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**TAXONOMIA E BIOLOGIA DOS *BRANCHIOSYLLIS* (ANNELIDA:SYLLIDAE)  
ASSOCIADOS A *CINACHYRELLA* (PORIFERA:DEMOSPONGIAE)**

Recife  
2021

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Dissertação apresentada ao Programa de Pós-graduação em Oceanografia da Universidade Federal de Pernambuco, como requisito parcial para a obtenção do Título de Mestre em Oceanografia.

Área de concentração: Oceanografia biológica.

Orientador: Prof. Dr. José Souto Rosa Filho.

Coorientadora: Profa. Dra. Karla Paresque.

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À minha família, à todos que fizeram  
parte disso. E à todos que sonham em fazer ciência.

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“In nature nothing exists alone”(CARSON, p.51,1962).

## RESUMO

As esponjas são organismos bentônicos que ocorrem em todos os ambientes aquáticos, onde desempenham diversos papéis ecológicos, entre eles servir de abrigo, local de reprodução e alimento para diversos grupos de organismos, colaborando significativamente para a manutenção da biodiversidade. Os *Branchiosyllis* fazem parte da família de anelídeos mais comumente encontrados associados as esponjas. Sendo também um gênero pouco conhecido para o nosso litoral, este estudo descreve uma nova espécie de *Branchiosyllis* associada a esponjas na Praia do Paiva na cidade de Santo Agostinho do estado de Pernambuco, Brasil. Em Agosto de 2019, milhares de toneladas de petróleo bruto de uma fonte não identificada apareceram na costa brasileira, causando o mais grave desastre ambiental que já impactou o Oceano Atlântico Sul. A praia do Paiva, que tem alguns dos recifes de corais tropicais mais bem preservados da costa brasileira. O capítulo 2 já foi publicado e revela que após o derramamento de óleo, foram encontradas esponjas com manchas de óleo em sua superfície e em seus canais, e foram identificadas gotículas de óleo entre os grãos do sedimento acumulado dentro desses canais. Durante este mesmo período, os anelídeos que são base desse estudo e estavam em associação com as esponjas tinham gotículas de óleo na superfície do corpo ou em sua faringe. Os testes de solubilidade utilizando óleo mineral e espectros Raman indicaram que estes óleos gotas, encontradas tanto nas esponjas como nas poliaquetas, tinham produtos químicos similares características do petróleo bruto que se derramou na praia. Seguindo o desastre, a abundância de *Branchiosyllis* diminuiu drasticamente, embora não houvesse mudança significativa no tamanho médio dos indivíduos. Em dezembro de 2019, a densidade de poliaquetas foi significativamente menor do que nos meses anteriores ( $107,9 \pm 28,31$  ind.10 mL<sup>-1</sup> de esponja em agosto de 2019 vs.  $18,62 \pm 35,48$  ind.10 mL<sup>-1</sup> de esponja em dezembro 2019). Esta redução abrupta da abundância sem mudança no tamanho médio do indivíduos indicam que a mortalidade afetou todas as classes de tamanho (idade) de forma semelhante, o que é típico dos impactos antropogênicos, ao invés da mortalidade natural. Fica assim claro que a contaminação de poliaquetas com óleo cru aumentou a mortalidade, causando uma significativa redução das populações de *Branchiosyllis* dos recifes de coral da praia do Paiva, seguindo o derramamento de óleo de 2019.

Palavras-chave: esponja; anelídeo; associação; óleo

## ABSTRACT

Sponges are benthic organisms that occur in all aquatic environments, where they play diverse ecological roles, including serving as shelter, breeding sites and food for various groups of organisms, collaborating significantly to the maintenance of biodiversity. The *Branchiosyllis* are part of the family of annelids most commonly found associated with sponges. Being also a little known genus for our coastline, this study describes a new species of *Branchiosyllis* associated with sponges in Praia do Paiva in the city of Santo Agostinho in the state of Pernambuco, Brazil. In August 2019, thousands of tonnes of crude oil from an unidentified source appeared on the Brazilian coast, causing the most serious environmental disaster ever to impact the South Atlantic Ocean. Paiva Beach, which has some of the best-preserved tropical coral reefs on the Brazilian coast. Chapter 2 has already been published and reveals that after the oil spill, sponges were found with oil stains on their surface and in their channels, and oil droplets were identified among the grains of sediment accumulated within these channels. During this same period, the annelids that are the basis of this study and were in association with the sponges had oil droplets on their body surface or in their pharynx. Solubility tests using mineral oil and Raman spectra indicated that these oil droplets, found on both the sponges and the polychaetes, had similar chemicals characteristic of the crude oil that washed up on the beach. Following the disaster, the abundance of *Branchiosyllis* decreased dramatically, although there was no significant change in the average size of individuals. In December 2019, the density of polychaetes was significantly lower than in previous months ( $107.9 \pm 28.31$  ind.  $10 \text{ mL}^{-1}$  sponge in August 2019 vs.  $18.62 \pm 35.48$  ind.  $10 \text{ mL}^{-1}$  sponge in December 2019). This abrupt reduction in abundance without change in mean size of individuals indicate that mortality affected all size (age) classes similarly, which is typical of anthropogenic impacts rather than natural mortality. It is thus clear that contamination of polychaetes with crude oil increased mortality, causing a significant reduction in *Branchiosyllis* populations of Paiva Beach coral reefs following the 2019 oil spill.

Keywords: sponge; annelid; association; oil.

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## 1 INTRODUÇÃO GERAL

Nos ambientes marinhos, e particularmente no bentos, as interações biológicas são muito abundantes e responsáveis pela estruturação de muitas comunidades (WOODING, 1998; LEVINTON, 2018). Membros de espécies diferentes podem viver em associação muito estreita, até mesmo uma dentro da outra. Tais relacionamentos íntimos são exemplos de simbiose, que literalmente significa “viver junto”. Tradicionalmente se divide a simbiose em diferentes categorias conforme os organismos envolvidos se beneficiam ou sofrem com o relacionamento (PEARSE, 1950). Nas relações comensais, uma espécie obtém alimento, abrigo, ou algum outro benefício, sem afetar a outra espécie. Por outro lado, às vezes o simbionte, geralmente o menor organismo da relação, se beneficia à custa do hospedeiro, isso é chamado de parasitismo. Anulando a unilateralidade das relações simbióticas, o mutualismo é o tipo de simbioses onde ambas as partes se beneficiam (CASTRO E HUBER, 2012).

As esponjas são animais bentônicos que ocorrem em todos os ambientes marinhos (HOOPER E LÉVI, 1994) e desempenham diversos papéis ecológicos, entre eles servir de abrigo, local de reprodução e alimento para diversos grupos de organismos, colaborando significativamente para a manutenção da biodiversidade marinha (WULFF 2001; FIORE E JUTTE 2010). Entre os organismos mais abundantes e diversos associados a esponjas, de forma obrigatória ou não, estão os anelídeos, que exploram as variadas estruturas internas dos poríferos, como canais, sulcos e câmaras (MARTIN E BRITAYEV 1998; NEVES E OMENA 2003; HICKMAN ET AL 2004). Para os anelídeos as esponjas são ‘verdadeiros hotéis’ (PEARSE, 1950), provendo local para reprodução, proteção contra predação e local de reprodução (WESTINGA E HOETJES, 1981; ÇINAR ET AL., 2002; WULFF, 2006; HUANG ET AL., 2008).

Dentre esses anelídeos estão os Syllidae que são frequentemente encontrados associados a esponjas marinhas (MARTIN E BRITAYEV, 1998; CAPA ET AL., 2001), sendo provavelmente os anelídeos com maior frequência de simbiose com esponjas nos mares tropicais, subtropical e temperados (MARTIN ET AL 2003). A família Syllidae é composta por 79 gêneros e muitas das espécies de sillídeos são registrados como inquilinos de esponjas, podendo a relação ser específica, quando uma cada espécie de poliqueta ocorre em apenas uma espécie de porífera, ou genérica, quando os poliquetas de uma espécie ocorrem em diversas espécies de esponjas.

Por exemplo *Branchiosyllis oculata* San Martin 2013 que ocorre em esponjas, hydrozoários, algas e corais mortos localizados até cerca de 50 metros de profundidade (San Martin et al 2013). Apesar de abundantes em associação com esponjas, ainda pouco se sabe sobre a biologia dos Syllidae sobre suas interações com outras espécies (ÇINAR E GÖNLÜGÜR-DEMIRCI 2005, SERRANO ET AL. 2006, MUSCO ET AL.2009).

Pertencente a família Syllidae, o gênero *Branchiosyllis* Ehlers, 1887, que possui 27 espécies, e em muitas espécies do gênero são observadas em associação com esponjas marinhas, por exemplo, espécies como *Branchiosyllis tamandarensis*, *Branchiosyllis belchiori* e *Branchiosyllis gonzaguinhai* tem sido citadas em associação com as esponjas da espécies *Tedania ignis* Duchassaing & Michelotti, 1864 e da espécie *Haliclona caerulea* Hechtel, 1965, respectivamente. (PARESQUE ET AL 2016; NASCIMENTO, 2019). Também já se indentificou a associação de *Branchiosyllis oculata* em esponjas da espécies *Cinachyrella alloclada* (PAWLIK, 1983).

