UNIVERSIDADE FEDERAL DO ESTADO DO RIO DE JANEIRO – UNIRIO
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
BIODIVERSIDADE NEOTROPICAL

Geovanna Theobald Borsato

Primeiro registro de *Ostreopsis lenticularis* e diversidade de espécies bentônicas de *Prorocentrum* (Dinophyceae) no Sudoeste do Atlântico

Rio de Janeiro

2022

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Biológicas (Biodiversidade Neotropical) da Universidade Federal do Estado do Rio de Janeiro como requisito para a obtenção do grau de Mestre em Ciências Biológicas.

Orientadora: Profª. Drª. Silvia Mattos Nascimento

Rio de Janeiro

2022

Borsato, Geovanna Theobald
BB738 Primeiro registro de Ostreopsis lenticularis e diversidade de espécies bentônicas de Prorocentrum (Dinophyceae) no Sudoeste do Atlântico / Geovanna Theobald Borsato. -- Rio de Janeiro, 2022.
109 f

Orientadora: Silvia Mattos Nascimento.
Dissertação (Mestrado) - Universidade Federal do Estado do Rio de Janeiro, Programa de Pós-Graduação em Ciências Biológicas, 2022.

1. Dinoflagelados bentônicos. 2. Ostreopsis. 3. Prorocentrum. 4. Taxonomia integrada. 5. Abundância. I. Nascimento, Silvia Mattos, orient. II. Título.

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Banca Examinadora

Silvia M. Nascimento

Profª. Drª. Silvia Mattos Nascimento (Orientadora)

Universidade Federal do Estado do Rio de Janeiro

FRAGA RIVAS, SANTIAGO (FIRMA)

Firmado digitalmente por FRAGA RIVAS, SANTIAGO (FIRMA)
Fecha: 2022.08.18 23:32:01 +02'00'

Dr. Santiago Fraga

Centro Oceanográfico de Vigo, Instituto Español de Oceanografía

Joel

Prof. Dr. Joel Campos de Paula

Universidade Federal do Estado do Rio de Janeiro

Agradecimentos

Agradeço à minha querida orientadora, professora Silvia Nascimento, pela orientação, dedicação, compreensão e apoio ao longo da construção deste trabalho. Obrigada por permitir minha participação no Laboratório de Microalgas Marinhas da UNIRIO, o qual sinto muito orgulho de fazer parte. Obrigada por compartilhar conosco o seu amor pelo mundo dos dinoflagelados, que possamos descobrir muito mais sobre ele juntas.

Agradeço à UNIRIO e a equipe docente do Programa de Pós-Graduação em Ciências Biológicas, os quais contribuíram para a minha formação. Agradeço à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela bolsa concedida, indispensável para a realização do mestrado.

Agradeço ao professor Fabiano Salgueiro, grande parceiro e colaborador do Laboratório de Microalgas Marinhas, responsável pelas análises moleculares realizadas neste estudo. Obrigada por nos ajudar nesta tarefa tão difícil que é a identificação desses pequenos organismos. Agradeço à professora Maria Lucia Lorini pelas sugestões dadas no exame de qualificação.

Agradeço ao professor Santiago Fraga por avaliar este trabalho, anteriormente como parte da banca do exame de qualificação e no momento como membro da banca examinadora. Aproveito este espaço para agradecê-lo por toda a sua contribuição na área dos dinoflagelados bentônicos, estudar estes pequenos organismos é um verdadeiro desafio. Agradeço ao professor Joel C. de Paula por aceitar participar como membro da banca examinadora deste trabalho e por identificar as espécies de macroalgas utilizadas neste estudo. Também agradeço às professoras Christina W. C. Branco e Priscila K. Lange por avaliarem este trabalho como membros suplentes da banca. É um imenso prazer poder compartilhar este estudo com vocês!

Agradeço ao professor Carlos E. L. Ferreira e aos demais participantes da equipe do Programa de Pesquisa Ecológica de Longa Duração – Ilhas Oceânicas Brasileiras pela coleta das amostras nos arquipélagos de São Pedro e São Paulo, Fernando de Noronha e Ilha da Trindade. Agradeço ao professor Marcelo H. P. da Silva do Instituto Militar de Engenharia – IME por permitir o uso do microscópio eletrônico de varredura desta instituição, ferramenta muito importante para a realização deste trabalho.

Agradeço à minha família, especialmente minha mãe, Rosimere, por toda força, apoio e compreensão nessa caminhada. Obrigada por estar sempre ao meu lado, me aconselhando e torcendo por mim. Agradeço à minha sobrinha, Meghan, pelas boas risadas e momentos divertidos que foram muito importantes para mim na reta final do mestrado. Agradeço ao meu companheiro de vida e de laboratório, Rodrigo, e aos seus pais, Marisa e Adauto, que me apoiaram e me acolheram com muito carinho. Rodrigo, obrigada por tudo, por me apresentar o mundo dos dinoflagelados, me ensinar a isolar células na iniciação científica, me ensinar a preparar amostras para a observação em microscópio eletrônico de varredura e, principalmente, estar ao meu lado nessa caminhada com tantos obstáculos. Você foi essencial.

Obrigada a todos!

Sumário

Resumo

Os dinoflagelados são componentes importantes dos ecossistemas marinhos, constituindo a base das teias alimentares. Espécies da assembleia de dinoflagelados bentônicos são encontradas associadas a diferentes substratos, como macroalgas e, sob certas circunstâncias, a composição e a abundância dessa assembleia pode variar, podendo ocorrer a proliferação e o acúmulo dessas microalgas, eventos conhecidos como florações. Essas florações podem ser prejudiciais ao ecossistema marinho, à saúde humana e à economia. O objetivo do presente estudo foi realizar uma investigação taxonômica da espécie *Ostreopsis lenticularis* encontrada em Fernando de Noronha e de espécies bentônicas de *Prorocentrum* nas regiões nordeste, sudeste e nas ilhas oceânicas do Brasil, além de avaliar a abundância das espécies nesses locais. Uma revisão global da riqueza de espécies bentônicas de *Prorocentrum* também é apresentada. Análise morfológica e molecular (LSU e ITS rDNA) de células de campo confirmaram a identidade de *Ostreopsis lenticularis* em Fernando de Noronha, sendo essas as primeiras sequências dessa espécie no Oceano Atlântico. Abundâncias de até 7.6 x 10^4 céls.gPUmacroalga^{-1} de *O. lenticularis* foram registradas. Espécies bentônicas de *Prorocentrum* foram encontradas em doze sítios na costa e três ilhas oceânicas do Brasil. Ao todo, onze espécies bentônicas desse gênero foram encontradas, três na região nordeste, quatro no sudeste e onze nas ilhas oceânicas. Fernando de Noronha apresentou o maior número de espécies (11) e pode ser considerado um *hotspot* de diversidade de espécies bentônicas de *Prorocentrum*, com riqueza comparada às áreas mais diversas do mundo. As espécies *P. borbonicum, P. hoffmannianum, P. lima* e *P. rhathymum* foram identificadas através de análise morfológica e molecular (LSU e ITS rDNA) combinada, enquanto *P.* cf. *caipirignum, P.* cf. *concavum, P.* cf. *fukuyoi, P.* cf. *norrisianum, P. panamense* e *P.* cf. *sculptile* foram identificados a partir de observação em microscopia eletrônica de varredura e/ou microscopia ótica. *Prorocentrum lima* foi onipresente e apresentou as maiores abundâncias, de até 1.9 x 10^4 céls.gPUmacroalga^{-1} na região nordeste, enquanto as demais espécies não ultrapassaram 1 x 10^3 céls.gPUmacroalga^{-1}. As abundâncias das espécies bentônicas de *Prorocentrum*, especialmente *P. lima*, encontradas nesse estudo podem ser potencialmente prejudiciais aos organismos marinhos e à saúde humana nessas regiões.

Palavras-chave: Dinoflagelados bentônicos, Taxonomia, Filogenia, Abundância, Oceano Atlântico

Abstract

Dinoflagellates are important components of marine ecosystems, forming the basis of marine food webs. Species of the benthic dinoflagellate assemblage are found associated with different substrates, such as macroalgae and, under certain circumstances, the composition and abundance of this assemblage may vary, and proliferation and accumulation of these microalgae may occur, events known as blooms. These blooms can be harmful to the marine ecosystem, human health and the economy. The aim of the present study was to perform a taxonomic investigation of the species *Ostreopsis lenticularis* found at Fernando de Noronha and of benthic species of *Prorocentrum* in the Northeast, Southeast and oceanic islands of Brazil, in addition to evaluating the abundance of the species in these locations. A global review of benthic *Prorocentrum* species richness is also presented. Morphological and molecular (LSU and ITS rDNA) analysis of field cells confirmed the identity of *Ostreopsis lenticularis* at Fernando de Noronha, and the first genetic sequences of this species in the Atlantic Ocean were provided. Abundances up to 7.6 x 10^4 cells *O. lenticularis* per gFWmacroalgae^{-1} were recorded. Benthic species of *Prorocentrum* were found at twelve sites off the coast and three oceanic islands of Brazil. Altogether, eleven benthic species of this genus were found, three at the Northeast region, four at the Southeast and eleven at the oceanic islands. Fernando de Noronha presented the largest number of species (11) and can be considered a *hotspot* of diversity of benthic *Prorocentrum*, with species richness compared to the more diverse areas in the world. The species *P. borbonicum, P. hoffmannianum, P. lima* and *P. rhathymum* were identified through combined morphological and molecular (LSU and ITS rDNA) analysis, while *P.* cf. *caipirignum, P.* cf. *concavum, P.* cf. *fukuyoi, P.* cf. *norrisianum, P. panamense* and *P.* cf. *sculptile* were identified through observation in scanning electron microscopy and/or light microscopy. *Prorocentrum lima* was ubiquitous and presented the highest abundances, up to 1.9 x 10^4 cells.gFWmacroalgae^{-1} in the Northeast region, while the other species did not exceed 1 x 10^3 cells.gFWmacroalgae^{-1}. The abundances of benthic species of *Prorocentrum*, particularly *P. lima*, found in this study could potentially be harmful to marine organisms and human health in these regions.

Key words: Benthic dinoflagellates, Taxonomy, Phylogeny, Abundance, Atlantic Ocean

Introdução Geral

Os dinoflagelados são componentes importantes dos ecossistemas marinhos, devido a capacidade de inúmeras espécies realizarem fotossíntese e integrarem teias alimentares. Os dinoflagelados bentônicos constituem um grupo de microalgas marinhas que vivem associadas a diferentes substratos como macroalgas, recifes de coral, entre grãos de areia, sedimentos e detritos presentes no ambiente marinho, ocupando uma grande variedade de micronichos fornecidos por esses habitats (Tester et al., 2014; Yong et al., 2018). Esse grupo é encontrado em regiões tropicais, subtropicais e temperadas (Vila et al., 2001; Parsons e Preskitt, 2007; Aligizaki et al., 2009; Okolodkov et al., 2014; Boisnoir et al., 2019; Accoroni et al., 2020; Bravo et al., 2020), entretanto, alguns gêneros de dinoflagelados são mais diversos em áreas tropicais, como o gênero *Prorocentrum* (Ehrenberg, 1834) que apresenta elevada diversidade na região do Caribe (Faust, 1990, 1993a, 1993b, 1994, 1997).

Sob certas circunstâncias, relacionadas à fatores abióticos e bióticos do ambiente, a composição e a abundância da assembleia de dinoflagelados bentônicos pode variar, podendo ocorrer a proliferação e o acúmulo dessas microalgas, eventos conhecidos como florações (Berdalet et al., 2016). Os eventos de floração são considerados nocivos, pois inúmeras espécies de microalgas produzem toxinas naturais potentes e persistentes que podem ser prejudiciais ao ecossistema marinho, à saúde humana e à economia (Berdalet et al., 2016). Além disso, quando grandes florações se deterioram, a degradação da elevada biomassa de microalgas realizada por bactérias pode reduzir as concentrações de oxigênio no ambiente marinho, causando hipóxia e consequente mortalidade de organismos co-ocorrentes (Berdalet et al., 2016).

Os gêneros *Ostreopsis* (Schmidt, 1901) e *Prorocentrum* estão entre os principais constituintes da assembleia de dinoflagelados bentônicos. Atualmente, o gênero *Ostreopsis* é composto por 11 espécies, nomeadas *O. siamensis* (Schmidt, 1901), *O. ovata* (Fukuyo, 1981), *O. lenticularis* (Fukuyo, 1981), *O. heptagona* (Norris et al., 1985), *O. mascarenensis* (Quod, 1994), *O. labens* (Faust e Morton, 1995), *O. belizeanus* (Faust, 1999), *O. caribbeanus* (Faust, 1999), *O. marina* (Faust, 1999), *O. fattorussoi* (Accoroni et al., 2016) e *O. rhodesae* (Verma et al., 2016). Várias espécies desse gênero são consideradas potencialmente tóxicas, devido a produção de análogos da palitoxina, considerada uma das toxinas biológicas mais potentes, entretanto, a toxicidade de algumas espécies continua incerta (Pavaux et al., 2020). A espécie *Ostreopsis* cf. *ovata* é uma das principais espécies tóxicas de dinoflagelados bentônicos, produzindo ovatoxinas (Tartaglione et al., 2017; Pavaux et al., 2020).

Ostreopsis cf. *ovata* foi encontrado em diversos locais da costa brasileira (Nascimento et al., 2012a, 2020; Gómez et al., 2017; Tibiriçá et al., 2019), sendo, por muitos anos, a única espécie de *Ostreopsis* registrada no Brasil. Espécies desse gênero podem atingir elevadas densidades em regiões tropicais, subtropicais e temperadas, especialmente no Mar Mediterrâneo (Gladan et al., 2019). No Brasil, florações de *Ostreopsis* cf. *ovata* foram encontradas no Rio de Janeiro (Nascimento et al., 2010), no Arquipélago de São Pedro e São Paulo (Nascimento et al., 2012b) e no Paraná (Tibiriçá et al., 2019). Florações dessa espécie estão relacionadas a efeitos negativos e mortalidade de organismos marinhos (Granéli et al., 2002; Vale e Ares, 2007; Shears e Ross, 2009; Blanfuné et al., 2012), assim como impactos na saúde humana (Durando et al., 2007; Tubaro et al., 2011; Vila et al., 2016; Pavaux et al., 2020).

O gênero *Ostreopsis* possui morfologia dinoconte, com epiteca e hipoteca constituídas por placas de diferentes formas e tamanhos. A identificação morfológica das espécies de *Ostreopsis* baseia-se em características como forma e tamanho das células, padrão das placas tecais, forma e tamanho das principais placas tecais (Penna et al., 2005) e a presença de poros com diferentes tamanhos na superfície tecal (Fukuyo, 1981; Chomérat et al., 2019). Entretanto, existe alta variabilidade morfológica, com variações na forma e pequenas diferenças em certas placas tecais, além da variabilidade das dimensões das células, dentro de uma mesma espécie (Penna et al., 2005). Portanto, análises morfológicas e moleculares combinadas são fundamentais para a correta identificação das espécies de *Ostreopsis*.

Atualmente, o gênero *Prorocentrum* é composto por 84 espécies (Guiry e Guiry 2022), das quais aproximadamente metade são bentônicas. Inúmeras espécies foram descritas a partir de regiões tropicais (Faust, 1990; 1993a, 1993b, 1994, 1997) e constituem uma parte significativa das assembleias de dinoflagelados bentônicos nessas regiões, ocorrendo com espécies de *Gambierdiscus*, *Ostreopsis*, *Coolia* e *Amphidinium* (Tindall e Morton, 1998; Delgado et al., 2006; Richlen e Lobel, 2011; Skinner et al., 2013; Okolodkov et al., 2014; Boisnoir et al., 2019). Assim como em *Ostreopsis*, diversas espécies de *Prorocentrum* são consideradas potencialmente tóxicas. A produção de toxinas diarreicas, como o ácido ocadáico e seus análogos, dinofisistoxinas, foi relatada em espécies do complexo *P. lima* (Ehrenberg, Stein, 1878), *P. rhathymum* (Loeblich et al., 1979), *P. emarginatum* (Fukuyo, 1981), *P. concavum* (Fukuyo, 1981), *P. hoffmannianum* (Faust, 1990), *P. borbonicum* (Ten-Hage et al., 2000) e *P. caipirignum* (Nascimento et al., 2017) (Nakajima et al., 1981; Murakami et al., 1982; Dickey et al., 1990; Morton et al., 1994; Holmes et al., 2001; Faust et al., 2008; Caillaud et al., 2010; Nascimento et al., 2017; Nishimura et al., 2020). Essas toxinas são responsáveis pela intoxicação diarreica por moluscos (Diarrhetic Shellfish Poisoning – DSP), uma doença gastrointestinal causada pelo consumo de moluscos contaminados pelo acúmulo de tais toxinas (Yasumoto et al., 1978; Lee et al., 2016).

Prorocentrum possui morfologia desmoconte, apresentando duas placas laterais unidas por uma sutura sagital e pequenas placas na área periflagelar. A identificação morfológica das espécies de *Prorocentrum* baseia-se em características como forma e tamanho das células, ornamentação da superfície celular, padrão de poros, morfologia da banda intercalar e o número, forma e organização das pequenas placas e estruturas (espinhos e protuberâncias) da área periflagelar (Hoppenrath et al., 2013). A área periflagelar é considerada uma característica taxonômica valiosa, enquanto outros caracteres morfológicos, como forma e padrão de poros, podem variar em algumas espécies ou apresentar diferenças sutis entre as espécies (Hoppenrath et al., 2013). Dados moleculares são, portanto, necessários, em combinação com estudos morfológicos para identificar inequivocamente as espécies de *Prorocentrum*.

A correta identificação de dinoflagelados bentônicos, especialmente espécies tóxicas, é o primeiro passo para o conhecimento de sua distribuição geográfica, quantificação de suas populações, caracterização das toxinas produzidas e dos riscos associados à sua presença, tanto para os ecossistemas marinhos, quanto à saúde humana e à economia.

O objetivo do presente estudo foi realizar uma investigação taxonômica da espécie *Ostreopsis lenticularis* encontrada no Arquipélago de Fernando de Noronha e de espécies bentônicas do gênero *Prorocentrum* em doze locais da costa e três ilhas oceânicas do Brasil, além de avaliar a abundância das espécies nesses locais. Uma revisão global da riqueza de espécies bentônicas de *Prorocentrum* também é apresentada.

Esta dissertação foi dividida em dois capítulos, sendo o primeiro "*Ostreopsis lenticularis* Y. Fukuyo (Dinophyceae, Gonyaulacales) from the South Atlantic Ocean: morphological and molecular characterization" publicado em 2020 na revista Marine

Pollution Bulletin e o segundo “Taxonomy and abundance of benthic *Prorocentrum* (Dinophyceae) species from the tropical and subtropical Southwest Atlantic Ocean including a review of their global diversity” que está em preparação para posterior submissão na revista Harmful Algae.

Capítulo 1

Marine Pollution Bulletin 158 (2020) 111441

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Ostreopsis lenticularis Y. Fukuyo (Dinophyceae, Gonyaulacales) from the South Atlantic Ocean: morphological and molecular characterization

Geovanna Theobald Borsato[a], Fabiano Salgueiro[b,*], Cristhian Gomes Tavares da Silva[b,c], Adriana D. Menezes-Salgueiro[c], Silvia M. Nascimento[a,*]

[a] *Laboratório de Microalgas Marinhas, Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Av. Pasteur, 458, 314-B, Rio de Janeiro, RJ 22290-240, Brazil*
[b] *Laboratório de Biodiversidade e Evolução Molecular, Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Av. Pasteur, 458, 512, Rio de Janeiro, RJ 22290-240, Brazil*
[c] *Laboratório de Biotecnologia Vegetal, Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (IFRJ), Rua Senador Furtado, 121, 112. Maracanã, Rio de Janeiro, RJ 20270-021, Brazil*

ARTICLE INFO

Keywords:
Benthic dinoflagellates
Harmful algal blooms
Phylogeny
Taxonomy
Brazil
Atlantic Ocean

ABSTRACT

Ostreopsis is a benthic dinoflagellate genus comprising eleven species including seven that are toxigenic. *Ostreopsis lenticularis* was first described from French Polynesia and is widely distributed in many insular locations from the Pacific Ocean. The current study presents morphological and molecular evidence of the presence of *O. lenticularis* at the Fernando de Noronha Archipelago, South Atlantic. Light microscopy and scanning electron microscopy of field cells showed morphological features in agreement with the description of *O. lenticularis*. Cells were broadly oval, 66.0–117.5 µm in dorso-ventral diameter, 50.0–92.5 µm in width, with thecal plates presenting two kinds of pores. Phylogenetic analyses based on LSU (D1-D3) and ITS sequences from field cells from Fernando de Noronha clustered with *O. lenticularis* sequences from elsewhere. The species was present in low to high abundances. The current study expands the distribution of *O. lenticularis* to Fernando de Noronha Archipelago in the South Atlantic Ocean.

1. Introduction

The genus *Ostreopsis* J. Schmidt is widely known for its bloom events and the ensuing impacts on marine ecosystems and human health. These impacts are mainly due to the toxic effects produced by some species of *Ostreopsis* that synthesize analogs of palytoxin, known as ovatoxins, ostreocins and mascarenotoxins (Berdalet et al., 2017). Initially identified in tropical areas (Schmidt, 1901; Fukuyo, 1981; Faust, 1999), this genus has also been later found in temperate regions (Vila et al., 2001; Rhodes et al., 2010; Berdalet et al., 2017). Currently, the genus is composed of eleven species, named *O. siamensis* J. Schmidt (Schmidt, 1901), *O. lenticularis* Y. Fukuyo (Fukuyo, 1981), *O. ovata* Y. Fukuyo (Fukuyo, 1981), *O. heptagona* D.R. Norris, J.W. Bomber & Balech (Norris et al., 1985), *O. mascarenensis* J.P. Quod (Quod, 1994), *O. labens* M.A. Faust & S.L. Morton (Faust and Morton, 1995), *O. belizeana* M.A. Faust, *O. caribbeana* M.A. Faust, *O. marina* M.A. Faust (Faust, 1999), *O. fattorussoi* Accoroni, Romagnoli & Totti (Accoroni et al., 2016) and *O. rhodesiae* Verma, Hoppenrath & Murray (Verma et al., 2016).

The identification of *Ostreopsis* species is based on morphological characteristics such as cell shape and size, pattern, shape and size of the main thecal plates (Penna et al., 2005), in addition to different pore sizes on the theca surface (Fukuyo, 1981). However, the thecal plate pattern is very similar in all species of the genus, except in *O. heptagona* (Penna et al., 2005). In addition, some characteristics used to distinguish *Ostreopsis* species such as cell shape and size, and shape of certain thecal plates have been shown to vary within the same species (Penna et al., 2005). Sequences of the ITS1-5.8S-ITS2 region (ITS), the D1-D3 and D8-D10 domains of the LSU rDNA have been used for identification of *Ostreopsis* species in combination with morphometry (Penna et al., 2005; Accoroni et al., 2016; Verma et al., 2016; Chomérat et al., 2019, 2020).

Ostreopsis lenticularis was described by Fukuyo (1981) from French Polynesia and New Caledonia. This species is widely distributed in tropical areas of the world's oceans, but, to date, there are still no molecular data to support its identification in the Caribbean Sea and the Atlantic Ocean. The species was recently reinvestigated by Chomérat et al. (2019) who provided reference molecular sequences for *O.*

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* Corresponding authors.
E-mail addresses: fabiano.salgueiro@gmail.com (F. Salgueiro), adriana.salgueiro@ifrj.edu.br (A.D. Menezes-Salgueiro), silvia.nascimento@unirio.br (S.M. Nascimento).

https://doi.org/10.1016/j.marpolbul.2020.111441
Received 16 May 2020; Received in revised form 3 July 2020; Accepted 4 July 2020

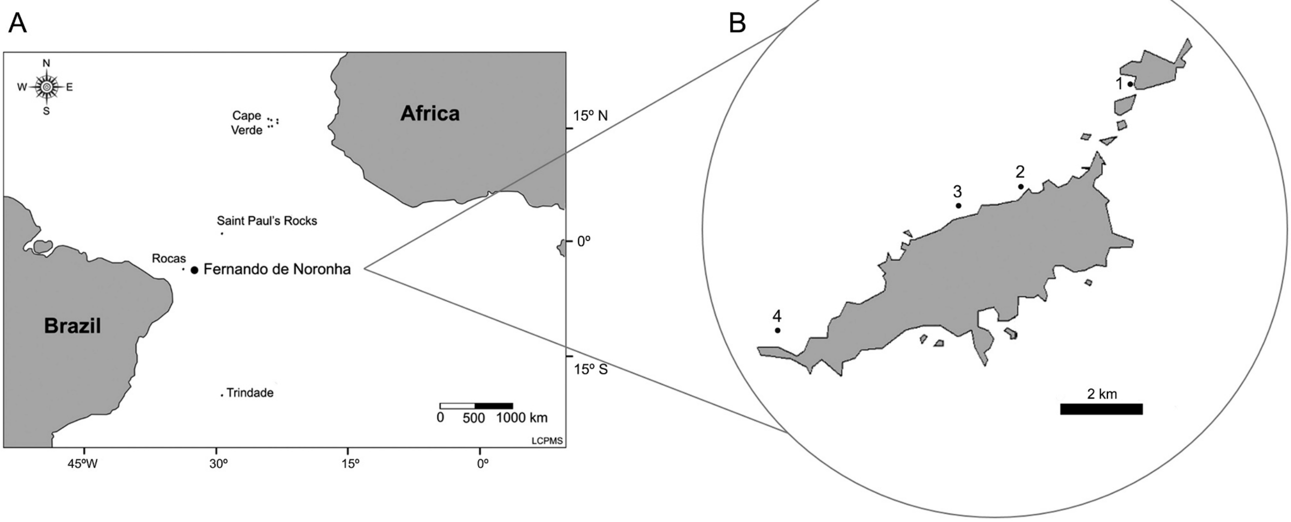


Fig. 1. (A) Location of the Fernando de Noronha Archipelago in the South Atlantic Ocean. (B) Sampling sites at Fernando de Noronha: (1) Cagarras, (2) Conceição, (3) Boldró, (4) Ponta da Sapata.

