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Doutorado em Ecologia: Teoria, Aplicação e Valores

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MODERN SPATIAL DISTRIBUTION OF DINOFLAGELLATES AND NON-POLLEN PALYNOMORPHS IN A NEOTROPICAL ESTUARY (NORTHEASTERN BRAZIL)

Salvador, julho de 2023

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Tese apresentada ao Programa de Pós-Graduação em Ecologia: Teoria, Aplicação e Valores, como parte dos requisitos exigidos para obtenção do título de Doutor em Ecologia: Teoria, Aplicação e Valores.

Orientador: Dr. DORIEDSON FERREIRA GOMES

Co-orientadora: Dr. ANNE DE VERNAL

Salvador, julho de 2023

DEDICATÓRIA

No matter gay, straight, or bi', lesbian, transgender life

I'm on the right track, baby, I was born to survive

No matter Black, white or beige, chola, or Orient' made

I'm on the right track, baby, I was born to be brave

Lady Gaga

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Passado, presente e futuro: o que comunidades podem revelar sobre os ambientes?

É esperado que as mudanças climáticas afetem diretamente a comunidade fitoplanctônica por meio do aumento da temperatura e estratificação superficiais, afetando a circulação oceânica e causando fenômenos chamados de ressurgência que podem intensificar florações algais nocivas. Algumas respostas fundamentais já estão bem sedimentadas na literatura como projeções de aumento da estratificação superficial da água que favorece o aumento da presença de cianobactérias e dinoflagelados e aumento da precipitação como um dos reguladores da ecologia fitoplanctônica. Tais fatores que influenciam o resultado de dinâmicas de florações não são universais, portanto demandam caracterizações locais e temporais.

O estudo realizado no estuário da Baía de Camamu permitiu, dentre outras coisas, conhecer melhor e documentar a distribuição de uma assembleia bentônica, ou seja, de um conjunto de organismos que vive no sedimento que compõe o fundo dos ambientes aquáticos. Neste trabalho, focamos nos organismos conhecidos como microfósseis, pois possuem a capacidade de permanecer fossilizados no ambiente durante longos intervalos de tempo graças à sua composição orgânica super resistente que lhes confere preservação. Os microfósseis chamados de palinomorfos são organismos de parede orgânica, como pólen, cistos de dinoflagelados e testas de foraminíferos que resistem a um tratamento que envolve ataques químicos com ácidos, filtragens e lavagens. Até o começo do século passado, os micropaleontologistas considerados pré-Quaternáio, ou seja, que estudam períodos geológicos mais antigos, descreviam todos os objetos que encontravam nas lâminas palinológicas. Isso mudou a partir do início de 1900 após as publicações dos palinógos pós-Quaternário, que estudam o período geológico mais recente da Terra, que passaram a priorizar e focar em pólen e esporos. Apesar desses últimos serem bons indicadores ambientais, sua origem terrestre não informa sobre condições locais do ambiente em que são encontrados, diferentemente dos palinomorfos não-polínicos, ignorados pelos quaternaristas, que respondem localmente à características do ambiente graças à sua origem aquática. Neste trabalho, nós mostramos a distribuição espacial desses organismos ao longo de dois principais rios que compõem o estuário: o rio Maraú e o rio Serinhaém. Este último foi um grau mais frio e também mais salino que o rio Maraú, características essenciais para diversas espécies. Os palinomorfos considerados de água salgada, como foraminíferos, fragmentos de animais e alguns cistos de dinoflagelados dominaram a assembleia de amostras coletadas em regiões de maior salinidade como a “boca" do estuário, onde a influência do mar é maior. Já os palinomorfos de água doce, como as algas verdes, e cianobactérias foram encontrados nas estações localizadas à montante, ou seja, em direção contrária ao mar. Esse resultado reforça a resposta local que esses organismos demonstram em relação às características do ambiente e, por isso, são ferramentas valiosas na reconstrução de ambientes no passado, através de técnicas matemáticas e estatísticas que permitem inferir o valor de uma determinada propriedade do ambiente a partir dos organismos da assembleia moderna que nele se encontram. Além disso, com ferramentas estatísticas específicas é possível modelar a resposta das espécies a valores hipotéticos de uma determinada variável, como a temperatura por exemplo, permitindo reconstruções futuras em cenários de aquecimento global.

Segundo programas globais de monitoramento a frequência de marés vermelhas e florações de microalgas nocivas/tóxicas está aumentando em regiões costeiras como reflexo do acréscimo de nutrientes de origem antrópica. Exemplo disso é a relação entre o aumento da população de Hong Kong (China) e o aumento na frequência de marés vermelhas. Nesta tese apresentamos dois eventos de altas concentrações da mesma espécie de dinoflagelado produtoras de toxinas; uma planctônica, ou seja, observada na superfície da água em forma de espuma e outra bentônica, no sedimento superficial, representando risco potencial à saúde humana, dado consumo de pescados na região, alêm dos efeitos ecossistêmicos. Entender a distribuição passada desses organismos pode contribuir com estratégias futuras de redução de seus efeitos nocivos.

Resumo: O aumento da temperatura em consequência das mudanças climáticas globais tem sido fonte de preocupação e investigação em diversas áreas do conhecimento. Em escala local, as consequências da pressão antrópica sobre os ambientes também afetam e alteram a composição e distribuição de comunidades biológicas, cujas dinâmicas são mecanismos de regulação e ciclagem de nutrientes na base de complexos tróficos. A composição de comunidades fitoplanctônicas compreende espécies capazes de florescer, gerando consequências ao equilíbrio ecossistêmico e à saúde humana, em casos que envolvem espécies produtoras de toxinas que podem ser transferidas através da cadeia alimentar. O objetivo deste trabalho, portanto, é utilizar a dinâmica espacial de assembleias palinológicas na compreensão de aspectos ecológicos como a dinâmica successional e os processos do ambiente que afetam a sua distribuição e composição. Esta tese compreende um capitulo introdutório, um capítulo relacionado à floração planctônica em forma de pluma com descoloração da água causada pela co-ocorrência de duas espécies de dinoflagelado produtores de toxina; um capítulo descrevendo a distribuição bentônica incomum dessa mesma espécie em altas concentrações no sedimento superficial de uma região urbanizada, onde há pesca e consumo de mariscos; um capítulo modelando a distribuição local espacial de palinomorfos não polínicos em função dos gradientes ambientais estuarinos e, por fim um capítulo de conclusão e integração das informações. A floração combinada de *Prorocentrum lima* e *Prorocentrum rhathymum* em magnitude de milhões de células por litro em forma de espuma com descoloração da água representa uma novidade para o Atlântico do Sul e Central, onde essas espécies estão mais abundantemente distribuídas. A ocorrência após tratamento palinológico de *Prorocentrum lima* em areia como substrato também representa uma novidade na literatura. A distribuição de algas verdes e cianobactérias nos sítios de menor salinidade e maior disponibilidade de luz e nutrientes, bem como a distribuição dos membros marinhos da assembleia em sítios de maior salinidade corroboram a resposta local da assembleia em função das características do ambiente estuarino. A mudança de dominância de algas verdes na assembleia moderna em comparação com a dominância de fungos e cianobactéria demonstram potenciais variações no ambiente que podem ser associadas à ocupação humana. A resposta desses organismos às mudanças do ambiente representam uma contribuição relevante no atual cenário de mudanças climáticas globais, ja que esses organismos desempenham papeis biogeoquímicos centrais no funcionamento dos ecossistemas tanto em nível local quanto regulatório de larga escala.

Palavras-chave: Fitoplâncton, Florações Algais Nocivas, Palinomorfos Não Polínicos.

Abstract: Increasing temperatures as a consequence of the global climate changes has been considered as a central topic for many sciences. In local scales, the consequences of the anthropogenic pressure against the environment also affect and alter the composition and distribution of biological communities. The dynamics of such communities are also mechanisms of nutrient regulation and cycling within the basis of the trophic complex. The composition of phytoplanktonic communities relies also on bloom-forming species, leading to consequences for the ecosystem balance and human health, given the ability of some species to produce toxins that may be transferred through the food web. Hence, we aim to use the spatial dynamics of the palynomorph assemblages to understand ecological aspects, such as the succession dynamics and the environmental characteristics affecting their composition and distribution. The present thesis is composed of an introductory chapter, a chapter describing a planktonic foam-plume bloom of co-occurring toxin-producing dinoflagellates; another chapter describing the unusual benthic high concentration of the same species complex within surface sediments of an urbanized area, where there are fishing and shellfish consumption; another chapter modelling the local distribution of non-pollen palynomorphs alongside the estuarine gradients; and finally a conclusion chapter reuniting and bonding the informations. The combined bloom of *Prorocentrum lima* and *Prorocentrum rhathymum* in the magnitude of millions of cells per litre represents a novelty for the South and Central Atlantic, where these species are abundantly distributed. The occurrence of *P. lima* in sand substrate after palynological treatment is also a novelty. The distribution of green algae and cyanobacteria in sites with decreased salinity and increased light and nutrient availability, as well as the distribution of the marine members in sites with higher salinity corroborates the local response of the assemblage to the characteristics of the estuarine environment. The dominance change from green algae in the modern assemblage to cyanobacteria and fungal spores in the fossil assemblage suggests potential environmental variations related to human occupation. The response of these organisms represents a relevant contribution in the actual scenario of global climate changes, since the role these organisms play in local and large scale biogeochemical processes.

KEYWORDS: Phytoplankton, Harmful Algae Bloom, Benthic Harmful Algae Bloom.

SUMMARY

1. General introduction, objectives and methods

2. **Chapter 1: First record of a planktonic water-discolouring bloom of the toxic dinoflagellates *Prorocentrum lima* complex *and Prorocentrum rhatymum* (Dinophyceae) in the Atlantic Ocean.**

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Structure of the thesis

This thesis is structured in three chapters, presented after a general background on phytoplankton ecology emphasizing the ecology of dinoflagellates and non-pollen palynomorphs.

Chapter 1: (technical note)

First record of a planktonic water-discolouring bloom of the toxic dinoflagellates Prorocentrum lima complex and Prorocentrum rhatymum (Dinophyceae) in the Atlantic Ocean.

Prorocentrum is a genus of desmokont dinolagellates comprising planktonic and epiphytic species widely distributed in coastal waters. *Prorocentrum lima* is mostly documented attached to macroalgae and associated to diarrheic events. *Prorocentrum rhathymum* has been associated with red tides in Mexico and water discolouration during a bloom in a lagoon of India. Here we aim to document the observation of a foam-plume aggregation of a toxigenic species in an area where coastal communities depend on fishing and consume shellfish. We collected a sample of the plume by manually grabbing the foam and adjacent water into a plastic container which was kept frozen until analysis. The estimated concentration of *P. rhathymum* in the foam plume was 2.041.665 cells/L and *Prorocentrum lima* complex was 1.691.670 cells/L. Under light microscopy the cells were generally well-preserved, most of them still hosting internal structures like chloroplasts, starch sheath, nucleus, mucocysts and pyrenoid in the case of *P. lima*. Some empty body sheaths were also noticed. The plume was collected in the upstream section of Serinhaém River, near the main town of the estuary developed on the margins of the river. The water surface temperature was 25.3ºC, the salinity was 15.6 PSU, the pH was 6.58 and the depth was 11.6 m. Total phosphorus and total nitrogen were 1.30 µM/L and 19.6 µM/L, respectively and phosphate 0.3 µM/L. Blooms are generally associated to low turbulence, high nutrient and light conditions. Nitrogen and phosphorus are limiting nutrients affecting dinoflagellates growth and development, as well as nitrogen enrichment is associated to dinoflagellate blooms.

Chapter 2: (original article)

High concentrations of the toxic epiphytic dinoflagellate Prorocentrum lima complex (Dinophyceae) in surface sediments from northeastern Brazil

Prorocentrum lima is a photoautotrophic dinoflagellate that includes several cryptic species documented to dwell epiphytically attached to epibiont macroalgae, and capable to dwell as a motile cell in the microplankton. The toxicity and abundance of *P. lima* in tropical coastal waters have motivated surveys and monitoring in areas of seafood consumption because of the risk to human health and ecosystem balance. In this study, we collected twenty box cores to recover surface sediment samples in two main rivers of Camamu Bay, with the aim to document the occurrence of *P. lima* along estuarine gradients and examine the morphology of the resistant organic walled cells. Hence, the surface sediment samples were processed following standard palynological techniques. The cells were generally well-preserved and show a large v-shape periflagellar platelet, pyrenoid and starch ring, kidney-shaped thecal pores, and varying texture of the wall surface. The highest concentrations of *P. lima* in the sediment was observed upstream of the Serinhaém River, where up to four thousand cells per gram of dried sediment were recovered. The specimens from Camamu Bay were characterized by mean size (39.6 μm length, 28.2 μm width) and morphology (oblong-ovate, laterally flat with kidney-shape pores), comparable to strains, which were described from Paranaguá Bay as a distinct type and possibly a new species. The sites with the highest concentrations are located near Ituberá town, one of the main towns of Camamu Bay, receiving tributaries and pollution, in addition to being an area of consumption of fish and mollusks. Therefore, we recommend prospections on the toxin content of the organisms from Camamu and the feeding habits of organisms from superior trophic levels.

Chapter 3: (original article)

Dinocysts and other non-pollen palynomorphs as indicators of local environmental conditions in a neotropical estuary (Camamu Bay, Brazil)

Non-pollen palynomorphs (NPP) are organic-walled remains of several aquatic and terrestrial organisms often found in palynological slides but rarely reported. Regardless pollen palynomorphs, the remains belonging to the aquatic environment respond to local conditions and thus may offer valuable information of past conditions and environmental changes. In this study, we analyzed the palynological content of surface sediment samples collected by box coring in the two main rivers of Camamu Bay, with the aim to document the distribution and spatial dynamics of non-pollen microfossils along estuarine environmental and nutritional gradients. After processing the samples following standard palynological techniques, we identified 69 NPP taxa, comprising dinoflagellate cysts, diatoms, green algae, cyanobacteria, zoological remains and fungal spores. The assemblage of the downstream sites were dominated by dinoflagellate cysts in Serinhaém river and green algae in Maraú associated to the decreased salinity and higher nutrient concentrations, whereas the marine section was dominated by foraminiferal linings, zoological remains and fungal spores. The reduced model composed of salinity, temperature, sediment type, silicate, phosphorus and N:P ratio explained of the variability in species data with both axis being statistically significant. The chemical stoichiometry between Nitrogen and Phosphorus can be limiting for phytoplankton growth and dynamics, as well as it indicates potential eutrophication in several systems. Dinocysts are documented to be less abundant in tropical estuarine environments due to the high sedimentation rates and turbulence conditions. The photoautotrophic dinoflagellate *Prorocentrum lima* dominated the dinoflagellate assemblage of the shallow, mesohalyne and nitrogen-rich waters of the upstream section of Serinhaém river. High abundances of photoautotrophic dinoflagellates are expected to be found in shallow coastal areas due to stability, light and nutrient conditions. The distribution of the non-pollen palynomorphs from Camamu Bay responded to the spatial variation of environmental conditions and nutrient stoichiometries and may be used on further paleoecological reconstruction.

1. General introduction, objectives and methods

1.1 Phytoplankton, nutrient cycling and climate change

The relationship between global climate changes and human impacts have been a central topic in many sciences, as the response of the environment to the disturbing effect of rising temperatures is far less known (IPCC, 2007). The ecological dynamics and ecosystem function reflect the context of a planet dominated by humans (Morris, 2011), which alter, affect or destroy ecosystems since Pré-History (Doughty, Wolf and Field, 2010). The impacts can be direct on habitats, such as changes in species composition by removal or introduction and connectivity (Sutherlands, et al., 2013); or the impacts can be indirects through alteration of biogeochemistry of the oceans and atmosphere leading to climatic unbalance (He-man and Reichstein, 2008). Within the oceans, increasing green house effect gases lead to the elevation of sea surface temperature, to pH reduction and to changes in vertical mixing or resurgence (Pachauri et al., 2014). Understanding these processes is relevant to determine how fast ecosystems respond to climate changes and how much past changes can offer evidences on ecological reaction for future interpretations and predictions of a planet in constant heating (Sutherlands et al., 2013). The trade-off relationship between phytoplankton and climate dynamics is well known along the Mesozoic and Cenozoic because the main phytoplankton groups (diatoms, dinoflagellates and cocolitophorids) preserved in sedimentary records are still occurring on modern water and sediments (Burnett et al., 2020).

Phytoplankton communities are composed of microscopic unicellular organisms, mostly photosynthesizing, which are responsible for up to 45% of the annual global primary productivity, even representing only 1% of Earth's photosynthetic biomass (Falkowsky et al., 2014). Although their fundamental role on global carbon dynamics, these organisms have been relatively less studied than terrestrial individuals, especially regarding biodiversity and ecosystem services.

The distribution and community structuring of phytoplankton as a response to the local environment is well documented worldwide, given the role these organisms play on carbon sequestration and dynamics within sediments and deep ocean transportation, as well as due to its contribution on sequestrating approximately 20-35% of the global CO_2 emissions (Burrows et al., 2014; Martin et al., 2011; Khatiwala et al., 2009). The species composing the phytoplankton community requires other key nutrients from the protoplasm to grow and develop, which in turns lead to nitrogen, phosphorus and silicate cycling and dynamics, affecting an entire trophic complex as they act like primary producers. The nutrients, such as Nitrogen, are as important as they are often correlated to harmful algae blooms dominated by dinoflagellates when the Nitrogen sources are high (Anderson et al., 2018; Zhou et al., 2008).

1.2 Dinoflagellates and dinocysts

Dinoflagellates (Figure 1) are one of the most abundant and diverse members of the phytoplankton community (Fensome et al., 1996). These microscopic protists are widely distributed along several types of aquatic environments, i.e. lakes, estuaries, epicontinental waters and oceans, from warm tropical ecogeographic regions to frozen polar environments (Matthiessen et al., 2005). Dinoflagellates are generally biflagellate composed of thecal plates, however they can also be athecate, coccoid, amoeboid and filamentous (Spector, 1984). Their life and nutrition strategies can vary from photoautotrophic to symbionts or parasites (Fensome et al., 1996). Approximately 50% of the species are photoautotrophic and represent an important contributor on primary production together with diatoms and cocolitophorids (de Vernal & Marret, 2007). During a specific part of their sexual reproduction, some species are able to produce a diploid cell, involved by a organic matter capsule, also known as cyst, allowing the organism to survive during a dormancy period (Wall & Dale, 1968), generally when environmental conditions are not suitable or disturbed. Approximately 15% of the 2.000 extent species are documented to produce resting cysts that are considered important ecological sources of recurring blooms, as it can fossilize and remain in the environment for millions of years (Matsuoka and Fukuyo, 2003).

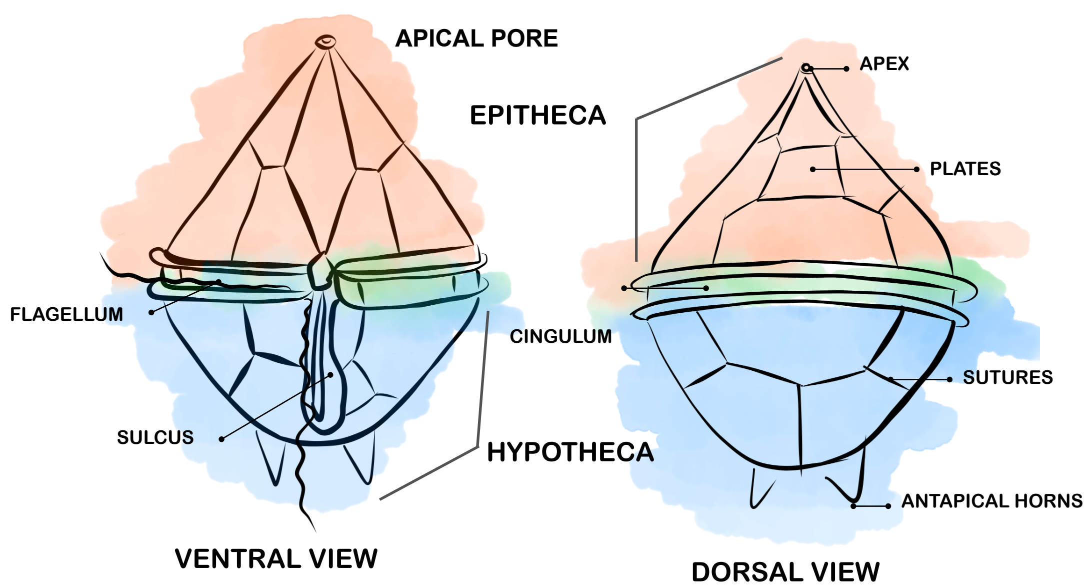

Figure 1: Main morphological characteristics of dinoflagellates. Adapted from Fensome et al., 1996.

A wide known proxy to reconstruct Quaternary marine paleoenvironments are cysts of dinoflagellates (de Vernal et al., 2005; Radi and de Vernal, 2008; Verve and Louwye, 2010). Modern cysts distribution have been used to reconstruct sea level changes, biogeography and hydrographic conditions of ocean surface, such as salinity, temperature, ice cover and productivity (de Vernal and Marret, 2007). Several studies have been developed during the past 30 years on the subject of climate changes and global warming, especially regarding cyst distributions in boreal-polar latitudes, more sensible to these alterations (Rochon and Marret, 2004), considering that orbital variations of the planet can affect solar light distribution over the surface and, thus, climate (Wright, 2004). The main techniques used to reconstruct past conditions have been based on a multiproxy approach, with foraminifer fossil shells being the most common target species to measure marine isotope stage (MIS), used to establish stratigraphic relationships along the Quaternary based on δ18O, for example (Wright, 2004).

A taxonomical challenging feature of dinoflagellates is the capacity of a single vegetative species to produce morphologically dissimilar cysts depending on salinity and temperature conditions (Ellegaard et al., 2002; Zonneveld & Susek, 2006). As evolutive unities, notwithstanding, cyst morphotypes are able to provide details about evolutionary processes, which are not provided by the modern thecas (Wall & Dalle, 1968). This way, the cyst mechanism is an adaptive strategy of dispersion that may lead to speciation and promote temporal or geographical isolation, as much as encystment and excystment occurs under defined environmental conditions, likewise temperature (Wall & Dale, 1968). This means that allying sedimentary records with modern planktonic collections creates the potential to understand the cyst stage evolution in dinoflagellate natural life history and establish connections, correlations and comparisons to determine the level and nature of systematic variations shown by modern cysts and their affinities to modern dinoflagellates. Most of our knowledge on these relations are based on polar, Arctic assemblages.

1.3 Non-pollen palynomorphs

Non-pollen palynomorphs (NPP) are organic-walled microfossil organisms belonging to a variety of taxa ranging in size from 5 to 250 micrometers that are observed in palynological slides after chemical treatments involving acid digestion, sieving and sonication (Jansonius and McGregor 1996, Shumilovskikh et al., 2021). The history of the studies on pre-Quaternary and Quaternary paleoecological entities reveals a distinction on the importance given to pollen and non-pollen objects by the scientists of the 1840-1850s and those from early 1900s (O`Keefe et al., 2021). It is argued that the pollen records became the focus of paleopalynology after the publications on Quaternary paleoecology in the beginning of the last century (O`Keefe et al., 2021), whereas the early paleoecologists were describing dinoflagellate cysts and achritarchs

(Ehrenberg, 1837), foraminiferal linings, some types of chlorophytes and prasinophytes in 1852, scolecodonts or polychaete jaws in 1854 (Sarjeant, 2002, O`Keefe et al., 2021) and copepod eggs, Rhabdocoelan oocytes and tested amoebae in 1900 (Rudolph, 1917). Later in the 1970s, the extra objects on Quaternary pollen slides started, once more, to yield the attention with systematic documentation by the pioneering work of Bas van Geel (1972). From this, it was created a list of morphotypes, not necessarily holotypes, but identifying acronyms providing descriptions of homogeneous objects found in palynological slides (Shumilovskikh et al., 2021).

1.4 Objectives

1.4.1 General objective

Document, describe and analyze the modern distribution and dynamics of the non-pollen palynomorph assemblages from Camamu Bay alongside the environmental and nutritional gradients.

1.4.2 Specific objectives

- Document for the first time in the Atlantic a water-discolouring planktonic co-occurring bloom of the toxic dinoflagellates *Prorocentrum lima* and *Prorocentrum rhathymum;*
- Describe and analyze for the first time high concentrations of the dinoflagellate *Prorocentrum lima* in surface sediments instead of macroalgae and the potential factors driving the high accumulations at the time it was observed;
- Describe and analyze the benthic distribution of dinoflagellates, dinocysts and palynomorphs of surface sediments alongside environmental and nutritional gradients.

1.5 Methods

1.5.1 Study area

Camamu Bay (Figure 2) is an estuarine system located in the State of Bahia, Brazil (13º40.2’S; 38º55.8'W and 14º12.6'S; 39º 9.6'W). It is characterized as a species-rich ecosystem representing an important regional center of economic activities based around coastal tourism and fishing (Amorim, 2005). Three main rivers flow toward the Atlantic Ocean downstream in the southeastern. The water flow are chiefly the result of the ebb tide, with a maximum seaward velocity of 84 cm/s (Amorim, 2005); the tidal regime is semidiurnal with a maximum and minimum amplitude of 2.2 m and 0 m, respectively (Lenz, 2008). Easterly and southeasterly winds predominate in the dry and rainy season, with average velocities of 2.7 m/s and 2.3 m/s, respectively and the annual mean temperature ranges from 21ºC to 25ºC (Amorim, 2005).

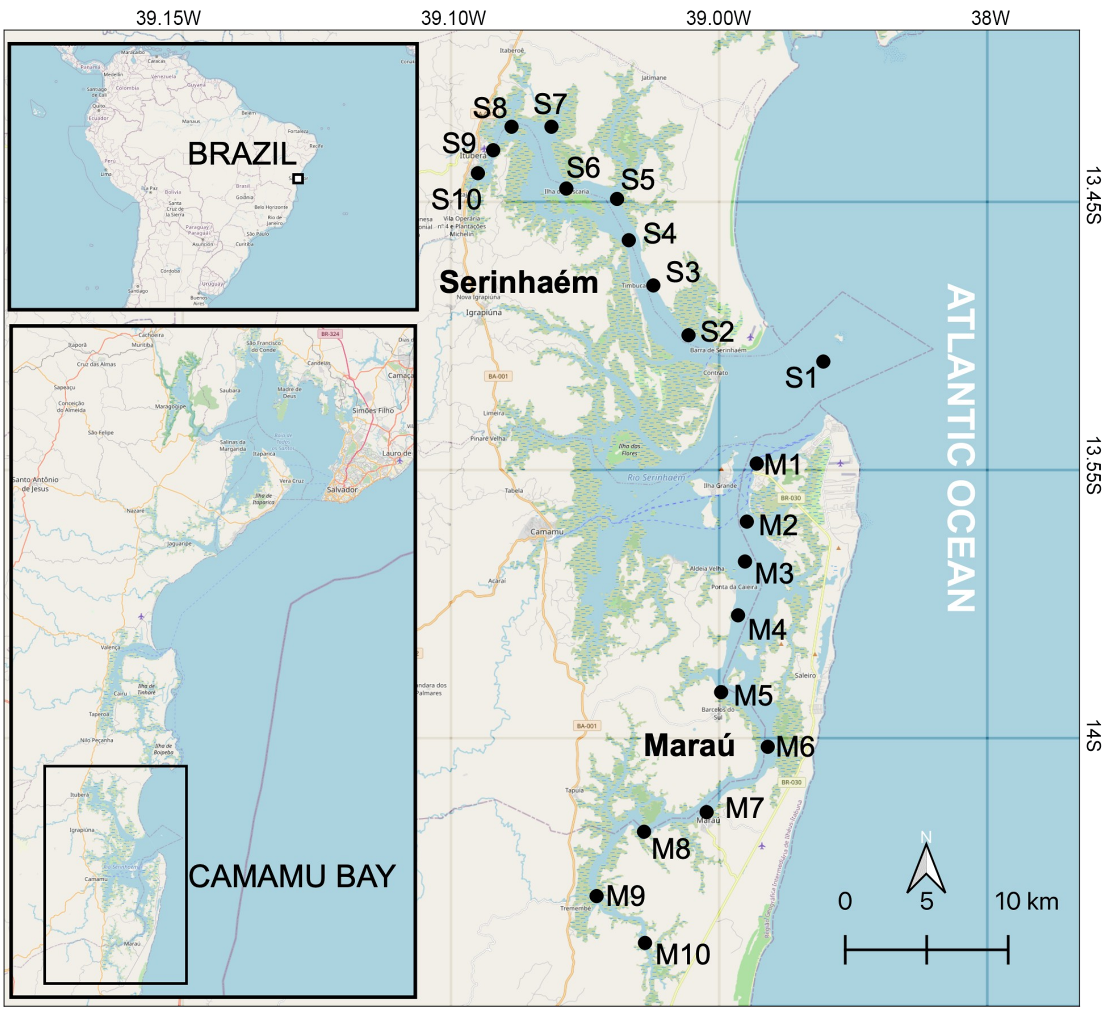

Figure 2: Geographic location of the sampled sites in the Camamu Bay.

Camamu Bay (Figure 3) receives 2.570 mm of rainfall annually (ANA, 2005), typically over two rainy periods. The main rainy period occurs from March to July, with a maximum monthly rainfall in excess of 260 mm (July), and may average ten days of rain in a single month. A second rainy period occurs from August to February, for which the maximum rainfall that may exceed 230 mm (November), and averages eight days of precipitation per month (ANA, 2005). In the sample collection the values of precipitation reached 25 mm in the first rainy campaign and 0 mm in the dry one. There is no data available for the sample collection in the second rainy period, in addition 0 mm of precipitation for the second dry campaign were observed (INMET, 2020).

Figure 3: Camamu Bay during the sampling collection.

The area is located under a state and federal conservation unity, namely “APA Baía de Camamu” (extant area of 384 km²). The ecological importance relies upon the existence of preserved restinga, 40% of mangrove covering (Figure 4) and remaining fragments of Atlantic rainforest (Bahia, 2002). The main anthropogenic pressures inside this conservation area are mangrove deforestation, permanent invasion of preserved areas, drainage of flooded areas due to condominium implementation, disorderly soil occupation and replacement of native Atlantic rainforest vegetation, restinga and mangroves to coconut monocultures (Ecotema, 2002).

Figure 4: The dense mangrove vegetation around Maraú River.

1.5.2 Sampling

In September 2021, we made a first 34 km transect and sampled ten stations distributed along the River Maraú to the south and a second 30 km transect to recover samples from ten stations sites alongside Serinhaém River to the north (Fig. 1; Table 1). The surface sediment was collected by box corer, then stored in plastic bottles and preserved with formaldehyde (4%). Some in situ environmental variables were measured, such as water surface temperature (WST), water depth using an infra-red depth gauge, water transparency with a Secchi disk, pH and salinity with a Horiba multiparameter probe (see Appendix 1). The samples collected for nutrient concentration analysis were immediately cooled in a dark thermic box and frozen until measurements in laboratory. Nutrient concentration, including silicate, soluble reactive phosphorus, total phosphorus, total nitrogen and dissolved inorganic nitrogen (DIN; the sum of ammonium ion, nitrate and nitrite) were calculated comparing the light absorbance of the samples after reaction with a set of four controlled concentrations under spectrophotometry based on a reference set of 0, 1, 5 and 10 μM for nitrogen and phosphate series (NO2, NO3, NH4 and PO4) and 0,10, 20 and 40 μM for silicate at 540 and 810 nm (Grasshoff, 1966; Wood et al., 1967; Koroleff, 1976). The organic matter and organic carbon were estimated based on loss ignition indicated for non-calcareous samples (Allen et al., 1974). We calculated the weight of bulk density of fresh sample, the dry weight after 12 hours in an air-circulated oven and the loss on ignition after 6h at 550ºC following the methodology and calculation described in Berglund (1987).

