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TUBES OF CERIANTHARIA (CNIDARIA; ANTHOZOA): A MARINE HABITATION

FROM ITS CONSTRUCTION TO ITS GUESTS

Botucatu - SP
Hellen Ceriello | PhD thesis | Sept. 2022

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TUBES OF CERIANTHARIA (CNIDARIA; ANTHOZOA): A MARINE HABITATION FROM ITS CONSTRUCTION TO ITS GUESTS

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DEDICATÓRIA

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“Malfeito feito!”

(Mapa do Maroto)

TUBES OF CERIANTHARIA (CNIDARIA; ANTHOZOA): A MARINE HABITATION FROM ITS CONSTRUCTION TO ITS GUESTS

Abstract

This study assessed marine biodiversity of microbes and metazoans found in ceriantharian tubes via species characterization, using both species traditional recognition and metagenomics of environmental DNA (eDNA) methods, and it also described ceriantharian tube-building behavior patterns. Using the traditional method for species characterization, 14 taxa of crustaceans were identified, including one novel species, *Podocerus carmelina* **sp. nov.**, found in *Ceriantheomorphe brasiliensis* tubes (Mello-Leitão, 1919) (n = 3) and in unidentified tubes (n = 5). Also, two new location records for previously known crustacean species were reported as well as an identification key for *Podocerus* species with type localities in the Atlantic Ocean was provided. Analyses of eDNA metagenomics in tubes of *C. brasiliensis* (n = 2) and *Ceriantheopsis lineata* Stampar, Scarabino, Pastorino & Morandini, 2015 (n = 2) showed that these ceriantharian tubes are composed of 79% microbial families and 21% metazoan families. Moreover, tubes from different locations presented a higher metazoan diversity than the microbial that nearly remained unaltered, indicating that, although microbial families are abundant in ceriantharian tubes, they are uniform throughout different locations. The ceriantharian tube-building research revealed that *Botrucnidifer norvegicus* Carlgren, 1912 can build ramified tubes housing two polyps, adult *C. brasiliensis* lacks substrate selection mechanisms and sediment capture, is able to build more than a single tube throughout life, usually vertically oriented, and its behavior may differ from the species *Isarachnanthus nocturnus* (den Hartog, 1977) and *Cerianthus vogti* Danielssen, 1890. The ceriantharian tube is critical in controlling the internal water pressure in ceriantharians in addition to providing protection. In conclusion, this research demonstrated that, other than biodiverse microhabitats, ceriantharian tubes are potential tools for basic research and biodiversity surveys, driving studies of ecology, behavior, life cycles and cnidae toxicity.

Keywords: behavior, bioconstruction, eDNA, marine biodiversity, metagenomics, microhabitat.

Summary

Introduction

Coral reefs are recognized as valuable sources of biological diversity. As such, they play an important role in marine biodiversity as they provide suitable feeding locations and habitats for a wide variety of species [1]. Despite this, competition between reef-dwelling organisms is likely to occur, mainly over recruitment space which is the most disputed resource in coral reefs [2]. The availability of these areas is recognized as a limiting factor for species that rely on reef establishment to complete their life cycle. As a result, the shortage of dwelling spaces, along with the high demand for reefs, may drive the search for alternative settlement habitation structures [2].

In this scenario, some studies on Ceriantharia (Cnidaria; Anthozoa), also known as tube-dwelling anemones (e.g. 3–4), suggest that ceriantharian tubes (Fig. 1) can provide suitable habitats for the survival of various marine invertebrate species, acting as alternative substrates for these organisms. The findings in these investigations assist in understanding if such structures have an ecological function similar to that observed in coral reefs, although in a smaller scale, and may thus be designated as rich microhabitats.

Fig. 1 Several tubes of *Ceriantheomorphe brasiliensis*, two of which had polyps extending outwards (Photo: Marcelo Krause).

Although groups such as Bryozoa, Crustacea, Phoronida, Mollusca, and Sipuncula have been observed anchored on the ceriantharian tube [3–4–5–6–7], knowledge on the species that can use this microhabitat is still very limited, particularly when restricted to a macroscopic view. As a result, there is still a biased perception of the variety and ecological significance of these structures.

Currently, it is estimated that the number of worldwide known species is considerably lower than the real biological variety in the planet, reflecting the gap of biodiversity information while emphasizing the need for more studies that provide registration of the numerous undiscovered species. As shown by some taxonomic studies (e.g. 8–9), there are around 8.7 million eukaryotic species worldwide, although only approximately 1.6 million have been discovered. Furthermore, when it comes to marine animals, records suggest that only 9% of all species on the planet are known [8–10]. Nonetheless, while no precise estimate of the number of prokaryotic species exists, Gilbert et al. [11] found nearly 6 million bacterial taxa at the species or genus level in their investigation and yet they support the idea that this estimate is still lower than the predicted bacterial abundance globally.

Even so, the relevance of biodiversity records extends beyond demonstrating the richness of species in a given space/time to comparing reports of new species with older records, providing an abundance projection of known species, demonstrating the loss of current biodiversity, and estimating future extinction rates [8–9–12]. Some strategies are used to recognize biodiversity, such as species traditional recognition and recognition based on genomic data.

Species traditional recognition

Species traditional recognition focuses on morphological characters which, although effective in the field, have disadvantages. For example, the taxonomy of some worm groups (e.g. Nematoda) is reliant on electron microscopy methods, which are both costly and time-consuming to employ [13–14]. Another issue is that traditional identification may be misleading because species recognition and determination are credited based on the perspective of its identifier [15]. On the other hand, species identification based on molecular

data has gained interest among researchers since its information arrangement allows more consistent identifications [16]. Thus, the methodology in this work was based on environmental DNA (eDNA) metagenomics to identify molecular data from species in ceriantharian tubes and characterize them.

eDNA and Metagenomics

Because of the high phenotypic plasticity found in several species from a number of taxonomic groups, recognizing global biodiversity only by traditional methods has been a major challenge [17]. A greater challenge yet, is attempting to identify a species when it is impossible to visually confirm their presence in a particular environment.

As Gilbert et al. [11] have already pointed out, the diversity of microbial species is vast, as microorganisms can be found in the most diverse ecosystems, making accurate quantification difficult. However, combining eDNA analysis with metagenomics approach enabled a better understanding of the variety of organisms found in a given environment.

The set of genomic DNA of an entire community of organisms obtained from the same site, such as water droplets, soil, and substrates, is referred to as eDNA [18–19–20–21]. Metagenomics, otherwise, refers to the technique that allows eDNA extraction [22].

In the last 10 years, the metagenomic approach has been widely used, integrating knowledge in ecological, microbiological, industrial, and health fields [23–24]. In addition to the fact that it is a rather rapid technique to conduct, it also allows the identification of microorganisms using a small amount of material from the most diverse environments, including microhabitats [25].

This approach has a variety of applications, including the identification of microorganisms of importance to the human population without the need for their cultivation in laboratory [25], the registration of species, including unknown organisms [26–27–28], and the recognition of threatened species [29] as well as the monitoring of past and present biodiversity [30], thus assisting in the area of taxonomy.

Currently, metagenomic research employing eDNA has been conducted with fungi, viruses, gastrointestinal microorganisms and microorganisms found

in wounds, bacteria, archaea [31–32–33–34–35–36], and also in aquatic ecosystems, providing a wealth of information on microorganisms that live in these habitats [28–37–38–39–40–41].

As there may be more than a million species in a gram of sediment or in a single drop of water, marine microbial metagenomics is one of the most data-rich fields [18], revealing the immense species variety found in marine ecosystems [42–43]. Moreover, metagenomics often records microbial communities associating with other organisms, such as anthozoans [44–45]. Thus, the qualitative metagenomic analysis of ceriantharian tubes could provide molecular data on species composition heterogeneity, including fragmented specimens, and organic compounds found amidst the sediments adhered to this structure/substrate, similar to the studies by Wei et al. [46] with sediments found in the Yellow Sea in China, and Grassle and Maciolek [47] that were able to estimate species richness found in deep seas through the evaluation of seabed samples.

Marine Bioconstructions

Elevated and often multi-layered structures created by benthic animals, from the aggregation and accumulation of components found both on the seafloor or synthesized by themselves, are common in the marine ecosystem [48]. Because of their building activities, such animals are considered as bioconstructors or ecosystem engineers [49], and structures formed by their activities are referred to as bioconstructions or biogenic reefs [50]. Similar to coral and algae reefs, the ceriantharian tube is a biogenic structure, although soft, produced by "cementing" activities in which small sediment fragments are adhered to cnida filaments produced by the bioconstructor, like in the process of tube bioconstruction by tube polychaetes [51]. Additionally, ceriantharian tubes are built on unconsolidated substrates and may be one of the few alternatives of consolidated structures for species establishment in these habitats [3].

Bioconstructions increase spatial complexity and provide secondary substrates for species establishment throughout several generations, as such structures may be modified and maintained by bioconstructors other than those who built them initially [52–53]. Thus, bioconstructions play an essential role in

terms of species diversity found in marine ecosystems, generating and sustaining biodiversity, and hence contributing to the maintenance of ecosystems' high levels.

It is not uncommon to investigate fauna found in biogenic reefs, and it has been previously done in numerous groups such as algae [54], polychaetes [55–56–57], and cnidarians [58]. However, the use of molecular tools to conduct fauna survey in ceriantharian bioconstructions is unprecedented and has the potential to contribute to the identification of biodiversity in these microhabitats as well as to possibly designate them as biodiversity hotspots.

Is Ceriantharia's tube-building behavior inherited?

Stampar et al. [59] investigated the ultrastructure and ptychocyst production/release for ceriantharian tube construction. However, changes in tube formation in response to diverse substrates were not addressed, and one of the current questions is whether the polyp is likely to select sediments during tube building. If so, is this an intentional or random choice? These guiding questions are rather relevant because the composition of organisms using the tube as a substrate may be modified by the polyp's own performance.

Based on the scenario described above, the goal in this study was to better understand the role of ceriantharian tubes as microhabitats for the management of marine biodiversity. To do so, metazoan and microbial diversity found in ceriantharian tubes of *Ceriantheomorphe brasiliensis* (Mello-Leitão, 1919) and *Ceriantheopsis lineata* Stampar, Scarabino, Pastorino & Morandini, 2015 was identified, the changes in species composition in relation to the ecosystem in which these bioconstructions were built was investigated, and the tube-building behavior of *Botrucnidifer norvegicus* Carlgren, 1912 and *C. brasiliensis* was described based on observations in laboratory and in natural habitat (only the latter).

Objectives

The overall goal in this study was to look into the potential of the ceriantharian tube as marine biodiversity rich microhabitat by describing and

comparing species composition associated with ceriantharian tubes of different species and from different locations, as well as describing ceriantharian tube-building behaviors. To do so, the following specific goals were designed:

- Fauna survey of species, including both metazoan and microbial organisms, found in/on ceriantharian tubes;
- Comparison of fauna found in/on ceriantharian tubes of different species and from different locations;
- Description of sediment use patterns for the construction of ceriantharian tubes.

This thesis was devided into two main sections: Behavior and Biodiversity.

The behavior section includes two chapters: (1) description of the behavior of *Ceriantheomorphe brasiliensis* in natural habitat and during tube construction under laboratory conditions; (2) description of the behavior of species *Botrucnidifer norvegicus* while anchored to the skeleton of dead colonies of a deep-sea coral.

The biodiversity section includes two chapters: (1) Crustacea biodiversity found in ceriantharian tubes and identified using a species traditional identification method; (2) microbial and metazoan families' diversity found in ceriantharian tubes of species *C. brasiliensis* and *Ceriantheopsis lineata*, using an eDNA metagenomics approach.

CHAPTER 1. *Behavior*

1.1. *Ceriantheomorphe brasiliensis* (Cnidaria; Ceriantharia): how does it behave?

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Abstract

Behavior research on Cnidaria, particularly Ceriantharia (Cnidaria, Anthozoa), is uncommon in general. Although ceriantharians or tube-dwelling anemones are known to build soft tubes, their tube-building behaviors remain unknown. In this study, however, we describe the tube-building behavior of *Ceriantheomorphe brasiliensis* (Mello-Leitão, 1919) for the first time as well as detail its behavior in natural habitat, including illustrations of live specimens. Our results showed that *C. brasiliensis* can build more than one tube throughout life, the tubes are usually “L-shaped”, longer than the polyp, and vertically oriented when built in deeper substrates, but horizontally oriented when built in shallower substrates. During tube construction, the polyp does not feed nor use its tentacles to catch or selected specific sediment particle sizes for tube construction. Given the tube’s vertical orientation, it is possible that *C. brasiliensis*’s tube-building behavior differs from that of other species. Although this study only included a single specimen, our results were consistent and suggest that the behaviors observed can occur on occasion. Furthermore, this research contains useful information that may guide future tube-building behavior studies on Ceriantharia which are, currently, nonexistent.

Keywords: Anthozoa, behavior, bioconstruction, tube-dwelling anemone, tube-building.

Introduction

Elevated and sometimes multi-layered structures are commonly found in the marine ecosystem. Those can be built by benthic animals from the aggregation and accumulation of components found on the seabed or synthesized by themselves [48]. Because of their building activities, such animals are considered as bioconstructors or ecosystem engineers [49], and structures formed by their activities are referred to as bioconstructions or biogenic reefs [50]. For the most part, biconstructions are created to provide refuge and protection for their builders, such as coral reefs built by some anthozoans and tubes built by tubeworms [51] and ceriantharians (Cnidaria; Anthozoa).

The ability of building tubes dates back to the Cambrian period [60] and has been preserved by a wide range of animal groups (e.g. Annelida, Crustacea, Cnidaria) across time [61–62].

Ceriantharians, also known as tube-dwelling anemones, can build soft and flexible tubes by adhering cnidae filaments (ptychocysts) to sediments [6–63–64] on soft bottoms or on stony corals [65].

Although the components utilized to construct ceriantharian tubes are mostly the same for all Ceriantharia members, the shape, length, and thickness of the tubes may differ depending on the species that builds them [59]. Cerianthidae members, for example, build single heavy tubes with only one opening [59–66–67–68], whereas Arachnactidae members build delicate tube systems with few layers overlapped and several openings [6–69–70], and Botrucnidiferidae either build single tubes or tube compounds that lodge ceriantharian clusters [65–71].

In general, research on the building of soft tubes, as well as behavioral studies on cnidarians, are still rare (e.g. 59–72), as are behavior studies on Ceriantharia mainly because, when found, they hide at the slightest disturbance making it hard to sample [6–65]. Stampar et al. [59], for example, investigated the ultrastructure and ptychocyst production/release in the tube-building process of *Ceriantheomorphe brasiliensis* (Mello-Leitão, 1919) [73]. However, this study did not address the behavioral patterns involved in the tube-building process, leaving concerns unsolved. Another concern is whether ceriantharians can build their tubes in hard substrate rather than in soft ones. To date, there

has only been a single record of a ceriantharian species, *Botrucnidifer norvegicus* Carlgren, 1912 [74], occurring in hard substrate [65]. Thus, to fill these information gaps, this work aims to detail the entire tube-building process of *C. brasiliensis*, including behavioral characteristics observed under laboratory conditions in both soft and hard substrates, as well as to describe its behavior in natural environment.

Material and methods

In situ

The behavior of *Ceriantheomorphe brasiliensis* in natural environment was observed on October 15, 2020 in São Sebastião, Southeast Brazil (23°49'43.5"S 45°25'21.9"W), at 6 m depth. The specimen was uninterruptedly filmed for 6 hours during the day using a GoPro Hero 8 Black camera installed atop a structure built out of thermoplastic pipes having a 2 Kg dumbbell attached to keep it from floating (Fig. 1).

Fig. 1 Underwater filming of *Ceriantheomorphe brasiliensis* behavior in natural habitat

Ex situ

A single polyp of *Ceriantheomorphe brasiliensis* (~ 11.4 cm) and its tube were sampled by SCUBA dive on December 11, 2018. The specimen, lodged in

its tube, was extracted from sandy bottom (fine sand) in Pântano do Sul, southern Brazil (27°47.059'S 48°30.429'W), at 4 m depth, with the seawater temperature ranging from 22ºC to 24°C, and placed in a plastic bag. After collection, the animal, still inside the tube, was moved to an open jar filled with 15 cm of sand sampled from the collection site, and put on the surface of this substrate. The jar was placed inside a closed-system aquarium (40 cm x 30 cm x 20 cm) with aeration at the water surface, filled with 20L of seawater from natural habitat (salinity of 30 ppt), and temperature was kept the same as in natural habitat (~ 24ºC). The animal was acclimatized for 46 days and daily fed on newly hatched nauplii of *Artemia* sp. three times a day. Only two days before experiments its feeding was suspended.

Then, the polyp was manually removed from its prior tube and placed in an aquarium with the same acclimatization conditions, including the feeding regimen. During experiments, aquarium water was changed once a week.

In the first experiment, it was evaluated whether the polyp had the same tube-building behavior in different substrates and if the use of sediment particles is random or selected. To do so, the animal was placed horizontally in jars (15 cm deep and 20 cm wide) with two distinct types of substrates (fine sand and fragmented mollusk shells), one for each jar, obtained from the collection site, and its tube-building behavior was filmed and described while in each substrate. After a new tube was built, the animal was given a one-week interval before the experiment was repeated. Each experiment had three replicates, and at the end of the third replicate, for each substrate, a new substrate was provided.

We also observed tube-building behavior in shallower substrates to verify if substrate depth could affect this behavior. This experiment was carried the same way as in deeper substrates, only in shallower jars (2 cm deep and 30 cm wide).

All experiments were filmed by a Panasonic HCM80 camera, set to standard timelapse mode, mounted on a tripod and placed outside the aquarium, 24 hours a day for seven days. At every day, the animal spent 12 hours under moderate light intensity and 12 hours in the dark, allowing eventual nocturnal activities to be investigated.

The all occurrence and instantaneous sampling methods were used to describe behaviors following Altmann [75].

Results

Observations *in situ* and *ex situ* yielded a total of 2.022 hours of footage, resulting in the following behavior descriptions:

In situ

The ceriantharian tube was in a vertical position and extended from the soft sandy bottom where it was built. The bottom was covered by algae, seagrass and mollusk shell debris. The ceriantharian column was kept inside the tube while its tentacles were distended and exposed outside. Tentacles only moved along with water current that constantly brought sediment, leaves and algae stalks towards the tentacular disk. However, we did not observe tentacles trying to capture any bottom component that was brought to them nor the polyp hid inside the tube when they touched its tentacles.

Fishes of the species *Lutjanus synagris* (Linnaeus, 1758) [76], *Diplectrum formosum* (Linnaeus, 1766) [77], *Eucinostomus argenteus* Baird & Girard, 1855, *Haemulon parra* (Desmarest, 1823), *Mugil curema* Valenciennes, 1836, *Orthopristis ruber* (Cuvier, 1830), *Anisotremus virginicus* (Linnaeus, 1758) [76], and *Eugerres brasilianus* (Cuvier, 1830) were observed swimming and feeding on the bottom nearby the tentacular disk but never close enough to touch polyp's tentacles. The crab species *Charybdis hellerii* (A. Milne Edwards, 1867) [78] was also observed passing by the polyp and briefly touching its tentacles as it walked away. The polyp did not try to capture any fish or the crab nor the fishes and crab interacted with the polyp.

When the polyp was entirely hidden inside the tube, *D. formosum* and *H. parra* fed closer to the tube, whereas *A. virginicus* fed both nearer and on the tube.

Tube-building in sand (*Ex situ*)

Ceriantheomorphe brasiliensis can burrow into deeper substrates, lifting its body and building an early vertical tube in about 4 h to 17 h. First, the polyp took 17 h to build its tube and staying erect, then 8 h, and finally 4 h. However, in shallower substrates, the polyp does not lift its body, but instead it builds a horizontally oriented tube on the surface of the substrate in about 10 h.

We separated the entire tube-building process of *C. brasiliensis* (Fig. 2a–c) into the following topics:

Tube removal

The animal contracts its column and tentacles shortly after being removed from its previous tube, reducing its total body length by half (~ 5.7 cm, tentacular disk and column). Although we have removed the polyp from its previous tube, a thin and translucent ptychocyst filaments membrane, reminiscent from the tube, was observed covering the animal's column. This membrane is carried down and removed by successive column contractions and distensions while the polyp is laying on the substrate. However, in one of the replicates, the polyp used its marginal tentacles to assist in this process. In this case, in addition to the contraction and distention movements, the polyp places one or more marginal tentacles on each side of its column and pushes the membrane down to approximately 1/3 of the column.

Burrowing into the substrate

After the ptychocyst filaments membrane is removed, the polyp gradually distends its body to its fullest (~ 11.4 cm) and moves its marginal tentacles from side to side, aggregating sediment particles and forming a soft "sandbank" under the tentacular disk. This "sandbank" later assists in the column lifting.

During tentacular movement, the polyp reclines its column, keeping both the aboral region (i.e. column posterior end) and the tentacular disk on the substrate. Then, it rotates and spins these body regions (Fig. 2a), and burrows into the substrate with the aboral end (Fig. 2b). While rotating and spinning, “sacks” of ptychocyst filaments are released by the aboral region.

Tube layers

Each ptychocyst sack is carried from the aboral end up (Fig. 2c) by successive waves in the column wall associated with column expansions, and coats the column entirely as if dressing it. At each wave, a portion of the aboral end is withdrawn from the early burrow, and one sack is released. Immediately after, the aboral region is reinserted into the burrow, “hammering” it, then the

column briefly expands, shaping ptychocyst sacks on it before the cycle restarts.

Approximately after 14 cycles, the whole column is lined by uniform layers of ptychocyst sacks with sediment particles adhered to them. It is worth noting that the addition of new ptychocyst filaments layers occurs beneath the newly constructed ones, i.e. from the inside out.

Column lifting

Once the column is lined with a few layers of ptychocyst filaments and sediment, and part of the aboral end is more deeply burrowed into the substrate, the polyp carries all layers downward, much as it does when removing the reminiscent membrane from a previous tube. However, in this case, the layers are not put away but, instead, they fill up the burrow (~ 5 cm) into which the aboral end is inserted. The accumulation of ptychocyst layers creates an initial tube. The polyp then gradually bends its column up until it stays completely erect. Finally, by adding more layers, the tube can be lengthened and thickened. This addition, however, is variable and inconstant.