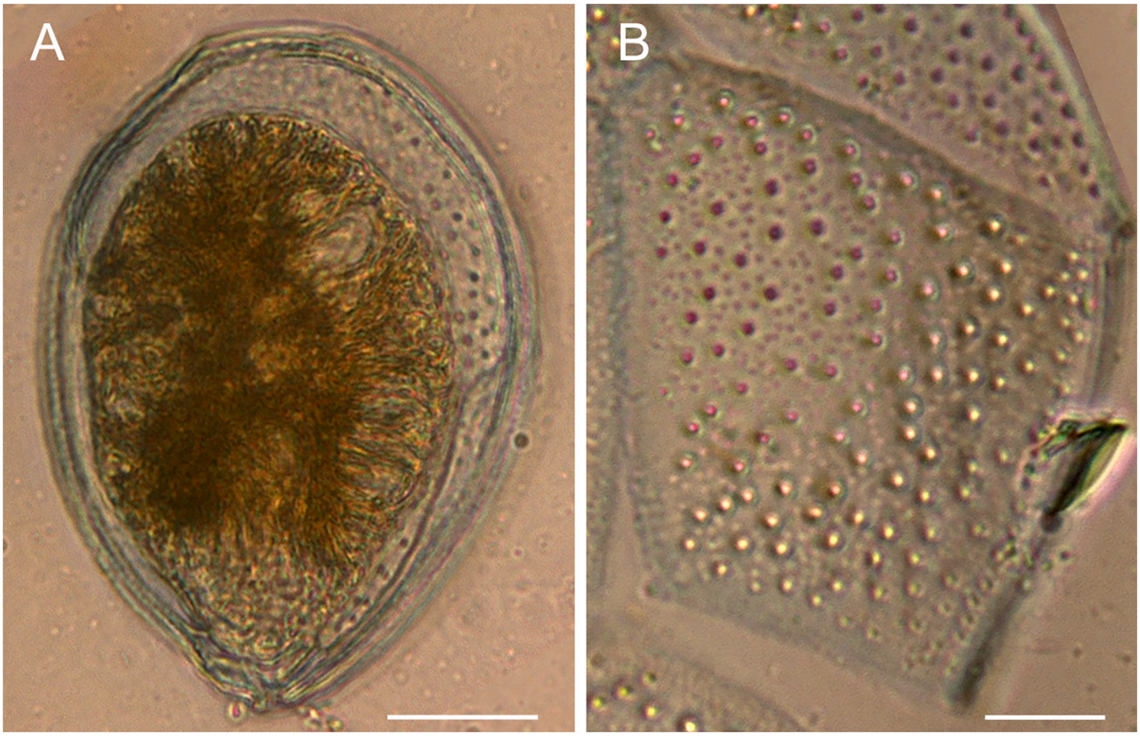


Fig. 2. Light microscopy images of *O. lenticularis* from Fernando de Noronha. A–B: apical view; B: detail of the thecal surface with pores of two sizes. Scale bars: A: 20 μm; B: 10 μm.

lenticularis from the species type locality and substantiated the morphological features presented by Fukuyo (1981) in the original description of the species. Moreover, Chomérat et al. (2019) confirmed that the presence of two kinds of thecal pores is a reliable taxonomic feature that can distinguish *O. lenticularis* from other similar species in the genus, as indicated originally by Fukuyo (1981). The current study reports the presence of *O. lenticularis* at the Fernando de Noronha Archipelago in the South Atlantic Ocean, providing morphological and molecular data.

2. Material and methods

2.1. Sample collection

Macroalgal samples were collected in sealable plastic bags from a depth of 2–4 m by scuba diving from four sites: Cagarras (3° 48′ 52″ S, 32° 23′ 25″ W), Conceição (3° 50′ 21″ S, 32° 24′ 54″ W), Boldró (3° 50′ 41.25″ S, 32° 25′ 48.32″ W) and Ponta da Sapata (3° 52′ 29″ S, 32° 28′ 28″ W) at the Fernando de Noronha Archipelago (Fig. 1) in October 2018. Sampling campaigns were conducted in accordance with the Chico Mendes Institute for Biodiversity Conservation (ICMBio

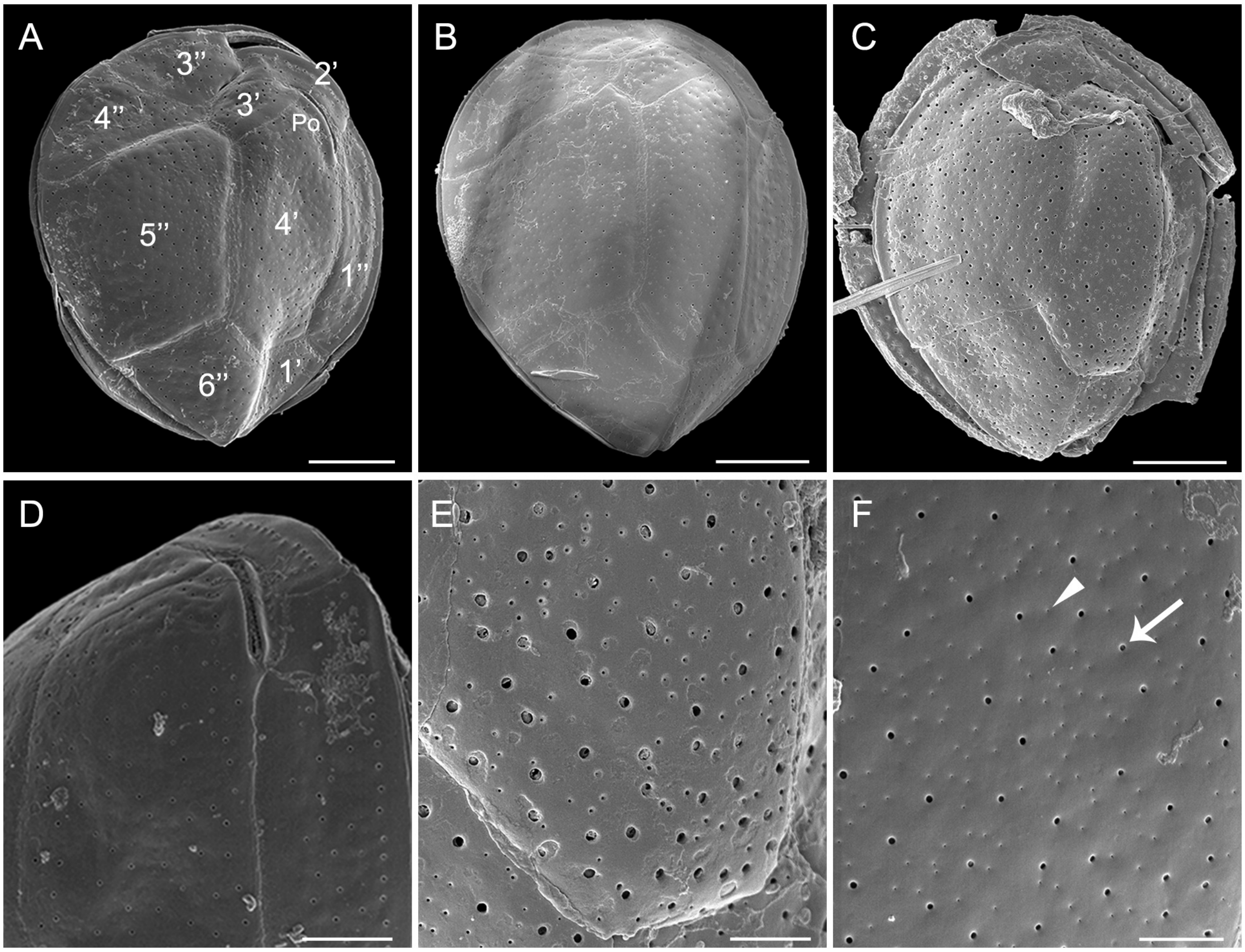


Fig. 3. Scanning electron microscopy images of *O. lenticularis* from Fernando de Noronha. A–C: apical view; D: lateral apical view; E–F: detail of the thecal surface with pores of two sizes, arrow indicates large pores and arrow head small pores; C, E: senescent cell. Scale bars: A–C: 20 μm; D: 10 μm; E–F: 5 μm.

authorization for scientific activity no 41327-14). Macroalgae samples were vigorously shaken for 2 min to detach the associated epiphytic cells, that were preserved with neutral Lugol iodine solution. The epiphyte suspension was sieved through a 255 μm mesh to remove macroalgae debris.

2.2. *Light and scanning electron microscopy*

Lugol preserved *O. lenticularis* cells were observed using an upright light microscope (ImagerA2, Zeiss, Germany) equipped with phase contrast. The dorso-ventral diameter (DV) (or depth) and width (W) of *O. lenticularis* cells were measured with a microscope eyepiece ruler. For scanning electron microscopy (SEM) observation, Lugol preserved *O. lenticularis* cells were filtered through Durapore membrane filters (Millipore, USA), rinsed twice with distilled water and dehydrated in a series of 30, 50, 70, 80, 95 and 100% (100% etOH twice) ethanol followed by hexamethyldisilazane. Filters containing the cells were coated with nickel and gold with a sputter coater (Leica EM ACE 600, Germany) and observed with a Micro Quanta FEG 250 scanning electron microscope (FEI Company, Hillsboro, Oregon, USA) at Instituto Militar de Engenharia (IME), Rio de Janeiro, Brazil. A modified Kofoid tabulation system (Kofoid, 1909) as described in Besada et al. (1982) was followed to name the plates and enable comparisons with other genera.

2.3. *Molecular characterization of field specimens*

First, a single-cell PCR approach was tested. However, no amplicons were obtained from PCRs. Then, to increase the amount of DNA, approximately five hundred cells of *O. lenticularis* were isolated from Lugol-preserved samples collected at Fernando de Noronha by micropipeting under an inverted light microscope (Zeiss, Primovert, Germany). Cells were rinsed into several drops of filtered and autoclaved seawater, transferred to a 1.5 mL microtube and centrifuged at 5000 ×*g* for 15 min. The supernatant was discarded and the cell pellet was used for total genomic DNA extraction using the commercial kit NucleoSpin® Plant II (Macherey–Nagel, Germany) following the manufacturer's instructions. The extracted DNA solution was then stored at −20 °C.

Two ribosomal DNA (rDNA) *loci* were analyzed: the D1-D3 region of the large subunit (LSU) and the Internal Transcribed Spacer (ITS = ITS1–5.8S-ITS2). Initially several primer pairs available in the literature were unsuccessfully tested: ITSAxITSB (Sato et al., 2011), ITS1xITS4 (White et al., 1990), ITScxITSd (Leaw et al., 2001), 25F1x25R1 (Kogame et al., 1999), D1RxD3Ca (Scholin et al., 1994), D1RxLSUB (Scholin et al., 1994; Litaker et al., 2003), D1RxD3B (Scholin et al., 1994; Nunn et al., 1996) and FD8xRB (Chinain et al., 1999). Thus, two new primer pairs were designed based on LSU and ITS sequences of *O. lenticularis* available from GenBank using the standard parameters of the Geneious Prime v2020.1 software (www.geneious.com). Forward and reverse primers were designed within conserved regions of the multiple sequence alignment of the *O. lenticularis* rDNA.

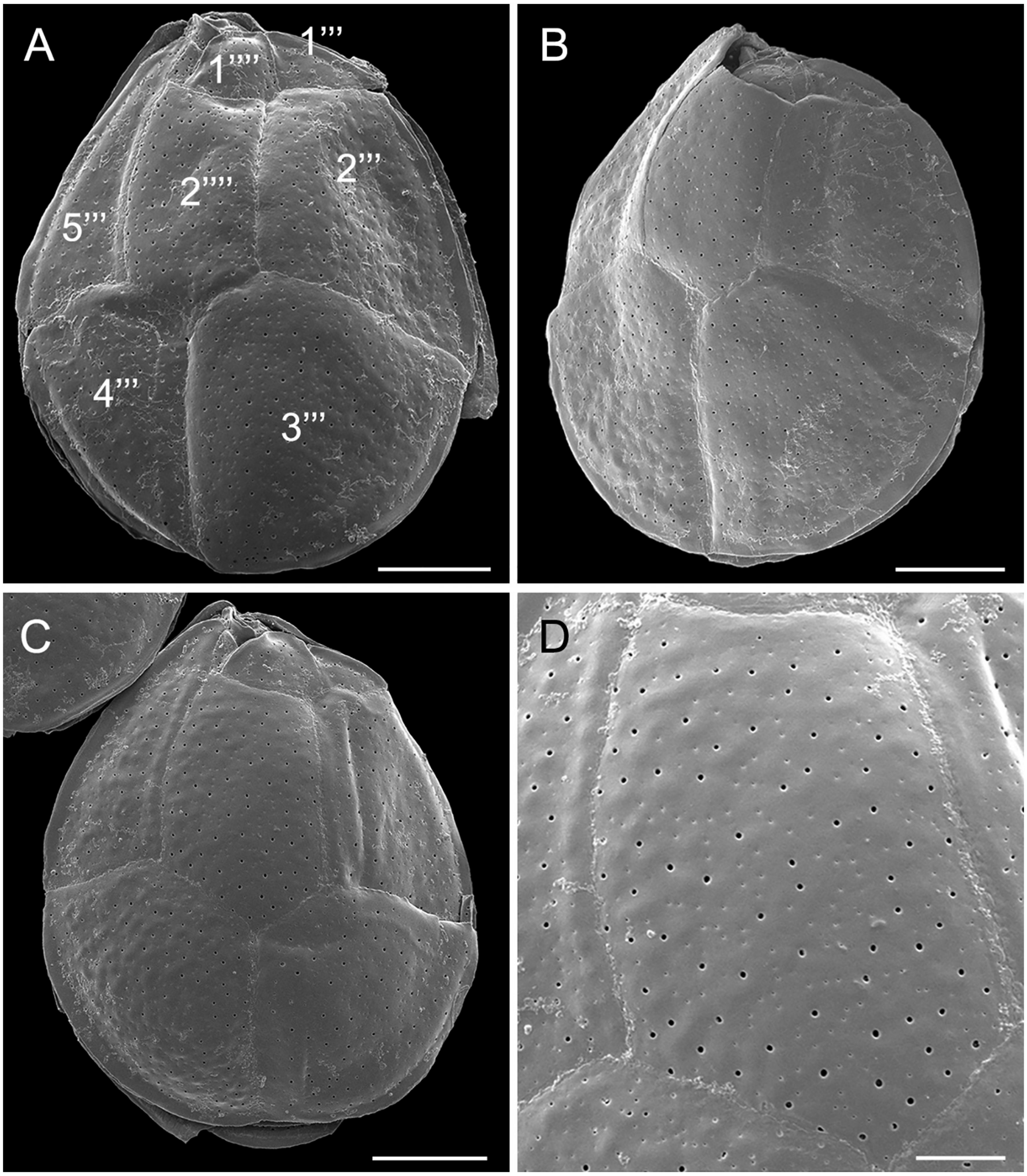


Fig. 4. Scanning electron microscopy images of *O. lenticularis* from Fernando de Noronha. A–C: antapical view; D: detail of the thecal surface showing two kinds of pores. Scale bars: A–C: 20 μm; D: 5 μm.

The LSU D1-D3 region was amplified using the pair of primers O.lent_LSU_D1_F1/O.lent_LSU_D3_R1 (5′-GCAAGTAATTGCGAATGATTGC-3′/5′-TCACCATCTTTCGGGTCCTG-3′) and the ITS region was amplified using the pair of primers O.lent_ITS_F1/O.lent_ITS_R1 (5′-TCTGGGCCACAACATCTCAA-3′/5′-GCATCACAGCAAACTACATGAT-3′).

The amplification reaction mixture of 25 μL contained 1 unit (U) Taq DNA polymerase (Thermo Scientific Inc., USA), 1 × reaction buffer with NH_4SO_4, 2.5 mM $MgCl_2$, 0.16 mM dNTPs (Thermo Scientific, USA), 8 pmol of each primer, 0.2 mg of Bovine Serum Albumin (BSA) and 3.0 μL of unquantified genomic DNA. Reactions with LSU and ITS primers comprised a touchdown PCR procedure with an initial 5 min heating step at 95 °C, followed by 10 cycles of 95 °C for 1 min, 50 °C–45 °C (decreasing 0.5 °C per cycle) for 1 min, 72 °C for 1 min; 40 cycles of 95 °C for 1 min, 45 °C for 1 min, 72 °C for 1 min; and a final extension at 72 °C for 5 min. PCR products were purified and sequenced by Macrogen Inc. (Seoul, Korea) in both directions using the PCR primers and the traditional Sanger sequencing method.

Sequence reads were manually checked and edited using Chromas v2.6.6 (http://technelysium.com.au/wp/chromas) and MEGA v7.0 (Kumar et al., 2016) programs. The electropherograms were carefully checked (for both forward and reverse reads) to avoid the insertion of artificial sequence variations. The new sequences obtained in the present study were first BLAST searched against the GenBank database (www.ncbi.nlm.nih.gov/blast) to test for sequence homology with non-target taxa. The sequences were then aligned with other *Ostreopsis* sequences retrieved from GenBank using MAFFT v7.0 (Katoh and Standley, 2013) with default settings. Phylogenetic analyses were conducted separately for each molecular marker. Sequences of *Coolia monotis* were used as outgroup.

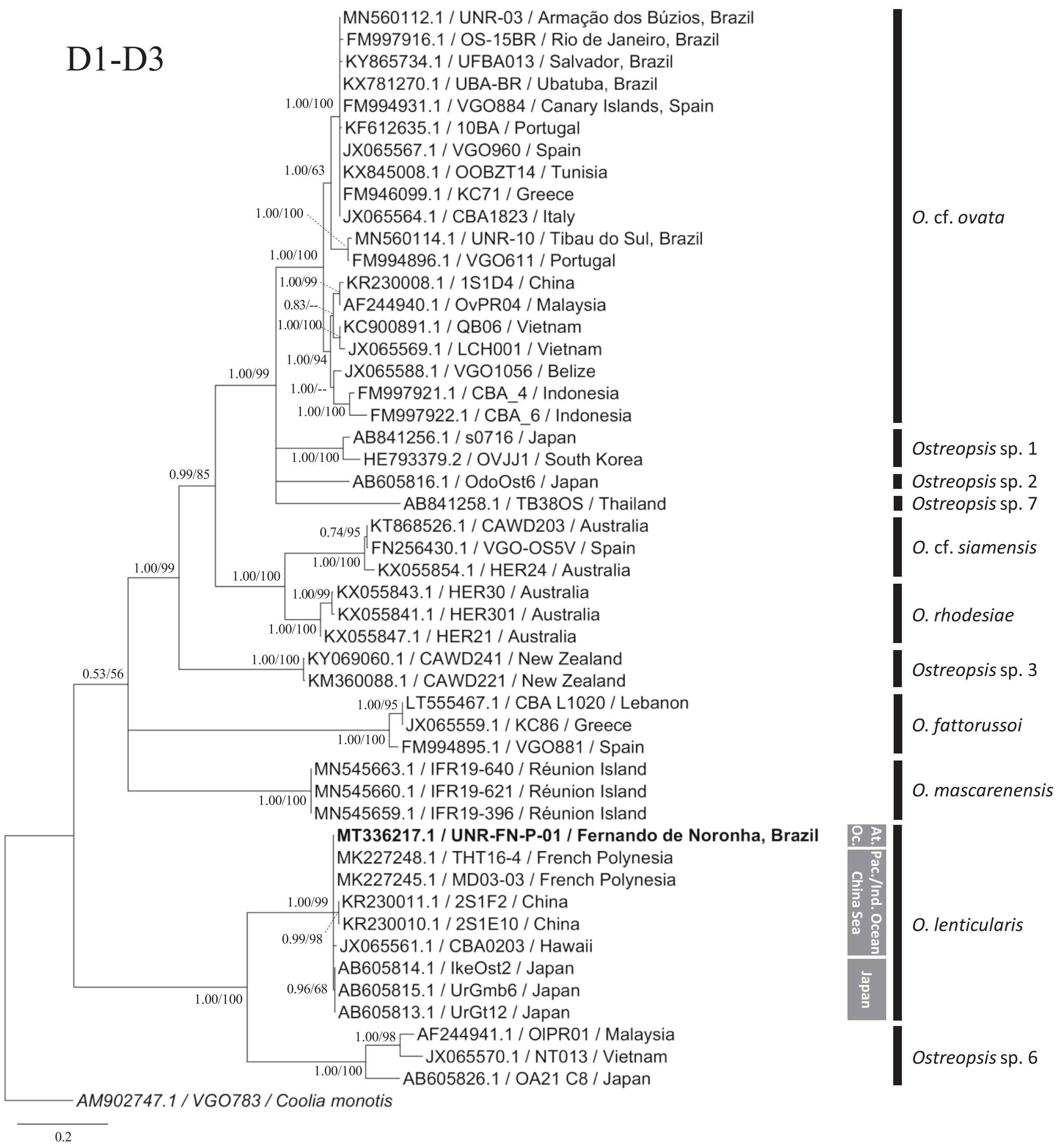


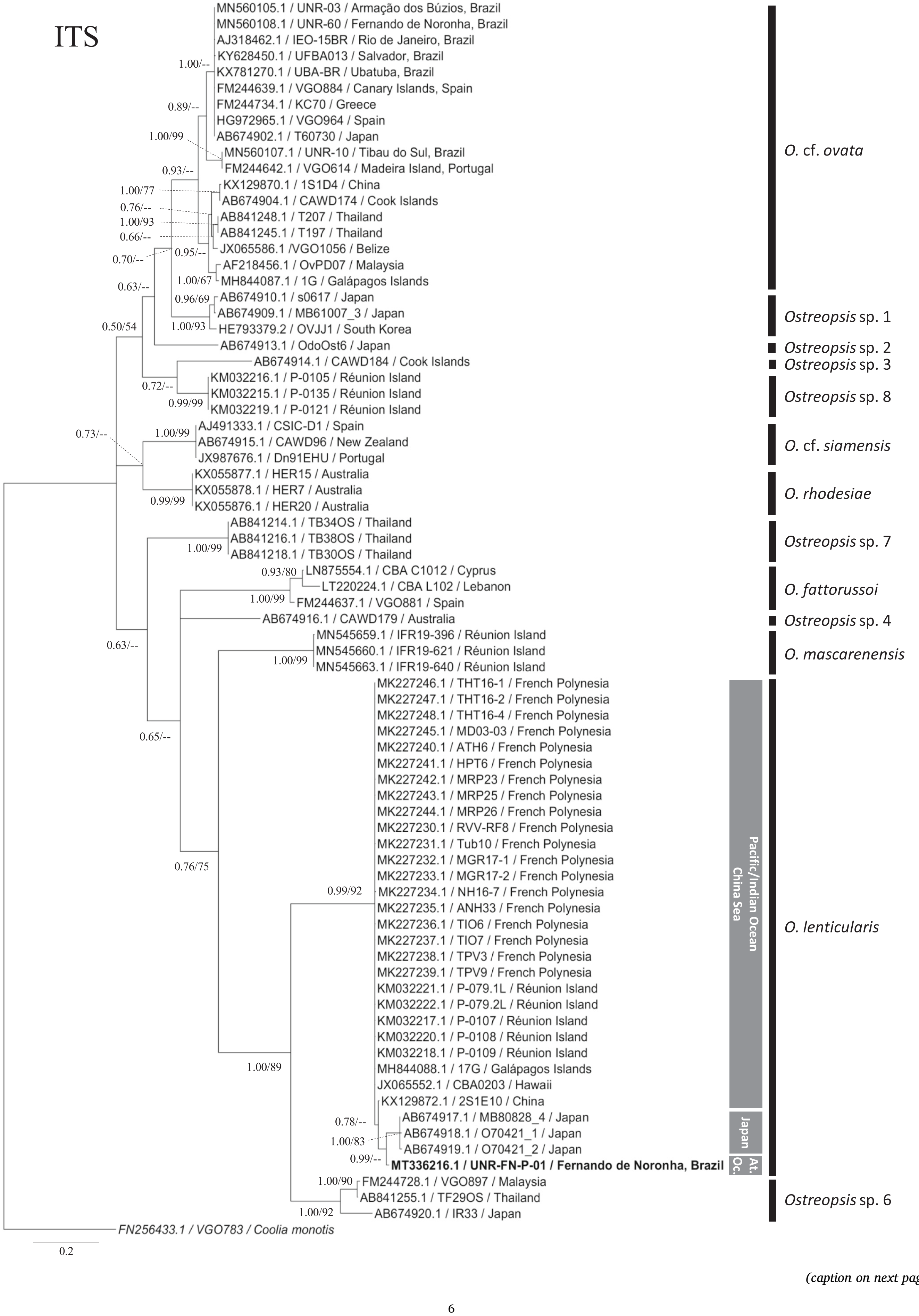
Fig. 5. Bayesian Inference phylogenetic tree based on LSU rDNA D1-D3 sequences of several *Ostreopsis* strains. Operational taxonomic units (OTUs) are identified by: GenBank accession number\strain name\locality. Numbers at nodes represents posterior probability from BI and bootstrap values from ML analyses, respectively (cut-off = 50% for both analyses). For the *O. lenticularis* clade, the vertical gray bars compile the geographic origin of the strains (At. Oc = Atlantic Ocean). The new *O. lenticularis* sequence (MT336217.1) is displayed in bold.

The MEGA software was used to select the best-fit model of nucleotide substitution (T92 + G + I for both markers) and construct maximum likelihood (ML) phylogenetic trees with 1000 bootstrap (BS) replications. The phylogenetic relationships were also examined using Bayesian inference (BI) with MrBayes v3.2.7a (Ronquist et al., 2012). To sample across nucleotide substitution models, the command "lset nst = mixed" was used before running the analysis. Markov Chain Monte Carlo procedure consisted of two independent trials with four chains each. Each chain was run for 1.000.000 generations and sampled every 100th cycle. Posterior probability (PP) values for the resulting 50% majority rule consensus tree were estimated after discarding the first 10% of trees as burn-in.