1.5.3 Palynological treatment

To concentrate the organic remains from the sediment, we used the palynological techniques (Figure 5) described in de Vernal et al. (2009). A volume of 5 cc of wet sediment was dried and a tablet of *Lycopodium clavatum* (batch: 2013001; n = 27,560 spores/tablet) was added to each sample for further concentration calculation. The sediment was wet sieved through two different size mesh sieves (106 μm and 10 μm) after deflocculation by adding soap and distilled water until the clay and small silt content was drained out. The chemical treatment included repeated digestion with cold HCl (10%) and cold HF (49%) to dissolve carbonate and silicate particles respectively. A solution of KOH (5-10%) was added to dissolve the labile organic compounds and the sample was finally ultrasonified for 20 seconds prior to a last sieving at 10 μm. A drop of the residue was mounted between slide and cover-slide in gelatine and the remaining residue was preserved in water with phenol (10%).

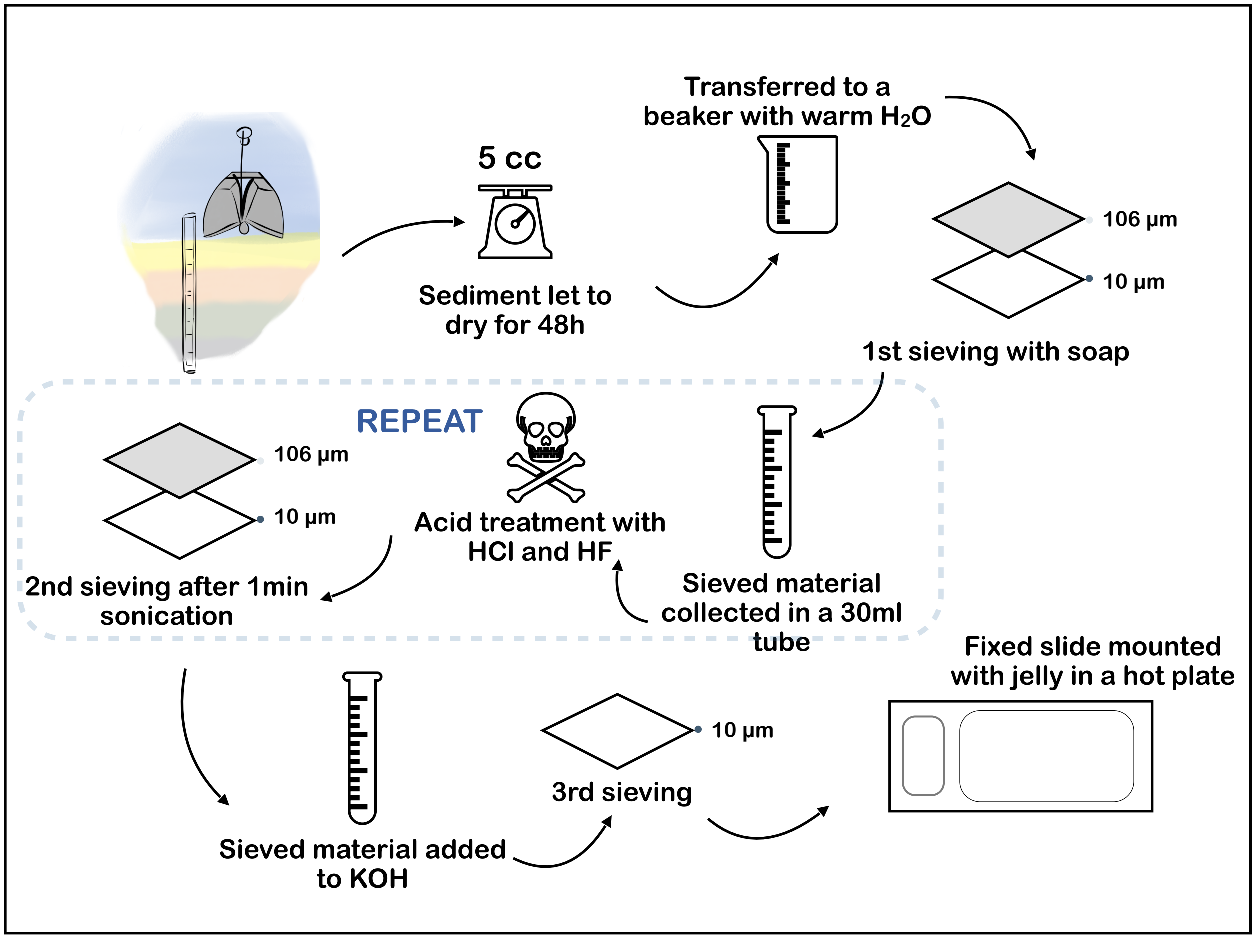

Figure 5: Schematic representation of the standard palynological treatment.

Chapter 1

First record of a planktonic water-discolouring bloom of the toxic dinoflagellates *Prorocentrum lima* complex and *Prorocentrum rhatymum* (Dinophyceae) in the Atlantic Ocean

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First record of a planktonic water-discolouring bloom of the toxic dinoflagellates *Prorocentrum lima* complex and *Prorocentrum rhatymum* (Dinophyceae) in the Atlantic Ocean

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Abstract: *Prorocentrum* is a genus of desmokont dinolagellates comprising planktonic and epiphytic species widely distributed in coastal waters. *Prorocentrum lima* is mostly documented attached to macroalgae and associated to diarrheic events. *Prorocentrum rhathymum* has been associated with red tides in Mexico and water discolouration during a bloom in a lagoon of India. Here we aim to document the observation of a foam-plume aggregation of a toxigenic species in an area where coastal communities depend on fishing and consume shellfish. We collected a sample of the plume by manually grabbing the foam into a plastic container which was kept cooled until analysis. The estimated concentration of *P. rhathymum* in the foam plume was 2.041.665 cells/L and *Prorocentrum lima* complex was 1.691.670 cells/L. Under light microscopy the cells were generally well-preserved, most of them still hosting internal structures like chloroplasts, starch sheath, nucleus, mucocysts and pyrenoid in the case of *P. lima*. Some empty body sheaths were also noticed. The plume was collected in the upstream section of Serinhaém River, near the main town of the estuary developed on the margins of the river. The water surface temperature was 25.3ºC, the salinity was 15.6 PSU, the pH was 6.58 and the depth was 11.6 m. Total phosphorus and total nitrogen were 1.30 μM/L and 19.6 μM/L, respectively and phosphate 0.3 μM/L. Blooms are generally associated to low turbulence, high nutrient and light conditions. Nitrogen and phosphorus are limiting nutrients affecting dinoflagellates growth and development, as well as nitrogen enrichment is associated to dinoflagellate blooms.

INTRODUCTION

Prorocentrum is a genus of morphologically distinct dinoflagellates (desmokont flagellation), widely distributed from tropical to polar environments inhabiting mostly planktonic but also benthic habitats and documented as important contributors of the marine food web (Dodge, 1965; Dodge, 1975; Faust, 1993). The benthic species are commonly found attached to substrates in shallow waters, such as macrophytes, coral reefs, sand and rocks by the production of mucous and polysaccharide filaments (Honsell et al., 2013). However, epiphytic dinoflagellates are flagellated during their vegetative life cycle and thus fully capable of detaching from the substrate and dwell planktonically free (Durán-Riveroll et al., 2019). The abundant and diverse distribution of benthic species in shallow tropical waters indicates that the cell growth and development is favoured by elevated water temperatures (Durán-Riveroll et al., 2019). The response of natural communities or populations to varying temperatures is a key aspect in a global warming scenario that is still uncertain (IPCC, 2022).

Coastal regions throughout the world have experienced an escalation in the incidence of toxic or Harmful Algal Blooms (HABs) over the last several decades (Anderson, 2002). The intensity and frequency of occurrence of blooms are recognized as serious ecological issues (Hallegraeff, 1993; Anderson, 1997, 2009, McGowan et al., 2017) given the potential alteration of marine habitats and trophic structures (Anderson, 2002). The blooms are also related to economic losses to local aquaculture of scallops and fishes (Wu et al., 2001; Yu and Hao, 2009). Harmful Algae Blooms are mostly associated to planktonic species, however benthic species may form Benthic Harmful Algae Blooms (BHABs), when high aggregations of toxigenic species accumulate and release toxins that may be transferred via the food web, causing fauna and eventually human intoxication (Berdalet et al., 2016). Approximately 34 benthic dinoflagellate species are able to produce toxins (Moestrup and Calado, 2018), among them *Prorocentrum lima* Ehrenberg Stein 1878 emend. Nagahama et al., 2011 was associated to diarrheic shellfish poisoning events in coastal areas of Japan (Koike et al., 1998), United Kingdom (Foden et al. 2005), Canada (Lawrence et al.,1998) and Argentina (Gayoso et al. 2002); and *Prorocentrum rhathymum* Loeblich III, Sherley & Schmidt (Loeblich et al. 1979), a tychoplanktonic toxigenic species formerly identified by the synonym *P. mexicanum* Gárate-Lizárraga and Martínez-López (1997), associated to red tides in Mexico (Ramírez-Camarena et al. 1999) and water discolouration during a bloom in the Bangaran lagoon, India (Thomas and Padmakumar, 2020). In the subtropical and tropical Atlantic Ocean *Prorocentrum lima* and *P. rhathymum* were described, among other species, as important contributors for ciguatera and were highly abundant in the Gulf of Cariaco, northeastern Venezuela (Navarro-Vargas et al., 2014), in the Caribbean Sea coast of Colombia (Arbelaez et al., 2017) and in the reef zone of the mesoamerican reef system, including the Gulf of Mexico (Estrada-Vargas et al., 2017). In the South Atlantic waters the distribution and ecology of *Prorocentrum* species from benthic habitats is incipient with most of the studies focusing on toxicity and growth based on culture isolation and collection from macrophytes (Hoppenrath et al., 2013; Nascimento et al., 2016; Moreira-Gonzáles et al., 2019, Durán-Riveroll et al., 2019). *P. lima* is reported from the Brazilian latitudes 8ºS to 27ºS always attached to macroalgae (Cardoso, 1998; Nascimento et al., 2008; Nascimento et al., 2016). The distribution of these species are thus hotspots of ecosystem balance and dynamics, human health, local economy of coastal communities, and seafood industries (Wells et al., 2015; Berdalet et al., 2016).

Here we aim to describe for the first time to the best of our knowledge in the Atlantic Ocean a co-occurring planktonic bloom of the benthic epiphytic dinoflagellates *P. lima* and *P. rhathymum* observed within a mucilaginous water-discolouring foam-plume in a tropical estuarine system.

METHODOLOGY

Camamu Bay (Fig. 1) is an estuarine system located in the State of Bahia, Brazil (13º40.2'S; 38º 55.8'W and 14º 12.6'S; 39º 9.6'W). It is characterized as a rich species diversity and represents an ecosystem important as regional center of economic activities based around coastal tourism and fishing (Amorim, 2005). The precipitation mean is 2,570 mm of rainfall annually (ANA, 2005), typically over two rainy periods. The main rainy period occurs from March to July, with a maximum monthly rainfall of 260 mm (July) and the second rainy period occurs from August to February, for which the maximum rainfall that may exceed 230 mm (November)(ANA, 2005). The surrounding vegetation is 40% composed by mangrove, in addition to remaining fragments of Atlantic Forest, comprising a state and federal conservation unit (Bahia, 2002). The main urban areas are Ituberá (population: 28,740), located upstream Serinhaém river and Maraú (45,523) located in the middle section of Maraú river. The place where the plume was observed is located upstream Serinhaem river near Ituberá town (13°43’38.8"S; 39°08’04.0”W).

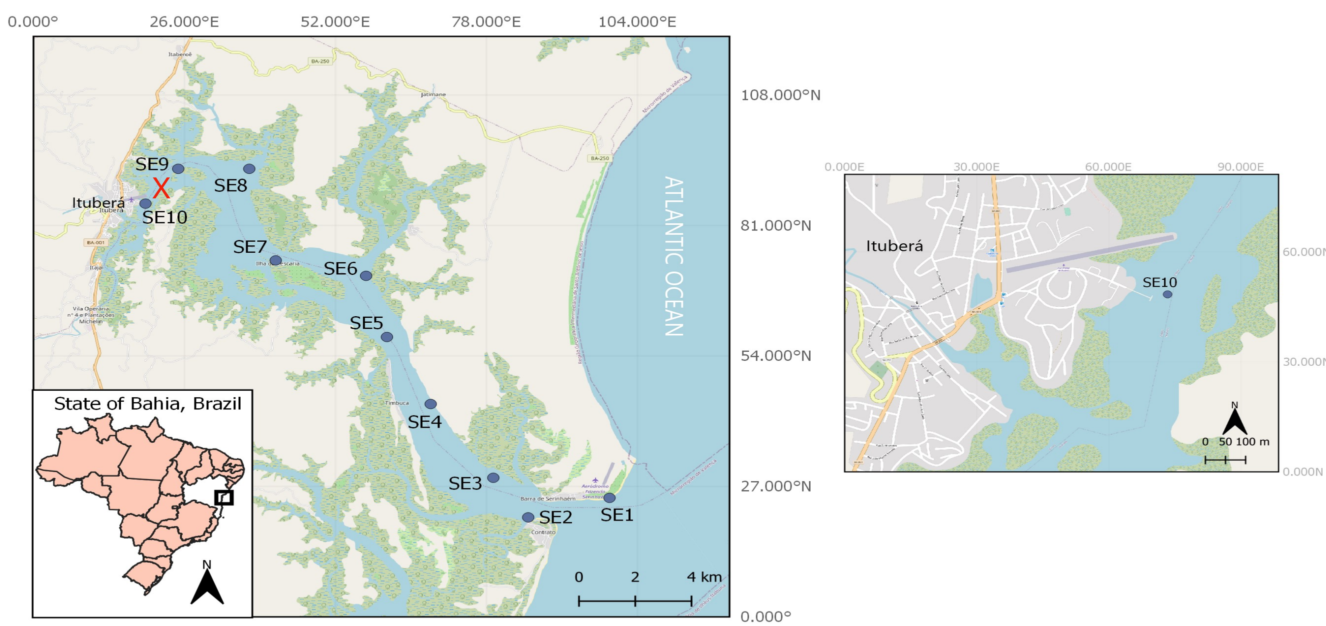

Figure 1: Geographic location of the plume (red X) observed between the sites S10 and S9. The town of Ituberá is also highlighted to illustrate the distance to the main urban anthropogenic pressure.

The sample was collected on September 3rd 2021 by manually grabbing the foam into a plastic container without adding any preservative solution until first analyses. The sample was cooled immediately after collection. Some in situ environmental variables were measured, such as water surface temperature with a thermometer, depth with an infra-red depth gauge, water transparency (WT hereafter) with a Secchi disk and pH, temperature and salinity with a Horiba multiparameter probe. Samples for posterior laboratory nutrient concentration analysis were immediately cooled inside a dark thermic box and frozen until analysis. Nutrient concentration, including silicate, soluble reactive phosphorus, total phosphorus, total nitrogen, ammonium and nitrate were calculated comparing the light absorbance of the samples reaction with a set of four controlled concentrations under spectrophotometry (Grasshoff, 1966; Wood et al., 1967; Koroleff, 1976). For each sampling point, we recorded the geographical coordinates using a GPS Garmin e-Trex. To estimate the cell concentration per litre we used the standard Utermöhl method for planktonic analysis (Utermöhl, 1931, 1958) by adding 2ml of homogenized sample in a sedimentation chamber and counting the cells under the light inverted microscope at 400x magnification. Considering the high concentration we counted 30 random transects of 0.1924 mm and estimated the concentration by extrapolation as follows:

$$C = N * (Ba/Bc)/V$$

Where: C = concentration of cells per ml (multiplied by 1.000 to get per liter);
N = number of counted cells;
Ba = Bottom area of the chamber (50.265 mm^2);
Bc = Bottom area counted (5.772 mm^2);
V = Volume of the chamber (2 ml).

To ensure an accurate taxonomical identification we observed a subsample under scanning electron microscopy following standard alcohol dehydration series and CO_2 critical point drying. The species identification criteria were number and disposition of the periflagelar platelets, number and disposition of surface trichocyst pores, presence and size of the anterior spine, and length-to-width ratio, features often used to determine species level, since they are demonstrated to reflect intraespecific regularity (Dodge, 1975; Hoppenrath et al., 2013). As we do not have replicates of the plume we limit our interpretation to a local specific frame of the environment at a particular time, so we do not aim to conclude any mechanistic relationship between the high concentrations and the environmental variables.

RESULTS

During a sampling campaign alongside Serinhaém River on September 4th 2021 we observed a foam plume of eight metres long and one meter wide. The coloration was brown with a greasy aspect (fig. 2). Other smaller similar foam plumes were observed upwards from this point until the town of Ituberá, however they were not sampled because we did not design the sampling collection for this purpose.

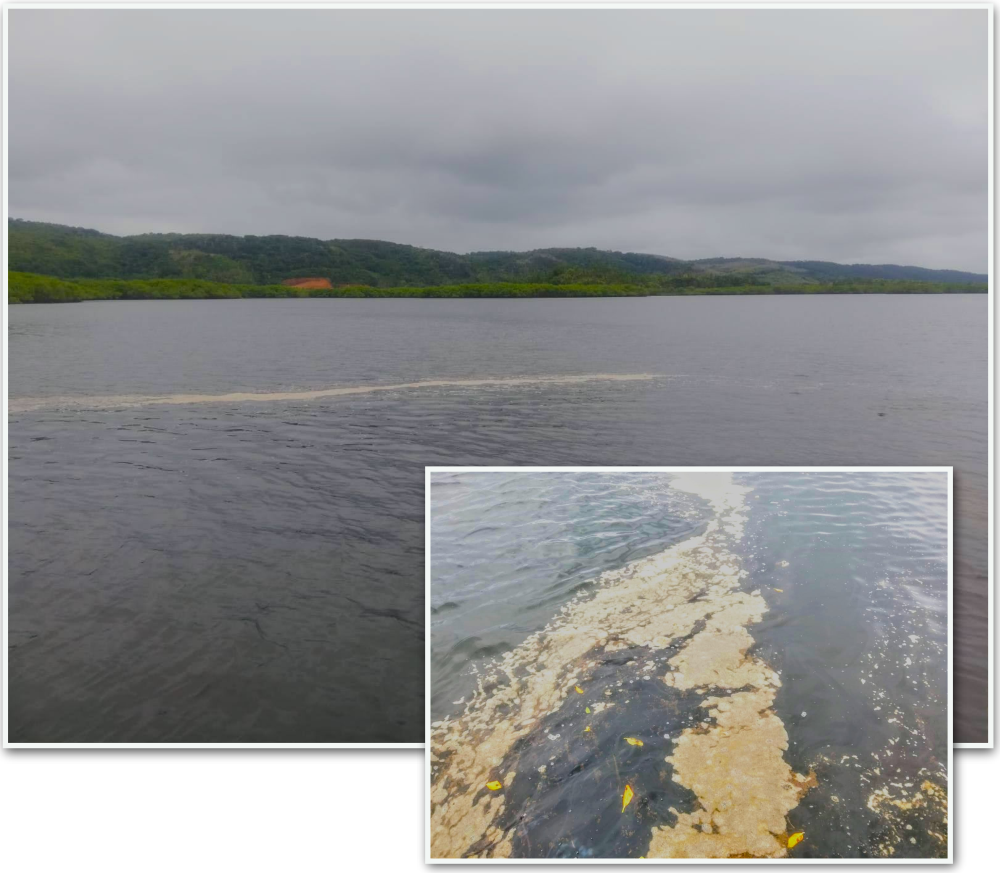

Figure 2: Mucilagenous water discolouring bloom of *Prorocentrum lima* and *Prorocentrum rhatymum* in Camamu Bay.

The estimated concentration of *Prorocentrum rhathymum* in the foam plume sample was 2.041.665 cells/L and *Prorocentrum lima* 1.691.670 cells/L. Under light microscopy the cells were generally well-preserved, most of them still hosting internal structures like chloroplasts, starch sheath, nucleus, mucocysts and pyrenoid in the case of *P. Lima. S*ome empty thecae were also noticed. Under scanning electron microscopy the cells were generally oblong-ovate or elliptical composed by a ventral plate sagittally connected to a dorsal plate by a suture, which is more visible in *P. rhathymum.* The size of the plates was 36 to 42 µm in length, 17 to 33 µm width, 3 to 7 µm thickness (mean = 5.87; sd = 1.96) and 1.25 to 2.35 µm length/width ratio in *P. lima*. The size of the plates was 36 to 42 µm in length, 24 to 30 µm in width and a length/width ratio of 1.4 µm. The surface ornamentation of *P. rhathymum* was foveate and reticulated-foveate with the pores disposed in lines radiating from the centre of the cell perpendicular to the margin. The number of pores for *P. Lima* was 80 and for *P. rhathymum* the lines emerging from the right valve was composed of six and seven pores. The aggregations observed were composed of several cells connected with mucilage straps. Several diatom types were also noticed within the foam plume dominated by penate morphotypes.

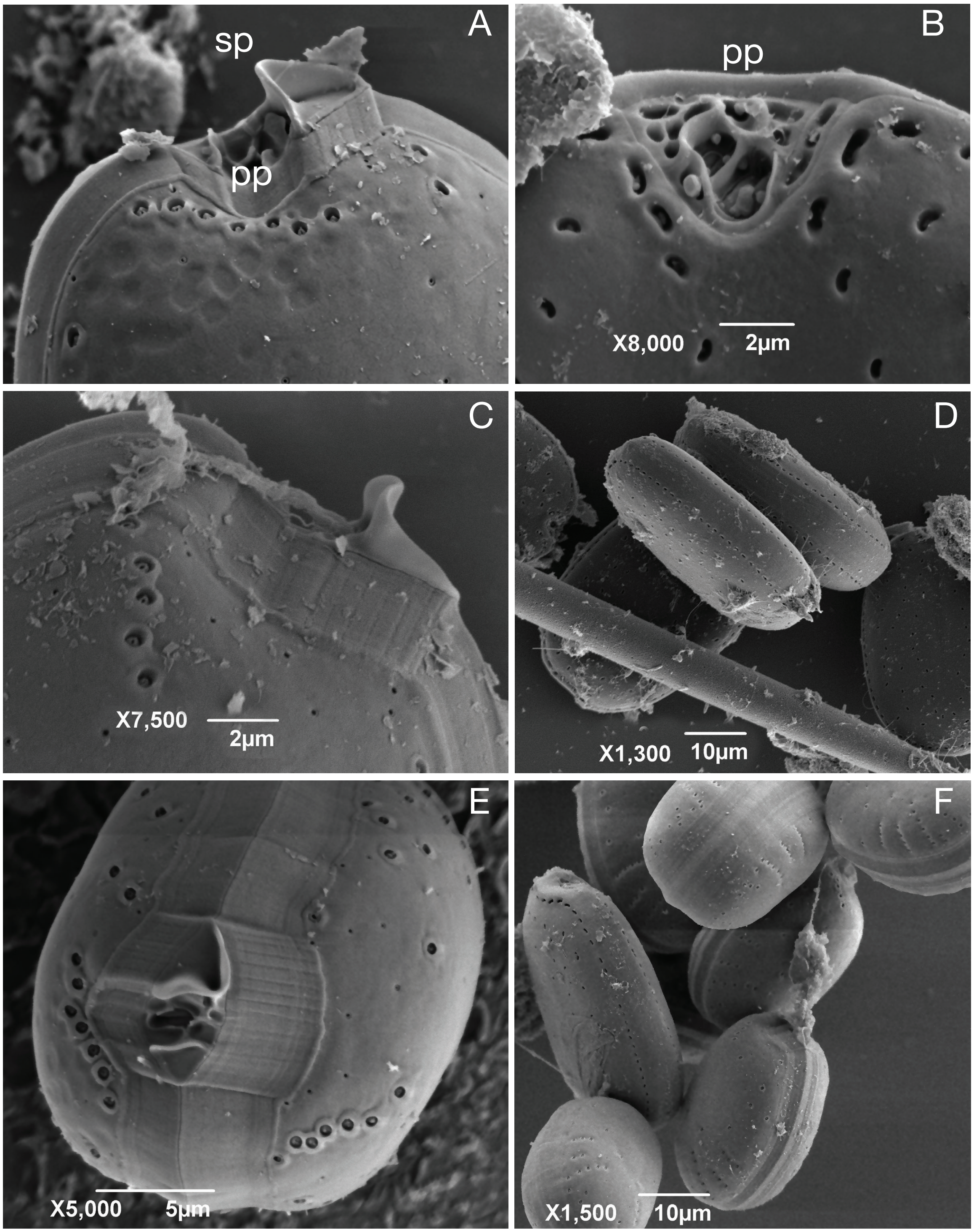

Figure 3: Scanning electron microscopy of the foam-plume sample. A) Anterior ventral plate of *Prorocentrum rhathymum* evidencing the periflagellar platelet (pp), spine (sp), foveate pores in a row and sculpted squares on the smooth surface. B) *Prorocentrum lima* periflagelar platelet and kidney-shaped pores. C) Highlight of the spine in *P. rhathymum*. D) Two coupled cells of *P. lima* with visible polysaccharide filaments. E) Polar view of *P.rhathymum* evidencing the connection between the plates. F) *P. lima* and *P. rhathymum* co-occurrence.

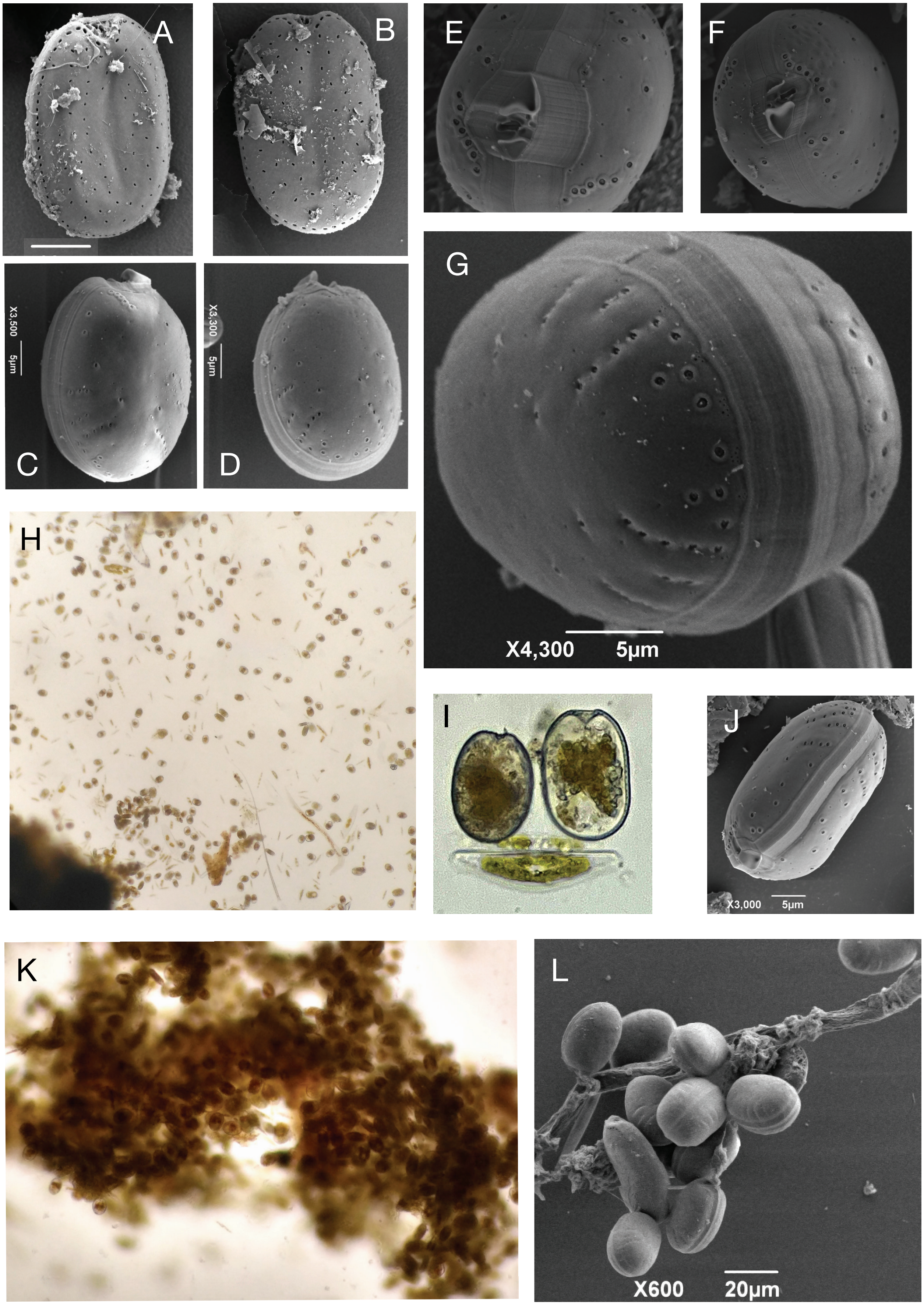

Figure 4: A, B) Ventral plates of *P. lima* complex; C, D) Ventral plates of *P. rhathymum*; E, F) anterior polar view of *P. rhathymum*; G) Posterior polar view of *P. rhathymum* evidencing the sagittal suture connecting the plates, and the pores irradiating from the center. H) Light micrography under 200X; I) Light micrography of randomly arranged specimens *P. rhathymum, P. lima* and a penate diatom type with visible chloroplasts and starch ring. J) Lateral view of P. rhathymum. K,L) Aggregations of *Prorocentrum* under K) light microscopy and under L) SEM showing the attachment to a debris.

At the time we collected the sample, the water surface temperature was 25.3ºC, the water surface salinity was 15.6, the pH was 6.58 and the depth was 11.6 m. Total phosphorus and total nitrogen was 1.30 and 19.6 μM/L, respectively and phosphate 0.3 μM/L.

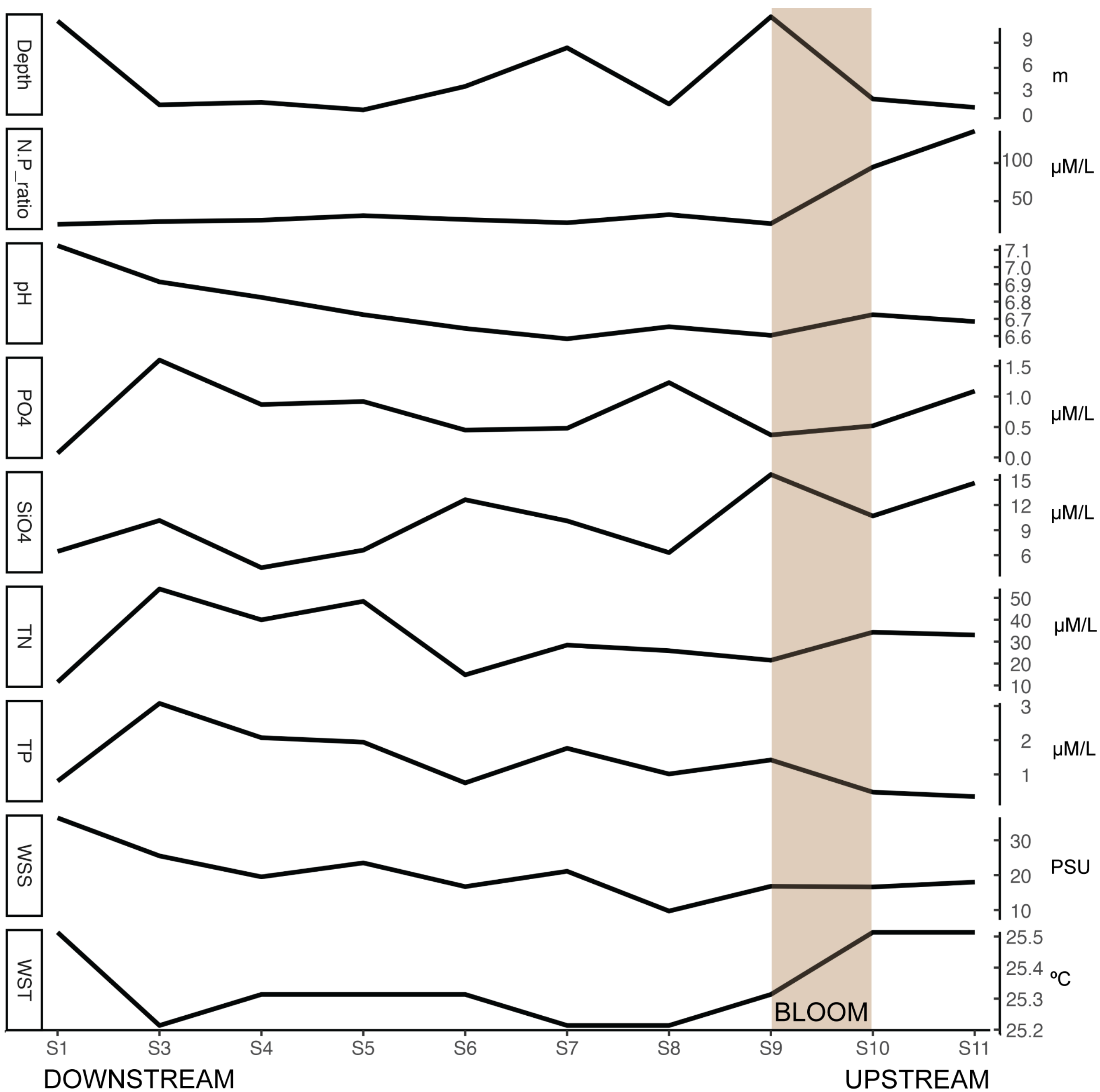

Figure 5: Environmental and nutritional variation alongside the river with a highlight where the bloom was sampled where the Nitrogen starts to increase.

