UNIVERSIDADE ESTADUAL PAULISTA ‘JULIO DE MESQUITA FILHO’

INSTITUTO DE BIOCIÊNCIAS - CAMPUS DE BOTUCATU

ROMULO AUGUSTO DE OLIVEIRA BAZZO



Taxonomia Integrativa e Padrões de Distribuição das Esponjas Calcareas (Porifera, Calcarea) da Ecorregião Marinha Sudeste do Brasil

BOTUCATU

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Dissertação apresentada à Universidade Estadual Paulista (UNESP), Instituto de Biociências, Botucatu, para a obtenção do título de Mestre em Zoologia.

Orientador: Dr. Sérgio Nascimento Stampar

Coorientadora: Dra. Fernanda Correia Azevedo

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I live by the ocean

And during the night

I dive into it

Down to the bottom

Underneath all currents

And drop my anchor

And this is where I'm staying

This is my home

Björk

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Taxonomia Integrativa e Padrões de Distribuição das esponjas calcareas (Porifera, Calcarea) da ecorregião marinha Sudeste do Brasil

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Resumo

As esponjas marinhas desempenham funções ecológicas essenciais nas comunidades bentônicas dos ecossistemas costeiros; entretanto, apesar de seus relevantes serviços ecossistêmicos, o conhecimento relativo à composição taxonômica, riqueza específica, distribuição geográfica e afinidades biogeográficas do filo Porifera permanece desigual entre as diferentes ecorregiões marinhas globais. A classe Calcarea, composta por mais de 800 espécies descritas e reconhecidas cientificamente, enfrenta desafios adicionais relacionados ao seu porte reduzido e à preferência por habitats crípticos de baixa luminosidade, características que dificultam a exploração desse grupo. Nos últimos anos, esforços de amostragem na região neotropical têm contribuído para a descrição de uma expressiva gama de novas espécies, bem como para a ampliação das áreas conhecidas de dezenas de táxons; contudo, no Brasil, dados do World Porifera Database revelam lacunas significativas ao longo da faixa litorânea dos estados do Paraná, Rio Grande do Sul e em grande parte do litoral de São Paulo. A presente dissertação está organizada em três capítulos: o primeiro aborda padrões de endemismo de Calcarea no Atlântico Ocidental; o segundo dedica-se à taxonomia integrativa dos exemplares coletados na porção norte do litoral paulista; e o terceiro consiste em uma nota sobre a ocorrência da espécie exótica invasora *Heteropia glomerosa* em três praias de São Paulo.

Palavra-chave: Zoologia, Esponjas Calcareas, Litoral Paulista, Biodiversidade, Taxonomia

Taxonomia Integrativa e Padrões de Distribuição das esponjas calcareas (Porifera, Calcarea) da ecorregião marinha Sudeste do Brasil

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Abstract

Marine sponges perform essential ecological functions within benthic communities of coastal ecosystems; however, despite their significant ecosystem services, knowledge concerning the taxonomic composition, species richness, geographic distribution, and biogeographic affinities of Phylum Porifera remains uneven across global marine ecoregions. Class Calcarea, which comprises over 800 scientifically described and recognized marine species, faces additional challenges related to its diminutive body size and preference for cryptic, low-light habitats, traits that hinder comprehensive exploration of this group. In recent years, sampling efforts in the Neotropical region have contributed to the description of a substantial array of new species and to the expansion of known distributional ranges for dozens of taxa; nevertheless, in Brazil, data from the World Porifera Database reveal significant knowledge gaps along the coastline of Paraná, Rio Grande do Sul, and much of São Paulo. This dissertation is organized into three chapters: the first addresses endemism patterns of Calcarea in the Western Atlantic; the second is devoted to the integrative taxonomy of specimens collected from the northern portion of the São Paulo coast; and the third presents a brief note on the occurrence of the exotic invasive species *Heteropia glomerosa* on three beaches in São Paulo.

Keyword: Zoology; Calcareous sponges; São Paulo Coast; Biodiversity; Taxonomy

Introdução Geral

As esponjas fazem parte da complexa comunidade bentônica dos ecossistemas marinhos. Elas fornecem habitat e suporte para diversos animais (Miller et al., 2012; Padua, Lanna and Klautau, 2013; Kersken et al., 2014; Ribeiro et al., 2018; García-Hernández et al., 2019), servem como fonte de alimento (Pawlik, 1998; McClintock, 2005; Ruzicka and Gleason, 2008) e desempenham um papel significativo no fluxo de matéria orgânica dissolvida na água (de Goeij et al., 2013). Apesar de seus importantes serviços ecossistêmicos, o conhecimento sobre a composição, riqueza, distribuição e afinidades biogeográficas das espécies permanece desigual entre as ecorregiões marinhas ao redor do mundo (Van Soest et al., 2012). Este é um problema com diversos fatores atuantes, entre eles a dificuldade em treinar taxonomistas locais, produzir conhecimento em forma de artigos, guias, livros, fatores político-econômicos entre outros (Miloslavich et al., 2011).

Sobre o filo Porifera existem desafios para uma melhor compreensão biogeográfica e nem todas as classes de esponjas recebem a mesma atenção (Van Soest et al., 2012). Entre elas, a classe Calcarea é composta por mais de 800 espécies com descrições aceitas pela comunidade científica (de Voogd et al., 2025). São animais exclusivamente marinhos, pequenos, com uma preferência por habitats crípticos, pouco iluminados. Tais características são desafiadoras para uma difusão adequada de conhecimentos gerados na academia para além dos especialistas do grupo. Apesar disso, esforços de amostragem no neotrópico, nas últimas décadas, têm contribuído para a descrição de uma expressiva variedade de novas espécies par a ciência (Azevedo et al., 2017; Cavalcanti et al., 2013; Klautau et al., 2017; Lanna et al., 2007, 2009), bem como para a expansão da área de distribuição de dezenas de espécies (Cóndor-Luján et al., 2018; Fontana et al., 2018; Lopes et al., 2018; Lopes et al., 2025). No Brasil, de acordo com as informações contidas no banco de dados World Porifera Database (https://www.marinespecies.org/porifera/), não há registros de ocorrência de esponjas calcareas em toda a faixa litorânea dos estados do Paraná, Rio Grande do Sul e em grande parte do litoral de São Paulo (Figura 1A).

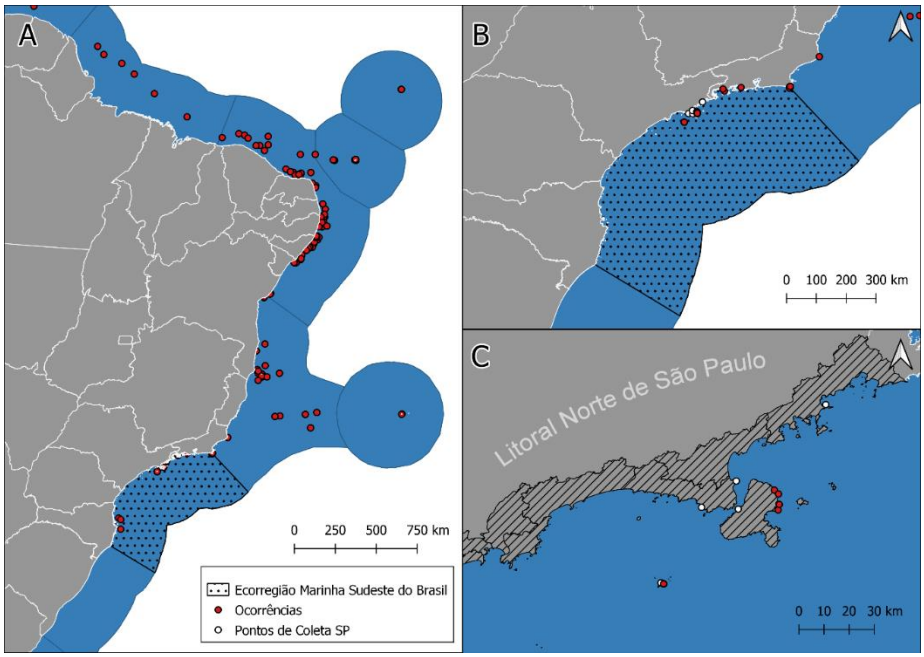


Figura 1. Mapa contendo a classificação das ecorregiões marinhas ao longo da costa brasileira de acordo com a proposta de Spalding et al. (2007). A – Esforço amostral das esponjas calcareas no Brasil sendo representado pelos círculos vermelhos. B – Destaque para a ecorregião marinha do Sudeste do Brasil, a qual contempla os estados do Rio de Janeiro, São Paulo, Paraná e Santa Catarina. C – Destaque para o Litoral Norte de São Paulo, contendo os círculos brancos representando as localidades amostradas neste projeto.

Na ecorregião marinha Sudeste do Brasil (Figura 1B), a área mais ao norte, incluindo o estado do Rio de Janeiro, possui dados de ocorrência para as esponjas calcareas resultantes de amostragens mais frequentes. Em cenários como esse, investigar áreas pouco amostradas dentro dessa ecorregião pode revelar padrões de distribuição que sustentam ou desafiam a proposta de delimitação espacial atual das espécies segundo Spalding et al. (2007). Uma dessas áreas pouco exploradas faz parte do litoral do estado de São Paulo, caracterizado por uma faixa de 880 km de paisagens costeiras diversificadas entre serra do mar, bancos de areia e manguezais (Ab'Saber, 1955). Uma análise das principais coleções marinhas do estado de São Paulo revelou uma notável escassez de material do filo Porifera depositado em comparação com outros grupos taxonômicos (Borges et al., 2022). Neste estudo, realizamos levantamentos na porção norte da costa de São Paulo (Figura 1C) em praias ou pontos ainda não documentados quanto à presença de esponjas calcareas.

Atualmente, um total de 11 espécies distribuídas em 10 gêneros são documentadas para uma área restrita da costa de São Paulo, são elas: *Amphoriscus pedunculatus* Klautau, Cavalcanti & Borojevic, 2017, *Arturia alcatraziensis* Lanna, Rossi, Cavalcanti, Hajdu & Klautau, 2007, *Clathrina aurea* Solé-Cava, Klautau, Boury-Esnault, Borojevic & Thorpe, 1991, C. *conifera* Klautau & Borojevic, 2001, C. *primordialis* (Haeckel, 1872), *Heteropia glomerosa* (Bowerbank, 1873), *Leucandra serrata* Azevedo & Klautau, 2007, *Leucascus*

roseus Lanna, Rossi, Cavalcanti, Hajdu & Klautau, 2007, *Neoernsta tetractina* (Klautau & Borojević, 2001), *Paraleucilla magna* Klautau, Monteiro & Borojevic, 2004, *Sycon pentactinale* Rossi, Farina, Borojevic & Klautau, 2006, *Leucosolenia* e *Grantia* (Custódio & Hajdu, 2011; Klautau et al., 2017, 2020; Lanna et al., 2007; Muricy et al., 2011). Registros de esponjas calcareas no estado de São Paulo remontam às décadas de 1970s (Borojevic, 1971), 2000s (Lanna et al., 2007), e 2010s (Oricchio et al., 2019; Cavalcanti et al., 2020) restritos aos municípios de São Sebastião, Ilhabela e Ubatuba.

O primeiro capítulo apresenta o estudo sobre os padrões de endemismo de esponjas calcareas no Atlântico Ocidental, com intenção de submissão à revista Biota Neotropica (ISSN-online: 1676-0611). O segundo capítulo trata das identificações, por meio de abordagem integrativa, dos espécimes coletados no presente estudo na costa de São Paulo. O terceiro capítulo traz um "short communication" sobre a presença da espécie exótica invasora *Heteropia glomerosa* em três praias de São Paulo, o qual foi submetido à revista Zoologia (ISSN-online: 1984-4689).

Chapter 01: Areas of Endemism of Calcareous Sponges (Porifera: Calcarea) in the Western Atlantic

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Abstract

Calcareous sponges are marine animals with globally underestimated biodiversity. While some parts of the world have a satisfactory sample marine ecoregion, in neotropics many gaps persist. We investigated Western Atlantic distribution patterns using Geographic Interpolation of Endemism (GIE) on. a dataset of 78 species and 295 occurrence records endemics to the region, identifying 17 areas of endemism (AoEs). Notably, the Brazilian coast has more AoEs than the Caribbean, contrasting with diversity patterns observed in other marine taxa (e.g., reef fishes, sea anemones) where the Caribbean serves as the most diverse hotspot in the Atlantic. This discrepancy likely stems from higher sampling effort and the presence of established taxonomic research groups in Brazil, which have advanced local biodiversity knowledge. Mapping AoEs revealed priority regions for future sampling. From a conservation perspective, several Brazilian AoEs overlap with Marine Protected Areas (MPAs), reinforcing the importance of maintaining these protections.

Keywords: biogeography; benthic animals; kernel interpolation

Introduction

Calcareous sponges constitute one of the four living classes of the phylum Porifera, the only one consisting exclusively of marine animals in which the skeletal structures are entirely formed by calcium carbonate. Due to the difficulty of recognizing and collecting these sponges in their environments, studying this group poses several challenges to understanding their life habits, reproduction, ecology, and evolutionary history, requiring further attention from taxonomists. Currently, more than 800 species are described and recognized by science (de Voogd *et al.*, 2025). Regarding their distribution in marine environments, there are few marine ecoregions where the biodiversity of this group is well-documented leaving many locations yet to be investigated (Van Soest *et al.*, 2012). Due to their morphological characteristics, which exhibit a high degree of homoplasy, many species have been considered cryptic (Manuel *et al.*, 2003; Voigt, Wülfing and Wörheide, 2012). For a long time, they were thought to have cosmopolitan distribution. However, advances in molecular studies have revealed that distant populations previously classified as the same species represent distinct taxa (Solé-Cava *et al.*, 1991; Klautau, Solé-Cava and Borojevic, 1994) (e.g., *Borojevia cerebrum* (Haeckel, 1872) in the Mediterranean and *Borojevia brasiliensis* (Solé-Cava, Klautau, Boury-Esnault, Borojevic & Thorpe, 1991) in Brazil). Another factor supporting the suspicion of its restricted distribution is the behavior of larval stage of sponges, their only phase with mobility in the water column, which is often short-lived before settling on the substrate (Maldonado, 2006).

In the Neotropical region, significant knowledge gaps remain, with some ecoregions lacking any records of these animals. In recent years, increasing efforts in biodiversity studies and taxonomic descriptions have aimed to address this issue (Azevedo *et al.*, 2017; Cóndor-Luján *et al.*, 2018; Lopes *et al.*, 2018; Fonseca, Cóndor-Luján and Cavalcanti, 2023). The most extensively sampled ecoregions are Southeastern Brazil (18 species) and Eastern Brazil (38 species) (Lopes et al., 2025), a pattern related to the presence of active research groups in these areas. As a result, Brazilian institutions and museums hold specimens from the region deposited in scientific collections, such as of the Federal University of Rio de Janeiro (UFRJPOR), Federal University of Bahia (UFBAPOR), and Museu Nacional (MNRJ). Additionally, some specimens are housed in European institutions, including the Muséum National d'Histoire Naturelle, Paris (MNHN) and the Zoological Museum Amsterdam (ZMA). Many specimens remain undescribed in the Brazilian collections, with the potential to fill gaps of the biodiversity knowledge in the region. Notably, some materials were described for the Guyana Shelf (Van Soest, 2017). Using occurrence data from these studies and databases of biodiversity (GBIF, WPD and

OBIS) we compiled a georeferenced database to analyze the distribution patterns of calcareous sponges in neotropics.

Studies on area of endemism are common in the field of historical biogeography, as they provide a way to understand current distribution patterns in conjunction with the movement of tectonic plates that have shaped environments and, consequently, life on Earth (Morrone, 1994). For instance, the Caribbean, in the Atlantic region, maintained a continuous connection with the Pacific Ocean for millions of years during the Eocene (Bartonian, 40 Ma), allowing gene flow between populations from both oceans. It was only around the Pliocene (2.8 Ma), with the formation of the Isthmus of Panama, that this region became isolated (O'Dea et al., 2016). In terms of biodiversity, the Caribbean is considered the most diverse region in the Atlantic (Yasuhara *et al.*, 2022). As for sponges, diversity in the region is influenced by two key factors: the extent of sampling efforts and the taxonomic effort on the collected samples (Miloslavich et al., 2010; Oliveira et al., 2024). Considering this, we conducted an analysis of all recorded occurrences of calcareous sponges to identify areas of endemism in the Western Atlantic Ocean (7°42' N to 45°12' W) and to investigate the potential biogeographic processes underlying these patterns. Additionally, we examined whether the areas of endemism identified along the Brazilian coastline fall under marine protected areas, to evaluate the extent of their conservation coverage.

Materials and Methods

Study Area and Records

The geographical boundaries of this study were defined in the Western Atlantic Ocean, with northernmost (32°18′N) and southernmost (59°27′S) covering three realms: Temperate North Atlantic, Western Tropical Atlantic, and Temperate South America. Species occurrence data were extracted from the World Porifera Database (WPD, https://www.marinespecies.org/porifera/), Global Biodiversity Information Facility (GBIF, https://www.gbif.org), Ocean Biodiversity Information System (OBIS, https://obis.org), and specialized literature. To prevent duplications bias, a meticulous visual check and manual editing was conducted to eliminate redundancies, resulting in the analysis of a total of 293 records. A total of 92 species (Table 1), including 3 exotic invasive species, are found in the region, but only 78 species were analyzed. The remaining species also occur outside the study area and were not included, as their natural distribution extends beyond the defined boundaries. Additionally, 32 records for the 3 exotic invasive species were mapped to check their proximity to areas of endemism and marine protection areas.

Geographical Interpolation of Endemism (GIE)

The GIE method is used to delimit areas of endemism by applying Kernel interpolation to estimate the congruence of species distribution within the study area. The approach involves using a Gaussian function on occurrence records to generate centroids for each species' distribution. After identifying the centroids, Kernel interpolation assigns density values to the congruence of these areas. The results are then visualized on a map, where densities reveal the significance of endemism areas (Oliveira, Brescovit and Santos, 2015). Areas of endemism were defined by the criterion of quantifying species occurrences, where the distance between occurrence points generates the distribution area. It was determined that two species must occur in the same area to establish an area of endemism (Crother & Murray, 2011). The GIE analysis was performed using the BioDinamica package (Oliveira et al., 2019) within the Dinamica EGO 7 software (https://csr.ufmg.br/dinamica/), v7.4.0, and for map elaboration the results were processed in QGIS (https://qgis.org), v3.26.1.

Marine Protected Areas (MPAs)

Brazilian MPAs were obtained using the 'Painel de Unidades de Conservação Brasileiras' platform from the Ministry of the Environment and Climate Change (https://cnuc.mma.gov.br/powerbi), while graphical representation of the units was obtained from the federal government's open data database (https://dados.gov.br/dados/conjuntos–dados/unidadesdeconservacao).

Table 1. Calcareous sponge species from the Western Atlantic Ocean, including marine ecoregion, depth in meters, and year of the record. Species not included in the analysis have an asterisk (*).

Species	References	Marine Ecoregion	Bathymetry	Year
Subclass Calcaronea				
*Leuconia joubini** (Topsent, 1907)	Burton, M. (1932)	Malvinas/Falklands	–	–
Amphoriscus ancora Van Soest, 2017	Van Soest (2017)	Guianan	35	1970
Amphoriscus pedunculatus Klautau, Cavalcanti & Borojevic, 2017	Klautau et al. (2017)	Southeastern Brazil	6 – 13	1988 2002 2008
Amphoriscus synapta (Schmidt in Haeckel, 1872)	Haeckel (1872)	Eastern Brazil	–	–
Amphoriscus testiparus (Haeckel, 1872)	Haeckel (1872)	Greater Antilles	–	–
Amphoriscus urna Haeckel, 1872	Haeckel (1872)	Southern Caribbean	–	–
Leucilla antillana Cóndor-Luján, Louzada, Hajdu & Klautau, 2018	Cóndor-Luján et al. (2018)	Southern Caribbean	8.6	2011

Species	References	Marine Ecoregion	Bathymetry	Year
Leucilla micropilosa Cóndor-Luján, Louzada, Hajdu & Klautau, 2018	Cóndor-Luján et al. (2018)	Southern Caribbean	9.9	2011
Leucilla sacculata (Carter, 1890)	Carter (1890)	Fernando de Noronha and Atoll das Rocas	–	–
*Leucilla uter** Poléjaeff, 1883	Borojevic & Peixinho (1976), Poléjaeff (1883)	Bermuda Northeastern Brazil Eastern Brazil	11 – 65	1965 1967 1968 1969
Paraleucilla incomposita Cavalcanti, Menegola & Lanna, 2014	Cavalcanti et al. (2014), Chagas & Cavalcanti (2017)	Eastern Brazil	1 – 3	2012
Paraleucilla oca Cavalcanti, Menegola & Lanna, 2014	Cavalcanti et al. (2014)	Eastern Brazil	1	2011
Paraleucilla perlucida Azevedo & Klautau, 2007	Azevedo & Klautau (2007), Cóndor-Luján et al. (2019)	Southeastern Brazil Sao Pedro and Sao Paulo Islands	1 – 3	2004 2005
Paraleucilla solangeae Cavalcanti, Menegola & Lanna, 2014	Cavalcanti et al. (2014)	Eastern Brazil	23	2008
Paraleucilla sphaerica Lanna, Cavalcanti, Cardoso, Muricy & Klautau, 2009	Lanna et al. (2009)	Northeastern Brazil	13 – 20	2004 2007
Grantia atlantica Ridley, 1881	Borojevic & Peixinho (1976), Ridley (1881)	Eastern Brazil	65 – 71	1965 1967 1968
*Grantia hirsuta** (Topsent, 1907)	Burton, M. (1932)	Malvinas/Falklands	2 - 79	1926 1927
Grantia kempfi Borojevic & Peixinho, 1976	Borojevic & Peixinho (1976), Van Soest (2017)	Guianan Amazonia Northeastern Brazil Eastern Brazil	19 – 73	1965 1966 1967 1968 1969 1970
Leucandra amorpha (Poléjaeff, 1883)	Poléjaeff (1883)	Bermuda	–	–
Leucandra barbata (Duchassaing & Michelotti, 1864)	Borojevic & Peixinho (1976), Duchassaing De Fonbressin & Michelotti (1864), Laubenfels (1936)	Floridian Northeastern Brazil Eastern Brazil	17 – 65	1932 1968
Leucandra caribea Cóndor-Luján, Louzada, Hajdu & Klautau, 2018	Cóndor-Luján et al. (2018)	Southern Caribbean	13.9	2011
Leucandra crassior (Ridley, 1881)	Ridley (1881)	Eastern Brazil	71	
Leucandra crustacea (Haeckel, 1872)	Haeckel (1872) Laubenfels (1950)	Southern Caribbean Bermuda	–	1947
Leucandra curva (Schuffner, 1877)	Schuffner (1877),	Eastern Caribbean	–	–

Species	References	Marine Ecoregion	Bathymetry	Year
*Leucandra hentschelii** Brøndsted, 1931	Borojevic & Peixinho, (1976), Van Soest (2017),	Northeastern Brazil Guianan	0.3 – 85	1970
Leucandra multiformis (Poléjaeff, 1883)	Poléjaeff (1883),	Bermuda	–	–
*Leucandra rudifera** (Poléjaeff, 1883)	Moraes et al. (2006), Muricy et al. (1991), Poléjaeff (1883)	Bermuda Eastern Brazil Trindade and Martin Vaz Islands	65	–
Leucandra serrata Azevedo & Klautau, 2007	Azevedo & Klautau, (2007), Fonseca et al. (2023), Ignacio et al. (2010), Lanna et al. (2009)	Northeastern Brazil Eastern Brazil Southeastern Brazil	1 – 3	2003 2004 2007 2013 2016 2017
Leucandra typica (Poléjaeff, 1883)	Poléjaeff (1883)	Bermuda	58	1873
Leucandrilla quadriradiata Cóndor-Luján, Louzada, Hajdu & Klautau, 2018	Cóndor-Luján et al. (2018)	Southern Caribbean	3 – 5	2011
Grantessa anisactina Borojevic & Peixinho, 1976	Borojevic & Peixinho (1976)	Northeastern Brazil	42	1965
Grantessa tumida Cóndor-Luján, Louzada, Hajdu & Klautau, 2018	Cóndor-Luján et al. (2018)	Southern Caribbean	3 – 6.3	2011
Sycettusa pelagica Ridley 1881	Ridley (1881)	Eastern Brazil	71	–
Vosmaeropsis complanatispinifera Cavalcanti, Bastos & Lanna, 2015	Cavalcanti et al. (2015)	Northeastern Brazil	65	–
*Vosmaeropsis levis** Hôzawa, 1940	Borojevic (1971), Hôzawa (1940)	Southeastern Brazil	–	–
Vosmaeropsis recruta Cavalcanti, Bastos & Lanna, 2015	Cavalcanti et al. (2015)	Eastern Brazil	–	–
*Leucosolenia discovery** Jenkin, 1908	Burton, M. (1932)	Malvinas/Falklands	2	1926
*Leucosolenia falklandica** Breitfuss, 1898	Breitfuss, L.L. (1898)	Malvinas/Falklands	–	–
Leucosolenia horrida (Schmidt in Haeckel, 1872)	Haeckel (1872)	Floridian Northern Gulf of Mexico	–	–
Leucosolenia salpinx Van Soest, 2017	Van Soest (2017)	Guianan	28 – 39	1970
Sycetta papillata Mattedi & Cavalcanti, 2024	Mattedi & Cavalcanti (2024)	Eastern Brazil	0.5 – 25	1995
Sycon acanthoxea (Little, 1963)	Little (1963)	Northern Gulf of Mexico		
*Sycon ampulla** (Haeckel, 1870)	Haeckel (1872)	Southern Caribbean Southeastern Brazil	–	–
Sycon avus Chagas & Cavalcanti, 2017	Fonseca et al. (2023)	Eastern Brazil	1	2016
Sycon barbadense (Schuffner, 1877)	Schuffner (1877)	Eastern Caribbean		
Sycon bellum Chagas & Cavalcanti, 2017	Chagas & Cavalcanti (2017)	Eastern Brazil		2014
Sycon brasiliense Borojevic, 1971	Borojevic (1971)	Southeastern Brazil		–

Species	References	Marine Ecoregion	Bathymetry	Year
Sycon conulosum Cóndor-Luján, Louzada, Hajdu & Klautau, 2018	Cóndor-Luján et al. (2018)	Southern Caribbean	3 – 5	2011
Sycon frustulosum Borojevic & Peixinho, 1976	Borojevic & Peixinho (1976)	Northeastern Brazil		–
*Sycon incrustans** Breitfuss, 1898	Burton, M. (1934)	Malvinas/Falklands	7	1902
Sycon magnapicale Cóndor-Luján, Louzada, Hajdu & Klautau, 2018	Cóndor-Luján et al. (2018)	Southern Caribbean	14.9	2011
Sycon pentactinale Rossi, Farina, Borojevic & Klautau, 2006	Rossi et al. (2006)	Southeastern Brazil	2	–
Subclass Calcinea				
Arturia alcatraziensis (Lanna, Rossi, Cavalcanti, Hajdu & Klautau, 2007)	Fontana et al. (2018), Lanna et al. (2007)	Southeastern Brazil Southern Caribbean	8 – 11	2002 2013
Arturia vansoesti Cóndor-Luján, Louzada, Hajdu & Klautau, 2018	Cóndor-Luján et al. (2018)	Southern Caribbean Eastern Caribbean	3 – 10	2011 2013
Borojevia aspina (Klautau, Solé-Cava & Borojevic, 1994)	Klautau et al. (1994)	Southeastern Brazil	2 – 10	1993
Borojevia brasiliensis (Solé-Cava, Klautau, Boury-Esnault, Borojevic & Thorpe, 1991)	Solé-Cava et al. (1991)	Southeastern Brazil	2 – 10	1989
Borojevia crystallina Fontana, Cóndor-Luján, Azevedo, Pérez & Klautau, 2018	Fontana et al. (2018)	Eastern Caribbean	12	2011
Borojevia tenuispinata Azevedo, Padua, Moraes, Rossi, Muricy & Klautau, 2017	Azevedo et al. (2017), Fonseca et al. (2023)	Sao Pedro and Sao Paulo Islands Eastern Brazil	1 – 17	2004 2011 2013
Borojevia trispinata Azevedo, Padua, Moraes, Rossi, Muricy & Klautau, 2017	Azevedo et al. (2017), Fonseca et al. (2023)	Sao Pedro and Sao Paulo Islands Eastern Brazil	1 – 10	2011 2013
Clathrina angraensis Azevedo & Klautau, 2007	Azevedo & Klautau (2007)	Southeastern Brazil	2	2003
*Clathrina aurea** Solé-Cava, Klautau, Boury-Esnault, Borojevic & Thorpe, 1991	Fontana et al. (2018), Pérez et al. (2017), Solé-Cava et al. (1991)	Southeastern Brazil Eastern Brazil Eastern Caribbean	1 – 18.8	1989 2008 2011 2013 2015
Clathrina curacaoensis Cóndor-Luján, Louzada, Hajdu & Klautau, 2018	Cóndor-Luján et al. (2018)	Southern Caribbean	3 – 10	2011
Clathrina cylindractina Klautau, Solé-Cava & Borojevic, 1994	Fontana et al. (2018), Klautau et al. (1994)	Eastern Brazil Eastern Caribbean	14	1989 2015
Clathrina delicata Fontana, Cóndor-Luján, Azevedo, Pérez & Klautau, 2018	Fontana et al. (2018), Muricy et al. (2024)	Eastern Caribbean Fernando de Noronha and Atoll das Rocas	11	2015 2022
Clathrina globulosa Cóndor-Luján, Louzada, Hajdu & Klautau, 2018	Cóndor-Luján et al. (2018)	Southern Caribbean	10	2011

Species	References	Marine Ecoregion	Bathymetry	Year
Clathrina hondurensis Klautau & Valentine, 2003	Cóndor-Luján et al. (2018), Klautau & Valentine (2003), Rützler et al. (2014)	Western Caribbean Southern Caribbean	1 – 25	1935 1975 2006 2009 2011 2012
Clathrina insularis Azevedo, Padua, Moraes, Rossi, Muricy & Klautau, 2017	Azevedo et al. (2017), Cóndor-Luján et al. (2018), Fontana et al. (2018), Muricy et al. (2024)	Fernando de Noronha and Atoll das Rocas Southern Caribbean Eastern Caribbean	14.9 – 15	2011 2013 2022
Clathrina lutea Azevedo, Padua, Moraes, Rossi, Muricy & Klautau, 2017	Azevedo et al. (2017), Cóndor-Luján et al. (2018), García-Hernández et al. (2019)	Southern Caribbean Eastern Brazil Fernando de Noronha and Atoll das Rocas Greater Antilles	3 – 10.9	1999 2005 2011 2013 2015
Clathrina mutabilis Azevedo, Padua, Moraes, Rossi, Muricy & Klautau, 2017	Azevedo et al. (2017), Fontana et al. (2018)	Fernando de Noronha and Atoll das Rocas Eastern Caribbean	6 – 18.8	2011 2013 2015
Clathrina robusta Fonseca, Cóndor-Luján & Cavalcanti, 2023	Fonseca et al. (2023)	Eastern Brazil	1 – 6	2006 2014 2016
Clathrina smaragda Lopes, Padua, Cóndor-Luján & Klautau, 2018	Lopes et al. (2018)	Floridian	10 – 15	2015
Clathrina soluta Fonseca, Cóndor-Luján & Cavalcanti, 2023	Fonseca et al. (2023)	Eastern Brazil	1 – 2	2013 2016 2017
Clathrina zelinhae Azevedo, Padua, Moraes, Rossi, Muricy & Klautau, 2017	Azevedo et al. (2017)	Fernando de Noronha and Atoll das Rocas	3	2011
Janusya trindadensis (Azevedo, Padua, Moraes, Rossi, Muricy & Klautau, 2017)	Azevedo et al. (2017)	Trindade and Martin Vaz Islands	13	2003
Nicola tetela (Borojevic & Peixinho, 1976)	Borojevic & Peixinho (1976), Cóndor-Luján & Klautau (2016)	Eastern Brazil Southern Caribbean	5.6 – 47	1968 2011
Ascandra alba Fonseca, Cóndor-Luján & Cavalcanti, 2023	Fonseca et al. (2023)	Eastern Brazil	6	2007
Neoernsta adunca (Fontana, Cóndor-Luján, Azevedo, Pérez & Klautau, 2018)	Fontana et al. (2018)	Eastern Caribbean	18.8	2015
Neoernsta citrea (Azevedo, Padua, Moraes, Rossi, Muricy & Klautau, 2017)	Azevedo et al. (2017)	Fernando de Noronha and Atoll das Rocas	0.5 – 5	1999 2011