3. Results

3.1. Morphology

Ostreopsis lenticularis cells from Fernando de Noronha were broadly oval or oval in apical and antapical views (Figs. 2A, 3A–C, 4A–C). Cells were highly compressed antero-posteriorly. The dorsoventral diameter (DV) varied between 66.0 and 117.5 μm (mean ± standard deviation: 92.0 ± 13.4 μm, $n = 197$), width (W) ranged from 50.0 to 92.5 μm



(caption on next page)

Fig. 6. Bayesian Inference phylogenetic tree based on ITS rDNA sequences of several *Ostreopsis* strains. Operational taxonomic units (OTUs) are identified by: GenBank accession number\strain name\locality. Numbers at nodes represents posterior probability from BI and bootstrap values from ML analyses, respectively (cut-off = 50% for both analyses). For the *O. lenticularis* clade, the vertical gray bars compile the geographic origin of the strains (At. Oc = Atlantic Ocean). The new *O. lenticularis* sequence (MT336216.1) is displayed in bold.

Table 1
Dorso-ventral (DV) diameter and width (W) in µm, and DV/W ratio of cultured and field cells of *Ostreopsis lenticularis* from diverse locations showing range of dimensions and mean ± standard deviations values in brackets.

Sample location	DV	W	DV/W	Origin	Reference
Fernando de Noronha Archipelago, Brazil	66.0–117.5 (92.0 ± 13.4; *n* = 197)	50.0–92.5 (69.5 ± 12.5; *n* = 197)	1.1–1.8 (1.3 ± 0.1; *n* = 197)	Field	Current study
Gambier Islands and Society Islands, French Polynesia/New Caledonia, France	60–100	45–80		Field	Fukuyo (1981)
Nha Trang Bay, Vietnam	60–80	50–80		Field	Larsen and Nguyen (2004)
Hainan Islands, China (Cells in LM)	68.0–113.5 (80.3 ± 8.1; *n* = 81)	56.5–97.3 (68.2 ± 7.9; *n* = 81)	1.0–1.4 (1.2 ± 0.1; *n* = 81)	Culture (2S1F2, 2S1E10)	Zhang et al. (2018)
Hainan Islands, China (Cells in SEM)	72.6–121.3 (95.6 ± 13.7; *n* = 39)	60.6–98.2 (79.5 ± 10.7; *n* = 39)	1.1–1.4 (1.2 ± 0.1; *n* = 39)	Culture (2S1F2, 2S1E10)	Zhang et al. (2018)
Revillagigedo Archipelago, Mexico	65–100 (*n* = 30)	50–80 (*n* = 30)		Field	Gárate-Lizárraga et al. (2018)
Nuku Hiva Island, Marquesas Archipelago, French Polynesia, France	73.0–94.4 (81.2 ± 5.7; *n* = 22)	58.0–78.2 (67.5 ± 6.1; *n* = 22)	1.08–1.32 (1.21 ± 0.01; *n* = 22)	Field	Chomérat et al. (2019)
Tahiti Island, Society Archipelago, French Polynesia, France	60.5–89.3 (80.3 ± 7.5; *n* = 30)	56.1–73.4 (65.8 ± 5.4; *n* = 30)	1.08–1.36 (1.22 ± 0.08; *n* = 27)	Culture (THT16–4)	Chomérat et al. (2019)
Cape Verde Archipelago, Macaronesia, Central Eastern Atlantic	60–105	53–74	1.13–1.42	Field	Soler-Onís and Fernández-Zabala (2019)

(mean 69.5 ± 12.5 µm, *n* = 197) and the mean DV/W ratio was 1.3 ± 0.1 (1.1–1.8). The thecal surface was smooth with numerous circular pores with two size classes, large and small pores, spread over all the thecal plates (Figs. 2B, 3E–F, 4D). Small pores were abundant, but due to their small size, they could be observed only at high magnifications in light microscopy (Fig. 2B) and with SEM (Figs. 3F, 4D).

The thecal plate pattern was APC 4′, 6″, 6c, ?s, 5‴, 2⁗, and thecal plates were clearly visible in SEM (Figs. 3A–D, 4A–C). The apical pore complex (APC) consisted of a narrow, elongated and slightly curved Po plate, bearing a slit and extending dorsally beyond the APC (Fig. 3D). There were four apical plates, considering the system by Besada et al. (1982). The fourth apical plate (4′) was elongated and hexagonal, located mostly on the left side of the epitheca (Fig. 3A–C). The second apical plate (2′) was narrow and elongated and was located below the APC, reaching approximately plate 3′ (Fig. 3D). Plate 2′ was the smallest of the apical series and of the entire epitheca. The third apical plate (3′) was irregularly hexagonal, in contact with 4′, 2″, 3″, 4″ and 5″, and with a small suture with the Po (Fig. 3A–B). There were six precingular plates. Plates 2″, 3″, 4″ and 6″ were irregularly quadrangular, while plates 1″ and 5″ were pentagonal, with plate 5″ being the largest plate of the precingular series and of the entire epitheca (Fig. 3A–C).

In the hypotheca, there were five postcingular plates. The first postcingular plate (1‴) was triangular, being the smallest of the postcingular series and the entire hypotheca (Fig. 4A–C). Plate 2‴ was pentagonal, while plates 3‴, 4‴ and 5‴ were four sided, with 2‴, 3‴ and 4‴ occupying the largest area of the hypotheca (Fig. 4A–C). The fifth postcingular plate (5‴) was narrow and elongated compared to other postcingular plates (Fig. 4A–C). In the antapical series, the first antapical plate (1⁗) was small and four sided, while plate 2⁗ presented an elongated pentagonal shape, being in contact with plates 2‴, 3‴, 4‴, 5‴ and 1⁗ (Fig. 4A–D).

3.2. Molecular phylogeny

The phylogenetic analysis inferred from the D1-D3 LSU locus encompassed one new sequence generated in the present study (MT336217.1) and 50 sequences retrieved from GenBank. The final alignment included 729 aligned nucleotides and 618 variable sites. The ITS analysis encompassed one new sequence of the present study (MT336216.1) and 76 sequences retrieved from GenBank, generating a final alignment with 401 nucleotides and 357 variable sites.

The topology of the BI and ML trees agreed in general with previous studies on Ostreopsis species (Accoroni et al., 2016; Verma et al., 2016; Zhang et al., 2018; Chomérat et al., 2019, 2020; Nascimento et al., 2020) for both D1-D3 LSU (Fig. 5) and ITS (Fig. 6). For both markers, the main clades, formed by sequences of the different *Ostreopsis* species available from GenBank, were retrieved with high support values, particularly in the Bayesian Inference analysis.

The D1-D3 LSU (MT336217.1) and ITS (MT336216.1) sequences obtained in this study clustered with a strong support (D1-D3 LSU: PP = 1/BS = 99; ITS: PP = 0,99/BS = 92) in a clade including all *O. lenticularis* sequences available from GenBank, that originated from the Indian and Pacific Oceans (China, French Polynesia, Galápagos Islands, Hawaii, Japan and Réunion Island, Figs. 5 and 6). The D1-D3 sequence (MT336217.1) of *O. lenticularis* from Fernando de Noronha was identical to those of strains THT16-4 (MK227248.1) and MD03-03 (MK227245.1), both from Tahiti, French Polynesia, while the ITS sequence (MT336216.1) of *O. lenticularis* from Fernando de Noronha was different from all other sequences available from GenBank. The ITS sequence from Brazil presented 10 polymorphic sites when compared to the closer genetically related *O. lenticularis* sequences available from GenBank, including 2 indels and 8 SNPs (6 G/A, 1 G/T and 1 A/T).

3.3. Ecology

Ostreopsis lenticularis was found epiphytically on the macroalgae *Canistrocarpus cervicornis* (F.T. Kützing) De Paula & De Clerck (De Clerck et al., 2006), *Canistrocarpus crispatus* (J.V. Lamouroux) De Paula & De Clerck (De Clerck et al., 2006), *Dictyota pinnatifida* F.T. Kützing (Kützing, 1859), *Dictyopteris delicatula* J.V. Lamouroux (Lamouroux, 1809) and *Bryothamnion triquetrum* (S.G. Gmelin) M.A. Howe (Howe, 1915) with *Ostreopsis* cf. *ovata* Y. Fukuyo (Fukuyo, 1981), and species of *Gambierdiscus* R. Adachi & Y. Fukuyo (Adachi and Fukuyo, 1979), *Prorocentrum* C.G. Ehrenberg (Ehrenberg, 1834), *Coolia* A. Meunier (Meunier, 1919), *Amphidinium* É. Claparède & J. Lachmann (Claparède and Lachmann, 1859), *Sinophysis* D. Nie & C.-C. Wang (Nie and Wang, 1944) and *Cabra* S. Murray & D.J. Patterson (Murray and Patterson, 2004). *Ostreopsis lenticularis* was observed in abundances that varied between 6 and 7.6 × 10^4 cells g^{-1} Fresh weight (FW) macroalgae. Seawater temperature at Fernando de Noronha Archipelago varies from 26.3 to 28.3 °C (Sea Surface Temperature).

4. Discussion

The morphology of *O. lenticularis* cells from Fernando de Noronha, such as the broadly oval cell shape and the pattern and ornamentation of the thecal plates are in accordance with the original description of *O. lenticularis* by Fukuyo (1981). The dorsoventral diameter (DV) and the width (W) of *O. lenticularis* cells from Fernando de Noronha showed maximum values higher than that reported for cells from French Polynesia (Fukuyo, 1981; Chomérat et al., 2019), Vietnam (Larsen and Nguyen, 2004), Revillagigedo Archipelago, Mexico (Gárate-Lizárraga et al., 2018) and the Cape Verde Archipelago, Atlantic Ocean (Soler-Onís and Fernández-Zabala, 2019) (Table 1). Cell dimensions were closer to that of cells from Hainan Island, South China Sea (Zhang et al., 2018) (Table 1). The larger dimensions observed in cells from the current study may be related to the age of cells, as many senescent cells as well as empty theca, were observed, which may indicate that cells were likely at the decline phase of a bloom. A relationship between cell dimensions of *Ostreopsis* cf. *ovata* and bloom phases has been presented by Accoroni et al. (2012) and cells in the final phase of the bloom had a significantly longer DV than those in both the initial and in the proliferation phases. It is possible that the same pattern occurs in *O. lenticularis*.

As described by Fukuyo (1981), the species *O. lenticularis* exhibits two kinds of pores with distinct sizes, large and small pores, spread across all the thecal plates. This characteristic was observed in cells from the type locality (Tahiti Island) in the Society Archipelago, Gambier Islands and New Caledonia (Fukuyo, 1981) as well as from Nha Trang Bay, Vietnam (Larsen and Nguyen, 2004), the Réunion Island, Western Pacific (Carnicer et al., 2015), the Revillagigedo Archipelago in Mexico, Eastern Pacific (Gárate-Lizárraga et al., 2018), the Gambier, Australes and Marquesas Archipelagos, French Polynesia (Chomérat et al., 2019) and the Cape Verde Archipelago in Macaronesia, Eastern Atlantic (Soler-Onís and Fernández-Zabala, 2019). The presence of three distinct pores, “mini pores”, and larger pores that were round or oblong were observed in *O. lenticularis* strains from Hainan Island, China (Zhang et al., 2018). Oblong pores have also been observed in strains of *O. lenticularis* from French Polynesia, but this feature was unusual and found only in some thecal plates in cells in laboratory cultures and was likely a result of morphological plasticity of the large pores (Chomérat et al., 2019). In the current study, only two pore sizes were observed in all cells examined.

Ostreopsis lenticularis shares most of its morphological characteristics (cell shape and size and the thecal plate pattern) with *O. siamensis*. However, *O. lenticularis* differs from *O. siamensis* by the presence of thecal pores with two distinct sizes, spread across all thecal plates and the absence of a body undulation (Fukuyo, 1981). Chomérat et al. (2019) have recently assigned a genotype to *O. lenticularis*, providing reference molecular sequences for this species and showed that the presence of two kinds of thecal pores is a reliable taxonomic feature that distinguishes this species from others with similar morphology. *Ostreopsis siamensis*, *O. marina* and *O. labens* are similar in cell shape and size to *O. lenticularis*, however, according to the original descriptions of these species, or interpretation (by Fukuyo, 1981) for *O. siamensis*, all present only one kind of pore. These species have not been carefully reinvestigated yet and this character still needs to be confirmed.

The molecular data confirms the presence of *O. lenticularis* in French Polynesia, Réunion Island, Galápagos, Hawaii, Japan and Hainan Island, China (Chomérat et al., 2019). The current study expands the distribution of *O. lenticularis* to Fernando de Noronha Archipelago at the South Atlantic Ocean, providing molecular and morphological evidence of its presence. *Ostreopsis* cf. *ovata* was the only *Ostreopsis* species previously reported in the South Atlantic (Nascimento et al., 2012a, 2012b, 2020; Gómez et al., 2017; Mendes et al., 2017; Tibiriçá et al., 2019), including at the Fernando de Noronha Archipelago (strain UNR-60, Nascimento et al., 2020). Interestingly the presence of *O. lenticularis* has been confirmed so far solely from insular sites.

The phylogenetic analyses based on D1-D3 LSU and ITS sequences corroborate the morphological results of the present study, confirming the presence of *O. lenticularis* at Fernando de Noronha. The analysis of the ITS locus suggests a closer phylogenetic relationship between the Brazilian sequence (MT336216.1) and the *O. lenticularis* strains from Japan (Fig. 6). However, this relationship is not supported when D1-D3 LSU sequences are taken into account (Fig. 5). Thus, the phylogeographic relationship between this lineage of *O. lenticularis* from the South Atlantic Ocean (Fernando de Noronha, Brazil) and those from the Indian and Pacific Ocean remains unclear.

Despite the lack of molecular data, the presence of *O. lenticularis* in Nha Trang Bay, Vietnam (Larsen and Nguyen, 2004), in the Revillagigedo Archipelago, Mexico (Gárate-Lizárraga et al., 2018) and the Cape Verde Archipelago, Atlantic Ocean (Soler-Onís and Fernández-Zabala, 2019) is supported by the morphological description presented in these studies (cell size and shape and the presence of two kinds of pores in thecal plates).

In the Caribbean Sea, the presence of *O. lenticularis* has been reported in several locations, such as the Virgin Islands (Carlson and Tindall, 1985), Puerto Rico (Ballantine et al., 1988; Tosteson et al., 1989), Belize (Faust, 1995), Cuba (Delgado et al., 2006) and Venezuela (Marchan-Álvarez et al., 2017) based on morphological observation of specimens. A series of inaccurate identifications has been made in the Caribbean (see Chomérat et al., 2019), e.g. Faust et al. (1996) identified cells with a single pore size as *O. lenticularis* while other cells that clearly presented two kinds of pore were identified as *O. siamensis*, and probably corresponded to *O. lenticularis*. Moreover, *O. labens* and *O. marina* have been described from the Caribbean Sea by Faust and Morton (1995) and Faust (1999) with no molecular data associated to the morphological description. The morphological features that distinguish these species from other morphologically similar *Ostreopsis* species were not presented, creating uncertainty about the identification of specimens in that region based only on morphological observations. Therefore, the presence of *O. lenticularis* in the Caribbean Sea still needs molecular support, although it is likely that the species is present, considering Fig. 8 in Faust et al. (1996).

The presence of *O. lenticularis* is confirmed by morphological and molecular data at the Fernando de Noronha Archipelago, South Atlantic Ocean. The species was found in bloom abundances (maximum of 7.6 × 10^4 cels^{-1}gFW macroalgae) and was probably at the declining phase of the bloom. Harmful ecological effects such as anoxia may occur due to its presence in high abundances. *Ostreopsis lenticularis* isolates from the Pacific Ocean were not toxic (Sato et al., 2011 as *Ostreopsis* sp. 5, Chomérat et al., 2019), but there are no toxin analysis of genetically identified strains from the Atlantic Ocean and the Caribbean Sea. As shown for *Ostreopsis* cf. *ovata*, some populations (subclades) have shown to be highly toxic while others are nearly non-toxic. In the future it will be desirable to perform toxin analysis of genetically identified clones from the Atlantic and the Caribbean to confirm the toxicity status of the species.

CRediT authorship contribution statement

Geovanna Theobald Borsato:Investigation, Formal analysis, Writing - original draft.**Fabiano Salgueiro**:Formal analysis, Writing - original draft, Writing - review & editing, Resources, Supervision, Funding acquisition.**Cristhian Gomes Tavares da Silva**:Investigation, Writing - original draft.**Adriana D. Menezes-Salgueiro**:Resources, Supervision, Funding acquisition.**Silvia M. Nascimento**:Conceptualization, Writing - original draft, Writing - review & editing, Resources, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Carlos E. L. Ferreira (C.E.L.F.) for sample collection and Joel C. de Paula for the identification of macroalgae species. Flávio J. H.T.V. Ramos from Instituto Militar de Engenharia is acknowledged for assistance with SEM. Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) provided financial support for the research project PELD ILOC/CNPQ 441241/2016-6 to C.E.L.F. Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) provided support via research grant E-26/111.925/2012 to S.M.N. and E-26/202.800/2015 to F.S. Additional financial support was provided by Universidade Federal do Estado do Rio de Janeiro (UNIRIO, Edital IN-UNIRIO 01/2018) to F.S. Authors are grateful to CNPq/CAPES/FAPs/BC-Fundo Newton - Pesquisa Ecológica de Longa Duração for a scientific initiation scholarship (IC) to G.T.B. 88887.198477/2018-00 and to Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (IFRJ) for an IC scholarship to C.G.T.S.

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Capítulo 2

Taxonomy and abundance of benthic *Prorocentrum* (Dinophyceae) species from the tropical and subtropical Southwest Atlantic Ocean including a review of their global diversity

Geovanna Theobald Borsato[a], Fabiano Salgueiro[b], Gabriela A. L. De'Carli[a], Agatha M. Morais[a], Amanda S. Goulart[b], Silvia M. Nascimento[a,*]

a. Laboratório de Microalgas Marinhas, Universidade Federal do Estado do Rio de Janeiro - UNIRIO, Av. Pasteur, 458, Urca, Rio de Janeiro, 22290-240, RJ, Brazil

b. Laboratório de Biodiversidade e Evolução Molecular, Universidade Federal do Estado do Rio de Janeiro - UNIRIO, Av. Pasteur, 458, Urca, Rio de Janeiro, 22290-240, RJ, Brazil

*E-mail address: Silvia Mattos Nascimento (silvia.nascimento@unirio.br)

Abstract

In the tropical South Atlantic Ocean, studies on the taxonomy and abundance of benthic harmful algae species are scarce and the region has been largely under investigated. In this study, morphological descriptions, molecular data (LSU and ITS rDNA) and abundance data of benthic *Prorocentrum* species from the Northeast and Southeast coast of Brazil and three oceanic islands in the South Atlantic Ocean were provided. Moreover, a global review of benthic *Prorocentrum* species richness was presented. Eleven benthic *Prorocentrum* species were found, three in the Brazilian Northeast, four in the Southeast and up to eleven in the oceanic islands. Morphological and molecular data on *P. borbonicum*, *P. hoffmannianum*, *P. lima* species complex and *P. rhathymum* were provided, while *P.* cf. *caipirignum*, *P.* cf. *concavum*, *P.* cf. *fukuyoi*, *P.* cf. *norrisianum*, *P. panamense*, *P.* cf. *sculptile* and *Prorocentrum* sp. were tentatively identified through morphological analysis using scanning electron or light microscopy.

Strains of *P. borbonicum*, *P. hoffmannianum*, *P. lima* complex and *P. rhathymum* were established. *Prorocentrum lima* complex was found in all investigated sites, in abundances up to 20.000 cells.gFW^{-1} at the Northeast Brazil, while maximum abundance of all the remaining species did not exceed 1.000 cells.gFW^{-1}. Fernando de Noronha archipelago can be considered a hotspot of benthic *Prorocentrum* species diversity, with eleven species registered. In the context of climate change, this tropical area should be the focus of research studies, as declines in the diversity of tropical species are expected with increasing ocean temperature.

Keywords: Biodiversity, Dinoflagellate, Harmful Algae Blooms, Morphology, Phylogeny

1.0 Introduction

Currently, the genus *Prorocentrum* Ehrenberg (1834) encompasses 84 valid species (Guiry and Guiry, 2022) and half of these are benthic and can be found associated with macrophytes, coral reefs, among sand grains and debris in the marine environment (Fukuyo, 1981; Faust, 1990; Tindall and Morton, 1998; Ten-Hage et al., 2000; Richlen and Lobel, 2011; Yong et al., 2018). Benthic *Prorocentrum* species have been reported to produce toxins, such as okadaic acid (OA) and its analogues, dinophysistoxins (DTXs) (Nakajima et al., 1981; Murakami et al., 1982; Holmes et al., 2001; Caillaud et al., 2010; Nascimento et al., 2017; Nishimura et al., 2020), responsible for diarrhetic shellfish poisoning (DSP) (Murakami et al., 1982).

Morphologically, *Prorocentrum* species have two lateral plates joined by a sagittal suture and small platelets in the periflagellar area at the anterior end of the cell (Hoppenrath et al., 2013). The taxonomy of this genus is based on morphological criteria such as cell shape and size, cell surface ornamentation and pore pattern, intercalary band morphology, and number, shape and organization of platelets and structures (spines, collars and protrusions) in the periflagellar area (Hoppenrath et al., 2013). The periflagellar area is considered a valuable and promising taxonomic feature in the genus, while other morphological characters, such as cell shape and pore pattern may be variable in some species or present subtle differences among species (Hoppenrath et al., 2013). The analysis of molecular data is therefore necessary, in combination with morphological studies, to unambiguously identify the species of *Prorocentrum* and other dinoflagellates (Nagahama et al., 2011; Nascimento et al., 2017, 2019; Jang et al., 2018; Lim et al., 2019; Chomérat et al., 2020).

Prorocentrum species generally constitute a significant part of benthic dinoflagellate assemblages in tropical and subtropical areas and occurs with several species of the genera *Amphidinium* Claperède & Lachmann, *Coolia* A. Meunier, *Gambierdiscus* R. Adachi & Y. Fukuyo and *Ostreopsis* J. Schmidt (Tindall and Morton, 1998; Delgado et al., 2006; Richlen and Lobel, 2011; Boisnoir et al., 2019). Many studies have revealed high diversity of *Prorocentrum* in benthic reef ecosystems in the Pacific and Indian Oceans, and particularly in the Caribbean Sea, where numerous benthic *Prorocentrum* species have been described (Faust, 1990, 1993a, 1993b, 1997, 1994; Faust et al., 2008). The Pacific Ocean was the type locality of other benthic *Prorocentrum* species (Fukuyo, 1981; Mohammad-Noor et al., 2007; Hoppenrath and Leander, 2008; Lim et al., 2019), while a smaller number has been described from the Indian Ocean (Ten-Hage et al., 2000; Chomérat et al., 2012). Only one benthic *Prorocentrum* species has been described from the South Atlantic Ocean (Nascimento et al., 2017).

In the Atlantic Ocean, studies have focused on the occurrence and toxicity of *Prorocentrum lima* (Ehrenberg) F. Stein (1878) in temperate areas, such as the United States (Morton et al., 1999; Maranda et al., 2007a, 2007b), Canada (Lawrence et al., 2000; Levasseur et al., 2003), United Kingdom (Foden et al., 2005; Nascimento et al., 2005), Ireland (Kilcoyne et al., 2020), Portugal (Vale et al., 2009), Spain (Bravo et al., 2001) and Argentina (Gayoso et al., 2002). Some studies in the temperate Atlantic have also evaluated the distribution and diversity of other benthic *Prorocentrum* species, such as in France (Chomérat et al., 2010; 2011) and the Bay of Biscay (Laza-Martinez et al., 2011; David et al., 2014). In the tropical Atlantic, apart from the Caribbean Sea, studies on the diversity and abundance of benthic *Prorocentrum* species are scarce and the region has been investigated only recently (Nascimento et al., 2016, 2017; Gómez et al., 2017; Fernández-Zabala et al., 2019; Mendes et al., 2019; Moreira-González et al., 2019a, 2019b, 2020).

The factors that affect species distribution constitute an important issue not yet fully understood in ecology (Araújo and Guisan, 2006), however, changes in species distribution are increasingly a reality, and are usually strongly linked to the recent increase in global temperature (Lenoir and Svenning, 2015). Climate change is leading to a reorganization of species as they keep up with changes in temperature. Studies have shown that warmer temperate regions will receive an influx of species from the tropics, that will experience species loss. This change in distribution will lead to new biotic interactions and new community structures, with consequences for functioning of ecosystems (Antão et al., 2020). In this context, harmful benthic species are expected to expand their distribution and have greater growth potential, increasing overall cell abundance (Tester et al., 2020).

The aim of this study was to perform taxonomic study of benthic *Prorocentrum* species from Brazil, including the analysis of field and isolated cells kept in culture. Morphological descriptions and molecular data were provided for benthic *Prorocentrum* species from the Brazilian Northeast and Southeast coast, as well as from three oceanic islands of the South Atlantic. Information on *Prorocentrum* species abundance at each sampling site is presented and can be used to gain insight on the potential risk of toxin bioaccumulation in the marine fauna as well as on seafood safety. A global review of benthic *Prorocentrum* species richness is also presented.

2.0 Materials and Methods

2.1 Sample collection and treatment

Macroalgal samples were collected by diving from 12 locations in the coast (Northeast and Southeast regions, from latitude 00°55'10" N to 23°00'03" S), two archipelagos and one oceanic island in Brazil (Supplementary Table S1, Figure 1). In the present study, the archipelagos will be referred to as islands, because they are being treated as a biogeographic unit. In total, 632 macroalgae samples were collected, 487 from the Southeast coast, 88 from the Northeast coast and 57 from the oceanic islands. Macroalgae species varied between sampling sites and dominant species at the time of sampling at each site were collected (Table S2). Macroalgae were placed in sealable plastic bags and were vigorously shaken for two minutes to detach the associated epiphytic cells. The epiphytic suspensions were preserved with 1% neutral Lugol iodine solution for species identification and quantification in the laboratory. Date of sampling, depth, seawater temperature, measured in the field at the time of sampling or estimated from satellites and salinity (estimated from satellites) from each sampling site are presented in table S1.