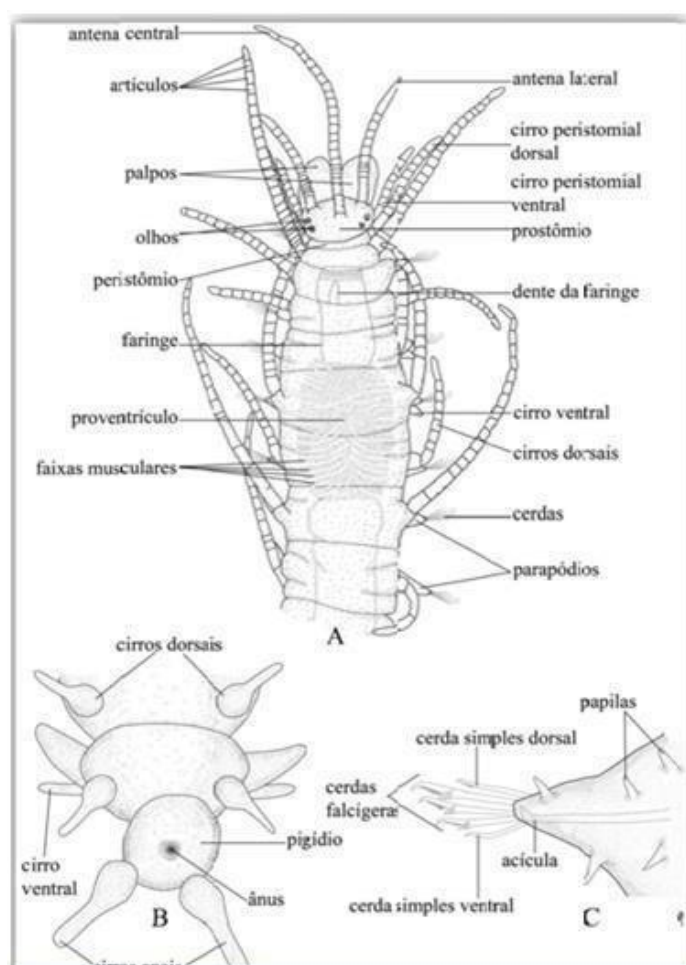
Os poliquetas do gênero *Branchiosyllis*, têm geralmente tamanho relativamente pequenos, medindo entre 5 e 100 mm (NASCIMENTO, 2019).

Segundo a descrição morfológica realizada por Ehlers em 1887 com exemplares da espécie diagnose *Branchiosyllis oculata* o corpo dos *Branchiosyllis* é comumente mais largo no nível do proventrículo e afila aos poucos em direção ao pigídio. O corpo é geralmente subcilíndrico, curvado dorsalmente e planoventralmente. Algumas espécies são achatados dorso-ventralmente achatados, e *B. orbiniiformis* San Martín, Hutchings e Aguado (2008) é lateralmente comprimida com os parapódios direcionados para cima.

O prostômio é bem desenvolvido, com formatos variados, desde aproximadamente semi-circular a subpentagonal. A maioria das espécies possui um par de palpos anteriormente, com formato aproximadamente cônico e voltado para frente na maioria das espécies, com

grau de fusão variável, desde totalmente fundidos até totalmente livres um do outro. Quatro olhos relativamente complexos, lenticulados (VERGER-BOCQUET, 1983) estão presentes na maioria das espécies e, por vezes, também há duas manchas ocelares anteriores, excepcionalmente, algumas espécies não apresentam olhos nem manchas ocelares. Em geral, os *Branchiosyllis* e os silídeos possuem ainda três antenas no prostômio, duas laterais e uma central, sendo que o ponto de inserção, comprimento e forma variam entre espécie.

Figura 1 - Morfologia geral dos Syllidae



Fonte: Paresque, 2014.

O peristômio normalmente pode ser visto tanto dorsal, quanto ventralmente, onde se encontra bem desenvolvido, formando a boca (Figura 1). Em alguns táxons, o peristômio pode ser recoberto dorsalmente pelo prostômio ou pelo setígero 1 enquanto em outros, o peristômio pode formar um lóbulo nucal e recobrir a parte posterior do prostômio. Assim como o prostômio, o peristômio é aqueto e possui 1–2 pares de cirros peristomiais lateralmente. Os órgãos nucais, localizados dorso-lateralmente, entre o prostômio e o peristômio, apresentam-se como projeções nucais, ovais a digitiformes, estendendo-se em

direção posterior, de extensão variável. Outras partes do corpo, como palpos, prostômio, peristômio, apêndices e superfícies dorsal e ventral também podem apresentar ciliação, formando faixas ciliadas ou tufo dispersos.

A segmentação é homônoma. Características como dos cirros dorsais, e de cerdas e acículas por parapódio, geralmente variam entre as regiões do corpo. O pigídio normalmente possui um par de cirros anais semelhantes aos cirros dorsais posteriores (Figura 1). Os parapódios são unirremes, com os notopódios reduzidos, sem cerdas ou acículas, apenas portando cirros dorsais, usualmente bem desenvolvidos. Os cirros ventrais geralmente são ovais ou digitiformes, mais curtos do que os lóbulos parapodiais. Internamente, os parapódios são sustentados por acículas, de número e morfologia variáveis.

As cerdas compostas são principalmente em forma de ungula virada a 90° em relação as hastes, sendo essa característica classificatória do gênero (GÓNGORA-GARZA ET AL., 2011). Durante o período reprodutivo, nos segmentos sexuais (epigamia) ou nos estolões (esquizogamia), os parapódios adquirem notoacícula e notocerdas para natação. (PANDIAN, 2019). As cerdas simples dorsal e ventral podem estar presentes desde o primeiro setífero, mas geralmente estão presentes nos setíferos mais posteriores e são mais finas do que as hastes das cerdas compostas no mesmo fascículo. Podem ser unidentadas, bidentadas ou bifidas, como usam espinulação subdistal, retas ou sigmoides.

Antenas, cirros peristomiais, cirros dorsais e cirros anais, são denominados como “apêndices”, possuem diferentes formatos e são articulados. Esses artículos são caracterizados como moniliformes, são divididos em artículos esféricos, cúbicos ou achatados, o número de artículos não necessariamente refletindo o tamanho relativo entre os apêndices, uma vez que o tamanho dos artículos pode variar de acordo com o apêndice e ao longo do mesmo.

A faringe estende-se por um número variável de segmentos a partir da boca, esta localizada ventralmente no peristômio. Anteriormente, a margem pode ser circundada por uma coroa de papilas (normalmente 10) e uma densa franja de cílios. Comumente possui um dente. Posterior a faringe localiza-se o proventrículo que desempenha importante papel endócrino, secretando hormônios relacionados ao controle dos ciclos reprodutivos (FRANKE, 1999), e digestivo, agindo com uma bomba de sucção (FAUCHALD E JUMARS, 1979), e é considerado a principal sinapomorfia da família (GLASBY, 1993).

Além dos caracteres morfológicos, utilizados para definição de espécies, gêneros e até mesmo subfamílias, os aspectos reprodutivos também são importantes principalmente para classificação das subfamílias, sendo que os sistemas de incubação e tipos de estolões representam valiosas informações filogenéticas (AGUADO *et al.*, 2012).

No ano de 2019 aproximadamente 5.000 toneladas de petróleo bruto atingiu mais de 1.000 localidades em 11 estados ao longo de 4.334 km da costa brasileira (Brum *et al.*, 2020;

Lourenço et al., 2020), causando o impacto ambiental mais extenso e mais severo na costa brasileira, no Oceano Atlântico Sul e nos oceanos tropicais até agora (Soares et al., 2020a,b)

Acontecimentos com estas proporções envolvendo são um dos impactos ambientais mais destrutivos nos ecossistemas marinhos, particularmente nas zonas costeiras, e podem afectar as populações humanas, o ambiente físico e a biota a todos os níveis tróficos (WEISS, 2015; MCLACHLAN E DEFEO, 2018) devido a elevada toxicidade dos componentes presentes nestas substâncias, principalmente hidrocarbonetos, que podem representar 90% do total de compostos químicos (TISSOT E WELTE, 1984; UNEP, 1992; NRC, 2003).

Os efeitos dos compostos derivados do petróleo nos organismos podem ser letais ou subletais, impactando a natação, as capacidades de captura e predação de presas, e o sucesso da reprodução, levando assim a mudanças nas comunidades e/ou populações (WEISS, 2014; TORREIRO-MELO ET AL., 2015). A toxicidade do petróleo para os organismos marinhos é influenciada pelas propriedades físicas e químicas do petróleo, características

uma coroa de papilas (normalmente 10) e uma densa franja de cílios. Comumente possui um dente. Posterior a faringe localiza-se o proventrículo que desempenha importante papel endócrino, secretando hormônios relacionados ao controle dos ciclos reprodutivos (FRANKE,1999),e digestivo,agindo com uma bomba de sucção (FAUCHALD E JUMARS, 1979), e é considerado a principal sinapomorfiada família (GLASBY, 1993).

Além dos caracteres morfológicos, utilizados para definição de espécies, gêneros e até mesmo subfamílias, os aspectos reprodutivos também são importantes principalmente para classificação das subfamílias, sendo que os sistemas de incubação e tipos de estolões representam valiosas informações filogenéticas (AGUADO *et al.*,2012).

No ano de 2019 aproximadamente 5.000 toneladas de petróleo bruto atingiu mais de 1.000 localidades em 11 estados ao longo de 4.334 km da costa brasileira (BRUM *et al.*, 2020; LOURENÇO *et al.*, 2020), causando o impacto ambiental mais extenso e mais severo na costa brasileira, no Oceano Atlântico Sul e nos oceanos tropicais até agora (SOARES *et al.*, 2020a,b).

Acontecimentos com estas proporções envolvendo são um dos impactos ambientais mais destrutivos nos ecossistemas marinhos, particularmente nas zonas costeiras, e podem afectar as populações humanas, o ambiente físico e a biota a todos os níveis tróficos (WEISS, 2015; MCLACHLAN E DEFEO, 2018) devido a elevada toxicidade dos componentes presentes nestas substâncias, principalmente hidrocarbonetos, que podem representar 90% do total de compostos químicos (TISSOT E WELTE, 1984; UNEP, 1992; NRC, 2003).

Os efeitos dos compostos derivados do petróleo nos organismos podem ser letais ou subletais,

impactando a natação, as capacidades de captura e predação de presas, e o sucesso da reprodução, levando assim a mudanças nas comunidades e/ou populações (WEISS, 2014; TORREIRO-MELO *et al.*, 2015). A toxicidade do petróleo para os organismos físicas e químicas da água, hidrodinâmica local, via de exposição e hábito de alimentação dos animais (KNEZOVICH, 1987; WOLFE *et al.*, 1998; WEISS, 2014).

Neste sentido, é de elevada importância avaliar os impactos agudos (curto prazo) dos derrames de petróleo na biodiversidade marinha (WEISS, 2014; YUEWEN E ADZIGBLI, 2018). Então, considerando que há um desconhecimento da fauna associada a esponjas do gênero *Cinachyrella* na Praia do Paiva esse estudo tratou de descrever uma nova espécie de *Branchiosyllis* para conhecimento científico e ainda revelou consequências sofridas pelo gênero após a ingestão de petróleo cru.