In general, all tubes were slightly longer (~ 12.5 cm) than their builders (~ 11.4 cm when distended). Tubes built in deeper substrate were L-shaped, 0.1 mm thick and slightly protruded from the substrate.

When stressed by necessary experimental manipulation, the polyp coils the tips of its marginal tentacles and then retracts whole tentacles. Simultaneously, its column contracts, reducing its length by half, and the polyp hides inside the tube but retains the tips of its tentacles outside.

During tube construction, *Ceriantheomorphe brasiliensis* is active both during day and night, but does not feed despite intense tentacular movements in response to the presence of food. Feeding is only resumed six days after a new tube is built.

Tube-building in mollusk shells (*Ex situ*)

The tube-building behavior observed in mollusk shells substrates (see supplementary material) is quite similar to that described in sand, except for: in mollusk shells substrates, *C. brasiliensis* does not create a sandbank under its tentacular disk, and during burrowing in shallow substrate, marginal tentacles in

the first cycle are kept completely coiled (Fig. 3a). Also, the polyp does not carry layers of ptychocyst sacks with shell fragments downward the aboral end.

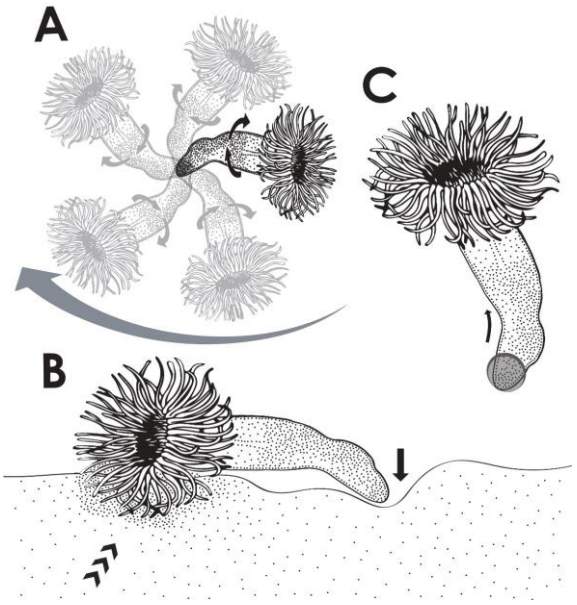

Fig. 2 Tube-building process of *Ceriantheomorphe brasiliensis* (length 11 cm – 11.4 cm) in sandy substrate. **a** Movements of rotation (black arrow) and spinning (grey arrow); **b** Polyp burrowing into the substrate (full arrow) and creating "sandbank" underneath the tentacular disk (dashed arrow); **c** Ptychocyst sack (in grey) carried up the polyp

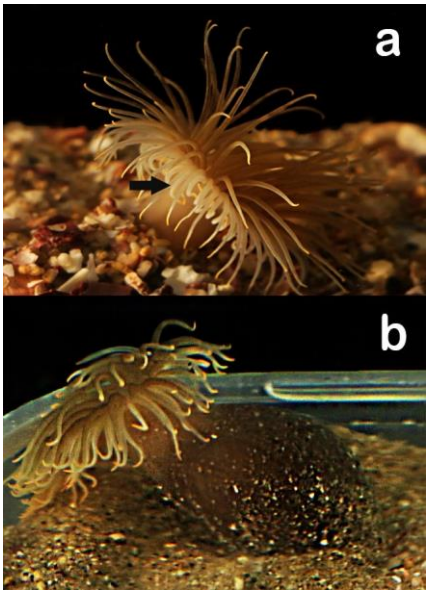

Fig. 3 *Ceriantheomorphe brasiliensis* (length 11 cm – 11.4 cm): **a** Arrow indicating first cycle of marginal tentacles coiled (in hard substrate); **b** Polyp in a roundish shape (in sandy substrate).

For both substrates, regardless of the depth, when the polyp did not build a new tube, it completely filled its column with water, molding its body into a roundish shape (Fig. 3b).

Table 1 shows a comparison of some behavioral aspects observed in both substrates in this investigation.

Table 1. Comparison of *Ceriantheomorphe brasiliensis* behavioral patterns in different substrates during tube-building.

BEHAVIORAL CHARACTERISTICS	DEEP SUBSTRATE		SHALLOW SUBSTRATE	
	Sand	Mollusk shells	Sand	Mollusk shells
Feeding	No	No	No	No
Burrowing	Yes	Yes	Yes	Yes
Tube-building duration	4 h – 17 h	16 h	10 h	8 h
Tube orientation	Vertical	Vertical	Horizontal	Horizontal
Column lining	$^2/_3$ of the column	$^2/_3$ of the column	$^2/_3$ of the column	$^2/_3$ of the column
Nocturnality	Yes	Yes	Yes	Yes

Discussion

Like other bioconstructors (e.g. some amphipods and insects) [79–80] *Ceriantheomorphe brasiliensis* can build more than one tube throughout life. However, it does not feed during tube construction as some other organisms (e.g. Polychaeta and Crustacea) [81–82].

Although fishes *A. virginicus*, *D. formosum*, *E. argenteus*, *E. brasilianus*, *H. parra*, *L. synagris*, *M. curema*, and *O. ruber* were observed swimming near the ceriantharian polyp, none of them approached it when its tentacular disk was outside the tube, avoiding tentacles. Likewise, a similar behavior was observed by Randall and Fautin [83] with fish species *Ostorhinchus nanus* (Allen, Kuiter & Randall, 1994) [84] in association with a non-identified ceriantharian. Like other cnidarians, ceriantharians are carnivorous and have nematocysts (stinging cells) in their tentacles [85]. In general, nematocysts are triggered by touch, discharging toxins [86] that may represent danger to some species, thus keeping them from approaching the tentacles more closely. On the other hand, although common but generally understudied in the last three decades, some fish species (e.g. cod, haddock, scup) are able to graze ceriantharian tentacles and prey on whole ceriantharians [85–87–88–89]. Interestingly, Ates [89] proposed that Ceriantharia evolved the traits of building long tubes and the ability to quickly withdraw into them as a defense mechanism against predation by fishes. Despite that, some fish species are

adapted to live in a harmonic association with anthozoans, such as the well-known clownfish-anemone relationship (e.g. 90–91–92). However, this does not seem to be the case in our investigation because fish species in our study did not interact with the ceriantharian, thus no symbiotic relationship was observed. Despite that, it is typical that species interact with others in the community. For example, *Haemulon parra*, *Anisotremus virginicus* and *Mugil curema* are mutualistics with shrimps and other fish species in cleaning symbiosis [93–94–95–96–97] *Diplectrum formosum* associates with several organisms [97] and is also a commensal with sea stars [97–100], *Eucinostomus argenteus* has a mimicry relationship with snook fishes [101], *Lutjanus synagris* and *Mugil curema* are susceptible to parasitism by gnathiid isopods and copepods, respectively [102–103]. The worldwide invasive crab *Charybdis hellerii* has also a parasitic relationship, but with barnacles and nemerteans [104–105–106]. All these types of interactions and several others offer resources or services to organisms in the community, helping to structure ecosystems and contributing to maintaining biodiversity high-levels [107–108].

When the ceriantharian was completely hidden inside its tube, *A. virginicus* not only swam closer to the tube but also fed on it. However, ceriantharian tubes have filaments of ptychocyst as their main component that, as a cnida, may have a certain level of toxicity (HC pers. obs.). To our knowledge, there is only a single report [109] on ptychocysts found in stomach contents of fishes (butterflyfish), but the authors did not discuss the implication of this particular finding. Some fish species prey on ceriantharians [89], indicating that they are well adapted to this kind of food. Likewise, we assume that species able to prey on ceriantharian tubes are morphologically or physiologically adapted to a possible toxicity by ptychocysts and therefore they are not negatively affected by it. Yet, more studies are needed to further investigate this concern. Moreover, the diet of *A. virginicus* is mainly based on small benthic organisms (e.g. crustaceans, polychaetes, echinoderms) [110] of which are commonly found on ceriantharian tubes [3]. Thus, it is likely that *A. virginicus* accidentally prey on ceriantharian tubes while foraging after its usual food.

Some groups of marine invertebrates (e.g. Bryozoa, Phoronida, Mollusca, Crustacea and Polychaeta) have been commonly found anchored on

the surface of ceriantharian tubes or between layers of ptychocyst filaments [3–4–6–7]. However, most of these studies do not explain how species settle on the tubes — Is it accidentally, by choice or were the species captured and put on the tube by the ceriantharian? — on the other hand, our findings help fill this gap. We observed that adult *C. brasiliensis* does not actively catch sediment particles with its tentacles as some animal groups (e.g. amphipods and polychaetes) that not only select particle sizes but also arrange them in their tubes in a specific manner [81–82]. Therefore, it is unlikely that there is any selection or preference between particles used as structural components for tube construction during the adult stage. Also, after the polyp completely lifts its column from the substrate, it stops spinning over it and the random addition of sediment particles to the tube ends. This implies that there must be other means for sediment and species buildup on the tube post column lifting, and we have two hypotheses for it: 1) Water currents commonly move species and sediment in marine environments, leading to their deposition on surfaces and recruitment [54]. Thereby, it is possible that water motion in natural environment could also contribute to the accidental accumulation of sediments and fouling of benthic species on ceriantharian tubes; 2) Except for species that could be accidentally adhered to the tube during the spinning of the polyp, specimens found on the surface or between layers of ceriantharian tubes recruit this substrate actively.

Water currents and sediments brought to the tentacular disk do not seem to represent a disturbance to *C. brasiliensis* in its natural habitat as it does not hide within the tube when in close contact with them. Otherwise, when extended, tentacles act as sensitive indicators of current direction.

According to Cummins and Lauff [111], and McLachlan [112], the distribution of some tubicolous larvae is related to the size of sediment particles found in the substrate in which they build their tubes. Some chironomid larvae, for example, prefer to recruit substrates with smaller sediment particles rather than substrates with larger ones that cannot be used for tube construction [112]. Inversely, some other species prefer substrates with larger particles [113]. Thus, it is evident that the occurrence of some species in certain regions is related to the substrate composition in which they are found. Although the specimen in our research was an adult, ceriantharian settlement occurs

predominantly during the pelagic larval stage [114] in which suitable recruitment areas may be selected. Thus, further studies with Ceriantharia larvae are crucial to assess the likelihood of substrate preference during larval stage.

Like most worms and some anthozoans, the behavior of repeatedly contracting and distending the column [115–116] also contributes to the burrowing of *C. brasiliensis* polyp into the substrate. Moreover, we observed that temporary column expansion causes prior tube layers to enlarge, allowing the synthesis of new layers beneath the preceding ones, thickening the tube from the inside out [63]. Also, as previously indicated by Arai [117], wave motions in the column wall aid in carrying ptychocyst sacks up, assisting in the lining process.

The continuous contact of tentacles with the substrate during the initial tube-building process probably triggers the discharge of spirocyst capsules, which are abundant in tentacles. Spirocysts are a kind of cnida containing spiraled filaments with adhesive properties that help in prey capture [118]. Due to their adhesive property, spirocyst filaments may bind sand grains together, forming a small "sandbank" on the surface of the substrate surrounding the area where the tube is built. Moreover, our findings support those by Stampar et al. [59] on the adhesive properties of ptychocyst filaments, as we noticed numerous sediment particles adhered to newly released filaments.

According to studies by Ólafsson and Paterson [119] and Meadows et al. [120] with insect tubicolous larvae, crustaceans and polychaetes, sediments cohesion by biological action can stabilize the surface of the substrate [121], contributing to species establishment and foundation in the bottom. Although we have not tested this subject, binding sediment particles on the surface of substrates during tube-building appears to have the potential to stabilize, at some level, portions of substrates containing ceriantharian tubes, and it is a subject worth future investigation.

While laid on the substrate, the accumulation of ptychocyst layers and sediments along the polyp's column appears to inhibit the ceriantharian from rising upright. On the other hand, carrying layers downward is possibly a solution for this obstacle. Similar to *Botrucnidifer norvegicus* [65], *C. brasiliensis* does not use its tentacles to carry ptychocyst layers down, and can also build tubes in hard substrates (e.g. mollusk shells).

The initial tube layers lining the polyp column are extremely thin and fragile, and would most likely not provide adequate protection. When they are transported downwards the aboral end, however, they act as a support, promoting column raising and assisting the polyp to remain erect. After the tube is formed, additional filament layers can be added to thicken it and give further protection. Adding new layers also allows the tube to advance from the aboral end up and, as a result, a portion of the tube may protrude from the surface of the substrate, as seen in other groups of bioconstructors (e.g. polychaetes) that build their tubes from the bottom up [122].

Our results support those by Robinson [123] relating the tube length to protection. According to Robinson [123], the longer the tubes are or the more deeply burrowed into the substrate they are the greater will be the protection provided. Although the tubes built in our study were nearly as long as the polyp that they sheltered, we noticed that when the ceriantharian is under stress it contracts its body, decreasing its length by half. Under this condition, the tube becomes twice as long as the polyp and can provide it greater protection. Regardless, tentacle tips were occasionally observed outside the tube; perhaps because our observations ended before the polyp could burrow more deeply allowing it to hide completely.

In accordance with Child's reports [124–125], we observed that the column of *C. brasiliensis* becomes roundish when outside a tube for too long. This finding suggests that the ceriantharian tube plays an essential role in regulating body's internal water pressure, which affects body form. Without the tube, the ceriantharian is incapable of regulating internal water pressure, causing the column and tentacles to become entirely filled with water and swollen, preventing the animal from feeding. However, in typical conditions (i.e. housed inside a tube), the tube wall offers support to the column, enabling it to contract and distend as needed, as well as the tentacles. Thus, the body wall does not have to bear the full pressure on its own, allowing the ceriantharian to maintain its regular body form and feeding activity stabilized.

Experiments carried out in both shallow and deep substrates with mollusk shells and sand revealed identical results in terms of tube-building behavior. However, minor differences in tube orientation were identified. In deep substrates, the polyp can burrow into the bottom and build the tube in a vertical

position. On the other hand, *C. brasiliensis* cannot burrow deep enough into shallow substrates, building a horizontally oriented tube. These findings suggest that, like some insect tubicolous larvae, the orientation of *C. brasiliensis* tubes is limited by the depth of sediment during tube construction, as well as by the effectiveness of burrowing [126]. In contrast, whereas tube systems built by Arachnactidae members (e.g. *Isarachnanthus nocturnus* (den Hartog, 1977) [69] and *Cerianthus vogti* Danielssen, 1890 [127]) are typically found in deep soft substrates, they are rarely seen in a vertical position [69–128]. Because their tubes are mostly found horizontally oriented, we speculate that Arachnactidae tube-building behaviors differ from that of *C. brasiliensis* (Cerianthidae). Although the substrate depth may impact on tube orientation, the cylindrical shape of the tube remains unchanged.

In general, observing Ceriantharia behavior *in situ* is difficult, especially because ceriantharians are hard to find, sample, and handle owing their hideaway behavior at the least disturbance. As a result, information on this matter is scarce, and our observations accompanied by photos *in situ* are the only ones reported so far. On the other hand, observations of sampled Ceriantharia in aquaria may help in expanding our understanding about Ceriantharia peculiar behaviors. Even though this study only included a single specimen for tube-building behavior description, it contains useful information that may guide future tube-building behavior studies on Ceriantharia, which are, currently, nonexistent. Although we cannot determine if such behavior is typical or not, we observed it three times for each experiment, providing consistency to our results and suggesting that it can occur on occasion. Besides, to diminish pseudoreplication effects, after the conclusion of each experiment replicate the animal was given a one-week interval, avoiding biases. Still, further observations of tube-building behaviors in other ceriantharian species (e.g. *B. norvegicus*, *C. vogti*, *I. nocturnus*) could provide additional support to our preliminary findings.

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SUPPLEMENTARY MATERIAL

Mollusk Shells

Deep substrate

No reminiscent ptychocyst membrane from a previous tube was observed.

As soon as the polyp is placed on the substrate, its column and tentacles distend, increasing the length of its body. The column then repeatedly contracts and distends longitudinally, allowing the polyp to rotate and spin its body across the substrate. During this process, ptychocyst filaments are synthesized in the column's aboral end and carried upward with shell fragments adhered to them. Moreover, the polyp completely coils the whitish marginal tentacles in the first cycle (outermost cycle) of the tentacular disk and burrows into the substrate with its aboral end while spinning and rotating, gradually inserting the column end in it.

Once the column end is burrowed into the substrate, both rotation and spinning movements stop. The column, on the other hand, continues to contract and distend, while ptychocyst filaments and shell fragments are carried up, producing a delicate lining. As the polyp contracts and distends its column, it also burrows deeper into the substrate assisting the column lifting.

Within 16 hours, $^{2}/_{3}$ of the column is completely inserted into the substrate, forming a burrow lined with ptychocyst filaments that the polyp uses as habitation. Lastly, some shell fragments were observed adhered to the tentacular crown above the substrate.

Shallow substrate

No reminiscent ptychocyst membrane from a previous tube was observed.

In shallow substrates, the polyp reduces its body length by half rather than increasing it as it does in deep substrates. In this condition, it gradually distends and spins. Only when the spinning stops, the polyp repeatedly “hammers” the substrate with its aboral end, burrowing into it. Simultaneously, sacks of ptychocyst filaments are released/produced in this body region and transported up the column, carrying shell fragments with them. Each sack is carried upward and molded in the column by mild column expansions. At every expansion, one sack is released while part of the aboral region is withdrawn from the early burrow. Within approximately 8 hours, a lining of ptychocyst filaments and shell fragments fully covers $^{2}/_{3}$ of the column, forming an inconspicuous tube that is remarkably similar to the substrate surface and horizontally oriented.

Despite tube construction, the ceriantharian does not lift its column, but instead, it continues to "hammer" the substrate. Then, it slips out of the horizontal tube and remains on the substrate with its column filled with water and completely distended, and tentacles contracted. Lastly, the ceriantharian does not exhibit selection or preference for different sizes of shell fragments, does not feed during tube construction, and remains active at night.

1.2. **Corals as substrate for tube-dwelling anemones**

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Abstract

This study is about seven individuals of *Botrucnidifer norvegicus* Carlgren, 1912 (Anthozoa, Ceriantharia) that were attached to a stony deep-sea coral, *Desmophyllum pertusum* (Linnaeus, 1758) (Anthozoa, Scleractinia). They were collected during surveys at Agdenes, Trondheimsfjord. We included photographs of live specimens, discussed the implications of this association, and described the species interaction and the behavior of the ceriantharian, which we discussed in light of previous published information.

Keywords: anchoring, association, behavior, Ceriantharia, epibiosis, Scleractinia.

Introduction

The subclass Ceriantharia (Cnidaria; Anthozoa) comprises a group of marine tube-dwelling animals best known as ceriantharians. They are found in soft substrates worldwide, and are divided over only three families: Arachnactidae, Botrucnidiferidae, and Cerianthidae [129].

Although members of Cerianthidae and Arachnactidae have been more commonly studied, given a number of reports on them addressing morphologic, genetic, and phylogenetic studies (e.g. 128–130–131–132–133), little is known about their life habits and/or interactions with other species [3], and their behavior [134]. Likewise, studies entirely focused on Botrucnidiferidae are even fewer and infrequent, leaving a knowledge gap concerning this family [135].

Studies mentioning Botrucnidiferidae date back to the 1800s, when van Beneden [136] first proposed a classification for Ceriantharia based on their larval forms, in which Botrucnidiferidae was distinguished from other families by a particular internal structure, similar to a grape bunch, later termed botrucnid [137], and only found in this family. Later, during the 1900s, some studies on Ceriantharia mentioned Botrucnidiferidae (e.g. 74–138–139–140–141–142–143–144) but none was specifically dealing with this family. One century later, studies reporting Botrucnidiferidae have reappeared, resulting in new information on some species of this family, mainly those of the deep-sea genus *Botrucnidifer* Carlgren, 1912 [74–136].

Its type species, *Botrucnidifer norvegicus* Carlgren, 1912 [74], is rarely found and difficult to sample, due to its behavior of retracting inside tubes at the slightest disturbance, as most ceriantharians. Issues like these prevent that knowledge about Botrucnidiferidae becomes easy to obtain. To date, the Botrucnidiferidae have some information about their larval forms, characteristic structures [114–136–137–138–144–145], and their tube ultrastructure [59]. In addition, there is recent ecological data on new localities and depths [136–145–146–147], and the occurrence on living substrata [74–148]. The most recent studies focused entirely on Botrucnidiferidae reported on a new species of *Botruanthus* McMurrich, 1910 [140–148] from the Golf of Mexico, and on coloniality in *Botrucnidifer norvegicus* [71].

In the present study, we describe the association between *B. norvegicus* and a stony deep-sea coral of the order Scleractinia. We also discuss the

ecological implications of this association and provide information about its interspecific interaction and the behavior of the ceriantharian.

Material and methods

A total of seven specimens of the species *Botrucnidifer norvegicus* were found on dead colonies of the scleractinian *Desmophyllum pertusum* (Linnaeus, 1758) [76], previously known as *Lophelia pertusa* [149]. The samples were dredged with a triangular dredge at Agdenes, Trondheimsfjord (63º39'5.6412" N 9°46'0.9012" E), from 110 to 220 m depth on 11 June 2020. The dead coral was kept in an aquarium with running sea water, where specimens were observed and photographed (Fig. 1a–b) with an Olympus Mju camera. Tubes with ceriantharian polyps were removed from the corals and placed in a petri dish for photography and filming using a MC170HD CMOS camera equipped with a Leica Z6 Apo lens and Leica LAS imaging software. Specimens were filmed up to about four minutes.

Specimens were preserved in 96% ethanol and deposited in the marine invertebrate collection at Norwegian University of Science and Technology (NTNU University Museum), Trondheim [150] under the registration numbers NTNU-VM 76342-76347.