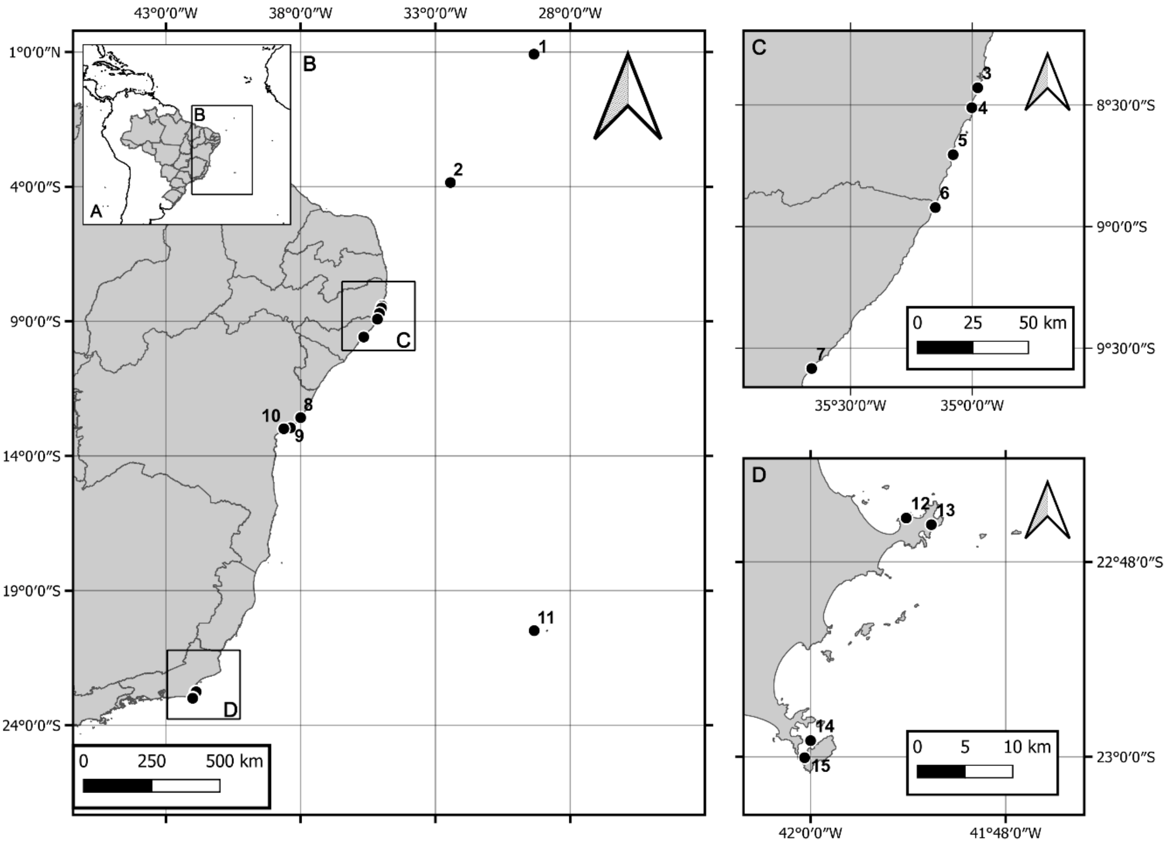

Figure 1. Map showing sampling sites at the coast and oceanic islands of Brazil. A. Brazil, Atlantic Ocean, B. Coastal and oceanic area, C. Some sites at the Northeast coast, D. Sites at the Southeast coast. 1. Saint Paul's Rocks Archipelago; 2. Fernando de Noronha Archipelago; 3. Muro Alto; 4. Porto de Galinhas; 5. Carneiros; 6. Maragogi; 7. Garça Torta; 8. Forte; 9 Placaford; 10. Penha; 11. Trindade Island; 12. Tartaruga; 13. Forno (AB); 14. Forno (AC); 15. Cabo Frio.

At six of the 15 sites, in addition to the samples preserved with Lugol, epiphytes suspensions were kept alive for the isolation of strains and the establishment of cultures in the laboratory, according to Nascimento et al. (2017). The place of origin of each strain is presented in table S1. Cultures were maintained in L1 medium (Guillard and Hargraves, 1993) that was modified by omitting silicate, nickel, vanadium and chromium. They were kept in a temperature-controlled cabinet at 24 ± 2 ºC with a light/dark cycle of 12 h/12 h and a photon flux density of 70 µmol m^{-2} s^{-1}, provided by cool-white fluorescent tubular lamps. Photosynthetically active radiation was measured with a QSL-100 quantum sensor (Biospherical Instruments, San Diego, California, USA).

2.2 Morphological characterization

Cultured cells and Lugol preserved field cells were observed by light microscopy (LM) and scanning electron microscopy (SEM). Cells were observed in a light microscope (ImagerA2, Zeiss, Germany) and images were captured with an AxioCam ICc1 digital camera (Zeiss, Germany). For observation of *Prorocentrum* cells kept in culture in a scanning electron microscope, cells were fixed with 2% glutaraldehyde SEM grade (Merck, Darmstadt, Germany) for 1 hour. Cells were then filtered through Durapore membrane filters (Millipore, USA), rinsed twice with distilled water for 10 minutes and subjected to dehydration through a series of ethanol (10, 30, 50, 70, 80, 95 and twice at 100%, 10 min each step), followed by three drops of hexamethildisilazane for 15 min.

Field cells preserved with Lugol were isolated under observation in LM and transferred to a 1.5 ml tube with filtered and autoclaved seawater. The isolated field cells were then filtered through Durapore membrane filters, rinsed once with distilled water and dehydrated in an ethanol series, as described above. After being air-dried overnight, culture and field cells were coated with nickel or gold with a sputter coater (Leica EM ACE 600, Germany) and observed with a Micro Quanta FEG 250 scanning electron microscope (FEI Company, Hillsboro, Oregon, USA). Scanning electron microscopy images were presented on a black background using Adobe Photoshop CS6.

Cultured cells were preserved with Lugol for length (L) and width (W) measurement using Axiovision software (Zeiss, Germany). The L and W of the Lugol preserved field cells were measured using the Axiovision software or with a microscope eyepiece ruler (Imager A2 or Primovert, Zeiss, Germany).

2.3 Molecular characterization

Exponentially growing cells of strains UNR-11, UNR-12, UNR-15, UNR-35, UNR-44, UNR-68, UNR-72 and UNR-86 were harvested in 2 ml centrifuge tubes by centrifugation at 5000 × g for 15 min for DNA extraction. The supernatant was discarded and the cell pellet was stored at -80 °C for further analysis. Genomic DNA was extracted using the commercial kit NucleoSpin® Plant II (Macherey-Nagel, Germany) following the manufacturer's instructions, and then stored at -20 °C. DNA quantification was performed using Qubit® 3.0 Fluorometer (Life Technologies) and checked by electrophoresis on a 1% (w/v) agarose gel in 1XTAE buffer (Tris-acetate-EDTA) stained with GelRed (Biotium Inc., USA).

Two ribosomal DNA (rDNA) loci were analyzed: the Internal Transcribed Spacer (ITS = ITS1-5.8S-ITS2) and the D1-D3 domain of the Large Subunit (LSU) ribosomal DNA. To amplify the ITS and the LSU loci, first, several pairs of primers frequently used in the literature were tested, such as: ITSAxITSB (Sato et al., 2011), ITS1xITS4 (White et al., 1990), ProITS-FxProITS-R (Nagahama et al., 2011), D1RxD2C (Scholin et al., 1994a), D1RxD3Ca (Scholin et al., 1994b), D1RxLSUB (Scholin et al., 1994a; Litaker et al., 2003) and D1RxD3B (Scholin et al., 1994a; Nunn et al., 1996). The ITS sequences of *P. borbonicum* Ten-Hage, Turquet, Quod, Puiseux-Dao & Couté (2000) strain UNR-12 and *P. rhathymum* AR Loeblich, JL Sherley & RJ Schmidt (1979) strain UNR-11 were obtained using the pair of primers ITS1xITS4 (White et al., 1990) and the LSU sequences were obtained using the pair of primers D1RxD2C (Scholin et al., 1994a). Regarding the *P. hoffmannianum* MA Faust (1990) strain UNR-44 and the *P. lima* (Ehrenberg) F. Stein (1878) species complex strains UNR-15, UNR-35, UNR-68, UNR-72 and UNR-86 sequences were obtained using four new pair of primers (Table 1) designed based on ITS and LSU sequences of the *P. lima* – *P. caipirignum* – *P. hoffmannianum* clade available from GenBank, using the standard parameters of the Geneious Prime v2020.2.4 software (www.geneious.com). Forward and reverse primers were designed within conserved regions of the ITS and LSU multiple sequence alignment.

Table 1. PCR primers designed in the present study to amplify the ITS and D1-D3 LSU loci of *P. hoffmannianum* and *P. lima* complex strains

Target locus	Primer name and primer sequence (5'-3')	Expected size
ITS (ITS1-5.8S-ITS2)	PLSC_ITS_F1 GTTGATTACGTCCCTGCCCT PLSC_ITS_R1 ATGAAAGCCACCACCACCTT	~1000bp
	PLSC_ITS_F2 TGCACATCAGGGCACATTAT PLSC_ITS_R2 TTCACTGGCCTAACATCGTG	~550bp
LSU (D1-D3 domain)	PLSC_D1D3_F1 CAGGATTCCGTGAGCCAACA PLSC_D1D3_R1 AGGGAAACTTCGGAGGGAAC	~1250bp
	PLSC_D1D3_F2 TCAGTAATGGCGAATGAACG PLSC_D1D3_R2 TCGGAGGGAACCAGCTACTA	~1200bp

The amplification reaction mixture of 25 µL contained 1 unit (U) Taq DNA polymerase (Thermo Scientific Inc, USA), 1x reaction buffer with NH_4SO_4, 2.5 mM $MgCl_2$, 0.16 mM dNTPs (Thermo Scientific Inc., USA), 8 pmol of each primer, 0.2 mg of Bovine Serum Albumin (BSA) and ~10 ng of genomic DNA. For the *P. borbonicum* and *P. rhathymum* strains the PCR cycling comprised an initial 5 min heating step at 95 °C, followed by 40 cycles of 95 °C for 1 min, 45 °C (for ITS) or 58 °C (for LSU) for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 5 min. Regarding the *P. hoffmannianum* and *P. lima* species complex strains the PCR cycling comprised an initial 5 min heating step at 95 °C, followed by 10 cycles of 95 °C for 1 min, touchdown of 60°C-55°C (Δ = -0,5 ºC/cycle) for 1 min, 72 °C for 90 sec, 35 cycles of 95 °C for 1 min, 55 °C for 1 min, 72 °C for 90 sec and a final extension at 72 °C for 5 min. PCR products were checked by electrophoresis on 1% (w/v) agarose gel in 1X TAE buffer stained with GelRed. PCR products were purified and sequenced by Macrogen Inc. (Seoul, Korea) in both directions, using the same PCR primers, through the traditional capillary sequencing method.

Sequence reads were manually checked and edited using Chromas 2.6.6 (http://technelysium.com.au/wp/chromas) and MEGA 7.0 (Kumar et al., 2016) programs. The new sequences obtained in the present study were first BLAST searched against the GenBank database (www.ncbi.nlm.nih.gov/blast) to test for sequence homology with non-target taxa. All sequences obtained in this study have been deposited in GenBank.

The sequences were then aligned with others *Prorocentrum* sequences retrieved from GenBank using MAFFT 7.0 (Katoh and Standley, 2013) with default settings. Phylogenetic analyses were conducted separately for each molecular marker. Sequences of *Adenoides eluden* were used as outgroup. The MEGA software was used to select the best-fit model of nucleotide substitution (T92+G+I for both markers) and construct maximum likelihood (ML) phylogenetic trees with 1000 bootstrap (BS) replications. The phylogenetic relationships were also examined using Bayesian inference (BI) with MrBayes 3.2.7a (Ronquist et al., 2012). To sample across nucleotide substitution models, the command “lset nst=mixed” was used before running the analysis. Markov Chain Monte Carlo procedure consisted of two independent trials with four chains each. Each chain was run for 1.000.000 generations and sampled every 100th cycle. Posterior probability (PP) values for the resulting 50% majority rule consensus tree were estimated after discarding the first 10% of trees as burn-in.

2.4 Abundance data

Benthic dinoflagellates of the genus *Amphidinium*, *Cabra* Murray and Patterson (2004), *Coolia, Gambierdiscus*, *Ostreopsis*, *Prorocentrum* and *Sinophysis* D. Nie & C.-C. Wang (1944) were quantified in all Lugol preserved field samples using a Sedgewick Rafter or sedimentation chamber and observation in a light inverted microscope (Primovert, Zeiss, Germany). The fresh weight of each macroalgae sample was measured and the abundance of benthic dinoflagellates was determined as cells gFresh Weight macroalgae^{-1}. The relative abundance (%) of the genus and each species of *Prorocentrum* to the total benthic dinoflagellates abundance was calculated. *Prorocentrum* abundance was not determined at the Carneiros.

The morphological features on which *P. emarginatum* Y. Fukuyo (1981), *P. fukuyoi* Murray S. et Nagahama Y. (2007) and *P. sculptile* M.A. Faust (1994) are reliably distinguished are not yet clear (Hoppenrath et al., 2013) and not positively recognized through observation using the light microscope, therefore, cell dimensions and abundance data for these species from field samples were presented combined. Morphological descriptions of field cells from *P.* cf. *fukuyoi* and *P.* cf. *sculptile* were based on observation of SEM images.

3.0 Results

Ten strains of benthic *Prorocentrum* species were established (Table S1), and eight of them were identified through combined morphological (LM and SEM) and molecular (ITS and LSU rDNA) analysis. These strains include the species *P. borbonicum* (strain UNR-12), *P. hoffmannianum* (UNR-44), *P. lima* species complex (UNR-15, UNR -35, UNR-68, UNR-72 and UNR-86) and *P. rhathymum* (UNR-11). The two remaining strains, *P. borbonicum* (UNR-13) and *P. hoffmannianum* (UNR-29), were identified based on morphology. Moreover, field cells of *P.* cf. *caipirignum*, *P.* cf. *fukuyoi*, *P. panamense* and *P.* cf. *sculptile* were tentatively identified through observation of cell morphology on LM and SEM and *P.* cf. *concavum* and *P.* cf. *norrisianum* through LM and no sequences was obtained for these morphospecies. One species was not identified (*Prorocentrum* sp.).

3.1 Morphology and distribution

Dimensions of strains and field cells of *Prorocentrum* species are presented in table 2.

Prorocentrum borbonicum Ten-Hage, Turquet, Quod, Puiseux-Dao & Couté, 2000

Prorocentrum borbonicum cells from strains UNR-12 (Fig. 2A) and UNR-13 (Fig. 2B-E) were oblong to ovoid and symmetric. Thecal surface foveate with scattered pores, without a marginal row of pores (Fig. 2B-D). Pores round and of two size classes, both located inside depressions (Fig. 2B-D), that were deeper at the center of the right thecal plate (Fig. 2B). Intercalary band horizontally striated (Fig. 2D). Periflagellar area wide and V-shaped excavating the right thecal plate (Fig. 2E). Cells with many golden-brown chloroplasts and a centrally located pyrenoid (Fig. 2A).

Distribution: Maragogi (sequence obtained), Fernando de Noronha and Trindade (morphospecies).

Prorocentrum caipirignum S. Fraga, M. Menezes et S. Nascimento, 2017

Prorocentrum cf. *caipirignum* field cells were oblong and symmetric (Fig. 2F). Thecal surface smooth with scattered round to oblong pores, except in the central area, and a marginal row of pores (Fig. 2F). *Prorocentrum* cf. *caipirignum* cells from Fernando de Noronha presented a larger number of pores relative to the original description of the species. Periflagellar area V-shaped excavating the right thecal plate (Fig. 2F).

Distribution: Fernando de Noronha and Cabo Frio (morphospecies).

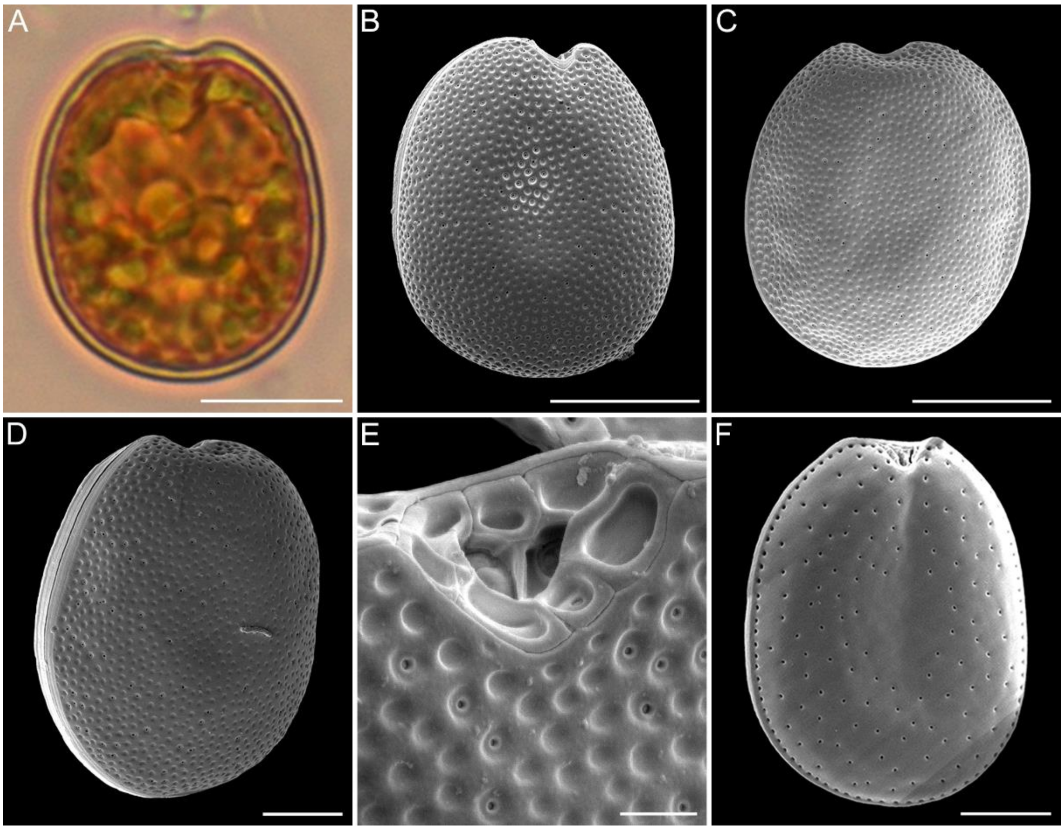

Figure 2. (A) Light and (B-F) scanning electron microscopy of *Prorocentrum* species. (A-E) *Prorocentrum borbonicum*: (A) Strain UNR-12, (B-E) Strain UNR-13. (F) *Prorocentrum* cf. *caipirignum* Fernando de Noronha field cell. (A, B, F) Right thecal plate view, (C) Left thecal plate view, (D) Lateral view, (E) Detail of the periflagellar area. Scale bars: (A-C, F) 10 µm (D) 5 µm (E) 1 µm.

Prorocentrum emarginatum Y. Fukuyo, 1981, *Prorocentrum fukuyoi* Murray S., et Nagahama Y., 2007 and *Prorocentrum sculptile* M.A. Faust, 1994 group

Prorocentrum cf. *fukuyoi* field cells were round to oval and slightly asymmetric (Fig. 3A-E). Thecal surface smooth with pores round and of two size classes (Fig. 3B-E), which in some specimens clearly arranged in radial rows (Fig. 3E). Marginal row of pores present and the center of the thecal plates devoid of pores (Fig. 3B-E). Periflagellar area deep, narrow and V-shaped excavating the right thecal plate, with a wing (Fig. 3B-C).

Distribution: St. Paul’s Rocks, Fernando de Noronha, Porto de Galinhas, Maragogi, Penha, Trindade, Tartaruga, Forno (AC) and Cabo Frio (morphospecies).

Prorocentrum cf. *sculptile* field cells were oval and asymmetric (Fig. 3F-I). Thecal surface ornamentation was variable, and the cells were strong (Fig. 3F-G), slight (Fig. 3H) or faint (Fig. 3I) foveate. In cells with a slight or faint foveate ornamentation, depressions were visible in the margin of the thecal plates and pores arranged in radial rows (Fig. 3H-I). Pores round and of two size classes, and the center of the thecal plates devoid of pores (Fig. 3F-I). Periflagellar area deep, narrow and V-shaped excavating the right thecal plate, with a wing (Fig. 3F).

Distribution: Fernando de Noronha (morphospecies).

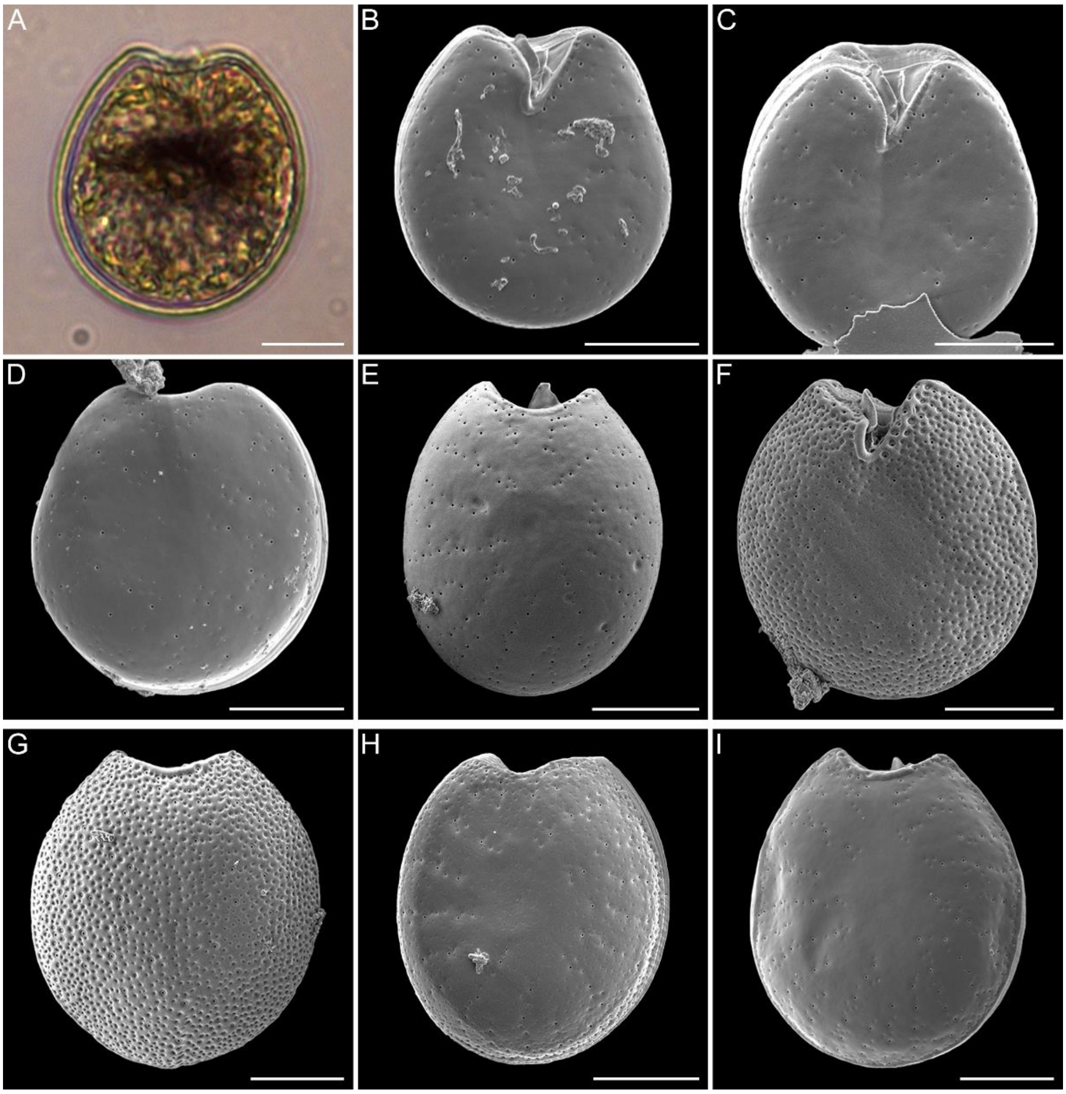

Figure 3. (A) Light and (B-I) scanning electron microscopy of *Prorocentrum emarginatum*/*fukuyoi*/*sculptile* group (Fernando de Noronha field cells). (A-E) *Prorocentrum* cf. *fukuyoi*, (F-I) *Prorocentrum* cf. *sculptile*. (B-C, F) Right thecal plate view, (D-E, G-I) Left thecal plate view. Scale bars: 10 µm.

Prorocentrum hoffmannianum M.A. Faust, 1990 emend. S. Fraga (2018)

Prorocentrum hoffmannianum cells of strains UNR-29 (Fig. 4A-E) and UNR-44 and field cells from St. Paul’s Rocks and Fernando de Noronha (Fig. 4F-I) were symmetric, ovoid to broadly oval, narrow at the anterior end and wider in the middle region of the cell, with a rounded posterior margin. Most field cells from Trindade were wider below the middle of the cell (Fig. 4J-L). Thecal surface reticulate-foveate with scattered pores, except in the central area, and a marginal row of pores (Fig. 4B-D, G-H, J-K). Some depressions with one or two round pores, or two pores merged and a larger elongated pore (oblong or kidney-shaped) was evident (Fig. 4E, I). Periflagellar area V-shaped excavating the right thecal plate (Fig. 4A-C, E-F, G, J, L). Cells with many golden-brown chloroplasts and a centrally located pyrenoid (Fig. 4A, F).