Species	References	Marine Ecoregion	Bathymetry	Year
Neoernsta multispiculata (Azevedo, Padua, Moraes, Rossi, Muricy & Klautau, 2017)	Azevedo et al. (2017)	Fernando de Noronha and Atoll das Rocas	3	2011
Neoernsta quadriradiata (Klautau & Borojević, 2001)	Klautau & Borojevic (2001)	Eastern Brazil	–	1999
Neoernsta rocasensis (Azevedo, Padua, Moraes, Rossi, Muricy& Klautau, 2017)	Azevedo et al. (2017)	Fernando de Noronha and Atoll das Rocas	0.5 – 2	2011
Neoernsta sanctipauli (Azevedo, Padua, Moraes, Rossi, Muricy & Klautau, 2017)	Azevedo et al. (2017)	Sao Pedro and Sao Paulo Islands	10	2011
Neoernsta solaris (Azevedo, Padua, Moraes, Rossi, Muricy & Klautau, 2017)	Azevedo & Klautau (2007)	Trindade and Martin Vaz Islands	0.1	2003
Neoernsta tetractina (Klautau & Borojević, 2001)	Klautau & Borojevic (2001)	Southeastern Brazil	–	1999
Ascaltis agassizii Haeckel, 1872	Haeckel (1872)	Floridian Northern Gulf of Mexico	–	–
Bidderia bicolora Lopes, Cóndor-Luján, Azevedo, Pérez & Klautau, 2018	Lopes et al. (2018)	Eastern Caribbean	13 – 20	2015
Leucascus albus Cavalcanti, Rapp & Klautau, 2013	Cavalcanti et al. (2013)	Eastern Brazil	12	1987 1988
Leucascus roseus Lanna, Rossi, Cavalcanti, Hajdu & Klautau, 2007	Cavalcanti et al. (2013) Lanna et al. (2007, 2009)	Southeastern Brazil Eastern Brazil Northeastern Brazil	8 – 14	1996 1997 2002 2007
*Leucaltis clathria** Haeckel, 1872	Borojevic & Peixinho (1976), Fontana et al. (2018), Haeckel (1872) Van Soest (2017)	Floridian Amazonia Fernando de Noronha and Atoll das Rocas Northeastern Brazil Eastern Brazil Guianan	21 – 93	1961 1965 1967 1968 1970 2'015
Leucaltis corticata (Haeckel, 1872)	Haeckel (1872)	Eastern Caribbean Greater Antilles	–	–

Species	References	Marine Ecoregion	Bathymetry	Year
Leucetta floridana (Haeckel, 1872)	Haeckel (1872), Laubenfels, (1950), Lehnert & van Soest (1998), Moraes et al. (2006), Valderrama et al. (2009), Lanna et al. (2009), Rützler et al. (2014), Pérez et al. (2017)	Floridian Bermuda Greater Antilles Fernando de Noronha and Atoll das Rocas Northeastern Brazil Eastern Brazil Southwestern Caribbean Southern Caribbean	9 – 101	1947 1979 1993 1995 1997 2000 2003 2004 2007 2013
Leucetta imberbis (Duchassaing & Michelotti, 1864)	Duchassaing De Fonbressin & Michelotti (1864), Lehnert & Van Soest (1998)	Eastern Caribbean Greater Antilles	10 – 25	1993
Leucetta potiguar Lanna, Cavalcanti, Cardoso, Muricy & Klautau, 2009	Lanna et al. (2009)	Northeastern Brazil	10	2007
*Rowella haeckeliana** (Poléjaeff, 1883)	Burton (1932)	Malvinas/Falklands	73 - 74	1927
*Rowella simplicissima** (Burton, 1932)	Burton (1932)	Malvinas/Falklands	74	1927

Results

A total of 17 areas of endemism (AoEs) were identified (Figure 1), with a predominance of 7 AoEs in the class interval (0–50 km), which includes 32 species (Table 2). In the second interval (51–250 km), a single AoE was detected along the southeastern coast of Brazil (Figure 1C), containing 3 species. The third interval (251–700 km) revealed 4 AoEs, showing connections between oceanic islands in both the Caribbean and Brazil (Figure 1D), with a total of 17 species. In the fourth-class interval (701–1200 km), only 1 AoE was observed along the northeastern coast of Brazil (Figure 1E), with 6 species. In the fifth-class interval (1201–1800 km), 2 AoEs were identified (Figure 1F), with patterns like those in the third-class interval (251–700 km), were species found over long distances between oceanic islands in the Caribbean. Additionally, the first interaction between the northeastern and southeastern regions of the Brazilian coast was observed, with a total of 9 species. In the sixth-class interval (1801–2500 km), a single AoE was recorded (Figure 1G), marking the first connection between the Caribbean and northeastern Brazil, with 10 species. Finally, species with broad geographic distributions formed deeper connections between southeastern Brazil and the Caribbean, with 9 species (Figure 1H).

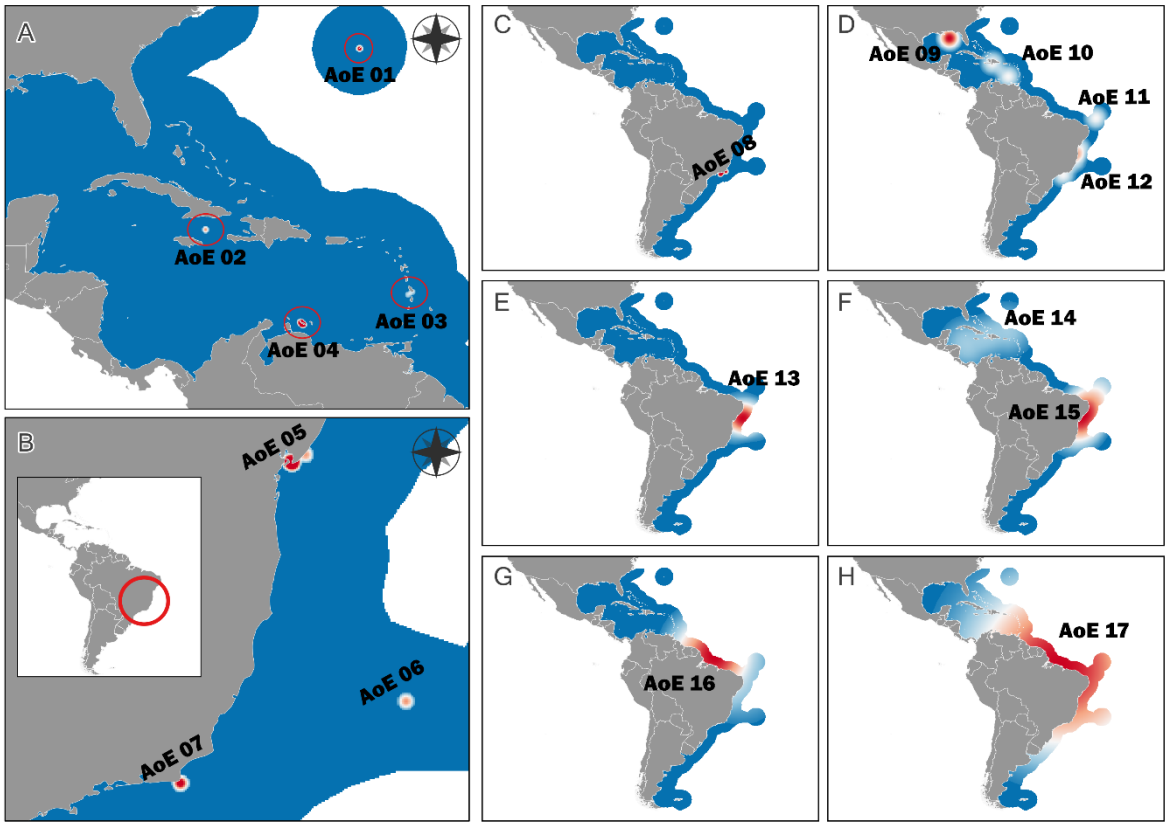


Figure 1. Areas of endemism (AoEs) by class ranges. A – 4 AoEs in the Caribbean (0–50 km); 1B = 3 AoEs along the Brazilian coast (0–50 km); C – 1 AoE in southeastern Brazil (51–250 km); D – 2 AoEs in the Caribbean and 2 AoEs along the Brazilian coast (251–700 km); E – 1 AoE in northeastern Brazil (701–1200 km); F – 1 AoE connecting Caribbean islands and 1 AoE connecting eastern Brazil (1201–1800 km); G – 1 AoE at the Amazon estuary, representing the first Brazil-Caribbean connection (1801–2500 km); H – Broad southeastern Brazil-Caribbean connections (2500+ km).

The clustering results of the species on the maps were tabulated, and the AoEs were named in order of appearance, starting from the Caribbean and extending to Brazil. Table 2 presents the relationship between the AoEs and the species that comprise them. It is important to note that, despite the overlap of different class intervals, the species were listed based on their first appearance in the results.

Table 2. Species composition of Areas of Endemism (AoE), listed in order of appearance from the Caribbean to Brazil.

Classes	Species
Class 1	0 – 50 km
AoE 1	*Leucandra amorpha*, *Leucandra multiformis*, *Leucandra typica*
AoE 2	*Amphoriscus testiparus*, *Leucaltis corticata*
AoE 3	*Borojevia crystallina*, *Neoernsta adunca*
AoE 4	*Clathrina curacaoensis*, *Clathrina globulosa*, *Grantessa tumida*, *Leucandra caribea*, *Leucandrilla quadriradiata*, *Leucilla antillana*, *Leucilla micropilosa*, *Sycon conulosum*, *Sycon magnapicale*
AoE 5	*Ascandra alba*, *Clathrina robusta*, *Clathrina soluta*, *Sycetta papillata*, *Sycon avus, Sycon bellum*, *Paraleucilla oca*, *Paraleucilla solangeae*
AoE 6	*Leucandra crassior*, *Sycettusa pelagica*
AoE 7	*Leucascus albus*, *Neoernsta quadriradiata*, *Vosmaeropsis recruta*
Class 2	50 – 250 km
AoE 8	*Borojevia aspina, Borojevia brasiliensis*, *Clathrina angraensis*, *Sycon brasiliense*
Class 3	250 – 700 km
AoE 9	*Ascaltis agassizii*, *Leucosolenia horrida*, *Sycon acanthoxea*
AoE 10	*Amphoriscus urna*, *Arturia vansoesti*
AoE 11	*Clathrina zelinhae*, *Leucetta potiguar*, *Leucilla sacculata*, *Neoernsta sanctipauli*
AoE 12	*Amphoriscus pedunculatus*, *Amphoriscus synapta*, *Neoernsta tetractina*, *Paraleucilla incomposita*, *Sycon pentactinale*
Class 4	700 – 1200 km
AoE 13	*Grantessa anisactina*, *Leucandra crassior*, *Neoernsta citrea*, *Neoernsta rocasensis*, *Sycettusa pelagica*, *Sycon frustulosum*
Class 5	1200 – 1800 km
AoE 14	*Bidderia bicolora*, *Clathrina hondurensis*, *Clathrina smaragda*, *Leucandra curva*, *Leucetta imberbis*, *Sycon barbadense*
AoE 15	*Grantia atlantica*, *Neoernsta multispiculata*
Class 6	1800 – 2500 km
AoE 16	*Amphoriscus ancora*, *Clathrina cylindractina*, *Clathrina delicata*, *Clathrina mutabilis*, *Grantia kempfi*, *Janusya trindadensis*, *Leucosolenia salpinx*, *Neoernsta solaris*, *Vosmaeropsis complanatispinifera*
Class 7	> 2500 km
AoE 17	*Arturia alcatraziensis, Borojevia tenuispinata*, *Borojevia trispinata, Clathrina lutea*, *Clathrina insularis*, *Leucandra barbata*, *Leucascus roseus*, *Nicola tetela*, *Paraleucilla perlucida*

While individual class intervals reveal regional groupings, the consensus map (Figure 2A) overlays all identified AoEs. Our results show a higher intensity of clustering along the Brazilian coast compared to the Caribbean. Additionally, a map overlaying Marine Protected Areas and AoEs (Figure 2B) was created for the Brazilian coast to visualize the extent of protection in these areas.

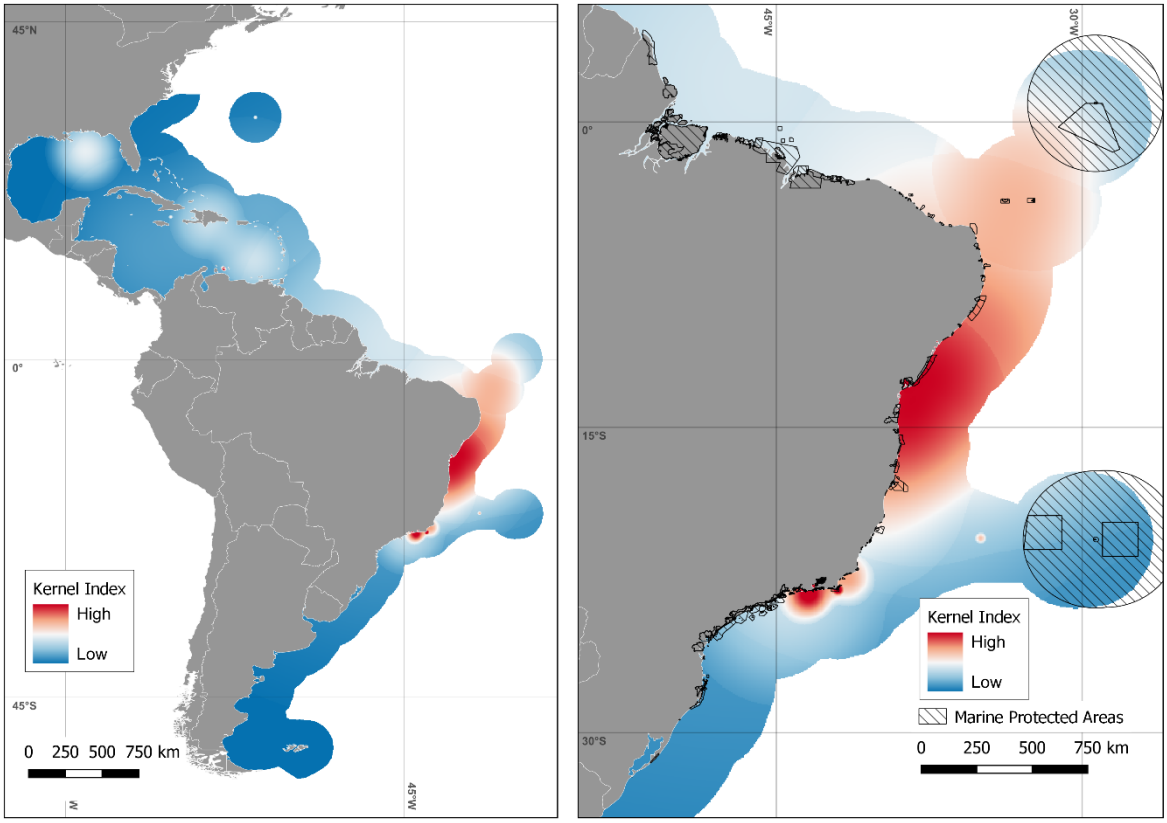


Figure 2. Map of areas of endemism in the Western Atlantic identified using GIE. A – Consensus across seven class intervals reveals a high concentration of AoEs along the Brazilian coastline. B – Detail of the Brazilian coastline with 220 marine protected areas.

In addition to the local biodiversity, three exotic invasive species have been documented along the Brazilian coast: *Heteropia glomerosa*, *Sycettusa hastifera*, and *Paraleucilla magna*. Together, these species account for 32 occurrences over a range exceeding 2,800 km (Figure 3). The shown locations are near or within conservation units, including: Ceará State, near the Dunas do Litoral Oeste and Lagamar do Cauípe MPAs (Figure 3A); Alagoas State, near the Costa dos Corais MPA (Figure 3B); Bahia State, within the Baía de Todos os Santos MPA (Figure 3C); Rio de Janeiro State, in the Reserva Extrativista Marinha do Arraial do Cabo MPA (Figure 3D); Restinga da Marambaia, within the Orla Marítima da Baía de Sepetiba MPA (Figure 3E); Baía da Ilha Grande, within the Estação Ecológica de Tamoios MPA (Figure 3F); São Paulo State, in the Alcatrazes Archipelago MPA (Figure 3G); and Santa Catarina State, in the Parque Estadual da Serra do Tabuleiro MPA (Figure 3H).

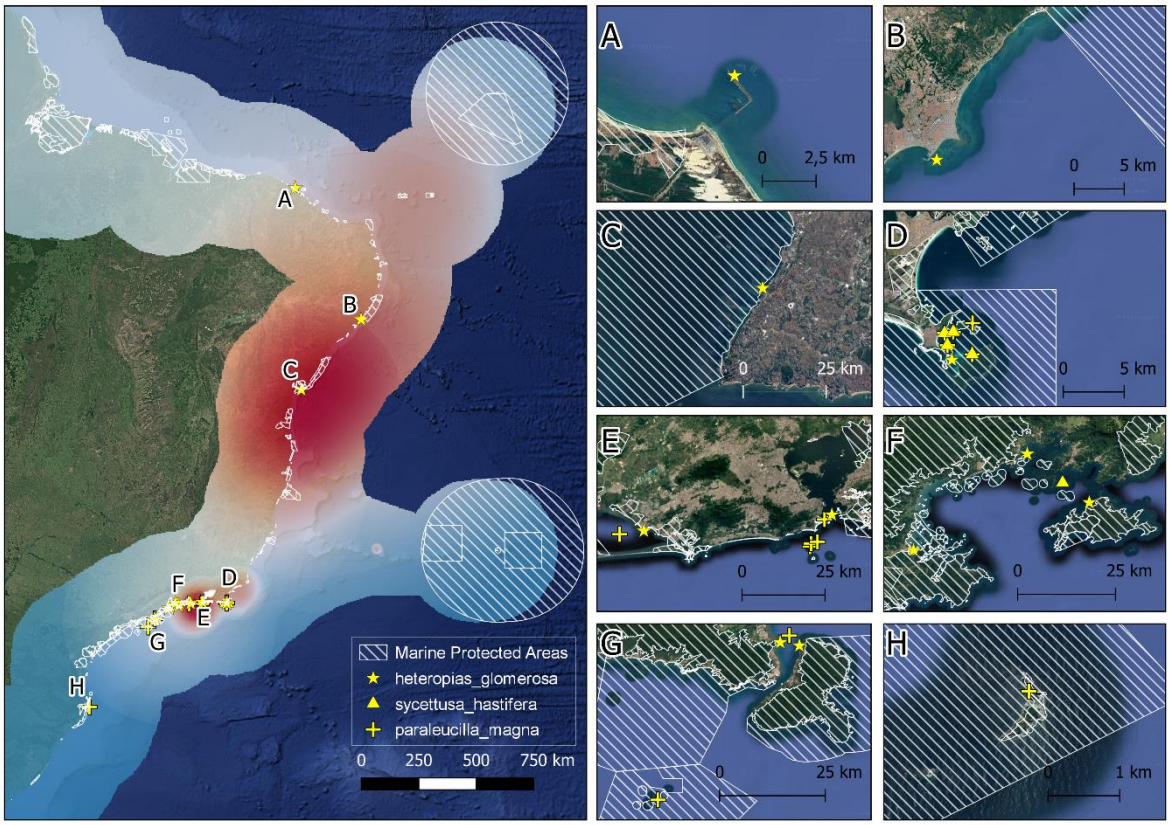


Figure 3. Map representing the Brazilian coastline with its Marine Protected Areas (MPAs) and areas of endemism, overlaid with the distribution of three invasive species: *Heteropia glomerosa* (star), *Paraleucilla magna* (triangle), and *Sycettusa hastifera* (cross). The visible regions include A – São Gonçalo do Amarante, Northeastern Brazil; B – Maceió, Eastern Brazil; C – Todos os Santos Bay, Eastern Brazil; D – Arraial do Cabo, Eastern Brazil; E – Sepetiba Bay (left) and Cagarras Islands Archipelago (right), Southeastern Brazil; F – Paraty (left) and Ilha Grande Bay (right), Southeastern Brazil; G – São Sebastião Channel (upper) and Alcatrazes Archipelago (lower), Southeastern Brazil; and H – Moleques do Sul Islands, Southeastern Brazil.

Discussion

Our results highlight how uneven sampling effort shapes regional biodiversity understanding. The most striking finding is the contrast between the two main regions: the Caribbean and Brazil. The Brazilian coastline contains significantly more Areas of Endemism (AoEs), distributed across the Southeastern, Eastern, and Northeastern coasts (Figure 2). As mentioned previously, taxonomic knowledge and funding have helped the discovery of calcareous sponges in the country. As such, when compared to the Caribbeans, the AoEs are less evident and sparse. While the Caribbean is recognized as one of the world's major marine biodiversity hotspots (Yasuhara et al., 2022), the biogeography of calcareous sponges reveals a pattern that differs from what is typically observed in this region. In contrast, several marine taxa – such as reef fish (Pinheiro et al., 2018), bivalves (Jablonski

et al., 2013), and sea anemones (Stampar et al., 2012; Targino and Gomes, 2020) – exhibit a pattern of diversity radiation in the Caribbean, with some species dispersing southward (Briggs and Bowen, 2013). However, in the case of calcareous sponges, our understanding of the region remains limited due to various factors.

A major limitation in studying calcareous sponges stems from their biology. These organisms predominantly inhabit photophobic and cryptic environments (Lopes, Pérez & Klautau, 2024; Muricy et al., 2024), making them easily overlooked without specialized training. Thus, capacity-building initiatives to train local specialists are critical for advancing taxonomic knowledge. Successful examples, such as the training of spongiologists on Martinique Island (Pérez et al., 2017), demonstrate how regional efforts can expand species inventories and accelerate new discoveries. However, while taxonomic expertise is vital, it is not the sole factor determining our ability to document biodiversity. Political and economic conditions across different countries in the region also play a major role in shaping scientific progress (Cavalcante, Negacz and Pattberg, 2025). Challenges beyond scientific development negatively impact the ability to understand regional biodiversity (Barber et al., 2014).

Despite the limitations, this work aims to understand potential patterns for the region. This is the first paper to employ the GIE technique to investigate patterns of areas of endemism in the marine environment, and therefore, several considerations merit special attention. First, bathymetry was not considered in the analysis; calcareous sponges are often classified as shallow-water organisms (0–100 m), and all occurrences for the group were included in the analysis. This does not imply that bathymetry cannot influence the formation and delimitation of AoEs, but its influence could not be quantified in this study. Second, unlike terrestrial or inland aquatic environments, where geographic barriers are evident, other forces govern species distribution in the marine realm, and these cannot be measured by the method used. One of the objectives in employing this methodology is its efficiency in dealing with complex areas, as kernel interpolation does not rely on grid cells (Oliveira, Brescovit and Santos, 2015), thereby fuzzy edges of the coastline are included in the process.

Class intervals represent the primary parameter that must be adjusted by researchers while working with GIE. In this case, we considered the known distances for some species in the region to define our class intervals (for example, *Clathrina lutea* is found in the Greater Antilles, Southern Caribbean, Fernando de Noronha and Atol das Rocas, and Eastern Brazil, with a maximum distance of 5,000 km). Most class intervals reveal a distribution pattern influenced by sampling efforts (Figure 1A–F); intervals 1 (0–50 km), 2 (50–250 km), 3 (250–

700 km), 4 (700–1200 km), and 5 (1200–1800 km) together include 61 species with isolated or very closely spaced occurrences, which interfere our understanding of the natural distribution of these sponges. Moreover, some AoEs within these intervals (AoE 11, AoE 13, AoE 14, and AoE 15) are clusters formed by species from oceanic islands; in these cases, the clustering resulted in an AoE containing species that had not been previously grouped, an effect produced by single or closely located occurrences. However, some AoEs in these intervals may reflect an environmental preference of these species; for instance, the species in AoE 7 (*Leucascus albus*, *Neoernsta quadriradiata*, and *Vosmaeropsis recruta*), located in Arraial do Cabo, occur in an area influenced by oceanic upwelling, resulting in colder waters compared to nearby locations.

Regarding the larger intervals (Figure 1 G–H), classes 6 (1800–2500 km) and 7 (<2500 km) group 17 species of calcareous sponges that exhibit one of three patterns: they may be species with few occurrences distributed over a wide coastal range (e.g., *Arturia alcatraziensis, Clathrina delicata,* and *Neoernsta solaris*); species with numerous occurrences throughout the region (e.g., *Clathrina lutea, Grantia kempfi,* and *Leucandra barbata*); or species represented by only a single occurrence within this cluster (e.g., *Janusya trindadensis*). These two classes form regional connections between the Caribbean and Brazil. Notably, calcareous sponges supposedly have a limited dispersal capacity during the larval phase; however, species such as *Clathrina aurea* Solé-Cava, Klautau, Boury-Esnault, Borojevic & Thorpe, 1991 display a distribution encompassing populations in the Caribbean, Brazil, and Peru, with the Caribbean and Brazilian populations being more closely related and sharing several common haplotypes (Cóndor-Luján et al., 2021). In this way, these sponges manage to persist over large distances despite the assumption of short larvae dispersal phase. Another important factor is the environmental barrier separating these regions, the Amazon-Orinoco plume. Spanning more than 2300 km, the discharge of 300,000 m^3/s freshwater and sediments creates a salinity gradient that could prevent gene flow between Caribbean and Brazil Provinces. Now the only organisms addressing the influence of this barrier with a chrono-phylogenetic approach are reef fishes and sardines (Araujo et al., 2022; Ferreira-Araújo et al., 2024). It remains unclear whether this salinity gradient affects the genetic flow of calcareous sponges, with two key hypotheses: either these species are resistant to the low salinity levels, or populations exist beyond the influence of this gradient, acting as a link bypassing the barrier above. The latter have a strong possibility since sponges are one of the animals that colonize the deep outer shelf (Collette and Rützler, 1977; Moura et al., 2016), forming The Great Amazon Reef System (GARS).

Although they are often considered shallow-water animals (0–100 m), there are records of calcareous sponges in abyssal and bathyal depths (Rapp, Janussen and Tendal, 2011; Rapp, 2013; Rapp et al., 2013). Therefore, questions regarding the influence of environmental factors are crucial for a better understanding of the distribution of these animals. Currently, the main sampling method is scuba diving, with many species recorded at depths ranging from 0.5 to 20 m. Despite the efficiency of scuba diving, the predomination of one method can bias the apparent natural distribution of these sponges, as deeper-water populations may exist but are not reachable. In addition, gene flow at shallow depths is known as some species have connections in distant locations (e.g. *Clathrina aurea*), on it is also essential to conduct surveys for deep-water sponges and to assess the possibility of gene flow between deep and shallow-water populations.

In terms of marine protected areas, the Brazilian coast features 220 conservation units, covering a total area of more than 960,000 km². These conservation units serve various purposes, with the majority designated for sustainable use – typically mapped into zones for fishing activities and areas where no activities are permitted. Furthermore, not all units have a management plan or management council, with only 93 MPAs having a management plan and 133 MPAs having a management council. Although the preservation of calcareous sponges is not specifically reinforced by management plans, the protection afforded to various groups of marine invertebrates in these areas is important for the conservation of the environment. In our analysis, we contrasted Brazil's MPAs with the AoEs generated by the GIE, and we also search the literature for all occurrences of exotic calcareous sponges to verify their presence in MPAs (Figure 3). Although there are no studies on the impacts of these exotic invasive species, monitoring them is an important step towards understanding their life cycle and interactions in the environment. As our map shows, all occurrences of exotic invasive calcareous sponges are near MPAs (Figure 3), also most of them are inside AoEs in Eastern Brazil and Southeastern Brazil.

Conclusion

The Neotropical region has achieved significant progress in our understanding of calcareous sponge fauna since the first comprehensive picture of the global biodiversity of Porifera by Van Soest et al. (2012). However, the constant discovery of new species is proof that the region is greatly underestimated and will soon have to undergo more extensive biogeographical analyses. Enhancing our knowledge of the natural extent of populations of species already described remains a challenge and a key factor in improving our

understanding of the distribution of calcareous sponges in the region. In the lightly better sampled areas, the presence of active research groups in Southeastern and Eastern Brazil is crucial for a more robust comprehension of local biodiversity. Moreover, mapping these AoEs facilitates the identification of regions that may be targeted for future sampling. Regarding local biodiversity conservation, the areas of endemism in Brazil are located within several MPAs, and thus, efforts to preserve these environments should be sustained, along with increased attention to species recognized as exotic within these conservation units.

References

Araujo, G. S., Rocha, L. A., Lastrucci, N. S., Luiz, O. J., Di Dario, F., & Floeter, S. R. (2022). The Amazon-Orinoco Barrier as a driver of reef-fish speciation in the Western Atlantic through time. *Journal of Biogeography*, *49*(8), 1407–1419. https://doi.org/10.1111/jbi.14398

Azevedo, F., & Klautau, M. (2007). Calcareous sponges (Porifera, Calcarea) from Ilha Grande Bay, Brazil, with descriptions of three new species. *Zootaxa*, *1402*(1). https://doi.org/10.11646/zootaxa.1402.1.1

Azevedo, F., Padua, A., Moraes, F., Rossi, A., Muricy, G., & Klautau, M. (2017). Taxonomy and phylogeny of calcareous sponges (Porifera: Calcarea: Calcinea) from Brazilian mid-shelf and oceanic islands. *Zootaxa*, *4311*(3), 301. https://doi.org/10.11646/zootaxa.4311.3.1

Barber, P. H., Ablan-Lagman, M. C. A., Ambariyanto, Berlinck, R. G., Cahyani, D., Crandall, E. D., Ravago-Gotanco, R., Juinio-Meñez, M. A., Mahardika, I. N., Shanker, K., Starger, C. J., Toha, A. H. A., Anggoro, A. W., & Willette, D. A. (2014). Advancing biodiversity research in developing countries: The need for changing paradigms. *Bulletin of Marine Science*, *90*(1), 187–210. https://doi.org/10.5343/bms.2012.1108

Borojecic, R., & Peixinho, S. (1976). Ésponges calcaires du nord-est du Brésil. *Bulletin Du Muséum National d'Histoire Naturelle*, *Zoologie*(279), 987–1036.

Borojevic, R. (1971). Eponges calcaires des côtes du Sud-Est du Brésil, épibiontes sur Laminaria brasiliensis et Sargassum cymosum. *Revista Brasileira de Biologia*, *31*, 525–530.

