Microscopic Diversity of Singapore Marine diatoms

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Abstract **–** Marine Diatoms are a dominant group of phytoplankton and one of the most ecologically important groups of organisms in the world. They are one of the most diverse lineages of eukaryotes. The tropical coastal diatom community in Singapore differs from temperate diatoms and open ocean diatom communities. While open ocean and temperate diatom communities have been heavily studied, little has been done to understand tropical coastal diatom diversity. This project uses light microscopy to identify the most common genera of marine diatoms collected in Pulau Kusu and Pulau Hantu, and compares two preservation methods use, Lugol's solution and 50% Ethanol.

Keywords – Diatoms, Light microscopy

1 INTRODUCTION

Diatoms, also called Bacillariophyceae, are group of unicellular photoautotrophic organisms with possibly over 100,000 species, representing one of the most diverse lineages of eukaryotes (Mann et *al*., 2008). They are ubiquitous in marine and freshwater habitats as well as terrestrial environments. Given their size and abundance, it has been estimated that planktonic diatoms, especially the ones found in shallow coastal areas, account for approximately one-fifth of global photosynthetic carbon fixation (Armbrust, 2009), making them an extremely important carbon sink.

Diatoms are heavily grazed upon by a plethora of marine heterotrophs, particularly copepods. Due to their fast reproduction and the fact that they are unicellular, diatoms are a good bioindicator of water quality, as exhibiting sensitive responses to environmental stimuli on an individual and community level (Bianchi et *al*., 2003). Diatoms are also a good bioindicator of ecosystem change, which is reflected in relatively rapid shifts in density and community composition (Leppard & Munawar, 1992).

Diatoms in tropical coastal waters refer to diatoms that live in the coastal region of the ocean between latitudes of 20°N and 20°S. Two characteristics make them particularly well suited to such environments: 1) diatoms are well adapted to growth with intermittently expose to high light levels characteristic of deeply mixed turbulent waters, 2) they are well adapted to pulsed availability of nutrients because they can storage nutrient in their often large central vacuole. The dynamic coastal environment selects for large diatoms, as large diatoms have higher maximum nutrient uptake rates and higher nutrient storage capacity compared to smaller diatoms, allowing them to take advantage of intermittent nutrient level fluctuations (Huete-Ortega et *al*., 2014). Larger species of diatoms also survive well in environments with varying light intensities, since their photosynthetic apparatus are less susceptible to damage by high light intensities (Key et *al*., 2010). Hence large diatom species are more abundant in coastal areas.

The dynamics of tropical diatom communities differ from temperate diatoms. In temperate areas, diatom communities show changes in composition and biomass driven by the changes in temperatures due to seasons. Equatorial waters are subjected to monsoons systems which have been shown to also influence phytoplankton dynamics. Miki et *al*. (2008) demonstrated a higher concentration of chlorophyll (a proxy for phytoplankton biomass) during the NE monsoon than the SW monsoon in the Sulu Sea off the southwest side of the Philippines. A similar trend in

chlorophyll concentration was also observed in the Strait of Malacca by Siswanto et *al* (2014).

So far, there has been limited research in diatoms of Singapore's waters. A study done by Tan, et *al*., 2016, which uses light microscopy, identified a large number of marine diatoms including pennate diatoms. However, these pennate diatoms were missing from another study conducted by Chenard et *al*., 2019 which uses high throughput sequencing of ribosomal gene marker 18S. This project aimed to create an image database of diatoms from Singapore's coastal waters from fresh and preserved samples.

2 MATERIAL & METHODS

Sampling

Surface water (~1m) samples were collected at two stations, Pulau Kusu (N 01°13.561', E 103°51.616') (Figure 1) and Pulau Hantu (N 01°13.593', E 103°44.804') February and March 2020 (Figure 2), using a 20 µm-mesh plankton net haul. Plankton cells smaller than 20 µm in diameter were filtered through the net, while cells bigger than 20 µm in diameter were concentrated and collected. This sampling process was done every month.