Distribution: St. Paul’s Rocks (morphospecies), Fernando de Noronha (sequence obtained and morphospecies) and Trindade (strain and morphospecies).

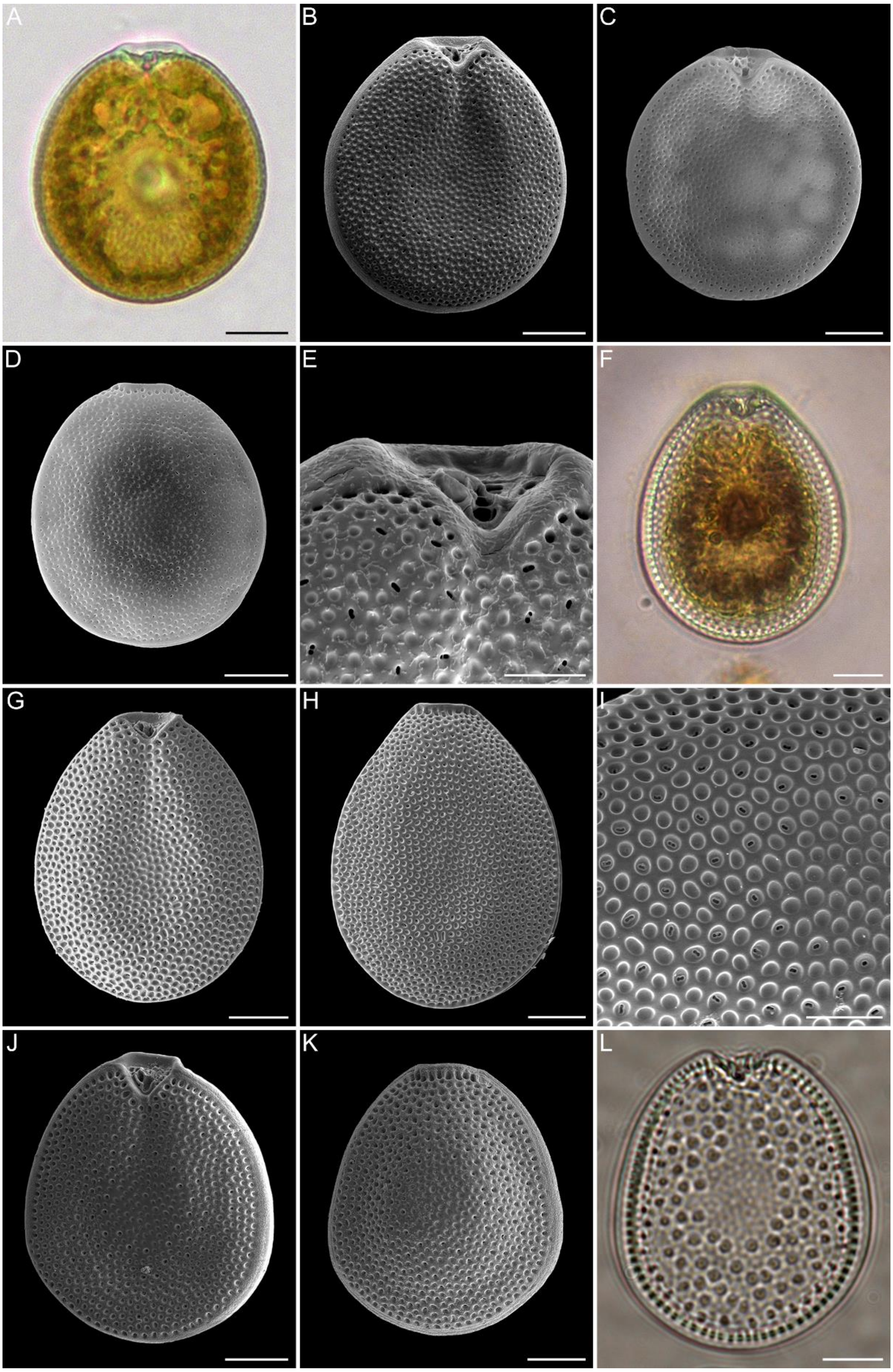

Figure 4. (A, F, L) Light and (B-E, G-K) scanning electron microscopy of *Prorocentrum hoffmannianum*. (A-E) Strain UNR-29, (F-I) Fernando de Noronha field cells, (J-L) Trindade field cells. (A-C, F-G, J, L) Right thecal plate view, (D, H, K) Left thecal plate view, (E) Detail of the periflagellar area, (I) Detail of the thecal surface. Scale bars: (A-D, F-H, J-L) 10 µm, (E) 4 µm, (I) 5 µm.

Prorocentrum lima species complex (Ehrenberg) F. Stein 1878 emend. Nagahama et al. (2011)

Prorocentrum lima cells of strains UNR-15 (Fig. 5A-D), UNR-35, UNR-68 and UNR-72 were symmetric, ovate, narrow at the anterior end and wider in the middle region, with maximum width below the cell center (Fig. 5A-C). Thecal surface smooth with scattered pores, except in the central area, and a marginal row of pores (Fig. 5B-C). Pores round to oblong (Fig. 5D). Periflagellar area V-shaped excavating the right thecal plate (Fig. 5B). Cells with many golden-brown chloroplasts and a centrally located pyrenoid (Fig. 5A).

Prorocentrum lima cells of strain UNR-86 were symmetric and broadly ovate, with maximum width in the middle region of the cell (Fig. 5E). Thecal surface smooth with scattered pores, except in the central area, and a marginal row of pores. Pores round to oblong. Periflagellar area V-shaped excavating the right thecal plate (Fig. 5E).

Prorocentrum lima field cells from Fernando de Noronha presented cell shapes that varied from narrow, almost oblong (morphotype 1, Fig. 5F-G) to ovate (morphotype 2, Fig. 5H) and broadly ovate (morphotype 3, Fig. 5I). Morphotype 3 was morphologically similar to cultured cells of strain UNR-86. Cells from all morphotypes presented a smooth thecal surface with scattered pores (mostly round, but also oblong), except in the central area, and a marginal row of pores (Fig. 5F-I). Periflagellar area V-shaped excavating the right thecal plate in cells of morphotypes 1 and 2 (Fig. 5F, H) and wide V-shaped in morphotype 3 (Fig. 5I). A central pyrenoid and many chloroplasts were present (Fig. 5A, E).

At all the remaining sampling sites, *P. lima* field cells were predominantly ovate, with cell shape similar to morphotype 2 cells from Fernando de Noronha.

Distribution: Present at all investigated sites.

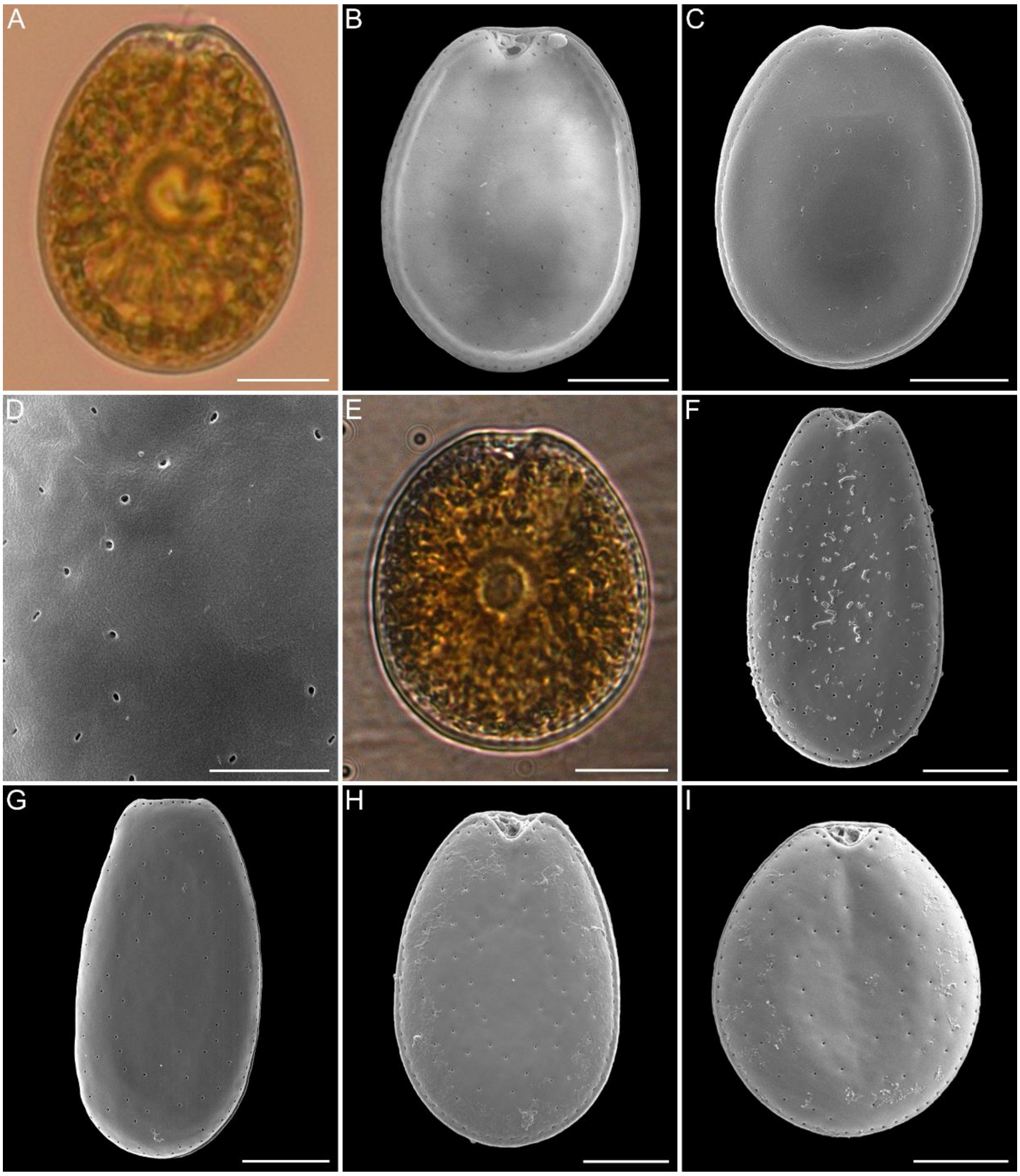

Figure 5. (A, E) Light and (B-D, F-I) scanning electron microscopy of *Prorocentrum lima* species complex. (A-D) Strain UNR-15, (E) Strain UNR-86, (F-I) Fernando de Noronha field cells. (A-B) Right thecal plate view of strain UNR-15, (C) Left thecal plate view of strain UNR-15, (D) Detail of the thecal surface of strain UNR-15, (E) Right thecal plate view of strain UNR-86, (F) Right thecal plate view of morphotype 1, (G) Left thecal plate view of morphotype 1, (H) Right thecal plate view of morphotype 2, (I) Right thecal plate view of morphotype 3. Scale bars: (A-C, E-I) 10 µm (D) 5 µm.

Prorocentrum panamense Grzebyk, Sako et Berland, 1998

Prorocentrum panamense field cells were heart-shaped and asymmetric. Thecal surface reticulate-foveate with scattered pores, except in the central area, and without a marginal row of pores. Pores round and located inside depressions. Depressions shallower towards the center of the thecal plates, that was devoid of pores and depressions. Periflagellar area linear and not excavated (Fig. 6A).

Distribution: Fernando de Noronha and Trindade (morphospecies).

Prorocentrum rhathymum A.R. Loeblich, J.L. Sherley & R.J. Schmidt, 1979

Prorocentrum rhathymum cells of strain UNR-11 (Fig. 6B-C) and field cells (Fig. 6D-F) were oval to oblong and asymmetric. Thecal surface smooth with a characteristic pore pattern, showing one apical row and several posterior radial rows in shallow furrows, present in both thecal plates (large pores located inside depressions) (Fig. 6B-C). Small pores scattered on the surface of cells (Fig. 6B-D). Both pores round (Fig. 6C-E). The center of the thecal plates was devoid of pores (Fig. 6C-E). Periflagellar area wide V-shaped excavating the right thecal plate, with a wing on platelet 1 and a short wing at the most ventral platelet (Fig. 6F). Cells with many golden-brown chloroplasts (Fig. 6B).

Distribution: Fernando de Noronha (morphospecies), Tartaruga (morphospecies) and Cabo Frio (sequence obtained).

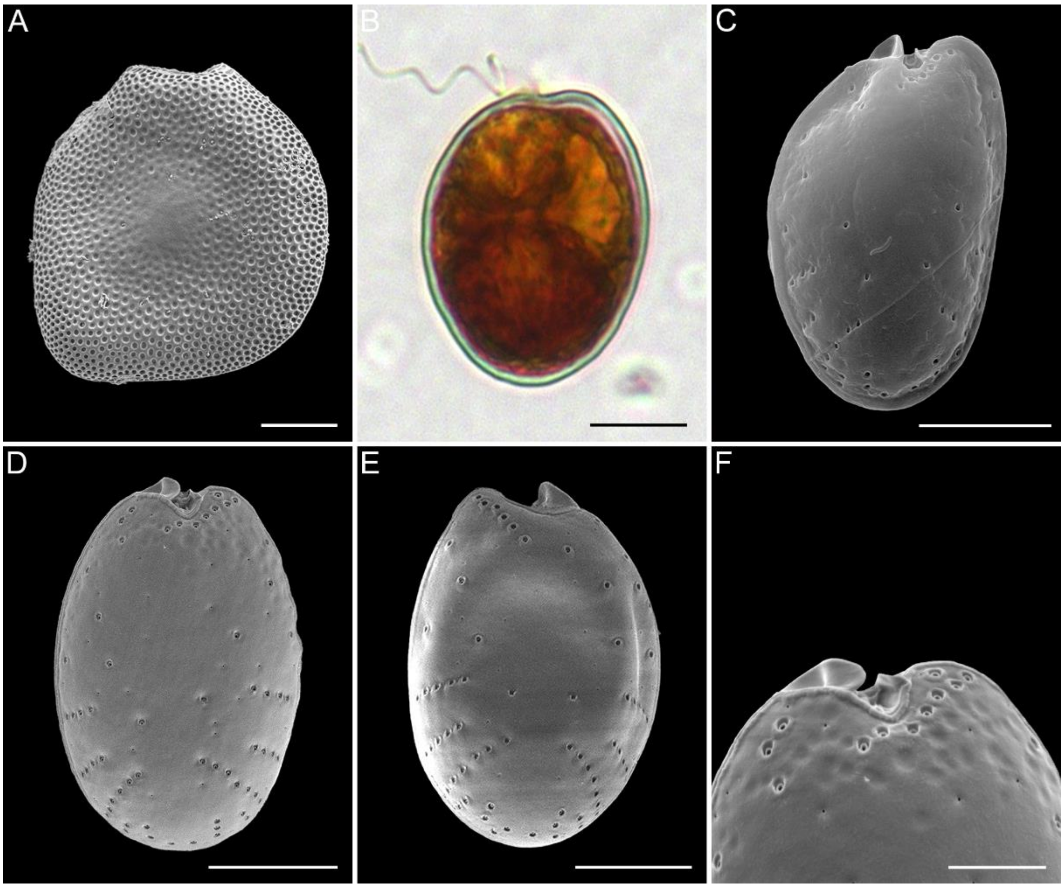

Figure 6. (A, C-F) Scanning electron and (B) light microscopy of *Prorocentrum* species. (A) *Prorocentrum panamense* Fernando de Noronha field cell. (B-F) *Prorocentrum rhathymum*: (B-C) Strain UNR-11, (D-F) Fernando de Noronha field cells. (A-D) Right thecal plate view, (E) Left thecal plate view, (F) Detail of the periflagellar area. Scale bars: (A-E) 10 µm (F) 4 µm.

Prorocentrum sp.

Prorocentrum sp. field cells were broadly oval to almost round and symmetric (Fig. 7A-C). Thecal surface reticulate-foveate with scattered pores, except in the central area, and a marginal row of pores was not visible (Fig. 7A-C). Pores round and located inside depressions (Fig. 7D), that were larger towards the periphery of the cell. Periflagellar area wide V-shaped, slightly excavating the right thecal plate (Fig. 7A).

Distribution: Fernando de Noronha and Trindade (morphospecies).

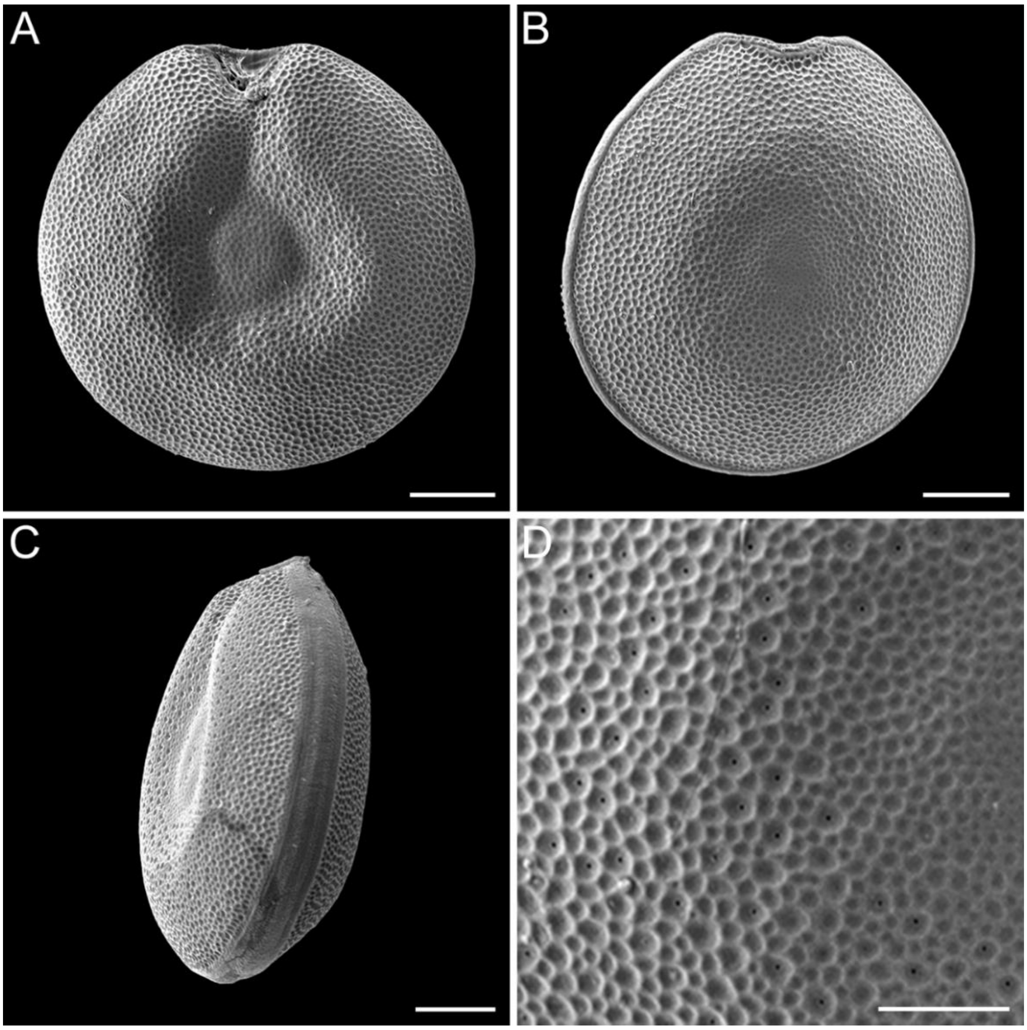

Figure 7. (A-D) Scanning electron microscopy of *Prorocentrum* sp. (A) Right thecal plate view, (B) Left thecal plate view, (C) Lateral view, (D) Detail of the thecal surface. Scale bars: (A-C) 10 µm (D) 5 µm.

Table 2. Dimensions of cultivated and field cells of *Prorocentrum* species, indicating site of origin, size range, mean value ± standard deviation and number of cells measured (n).

Species	Sampling sites	Length (L)	Width (W)	L/W	Origin
Prorocentrum borbonicum	Maragogi	23.4 - 26.8 (25.0 ± 0.8, n = 69)	19.6 - 22.8 (21.2 ± 0.8, n = 69)	1.12 - 1.24 (1.18, n = 69)	Strains UNR-12 and UNR-13
Prorocentrum cf. *caipirignum*	Fernando de Noronha	42.5 - 47.5 (44.7 ± 1.8, n = 22)	32.5 - 40.0 (36.0 ± 2.0, n = 22)	1.1 - 1.3 (1.2 ± 0.0, n = 22)	Field
Prorocentrum emarginatum/*fukuyoi*/ *sculptile* group	Fernando de Noronha	27.5 - 42.5 (33.8 ± 3.0, n = 120)	25.0 - 37.5 (29.8 ± 2.6, n = 120)	1.0 - 1.3 (1.1 ± 0.1, n = 120)	Field
	Trindade	28.0 - 37.0 (33.0 ± 4.0, n = 50)	23.0 - 31.0 (27.0 ± 3.0, n = 50)	1.0 - 1.3 (1.2 ± 0.1, n = 50)	Field
Prorocentrum hoffmannianum	Fernando de Noronha	45.0 - 50.0 (47.9 ± 1.7, n = 50)	37.5 - 42.5 (39.4 ± 1.5, n = 50)	1.1 - 1.3 (1.2 ± 0, n = 50)	Strain UNR-44
	Fernando de Noronha	47.5 - 57.5 (53.2 ± 2.3, n = 102)	35.0 - 45.0 (42.0 ± 2.2, n = 102)	1.2 - 1.4 (1.3 ± 0.1, n = 102)	Field
	Trindade	41.3 - 47.0 (44.2 ± 1.5, n = 35)	37.3 - 45.0 (39.8 ± 1.6, n = 35)	1.06 - 1.15 (1.11, n = 35)	Strain UNR-29
	Trindade	41.0 - 48.0 (45.0 ± 5.0, n = 49)	35.0 - 40.0 (37.0 ± 3.5, n = 49)	1.1 - 1.3 (1.2 ± 0.2, n = 49)	Field
Prorocentrum lima complex	Fernando de Noronha	35.0 - 37.5 (35.8 ± 1.2, n = 35)	27.5 - 32.5 (30.3 ± 1.5, n = 35)	1.1 - 1.3 (1.2 ± 0, n = 35)	Strain UNR-86
	Fernando de Noronha (morphotype 1)	32.5 - 47.5 (43.5 ± 3.1, n = 51)	22.5 - 25.0 (24.6 ± 1.0, n = 51)	1.3 - 2.0 (1.8 ± 0.1, n = 51)	Field
	Fernando de Noronha (morphotype 2)	32.5 - 47.5 (40.6 ± 5.1, n = 30)	27.5 (27.5 ± 0.0, n = 30)	1.2 - 1.7 (1.5 ± 0.2, n = 30)	Field
	Fernando de Noronha (morphotype 3)	32.5 - 47.5 (36.9 ± 2.4, n = 88)	30.0 - 37.5 (31.3 ± 1.9, n = 88)	0.9 - 1.6 (1.2 ± 0.1, n = 88)	Field
	Maragogi	36.4 - 41.1 (38.8 ± 1.1, n = 35)	27.6 - 31.9 (29.4 ± 1.1, n = 35)	1.25 - 1.39 (1.3, n = 35)	Strain UNR-15
	Trindade	30.0 - 42.0 (36.0 ± 3.8, n = 49)	20.0 - 29.0 (24.0 ± 6.5, n = 49)	1.3 - 1.8 (1.5 ± 0.5, n = 49)	Field
Prorocentrum panamense	Fernando de Noronha	45.0 - 55.0 (47.3 ± 2.7, n = 15)	42.5 - 50.0 (44.0 ± 2.1, n = 15)	1.0 - 1.3 (1.1 ± 0.1, n = 15)	Field
Prorocentrum rhathymum	Fernando de Noronha	30.0 - 35.0 (33.5 ± 1.4, n = 30)	20.0 - 25.0 (22.9 ± 1.7, n = 30)	1.3 - 1.8 (1.5 ± 0.1, n = 30)	Field
	Cabo Frio	28.0 - 32.0 (29.9 ± 0.8, n = 35)	18.8 - 25.7 (23.0 ± 1.3, n = 35)	1.19 - 1.49 (1.31, n = 35)	Strain UNR-11
Prorocentrum sp.	Fernando de Noronha	45.0-60.0 (53.4 ± 3.4, n = 37)	42.5-57.5 (50.1 ± 3.6, n = 37)	1.0-1.1 (1.1 ± 0.0, n = 37)	Field

3.2 Phylogenetic analysis

The ITS and LSU sequences were retrieved for seven strains of benthic *Prorocentrum* species: UNR-11, UNR-12, UNR-15, UNR-35, UNR-68, UNR-72 and UNR-86, and for the strain UNR-44 only the ITS sequence was obtained. The final alignment of the ITS sequences comparing 64 OTUs comprised 525 aligned nucleotides. The alignment of the LSU sequences comparing 85 OTUs included 478 aligned nucleotides. The general topology of the ML and BI trees agreed with previous studies (Luo et al., 2017; Moreira-González et al., 2019a; Chomérat et al., 2019; Lim et al., 2019; Nishimura et al., 2020) for ITS (Fig. 7) and LSU (Fig. 8). All species clades showed high support values (BS>95 and PP>0.95).