Briggs, J. C., & Bowen, B. W. (2013). Marine shelf habitat: Biogeography and evolution. *Journal of Biogeography*, *40*(6), 1023–1035. https://doi.org/10.1111/jbi.12082

Burton, M. (1932). Sponges. *Discovery Reports*. https://www.marinespecies.org/porifera/porifera.php?p=sourcedetails&id=7275

Carter, H. J. (1890). Porifera. In: Ridley, H.N.(ed.) Notes on the Zoology of Fernando do Noronha. *Journal of the Linnean Society*, *20*, 564–569.

Cavalcante, A. F. M., Negacz, K., & Pattberg, P. (2025). Biodiversity governance in Latin America: Analyzing the role and relevance of non-state and sub-national cooperative initiatives. *Biological Conservation*, *305*, 111053. https://doi.org/10.1016/j.biocon.2025.111053

Cavalcanti, F. F., Bastos, N., & Lanna, E. (2015). Two new species of the genus Vosmaeropsis Dendy, 1892 (Porifera, Calcarea), with comments on the distribution of V. sericata (Ridley, 1881) along the Southwestern Atlantic Ocean. *Zootaxa*, *3956*(4). https://doi.org/10.11646/zootaxa.3956.4.2

Cavalcanti, F. F., Menegola, C., & Lanna, E. (2014). Three new species of the genus Paraleucilla Dendy, 1892 (Porifera, Calcarea) from the coast of Bahia State, Northeastern Brazil. *Zootaxa*, *3764*(5), 537. https://doi.org/10.11646/zootaxa.3764.5.3

Chagas, C., & Cavalcanti, F. F. (2017). Taxonomy of calcareous sponges (Porifera, Calcarea) sampled on artificial substrates of a recreational marina in the Tropical Northeastern Brazilian coast. *Zootaxa*, *4363*(2). https://doi.org/10.11646/zootaxa.4363.2.2

Collette, B. B., & Rützler, K. (1977). *Reef fishes over sponge bottoms off the mouth of the Amazon River*. Proceedings, Third International Coral Reef Symposium, Miami, Florida: University of Miami.

Cóndor-Luján, B., Azevedo, F., Hajdu, E., Hooker, Y., Willenz, P., & Klautau, M. (2019). Tropical Eastern Pacific Amphoriscidae Dendy, 1892 (Porifera: Calcarea: Calcaronea: Leucosolenida) from the Peruvian coast. *Marine Biodiversity*, *49*(4), 1813–1830. https://doi.org/10.1007/s12526-019-00946-y

Cóndor-Luján, B., & Klautau, M. (2016). Nicola gen. nov. with redescription of Nicola tetela (Borojevic & Peixinho, 1976) (Porifera: Calcarea: Calcinea: Clathrinida). *Zootaxa*, *4103*(3), 230. https://doi.org/10.11646/zootaxa.4103.3.2

Cóndor-Luján, B., Leocorny, P., Padua, A., Azevedo, F., Seixas, V. C., Hooker, Y., Hajdu, E., Willenz, P., Pérez, T., & Klautau, M. (2021). Evolutionary history of the calcareous sponge Clathrina aurea: Genetic connectivity in the Western Atlantic and intriguing occurrence in the Eastern Pacific. *Marine Biology*, *168*(8), 127. https://doi.org/10.1007/s00227-021-03934-8

Cóndor-Luján, B., Louzada, T., Hajdu, E., & Klautau, M. (2018). Morphological and molecular taxonomy of calcareous sponges (Porifera: Calcarea) from Curaçao, Caribbean Sea. *Zoological Journal of the Linnean Society*, *183*(3), 459–525. https://doi.org/10.1093/zoolinnean/zlx082

Crother, B. I., & Murray, C. M. (2011). Ontology of areas of endemism: Ontology of areas of endemism. *Journal of Biogeography*, *38*(6), 1009–1015. https://doi.org/10.1111/j.1365-2699.2011.02483.x

de Voogd, N., Alvarez, B., Boury-Esnault, N., Cárdenas, P., Díaz, M.-C., Dohrmann, M., Downey, R., Goodwin, C., Hajdu, E., Hooper, J., Kelly, M., Klautau, M., Lim, S.-C., Manconi, R., Morrow, C., Pinheiro, U., Pisera, A., Ríos, P., Rützler, K., … Xavier, J. (2025).

World Porifera Database. Accessed at https://www.marinespecies.org/porifera on 2025-04-04. https://doi.org/10.14284/359

Duchassaing De Fonbressin, P., & Michelotti, G. (1864). *Spongiaires de la mer Caraïbe, par P. Duchassaing de Fonbressin et Giovanni Michelotti.* Les héritiers Loosjes,. https://doi.org/10.5962/bhl.title.11943

Ferreira-Araújo, T., Hollanda-Carvalho, P., Di Dario, F., Mendes, L. D. F., Oliveira, C., Gasparini, J. L., Rotundo, M. M., Macieira, R. M., & Lima, S. M. Q. (2024). Different roles of the Amazon-Orinoco barrier on the genetic structure of two sardine genera from the Western Atlantic Ocean. *Hydrobiologia*, *851*(10), 2429–2445. https://doi.org/10.1007/s10750-023-05468-0

Fonseca, E. S. M., Cóndor-Luján, B., & Cavalcanti, F. F. (2023). Calcareous sponges (Porifera, Calcarea) from the Northeastern Brazilian coast: Integrative taxonomy and support for the creation of a marine park. *Marine Biodiversity*, *53*(5), 67. https://doi.org/10.1007/s12526-023-01364-x

García-Hernández, J. E., Hammerman, N. M., Cruz-Motta, J. J., & Schizas, N. V. (2019). Associated organisms inhabiting the calcareous sponge Clathrina lutea in La Parguera, Puerto Rico. *Caribbean Journal of Science*, *49*(2–3), 239. https://doi.org/10.18475/cjos.v49i2.a12

Haeckel, E. (1872). Die Kalkschwämme. Eine Monographie in zwei Bänden Text und einem Atlas mit 60 Tafeln Abbildungen. *G. Reimer:Berlin*. https://www.marinespecies.org/porifera/porifera.php?p=sourcedetails&id=7557

Hôzawa, S. (1940). Reports on the calcareous sponges obtained by the Zoological Institute and Museum of Hamburg. *Science Reports of the Tôhoku Imperial University*, *15*, 131–163.

Ignacio, B. L., Julio, L. M., Junqueira, A. O. R., & Ferreira-Silva, M. A. G. (2010). Bioinvasion in a Brazilian Bay: Filling Gaps in the Knowledge of Southwestern Atlantic Biota. *PLoS ONE*, *5*(9), e13065. https://doi.org/10.1371/journal.pone.0013065

Jablonski, D., Belanger, C. L., Berke, S. K., Huang, S., Krug, A. Z., Roy, K., Tomasovych, A., & Valentine, J. W. (2013). Out of the tropics, but how? Fossils, bridge species, and thermal ranges in the dynamics of the marine latitudinal diversity gradient. *Proceedings of the National Academy of Sciences*, *110*(26), 10487–10494. https://doi.org/10.1073/pnas.1308997110

Klautau, M., & Borojecic, R. (2001). Sponges of the genus Clathrina Gray, 1867 from Arraial do Cabo, Brazil. *Zoosystema*, *3*, 395–410.

Klautau, M., Cavalcanti, F. F., & Borojevic, R. (2017). The new sponge species Amphoriscus pedunculatus (Porifera, Calcarea). *Zootaxa*, *4341*(1), 105. https://doi.org/10.11646/zootaxa.4341.1.9

Klautau, M., Solé-Cava, A. M., & Borojevic, R. (1994). Biochemical systematics of sibling sympatric species of Clathrina (Porifera: Calcarea). *Biochemical Systematics and Ecology*, *22*(4), 367–375. https://doi.org/10.1016/0305-1978(94)90027-2

Klautau, M., & Valentine, C. (2003). Revision of the genus Clathrina (Porifera, Calcarea). *Zoological Journal of the Linnean Society*, *139*(1), 1–62. https://doi.org/10.1046/j.0024-4082.2003.00063.x

Lanna, E., Cavalcanti, F. F., Cardoso, L., Muricy, G., & Klautau, M. (2009). Taxonomy of calcareous sponges (Porifera, Calcarea) from Potiguar Basin, NE Brazil. *Zootaxa*, *1973*(1), 1–27. https://doi.org/10.11646/zootaxa.1973.1.1

Lanna, E., Rossi, A. L., Cavalcanti, F. F., Hajdu, E., & Klautau, M. (2007). Calcareous sponges from São Paulo State, Brazil (Porifera: Calcarea: Calcinea) with the description of two new species. *Journal of the Marine Biological Association of the United Kingdom*, *87*(6), 1553–1561. https://doi.org/10.1017/S0025315407056871

Laubenfels, M. W. de. (1936). *A discussion of the sponge fauna of the Dry Tortugas in particular and the West Indies in general, with material for a revision of the families and orders of the Porifera* (Vol. 30). Carnegie Institution of Washington.

Lehnert, H., & van Soest, R. W. M. (1998). Shallow water sponges of Jamaica. *Beaufortia*, *48*(5), 71–103.

Little, F. J. Jr. (1963). The sponge fauna of the St. George's Sound, Apalache Bay, and Panama City Regions of the Florida Gulf Coast. *Tulane Studies in Zoology 11(2)*, 31–71.

Lopes, M. V., Cóndor-Luján, B., Azevedo, F., Pérez, T., & Klautau, M. (2018). A new genus of calcareous sponge discovered in the Caribbean Sea: Bidderia gen. nov. (Porifera, Calcarea, Calcinea). *Zootaxa*, *4526*(1). https://doi.org/10.11646/zootaxa.4526.1.4

Lopes, M. V., Padua, A., Azevedo, F., & Klautau, M. (2025). Integrative taxonomy of Calcarea (Porifera) from Espírito Santo, Eastern Brazil. *Zootaxa*, *5618*(2), 151–205. https://doi.org/10.11646/zootaxa.5618.2.1

Lopes, M. V., Padua, A., Cóndor-Luján, B., & Klautau, M. (2018). Calcareous sponges (Porifera, Calcarea) from Florida: New species, new records and biogeographical affinities. *Zootaxa*, *4526*(2), 127. https://doi.org/10.11646/zootaxa.4526.2.2

Lopes, M. V., Pérez, T., & Klautau, M. (2024). Cave-dwelling calcareous sponges (Porifera: Calcarea) from the Marquesas Islands, French Polynesia. *Zoological Journal of the Linnean Society*, *202*(3), zlae138. https://doi.org/10.1093/zoolinnean/zlae138

Maldonado, M. (2006). The ecology of the sponge larva. *Canadian Journal of Zoology*, *84*(2), 175–194. https://doi.org/10.1139/z05-177

Manuel, M., Borchiellini, C., Alivon, E., Le Parco, Y., Vacelet, J., & Boury-Esnault, N. (2003). Phylogeny and Evolution of Calcareous Sponges: Monophyly of Calcinea and Calcaronea, High Level of Morphological Homoplasy, and the Primitive Nature of Axial Symmetry. *Systematic Biology*, *52*(3), 311–333. https://doi.org/10.1080/10635150390196966

Mattedi, A., & Cavalcanti, F. F. (2024). Sycetta papillata sp. nov. (Porifera, Calcarea): The first record of Sycetta to the Southwestern Atlantic Ocean. *Zootaxa*, *5481*(3), 353–362. https://doi.org/10.11646/zootaxa.5481.3.4

Miloslavich, P., Díaz, J. M., Klein, E., Alvarado, J. J., Díaz, C., Gobin, J., Escobar-Briones, E., Cruz-Motta, J. J., Weil, E., Cortés, J., Bastidas, A. C., Robertson, R., Zapata, F., Martín, A., Castillo, J., Kazandjian, A., & Ortiz, M. (2010). Marine Biodiversity in the Caribbean: Regional Estimates and Distribution Patterns. *PLoS ONE*, *5*(8), e11916. https://doi.org/10.1371/journal.pone.0011916

Moraes, F. C., Ventura, M., Klautau, M., Hajdu, E., & Muricy, G. (2006). *Biodiversidade de esponjas das ilhas oceânicas brasileiras. Pp. 147–178 In: Alves, R.J.V. & Castro, J.W.A. (Eds.), Ilhas Oceânicas Brasileiras – da pesquisa ao manejo. Ministério do Meio Ambiente, Secretaria de Biodiversidade e Florestas, Brasília*. https://www.marinespecies.org/aphia.php?p=sourcedetails&id=220500

Morrone, J. J. (1994). On the Identification of Areas of Endemism. *Systematic Biology*, *43*(3), 438. https://doi.org/10.2307/2413679

Moura, R. L., Amado-Filho, G. M., Moraes, F. C., Brasileiro, P. S., Salomon, P. S., Mahiques, M. M., Bastos, A. C., Almeida, M. G., Silva, J. M., Araujo, B. F., Brito, F. P., Rangel, T. P., Oliveira, B. C. V., Bahia, R. G., Paranhos, R. P., Dias, R. J. S., Siegle, E., Figueiredo, A. G., Pereira, R. C., … Thompson, F. L. (2016). An extensive reef system at the Amazon River mouth. *Science Advances*, *2*(4), e1501252. https://doi.org/10.1126/sciadv.1501252

Muricy, G., Hajdu, E., Custódio, M. R., Klautau, M., Russo, C., & Peixinho, S. (1991). Sponge Distribution at Arraial do Cabo, SE Brazil. In: Magoon, O.T., Converse, H., Tippie, V., Tobin, L.T.& Clark, D. (Eds), Coastal Zone ’91. *Proceedings of the VII Symposium on Coastal and Ocean Management*, *2*, 1183–1196.

Muricy, G., Lage, A., Sandes, J., Klautau, M., Pinheiro, U., Laport, M. S., De Oliveira, B. F. R., Pequeno, C. B., & Lopes, M. V. (2024). Sponge Communities of Submarine Caves and Tunnels on the Fernando de Noronha Archipelago, Northeast Brazil. *Journal of Marine Science and Engineering*, *12*(4), 657. https://doi.org/10.3390/jmse12040657

O’Dea, A., Lessios, H. A., Coates, A. G., Eytan, R. I., Restrepo-Moreno, S. A., Cione, A. L., Collins, L. S., De Queiroz, A., Farris, D. W., Norris, R. D., Stallard, R. F., Woodburne, M. O., Aguilera, O., Aubry, M.-P., Berggren, W. A., Budd, A. F., Cozzuol, M. A., Coppard, S. E., Duque-Caro, H., … Jackson, J. B. C. (2016). Formation of the Isthmus of Panama. *Science Advances*, *2*(8), e1600883. https://doi.org/10.1126/sciadv.1600883

Oliveira, U., Azevedo, F., Dias, A., De Almeida, A. C. S., Senna, A. R., Marques, A. C., Rezende, D., Hajdu, E., Lopes-Filho, E. A. P., Pitombo, F. B., De Oliveira, G. M., Doria, J. G., Carraro, J. L., De-Paula, J. C., Bahia, J., De Araujo, J. M., Paresque, K., Vieira, L. M., Fernandes, L. M., … Venekey, V. (2024). Beta diversity and regionalization of the western Atlantic marine biota. *Journal of Biogeography*, *51*(8), 1469–1480. https://doi.org/10.1111/jbi.14837

Oliveira, U., Brescovit, A. D., & Santos, A. J. (2015). Delimiting Areas of Endemism through Kernel Interpolation. *PLOS ONE*, *10*(1), e0116673. https://doi.org/10.1371/journal.pone.0116673

Oliveira, U., Soares-Filho, B., Leitão, R. F. M., & Rodrigues, H. O. (2019). BioDinamica: A toolkit for analyses of biodiversity and biogeography on the Dinamica-EGO modelling platform. *PeerJ*, *7*, e7213. https://doi.org/10.7717/peerj.7213

Pérez, T., Díaz, M.-C., Ruiz, C., Cóndor-Luján, B., Klautau, M., Hajdu, E., Lobo-Hajdu, G., Zea, S., Pomponi, S. A., Thacker, R. W., Carteron, S., Tollu, G., Pouget-Cuvelier, A., Thélamon, P., Marechal, J.-P., Thomas, O. P., Ereskovsky, A. V., Vacelet, J., & Boury-Esnault, N. (2017). How a collaborative integrated taxonomic effort has trained new spongiologists and improved knowledge of Martinique Island (French Antilles, eastern Caribbean Sea) marine biodiversity. *PLOS ONE*, *12*(3), e0173859. https://doi.org/10.1371/journal.pone.0173859

Pinheiro, H. T., Rocha, L. A., Macieira, R. M., Carvalho-Filho, A., Anderson, A. B., Bender, M. G., Di Dario, F., Ferreira, C. E. L., Figueiredo-Filho, J., Francini-Filho, R., Gasparini, J. L., Joyeux, J., Luiz, O. J., Mincarone, M. M., Moura, R. L., Nunes, J. D. A. C. C., Quimbayo, J. P., Rosa, R. S., Sampaio, C. L. S., … Floeter, S. R. (2018). South-western Atlantic reef fishes: Zoogeographical patterns and ecological drivers reveal a secondary biodiversity centre in the Atlantic Ocean. *Diversity and Distributions*, *24*(7), 951–965. https://doi.org/10.1111/ddi.12729

Poléjaeff, N. (1883). Report on the Calcarea dredged by H.M.S.'Challenger' during the years 1873-1876. *Report on the Scientific Results of the Voyage of H.M.S. 'Challenger', 1873–76. Zoology*. http://www.19thcenturyscience.org/HMSC/HMSC-Reports/Zool-24/README.htm

Rapp, H. T. (2013). A monograph of the calcareous sponges (Porifera, Calcarea) of Greenland. *Journal of the Marine Biological Association of the United Kingdom*, *95*(7), 1395–1459. https://doi.org/10.1017/S0025315413001070

Rapp, H. T., Göcke, C., Tendal, O. S., & Janussen, D. (2013). Two new species of calcareous sponges (Porifera: Calcarea) from the deep Antarctic Eckström Shelf and a revised list of species found in Antarctic waters. *Zootaxa*, *3692*(1). https://doi.org/10.11646/zootaxa.3692.1.9

Rapp, H. T., Janussen, D., & Tendal, O. S. (2011). Calcareous sponges from abyssal and bathyal depths in the Weddell Sea, Antarctica. *Deep Sea Research Part II: Topical Studies in Oceanography*, *58*(1–2), 58–67. https://doi.org/10.1016/j.dsr2.2010.05.022

Ridley, S. O. (1881). XI. Spongida. Horny and Siliceous Sponges of Magellan Straits, S.W. Chili, and Atlantic off SW Brazil. In: Account of the Zoological Collections made during the Survey of H.M.S. "Alert" in the Straits of Magellan and on the Coast of Patagonia. Gunther, A. (Ed.). *Proceedings of the Zoological Society of London*, 107-141,.

Rossi, A. L., Farina, M., Borojevic, R., & Klautau, M. (2006). Occurrence of five-rayed spicules in a calcareous sponge: Sycon pentactinalis sp. Nov. (Porifera: Calcarea). *Cahiers de Biologie Marine*, *47*(3), 261-270.

Rützler, K., Piantoni, C., Van Soest, R. W. M., & Díaz, M. C. (2014). Diversity of sponges (Porifera) from cryptic habitats on the Belize barrier reef near Carrie Bow Cay. *Zootaxa*, *3805*(1), 1. https://doi.org/10.11646/zootaxa.3805.1.1

Schuffner, O. (1877). *Beschreibung einiger neuer Kalkschwämme*. WorldCat.

Solé-Cava, A. M., Klautau, M., Boury-Esnault, N., Borojecic, R., & Thorpe, J. P. (1991). Genetic evidence for cryptic speciation in allopatric populations of two cosmopolitan species of the calcareous sponge genusClathrina. *Marine Biology*, *111*(3), 381–386. https://doi.org/10.1007/BF01319410

Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdaña, Z. A., Finlayson, M., Halpern, B. S., Jorge, M. A., Lombana, A., Lourie, S. A., Martin, K. D., McManus, E., Molnar, J., Recchia, C. A., & Robertson, J. (2007). Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas. *BioScience*, *57*(7), 573–583. https://doi.org/10.1641/B570707

Stampar, S. N., Maronna, M. M., Vermeij, M. J., Silveira, F. L. D., & Morandini, A. C. (2012). Evolutionary diversification of banded tube-dwelling anemones (Cnidaria; Ceriantharia; *Isarachnanthus)* in the Atlantic Ocean. *PLoS One*, 7(7), e41091.

Targino, A. K. G., & Gomes, P. B. (2020). Distribution of sea anemones in the Southwest Atlantic: Biogeographical patterns and environmental drivers. *Marine Biodiversity*, *50*(5), 80. https://doi.org/10.1007/s12526-020-01099-z

Valderrama, D., Rossi, A. L., Solé-Cava, A. M., Rapp, H. T., & Klautau, M. (2009). Revalidation of *Leucetta floridana* (Haeckel, 1872) (Porifera, Calcarea): A widespread species in the tropical western Atlantic. *Zoological Journal of the Linnean Society*, *157*(1), 1–16. https://doi.org/10.1111/j.1096-3642.2009.00522.x

Van Soest, R. W. M. (2017). Sponges of the Guyana Shelf. *Zootaxa*, *4217*(1). https://doi.org/10.11646/zootaxa.4217.1.1

Voigt, O., Wülfing, E., & Wörheide, G. (2012). Molecular Phylogenetic Evaluation of Classification and Scenarios of Character Evolution in Calcareous Sponges (Porifera, Class Calcarea). *PLoS ONE*, *7*(3), e33417. https://doi.org/10.1371/journal.pone.0033417

Yasuhara, M., Huang, H.-H. M., Reuter, M., Tian, S. Y., Cybulski, J. D., O'Dea, A., Mamo, B. L., Cotton, L. J., Martino, E. D., Feng, R., Tabor, C. R., Reygondeau, G., Zhao, Q., Warne, M. T., Aye, K. K. T., Zhang, J., Chao, A., Wei, C.-L., Condamine, F. L., … Hong, Y. (2022). Hotspots of Cenozoic Tropical Marine Biodiversity. In S. J. Hawkins, A. J. Lemasson, A. L. Allcock, A. E. Bates, M. Byrne, A. J. Evans, L. B. Firth, E. M. Marzinelli, B. D. Russell, J. Sharples, I. P. Smith, S. E. Swearer, P. A. Todd, C. Lucas, & P. J. Mumby, *Oceanography and Marine Biology: An Annual Review, Volume 60* (1st ed., pp. 243–300). CRC Press. https://doi.org/10.1201/9781003288602-5

Chapter 02: Discovering the biodiversity of Calcareous Sponges (Porifera: Calcarea) along the Coast of São Paulo by integrative approaches using molecular and morphological data

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Abstract

The calcareous sponge (Calcarea) fauna along the northern coast of São Paulo, Brazil, has been documented since the 1970s, yet gaps remain in understanding its biodiversity and distribution. This study investigates the Calcarea diversity along the São Paulo coastline, focusing on molecular and morphological analyses to update taxonomic records and assess zoogeographical affinities. Sampling was conducted between 2023 and 2024 across five sites, including Ubatuba, São Sebastião, Ilhabela, and the Alcatrazes Archipelago, using scuba diving and intertidal collection methods. Specimens were examined morphologically via light and scanning electron microscopy, and molecularly through 28S (C-LSU) and ITS sequencing. We identified 4 species, including new records for the region, such as *Borojevia brasiliensis, B. trispinata, Clathrina antofagastensis* and *Leucandra serrata,* as well as a potential new species of *Clathrina.* Phylogenetic analyses revealed distinct clades, with some specimens forming sister groups to known species, while others exhibited significant genetic divergence. Additionally, invasive species *Heteropia glomerosa* was recorded, emphasizing the need for monitoring their spread. The study highlights São Paulo's role as a transitional zone between tropical and subtropical marine ecoregions, with species shared across South American coasts. However, endemic and rare taxa, such as S*ycon pentactinale* and the undescribed *Clathrina* sp., underscore the region's unique biodiversity. Despite these findings, sampling gaps persist in southern São Paulo and adjacent states, urging further exploration. This research contributes to the state's Porifera collection and supports conservation efforts by updating distribution data for invasive and endemic species, reinforcing the need for expanded taxonomic and ecological studies in underrepresented marine habitats.

Keywords: Benthos, Systematics, Distribution, Zoogeographic affinities, biogeographical patterns, Southeastern Brazil Ecoregion

Introduction

Records of calcareous sponges exist only on the northern coast of the state of São Paulo, with the first report by Borojevic in 1971 describing *Clathrina primordialis* (Haeckel, 1872) in Ubatuba. Advances in molecular studies have reclassified *Clathrina primordialis* from the Rio de Janeiro coast as *Clathrina cylindractina* Klautau, Solé-Cava & Borojevic, 1994, indicating that this record for Ubatuba deserves to be revisited. In 2004 the second species was described for the region: *Paraleucilla magna* Klautau, Monteiro & Borojevic, 2004, recorded in the Alcatrazes Archipelago and later recognized as an invasive species. In 2006, the third species, *Sycon pentactinale* Rossi, Farina, Borojevic & Klautau, 2006 with a rare spicule type, was described for São Sebastião.

A year later, the first taxonomic study dedicated to the Calcinea subclass was carried out from collections made in the Alcatrazes Archipelago culminating in the publication of five species: *Arturia alcatraziensis* (Lanna, Rossi, Cavalcanti, Hajdu & Klautau, 2007), *Leucascus roseus* Lanna, Rossi, Cavalcanti, Hajdu & Klautau, 2007, *Clathrina aurea* Solé-Cava, Klautau, Boury-Esnault, Borojevic & Thorpe, 1991, *C. conifera* Klautau & Borojevic, 2001, and *Neoernsta tetractina* (Klautau & Borojević, 2001).

Ten years later, *Amphoriscus pedunculatus* Klautau, Cavalcanti & Borojevic, 2017 was described based on specimens from São Sebastião and Arraial do Cabo. Oricchio et al. (2019) reported *Leucandra serrata* Azevedo & Klautau, 2007 and *Heteropia glomerosa* (Bowerbank, 1873) in Ilhabela and São Sebastião. Lastly, in 2020, a new record of *Paraleucilla magna* was reported for Ilhabela by Cavalcanti et al. (2020).

As a key step in advancing scientific knowledge lies in building accessible resources (Costello et al., 2010), this project was guided by the investigation of the São Paulo coastline to establish a Porifera collection, with emphasis on the class Calcarea, at the São Paulo State University. Part of this material also aims to expand the main zoological collection of the state, housed at the Museum of Zoology of the University of São Paulo (MZUSP), which, according to the latest survey, holds records for only 26 sponge species (Borges et al., 2022). The importance of carrying out such actions lies in the fact that, although traditional institutions are recognized as major centers of knowledge, Brazil has continental dimensions. The advancement of knowledge about the biodiversity of its marine territory is one of the reasons that justify the maintenance of existing marine protected areas, as well as the studies that support the creation of new ones. Additionally, regarding calcareous sponges, São Paulo state has records of two exotic invasive species, *Heteropia glomerosa* and *Paraleucilla magna*. By exploring areas with no previous records of calcareous sponges and finding these

species, we can update their known distribution ranges and keep the state institutions responsible for preserving these regions well-informed.

Materials and Methods

Study Area and Sampling

The study area is located along the São Paulo coast (23°22'03.0" to 25°18'52.5"S; 44°43'25.4" to 48°05'39.5"W), which extends for approximately 880 km and is part of the Southeastern Brazil marine ecoregion. The São Paulo coastline is subdivided according to the region's hydrographic micro-basins, being separated into three areas: northern coast, Baixada Santista, and southern coast. Sampling was focused on the northernmost portion of this coastline. Apart from the Alcatrazes Archipelago, where specimens were collected via scuba diving at a depth of 17 meters, all other samples were obtained from rocky shores during low tide. Sponges were collected using diving knives and immediately preserved in 96% ethanol. Samplings were conducted between June 2023 and April 2024. In June 2023, the first site sampled was Saco da Ribeira, Ubatuba (23°30'01.1"S – 45°07'05.7"W), where specimens of *Heteropia glomerosa* were found on natural substrate (Chapter 03). The second site was Praia das Cigarras, São Sebastião (23°43'42.8"S – 45°23'52.1"W), sampled in September 2023. The third site was Pedras Miudas Beach, Ilhabela (23°49'50.1"S – 45°23'27.9"W). The fourth site was Toque-Toque Pequeno Beach (23°49'25.5"S – 45°32'02.4"W), also sampled in September 2023. Finally, samples were collected in the Alcatrazes Archipelago (24°06'S, 45°42'W) in April 2024.

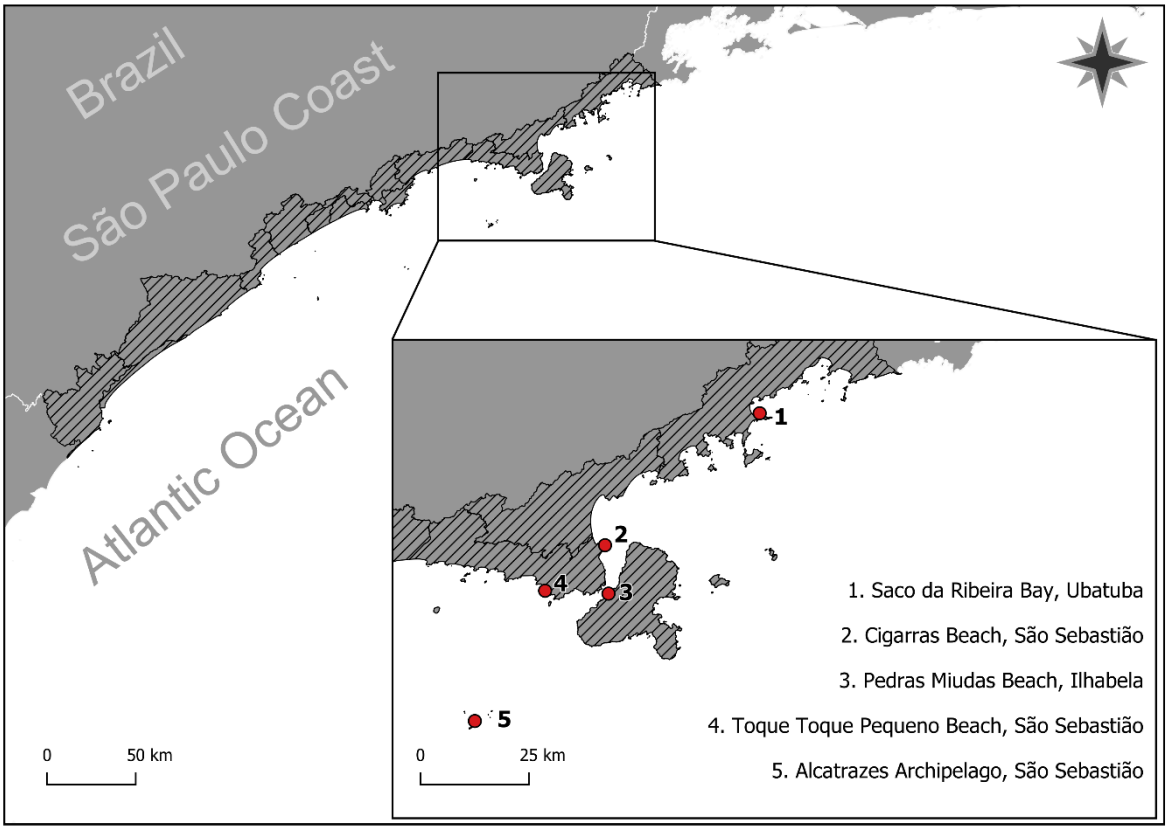


Figure 1. Map shows the area of sampling at São Paulo coast, with a zoom in the collected points, indicating the names sites and cities sampling.