Results and discussion

We observed seven individuals of *Botrucnidifer norvegicus* anchored to branches of dead colonies of the stony deep-sea coral *Desmophyllum pertusum*. Previously, Carlgren [74] reported on *B. norvegicus* occurring among branches of corals belonging to the scleractinian genera *Desmophyllum* Ehrenberg, 1834 [151], *Paragorgia* Edwards, 1857, and *Oculina* Lamarck, 1816 [152] (both alive and dead) from the Trondheimsfjord, but his survey did not result in detailed information and illustrations of the associations between the ceriantharians and their hosts.

Polyps of *B. norvegicus* in our study were lodged in tubes slightly longer than their builders (polyps: 30–40 mm; tubes: 40–50 mm), next to each other, and anchored to the skeleton of *D. pertusum*, with their tentacle discs close to

openings in the skeleton of their hosts. Some tubes were ramified and lodged two ceriantharian polyps (Fig. 1a, c). Furthermore, this is the first record of a ceriantharian species containing more than one single polyp in ramified tubes, which is exceptional because species that build tube systems usually lodge only one polyp per tube, such as *Isarachnanthus nocturnus* (den Hartog, 1977) [69] and *Cerianthus vogti* Danielssen, 1890 [69–127–128–70]. Unfortunately, our observations did not reveal how these polyps originated in the same tube but two hypotheses are that they originated from: 1) asexual reproduction, which is still relatively unknown in Ceriantharia; 2) settlement of two larvae (planulae?) of similar offspring in a restricted habitat, which fused their tubes during construction. In order to find the correct explanation, more research on the species' life cycle has to be performed.

Molodtsova [146] suggested the possibility of *B. norvegicus* being an obligate commensal of living deep-sea corals. We do not entirely agree with this hypothesis as we have observed *B. norvegicus* occurring only on dead colonies, similar to Jensen and Frederiksen [153]. Instead, we suggest that, when *B. norvegicus* is anchored to dead coral colonies or dead parts of living corals, it uses the bare coral skeleton as an anchoring substrate, not necessarily in connection with a symbiotic relationship. On the other hand, when anchored to living coral colonies, the ceriantharian could have an epibiotic relationship, however non-obligatory. There is no specific information on toxins in ceriantharians and scleractinians that could be harmful to the other, because they have not yet been encountered in aggressive interactions.

Most species of Ceriantharia typically burrow and build their tubes in soft substrates [6–67]. Conversely, this seems not to be the case of *B. norvegicus*, as most reports on it, including the present study, usually describe specimens occurring on cold-water stony coral reefs [74–146–153]. In general, such reefs are affected by human actions (e.g. dredging, pollution, trawling), which not only cause environmental disturbances but also contribute to severe damage, impacting the benthic fauna nearby [154–155–156–157–158–159]. As a consequence, it is expected that invertebrates that use dead coral skeletons or living corals as habitat become vulnerable to threats involving their hosts and may be compromised by their loss and instability [160–161].

Nothing is known about the life cycles and behavior of Botrucnidiferidae [70]. Our data, however, can contribute a bit to the latter. After dissecting the tubes of the seven polyps, we observed that, other than the tubes themselves, polyps of *B. norvegicus* have a thin and translucent membrane of ptychocyst filaments lining their whole body (tentacle disc and column) (Fig. 1d). This membrane has been moved halfway down the column (Fig. 1e), towards the aboral region, while the polyp rotates its body clockwise. One of the specimens first rotated its body clockwise and then counterclockwise after the membrane reached halfway its column. The ptychocyst membrane kept on sliding down the column regardless the direction in which the polyp rotated its body. Tentacles were not observed to help in moving the membrane down. In spite of this, after the membrane uncovered the tentacle disc, the marginal tentacles gradually extended and the tentacle disc became wide open.

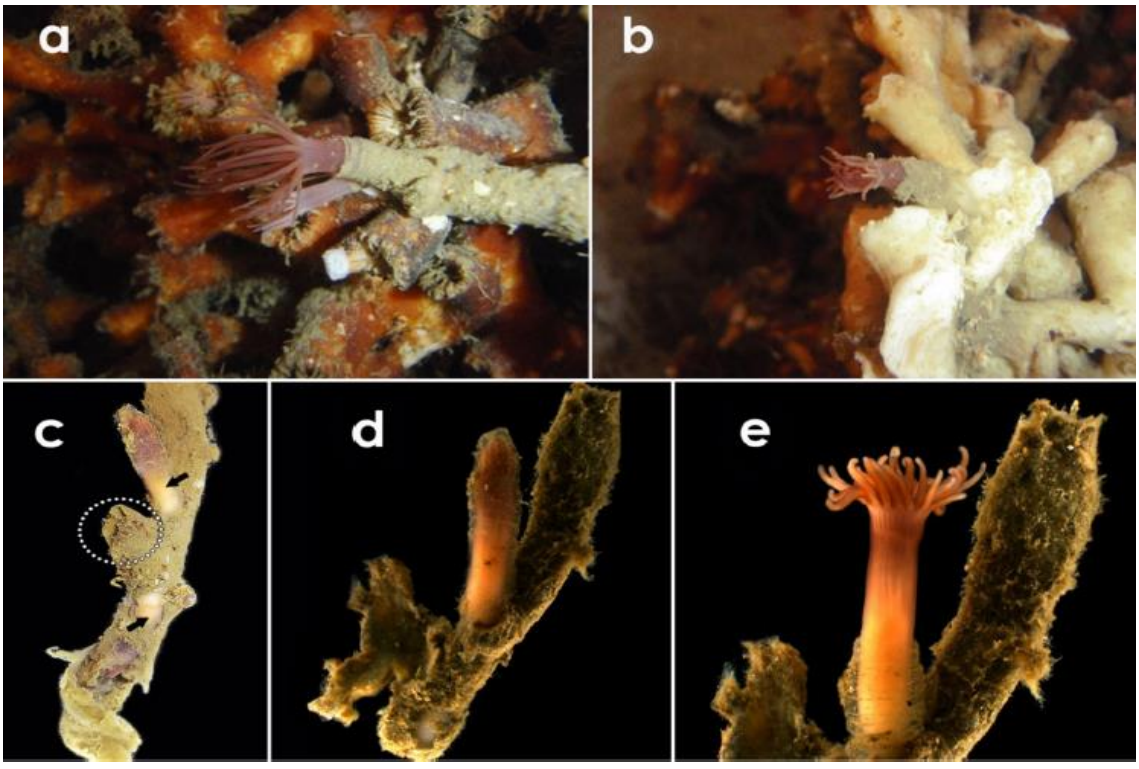

Fig.1 Individuals of *Botrucnidifer norvegicus* (length 30 mm – 40 mm) attached to dead *Desmophyllum pertusum* coral: **a** two polyps of *B. norvegicus* in a ramified tube; **b** one polyp of *B. norvegicus*; **c** ramified tube of *B. norvegicus*, housing two polyps — the circle indicates tube ramification and the arrows indicate polyps; **d** polyp of *B. norvegicus* with entire body covered by ptychocyst filaments membrane; **e** polyp of *B. norvegicus* with ptychocyst filaments membrane covering only half of its column.

Since information about Botrucnidiferidae is scattered and incomplete, our findings may contribute to fill and complete some behavioral and ecologic gaps in knowledge about this family. The present study showed that more investigations on species of this family are necessary, such as regarding their life cycle, behavior, feeding, tube construction, and possible associations with other animals.

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CHAPTER 2. *Biodiversity*

2.1. **Crustacea biodiversity in tubes of Ceriantharia (Cnidaria; Anthozoa), including the description of a novel species of Amphipoda from southeastern Brazil**

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Abstract

This study investigates ceriantharian tubes to better understand the biodiversity of marine benthic crustaceans. The inner and outer surfaces of eight tubes of Ceriantharia from the northern coast of the state of São Paulo, Brazil, were analyzed. We observed 14 taxa of crustaceans, including one novel species, *Podocerus carmelina* **sp. nov.** We also report two new location records for previously known species and provide an identification key for *Podocerus* species with type localities in the Atlantic Ocean. The new species can be easily differentiated from its congeners by the following features: pereonite 3 with weak dorsal carina; gnathopod 2 carpus indistinct, almost entirely fused to propodus, propodus bearing several facial short plumose setae, palm defined by a proximal blunt projection, with a subtriangular tooth medially, a “u-shaped” concavity and a crenulated lobe distally; pereonite 4 elongated, about 2.2 × deeper than wide; pereonites 5–6 anteroventral corner with a subacute projection; coxa 5 anteroventral corner with a notch, forming an acute spine; telson dorsal lobe with three stout setae, with naked lateral margins; uropod 2 outer ramus with one subapical long and stout plumose seta.

Keywords: benthic marine invertebrates, *Podocerus*, taxonomy, tube-dwelling anemone.

Introduction

Biodiversity represents the variety of life on Earth, including plants, animals, fungi, and bacteria, as well as the genetic variability at the species and individual levels [162]. High levels of biodiversity enhance ecosystem multifunctionality across trophic levels and habitats, increase ecosystem stability and productivity, maintain water quality, and help ecosystem recovery after disturbances [162–163–164–165].

Despite the significant importance of biodiversity, identifying the world's species remains one of the greatest challenges in taxonomy [166]. Although databases do exist with records of thousands of species (e.g. Ocean Biodiversity Information System - OBIS, World Register of Marine Species - WoRMS, Global Biodiversity Information Facility – GBIF), the number of currently known species represents a small fraction of the estimated number worldwide [8]. Marine species, for example, represent 65% of global biodiversity [167], and yet only 9% of them are known [8–10]. Moreover, the lack of studies on marine invertebrate diversity and demography compound the difficulty of assessing extinction risks and preparing conservation plans for invertebrate species of concern [168–169].

The paucity of studies into the biodiversity of benthic marine invertebrates is particularly concerning in the Brazilian context. The fauna of the Brazilian coast is among the most poorly studied in the world, a fact that is supported by the uptick in reports of new species, even in well-explored regions [166–167–170]. In particular, peracaridan amphipods (Crustacea) are among the most important members of the marine benthic macrofauna, with over 10.000 species identified worldwide. However, new species reports have risen significantly over the last 20 years [171], suggesting a taxonomic knowledge gap. These animals are quite diverse and abundant, with a wide distribution and bathymetry. They can be found in association with other invertebrates (e.g. cnidarians) or living freely on rock and sandy beaches, such as those of São Sebastião, on the northern coast of the state of São Paulo [171–172]. The beaches of São Sebastião have various substrates, ranging from mud to coarse sand, as well as rock fragments and shell debris [173–174].

Research into the benthic biodiversity in Brazil is most active along the country's southeastern coast, especially in the northern extent of this region.

However, while this area is considered a hotspot for marine research [175], many species remain unknown, while occurrence reports of known species have become less frequent.

Studies on tubes of tube-dwelling anemones (Anthozoa; Ceriantharia), however, have provided important insights into marine benthic biodiversity, as these structures can provide favorable habitats for several groups of marine invertebrates (e.g. Annelida, Crustacea, Mollusca), acting as small-scale biodiversity hotspots [3]. This highlights their importance in research on species biodiversity.

Associations between amphipods and anthozoans (Cnidaria) are not uncommon, though these are most often described as co-occurring with Actiniaria (sea anemones) [176–177–178–179–180], while associations with Ceriantharia are limited to a small number of studies (e.g. 3–5). Ceriantharian tubes are commonly overlooked, and collecting polyps from the tubes is challenging. These might be reasons for the underreporting of species associated with Ceriantharia, resulting in a poor understanding of the biodiversity living in and on them [3]. This study, therefore, aims to investigate ceriantharian tubes in order to advance the understanding of marine crustacean benthic biodiversity.

Material and methods

Eight tubes of Ceriantharia were collected from soft bottoms at depths of 3 m – 35 m by SCUBA diving off the coast of São Sebastião and Alcatrazes Island, both located along the northern coast of São Paulo state, Brazil, and in Laje de Santos on the southern coast of the same state. Each tube was collected and immediately stored in individual plastic bags containing only seawater. Two tubes from São Sebastião and one from Laje de Santos had one polyp each inside; these were removed from the tubes prior to analyses and morphologically identified to determine the species as *Ceriantheomorphe brasiliensis* (Mello-Leitão, 1919) [73], following Lopes et al. [133] and Stampar et al. [129]. The remaining five tubes, from Alcatrazes Island, contained polyps inside while in nature, but they escaped at the time of sampling. Consequently, identifying the tubes collected without polyps to species or genus level was not

possible. However, their heavy appearance suggested that they were built by members of the family Cerianthidae [59].

After sampling, all material was individually preserved in labeled jars containing 70% seawater-buffered ethanol solution.

First, tubes were longitudinally cut with scissors and opened. They were then placed inside bowls full of freshwater and fixed with acupuncture needles to black craft foam at the bottom of the bowls. The inner (smooth) and outer (rugged) surfaces of all the tubes were analyzed separately under an Opticam stereomicroscope.

The analysis was limited to crustaceans attached to the tubes, which were removed and measured using a stereomicroscope and an optical microscope. Afterward, all specimens were morphologically identified through the dissection of mouthparts and appendages on glycerin gel slides. The illustrations of *Podocerus carmelina* **sp. nov.** were made under an optical microscope with a Motic BA-310 camera lucida and digitized with CorelDRAW 2018. Setal/spine classification followed Garm and Watling [181], and the nomenclature of gnathopod palms was based on Poore and Lowry [182]. Moreover, we prepared an identification key for *Podocerus* species with type localities in the Atlantic Ocean.

All crustaceans examined in this study were deposited at the Crustacea Collections of the Universidade do Estado do Rio de Janeiro (UERJ).

Results

In total, we found 143 crustaceans from 14 different taxa, on eight ceriantharian tubes (Table 1). All the specimens analyzed were alive at the time of sampling but dead during our analyses.

Table 1. Crustacea taxa, grouped by orders, found on ceriantharian tubes.

CRUSTACEA ORDERS	TUBES OF CERIANTHARIA	
	Ceriantheomorphe brasiliensis (n = 3)	Unidentified tubes (n = 5)
Amphipoda	*Bemlos* aff. *unicornis* (n = 1) *Cymadusa* sp. (n = 1) Hyalidae sp. (n = 1) *Photis sarae* (n = 4)	*Caprella equilibra* (n = 13) *Ericthonius brasiliensis* (n = 4) *Jassa valida* (n = 1) *Monocorophium* sp. (n = 1) *Photis sarae* (n = 7) *Podocerus carmelina* **sp. nov.** (n = 82) *Pseudaeginella biscaynensis* (n = 4)
Isopoda	-	Asellota sp. (n = 1)
Leptostraca	-	*Nebalia* sp. (n = 1)
Tanaidacea	Apseudomorpha sp. (n = 1) Tanaidomorpha sp. (n = 19)	Tanaidomorpha sp. (n = 2)
	Total taxa: 6	Total taxa: 10

All crustaceans were found on the surface of the ceriantharian tubes, primarily in the softer areas, surrounded by ptychocyst filaments. Even so, all the taxa detected within the filaments were also found in areas free from them. Specimens that were more deeply embedded in the filaments were correspondingly more firmly anchored to tubes. Meanwhile, specimens with no filaments surrounding them remained free on the surface of the tubes without any adhesion.

Some specimens were found with their pereopods covered with filaments and were firmly attached to the tubes. Some Tanaidomorpha were observed with their gnathopods keeping them anchored to tubes but not as firmly attached as the species surrounded by ptychocyst filaments.

Although tubicolous, no tubes belonging to *Bemlos* aff. *unicornis*, *Cymadusa* sp., *Ericthonius brasiliensis* (Dana, 1853) [183], *Jassa valid*a Dana, 1853 [183], *Monocorophium* sp., *Photis sarae* Souza-Filho & Serejo, 2010 [184], or any of the tanaidaceans was found on or in the ceriantharian tubes.

Taxonomy

Subclass Eumalacostraca Grobben, 1892
Order Amphipoda Latreille, 1816
Suborder Senticaudata Lowry & Myers, 2013
Family Ampithoidae Boeck, 1871
Genus *Cymadusa* Savigny, 1816
***Cymadusa* sp.**

Material examined. One juvenile, in ethanol 70%, on *Ceriantheomorphe brasiliensis* tubes, São Sebastião, state of São Paulo, Brazil (UERJ 1321).
Remarks. This is a relatively abundant genus worldwide, commonly found in shallow waters and usually associated with algae, coral rubble, rhodolith banks, and sandstone reefs. Members of this genus are typically tubicolous. Currently, there are seven species of *Cymadusa* recorded in Brazil, ranging from southern to northeastern waters [171–185–186]. The individual found in this study was a juvenile, making species-level identification impossible.

Family Aoridae Stebbing, 1899
Genus *Bemlos* Shoemaker, 1925
Bemlos* aff. *unicornis

Material examined. One individual, in ethanol 70%, on *Ceriantheomorphe brasiliensis* tubes, Laje de Santos, state of São Paulo, Brazil (UERJ 1323).
Remarks. The specimen was damaged, thus hampering precise identification. Nevertheless, it appeared to be very similar to *Bemlos unicornis* [187]. This species is found in the Brazilian states of Paraná, Pernambuco, Alagoas, and São Paulo, where it is often found in association with algae, sponges, seagrasses, and hard substrates [171–188].

Family Caprellidae Leach, 1814
Genus *Caprella* Lamarck, 1801
Caprella equilibra Say, 1818 [189]

Material examined. Four individuals, in ethanol 70%, on unidentified ceriantharian tubes, Kitahara MV coll., Alcatrazes Archipelago, state of São Paulo, Brazil, May 22, 2019 (UERJ 1286); two individuals, in ethanol 70%, same sampling data (UERJ 1291); three individuals, in ethanol 70%, same sampling data (UERJ 1293); four individuals, in ethanol 70%, same sampling data (UERJ 1310).

Remarks. This is a cosmopolitan species. In Brazil, it is reported in the states of Rio de Janeiro, São Paulo, Paraná, Rio Grande do Sul, and Santa Catarina, typically found associated with algae, hydroids, bryozoans, sponges, and ascidians [171–190].

Genus *Pseudaeginella* Mayer, 1890

Pseudaeginella biscaynensis (McCain, 1968) [191]

Material examined. Two individuals, in ethanol 70%, on unidentified ceriantharian tubes, Kitahara MV coll., Alcatrazes Archipelago, state of São Paulo, Brazil, May 22, 2019 (UERJ 1287); two individuals, in ethanol 70%, same sampling data (UERJ 1294).

Remarks. This species can be found associated with seaweed, seagrass, sponges, and corals [192]. It has also been reported as occurring on the brown algae *Dictyota* spp., in shallow waters from Sebastião Gomes Reef and Abrolhos Bank, state of Bahia, Brazil [193]. This species was previously reported as occurring only in Bahia [171], with this specimen representing the first record from São Paulo state.

Family Corophiidae Leach, 1814

Genus *Monocorophium* Bousfield & Hoover, 1997

***Monocorophium* sp.**

Material examined. One juvenile, in ethanol 70%, on unidentified ceriantharian tubes, Kitahara MV coll., Alcatrazes Archipelago, state of São Paulo, Brazil, May 22, 2019 (UERJ 1315).

Remarks. *Monocorophium* is a shallow-water genus found in a wide range of substrata, including tubes, algae, bryozoans, and tunicates. Three species have

been reported in Brazil, from Pernambuco to Santa Catarina states [171]. Given the reduced size of the juvenile specimen in this study, it was not possible to identify it to species level.

Family Hyalidae Bulyčeva, 1957
Hyalidae sp.

Material examined. One juvenile, in ethanol 70%, on *Ceriantheomorphe brasiliensis* tubes, São Sebastião, state of São Paulo, Brazil (UERJ 1320).
Remarks. Hyalidae is a cosmopolitan family often found in intertidal zones and shallow littoral waters [194]. It includes eight species in Brazilian waters, which occur from the states of Maranhão to Rio Grande do Sul [171]. The only sampled specimen in our study was a tiny juvenile that was hard to identify beyond the family level.

Family Ischyroceridae Stebbing, 1899
Genus *Ericthonius* H. Milne Edwards, 1830
Ericthonius brasiliensis (Dana, 1853) [183]

Material examined. Four individuals, in ethanol 70%, on unidentified ceriantharian tubes, Kitahara MV coll., Alcatrazes Archipelago, state of São Paulo, Brazil, May 22, 2019 (UERJ 1312).
Remarks. This tubicolous fouling species has a broad geographic range, primarily because it can raft on floating objects [195]. In Brazil, it is known to occur in the states of Pernambuco, Bahia, Rio de Janeiro, and São Paulo [171].

Genus *Jassa* Leach, 1814
Jassa valida (Dana, 1853) [183]

Material examined. One juvenile, in ethanol 70%, on unidentified ceriantharian tubes, Kitahara MV coll., Alcatrazes Archipelago, state of São Paulo, Brazil, May 22, 2019 (UERJ 1316).
Remarks. *Jassa valida* is among the three species of the genus known to occur in Brazil, which has thus far been recorded from the coast of São Paulo, Paraná

and Rio de Janeiro states [171]. This species, originally described by Dana [183] on the coast of Rio de Janeiro, was recently resurrected by Conlan et al. [196] as a transhemispheric species with a disjunct known distribution in the northwestern Atlantic (from North Carolina to Florida) and southwestern Atlantic (from Rio de Janeiro to Santa Catarina), in addition to a ship-based record far off the coast of Chile. This genus is considered to be particularly important due to its high densities in both natural and artificial substrates and its role as a food source for many organisms (e.g. fishes, gray whales and invertebrates) [196–197–198–199–200–201]. Although the examined individual in our study was a juvenile, it was possible to confirm its identity based on a recent publication by Conlan et al. [196], which established a neotype for the species with material from the Brazilian state of Santa Catarina.

Family Photidae Boeck, 1871
Genus *Photis* Krøyer, 1842
Photis sarae Souza-Filho & Serejo, 2010 [171]

Material examined. One individual, in ethanol 70%, on unidentified ceriantharian tubes, Kitahara MV coll., Alcatrazes Archipelago, state of São Paulo, Brazil, May 22, 2019 (UERJ 1296); six individuals, in ethanol 70%, same sampling data; one individual, in ethanol 70%, on *Ceriantheomorphe brasiliensis* tubes, São Sebastião, state of São Paulo, Brazil (UERJ 1318); three individuals, in ethanol 70%, on *Ceriantheomorphe brasiliensis* tubes, Laje de Santos, state of São Paulo, Brazil (UERJ 1322).
Remarks. This species has already been reported in São Paulo state [3]. However, this is its first record from Alcatrazes Island. It was previously found in Guanabara Bay in the state of Rio de Janeiro [171], as well as in São Sebastião and Laje de Santos, both in São Paulo state [3].