**Esta dissertação foi dividida em três capítulos, que abordam diferentes aspectos dos *Branchiosyllis* (Polychaeta: Syllidae) associados as esponja *Cinachyrella* (Porifera: Desmospongiae) na praia do Paiva (PE, Brasil), quais sejam: O primeiro capítulo do texto refere-se ao levantamento bibliográfico o segundo capítulo refere-se ao artigo intitulado - ‘NEW SPECIES OF *BRANCHIOSYLLIS* EHLERS, 1887 ASSOCIATED WITH SPONGES *CINACHYRELLA* WILSON, 1925 FROM BRAZIL’, que apresenta descrição de uma nova espécie para o gênero; já o terceiro capítulo refere-se ao artigo - ‘EFFECTS OF CONTACT AND INGESTION OF CRUDE OIL BY THE SYMBIOTIC POLYCHAETE *BRANCHIOSYLLIS* SP. LIVING IN SPONGES *CINACHYRELLA* SP. AFTER 2019 OIL SPILL ON THE BRAZILIAN TROPICAL COAST’, que trata dos efeitos do derramamento de petróleo de 2019 sobre as populações e foi publicado na Science of The Total Environment.**

## 2 OBJETIVO

### 2.1 Objetivo geral

Descrever os poliquetas *Branchiosyllis* (Polychaeta: Syllidae) associados a esponja *Cinachyrella* (Porifera: Desmospongiae) na praia do Paiva (Pernambuco, Brasil), identificando as espécies presentes, suas variações temporais e o impacto do derramamento de petróleo de 2019 nas populações.

### 2.2 Objetivos específicos

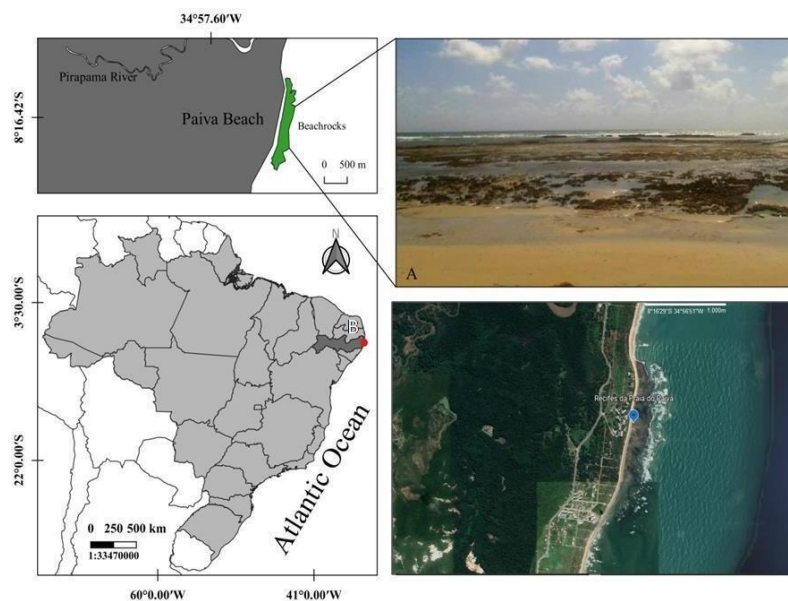
- Identificar as espécies de *Branchiosyllis* associadas a *Cinachyrella*;
- Descrever as variações temporais na estrutura das populações;
- Identificar o efeito do derramamento de petróleo de 2019 nas populações.

### 3 METODOLOGIA

#### 3.1 Local de estudo

A praia do Paiva localiza-se no litoral sul do Estado de Pernambuco, Município do Cabo, entre  $08^{\circ} 15' 76''$  e  $08^{\circ} 17' 89''$  S, e  $034^{\circ} 57' 30''$  W (Fig.2). É uma praia de mar aberto com aproximadamente 7,8 km de extensão (Holanda et al., 2020). A linha do recife mais próxima da praia tem até 2,5 km de comprimento e 1 km de largura e é colonizada por uma comunidade bentônica diversa (Vasconcelos et al., 2019). Na região regime de marés é do tipo meso-maré, dominada por ondas e sob a ação constante dos ventos alísios vindos principalmente de E-SE, no período de abril a setembro (mais intensos em agosto e setembro), e de N-NE, quando sopram de outubro a março (LGGM, 1997). O clima é caracterizado por ser tropical úmido com chuvas de outono, em prática existe dois períodos: período de chuva (outono/inverno) e períodos de seca (primavera/verão) (Alvarez, 2013).

Figura2. Mapa da praia do Paiva - A: vista geral dos recifes de coral da praia do Paiva.



Fonte: o Autor

### **3.2 Amostragem**

Mensalmente entre julho de 2019 e junho de 2020 (exceção foi março de 2020) trinta esponjas foram coletadas manualmente nos recifes intertidais da praia do Paiva. Antes da coleta, as esponjas eram cobertas por um saco plástico para evitar a perda de animais. No laboratório, o volume de cada esponja era medido (deslocamento da coluna de água em um cilindro graduado) a posteriori foram pesadas e tiveram os diâmetros e a altura medidos. Em seguida, as esponjas foram dissecadas sob estereomicroscópio para coletar os poliquetas presentes em seus canais e câmaras. Os poliquetas foram identificados e contados. O comprimento total de cada poliqueta foi medido usando um retículo micrométrico acoplado a ocular de um microscópio estereoscópico.

**4 NEW SPECIES OF *BRANCHIOSYLLIS* EHLERS, 1887 ASSOCIATED WITH SPONGES *CINACHYRELLA* WILSON, 1925 FROM BRAZIL**

**New species of *Branchiosyllis* Ehlers, 1887 associated with sponges *Cinachyrella* Wilson, 1925 from Brazil**

*Paper to be submitted for publication in the Zootaxa*

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**Abstract**

In this paper we describe a new species of the genus *Branchiosyllis* Ehlers, 1887 associated with sponges of the genus *Cinachyrella*. The collections were made at Praia do Paiva, southern coast of the state of Pernambuco, Brazil. We compare the species described here with their morphologically more similar congeners and provide a synoptic table of morphological variation among individuals of the type series, as well as an update of the key to *Branchiosyllis* recorded in Brazil already provided.

Key-words: Polychaeta, Biodiversity; Taxonomy; Intertidal; *Cynachirella*

## Introduction

The family Syllidae Grube, 1850 is composed by 79 genera and almost ~1100 (Martin *et al.* 2021; Read, G. and Fauchald, K.,2020) valid species occurring exclusively in marine habitats widely distributed in all oceans (San Martín 2003; Aguado and San Martín 2009; Aguado *et al.* 2012). The individuals belonging to this family can be found in muddy and sand bottom or under rocks, inhabit since the intertidal zone (e.g. coral reefs) to deep-sea ocean in hydrothermal vents (San Martín 2004; León-González *et al.* 2009; San Martín and Worsfold 2015).

The Syllidae have various feeding habits occupying different trophic guilds including carnivores, herbivores and detritivores (Fauchald and Jumars 1979; Giangrande *et al.* 2000). Many species of Syllidae can be found associated with other marine organisms, e.g. hydrozoan and bryozoan colonies, as symbionts with corals other polychaetes and sponges (Lopez *et al.* 2001; San Martín 2004; León-González *et al.* 2009; Lattig and Martin 2011; San Martín and Worsfold 2015).

This family is subdivided in 5 subfamilies: Anoplosyllinae Aguado and San Martín, 2009; Autolytinae Langerhans, 1879; Eusyllinae Malaquin, 1893; Exogoninae Langerhans, 1879 and Syllinae Grube, 1850. *Branchiosyllis* belongs to the subfamily Syllinae, characterized by having articulated appendages, free or partially fused palps in base, antennae and cirri (tentacular, dorsal e anal) articulated (San Martín *et al.* 2008; León-González *et al.* 2009).

The genus *Branchiosyllis* Ehlers, 1887 comprises 27 species (Nascimento *et al.* 2019), is easily recognized by the presence of claw-shaped falcigers (ungulae) in all the species (Gongora-Garza *et al.* 2011; Alvarez-Campos *et al.* 2012), being it considered the main diagnostic of the genus (Aguado and San Martín 2009; Alvarez-Campos *et al.* 2012). From Brazil, only six species of *Branchiosyllis* were recorded: *B. belchiori* Nascimento, Fukada e

Paiva (Nascimento et al. 2019); *B. diazi* Rioja, 1958 (Rullier and Amoureux 1979); *B. exilis* Gravier, 1900 (Nogueira 2000, 2006; Fukuda 2010) *B. gonzaguinhai* Nascimento, Fukada and Paiva (Nascimento et al. 2019); *B. oculata* Ehlers, 1887 (Rullier and Amoureux 1979; Neves and Omena 2003; Paiva et al. 2007) and *B. tamandarensis* Paresque, Fukada and Nogueira, 2016 (Paresque et al. 2016). Here, we describe a new species of genus *Branchiosyllis* associated with marine sponge *Cinachyrella* sp. sampled in sandstone reefs in northeastern Brazil.

### **Material and methods**

Sponges were manually collected on the intertidal reefs of Paiva beach (08° 15' 76" and 08° 17' 89" S, and 034° 57' 30" W)(Fig.3a). Prior to collection, sponges were covered with a plastic bag to prevent loss of animals. The sponges were then preserved in 70% alcohol until dissected under a microscope to collect the polychaetes present in their channels and chambers (Fig.3b). The total length of each polychaete was measured using an ocular micrometer attached to the microscope. The length of the specimens was measured from the tip of the palps to the tip of the pygidium, excluding the anal cirri; the width was measured at the proventricular level, excluding the parapodia. In addition, some specimens were examined using scanning electron microscopy (SEM). For SEM, specimens were first dehydrated in a graded series of increasing concentrations of ethanol (70-100%), critical point dried, coated with ~35 nm gold, and examined and photographed at the Technology Platforms Center/ FIOCRUZ.