Morphological analysis

The specimens were first examined under a stereomicroscope (Leica) and photographed. They were then sectioned tangentially, with one half preserved and the other used for subsequent analysis. For the spicule composition and cross section slides we followed standard procedures (Klautau and Valantine 2003) with a subsequent modification, the substitution of Canada balsam for Entellan (Merck) as the mounting medium for spicule slides (Cavalcanti, Menegola and Lanna, 2014). The slides were analyzed under a microscope (Nikon) and pictures were captured with a coupled camera (Motic Image Plus 2.0). For measurements the software ImageJ v1.54g was used. The results are presented in tabular form for each species, including the minimum (min), mean, standard deviation (SD), and maximum (max) values for both length and width, along with number of spicules analyzed (N). To investigate spicule size variation, we searched for the largest and smallest spicules in each category and randomly measured 18 additional ones. Some samples were taken micrographs of individual spicules with a scanning electron microscope (SEM; Zeiss EVO-LS15) at the Faculty of Sciences of the State University of São Paulo. For SEM preparation, stubs covered with double-sided carbon tape were fitted with coverslips. A

droplet of 100% alcohol containing dissociated spicules was placed on top and left to dry. Samples were then gold-coated (Au) before SEM analysis.

DNA extraction, amplification and sequencing

Genomic DNA was extracted using the Easypure Genomic DNA Kit (TransGen Biotech), the Nova Biotecnologia Extraction Kit, or the QuatroG Biotecnologia DNA Kit, and stored at –20 °C. For both Calcarea subclasses, the C-LSU region (28S) was amplified using the following primers: forward 5′-GAAAAGCACTTTGAAAAGAGA-3′ (Voigt & Wörheide, 2016) and reverse 5′-TCCGTGTTTCAAGACGGG-3′ (Chombard, Boury-Esnault & Tillier, 1998). When possible, samples of Calcinea were also amplified for the ITS region using the primers: forward 5′-TCATTTAGAGGAAGTAAAAGTCG-3′ and reverse 5′-GTTAGTTTCTTTTCCTCCGCTT-3′ (Lôbo-Hajdu et al., 2004). Polymerase Chain Reaction (PCR) master mixing contained 1x Buffer (10x Buffer kit, Ludwing), 2.5 mM $MgCl_2$, one unit of Taq DNA polymerase (Ludwing), 0.33 µM of each primer (Exxtend), 0.2 mM dNTP (Ludwing), 0.5 µg/µL of bovine serum albumin (BSA) and 50-100 ng of DNA in a volume of 25 µL. The amplification conditions were one cycle of 4 min at 94 ºC, 40 cycles of 1 min at 92 ºC, 1 min at 48 ºC, 50 ºC or 52 ºC and 1 min at 72 ºC, followed by a final cycle of 6 min at 72 ºC. PCR products were purified prior to shipment to IBTEC (Botucatu, Brazil), where sequencing was performed using an ABI 3500 Genetic Analyzer (ThermoFisher Scientific). Sequences were edited and trimmed in Geneious v11.1.5, and BLAST searches were conducted to confirm they originated from calcareous sponges.

Phylogenetic analysis

Previous sequences were retrieved from GenBank database and used with the new sequences to generate Maximum Likelihood (ML) phylogenetic trees; sequences are shown in Table 1. The online version of MAFFT v7 (Katoh et al., 2019) was used for the aligned of DNA sequences using the Q-INS-i strategy (Katoh & Toh, 2008). All other parameters were left in the default option. ML phylogenetic trees were constructed using IQ-TREE v2.4.0 (Minh et al., 2020). The best-fit substitution model was selected based on the Bayesian Information Criterion (BIC). For the C-LSU dataset, the TIM+F+I+G4 model was chosen, while the TN+F+I+G4 model was selected for the ITS dataset. Branch support for ML was obtained with the ultrafast bootstrap (Minh et al., 2013; Hoang et al., 2018) with 100,000 replicates. The final ML phylogenetic trees were rendered in FigTree v1.4.0 and

subsequently stylized using Adobe Photoshop. The uncorrected *p*-distance was calculated in MEGA X (Kumar et al. 2018) to estimate the intraspecific and interspecific variability.

Table 1. Species, Marine Ecoregions, Vouchers and GenBank Accession of all sequences used in the molecular tree reconstructions. Sequences generated in this study are bold.

Species	Marine Ecoregion	Voucher	GenBank Accession	
			C-LSU	ITS
Calcaronea				
Amphoriscus pedunculatus	Southeastern Brazil	UFRJPOR 6004	MH028217	-
Grantessa tumida	Southern Caribbean	UFRJPOR 6695	MF472617	-
Grantessa tumida	Southern Caribbean	UFRJPOR 6701	MF472616	-
Grantessa woerheidei	Southern Red Sea	RMNH 9586	MF872773	-
Grantia arctica	West Greenland Shelf	RMNH-FB04	MH385235	-
Grantia arctica	West Greenland Shelf	RMNH-FB25	MH385237	-
Grantia capillosa	West Greenland Shelf	RMNH-SA28	MH385238	-
Grantia capillosa	West Greenland Shelf	RMNH-SA29	MH385239	-
Grantia compressa	Southern Norway	RMNH-AA01	MH385240	-
Grantia compressa	Southern Norway	RMNH-SA39	MH385241	-
Grantia compressa	Celtic Seas	RMNH-SA80	MH385242	-
Grantia sp.	South and West Iceland	RMNH-SA105	MH385246	-
Grantia sp.	South and West Iceland	RMNH-SA129	MH385247	-
Heteropia glomerosa	Eastern Brazil	UFRJPOR 6151	MN402721	-
Heteropia glomerosa	Eastern Brazil	UFRJPOR 6191	MN402722	-
Heteropia glomerosa	Southeastern Brazil	UFRJPOR 7608	MN402723	-
Heteropia glomerosa	Southeastern Brazil	UFRJPOR 7609	MN402724	-
Heteropia glomerosa	Southeastern Brazil	**PPM 12**	*	-
Heteropia glomerosa	Southeastern Brazil	**TTP 39**	*	-
Heteropia minor	Papua	RMNH 1872	MF686066	-
Heteropia minor	Sunda Shelf/Java Sea	RMNH 3603	MF686067	-
Heteropia minor	Sunda Shelf/Java Sea	RMNH 9194	MF686068	-
Heteropia minor	Papua	RMNH 9349	MF686070	-
Leucandra ananas	Northern Norway and Finnmark	RMNH-SA136	MH385249	-
Leucandra ananas	Northern Norway and Finnmark	RMNH-SA139	MH385250	-
Leucandra aspera	South European Atlantic Shelf	RMNH-PT15	MH385251	-
Leucandra cavernicola	Marquesas	UFRJPOR 7517	OR987538	-
Leucandra cf. *gausapata*	Antarctic†	RMNH-HT15	MH385252	-
Leucandra mozambiquensis	Western and Northern Madagascar	ZMA 22408B	MF872766	-
Leucandra ornata	Mascarene Islands	UFRJPOR 8928	MW350116	-
Leucandra penicillata	West Greenland Shelf	RMNH FB40	MH385253	-
Leucandra penicillata	West Greenland Shelf	RMNH FB49	MH385254	-
Leucandra pilula	Seychelles	ZMA 10528	MF872767	-
Leucandra serrata	Southeastern Brazil	**Cig03**	*	-
***Leucandra* sp. 1**	Southeastern Brazil	**TTP04**	*	-
***Leucandra* sp. 1**	Southeastern Brazil	**TTP29**	*	-

Species	Marine Ecoregion	Voucher	GenBank Accession	
			C-LSU	ITS
***Leucandra* sp. 1**	Southeastern Brazil	**Cig01**	*	-
***Leucandra* sp. 2**	Southeastern Brazil	**A02**	*	-
Leucandra tahuatae	Marquesas	UFRJPOR 6454	MN422253	-
Leucilla antillana	Southern Caribbean	UFRJPOR 6768	MF472615	-
Leucilla mancoraensis	Central Peru	MNRJ 12967	MH028214	-
Leucilla micropilosa	Southern Caribbean	UFRJPOR 6755	MF472621	-
Paraleucilla dalmatica	Adriatic Sea	RMNH-PT03	MH385276	-
Paraleucilla dalmatica	Adriatic Sea	RMNH-PT05	MH385277	-
Paraleucilla dalmatica	Adriatic Sea	RMNH-PT08	MH385278	-
Paraleucilla erpenbecki	Western and Northern Madagascar	ZMA22409C	MF872728	-
Paraleucilla magna	South European Atlantic Shelf	RMNH-PT06	MH385279	-
Paraleucilla magna	South European Atlantic Shelf	RMNH-PT07	MH385280	-
Paraleucilla magna	South European Atlantic Shelf	RMNH-PT11	MH385281	-
Paraleucilla magna	South European Atlantic Shelf	RMNH-SA84	MH385282	-
Paraleucilla perlucida	São Pedro e São Paulo Archipelago	UFRJPOR 6515	MH028215	-
Paraleucilla* cf. *perlucida	Southeastern Brazil	**TTP42**	*	-
Paraleucilla* cf. *perlucida	Southeastern Brazil	**TTP43**	*	-
***Paraleucilla* sp. 1**	Southeastern Brazil	**A03**	*	-
***Paraleucilla* sp. 1**	Southeastern Brazil	**A16**	*	-
***Paraleucilla* sp. 1**	Southeastern Brazil	**A21**	*	-
***Paraleucilla* sp. 1**	Southeastern Brazil	**A48**	*	-
***Paraleucilla* sp. 2**	Southeastern Brazil	**Cig09**	*	-
Paraleucilla tarazonai	Central Peru	MNRJ11448	MH028216	-
Sycetta antarctica	Antarctic†	RMNH HT14	MH385287	
Sycetta antarctica	Antarctic†	RMNH HT16	MH385288	-
Sycetta asconoides	South and West Iceland	RMNH SA106	MH385289	-
Sycetta vinitincta	Lesser Sunda	RMNH 1873	MF686094	-
Sycettusa hastifera	Southern Red Sea	RMNH 9659	MF872777	-
Sycettusa hastifera	Northern and Central Red Sea	ZMA 13443	MF872778	-
Sycettusa hirsutissima	Southern Red Sea	RMNH 9588.1	MF872775	-
Sycettusa hirsutissima	Southern Red Sea	RMNH 10004	MF872774	-
Sycettusa simplex	Maldives	RMNH 10154.2	MF872780	-
Sycettusa simplex	Seychelles	ZMA 12446	MF872781	-
Sycettusa stauridia	Northern and Central Red Sea	ZMA 13664	MF872782	-
Sycettusa thompsoni	West Greenland Shelf	RMNH-FB48	MH385292	-
Sycettusa zanzibaris	Seychelles	ZMA 11568	MF872783	-
Sycon caminatum	Azores Canaries Madeira	RMNH-PT02	MH385301	-
Sycon ciliatum	Southern Norway	RMNH 127228	MK696121	-
Sycon conulosum	Southern Caribbean	UFRJPOR 6707	MF472620	-

Species	Marine Ecoregion	Voucher	GenBank Accession	
			C-LSU	ITS
Sycon karajakense	West Greenland Shelf	RMNH-SA48	MH385306	-
Sycon karajakense	West Greenland Shelf	RMNH-SA49	MH385307	-
Sycon magnapicale	Southern Caribbean	UFRJPOR 6748	MF472622	-
Sycon magnapicale	Southern Caribbean	UFRJPOR 6763	MF472623	-
***Sycon* sp. 1**	Southeastern Brazil	**SRB12**	*	-
***Sycon* sp. 1**	Southeastern Brazil	**SRBD06**	*	-
***Sycon* sp. 1**	Southeastern Brazil	**SRBD07**	*	-
***Sycon* sp. 1**	Southeastern Brazil	**SRBD09**	*	-
***Sycon* sp. 1**	Southeastern Brazil	**TTP50**	*	-
***Sycon* sp. 1**	Southeastern Brazil	**TTP51**	*	-
***Sycon* sp. 1**	Southeastern Brazil	**TTP53**	*	-
***Sycon* sp. 2**	Southeastern Brazil	**TTP40**	*	-
***Sycon* sp. 3**	Southeastern Brazil	**SRBD03**	*	-
Sycon spinispiculum	Southern Norway	RMNH-SA35	MH385311	-
Sycon spinispiculum	Southern Norway	RMNH-SA36	MH385312	-
Sycon villosum	Northern Norway and Finnmark	RMNH-SA24	MH385314	-
Calcinea				
Ascandra concorta	Western Mediterranean	UFRJPOR 6327	HQ589011	-
Ascandra corallicola	Southern Norway	UFRJPOR 6329	HQ589012	-
Borojevia aff. *aspina*	Southern Red Sea	KAUMM-1	KY366359	-
Borojevia aff. *aspina*	Southern Red Sea	SMF 11637	KY366400	KY366409
Borojevia cf. *aspina*	Eastern Brazil	UFRJPOR 5407	-	KX548912
Borojevia cf. *aspina*	Eastern Brazil	UFRJPOR 5492	-	KX548913
Borojevia cf. *aspina*	Eastern Brazil	UFRJPOR 5498	-	KX548914
Borojevia brasiliensis	Southeastern Brazil	**A49**	*	-
Borojevia brasiliensis	Eastern Brazil	UFRJPOR 5214	HQ589015	HQ588978
Borojevia brasiliensis	Eastern Brazil	UFRJPOR 5230	-	HQ588999
Borojevia brasiliensis	Southeastern Brazil	UFRJPOR 5406	-	KX548909
Borojevia cerebrum	Western Mediterranean	UFRJPOR 6322	HQ589008	-
Borojevia cerebrum	Adriatic Sea	IRB-CLB32	-	KP740031
Borojevia cerebrum	Adriatic Sea	IRB-CLB33	-	KP740032
Borojevia crystallina	Eastern Caribbean	UFRJPOR7937	MG644610	MG644615
Borojevia moana	Marquesas	UFRJPOR7524	OR987532	OR987539
Borojevia pirella	Mascarene Islands	RMNH11622A.1	MF872729	-
Borojevia pirella	Mascarene Islands	RMNH11622A.2	MF872730	-
Borojevia pirella	Mascarene Islands	RMNH11622B	MF872731	-
Borojevia tenuispinata	Southern Caribbean	UFRJPOR 6700	MF472611	MF472608
Borojevia trispinata	Eastern Brazil	UFBAPOR 4459	OQ198704	-
Borojevia trispinata	Eastern Brazil	UFBAPOR 4461	OQ198703	-
Borojevia trispinata	São Pedro and São Paulo Islands	UFRJPOR 6419	-	KX548918
Borojevia trispinata	São Pedro and São Paulo Islands	UFRJPOR 6487	-	KX548919
Borojevia tubulata	Maldives	RMNH10158	MF872732	-
Borojevia aff. *tubulata*	Seychelles	ZMA12435	MF872733	-
Brattegardia nanseni	Greenland†	UFRJPOR 6332	HQ589013	-

Species	Marine Ecoregion	Voucher	GenBank Accession	
			C-LSU	ITS
Clathrina aff. *adusta*	Northern Norway and Finnmark	SMF 11618	KY366387	-
Clathrina antofagastensis	Central Peru	MNRJ 13674	-	KF002722
Clathrina antofagastensis	Humboldtian	MNRJ 11294	-	KF002723
Clathrina antofagastensis	Humboldtian	MNRJ 9289	HQ589003	-
Clathrina antofagastensis	Southeastern Brazil	**A08**	*	-
Clathrina antofagastensis	Southeastern Brazil	**A32**	*	-
Clathrina blanca	Adriatic Sea	PMR 13744	-	KC479087
Clathrina blanca	Adriatic Sea	PMR 14307	-	KP740017
Clathrina clathrus	Adriatic Sea	PMR 14308	-	KC479089
Clathrina conifera	Southeastern Brazil	MNRJ 8991	HQ589010	-
Clathrina conifera	-	UFRJPOR5713	-	KP713807
Clathrina conifera	-	UFRJPOR6071	-	KP713810
Clathrina coriacea	Norway†	UFRJPOR 6330	HQ589001	HQ588986
Clathrina curacaoensis	Southern Caribbean	UFRJPOR 6734	-	MF472607
Clathrina cylindractina	Southeastern Brazil	UFRJPOR 5206	HQ589007	-
Clathrina cylindractina	Southeastern Brazil	UFRJPOR 5413	-	HQ588993
Clathrina fjordica	Chiloense	MNRJ 8143	-	HQ588984
Clathrina fjordica	Chiloense	MNRJ 9964	HQ589016	-
Clathrina* cf. *globulosa	Southeastern Brazil	**TTP07**	*	-
Clathrina* cf. *globulosa	Southeastern Brazil	**TTP12**	-	*
Clathrina* cf. *globulosa	Southeastern Brazil	**TTP14**	-	*
Clathrina helveola	Central and Southern Great Barrier Reef	G313680	-	HQ588988
Clathrina hispanica	Western Mediterranean	UFRJPOR 6308	-	KX548907
Clathrina cf. *hondurensis*	Adriatic Sea	PMR-14305	-	KC479086
Clathrina lacunosa	Northern Norway and Finnmark	UFRJPOR 6334	HQ589020	-
Clathrina mutabilis	Southern Caribbean	UFRJPOR 6741	MF472613	-
Clathrina primordialis	Adriatic Sea	IRB-CLB3	-	KP740016
Clathrina aff. *pulcherrima*	Western and Northern Madagascar	ZMA 22408C	MF872742	-
Clathrina purpurea	Sulawesi Sea/Makassar Strait	RMNH 6625	MF686058	-
Clathrina ramosa	Chiloense	MNRJ 10313	HQ589002	-
Clathrina robusta	Eastern Brazil	UFBAPOR 4557	OQ198710	-
Clathrina rodriguesensis	Mascarene Islands	RMNH 11670	MF872749	-
Clathrina rotundata	Southern Red Sea	SMF 11636	KY366399	-
Clathrina rowi	Southern Red Sea	SMF 11633	KY366395	-
Clathrina rowi	Northern and Central Red Sea	SMF 11634	KY366396	-
Clathrina rubra	Adriatic Sea	PMR 14306	-	KC479088
Clathrina sinusarabica	Northern and Central Red Sea	SMF 11630	KY366391	-
Clathrina sinusarabica	Northern and Central Red Sea	SMF 11631	KY366393	-
Clathrina smaragda	Floridian	UFRJPOR 8359	-	MG017974
Clathrina soluta	Eastern Brazil	UFBAPOR 4705	OQ198706	-

Species	Marine Ecoregion	Voucher	GenBank Accession	
			C-LSU	ITS
***Clathrina* sp. nov. 1**	Southeastern Brazil	**A23**	*	-
Clathrina sp.	Norwegian Sea†	UFRJPOR 6336	HQ589019	-
Clathrina williamsi	Great Australian Bight	SAMA-S1885	OR228945	
Neoernsta tetractina	Southeastern Brazil	UFRJPOR5183	HQ589021	-

New sequences are marked with an asterisk (*) in place of a GenBank accession number, as they are not yet available. An obelisk (†) was used for sequences with only locality, not marine ecoregion.

Results

Molecular results

The ML phylogeny tree using the ITS marker (Figure 2) recovered a newly sequenced *Clathrina* cf. *globulosa* specimens (TTP12 and TTP14) with 99% bootstrap support, forming a sister clade to *C. hispanica* Klautau & Valentine, 2003 and *C. blanca* (Miklucho-Maclay, 1868), which had 100% support. However, the support for the composition of the larger clade was low, at only 42%. Lastly, a neighboring clade with 99% bootstrap support was formed by *C. antofagastensis* Azevedo, Hajdu, Willenz & Klautau, 2009, *C. primordialis* (Haeckel, 1872), and *Clathrina* cf. *hondurensis* Klautau & Valentine, 2003.

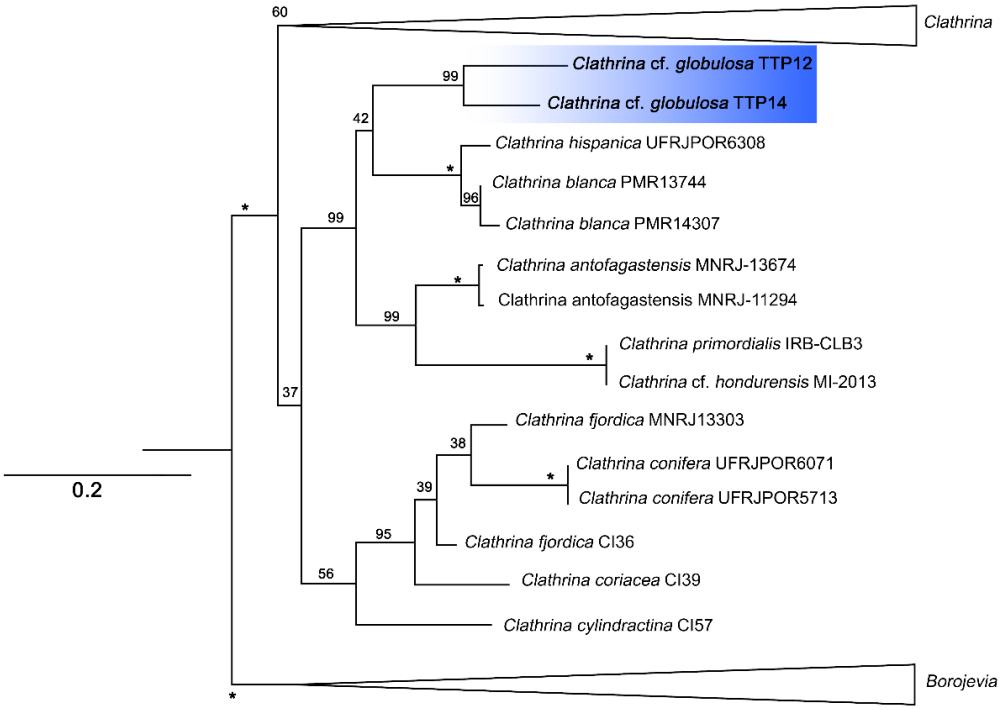


Figure 2. Maximum likelihood phylogeny of Calcinea using the ITS marker. Bold names are newly generated sequences. Bootstrap values are given on the branches. Bootstraps = 100% are presented with an asterisk (*).

The ML phylogeny tree using the C-LSU marker (Figure 3) recovered the typical division between the two subclasses, Calcinea and Calcaronea. On the Calcaronea side, two major clades formed by *Sycon* Risso 1827, *Sycettusa* Haeckel, 1872, and *Sycetta* Haeckel, 1872 species are observed. One clade includes our newly obtained sequences; with 75% bootstrap support, our *Sycon* spp. (*Sycon* sp. 1, *Sycon* sp. 2, and *Sycon* sp. 3) formed a clade with *Sycon ciliatum* (Fabricius, 1780), *Grantessa tumida* Cóndor-Luján, Louzada, Hajdu & Klautau, 2018, and a separate clade composed of *Sycettusa* and *Grantia* Fleming, 1828 species. The second major clade at the top contains our new sequences of *Heteropia glomerosa* specimens, with 88% bootstrap support and a *p*-distance of 0 to 1%. These are grouped with other *Heteropia glomerosa* sequences. A smaller clade at the top, with 30% bootstrap support, includes our new sequences of Leucosolenida Hartman, 1958 (A06, A10, A43, and PPM10). These specimens resemble *Vosmaeropsis* Dendy, 1893 and *Grantia*. Since we do not yet have a definitive identification, these sequences remain labeled under the order name Leucosolenida.

At the bottom, a split with 94% bootstrap support includes at the top our sequences for *Paraleucilla* cf. *perlucida* (TTP42 and TTP43), which form a clade with *Paraleucilla perlucida* Azevedo & Klautau, 2007, supported by 100% bootstrap and a *p*-distance of 2 to 7%. Other *Paraleucilla* sequences were grouped below, forming a clade with *Paraleucilla erpenbecki* Van Soest & De Voogd, 2018, with 93% bootstrap support. This clade also includes our *Paraleucilla* sp. 2 (Cig09) and *Paraleucilla* sp. 1 (A03, A16, A48, A21), which together show 75% bootstrap support and a *p*-distance of 3 to 4% between them, and a *p*-distance of 5 to 6% between them and *Paraleucilla erpenbecki*. Below, a clade with 82% bootstrap support includes *Paraleucilla dalmatica* Klautau, Imešek, Azevedo, Pleše, Nikolić & Ćetković, 2016 and three species of *Leucandra* Haeckel, 1872: *Leucandra mozambiquensis* Van Soest & De Voogd, 2018, *Leucandra pilula* Van Soest & De Voogd, 2018, and our new sequences, which form an independent clade of *Leucandra* sp. 1 (Cig01, TTP04, and TTP29) with 99% bootstrap support and 0% *p*-distance. At the end of the Calcaronea side, our new sequence of *Leucandra* sp. 2 (A02) formed an independent clade with 70% bootstrap support. Additionally, our sequence of *Leucandra serrata* Azevedo & Klautau (2007) formed a sister clade with *Sycon conulosum* Cóndor-Luján, Louzada, Hajdu & Klautau, 2018 and Leucilla antillana Cóndor-Luján, Louzada, Hajdu & Klautau, 2018, with 83% bootstrap support and a *p*-distance of 6%.

On the Calcinea side of the ML phylogenetic tree, our new sequence of *Borojevia brasiliensis* (Solé-Cava, Klautau, Boury-Esnault, Borojevic & Thorpe, 1991) grouped with

a previously published *Borojevia brasiliensis* sequence, with 98% bootstrap support and a *p*-distance of 5%. On the other side, *Clathrina antofagastensis* formed a clade with our new sequences (A08 and A32), showing 100% bootstrap support and a *p*-distance of 5% between them. Finally, our sequence of *Clathrina* sp. nov. 1 grouped with *Clathrina* aff. *pulcherrima* with 100% bootstrap support, and together they formed a clade with our new sequence of *Clathrina* cf. *globulosa*, supported by 97% bootstrap and a *p*-distance of 7 to 9%.

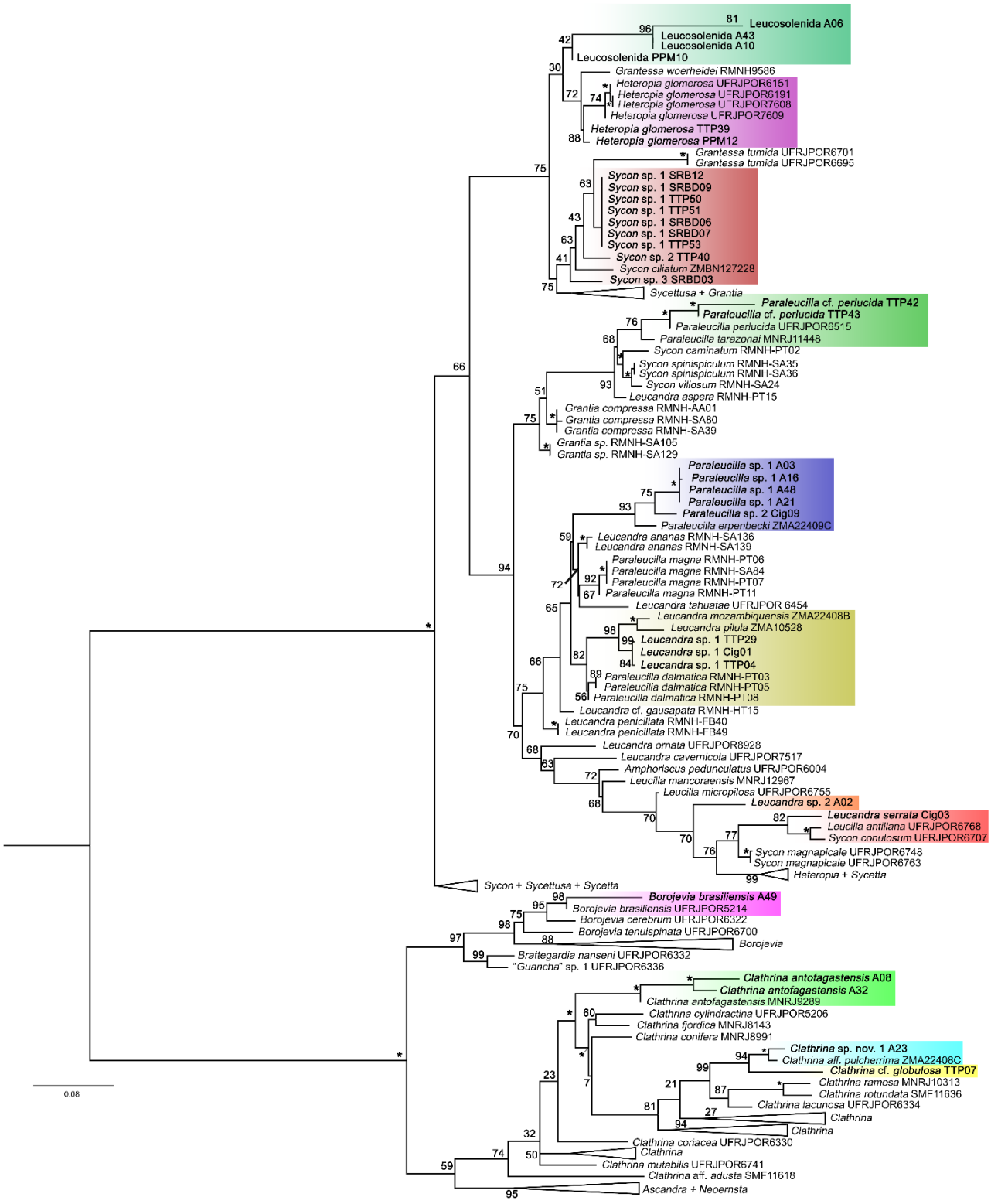


Figure 3. Maximum likelihood phylogeny of Calcarea using the LSU marker. Bold names are newly generated sequences. Bootstrap values are given on the branches. Bootstraps = 100% are presented with an asterisk (*).

Taxonomy and descriptions
Class CALCAREA Bowerbank, 1862

Subclass CALCINEA Bidder, 1898

Order CLATHRINIDA Hartman, 1958

Family CLATHRINIDAE Minchin, 1900

Genus *Borojevia* Klautau, Azevedo, Cóndor-Luján, Rapp, Collins & Russo, 2013

Diagnosis. "Calcinea in which sponge cormus comprises tightly anastomosed tubes. The skeleton contains regular (equiangular and equiradiate) triactines, tetractines, and tripods. The apical actine of the tetractines has spines. Aquiferous system asconoid" (Klautau *et al.* 2013).

Type species. *Ascaltis cerebrum* Haeckel, 1872 by original designation in Klautau *et al.* (2013).

***Borojevia brasiliensis* (Solé-Cava, Klautau, Boury-Esnault, Borojević & Thorpe, 1991)**

Synonyms. *Clathrina cerebrum*, *sensu* Borojević 1971: 526 [non *Clathrina cerebrum* (Haeckel, 1872)]. *Clathrina brasiliensis*, Solé-Cava et al. 1991: 382; Klautau et al. 1994: 372; Klautau & Borojević 2001: 403; Klautau & Valentine 2003: 11. *Borojevia brasiliensis*, Klautau et al. 2013: 458.

Material examined. Alcatrazes 49, Coral Garden at Alcatrazes Archipelago (24°05'48.3"S 45°41'41.6"W), 17 m depth, 25.iv.2024, colls. F. Azevedo and S. Stampar.

Morphology and anatomy. White *Borojevia* with a cormus composed of thin, regular, tightly anastomosed tubes. Oscula forms at the convergence points of the larger tubes across the body. The sponge retains its white coloration when preserved in ethanol. The skeleton comprises three types of spicules: tripods (not typical), regular triactines, and tetractines, all with conical actines. The apical actine of tetractines is ornamented with spines arranged apparently in three rows (tip is broken), exclusively near the tip (Fig. 4F).