Light Microscope

The Live samples were observed with CKX53 Olympus inverted light microscope. Pictures of live and preserved cells were captured with a SPOT digital camera. Preservation was also needed, as the sampling process usually requires an entire day and hence most of the microscopy observation could only be done in the following days.

Images were processed and identification plates mounted using InkScape free software (https://inkscape.org/).

Preservation

Lugol's Fixation

Adapted from Piganeau et *al.,* (2012): 0.2mL of the sample was aliquoted into a 1.5mL tube and fixed with Lugol's iodine solution (10g I2, 20g of KI, 200mL of ddH2O).

Ethanol preservation

Adapted from Lepestuar et *al*., (1993): 0.2mL of the sample was aliquoted into a 1.5mL tube with equal volumes of ethanol and water to make 50% ethanol.

Identification

Morphological identification was performed by comparing the images obtained against reference taxonomic books such as Identifying Marine Phytoplankton by Tomas, C. R.(1997) and Coastal Phytoplankton: Photo Guide for Northern European Seas by Kraberg, A et *al* (2010).

3 RESULTS AND DISCUSSION

Diatoms are categorized into centrics and pennates based on characteristics of valves (silica shell), including shape, ultrastructure and ornamentation. Centric diatoms possess radially organized valves with striae radiating from a central region or ring, whereas pennates possess elongated valves with striae oriented perpendicular to a midrib, like in a feather.

Among the pennate diatoms, the following genera were observed in both samples: *Pleurosigma*, *Cylindrotheca*, *Bacillaria*, *Pseudo-Nitzschia*, *Navicula*, *Achnanthes* and *Thalassionema* (Figure 3). A high diversity of centric diatoms was observed in the samples from both locations. The list of genera observed included: *Odonthella*, *Proboscia*, *Meuniera*, *Eucampia*, *Mediopyxis*, *Corethon*, *Chaetoceros*, *Bacteriastrum*, *Paralia*, *Lauderia*, *Leptocylindrus*, *Cerataulina*, *Dactyliosolen*, *Guinardia*, *Skeletonema*, *Coscinodiscus Actinoptychus* (Figures 4, 5 and 6).

Several genera identified by Tan et *al*., (2016) were also observed during this project, however the genera *Asterionellopsis*, *Dactyliosolen*, *Proboscia*, *Neocalyptrella*, *Corethron* and *Mediopyxis* were absent in 2016.

The following genera were observed during this project but absent in the molecular description of Chenard et *al*., (2019): *Achnanthes*, *Actinoptychus*, *Asterionellopsis*, *Bacillaria*, *Bacteriastrum*, *Corethron*, *Coscinodiscus*, *Dactyliosolen Eucampia*,

Guinardia, Lauderia, Meuniera, Mediopyxis, Navicula, Neocalyptrella, Paralia, Pleurosigma, Proboscia, Stephanopyxis and *Thalassionema.*

Fixing cells in Lugol's solution was more effective as a preservation method for light microscopy compared to saline ethanol. The differences in effectiveness of the two preservation methods can be observed in Figure 7, with (A-E) being ethanol preserved and (F-Z) being Lugol preserved. Most cells when preserved in Lugol's solution showed limited change in morphology. In contrast, in saline ethanol the few remaining cells often showed changes in morphology and chloroplast damage. Empty silica shells were often observed in samples preserved with saline ethanol. In saline ethanol, most of the observed diatoms were centric diatoms (*Coscinodiscus, Paralia, Cyclothela and Skeletenoma)* (Figure 7 A – E). In contrast, with Lugol's solution a larger variety of diatoms, including pennate diatoms like *Neocalyptrella* (Figure 7Y) and *Navicula* (Figure 7V and 7W) were able to be preserved.