Family Podoceridae Leach, 1814
Genus *Podocerus* Leach, 1814
Diagnosis. See Hughes [202]
***Podocerus carmelina* sp. nov.** (Fig. 1–5)

***Material examined*.** Holotype: male, dissected and illustrated, 3.3 mm, 30 m – 35 m depth, on ceriantharian tubes, Kitahara MV coll., Alcatrazes Archipelago, state of São Paulo, Brazil, May 22, 2019 (UERJ 2318). Paratypes: 24 individuals, in ethanol 70%, same sampling data as holotype (UERJ 1288); 14 individuals, in ethanol 70%, same sampling data as holotype (UERJ 1292); 14 individuals, in ethanol 70%, same sampling data as holotype (UERJ 1295); two individuals, in ethanol 70%, same sampling data as holotype (UERJ 1309); 27 individuals, in ethanol 70%, same sampling data as holotype (UERJ 1313).

Diagnosis. Head with short rostrum, lateral cephalic lobe rounded, with a short internal spine above it. Antenna 1 accessory flagellum 1-articulate. Right mandible incisor with five teeth, lacinia mobilis flabellate, palp article 3 apically setose with plumose setae. Left mandible incisor with six teeth, lacinia mobilis with three teeth, molar flake present. Gnathopod 1 palm not defined by a stout seta; dactylus shorter than palm, inner margin with three acute spines. Gnathopod 2 carpus indistinct, almost entirely fused to propodus; propodus bearing several facial short plumose setae, palm defined by a proximal blunt projection, with a subtriangular tooth medially, a “u-shaped” concavity, and a crenulated lobe distally. Pereonite 4 elongated, about 2.2 × deeper than wide. Pereonites 5 – 6 anteroventral corner with a subacute projection. Coxa 5 anteroventral corner with a notch, forming an acute spine.

Description. Based on male holotype (UERJ 2318).

Head. *Eyes:* medium and ovate, rostrum short, lateral cephalic lobe rounded, with a short internal spine above it. *Antenna 1*: peduncular article 1 about 1.4 × longer than wide, bearing three plumose facial setae, dorsal margin with one simple and two plumose setae, article 2 about 4.7 × longer than wide, dorsal apex three plumose setae, ventral margin setose with plumose setae, article 3 about 5.3 × longer than wide, dorsal margin with three plumose setae medially and one distally, ventral margin setose with plumose setae; accessory flagellum 1-articulate, 6.6 × longer than wide; primary flagellum 3-articulate, articles reducing in length, article 1 ventrally with plumose setae and one facial stout seta, article 2–3 with sparse setules. *Antenna 2*: peduncular articles 1 – 2 short, article 3 slightly wider than long, with four plumose facial setae, ventral margin

with eight plumose setae, article 4 about 3.7 × longer than wide, with seven plumose facial setae, dorsal margin with four setae medially and one distally, ventral margin with nine plumose setae, article 5 about 5.5 × longer than wide, with six plumose facial setae, dorsal margin with four setae, ventral margin with five plumose setae; flagellum 3-articulate, reducing in length, articles 1 – 3 with sparse stout and simple setae. *Right mandible*: incisor with five teeth, lacinia mobilis flabellate, with two small and three large teeth, molar triturative, flake present, setal accessory row with two stout serrate setae and two plumose setae; palp article 1 short, article 2 broad, about twice as long as wide, with nine plumose facial setae, medial margin with 10 plumose setae, article 3 about 0.7 × the length of article 2, apical margin truncated, weakly crenulated, strongly setose with plumose setae, bearing two facial rows of plumose setae. *Left mandible*: incisor with six teeth, lacinia mobilis slender, with three teeth, molar triturative, flake present, setal accessory row with two stout serrate setae and four plumose setae. *Maxilla 1*: outer plate with six stout apical setae, four of which bifid; palp 2-articulate, with facial row of plumose setae, lateral margin covered with setules, apical margin with four wedge-shaped stout setae. *Maxilla 2*: inner plate apically setose with plumose setae, medial margin with eight long setules and plumose setae distally; outer plate broader than inner, about 1.2 × longer than inner, apically setose with plumose setae. *Maxilliped*: inner plate broad, apically truncated, setose with plumose setae; outer plate with six facial proximal plumose setae and eight plumose facial setae medially, medial margin with six stout plumose setae, apically with four plumose setae; palp article 2 medial margin setose with plumose setae, lateral margin with two distal plumose setae, article 3 setose with medial, lateral and plumose facial setae, article 4 embedded, about twice as long as wide, apically with three stout and two plumose setae.

Pereon. *Gnathopod 1*: coxa rhomboidal, produced anteriorly; basis about 2.1 × longer than wide; ischium short, posterodistal corner with two setae; merus posterior margin with a weak lobe, bearing many long setae; carpus with three facial rows of long setae, posterior margin lobed, bearing long setae; propodus anterior margin with seven facial rows of setae; palm acute, setose, not defined by a stout setae, about 1.8 × longer than posterior margin of propodus; dactylus shorter than palm, inner margin with three acute spines. *Gnathopod 2*: coxa

subrectangular; basis about 2.7 × longer than wide, anterodistal corner with large produced lobe, posterodistal corner with one plumose seta; ischium short and subtriangular, posterodistal corner with two plumose setae; merus posterior margin with six plumose setae distally; carpus indistinct almost entirely fused to propodus; propodus subovate, anterior and posterior margins with sparse plumose setae, bearing several facial short plumose setae; palm setose, defined by a proximal blunt projection, with a subtriangular tooth medially, a "u-shaped" concavity and a crenulated lobe distally; dactylus slightly shorter than palm. *Pereopod 3*: coxa produced anteriorly, ventral margin with one short seta; basis about 1.7 × longer than wide, anterodistal corner with a produced lobe bearing four setae, posterodistal corner with four setae; ischium posterodistal corner with three setae; merus anterior margin distally produced, bearing four setae, posterior margin with two setae medially and five distally; carpus margins weakly setose, distal facial lobe produced; propodus anterior and posterior margins weakly setose; dactylus about 35% the length of propodus. *Pereonite 4*: elongated, about 2.2 × deeper than wide. *Pereopod 4*: coxa anteroventral margin convex; basis about 1.5 × longer than wide, anterior margin produced as a lobe, bearing eight setae, posterodistal corner with a row of four setae; ischium subrectangular, anterodistal corner with one seta, posterodistal corner with a row of four setae; merus anterior margin distally produced, bearing four setae, posterior margin with one medial and a distal seta; carpus weakly setose, distal facial lobe produced, anterior margin with a distal row of five setae, posterior margin with a distal row of six setae; propodus weakly setose, anterior margin with a distal row of six setae, posterior margin with a distal stout setae plus a short seta; dactylus about 55% the length of propodus. *Pereonite 5*: anteroventral corner with a subacute projection downward. *Pereopod 5*: coxa anteroventral corner with a notch, forming an acute spine; basis about 1.3 × longer than wide, anterodistal corner produced as a lobe bearing six setae, mesial margin slightly concave, with a small lobe bearing two setae, posterodistal corner with a row of four setae; ischium short and subrectangular, anterodistal corner with one seta, posterodistal corner with a row of four setae; merus anterior margin distally produced, bearing five setae; carpus weakly setose, distal facial lobe produced, anterior margin with a distal row of six setae, posterior margin with a distal row of four setae; propodus weakly setose;

dactylus about 45% the length of propodus, outer margin with one proximal and two distal setules, inner margin with one distal setule. *Pereonite 6*: bearing two lateral setae, anteroventral corner with a subacute projection downward. *Pereopod 6*: coxa subtrapezoidal; basis about 1.2 × longer than wide, anterior margin produced as a lobe, bearing two distal setae, mesial margin slightly concave with two distal setae; ischium short and subquadrate, anterodistal corner with two setae, posterodistal corner with one seta; merus anterior margin distally produced, bearing six setae; carpus weakly setose, distal facial lobe produced, anterior margin with a distal row of seven setae, posterior margin with a distal row of three setae; propodus anterior margin with four sets of setae until apex, posterior margin with three stout setae medially and two slender distally; dactylus about 50% the length of propodus, outer margin with one proximal and one distal setule, inner margin with one distal setule. *Pereopod 7*: coxa ventral margin with one setule; basis subquadrate, slightly longer than wide, anterodistal corner with two setae, posterior margin with three setae, posterodistal corner with one seta; ischium anterodistal corner with two setae, posterodistal corner with one seta; merus anterior margin distally produced, bearing seven setae, posterior margin with one distal seta; carpus weakly setose, distal facial lobe produced, anterior margin with a distal row of five setae, posterior margin with a distal row of six setae; propodus anterior margin with four sets of setae until apex, posterior margin with five sets of stout setae until apex; dactylus about 45% the length of propodus, outer margin with one proximal setule, inner margin with one distal setule.

***Pleon*.** *Pleonites 1 – 2*: with three lateral setae each. *Epimeral plate 1*: with rounded margins, bearing three lateral setae near dorsal margin. *Epimeral plate 2*: anterior margin weakly concave, ventral and posterior margins rounded, bearing three lateral setae near dorsal margin. *Epimeral plate 3*: anterior margin weakly concave, ventral and posterior margins rounded. *Uropod 1*: peduncle 2.8 × longer than wide, dorsomedial and dorsolateral margins with five and four stout setae with accessory seta, respectively; inner ramus slightly longer than peduncle and 1.3 × longer than outer ramus, dorsomedial and dorsolateral margins with seven and three stout setae with accessory seta, respectively, apically with three stout setae with accessory seta and one longer stout seta; inner ramus dorsolateral margin with two stout setae with accessory setae,

apically crenulated, bearing two stout setae with accessory seta and one longer stout seta. *Uropod 2*: peduncle slightly longer than wide, dorsomedial margin with one distal stout seta, dorsolateral margin with four stout setae; inner ramus about twice longer than peduncle and 1.4 × longer than outer ramus, dorsomedial margin with six stout setae, three of which with accessory seta, dorsolateral margin with three stout setae with accessory seta, bearing three stout subapical setae with accessory setae; outer ramus dorsal margin with three stout setae, bearing three stout subapical setae, one of which longer and apically plumose. *Uropod 3*: plate-like, with two dorsal, two apical, and one ventral seta. *Telson*: as long as wide, dorsal lobe with three stout setae, lower margin without lateral setae.

Type locality. Alcatrazes Archipelago, state of São Paulo, Brazil.
Distribution. Known only from the type locality.
Etymology. This species is named after the first author's grandmother, Carmelina Vieira.

Remarks. *Podocerus* is a shallow-water genus usually found associated with algae, bryozoans, floating debris, and fouling artificial structures [203]. There are three species of the genus known to inhabit Brazilian waters: *P. brasiliensis* [183] and *P. fissipes* Serejo, 1996 [204], recorded in Pernambuco, Rio de Janeiro, and São Paulo states, and *P. fulanus* J.L. Barnard, 1962 [205], recorded only in São Paulo state [171]. *Podocerus carmelina* **sp. nov.** is morphologically similar to *P. fissipes* due to the following characters: head with short rostrum, rounded lateral cephalic lobe; gnathopod 1, carpus shorter than propodus, with posterior lobe; dactylus inner margin with three acute spines; gnathopod 2 basis anterodistal corner with produced lobe, palm defined by a proximal projection, with a subtriangular tooth medially, and a crenulated lobe distally; uropod 1 longer than uropod 2, inner ramus longer than outer. However, the new species can be distinguished from *P. fissipes* by the following characters (characters of *P. fissipes* in parenthesis): gnathopod 2 propodus laterally setose with plumose setae above palm (laterally naked); palm defined by a short blunt projection (defined by an acute larger projection), medially with a single subtriangular tooth (with a bidentate tooth); pereonite 3 with weak

dorsal carina (without carina); pereopod 3 with anterodistal lobe (without lobe); uropods 1 – 2 inner rami with medial margin smooth (minutely serrated); telson dorsal lobe with three stout setae (with two setae). Hughes [206] discussed the morphological variations of *P. brasiliensis* reported in the literature and designated a neotype for the species. Considering the author's description, *P. carmelina* **sp. nov.** can be readily distinguished using the following characters: palm of gnathopod 2 with a defining proximal blunt projection, a subtriangular tooth medially, a "u-shaped" concavity, and a crenulated lobe distally. In contrast, according to the description of the neotype, the palm is smooth and almost straight. In addition, the gnathopod 1 palm is not defined by a stout seta, and the uropods 1 – 2 plus telson of the new species is less setose. The new species can also be easily differentiated from *P. fulanus* by the absence of dorsal projected carinae on pereonites 6 – 7 and pleonites 1 – 2.

Podocerus carmelina **sp. nov.** has a unique combination of characters that distinguishes it within the genus, such as pereonite 3 with weak dorsal carina; gnathopod 2 carpus indistinct, almost entirely fused to propodus, propodus bearing several facial short plumose setae, palm defined by a proximal blunt projection, with a subtriangular tooth medially, a "u-shaped" concavity and a crenulated lobe distally, a different pattern from its congeners; pereonite 4 elongated, about 2.2 × deeper than wide; pereonites 5 – 6 anteroventral corner with a subacute projection; coxa 5 anteroventral corner with a notch, forming an acute spine; telson dorsal lobe with three stout setae, with naked lateral margins; uropod 2 outer ramus with one subapical long and stout plumose seta.

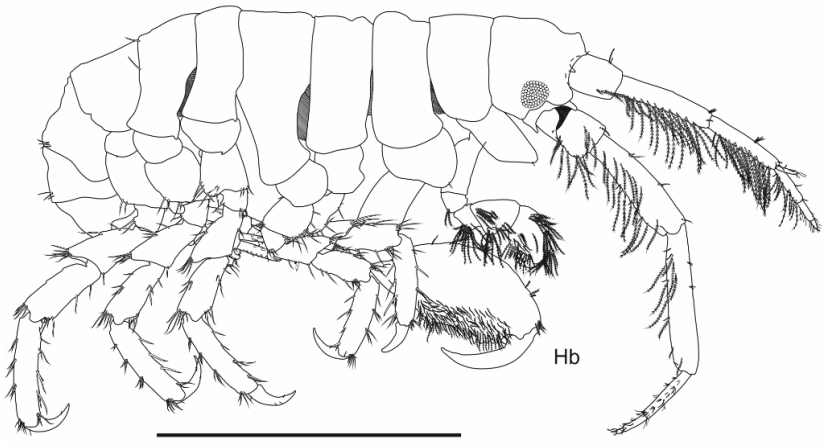

Fig. 1 *Podocerus carmelina* **sp. nov.**, male holotype, 3.3 mm, Alcatrazes Archipelago, state of São Paulo, Brazil (UERJ 2318). Hb, Habitus. Scale bar: 0.5 mm.

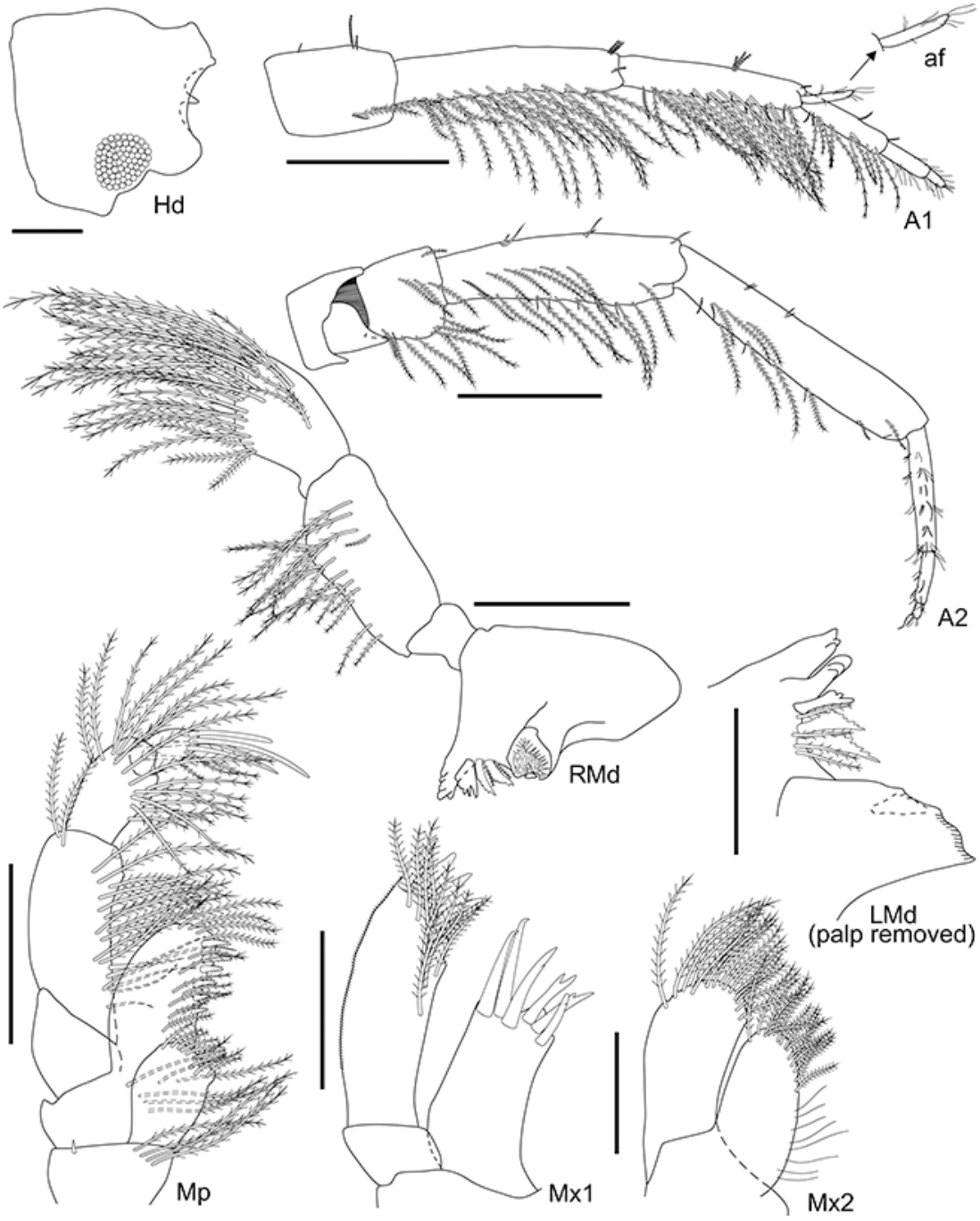

Fig. 2 *Podocerus carmelina* **sp. nov.**, male holotype, 3.3 mm, Alcatrazes Archipelago, state of São Paulo, Brazil (UERJ 2318). Hd, Head; A1 – 2, Antennae 1 – 2; LMd, Left Mandible; RMd, Right Mandible; Mx1 – 2, Maxillae 1 – 2; Mp, Maxilliped. Scale bars: 0.5 mm for A1 – 2 0.2 mm for RMd and Mp; 0.1 mm for Hd, Mx1 – 2 and LMd.

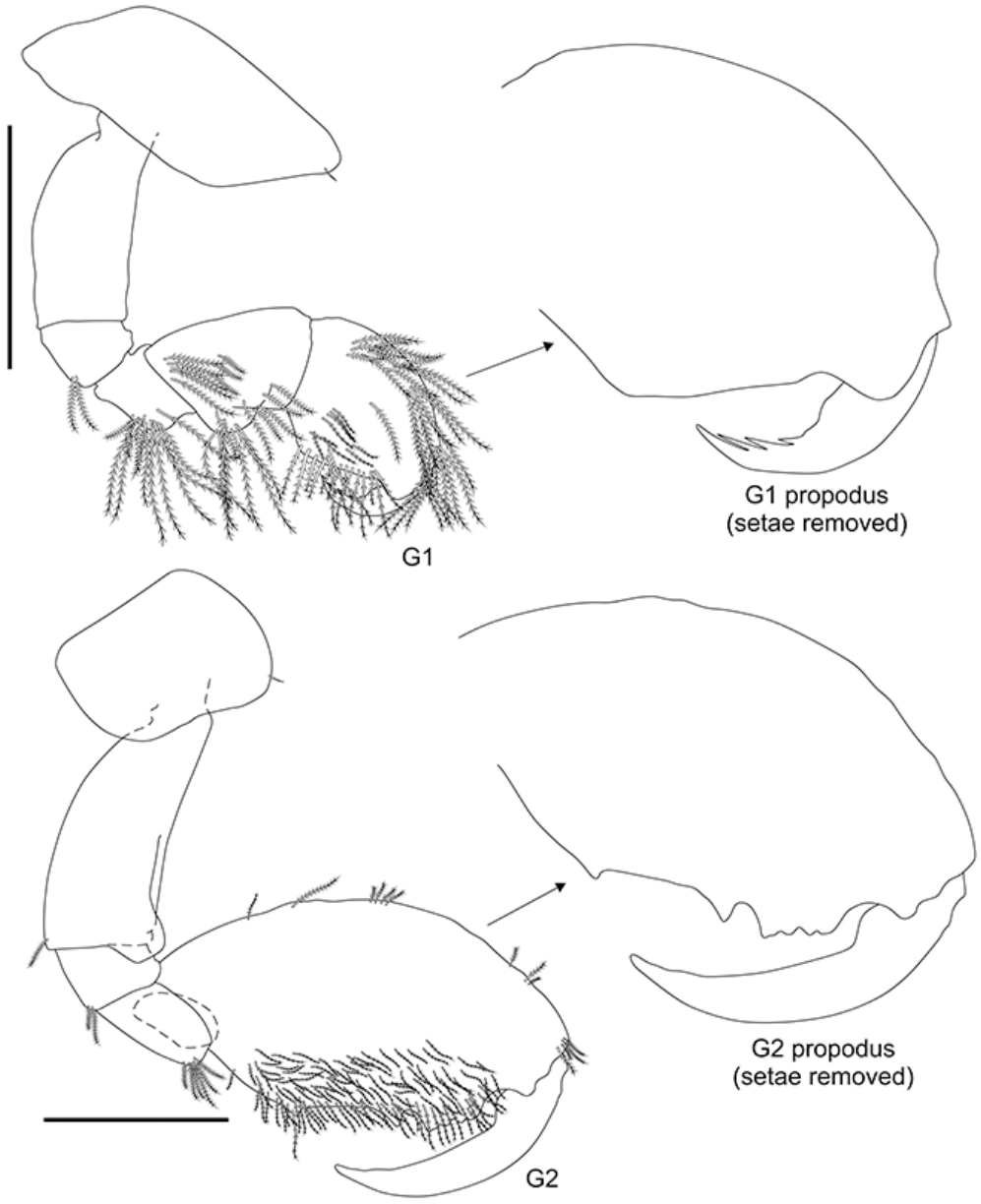

Fig. 3 *Podocerus carmelina* **sp. nov.**, male holotype, 3.3 mm, Alcatrazes Archipelago, state of São Paulo, Brazil (UERJ 2318). G1 – 2, Gnathopods 1 – 2. Scale bars: 0.5 mm.