Spicules (Table 2)

Tripods. Regular to subregular. They are like large triactines. Actines are conical and slightly undulated, with blunt tips. Size: 68.8 (± 6.3)/ 6.9 (± 1.2) μm (N = 20).

Triactines. Regular. Actines are conical and straight, with slightly blunt tips. Size: 73.3 (± 7.5)/ 6.7 (± 0.7) μm (N = 20).

Tetractines. Basal actines are similar to the triactines. The apical actine is shorter than the basal ones, conical and straight, with ornaments spines organized in 3 rows near the tip (Fig. 4F). Size: basal actines – 70 (± 5.9)/ 7.7 (± 1.2) µm (N = 14); apical actine – 11.5 (± 10.8)/ 6.1 (± 1.6) µm (N = 6).

Table 2. Spicule measurements of *Borojevia brasiliensis*. A = apical actine; B = basal actine; H = holotype.

Specimen	Spicule	Actine	Length (µm)				Width (µm)				N
			Min	**Mean**	SD	Max	Min	**Mean**	SD	Max	
A49	Tripod		55	**68.8**	6.3	86	5.6	**6.9**	1.2	10.1	20
	Triactine		46.8	**73.3**	7.5	80.1	4.7	**6.7**	0.7	7.5	20
	Tetractine	B	58.6	**70**	5.9	81.7	5.8	**7.7**	1.2	9.9	14
		A	6.3	**11.5**	10.8	35.5	4.6	**6.1**	1.6	9.6	06
MNHN-LBIM. C 1989.2 (H)*	Tripod		67	**81**	8.2	95.7	-	**11**	1.7	-	20
	Triactine		60.9	**78.2**	10.6	102.2	-	**10.8**	1.5	-	20
	Tetractine	B	56.5	**75.3**	10	91.3	-	**10.4**	1.3	-	20
		A	14.4	**36.4**	9.1	50	-	**8**	2.2	-	20
UFRJPOR8827 **	Tripod		75	**98.9**	9.1	112.5	12.5	**13.1**	1.1	15	20
	Triactine		50	**71.1**	9.3	87.5	7.5	**9**	1.3	10	20
	Tetractine	B	50	**73.8**	12.7	100	5	**9.4**	2	12.5	20
		A	35	**46.8**	4.5	52.5	5	**5.1**	0.3	6.3	20

*From Klautau & Valentine (2003), **From Lopes et al. 2025.

Remarks. *Borojevia brasiliensis* was found at the Alcatrazes Archipelago, attached to a small rocky fragment. Upon removing this fragment, the shape formed by the sponge around the substrate was visible. Some small fragments of algae and hydrozoans were also present. This is the first record of *Borojevia brasiliensis* for the São Paulo coastline, thus expanding the known distribution of the species in the Southeastern Brazil region.

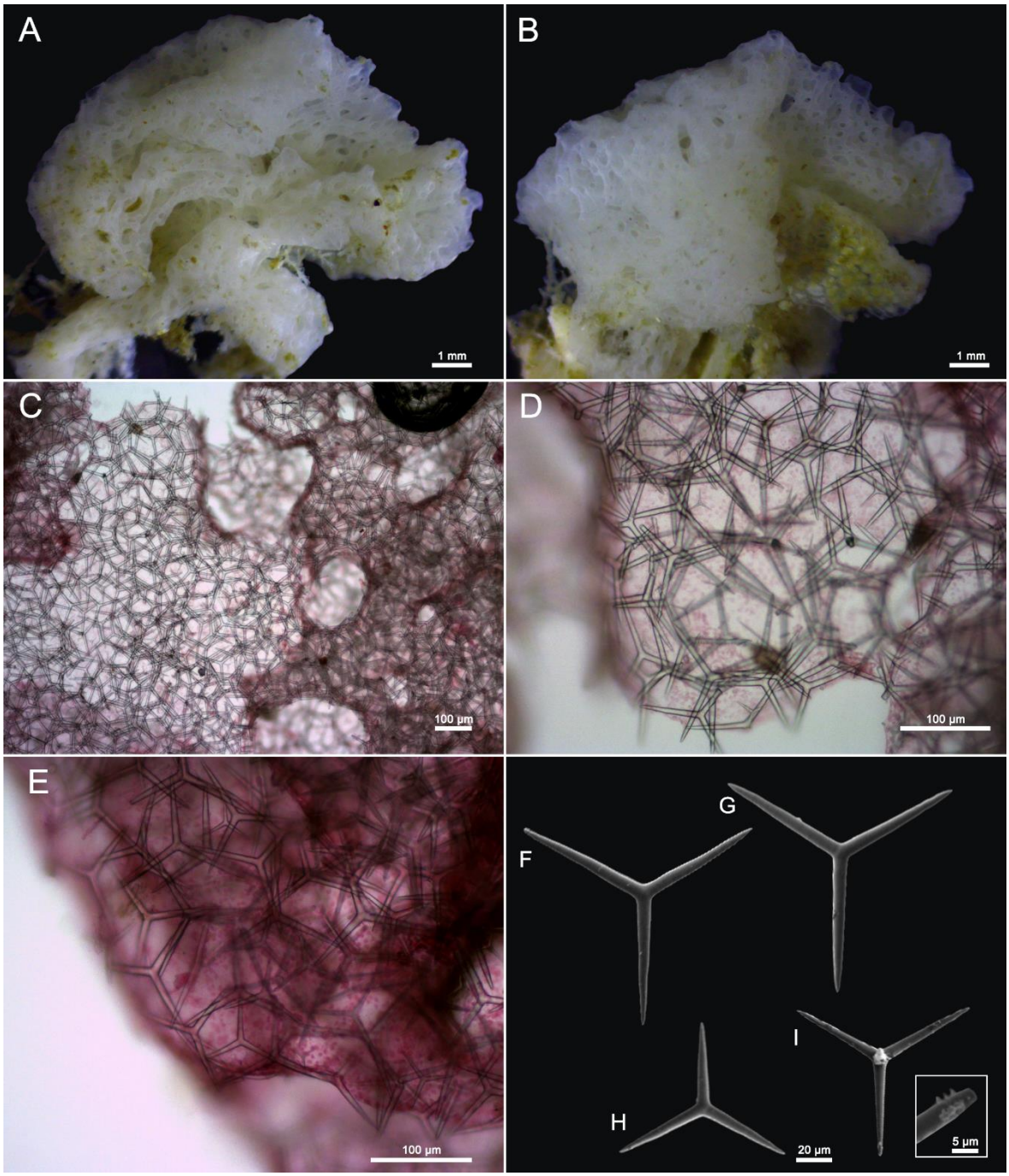


Figure 4. *Borojevia brasiliensis* (Alcatrazes 49). A – Specimen preserved in ethanol (frontal view). B – Posterior view of the specimen, showing the area adherent to the substrate. C – Tangential section of the skeleton. D – Close-up of the skeleton displaying two tetractines. E – Organization of the sponge's outer layer. F - G – Tripods; H – Triactine; I – Tetractine, inset shows the spines in the apical actine.

***Borojevia trispinata* Azevedo, Padua, Moraes, Rossi, Muricy & Klautau, 2017**

Synonyms. *Clathrina aspina*, Azevedo & Klautau 2007: 4, Klautau et al. 2013: 448. *Clathrina* aff. *aspina*, Rossi et al. 2011: 1028. *Borojevia aspina*, Imesek et al. 2014: 22, Lanna & Klautau 2015: 3, Azevedo et al. 2015: 792. *Borojevia* cf. *aspina*, Klautau et al. 2016: 5. *Borojevia trispinata*, Azevedo et al. 2017: 313, Fonseca et al. 2023:67, Lopes et al. 2025: 151.

Material examined. Alcatrazes 33, Coral Garden at Alcatrazes Archipelago (24°05'48.3"S 45°41'41.6"W), 17 m depth, 25.iv.2024, colls. F. Azevedo and S. Stampar.

Morphology and anatomy. White *Borojevia* with tightly anastomosed tubes. Color white in ethanol (Figure 5A). Soft to the touch. Asconoid aquiferous system.

Spicules (Table 3)

Triactines. Regular. Actines are conical, slightly undulated, with blunt to sharp tips. Size: 80.4 (± 7.5)/ 8 (± 1.2) µm (N = 20).

Tetractines. Regular. Basal actines are similar to the triactines. Apical actine are long, with three equidistant spines radially disposed in one row, some tetractines have smooth surfaces with no visible ornamentation. Size: 83 (± 11.7)/ 8.7 (± 1), 80.8 (±8.2)/ 9.3 (± 1.2), 20.3 (± 7)/ 6.5 (± 1.1) µm (N = 20).

Tripods. Regular to sagittal. Large actines, conical and straight, whit blunt to sharp tips. Size: 93.2 (± 9)/ 9.3 (± 1) µm (N = 20).

Table 3. Spicule measurements of *Borojevia trispinata*.

Specimen	Spicules	Actines	Length (µm)				Width (µm)				N
			Min	**Mean**	SD	Max	Min	**Mean**	SD	Max	
A33	Tripods		81.9	**93.2**	9	115.1	7.8	**9.3**	1	11.7	20
	Triactine		65.7	**80.4**	7.5	94.3	5.9	**8**	1.2	11	20
	Tetractine	Basal	53.5	**83**	11.7	96.5	6.4	**8.7**	1	10	20
		Apical	10.3	**20.3**	7	33.3	4.5	**6.5**	1.1	8.5	20
UFRJPOR6419 (H)*	Tripods		62.1	**72.2**	5.6	83.7	8.1	**9.5**	0.9	10.8	20
	Triactine		54	**64.8**	5.5	75.6	6.8	**8**	0.5	9.5	20
	Tetractine	Basal	54	**65.7**	5.4	75.6	6.8	**7.7**	0.6	8.1	20
		Apical	32.4	**48.5**	11.2	78.3	5.4	**5.7**	0.6	6.8	20
BMNH 1999.9.16.3 (H)**	Tripods		62.5	**78.8**	8.8	92.5	-	**9.5**	0.8	-	30
	Triactine		55	**70**	7.5	80	-	**6**	1	-	30
	Tetractine	Basal	52.5	**68.8**	7.8	82.5	-	**6**	1	-	30
		Apical	40	**50.3**	6.3	62.5	-	**5**	-	-	30

UFRJPOR4863***	Tripods		62.2	**73.4**	9	96	-	**8.6**	1.2	-	15
	Triactine		42	**70**	7	80	-	**7.4**	0.8	-	30
	Tetractine	Basal	52	**71.6**	6.4	82	-	**7.8**	0.8	-	30
		Apical	40	**54**	7.8	72	-	**7**	0.8	-	30
UFBAPOR4461****	Tripods		74.4	**86.1**	6.5	86.1	-			-	30
	Triactine		52.1	**71**	6.6	81.5		**8**	0.8		
	Tetractine	Basal	52.3	**69.8**	8	81.7	-	**8**	1.2	-	30
		Apical	9.1	**28.1**	16.8	69.9	-	**6.5**	1.6	-	30
UFRJPOR8820*****	Tripods		78.4	**91.9**	10.5	105.4	12.2	**12.8**	0.7	13.5	08
	Triactine		77.5	**87.3**	6.1	100	10	**11.1**	1	12.5	20
	Tetractine	Basal	75	**88.8**	6.7	100	10	**11.1**	1.2	12.5	20
		Apical	45	**61.1**	8.9	82.5	8.8	**10.1**	0.6	11.3	20

*From Azevedo et al. (2017), **From Klautau & Valentine (2003), ***From Azevedo & Klautau (2007), ****From Fonseca et al. 2023, *****From Lopes et al. 2025.

Remarks. The specimen was found entangled in algae, alongside hydrozoans, bryozoans, and small orange solitary ascidians (not shown). The identification of *Borojevia trispinata* is based primarily on the presence of tetractines with three robust spines arranged in a single radial row on the apical actine (Azevedo et al., 2017). This represents the fifth record of the species and extends its known distribution within the Southeastern Brazil marine ecoregion. The previous record in the region was from Ilha Grande Bay (Botinas Islands) as *Clathrina aspina* in Azevedo & Klautau (2007) and went to subsequent revaluation as *Borojevia trispinata* since Azevedo et al. (2017).

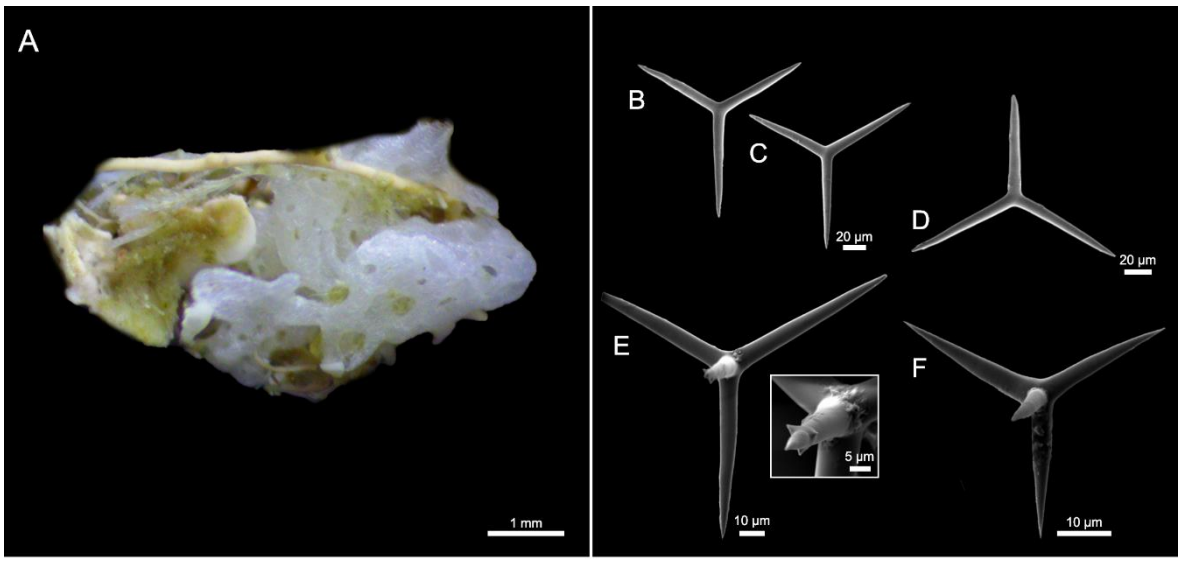


Figure 5. *Borojevia trispinata* (Alcatrazes 33). A – Specimen preserved in ethanol. B - C – Triactines; D – Tripod; E – Tetractine with ornaments, inset emphasizes the spines; F – Tetractine without spine in the apical actine.

Genus *Clathrina* Gray, 1867

Diagnosis. “Calcinea in which the cormus comprises anastomosed tubes. A stalk may be present. The skeleton contains regular (equiangular and equiradiate) and/or parasagittal triactines, to which diactines and tripods may be added. Asconoid aquiferous system” (Klautau et al. 2013).

Type species. *Grantia clathrus* Schmidt, 1864 by original designation.

***Clathrina antofagastensis* Azevedo, Hajdu, Willenz & Klautau, 2009**

Material examined. Alcatrazes 08 and Alcatrazes 32, Coral Garden at Alcatrazes Archipelago (24°05'48.3"S 45°41'41.6"W), 17 m depth, 25.iv.2024, colls. F. Azevedo and S. Stampar.

Morphology and anatomy. White *Clathrina* with greenish undertones. Cormus is formed by irregular and tightly anastomosed tubes. The skeleton is composed by triactines of two sizes. Asconoid aquiferous system.

Synonyms. *Clathrina antofagastensis* Azevedo et al., 2009: 4, Azevedo et al., 2015: 796.

Spicules (Table 4)

Triactine I. Regular to subregular. Actines are conical and slightly undulated, with blunt to sharp tips. Size: 63.1 (± 16.6)/ 6 (± 1) μm (N = 20)

Triactine II. Regular. Actines are small, conical to cylindrical, with blunt tips. Size: 22.4 (± 4.3)/ 4.2 (± 0.5) μm (N = 30)

Table 4. Spicule measurements of *Clathrina antofagastensis*. H = Holotype.

Specimen	Spicule	Length (µm)				Width (µm)				N
		Min	**Mean**	SD	Max	Min	**Mean**	SD	Max	
Clathrina antofagastensis (Alcatrazes 08)	Triactine I	27.1	**63.1**	16.6	78.3	4.2	**6.0**	1.0	8.1	30
	Triactine II	12.7	**22.4**	4.3	27.9	2.7	**4.2**	0.5	5.0	20
Clathrina antofagastensis (Alcatrazes 32)	Triactine I	45.6	**72.3**	10.5	81.3	4.1	**6.6**	0.9	7.4	20
	Triactine II	13.7	**31.3**	6.6	39.6	3.2	**5.0**	0.8	6.3	20
Clathrina antofagastensis (MNRJ 9289)* (H)	Triactine I	69.3	**104.1**	13.3	125.4	-	**10.5**	1.7	-	20
	Triactine II	23.1	**39.6**	11.1	59.4	-	**5**	1.4	-	20
Clathrina antogastensis (MNRJ 13125)**	Triactine I	32.5	**41.5**	4.7	50	-	**5.1**	0.3	-	30
	Triactine II	67.5	**84**	9	100	-	**8**	0.6	-	30

*From Azevedo et al. (2009), **From Azevedo et al. (2017).

Remarks. *Clathrina antofagastensis* was recorded at Alcatrazes Archipelago, representing the first report of this species in the Atlantic Ocean. Although uncommon, two calcinean sponges have been reported on both sides of South America, *Clathrina aurea* Solé-Cava, Klautau, Boury-Esnault, Borojevic & Thorpe, 1991 and *Neoernsta tetractina* (Klautau & Borojevic, 2001). The original description of *C. antofagastensis* from the Chilean coast notes the absence of water-collecting tubes, while specimens from the Peruvian coast possess them. Our specimens (Alcatrazes 08 and 32) also exhibit water-collecting tubes. The uncorrected *p*-distance between our sequences and *C. antofagastensis* (MNRJ 9289) was 6.9% and 5.3%, respectively. They also form a clade with 100% bootstrap support. Our specimens Triactine I are smaller in size when compared with the holotype, but the variation of Triactine II is inside the standard deviation. The measurements from the Peruvian coast are inside the standard deviation for both spicules (all specimens: Triactine I 32.5-**40.3**-1.9-50 x **5.5**-0.4; Triactine II 60-**77.5**-8.7-100 x **8**-0.2; Azevedo et al. 2017).

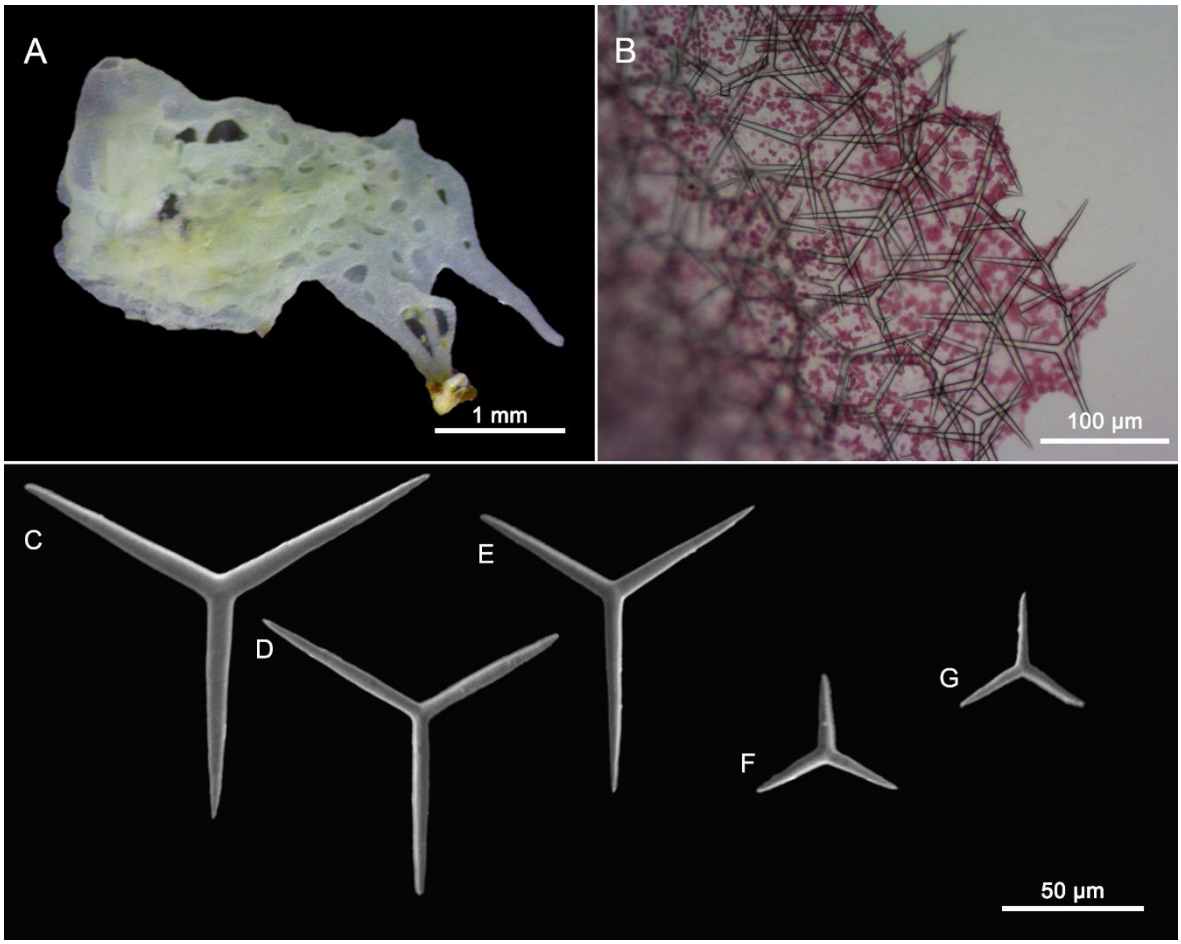


Figure 6. *Clathrina antofagastensis* (Alcatrazes 08). A – Preserved specimen in ethanol; B – Section showing spicule organization; C - E – Triactine I; F - G – Triactine II.

***Clathrina* sp. nov. 1**

Material examined. Alcatrazes 23, Coral Garden at Alcatrazes Archipelago (24°05'48.3"S 45°41'41.6"W), 17 m depth, 25.iv.2024, colls. F. Azevedo and S. Stampar.

Morphology and anatomy. White and translucid *Clathrina* with a stalk that diverges. The cormus has anastomosed tubes in a branch pattern that starts at the stalk and continues into the tips where oscula were found.

Spicules (Table 4)

Triactine I. Parasagittal. Actines are conical and slightly undulated, with blunt to sharp tips. The paired actines are shorter than the unpaired actine. Some spicules have one of the paired actines in another axis. Commun to the stalk. Size: 44.6 (± 4.2), 101.7 (± 26.8)/ 3.2 (± 0.4), 3.9 (± 0.5) µm (N = 20)

Triactine II. Parasagittal. Actines are conical and straight, with blunt to sharp tips. The paired actines are shorter than the unpaired actine. Commun to the cormus. Size: 43.5 (± 3.4), 146.6 (± 43.9)/ 4.9 (± 0.6), 5.8 (± 0.6) µm (N = 20)

Triactine tripod-like. Regular to subregular. Giant. Actines are conical to cylindrical and slightly undulated, with blunt tips. Size: 24.7 (± 11.6)/ 3.1 (± 0.4) µm (N = 14)

Table 4. Spicule measurements of *Clathrina* sp. nov. 1 and *Clathrina* aff. *pulcherrima*.

Specimen	Spicule	Actine	Length (µm)				Width (µm)				N
			Min	**Mean**	SD	Max	Min	**Mean**	SD	Max	
Clathrina sp. nov. 1	Parasagittal I	Paired	35,0	**44,6**	4,2	52,5	2,7	**3,2**	0,4	4,1	20
(Alcatrazes 23)		Unpaired	56,5	**101,7**	26,8	155,6	3,1	**3,9**	0,5	4,7	20
	Parasagittal II	Paired	36,0	**43,5**	3,4	49,8	4,0	**4,9**	0,6	6,5	20
		Unpaired	90,6	**146,6**	43,9	246,6	4,5	**5,8**	0,6	7,0	20
	Tripod-like		13,8	**24,7**	11,6	55,0	2,2	**3,1**	0,4	3,7	14
Clathrina aff. *pulcherrima* (ZMA 22408c)*	Parasagittal Triactine	Paired	61	**114**	-	140	7	**10.1**	-	12	20
		Unpaired	151	**226**	-	267	7.5	**11.5**	-	13	20
	Triactine		59	**101**	-	126	5	**6.3**	-	8	20

*From Van Soest & De Voogd (2018)

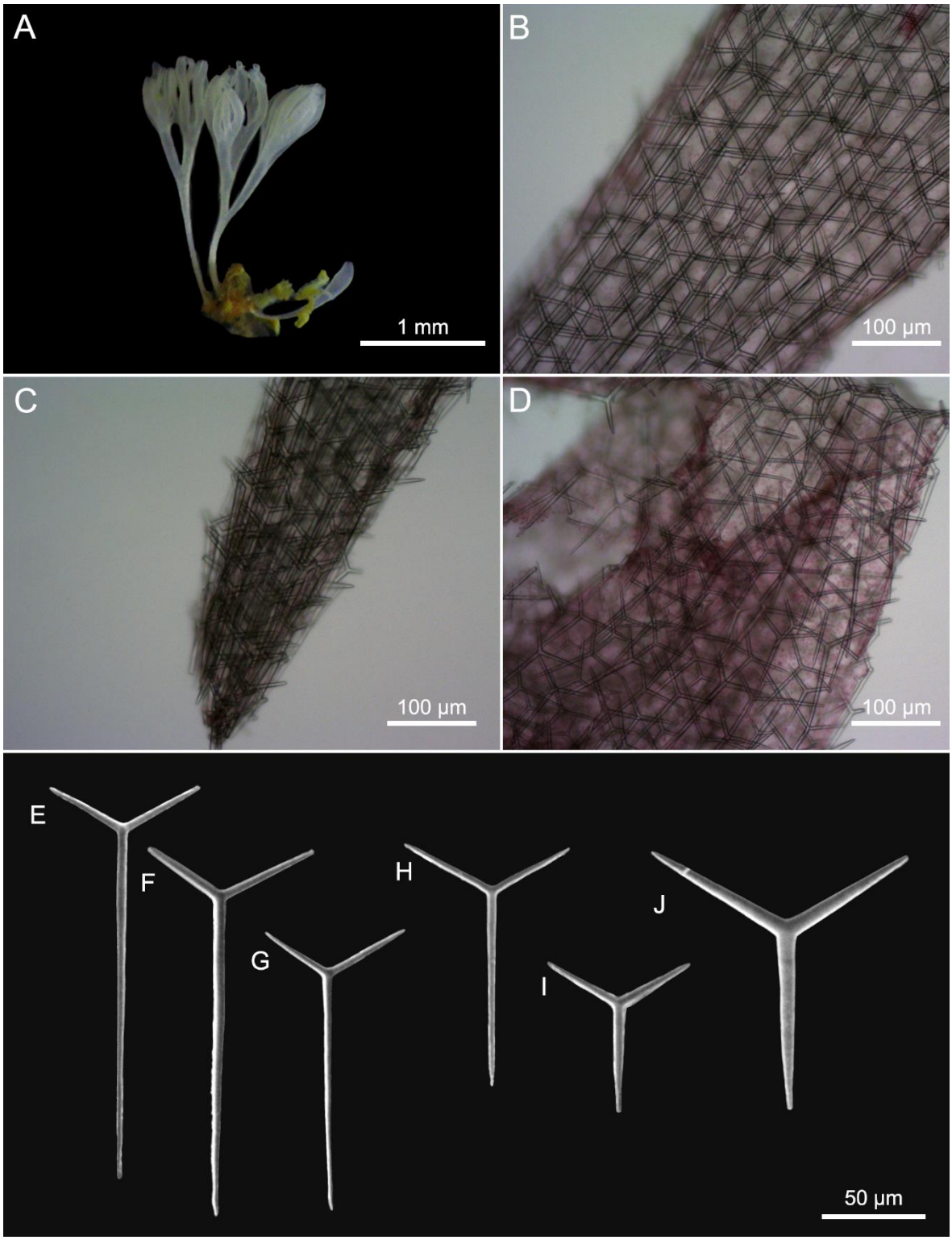


Figure 7. *Clathrina* sp. nov. 1 (Alcatrazes 23). A – Preserved specimen in ethanol; B – Skeleton organization showing the transition between stalk and cormus; C – Skeleton organization showing at the stalk; D - Skeleton organization of the cormus; E - G – Triactine I; H - I – Triactine II; J – Triactine III.

Remarks. The specimen analyzed here belongs to "*Guancha"*-like morphological group, and morphological comparisons were therefore conducted within this clade. Phylogenetic

reconstruction reveals that *Clathrina* sp. nov. 1 forms a supported clade (100% bootstrap) with *Clathrina* aff. *pulcherrima*. This clade is sister to a group containing an undescribed pedunculate *Clathrina* species, supported by a 94% bootstrap value. A neighboring clade (99% bootstrap) includes *Clathrina ramosa*, *Clathrina rotundata*, and *Clathrina lacunosa*, species not recorded along the Brazilian coast. The stalk trait shared among these taxa likely holds phylogenetic significance, as its signal is detectable in the C-LSU region.

***Clathrina* cf. *globulosa* Cóndor-Luján, Louzada, Hajdu & Klautau, 2018**

Material examined. TTP 10, Toque-Toque Pequeno Beach at São Sebastião – SP (23°49'25.0"S 45°32'02.0"W), 0.5 m depth, 30.ix.2023, colls. F. Azevedo and R. Bazzo. Additional material: TTP 07, TTP 08, TTP 09, TTP 11, TTP 12, TTP 13, TTP 14, same as TTP 10.

Morphology and anatomy. White *Clathrina*. The sponge has a short stalk and a dense oval cormus. The anastomosed tubes are small and with few gaps in between. There is a presence of osculum at the top. Asconoid aquiferous system.

Spicules (Table 5)

Triactine I. Parasagittal. Spicules are conical with some presenting a strong undulation near the center of one of the paired actines. The paired ones are smaller than the unpaired (Figure 8D). Blunt tips. Size: 58.6 (±8.1), 100.3 (±19.1)/ 7.6 (±1.5), 9.2 (2,5) μm (N = 12).

Triactine II. Parasagittal. Spicules are conical with slight undulations. The paired actines are almost the same size as the unpaired (Figure 8E-F). Blunt to sharp tips. Size: 36.5 (± 9.9), 62.5 (± 15.2)/ 6.9 (± 1.8), 7.5 (± 1.7) μm (N = 13).

Triactine III. Parasagittal to subregular. Spicules are conical. One of the paired actines is always shorter (Figure 8G). Blunt tips. Size: 45.3 (± 12,3), 67.8 (±25.2)/ 6.5 (±2), 5.9 (±2.1) μm (N = 12).

Triactine tripod-like. Robust. Spicules are conical and slightly straight (Figure 8H). Blunt tips. Size: 50.5 (± 14.8)/ 6.7 (± 1) μm (N = 10).

Table 5. Spicule measurements of *Clathrina* cf. *globulosa* (TTP 10) and UFRJPOR 6759. H = holotype.