DISCUSSION

The foam plume was collected at the upstream section of Serinhaem river, near the main town of the estuary, an urban center developed on the margins of the river. The demographic census conducted by the Brazilian Institute of Geography and Statistic (IBGE, 2016) reported that only 36% of the sanitary sewage in this area (Ituberá, population 21.913) is adequately treated before dumping it to the environment (IBGE, 2016). The same census reported 4.6 medical admissions per thousand inhabitants due to diarrhea infections, which is one of the main symptoms of diarrhetic shellfish poisoning (DSP) (Berdalet et al., 2016).

It has been discussed the stimulation and the frequency of HABs as a response to eutrophication linked to human-induced pressures associated to increasing population and animal or plant runoff from agriculture (Hallegraeff, 1993; Burkholder et al., 2006; Glibert et al., 2008). Among others, phosphorus and nitrogen are recognized as key factors regulating the growth of harmful algal species (Hodgkiss & Lu, 2004). Phosphorus and nitrogen limitation in aquatic ecosystems is generally assessed from the principal concept of a 16:1 threshold for N:P stoichiometry (Redfield, 1958). Variations in N:P ratios are associated to the synthesis or decomposition of organic matter and are documented as key inorganic elemental sources on phytoplankton biogeochemistry and productivity (Redfield, 1958; Falkowski, 2000). N-fixation and denitrification are hypothesized to regulate N:P ratio modulated by phytoplankton composition in ocean and coastal environments (Redfield, 1958; Falkowski, 1997; Falkowski, 2000). In estuarine systems phosphorus is assumed to be limiting for algal growth in the riverine freshwater section, as well as nitrogen in the marine section associated to coastal removal of nitrogen or denitrification (Redfield, 1958; Howarth and Marino, 2006). Therefore, we argue that the sites where the N:P ratio reached massive values higher than 100:1, such as the site where this bloom was found, indicate phosphorus limitation and nitrogen enrichment possibly associated to the anthropogenic pressure of the town located in the margins of the upstream section of Serinhaém river. We also noticed resorts and disordered or unplanned occupation with domestic precarious buildings advancing towards the mangrove and in most cases dumping the sewage directly into the river, which is also a source of inorganic elements. The characteristics measured in the site S9 during the bloom event match the conditions suggested as optimum for the formation and development of blooms in coastal waters, i.e. nutrient availability, low turbulence and light availability (Margalef, 1978).

The high abundance of *Prorocentrum* species in tropical waters is well documented, specially in tropical waters where temperature is known to benefit benthic dinoflagellates (Durán-Riveroll et al., 2019). To the best of our knowledge there is only one reported and photographed bloom of *P. rhathymum*, documented in the Bangaram lagoon, Lakshadweep Islands of India and the coloration of the bloom was reddish-brown with the same greasy aspect and mucilaginous aggregations on macroalgal debris (Thomas and Padmakumar, 2020). Coastal regions throughout the world have experienced an escalation in the incidence of toxic or harmful algal blooms over the last several decades (Anderson, 2002). The intensity and frequency of blooms are recognized as a serious ecological issue (Hallegraeff, 1993; Anderson, 1997, McGowan et al., 2017) due to the potential alteration of marine habitats and trophic structures

(Anderson, 2002). The effects of some taxa developing high biomass are related to the formation of foams or scums, the depletion of oxygen as blooms decay (Anderson, 2002). Understanding the development of these potentially toxigenic species in urban coastal areas involving consumption of seafood and fish reflect the relevance of the observation described herein, as well as the necessity of a biomonitoring program in the area.

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Chapter 2

High concentrations of the toxic epiphytic dinoflagellate *Prorocentrum lima* complex (Dinophyceae) in surface sediments from northeastern Brazil

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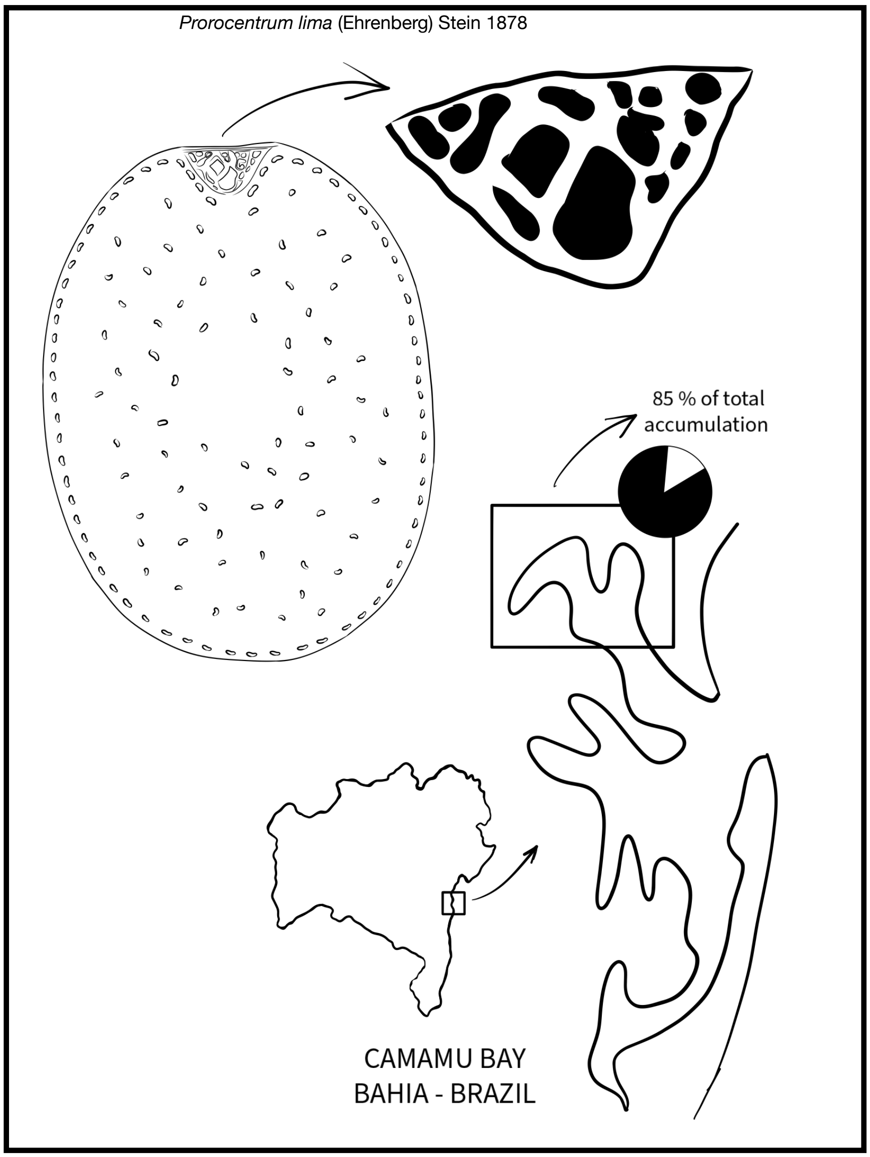

High concentrations of the toxic epiphytic dinoflagellate *Prorocentrum lima* complex (Dinophyceae) in surface sediments from northeastern Brazil

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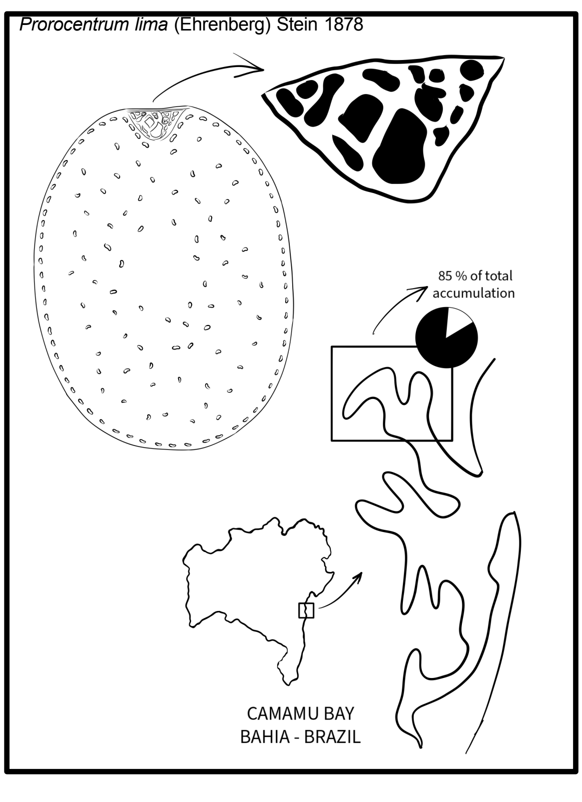
Abstract: Abstract: *Prorocentrum lima* is a phototrophic dinoflagellate that includes several cryptic species documented to dwell epiphytically attached to epibiont macroalgae, and capable of dwelling as a motile cell in the microplankton. The toxicity and abundance of *Prorocentrum lima* in tropical coastal waters have motivated surveys and monitoring in areas of seafood consumption because of the risk to human health and ecosystem balance. In this study, we collected twenty box cores to recover surface sediment samples in two main rivers of Camamu Bay, to document the occurrence of *Prorocentrum lima* complex along estuarine gradients and examine the morphology of the resistant organic walled cells. The surface sediment samples were processed following standard palynological techniques. The cells were generally well-preserved and showed a large v-shape periflagellar platelet, pyrenoid and starch ring, kidney-shaped thecal pores, and smooth wall surface. The highest concentrations of *Prorocentrum lima* in the sediment were observed upstream of the Serinhaém River, where up to four thousand cells per gram of dried sediment were recovered. The specimens from Camamu Bay were characterized by a mean size of 39.6 µm in length and 28.2 µm in width and a morphology oblong-ovate, laterally flat with kidney-shape pores, comparable to the strains, which were described from Paranaguá Bay as a distinct type and possibly a new species. The sites with the highest concentrations are located near Ituberá town, one of the main towns of Camamu Bay, receiving tributaries and pollution, in addition to being an area of consumption of fish and molluscs. Therefore, we recommend prospections on the toxin content of the organisms from Camamu and the feeding habits of organisms from superior trophic levels.

Key-words: Benthic harmful algae bloom, prorocentrales, dinocyst assemblage, estuary.

Graphic abstract:



1. INTRODUCTION

It is estimated that up to 90 dinoflagellates species produce toxins (Moestrup and Calado, 2018) and that among them 34 are benthic. Although harmful algal blooms (HABs) are mostly attributed to planktonic dinoflagellate species, benthic dinoflagellates may form Benthic Harmful Algae Blooms (BHABs), when high aggregations of toxigenic species accumulate and release toxins that may be transferred via the food web, causing fauna and eventually human intoxication, mainly due to ciguatera fish poisoning (CFP) and diarrhetic shellfish poisoning (DSP) (Berdalet et al., 2016). As a result, the distribution and composition of epibenthic species are also a source of concern for the ecosystem balance, human health, local economy of coastal communities, and seafood industries (Wells et al., 2015; Berdalet et al., 2016). In Latin America, where marine toxic species are abundant, there is a known relationship between human intoxication syndromes and toxin bioaccumulation through the food web, notably involving fishes (Sierra-Beltrán., 1998; García-Mendoza et al., 2016), octopuses and filter-feeding molluscs (Barón-Campis et al., 2014).

The order Prorocentrales forms a monophyletic group of benthic and planktonic species. It is morphologically distinct from other dinoflagellates because of the lack of cingular and sulcal plates, and of an apical instead of a ventral insertion of flagellae, which generates a desmokont flagellation that alters swimming displacement (Fensome et al., 1993; Hoppenrath et al., 2013). The morphotypes of *Prorocentrum lima* (Ehrenberg) Stein 1878 emend. Nagahama et al., 2011 are recommended to be referred to as belonging to the *P. lima* complex given the high morphological variability and intraspecific plasticity in terms of structure and ornamentation of this potential high diversity of cryptic species (Aligizaki, 2009; Durán-Riveroll et al., 2019). We will omit the word “complex” sometimes to achieve a better reading, however we follow the suggestion of Hoppenrath et al. (2013) until a molecular characterization, given the uncertainties regarding cryptic species plasticity. The distribution of the *P. lima* complex is cosmopolitan with documented descriptions and reports worldwide (Dodge, 1975; Faust, 1991). It has been reported as the most abundant toxigenic taxon and lives attached to seagrass or macroalgae from Caribbean waters, northern Venezuela (Navarro-Vargas et al., 2014), the Yucatan Mexican waters (Okolodkov et al., 2014) and the coast of United States (Mitchell, 1985).

The *P. lima* complex comprises okadaic acids and analogues, such as dinophysistoxins, borbotoxins and prorocentrolides (Faust, 1991; Hoppenrath et al., 2013). Because of the high consumption of seafood and fish in the Camamu Bay estuary, it is highly relevant to survey the distribution and occurrence of toxigenic species in relation to environmental changes. Several studies documented the morphological, taxonomical and physiological properties of *P. lima* strains from the central and South Atlantic Ocean based on culture isolation and collection from macrophytes (Hoppenrath et al., 2013; Nascimento et al., 2016; Moreira-Gonzáles et al., 2019, Durán-Riveroll et al., 2019). Direct observations in the environment are rare and, to our knowledge, our study is the first to provide information on morphological variations and local distribution from surface sediments, where blooms potentially develop. Therefore, our primary objective is to describe the morphology and distribution of *P. lima* at different stations of the Camamu Bay estuary.

2. METHODS

2.1 *STUDY AREA*

Camamu Bay (Fig. 1) is an estuarine system located in the State of Bahia, Brazil (13º40.2'S; 38º 55.8'W and 14º 12.6'S; 39º 9.6'W). It is characterized as a rich species diversity and represents an important center of economic activities based on tourism and fishing (Amorim, 2005). Three main rivers named Serinhaém, Maraú and Orojó flow toward the Atlantic Ocean in the center of the Bay. The surrounding vegetation is 40% composed of mangroves, in addition to the remaining fragments of Atlantic Forest protected as a conservation unit by the state and federal governments (Bahia, 2002). The main urban areas include Ituberá (population: 21.913), located upstream of the Serinhaém River and the city of Maraú (population: 24.580) located in the middle section of the Maraú River.

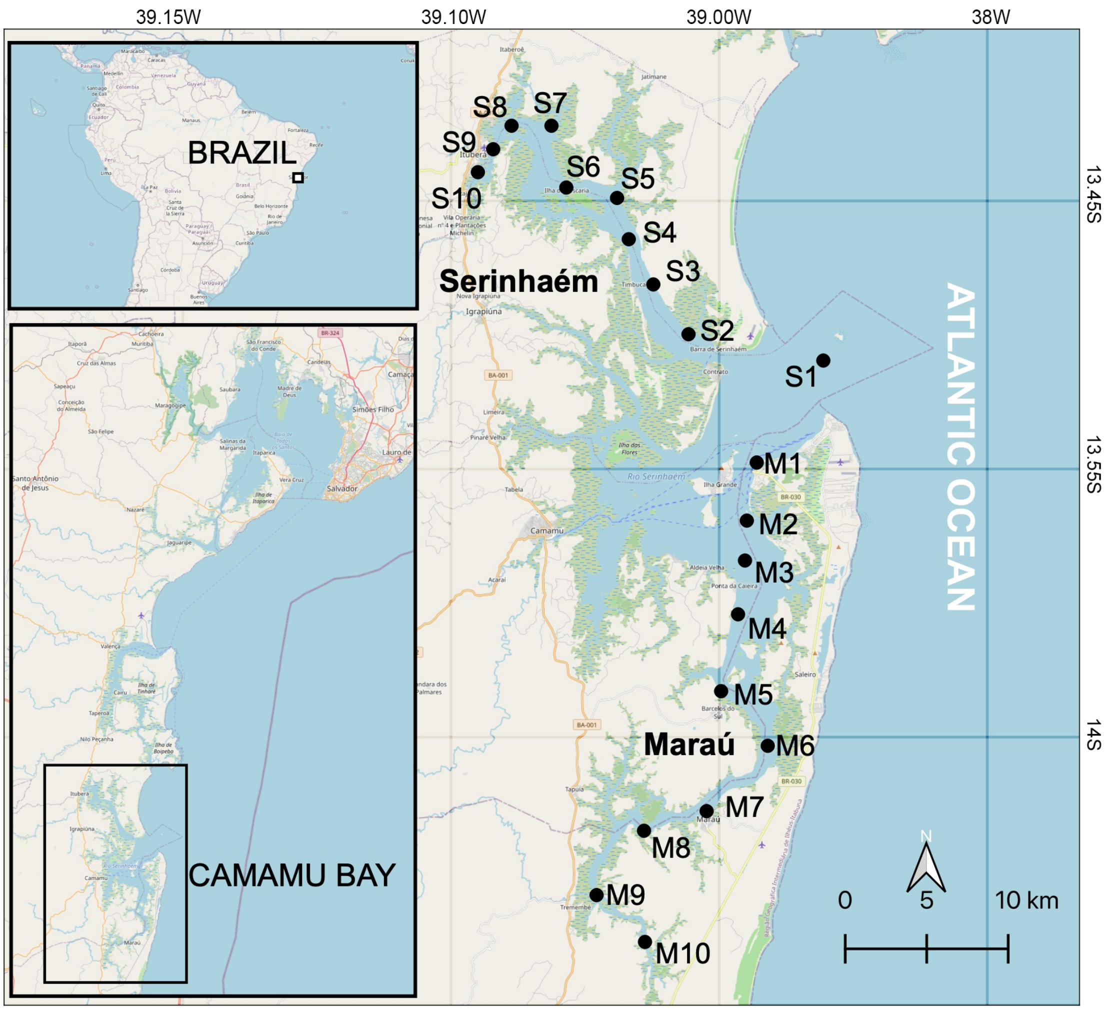


Figure 1: Geographic location of the sampled sites in the Camamu Bay.

2.2 *ENVIRONMENTAL CONTEXT*

The water flow results mainly from the ebb tide, with a maximum seaward velocity of 84 cm/s (Amorim, 2005). The tidal regime is semidiurnal with an amplitude ranging from 0 to 2.2 m (Lenz, 2008). Easterly and southeasterly winds predominate in the dry and rainy season, with average velocities of 2.7 m/s and 2.3 m/s, respectively, and the annual mean temperature ranges from 21 to 25ºC (Amorim, 2005) the entire year. Camamu Bay receives 2.570 mm of rainfall annually (ANA, 2005), typically over two periods. The main rainy season occurs from March to July, with a maximum monthly rainfall of 260 mm (July). From August to February, the maximum rainfall reaches up to 230 mm in November, and averages eight days of precipitation per month (ANA, 2005).

The granulometric fractions of the sediment resulting from erosion and transported by the river flow are mostly composed of medium-size sand and mud in the Serinhaém River and mostly composed of gravel, medium-fine sand grains in the Maraú River (unpublished data by Dr. Simone Moraes, personal communication).

2.3 *SAMPLING COLLECTION*

In September 2021, we made one 34 km-long transect and sampled ten stations distributed along the River Maraú to the south and a second 30 km-long transect to recover samples from ten stations alongside Serinhaém River to the north (Fig. 1; Table 1). The surface sediment was collected by box coring, stored in plastic bottles and preserved with formaldehyde (4%). Some *in situ* environmental variables were measured, such as surface water temperature (SWT), water depth using an infra-red depth gauge, turbidity with a Secchi disk, pH and salinity with a Horiba multiparameter probe (see Appendix 1). The samples collected for nutrient concentration analysis were immediately cooled in a dark thermic box and frozen until measurements in the laboratory. The concentration of nutrients, including silicate, soluble reactive phosphorus, total phosphorus, total nitrogen and dissolved inorganic nitrogen (DIN; the sum of ammonium ion, nitrate and nitrite) were calculated comparing the light absorbance of the samples after reaction with a set of four controlled concentrations under spectrophotometry based on a reference set of 0, 1, 5 and 10 μM for nitrogen and phosphorus series (NO_2, NO_3, NH_4 and PO_4) and 0,10, 20 and 40 μM for silicate at 540 and 810 nm (Grasshoff, 1966; Wood et al.,1967; Koroleff, 1976). The organic matter and organic carbon were estimated based on loss ignition as recommended for non-calcareous samples (Allen et al., 1974). We calculated the weight of bulk density of fresh samples (5cc), the dry weight after twelve hours in an air-circulated stove at 115ºC and the loss on ignition after 6h at 550ºC following the methodology and calculation described in Berglund (1987).

Table 1: Geographic coordinates and depth of the sampled sites

Site	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
longitude west	-38.986889	-38.992528	-38.993583	-38.997528	-39.007139	-38.980621	-39.015389	-39.051000	-39.077972	-39.050444
latitude south	-13.906306	-13.938389	-13.960417	-13.990111	-14.032528	-14.062667	-14.098722	-14.109528	-14.145028	-14.170861
Water depth (m)	11.60	1.00	6.90	7.50	3.40	1.60	5.10	5.60	0.90	0.60
Site	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**
longitude west	-38.949027	-39.025694	-39.045694	-39.059667	-39.066333	-39.095194	-39.103639	-39.126333	-39.136778	-39.145477
latitude south	-13.850016	-13.835472	-13.807972	-13.783	-13.760194	-13.754444	-13.720306	-13.720306	-13.733278	-13.745976
Water depth (m)	11.10	1.10	1.40	0.50	3.30	7.90	1.20	11.60	1.80	0.80

2.4*LABORATORY PREPARATION*

To concentrate the organic remains from the sediment, we used palynological techniques following the standard procedure described by de Vernal et al. (1999). A volume of 5 cm^3 of wet sediment was dried and a tablet of *Lycopodium clavatum* (batch: 2013001; n = 27.560 spores/tablet) was added to each sample for concentration calculation. The sediment was wet sieved through two different size mesh sieves (106 µm and 10 µm) after deflocculation by adding soap and distilled water until clay and small silt particles were drained out. The chemical treatment included repeated digestion with cold HCl (10%) and cold HF (48%) to dissolve carbonate and silicate particles, respectively. Finally, a solution of KOH (5-10%) was added to dissolve the labile organic compounds, followed by homogenizing with vortex and cleaning with ultrasound for 20 seconds. A drop of the residue was mounted between the slide and cover slide in gelatine and the remaining residue was preserved in water with phenol (10%).

2.5 *IDENTIFICATION, IMAGERY AND COUNTING*

The slides were scanned in optical microscopy. *Lycopodium clavatum* spores from the calibrated tablets were used as marker grains to estimate the concentration of *Prorocentrum lima* in the sediment samples. The spores added were stained in red to be distinguished from the spores originating from the surrounding vegetation. We counted the entire slide, with the same number of horizontal transects (20) at 400x magnification. The morphometric observations and measurements were made under transmitted light microscopy (Leica DM2500M) and the photographs were taken with a camera (Lumenera Infinity X). The samples were also examined in scanning electron microscopy (SEM) on a Hitachi S-3400N. A drop of the palynological residue was dried on a heating plate at 50ºC for twenty minutes. The sample was then metallized without using a CO_2 critical point.

The photographic plates of pictures taken in optical and electronic microscopy were made by scaling evenly all the specimens, so the differences in size were kept proportional. The identification criteria included shape, size, length-to-width ratio, the position and number of periflagellar platelets under SEM, and the disposition and number of pores (Hoppenrath et al., 2013). The concentration, expressed in number of cells per gram of dry sediment, was calculated as follows:

$$Np = Ne \times np \div ne$$

Where "Np" is the concentration of *P. lima* in the sample; "Ne" is the number of marker grains added to the initial sample prior to preparation; "np" is the number of individuals counted, "ne" is the number of counted marker grains. The concentration of cells per unit weight (g) was calculated by dividing "Np" by the initial weight of dried sediment.

2.6 *DATA ANALYSIS*

We conducted a Principal Component Analysis (PCA) as ordination method to extract most of the information from the dataset containing the environmental and nutrient variables and the concentration of *P. lima* per gram of dried sediment (Table 1, Appendix 1). The objective was to reduce the overall information into orthogonal dimensions explaining the maximum variance (Legendre and Legendre, 1998). This method demands transformed standardized variables to ensure the same range for all variables (Abdi and Williams, 2010). The quality of representation of a variable in each component was analyzed based on the square cosine of the eigenvalues (Abdi and Williams, 2010). The Pearson coefficient of correlation was calculated between the abundance of *Prorocentrum lima* and the environmental variables measured at the same sites. The analyses were conducted in the software R (version 4.2.1).

3. RESULTS

3.1 MORPHOLOGY AND MORPHOMETRICS

Under light microscopy, the cells of *Prorocentrum lima* were generally well-preserved, most of them still hosting internal organelles, such as pyrenoid (Plate 1, Fig.1), starch sheath ring (Figs.1-6; Figs.10-14), nucleus (Fig.1-5), mucocysts (e.g. Figs. 5,6), and some being empty body sheaths with a grey coloration (Fig.7), sometimes thecae still attached to the cytoplasm (Fig.12) during the process of ecdysis. The cytoplasm without the plates was also observed in a well-preserved state (Fig. 13) after palynological treatment. Under SEM the cells were generally oblong-ovate or elliptical, flattened laterally, composed by a ventral plate connected to a dorsal plate by a suture (intercalary band), an apical wide V-shaped periflagellar area with eight platelets around a main flagellar pore and a smaller accessory pore excavated on the right plate. The ornamentation was generally smooth with scattered pores always oblong or kidney-shaped around the plate except in the center. The marginal pores were also oblong with a kidney shape. Morphological variations included the posterior plate pointed instead of round (Fig.11), a round cell with uncommon periflagellar platelet (Fig. 8); a posteriorly sharpened cell with prominent edges around the theca (Fig.5). Some morphotypes were observed in an urn-shape form (Fig.9).

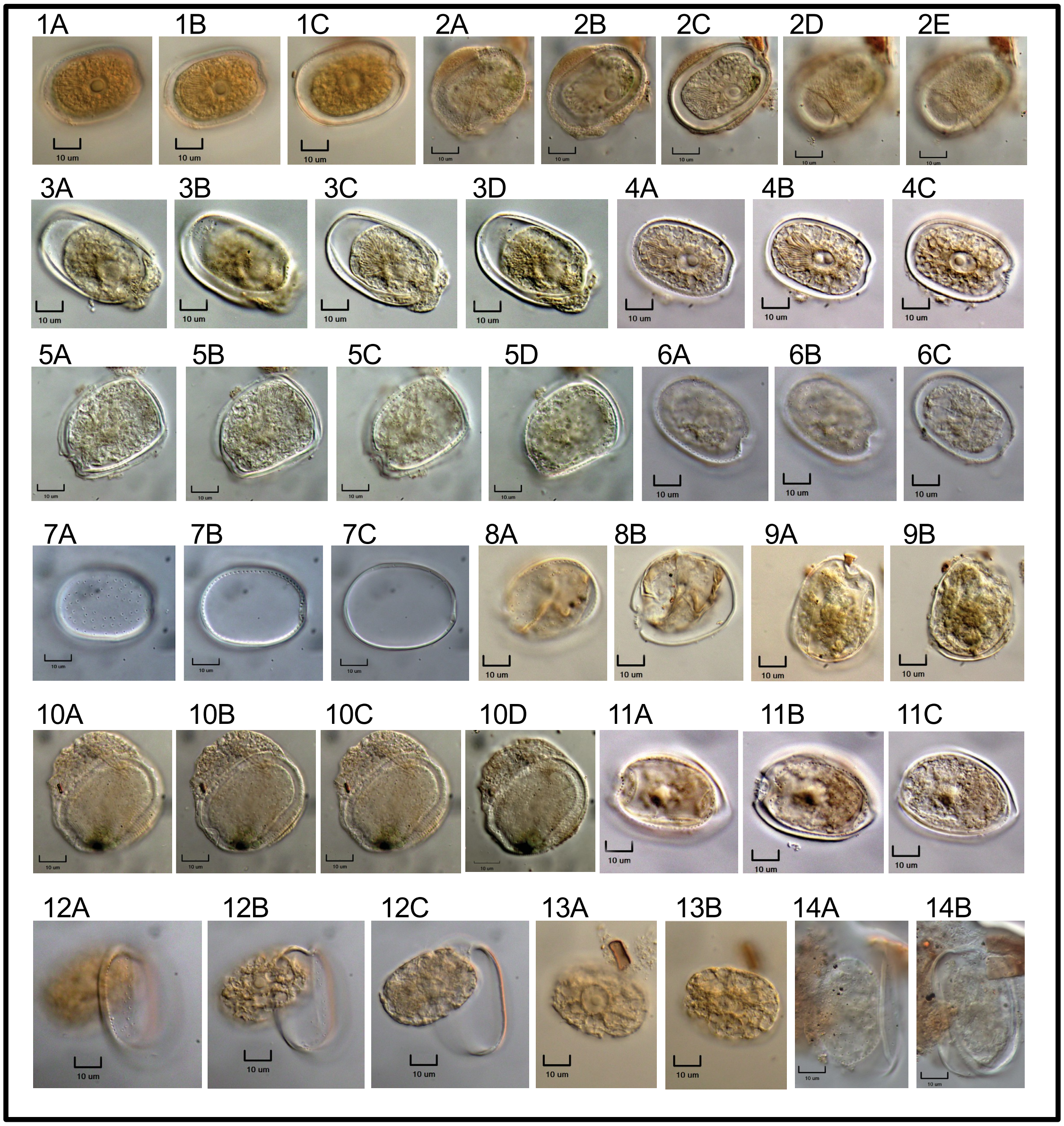


Plate 1: Selected morphotypes of *Prorocentrum lima* complex under light microscope. 1: Most common shape laterally flattened with the visible starch ring, chloroplasts, pyrenoid, nucleus and mucocyst; 2: Cell with visible internal features and uncommon enclosure; 3: Oblong-elongated theca with partial ecdysis through apical intercalary band disruption, visible pyrenoid ring and visible nucleus; 4: Most common shape laterally flattened with visible starch ring, pyrenoid, nucleus and mucocyst; 5: Urn-shaped cell flattened in the inferior antapical plate; 6: Smaller cell during ecdysis still attached to the thecae and visible rupture through the suture of the intercalary band; 7: Empty body sheath with visible pores along the thecal plate; 8: Uncommon cell shape also urn-involved with a geometric discontinuity in the surface of the ventral plate; 9: Urn-involved cell division with displaced pyrenoid and surface crack. 10: Cell with leaking cytoplasm still attached to the interior of the cell; 11: Deformed plate with anterior pointed shape instead of round and remarkable pyrenoid; 12: Vegetative cell during ecdysis still attached to the theca; 13: Preserved cytoplasm without theca after palynological treatment; 14: thick plate with vegetative cell.