4 FUTURE WORK

Although highly attractive, sequencing of phytoplankton samples has several challenges especially when applied in monitoring programs. One obstacle for molecular phytoplankton analysis arises from the lack of the classified sequences in public databases (e.g PR2 and Silva) with accurate taxonomic resolution. As demonstrated by Tan et *al.*, (2016) and our work, pennate diatoms are largely present in Singapore planktonic communities although missing in the dataset generate by Chenard et *al* (2019). The absence of pennates on this molecular study can be attributed to a primer bias which can reduce the actual diversity scene in community studied as proposed by Chenard et *al* but also absence of classified sequences in the databased used, especially considering the skew information available in literature regarding the phytoplanktonic community in Singapore marine waters. We propose that diatoms observed under light microscopy but not described by Chenard et *al*., (2019) can be obtained through the use of single cell PCR. Cells of a selected species can be identified under light microscope, manually isolated by suction and ran through single cell PCR in order to obtain their DNA sequence marker.

The manual single cell isolation technique requires high levels of skills by the operator and is highly time consuming. Since often sampling and processing occur at different days requiring the samples to preserve, we suggest the use of lugol instead of saline ethanol as more cells, from different genera were better preserved than when ethanol was applied. PCR from cells preserved in lugol's solution may offer a challenge as many steps involving the neutralizing of the lugol is required prior to the PCR (Hamilton et *al*., 2015). The standardization of this step would also require a further study as a single method for removing lugol is not available in literature.

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7 FIGURES

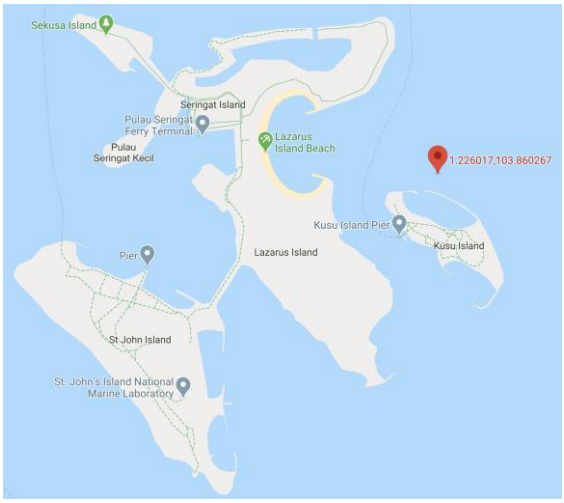

Figure 1. Sampling locations (Pulau Kusu)

Figure 2. Sampling locations (Pulau Hantu)

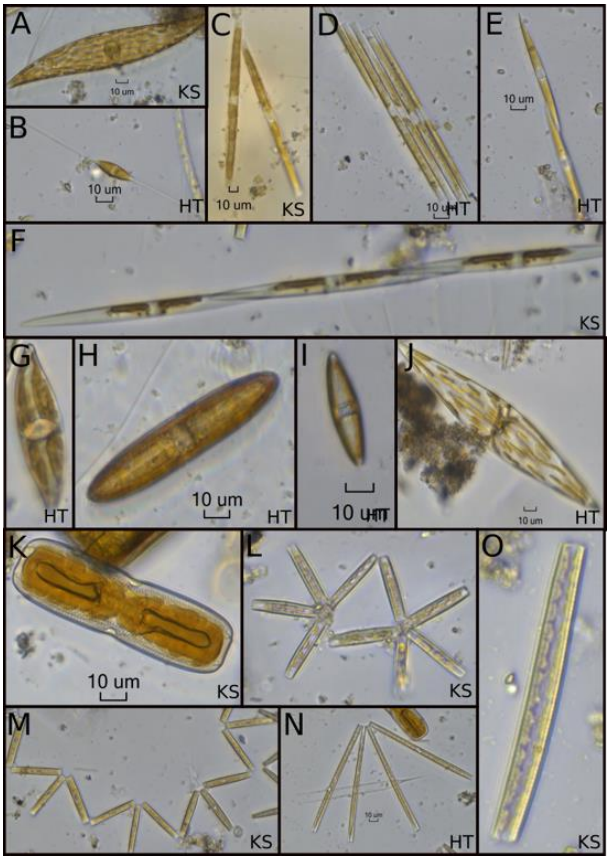

Figure 3. Pennate Diatoms: (A,G) *Pleurosigma*, (B) *Cylindrotheca*, (C, D) *Bacillaria*, (E, F) *Pseudo-Nitzschia*, (H, I) *Navicula*, (K) *Achnanthes*, (L-O) *Thalassionema*. KS: Kusu HT: Hantu.