Specimen	Spicule	Actine	Length (µm)				Width (µm)				N
			Min	**Mean**	SD	Max	Min	**Mean**	SD	Max	
Clathrina cf. *globulosa* (TTP10)	Parasagittal I	Paired	44,6	**58,6**	8,1	66,7	7,0	**7,6**	1,5	11,7	12
		Unpaired	71,6	**100,3**	19,1	138,1	6,9	**9,2**	2,5	16,5	12
	Parasagittal II	Paired	31,8	**36,5**	9,9	64,2	5,7	**6,9**	1,8	11	13
		Unpaired	41,8	**62,5**	15,2	85,4	5,4	**7,5**	1,7	10,4	13
	Parasagittal III	Paired	38,5	**45,3**	12,3	77,6	4,1	**6,5**	2	10,3	12
		Unpaired	41,9	**67,8**	25,2	128,6	3,8	**5,9**	2,1	10,4	12
	Tripod-like		35,3	**50,5**	14,8	85,9	5,3	**6,7**	1	8,7	10
UFRJPOR 6759* (H)	Triactine I		67.5	**87.2**	11.6	108	4.1	**5.3**	0.4	5.4	30
	Triactine II		45.9	**54.8**	4.6	62.1	5.4	**6.4**	0.9	8.1	30
	Triactine of the stalk	Paired	40.5	**51.8**	5.7	62.1	8.1	**8.1**	0	8.1	10
		Unpaired	94.5	**119.8**	21.6	151.2	5.4	**7.9**	1.8	9.4	7

*From Cóndor-Luján et al. (2018)

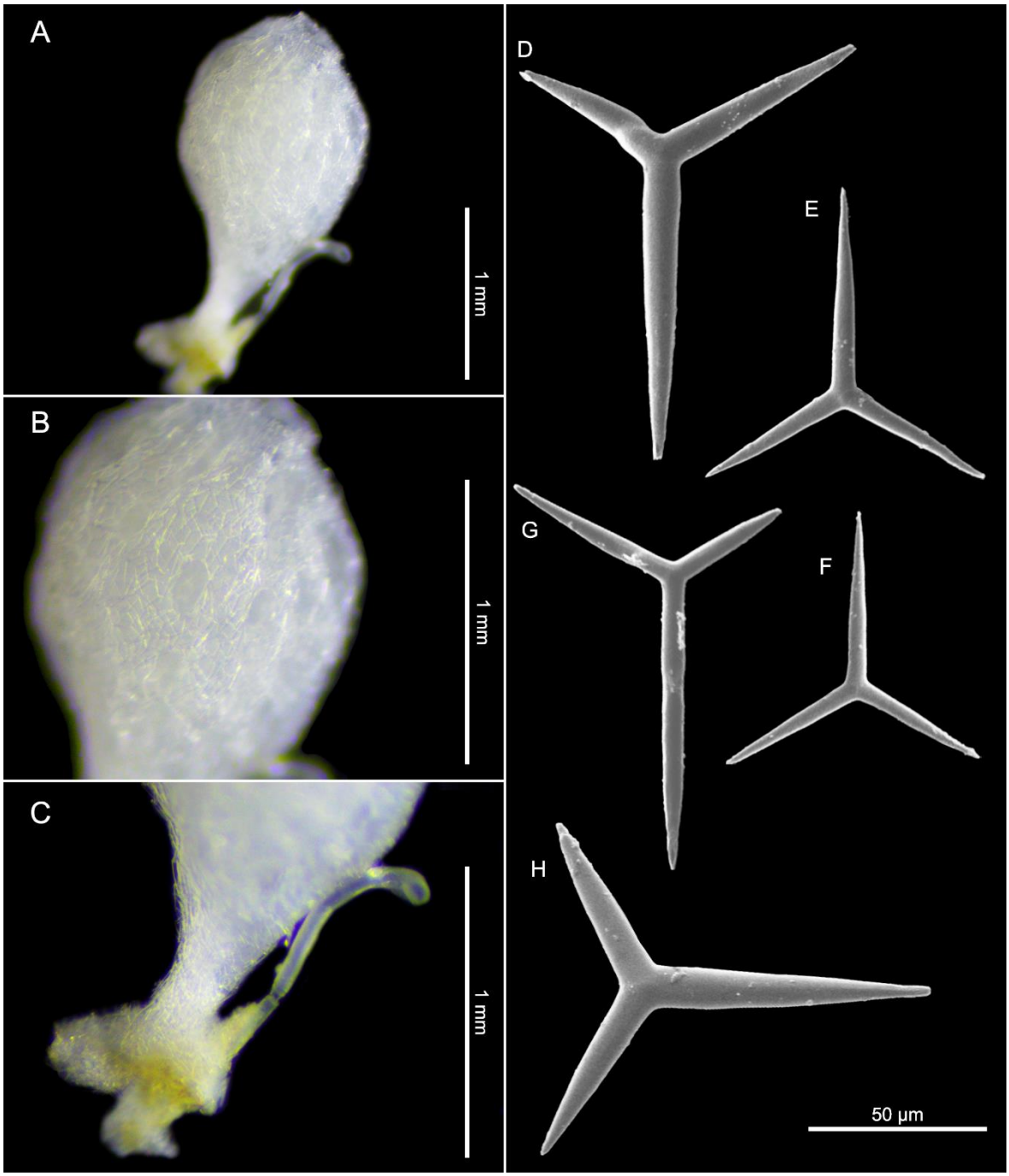


Figure 8. *Clathrina* cf. *globulosa* (TTP10). A – Preserved specimen in ethanol; B – Detail in the cortex region; C –Detail in the stalk; D – Triactine I; E - F – Triactine II; G – Triactine III; H – Tripod-like.

Remarks. *Clathrina* with a stalk are often referred to as "*Guancha*"-like, since all specimens of "*Guancha*" with only triactines were moved to *Clathrina* following Klautau et al. (2013). While examining *Clathrina* species that share this feature, we began to look for differences and affinities. *Clathrina arnesenae* (Rapp, 2006), *C. pellucida* (Rapp, 2006), and *C. camura* (Rapp, 2006) all have the typical parasagittal triactine in the stalk region, although with distinctions such as a 'U' shape in *C. arnesenae*, slightly undulated actines in *C. pellucida*,

and a 'horn shape' in *C. camura*, none of which match the spicules of our specimens. In Curaçao, *Clathrina globulosa* presents two types of regular triactines in the cormus and one category of parasagittal triactines in the stalk. Although our specimen has smaller spicules compared to *Clathrina globulosa*, it often presents a spherical, dense cormus with a stalk. We hypothesize that our individual is a juvenile and that some characteristics are still under development. For that reason, we were unable to separate the spicules into two categories based on body region (stalk and cormus).

Unfortunately, *Clathrina globulosa* does not have a molecular sequence available. In our case, TTP07 was found in a clade with *Clathrina* sp. nov. 1 (Alcatrazes 23) and *Clathrina* aff. *pulcherrima*, with a bootstrap value of 94%, the only relevant shared feature being the presence of a stalk. A neighboring clade (99% bootstrap) includes *Clathrina ramosa* (Azevedo, Hajdu, Willenz & Klautau, 2009), *Clathrina rotundata* Voigt, Erpenbeck & Wörheide, 2017, and *Clathrina lacunosa* (Johnston, 1842). In the ITS tree, TTP12 and TTP14 formed a sister clade to *Clathrina hispanica* Klautau & Valentine, 2003 and *Clathrina blanca* (Miklucho-Maclay, 1868), with a bootstrap value of 42%. As we are still uncertain whether this is the first recorded *Clathrina globulosa* outside of the Caribbean, for now the specimens will be called *Clathrina* cf. *globulosa.*

Family AMPHORISCIDAE, Dendy 1893

Genus *Paraleucilla* Dendy, 1893

Diagnosis. "Amphoriscidae with leuconoid organization. The thick wall is divided into two regions. The outer region is supported by the skeleton which remains essentially inarticulated, with the apical actines of cortical tetractines pointed inwards, and a layer of triactines and/or tetractines with the unpaired actine pointed outwards. The inner region of the choanoskeleton is intercalated between the original subatrial skeleton and the atrial one, and it is supported by large triactines and/or tetractines, that are scattered in disarray, and whose form is similar to the spicules found in the outer layer of the choanoskeleton, or inside the atrial skeleton. Because the original subatrial layer still remains in the outer part of the choanosome, facing the cortical tetractines, there are no typical subatrial spicules adjacent to the atrial skeleton" (Borojevic et al. 2002).

Type species. *Leucandra cucumis* Haeckel, 1872 by subsequent designation in Dendy (1892).

***Paraleucilla* sp. 1**

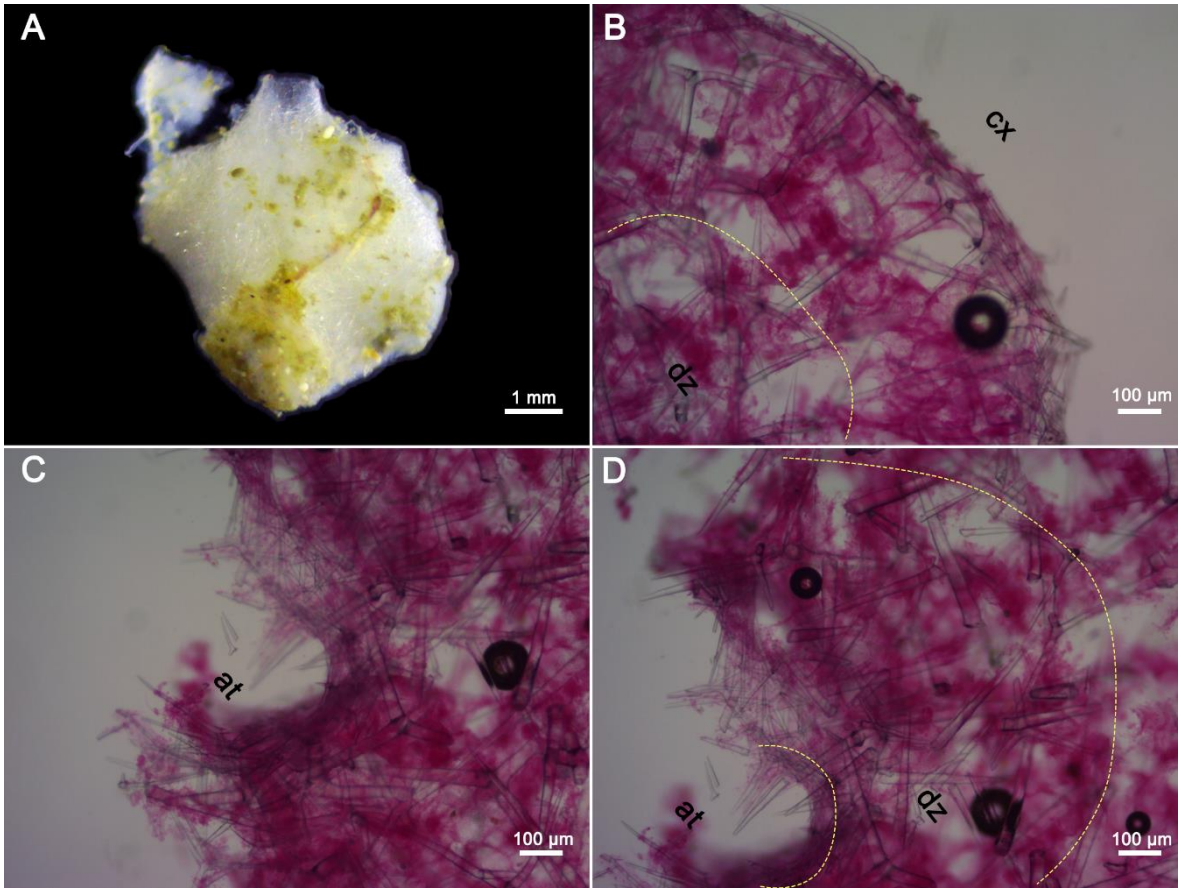


Figure 10. *Paraleucilla* sp. 1 (Alcatrazes 16). A – Preserved specimen in ethanol; B – Cross section of the skeleton showing the cortex (cx) and part of the disorganized zone (dz); C – Cross section of the atrium (at); D – Cross section center in the disorganized zone (dz) delimited by yellow dotted lines.

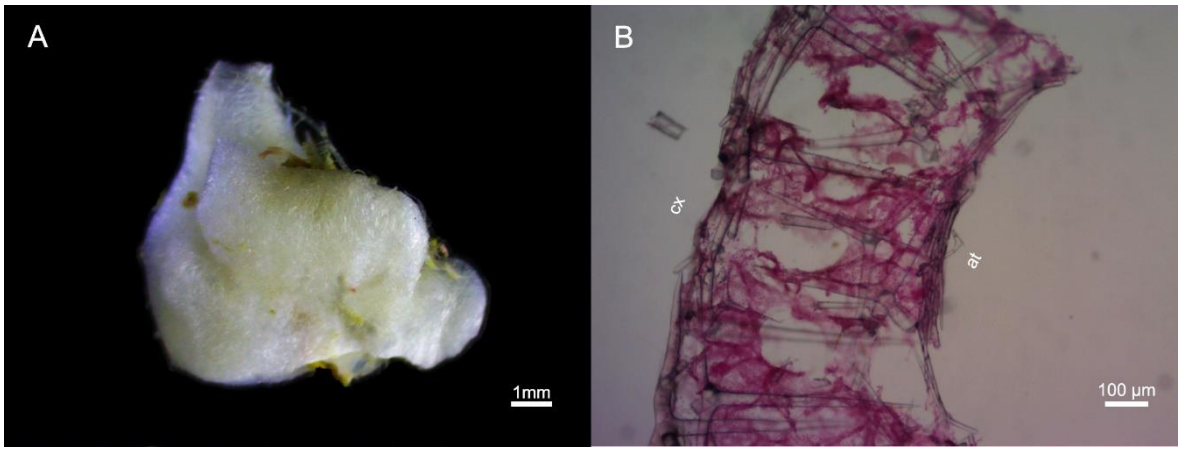


Figure 11. *Paraleucilla* sp. 1 (Alcatrazes 03). A – Preserved specimen in ethanol; B – Cross section of the skeleton showing spicule organization; cortex = cx; atrium = at.

Remarks. Most of the specimens sampled in Alcatrazes Archipelago belonged to a group of *Paraleucilla*-like sponges. Although the classification of *Paraleucilla* is based on a

developmental trait, namely a disorganized zone, this feature is not always visible. In our specimens, Alcatrazes 16 shows a disorganized zone (Figure 10B), while Alcatrazes 3 does not (Figure 11B). In the ML phylogenetic tree, this group formed a distinct clade with 100% bootstrap support and 0% *p*-distance. A sister clade included another *Paraleucilla*-like sponge found at Cigarras Beach (50 km apart), with 75% bootstrap support and 3 to 4% *p*-distance. Together, they formed a sister clade with *Paraleucilla erpenbecki*, supported by a 93% bootstrap value.

Family GRANTIIDAE Dendy, 1892

Genus *Leucandra* Haeckel, 1872

Diagnosis. "Grantiidae with sylleibid or leuconoid organization. Longitudinal large diactines, if present, are not restricted to the cortex, but lie obliquely across the external part of the sponge wall and protrude from the surface of the sponge" (Borojevic et al. 2002).

Type species. *Sycinula egedii* Schmidt, 1870 by subsequent designation in Dendy & Row (1913).

***Leucandra serrata* Azevedo & Klautau, 2007**

Material examined. Cigarras 03, Cigarras Beach at São Sebastião – SP (23°43'42.7"S 45°23'52.0"W), 0.5 m depth, 28.ix.2023, colls. F. Azevedo and R. Bazzo.

Morphology and anatomy. Whitish sponge with scattered beige patches. It is harsh to touch. The external layer exhibits diactines spicules penetrating the cortex and scattered ornamented microdiactines. A single osculum is surrounded by a fringe of trichoxeas. The cortical layer is composed of triactines and diactines. The choanosomal skeleton consists of giant triactines and tetractines surrounding the canals, with their apical actines projecting into the lumen. The atrial region contains tetractines and sparse triactines.

Spicules (Table 6)

Diactines. Large, actine are conical, fusiform and lightly curved, with sharp tips. Size: 880.4 (± 189.9)/ 28.6 (± 8.2) μm (N = 6).

Microdiactines. It is short and thin, with an ornamented tip (jagged) and the other tip is smooth, both tips are sharp (Figure 12C). Sometimes it does not have a jagged tip, being completely smooth. Size: 106.9 (± 36.7)/ 2.4 (± 0.9) μm (N = 20).

Cortical triactines. Regular to subregular. Paired actines can be curved, being larger than the unpaired actine (Figure 12F). Actines are cylindrical to slightly conical, with sharp tips. Size: 201.6 (± 68)/ 7.5 (± 1.9) µm (N = 20).

Choanosomal triactines. Subregular. Large, actines are conical, with sharp tips (Figure 12H). Paired actines are slightly longer than the unpaired actine. Size: 234.4 (± 91.8)/ 103.5 (± 44.5) µm (N = 20).

Canals tetractines. Sagittal. Rare type (not abundant). Actines are conical to cylindrical, whit sharp tips (Figure 12I). Paired actines are longer than the unpaired actine. Apical actine is short and sharp. Size: 155.5 (± 31.4)/ 173.1 (± 54.4)/ 34.4 (±16.2) µm (N = 8).

Atrial tetractines. Sagittal. Actines are cylindrical, with sharp tips. Paired actines are longer than unpaired actine (Figure 12J). Apical actine is shorter than the unpaired actine, has sharp, smooth tips. Size: 250 (± 42.4)/ 134 (± 48.2)/ 49.2 (±23.4) µm (N = 17).

Table 6. Spicule measurements of *Leucandra serrata* (Cigarras 03).

Spicules	Actines	Length (µm)				Width (µm)				N
		Min	Mean	SD	Max	Min	Mean	SD	Max	
Diactine		512.6	880.4	189.9	948.8	14.7	28.6	8.2	37.4	6
Microdiactine		67.6	106.9	36.7	241.1	2.4	0.9	0.9	5.5	20
Cortical triactine	Paired	1031	201.6	68.1	426	5.7	7.4	1.9	13.4	20
	Unpaired	51	103.5	44.5	231	6	8.5	2.4	16.2	20
Choanosomal triactine	Paired	103.7	234.4	91.8	446.6	11.3	20.2	7	35.3	20
	Unpaired	93.2	103.5	44.5	231	12.6	21.2	7.1	36.4	20
Canals tetractine	Paired	101.1	155.5	31.4	188.6	5.8	7.8	1.4	9.5	8
	Unpaired	126.4	173.1	54.4	305.9	4.4	7.8	2.6	12.2	8
	Apical	13.5	34.4	16.2	51.5	3.8	5.6	1.3	7.6	8
Atrial tetractine	Paired	167	250	42.4	319.5	5.5	7.1	0.9	9.5	17
	Unpaired	60	134	48.2	226.6	5.9	8	1.3	11.6	17
	Apical	14.9	49.2	23.4	100.4	4.1	6.2	0.8	7.3	17

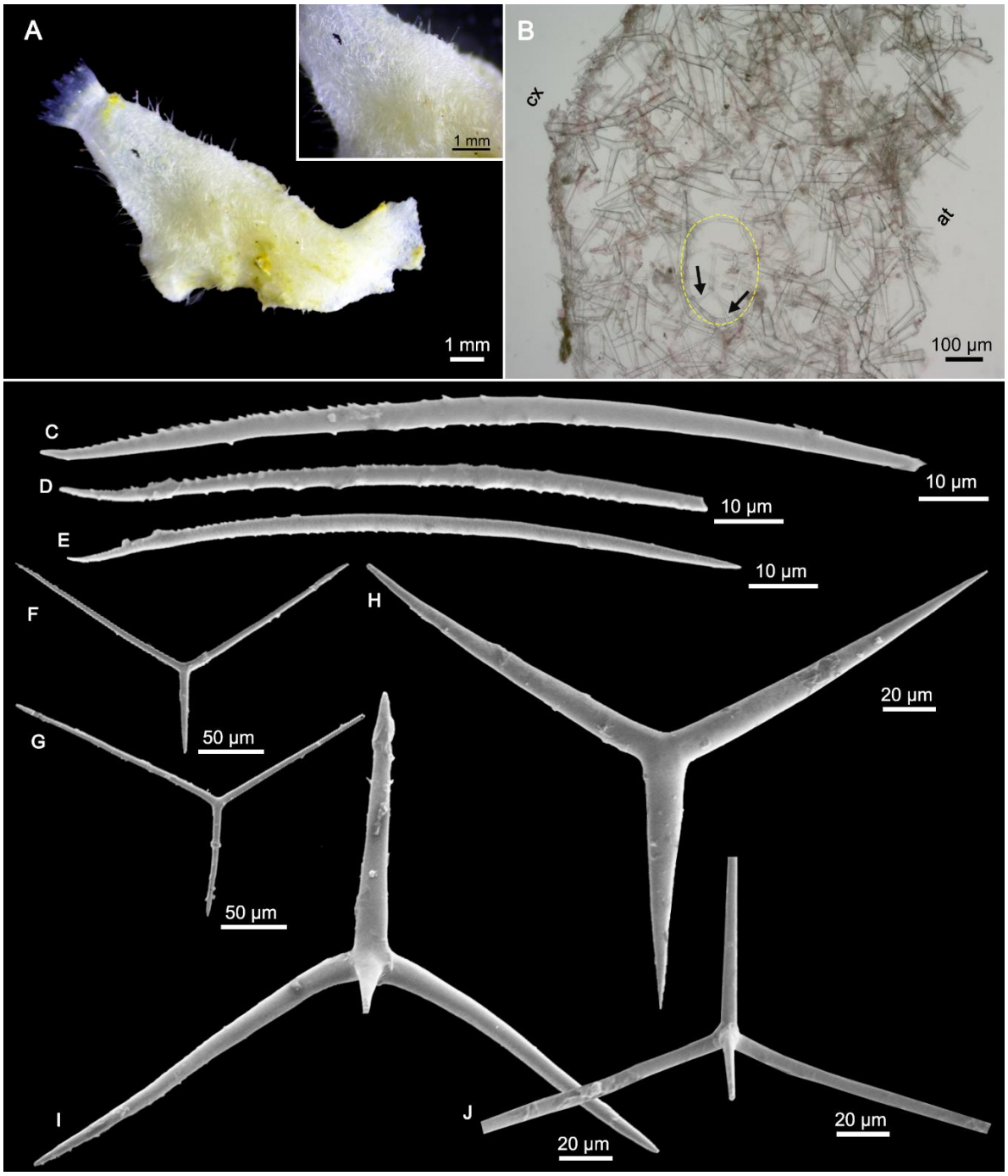


Figure 12. *Leucandra serrata* (Cigarras 03). A – Sponge preserved in ethanol, inset shows the hispid cortex; B – Tangential section of the skeleton (cx – cortex; at – atrium; yellow circle – canal; arrows – apical actine of canals tetractines); C - E – Microdiactines with ornamental spines; F - G – Cortical triactine; H – Choanosomal triactine; I – Canal tetractine; J – Atrial tetractine.

Remarks. *Leucandra serrata* was found on a beach with a rocky shore from São Sebastião. It is a sponge with a rough cortex. In addition to the appearance of the cortex, the sponge presents choanocyte canals with the apical actines of the tetractines protruding into the lumen. Also, the main characteristic of the species, the jagged ornamented microdiactines, was observed. This is the second record of *Leucandra serrata* for the region, the first being from Ilhabela (Oricchio *et al*., 2019). Both specimens were found in the São Sebastião Channel. Additionally, this species has already been recorded along the Brazilian

coast (Potiguar Basin - RN, Salvador -BA, Angra dos Reis - RJ, Sepetiba – RJ, and São Sebastião - SP), with the type locality near São Paulo State, in Ilha Grande Bay (Azevedo & Klautau, 2007). This is the first molecular record for the species. In our ML phylogenetic tree, it forms a sister clade with *Sycon conulosum* and *Leucilla antillana*, with 83% bootstrap support.

SYCONIDAE Poléjaeff, 1883

***Genus Sycon* Risso, 1827**

Diagnosis: "Syconidae with radial tubes partially or fully coalescent; distal cones are decorated by tufts of diactines. The inhalant canals are generally well defined between the radial tubes and are often closed at the distal end by a membrane that is perforated by an ostium, devoid of a skeleton. There is no continuous cortex covering the distal ends of the radial tubes. Skeleton of the atrium and of the tubes composed of triactines and/or tetractines" (Borojevic et al., 2002; Sanamyan & Sanamyan 2021).

***Sycon* sp. 3**

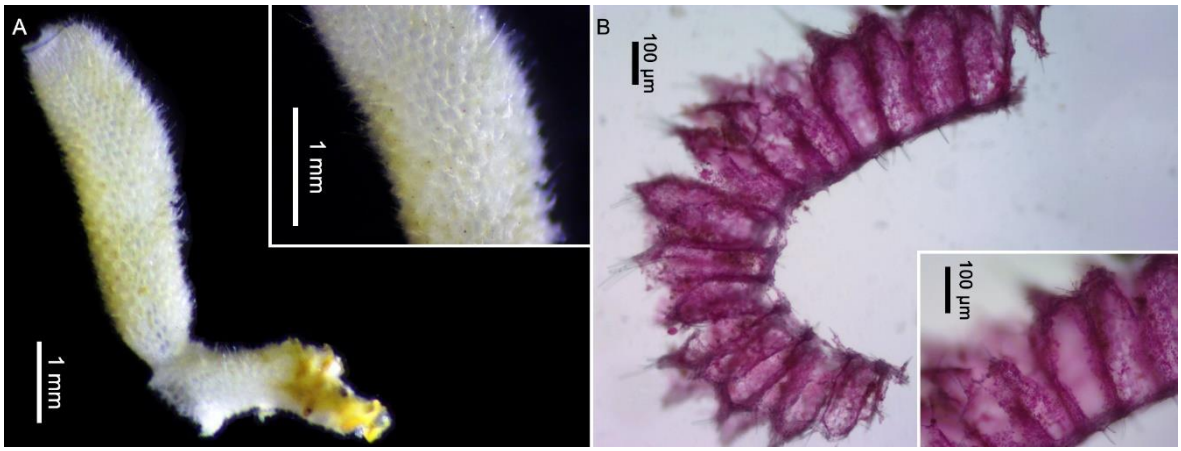


Figure 12. *Sycon* sp. 3. (SRBD03). A – Specimen preserved in ethanol, inset details of the cortex; B – Cross section of the skeleton showing the coalescent tubes with tufts of diactines at the tips, inset shows details of the tubes.

Remarks. *Sycon* is one of the most numerous described genera in Calcaronea, characterized by its tufts of diactines and syconoid aquiferous system. In our sampled sites, we found three distinct Sycon species based on molecular evidence (*p*-distance between them varies from 0 to 8%). At the moment, no further morphological analysis has been conducted. On the São Paulo coast, the only previously recorded species is *Sycon pentactinale* from São Sebastião.

Considerations about Calcarea Biodiversity at Southeastern Brazil Ecoregion

The calcareous sponge biodiversity in Southeastern Brazil remains insufficiently explored in the southern portions of the ecoregion. Along the São Paulo coastline, which can be divided into three main sections, advancements in biodiversity knowledge have been limited to the northern coast. The "Baixada Santista" and southern coastal regions still lack records of calcareous sponges in the literature. As part of ongoing efforts to investigate these areas, we conducted three sampling attempts: at Urubuqueçaba Island, Santos; Cabras Island, Itanhaém; and Enseada Beach, Ubatuba. Although no calcareous sponges were found during these efforts, their presence at these sites cannot be ruled out. In some cases, adverse weather conditions compromised the sampling effort. Notably, the site at Enseada Beach is considered particularly promising due to the presence of a subtidal rocky reef near the beach; however, underwater visibility was poor at the time of sampling. Another area that could not be accessed is part of the Cananéia-Iguape Coastal System (latitudes: 24º40'S – 25º20'S), a complex mangrove environment. Pérez et al. (2017) reported the presence of a *Sycon* sp. specimen in a mangrove habitat on Martinique Island, Caribbean, suggesting that the occurrence of calcareous sponges in brackish waters should be further investigated.

Apart of the São Paulo coast, three other Brazilian states are included in the Southeastern Brazil marine ecoregion: Rio de Janeiro, Paraná, and Rio Grande do Sul. Most of the calcareous sponge diversity reported for the ecoregion is from studies in Rio de Janeiro, where 25 species have been recorded. For the Paraná Coast, only *Clathrina conifera* record is currently available in the literature (Bumbeer et al., 2016). In Rio Grande do Sul, six species have been reported: *Ascandra ascandroides*, *Clathrina conifera*, *C. aurea*, *Paraleucilla magna*, *Sycon ampulla*, and *S. vigilans*. In the portion of the ecoregion corresponding to São Paulo State, species such as *Amphoriscus pedunculatus*, a rarely encountered genus, and *Sycon pentactinale*, characterized by the presence of pentactine spicules (a rare spicule type), suggest that poorly studied areas may conceal significant biodiversity representing distinct calcareous sponge lineages. In our study, we recorded species that expand their known distribution ranges, such as *Borojevia brasiliensis*, *B. trispinata*, *Clathrina antofagastensis, Clathrina* cf. *globulosa*, *Leucandra serrata*, and *Paraleucilla* cf. *perlucida*. With one new species of *Clathrina* yet to be named according to the International Code of the Zoological Nomenclature (ICZN). Additional specimens matched genera that are rarely reported from this region, including *Sycon* (three distinct species), *Leucandra* (two species), and *Paraleucilla* (two distinct species).

Zoogeographical Affinities of São Paulo Species

A total of 14 species were found along the São Paulo coast. These species have records across nine marine ecoregions, all located in South America. Seven of these ecoregions, Southwestern Caribbean, Eastern Caribbean, and Rio Grande as well as Central Peru, Humboldtian, São Pedro and São Paulo, and Southeastern Caribbean, share only 1 to 2 species in common with São Paulo, respectively. Northeastern Brazil shares 3 species, while Eastern Brazil has 5 species in common. It's an expected pattern of wide distribution in the Brazilian coastline since the nearby Eastern Brazil ecoregion has the greatest number of species in common. That is not true for the north neighboring ecoregion, Rio Grande with only 1 species in common (*Clathrina aurea*). Due to various reasons such as taxonomic effort and sampled effort, the Rio Grande ecoregion has some particularities in terms of its geography with most of the coastline being made of sand beaches, in this case calcareous sponges need a hard substrate to survival.