Individual measurements (n = 68) of the common type, which is the oblong-ovate laterally flattened with kidney-shape pores) ranged from 36 to 42 μm in length, 17 to 33 μm in width, 3 to 7 μm in thickness (mean = 5.87±1.96 μm) and 1.25 to 2.35 μm for the length-to-width ratio (mean = 1.41± 0.15 μm). Variations in cell size were also observed under SEM (e.g. Plate 2, figs. A-E). The surface of some specimens observed under SEM presented a ventral and/or dorsal convex shape (Plate 2, figs. A-J), whereas the surface of other specimens was smooth and flat (figs. K-Q). Some specimens also presented superficial wrinkles and changes in structural shape, likewise antapical flatness (fig. L), displacement of the intercalary band (figs. N, P and Q) and undetermined variations (figs. R-T).

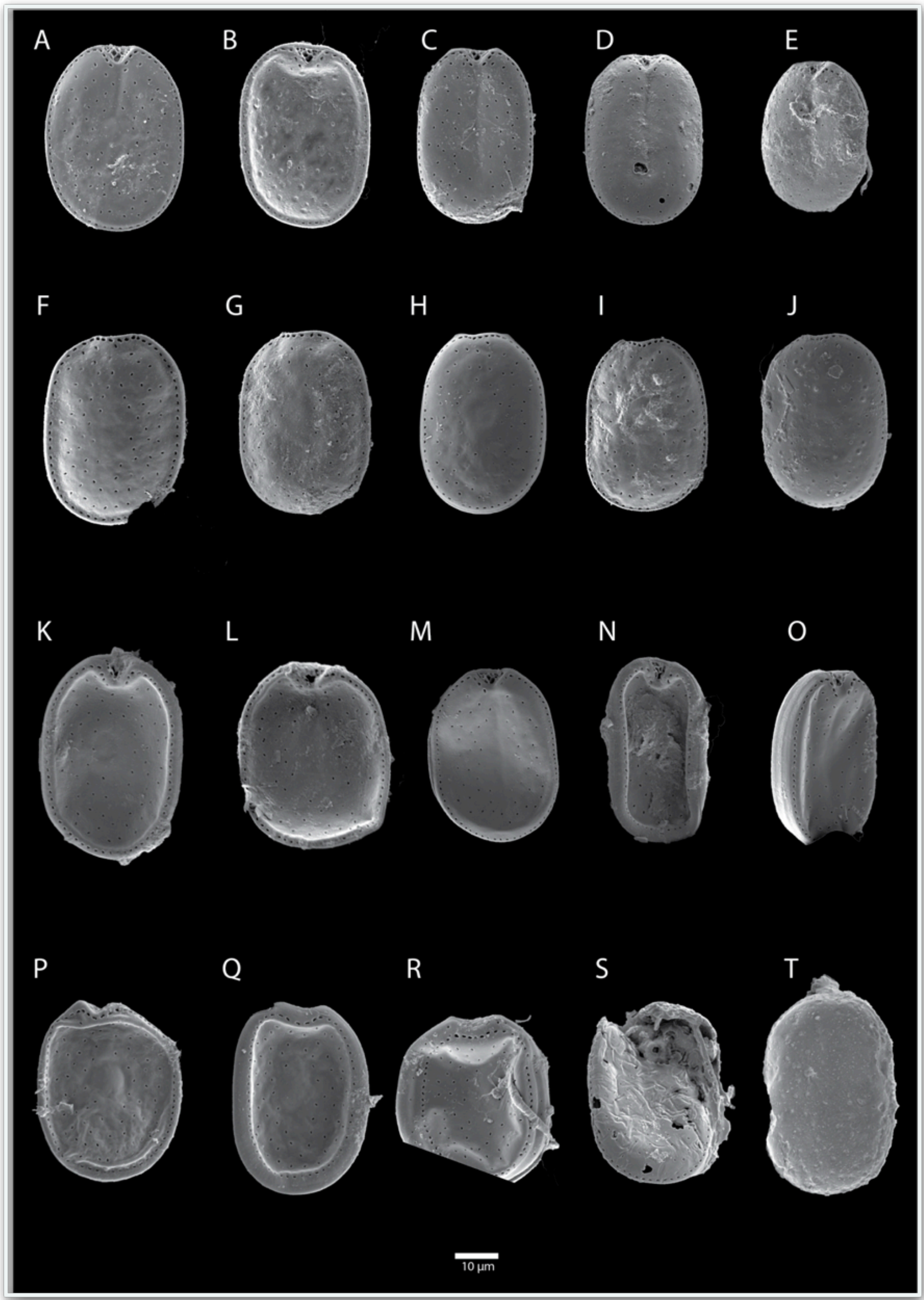


Plate 2: Scanning electron microscopy of selected cells evidencing shape, surface variations and structural diversity.

The cells of *Prorocentrum lima*, which average 39 µm ± 2 in length, 28 µm ± 2.8 in width and 1.4 µm ± 0.1 length-to-width ratio, are smaller than the strains described in peer-reviewed publication for the northern part of the South Atlantic Ocean and larger than those described in the southern part (Table 2).

Table 2: Comparison of the morphometrics of P. lima among described strains in the South Atlantic and the specimens from Camamu

Strain	Length µm	Width µm	L:W ratio µm	Shape	Pore type	Location	n	Reference
Our study	39.6 (±2.05)	28.2 (±2.87)	1.41 (±0.15)	Oblong-ovate (laterally flattened, wide)	Oblong, kidney-shape	Camamu Bay	68	This study
LM001	38.4 (±1.18)	26.2 (±0.86)	1.46 (±0.03)	Oblong-ovate (laterally flatten)	Oblong, kidney-shape	Paranaguá, Brazil	80	Moreira-Gonzáles et al., 2018
LM002	40.1 (±1.01)	27.5 (±0.71)	1.45 (±0.03)	Ovoid (laterally round, anteriorly narrow)	Round, ovoid, oblong and kidney-shape	Recife, Brazil	80	Moreira-Gonzáles et al., 2018
LM003	41.8 (±1.35)	26.6 (±0.71)	1.58 (±0.07)	Ovoid (laterally round, anteriorly narrow)	Oblong, kidney-shape	Cuba	80	Moreira-Gonzáles et al., 2018
UNR-01	38.4 (±2.4)	27.4 (±2.1)	1.40 (n/a)	Oblong-ovate (anteriorly narrow)	Circular to oblong	Rio de Janeiro, Brazil	30	Nascimento et al., 2016
UNR-09	38.4 (±1.4)	27.6 (±1.2)	1.30 (n/a)	Oblong-ovate (anteriorly narrow)	Circular to oblong	Rio de Janeiro, Brazil	30	Nascimento et al., 2016

3.2 ENVIRONMENTAL DATA

The water surface salinity (WSS) ranged from 3 to 29 psu in the Maraú River and from 8.5 to 35 psu in the Serinhaém River. The minimum salinity of 3 psu was observed upstream of the Maraú River (station M10) and the maximum was 35 psu in the turbulent downstream station of Serinhaém (S1), located in the open sea part of the estuary. The surface water temperature ranged from 25.2ºC to 26.7ºC, with the SWT at stations from Maraú being one degree warmer than those from Serinhaém. The pH ranged from 6.49 to 7.4, with values slightly more acid in Serinhaém (mean 6.7) than in Maraú (mean 7). The water depth ranged from 50 cm to 11.6 meters. The downstream stations M1 and S1 were the deepest (~11m both), and the upstream stations were the shallowest, reaching 60 cm at M10 and 80 cm at S10. The minimum depth of 50 cm was recorded in the middle of Serinhaém River, at station S4. The sediment fractions alongside Serinhaém were mostly composed of mud and medium-size sand, while it was dominated by gravel and medium-size sand in Maraú.

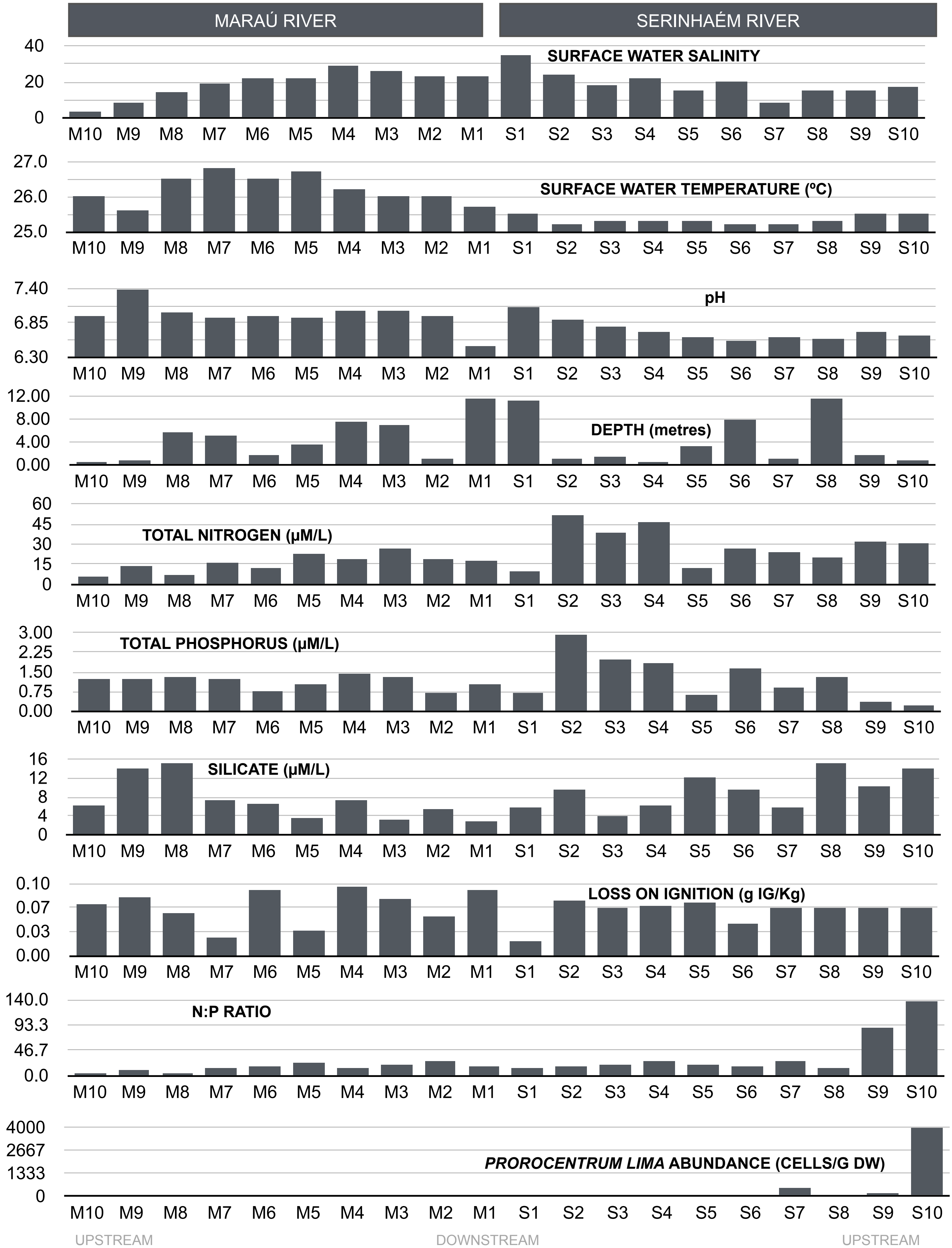


Figure 2: Environmental variation and nutrient stoichiometry alongside Camamu Bay. The measurements were taken on September 2021.

Total phosphorus ranged from 0.70 to 1.41 µM in the Maraú River, and from 0.2 to 2.95 µM decreasing upward throughout Serinhaém River. Total nitrogen varied in the two rivers, ranging from 6.2 to 26.7 µM alongside the Maraú River and from 9.6 to 52 µM alongside the Serinhaém River. Consequently, the stoichiometry between phosphorus and nitrogen, the N:P ratio, was different in the two rivers, varying from 5 to 27.6 µM in Maraú River and from 14 to 136.2 µM at the stations from the Serinhaém River. Dissolved inorganic nitrogen (DIN), which is the sum of nitrate, nitrite and ammonium ions ranged from 4.6 to 13.6 µM in the Maraú River and from 2.5 to 38.6 µM in Serinhaém River, with a maximum concentration at station S5 and a minimum at station S1. Silicate varied from 2 to 15 µM, increasing upwards. The loss on ignition, assumed to be correlated to organic carbon, ranged from 0.025 to 0.096 g IG/Kg (grams per ignited kilogram) along the Maraú River and from 0.021 to 0.075 g IG/Kg along Serinhaém River. Total nitrogen and orthophosphate were the only variables that correlate together (Figure 3).

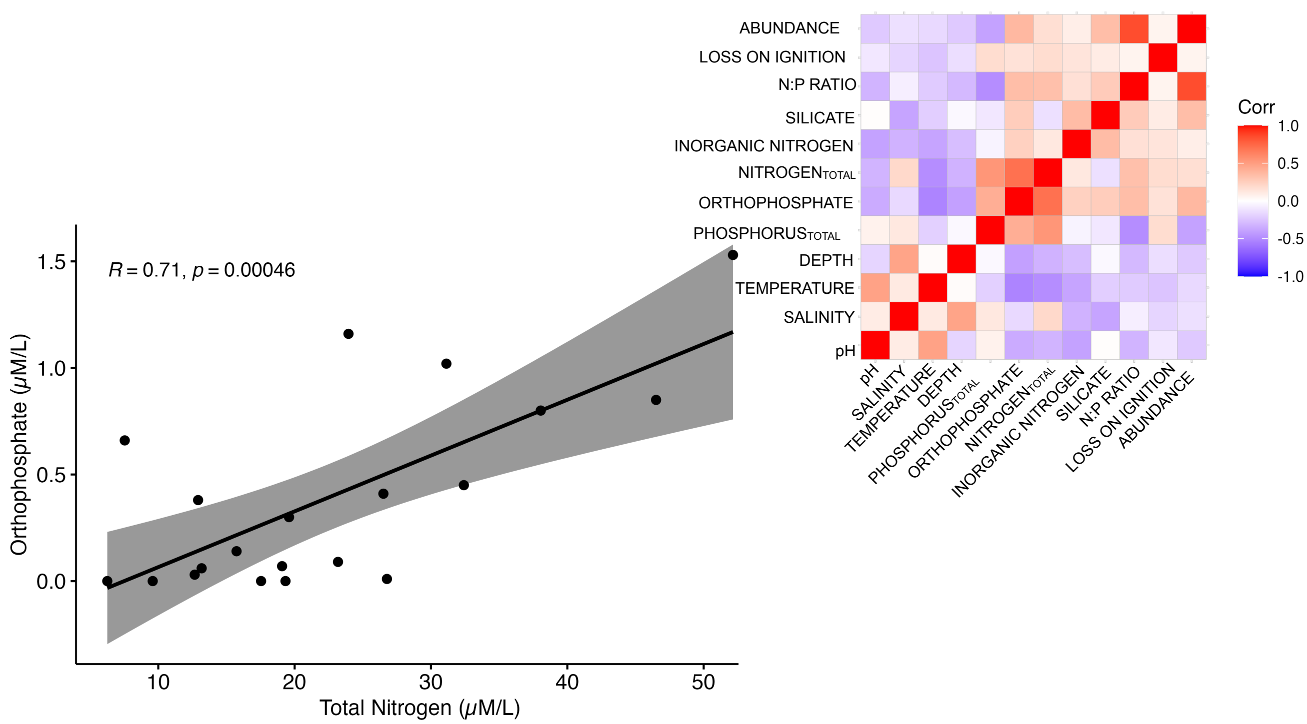


Figure 3: Scatter plot showing the positive correlation between total nitrogen on axis X and orthophosphate on axis Y. To the right, Pearson`s correlation matrix among the set of environmental variables, nutrient concentration and the distribution *P. lima* (as “abundance” on the plot). The coefficient values are indicated in the scale on the left side.

SPATIAL DISTRIBUTION

The concentration of cells belonging to the *P. lima* complex ranged from 0 to 4 per gram of dried sediment. The maximum was observed upstream of Serinhaém River at station S10, while rare specimens were observed in the Maraú River (Figure 4). Empty sheaths were observed at stations S8 (215 cell/g), S9 (68 cells/g) and S10 (596 cells/g). The cells showing morphological variations were mostly recovered at S10 (262 cells/g) with rare occurrences at other stations (M2, S4, S9 and S10).

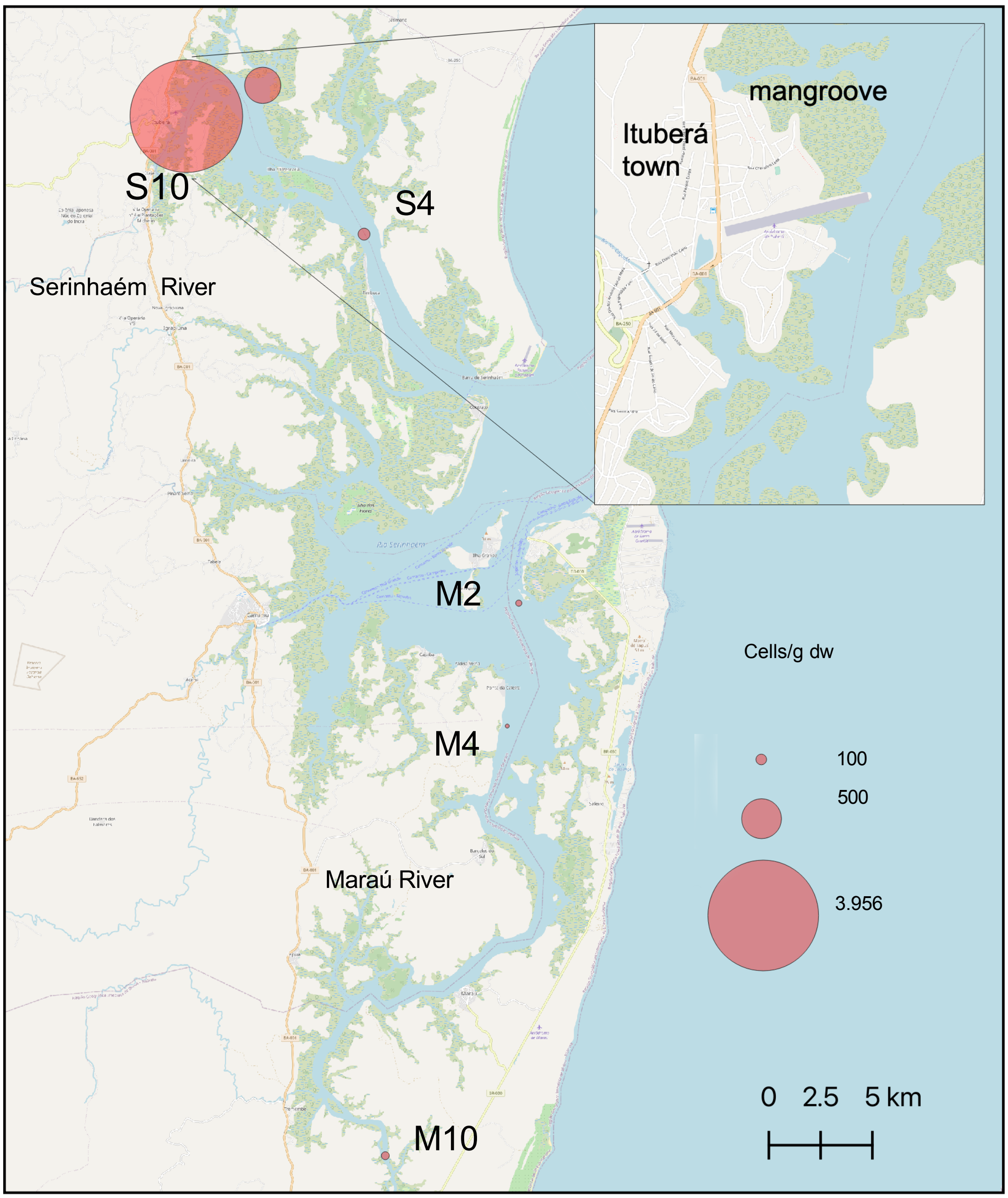


Fig. 4: Spatial distribution of *P. lima* complex alongside Camamu Bay estuarine section.

The first axis of the Principal Components Analysis (Fig. 5) explained about half (49.9%) of the variance in the data presented herein. Together, axes 1 and 2 explained 72% of the variance. Axis 1 shows an opposition between hydrological conditions (salinity, temperature, pH and depth), and nutrient concentration (nitrogen, phosphorus, orthophosphate, silicate and dissolved inorganic nitrogen), which recorded respectively positive and negative scores. Axis 2 shows an opposition between species abundance, N:P ratio, silicate, temperature, pH in the positive margin and salinity, nitrogen and phosphorus (total), loss on ignition and orthophosphate on the negative dimension. The abundance of *P. lima* is associated with high N:P ratios ($r^2 = 0.85$) and shows a negative relationship with salinity, temperature, pH and depth. The eigenvalue loadings were positive for all these environmental variables, whereas they were negative for all the nutrient concentrations, in addition to the abundance of *P. lima* complex. The quality of representation, based on the square cosine of the loadings, suggested only organic matter as a variable unlikely to be represented by the components and orthophosphate, N:P ratio and total nitrogen as the strongest contributors for the component (Fig. 5) expressed in the axis 1. The position of the elements in the ordination suggests that the high concentrations of *P. lima* are more likely to be associated to shallow, nutrient-rich stations where the salinity is mesohaline and the pH slightly acid. The ordination also demonstrates the low concentration of phosphorus in the upstream site, where the highest concentration of *P. lima* was recorded (S10), as it is orthogonally disposed on the opposite side of the total phosphorus gradient.

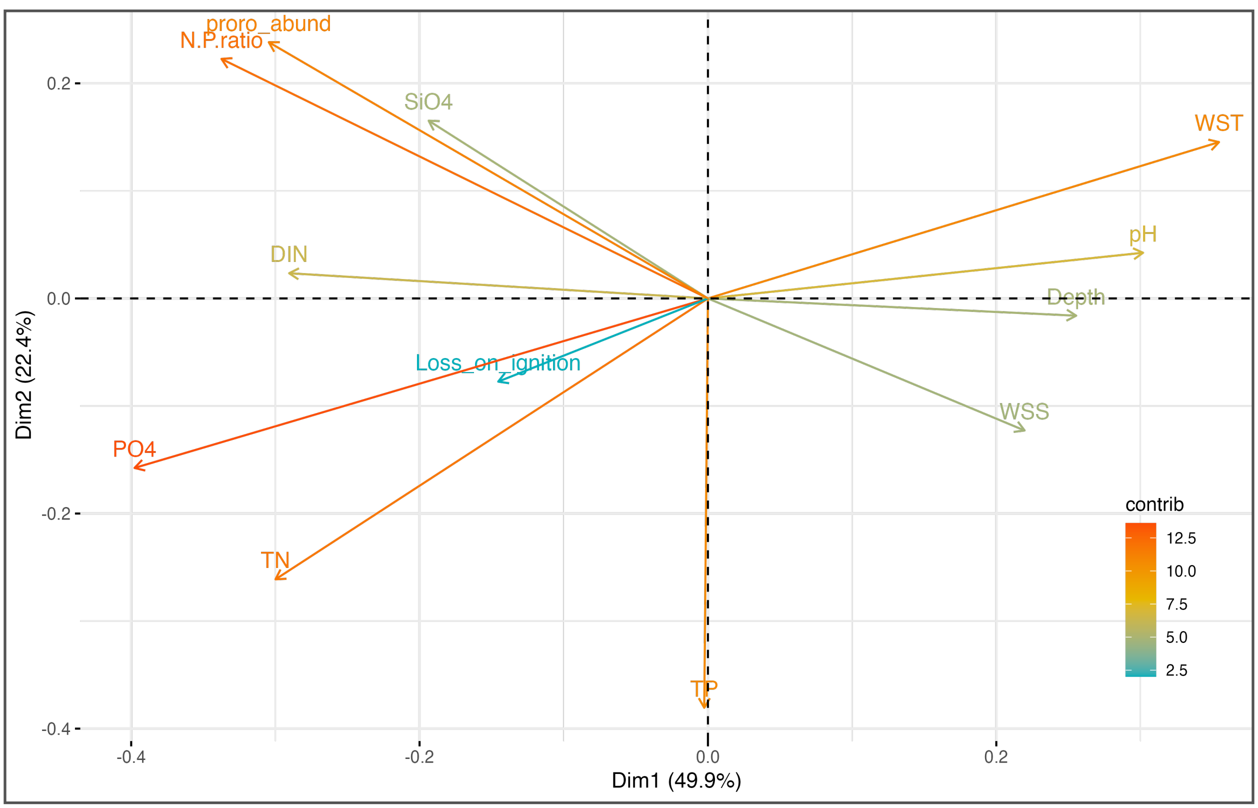


Figure 5: Ordination plot of the Principal Component Analysis showing the orthogonal arrangement explaining 72% of the data variation, half of it on the X axis. The colors represent the contribution of the variable in the components.

4. DISCUSSION

4..1 *MORPHOLOGY AND MORPHOMETRICS*

The specimens of the *Prorocentrum lima* complex found in Camamu Bay resisted the chemical treatment for palynological purposes, including HF and HCl, in addition to the mechanical treatments consisting in sieving and sonication. To our knowledge, this is the first report of vegetative or non-cyst forms of this species complex in palynological slides and surface sediment as substrate.

Our results indicate more rounded, smaller but larger, specimens of *Prorocentrum lima* in Camamu Bay compared to available described strains (Fig. 6) from Cuba (LM003; Moreira-Gonzáles et al., 2018) and Recife, Brazil (LM002; Moreira-Gonzáles et al., 2018). Our cells were slightly bigger and larger than the strains isolated from Rio de Janeiro (mean 38 μm and 27 μm; Nascimento et al., 2016) and from Paranaguá Bay, South of Brazil (LM001; Moreira-Gonzáles et al., 2018); The strain LM001 is the most similar to our specimens considering the combination of size and shape of the thecal pores. The strains from Recife and Rio de Janeiro are documented to have round, ovoid, oblong and kidney-shaped thecal pores (Nascimento et al., 2016), while the strains from Cuba and Paranguá are documented to have predominantly oblong, kidney-shaped pores (Moreira-Gonzáles et al., 2018), such as the specimens of this study. The smaller, kidney-shaped strain from Paranguá Bay is also suggested to be a new species lacking descriptions within the *P. lima* complex (Moreira-Gonzáles et al., 2018) since molecular studies have permitted differentiation from other morphologically similar species such as the new species *Prorocentrum caipirignum* (Nascimento, et al., 2017) formerly referred to as *P. lima*. The specimens from Camamu Bay were morphologically similar to the strain described genetically and physiologically in Paranguá Bay. This strain was reported to be less toxic to metanauplii zooplankton than the strains from Cuba and Recife, but showed a higher growth rate and higher production of mucilaginous material (Moreira-Gonzáles et al., 2018).

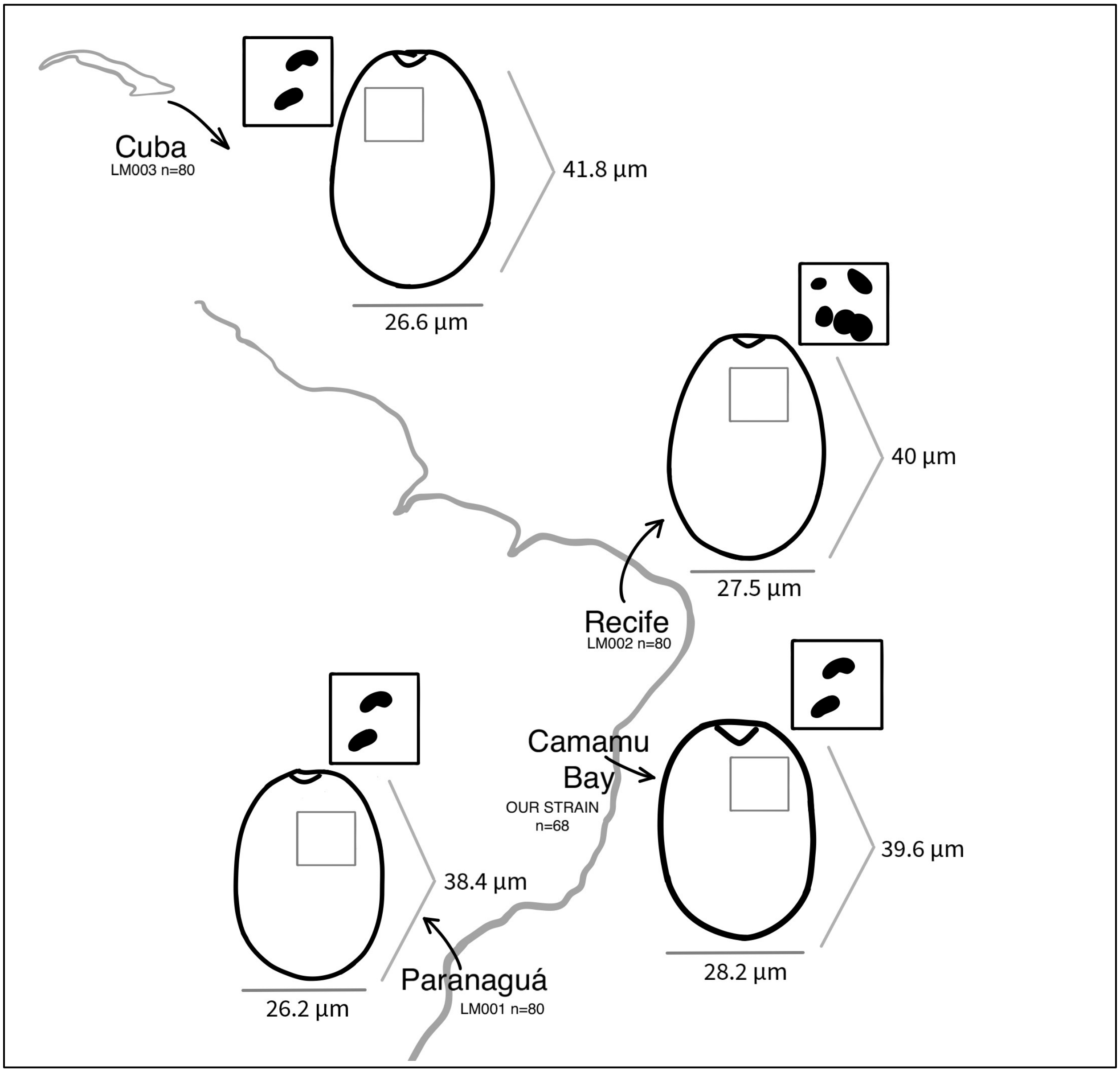


Figure 6: Conceptual illustration of the main differing characters in morphology among different geographic locations in the South Atlantic Ocean based on available peer- reviewed descriptions.

The SEM observations permitted to notice crumpled cells with no clear pattern (Plate 2, figs. K-R), while the others were superficially flat or convex. The first descriptions for this species reported a concave pattern in the middle of the anterior valve (Faust, 1991), but not flat or convex as we noticed. Initially, we hypothesized this would be related to the asexual reproduction during which a chain of thin-walled cysts forms, as described by Faust (1993). Hence, one cell may grow fitted on another and change its surface morphology. The other type of asexual reproduction by binary fission may explain the cells that are flat and smooth, since a daughter cell sheds the plates from its mother cell and produces two new valves (Faust, 1993) without contact and deformation. A triple-layer resting cyst via isogamous gametes was documented for this species by conjugation (Faust, 1993). However, there is no record of this form in modern surveys, suggesting that it is not preservable (Matsuoka and Fukuyo, 2003). After consulting several other dinoflagellate specialists we are convinced that the deformations observed in the surface of the thecae observed under SEM are likely associated to the stressful palynological treatment. However, there were indeed morphological variations that can not be associated to methodological issues, such as pore type, shape and size. Based on the evidences presented here we suggest prospect molecular and chemical characterizations to confirm the systematics erecting a new species within the complex with a potentially different toxin profile. Most knowledge about the *P. lima* complex is based on cultures and controlled essays from macroalgae, which means that little is known from the natural environments. Hence, this study describing morphological variations of *P. lima* from natural communities provides new information.