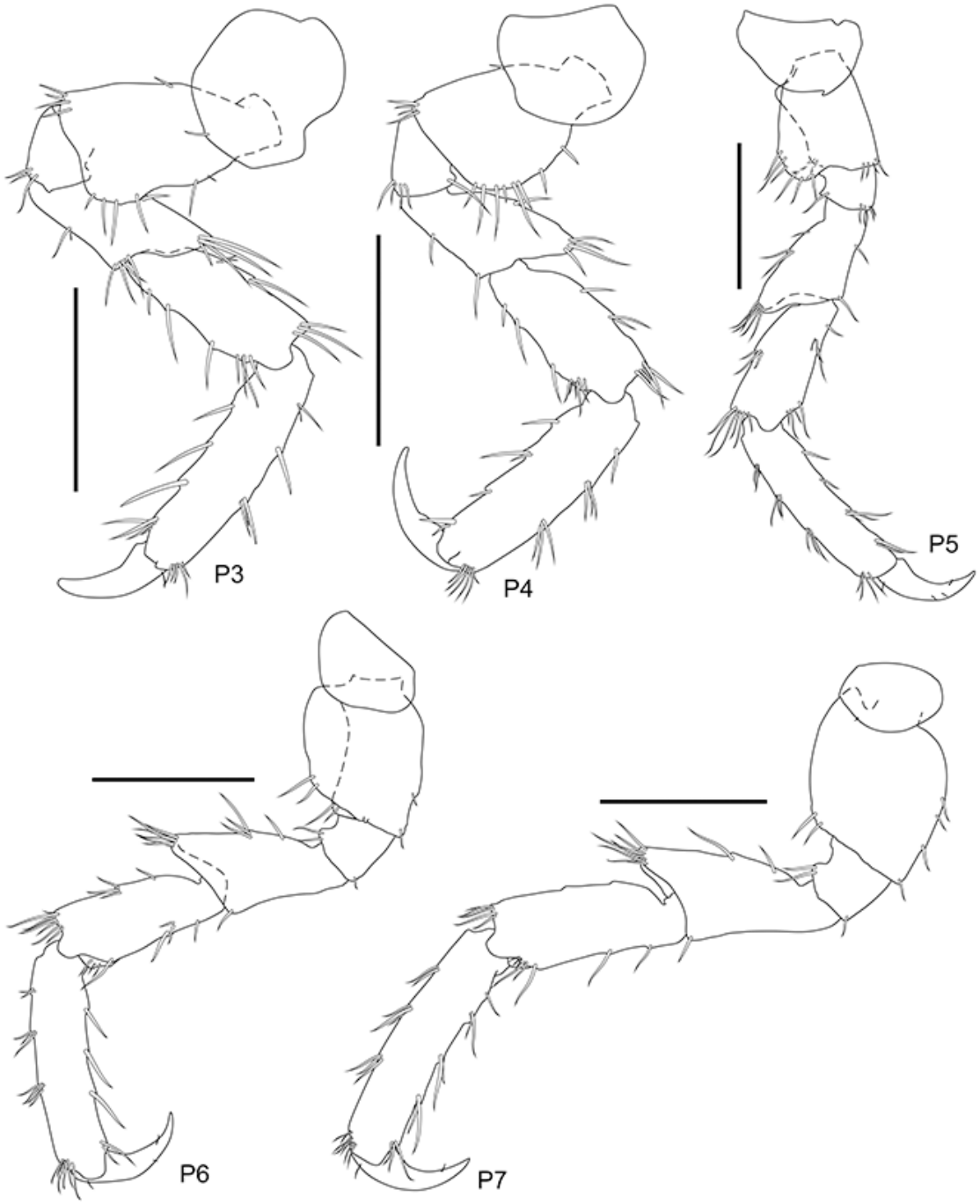

Fig. 4 *Podocerus carmelina* **sp. nov.**, male holotype, 3.3 mm, Alcatrazes Archipelago, state of São Paulo, Brazil (UERJ 2318). P3 – 7, Pereopods 3 – 7. Scale bars: 0.5 mm.

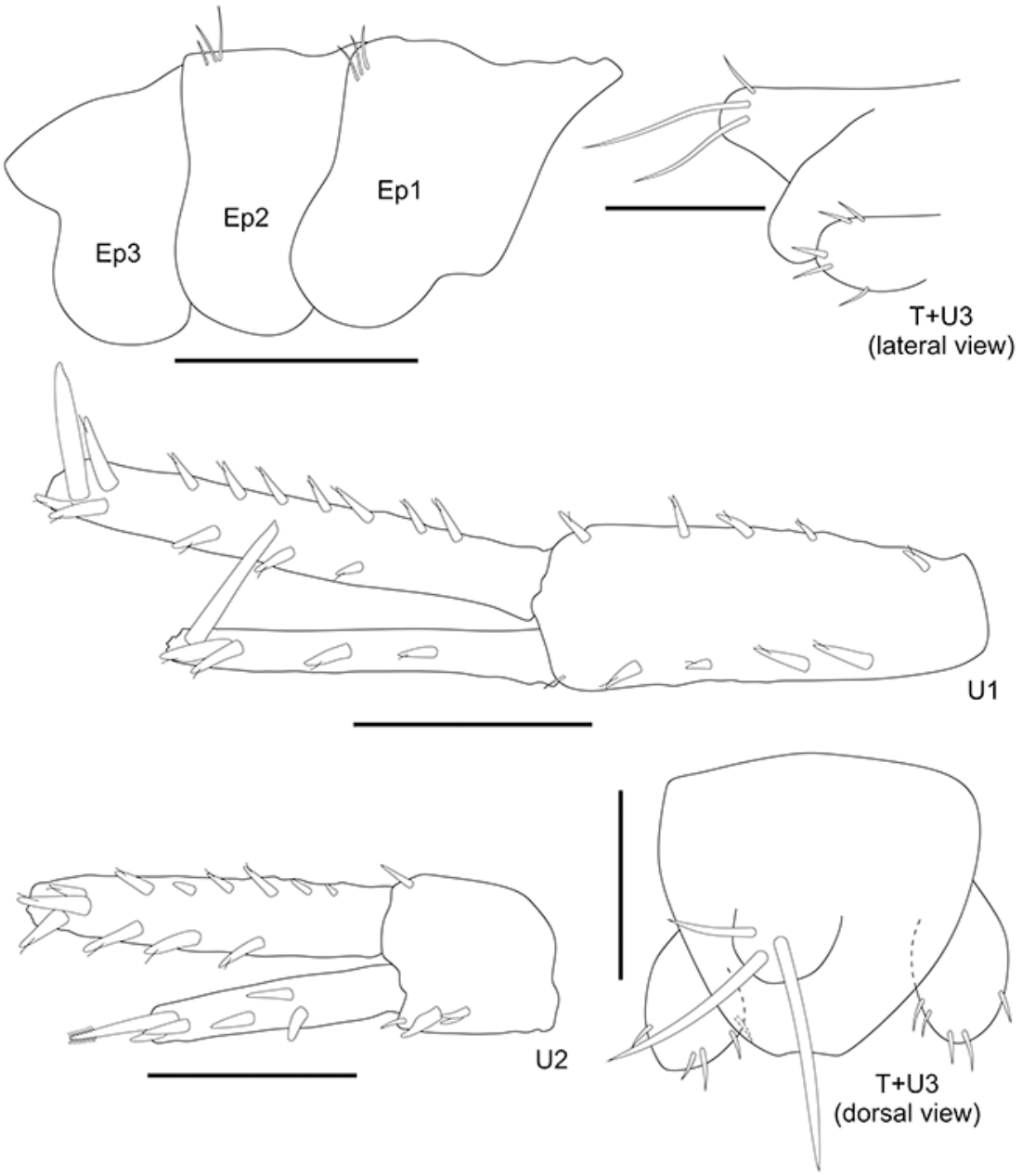

Fig. 5 *Podocerus carmelina* **sp. nov.**, male holotype, 3.3 mm, Alcatrazes Archipelago, state of São Paulo, Brazil (UERJ 2318). Ep1 – 3, Epimeral plates 1 – 3; U1 – 3, Uropods 1 – 3; T, Telson. Scale bars: 0.2 mm for Ep1 – 3 and U1 – 2; 0.1 mm for T and U3.

Key to species of *Podocerus* with a type locality in the Atlantic Ocean

1 Body dorsally smooth, without any carinae or projections 2

- Body with some dorsal carinae or projections 8

2 Pereonites and pleonites lacking dorsal or lateral slender or stout setae 3

- Pereonites and/or pleonites bearing dorsal or lateral slender or stout setae..... 6

3 At least one of uropods 1 – 2 with a peduncular interramal ventral spine..... *P. brasiliensis* Dana, 1853

- Uropods 1 – 2 lacking peduncular interramal ventral spine 4

4 Gnathopod 2 basis with an oblique facial row of plumose setae, merus, carpus, and propodus bearing dense setal fringe..... *P. senegalensis* Chevreux, 1925

- Gnathopod 2 basis without an oblique facial row of setae, merus, carpus, and propodus bearing weak to moderate setal fringe..... 5

5 Pereopods 3 – 7 basis expanded and short relative to gnathopod 2 basis length..... *P. chelonophilus* (Chevreux & Guerne, 1888)

- Pereopods 3 – 7 basis not expanded and elongate, almost the same length as gnathopod 2 basis ..... *P. pyurae* Griffiths, 1975

6 Pereonites 5 – 7 with slender or stout setae..... 7

- Pereonites 5 – 7 naked ..... *P. carmelina* **sp. nov.**

7 Gnathopod 2 propodus with two stout setae defining palm; telson apex with four stout setae..... *P. tachyrheo* Baldinger & Gable, 1994

- Gnathopod 2 propodus with one stout seta defining palm; telson apex with two stout setae..... *P. fissipes* Serejo, 1996

8 Coxa 1 entire 9

- Coxa 1 cleft..... *P. kleidus* Thomas & Barnard, 1992

9 Uropod 1 with a peduncular interramal ventral spine..... 10

- Uropod 1 without a peduncular interramal ventral spine ..... *P. schieckei* Ruffo, 1987

10 Pereonites 1 – 2 dorsally smooth, not produced 11

- Pereonites 1 – 2 dorsally produced..... *P. jareckii* Baldinger & Gable, 2002

11 Head with acute rostrum..... *P. delacruzi* Winfield, Ortiz & Ardisson, 2016

- Head with blunt rostrum ..... *P. lazowasemi* Baldinger & Gable, 2002

Order Isopoda Latreille, 1817
Suborder Asellota Latreille, 1802
Asellota sp.

Material examined. One individual, in ethanol 70%, on unidentified ceriantharian tubes, Kitahara MV coll., Alcatrazes Archipelago, state of São Paulo, Brazil, May 22, 2019 (UERJ 1314).
Remarks. This suborder is one of the most varied in isopod species, with a wide geographical and bathymetric distribution and a notable dominance in the deep sea [207–208].

Order Tanaidacea Dana, 1849
Suborder Apseudomorpha Sieg, 1980
Apseudomorpha sp.

Material examined. One individual, in ethanol 70%, on *Ceriantheomorphe brasiliensis* tubes, São Sebastião, state of São Paulo, Brazil (UERJ 1317).
Remarks. Apseudomorph tanaidaceans are very diverse, widely distributed, and known as benthic in shallow waters [209]. They can be found on silt, sand, or gravel and are also abundant on coral reefs, estuaries, and mangroves ranging from tropical to temperate waters [210].

Suborder Tanaidomorpha Sieg, 1980
Tanaidomorpha sp.

Material examined. One individual, in ethanol 70%, on unidentified ceriantharian tubes, Kitahara MV coll., Alcatrazes Archipelago, state of São Paulo, Brazil, May 22, 2019 (UERJ 1289); one individual, in ethanol 70%, same sampling data (UERJ 1308); 19 individuals, in ethanol 70%, on *Ceriantheomorphe brasiliensis* tubes, São Sebastião, state of São Paulo, Brazil (UERJ 1319).
Remarks. In general, tanaidomorphs exhibit tube-dwelling behaviors and have a diverse morphology and broad distribution [210].

Subclass Phyllocarida Packard, 1879
Order Leptostraca Claus, 1880
Suborder Nebaliacea Calman, 1904
Family Nebaliidae Samouelle, 1819
Genus *Nebalia* Leach, 1814
***Nebalia* sp.**

Material examined. One individual, in ethanol 70%, on ceriantharian tubes, Kitahara MV coll., Alcatrazes Archipelago, state of São Paulo, Brazil, May 22, 2019 (UERJ 1290).

Remarks. *Nebalia* can be found from shallow to bathyal zones, associated with estuarine mudflats, rocky shores, coral reefs, and sponges [211–212]. In Brazil, there is only one record of *Nebalia* sp. from the coast of Ubatuba, São Paulo state [213]. However, individuals of *Nebalia* have been collected elsewhere in the country in recent years, with locations including Icapuí beach, Fortaleza, Ceará; Abrolhos, Bahia, and Campos Basin and Itaipuaçu, Rio de Janeiro (ARS pers. obs.).

Discussion

This study reported a new crustacean species, as well as occurrences of known species associated with an alternative animal substrate, the ceriantharian tube. Our findings revealed that ceriantharian tubes may represent a biodiversity indicator for basic research and biodiversity surveys.

Our observations included specimens of Asellota sp., Hyalidae sp., *Caprella equilibra, Ericthonius brasiliensis, Monocorophium* sp., and *Nebalia* sp. associated with tubes of Ceriantharia. Although these taxa are commonly found in association with animal substrates [171–214–215–216–217–218–219–220–221–222], this is the first report of them occurring on ceriantharian tubes. This result indicates that ceriantharian soft tubes can be substrates of interest for some crustacean species. Though some of these taxa (e.g. genera *Nebalia* and *Monocorophium*) are commonly reported as having interspecific symbiotic relationships [223–224], we did not observe them in the natural environment

while alive, and therefore we cannot establish whether such symbiotic associations exist between them and Ceriantharia.

Our results confirm those of Ceriello et al. [3] on the use of ptychocyst filaments by marine benthic invertebrates as an anchoring method on ceriantharian tubes. However, we observed that this anchoring method was not a requirement for a successful establishment on tubes, as some specimens were observed in areas free from filaments, while others were found to use their gnathopods to remain anchored when they were not surrounded by filaments. Gnathopods are body structures with several functions, such as aiding in reproduction, defense, and are used during fights between males, representing sexual dimorphism [225–226–227–228]. They also have a fixation function, as males use them to hold onto females and carry them [229–230]. Some Tanaidomorpha in our study used these structures to remain attached to ceriantharian tubes, showing that gnathopods can also serve as anchoring structures on soft substrates. Still, individuals using ptychocyst filaments as an anchoring method were more sturdily fixed than those using gnathopods.

We reported the new amphipod species *P. carmelina* **sp. nov.** occurring at a depth of 35 m on the northern coast of São Paulo state, which may have remained unknown to date because research efforts tend to overlook Amphipoda from soft bottoms and waters deeper than 25 m [166–167–231]. As a result, it is likely that other amphipod species, including those occurring on animal substrates, are yet to be identified [232].

Acknowledgements

We would like to thank the Chico Mendes Institute for Biodiversity Conservation (ICMBio) for supporting research activities at the Alcatrazes Wildlife Refuge, the Center for Marine Biology for infrastructure support, as well as the reviewers and subject editor for their comments helping us improve this paper.

2.2. **Environmental DNA (eDNA) as a tool for characterization of marine biodiversity found in ceriantharian tubes (Cnidaria, Anthozoa) from South and Southeast Brazil**

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2. UNESP - Universidade Estadual Paulista, Instituto de Biociências, Departamento de Zoologia, Botucatu, Brazil.
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Abstract

In this study we aimed to analyze metazoan and microbial biodiversity found in ceriantharian tubes through environmental DNA (eDNA) metagenomics combined with shotgun sequencing. To do so, we sampled two tubes of *Ceriantheomorphe brasiliensis* (Mello-Leitão, 1919) in Santa Catarina and two tubes of *Ceriantheopsis lineata* Stampar, Scarabino, Pastorino & Morandini, 2015 in Alcatrazes archipelago, South and Southeast Brazil, respectively, via SCUBA dive. The eDNA found in the tubes was extracted by metagenomics technique, amplified and sequenced by shotgun sequencing on the Illumina HiSeq platform. Our results showed that this approach was efficient for assigning biodiversity to family level, providing a wider view of metazoan and microbial families found in ceriantharian tubes. Of all families found in this microhabitat, approximately 79% represented microbes and 21% metazoans, with Enterobacteriaceae and Branchiostomatidae, respectively, being the most prevalent ones for each group. Tubes from different locations presented a higher metazoan diversity than the microbial that nearly remained unaltered, indicating that, although microbial families are abundant in ceriantharian tubes, they are uniform throughout different locations.

Keywords: Metagenomics, Metazoa, MG-RAST, microbiome, shotgun, tube-dwelling anemone.

Introduction

Marine ecosystem has a wide diversity of habitats of which some are built by marine organisms known as bioconstructors or ecosystem engineers [49]. Their bioconstructions (e.g. coral reefs, tubes) can vary in shape and extension, be found from both shallow and deep-seas, and persist in the environment for a few years to millennia [50–53]. It has been demonstrated that bioconstructions alter hydrodynamic regimes and sedimentation, contribute to the maintenance and increase of biodiversity, and enhance habitat complexity as they provide three-dimensional refuge and alternative feeding, breeding and nursery grounds for a variety of marine organisms [49–85–233–234–235].

Tube-anemones (Ceriantharia) are marine autogenic structural bioconstructors (see definition in 234), i.e. they build elevated structures (soft tubes) from the adhesion of seabed sediments to a self-synthesized material called ptychocyst filament [6–131–236]. Ceriantharian tubes are usually built in soft bottoms and may represent one of the few alternatives of consolidated structures on unconsolidated bottoms, contributing to species establishment [3]. Ceriantharian tube research has revealed that tubes may host a variety of marine invertebrates, the majority of which use them as refuge and settling sites (e.g. phoronids), similar to the ecological function of coral and oyster reefs [4–237]. Thus acting as suitable alternative microhabitats [3–238] and pointing out their relevance as structures worth investigating in terms of species biodiversity and conservation. Despite that, knowledge about species found in this microhabitat remains quite limited, especially when restricted to a macroscopic view usually based on species traditional recognition method that relies only on morphological characters.

To the best of our knowledge, for nearly a century all studies reporting biodiversity co-occurring with ceriantharians have relied on traditional taxonomy methods for species identification [3–4–5–6–7–69–83–85–238–239–240–241–242–243–244–245–246–247–248–249–250–251–252–253–254–255].

However, although useful, this method has some limitations. As it is mainly based on morphological traits, species identification may be impacted by phenotypic plasticity and crypticity [256], as well as damaged or absent body structures may prevent or limit precise identifications. Moreover, the determination of species by this method is based on the perception of the

identifier which may differ among different identifiers, leading to uncertainties and disagreements [17]. Another issue concerns sampling efforts, such as some collection methods that are destructive to fauna (e.g. toxic, electricity, trawling). Additionally, species are often not found or are hard to be visually identified during surveys (e.g. species of small size, water clarity, turbidity), and, in some cases, they cannot be collected in the season of interest [257]. Ceriantharians, for example, are rather difficult animals to find and especially hard to sample given their ability to quickly hide inside their tubes when disturbed [65]. The use of genomic data obtained from ceriantharian tubes, however, seems to be an interesting alternative for resolving challenges like these.

There are several genomic approaches used for biodiversity identification, such as barcoding, metabarcoding and metagenomics. Put simply, barcoding is mostly used for single specimen assignments while metabarcoding is used for multiple specimens of interest. Metagenomics, however, does not restrict any taxon of interest. Instead, it allows the extraction of genomic material from entire communities through the DNA left by them in the environment (environmental DNA – eDNA) [22–258–259–260], giving a wider view of taxa found in a given habitat.

The eDNA approach has many advantages, including being a non-invasive, quick, and less costly technique when compared to other methods [22]. It requires less sampling effort, and is able to assess distribution, monitoring, and identification of a wide variety of taxa including cryptic, rare, threatened and invasive species, and microbial communities in several substrates and environments [261]. It is also useful to monitor biodiversity, report new occurrence records, and be used in evolutionary genomics [262–263–264–265–266]. However, most metagenomic eDNA studies investigating marine environments are focused on the diversity of enzymes, bioactive compounds, microbiota (e.g. 267–268), and we are not aware of a single study that uses this technique to investigate metazoan biodiversity. Furthermore, due the high concentration of eDNA found in substrates, it persists longer in bottoms than in water [269–270–271]. As a result, substrate eDNA is an appropriate tool for revealing past and present biodiversity [271]. Thus, in this study we aimed to identify microbial, including

Archaea and Bacteria, and metazoan marine biodiversity found in ceriantharian tubes using the eDNA metagenomics approach combined with shotgun sequencing.