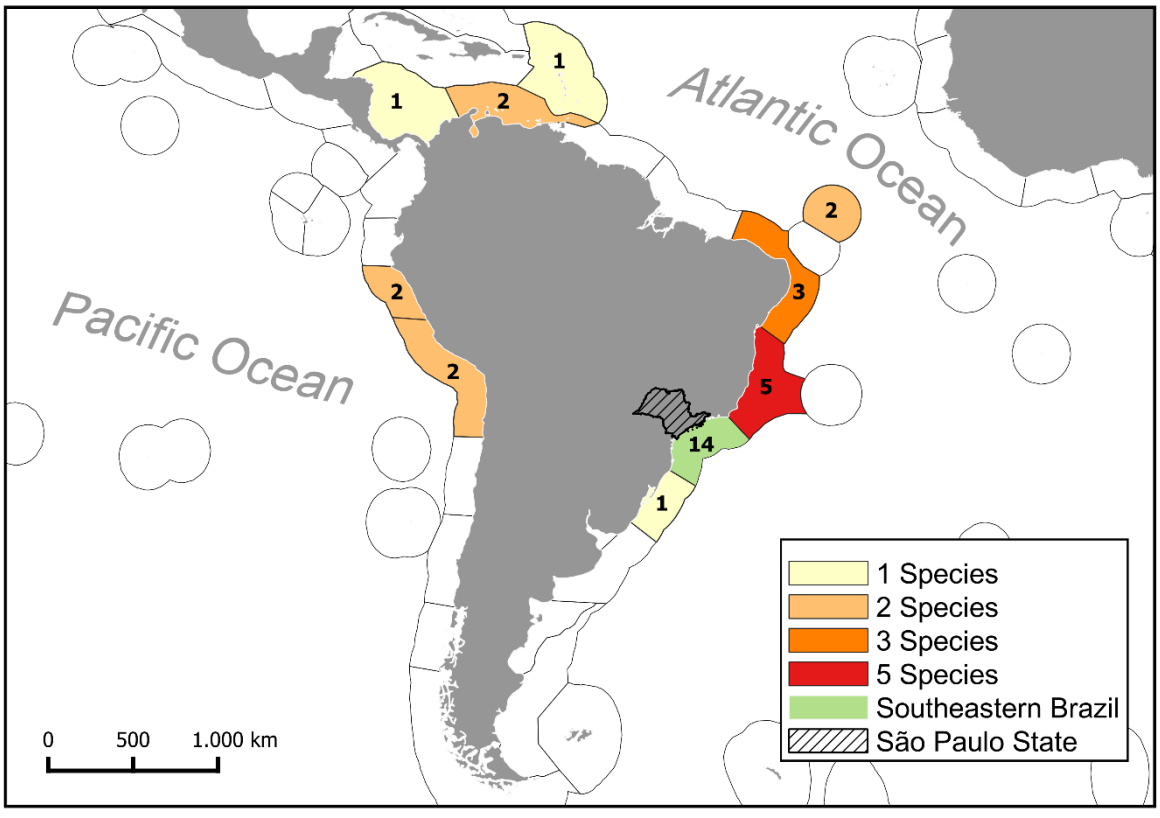


Figure 13. Maps of South America showing the marine ecoregions. Relation between species number found in São Paulo and the species number shared with other regions. At Southeastern Brazil the total number of species found in São Paulo coast, not included exotic invasive species.

When comparing the species, *Clathrina aurea* is the most widely distributed in the region, with records in Central Peru, Humboldtian, Rio Grande, Southeastern Brazil, Eastern

Brazil, Northeastern Brazil, Eastern Caribbean, and Southwestern Caribbean. The other two species connecting the Pacific and Atlantic are *Neoernsta tetractina* (Willenz & Hajdu, 2022) and *Clathrina antofagastensis* from our study. *Neoernsta tetractina* has records in Central Peru and Southeastern Brazil, while *Clathrina antofagastensis* has records in Central Peru, Humboldtian, and Southeastern Brazil. The Eastern Brazil shares the presence of *Amphoriscus pedunculatus*, *Borojevia brasiliensis*, *Borojevia trispinata*, *Leucandra serrata*, and *Leucascus roseus*. In Northeastern Brazil, the shared species are *Clathrina conifera*, *Leucandra serrata*, and *Leucascus roseus*. The oceanic ecoregion of São Pedro e São Paulo shares two species: *Borojevia trispinata* and *Paraleucilla perlucida*. Lastly, the Southeastern Caribbean has *Arturia alcatraziensis* and *Clathrina globulosa*. At last, the only species found in Southeastern Brazil with endemic records in São Paulo coast are *Sycon pentactinale* and *Clathrina* sp. nov. 1.

Conclusions

This study significantly advances our understanding of calcareous sponge (Calcarea) biodiversity along the northern coast of São Paulo, Brazil, through integrated morphological and molecular analyses. By revisiting historical records and describing new occurrences, we have expanded the known distribution of several species, including *Borojevia brasiliensis*, *B. trispinata*, *Clathrina antofagastensis*, and *Leucandra serrata*, while also identifying a potential new species of *Clathrina*. The discovery of *Clathrina antofagastensis* in the Atlantic Ocean highlights the importance of taxonomic revisions and molecular tools in resolving species distributions, particularly for trans-Pacific taxa.

Our phylogenetic analyses revealed distinct evolutionary lineages, with some specimens forming well-supported clades alongside known species, while others exhibited significant genetic divergence, suggesting possible cryptic diversity. The presence of invasive species (*Heteropia glomerosa*) underscores the need for continuous monitoring to assess their ecological impact on native marine communities.

Despite these findings, significant gaps remain in our knowledge of Calcarea diversity, particularly in the southern regions of São Paulo and adjacent states (Paraná and Rio Grande do Sul). The absence of records in areas such as Baixada Santista and the Cananéia-Iguape mangroves may reflect sampling limitations rather than true absence, emphasizing the need for further exploration in underrepresented habitats. Zoogeographically, the São Paulo coast serves as a transitional zone, sharing species with both tropical and subtropical marine ecoregions. Endemic taxa such as *Sycon pentactinale* and the undescribed *Clathrina* sp. nov. highlight the region's unique biodiversity, while widely distributed species like *Clathrina aurea* demonstrate connectivity across South American waters.

References

Azevedo, F., Hajdu, E., Willenz, P., & Klautau, M. (2009). *New records of Calcareous sponges (Porifera, Calcarea) from the Chilean coast*. https://doi.org/10.5281/ZENODO.187091

Azevedo, F., & Klautau, M. (2007). Calcareous sponges (Porifera, Calcarea) from Ilha Grande Bay, Brazil, with descriptions of three new species. *Zootaxa*, *1402*(1). https://doi.org/10.11646/zootaxa.1402.1.1

Azevedo, F., Padua, A., Moraes, F., Rossi, A., Muricy, G., & Klautau, M. (2017). Taxonomy and phylogeny of calcareous sponges (Porifera: Calcarea: Calcinea) from Brazilian mid-shelf and oceanic islands. *Zootaxa*, *4311*(3), 301. https://doi.org/10.11646/zootaxa.4311.3.1

Borges, M., Petti, M. A. V., Fukuda, M. V., Cassano, V., Fujii, M. T., & Amaral, A. C. Z. (2022). Marine planktonic and benthic organisms: An ocean of diversity in the collections of the State of São Paulo. *Biota Neotropica*, *22*(spe), e20221406. https://doi.org/10.1590/1676-0611-bn-2022-1406

Borojevic, R. (1971). Eponges calcaires des côtes du Sud-Est du Brésil, épibiontes sur Laminaria brasiliensis et Sargassum cymosum. *Revista Brasileira de Biologia*, *31*, 525–530.

Borojevic, R., Boury-Esnault, N., Manuel, M., & Vacelet, J. (2002). Order Leucosolenida Hartman, 1958. In J. N. A. Hooper, R. W. M. Van Soest, & P. Willenz (Eds.), *Systema Porifera* (pp. 1157–1184). Springer US. https://doi.org/10.1007/978-1-4615-0747-5_120

Bumbeer, J., Cattani, A. P., Chierigatti, N. B., & Rocha, R. M. D. (2016). Biodiversity of benthic macroinvertebrates on hard substrates in the Currais Marine Protected Area, in southern Brazil. *Biota Neotropica*, *16*(4). https://doi.org/10.1590/1676-0611-BN-2016-0246

Cavalcanti, F. F., Menegola, C., & Lanna, E. (2014). Three new species of the genus Paraleucilla Dendy, 1892 (Porifera, Calcarea) from the coast of Bahia State, Northeastern Brazil. *Zootaxa*, *3764*(5), 537. https://doi.org/10.11646/zootaxa.3764.5.3

Cavalcanti, F. F., Padua, A., Cunha, H., Halasz, M., Nikolić, V., Barreto, S., & Klautau, M. (2020). Population differentiation supports multiple human-mediated introductions of the transatlantic exotic sponge Paraleucilla magna (Porifera, Calcarea). *Hydrobiologia*, *847*(17), 3571–3590. https://doi.org/10.1007/s10750-020-04368-x

Chombard, C., Boury-Esnault, N., & Tillier, S. (1998). Reassessment of Homology of Morphological Characters in Tetractinellid Sponges Based on Molecular Data. *Systematic Biology*, *47*(3), 351–366. https://doi.org/10.1080/106351598260761

Cóndor-Luján, B., Louzada, T., Hajdu, E., & Klautau, M. (2018). Morphological and molecular taxonomy of calcareous sponges (Porifera: Calcarea) from Curaçao, Caribbean Sea. *Zoological Journal of the Linnean Society*, *183*(3), 459–525. https://doi.org/10.1093/zoolinnean/zlx082

Costello, M. J., Coll, M., Danovaro, R., Halpin, P., Ojaveer, H., & Miloslavich, P. (2010). A Census of Marine Biodiversity Knowledge, Resources, and Future Challenges. *PLoS ONE*, *5*(8), e12110. https://doi.org/10.1371/journal.pone.0012110

Fonseca, E. S. M., Cóndor-Luján, B., & Cavalcanti, F. F. (2023). Calcareous sponges (Porifera, Calcarea) from the Northeastern Brazilian coast: Integrative taxonomy and support for the creation of a marine park. *Marine Biodiversity*, *53*(5), 67. https://doi.org/10.1007/s12526-023-01364-x

Hoang, D. T., Chernomor, O., Von Haeseler, A., Minh, B. Q., & Vinh, L. S. (2018). UFBoot2: Improving the Ultrafast Bootstrap Approximation. *Molecular Biology and Evolution*, *35*(2), 518–522. https://doi.org/10.1093/molbev/msx281

Katoh, K., Rozewicki, J., & Yamada, K. D. (2019). MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics*, *20*(4), 1160–1166. https://doi.org/10.1093/bib/bbx108

Katoh, K., & Toh, H. (2008). Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. *BMC Bioinformatics*, *9*(1), 212. https://doi.org/10.1186/1471-2105-9-212

Klautau, M., Azevedo, F., Condor-Lujan, B., Rapp, H. T., Collins, A., & Russo, C. A. d. M. (2013). A Molecular Phylogeny for the Order Clathrinida Rekindles and Refines Haeckel's Taxonomic Proposal for Calcareous Sponges. *Integrative and Comparative Biology*, *53*(3), 447–461. https://doi.org/10.1093/icb/ict039

Klautau, M., & Borojecic, R. (2001). Sponges of the genus Clathrina Gray, 1867 from Arraial do Cabo, Brazil. *Zoosystema*, *3*, 395–410.

Klautau, M., Cavalcanti, F. F., & Borojevic, R. (2017). The new sponge species Amphoriscus pedunculatus (Porifera, Calcarea). *Zootaxa*, *4341*(1), 105. https://doi.org/10.11646/zootaxa.4341.1.9

Klautau, M., Monteiro, L., & Borojevic, R. (2004). *First occurrence of the genus Paraleucilla (Calcarea, Porifera) in the Atlantic Ocean: P. m a g n a sp. nov.* https://doi.org/10.5281/ZENODO.158320

Klautau, M., Solé-Cava, A. M., & Borojevic, R. (1994). Biochemical systematics of sibling sympatric species of Clathrina (Porifera: Calcarea). *Biochemical Systematics and Ecology*, *22*(4), 367–375. https://doi.org/10.1016/0305-1978(94)90027-2

Klautau, M., & Valentine, C. (2003). Revision of the genus Clathrina (Porifera, Calcarea). *Zoological Journal of the Linnean Society*, *139*(1), 1–62. https://doi.org/10.1046/j.0024-4082.2003.00063.x

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Lanna, E., Rossi, A. L., Cavalcanti, F. F., Hajdu, E., & Klautau, M. (2007). Calcareous sponges from São Paulo State, Brazil (Porifera: Calcarea: Calcinea) with the description of two new species. *Journal of the Marine Biological Association of the United Kingdom*, *87*(6), 1553–1561. https://doi.org/10.1017/S0025315407056871

Lôbo-Hajdu, G., Guimarães, A. C. R., Salgado, A., Lamarão, F. R. M., Vieiralves, T., Mansure, J. J., & Albano, R. M. (2004). Intragenomic, Intra- and Interspecific Variation in the rDNA ITS of Porifera Revealed by PCR-Singlestrand Conformation Polymorphism (PCR-SSCP). *Bolletino Dei Musei e Degli Istituti Biologici Dell'Universitá Di Genova*, *68*, 413–423.

Lopes, M. V., Padua, A., Azevedo, F., & Klautau, M. (2025). Integrative taxonomy of Calcarea (Porifera) from Espírito Santo, Eastern Brazil. *Zootaxa*, *5618*(2), 151–205. https://doi.org/10.11646/zootaxa.5618.2.1

Minh, B. Q., Nguyen, M. A. T., & Von Haeseler, A. (2013). Ultrafast Approximation for Phylogenetic Bootstrap. *Molecular Biology and Evolution*, *30*(5), 1188–1195. https://doi.org/10.1093/molbev/mst024

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., Von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Molecular Biology and Evolution*, *37*(5), 1530–1534. https://doi.org/10.1093/molbev/msaa015

Oricchio, F. T., Marques, A. C., Hajdu, E., Pitombo, F. B., Azevedo, F., Passos, F. D., Vieira, L. M., Stampar, S. N., Rocha, R. M., & Dias, G. M. (2019). Exotic species dominate marinas between the two most populated regions in the southwestern Atlantic Ocean. *Marine Pollution Bulletin*, *146*, 884–892. https://doi.org/10.1016/j.marpolbul.2019.07.013

Pérez, T., Díaz, M.-C., Ruiz, C., Cóndor-Luján, B., Klautau, M., Hajdu, E., Lobo-Hajdu, G., Zea, S., Pomponi, S. A., Thacker, R. W., Carteron, S., Tollu, G., Pouget-Cuvelier, A., Thélamon, P., Marechal, J.-P., Thomas, O. P., Ereskovsky, A. V., Vacelet, J., & Boury-Esnault, N. (2017). How a collaborative integrated taxonomic effort has trained new spongiologists and improved knowledge of Martinique Island (French Antilles, eastern Caribbean Sea) marine biodiversity. *PLOS ONE*, *12*(3), e0173859. https://doi.org/10.1371/journal.pone.0173859

Rossi, A. L., Farina, M., Borojevic, R., & Klautau, M. (2006). Occurrence of five-rayed spicules in a calcareous sponge: Sycon pentactinalis sp. Nov. (Porifera: Calcarea). *Cahiers de Biologie Marine*, *47*(3), 261-270.

Sanamyan, K., & Sanamyan, N. (2021). Notes on nomenclature of Sycetta Haeckel, 1872 and reinstating the family Syconidae Poléjaeff, 1883 (Calcarea, Porifera). *Zootaxa*, *5067*(1), 149–150. https://doi.org/10.11646/zootaxa.5067.1.12

Solé-Cava, A. M., Klautau, M., Boury-Esnault, N., Borojecic, R., & Thorpe, J. P. (1991). Genetic evidence for cryptic speciation in allopatric populations of two cosmopolitan species of the calcareous sponge genusClathrina. *Marine Biology*, *111*(3), 381–386. https://doi.org/10.1007/BF01319410

Van Soest, R. W. M., & De Voogd, N. J. (2018). Calcareous sponges of the Western Indian Ocean and Red Sea. *Zootaxa*, *4426*(1). https://doi.org/10.11646/zootaxa.4426.1.1

Voigt, O., & Wörheide, G. (2016). A short LSU rRNA fragment as a standard marker for integrative taxonomy in calcareous sponges (Porifera: Calcarea). *Organisms Diversity & Evolution*, *16*(1), 53–64. https://doi.org/10.1007/s13127-015-0247-1

Willenz, P., & Hajdu, E. (2022). Marine and Freshwater Sponges of Peru (Vol. 22). Royal Belgian Institute of Natural Sciences.

Chapter 03: New Records of Invasive Calcareous Sponge *Heteropia glomerosa* (Calcarea, Calcaronea, Leucosolenida, Heteropiidae) on the Coast of São Paulo, Brazil

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Abstract

In this work, we present evidence from our investigation in the coastline of São Paulo state, identifying the presence of the exotic invasive *Heteropia glomerosa* (Bowerbank, 1873), observed growing on natural substrate for the first time in the region. Previous records were observations on artificial substrate (PVC panels) from Ilhabela and São Sebastião. All records herein are from beaches never sampled before, which represents an advancement in understanding the potential for colonization of this species along the São Paulo coastline. Additionally, this finding on the natural substrate is noteworthy since previous observations along the Brazilian coast indicated that its colonization occurs most frequently on artificial substrates such as ropes, docks, wood, plastic, concrete and PVC panels and the favorite habitats were inside marinas, ports and harbors. These new records to São Paulo demonstrate the ability of this species to reach the southernmost coast. Notably, a specimen was found living in association with a cryptogenic black ascidia, *Phallusia nigra* Savigny, 1816, demonstrating an ecological interaction never mentioned in the literature. Further investigations are needed to understand the behavior of spread, other ecological interactions and evaluate possible environmental impacts of this calcareous sponge by including long-term environmental monitoring actions.

Keywords: alien species, marine invertebrates, natural substrate, southeastern ecoregion

Introduction

Heteropia glomerosa (Bowerbank, 1873) is one of the three exotic calcareous sponges record for the Brazilian coast. The other two are *Paraleucilla magna* Klautau, Monteiro & Borojevic, 2004 and *Sycettusa hastifera* (Row, 1909), (Cavalcanti et al. 2020; Klautau et al. 2020; Ribeiro et al. 2021). So far, non-positive or negative impacts have been reported to the biological communities living nearby or in association with these three calcareous sponges, nor environmental or economic damage.

According to COP 6 recommendation Decision VI/23 of the Conference of the Parties to the Convention on Biological Diversity, no proof of impact is required for classifying exotic species as invasive. Therefore, in the present study we are adopting the status of exotic invasive for *H. glomerosa*, but the same could be applied for the other two species (Dechoum et al., 2024). Additionally, recent papers using *H. heteropia* as a biological model for studies on reproduction (Calazans and Lanna, 2019), recruitment dynamics (Chagas et al., 2020) and integrative taxonomy (Klautau et al., 2020) have obtained results that corroborate this view.

Calazans and Lanna (2019) mentioned that *H. glomerosa* was very abundant in Todos os Santos Bay, Salvador, Bahia, and was generally found as large, conspicuous and easily accessible species. They also revealed its opportunistic life history strategy characterized by reproduction early in its life cycle, producing abundant propagules, giving the species the greatest reproductive effort among sponges. In addition, *H. glomerosa* showed continuous reproductive activity, which is a typical characteristic exhibited by the two other species of invasive exotic calcareous sponges, *P. magna* and *S. hastifera*. The authors suggested that the rainy season is the best time for field action efforts to prevent the expansion and establishment of the species.

Chagas, Barros and Cavalcanti (2020), studying the dynamic of recruitment of *H. glomerosa* found that the number of specimens of *H. glomerosa* found on the plates were extremely low. Also, they noticed thar no clear patterns of recruitment were observed for the species. Although no significant variation in the number of recruits over time was found, they observed the highest number of recruits between February and April 2016, mainly in the dry season. The authors hypothesized that the short period of immersion and the ecological requirements of an initially pre-established fouling community are necessary for the species to establish itself.

Klautau et al (2020) commented on the notorious abundance and fast spreading of *H. glomerosa* along the Brazilian coast since its first appearance in 2005 to the year of publication of the study in 2020, covering the distance of 2,900 km. In addition, the authors found that *H. glomerosa* met seven of the 10 criteria proposed by Chapman and Carlton (1991) for recognizing an exotic species and hypothesized that biofouling was the main source of the sponge's introduction into the Western Atlantic. They also discussed the species' ability to colonize geographical areas with very distinct seawater temperatures, such contrast is found mainly between the northeast and southeast coasts of Brazil.

Among the three calcareous sponges, *Heteropia glomerosa* is the most widely dispersed, being present in the northeastern, eastern and southeastern ecoregions of Brazil. The environment conditions and type of habitat varies significantly throughout this range, making it the most successful exotic invasive calcareous sponge to colonize such an extensive latitudinal gradient. In the southeastern ecoregions, records from the coast of the state of Rio de Janeiro have been known for at least 30 years (Klautau *et al*., 2020), while the presence of the species further south, on the coast of the state of São Paulo, was only confirmed in 2016 during a biofouling experiment (Oricchio *et al*., 2019).

Studies on ecological interactions with calcareous sponges are scarce. An investigation into the associated macrofauna was carried out with the species *Paraleucilla magna* (Pádua, Lanna and Klautau, 2013) in small scale, but no such study exists for *H. glomerosa* and *S. hastifera.* There is no data on the ecological association between *H. glomerosa* and the local fauna at any of the sites where the species occurs.

Although, Calazans and Lanna (2019) had reported the removal of the associated organisms (crustaceans, ascidians, algae and others) during the cleaning of *H. glomerosa* before histological procedure; also, Chagas, Barros and Cavalcanti (2020) had drawn attention to several organisms (colonial and solitary ascidians, demosponges and algal turfs, barnacles, serpulids and possibly hydroids) found colonizing the polyester cables on the artificial plates with the calcareous sponges; these examples do not mentioned evidence of direct interaction of these associated organisms with the sponge *H. glomerosa.*

In this note, we report the first occurrence of this calcareous sponge on natural substrates along the São Paulo coast, comment on the association with an ascidian, also considered as potentially exotic species and discuss potential implications for the local fauna.

Material and methods

Sampling sites

In this study, all samples were carried out at low tide, at depths of up to 0.5 meters in rocky shore environments. At Saco da Ribeira Bay (Figure 1A), the landscape consists of a bay with small boats and yachts in a highly polluted environment with suspended sediments (CETESB, 2024). Two samplings were conducted in this area, one in April and the other in October 2023. At Pedras Miudas Beach sampling occurred in September 2023. Pedras Miudas Beach (Figure 1B) is a small beach facing the São Sebastião Channel, an area with constant large ship traffic, close to Cabras Island, frequented by swimmers and small boats. At this site, one individual was found at the base of a *Phallusia nigra* Savigny, 1816, a cryptogenic sea squirt (Figure 2C).

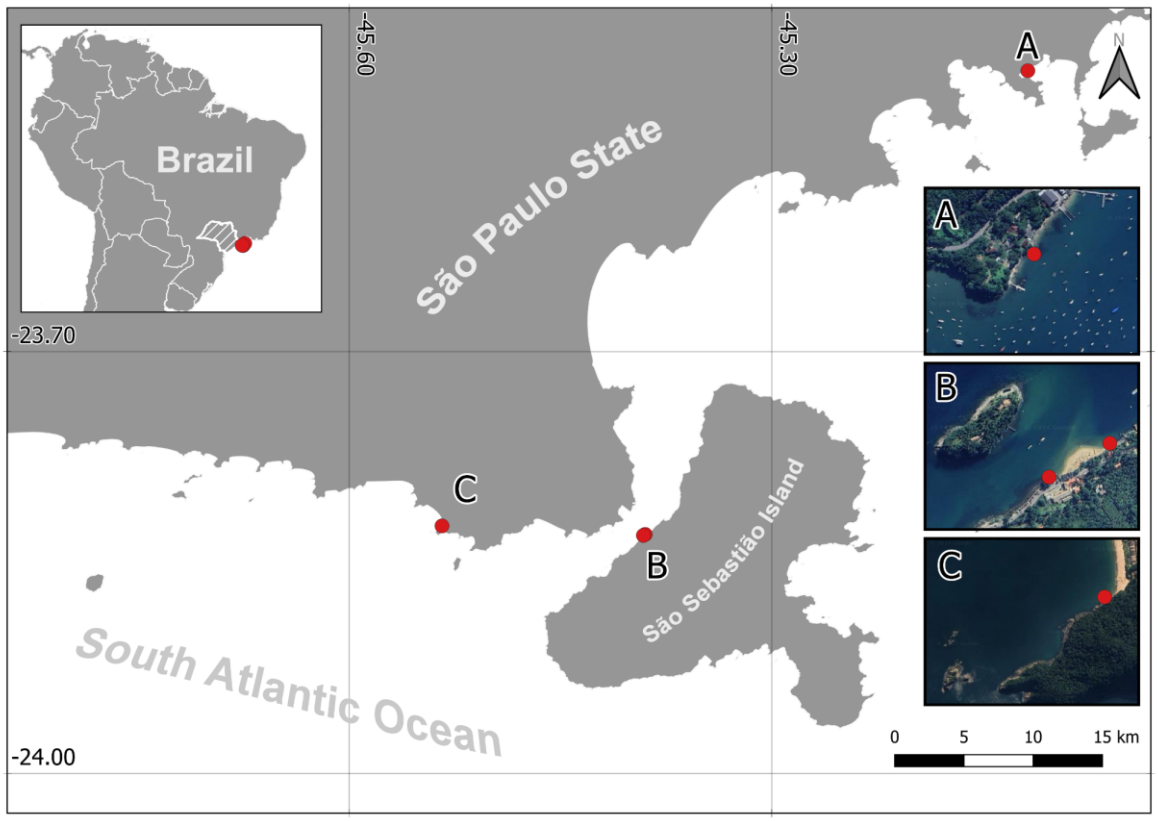


Figure 1. Map illustrating the study area located on the northern coastline of São Paulo State. A = Saco da Ribeira Bay, B = Pedras Miúdas Beach, C = Toque-Toque Pequeno Beach.

Collection and preservation

Rocky shore areas investigation and sponge sampling were conducted throughout 2023 in three municipalities along the coast of São Paulo State (Figure 1), which are included in the Southeastern Brazil marine ecoregion (Spalding *et al.*, 2007). A total of 23 specimens were collected: 13 specimens at Saco da Ribeira Bay (SRB) (23°30'00.6"S – 45°07'05.6"W) 9 specimens at Pedras Miudas Beach (PMB) (23°49'47.7"S – 45°23'23.4"W) and 1 specimen at Toque-Toque Pequeno Beach (TTPB) (23°49'25.6"S – 45°32'02.2"W). Samples were fixed in 96% alcohol and later transferred to 100% alcohol.

Morphological identification

The spicule composition of the specimens was analyzed under the microscope (Nikon), pictures were captured with a camera (Motic Image Plus 2.0) and measurements were done on computer using software ImageJ v1.54g. Only one specimen from each site was measured (Figure 2B). The results are presented in tabular form, including the minimum (min), mean, standard deviation (SD), and maximum (max) values for both measurements, along with the number of spicules analyzed (N) (Table 1). Identification followed standard protocols (Klautau and Valentine, 2003) with a subsequent modification, the substitution of Canada balsam for Entellan (Merck) as the mounting medium for spicule slides (Cavalcanti, Menegola and Lanna, 2014).

Genetic identification

When possible, DNA sequencing was performed to complement morphological analysis. DNA was extracted using an Easypure Genomic DNA Kit (TransGen Biotech). The C-region of the Large Subunit (LSU) rRNA was amplified by polymerase chain reaction (PCR) with the primers: fwd 50-GAAAAGCACTTTGAAAAGAGA-30 (Voigt and Wörheide, 2016) and rv 50- TCCGTG TTTCAAGACGGG-30 (Chombard, Boury-Esnault and Tillier, 1998). PCR mixes contained 1 buffer (Taq DNA Polimerase 5U, Ludwig), 0.2 mM dNTP, 2.5 mM MgCl2, 0.33 mM of each primer, one unit of Taq DNA polymerase (Ludwig) and 100–150 ng of DNA in a final volume of 25 μL. Amplification followed a first cycle of 4 min at 94°C, 1 min at 50°C and 1 min at 72°C, 35 cycles of 1 min at 92°C, 1 min at 50°C and 1 min at 72°C, with a final cycle of 6 min at 72°C. PCR product was purified using DNA Extraction and Purification Kit (NovaBiotecnologia). Sequences were trimmed and edited using Geneious v11.1.5. BLAST searches were performed to confirm the identity of the sequences (http://www.ncbi.nlm.nih. gov/blast/). Sequences from GenBank were combined with newly generated ones (Table 1) to construct a phylogenetic tree. DNA

alignments were performed using the online MAFFT v7 platform (Katoh et al., 2019) with the Q-INS-i strategy (Katoh & Toh, 2008). Default parameters were applied. Tree construction was performed in IQ-TREE v2.4.0 (Minh et al., 2020), applying the best-fit substitution model for the C-LSU dataset (TIM+F+I+G4), as determined by the Bayesian Information Criterion (BIC). Branch support was calculated using ultrafast bootstraps (Minh et al., 2013; Hoang et al., 2018) with 100,000 replicates. Final trees were rendered in FigTree v1.4.0 and stylized in Adobe Photoshop.

Table 1. Species, ecoregions, vouchers, and GenBank accessions used in phylogenetic analyses. New sequences in bold.

Species	Marine Ecoregion	Voucher Number	GenBank Accession (C-LSU)
Anamixilla torresi	Halmahera	RMNH 6572	MF686072
Grantessa tumida	Southern Caribbean	UFRJPOR 6701	MF472616
Grantessa woerheidei	Southern Red Sea	RMNH 9586	MF872773
Heteropia glomerosa	Southeastern Brazil	**TTP39**	*
Heteropia glomerosa	Southeastern Brazil	**PPM12**	*
Heteropia glomerosa	Eastern Brazil	UFRJPOR 6151	MN402721
Heteropia glomerosa	Eastern Brazil	UFRJPOR 6191	MN402722
Heteropia glomerosa	Eastern Brazil	UFRJPOR 7608	MN402723
Heteropia glomerosa	Eastern Brazil	UFRJPOR 7609	MN402724
Heteropia minor	Sulawesi Sea/Makassar Strait	RMNHPOR 1872	MF686070
Heteropia minor	Banda Sea	RMNHPOR 3603	MF686068
Heteropiidae sp.	St. Helena and Ascension Islands	RMNHPOR-SA86	MH385248
Sycettusa hastifera	Northern and Central Red Sea	ZMA 13443	MF872778
Sycettusa hirsutissima	Northern and Central Red Sea	RMNH 9588	MF872776
Sycettusa simplex	Maldives	RMNH 10154	MF872779
Sycettusa zanzibaris	Seychelles	ZMA 11568	MF872783
Uteopsis argentea	Sulawesi Sea/Makassar Strait	RMNHPOR 1871	MF686074
Uteopsis argentea	Sunda Shelf/Java Sea	RMNHPOR 2598	MF686075
Uteopsis argentea	Northeast Sulawesi	RMNHPOR 6593	MF686076

Results

Morphological evidence

The external shape of the sponge *Heteropia glomerosa* is very variable in the number, diameter and height of the tubes that are connected at the base (Figure 2B). It resembles a bunch of bananas (Figure 2A). The shape of each tube can also vary between cylindrical or pyriform, and straight or curved (Figure 2B). The ornamentation of the oscula can also present certain variation, with the crown being well developed, scarce or even absent. These variations can be seen within the same individual or among individuals. The color in some individuals is white, but in others it could be light beige or beige, mainly because of the accumulation of thin sediment and micro-incrustation on its external surface. The external wall of the body has an appearance of an armour because of the presence of large diactines disposed longitudinal side by side forming a rigid reinforcement (Figure 2C). The thickness of the wall is relatively thin, being a little bit wider than 100 µm (Figure 2D-E) on the contrast the species is composed of several types of spicules (Figure 3). Figure B shows one individual that was collected associated to a black solitary ascidian *Phallusia nigra*. All the characteristics and its variations commented herein are compatible with what was described in Klautau et al. (2020) for the Brazilian specimens.