4.2 *ENVIRONMENTAL VARIABLES AND NUTRIENT CONCENTRATIONS*

Our results illustrated different water properties at the study station of the rivers that compose the estuarine system of Camamu Bay. The difference in salinity recorded between the rivers may be explained by the freshwater budget in the various parts of the estuary. The mean temperature was relatively uniform, but it was one degree higher in the Maraú River (~26 ºC) than in the Serinhaém River (~25 ºC). Although information regarding spatial distribution of *P. lima* and corresponding hydrographic conditions in neotropical environments, most culture studies have been conducted at 24-25ºC with strains collected in environments where SWT ranged from 20ºC to 30ºC (Nascimento et al., 2016; Moreira- Gonzáles et al., 2019). Temperature and light conditions were suggested to be the main factors driving the growth of *P. lima* in the southern Mediterranean basin, dividing 0.33 times per day and reaching a maximum cell density of 32.019 cells/ml after 60 days of culture under 25-29ºC (Ben-Gharbia et al., 2016); In the Japanese coastal area the cell density per gram of wet algae reached 29.4 cells/g algae in waters 15 m deep under salinity and temperature of 32-34 and 21-25ºC respectively (Nishimura et al., 2020); In mussel farms of Magdalen Islands, eastern Canada, the maximum density of 9.671 cells/g of dry epibiont algae in the summer, when salinity ranged 27-31PSU and temperature of 17-21.5ºC (Levasseur et al., 2003). In the Kattegat-Skagerrak west cost of Sweden, the highest abundance of 1,700 cell/g of fresh weight macroalgae was recorded at 16ºC and 29 PSU (Álvarez et al., 2022). Our results showing maximum density of almost four thousand cells/g of dried sediment in areas characterized by 25.5ºC and 20 PSU in surface waters provide some new insights into the ecological affinities of the species.

4.3 *SPATIAL DISTRIBUTION*

The concentration of *Prorocentrum lima* reached 3,956 cells/g dw sediment in the upstream sites of the Serinhaém River, close to an urban area dumping an average effluent discharge of 18 m³/s (Amorim, 2005). In the stations from Maraú River, the maximum abundance did not exceed a hundred cells per gram of dried sediment. The results from the ordination analysis demonstrated a correlation between the abundance of *P. lima* and N:P ratio. Orthophosphate, total nitrogen, temperature and total phosphorus also seem to exert a role in the distribution of the species in the estuary. In estuarine systems phosphorus can be limiting, especially in freshwaters, while nitrogen is more critical in marine waters where coastal removal of nitrogen and denitrification (Howarth and Marino, 2006) affect algal growth. The nitrogen limitation in marine sections of estuaries is documented to be caused by high microbial rates and low N-fixing organisms associated with silicate reduction (Howarth and Marino, 2006; Tamminen and Andersen, 2007). Silicate is known to be a required nutrient for diatom physiology, but not for dinoflagellates (Hildebrand et al., 1997; Xu et al., 2022). However, it was demonstrated that other dinoflagellates, such as *Akashiwo sanguinea* are favoured in the end of diatom blooms (Cloern et al., 2005; Jester et al., 2009).

According to the demographic census conducted by the Brazilian Institute of Geography and Statistic, only 36% of the sanitary sewage of the town bordering the upstream section of Serinhaém (Ituberá, population 21.913) is adequately treated before dumping it to the environment (IBGE, 2016). The same census reported 4,6 medical admissions per thousand inhabitants due to diarrhea infections (IBGE, 2016), which is one of the main symptoms of diarrhetic shellfish poisoning (DSP) (Berdalet et al., 2016). Hence, the high concentration of a toxigenic species near the town of Ituberá is a source of regional concern having consequences for management since the area is used to cultivate fish and other seafood, including benthic fauna that are ingested by humans. We thus suggest that special attention should be paid to BHABs in the region in order to prevent human health risks and economic losses as demonstrated in other studies (Berdalet et al., 2016; Durán- Riveroll et al., 2019).

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Appendix

Table 1:

Matrix of environmental variables, nutrient concentration and P. lima abundance alongside Camamu Bay

Site	pH	WSS	WST	Depth	TP	PO4	TN	DIN	SiO4	N:P ratio	OM	P. *lima*
M10	6.95	3.10	26.00	0.60	1.24	0.00	6.26	13.65	6.33	5.04	0.07	0
M9	7.40	8.30	25.60	0.90	1.20	0.06	13.18	11.70	14.19	10.96	0.08	66
M8	7.02	14.60	26.50	5.60	1.31	0.66	7.54	10.27	15.11	5.76	0.06	0
M7	6.94	18.80	26.80	5.10	1.23	0.14	15.74	12.08	7.36	12.78	0.02	3
M6	6.97	21.80	26.50	1.60	0.79	0.03	12.67	8.75	6.53	16.08	0.09	41
M5	6.94	22.40	26.70	3.40	1.00	0.09	23.18	4.66	3.40	23.18	0.03	10
M4	7.04	29.10	26.20	7.50	1.41	0.07	19.08	6.49	7.40	13.49	0.10	0
M3	7.04	25.60	26.00	6.90	1.30	0.01	26.77	11.22	3.31	20.61	0.08	25
M2	6.95	22.80	26.00	1.00	0.70	0.00	19.33	8.43	5.50	27.58	0.05	31
M1	6.49	22.60	25.70	11.60	1.05	0.00	17.54	7.83	2.67	16.73	0.09	0
S1	7.10	35.20	25.50	11.10	0.68	0.00	9.59	2.48	5.94	14.10	0.02	0
S2	6.89	24.30	25.20	1.10	2.95	1.53	52.15	12.25	9.65	17.69	0.08	0
S3	6.80	18.30	25.30	1.40	1.95	0.80	38.05	12.59	3.99	19.56	0.07	62
S4	6.70	22.30	25.30	0.50	1.82	0.85	46.51	18.42	6.09	25.56	0.07	0
S5	6.62	15.50	25.30	3.30	0.63	0.38	12.92	38.59	12.14	20.40	0.07	0
S6	6.56	19.90	25.20	7.90	1.64	0.41	26.51	19.15	9.60	16.20	0.05	0
S7	6.63	8.50	25.20	1.20	0.89	1.16	23.95	9.82	5.80	26.79	0.07	538
S8	6.58	15.60	25.30	11.60	1.30	0.30	19.59	9.35	15.16	15.08	0.07	132
S9	6.70	15.40	25.50	1.80	0.36	0.45	32.41	15.24	10.19	89.15	0.07	176
S10	6.66	16.80	25.50	0.80	0.23	1.02	31.13	15.77	14.14	136.20	0.07	3956

Chapter 3

DINOCYSTS AND OTHER NON-POLLEN PALYNOMORPHS AS INDICATORS OF LOCAL ENVIRONMENTAL CONDITIONS IN A NEOTROPICAL ESTUARY (CAMAMU BAY, BRAZIL)

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DINOCYSTS AND OTHER NON-POLLEN PALYNOMORPHS AS INDICATORS OF LOCAL ENVIRONMENTAL CONDITIONS IN A NEOTROPICAL ESTUARY (CAMAMU BAY, BRAZIL)

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Abstract: Non-pollen palynomorphs (NPP) are organic walled remains of several aquatic and terrestrial organisms often found in palynological slides but rarely reported. The remains belonging to the aquatic organisms respond to local conditions and thus may offer valuable information on past conditions and environmental changes. In this study, we analyzed the palynological content of surface sediment samples collected by box coring in the two main rivers of Camamu Bay, with the aim of documenting the distribution of NPP along estuarine environmental and nutritional gradients. After processing the samples following standard palynological techniques, we identified 69 NPP taxa, comprising 40 divisions represented by dinoflagellate cysts, foraminifer linings, green algae, cyanobacteria, ciliates, scolecodonts, amoebozoans, zoological fragments and fungal spores. Fungal spores dominated in almost every station. The upstream stations of both rivers were characterized as shallow, with lower salinity and higher nutrient ratios compared to the downstream stations. The minimum salinity reached 3 at Maraú where the assemblage was 40% composed of chlorophytes, whereas the dominance changed to foraminiferal linings in the downstream stations, where salinity peaked 35 and N:P ratio was less than 40 µM. Salinity, temperature, sediment type, silicate, phosphorus and N:P ratio were the most parsimonious set of variables to explain the variability in taxa distribution.The local responses of new and former taxa presented here may be used on further paleoecological reconstructions, as well as they represent information still very scarce in the region.

Key-words: surface sediment; benthic assemblages; micropaleontology; biogeochemistry

1. INTRODUCTION

Non-pollen palynomorphs (NPP) are organic-walled microfossil organisms belonging to a variety of taxa ranging in size from 5 to 250 micrometers and observed in palynological slides after chemical treatments involving acid digestion, sieving and sonication (Jansonius and McGregor 1996, Shumilovskikh et al., 2021). The history of paleoecological studies shows that pollen records became the focus of paleopalynology since the 20th century (cf. O`Keefe et al., 2021). Nonetheless, the early paleoecologists described dinoflagellate cysts and acritarch (Ehrenberg, 1837), foraminiferal linings, some types of chlorophytes and prasinophytes, scolecodonts or polychaeta jaws (Sarjeant, 2002, O`Keefe et al., 2021) and copepod eggs, Rhabdocoelan oocytes and tested amoebae (Rudolph, 1917) since the 19th Century, but mostly from marine sediments. In the 1970s, the extra objects on Quaternary pollen slides prepared from lake sediments or peat attracted the attention of palynologists through the systematic documentation by the pioneering work of Bas van Geel (van Geel, 1972). From this, a list of morphotypes with formal to informal descriptions of taxa found in palynological slides was developed (Shumilovskikh et al., 2021).

The NPP assemblages encompass highly diverse and taxonomically heterogeneous organisms that may reflect diverse local habitat conditions (van Geel, 2001) and may provide information on past human impact on ecosystems, such as fire, soil erosion and grazing pressure (Gauthier and Bapicot, 2021). The NPP may include membranes or envelopes produced by several types of organisms, such as green algae colonies or coenobium, lorica of tintinnids and rotifers, thecamoebians; foraminiferal linings, body fragments of insects such as hair, wing, leg or claw of arthropods; cysts of dinoflagellates, tardigrades, helminths and fungal spores (Shumilovskikh et al., 2021).

Some NPP have been documented in a few studies conducted in South America. Southern Brazilian peats and lake sediment of Holocene age revealed abundant fungal spores (Medeanic and Silva, 2010) and freshwater algal palynomorphs (Medeanic, 2006). Late Glacial and Holocene records of the Neotropical region of Venezuela, also indicate occurrences of fungal palynomorphs (Montoya et al., 2012). Studies of recent NPP assemblages documenting their relationship with local environmental conditions and nutrient stoichiometry are rare, especially in estuarine systems. The only systematic survey of algal palynomorph assemblages from modern sediments in relation to environmental conditions was conducted in different water bodies of Latvia, northern Europe (Stivrins et al., 2022), in a hydrological and climatic context distinct from neotropical environments. Here we aim to document the distribution of NPP alongside estuarine gradients of environmental conditions and nutrient concentrations in Camamu Bay, northeastern Brazil.

2. MATERIAL AND METHODS

2.1 STUDY AREA

Camamu Bay is an estuarine system with an extant area of 384 km² located in the state of Bahia, Brazil (13º40.2'S; 38º55.8'W and 14º12.6'S; 39º9.6'W) within a federal conservation area, which is 40% covered by mangrove vegetation and remaining Atlantic Forest. It is an important regional center of economic activities based on coastal tourism and fishing (Amorim et al., 2015). The larger rivers of the estuarine system are Maraú River to the south and the Serinhaém River to the north. Both rivers presently drain towns with unplanned settling urbanization, which lead to high amounts of untreated domestic wastes in addition to the natural terrestrial runoff caused by the frequent rain of a tropical climate. The main urban areas are Ituberá (population in 2022 was 21,913 inhabitants), located upstream Serinhaém River, and Maraú (population in 2022 was 24,580 inhabitants) located in the middle section of Maraú River (Figure 1).

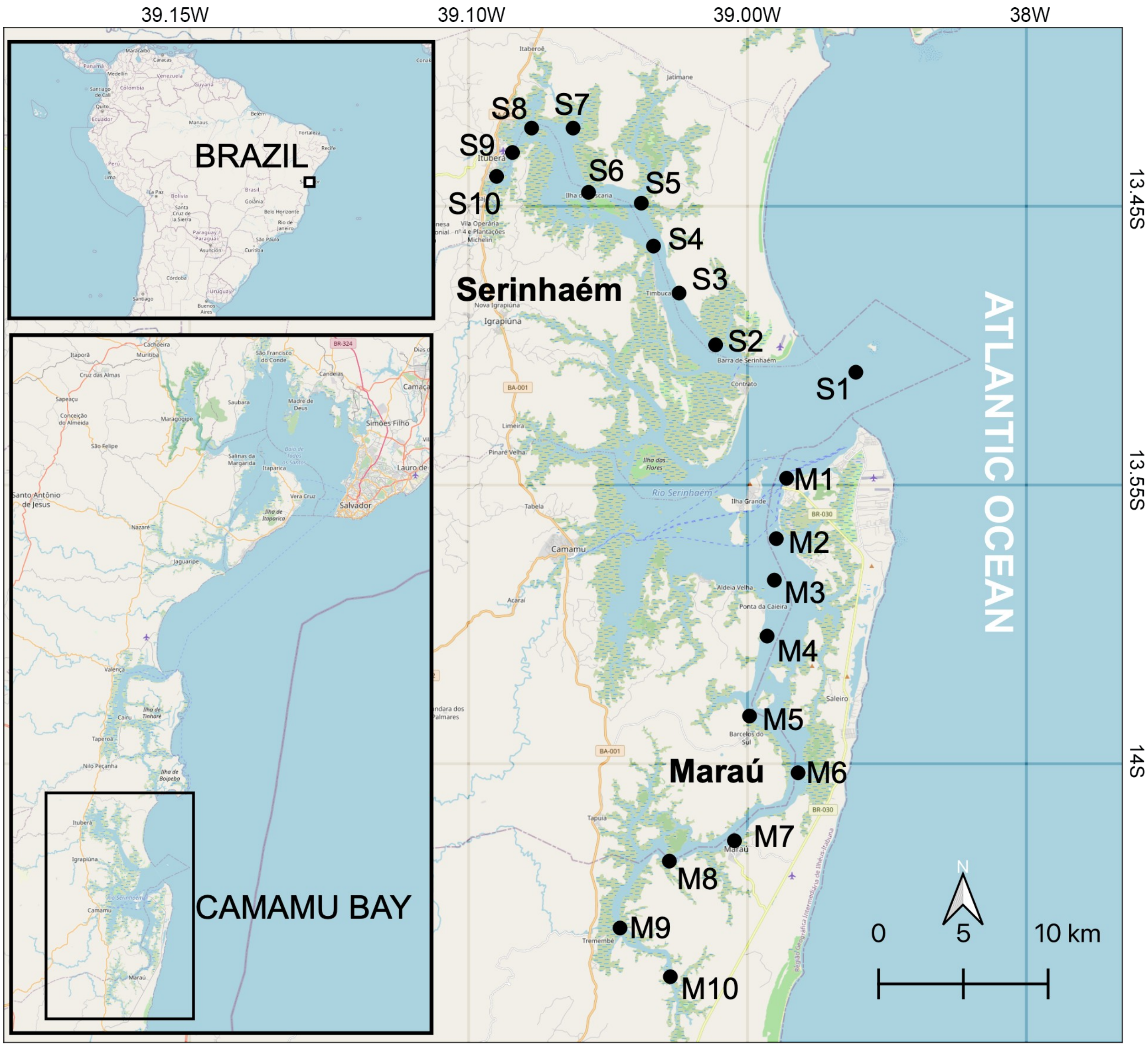


Figure 1: Geographical location of the sampled sites in the Camamu Bay. Ituberá town is located in the upper part of the map near the station S10.

2.2 *ENVIRONMENTAL CHARACTERISTICS*

In the study area, the precipitation is distributed typically over two periods: a rainy season from March to July with maximum monthly rainfall of 260 ± 115 mm in July and a dry season from August to February, when the mean precipitation tends to be 30% lower than the rainy period with maximum monthly rainfall of 235 ± 94 mm in November (Amorim, 2005). The mean annual cumulative precipitation is 2570 mm based on 40 year-long historical pluviometry data (ANA, 2005).

The seaward currents maximum velocity is 84 cm/s (Amorim, 2005) and the tidal regime is semidiurnal with a maximum and minimum amplitude of 2.2 m and 0 m, respectively (Lenz, 2008). Easterly and southeasterly winds predominate in the dry and rainy seasons, with average velocities of 2.7 m/s and 2.3 m/s, respectively. The annual mean sea-surface temperature ranges from 21ºC to 25ºC (Amorim, 2005).

The granulometry of the sediment are mostly medium-size sand and mud in the Serinhaém River and mostly gravel, medium-fine sand in the Maraú River (unpublished data by Simone Moraes, personal communication).

2.3 *SAMPLING*

In September 2021, we sampled surface sediments with a box core at twenty sites along two transects, one in the Maraú River (34 km) and in the other in the Serinhaém River (30 km). The samples were stored in plastic bottles and preserved with formaldehyde (4%). Some in situ environmental variables were measured. The water depth measured with an infrared depth gauge and the turbidity estimated with a Secchi disk. The pH, temperature and salinity were measured with a Horiba multiparameter probe. Samples for further laboratory nutrient concentration analysis were immediately cooled in a dark thermic box and frozen. The concentration of nutrients, including silicate, soluble reactive phosphorus, total phosphorus, total nitrogen, ammonium and nitrate were calculated comparing the light absorbance of the sample reaction with a set of four controlled concentrations under spectrophotometry (Grasshoff, 1966; Wood et al., 1967; Koroleff, 1976). The N:P ratio was calculated (Redfield, 1958) to understand the stoichiometry alongside the estuary and relationship with the biological assemblage at the time they were collected. The organic matter and organic carbon were estimated based on loss ignition (Allen et al., 1974). We calculated the weight of bulk density of fresh sample, the dry weight after 12 hours in an air-circulated oven and the loss on ignition after 6 h at 550ºC following the methodology and calculation described in Berglund (1987). For each sampling site, we recorded the geographical coordinates using a GPS Garmin e-Trex. Data are reported in the supplementary materials.

2.4 *PALYNOLOGICAL TREATMENT*

To concentrate the organic remains from the sediment, we used the palynological techniques described in de Vernal et al. (1999). A volume of 5 cc of wet sediment was dried and a tablet of *Lycopodium clavatum* (batch: 2013001; n = 27,560 spores/tablet) was added to each sample for further concentration calculation. The sediment was wet sieved through two different size mesh sieves (106 µm and 10 µm) after deflocculation by adding soap and distilled water until the clay and small silt content was drained out. The chemical treatment included repeated digestion with cold HCl (10%) and cold HF (48%) to dissolve carbonate and silicate particles respectively. A solution of KOH (5-10%) was added to dissolve the labile organic compounds. Finally, the sample was ultrasonified for 20 seconds prior to a last sieving at 10 µm. A drop of the residue was mounted between slide and cover-slide in gelatine and the remaining residue was preserved in water with phenol (10%).

2.5 *IMAGERY AND IDENTIFICATION*

The slides were scanned in optical microscopy. *Lycopodium clavatum* spores from calibrated tablets were used as a marker grain to estimate the concentration of NPP per gram of dried sediment. The spores added were stained in red to be distinguished from the spores originating from the surrounding vegetation. The entire slides were scanned at 400x magnification. The images were taken under transmitted light microscopy (Leica DM 2500M) coupled with a high-resolution camera (Lumenera Infinity X). The photographic plates were made by scaling all the specimens to visually ensure proportional size classes. The identification criteria included shape, size, color and ornamentation.

Table 1: Groups of NPP analyzed in Camamu Bay samples.

GROUP	DESCRIPTION	SIZE (µm)	REFERENCE
DINOCYSTS	Organic-walled cysts and vegetative cells	20-80	Fensome, 1993, de Vernal et al., 2007, de Vernal et al., 2001, Zoneveld and Pospelova, 2015.
ACRITARCHS	Uncertain affinity organisms	10-60	Evitt, 1963, Strother 1996.
GREEN ALGAE	Filamentous, colonial or coccoid remains of Chlorophyte, Charophyte and Ocrophye.	2-30	van Geel et al. (1983), Batten (1996), Jankovská and Komárel (1995), Shumilovskikh et al., 2021.
CYANOBACTERIA	Sheaths, akinetes and heterocysts of cyanobacteria	10-40	van Geel et al. 1989, van Geel et al., 1983, Shumilovskikh et al., 2021
FORAMINIFERAL LININGS	Organic walled remains of Foraminifera after shell dissolution.	20-80	de Vernal 2009, Stancliffe, 1989, Mudie and Yanko-Hombach, 2019.
CILIATES	Loricae of tintinnids and cysts of other ciliates.	15-100	Dolan and Pierce, 2013.
TESTATED AMOEBOZOANS	Decay-resistant shell	30-100	Payne et al., 2012.
SCOLECODONTS	Polychaete jaws.	30-80	Mudie et al., 2021, Szaniawski, 1996.

ZOOLOGICAL REMAINS	Chitinous remains (exoskeleton, legs, eggs, mandibles, claws, hair and oocytes) of Copepod, Rotifer and Arthropod organisms.	20-100	Van Geel et al, 1983, Knapp et al., 2001, Ruttner-Kolisko, 1974).
FUNGI	Coprophilous, saprotrophic, mycorrhizal, carbonicolous and freshwater fungi.	5-30	Elsik et al, 1983, Kalgutkar and Jansonius (2000).

The identification was made under light microscopy at 400x and 1000x magnification following the mentioned references (table 1). Dinocysts were identified at the species level when possible, however some specimens were described as round brown cysts due to uncertainties. Fungal remains were counted as fungi with no attempt at more specific identifications. Green algae and cyanobacteria were identified at the most specific level possible. Foraminiferal linings were differentiated as uniseriate, trochospiral or planospiral. Ciliates were differentiated between cyst and lorica. Scolecodonts were counted with no attempt at more specific identification, as well as the fragment parts and eggs. The concentration was expressed in number of cells per gram of dried sediment, and it was calculated by extrapolation as follows:

$$Np = Ne \times np \div ne$$

Where "Np" is the number of palynomorphs; "Ne" is the number of marker grains added to the initial sample prior to preparation; "np" is the number of individuals counted, and "ne" is the number of counted marker grains. The calculation to estimate the concentration of cells per unit volume was done by dividing "Np" by the initial volume of dried sediment.

2.6 *STATISTICAL ANALYSIS*

A raw compositional site-species matrix was initially submitted to a detrended correspondence analysis (DCA) to select the most adequate ordination method. If the length of the first axis is less than two standard deviation units, it is suggested a linear relationship among the assemblage and the environmental gradients. In the case of more than two standard deviation units, the distribution is likely unimodal (Ter Braak, 1987). Our data set indicates a length of the first DCA axis (2.23 sd units) supporting the decision to conduct a Canonical Correspondence Analysis (CCA). A variance inflation factor function was applied with an upper limit of ten units (Groß, 2003). To overcome potential overinflated variables, a stepwise selection method with *Akaike Information Criterion (*AIC) algorithm and permutation test with 999 iterations were applied. This approach permits to identify the set of variables explaining the maximum variance on species data (Blanchet et al., 2008). An alternative contingency table was created by reducing the species following the criteria of presence in at least two of the twenty sites, resulting in a total of 35 palynomorphs from 52. Analysis of variance (ANOVA) with

Monte-Carlo permutation test (999 permutations) was made to test the statistical significance of the results. We calculated the Pearson`s coefficient of correlation among the environmental variables, nutrient concentrations and the non-pollen palynomorph assemblage. The software R (version 4.2.1) was used to carry out the whole analysis (R Core Team, 2022).

3. RESULTS

3.1 NON-POLLEN PALYNOMORPH TAXA: TAXONOMY AND OCCURRENCES

We identified 52 non-pollen palynomorph morphotypes comprising 14 divisions or Phylum, 16 Classes, 22 Orders, 30 Families and 31 Genus.

3.1.1 DINOCYSTS

Division Dinoflagellata (Bütschli, 1885) Fensome et al., 1993
Subdivision Dinokaryota Fensome et al., 1993
Class Dinophyceae Pascher, 1914
Order Prorocentrales Lemmermann, 1910
Family Prorocentraceae Stein, 1883
Genus *Prorocentrum* Ehrenberg, 1834

***Prorocentrum lima* complex (Ehrenberg) F. Stein, 1878 – Plate 4, figs. 48-61**
Description: Oblong-ovate cells laterally flattened measuring 36 to 42 µm in length, 17 to 33 µm in width (n = 68), composed by a ventral plate sagittally connected to a dorsal plate by a suture (intercalary band). The wide V-shaped apical periflagellar area contained eight platelets around a main flagellar pore and a smaller accessory pore excavated on the right plate. The ornamentation was generally smooth with scattered pores always oblong or kidney-shaped around the plate except in the center. The marginal pores were also kidney shaped. Morphological variations included a posterior plate with a pointed instead of round contour (Fig. 58), a round cell with uncommon periflagellar platelet (Fig. 55), a posteriorly sharpened cell with prominent edges around the theca (Fig. 52) and an urn-shape form (Fig.49, 52).

Ecology: Photoautotrophic epiphytic dinoflagellate widely distributed from tropical to polar environments inhabiting mostly planktonic but also benthic habitats (Dodge, 1965; Dodge, 1975; Faust, 1993). The benthic species are commonly found attached to substrates in shallow waters, such as macrophytes, coral reefs, sand and rocks by the production of mucous and polysaccharide filaments (Honsell et al., 2013). Epiphytic dinoflagellates are flagellated during their vegetative life cycle and thus fully capable of detaching from the substrate and dwell planktonically given the motile capability (Durán-Riveroll et al., 2019).

Occurrence: High concentrations (up to 4000 cells per gram) at the upstream section of Serinhaém River (stations S10, S9, S8) and less than 100 cells at the other locations.

Remarks: The *P. lima* complex comprise okadaic acids and analogues, likewise dinophysistoxins, borbotoxins and prorocentrolides (Faust, 1991; Hoppenrath et al., 2013).

Prorocentrum rhathymum **Loeblich III, Sherley & Schmidt (Loeblich et al. 1979)**
Description: Foveate and reticulated-foveate cells measuring 36 to 42 µm in length, 24 to 30 µm in width and a length/width ratio of 1.4 µm. The pores were disposed in lines radiating from the center of the cell perpendicular to the margin. The pore lines emerging from the right valve were composed of six and seven spherical small pores.

Ecology: Tychoplanktonic toxigenic species formerly identified as *P. mexicanum* Gárate-Lizárraga and Martínez-López (1997), associated to red tides in Mexico (Ramírez-Camarena et al. 1999) and water discoloration during a bloom in the Bangaran lagoon, India (Thomas and Padmakumar, 2020). In the subtropical and tropical Atlantic Ocean *P. rhathymum* was described, among other species, as an important contributor for ciguatera poisoning. High abundances are reported in the Gulf of Cariaco, northeastern Venezuela (Navarro-Vargas et al., 2014), in the Caribbean Sea coast of Colombia (Arbelaez et al., 2017) and in the reef zone of the meso-American reef system, including the Gulf of Mexico (Estrada-Vargas et al., 2017).

Occurrence: 108 cells/g dw at the upstream section of Serinhaém River (station S8).

Remarks: this species was also observed in a planktonic foam-plume bloom during the collection, presenting water discoloration in the same upstream area reaching millions of cells per liter.

Prorocentrum **sp. (?leve) - Plate II, fig. 22.**
Description: round wide cell with visible periflagellar area and hidden platelets. A sequence of round to elliptical marginal pores is visible, in the apical collar inclusively. There was no visible pyrenoid or starch ring. Within the cytoplasm it was noticed spherical small structures regular in shape and size resembling chloroplasts. An unclear transparent sheath covered the cell with an apical disruption. The difficulty to observe the periflagellar platelet and the position of the pyrenoid hampers the species identification.

Occurrence: rare occurrences in the upstream section of Serinhaém River (station S10).

Order Peridiniales Haeckel 1894
Family Podolampadaceae Er. Lindem. 1928
Genus *Cabra* Murray&Patterson 2004

Cabra matta **Sh.Murray & D.J.Patterson 2004 – Plate II, fig. 14**
Description: The cell was round in the middle with a crown-shape apical structure and three spines in the antapical portion of the cell in lateral view. The size was 28 µm in length, 30 µm in width. A golden-brownish content was visible, including the round

nucleus dorsally located in the hyposome. In a deep field contrast the cingulum was observed widely descending towards the left side of the cell. Some pores, mostly foveate, were observed in the surface of the theca, which was not clear enough to describe the disposition of the plates.

Ecology: Marine species documented to occur on coral reefs, sand grains and macrophytes from tropical and sub-tropical Australia (Selina et al., 2015), coastal waters of Korea (Shah et al., 2013) and Gulf of Mexico (Okolodkov et al., 2007).

Occurrence: Rare specimens at station S7.

Order Peridiniales Haeckel 1894
Family Protoperidiniaceae Fensome et al., 1993
Genus *Archaeperidinium* Jörgensen 1912 emend. A. Yamaguchi, Hoppenrath, Pospelova, T. Horiguchi & B.S. Leander 2011

Cyst of *Archaeperidinium* sp 1 (?*minutum*) - Plate I, fig. 6.
Description: Brown round cell of approximately 37 µm in diameter. The thick wall is ornamented with short acuminate dissimilar hollow processes. The cell content was brownish with several round structures like vacuoles. The cell wall seems to have layers with an opening on the left side. The theropylic archeopyle is not visible. The distinctive character of this morphotype is the medium hollow processes and overlapping layers around the wall.

Ecology: The genus *Archaeperidinium* is documented to occur in estuarine waters in several geographical locations, especially in the North Pacific along the British Columbia coasts (Mertens et al., 2012).

Occurrence: Peaked 289 cells/g dw in the middle of Serinhaém River (stations S4 and S5) and recorded less than a hundred cells per gram in the upstream section (stations S8 and S9).

Remark: see doi: 10.1111/pre.12081 fig. 33.

Round brown cyst type 1 (?*Archaeperidinium* ?*saanichi*) - Plate I, fig. 10
Description: Round light brown cell (~40 µm diameter) with irregular dissimilar short processes and the same multilayer-type wall. Compared to *Archaeperidinium sp1*. mentioned above, this cell presented a similar internal content but different shape, given the flatness of the antapical part in deep contrast and smoothness of the surface observed in focus contrast. The distinctive characteristics of this type are the solid processes, the light brown color and the sharpness of the inferior cell wall compared to *Archaeperidinium* sp1.

Occurrence: Rare specimens in the upstream section of Serinhaém River (station S9).

Remarks: Ornamentation resembling that of *Bitectatodinium spongium* (doi.org/10.1016/S0034-6667(99)00007-X). This is also similar to cyst type L described by Price and Pospelova (2011) in doi:10.1016/j.marmicro.2011.03.003.

Round brow cyst type 2 (?*Protoperidinium* sp.) - Plate I, fig. 1.
Description: Round brown cell measuring 28 μm in diameter, comprising inner content with a structure resembling a pyrenoid starch ring in the middle and chloroplasts within the cytoplasm. The surface is composed of plates with wave-shaped edges fitting together (Fig. 1A). Inside the cyst a thick grey wall is visible (Fig. 1C) hosting a yellow sphere laterally compressed or excavated and an uneven surface. The distinctive character of this morphotype is the arrangement of the thecal plates, giving the cell a flower shape and the triple layer composition.

Occurrence: Rare specimens in the middle of Serinhaém River (station S4).

Round brown cyst type 3 (?*Protoperidinium*) - Plate I, fig. 5.
Description: Round yellow cell of approximately 30 μm in diameter, with cryptopilic archeopyle. The inner cell content was composed of three round smooth structures (Fig. 5B), which is also the distinctive feature of this type.

Occurrence: Rare specimens in the riverine freshwater section of Maraú river (M9).

Round brown cyst type 4 (?*Brigantedinium*) - Plate I, fig. 7.
Description: Round brown cell like a sheath of 33 μm in diameter, laterally sharpened in ventral view and an inner cell of 28 μm diameter composed of a thick wall, cytoplasm, eyespot in the right side and nucleus. This inner cell has been observed without the brown sheath (cf. fig. 62-67). The distinctive character of this type is the lateral fold in the pericoel and the thick wall cell inside.