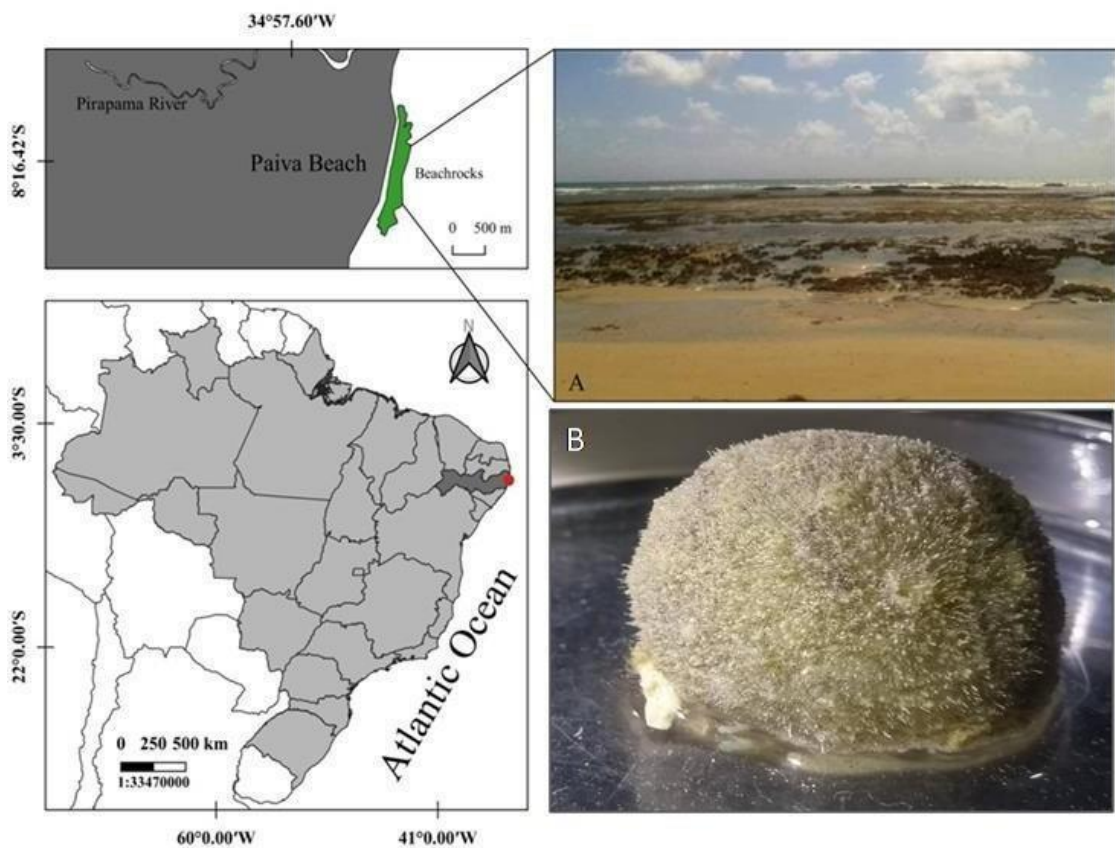


Fig 1 Map of Paiva beach (red circle). A: overview of the Paiva beach coral reefs, B : *Cynachirella* sp.

## Results

Family Syllidae Grube, 1850

Subfamily Syllinae Grube, 1850

Genus *Branchiosyllis* Ehlers,

1887

Type species: *Branchiosyllis oculata* Ehlers, 1887

**Diagnosis (San Martin, 2013).** Relatively medium-sized to large body, subcylindrical dorso-ventrally or, more rarely, laterally flattened. Palps free or fused at bases. Prostomium with four eyes, occasionally also with two anterior eyespots, three antennae and two palps. Peristomium with two pairs of peristomial cirri. Antennae, peristomial, dorsal and anal cirri articulated. Parapodial lobes sometimes with branchiae. Compound

chaetae as falcigers with regular blades and with modified, claw-shaped blades (ungulae), at least on part of body. Simple chaetae not known, apparently altogether absent. Pharynx with single anterior tooth; surrounded by crown of about 10 soft papillae. Two anal cirri. Proventricle usually of approximately same length as pharynx. Reproduction by acephalous stolon.(San Martin, 2013)

**Updated key to the species of genus *Branchiosyllis* currently recorded from Brazilian waters**

|                                                                                                                       |                              |
|-----------------------------------------------------------------------------------------------------------------------|------------------------------|
| 1 Falciger present                                                                                                    | 2                            |
| - Ungulae present                                                                                                     | 3                            |
| 2(1) Flattened body. Branchiae present                                                                                | <i>B. diazi</i>              |
| - Cylindrical body. Branchiae absent                                                                                  | <i>B. exilis</i>             |
| 3(1) Branchiae with one lobe                                                                                          | 4                            |
| - Branchiae multi-lobed                                                                                               | 5                            |
| 4(3) Branchiae dome or slightly flattened                                                                             | <i>B. oculata</i>            |
| 5(3) Branchiae with up to five flattened body lobes.                                                                  | <i>B.</i>                    |
| <i>Gonzaguinhai</i>                                                                                                   |                              |
| Branchiae with up to six lobes. Subcylindrical body                                                                   | <i>B. Tamandarensis</i>      |
| -Branchiae dome shaped on the second and multilobes with four or more lobes from the third parapodium. Flattened body | <i>Branchiosyllis</i> sp. n. |
| - Branchiae pyriform. Subcylindrical body                                                                             | <i>B. belchiori</i>          |

***Branchiosyllis sp. n.***

Type series. All individuals from Atlantic Ocean, Brazil, state of Pernambuco, Cabo de Santo Agostinho, Praia da Reserva do Paiva (08° 17' 89" S, 34° 57' 30" W) intertidal reefs, associated with *Cynachirella* sp.

Table 1. Morphological variations among select characters of the type series *Branchiosyllis sp. n.* .

| <i>Branchiosyllis</i><br><i>sp. n.</i>              | Holotype | Paratype1 | Paratype2 | Paratype3 | Paratype4 | Paratype5 | Paratype6 |
|-----------------------------------------------------|----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Length x<br>width(mm)                               | 1,7x0,3  | 6x0,74    | 5x0,68    | 5x0,7     | 4x0,63    | 6x0,72    | 5x0,7     |
| Length of<br>pharynx<br>(chaetigers)                | 6        | 6         | 4         | 6         | 4         | 6         | 5         |
| Length of<br>proventricle<br>(chaetigers)           | 7        | 6         | 6         | 7         | 5         | 5         | 7         |
| N. of muscle<br>cell rows of<br>the<br>proventricle | 24       | 24        | 23        | 24        | 24        | 23        | -         |
| N. of ungula<br>per<br>parapodium                   |          |           |           |           |           |           |           |
| Anterior body                                       | 3-4      | 4         | 6         | 4-5       | 3-4       | 4         | 3         |
| Midbody                                             | 3-4      | 4         | 4-6       | 4-5       | 3-5       | 3-4       | 3         |
| Posteriorbody                                       | 3        | 5         | 4-5       | 4         | 4         | 3-4       | 3         |
| Number of<br>aciculae per<br>parapodium             |          |           |           |           |           |           |           |
| Anterior body                                       | 2        | 2         | 2         | 2         | 2         | 2         | 2         |
| Midbody                                             | 1        | 1         | 1         | 1         | 1         | 1         | 1         |
| Posteriorbody                                       | 1        | 1         | 1         | 1         | 1         | 1         | 1         |

Tabela 2. Morphological variations among select characters of the type series *Branchiosyllis* sp. n.

| <i>Branchiosyllis</i> sp.n.       |                       |                  |                       |                       |                       |                       |                       |
|-----------------------------------|-----------------------|------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Type serie                        | Holotype              | Paratype1        | Paratype2             | Paratype3             | Paratype4             | Paratype 5            | Paratype 6            |
| Number of chaetigers              | 54                    | 61               | 63(broken)            | 75                    | 31                    | 72                    | 61                    |
| Number of articles                |                       |                  |                       |                       |                       |                       |                       |
| Central antennae                  | 13                    | 0                | 12                    | 12                    | 10                    | 5(broken)             | 10                    |
| Lateral antennae                  | 13,13                 | 0                | 13,14                 | 10,10                 | 0,12                  | 14,11                 | 0,9                   |
| Peristomial dorsal cirri          | 20,19                 | 0                | 0,21                  | 15,21                 | 20,<br>10(broken)     | 20, 20+               | 19, 0                 |
| Peristomial ventral cirri         | 10, 10                | 0                | 0,12                  | 0                     | 0, 10                 | 12,12                 | 11, 0                 |
| Dorsal cirri 1                    | 20,17                 | 0                | 20,22                 | 13,20                 | 12,8                  | 22,21                 | 20, 0                 |
| Dorsal cirri 2                    | 14+,0                 | 0                | (9),16                | 20,28                 | 14,15                 | 15,17                 | 15, 0                 |
| Dorsal cirri 3                    | 24, 20                | 0                | (10),30               | 0,35                  | 18,15                 | 23, 20                | 27, 0                 |
| Dorsal cirri 4                    | 24, 20                | 0,3              | (6),22                | 0                     | 12,16                 | 20,21                 | 17, 0                 |
| Dorsal cirri 5                    | 23,16                 | 0,2              | (10),20               | 0                     | 14,12                 | 18,22                 | 16, 0                 |
| Long cirri, midbody               | 11+,20,0,2<br>3,16,0  | 28,<br>0,0,0,0,0 | 23,20,16<br>,16,23,17 | 25,22,23<br>,25,24,0  | 12,11,11<br>,11,10,10 | 16,19,15,1<br>6,12,19 |                       |
| Short cirri, midbody              | 19,15,17,1<br>5,14,16 | 0                | 18,15,12<br>,0,0,(9)  | 22,20,30<br>,28,28,27 | 9,5,7,10,<br>8,10     | 14,19,14,1<br>0,16,14 | 16,19,22,2<br>2,18,14 |
| Anal cirri                        | 0                     | 0                | 0                     | 8                     | 10                    | 6                     | 12                    |
| Number of ungulae per parapodium  |                       |                  |                       |                       |                       |                       |                       |
| Anterior body                     | 3/4                   | 4                | 6                     | 4-5                   | 3/4                   | 4                     | 3                     |
| Midbody                           | 3/4                   | 4                | 4-6                   | 4,5                   | 3-5                   | 3/4                   | 3                     |
| Posterior body                    | 3                     | 5                | 4-5                   | 4                     | 4                     | 3/4                   | 3                     |
| Number of aciculae per parapodium |                       |                  |                       |                       |                       |                       |                       |
| Anterior body                     | 2                     | 2                | 2                     | 2                     | 2                     | 2                     | 2                     |
| Midbody                           | 1                     | 1                | 1                     | 1                     | 1                     | 1                     | 1                     |
| Posterior body                    | 1                     | 1                | 1                     | 1                     | 1                     | 1                     | 1                     |
| <b>BR</b>                         |                       |                  |                       |                       |                       |                       |                       |
| (p1)                              | absent                | -                | -                     | absent                | absent                | -                     | absent                |
| (p2)                              | Dome-shaped           | -                | -                     | dome-shapd            | dome-shapd            | --                    | -                     |
| (p3)                              | _____                 | multilob         | multilob              | multilob              | multilob              | multilob              | -                     |