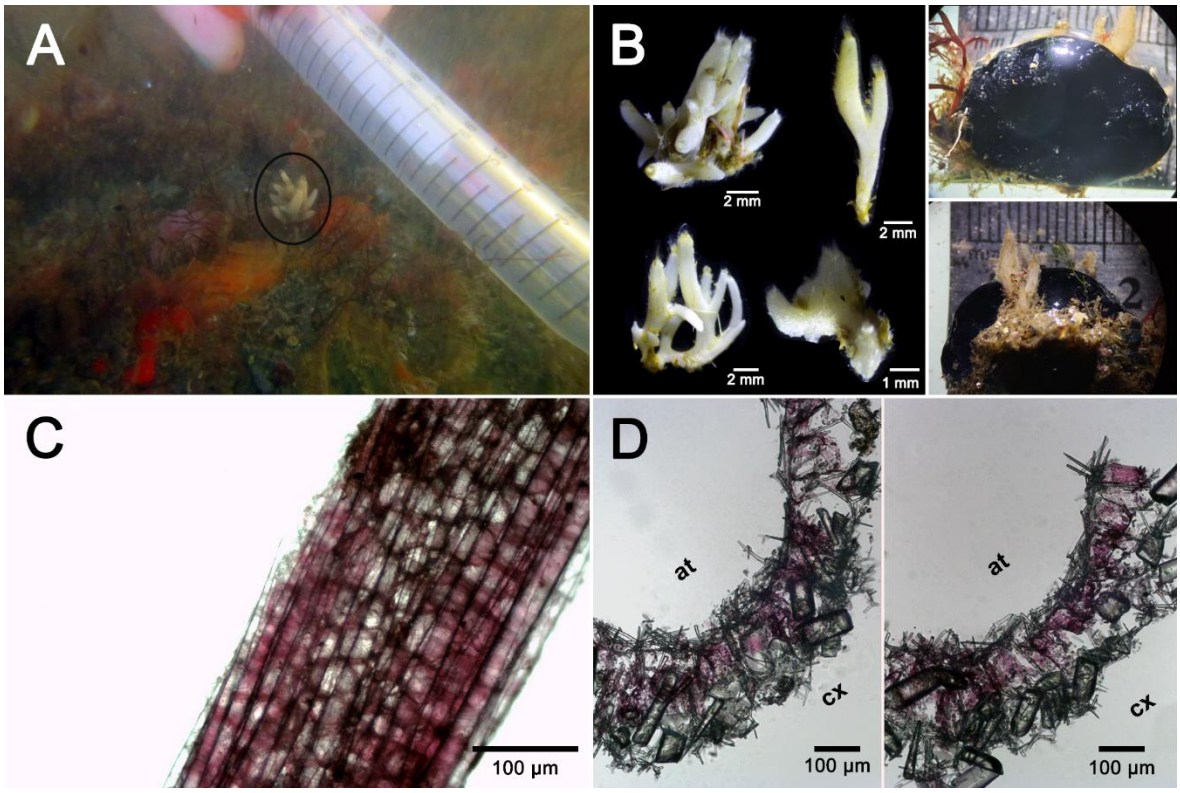


Figure 2. Morphological aspects of *Heteropia glomerosa* (SRB07). (A) *Heteropia glomerosa in vivo*, observed on a rocky shore at SRB, black circle around the sponge. (B)

Specimens of *H. glomerosa* preserved in ethanol (SRB01, PPM06, TTP39), inset shows *H. glomerosa* associated with a *Phallusia nigra*. (C) Section showing the cortex diactines of the sponge. (D) cross-section showing the skeleton organization of *H. glomerosa* (PPM04), cx = cortex and at = atrium.

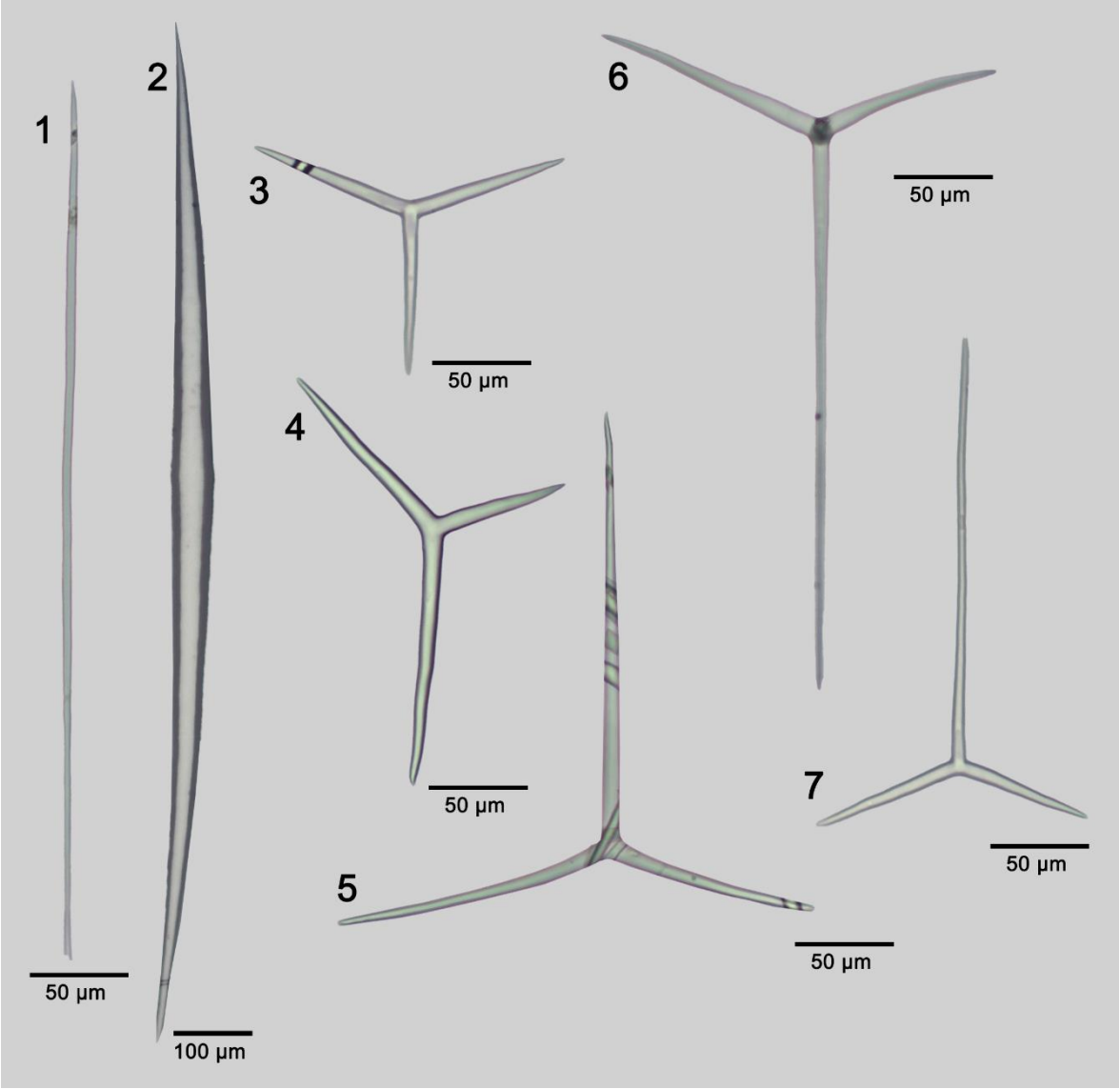


Figure 3. Spicules of *Heteropia glomerosa* (PPM06). 1 – cortical trichoxeas; 2 – cortical diactine; 3 – cortical triactine; 4 – subcortical pseudosagittal triactine; 5 – subatrial triactine; 6 – atrial tetractine; 7 – atrial triactine.

The specimen from which the spicules were prepared has seven distinct categories (Figure 3), and this composition is in line with what has been documented for the species. Likewise, in general, the dimensions of the seven categories of spicules are within the expected range for the species, with a few exceptions. The values of the specimens from Brazil (Ceará, Bahia, Arraial do Cabo) are the closest of the data presented here. However, our values are lower than those of the holotype. Some differences were observed in the sizes

of the diactines and in the apical of tetractines. The values of the diactines from our specimens were higher than the values for the specimen from Ceará. Also, the apical of the tetractines of our specimens (16.8 μm) were closest to those from the species *Uteopsis argentea* (18.4 μm). Therefore, spicule size is another characteristic evaluated in this study that supports our identification.

Table 2. Spicules measurements of the specimens of *Heteropia glomerosa*. SRB = Saco da Ribeira Bay, PMB = Pedras Miudas Beach, TTPB = Toque-Toque Pequeno Beach.

Specimen/Spicule	Actine	Length (µm)				Width (µm)				N
		Min	Mean	SD	Max	Min	Mean	SD	Max	
Cortical Diactine										
SRB 01		303	1095.8	489	2251.5	9.4	44.9	17.2	74.1	20
PMB 06		512.6	1301.4	574.9	2875.5	24.5	59.5	12.6	77.1	20
TTPB 39		405.8	789.1	267	1666.1	19.2	39.7	9.2	51.5	20
Cortical Triactine										
SRB 01	Paired	51.3	89.6	25.4	138.7	5.9	6.9	0.9	9.1	20
PMB 06		53.9	121.2	29.8	159.1	4.7	8.9	1.7	11.9	20
TTPB 39		59.9	94.6	17.4	123.1	4	6.6	1	8.2	20
SRB 01	Unpaired	43.4	68.7	12.8	91.1	4.9	6.7	1	8.4	20
PMB 06		45.5	77	17.6	107.6	5.7	8	1.7	11.9	20
TTPB 39		23	74	22.4	116.3	5	6.1	0.9	9.3	20
Subcortical Pseudosagittal Triactine										
SRB 01	Paired 1	39.3	104	23.9	135.9	4.5	6.6	0.9	9.3	20
PMB 06		92.3	135.1	23.3	198.6	6.2	10	1.4	11.2	20
TTPB 39		54.5	112.5	23.5	144	4.7	7.1	1.1	8.7	20
SRB 01	Paired 2	35.6	78.3	20.2	110.2	5.3	7.1	1.1	10.3	20
PMB 06		55.4	97.6	19.1	147.9	5.7	9.4	1.6	12.1	20
TTPB 39		52.1	82.5	14.6	118.5	4.4	6.9	1.4	9.9	20
SRB 01	Unpaired	20.1	61.9	30.1	149.6	4.5	7.1	1	8.2	20
PMB 06		19.5	72.9	18	103.7	5.9	9.3	1.5	11.9	20
TTPB 39		32.2	70.7	16.9	98.5	3.9	7.7	1.4	9.2	20
Subatrial Triactine										
SRB 01	Paired	58.9	90.6	18.5	134.1	4.4	6.3	1	8.5	20
PMB 06		64.1	105.8	32.2	180	4.4	7.6	1.5	10.8	20
TTPB 39		58.6	94.5	15	118.9	4.9	6.2	0.6	7.3	20
SRB 01	Unpaired	86.4	165.6	43	265.9	5.6	7.4	1.1	9.9	20
PMB 06		118.5	188.3	60.5	376.1	3.8	7.6	2.2	13.2	20
TTPB 39		102.6	152.9	31.3	219.8	4.9	6.5	1.1	10.1	20
Atrial Triactine										
SRB 01	Paired	44.2	82.2	15.8	112.9	4.5	6.5	0.9	8.4	20
PMB 06		47.5	81.7	16.4	118	5.1	6.6	0.8	8.3	20
TTPB 39		67.3	90	14.9	128	5.5	6.7	1	9.3	20
SRB 01	Unpaired	56.6	94.5	27.2	185.3	5.1	7	0.9	8	20
PMB 06		76.9	100.7	16.2	136.1	4.9	6.3	0.8	8.2	20
TTPB 39		73.8	125.9	22.9	149.7	4.7	6.3	0.8	8.3	20
Atrial Tetractine										
SRB 01	Paired	63.9	113.7	22.8	152.2	5.4	17.9	1.4	10.9	20
PMB 06		83.9	132	23.7	176.9	5.8	9.1	1.3	11.6	20
TTPB 39		77.2	121,6	20,2	156,2	5	7	0,9	8,6	20
SRB 01	Unpaired	81.7	157.6	49.2	299.8	5.6	7.5	1.2	10.1	20
PMB 06		138.2	197.1	58.4	304.5	7	7.8	0.9	10.7	20
TTPB 39		67.2	159.8	51.2	239	4.8	6.4	0.9	8.7	20
SRB 01	Apical	8.1	17.9	10.1	50.1	3.5	5.4	0.8	6.6	20
PMB 06		13.1	28.2	16	73.5	5.2	6.4	0.9	8.6	20
TTPB 39		9.5	16.8	6.7	35.8	3.8	4.7	0.6	5.9	20

Genetic evidence

Our molecular tree contains two specimens (PPM12 and TTP39) recently collected and analyzed in the present study (Figure 4). These two specimens were identified as *Heteropia glomerosa* based on morphological evidence. The phylogenetic reconstruction using the molecular marker C-LSU grouped all sequences of *H. glomerosa* retrieved from Genbank with our two specimens in the same clade. Additionally, the calculation of *p*-distance values shows an intraspecific variation of 0-1%, which corroborates our morphological findings, confirming the specific status of these two specimens as *H. glomerosa.* Therefore, this constitutes our genetic evidence that is in line with morphological data, allowing us to extrapolate the species determination to the other 21 specimens.

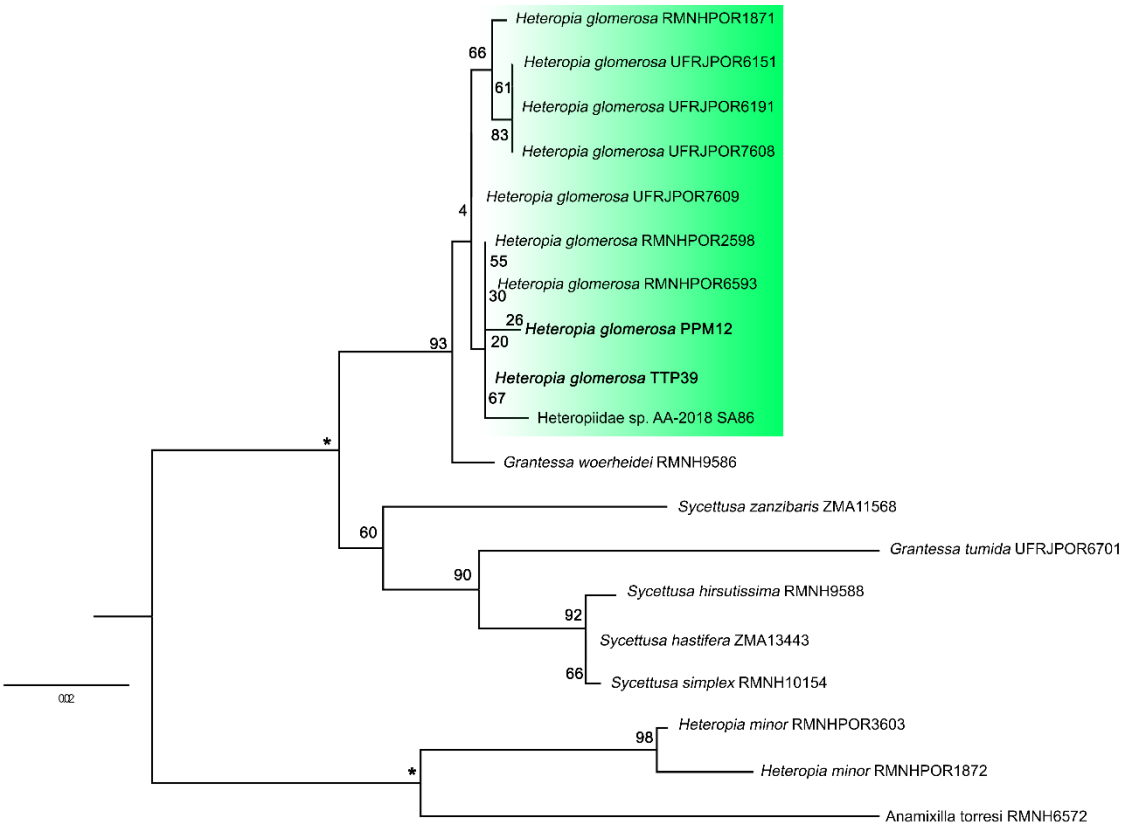


Figure 4. Maximum likelihood phylogenetic tree constructed with the C-LSU sequences of Heteropiidae and Jenkinidae. Bootstrap values are given on the branches. New sequences are bold. Bootstraps = 100% are presented with an asterisk (*).

Discussion

Among the records of *Heteropia glomerosa* along the Brazilian coast, it is common to observe the species growing on artificial substrates from 1–10 m of depth, in port areas and in mollusc farms. (Klautau et al., 2020). However, up to now there are no records of the species on ship hulls, which are the main suspected vectors for the spread of invasive calcareous sponges, such as *Paraleucilla magna* and *Sycettusa hastifera* (Ribeiro, Padua and Klautau, 2022).

Currently, there is no satisfactory understanding of the potential threat of these *H. glomerosa*'s bioinvasion to the environment, economy or biological community. These animals exhibit remarkable growth, reproduction, and colonization at artificial environments, yet little is known about how these traits might affect the local community were this calcareous sponge lives.

Therefore, close observation and the recording of new localities are essential to understanding the scope of its distribution. Research into population genetics could reveal whether these invasions are the result of one or multiple events and pinpoint where their distribution began, just as was done for *Paraleucilla magna* (Cavalcanti et al., 2020). Our observations on natural substrates are an important record for future investigations, as *Heteropia glomerosa* was observed interacting with the local fauna and may be competing for space with some native species.

Despite of our record of the association between *H. glomerosa* and *Phallusia nigra* what catches our attention is that the black solitary ascidian is under suspicion also to be an exotic invasive species in the Atlantic Ocean. *Phallusia nigra* Savigny, 1816 was first described for the Red Sea, then recorded for the Indian, Pacific, Atlantic and Mediterranean Oceans (WoRMS, OBIS and GBIF). On the Brazilian coast, it has been classified as a cryptogenic species, which means that its native distribution is already unknown or doubtful and recorded for the states of Ceará, Alagoas, Espírito Santo, Bahia, Rio de Janeiro and São Paulo (SiBBr, Rocha et al., 2011).

On the other hand, evidence from population genetic has pointed to a possible evolutionary origin of the species *Phallusia nigra* in the Western Atlantic, as populations over 8,000 km have been shown to be a single evolutionary lineage, with high gene flow, a high level of heterozygosity and a wide dispersal capacity of the larvae from Miami to São Paulo (Nóbrega et al., 2004; Rocha et al., 2012; Vandepas et al., 2015). According to the

GBIF database, the species has its northernmost distribution in Bermuda and its southern limit in São Paulo, Brazil.

To conclude, we reinforce what Klautau et al. (2020) state about the importance of new studies using *H. glomerosa* as a biological model in studies of population genetics, phylogeography, larval behavior, ecological niche and biological interactions for a better understanding of its biological potential as an exotic invasive species. This set of studies could provide us with relevant information for drawing up action plans in the event of a negative impact.

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References

Calazans, V. P. S. B., & Lanna, E. (2019). Influence of endogenous and exogenous factors on the reproductive output of a cryptogenic calcareous sponge. *Marine Biodiversity*, *49*(6), 2837–2850. https://doi.org/10.1007/s12526-019-01013-2

Cavalcanti, F. F., Menegola, C., & Lanna, E. (2014). Three new species of the genus Paraleucilla Dendy, 1892 (Porifera, Calcarea) from the coast of Bahia State, Northeastern Brazil. *Zootaxa*, *3764*(5), 537. https://doi.org/10.11646/zootaxa.3764.5.3

Cavalcanti, F. F., Padua, A., Cunha, H., Halasz, M., Nikolić, V., Barreto, S., & Klautau, M. (2020). Population differentiation supports multiple human-mediated introductions of the transatlantic exotic sponge Paraleucilla magna (Porifera, Calcarea). *Hydrobiologia*, *847*(17), 3571–3590. https://doi.org/10.1007/s10750-020-04368-x

CETESB. (2024). *Qualidade das Praias Litorâneas no Estado de São Paulo 2023*. Companhia Ambiental do Estado de São Paulo. <http://cetesb.sp.gov.br/praias/publicacoes-relatorios/> ISBN 978-65-5577-085-8

Chagas, C., Barros, F., & Cavalcanti, F. F. (2020). Temporal variation in the recruitment of calcareous sponges (Porifera, Calcarea) in Todos os Santos Bay, tropical Brazilian coast. *Journal of the Marine Biological Association of the United Kingdom*, *100*(7), 1063–1070. https://doi.org/10.1017/S0025315420001095

Chombard, C., Boury-Esnault, N., & Tillier, S. (1998). Reassessment of Homology of Morphological Characters in Tetractinellid Sponges Based on Molecular Data. *Systematic Biology*, *47*(3), 351–366. https://doi.org/10.1080/106351598260761

Dechoum, M. S., Junqueira, A. D. O. R., Orsi, M. L., Ziller, S. R., Pivello, V. R., Zenni, R. D., Thomaz, S. M., Fonseca, A. C., Vitule, J. R. S., Barros, F., Ivanauskas, N. M., Creed, J., Brito, M. F. G., Bergallo, H. G., Rocha, R. M., & Galheigo, F. A. (2024). Thematic assessment report on invasive alien species in Brazil: Summary for policymakers. *Biota Neotropica*, *24*(2), e20241645. https://doi.org/10.1590/1676-0611-bn-2024-1645

Hoang, D. T., Chernomor, O., Von Haeseler, A., Minh, B. Q., & Vinh, L. S. (2018). UFBoot2: Improving the Ultrafast Bootstrap Approximation. *Molecular Biology and Evolution*, *35*(2), 518–522. https://doi.org/10.1093/molbev/msx281

Katoh, K., Rozewicki, J., & Yamada, K. D. (2019). MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics*, *20*(4), 1160–1166. https://doi.org/10.1093/bib/bbx108

Katoh, K., & Toh, H. (2008). Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. *BMC Bioinformatics*, *9*(1), 212. https://doi.org/10.1186/1471-2105-9-212

Klautau, M., Cóndor-Luján, B., Azevedo, F., Leocorny, P., Brandão, F. D. A. R., & Cavalcanti, F. F. (2020). *Heteropia glomerosa* (Bowerbank, 1873) (Porifera, Calcarea, Calcaronea), a new alien species in the Atlantic. *Systematics and Biodiversity*, *18*(4), 362–376. https://doi.org/10.1080/14772000.2020.1758826

Klautau, M., & Valentine, C. (2003). Revision of the genus Clathrina (Porifera, Calcarea). *Zoological Journal of the Linnean Society*, *139*(1), 1–62. https://doi.org/10.1046/j.0024-4082.2003.00063.x

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Minh, B. Q., Nguyen, M. A. T., & Von Haeseler, A. (2013). Ultrafast Approximation for Phylogenetic Bootstrap. *Molecular Biology and Evolution*, *30*(5), 1188–1195. https://doi.org/10.1093/molbev/mst024

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., Von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Molecular Biology and Evolution*, *37*(5), 1530–1534. https://doi.org/10.1093/molbev/msaa015

Nóbrega, R., Solé-Cava, A. M., & Russo, C. A. M. (2004). High genetic homogeneity of an intertidal marine invertebrate along 8000 km of the Atlantic coast of the Americas. *Journal of Experimental Marine Biology and Ecology*, *303*(2), 173–181. https://doi.org/10.1016/j.jembe.2003.11.009

Oricchio, F. T., Marques, A. C., Hajdu, E., Pitombo, F. B., Azevedo, F., Passos, F. D., Vieira, L. M., Stampar, S. N., Rocha, R. M., & Dias, G. M. (2019). Exotic species dominate marinas between the two most populated regions in the southwestern Atlantic Ocean. *Marine Pollution Bulletin*, *146*, 884–892. https://doi.org/10.1016/j.marpolbul.2019.07.013

Padua, A., Lanna, E., & Klautau, M. (2013). Macrofauna inhabiting the sponge *Paraleucilla magna* (Porifera: Calcarea) in Rio de Janeiro, Brazil. *Journal of the Marine Biological Association of the United Kingdom*, *93*(4), 889–898. https://doi.org/10.1017/S0025315412001804

Ribeiro, B., Padua, A., & Klautau, M. (2022). Sessile exotic species moving around: Calcareous sponges on boat hulls. *Marine Biodiversity*, *52*(1), 3. https://doi.org/10.1007/s12526-021-01249-x

Rocha, R. M., Bonnet, N. Y. K., Baptista, M. S., & Beltramin, F. S. (2012). Introduced and native Phlebobranch and Stolidobranch solitary ascidians (Tunicata: Ascidiacea) around Salvador, Bahia, Brazil. *Zoologia (Curitiba)*, *29*(1), 39–53. https://doi.org/10.1590/S1984-46702012000100005

Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdaña, Z. A., Finlayson, M., Halpern, B. S., Jorge, M. A., Lombana, A., Lourie, S. A., Martin, K. D., McManus, E., Molnar, J., Recchia, C. A., & Robertson, J. (2007). Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas. *BioScience*, *57*(7), 573–583. https://doi.org/10.1641/B570707

Vandepas, L. E., Oliveira, L. M., Lee, S. S. C., Hirose, E., Rocha, R. M., & Swalla, B. J. (2015). Biogeography of *Phallusia nigra:* Is It Really Black and White? *The Biological Bulletin*, *228*(1), 52–64. https://doi.org/10.1086/BBLv228n1p52

Voigt, O., & Wörheide, G. (2016). A short LSU rRNA fragment as a standard marker for integrative taxonomy in calcareous sponges (Porifera: Calcarea). *Organisms Diversity & Evolution*, *16*(1), 53–64. https://doi.org/10.1007/s13127-015-0247-1

Referências da Introdução Geral

AB'SÁBER, A. N. Contribuição à Geomorfologia do Litoral Paulista. **Revista Brasileira de Geografia**, v. 17, n. 1, p. 3–48, mar. 1955.

AZEVEDO, F. et al. Taxonomy and phylogeny of calcareous sponges (Porifera: Calcarea: Calcinea) from Brazilian mid-shelf and oceanic islands. **Zootaxa**, v. 4311, n. 3, p. 301, 24 ago. 2017.

BORGES, M. et al. Marine planktonic and benthic organisms: an ocean of diversity in the collections of the State of São Paulo. **Biota Neotropica**, v. 22, n. spe, p. e20221406, 2022.

CAVALCANTI, F. F. et al. Population differentiation supports multiple human-mediated introductions of the transatlantic exotic sponge Paraleucilla magna (Porifera, Calcarea). **Hydrobiologia**, v. 847, n. 17, p. 3571–3590, out. 2020.

CAVALCANTI, F. F.; RAPP, H. T.; KLAUTAU, M. Taxonomic revision of Leucascus Dendy, 1892 (Porifera: Calcarea) with revalidation of Ascoleucetta Dendy & Frederick, 1924 and description of three new species. **Zootaxa**, v. 3619, n. 3, p. 275–314, 28 fev. 2013.

CÓNDOR-LUJÁN, B. et al. Morphological and molecular taxonomy of calcareous sponges (Porifera: Calcarea) from Curaçao, Caribbean Sea. **Zoological Journal of the Linnean Society**, v. 183, n. 3, p. 459–525, 28 jun. 2018.

CUSTÓDIO, M. R.; HAJDU, E. Checklist de Porifera do Estado de São Paulo, Brasil. **Biota Neotropica**, v. 11, n. suppl 1, p. 427–444, dez. 2011.

DE GOEIJ, J. M. et al. Surviving in a Marine Desert: The Sponge Loop Retains Resources Within Coral Reefs. **Science**, v. 342, n. 6154, p. 108–110, 4 out. 2013.

DE VOOGD, N. et al. **World Porifera Database. Accessed at https://www.marinespecies.org/porifera**. 2025. Disponível em: <https://www.marinespecies.org/imis.php?dasid=546&doiid=359>. Acesso em: 26 jan. 2025

FONTANA, T. et al. Diversity and distribution patterns of Calcareous sponges (subclass Calcinea) from Martinique. **Zootaxa**, v. 4410, n. 2, p. 331, 17 abr. 2018.

GARCÍA-HERNÁNDEZ, J. E. et al. Associated organisms inhabiting the calcareous sponge Clathrina lutea in La Parguera, Puerto Rico. **Caribbean Journal of Science**, v. 49, n. 2–3, p. 239, 22 out. 2019.

KERSKEN, D. et al. The infauna of three widely distributed sponge species (Hexactinellida and Demospongiae) from the deep Ekström Shelf in the Weddell Sea, Antarctica. **Deep Sea Research Part II: Topical Studies in Oceanography**, v. 108, p. 101–112, out. 2014.

KLAUTAU, M. et al. *Heteropia glomerosa* (Bowerbank, 1873) (Porifera, Calcarea, Calcaronea), a new alien species in the Atlantic. **Systematics and Biodiversity**, v. 18, n. 4, p. 362–376, 18 maio 2020.

KLAUTAU, M.; CAVALCANTI, F. F.; BOROJEVIC, R. The new sponge species Amphoriscus pedunculatus (Porifera, Calcarea). **Zootaxa**, v. 4341, n. 1, p. 105, 30 out. 2017.

LANNA, E. et al. Calcareous sponges from São Paulo State, Brazil (Porifera: Calcarea: Calcinea) with the description of two new species. **Journal of the Marine Biological Association of the United Kingdom**, v. 87, n. 6, p. 1553–1561, dez. 2007.

LANNA, E. et al. Taxonomy of calcareous sponges (Porifera, Calcarea) from Potiguar Basin, NE Brazil. **Zootaxa**, v. 1973, n. 1, p. 1–27, 9 jan. 2009.

LOPES, M. V. et al. Calcareous sponges (Porifera, Calcarea) from Florida: new species, new records and biogeographical affinities. **Zootaxa**, v. 4526, n. 2, p. 127, 29 nov. 2018.

LOPES, M. V. et al. Integrative taxonomy of Calcarea (Porifera) from Espírito Santo, Eastern Brazil. **Zootaxa**, v. 5618, n. 2, p. 151–205, 2 abr. 2025.

MCCLINTOCK, J. B. Ecology of Antarctic Marine Sponges: An Overview. **Integrative and Comparative Biology**, v. 45, n. 2, p. 359–368, 1 abr. 2005.

MILLER, R. J. et al. Structure-Forming Corals and Sponges and Their Use as Fish Habitat in Bering Sea Submarine Canyons. **PLoS ONE**, v. 7, n. 3, p. e33885, 21 mar. 2012.

MURICY, G. et al. **Catalogue of Brazilian Porifera**. Rio de Janeiro: Museu Nacional, 2011.

ORICCHIO, F. T. et al. Exotic species dominate marinas between the two most populated regions in the southwestern Atlantic Ocean. **Marine Pollution Bulletin**, v. 146, p. 884–892, set. 2019.

PADUA, A.; LANNA, E.; KLAUTAU, M. Macrofauna inhabiting the sponge *Paraleucilla magna* (Porifera: Calcarea) in Rio de Janeiro, Brazil. **Journal of the Marine Biological Association of the United Kingdom**, v. 93, n. 4, p. 889–898, jun. 2013.

PAWLIK, J. R. Coral reef sponges: Do predatory fishes affect their distribution? **Limnology and Oceanography**, v. 43, n. 6, p. 1396–1399, set. 1998.

RIBEIRO, B. et al. Exploitation of micro refuges and epibiosis: survival strategies of a calcareous sponge. **Journal of the Marine Biological Association of the United Kingdom**, v. 98, n. 3, p. 495–503, maio 2018.

RUZICKA, R.; GLEASON, D. F. Latitudinal variation in spongivorous fishes and the effectiveness of sponge chemical defenses. **Oecologia**, v. 154, n. 4, p. 785–794, jan. 2008.

SPALDING, M. D. et al. Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas. **BioScience**, v. 57, n. 7, p. 573–583, 1 jul. 2007.

VAN SOEST, R. W. M. et al. Global Diversity of Sponges (Porifera). **PLoS ONE**, v. 7, n. 4, p. e35105, 27 abr. 2012.