Occurrence: Low concentration (< 100 cells/g) in the intermediate and upstream zones of Serinhaém River (stations S5, S9 and S10).

Cyst of *Parvodinium* sp. - Plate II, fig. 26, 27.
Description: White armored cell encompassing conical epitheca with acuminate apex, wide thick cingulum excavated (dorsal view fig. 26A) and semicircular hypotheca. The presence of spines in the hypotheca is not visible. The shape of the antapical plates was hexagonal with smooth surface and sutures. Large sulcal plates were observed (as in fig. 27A). The size of the cell was approximately 28 μm length and 30 μm width. Intercalary plate and theropylic archeopyle noticed (fig. 26B-C). The specific identification depends on the visibility of the plates and sulcal excavation.

Ecology: Several species described in every brazilian region (Menezes, M., Branco, S. 2020. *Parvodinium* in Flora do Brasil 2020. Jardim Botânico do Rio de Janeiro. http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB124411).

Occurrence: Local occurrence at station S7.

Family Protoperidiniaceae Bujak and Davies
Genus *Votadinium* Reid, emend. 1977

***Votadinium* sp 1. - Plate I, fig. 11,12.**
Description: Brown cyst similar to *V. calvum* and *Protoperidinium oblongum* in shape and size (~50 µm length, 42 µm width) but bearing a different archeopyle, i.e., intercalary but not involving apical section. The endocoel of *Votadium calvum* is described to have curved epitheca, whereas it is more pointed and straight in *P. oblongum* (Bringué et al., 2018). The cell wall surface was reticulate instead of smooth, with a central pyrenoid elevation, ventral-to-dorsal compression, cingulum excavation and parasulcus with shallow depression. The archeopyle of the specimen represented in fig. 12 was smaller and round, contrasting to the morphological descriptions of *V. calvum*.
Original diagnosis (Reid 1977). "Peridinoid-shaped cysts with a rounded apex or elongate apical horn and with two antapical, rounded horns or lobes separated by an antapical depression which may be deep or shallow. Test compressed dorsoventrally, no evidence for a girdle. Archeopyle intercalary."

Occurrence: Rare specimens in the middle section of Maraú river (station M6).

Remarks: See : https://doi.org/10.1016/j.pocean.2018.12.007 (Figure 39).

***Votadinium* sp 2. - Plate II, fig. 35.**
Description: Spherical cell measuring 32 µm length and 35 µm width, compressed dorsoventraly with mucilage around. The sulcal depression seems less excavated compared to most of the *Votadinium* species. The epitheca was semicircular with a pylome-type dot and several random pores.

Occurrence: Rare specimens in the marine section of Serinhaém River (S3).

Family Protoperidiniaceae Balech, 1988 nom. cons.
Genus *Islandinium* Head et al., 2001

cf. *Islandinium brevispinosum* Pospelova and Head, 2002 – Plate I, fig. 13.
Description: Brown cyst of ~40 µm in diameter, spheroidal with slight compression inferiorly. The cell was covered by dense spines distributed all over the cell and granulated surface. The archeopyle was saphopylic located in the apical part of the cell.

Ecology: Estuarine species documented to occur in shallow, nutrient-rich waters from temperate to polar environments (Pospeslova and Head, 2002).

Occurrence: Local occurrence in the upstream section of Serinhaém River (station S9).

Order Gonyaulacales Taylor, 1980
Family Ostreopsidaceae Lindemann, 1928
Genus *Coolia* Meunier, 1919

***Coolia monotis* complex Meunier, 1919 – Plate II, fig. 25.**
Description: Round, small cell (~20 µm diameter) slightly compressed laterally in the apical part of the cell. Pores were observed in the anterior plate (Fig. 25A). The cingulum excavation is visible through the discontinuity of the wall in the apical portion extremity. The thecal plates 1’”, 2’”, 3’”,4’”,5’”and 2’”” slightly visible.

Ecology: Marine benthic species mostly documented in the Gulf of Mexico and Caribbean waters and several substrates, such as macrophytes and reef zones (Okolodkov et al., 2007).

Occurrence: Rare specimens at the upstream section of Serinhaém River (station S10).

Family Gonyaulacaceae Lindemann 1928
Genus *Spiniferites* Mantell, 1850 emend. Sarjeant 1970

***Spiniferites* cf. *bentorii* (Rossignol) Wall & Dale 1970 – Plate II, fig. 39.**
Description: Ovoid pear-shaped cell measuring 42 µm long and 40 µm wide with clear cell wall and proximochorate processes. The surface and archeopyle are not clear due to preservation and mucilage. The gonal and intergonal processes were long and solid with bifurcating tips. Antapical flange not visible.

Ecology: Distributed in coastal environments from equatorial to polar regions (Zonneveld et al. 2013).

Occurrence: Rare occurrence in the middle of Maraú River (station M6).

Family Pyrophacaceae Lindemann, 1928
Genus *Pyrophacus* Stein, 1883

***Pyrophacus horologium* F. Stein, 1883 – Plate III, fig. 41.**
Description: Discoid cell with even epitheca and hypotheca, equatorial narrow cingulum, short sulcus and standard plate tabulation 5-9', 0-8a, 7-15 '',9-16c, 8-17''', 0-9p, 3-7'''' (Taylor, 1976).

Ecology: marine (no information found)

Occurrence: Rare specimens in the marine section of Serinhaém River (S2).

Order Gymnodiniales Apstein, 1909
Family Gymnodiniaceae

Hyaline cyst with gymnodinioid-shaped content – Plate II, fig. 24.
Description: Spherical hyaline sheath of 30 µm in diameter with operculum-type aperture in the inferior part of the cell. The inner content was a green gymnodinioid cell with granulated surface, visible equatorial cingulum and parasulcus definition in the center.

Occurrence: low concentrations in the middle section of both rivers (stations M6 and S6), peaked 108 cells/g dw in the upstream section of Serinhaém river (S7).

Remarks: see *Gymnodinium litoralis* (Figure 5-G at doi:10.1016/j.hal.2011.08.008).

Order Amphidiniales Moestrup & Calado, 2018
Family Amphidiniaceae Moestrup & Calado, 2018
Genus *Amphidinium* Claperède & Lachmann, 1859

***Amphidinium* sp. - Plate II, fig. 33.**
Description: Elliptical green gymnodinioid cell of 55 µm length, 40 µm width, with a yellow central pyrenoid right above the cingulum excavation, sulcal excavation, elliptical thin chloroplasts with pronounced plastid contours. Horn-based epitheca pointed to the right and semicircular hypotheca.

Occurrence: Local occurrences in the upstream section of Serinhaém River (station S7).

Class Dinoflagellata incertain sedis
Genus *Dubridinium* P.C.Reid, 1977

***Dubridinium* sp. - Plate I, fig. 4, 9.**
Description: Brown partially translucid semispherical cyst elongated with a triangular opening and smooth surface. Maximum linear dimension 52 µm, orientation unclear.

Occurrence: rare specimens in the intermediate section of Maraú River (station M6).

Remarks: See also *Quinquecuspis concreta* in different view (https://doi.org/10.1016/j.pocean.2018.12.007).

3.1.2 *ACRITARCH AND UNCERTAIN IDENTIFICATION*

Group Acritarcha Evitt 1963

Subgroup Polygonomorphitae Downie et al. 1963
Genus *Polygonium* Vavrodova (1966) emend. Sarjeant & Stancliffe

***Polygonium* sp.** - Plate I, fig. 2.
Description: Yellow polygonomorph hollow vesicle of 22 µm in diameter ornamented with long obliquous spines. Thick spheres appear in the deep field observation. The spines are smooth, and the tips folded.

Occurrence: rare specimens.

Family Incertae Sedis
Genus *Pseudoschizaea* Thiergart and Frantz ex R. Potonie emend. Christopher

***Pseudoschyzaea circulina* Rossignol ex Christoph - Plate V, fig. 89**
Description: Spherical grey theca in polar view measuring 39 µm in diameter with sequential rings emerging towards the middle of the cell interrupted by a sequence of small wholes like pores. The original description mentions the polar area of both hemispheres as "pattern of loops, curls, spirals or bars" (Christopher, 1976). This is the distinguishing character separating *P. rubina* from *P. circulina* wich is described to have an ornamentation based on an "ornamentation with a series of anastomosing and bifurcating muri which form a mazelike polar complex. The polar complex may, in some specimens, resemble a foveolate or fenestrate thickening covering the pole" (Christopher, 1976).

Ecology: Freshwater distribution (Rossignol, 1962), observed in the south of Brazil.

Occurrence: < 50 specimens/g at the upstream stations of Maraú River, < 100 tests/g in the middle section of Serinhaém (Station S5).

Round pellicle greyish cyst – Plate IV, fig. 62-66
Description: Pellicle cyst of approximately 30 µm in diameter, thick wall generally spherical but in some cases flattened in one side, in one case forming a pentagonal shape. In the surface of the wall uncountable small pores with prominent contour distribute all over the cell. The inner cell content (Fig. 62A) comprises vesicle structures, accumulation body and a nucleus measuring more than one third the cell diameter. In some specimens the cell wall was straightly flattened. A granulation was noticed in the cytoplasm in dorsal view.

Occurrence: > 300 cells/g in the marine downstream section (stations S2 and S3) and > 100 cells/g at the upstream section (S7) of Serinhaém River.

Remarks: Except for the pores on the surface, the thick cell wall and the visible structures resemble the live pellicle resting cyst of *Alexandrium taylori* Balech, originally documented to encompass a thickened wall, greyish granular content and flattened shape after some months of culturing (Bravo et al., 2006, (DOI: 10.1080/09670260600810360). However, a greyish pellicle resting cyst is also produced by many *Scripsiella* species, also including an orange or red accumulation body (Orlova et al., 2004), in this case some species bear ornaments like short spines arising from the outer wall.

3.1.3 *GREEN ALGAE*

Division Chlorophyta Pascher, 1914
Class Chlorophyceae Wille, 1884
Order Chlamydomonadales F.E.Fritsch
Family Volvocaceae Ehrenberg 1834
Genus *Pandorina* Bory, 1826

***Pandorina elegans* (Ehrenberg) Dujardin 1841 – Plate V, fig. 68, 69.**
Description: Small coenobia of ~10 µm in diameter with 21 spherical green individual cells placed very close to each other in ventral view. The hyaline mucilage bond is visible. Coenobia without a delimited wall. Flagella and pyrenoid are not visible.

Ecology: Cosmopolitan in freshwater (Coleman, 1959).

Occurrence: >1000 coenobias/g dw in the middle section Serinhaém River (station S5) and in the upstream section of Maraú (station M9). Present in half of the twenty analyzed samples.

Remarks: The structure of extra-cellular matrix of the vegetative colony is the distinguishing character compared to *Eudorina* (Nozaki and Kuroiwa, 1992).

***Pandorina morum* (O.F.Müller) Bory 1826**
Description: Coccoid small coenobia measuring 12-20 µm in diameter and triangular individuals in number of 8 or 16.

Ecology: Freshwater toxin producing species.

Occurrence: Almost a thousand coenobias per gram in the marine section of Serinhaém River (station S2).

Family Sphaerocystidaceae Fott ex P.M.Tsarenko 1990
Genus *Sphaerocystis* R.Chodat, 1897

***Sphaerocystis schroeteri* Chodat 1897 (holotype) - Plate V, fig. 99.**
Description: Mucilaginous colony of approximately 25 µm in diameter of several spherical green individuals. The original description mentions cup-shaped choloroplasts (Chodat, 1987), which are partially visible in our samples.

Ecology: Freshwater (John, Whitton & Brook, 2011).

Occurrence: Rare specimens in the upstream section of Maraú River (M7-M10).

Order Oedogoniales Heering 1914
Family Oedogoniaceae de Bary ex Hirn 1900
Genus *Oedogonium* Link ex Hirn, 1900

***Oedogonium* sp.**
Description: Narrow cylindrical filamentous algae composed of cellulose, pectose and chitin. n apical round tip filament followed by three colorless capsules with symmetrical elliptical eggs divided in the middle, with a sphere in the middle.

Ecology: Freshwater algae

Occurrence: Almost 200 filaments/g in the upstream section of Maraú River (M9), in the intermediate section of Serinhaém (S4) and in the upstream section of the same river (S7).

Division Charophyta Cavalier-Smith
Class Zygnematophyceae van den Hoek et al., 1995
Order Spirogyrales S.Hess & J.de Vries 2022
Family Spirogyraceae Bessey 1907
Genus *Spirogyra* Link, 1820, nom. Cons.

***Spirogyra* sp.1 - Plate VI, fig. 113**
Description: Ellipsoid hyaline zigopspore, elongated, square tips and smooth surface slightly ornamented (similar to the original descriptions Van Geel & Van Der Hammen, 1978). Another orientation is shown in figure 124.

Ecology: Shallow stagnant freshwater algae, also documented in soil (Van Geel & Van Der Hammen, 1978).

Occurrence: Up to 300 zygospores per gram in the upstream section of Serinhaém River (S3) and rare occurrence in several adjacent stations.

***Spirogyra* sp.2**
Description: Cylindric filamentous unbranched uniseriate algae with zig-zag-like content as distinctive character. Transparent akinet, two-layer inner cell.

Ecology: Freshwater habitats, wide distribution (Guiry & Guiry, 2022).

Occurrence: Up to four thousand filaments per gram dry sediment in the upstream station of Maraú River (M10). Rare specimens in the middle section of Serinhaém River (stations S4 and S5).

Order Desmidiales Bessey 1907
Family Desmidiaceae Ralfs 1848
Genus *Staurastrum* Meyen ex Ralfs, 1848

***Staurastrum anatinum* f. *denticulatum* (G.M.Smith) A.J.Brook 1959 – Plate V, fig. 101**
Description: Triangular, vertical radial symmetric cell in apical view, longer than wide measuring ~50 µm in length and 30 µm in width. The angle terminations were ornamented with curved intramarginal granules and bear three terminal spines.

Ecology: Freshwater, lagoons and reservoirs (Bicudo et al., 2007).

Occurrence: Rare specimens in the upstream section of Maraú River (M9).

Class Coleochaetophyceae C.Jeffrey 1982
Order Coleochaetales Chadefaud 1960
Family Coleochaetaceae Nägeli 1847
Genus *Coleochaete* Brébisson, 1844, nom. cons.

***Coleochaete* sp.**
Description: Colonial branched filaments embedded in mucilage, irregular individuals, oogonia cells bearing single or double pyrenoid.

Ecology: Widely distributed from tropical to polar environments attached to submerged macrophytes in freshwater systems (John, 2002).

Occurrence: Rare specimens in the upstream section of Serinhaém and downstream Maraú River.

Division Ochrophyte
Class Eustigmatophyceae D.J.Hibberd & Leedale 1971
Order Eustigmatales D.J.Hibberd 1981
Family Chlorobotryaceae Pascher 1915
Genus *Chlorobotrys* Bohlin, 1901

***Chlorobotrys regularis* (West) Bohlin 1901 – Plate V, fig. 67.**

Description: Coccoid spherical small cell measuring 10 µm in diameter. In the original description the cell was “colourless, smooth and relatively thick and firm” (Hibberd, 1971). The individual spherical small cells were uncountable due to the mucilage that may overlap upon the cytoplasm content.

Ecology: Ubiquitous wide distribution in acid waters (Hibberd, 1974).

Occurrence: More than a thousand cells per gram in the upstream section of Maraú (M9) and in the intermediate section of Serinhaém River (S4), ~700 cells/g at station S2 and less than 200 cells/g in the adjacent upstream stations of Maraú.

3.1.4 *CYANOBACTERIA*

Division Cyanobacteria Stanier ex Cavalier-Smith 2002
Class Cyanophyceae Schaffner 1909
Order Chroococcales Schaffner 1922
Family Chroococcaceae Rabenhorst 1863
Genus *Chroococcus* Nägeli, 1849

***Chroococcus* sp. - Plate V, fig. 95.**
Description: Hyaline mucilaginous colony, homogeneous, delimited colorless wall visible, hosting two distant symmetrical individual envelopes measuring ~15 µm long and 13 µm wide, green, hemispherical to irregular shape, more or less spherical in the outer extremity, divided by a section line in the middle.

Ecology: Freshwater, wide distributed, documented on metaphyton, soil, thermal and aerophytic environments (Komárek, 2003).

Occurrence: Present in low concentrations in the entire estuary, reaching maximum 108 colonies per gram in the middle section of Maraú River (M6).

Family Microcystaceae Elenkin 1933
Genus *Microcystis* Lemmermann, 1907, nom. et typ. Cons.

***Microcystis* sp. - Plate Vi, fig. 122**
Description: Gelatinous colonies hosting uncountable discoid and spherical individual cells. Mucilage present.

Ecology: Bloom forming species in freshwater reservoirs, also documented epiphytically and epilithically (Strunecky et al., 2023).

Occurrence: Very abundant in the upstream section of both rivers, peaking > 3000 colonies per gram at station S10.

Order Nostocales Borzì 1914
Family Aphanizomenonaceae Elenkin 1938
Genus *Anabaena* Bory ex Bornet & Flahault, 1886, nom. cons.

***Anabaena* sp.**
Description: Elliptical empty and colorless akinetes similar to those described as HdV-601 (Ralska-Jasiewiczowa and van Geel, 1992; van Geel et al., 1994).

Ecology: Aquatic and terrestrial habitats, used as proxy of phosphate-eutrophication of lakes related to fertilization use and farming (van Geel et al., 1994, Shumilovskikh et al., 2021).

Occurrence: Less than a hundred specimens in the middle section Serinhaém River (stations S3 and S5).

Genus *Gloeotrichia* J.Agardh ex Bornet & Flahault, 1886

***Gloeotrichia* sp.**
Description: Green-yellowish colonial filamentous algae comprising a basal heteropolar heterocyte and a long gelatinous transparent sheath containing vegetative content.

Ecology: Freshwater species with limited area distribution, several considered periphytic (Strunecky et al., 2023).

Occurrence: Less than a hundred colonies per gram in the Maraú River (stations M2, M6 and M7).

***Gloeotrichia* empty sheath**
Description: Transparent, empty body sheaths similar to the description for HdV-146 (van Geel, 1989).

Occurrence: Up to 700 sheaths per gram in the intermediate section of Serinhaém (station S4) and rare distribution in several other stations (stations M7, M8, S2, S6).

Family Nodulariaceae Elenkin 1916
Genus *Nodularia* Mertens ex Bornet & Flahault, 1886, nom. cons.

***Nodularia* sp.**
Description: Long colonial filaments, green, isopolar, unbranched, difluent two-layered structure and spherical basal heterocyte.

Ecology: Brackish or slightly saline coastal waters, mostly cosmopolitan species (Strunecky et al., 2023).

Occurrence: Highest concentrations in the upstream section of both rivers (stations M7 and S7), rare specimens at S5 and M2.

Family Nostocaceae Eichler 1886
Genus *Nostoc* Vaucher ex Bornet & Flahault, 1886

***Nostoc* sp.**
Description: Filamentous colony forming a chain-like sequence of individual spherical cells compressed in the middle. See Nostoc commune Vaucher ex Bornet & Flahault 1888.

Ecology: Epiphytic and epilithic in soil and freshwater biotopes, including unpolluted lakes and ponds (Strunecky et al., 2023).

Occurrence: Up to four hundred colonies per gram in the upstream section of Serinhaém River (station S7) and rare distribution in stations S3, S8 and S9.

Order Oscillatoriales Schaffner 1922
Family Microcoleaceae O.Strunecky, J.R.Johansen & J.Komárek 2013
Genus *Planktothrix* Anagnostidis & Komárek, 1988

***Planktothrix isothrix* (Skuja) Komárek & Komárková 2004**
Description: Cylindrical straight individual sheath, isopolar, slightly pointed round tips without calyptrate structure. Greenish chain of cells wider than long.

Ecology: Freshwater.

Occurrence: > 300 sheaths per gram in the intermediate section of Serinhaem River (stations S5 and S7). Rare specimens in other stations.

Family Oscillatoriaceae Engler 1898
Genus *Phormidium* Kützing ex Gomont, 1892

***Phormidium* sp. - Plate 6, fig. 125**
Description: Filamentous, unbranched golden sheath, mostly ended in an open trichome calyptrate. Heterocyte and akinete not present. The interior is composed of sequenced individuals wider than long.

Ecology: Stagnant and stream waters, marine, littoral and harsh environments (Strunecky et al., 2023).

Occurrence: Upstream section of Maraú river and several stations of Serinhaém reaching up to 400 filaments per gram in the upstream section of Serinhaém (S7).

3.1.5 *PALYNOFORAMINIFERA*

Division Foraminifera d'Orbigny, 1826
Order Foraminiferida d'Orbigny, 1826
Class Globothalamea Pawlowski, Holzmann & Tyszka, 2013
Class Nodosariata Mikhalevich, 1992 emend. Rigaud et al., 2015

Foraminiferal lining type 1 – Plate VII, fig. 142
Description: Foraminiferal organic lining uniserial and biserial mostly light brown.

Foraminiferal lining type 2 – Plate VII, fig. 141
Description: Foraminiferal organic lining trochospiral and planispiral, generally well preserved, light brown with other varying color, surface and size.

Ecology: Marine organic biogenic producer used as proxy of $CaCO_3$ dissolution (de Vernal et al., 2015). Also indicator of marine and brackish waters (Matsuoka and Ishii, 2018).

Occurrence: More than 300 linings of the type plano- or trochospiral in seven of the ten stations of Serinhaém River, peaking ~2000 linings per gram at S4. Up to 400 linings of the type 1 per gram in the downstream section of both rivers (stations M4 and S3).

3.1.6 *CILIATES*

Division Ciliophora Doflein 1901
Class Spirotrichea Bütschli 1889
Order Tintinnida Kofoid & Campbell 1929
Family Codonellidae Kent, 1881

Tintinnid lorica
Description: Varying vase-shaped cell, different size classes and surface ornamentation.

Ecology: Marine organisms, although some species dwell in brackish freshwater environments, widely distributed biogeographically (Balkis, 2004). Tintinnids are considered indicators of eutrophication in freshwater lakes associated to low levels of dissolved oxygen (Barbieri and Orlandi, 1989, Shumilovskikh et al., 2021).

Occurrence: Up to 400 lorica per gram of dried sediment in the upstream section of Maraú River (M9) and 145 in the middle of Serinhaém (S4). Rare specimens in other stations.

Class Ciliatea Doflein 1901
Order Oligotrichida Bütschli, 1887
Family Strombidiidae Fauré-Fremiet, 1970
Genus *Strombidium* Claparède & Lachmann, 1859

***Strombidium* cyst – Plate VIII, fig. 146**
Description: Flask-shaped resting cyst measuring ~28 μm long and 30 μm wide. Plug not present. Structures around the cell wall similar to spines and an opening in the surface were noticed.

Ecology: Planktonic species inhabiting ocean and lake environments (Fenchel, 1987). Resting cyst described for many species (Reid, 1987).

Occurrence: ~100 cysts/g in the intermediate section of Serinhaém River and rare distribution in the upstream sites of the same river (S9 and S10).

Cyst type P of Reid
Description: Hyaline transparent tintinnid flask-shape cyst bearing a pentagonal star-shape opening. Sharpened in the inferior portion.

Occurrence: Less than a hundred individuals per gram in the upstream section of Serinhaém River (S3 and S4).

3.1.7 *TESTATE AMOEBOAZOANS*

Division Protozoa incertae sedis
Family Arcellidae Ehrenberg 1843
Genus *Arcella* Ehrenberg 1832

***Arcella* sp.**
Description: Spherical hyaline testate amoeba of varying size classes with round aperture of half-cell diameter.

Ecology: Documented to inhabit freshwater systems with high amounts of organic matter (Medioli e Scott, 1988).

Occurrence: ~100 tests per gram at stations S4, S7 and S9 only alongside Serinhaém.

Division Cercozoa Cavalier-Smith 1998
Class Filosa Cavalier-Smith 2003
Order Aconchulinida De Saedeleer 1934

Family Paulinellideae De Saedeleer 1934
Genus *Paulinella* Lauterborn, 1895

***Paulinella* sp. (cf.*micropora*) - Plate X, fig. 182.**
Description: Oval hyaline amoeboid sheath-like test of 37 μm in diameter, hosting a green elongated chromatophore from an endosymbiont cyanobacteria.

Ecology: Heterotrophic amoeba capable of photosynthesis from symbiont cyanobacteria chromatophore described in freshwater environments (Lhee et al., 2019).

Occurrence: Local occurrence of approximately 100 tests per gram in the upstream section of Serinhaém River (S7).

Class Gromiidea Cavalier-Smith, 2003
Order Gromiida Claparède & Lachmann, 1858
Family Gromiidae Ruess 1862
Genus *Gromia* Dujardin 1835

***Gromia* sp. - Plate Vii, fig. 140**
Description: Amphora-like shell slightly pointed in the inferior portion, measuring 60 μm in width. The surface is composed of irregular lines forming whole-like spaces.

Occurrence: ~100 shells/g at M4 and S7.

3.1.8 *SCOLECODONTS*

Division Annelida Lamarck, 1809
Class Polychaeta Grube, 1850

Scolecodont – Plate VII, fig. 131.
Description: Several jaw and mandible types of polychaeta.

Occurrence: < 100 pieces per gram in the downstream section of Serinhaém river, reaching up to 300 pieces per gram upstream (station S7).

3.1.9 *ZOOLOGICAL REMAINS*

Division Arthropoda von Siebold, 1848
Class Copepoda Milne Edwards, 1840
Order Cyclopoida Burmeister, 1834
Family Ergasilidae Burmeister, 1835

Ergasilidae body fragments and eggs – Plate VII, X, fig. 138, 186.
Description: Several parts of copepod organisms, mostly eggs that are large reaching 100 µm wide, greyish, brain-shaped, bearing hollow spines. Body parts comprise antennules (Fig. 186), hair and legs (Fig. 127). Spermatophores were also noticed (173) yielding a hyaline sheath hosting an orange, thin, long filament with striations in the middle.

Ecology: The majority (three quarters) found in freshwater and the extant in marine or salty habitats (Huys and Boxshall, 1991).

Occurrence: Rare occurrence in the upstream section of both rivers (stations M9, M10, S9, S10).

Division Platyhelminthes Minot 1876
Order Rhabdocoela Ehrenberg, 1831
Class Turbellaria Ehrenberg, 1831

Oocytes of Rhabdocoela – Plate IX, fig. 166
Description: Yellow vase-shaped oocyte of varying size classes with well-defined cell wall, peduncle visible in the inferior portion of the cell and smooth translucid surface with cracks.

Ecology: Cosmopolitan, freshwater distribution used in palynological reconstructions of environmental conditions given their restriction to specific habitats (Shumilovskikh et al., 2021).

Occurrence: Up to 400 eggs/g and 100 eggs/g in the freshwater section of both rivers (S7 and M10, respectively).

Undetermined animal remains – Plate VII, fig. 132
Description: Several fragments or body parts of undetermined zoological organisms. Hair, jaws, griffes, Coleoptera wings, teeth and legs included.

Occurrence: Up to 400 and 300 pieces/g in the upstream section of both rivers (S7 and M10, respectively).

Undetermined eggs – Plate IX, X, fig. 176-180, 187, 188.
Description: A miscellanea of size, shape, ornamentation, surface and apertures. Probably eggs from Rotifer, Copepod and Cladocera organisms.

Occurrence: High abundance up to 1.200 eggs/g in the downstream section of Serinhaém River. Not observed in the samples from Maraú.

3.1.10 *FUNGI*

Division Glomeromycota C. Walker & A. Schüßler
Division Ascomycota Whittaker, 1959

Fungal ascospores and glomus – Plate VIII, fig. 152.
Description: Glomus chlamydospore oval to spheric, yellow-brownish, globose with tick wall and connected coenocytic hyphae of 40 µm in diameter. The ascospores were generally small dark-brown opaque and varying size. Saprobic conidiospore fungi were septate long (similar to HdV-77A, van Geel) or spiral (similar to HdV-30, van Geel). Wood decay and freshwater ascospores and conidiospores were also noticed, showing light and dark brown coloration.

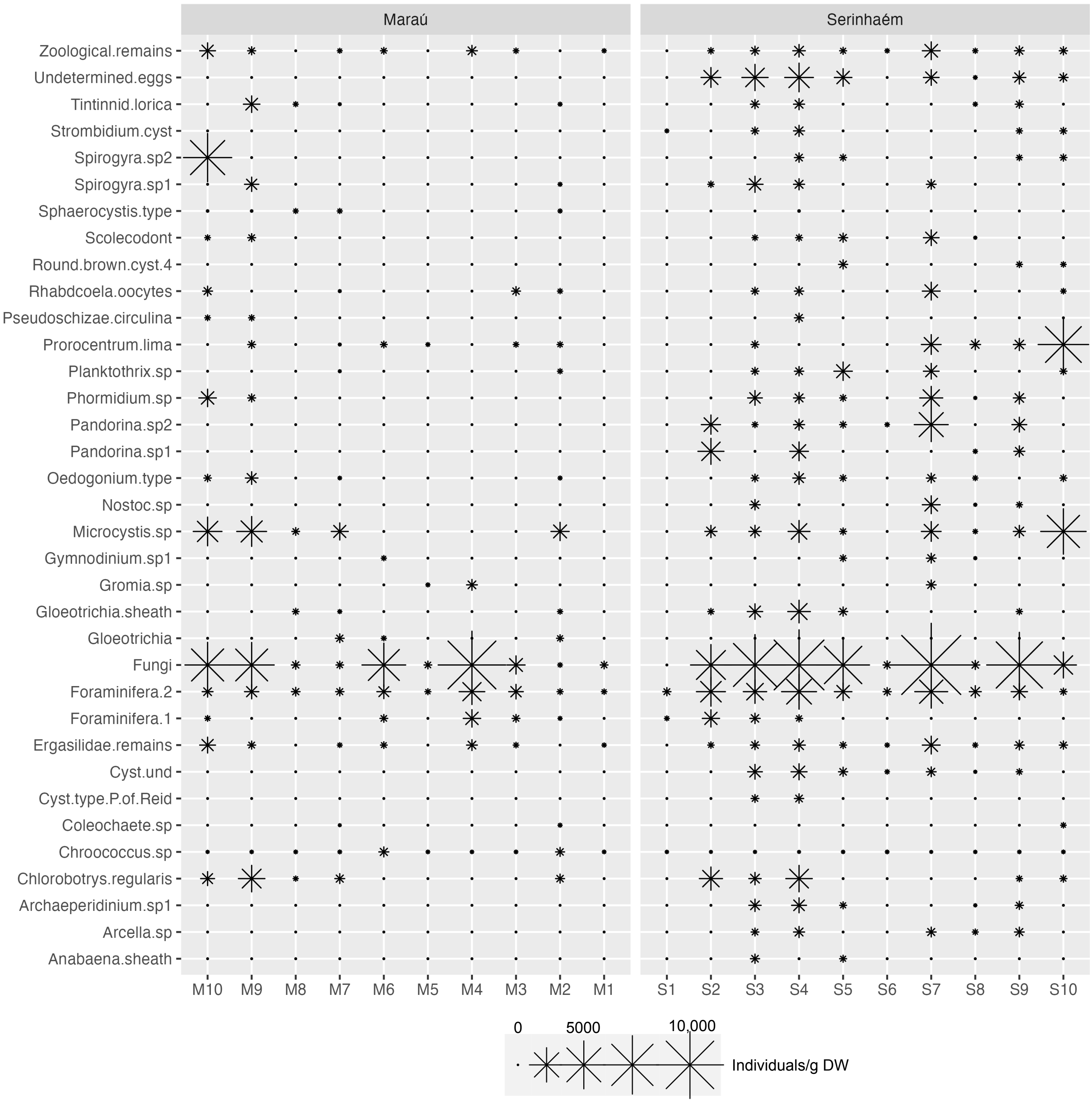
Ecology: Sapotrophic fungi may reflect changes in organic matter, and some are used in palynological studies due to their specific occurrence in specific environments (Shumilovskikh et al., 2021). Carboniculous fungi are used as indicators of fire, given their preference on burned substrates (Shumilovskikh et al., 2021).