**Description.** Medium-sized syllid, largest specimen analyzed with 75 chaetigers measuring ~5.0mm long and ~0.7mm wide; body whitish in color without differentiated pigmentation. Flattened shaped body. Palps triangle slightly oval. Prostomium rectangular oval, about as long as palps with two small pairs of eyes in trapezoidal arrangement; median antenna (with up to 13 articles) inserted near anterior margin of prostomium slightly posteriorly inserted to lateral antennae, slightly shorter than combined length of prostomium and palps; lateral antennae inserted in anterior margin of prostomium, with 13-16 articles(Fig.4.A). Peristomium shorter than anterior chaetigera; dorsal peristomial cirri longer than antennae, with more or less 20-30 articles each; ventral peristomial cirri approximately with equal length to antennae, with ~10-14 articles each. Branchiae on chaetiger 1 absent and dome-shaped or multilobed in the second chaetiger, following chaetigers with multilobed in the median and posterior part, with at least 4 lobes(Fig.4.D- E). Dorsal cirri of chaetiger 1 similar in length to dorsal peristomial cirri, with 13-20 articles; dorsal cirri of anterior and median regions with lengths not indicating alternation, the longest with up to 11-25 articles(Table.2); ventral cirri approximately same length as parapodial lobes, or slightly longer, conical to digitiform, progressively more distally tapered. Antennae, peristomial cirri and dorsal cirri along body with cirrophores; dorsal cirri with basal more flattened articles, distal articles generally rectangular. Chaetiger 1 only with presetal lobe, post setal and ventral lobe absent, from the second parapodia onwards it has the three lobes (Fig.4.B). Anterior parapodia with 3-6 unguiae each, unidentate bristles, bent at 90° to stalks; last parapodia, usually in formation, with 3-5 ungules each(Fig.4.C. Table.1). Anterior parapodia with 2 aciculae each, one subdistally oblique and another straight, distally tapered; from the midbody parapodia with 1 straight and smooth acicula per parapodium up to the last chaetiger (Table.1). Pygidium semicircular, with two articulated anal cirri slightly shorter than posterior dorsal cirri. Pharynx extending for 4-7 segments,

opening surrounded by 10 papillae; large, conical, pointed tooth in median position (Table.1); proventriculus occupying 5-7 segments, with more or less 24 rows of muscular cells (Table.1; Table.2).

Remarks: *Branchiosyllis* sp. n. resembles *B. australis* Hartmann-Schröder, 1981, *B. belchiori*, *B. gonzaguinhai*, *B. oculata* and *B. tamandarensis* in having unmodified unguiae and present branchiae. However, *Branchiosyllis australis* has been reported from Western Australia and the Philippines; it also has dome-shaped branchiae. *B. australis* still has 4-6 unguiae on each anterior parapod and 2-3 unguiae on each posterior parapod; pharynx through 5 chaetigers, proventriculus through 6-7 chaetigers and 26-30 rows of muscle cells in proventriculus.

*Branchiosyllis oculata* occur in the North Atlantic region, the Gulf of Mexico, and the Caribbean Sea and have branchiae dome or slightly flattened. Still *B. oculata* has 3-5 unguiae on each anterior parapodium and 3-5 unguiae on each posterior parapodium; pharynx through chaetigers, proventriculus through 8 chaetigers and 22 rows of muscle cells in proventriculus.

*Branchiosyllis belchiori*, *Branchiosyllis gonzaguinhai*, and *Branchiosyllis tamandarensis* are the only ones of those species described from the South Atlantic, specifically from the states of Paraíba, Pernambuco, and Bahia, Northeast Brazil, and so far is only known from the original description. However, *B. belchiori* and *B. gonzaguinhai* has ovate to pyriform and multilobate with more than 5 lobes, and *B. belchiori* has 3-4 unguates on the anterior parapodia, 3-5 on the parapodia of the median part of the body and 2-3 on the posterior part with a proventriculus that occupies 3 segments and has 21-24 rows of muscular cells; *B. gonzaguinhai* has unguates in the anterior parapodia 4-5, 4-6 in the parapodia of the middle part of the body and 4-6 in the posterior part, its proventriculus occupies 4-5 chaetigers with 21-24 rows of muscular cells.

While *B.tamandarensis* possesses multilobed branchiae it starts from the second chaetiger and has 4-6 unions on each anterior parapodium and 2-3 unions on each posterior parapodium; pharynx through 5 chaetigers, proventriculus through 3.5-5 chaetigers and 25-30 rows of muscle cells in proventriculus,

Relating comparatively *Branchiosyllis sp. n.* with the species mentioned above it is observed that *Branchiosyllis sp. n.* has dome-shaped branchiae in the second parapodium and multilobed branchiae from the third parapodium onwards in relation to the formation of the parapodium it is observed that in the anterior parapodium it has 3-4 ungules and posterior with 3-6, it has extended pharynx 4-7, the proventriculum occupied of 5-7 segments and has 24 rows of muscular cells.

Table 3. Morphological comparison among species of *Branchiosyllis* occurring in Brazilian waters

|                                                   | <i>B.<br/>belchiori</i>                                  | <i>B.<br/>diazii</i>                                          | <i>B.<br/>exilis</i>                                                                                                                                                                                                         | <i>B.<br/>gonzaguinhai</i>                         | <i>B.<br/>oculata</i>                                              | <i>B.<br/>tamandarensis</i>     | <i>Branchiosyllis</i> sp. n.            |
|---------------------------------------------------|----------------------------------------------------------|---------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------|--------------------------------------------------------------------|---------------------------------|-----------------------------------------|
| <b>Original description</b>                       | Nascimento, Fukada&Paiva, 2019                           | Rioja, 1958                                                   | Gravier, 1900                                                                                                                                                                                                                | Nascimento, Fukada&Paiva, 2019                     | Ehlers, 1887                                                       | Paresque,Fukada&Nogueira 2016   | This manuscript                         |
| <b>Reference</b>                                  | Nascimento et al 2019                                    | Alvares et al. 2012                                           | San Martín et al. 2008                                                                                                                                                                                                       | Nascimento et al 2019                              | San Martín et al. 2008                                             | Paresque et al 2016             | This manuscript                         |
| <b>Body</b>                                       | Subcylindrical                                           | Flattened                                                     | Cylindrical                                                                                                                                                                                                                  | Flattened                                          | Flattened                                                          | Flattened                       | Flattened                               |
| <b>Eyespots</b>                                   | -                                                        | -                                                             | Absent                                                                                                                                                                                                                       | -                                                  | -                                                                  | Absent                          | Present                                 |
| <b>Branchiae</b>                                  | Present                                                  | Present                                                       | Absent                                                                                                                                                                                                                       | Present                                            | Present                                                            | Present                         | Present                                 |
| <b>Falcigers</b>                                  | Absent                                                   | Present                                                       | Present                                                                                                                                                                                                                      | Absent                                             | Absent                                                             | Absent                          | Absent                                  |
| <b>Ungulae</b>                                    | All parapodia                                            | Posterior parapodia                                           | Posterior parapodia                                                                                                                                                                                                          | All parapodia                                      | All parapodia                                                      | All parapodia                   | All parapodia                           |
| <b>Pharynxlength (segments)</b>                   | 3.5                                                      | 7-8                                                           | 5-6                                                                                                                                                                                                                          | 5                                                  | 6                                                                  | 5                               | 4-7                                     |
| <b>Proventriclelength (segments)</b>              | 3                                                        | 7-8                                                           | 7-8                                                                                                                                                                                                                          | 4                                                  | 8                                                                  | 3.5-5                           | 5-7                                     |
| <b>Number of muscle cell rows in proventricle</b> | 24-27                                                    | -                                                             | 27-30                                                                                                                                                                                                                        | 24                                                 | 22                                                                 | 25-30                           | 23-25                                   |
| <b>Habitat</b>                                    | Associated with the sponge <i>Haliclona caerulea</i>     | Intertidal, among algae and in sand                           | Shallow waters, on sponges, algae, gorgonians and coral rubble<br>Circumtropical, warmer areas of theMediterranean Sea, Australia (North and central WesternAustralia, South Australia, New South Wales, Northern Territory) | On and with in sponges                             | Intertidal, sand, algae, coral Rubble, sponge                      | Associated sponges              | With in sponges <i>Cinachyrella</i> sp. |
| <b>Distribution</b>                               | Brazil (Todos os Santos Bay, Fernando de Noronha Island) | Gulf of México (Verde Island, Veracruz), and Brazil ( Recife) |                                                                                                                                                                                                                              | Brazil (Fernando de Noronha Islandand Rocas Atoll) | Gulf of México and Caribbean Sea (Florida,Cuba, México, Venezuela) | Brazil (Paraíba and Pernambuco) | Brazil (Pernambuco)                     |