Material and methods

Tube collection

Four tubes of Ceriantharia (approximately 50 cm long and 4.5 cm wide) were manually extracted from fine sand soft bottoms via SCUBA diving. Two tubes of *Ceriantheomorphe brasiliensis* (Mello-Leitão, 1919) [73] were collected from Florianópolis, Santa Catarina State (SC), South Brazil (27°47.059'S 48°30.429'W), from 4 m depth, and other two tubes of *Ceriantheopsis lineata* Stampar, Scarabino, Pastorino & Morandini, 2015 [68] were collected from Alcatrazes archipelago (AK), São Paulo State, Southeast Brazil (24° 6.004'S 45° 41.904'W), from 35 m depth. Originally, the tubes were sampled on 11 December 2018 (Santa Catarina) and 22 May 2019 (Alcatrazes) for purposes other than eDNA research, and after collection they were stored in 70% ethanol.

eDNA extraction and Shotgun sequencing

Tube samples from each collection area were thoroughly homogenized and ethanol drained. DNA extraction was performed using approximately 0.5 g of homogenized tubes with a DNA Isolation Kit (Qiagen) following the manufacturer's protocol.

For the shotgun sequencing, a total of 1 µg of DNA per sample was used as input material for library preparation. Genomic libraries were built using the NEBNext® Ultra™ DNA Library Prep Kit for Illumina. After this, DNA samples were fragmented, and then the DNA fragments were end-polished, A-tailed and ligated with the full-length adaptors for Illumina sequencing for further PCR amplification. Then, the PCR products were purified, and the libraries were analyzed for size distribution using an Agilent 2100 Bioanalyzer and quantified using real-time PCR. The clustering of the index-coded samples was performed on a cBot Cluster Generation System according to the manufacturer's

instructions. After cluster generation, the libraries were pooled and sequenced on an Illumina HiSeq 4000 platform using a 150 bp single-end chemistry.

Data analysis

eDNA datasets, from each sample, were separately processed by the online metagenomics RAST server (MG-RAST), using default parameters for taxonomic characterization of the metagenomic data, as proposed by Meyer et al. [272] and Glass et al. [273]. For taxa assignment, we considered a 97% similarity filter, as recommended by Konstantinidis and Tiedje [274], in which microbes (Archaea and Bacteria) and metazoans were assigned to the level of family.

Bioinformatics

DNA sequence annotations (Archaea, Bacteria and Eukaryota) were downloaded from MG-RAST server in the format of comma-separated values (csv) files. For each csv file, MG-RAST returned multiple hits (reference species) for a single DNA sequence, resulting in duplicates that were separated by a new line.

Analyses of csv data files were conducted in R 4.2.1 [275], and the following packages were used in the data cleaning process: dplyr [276], tidyr [277], and plyr [278].

After being cleaned, all csv data files were examined to determine which reference species appeared more than once in the dataset. This procedure counted all repeats and then eliminated them, resulting in a dataset in which each reference species only appeared once. Then, all reference species were filtered again, this time using habitat and kingdom filters, such that only species from the marine habitat and the Animalia Kingdom remained, resulting in a new dataset.

To obtain taxonomic information down to the family level, the datasets of marine metazoan species were compared to the online databases Barcode of Life Data System (BOLD) and World Register of Marine Species (WoRMS), via taxize package [279], and generating separate files. Since both databases returned different numbers of hits, the generated files were combined into a single one from which the overall number of families was determined. The same

protocol proposed for metazoan data (Fig. 1) was applied to microbial data, however, hits were matched to available data in the National Center for Biotechnology Information (NCBI) online database, and reference species did not have habitat filter. After data cleaning and family counting, we compiled a total of five final datasets.

Taxonomic identification results by family were visualized with the ggplot2 package [280]. All data files originated by MG-RAST were checked for errors and absence of values (i.e. NA data) following the protocol devised by the authors (Fig. 1).

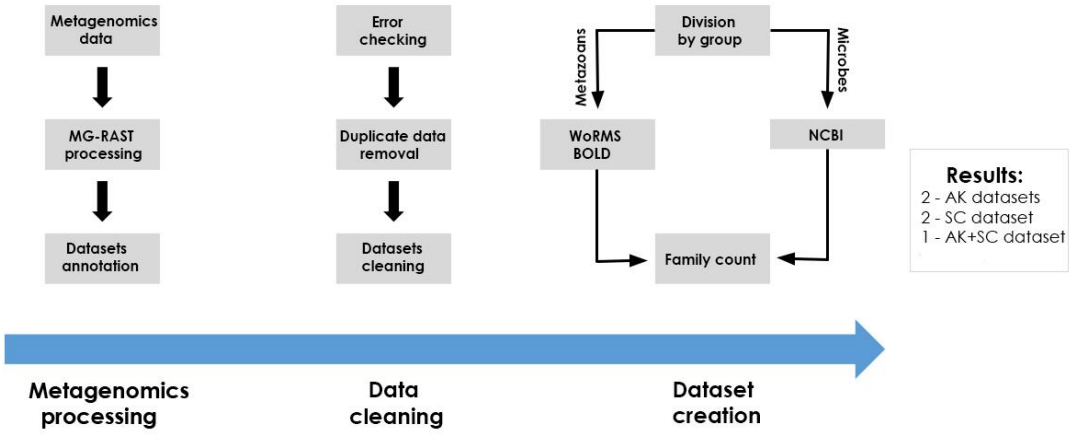

Fig. 1 Protocol for taxonomic assignment and analyses of eDNA sequences. AK indicates datasets of tubes of *Ceriantheopsis lineata* sampled in Alcatrazes archipelago whereas SC indicates datasets of tubes of *Ceriantheomorphe brasiliensis* sampled in Santa Catarina.

Results

We have not used any specific protocol for eDNA extraction in ceriantharian tubes and in no case was Ceriantharia DNA amplified. However, we obtained relevant data about several other marine organisms.

In total, for both locations, we obtained 73.303.984 sequences of which 19.433.149 (26.51%) failed to pass quality control by MG-RAST pipeline and were removed, virus sequences were not considered in our analysis and 5.220 sequences were removed, remaining 53.865.615 sequences. Of these, 2.540.074 were unknown to databases available on MG-RAST (e.g. NCBI, Reference sequence - RefSeq, National Institutes of Health (NIH), Genetic sequence database - GenBank, TrEMBL protein sequence database), and only

51.325.541 sequences (70.02%) were considered for taxa assignment, resulting in about 30% data loss (Fig. 2a).

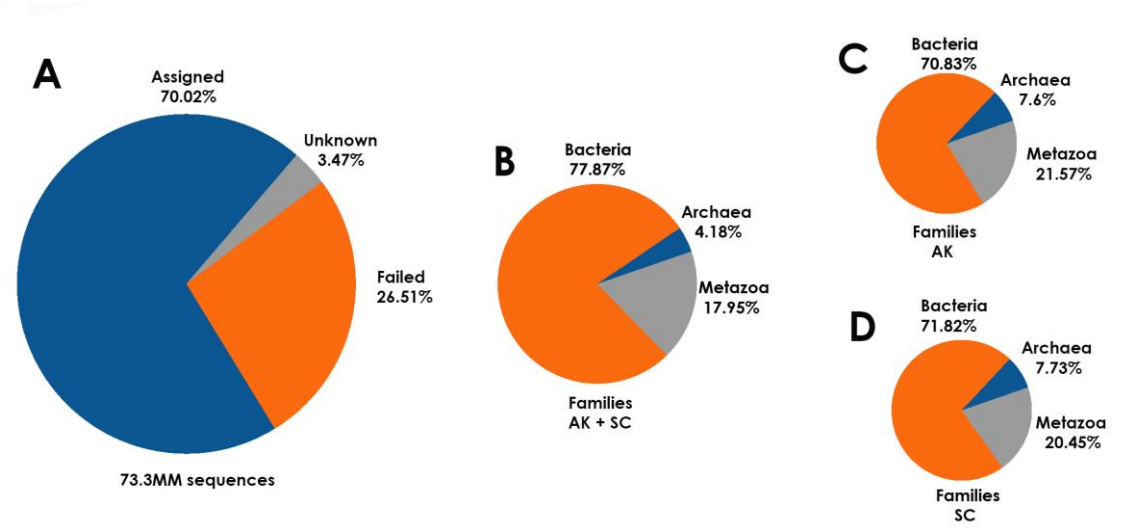

Fig 2. eDNA sequences and taxa assignment of organisms found in ceriantharian tubes of *Ceriantheopsis lineata* from Alcatrazes archipelago (AK) and tubes of *Ceriantheomorphe brasiliensis* from Santa Catarina (SC), Brazil: **a** total of eDNA sequences of tubes from AK and SC, recovered from the shotgun sequencing with taxa assignment by MG-RAST pipeline; **b** metazoan and microbial families from AK and SC tubes, combined; **c** metazoan and microbial families from AK tubes; **d** metazoan and microbial families from SC tubes.

eDNA obtained from ceriantharian tubes sampled from AK contained 33.958.392 reads of which 8.899.863 (26.21%) failed to pass quality control and were removed, as well as 2.227 virus sequences, remaining 25.056.302 sequences. Of these, 1.149.370 were unknown to databases available, and only 23.906.932 sequences (70.40%) were considered for taxa assignment. As to eDNA obtained from ceriantharian tubes from SC, they contained 39.345.592 reads of which 10.533.286 (26.77%) failed to pass quality control and were removed, as well as 2.993 virus sequences, remaining 28.809.313 sequences. Of these, 1.390.704 were unknown to databases available and only 27.418.609 reads (69.69%) were considered for taxa assignment.

The community analysis from both AK and SC revealed a similar domain proportion; SC in parenthesis: 70.83% (71,82%) represented Bacteria, followed by 21.57% (20,45%) representing Eukaryota (only metazoan), and 7.60% (7,73%) Archaea.

In total, we found 133 metazoan families and 320 microbial families (Supplementary material 1; Fig. 2b) in which the 10 most prevalent metazoan and microbial families are shown in table 1.

Table 1. Ten most metazoans and microbial families and respective phyla occurring in ceriantharian tubes from Alcatrazes archipelago and Santa Catarina, Brazil, and their frequency in percentage (Freq %) between samples.

Phylum	Metazoa	Freq %	Phylum	Microbes	Freq %
Chordata	Branchiostomatidae	10.53	Proteobacteria	Enterobacteriaceae	100
Porifera	Plakinidae	8.27	Firmicutes	Bacillaceae	82
Cnidaria	Acroporidae	4.51	Firmicutes	Streptococcaceae	70
Chordata	Cionidae	4.51	Firmicutes	Lactobacillaceae	58
Porifera	Niphatidae	4.51	Firmicutes	Staphylococcaceae	53
Mollusca	Onchidiidae	4.51	Proteobacteria	Burkholderiaceae	52.5
Porifera	Oscarellidae	4.51	Proteobacteria	Vibrionaceae	48.44
Mollusca	Vermetidae	4.51	Firmicutes	Enterococcaceae	41
Cnidaria	Edwardsiidae	3.76	Actinobacteria	Mycobacteriaceae	35.62
Echinodermata	Antedonidae	3.01	Proteobacteria	Pasteurellaceae	33

There was a total of 88 metazoan families and 320 microbial families (31 Archaea and 289 Bacteria) in ceriantharian tubes from the Alcatrazes archipelago, with Plakinidae being the most prevalent metazoan family (9,1%) and Enterobacteriaceae the most prevalent microbial one (50%) (Supplementary material 2; Fig. 2c). Ceriantharian tubes from Santa Catarina included a total of 82 metazoan families and 319 microbial families (31 Archaea and 288 Bacteria), with Branchiostomatidae being the most common metazoan family (9.76%) and Enterobacteriaceae the most common microbial family (50.47%) (Supplementary material 3; Fig. 2d).

The diversity of microbial families from both sites was 78.43% higher than that of metazoan families (21.57%). On the other hand, metazoan families from both locations shared 27,82% similarity while microbial families shared 99.7% similarity (Fig 3a–d).

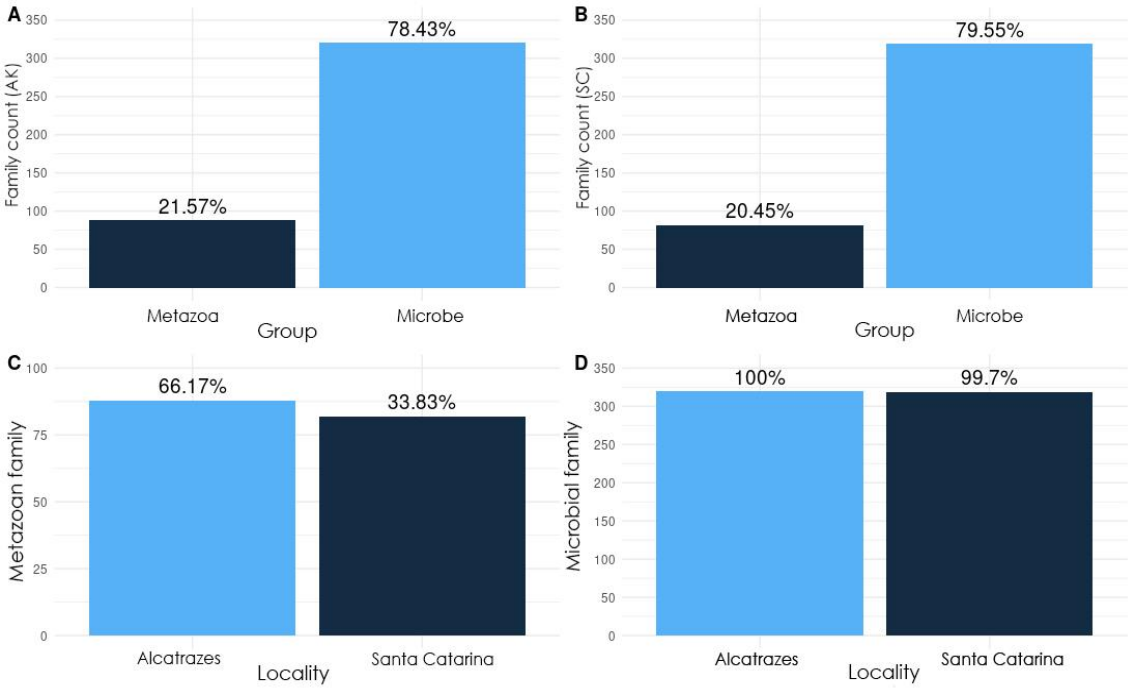

Fig. 3 Diversity and similarity of organisms found in ceriantharian tubes of *Ceriantheopsis* lineata from Alcatrazes arquipelago (AK) and *Ceriantheomorphe brasiliensis* from Santa Catarina (SC), Brazil: **a** metazoan and microbial diversity from AK tubes; **b** metazoan and microbial diversity from SC tubes; **c** metazoan similarity between AK and SC; **d** microbial similarity between AK and SC.

Discussion

We investigated the metazoan and microbial biodiversity found in tubes of *Ceriantheopsis lineata* from Alcatrazes arquipelago, São Paulo State, and *Ceriantheomorphe brasiliensis* from Florianópolis, Santa Catarina State. Our findings showed that the majority of organisms associated with ceriantharian tubes are represented by microbes whose composition is nearly identical for both locations in this study. Inversely, metazoan composition differs between locations, but is less abundant, and this may be owned to the short length of ceriantharian tubes (up to 1 m) when compared to other long extended bioconstructions, such as coral and oyster reefs, which are known to contain several metazoan taxa [237–266]. When the area extension decreases, the abundance of smaller organisms, including microbes, increases quicker while their diversity diminishes. In contrast, the abundance of larger organisms, such as metazoans, decreases while their diversity increases [286–287–288–289]. Despite this, although the ceriantharian tube has a less extensive area than reefs', they contain significant metazoan biodiversity and similar bacterial

composition. Thus, playing a similar role to reefs, but on a smaller scale, acting as mini-reefs.

Studies on metagenomics in coral reefs worldwide typically annotate Bacteria as the most abundant domain of which phylum Proteobacteria is usually the most common to occur, and contribute to glutamate synthases and nitrogen fixation [281–282–283–284–285]. Likewise, our analyses showed that this phylum is also the most abundant in ceriantharian tubes, however its function in tubes is still undetermined, as does its presence in polyps.

Substrate eDNA is rich in extracellular DNA. As a result, it is considered as a DNA global reserve and can be used as a biodiversity inventory, as well as a species archive [265]. Our analyses revealed a higher concentration of microbes (78.43%) than metazoans (21.57%). As we sampled a large number of microbes, we may hypothesize that, other than the short length of the ceriantharian tube, the low percentage of metazoa can also be attributed to eDNA consumption. As eDNA is a source of carbon, nitrogen, and phosphorus, which microorganisms use for nutrition or as energy source [265], its consumption aids in its degradation; hence, the higher the concentration of microorganisms in the substrate, the lower the concentration of eDNA [270–290]. Furthermore, as our analyses took an average of three years from material collection to eDNA extraction, it allowed microorganisms to consume part of the genetic material for longer. Despite this, we still managed to obtain relevant biodiversity data.

Directly sequencing metagenomic eDNA from ceriantharian tubes using a shotgun approach was inefficient for assigning biodiversity at species level. However, this technique provided a wider view of microbial and metazoan families found in this microhabitat, revealing a number of families that were previously unknown to occur in ceriantharian tubes by traditional identification methods based on direct observation. Moreover, given that metabarcoding has shown effectiveness in detecting species diversity from a variety of organisms and environments [291–292–293–294], we suggest that the combination of metagenomics and metabarcoding approaches may yield complementary results, one being wider and the other more specific, respectively. Thus, providing a more accurate characterization and including more specific taxonomic levels (e.g. genus and species).

In general, we had a data loss of around 30%, which we attributed mostly to incomplete databases. When comparing generated genetic sequences to those available in databases, many of the sequences are removed owing to a lack of correlation, and because most species' genetic material was not uploaded to these bases, making species identification challenging [257–266–295]. Thus, little is known about marine biodiversity, particularly eukaryotic [296]. Other than that, species identification techniques, including molecular ones, might fail and also cause some data loss [297]. Another problem is the use of broader similarity filters (e.g. under 97%), allowing highly identical sequences to be recognized as the same species, which, in most cases, results in erroneous identifications [237–274–297]. Additionally, identifying new species without making their morphological diagnoses and/or depositing their genetic sequences in databases prevents the species from being known by anyone other than those who collected them, making it impossible to identify them using both traditional identification methods and genetic data [296].

We did not compare sediment biodiversity to biodiversity found in ceriantharian tubes, thus we cannot infer that organisms of families found on tubes are tube-specific. However, further studies comparing taxa composition in both substrates may help reveal whether tube microhabitats could support some taxa better than other existing habitats. Another study worth exploring concerns the ceriantharian tube duration in the environment and how its duration would impact on species that make use of it — Do these species use it for a short period of time or in long-term? Do they have short or long life-cycles on the tube?

Climate change has had a severe influence on all ecosystems across the world, causing habitat loss and leading to the decrease of species richness [298]. Because species require appropriate shelter, feeding and breeding sites, and places to hide from predators, habitat loss is a major threat to biodiversity [299–300]. On the other hand, bioconstructors create new spaces and habitats for species colonization, altering their distribution and abundance. In this way, bioconstructions improve environmental heterogeneity and complexity, enhancing community dynamics and contributing to species coexistence and diversity [301–302]. Our study revealed that the ceriantharian

tube is a suitable microhabitat for a variety of metazoan and microbial taxa. Thus, it is worth the effort to do additional research on biogenic structures built by other organisms as alternative settling areas to ease the diminishing of habitats caused by antropic actions, and contributing to species conservation.

Supplementary material 1. Families of metazoans and microbes (Archaea and Bacteria) found in ceriantharian tubes of *Ceriantheopsis lineata* from Alcatrazes archipelago and *Ceriantheomorphe brasiliensis* from Santa Catarina, Brazil, combined. n indicates how often each family appears in the samples, and frequency (Freq) represents how frequently each family appears in both samples.