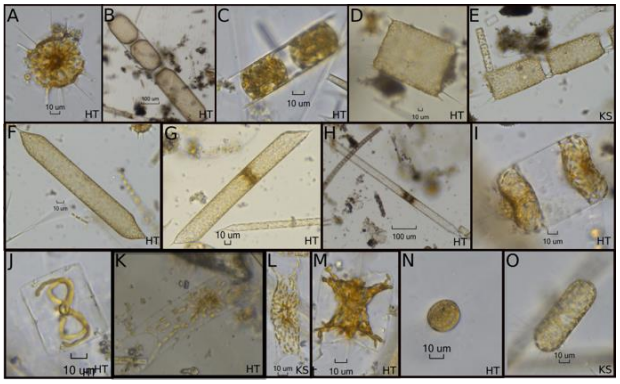

Figure 4. Centric Diatoms: (A-E) *Odonthella*, (F-H) *Proboscia*, (J) *Meuniera*, (K,L) *Eucampia*, (M) *Mediopyxis* (N,O) *Corethon* KS: Kusu HT: Hantu

Figure 5. Centric Diatoms: (A-F) *Chaetoceros*, (G,H) *Bacteriastrum*, (I,J) *Paralia*, (K,L) *Lauderia*, (M, O) Leptocylindrus, (N) Cerataulina (P,R) Dactyliosolen, (Q) Guinardia, (S) Skeletonema. KS: Kusu HT: Hantu

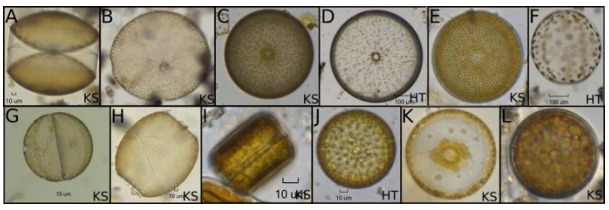

Figure 6. Centric Diatoms: (A-I) *Coscinodiscus* (L) *Actinoptychus*. KS: Kusu HT: Hantu

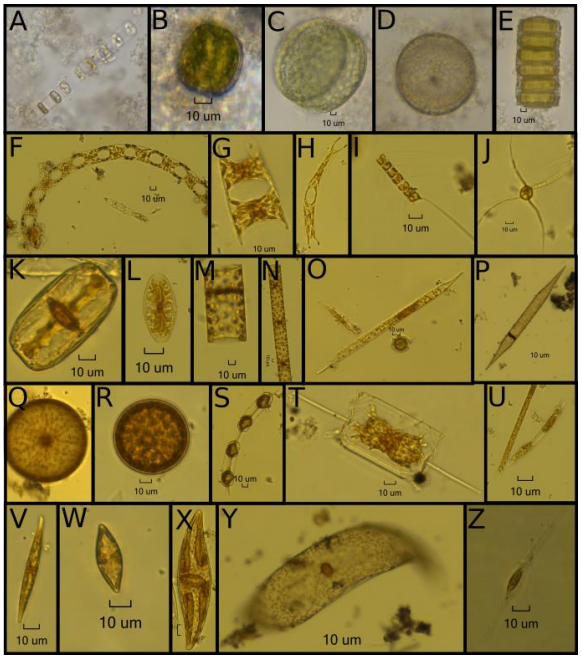

Figure 7 Preservation methods: (A-E): Cells fixed in 50% ethanol, (F-Z) Cells fixed in Lugol's solution: (A) *Skeletonema*, (B) *Cycotella*, (C,D) *Coscindodiscus*, (E) *Paralia*, (F-H) *Eucampia*, (I,J) *Chaetoceros*, (K, L) *Thalassiosira* (M,N) Guinardia (O,P) *Proboscia*, (Q,R) *Coscinodiscus*, (S,T) *Ditylum*, (U) *Stephanopyxis*, (V, W) Navicula, (X) *Pleurosigma*, (Y) *Neocalyptrella*, (Z) *Cylindrotheca* KS: Kusu HT: Hantu