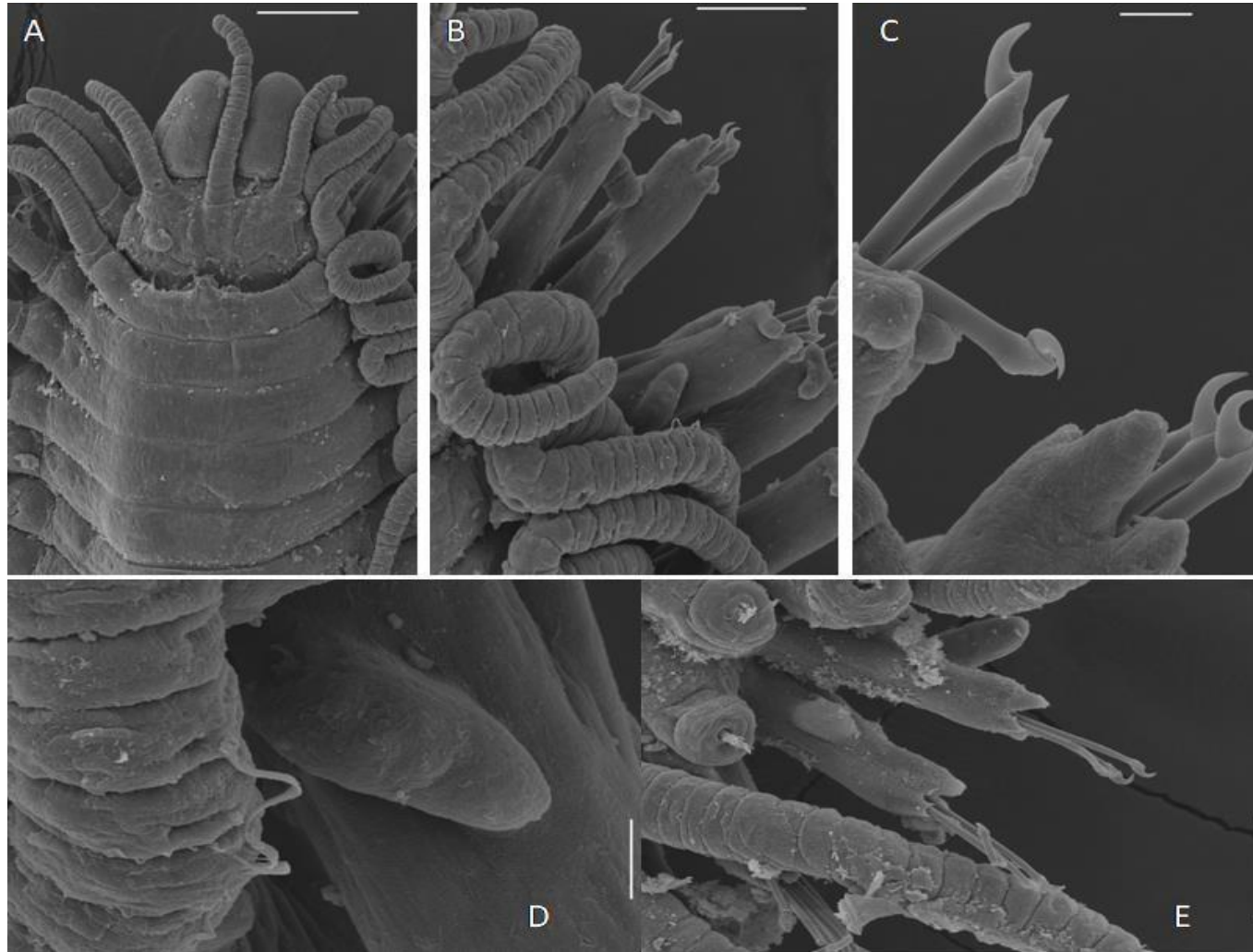


Fig 2.A, anterior region, dorsal view; B-C detail of the anterior parapodia; D, Branchiae multilobed ;E First parapodia with branchiae ausent, second parapodia with branchiae dome-shaped; Scale bars: A = 100  $\mu\text{m}$ ; B-C = 10  $\mu\text{m}$ ; D= 100  $\mu\text{m}$ ;E= 50  $\mu\text{m}$ .

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## 5 EFFECTS OF CONTACT AND INGESTION OF CRUDE OIL BY THE SYMBIOTIC POLYCHAETE

*BRANCHIOSYLLIS* SP. LIVING IN SPONGES *CINACHYRELLA* SP. AFTER THE 2019 OIL SPILL ON THE BRAZILIAN TROPICAL COAST

**Effects of contact with crude oil and its ingestion by the symbiotic polychaete *Branchiosyllis* living in sponges (*Cinachyrella* sp.) following the 2019 oil spill on the tropical coast of Brazil**

Paper published in the journal Science of The Total Environment

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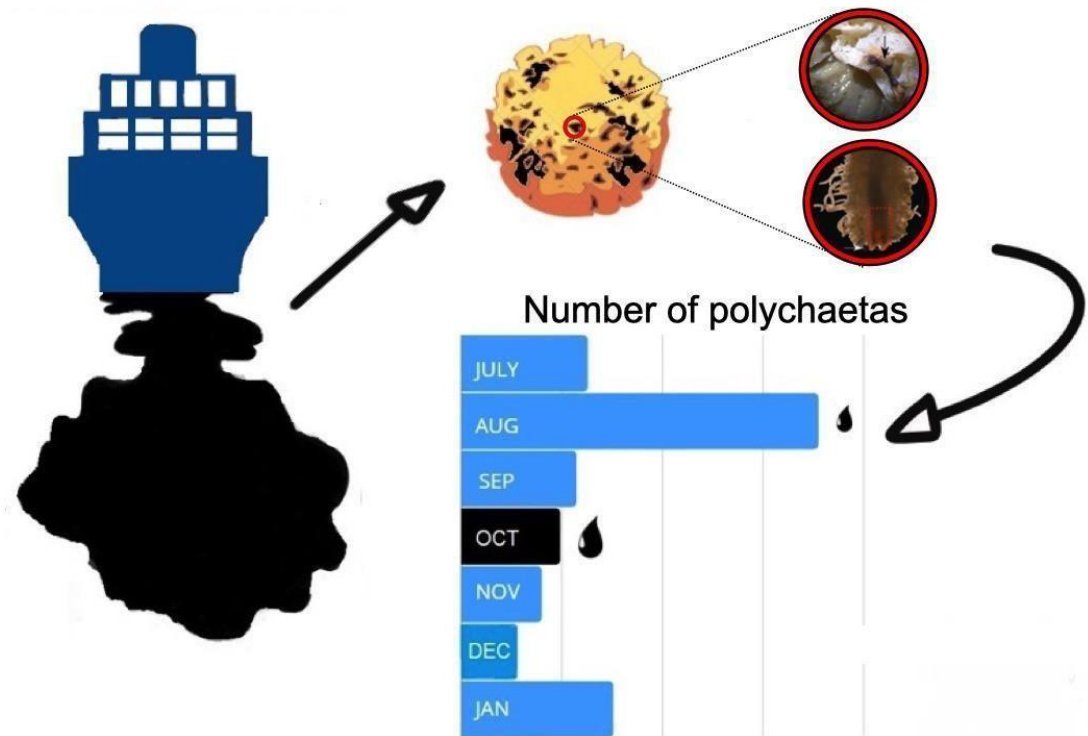
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### Highlights

- Coral reefs on the Brazilian coast were impacted by an oil spill in 2019.
- Oil stains and droplets were found on the sponges and polychaetes.
- The polychaetes had ingested crude oil.
- Significant reduction in the polychaete population following the oil spill.



## Abstract

In August 2019, thousands of tons of crude oil from an unidentified source began washing up on the Brazilian coast, causing the most severe environmental disaster that has ever impacted the South Atlantic Ocean. Paiva beach, which has some of the best-preserved tropical coral reefs on the Brazilian coast, was one of the coastal environments most severely affected by this oil. We report on the impact of the disaster on the local population of the symbiotic polychaete *Branchiosyllis* spp. associated with the sponge *Cinachyrella* sp. Following the oil spill sponges were found with oil stains on their surface and in their channels, and oil droplets were identified among the grains of the sediment accumulated within these channels. During this same period, the polychaetes in sponges had oil droplets on the surface of the body or in their pharynxes. Solubility tests using mineral oil and Raman spectra indicated that these oil droplets, found in both the sponges and the polychaetes, had

similar chemical characteristics to those of the crude oil that washed up on the beach. Following the disaster, the abundance of *Branchiosyllis* declined sharply, although there was no significant shift in the mean size of individuals. By December 2019, the density of polychaetes was significantly lower than in the preceding months ( $107.9 \pm 28.31$  ind. $10\text{ mL}^{-1}$  of sponge in August 2019 vs.  $18.62 \pm 35.48$  ind. $10\text{ mL}^{-1}$  of sponge in December 2019). This abrupt reduction in abundance with no change in the mean size of the individuals indicates that mortality affected all size (age) classes similarly, which is typical of anthropogenic impacts rather than natural mortality. It is thus clear that the contamination of polychaetes with crude oil increased mortality, causing a significant reduction in the *Branchiosyllis* populations of the coral reefs of Paiva beach following the 2019 oil spill.

## 1. Introduction

In 2019, approximately 5,000 tons of crude oil of unknown origin washed ashore at more than 1,000 localities in 11 states along 4,334 km of the northern and eastern coasts of Brazil (Brum et al, 2020; Lourenço et al., 2020). This caused the most extensive and severe environmental impact ever recorded on the Brazilian coast, in the South Atlantic Ocean, and in the world's tropical oceans (Soares et al., 2020a, b). This oil impacted mangroves, beaches, intertidal coral reefs (Magris and Giarrizzo, 2020), and seagrass meadows (Magalhães et al., 2020). This oil had an immediate impact on local economic activities (Araújo et al., 2020), the aquatic fauna (Brazilian Navy, 2019; Craveiro et al., 2021), and human health (Pena et al., 2020).

The coast of Pernambuco state, in tropical northeastern Brazil, is bordered by shallow coral reefs that run parallel to the coastline in outcrops or elongated banks, either adjacent to the shoreline or at depths of 5–10 m (Laborel-Deguen et al., 2019). Sponges of the genus *Cinachyrella* (Porifera: Desmospongiae) are very abundant on these shallow reefs, occurring at densities of 3.75–9.75 ind.m<sup>-2</sup> (Marinho, 2018), and are colonized by dense populations (up to 20 polychaetes per sponge) of the symbiotic polychaete *Branchiosyllis* (Polychaeta: Syllidae). In July 2019, a research project was initiated on Paiva beach, on the central Pernambuco coast, which focused on the taxonomy and population dynamics of the *Branchiosyllis* associated with *Cinachyrella*. In early September, patches of crude oil began to be observed on the beaches of Pernambuco, and on October 21st, during the collection of samples on Paiva beach, large quantities of oil began to wash up on the beach. Most of the estimated 1,676 tons of oil that reached the coast of Pernambuco arrived between October 19th and 28th 2019 (Brazilian Navy, 2019; SPG, 2019; Câmara et al., 2020). When processing the samples collected after this period, we observed many polychaetes stained with oil, which was also found in their pharynxes.