Metazoa	n	Freq	Microbes	n	Freq
Branchiostomatidae	14	10,53%	Enterobacteriaceae	321	100 %
Plakinidae	11	8,27%	Bacillaceae	263	82%
Acroporidae	6	4,51%	Streptococcaceae	224	70%
Cionidae	6	4,51%	Lactobacillaceae	186	58%
Niphatidae	6	4,51%	Staphylococcaceae	170	53%
Onchidiidae	6	4,51%	Burkholderiaceae	168	53%
Oscarellidae	6	4,51%	Vibrionaceae	155	48%
Vermetidae	6	4,51%	Enterococcaceae	131	41%
Edwardsiidae	5	3,76%	Mycobacteriaceae	114	36%
Antedonidae	4	3,01%	Pasteurellaceae	106	33%
Architeuthidae	4	3,01%	Pseudomonadaceae	95	30%
Brachionidae	4	3,01%	Clostridiaceae	88	28%
Bugulidae	4	3,01%	Yersiniaceae	86	27%
Caligidae	4	3,01%	Brucellaceae	82	26%
Daphniidae	4	3,01%	Bacteroidaceae	76	24%
Dictyodendrillidae	4	3,01%	Neisseriaceae	74	23%
Elopidae	4	3,01%	Listeriaceae	68	21%
Flustrellidridae	4	3,01%	Moraxellaceae	67	21%
Haliotidae	4	3,01%	Bifidobacteriaceae	66	21%
Harrimaniidae	4	3,01%	Mycoplasmataceae	66	21%
Laqueidae	4	3,01%	Lachnospiraceae	64	20%
Limulidae	4	3,01%	Roseobacteraceae	62	19%
Lineidae	4	3,01%	Streptomycetaceae	59	18%
Metridiidae	4	3,01%	Campylobacteraceae	56	18%
Mopaliidae	4	3,01%	Corynebacteriaceae	56	18%
Nereididae	4	3,01%	Rhizobiaceae	50	16%
Pectinidae	4	3,01%	Helicobacteraceae	49	15%
Plakobranchidae	4	3,01%	Shewanellaceae	49	15%
Ptychoderidae	4	3,01%	Borreliaceae	48	15%
Pyuridae	4	3,01%	Xanthomonadaceae	47	15%
Raspailiidae	4	3,01%	Anaplasmataceae	44	14%
Strongylocentrotidae	4	3,01%	Oscillospiraceae	44	14%
Trichoplacidae	4	3,01%	Prevotellaceae	44	14%
Watersiporidae	4	3,01%	Chlamydiaceae	42	13%
Mytilidae	4	3,01%	Peptostreptococcaceae	42	13%
Machilidae	3	2,26%	Francisellaceae	41	13%

Acipenseridae	2	1,50%	Flavobacteriaceae	40	13%
Ammotheidae	2	1,50%	Fusobacteriaceae	40	13%
Anisakidae	2	1,50%	Comamonadaceae	36	11%
Aplysiidae	2	1,50%	Actinomycetaceae	34	11%
Aplysinidae	2	1,50%	Rickettsiaceae	34	11%
Ascidiidae	2	1,50%	Micrococcaceae	32	10%
Asteriidae	2	1,50%	Synechococcaceae	32	10%
Callyspongiidae	2	1,50%	Nitrobacteraceae	30	9%
Cardiidae	2	1,50%	Peptoniphilaceae	30	9%
Cephalothricidae	2	1,50%	Prochlorococcaceae	30	9%
Cephalotrichidae	2	1,50%	Thermoanaerobacteraceae	28	9%
Chondrillidae	2	1,50%	Chlorobiaceae	26	8%
Cucumariidae	2	1,50%	Morganellaceae	26	8%
Cypridinidae	2	1,50%	Erwiniaceae	26	8%
Dentaliidae	2	1,50%	Sphingomonadaceae	26	8%
Discosomatidae	2	1,50%	Sulfolobaceae	25	8%
Dugongidae	2	1,50%	Rhodobacteraceae	25	8%
Gadilidae	2	1,50%	Veillonellaceae	24	8%
Gorgoniidae	2	1,50%	Nocardiaceae	23	7%
Hutchinsoniellidae	2	1,50%	Desulfovibrionaceae	20	6%
Loxosomatidae	2	1,50%	Acetobacteraceae	20	6%
Molidae	2	1,50%	Alcaligenaceae	18	6%
Nautilidae	2	1,50%	Erysipelotrichaceae	18	6%
Nephtheidae	2	1,50%	Legionellaceae	18	6%
Oikopleuridae	2	1,50%	Paenibacillaceae	18	6%
Onchocercidae	2	1,50%	Pectobacteriaceae	18	6%
Orbiniidae	2	1,50%	Thermoanaerobacterales	18	6%
Ostreidae	2	1,50%	Thermococcaceae	18	6%
Podospongiidae	2	1,50%	Aeromonadaceae	16	5%
Pollicipedidae	2	1,50%	Aphanothecaceae	16	5%
Scyliorhinidae	2	1,50%	Caulobacteraceae	16	5%
Spadellidae	2	1,50%	Geobacteraceae	16	5%
Suberitidae	2	1,50%	Methylobacteriaceae	16	5%
Synbranchidae	2	1,50%	Methanobacteriaceae	16	5%
Terebellidae	2	1,50%	Methanococcaceae	16	5%
Tethyidae	2	1,50%	Coxiellaceae	15	5%
Tetraclitidae	2	1,50%	Thermaceae	15	5%
Tettigoniidae	2	1,50%	Oxalobacteraceae	14	4%
Triakidae	2	1,50%	Synergistaceae	14	4%
Albulidae	2	1,50%	Desulfurococcaceae	14	4%
Amphiuridae	2	1,50%	Thermoproteaceae	14	4%
Aphrocallistidae	2	1,50%	Frankiaceae	13	4%
Arbaciidae	2	1,50%	Propionibacteriaceae	13	4%
Blenniidae	2	1,50%	Selenomonadaceae	12	4%
Briareidae	2	1,50%	Sphingobacteriaceae	12	4%
Bythograeidae	2	1,50%	Tannerellaceae	12	4%

Caproidae	2	1,50%	Thermotogaceae	12	4%
Carapidae	2	1,50%	Methanosarcinaceae	12	4%
Chaetodermatidae	2	1,50%	Acidithiobacillaceae	10	3%
Chaetodontidae	2	1,50%	Acidobacteriaceae	10	3%
Cladopathidae	2	1,50%	Aquificaceae	10	3%
Faviidae	2	1,50%	Bartonellaceae	10	3%
Gigantactinidae	2	1,50%	Chromatiaceae	10	3%
Irciniidae	2	1,50%	Dehalococcoidaceae	10	3%
Keratoisididae	2	1,50%	Ectothiorhodospiraceae	10	3%
Monognathidae	2	1,50%	Eubacteriaceae	10	3%
Mugilidae	2	1,50%	Leptotrichiaceae	10	3%
Muricidae	2	1,50%	Methylophilaceae	10	3%
Otariidae	2	1,50%	Micromonosporaceae	10	3%
Paralichthyidae	2	1,50%	Porphyromonadaceae	10	3%
Parazoanthidae	2	1,50%	Rhodospirillaceae	10	3%
Petrosiidae	2	1,50%	Methanocaldococcaceae	10	3%
Poeciliidae	2	1,50%	Pseudonocardiaceae	9	3%
Poritidae	2	1,50%	Acholeplasmataceae	8	3%
Soleidae	2	1,50%	Anaeromyxobacteraceae	8	3%
Stylephoridae	2	1,50%	Chromobacteriaceae	8	3%
Tetillidae	2	1,50%	Eggerthellaceae	8	3%
Tetrabrachiidae	2	1,50%	Hafniaceae	8	3%
Trachipteridae	2	1,50%	Halieaceae	8	3%
Trichodontidae	2	1,50%	Hydrogenothermaceae	8	3%
Urechidae	2	1,50%	Leptospiraceae	8	3%
Uristidae	2	1,50%	Nitrosomonadaceae	8	3%
Veneridae	2	1,50%	Oceanospirillaceae	8	3%
Xenoturbellidae	2	1,50%	Pelagibacteraceae	8	3%
Artemiidae	1	0,75%	Phyllobacteriaceae	8	3%
Loliginidae	1	0,75%	Pseudoalteromonadaceae	8	3%
Ophiocomidae	1	0,75%	Thiovulaceae	8	3%
Ophiotomidae	1	0,75%	Treponemataceae	8	3%
Palaemonidae	1	0,75%	Zymomonadaceae	8	3%
Salmonidae	1	0,75%	Haloarculaceae	8	3%
Suidae	1	0,75%	Nostocaceae	7	2%
Tachyglossidae	1	0,75%	Rhodothermaceae	7	2%
Tenebrionidae	1	0,75%	Weeksellaceae	7	2%
Tephritidae	1	0,75%	Aerococcaceae	6	2%
Tetrahymenidae	1	0,75%	Alicyclobacillaceae	6	2%
Thelyphonidae	1	0,75%	Aphanizomenonaceae	6	2%
Viperidae	1	0,75%	Arcobacteraceae	6	2%
Xenodermidae	1	0,75%	Atopobiaceae	6	2%
Zeidae	1	0,75%	Brachyspiraceae	6	2%
Astrangiidae	1	0,75%	Carnobacteriaceae	6	2%
Chromulinaceae	1	0,75%	Cellvibrionaceae	6	2%
Emplectonematidae	1	0,75%	Chloroflexaceae	6	2%

Hymenolepididae	1	0,75%	Coriobacteriaceae	6	2%
Maldanidae	1	0,75%	Deinococcaceae	6	2%
Mermithidae	1	0,75%	Erythrobacteraceae	6	2%
Stichopodidae	1	0,75%	Fervidobacteriaceae	6	2%
Terebridae	1	0,75%	Halomonadaceae	6	2%
			Marinobacteraceae	6	2%
			Microbacteriaceae	6	2%
			Microcoleaceae	6	2%
			Pirellulaceae	6	2%
			Planctomycetaceae	6	2%
			Stappiaceae	6	2%
			Syntrophomonadaceae	6	2%
			Xanthobacteraceae	6	2%
			Archaeoglobaceae	6	2%
			Halobacteriaceae	6	2%
			Haloferacaceae	6	2%
			Acidaminococcaceae	4	1%
			Alcanivoracaceae	4	1%
			Alteromonadaceae	4	1%
			Aurantimonadaceae	4	1%
			Beijerinckiaceae	4	1%
			Brevibacteriaceae	4	1%
			Cardiobacteriaceae	4	1%
			Clostridiales	4	1%
			Coprobacillaceae	4	1%
			Cytophagaceae	4	1%
			Desulfitobacteriaceae	4	1%
			Desulfobacteraceae	4	1%
			Desulfohalobiaceae	4	1%
			Desulfuromonadaceae	4	1%
			Dictyoglomaceae	4	1%
			Gallionellaceae	4	1%
			Gordoniaceae	4	1%
			Hyphomicrobiaceae	4	1%
			Hyphomonadaceae	4	1%
			Idiomarinaceae	4	1%
			Intrasporangiaceae	4	1%
			Kosmotogaceae	4	1%
			Maricaulaceae	4	1%
			Metamycoplasmataceae	4	1%
			Methylococcaceae	4	1%
			Microscillaceae	4	1%
			Myxococcaceae	4	1%
			Nautiliaceae	4	1%
			Nitrospiraceae	4	1%
			Nocardioidaceae	4	1%

	Nocardiopsaceae	4	1%
	Opitutaceae	4	1%
	Peptococcaceae	4	1%
	Piscirickettsiaceae	4	1%
	Psychromonadaceae	4	1%
	Roseiflexaceae	4	1%
	Spirochaetaceae	4	1%
	Spirosomaceae	4	1%
	Streptosporangiaceae	4	1%
	Thiotrichaceae	4	1%
	Tropherymataceae	4	1%
	Zoogloeaceae	4	1%
	Methanocellaceae	4	1%
	Methanomicrobiaceae	4	1%
	Methanoregulaceae	4	1%
	Natrialbaceae	4	1%
	Thermoplasmataceae	4	1%
	Acaryochloridaceae	2	1%
	Acidimicrobiaceae	2	1%
	Acidothermaceae	2	1%
	Ahrensiaceae	2	1%
	Akkermansiaceae	2	1%
	Amoebophilaceae	2	1%
	Archangiaceae	2	1%
	Azonexaceae	2	1%
	Azospirillaceae	2	1%
	Bdellovibrionaceae	2	1%
	Beutenbergiaceae	2	1%
	Bruguierivoracaceae	2	1%
	Calditerrivibrionaceae	2	1%
	Catenulisporaceae	2	1%
	Cellulomonadaceae	2	1%
	Chitinophagaceae	2	1%
	Chrysiogenaceae	2	1%
	Chthoniobacteraceae	2	1%
	Coleofasciculaceae	2	1%
	Colwelliaceae	2	1%
	Conexibacteraceae	2	1%
	Coprothermobacteraceae	2	1%
	Cyanothecaceae	2	1%
	Cyclobacteriaceae	2	1%
	Deferribacteraceae	2	1%
	Dermabacteraceae	2	1%
	Dermacoccaceae	2	1%
	Desulfarculaceae	2	1%
	Desulfobulbaceae	2	1%

	Desulfocapsaceae	2	1%
	Desulfomicrobiaceae	2	1%
	Desulfosudaceae	2	1%
	Desulfotomaculaceae	2	1%
	Elusimicrobiaceae	2	1%
	Endomicrobiaceae	2	1%
	Entomoplasmataceae	2	1%
	Eubacteriales	2	1%
	Fastidiosibacteraceae	2	1%
	Ferrimonadaceae	2	1%
	Fibrobacteraceae	2	1%
	Gemmataceae	2	1%
	Gemmatimonadaceae	2	1%
	Geodermatophilaceae	2	1%
	Geovibrionaceae	2	1%
	Gloeobacteraceae	2	1%
	Glycomycetaceae	2	1%
	Hahellaceae	2	1%
	Halanaerobiaceae	2	1%
	Halobacteroidaceae	2	1%
	Halothiobacillaceae	2	1%
	Herpetosiphonaceae	2	1%
	Jonesiaceae	2	1%
	Kangiellaceae	2	1%
	Kineosporiaceae	2	1%
	Kofleriaceae	2	1%
	Kribbellaceae	2	1%
	Ktedonobacteraceae	2	1%
	Kytococcaceae	2	1%
	Lentisphaeraceae	2	1%
	Magnetococcaceae	2	1%
	Mariprofundaceae	2	1%
	Marivirgaceae	2	1%
	Merismopediaceae	2	1%
	Methylacidiphilaceae	2	1%
	Methylocystaceae	2	1%
	Microcystaceae	2	1%
	Moritellaceae	2	1%
	Mycoplasmoidaceae	2	1%
	Nakamurellaceae	2	1%
	Nannocystaceae	2	1%
	Natranaerobiaceae	2	1%
	Nitratiruptoraceae	2	1%
	Oscillatoriaceae	2	1%
	Oscillochloridaceae	2	1%
	Paludibacteraceae	2	1%

Parachlamydiaceae	2	1%
Parvibaculaceae	2	1%
Parvularculaceae	2	1%
Petrotogaceae	2	1%
Porticoccaceae	2	1%
Promicromonosporaceae	2	1%
Puniceicoccaceae	2	1%
Rhodocyclaceae	2	1%
Rikenellaceae	2	1%
Rubrobacteraceae	2	1%
Saccharospirillaceae	2	1%
Sanguibacteraceae	2	1%
Segniliparaceae	2	1%
Sirenicapillariaceae	2	1%
Solibacteraceae	2	1%
Sphaerobacteraceae	2	1%
Spiroplasmataceae	2	1%
Spongiibacteraceae	2	1%
Sporomusaceae	2	1%
Sulfurospirillaceae	2	1%
Sulfurovaceae	2	1%
Symbiobacteriaceae	2	1%
Syntrophaceae	2	1%
Syntrophobacteraceae	2	1%
Syntrophotaleaceae	2	1%
Thermincolaceae	2	1%
Thermodesulfovibrionaceae	2	1%
Thermomicrobiaceae	2	1%
Thermomonosporaceae	2	1%
Thermosediminibacteraceae	2	1%
Thermosynechococcaceae	2	1%
Thiobacillaceae	2	1%
Trueperaceae	2	1%
Tsukamurellaceae	2	1%
Turicibacteraceae	2	1%
Verrucomicrobia subdivision 3	2	1%
Verrucomicrobiaceae	2	1%
Victivallaceae	2	1%
Waddliaceae	2	1%
Acidilobaceae	2	1%
Cenarchaeaceae	2	1%
Ferroplasmaceae	2	1%
Halorubraceae	2	1%
Methanocorpusculaceae	2	1%
Methanopyraceae	2	1%
Methanospirillaceae	2	1%

	Methanothermaceae	2	1%
	Methanotrichaceae	2	1%
	Nanoarchaeaceae	2	1%
	Nitrosopumilaceae	2	1%
	Picrophilaceae	2	1%
	Pyrodictiaceae	2	1%
	Thermofilaceae	2	1%
	Leptolyngbyaceae	1	0%

Supplementary material 2. Families of metazoans and microbes (Archaea and Bacteria) found in ceriantharian tubes of *Ceriantheopsis lineata* from Alcatrazes archipelago (AK). Frequency (Freq) represents the percentage of observations regarding the total number of families.

Alcatrazes (AK)					
		Microbes			
Metazoa		**Archaea**		**Bacteria**	
	Freq		**Freq**		**Freq**
Plakinidae	9%	Sulfolobaceae	42%	Enterobacteriaceae	55%
Branchiostomatidae	7%	Thermococcaceae	29%	Bacillaceae	45%
Cionidae	5%	Methanobacteriaceae	26%	Streptococcaceae	39%
Elopidae	5%	Methanococcaceae	26%	Lactobacillaceae	32%
Niphatidae	5%	Desulfurococcaceae	23%	Staphylococcaceae	29%
Oscarellidae	5%	Thermoproteaceae	23%	Burkholderiaceae	29%
Acipenseridae	2%	Methanosarcinaceae	19%	Vibrionaceae	27%
Acroporidae	2%	Methanocaldococcaceae	16%	Enterococcaceae	23%
Ammotheidae	2%	Haloarculaceae	13%	Mycobacteriaceae	19%
Anisakidae	2%	Archaeoglobaceae	10%	Pasteurellaceae	18%
Antedonidae	2%	Halobacteriaceae	10%	Pseudomonadaceae	17%
Aplysiidae	2%	Haloferacaceae	10%	Clostridiaceae	15%
Aplysinidae	2%	Methanocellaceae	6%	Yersiniaceae	15%
Architeuthidae	2%	Methanomicrobiaceae	6%	Brucellaceae	14%
Ascidiidae	2%	Methanoregulaceae	6%	Bacteroidaceae	13%
Asteriidae	2%	Natrialbaceae	6%	Neisseriaceae	13%
Brachionidae	2%	Thermoplasmataceae	6%	Listeriaceae	12%
Bugulidae	2%	Acidilobaceae	3%	Moraxellaceae	12%
Caligidae	2%	Cenarchaeaceae	3%	Bifidobacteriaceae	11%
Callyspongiidae	2%	Ferroplasmaceae	3%	Mycoplasmataceae	11%
Cardiidae	2%	Halorubraceae	3%	Lachnospiraceae	11%
Cucumariidae	2%	Methanocorpusculaceae	3%	Roseobacteraceae	11%
Cypridinidae	2%	Methanopyraceae	3%	Streptomycetaceae	10%
Daphniidae	2%	Methanospirillaceae	3%	Campylobacteraceae	10%
Dentaliidae	2%	Methanothermaceae	3%	Corynebacteriaceae	10%
Dictyodendrillidae	2%	Methanotrichaceae	3%	Rhizobiaceae	9%
Discosomatidae	2%	Nanoarchaeaceae	3%	Borreliaceae	8%
Dugongidae	2%	Nitrosopumilaceae	3%	Helicobacteraceae	8%
Edwardsiidae	2%	Picrophilaceae	3%	Shewanellaceae	8%
Flustrellidridae	2%	Pyrodictiaceae	3%	Xanthomonadaceae	8%
Gadilidae	2%	Thermofilaceae	3%	Anaplasmataceae	8%
Gorgoniidae	2%			Oscillospiraceae	8%
Haliotidae	2%			Prevotellaceae	8%
Harrimaniidae	2%			Chlamydiaceae	7%
Hutchinsoniellidae	2%			Peptostreptococcaceae	7%
Laqueidae	2%			Flavobacteriaceae	7%
Limulidae	2%			Francisellaceae	7%

Lineidae	2%	Fusobacteriaceae	7%
Loxosomatidae	2%	Comamonadaceae	6%
Metridiidae	2%	Actinomycetaceae	6%
Molidae	2%	Rickettsiaceae	6%
Mopaliidae	2%	Micrococcaceae	6%
Nautilidae	2%	Synechococcaceae	6%
Nephtheidae	2%	Nitrobacteraceae	5%
Nereididae	2%	Peptoniphilaceae	5%
Onchidiidae	2%	Prochlorococcaceae	5%
Onchocercidae	2%	Thermoanaerobacteraceae	5%
Orbiniidae	2%	Chlorobiaceae	4%
Ostreidae	2%	Morganellaceae	4%
Pectinidae	2%	Erwiniaceae	4%
Plakobranchidae	2%	Rhodobacteraceae	4%
Podospongiidae	2%	Sphingomonadaceae	4%
Pollicipedidae	2%	Veillonellaceae	4%
Ptychoderidae	2%	Desulfovibrionaceae	3%
Pyuridae	2%	Nocardiaceae	3%
Raspailiidae	2%	Acetobacteraceae	3%
Scyliorhinidae	2%	Alcaligenaceae	3%
Spadellidae	2%	Erysipelotrichaceae	3%
Strongylocentrotidae	2%	Legionellaceae	3%
Suberitidae	2%	Paenibacillaceae	3%
Synbranchidae	2%	Pectobacteriaceae	3%
Terebellidae	2%	Thermoanaerobacterales	3%
Tethyidae	2%	Aeromonadaceae	3%
Tetraclitidae	2%	Aphanothecaceae	3%
Tettigoniidae	2%	Caulobacteraceae	3%
Triakidae	2%	Geobacteraceae	3%
Trichoplacidae	2%	Methylobacteriaceae	3%
Watersiporidae	2%	Coxiellaceae	2%
Artemiidae	1%	Oxalobacteraceae	2%
Cephalothricidae	1%	Synergistaceae	2%
Cephalotrichidae	1%	Thermaceae	2%
Chondrillidae	1%	Frankiaceae	2%
Loliginidae	1%	Propionibacteriaceae	2%
Oikopleuridae	1%	Selenomonadaceae	2%
Ophiocomidae	1%	Sphingobacteriaceae	2%
Ophiotomidae	1%	Tannerellaceae	2%
Palaemonidae	1%	Thermotogaceae	2%
Salmonidae	1%	Acidithiobacillaceae	2%
Suidae	1%	Acidobacteriaceae	2%
Tachyglossidae	1%	Aquificaceae	2%
Tenebrionidae	1%	Bartonellaceae	2%
Tephritidae	1%	Chromatiaceae	2%
Tetrahymenidae	1%	Dehalococcoidaceae	2%

Thelyphonidae	1%	Ectothiorhodospiraceae	2%
Vermetidae	1%	Eubacteriaceae	2%
Viperidae	1%	Leptotrichiaceae	2%
Xenodermidae	1%	Methylophilaceae	2%
Zeidae	1%	Micromonosporaceae	2%
		Porphyromonadaceae	2%
		Rhodospirillaceae	2%
		Acholeplasmataceae	1%
		Anaeromyxobacteraceae	1%
		Chromobacteriaceae	1%
		Eggerthellaceae	1%
		Hafniaceae	1%
		Halieaceae	1%
		Hydrogenothermaceae	1%
		Leptospiraceae	1%
		Nitrosomonadaceae	1%
		Nostocaceae	1%
		Oceanospirillaceae	1%
		Pelagibacteraceae	1%
		Phyllobacteriaceae	1%
		Pseudoalteromonadaceae	1%
		Pseudonocardiaceae	1%
		Thiovulaceae	1%
		Treponemataceae	1%
		Zymomonadaceae	1%
		Aerococcaceae	1%
		Alicyclobacillaceae	1%
		Aphanizomenonaceae	1%
		Arcobacteraceae	1%
		Atopobiaceae	1%
		Brachyspiraceae	1%
		Carnobacteriaceae	1%
		Cellvibrionaceae	1%
		Chloroflexaceae	1%
		Coriobacteriaceae	1%
		Deinococcaceae	1%
		Erythrobacteraceae	1%
		Fervidobacteriaceae	1%
		Halomonadaceae	1%
		Marinobacteraceae	1%
		Microbacteriaceae	1%
		Microcoleaceae	1%
		Pirellulaceae	1%
		Planctomycetaceae	1%
		Rhodothermaceae	1%
		Stappiaceae	1%