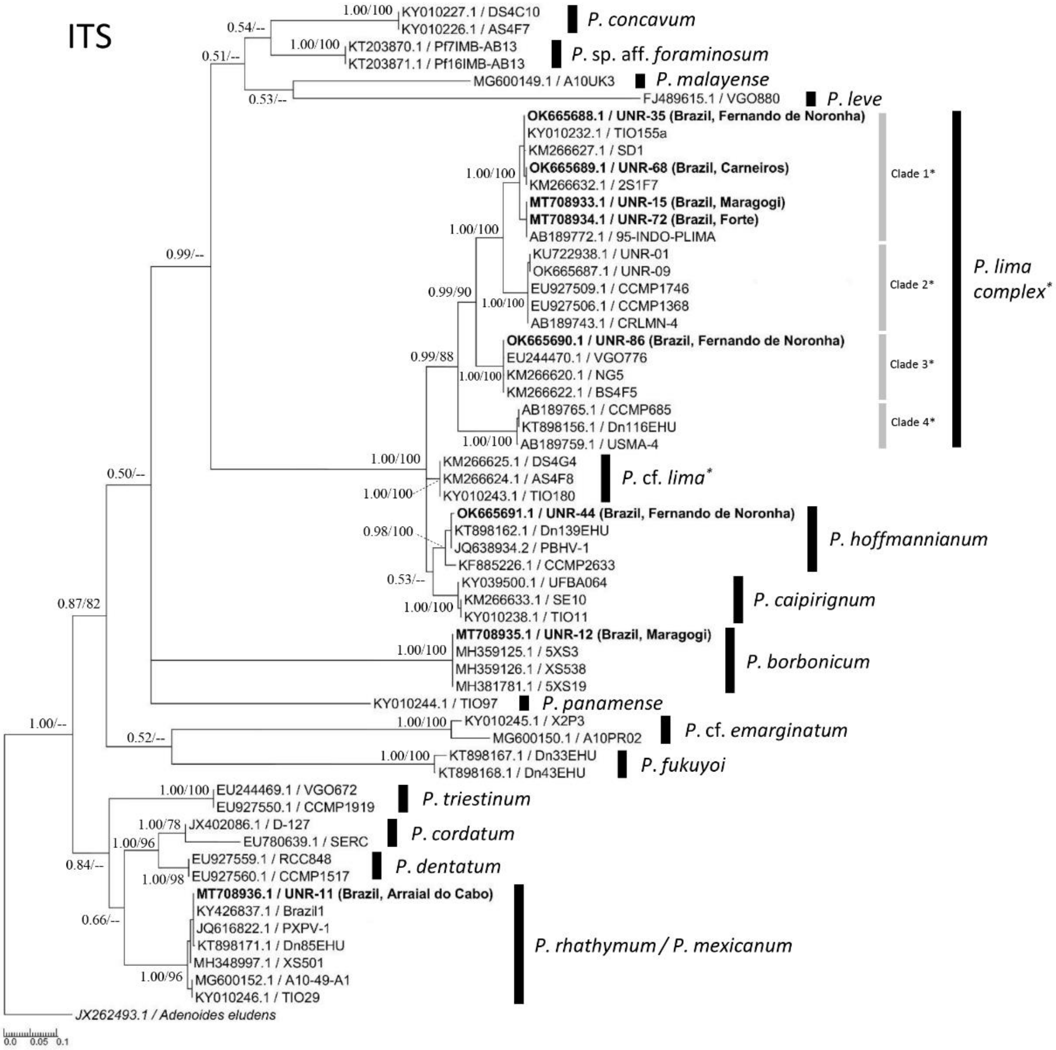

Figure 8. Bayesian Inference phylogenetic tree based on ITS rDNA sequences of *Prorocentrum* species. Operational taxonomic units (OTUs) are identified by: GenBank accession number \ strain name (locality, for Brazilian strains). Numbers at nodes represents posterior probability from BI and bootstrap values from ML analyses, respectively (cut-off = 50% for both analyses). New sequences published in this study are displayed in bold (UNR-11, UNR-12, UNR-15, UNR-35, UNR-44, UNR-68, UNR-72 and UNR-86). *Clades and taxa names according to Nishimura et al. (2020).

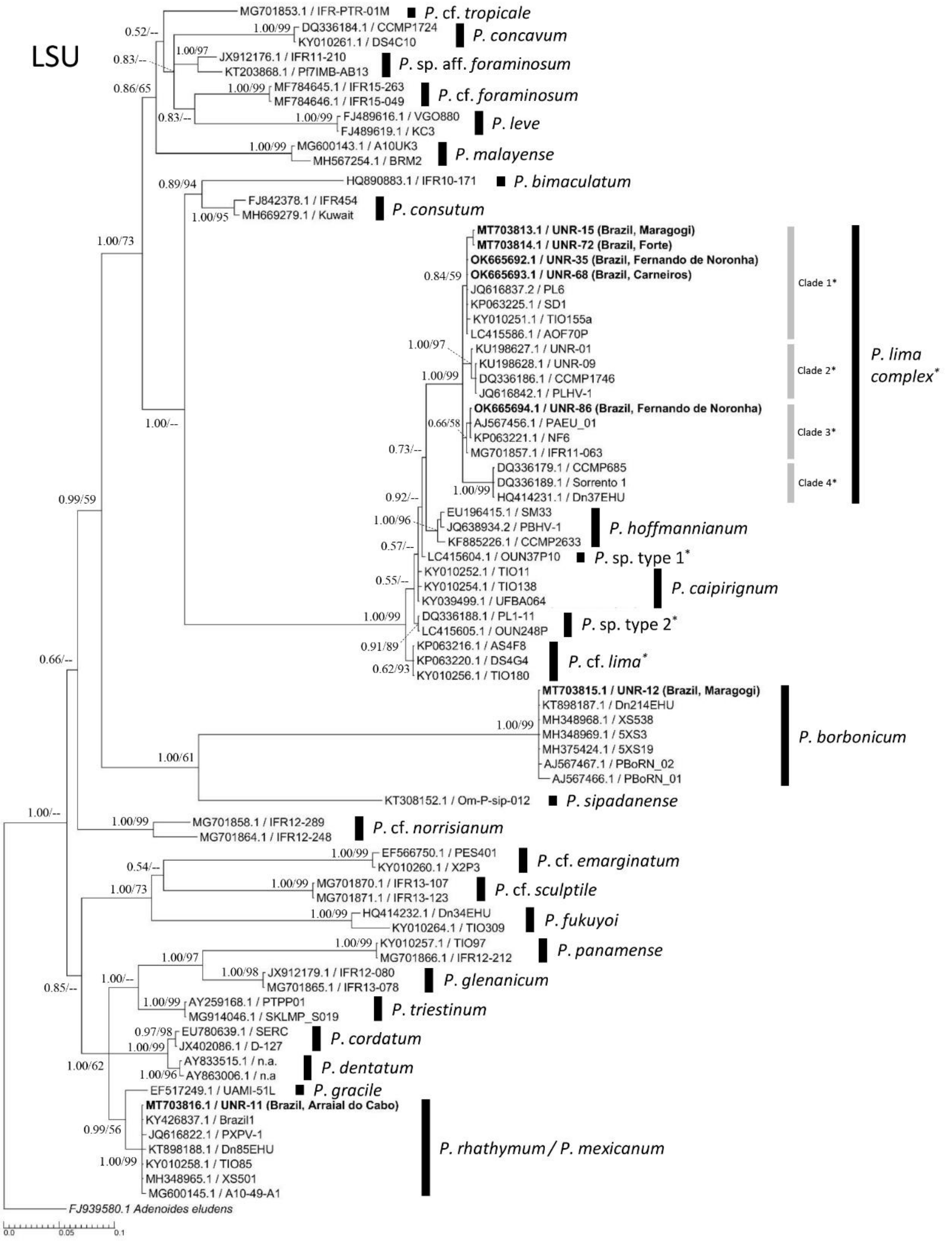

Figure 9. Bayesian Inference phylogenetic tree based on LSU D1-D3 rDNA sequences of *Prorocentrum* species. Operational taxonomic units (OTUs) are identified by: GenBank accession number \ strain name (locality, for Brazilian strains). Numbers at nodes represents posterior probability from BI and bootstrap values from ML analyses, respectively (cut-off = 50% for both analyses). New sequences published in this study are displayed in bold (UNR-11, UNR-12, UNR-15, UNR-35, UNR-68, UNR-72 and UNR-86). *Clades and taxa names according to Nishimura et al. (2020).

The phylogenetic analysis based on ITS and LSU loci grouped the sequences of five *Prorocentrum* strains from Brazil in the *P. lima* species complex (PLSC) clade. Considering the four clades of the PLSC by Nishimura et al. (2020), the strains UNR-15, UNR-35, UNR-68 and UNR-72 grouped in clade 1, while the strain UNR-86 grouped in clade 3 (Fig. 8 and 9), and corresponds to the morphospecies "*P. arenarium"* M.A Faust (1994), that was synonymized with *P. lima* by Nagahama et al., (2011).

Two other *Prorocentrum* species were identified based on ITS and LSU sequences. Strain UNR-11 clustered within the clade of *P. rhathymum*/*P. mexicanum* and strain UNR-12 clustered within the clade of *P. borbonicum* (Fig. 8 and 9). Moreover, the strain UNR-44 was identified as *P. hoffmannianum* based on the ITS-rDNA locus (Fig. 8).

3.3 Abundance

The abundance of the genus and species of *Prorocentrum* was determined in 14 of the 15 sampling sites. The genus *Prorocentrum* was present at all investigated sites (Table S3, Fig. 10). *Prorocentrum* showed high mean abundances of 1.916 and 7.137 cells g^{-1} FW at Porto de Galinhas and Forte, respectively (Table S3, Fig. 10A). Mean *Prorocentrum* abundances of 760 and 1.374 cells g^{-1} FW were found at the oceanic islands of Trindade and Fernando de Noronha, respectively (Table S3, Fig. 10A). At Fernando de Noronha, this genus represented 58% of the mean abundance of benthic dinoflagellates (Fig. 10B). *Prorocentrum* showed mean abundances between 100 and 500 cells g^{-1} FW at Maragogi, Tartaruga, Penha and Cabo Frio (Table S3, Fig. 10A). At Cabo Frio, *Prorocentrum* represented 79% of the total mean dinoflagellate abundance (Fig. 10B). Saint Paul's Rocks, as well as the coastal stations of Muro Alto, Garça

Torta, Placaford, Forno (AB) and Forno (AC) presented lower *Prorocentrum* mean abundances, below 100 cells g^{-1} FW (Table S3, Fig. 10A).

Maximum *Prorocentrum* abundances higher than 1.000 cells g^{-1} FW were found at Cabo Frio (2.353 cells g^{-1} FW), Trindade (2.590 cells g^{-1} FW), Fernando de Noronha (3.738 cells g^{-1} FW), Tartaruga (7.722 cells g^{-1} FW), Porto de Galinhas (8.527 cells g^{-1} FW) and Forte (19.829 cells g^{-1} FW) (Table S3, Fig. 10A). At Maragogi and Penha maximum abundances were 550 cells g^{-1} FW and 703 cells g^{-1} FW, respectively, while at St. Paul's Rocks, Muro Alto, Garça Torta, Placaford, Forno (AB) and Forno (AC) maximum abundances were lower than 500 cells g^{-1} FW (Table S3, Fig. 10A). *Prorocentrum* represented more than 80% of the mean abundance of benthic dinoflagellates at St. Paul's Rocks, Fernando de Noronha, Placaford, Penha, Tartaruga, Forno (AC) and Cabo Frio (Fig. 10B).

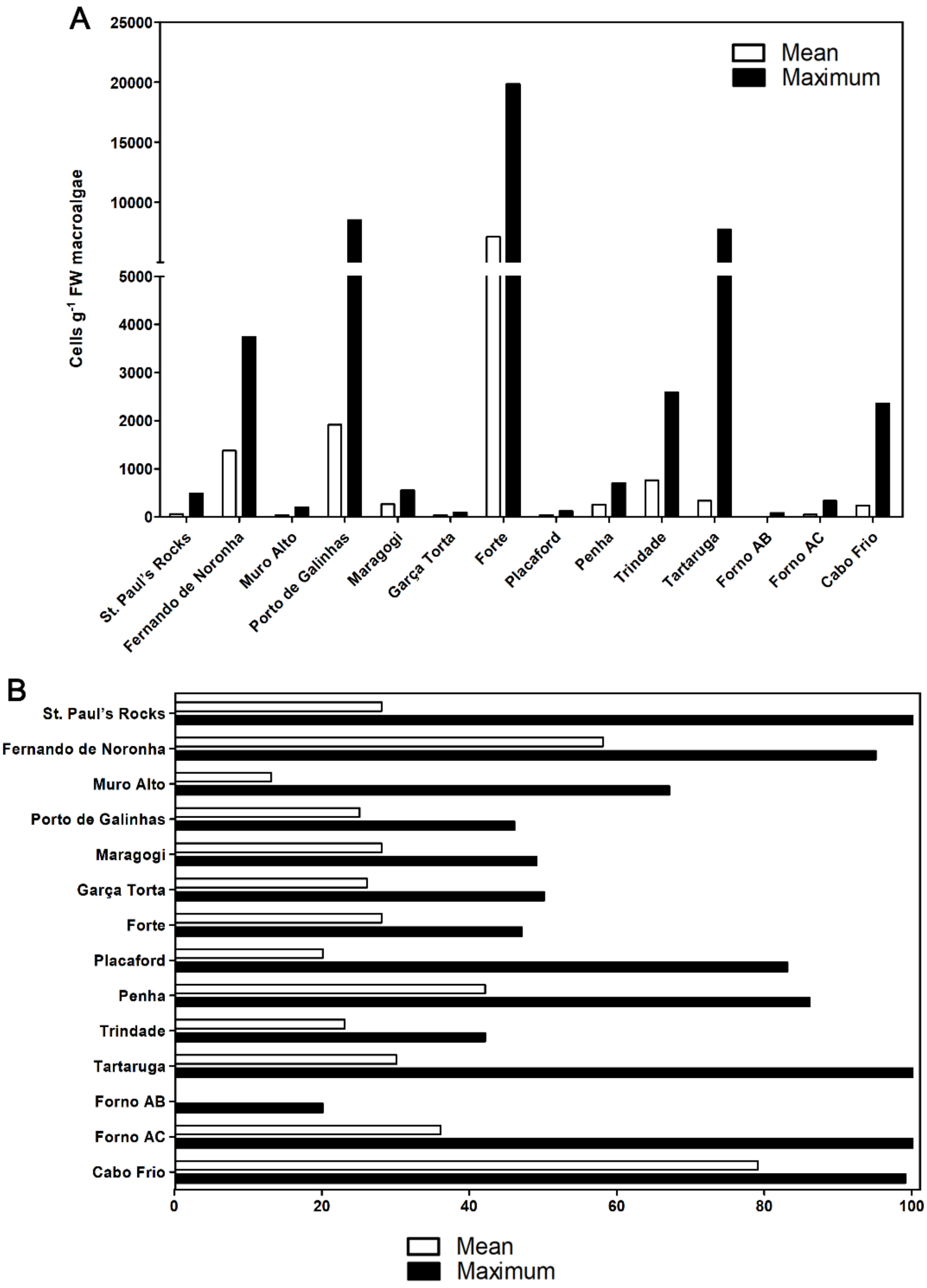

Figure 10. *Prorocentrum* abundance values at 11 sites on the coast and three oceanic islands of Brazil. (A) Mean and maximum abundance (cells g^{-1} FWmacroalgae); (B) Mean and maximum relative abundance (%) or the contribution of the genus to total benthic dinoflagellate abundance.

Prorocentrum lima was the only species observed at all sites (Table S3, Supplementary Figure S1A). Mean *P. lima* abundance ranged between one at Forno

(AB) and 7.099 cells g^{-1} FW at Forte. Peak abundances of *P. lima* were noteworthy at Forte (19.487 cells g^{-1} FW) and Porto de Galinhas (8.389 cells g^{-1} FW) (Table S3, Fig. S1A). Other sites with high maximum abundances include Trindade (1.984 cells g^{-1} FW), Fernando de Noronha (2.141 cells g^{-1} FW), Cabo Frio (2.288 cells g^{-1} FW) and Tartaruga (6.746 cells g^{-1} FW) (Table S3, Fig. S1A). *Prorocentrum lima* represented, on average, more than 20% of the benthic dinoflagellate abundance in eight of the 14 sites (Fig. S1B). *Prorocentrum emarginatum*/*fukuyoi*/*sculptile* group were found at nine of the 14 sites, in general at low abundances (Table S3) and representing a minor component of total benthic dinoflagellates abundance (between 1 and 17%). Mean abundance of this group ranged between zero at Forno (AB) and 243 cells g^{-1} FW at Trindade. Peak abundance was found at Tartaruga (977 cells g^{-1} FW) (Table S3).

Prorocentrum hoffmannianum was registered only at the oceanic islands with mean abundances ranging between two at St. Paul's Rocks and 191 cells g^{-1} FW at Fernando de Noronha, where peak abundance of 740 cells g^{-1} FW was recorded (Table S3). *Prorocentrum rhathymum* was found at Fernando de Noronha and at sampling sites on the Southeast coast of Brazil. Mean *P. rhathymum* abundance ranged between 0.2 at Forno (AC) and 83 cells g^{-1} FW at Fernando de Noronha, where maximum abundance of 586 cells g^{-1} FW was registered (Table S3). All other *Prorocentrum* species presented mean abundances lower than 70 cells g^{-1} FW (Table S3).

4.0 Discussion

4.1 Taxonomy and distribution

Prorocentrum borbonicum

Morphology of *P. borbonicum* cells was in agreement with the original description of the species (Ten-Hage et al., 2000), with cell dimensions slightly higher.

Prorocentrum borbonicum was described from Réunion Island, Indian Ocean (Ten-Hage et al., 2000) and, so far, has been found in few locations, such as Colombia (Arteaga-Sogamoso et al., 2022), Greece (Aligizaki et al., 2009), Tanzania (Hansen et al., 2001) and China (Zou et al., 2022). In Brazil, the species was positively identified with molecular data in the current study at Maragogi and Recife (Tibiriçá et al., 2020) at the Northeast coast and as a morphospecies in Fernando de Noronha and Trindade (Table S2 and S4).

Prorocentrum caipirignum

Morphology of *P.* cf. *caipirignum* was in agreement with the original description of *P. caipirignum* (Nascimento et al., 2017), except that cells from Fernando de Noronha presented a larger number of pores at the thecal surface. The length and width range of the Fernando de Noronha field cells were also higher than those presented in the original description. *Prorocentrum caipirignum* was described from Brazil (Nascimento et al., 2017), found at the Southeast (Arraial do Cabo and Angra dos Reis) and Northeast (Bahia and Recife) coasts (Nascimento et al., 2017; Tibiriçá, 2020) and, in this study, *P.* cf. *caipirignum* cells were found in Fernando de Noronha and Cabo Frio (Table S4). The current distribution of the species includes Cuba (Herrera-Sepúlveda et al., 2015), Malaysia (Lim et al., 2019), China (Zhang et al., 2015; Luo et al., 2017; Zou et al., 2022) and Japan (Nishimura et al., 2020) (Table S2).

Prorocentrum emarginatum/*fukuyoi/sculptile* group

Prorocentrum emarginatum, *P. fukuyoi* and *P. sculptile* form a problematic group of species with similar morphology and the features that can reliably distinguish them are not yet clear (Hoppenrath et al., 2013). Specimens with radiating lines of pores and a completely smooth surface have been considered by many authors as *P. emarginatum*, however, this is in contradiction with Fukuyo's original description

(Fukuyo, 1981) and these specimens likely correspond to a species in the *P. fukuyoi* complex (Chomérat et al., 2019). *Prorocentrum sculptile* was described with a rugose surface and shallow round to oblong depressions evenly distributed (Faust, 1994), however, a recent study showed that in young specimens the foveate ornamentation was visible only on the margin of the thecal plate, while the center was smooth and devoid of depressions and that the pores were arranged in radiating lines (Chomérat et al., 2019). Moreover, the absence of molecular data associated with the original description of *P. emarginatum* (Fukuyo, 1981) and *P. sculptile* (Faust, 1994), and the considerable genetic variability observed in this group does not allow to conclude in the identity of the specimens (Laza-Martinez et al., 2011; Chomérat et al., 2019). In the current study, only field cells of this group from two sites (Fernando de Noronha and Trindade) were observed using SEM. Cells with a smooth surface were tentatively identified as *P.* cf. *fukuyoi*, while cells with a strong, slight or faint foveate ornamentation were identified as *P.* cf. *sculptile*. As molecular data from these specimens are not available, positive identification was not possible.

Prorocentrum emarginatum was described from the Ryukyu Islands, Japan (Fukuyo, 1981), while *P. fukuyoi* was described from Sydney, Australia (Murray et al., 2007) and *P. sculptile* from Belize (Faust, 1994). Specimens from the *P. emarginatum*/*fukuyoi*/*sculptile* group have been recorded in several locations in the world's oceans (Table S2). In Brazil, *P. emarginatum* was positively found in the Northeast coast (Bahia), with molecular data associated to the morphological description (Mendes et al., 2019), and *P.* cf. *fukuyoi* was reported from the South coast (Paranaguá Bay), although no details on cell morphology or molecular data were presented (Moreira-González et al., 2020). In this study, this group was reported for other six sites at the Northeast and Southeast coasts, as well as three oceanic islands (Table S2 and S4).

Prorocentrum hoffmannianum

Morphology of *P. hoffmannianum* cells kept in culture and from field samples was in agreement with the redescription of the species (Rodríguez et al., 2018). Cell shape was variable, from ovoid to wider towards the posterior end of the cell at different sampling sites, and all cells examined presented a foveate cell surface. Strain UNR-29, isolated from Trindade Island, corresponds to the Belize ribotype sensu Herrera-Sepúlveda et al. (2015). The species was described from Belize (Faust, 1990), that is also the type locality of *P. belizeanum* (Faust, 1993a) and *P. maculosum* (Faust, 1993b) that are now considered synonyms of *P. hoffmannianum* (Herrera-Sepúlveda et al., 2015; Rodríguez et al., 2018).

Prorocentrum hoffmannianum has a broad distribution and was reported from Caribbean Sea (Faust, 1990; Delgado et al., 2006; Almazán-Becerril et al., 2015; Herrera-Sepúlveda et al., 2015; Aguilar-Trujillo et al., 2017; Irola-Sansores et al., 2018; Chomérat et al., 2019; Gómez and Pineda, 2019; Arbeláez et al., 2020; Cembella et al., 2021), Atlantic (Herrera-Sepúlveda et al., 2015; Rodríguez et al., 2018; Moreira-González et al., 2019b, Fernández-Zabala et al., 2019, 2022; Accoroni et al., 2020), Indian (Hansen et al., 2001; Hoppenrath et al., 2013) and Pacific (Grzebyk et al., 1998; Pearce and Hallegraeff, 2004; Parsons and Preskitt, 2007; Rhodes and Smith, 2019) oceans (Table S2). In Brazil, *P. hoffmannianum* was found at Trindade Island (Nascimento and Santiago, 2016) and the Northeast (Recife) and Southeast (Angra dos Reis) coasts (Tibiriçá et al., 2020), and this study expands its distribution to the oceanic islands of Fernando de Noronha and St. Paul’s Rocks (Table S2).

Prorocentrum lima species complex

Prorocentrum lima species complex was ubiquitous in this study, found at all investigated sites. *Prorocentrum lima* complex has a wide distribution in tropical, subtropical and temperate zones, along the Atlantic, Indian and Pacific Oceans (see Table S2). Four of the five Brazilian *P. lima* strains grouped in clade 1 of the *P. lima* species complex sensu Nishimura et al. (2020), whereas one strain grouped in clade 3. Strains from clade 1 have been recorded from locations in the Atlantic (Table S4 by Nishimura et al., 2020), Indian (Oyeku and Mandal, 2021) and Pacific (Murray et al., 2009, 2018; Zhang et al., 2015; Luo et al., 2017; Nishimura et al., 2020) oceans.

Strain UNR-86, that grouped within clade 3 of the *P. lima* species complex, has a broad oval cell shape, wider than *P. lima* strains from clade 1. This morphology corresponds to the species *P. arenarium* M.A Faust (1994), that is currently considered a synonym of *P. lima*. In Brazil, sequences belonging to clade 3 were recorded only from Fernando de Noronha. At this oceanic island, the cell shape, particularly cell width, of *P. lima* was variable, and three morphotypes (narrow, ovate and broadly ovate) were recognized. This island was the only site that presented narrow and broadly ovate cells, while at all the remaining sites *P. lima* cells were ovate. Sequences of *P. lima* clade 3 were found at Martinique Island (Chomérat et al., 2019), Canary Islands (Rodríguez et al., 2018), Réunion Island (Nishimura et al., 2020), Rawa Island, Malaysia (Lim et al., 2019) and Hainan Island, China (Zhang et al., 2015) and Australia (Murray et al., 2009).

Prorocentrum panamense

Morphology of *P. panamense* cells was in agreement with the original description of the species (Grzebyk et al., 1998), with slightly larger dimensions. *Prorocentrum panamense* was described from Panama (Grzebyk et al., 1998) and, to date, has been found in a few locations, such as Martinique Island (Chomérat et al., 2019), Canary Islands (Fernández-Zabala et al., 2019; Gaiani et al., 2022), Réunion Island (Hansen et al., 2001), China (Luo et al., 2017) and Brazil, where the species was found at the Northeast (Recife) coast (Tibiriçá et al., 2020) and at the Fernando de Noronha e Trindade Islands (Table S2).

Prorocentrum rhathymum

Morphology of *P. rhathymum* cells kept in culture and from field samples was in agreement with the description of the species (Loeblich et al., 1979). *Prorocentrum rhathymum* was described from the Virgin Islands, USA (Loeblich et al., 1979) and was later considered synonymous with *P. mexicanum* (Steidinger, 1983; Faust, 1990), however, Cortés-Altamirano and Sierra-Beltrán (2003) reestablished *P. rhathymum* as an independent species. However, later on, Gómez et al. (2017) presented morphological and molecular data seeking to reestablish synonymy. Therefore, the identification of these species remains confusing and, in this study, we chose to follow the taxonomic identification of Hoppenrath et al. (2013). As highlighted by Chomérat et al. (2019) and observed in this study (Figs. 8 and 9), all sequences attributed to *P. rhathymum* or *P. mexicanum* are very similar and through them it is not possible to distinguish these species.