The present study reports on the ingestion of crude oil by *Branchiosyllis* spp. (a group of four new species that are currently being described) associated with *Cinachyrella* sp. from the reefs of Paiva beach following the 2019 oil spill and the immediate effects of this disaster on the structure of the local polychaete population. Paiva beach has one of the best-preserved areas of coral reef in Brazil, and the results of the present study will provide important insights into the effects of this disaster on the fauna of the tropical coral reefs of the Brazilian coast. These findings also expand existing scientific knowledge on the effects of contamination from oil spills on the benthic fauna of coastal coral reefs in the Tropics.

## **2. Material and Methods**

Paiva Beach (8°16'46.4" S, 34°56'47.1" W) is an open, sandy oceanic beach approximately 7.8 km long (Holanda et al., 2020; Figure 1), with several discontinuous lines of rocky reefs in the nearshore waters running parallel to the shoreline (Laborel, 1970). The reef line closest to the beach is at least 2.5 km long and 1 km wide and is colonized by a diverse benthic community (Vasconcelos et al., 2019).

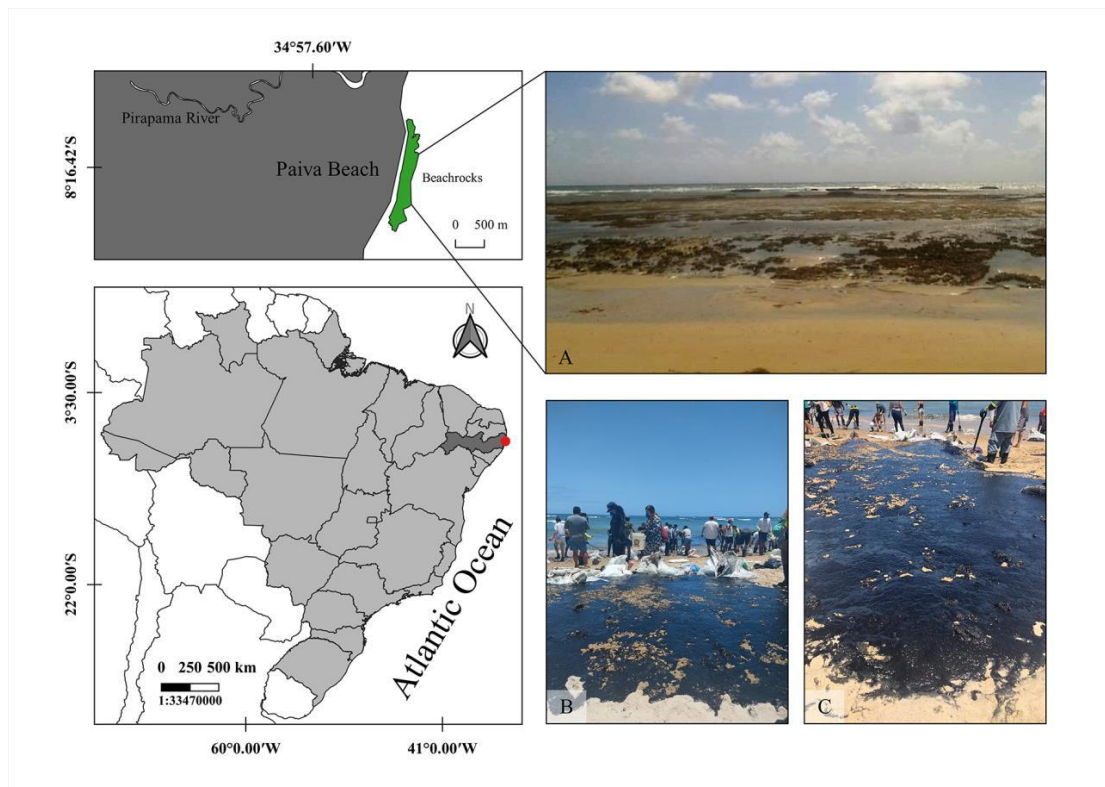


Figure 1 – Map of Paiva beach (red circle). A: overview of the Paiva beach coral reefs before the oil spill, B and C: popular mobilization for cleaning oil residues in Paiva beach (Photos: NGO XôPlástico).

Monthly, between July 2019 (three months before the 2019 oil spill) and January 2020 (three months after the disaster), 30 sponges were collected manually each month from the intertidal reefs of Paiva Beach. Prior to removal, each sponge was wrapped in a plastic bag to avoid any loss of the associated fauna. In the laboratory, the volume of each sponge was measured by verifying its displacement of the water column in a measuring cylinder graduated in millimeters. Each sponge was then dissected under a stereomicroscope to collect the polychaetes found in its channels and chambers. These polychaetes were identified and counted, and the total length of each individual was measured using an ocular micrometer attached to a stereoscopic microscope. The size (mm) and the density (number of individuals per 10 mL of sponges -  $\text{ind.}10 \text{ mL}^{-1}$  of sponge) of

the polychaetes were compared among months using a one-way ANOVA (after  $\log(x+1)$  transforming the data), followed by pairwise comparisons using Tukey's *post hoc* test. A significance level of 5% was considered in these analyses.

A solubility test using commercial mineral oil was used to identify the chemical characteristics of the oil found in the sediments and in the polychaetes themselves. The vibrational Raman spectra of these samples were also measured using Horiba iHR320 spectrophotometer ( $\lambda = 671 \text{ nm}$ ) between  $100 \text{ cm}^{-1}$  and  $1000 \text{ cm}^{-1}$ . This technique is often used to characterize the chemical profiles or fingerprints of oils, petroleum, and its derivatives (Kuptsov and Arbuzova, 2011). Spectra were obtained from the oil droplets found on the surface of the polychaete and in their pharynxes. The Raman spectrum of the crude oil washed up on Paiva beach during the same period was also determined for comparison.

### 3. Results and Discussion

Many of the sponges collected in November and December 2019 were stained with oil both externally and internally, and oil droplets were found interspersed among the particles of sediment accumulated on and inside the sponges (Figure 2). Many of the *Branchiosyllis* specimens collected during these months also had oil stains or droplets on their bodies or in their pharynxes (Figure 2). The mineral oil solubilized these droplets, confirming the non-polar nature of the chemical compounds they contained. The Raman spectrum profile of the crude oil samples collected from the beach was characterized by two bands at 120–274  $\text{cm}^{-1}$  and 690–875  $\text{cm}^{-1}$ , which are related to the vibrations of hydrocarbon chains and other organic groups (Larkin, 2011). This same profile was observed in the oil droplets collected from the polychaetes (Figure 3). Overall, then, the results of the solubility test and the Raman spectra indicated that the oil found in the sponge and polychaete samples was similar to the crude oil washing up on the beach

during the study period. This indicates that the polychaetes were coming into contact with and ingesting the crude oil that washed up on the beach.

After crude oil is spilled in the sea, the mechanical action of breaking waves and the turbulence of the water break it up into small droplets, ranging in size from 1  $\mu\text{m}$  to 100  $\mu\text{m}$  (Forrester, 1971; Li et al., 2017), which are dispersed in the water column (Delvigne, 1987; 1988). Sponges are effectively water pumps, which can filter a volume of water equivalent to that of their body every 7 s (Reiswig, 1971; Bergquist, 1978). When filtering sponges can ingest particles ranging in size from 0.2  $\mu\text{m}$  to 50  $\mu\text{m}$ , which means that they intercept pollutants in both dissolved and particle form (Yahel et al., 2006; Girard et al., 2020). The oil found in the sponges collected from Paiva beach in November and December 2019 would thus have been derived from the filtering activity of the sponges, which would have captured the oil droplets floating in the water column following the disaster. The polychaetes, in turn, must have come into contact with the oil droplets filtered by the sponges.

