study were influenced by sediment grain sizes and salinity<sup>[37,35,38]</sup>, similar to findings by Mohamed *et al.*<sup>[39]</sup> which showed the significant influence of salinity and sediment grain-size on the distribution of agglutinated foraminiferal assemblages in the Sedili Besar River and its offshore area (SE Malaysia).

The composition of benthic foraminiferal assemblages in this study was of a typical marine environment. The dominance of *Ammonia* sp. in terms of abundance in Teluk Bahang, Teluk Ketapang, Teluk Aling and Pantai Acheh in comparison with low abundance of other taxa

suggests a disturbed environment. Therefore, additional baseline data of this kind for foraminiferal assemblages and physicochemical parameters involving analyses of water and sediment quality is needed to provide more accurate information on the diversity of these benthic meiofauna and their interactions with the environment that may not be detected by monitoring environmental parameters alone.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgements

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### Comments

#### Background

In the tropical Malaysian peninsula, previous studies have provided some information of benthic foraminifera around coastal area. However, it is unknown the classification of foraminiferal taxa and their spatial and temporal distribution in the west coast of the Malaysian peninsula. Moreover, the foraminifera are also influenced by environment and have been used to interpret environmental change, so it has become a hot research area. This paper offers new sights into relevant areas of research.

#### Research frontiers

Until now, It is unknown the classification of foraminiferal taxa and their spatial and temporal distribution in the west coast of the Malaysian peninsula. This study was done concurrently with detailed characterization of the environment inhabited by foraminifera to investigate potential physicochemical factors that could explain patterns of foraminifera assemblage structure at multiple spatial and temporal scales.

#### Related reports

The methods and materials have taken reference of other workers' researches. Similar studies were undertaken in the northern part of Malaysia, e.g. investigations in benthic foraminifera from mangrove and estuarine environments. It is noticeable that this paper has taken into consideration the foraminiferal taxa in the west coast of the Malaysian peninsula, which is relatively lesser known in this area.

### Innovations and breakthroughs

This study is a first account of the benthic foraminiferal assemblages and their distribution across time and space in coastal waters around the Penang National Park (NW Malaysia). The results proved that a total of 14 benthic foraminiferal genera obtained from two major groups belonging to the calcareous and agglutinated groups have been identified at all four sampling locations throughout the sampling period. The abundance of 13 out of 14 species were significantly affected by different sampling sites and times.

### Applications

The data in this paper offered the basic biological and physiological information about benthic foraminiferal assemblages and their distribution across time and space in coastal waters around the Penang National Park, in combination with physiochemical data of surrounding environments. The multi-faceted considerations included in this study well engender deeper understandings of the ecosystem itself as a whole.

### Peer review

Benthic foraminiferal taxa and their temporal and spatial distribution are scarcely studied in Malaysia. This paper is proved to be valuable for relevant fields of studies, wherein results elaborate the research aims and discussion has clearly explained the hypotheses.

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