Prorocentrum rhathymum has a wide distribution and was found in the Atlantic, Indian and Pacific oceans (Table S2). In Brazil, *P. rhathymum* was found in Bahia (Mendes et al., 2014) and in Pernambuco (Nascimento, 2006; Tibiriçá, 2020) at the Northeast coast and in Arraial do Cabo (Nascimento, 2006) at the Southeast coast. The present study extends the distribution of the species to two other sites at the Southeast and at Fernando de Noronha (Table S4).

Prorocentrum sp.

Nearly round *Prorocentrum* specimens with a foveate cell surface and pores of one size class were observed at Fernando de Noronha and Trindade. Cells presented a thecal surface pattern that was similar to *P. concavum,* but were wider than this species. Further studies including molecular analysis will be necessary to confirm species identity.

4.2 Global benthic *Prorocentrum* species richness

Species of the genus *Prorocentrum* have a wide geographic distribution and can contribute a considerable part of the benthic dinoflagellates assemblage. Altogether, 11 benthic *Prorocentrum* species were found in the present study, seven morphospecies and four species identified based on morphology and molecular sequences. As expected, a clear latitudinal gradient in species richness was observed. The largest number of species was found at the oceanic island of Fernando de Noronha (03º50'S, 11 species), followed by sites at the Northeast coast (08º04'S - 18º02'S, up to 10 species), Southeast coast (22°45'S - 23°04'S, up to 5 species) and the South (25º30'S) coast presented fewest species numbers (4 species, Table S3). On the Northeast coast, the Maragogi site presented the largest total number of species (3), while in Cabo Frio, located on the Southeast coast, four species were found in total (Table S4).

One of the most general biogeographical patterns shows that insular habitats tend to have lower species richness compared to continental habitats. Islands are isolated and small compared to continents and have limited habitats and resources diversity (Brown and Lomolino, 2006). However, in the current scenario of coastal ecosystems degradation and consequent diversity loss, oceanic islands can be considered particular environments, as they have pristine or nearly pristine conditions, and are subjected to lower impacts caused by human activities (e.g. coastal eutrophication) (Quimbayo et al., 2019).

Species richness varied among the three oceanic islands studied, and while only three benthic *Prorocentrum* species were recorded at St. Paul's Rocks, seven were found at Trindade and 11 at Fernando de Noronha. Among the oceanic islands investigated in the current study, Fernando de Noronha is the largest (26 km²) and less isolated (345 km away from the continent), while St. Paul's Rocks has the smallest area (3.5 km²) and is 945 km away from mainland. Trindade is the island located more distant from the continent (1160 km) and presents intermediate area (10.2 km²). Regarding the time since formation, it is expected that older islands will have higher species richness, as there is more time for migrant arrivals and speciation (Whittaker et al., 2008). Fernando de Noronha is the older island (12.3 Ma), while St. Paul's Rocks is approximately 9 Ma old and Trindade has a more recent origin (3.7 Ma).

Fernando de Noronha, the largest, closest to the continent, and older island, showed the highest benthic *Prorocentrum* species richness, in accordance with the theory of island biogeography, that postulates that larger islands should maintain a greater number of species and offer more habitat heterogeneity, while more isolated islands should receive fewer immigrants and, consequently, maintain fewer species (MacArthur and Wilson, 1967). Saint Paul's Rocks consists of a remote group of ten small islands located close to the axis of the Mid-Atlantic Ridge and corresponds to the emerged summits of a meso-oceanic chain. This remote site presented the lowest benthic *Prorocentrum* species richness, following the predictions of island biogeography theory. Moreover, the island is subjected to severe sea and wind conditions with high hydrodynamics that likely have a negative effect on the association of benthic microalgae with their substrates. Trindade is the youngest and most distant island from the continent, with intermediary area and benthic *Prorocentrum* species richness. Its location further south, at 20° S latitude, relative to Fernando de Noronha

(03º50’S) and St. Paul’s Rocks (00°55’N), may partly explain the intermediary species richness.

Moreover, in the current study the sampling effort was uneven and some sites were more intensively studied than others. While the Southeast coast has been more intensively sampled, with 487 samples analyzed, covering all seasons, fewer samples (88) have been investigated from the Northeast coast and various sites were sampled only once. Noteworthy is the fact that the oceanic islands were the sites with fewer (57) and less frequent sampling and, despite of that, Fernando de Noronha and Trindade showed high species richness.

The geographic distribution of marine benthic *Prorocentrum* species was compiled from 87 studies performed worldwide (Table S2). A few issues arise from the compiled data, such as the taxonomic constrains and the uneven sampling effort in diverse areas of the world’s oceans. First, to discuss species distribution patterns requires a well-defined species concept (Lundholm and Moestrup, 2006). Molecular data greatly advanced the understanding of protist distribution and species number (Foissner, 2008), but genetic sequences are still not available from many sites that have been investigated worldwide. Therefore, the reliability of species identification can be problematic, particularly for close related species that are difficult to distinguish (e.g. *P emarginatum*, *P. fukuyoi* and *P. sculptile*). Moreover, there are uncertainties regarding species delimitations, and some species may present considerable intraspecific morphological variability, while in other cases morphologies of distinct species may largely overlap (Hoppenrath et al., 2014). Molecular evidence has led to the synonymy of a few benthic *Prorocentrum* species, e.g. *P. belizeanum* and *P. hoffmannianum* (Herrera-Sepúlveda et al., 2015), and later Rodríguez et al. (2018) showed that *P. maculosum* is also a synonym of *P. hoffmannianum*. Besides the taxonomic constrains, another issue to be considered is the fact that many regions have been undersampled or sampled infrequently and only a few areas have been sampled intensively enough to give a reliable estimate of the real species diversity present (Hoppenrath et al., 2014).

Despite of those drawbacks, there is sufficient evidence for the existence of several distribution patterns in marine phytoplankton (Lundholm and Moestrup, 2006) and the examination of the biogeography of marine benthic *Prorocentrum* species, compiled from the literature in table S2 also revealed a few patterns. As expected, higher species richness was found in the tropics and subtropics, with a decrease in the number of species reported towards the temperate latitudes (Table S2). At latitudes higher than 38^{o} N most sites investigated reported *P. lima* as the only benthic *Prorocentrum* species present. At the South hemisphere *P. lima* was the only species found in places like Argentina and temperate New Zealand (Table S2).

Places that have been more intensively studied clearly presented higher species richness. This is the case of Belize, in the Caribbean Sea, the location with the highest number of species (16, Table S2). Morphospecies of benthic dinoflagellates have been investigated in Belize since the 1990s by Faust and this is the type locality of 15 benthic *Prorocentrum* species. Among them, the species *P. reticulatum* and *P. sabulosum* have been recorded only from their type locality and further investigation using molecular and morphological characterization is necessary. Other studies in Mexico and Colombia, in the Caribbean Sea have also shown a high morphospecies richness, with 12 and eight species registered, respectively (Table S2). Recently, Chomérat et al. (2019) provided molecular and morphological descriptions to specimens of *P.* cf. *tropicale*, *P.* cf. *foraminosum* and *P. norrisianum*, that have been described from Belize without an associated molecular sequence, and the morphospecies *P.* cf. *ruetzlerianum* from Anse Dufour, in the Martinique Islands, East Caribbean. Twelve species were putatively identified from the same site at the Martinique Islands, eleven using partial LSU rDNA, and 1 morphospecies (Chomérat et al., 2019), confirming the Caribbean Sea is a hot spot of benthic *Prorocentrum* species diversity.

In the tropical Réunion Islands in the Indian Ocean, in Malaysia and in Hainan and Xisha Islands in China, in latitudes 16 - 21ºN, between seven and nine species of benthic *Prorocentrum* were registered (Table S2), reflecting their high species diversity, but also a higher number of studies that included an integrated taxonomical approach combining molecular and morphological analysis to study benthic dinoflagellates. Kuwait is also noteworthy for its high number of benthic *Prorocentrum* species (eight, Table S2), likely related to its subtropical location, shallow and protected coastline and by the number of studies performed.

A comprehensive study of *Prorocentrum* species has been performed in Japan, along 27 sampling sites from subboreal, temperate and subtropical latitudes, comprising the molecular analysis of 244 isolated strains (Nishimura et al., 2020). Eight benthic *Prorocentrum* species have been reported from Japan, a number comparable to that found in tropical and subtropical latitudes elsewhere (Table S2). This high species richness is likely a consequence of the impressive sampling effort, and the use of molecular analysis, that largely improved the capability of species recognition, including two new phylotypes, *Prorocentrum* sp. type 1 and *Prorocentrum* sp. type 2, that were found in the subtropical zone of Japan (Nishimura et al., 2020).

Prorocentrum lima is considered a cosmopolitan species and was found in nearly all the sites that have been investigated, from tropical to temperate latitudes (Table S2) and is frequently the only species reported in temperate latitudes. However, a high genetic variability is found in the *P. lima* species complex, that comprises four major clades (Nishimura et al., 2020). Clade 4 is restricted to temperate latitudes and presents high genetic divergence relative to clades 1, 2 and 3, compatible to that between different species (Nishimura et al., 2020). Clade 3 is consistently separated in *P. lima* phylogenies (Nascimento et al., 2016, 2017; Nishimura et al., 2020) and cells of this clade can be morphologically distinguished by its broader cell width, corresponding to the morphospecies *P. arenarium*. Therefore, the *P. lima* species complex comprises different lineages that altogether present a cosmopolitan distribution.

The morphologically similar *P. emarginatum P. sculptile* and *P. fukuyoi* have been frequently reported from many locations around the world, and until this group receives a detailed study, their identification and geographic distribution will be uncertain.

There is a clear bias caused by taxonomic impediment, as areas that have been more studied by specialists in the field present higher species richness, due to a higher sampling effort and improved capability of species recognition, that includes the use of rDNA sequence data and, more recently, high-throughput sequencing, using metabarcoding techniques (e.g. Smith et al., 2017). There is a need to train taxonomists in regions of the world from where few or no data are available. The current taxonomic knowledge on the geographic distribution of benthic *Prorocentrum* species is almost certainly heavily underestimated. More studies using morphological and molecular data are necessary and are expected to reveal higher diversity in different regions of the world, particularly in those poorly studied.

4.3 Abundance and potential associated risks

Precise data on cell abundances are crucial for establishing alarm concentration levels for potential harmful algal bloom events and the associated risks worldwide. In addition, exposure to low or intermediate concentrations of toxic species for long periods can also be harmful to benthic fauna, considering the initial condition of the exposed individuals (Neves et al., 2020). However, few data on the abundance of benthic *Prorocentrum* species are available, especially for the South Atlantic, and include few species such as *P. lima* complex/ *P.* cf. *lima* (Gayoso et al., 2002; Brustolin et al., 2014; Moreira-González et al., 2020, 2021) and *P.* cf. *fukuyoi* (Moreira-González et al., 2020, 2021).

The *P. lima* species complex has been in a few occasions associated to the accumulation of okadaic acid (OA) in mollusks in different regions of the world (e.g. Marr et al., 1992; Gayoso et al., 2002; Nascimento et al., 2005; Maranda et al., 2007b) and was considered a potential causative agent of DSP. In Paranaguá Bay at Southern Brazil, Moreira-González et al. (2021) found low to moderate concentrations of OA in marine benthic fauna (such as bivalves, gastropods, squid, crabs, echinoderms and fish) and indicated *P. lima* as the main vector of diarrhetic toxins, as a strong positive correlation between *P. lima* complex cell abundance and OA concentrations in microphytobenthic samples, with minimal contribution from the *Dinophysis* genus, if any, during the study.

Accumulation of DSP toxins in the mollusk *Mytilus* (33.5 ng g^{-1} of OA and 21.2 ng g^{-1} of DTX-1) and *Aulacomya* (150 ng g^{-1} of OA and 94.0 ng g^{-1} of DTX-1) was associated with *P. lima* abundances of 750 and 1.785 cells g^{-1}FWmacroalgae, respectively, in Argentina (Gayoso et al., 2002). In *Aulacomya*, OA concentrations were close to the regulatory guidance level by the U.S. Food and Drug Administration (160 ng OA equivalents per gram of meat) (FDA, 2020) to prevent acute intoxication by OA in human consumers. In Southern Brazil, highest OA concentrations were found in fish (maximum of 60.2 ng g^{-1}) and in bivalves (maximum of 23.4 ng g^{-1}), when *P. lima* abundances were on average lower than 500 cells g^{-1} FW, and in the absence of *Dinophysis* (Moreira-González et al., 2021).

Marine benthic organisms, besides acting as vectors of DSP toxins through the trophic webs, can be negatively affected by exposure to these toxins. Okadaic acid, from *P. lima* cells, can affect marine fish species at different life stages, from embryos to adults (Corriere et al., 2021). For example, Escoffier et al. (2007) observed a reduction in the survival rate of fish embryos, as well as a delay in embryonic development and an increase in the organ areas of surviving embryos. Behavioral changes in juveniles such as jumps, fast left-to-right turns, surface swims, poor feeding reflexes, inactivity and death have also been recorded (Ajuzie, 2008). Other organisms, such as mussels, can also be affected as demonstrated by Neves et al. (2019), where the bivalve *Perna perna* exposed to *P. lima* had increased reaction time and the number of stimuli necessary for the valve closure response, as well as increased phagocytic activity, decreased number and size of circulating hemocytes, among other damages.

In the current study, *P. lima* abundances higher than 500 cells g^{-1} FW were found at eight of the 14 investigated sites, indicating the potential accumulation of DSP toxins in the benthic fauna. At sites where high *P. lima* abundances (>1.000 cells g^{-1} FW) were found, the ecological and human health risks are higher. In some places, *P. lima* species complex was found together with *P. caipirignum* and *P. hoffmannianum*, which, in addition to OA, DTXs and their ester derivatives, produce a suite of toxic compounds such as belizeanic acid (Cruz et al., 2008), corozalic acid (Napolitano et al., 2010), fast-acting toxin prorocentrolide (Nascimento et al., 2017). Therefore, there is a need to monitor species abundances in order to prevent the risks associated to the chronic and acute exposure to OA and other toxins, ensuring seafood safety.

5.0 Conclusion

This is the first study on the diversity of benthic *Prorocentrum* species in different regions of Brazil, reporting on species abundance, a scarce data globally and particularly at the South Atlantic Ocean. Altogether, 11 species were found, three in the Northeast coast, four in the Southeast coast and up to 11 in the Brazilian oceanic islands. The highest species richness was found at Fernando de Noronha, that can be considered a hotspot of benthic *Prorocentrum*. *Prorocentrum borbonicum, P. hoffmannianum, P. lima* species complex and *P. rhathymum* were identified using molecular methods and morphology. Furthermore, field cells of *P.* cf. *caipirignum*, *P.* cf. *fukuyoi*, *P.* cf. *panamense* and *P.* cf. *sculptile* were provisionally identified through observation of cell morphology using SEM images and *P.* cf. *concavum* and *P.* cf. *norrisianum* through optical microscopy and further studies are needed to confirm their identities.

Different morphotypes of the *P. lima* species complex were observed at Fernando de Noronha and rDNA sequences from clades 1 and 3 were found. *Prorocentrum lima* was the only species recorded at all investigated sites and at certain places the abundance of this species may pose a risk to the marine ecosystem and human health. The research on benthic dinoflagellates of Brazil is at early ages and have revealed a high species richness, with sites that are hotspots of biodiversity. This is not surprising as Brazil is a megadiverse country, and hosts between 15 and 20 per cent of the world’s biological diversity. The research on benthic dinoflagellates in Brazil is quite challenging, considering the vast size of the country. Further studies that expand the spatial and temporal scales investigated are needed, including a large number of strains isolated from diverse areas, applying integrated taxonomy and including the investigation of their toxic secondary metabolites are highly desirable.

Acknowledgements

We thank Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) for the support through research grants to S.M.N. (SEI-

260003/002134/2021) and F.S. (E-26/010.001828/2019) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support to the project “Monitoramento de Longa Duração de Comunidades Recifais das Ilhas Oceânicas Brasileiras” (441241/2016-6) of Programa de Pesquisa Ecológica de Longa Duração Ilhas Oceânicas (PELD-ILOC) by Carlos E. L. Ferreira. Additional financial support was provided by the Universidade Federal do Estado do Rio de Janeiro (UNRIO, Edital PPQ-UNIRIO nº 04/2020) to F.S. We thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and CNPq for the master's scholarships granted to G.T.B (88887.481828/2020-00, CAPES) and G.A.L.C. (CNPq), and to UNIRIO for the IC scholarships granted to A.M.M and A.S.G. The authors also thank the students Tainá C. Santiago, Alice G. Alencar, Annaliza C. M. Souza, Erick L. C. Queiroz and Priscila O. Monteiro for their contribution in obtaining the data presented here, PELD-ILOC field team for collecting the samples at the oceanic islands, Dr. Joel C. de Paula for identifying the macroalgae analyzed in this study, MSc. Rodrigo A. F. da Silva for assisting with the map figure and Dr. Marcelo H. P. da Silva from Instituto Militar de Engenharia (IME) for allowing the use of the institution's scanning electron microscope.

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Supplementary Materials

Table S1. Summary of samples analyzed in this study, indicating the sampling sites, date of collection, sample origin (strain or field), depth of collection, water temperature range and mean value and salinity at each site.

Sampling sites	Date	Origin	Depth	Seawater Temperature (°C) Minimum - Maximum (Mean)	Mean Annual Sea Surface Salinity
Saint Paul’s Rocks Archipelago (00°55'10" N, 29°20'33" W)	November 2017, September 2018	Field	3 - 18 m	26.1 - 27. 9 (27.0)*	35.8
Fernando de Noronha Archipelago (03º50'52.3" S, 32º26'31.8" W)	October 2017, March and October 2018	Strains UNR-35, UNR-44, UNR-86 and Field	4 - 15 m	19.4 - 31.8 (27.6)	36.1
Muro Alto, Pernambuco (08°25'45" S, 34°58'40" W)	July 2012	Field	1 - 2 m	26.0 - 28.8 (27.4)*	36.4
Porto de Galinhas, Pernambuco (08°30'40" S, 35°00'7" W)	January 2013	Field	1 - 2 m	25.9 - 28.7 (27.3)*	36.4
Carneiros, Pernambuco (08°42'16" S, 35°04'43" W)	November 2016	Strain UNR-68	1 - 2 m		
Maragogi, Alagoas (08º55'18" S, 35°09'05.8" W)	January 2015	Strains UNR-12, UNR-13, UNR-15 and Field	1 - 2 m	25.9 - 28.5 (27.2)*	36.5
Garça Torta, Alagoas (09°35'00" S, 35°39'35" W)	July 2012	Field	1 - 2 m	25.9 - 28.2 (27.0)*	36.6
Forte, Bahia (12°34'41.0" S, 38°00'04.7" W)	January 2013	Strain UNR-72 and Field	1 - 2 m	25.3 - 28.2 (26.5)*	36.8

Placaford, Bahia (12°57'20" S, 38°22'45" W)	July 2012	Field	1 - 2 m	25.3 - 28.1 (26.4)*	36.9
Penha, Bahia (12°59'27" S, 38°37'29" W)	July 2012	Field	1 - 2 m	25.3 - 28.4 (26.8)*	36.9
Trindade Island (20°29'22.2" S, 29°20'04.2" W)	October 2015, September 2014 and 2017	Strain UNR-29 and Field	7 - 14 m	23.4 - 27.7 (25.5)	37.0
Tartaruga, Armação dos Búzios, Rio de Janeiro (22°45'18" S, 41°54'7" W)	June and September 2006; January, March-November 2007; February, April, May, September, October and December 2012; January-May 2013; February 2014	Field	1 - 2 m	21.2 - 25.2 (23.0)*	36.3
Forno, Armação dos Búzios - AB, Rio de Janeiro (22°45'42.5" S, 41°52'34.28" W)	May, September, October and December 2012; January-May 2013; February and December 2014	Field	1 - 2 m	21.2 - 25.2 (23.0)*	36.3
Forno, Arraial do Cabo - AC, Rio de Janeiro (22º59' S, 42º00' W)	July, September, November and December 2006; January-October 2007	Field	1 - 2 m	17.5 - 26.8 (23.2)	36.3
Cabo Frio Island, Arraial do Cabo, Rio de Janeiro (23°00'03" S, 42°00'22" W)	March 2007, October 2015	Strain UNR-11 and Field	1 - 2 m		

*Sea Surface Temperature estimated by satellites.

Table S2. Benthic *Prorocentrum* species found at the Northeast, Southeast and oceanic islands of Brazil and in various places of the world, including species richness at each place and the substrate that has been investigated in each study. The asterisk denotes that the species are synonyms and therefore only one of them was counted in the species richness. Species names in bold denote its type locality.

Place	Latitude	Number of species	Benthic species	Substrates	References
South Atlantic Ocean					
Saint Paul's Rocks, Brazil	**00°55'N**	3	*P.* cf. *fukuyoi*, *P. hoffmannianum*, *P. lima*	Macroalgae (*Dictyota*)	Current study
Fernando de Noronha, Brazil	03°50'S	11	*P.* cf. *borbonicum*, *P.* cf. *caipirignum*, *P.* cf. *concavum*, *P.* cf. *fukuyoi*, *P. hoffmannianum*, *P. lima*, *P.* cf. *norrisianum*, *P. panamense*, *P. rhathymum*, *P. sculptile*, *Prorocentrum* sp.	Macroalgae (*Bryothamnion*, *Canistrocarpus*, *Dictyopteris*, *Dictyota*, *Jania*, *Stypopodium*, *Tricleocarpa*)	Current study
Northeast Brazil	08°04'S - 18°02'S	10	*P. belizeanum**, *P. borbonicum*, *P. caipirignum*, *P. emarginatum*, *P.* cf. *fukuyoi*, *P. hoffmannianum*, *P. leve*, *P. lima*, *P. panamense*, *P. rhathymum*, *Prorocentrum* sp. type 2	Macroalgae (*Agardhiella*, *Amansia*, *Amphiroa*, *Bryopsis*, *Canistrocarpus*, *Dictyopteris*, *Dictyota*, *Gelidiella*, *Gelidium*, *Gracilaria*, *Halimeda*, *Hypnea*, *Lobophora*, *Ochtodes*, *Padina*, *Plocamium*, *Sargassum*)	Current study, Nascimento (2006), Mendes et al. (2014, 2019), Nascimento and Santiago (2016), Nascimento et al. (2017) Tibiriçá (2020)
Trindade, Brazil	20°29'S	7	*P.* cf. *borbonicum*, *P.* cf. *fukuyoi*, *P. hoffmannianum*, *P. lima*, *P.* cf. *norrisianum*, *P. panamense*, *Prorocentrum* sp.	Macroalgae (*Canistrocarpus*, *Dictyopteris*, *Dictyota*)	Current study, Nascimento and Santiago (2016)
Southeast Brazil	22°45'S - 23°04'S	5	***P. caipirignum***, *P.* cf. *fukuyoi*, *P. hoffmannianum*, *P. lima*, *P. rhathymum*	Macroalgae (*Amphiroa*, *Arthrocardia*, *Canistrocarpus*, *Caulerpa*, *Cladophora*, *Cladophoropsis*, *Codium*, *Dictyopteris*, *Dictyota*, *Hincksia*, *Hypnea*, *Jania*, *Laurencia*, *Padina*, *Plocamium*, *Sargassum*, *Spyridia*, *Ulva*, *Zonaria*)	Current study, Nascimento (2006), Nascimento and Santiago (2016), Nascimento et al. (2016, 2017), Tibiriçá (2020)
South Brazil	25°30'S	4	*P.* cf. *fukuyoi*, *P. lima*, *P.* cf. *lima* morphotype 5, *Prorocentrum* sp.	Macroalgae (*Acanthophora*, *Chaetomorpha*, *Hypnea*, *Ulva*), seagrass (*Halodule*), sediment and bare sand	Moreira-González et al. (2019a, 2019b, 2020)
Golfo San José and Golfo Nuevo, Argentina	42°15'S - 42°46'S	1	*P. lima*	Macroalgae (*Anotrichium*, *Ceramium*, *Dictyota*, *Ulva*)	Gayoso et al. (2002)
Caribbean Sea					
Venezuela	10°N	4	*P. concavum*, *P. emarginatum*, *P. lima*, *P. rhathymum*		Sunesen et al. (2021 and references therein)
Barú Island, Bahía Chengue and Concha Bay, Colombia	10°08'N - 11°20'N	8	*P. borbonicum*, *P.* cf. *concavum*, *P. emarginatum*, *P. hoffmannianum*, *P. lima*, *P. rhathymum*, *Prorocentrum* sp. 1, *Prorocentrum* sp. 2	Seagrass (*Syringodium*, *Thalassia*)	Gómez and Pineda (2019), Arbeláez et al. (2020), Arteaga-Sogamoso et al. (2022)
Martinique Island, Overseas France	14°31'N	12	*P. concavum*, *P.* cf. *foraminosum*, *P.* cf. *fukuyoi*, *P. glenanicum*, *P. hoffmannianum*, *P. lima*, *P.* cf. *maculosum**, *P.* cf. *norrisianum*, *P. panamense*, *P. rhathymum*, *P.* cf. *ruetzlerianum*, *P.* cf. *sculptile*, *P.* cf. *tropicale*	Surface sediment	Chomérat et al. (2019)
Carrie Bow Cay and Twin Cays, Belize	16°48'N - 18°48'N	16	***P. arenarium****, ***P. belizeanum****, ***P. caribbaeum***, *P. concavum*, ***P. elegans***, *P. emarginatum*, ***P. foraminosum***, ***P. formosum***, ***P. hoffmannianum***, ***P. leve***, *P. lima*, ***P. maculosum****, *P. mexicanum*, ***P. norrisianum***, ***P. reticulatum***, ***P. ruetzlerianum***, ***P. sabulosum***, ***P. sculptile***, ***P. tropicale***	Floating detritus, surface sediment, colored sand and coral rubble	Faust (1990, 1993a, 1993b, 1994, 1997), Faust et al. (2008)