Occurrence: Present in the entire estuary mostly in high concentrations, except in the marine station of Serinhaém river (S1). Peaked up to 7000 spores/g in the middle section of both rivers (M4 and S4).

3.2 *THE NPP ASSEMBLAGES*

The concentration of NPP ranged from 0 to 11,080 specimens per gram. Fungal spores were the most abundant group, reaching more than two thousand spores per gram in ten of the twenty stations. The relative contribution of fungal spores reached a maximum of 81% in the middle of Maraú (M4 and M6) and 74% upstream Serinhaém River (S9). Cysts and theca of dinoflagellates were mostly observed in low concentrations, except *Prorocentrum lima* that reached up to four thousand cells per gram in the upstream station of Serinhaém River (S10). Dinocysts dominated in the upstream assemblage of Serinhaém (46% at S10, 25% at S8). Among the green algae, the genus *Pandorina* predominated in the group of chlorophytes reaching maximum 1600 coenobias/g in the downstream stations of Maraú River and *Spirogyra* in the group of charophytes reaching up to 3600 cells/g. This group reached its maximum at the upstream section of Maraú River (40% at M10). Cyanobacteria represented 72% of the assemblage in the downstream station M2 and 61% in the intermediate station M7, whereas its relative abundance in Serinhaém never exceeded 10%, except at S10 (37%). *Microcystis* sp. and *Phormidium* sp. were the most abundant representatives, with a maximum concentration in the upstream section of Serinhaém River (> 3000 cells/g at S10). Foraminiferal linings of the planospiral and trochospiral types dominated in the middle of both rivers (951 linings/g at M4 and 1830 linings/g at S4), however their relative abundance reached maximum of 86% in the downstream-most station M1, and minimum of 1% and 2% in the upstream-most stations of both rivers (M10 and S10). Ciliates were also more abundant in the middle of Serinhaém River. Ciliates, testate amoebozoans and scolecodonts contributed punctually to the assemblage, reaching maximum relative abundance of 9%, 5% and 2%, respectively. Copepod eggs and several body fragments reached maximum concentrations in the upstream stations M10 (~300 fragments/g) and S7 (~430 fragments/g), representing relative abundance of 21% in the downstream station of Maraú.

Table 2: Distribution of NPP taxa alongside Camamu Bay.



3.4 *ENVIRONMENTAL CHARACTERISTICS*

The main rivers composing the estuary presented contrastive characteristics although being adjacently located. Salinity ranged from 3 to 29 psu in Maraú River and from 8.5 to 35 psu in Serinhaém River, the minimum was observed upstream the Maraú River (station M10, 3 psu) and the maximum was observed in the downstream station of Serinhaém (S1, 35 psu). The surface temperature ranged from 25.2ºC to 26.7ºC, with the stations from Serinhaém being one degree colder than those from Maraú. The pH ranged from 6.49 to 7.4. The downstream stations M1 and S1 were the deepest (~11m both), whereas the upstream stations were the shallowest, reaching 60 cm at M10 and 80 cm at S10. The minimum depth of 50 cm was recorded in the middle of Serinhaém River, at station S4. Gravel and medium sand were the predominant sediment type alongside Maraú, whereas Serinhaém was mostly composed of mud and medium sand.

The nutrient concentrations were higher in Serinhaém river compared to Maraú (Fig. 2). Total phosphorus ranged from 0.2 to 2.95 µM decreasing upward throughout Serinhaém River and from 0.70 to 1.41 µM in Maraú. Total nitrogen varied discrepantly between both rivers, ranging from 9.6 to 52 µM alongside Serinhaém and from 6.2 to 26.7 µM alongside Maraú. Consequently, the stoichiometry between phosphorus and nitrogen, the N:P ratio, was also contrastive between the rivers, fluctuating between 5 to 27.6 µM in Maraú and from 14 to 136.2 µM in the stations from Serinhaém, where it increased drastically upstream. Dissolved inorganic nitrogen (the sum of nitrate, nitrite and ammonium ion) ranged from 4.6 to 13.6 µM in Maraú and from 2.5 to 38.6 µM in Serinhaém, with the maximum concentration in the middle of Serinhaém River (S5) and the minimum in the marine section of the same river (station S1). Silicate was distributed similarly between the rivers varying from 2 to 15 µM decreasing towards the open ocean. The organic matter loss on ignition ranged from 0.025 to 0.096 g IG/Kg (grams per ignited kilogram) along Maraú and from 0.021 to 0.075 g IG/Kg along Serinhaém River.

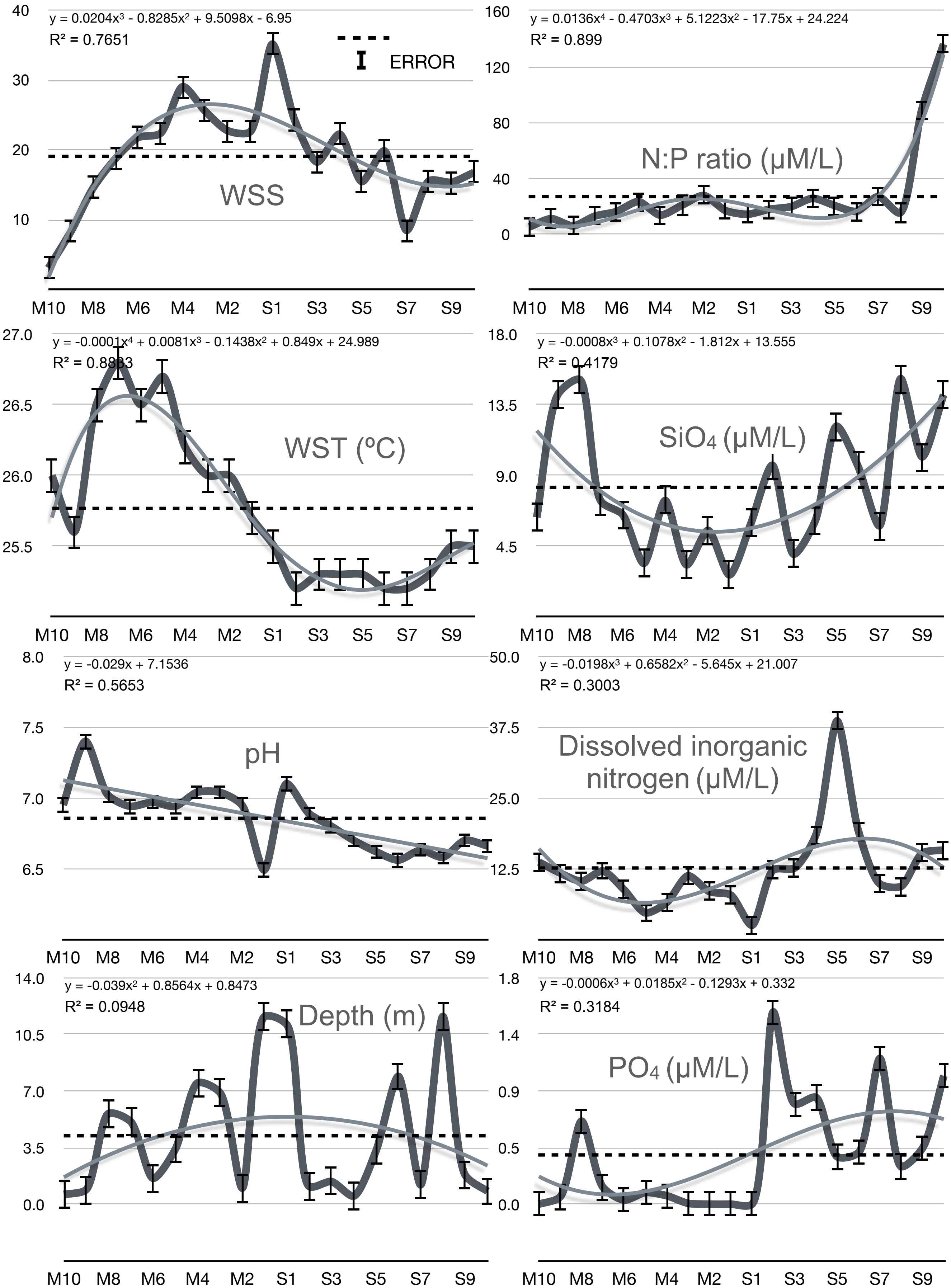


Figure 2: Spatial variation of environmental variables and nutrient concentration alongside Camamu Bay. The thiner trendline was adjusted based on polynomial curves, except for pH (linear). The equation and R^2 are shown in the superior left side.The dash line is the average.

SPATIAL DISTRIBUTION AND LOCAL CHARACTERISTICS

The Canonical Correlation Analysis performed on the entire set of NPP and environmental variables explained 61% of the variability in species data taking into consideration the variable with a variance inflation factor (VIF) of more than 10. The results indicate that an inflation may lead to meaningless interpretation of the results. The forward selection method salinity, total nitrogen, total phosphorus, N:P ratio, silicate, pH and sediment type as the best set of variables to explain species distributions, based on maximum likelihood (AIC) algorithm criteria. On this basis, the selected set of variables explains 67% of the variability in species data (R^2_{adj} = 0.667, p = 0.001, after 999 permutations) without inflation in the model.

Table 3: Parameters used to select the variables according to forward selection algorithm.

Variable	AIC	F	Significance	VIF (all variables)	VIF (forward selected)
Silicate	182.43	2.55	0.017	20.4	3
Sediment type	180.06	2.60	0.008	21.6	6.8
N:P ratio	178.25	2.93	0.010	43.1	12.3
Nitrogentotal	176.94	2.34	0.032	119.2	9
pH	175.47	2.27	0.047	40.2	3.5
$Phosphorus_{total}$	172.72	2.94	0.038	137.1	15
Loss on ignition	169.34	3.08	0.032	18.9	3.4
Salinity	167.15	2.09	0.082	54.4	6.9
Temperature	168.31	1.47	0.170	48.5	Not selected
Depth	168.35	1.45	0.185	129.3	Not selected
Inorganic Nitrogen	169.43	0.90	0.409	32.8	Not selected
Orthophosphate	169.35	0.94	0.411	86.5	Not selected

AIC = *Akaik* information criteria; F statistics = variance of the group means; VIF = Variance Inflation Factor.

The ordination biplot scaled by species (Figure 3) shows all the NPP taxa within the positive margins of both axis, except *Microcystis sp.* It is possible to notice the gradient limits of Serinhaem River represented by the first and last stations oppositely displaced in the axis 1, as well as the stations of Maraú River displaced in the axis 2. Most of the taxa are grouped in the marine section of the estuary, such as some dinoflagellate cysts, scolecodonts, some algae and zoological remains. *Prorocentrum lima* complex and *Coleochaete* sp appears in the opposite side of the gradient in the first axis, associated to the upstream site of Serinhaém (S10), whereas *Spirogyra* sp, appears in the opposite side of the gradient in the axis 2, associated to the upstream site of Maraú River (M10).

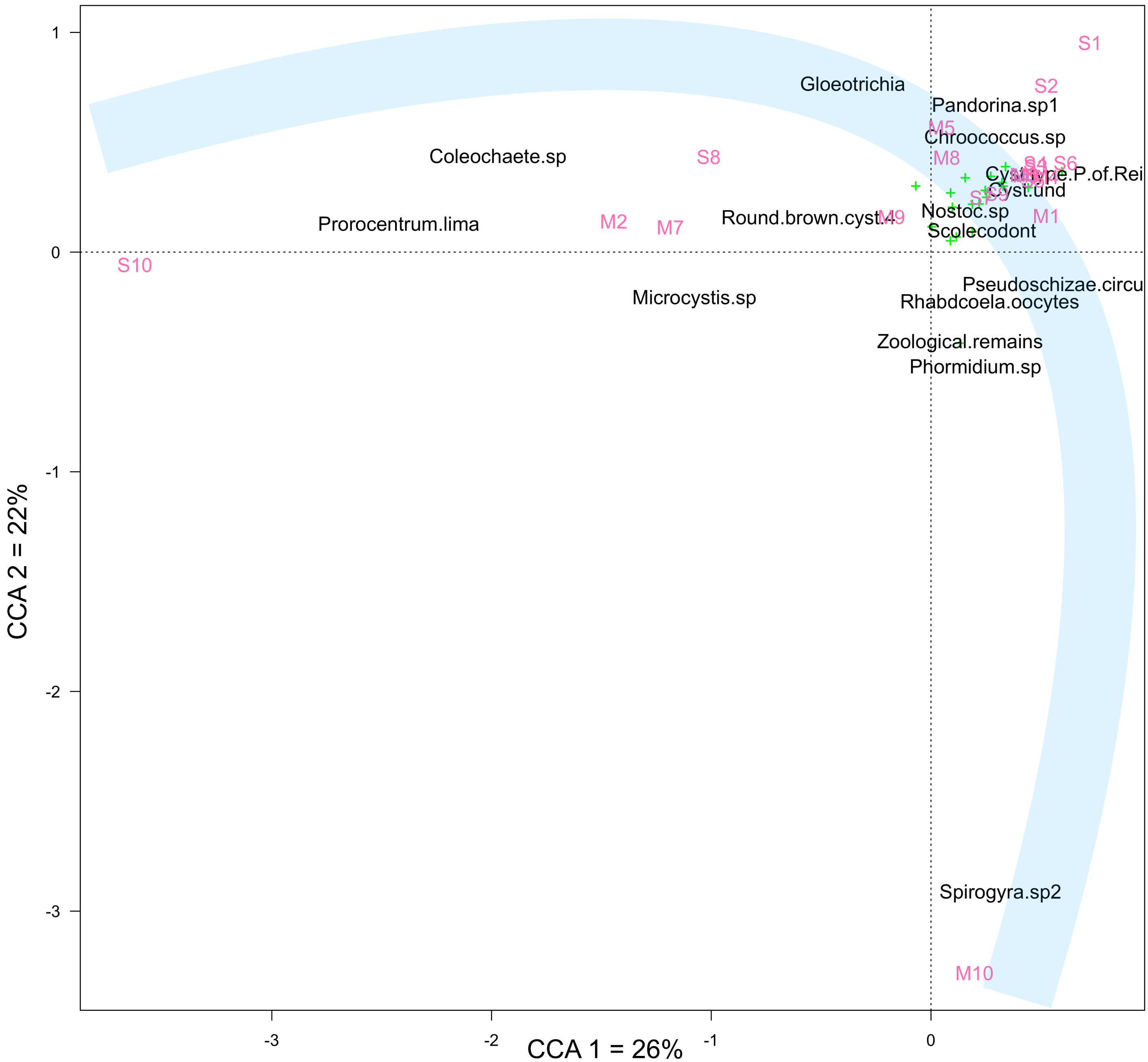


Figure 3: Ordination plot from Canonical Correspondence Analysis showing the distribution of the NPP taxa alongside sampled sites. The gradient was highlighted in blue.

The ordination biplot with the forward selected variables scaled by sites (Figure 4) shows the same arrangement of the gradient. On axis 1, it is possible to see the intermediate and upstream stations of both rivers in the negative side associated to the nutrient gradient. The upstream-most stations S10 and M10 appears in the negative side of the axis 2, that seems to be the gradient associated to salinity, pH and organic matter loss on ignition. The upstream site of Serinhaém river (S10) was grouped on the right superior quadrant representing the silicate and the N:P ratio gradients associated to the charophyte *Coleochate* and the toxigenic epiphytic dinoflagellate *Prorocentrum lima*. Scolecodonts, zoological remains, Rhabdocoelan oocytes, *Spirogyra* sp1, *Anabaena* sheaths and *Pseudoschizae circulina* were grouped more likely to be found on middle sand sediments and mesohalyne salinity. The filamentous *Spirogyra* type was grouped with the upstream-most station of Maraú (M10) suggesting its freshwater preference. Both foraminiferal lining types were grouped close to each other associated to marine stations, increased values of organic matter and other NPP such as ciliates and testated amoebozoans. The only variables in the negative threshold were the sediment type sand and the organic matter loss on ignition.

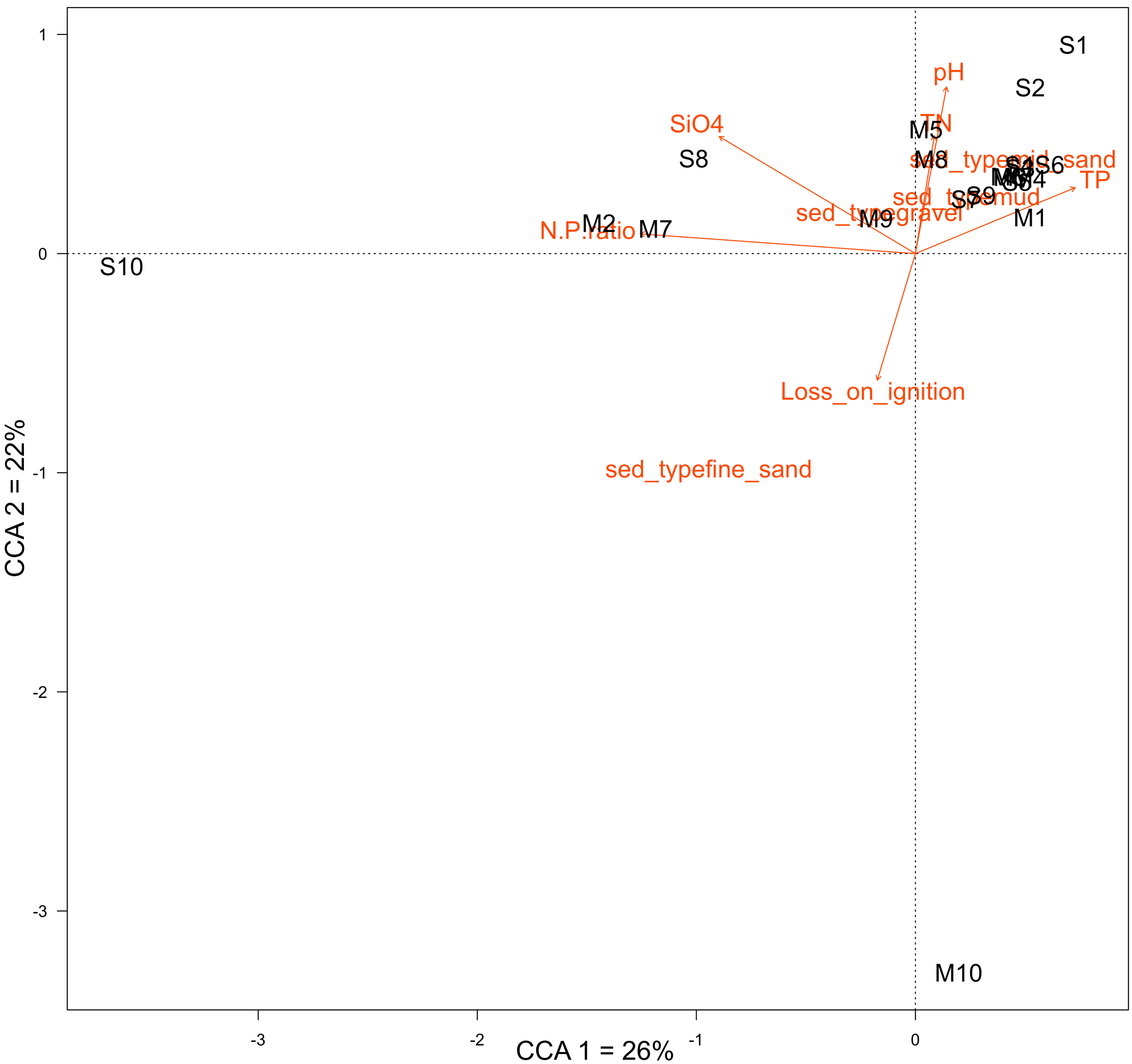


Figure 4: Ordination plot showing the scores of the forward selected variables.

In general, the NPP taxa presented low correlations regarding the set of environmental and nutritional variables (Figure 5). The highest correlations yielded *Pandorina* sp1 and total phosphorus (0.69), foraminiferal linings and orthophosphate (0.68), *Pandorina* sp1 and total nitrogen (0.73), undetermined eggs and total nitrogen (0.71), *Planktothrix* sp. and dissolved inorganic nitrogen (DIN, 0.69), round brown cyst and DIN (0.83), *Microcystis* sp. and N:P ratio (0.74), *Coleochaete* sp. and N:P ratio (0.72), *P. lima* and N:P ratio (0.85).

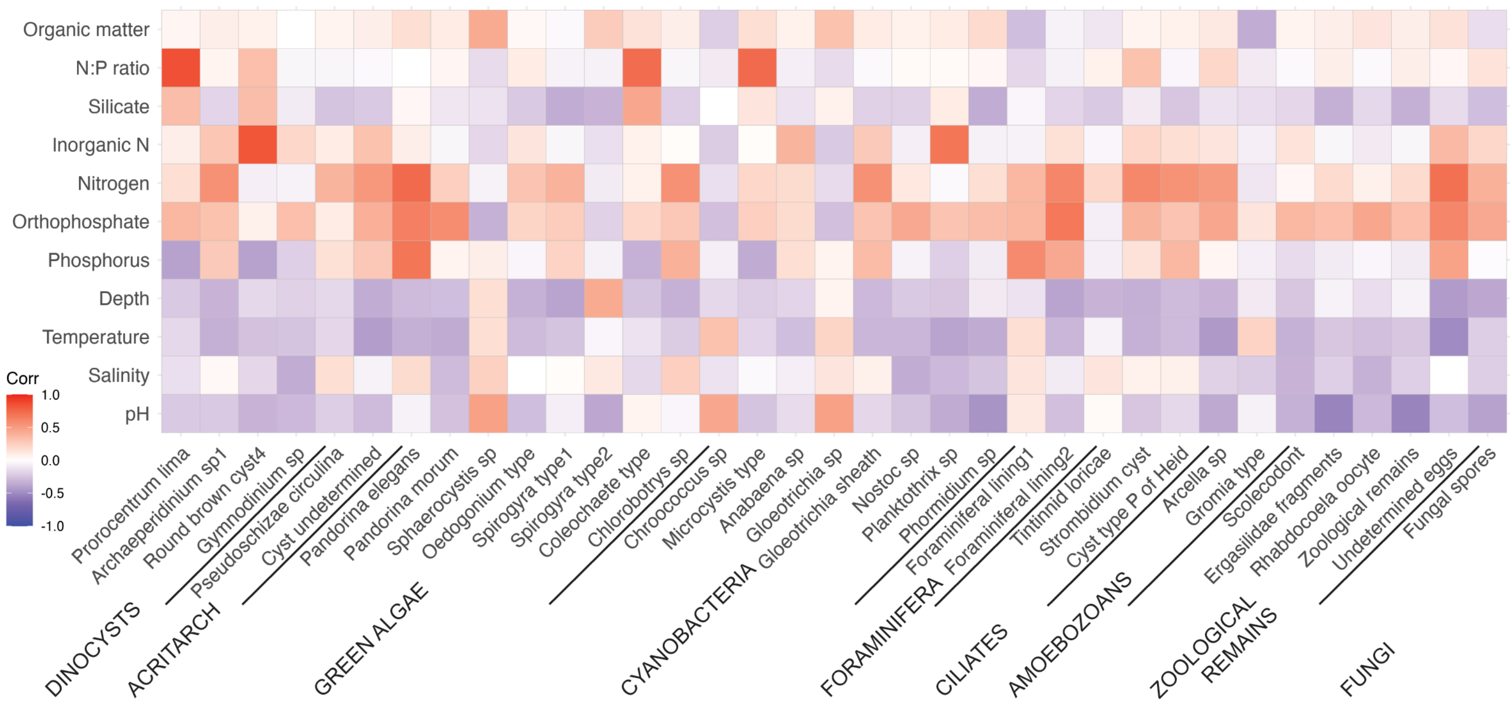


Figure 5: CCA biplots constrained by species (left) and by sites (right). On the bottom, the correlation matrix between environmental variables, nutrient concentrations and the NPP. The scale on the right represent the Pearson`s correlation coefficient.

4. DISCUSSION

In this study we analyzed up to 20GB of information including digital images of more than a hundred non-pollen palynomorphs of several groups distributed alongside 20 sampling sites covering a transect of 64 km along the main rivers of the third largest Bay of Brazil. The spatial resolution information presented herein on local responses of the non-pollen palynomorphs constrained to environmental gradients and nutrient concentrations represent a contribution to the micropaleontogical knowledge of the region, as well as it represents a novelty for the South Atlantic.

The non-pollen palynomorph assemblages described in this study encompassed organisms from 14 Phylum. Among them, we identified 25 dinocyst taxa belonging to the order Prorocentrales, Dinophysiales, Amphidiniales, Gonyaulacales, Peridiniales, Gymnodiniales. This group represented the highest number of species, however, 18 of them were rare, occurring only in one of the twenty sites and excluded from the ecological analysis. Some vegetative stage forms were observed, such as the tychoplanktonic *Prorocentrum rhathymum,* the benthic *Cabra Matta* and the epiphytic toxigenic *Prorocentrum lima* that dominated the assemblage from the upstream section of Serinhaém River (up to 4000 cells/g). This species complex resisted the chemical treatment during the palynological preparation, including digesting acids, sieving and sonication, representing the first report of vegetative or non-cyst forms in palynological slides based on surface sediment to the best of our knowledge. We dedicated a separate publication to highlight this unusual report of sand dwelling *Prorocentrum lima* that is morphologically dissimilar compared to other peer-reviewed descriptions for the South Atlantic and Caribbean waters. High abundances of photoautotrophic dinoflagellates are expected to be found in shallow coastal areas due to stability, light and nutrient conditions (Radi et al., 2007). The heterotrophic dinoflagellate *Brigantedinium* sp. occurred in low abundances in the upstream sites of Serinhaém river S9 and S10 and *Parvodinium* sp. occurred at S8 (215 cysts/g dw). Marine environments and heterotrophic dinocysts are usually associated, since they are widely used as proxies of sea surface salinity and temperature, ice cover and productivity (de Vernal and Marret, 2007). Those upstream sites of Serinhaém presented salinity values of 15 PSU, which is high compared to 3 PSU in the opposite riverine section (M10). The site S7 was less salty than the upstream-most sites S10 and S9. This represents a rupture in the gradient which we hypothesize it is related to the drainage of a secondary river transporting more freshwater to a certain point of the main river that is less affected by the tide circulation. A round process-based thick wall cell was found abundantly distributed in Serinhaém River, however no identification was possible to be attributed due to no information in available described species (Plate IV, fig. 62-66). Except for the pores on the surface, the thick cell wall and the visible structures resemble the live pellicle resting cyst of *Alexandrium taylori* Balech, originally documented to encompass a thickened wall, greyish granular content and flattened shape after some months of culturing (Bravo et al., 2006). *Pyrophacus horologium* was observed in the surface sediment of the coastal zone of Serinhaém river. This same species was also documented in a planktonic survey conducted in the riverine section of Maraú (da Silva Nunes et al., 2019). Dinocysts are documented to be less abundant in tropical estuarine systems due to the high sedimentation rates and turbulence conditions, which is a matter of investigations over the five last decades (Furio et al., 2006; Baula et al., 2011). In surface sediments of the tropical Kadan Island, Myanmar a similar pattern regarding low concentrations of dinocysts was described associated to increased sediment loads during the rainy season that leads to high sedimentation rates (Matsuoka et al., 2018). The low concentration of photoautotrophic or mixotrophic organisms were also associated to reduced irradiance when sediments are abundantly transported via fluvial enrichment during raining seasons (Maung-Saw-Htoo-Thaw et al., 2017). In addition, chemical components within the sediment are known to inhibit preservation in mangrove environments (Furio et al., 2006; Baula et al., 2011), such as Camamu Bay that is 40% composed of mangrove vegetation.

The chlorophytes were generally round to elliptical coenobias with varying green round individuals, e.g. *Pandorina* sp. (Plate V, fig. 67). Charophytes and

Phragmoplastophytes varied from moon-shape elongated filaments to mirrored desmid symmetric shapes, such as *Staurastrum anatinum* (Plate V, fig. 101). The filamentous algae *Spirogyra* sp. was the most abundant green algae reaching 3.592 cells/g dw in the upstream site of Maraú river (M10), where the relative abundance of green algae was also the highest (40%). The acritarch *Pseudoschizeae circulina* (Plate V, fig. 90) of possible assignment within the green algae was observed in the middle section of Maraú river (M5), upstream Serinhaém (S9 and S10) and it has been documented in non-palynologic samples from the Doce river, Espirito Santo (Ferrazzo et al., 2008), sedimentary profiles collected in Rio Grande do Sul (Roth and Forscheitter, 2016) and surface sediments from a lagoon of Rio de Janeiro (Misumi et al., 2018) suggesting a wide distribution in different climate regimes and habitat structures. Green algae are mostly represented in palynological surveys by species that were not observed herein, such as *Pediastrum*, *Tetraedron* and *Botryococcus* (Batten, 1996; Shumilovskikh et al., 2021). The abundance and frequency of Desmidiales representatives such as *Cosmarium sp*. and *Staurastrum anatinum* in our samples were low. This group is more likely to be found in freshwater lakes and peat bogs when studied as NPP microfossil for paleoecological purposes (Shumilovskikh et al., 2021), generally indicating human impact on lagoons (McCarthy et al., 2018). The filamentous algae *Spirogyra sp.* was the most abundant green algae in the upstream site of Maraú river (M10) with up to four thousand cells per gram, where the relative abundance of green algae was also the highest (40%). This finding in the most freshwater site of Camamu Bay corroborates the shallow, oxygen-rich and stagnant conditions where Zignematales representatives are documented to inhabit (Johnson, 2002). The relative abundance of green algae was also high downstream Maraú river, which is possibly related to the discharge of Orojó River, the upsampled river of the middle part of the estuary transporting the organic content to the sites located in Maraú by advection or local circulation influenced by the tide dynamics.

Cyanobacteria represented 71% of the total assemblage in two samples from Maraú. The most abundant representative was the neurotoxic and hepatotoxic bloom-forming *Microcystis* sp. with 3.169 coenobias/g at S10, comprehending different morphospecies generally round, with individual green cells varying in shape and size. Transparent akinetes of the *Gloeotrichia* sp. were abundant in the middle section of Serinhaém (S5, 723 akinetes/g dw), only observed as filaments instead of the entire colony. These forms were formerly described as rapid-growth, bloom-forming and toxic (see HdV-146 and Hdv-601, Van Geel et al., 1989), which are considered to be proxy of phosphate-eutrophication in lakes associated to fertilization use (Van Geel et al., 1994). Other less abundant cyanobacteria included the filamentous photosynthetic, terrestrial and aquatic *Nostoc* sp., the filamentous Phormidium sp. with trichome caliptrate (Plate VI, fig. 125), the bloom forming freshwater *Planktothrix* sp. and *Tolypothrix* types. The cyanobacteria remains of this study were generally in the form of akinetes and sheaths, mainly dominated by *Gloetrichia* sp. and *Anabaena* sp.

The concentration of planoespiral or trochoespiral foraminiferal linings represented 82% of the assemblage in the downstream section of Serinhaém River (S1), confirming the marine preference and local response of these organisms. Paleoforaminifera are marine organic biogenic producers used as proxy of $CaCO_3$ dissolution (de Vernal et al., 1992), marine and brackish deposits (Matsuoka and Ishii, 2018, Stancliffe, 1989, Antunes e Melo, 2001). Rhabdocoelan oocytes were recorded in our samples, more abundantly distributed in the freshwater parts of both rivers. This NPP type was described as HdV-353 (van Geel et al., 1981) and it is documented to be a good proxy for paleoenvironmental reconstructions due to its specific preference to certain habitat conditions (Shumilovskikh et al., 2021). Among the fragments of copepod, we identified eggs and spermatophores usually used as indicators of the presence of open waters (Prager et al., 2012, Mudie et al., 2021).