	Syntrophomonadaceae	1%
	Weeksellaceae	1%
	Xanthobacteraceae	1%
	Acidaminococcaceae	1%
	Alcanivoracaceae	1%
	Alteromonadaceae	1%
	Aurantimonadaceae	1%
	Beijerinckiaceae	1%
	Brevibacteriaceae	1%
	Cardiobacteriaceae	1%
	Clostridiales	1%
	Coprobacillaceae	1%
	Cytophagaceae	1%
	Desulfitobacteriaceae	1%
	Desulfobacteraceae	1%
	Desulfohalobiaceae	1%
	Desulfuromonadaceae	1%
	Dictyoglomaceae	1%
	Gallionellaceae	1%
	Gordoniaceae	1%
	Hyphomicrobiaceae	1%
	Hyphomonadaceae	1%
	Idiomarinaceae	1%
	Intrasporangiaceae	1%
	Kosmotogaceae	1%
	Maricaulaceae	1%
	Metamycoplasmataceae	1%
	Methylococcaceae	1%
	Microscillaceae	1%
	Myxococcaceae	1%
	Nautiliaceae	1%
	Nitrospiraceae	1%
	Nocardioidaceae	1%
	Nocardiopsaceae	1%
	Opitutaceae	1%
	Peptococcaceae	1%
	Piscirickettsiaceae	1%
	Psychromonadaceae	1%
	Roseiflexaceae	1%
	Spirochaetaceae	1%
	Spirosomaceae	1%
	Streptosporangiaceae	1%
	Thiotrichaceae	1%
	Tropherymataceae	1%
	Zoogloeaceae	1%
	Acaryochloridaceae	0%

	Acidimicrobiaceae	0%
	Acidothermaceae	0%
	Ahrensiaceae	0%
	Akkermansiaceae	0%
	Amoebophilaceae	0%
	Archangiaceae	0%
	Azonexaceae	0%
	Azospirillaceae	0%
	Bdellovibrionaceae	0%
	Beutenbergiaceae	0%
	Bruguierivoracaceae	0%
	Calditerrivibrionaceae	0%
	Catenulisporaceae	0%
	Cellulomonadaceae	0%
	Chitinophagaceae	0%
	Chrysiogenaceae	0%
	Chthoniobacteraceae	0%
	Coleofasciculaceae	0%
	Colwelliaceae	0%
	Conexibacteraceae	0%
	Coprothermobacteraceae	0%
	Cyanothecaceae	0%
	Cyclobacteriaceae	0%
	Deferribacteraceae	0%
	Dermabacteraceae	0%
	Dermacoccaceae	0%
	Desulfarculaceae	0%
	Desulfobulbaceae	0%
	Desulfocapsaceae	0%
	Desulfomicrobiaceae	0%
	Desulfosudaceae	0%
	Desulfotomaculaceae	0%
	Elusimicrobiaceae	0%
	Endomicrobiaceae	0%
	Entomoplasmataceae	0%
	Eubacteriales	0%
	Fastidiosibacteraceae	0%
	Ferrimonadaceae	0%
	Fibrobacteraceae	0%
	Gemmataceae	0%
	Gemmatimonadaceae	0%
	Geodermatophilaceae	0%
	Geovibrionaceae	0%
	Gloeobacteraceae	0%
	Glycomycetaceae	0%
	Hahellaceae	0%

	Halanaerobiaceae	0%
	Halobacteroidaceae	0%
	Halothiobacillaceae	0%
	Herpetosiphonaceae	0%
	Jonesiaceae	0%
	Kangiellaceae	0%
	Kineosporiaceae	0%
	Kofleriaceae	0%
	Kribbellaceae	0%
	Ktedonobacteraceae	0%
	Kytococcaceae	0%
	Lentisphaeraceae	0%
	Leptolyngbyaceae	0%
	Magnetococcaceae	0%
	Mariprofundaceae	0%
	Marivirgaceae	0%
	Merismopediaceae	0%
	Methylacidiphilaceae	0%
	Methylocystaceae	0%
	Microcystaceae	0%
	Moritellaceae	0%
	Mycoplasmoidaceae	0%
	Nakamurellaceae	0%
	Nannocystaceae	0%
	Natranaerobiaceae	0%
	Nitratiruptoraceae	0%
	Oscillatoriaceae	0%
	Oscillochloridaceae	0%
	Paludibacteraceae	0%
	Parachlamydiaceae	0%
	Parvibaculaceae	0%
	Parvularculaceae	0%
	Petrotogaceae	0%
	Porticoccaceae	0%
	Promicromonosporaceae	0%
	Puniceicoccaceae	0%
	Rhodocyclaceae	0%
	Rikenellaceae	0%
	Rubrobacteraceae	0%
	Saccharospirillaceae	0%
	Sanguibacteraceae	0%
	Segniliparaceae	0%
	Sirenicapillariaceae	0%
	Solibacteraceae	0%
	Sphaerobacteraceae	0%
	Spiroplasmataceae	0%

	Spongiibacteraceae	0%
	Sporomusaceae	0%
	Sulfurospirillaceae	0%
	Sulfurovaceae	0%
	Symbiobacteriaceae	0%
	Syntrophaceae	0%
	Syntrophobacteraceae	0%
	Syntrophotaleaceae	0%
	Thermincolaceae	0%
	Thermodesulfovibrionaceae	0%
	Thermomicrobiaceae	0%
	Thermomonosporaceae	0%
	Thermosediminibacteraceae	0%
	Thermosynechococcaceae	0%
	Thiobacillaceae	0%
	Trueperaceae	0%
	Tsukamurellaceae	0%
	Turicibacteraceae	0%
	Verrucomicrobia	0%
	Verrucomicrobiaceae	0%
	Victivallaceae	0%
	Waddliaceae	0%

Supplementary material 3. Families of metazoans and microbes (Archaea and Bacteria) found in ceriantharian tubes of *Ceriantheomorphe brasiliensis* from Santa Catarina (SC). Frequency (Freq) represents the percentage of observations regarding the total number of families.

Santa Catarina (SC)					
		Microbes			
Metazoa		**Archaea**		**Bacteria**	
	Freq		**Freq**		**Freq**
Branchiostomatidae	10%	Sulfolobaceae	39%	Enterobacteriaceae	56%
Vermetidae	6%	Thermococcaceae	29%	Bacillaceae	46%
Acroporidae	5%	Methanobacteriaceae	26%	Streptococcaceae	39%
Mytilidae	5%	Methanococcaceae	26%	Lactobacillaceae	33%
Onchidiidae	5%	Desulfurococcaceae	23%	Staphylococcaceae	30%
Edwardsiidae	4%	Thermoproteaceae	23%	Burkholderiaceae	29%
Machilidae	4%	Methanosarcinaceae	19%	Vibrionaceae	26%
Plakinidae	4%	Methanocaldococcaceae	16%	Enterococcaceae	23%
Albulidae	2%	Haloarculaceae	13%	Mycobacteriaceae	20%
Amphiuridae	2%	Archaeoglobaceae	10%	Pasteurellaceae	18%
Antedonidae	2%	Halobacteriaceae	10%	Pseudomonadaceae	16%
Aphrocallistidae	2%	Haloferacaceae	10%	Clostridiaceae	15%
Arbaciidae	2%	Methanocellaceae	6%	Yersiniaceae	15%
Architeuthidae	2%	Methanomicrobiaceae	6%	Brucellaceae	14%
Blenniidae	2%	Methanoregulaceae	6%	Bacteroidaceae	13%
Brachionidae	2%	Natrialbaceae	6%	Neisseriaceae	13%
Briareidae	2%	Thermoplasmataceae	6%	Listeriaceae	12%
Bugulidae	2%	Acidilobaceae	3%	Bifidobacteriaceae	11%
Bythograeidae	2%	Cenarchaeaceae	3%	Moraxellaceae	11%
Caligidae	2%	Ferroplasmaceae	3%	Mycoplasmataceae	11%
Caproidae	2%	Halorubraceae	3%	Lachnospiraceae	11%
Carapidae	2%	Methanocorpusculaceae	3%	Roseobacteraceae	11%
Chaetodermatidae	2%	Methanopyraceae	3%	Streptomycetaceae	10%
Chaetodontidae	2%	Methanospirillaceae	3%	Campylobacteraceae	10%
Cionidae	2%	Methanothermaceae	3%	Corynebacteriaceae	10%
Cladopathidae	2%	Methanotrichaceae	3%	Helicobacteraceae	9%
Daphniidae	2%	Nanoarchaeaceae	3%	Rhizobiaceae	9%
Dictyodendrillidae	2%	Nitrosopumilaceae	3%	Shewanellaceae	9%
Faviidae	2%	Picrophilaceae	3%	Borreliaceae	8%
Flustrellidridae	2%	Pyrodictiaceae	3%	Xanthomonadaceae	8%
Gigantactinidae	2%	Thermofilaceae	3%	Anaplasmataceae	8%
Haliotidae	2%			Oscillospiraceae	8%
Harrimaniidae	2%			Prevotellaceae	8%
Irciniidae	2%			Chlamydiaceae	7%
Keratoisididae	2%			Francisellaceae	7%
Laqueidae	2%			Peptostreptococcaceae	7%
Limulidae	2%			Flavobacteriaceae	7%

Lineidae	2%	Fusobacteriaceae	7%
Metridiidae	2%	Comamonadaceae	6%
Monognathidae	2%	Actinomycetaceae	6%
Mopaliidae	2%	Rickettsiaceae	6%
Mugilidae	2%	Micrococcaceae	6%
Muricidae	2%	Synechococcaceae	6%
Nereididae	2%	Nitrobacteraceae	5%
Niphatidae	2%	Peptoniphilaceae	5%
Oscarellidae	2%	Prochlorococcaceae	5%
Otariidae	2%	Erwiniaceae	5%
Paralichthyidae	2%	Sphingomonadaceae	5%
Parazoanthidae	2%	Thermoanaerobacteraceae	5%
Pectinidae	2%	Chlorobiaceae	5%
Petrosiidae	2%	Morganellaceae	5%
Plakobranchidae	2%	Nocardiaceae	5%
Poeciliidae	2%	Rhodobacteraceae	5%
Poritidae	2%	Veillonellaceae	4%
Ptychoderidae	2%	Acetobacteraceae	4%
Pyuridae	2%	Desulfovibrionaceae	3%
Raspailiidae	2%	Alcaligenaceae	3%
Soleidae	2%	Erysipelotrichaceae	3%
Strongylocentrotidae	2%	Legionellaceae	3%
Stylephoridae	2%	Paenibacillaceae	3%
Tetillidae	2%	Pectobacteriaceae	3%
Tetrabrachiidae	2%	Thermoanaerobacterales	3%
Trachipteridae	2%	Aeromonadaceae	3%
Trichodontidae	2%	Aphanothecaceae	3%
Trichoplacidae	2%	Caulobacteraceae	3%
Urechidae	2%	Coxiellaceae	3%
Uristidae	2%	Geobacteraceae	3%
Veneridae	2%	Methylobacteriaceae	3%
Watersiporidae	2%	Thermaceae	3%
Xenoturbellidae	2%	Frankiaceae	2%
Astrangiidae	1%	Oxalobacteraceae	2%
Cephalothricidae	1%	Propionibacteriaceae	2%
Cephalotrichidae	1%	Synergistaceae	2%
Chondrillidae	1%	Selenomonadaceae	2%
Chromulinaceae	1%	Sphingobacteriaceae	2%
Emplectonematidae	1%	Tannerellaceae	2%
Hymenolepididae	1%	Thermotogaceae	2%
Maldanidae	1%	Acidithiobacillaceae	2%
Mermithidae	1%	Acidobacteriaceae	2%
Oikopleuridae	1%	Aquificaceae	2%
Stichopodidae	1%	Bartonellaceae	2%
Terebridae	1%	Chromatiaceae	2%
		Dehalococcoidaceae	2%

	Ectothiorhodospiraceae	2%
	Eubacteriaceae	2%
	Leptotrichiaceae	2%
	Methylophilaceae	2%
	Micromonosporaceae	2%
	Porphyromonadaceae	2%
	Pseudonocardiaceae	2%
	Rhodospirillaceae	2%
	Acholeplasmataceae	1%
	Anaeromyxobacteraceae	1%
	Chromobacteriaceae	1%
	Eggerthellaceae	1%
	Hafniaceae	1%
	Halieaceae	1%
	Hydrogenothermaceae	1%
	Leptospiraceae	1%
	Nitrosomonadaceae	1%
	Oceanospirillaceae	1%
	Pelagibacteraceae	1%
	Phyllobacteriaceae	1%
	Pseudoalteromonadaceae	1%
	Rhodothermaceae	1%
	Thiovulaceae	1%
	Treponemataceae	1%
	Weeksellaceae	1%
	Zymomonadaceae	1%
	Aerococcaceae	1%
	Alicyclobacillaceae	1%
	Aphanizomenonaceae	1%
	Arcobacteraceae	1%
	Atopobiaceae	1%
	Brachyspiraceae	1%
	Carnobacteriaceae	1%
	Cellvibrionaceae	1%
	Chloroflexaceae	1%
	Coriobacteriaceae	1%
	Deinococcaceae	1%
	Erythrobacteraceae	1%
	Fervidobacteriaceae	1%
	Halomonadaceae	1%
	Marinobacteraceae	1%
	Microbacteriaceae	1%
	Microcoleaceae	1%
	Nostocaceae	1%
	Pirellulaceae	1%
	Planctomycetaceae	1%

	Stappiaceae	1%
	Syntrophomonadaceae	1%
	Xanthobacteraceae	1%
	Acidaminococcaceae	1%
	Alcanivoracaceae	1%
	Alteromonadaceae	1%
	Aurantimonadaceae	1%
	Beijerinckiaceae	1%
	Brevibacteriaceae	1%
	Cardiobacteriaceae	1%
	Clostridiales	1%
	Coprobacillaceae	1%
	Cytophagaceae	1%
	Desulfitobacteriaceae	1%
	Desulfobacteraceae	1%
	Desulfohalobiaceae	1%
	Desulfuromonadaceae	1%
	Dictyoglomaceae	1%
	Gallionellaceae	1%
	Gordoniaceae	1%
	Hyphomicrobiaceae	1%
	Hyphomonadaceae	1%
	Idiomarinaceae	1%
	Intrasporangiaceae	1%
	Kosmotogaceae	1%
	Maricaulaceae	1%
	Metamycoplasmataceae	1%
	Methylococcaceae	1%
	Microscillaceae	1%
	Myxococcaceae	1%
	Nautiliaceae	1%
	Nitrospiraceae	1%
	Nocardioidaceae	1%
	Nocardiopsaceae	1%
	Opitutaceae	1%
	Peptococcaceae	1%
	Piscirickettsiaceae	1%
	Psychromonadaceae	1%
	Roseiflexaceae	1%
	Spirochaetaceae	1%
	Spirosomaceae	1%
	Streptosporangiaceae	1%
	Thiotrichaceae	1%
	Tropherymataceae	1%
	Zoogloeaceae	1%
	Acaryochloridaceae	0%

	Acidimicrobiaceae	0%
	Acidothermaceae	0%
	Ahrensiaceae	0%
	Akkermansiaceae	0%
	Amoebophilaceae	0%
	Archangiaceae	0%
	Azonexaceae	0%
	Azospirillaceae	0%
	Bdellovibrionaceae	0%
	Beutenbergiaceae	0%
	Bruguierivoracaceae	0%
	Calditerrivibrionaceae	0%
	Catenulisporaceae	0%
	Cellulomonadaceae	0%
	Chitinophagaceae	0%
	Chrysiogenaceae	0%
	Chthoniobacteraceae	0%
	Coleofasciculaceae	0%
	Colwelliaceae	0%
	Conexibacteraceae	0%
	Coprothermobacteraceae	0%
	Cyanothecaceae	0%
	Cyclobacteriaceae	0%
	Deferribacteraceae	0%
	Dermabacteraceae	0%
	Dermacoccaceae	0%
	Desulfarculaceae	0%
	Desulfobulbaceae	0%
	Desulfocapsaceae	0%
	Desulfomicrobiaceae	0%
	Desulfosudaceae	0%
	Desulfotomaculaceae	0%
	Elusimicrobiaceae	0%
	Endomicrobiaceae	0%
	Entomoplasmataceae	0%
	Eubacteriales	0%
	Fastidiosibacteraceae	0%
	Ferrimonadaceae	0%
	Fibrobacteraceae	0%
	Gemmataceae	0%
	Gemmatimonadaceae	0%
	Geodermatophilaceae	0%
	Geovibrionaceae	0%
	Gloeobacteraceae	0%
	Glycomycetaceae	0%
	Hahellaceae	0%

	Halanaerobiaceae	0%
	Halobacteroidaceae	0%
	Halothiobacillaceae	0%
	Herpetosiphonaceae	0%
	Jonesiaceae	0%
	Kangiellaceae	0%
	Kineosporiaceae	0%
	Kofleriaceae	0%
	Kribbellaceae	0%
	Ktedonobacteraceae	0%
	Kytococcaceae	0%
	Lentisphaeraceae	0%
	Magnetococcaceae	0%
	Mariprofundaceae	0%
	Marivirgaceae	0%
	Merismopediaceae	0%
	Methylacidiphilaceae	0%
	Methylocystaceae	0%
	Microcystaceae	0%
	Moritellaceae	0%
	Mycoplasmoidaceae	0%
	Nakamurellaceae	0%
	Nannocystaceae	0%
	Natranaerobiaceae	0%
	Nitratiruptoraceae	0%
	Oscillatoriaceae	0%
	Oscillochloridaceae	0%
	Paludibacteraceae	0%
	Parachlamydiaceae	0%
	Parvibaculaceae	0%
	Parvularculaceae	0%
	Petrotogaceae	0%
	Porticoccaceae	0%
	Promicromonosporaceae	0%
	Puniceicoccaceae	0%
	Rhodocyclaceae	0%
	Rikenellaceae	0%
	Rubrobacteraceae	0%
	Saccharospirillaceae	0%
	Sanguibacteraceae	0%
	Segniliparaceae	0%
	Sirenicapillariaceae	0%
	Solibacteraceae	0%
	Sphaerobacteraceae	0%
	Spiroplasmataceae	0%
	Spongiibacteraceae	0%

	Sporomusaceae	0%
	Sulfurospirillaceae	0%
	Sulfurovaceae	0%
	Symbiobacteriaceae	0%
	Syntrophaceae	0%
	Syntrophobacteraceae	0%
	Syntrophotaleaceae	0%
	Thermincolaceae	0%
	Thermodesulfovibrionaceae	0%
	Thermomicrobiaceae	0%
	Thermomonosporaceae	0%
	Thermosediminibacteraceae	0%
	Thermosynechococcaceae	0%
	Thiobacillaceae	0%
	Trueperaceae	0%
	Tsukamurellaceae	0%
	Turicibacteraceae	0%
	Verrucomicrobia	0%
	Verrucomicrobiaceae	0%
	Victivallaceae	0%
	Waddliaceae	0%

General conclusion

Many species remain undiscovered due to a paucity of research in bioconstructions, such as biogenic microhabitats, that are home to a broad diversity of organisms. This study, however, helped reveal known and unknown organisms living in ceriantharian tubes.

We have reported crustacean biodiversity found on ceriantharian tubes using traditional taxonomic research, and described the new species *Podocerus carmelina* **sp. nov.,** as well as new location records for previously known crustacean species. These findings indicate that some crustacean species may be interested in soft ceriantharian tubes as microhabitats. Additionally, we investigated further species that cannot be easily visualized or are too damaged to be morphologically identified through metagenomic studies, helping to demonstrate that ceriantharian tubes are, other than biodiverse microhabitats, potential tools for basic research and biodiversity surveys.

The use of metagenomic eDNA sequenced by shotgun technique was inefficient for assigning biodiversity found in ceriantharian tubes to species level. However, this approach provided a wider view of microbial and metazoan families found in this microhabitat, revealing a number of families that were previously unknown to occur in ceriantharian tubes by traditional identification methods based on direct observation. Thus, contributing to knowledge on marine biodiversity.

Behavioral and ecological studies with Ceriantharia are scattered and incomplete, however, our findings contributed to fill and complete some gaps in knowledge about this group.

The ceriantharian tube-building research revealed that adult *Ceriantheomorphe brasiliensis* lacks substrate selection mechanisms and sediment capture. Moreover, this study indicated that species found on ceriantharian tubes are either accidentally carried by marine currents or actively recruit these substrates rather than being recruited by the ceriantharian itself.

Both *C. brasiliensis* and *Botrucnidifer norvegicus* can build tubes in hard substrate, and the latter can also share them with other specimens of the same species by building tubes with more than one opening, similar to *Isarachnanthus nocturnus* and *Cerianthus vogti*. Furthermore, because the ceriantharian species *B. norvegicus, I. nocturnus* and *C. vogti* build horizontally

oriented tube systems while *C. brasiliensis* most commonly build vertical oriented single tubes with only one opening, it is likely that the tube-building behavior of *C. brasiliensis* differs from those of these species.

Other than protection, the ceriantharian tube is critical in controlling the internal water pressure of ceriantharians. Without it, the animal not only becomes more exposed to predators, but it also cannot feed, putting its life in danger.

Lastly, there is a significant knowledge gap in the Ceriantharia group, and this work highlighted relevant concerns that need further exploration, such as symbiotic relationships, larval dispersal, sediment surface stabilization by ceriantharian tubes mats, and reproduction. All of these topics are essential for obtaining a better understanding of ceriantharian ecology, behavior, and life cycles.

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