Virgin Islands, United States	18°N	3	*P. concavum*, *P. lima*, *P. rhathymum*	Macroalgae (*Dictyota*, filamentous algae) and surface water	Loeblich et al. (1979), Kohler and Kohler (1992)
Quintana Roo and Yucatan, Mexico	18°N - 22°N	12	*P. arenarium**, *P. belizeanum**, *P. caribbaeum*, *P. concavum*, *P. emarginatum*, *P. fukuyoi*, *P. foraminosum*, *P. hoffmannianum*, *P. lima*, *P.* cf. *leve*, *P. maculosum**, *P. rhathymum*, *P. sculptile*, *P.* cf. *sipadanense*, *Prorocentrum* sp.	Macroalgae (*Acanthophora*, *Acetabularia*, *Acicularia*, *Amphiroa*, *Avrainvillea*, *Batophora*, *Bryothamnion*, *Caulerpa*, *Ceramium*, *Chaetomorpha*, *Chondria*, *Codium*, *Dictyota*, *Digenia*, *Enteromorpha*, *Eucheuma*, *Gracilaria*, *Halimeda*, *Halymenia*, *Heterosiphonia*, *Jania*, *Laurencia*, *Padina*, *Penicillus*, *Sargassum*, *Stypopodium*, *Udotea*), seagrass (*Halodule*, *Ruppia*, *Syringodium*, *Thalassia*), sponge and sediment	Okolodkov et al. (2014), Almazán-Becerril et al. (2015), Aguilar-Trujillo et al. (2017), Irola-Sansores et al. (2018), Cembella et al. (2021)
Veracruz Reef System, Veracruz, Mexico	19°11'N	2	*P. hoffmannianum*, *P. lima*	Macroalgae (*Laurencia*, *Padina*, brown algae, green algae) and seagrass (*Thalassia*)	Cembella et al. (2021)
Cienfuegos and Havana, Cuba	21°55'N - 23°N	5	*P. belizeanum**, *P. caipirignum* (as *P. maculosum**), *P. concavum*, *P. hoffmannianum*, *P. lima*, *P. mexicanum*	Macroalgae (*Chaetomorpha*, *Dictyota*, *Padina*, *Ulva*, brown macroalgae) and sediment	Delgado et al. (2006), Herrera-Sepúlveda et al. (2015), Moreira-González et al. (2019a,b)
North Atlantic Ocean					
Florida, United States	24°50'N - 25°15'N	4	*P.* cf. *emarginatum*, *P. hoffmannianum*, *P. lima*, *P. rhathymum*	Macroalgae (*Acanthophora*, *Ceramium*, *Halimeda*, *Hypnea*, *Palisada*, *Polysiphonia*) and seagrass (*Ruppia*, *Syringodium*, *Thalassia*)	Herrera-Sepúlveda et al. (2015), Accoroni et al. (2020)
Canary Islands, Overseas Spain	27°38'N - 29°13'N	4	*P. hoffmannianum*, *P. lima*, *P. maculosum**, *P. panamense*, *P. rhathymum*	Macroalgae (*Asparagopsis*, *Caulerpa*, *Colpomenia*, *Cystoseira*, *Dictyota*, *Galaxaura*, *Halimeda*, *Halopteris*, *Jania*, *Lobophora*, *Padina*, *Sargassum*, *Zonaria*) and artificial substrates	Rodríguez et al. (2018), Fernández-Zabala et al. (2019, 2022), Gaiani et al. (2022)
Lisbon, Portugal	38°41'N	1	*P. lima*	Water	Vale et al. (2009)
Maine, United States	41°23'N - 44°27'N	1	*P. lima*	Oyster cages, lines, buoys, rafts, macroalgae (*Ascophyllum*, *Fucus*) and seagrass (*Zostera*)	Maranda et al. (2007a)
Ria de Vigo and Ria de Pontevedra, Spain	42°15'N	1	*P. lima*	Algae and sandy/algae	Bravo et al. (2001)
Bay of Biscay (south-eastern), Spain	43°17'N - 43°35'N	5	*P. elegans*, *P. emarginatum/fukuyoi* group, *P. leve*, *P. lima*, *P. rhathymum*	Macroalgae (*Cladostephus*, *Corallina*, *Cystoseira*, *Dictyota*, *Gelidium*, *Plocamium*, *Stypocaulon*) and water column	Laza-Martinez et al. (2011), David et al. (2014)
Nova Scotia and Quebec, Canada	44°27'N - 47°60'N	2	*P. lima*, *P. mexicanum*	Epibionts (composed of hydrozoans and macroalgae intertwined around a mussel sock), mussels and water	Marr et al. (1992), Lawrence et al. (2000), Levasseur et al. (2003)
Gironde and Brittany, France	44°38'N - 47°46'N	6	***P. consutum***, *P.* cf. *fukuyoi*, ***P. glenanicum***, *P. leve*, *P. lima*, ***P. pseudopanamense***	Sandy sediment, sediment and water	Chomérat et al. (2010, 2011), Mertens et al. (2017)
Dorset, United Kingdom	50°37'N	1	*P. lima*	Macroalgae (*Ascophyllum*, *Calliblepharis*, *Ceramium*, *Chaetomorpha*, *Ectocarpaceae* spp., *Enteromorpha*, *Fucus*, *Gracilaria*, *Sargassum*, *Ulva*) and seagrass (*Ruppia*, *Zostera*) and water	Nascimento et al. (2005), Foden et al. (2005)
Ireland	51°N - 52°N	1	*P. lima*		Kilcoyne et al. (2020)

Helgoland, Germany	54°11'N	1	***P. clipeus***	Sand	Hoppenrath et al. (2000)
Mediterranean Sea					
Mellah lagoon, Algeria	36.54°N	1	*P. lima*	Surface water	Draredja et al. (2019)
Bizerte Bay and Lagoon, Tunisia	37°10'N - 37°16'N	4	*P. concavum*, *P. emarginatum*, *P. lima*, *P. rhathymum*	Macroalgae (*Ulva*) and seagrass (*Cymodocea*, *Zostera*)	Ben Gharbia et al. (2019)
Greece	40°N - 41°N	5	*P. borbonicum*, *P. emarginatum*, *P. leve*, *P. lima* complex, *P. rhathymum*	Macroalgae (*Codium*, *Corallina*, *Cystoseira*, *Padina*, *Ulva*), sediment and water column	Aligizaki et al. (2009)
Catalonia, Spain	41°N - 42°N	1	*P. lima*	Macroalgae (*Corallina*, *Dictyota*, *Dilophus*, *Halopteris*, *Jania*), sediment and surface water	Vila et al. (2001)
Catalonia, Languedoc, Provence, French Riviera and Corsica, France	41°24'N - 43°46'N	1	*P. lima*	Macroalgae (*Asparagopsis*, *Caulerpa*, *Ceramium*, *Cladophora*, *Cladostephus*, *Corallina*, *Cystoseira*, *Dictyopteris*, *Dictyota*, *Gelidiella*, *Gelidium*, *Haliptylon*, *Halopitys*, *Halopteris*, *Jania*, *Laurencia*, *Liagora*, *Padina*, *Parviphycus*, *Plocamium*, *Polysiphonia*, *Taonia*, *Ulva*, *Womersleyella*)	Blanfune et al. (2015)
Genoa and Naples, Italy	44ºN	1	***P. lima***	Macroalgae (*Halopteris*) and water	Nagahama and Fukuyo (2005), Giussani et al. (2017)
South Indian Ocean					
Zanzibar, Tanzania	06°S	7	*P. borbonicum*, *P. concavum*, *P. elegans*, *P. emarginatum*, *P. hoffmannianum* (as *P. belizeanum** and *P. maculosum**), *P. lima*, *P. mexicanum*	Dead coral with algal turf and water	Hansen et al. (2001)
Ste. Marie, Madagascar	16°S - 17°S	2	*P. arenarium**, *P. concavum*, *P. lima*	Benthic samples and water	Hansen et al. (2001)
Broome, Australia	17°97'S	2	*P. concavum*, *P. malayense*	Sandy sediment	Verma et al. (2019)
Mauritius Island	20°S	6	*P. belizeanum**, *P. concavum*, *P. elegans*, *P. emarginatum*, *P. hoffmannianum*, *P. lima*, *P. mexicanum*	Macroalgae (*Gracilaria*, *Hypnea*, *Jania*) and water	Hansen et al. (2001)
Réunion Island, Overseas France	20°55'S - 21°09'S	9	*P. arenarium**, *P. belizeanum**, ***P. borbonicum***, *P. concavum*, *P. elegans*, *P. emarginatum*, *P. hoffmannianum*, *P. lima*, *P. mexicanum*, *P. panamense*, *P. sculptile*	Dead coral with algal turf and water	Ten-Hage et al. (2000), Hansen et al. (2001)
North Indian Ocean					
Tamil Nadu, Bay of Bengal	9.281933°N	1	*P. lima*	Water	Oyeku and Mandal (2021)
Dhofar, Oman	16°49'N - 16°58'N	1	*P. sipadanense*	Macroalgae (*Padina*) and sediment	Saburova and Chomérat (2016)

Kuwait	28°30'N - 29°30'N	8	*P. arenarium**, ***P. bimaculatum***, *P. concavum*, *P. consutum*, *P. emarginatum*, *P. fukuyoi*, *P. lima*, *P. norrisianum*, *P. rhathymum*	Sediments	Saburova et al. (2009), Chomérat et al. (2012 and references therein)
South Pacific Ocean					
Queensland and New South Wales, Australia	11°S - 34°S	5	*P. concavum* (as ***P. faustiae****), ***P. fukuyoi***, *P. hoffmannianum*, *P. lima*, *P. rhathymum*	Macroalgae and sediment	Morton and Tindall (1995), Morton (1998), Murray et al. (2007, 2009), Ajani et al. (2011)
French Polynesia, Overseas France	~17°S	2	***P. concavum***, *P. mexicanum*	Macroalgae (*Amphiroa*, *Jania*, *Turbinaria*)	Fukuyo (1981), Grzebyk et al. (1998)
New Caledonia, Overseas France	~20°S	1	***P. concavum***	Macroalgae (*Amphiroa*, *Jania*, *Turbinaria*)	Fukuyo (1981)
New Zealand subtropical zone	29°S	5	*P.* cf. *emarginatum*, *P. fukuyoi*, *P. hoffmannianum*, *P. lima*, *P. maculosum**, *P. rhathymum*	Macroalgae and sediment	Rhodes and Smith (2019 and references therein)
New Zealand temperate zone	35°S - 45°S	1	*P. lima*	Macroalgae on mussel growing lines	Rhodes and Smith (2019 and references therein)
Anson's Bay and Little Swanport, Tasmania	41°03'S - 42°50'S	2	*P. lima*, *P. mexicanum*	Water, seagrass and benthos samples	Pearce et al. (2001)
Magallanes, Chile	50°S - 55°S	1	*P. lima*	Sediments	Salgado et al. (2012), Frangópulos et al. (2016)
North Pacific Ocean					
Singapore Strait	01°N	3	*P. concavum*, *P. emarginatum*, *P. lima*	Sediments and water	Tan et al. (2016)
Sabah, East Malaysia	04°06.72"N - 07°38.65"N	9	*P. arenarium**, *P. concavum*, *P. emarginatum*, *P.* cf. *faustiae**, *P. foraminosum*, *P. formosum*, *P. lima*, *P. norrisianum*, *P. rhathymum*, *P. sculptile*, ***P. sipadanense***	Macroalgae (*Padina*, *Sargassum*), seagrasses and dead corals	Mohammad-Noor et al. (2007)
Rawa Island, Peninsular Malaysia	05°57'N	7	*P. caipirignum*, *P. concavum*, *P.* cf. *emarginatum*, *P.* cf. *foraminosum*, *P. lima*, ***P. malayense***, *P. mexicanum*	Seaweeds, coral rubble and sediment	Lim et al. (2019)
Contadora Island, Panama	08°38'N	3	*P. concavum*, *P. hoffmannianum* (as *P. maculosum**), ***P. panamense***	Turf algae	Grzebyk et al. (1998)
Xisha Islands, China	15°46'N	7	*P. borbonicum*, *P. caipirignum*, *P. concavum*, *P. elegans*, *P.* cf. *emarginatum*, *P. lima* complex, *P. rhathymum*	Macroalgae, seagrasses, dead coral reefs and sediments	Zou et al. (2022)
Baja California Sur, Mexico	24°09'N	1	*P. lima*	Macroalgae (*Sargassum*, *Ulva*) and sediment with detritus	Cembella et al. (2021)
Johnston Atoll, United States	16°44'N	4	*P. concavum*, *P. emarginatum*, *P. lima*, *P.* cf. *rhathymum*	Macroalgae (*Caulerpa*)	Richlen and Lobell (2011)

Hainan Island, China	18°09'N - 18°18'N	8	*P. caipirignum* (as *P. lima* morphotype 4 and *P.* cf. *maculosum**), *P. concavum*, *P.* cf. *emarginatum*, *P. fukuyoi*, *P. lima*, *P. panamense*, *P. rhathymum*, *Prorocentrum* morphotype 5	Macroalgae (*Caulerpa*, *Colpomenia*, *Dictyota*, *Padina*, *Sargassum*, *Spatoglossum*, *Ulva*), seagrass (*Halophila*), dead coral reef and sandy sediments	Zhang et al. (2015), Luo et al. (2017)
Hawaiʻi Island, United States	19°30'N	6	*P. belizeanum**, *P. concavum*, *P. emarginatum*, *P. hoffmannianum*, *P. lima*, *P. mexicanum*, *P. ruetzlerianum*	Macroalgae (*Acanthophora*, *Ahnfeltiopsis*, *Amansia*, *Amphiroa*, *Anotrichium*, *Caulerpa*, *Centroceras*, *Ceramium*, *Coelothrix*, *Dictyopteris*, *Enteromorpha*, *Galaxaura*, *Gelidiella*, *Gelidiopsis*, *Gelidium*, *Gracilaria*, *Grateloupia*, *Haloplegma*, *Halymenia*, *Martensia*, *Portieria*, *Pterocladiella*, *Tolypiocladia*, *Trichogloea*, *Tricleocarpa*, *Turbinaria*, *Ulva*), rock and sand	Parsons and Preskitt (2007)
Guangxi, China	21°01'N - 21°25'N	3	*P. caipirignum* (as *P.* cf. *maculosum**), *P. fukuyoi*, *P. lima*	Macroalgal, seagrass, dead coral reef and sandy sediments	Luo et al. (2017)
Ryukyu Islands, Japan	~25°N	2	***P. concavum*, *P. emarginatum***	Macroalgae (*Amphiroa*, *Jania*, *Turbinaria*)	Fukuyo (1981)
Japan	26°14'N - 43°12'N	8	*P. caipirignum*, *P.* cf. *concavum*, *P.* cf. *emarginatum*, *P.* cf. *fukuyoi*, *P. lima* complex, *P.* cf. *rhathymum*, *Prorocentrum* sp. type 1, *Prorocentrum* sp. type 2	Macroalgae (*Codium*, *Coccophora*, *Dichotomaria*, *Distromium*, *Gelidium*, *Gracilaria*, *Grateloupia*, *Heterochordaria*, *Laurencia*, *Lobophora*, *Padina*, *Palisada*, *Ptilonia*, *Sargassum*, *Turbinaria*, *Ulva)*	Nishimura et al. (2020)
Jeju Island, Korea	33°12'N - 33°32'N	5	*P. concavum*, *P. emarginatum*, *P. fukuyoi*, *P. lima*, *P. rhathymum*	Macroalgae *(Champia*, *Chondrus*, *Cladophora*, *Codium*, *Colpomenia*, *Corallina*, *Dictyopteris*, *Ecklonia*, *Enteromorpha*, *Galaxaura*, *Gelidium*, *Gracilaria*, *Grateloupia*, *Hizikia*, *Hypnea*, *Jania*, *Martensia*, *Plocamium*, *Pterocladiella*, *Sargassum*, *Ulva*, *Wrangelia*)	Shah et al. (2013)
Zhitkova Bay and Sobol Bight, Sea of Japan	43°02'N - 43°04'N	4	*P. foraminosum*, *P. fukuyoi*, *P. lima*, *Prorocentrum* sp.	Macroalgae (*Ceramium*, *Chondria*, *Chordaria*, *Cladophora*, *Coccophora*, *Codium*, *Costaria*, *Cystoseira*, *Dasya*, *Dictyopteris*, *Grateloupia*, *Laurencia*, *Neorhodomela*, *Neosiphonia*, *Prionitis*, *Ptilota*, *Tichocarpus*, *Ulva*) and seagrass	Selina and Levchenko (2011), Kameneva et al. (2015)
British Columbia, Canada	49°0.0'N	1	***P. tsawwassenense***	Sand	Hoppenrath and Leander (2008)

Table S3. Minimum, maximum and mean abundances (in parentheses) of each *Prorocentrum* species, as well as total abundance of the genus *Prorocentrum* (cells g^{-1} FWmacroalgae) at eleven sites at the coast and three oceanic islands of Brazil. *Not identified.

Sampling sites	*P*. cf. *borbonicum*	*P.* cf. *caipirignum*	*P*. cf. *concavum*	*P*. *emarginatum*/*fukuyoi* / *sculptile* group	*P*. *hoffmannianum*	*P*. *lima* complex	*P*. cf. *norrisianum*	*P*. *panamense*	*P*. *rhathymum*	*Prorocentrum* sp.	*Prorocentrum* NI*	*Prorocentrum* abundance
Saint Paul's Rocks				0 - 471 (54)	0 - 15 (2)	0 - 19 (5)					0 - 1 (0)	0 - 482 (62)
Fernando de Noronha	0 - 142 (25)	0 - 164 (15)	0 - 188 (16)	0 - 621 (158)	0 - 740 (191)	0 - 2141 (404)	0 - 221 (31)	0 - 86 (5)	0 - 586 (83)	0-117 (17)	0 - 242 (25)	143 - 3738 (1374)
Muro Alto						0 - 197 (31)						0 - 197 (31)
Porto de Galinhas				0 - 139 (48)		76 - 8389 (1804)					0 - 444 (64)	76 - 8527 (1916)
Maragogi				0 - 188 (36)		0 - 550 (227)						31 - 550 (263)
Garça Torta						0 - 91 (33)						0 - 91 (33)
Forte						1026 - 19487 (7099)					0 - 342 (38)	1067 - 19829 (7137)
Placaford						0 - 113 (35)					0 - 17 (1)	0 - 117 (37)
Penha				0 - 20 (4)		3 - 684 (236)					0 - 68 (14)	5 - 703 (254)
Trindade	0 - 37 (5)			0 - 826 (243)	0 - 714 (163)	0 - 1984 (347)	0 - 13 (1)				0 - 19 (1)	0 - 2590 (760)
Tartaruga				0 - 977 (45)		0 - 6746 (286)			0 - 41 (1)		0 - 81 (3)	0 - 7722 (334)
Forno, AB						0 - 79 (1)						0 - 79 (1)
Forno, AC				0 - 140 (4)		0 - 305 (36)			0 - 7 (0)		0 - 77 (3)	0 - 333 (43)
Cabo Frio		0 - 16 (4)		0 - 15 (3)		1 - 2288 (204)			0 - 58 (8)		0 - 55 (20)	1 - 2353 (238)

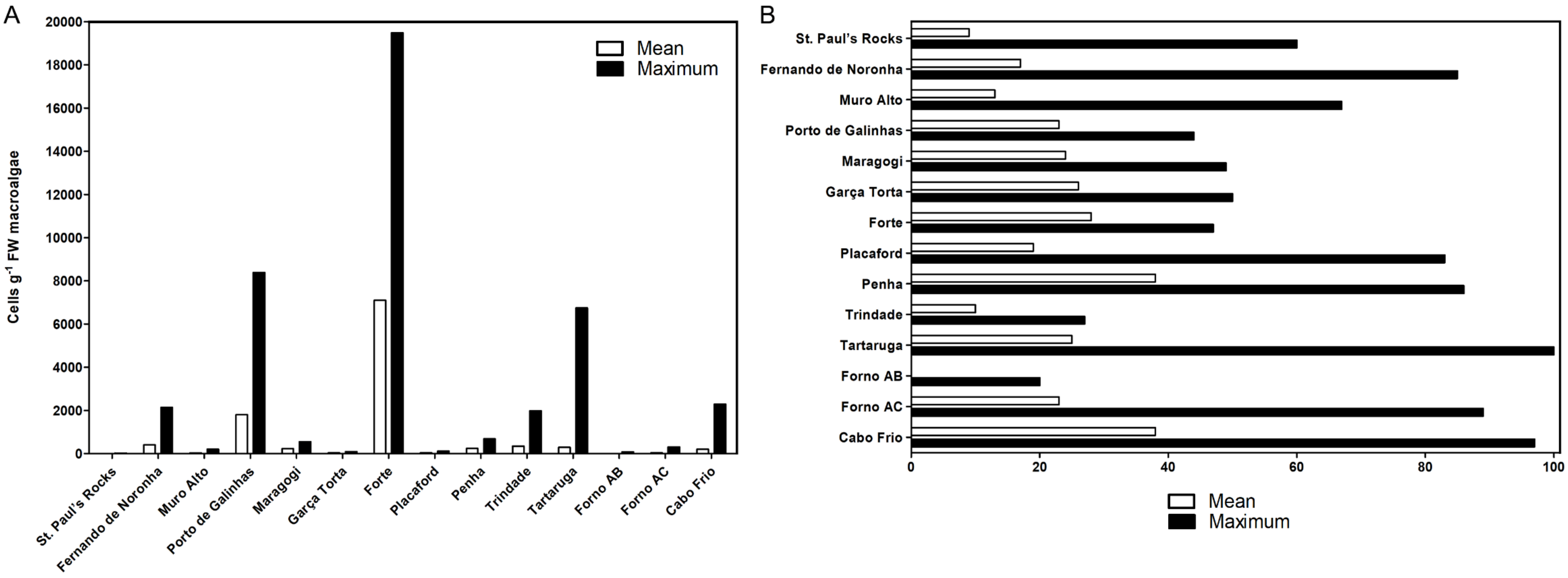

Figure S1. *Prorocentrum lima* abundance values at eleven sites on the coast and three oceanic islands of Brazil. (A) Mean and maximum abundance (cells g^{-1} FWmacroalgae); (B) Mean and maximum relative abundance (%) or the contribution of the species to total benthic dinoflagellate abundance.

Table S4. Summary of the benthic *Prorocentrum* species found at each site analyzed in the Northeast and Southeast coasts and oceanic islands of Brazil.

Coast or oceanic island of Brazil	Sampling sites	*P. borbonicum* or *P.* cf. *borbonicum*	*P.* cf. *caipirignum*	*P.* cf. *concavum*	*P. emarginatum/fukuyoi / sculptile* group	*P. hoffmannianum*	*P. lima* complex	*P.* cf. *norrisianum*	*P. panamense*	*P. rhathymum*	*Prorocentrum* sp.	*Prorocentrum* NI*
Oceanic islands	Saint Paul's Rocks				X	X	X					X
	Fernando de Noronha	X	X	X	X	X	X	X	X	X	X	X
Northeast coast	Muro Alto						X					
	Porto de Galinhas				X		X					X
	Maragogi	X			X		X					
	Garça Torta						X					
	Forte						X					X
	Placaford						X					X
	Penha				X		X					X
Oceanic island	Trindade	X			X	X	X	X	X		X	X
Southeast coast	Tartaruga				X		X			X		X
	Forno, AB						X					
	Forno, AC				X		X			X		X
	Cabo Frio		X		X		X			X		X

Conclusões Gerais

- Este trabalho contribuiu para o conhecimento da diversidade e distribuição geográfica dos gêneros de dinoflagelados bentônicos *Ostreopsis* e *Prorocentrum* no Brasil.
- Este foi o primeiro registro da espécie *Ostreopsis lenticularis* no Oceano Atlântico, combinando dados morfológicos e moleculares, apresentando as primeiras sequências genéticas desta espécie nesta região. A espécie foi encontrada em elevadas abundâncias (até 7.6 x 10^4 céls. gPUmacroalga^{-1}) em Fernando de Noronha.
- Este estudo abordou dados morfológicos, moleculares e de abundância das espécies bentônicas de *Prorocentrum* no Brasil, contemplando diferentes regiões do país.
- A riqueza de espécies bentônicas de *Prorocentrum* foi maior em Fernando de Noronha, onde onze espécies foram encontradas, sendo um *hotspot* de diversidade, apresentando número de espécies entre os mais altos do mundo. Nas demais ilhas oceânicas, sete espécies foram encontradas em Trindade e três em São Pedro e São Paulo. Considerando a área costeira, três espécies foram observadas na região nordeste e quatro na região sudeste.
- *Prorocentrum borbonicum*, *P. hoffmannianum*, *P. lima* complex e *P. rhathymum* foram identificados através de análise morfológica e molecular combinada, enquanto *P.* cf. *caipirignum, P.* cf. *concavum, P.* cf. *fukuyoi, P.* cf. *norrisianum, P. panamense* e *P.* cf. *sculptile* foram identificados a partir da observação em microscopia eletrônica de varredura e/ou microscopia ótica.
- *Prorocentrum lima* foi a única espécie encontrada em todos os sítios investigados, apresentando as maiores abundâncias, de até 1.9 x 10^4 céls. gPUmacroalga^{-1} na região nordeste, enquanto a abundância máxima das demais espécies não ultrapassou 1 x 10^3 céls. gPUmacroalga^{-1}.
- *Prorocentrum borbonicum, P. caipirignum, P. hoffmannianum, P. lima* complex e *P. rhathymum* produzem toxinas diarreicas, estando todas presentes na costa e/ou ilhas oceânicas do Brasil.
- As abundâncias das espécies bentônicas de *Prorocentrum*, especialmente *P. lima*, podem ser potencialmente prejudiciais à essas regiões, tanto para o ecossistema, a partir da transferência de toxinas pelas teias alimentares

bentônicas e a exposição dos organismos marinhos à toxinas diarreicas, quanto para a saúde humana, devido ao consumo de organismos marinhos contaminados.

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