The stoichiometry of N:P was the main gradient explaining the variation on non-pollen palynomorphs concentrations in Camamu Bay, according to the CCA results. Variations in N:P ratio are associated with synthesis or decomposition of organic matter and are documented as key inorganic elemental sources on phytoplankton biogeochemistry and productivity (Redfield, 1956; Falkowski, 2000). From the principal concept of a 16:1 threshold for N:P stoichiometry arose the characterization of phosphorus and nitrogen limitation in aquatic ecosystems (Redfield, 1956) and later for ocean and coastal environments (Falkowski, 1997) where N-fixation and denitrification are hypothesized to regulate N:P ratio modulated by phytoplankton composition (Redfield, 1958; Falkowski, 2000). In estuarine systems phosphorus is assumed to be limiting for algal growth in the riverine freshwater section, as well as nitrogen in the marine section associated to coastal removal of nitrogen or denitrification (Redfield, 1958; Howarth and Marino, 2006). In our sites the increase in N:P ratio was recorded in the upstream section of Serinhaém river, where most of the dinoflagellates were found, in addition to toxic bloom-forming N-fixing cyanobacterias such as *Microcystis* sp., *Phormidium* sp. and *Nostoc* sp. Lower amounts of N-fixing cyanobacteria are associated to nitrogen limitation and removal in marine systems (Howarth and Marino, 2006; Tamminen and Andersen, 2007), so we argue that the Cyanobacteria assemblages of Camamu Bay responded to local nutrient stoichiometries and thus represent potential value as environmental indicators even being receiving less attention in palynological studies. Therefore, the sites where the N:P ratio reached massive values higher than 100:1 indicate phosphorus limitation and nitrogen enrichment possibly associated to the anthropogenic pressure. The main town located in the margins of the upstream section of Serinhaém river (Ituberá, population 21.913, effluent discharge of 18.08 m^3/s, 36% of sewage treatment before dump). Increasing nitrogen enrichment may lead to eutrophication (Boesch, 2000), which we address as a concern not only for the ecosystem balance and human health, but also to the economic activities around fishing and tourism (Costanza et al., 1997) that local communities are dependent on. Contrastively, in most sites of the Maraú river, specifically in the mixing zone, the N:P ratio was inferior than 16:1 indicating either elements were limiting at the time they were sampled. Silicate was also indicated as a significant response variable in the same direction of N:P ratio and higher values in the upstream sites of both rivers. This nutrient is known to limit diatoms (Capellaccii et al 2012) but not dinoflagellates growth and it is usually integrated to the Redfield ratio as N:P:Si on studies with natural phytoplankton assemblages (Redfield, 1956). It has been demonstrated that diatom blooms can benefit dinoflagellates due to post-bloom nutrient dynamics (Cloern et al., 2005, Matsubara et al., 2008, Du et al., 2011). High abundance of diatoms also implies increased prey availability for heterotrophic dinoflagellates, so it would be reasonable to associate the silicate concentrations with an indirect effect on the dinoflagellate community. Although not considered in our analysis diatoms were observed in some sites, reaching 1.614 frustrules/g dw of the penate morphotype at S7 and 23,280 empty sheath-like cf.*Diploneis* sp. at M10. Hence, the forward selection method indicated silicate as a significant variable explaining the NPP distribution after 999 permutations with a gradient in the same upward direction as the N:P ratio and associated with fine sediment fraction.

Finally, the distribution of the non-pollen palynomorphs from Camamu Bay responded to the spatial variation of hydrological conditions and nutrient stoichiometries and may be used on further paleoecological reconstruction of the area from a core collected in the same occasion of the samples analyzed herein. There are also several morphotypes unknown that were not found within the available literature, such as the spherical, thick wall and reduced processes abundant in Serinhaém river.

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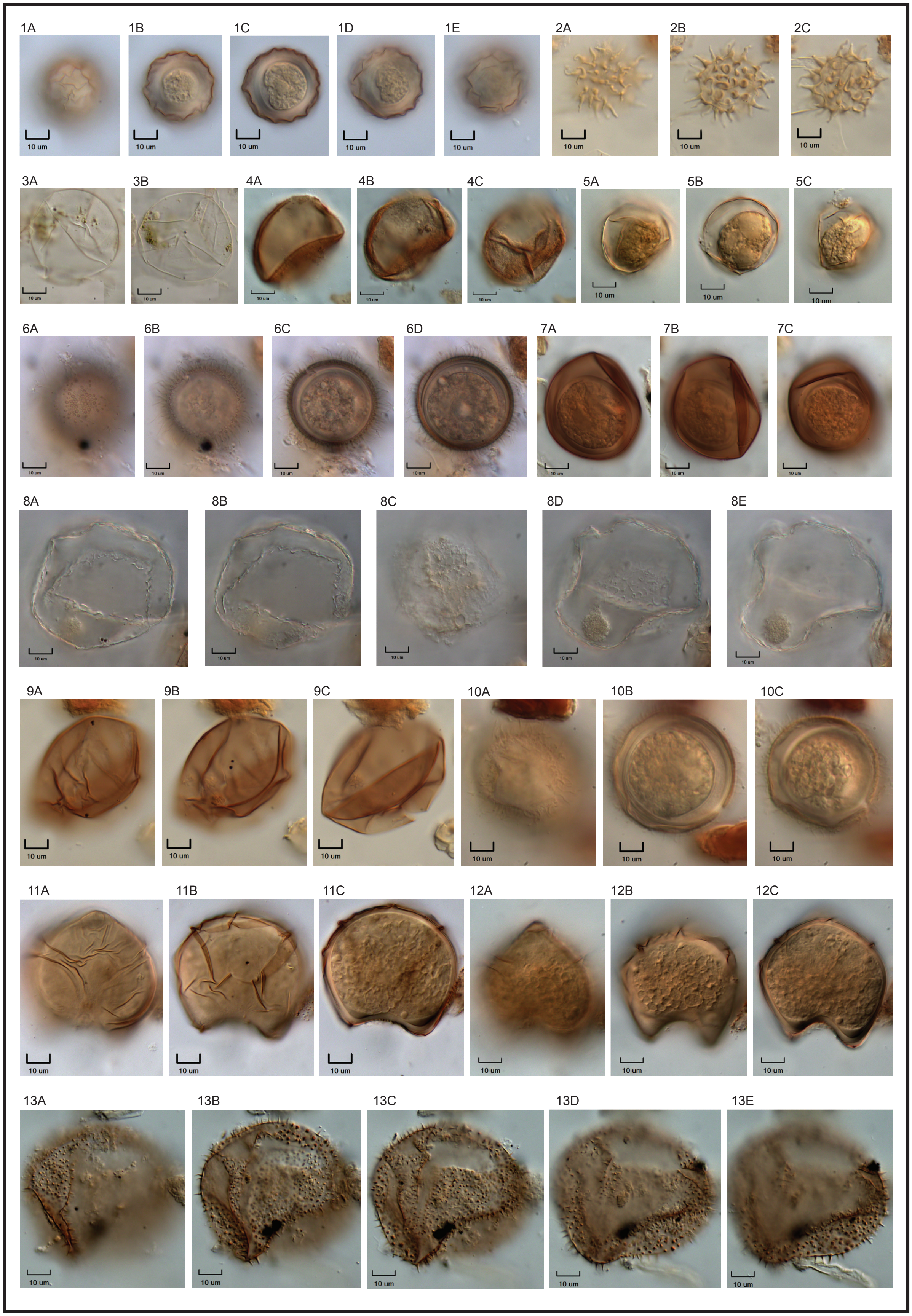
APPENDIX

Appendix 1: Plates of the NPP taxa of Camamu Bay.

Plate I



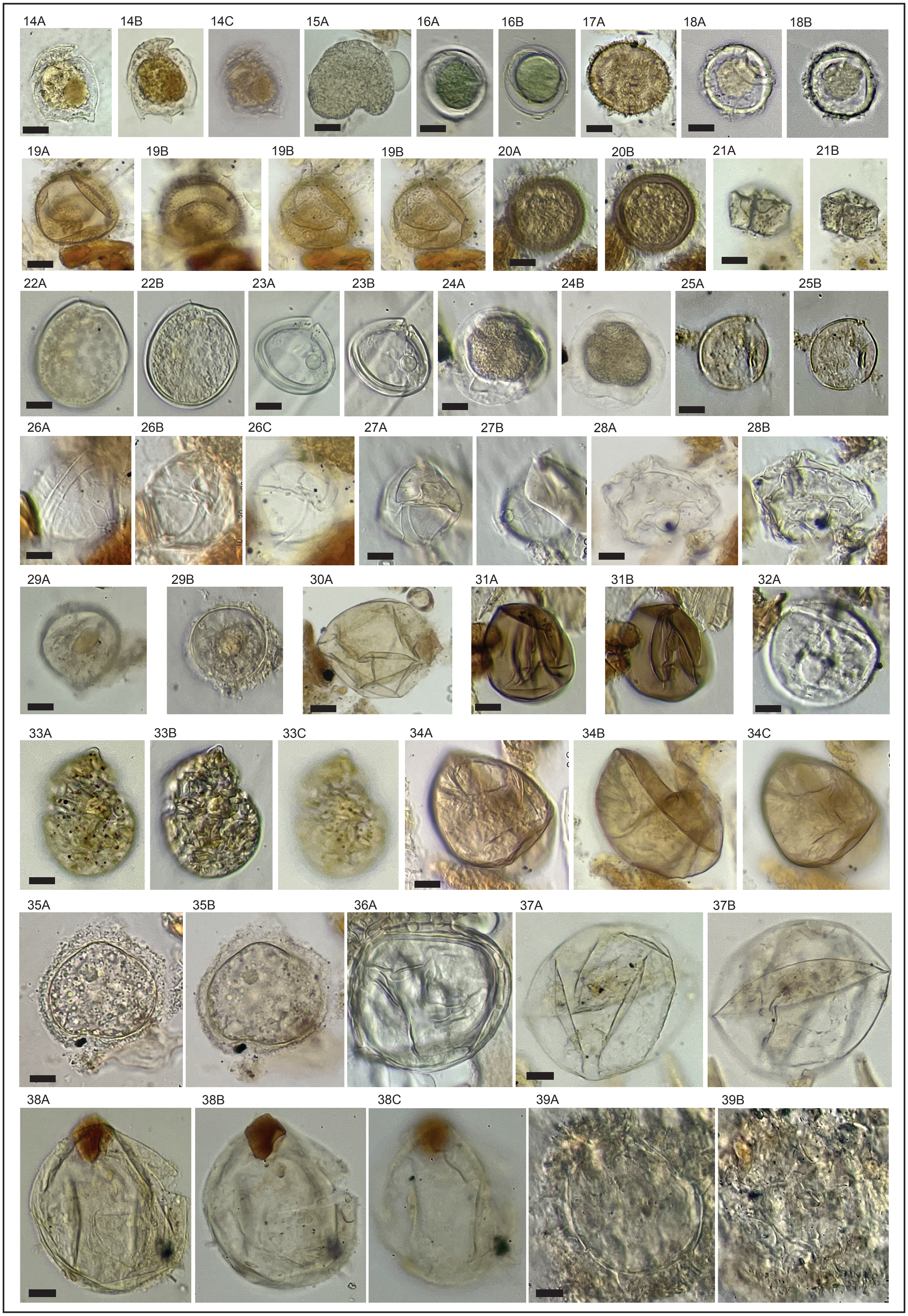

14A
14B
14C
15A
16A
16B
17A
18A
18B
19A
19B
19B
19B
20A
20B
21A
21B
22A
22B
23A
23B
24A
24B
25A
25B
26A
26B
26C
27A
27B
28A
28B
29A
29B
30A
31A
31B
32A
33A
33B
33C
34A
34B
34C
35A
35B
36A
37A
37B
38A
38B
38C
39A
39B

Plate III

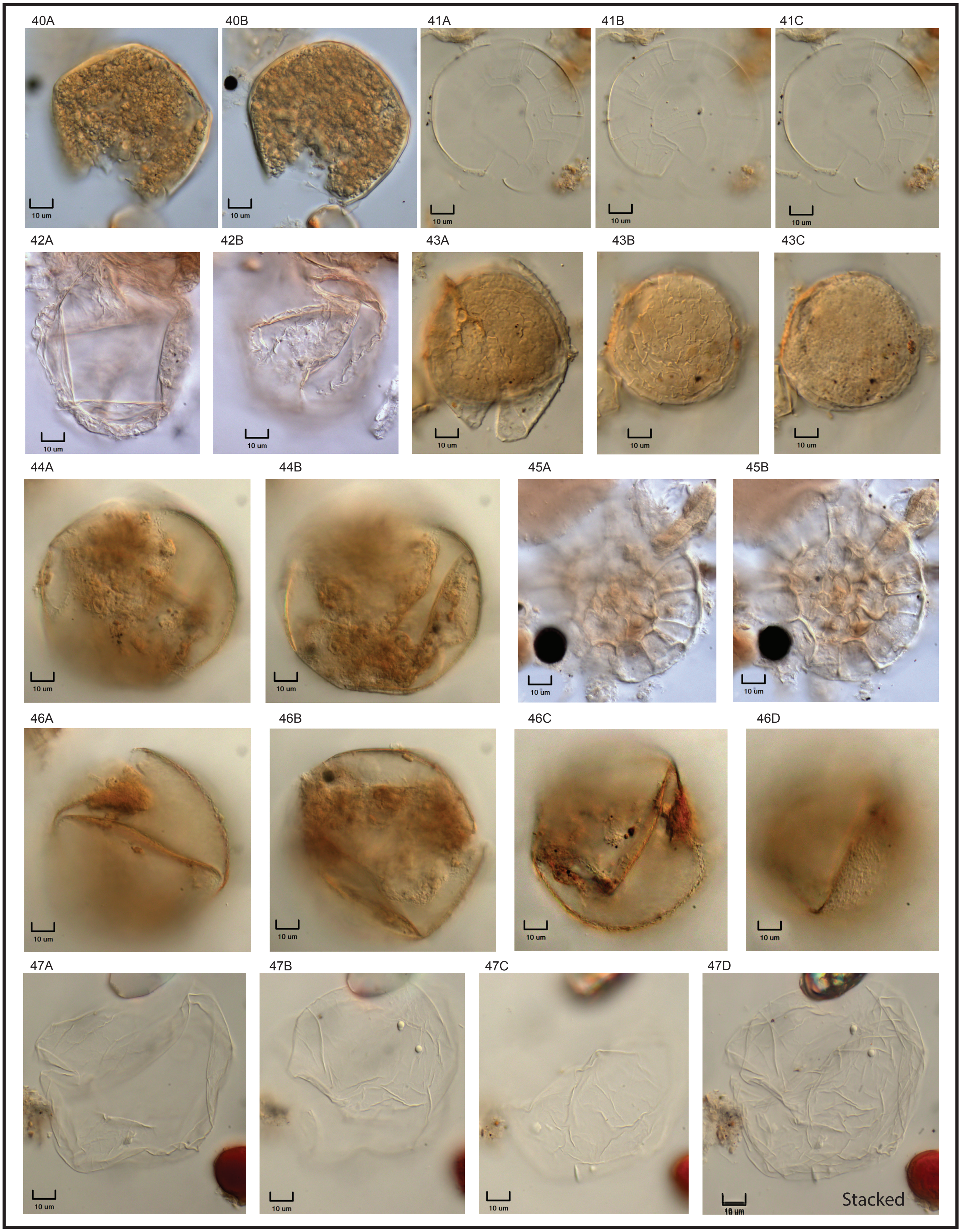
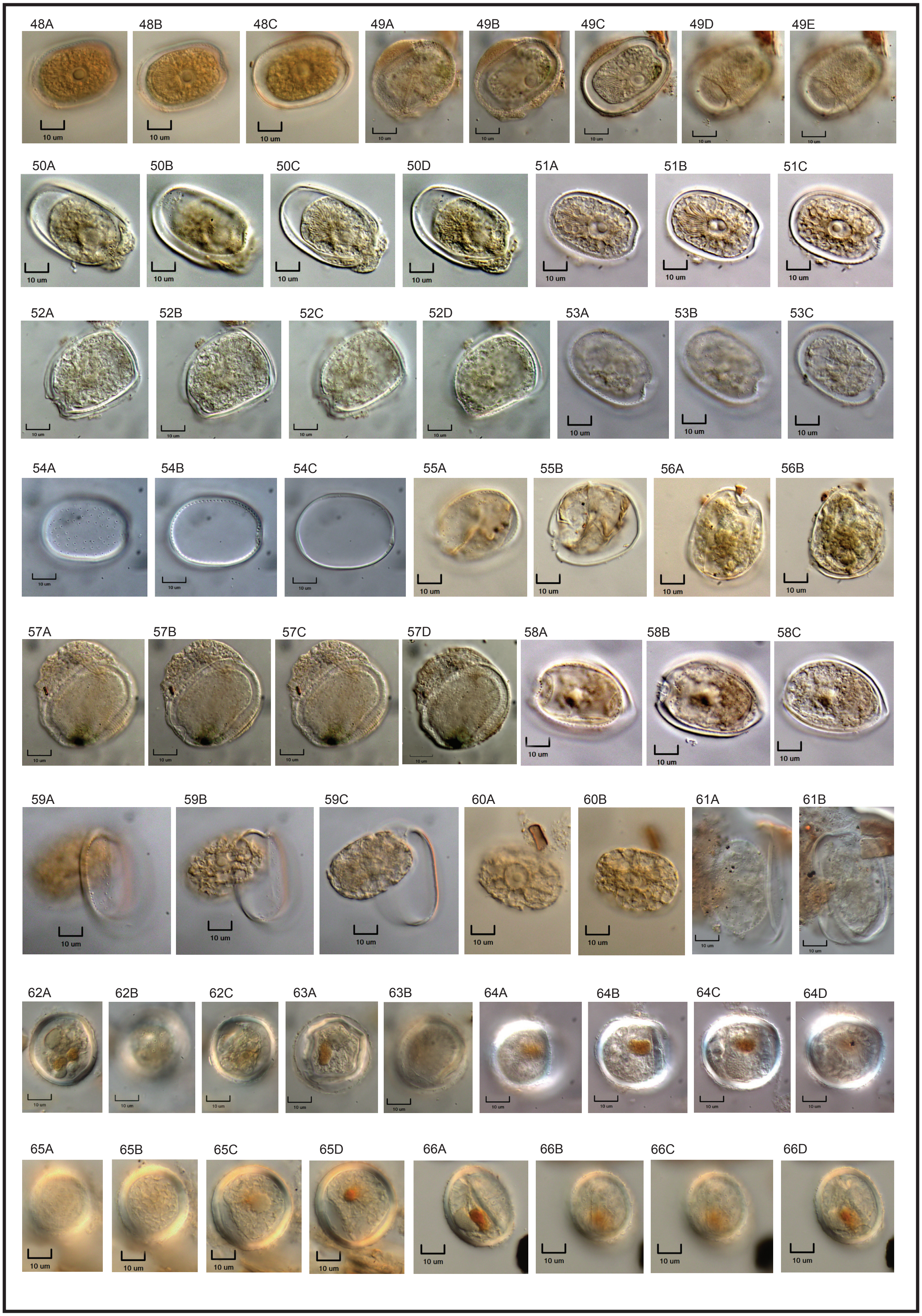

40A
40B
41A
41B
41C
42A
42B
43A
43B
43C
44A
44B
45A
45B
46A
46B
46C
46D
47A
47B
47C
47D
Stacked

Plate IV



48A
48B
48C
49A
49B
49C
49D
49E
50A
50B
50C
50D
51A
51B
51C
52A
52B
52C
52D
53A
53B
53C
54A
54B
54C
55A
55B
56A
56B
57A
57B
57C
57D
58A
58B
58C
59A
59B
59C
60A
60B
61A
61B
62A
62B
62C
63A
63B
64A
64B
64C
64D
65A
65B
65C
65D
66A
66B
66C
66D
10 um

Plate V

67A 67B 68A 68B 69A 69B 70A 70B 70C 71A 71B 72A 72B
73A 73B 73C 74A 74B 75A 75B 76A 76B
77A 77B 78A 78B 79A 79B 80A 81A 82A 82B
83A 83B 84A 84B 85A 85B 86A 867 878A
89A 89B 90A 90B 90C 91A 91B
92A 92B 93A 94A 94B 95A 95B
96A 96B 97A 97B 98A 98B
99A 100A 101A 102A 103A 103B
10 um

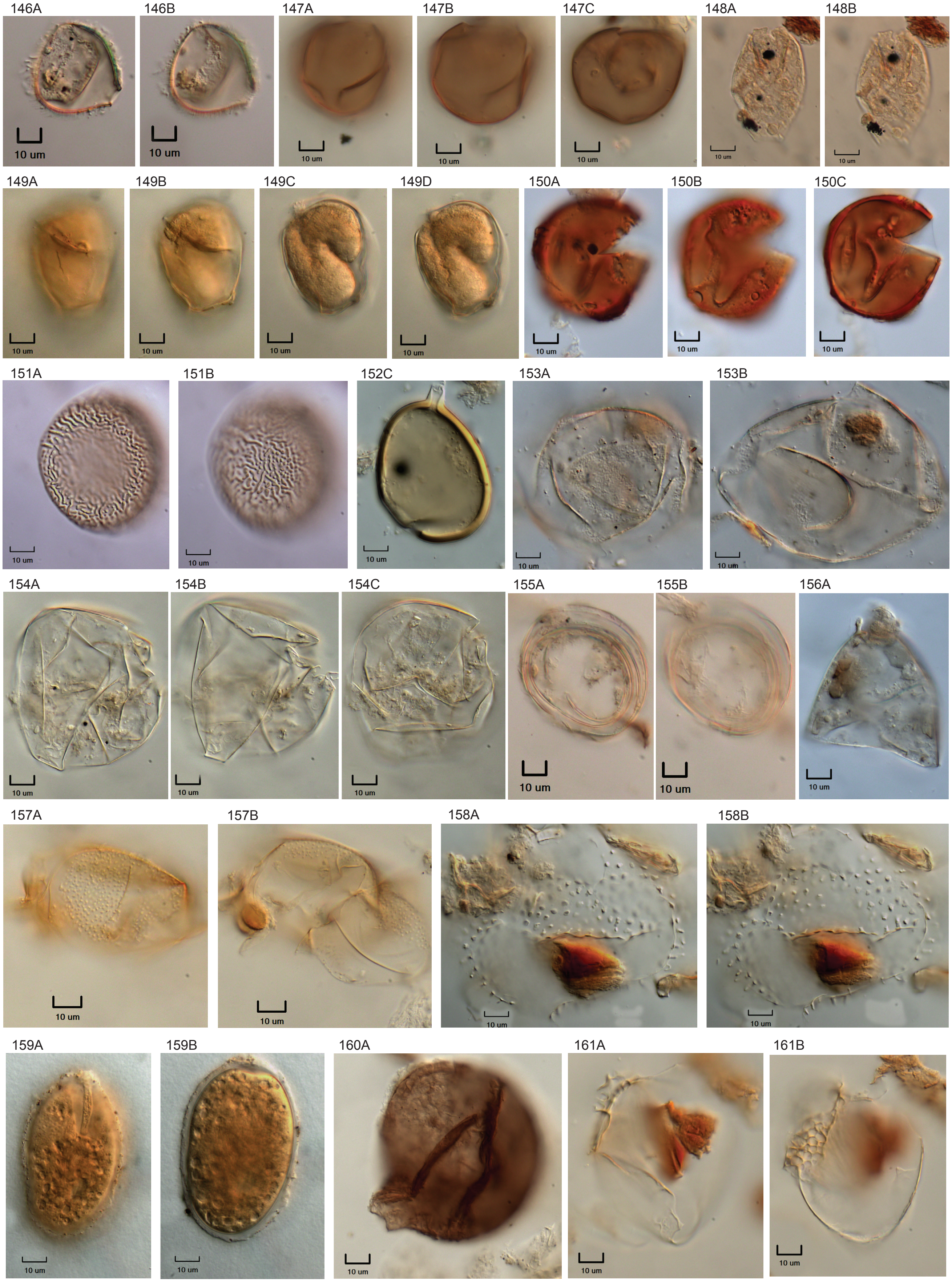
Plate VI


104A
104B
105A
106A
106B
107A
108A
109A
109B
110A
110B
111A
111B
112A
112B
113A
113B
114A
114B
115A
116A
117A
117B
118A
118B
119A
119B
120A
120B
121A
122A
123A
124A
125A
126A

Plate VII


127A
128A
128B
129A
129B
130A
130B
131A
131B
132A
132B
133A
133B
134A
134B
135A
136A
136B
137A
137B
138A
139A
139B
140A
141A
141B
142A
143B
144A
144B
145A
145B

Plate VIII


146A
146B
147A
147B
147C
148A
148B
149A
149B
149C
149D
150A
150B
150C
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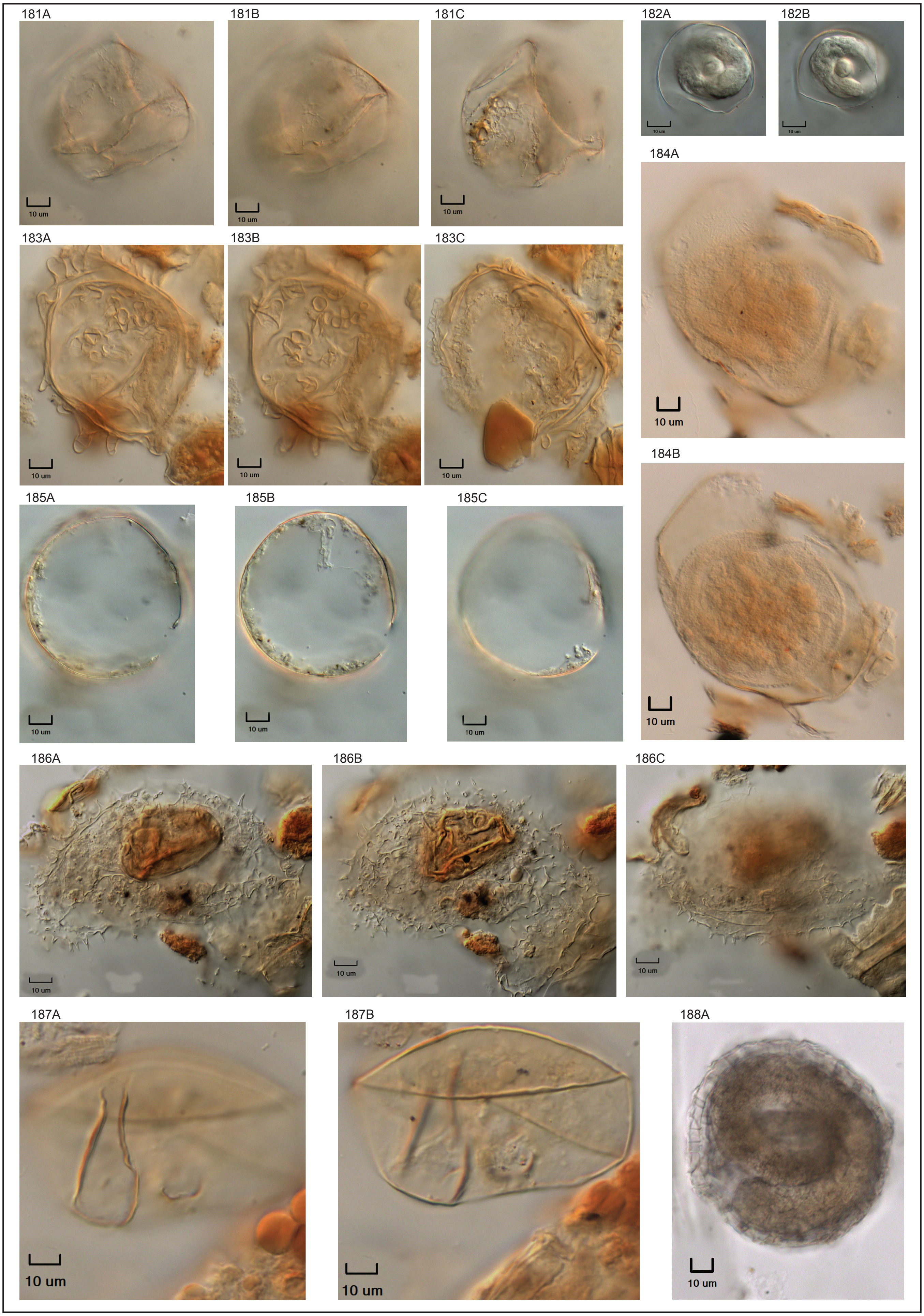
151B
152C
153A
153B
154A
154B
154C
155A
155B
156A
157A
157B
158A
158B
159A
159B
160A
161A
161B
10 um

Plate IX


162A
162B
163A
163B
164A
164B
165A
165B
166A
166B
167A
167B
168A
168B
168C
169A
169B
170A
171A
171B
172A
173A
174A
175A
175B
176A
176B
177A
178A
179A
179B
180A

Plate X


181A
181B
181C
182A
182B
183A
183B
183C
184A
184B
185A
185B
185C
186A
186B
186C
187A
187B
188A
10 um

5. General conclusions

Conclusions and future directions

- The chapters composing this thesis integrate the relationship water column-sediment providing information on processes of both habitat structures;
- The documentation of a harmful algae bloom with high concentrations within planktonic-free foams represents a novelty in terms of substrate descriptions for *Prorocentrum* species, that have been documented to be epiphytic, epilithic, to dwell in intersticial water, sediment, coral reefs but rarely seen on floating foams worldwide and never documented on Brazilian or South American waters.
- The abundant occurrence of the dinoflagellate *Prorocentrum lima* distributed as in the water column as in the sediment assemblages demonstrate an important aspect of the environment in Camamu Bay. The toxigenic potential of this species complex was highlighted in this thesis, so its distribution in high concentrations should be a matter of continuous monitoring, specially in areas of fish and shellfish consumption;
- Our results describing benthic high concentrations of *Prorocentrum lima* complex within palynological assemblages indicates morphological variations among the specimens observed in Camamu Bay and among the specimens from other researches. Size, shape and pore shape are intraspecific distinctive morphological features that may indicate possibly new species within the complex, given the known high diversity of cryptic species within the complex. Furthermore, we address the necessity of molecular phylogenetic studies from cultured strains to understand whether the morphological distinctions highlighted here are merely phenotypic plasticity or if they represent a new species, as it was also suggested by other researchers. E.g. the recently described *Prorocentrum caipirignum* from Brazilian waters, which was erected as a new species formerly described as a *P. lima* morphospecies.
- Dinocysts are common NPP taxa often found in palynological slides, however, vegetative cells of *Prorocentrum lima* were not found on other documented assemblages that we have read to cite in this thesis. That is why the presence of such high concentrations were a surprise in the occasion of the first observation. We thus also document the chemical resistance capability of the thecal plates and the organic structures such as the cytoplasm and pyrenoid of *P. lima,* which we address as a contribution to the micropaleontological knowledge.
- The knowledge on NPP taxa provided in this thesis reinforces the advantages of their use as multi-proxies in neoecological and paleoecological Quaternary studies, as their local responses are potentially informative on ecosystem dynamics and functioning;
- Non-pollen palynomorphs from the modern sediments of Camamu Bay responded to local characteristics and, thus represent valuable tools for further paleoecological reconstructions of the area, indicating freshwater and marine conditions, nutrient requirement and possibly human-induced pressures, such as phosphorus enrichment from farming.

- Our future directions regarding the NPP assemblages is already in course. We have collected one core in each river (35 and 45 cm) that we intend to use in a paleoecological reconstruction and describe its stratigraphic NPP succession in the last hypothetical two hundred years. We did not have resources to conduct the radiocarbon dating, so this scale is based on estimates from the age model of another core collected in the same region, and the known sedimentation rate of the area. We are prospecting resource funding to proceed the proper dating and age model calibration. In this multi-proxy project we also intend to use foraminiferal tests from the sediment fraction > 106 micrometers that was separated during the palynological process.
- Other NPP appeared in our samples, such as the round grey object that we grouped as acritarch, but it is probably a dinocyst. Several visible structures, such as accumulation body, surface protuberances like pores and thick wall seems like *Scrippsiella* or *Alexandrium* species. We intend to put some attention to its occurrence and description.
- We address, finally, that some attention should be paid to the health care in this region considering the fishing and shellfishing activities that are the subsistence basis for many families. We suggest a multidisciplinar ethnoscientific approach with the involved actors of the area (coastal community, fishermen, shellfish gatherers, farmers, public leaders, health professionals etc) to alert about the risks of consuming food potentially contaminated with toxins depending on the concentrations of certain organisms, as well as to protect the river, the mangrove and avoid HABs.