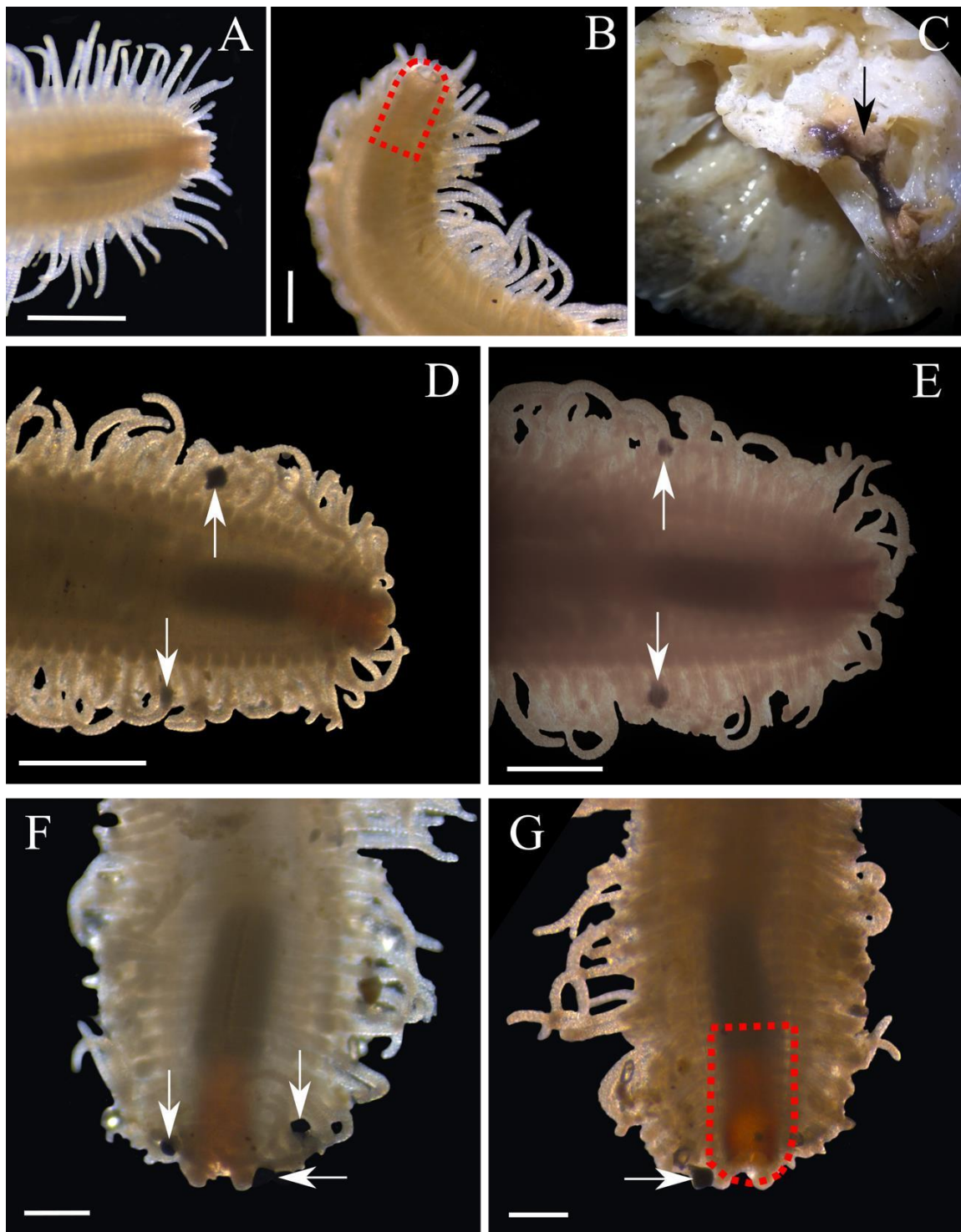


Figure 2 - *Branchiosyllis* spp. (anterior view) without oil (red rectangle indicates the pharynx) (A, B), oil in the channels of *Cinachyrellasp.* (the black arrow indicates the oil stain) (C), oil stains on *Branchiosyllis* spp. (white arrows indicate oil droplets) (D, E, F), *Branchiosyllis* spp. with oil on the surface (white arrow) and with oil in the pharynx (red rectangle) (G). Figure bar scales: A,D,E: 0.5 mm, B,F,G: 0.2 mm.

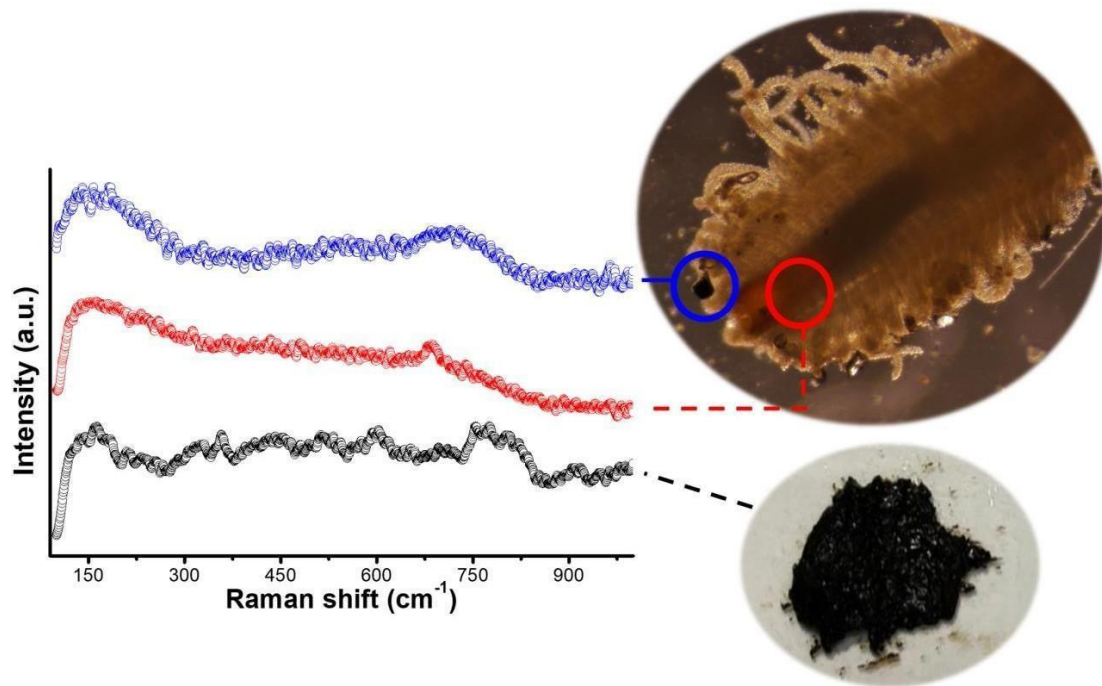


Figure 3 – Raman spectra of the crude oil (black line), and of the oil in the pharynx (red line) and on the surface (blue line) of the polychaetes.

Two months after the disaster, in December 2019, the number of polychaetes had decreased significantly in comparison with the previous months (Figure 3). Despite this decline in abundance, the mean size of the polychaetes had not changed significantly, which indicates that mortality occurred in all size (age) classes, that is, that the entire population was affected (Brey, 2001; Begon et al., 2006). This abrupt decline in the numbers of individuals of all size classes is typical of anthropogenic impacts, rather than natural mortality (Rumhill, 1990; Caddy, 1996). In invertebrates, the consequences of contamination with crude oil depend on the characteristics of the oil, the feeding habits of the species, and the age of the animals (Knezovich et al., 1987; Wolfe et al., 1998; Weiss, 2014). The 2019 Brazilian oil spill involved heavy oil typical of Venezuelan sources (Oliveira et al., 2020), which has a high potential toxicity (due to its light hydrocarbon content) and is difficult to dispose of, due to its relatively solid texture (Lourenço et al., 2020).

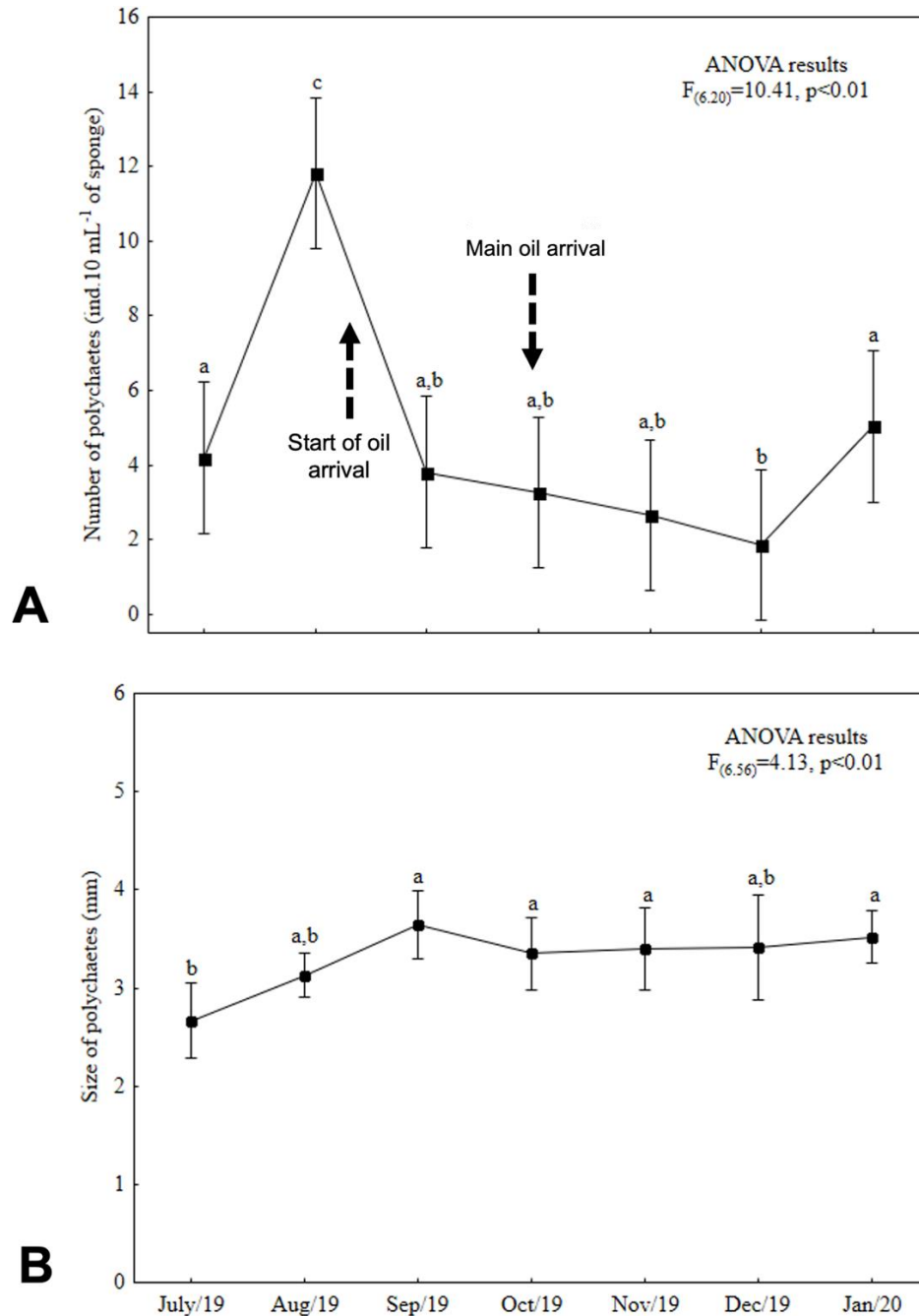


Figure 4 – Abundance (mean  $\pm$  standard deviation) (A) and size (mean  $\pm$  standard deviation) (B) of *Branchiosyllis* spp. associated with sponges *Cinachyrella* sp. in Paiva beach coral reefs. Vertical bars indicate standard deviation. Letters indicate the results of Tukey's tests.

The contamination of benthic polychaetes by hydrocarbons can occur through either

the absorption of dissolved compounds through the body wall or the ingestion of contaminated material (Weston, 1990; Weston et al., 2000). In polychaetes, the uptake of hydrophobic compounds from ingesta has been reported as a major contributor to the animal's total toxin load (Kaag et al., 1998; Selck et al. 2003). In polychaetes, the accumulation of organic contaminants is highest in sediment feeders, in particular selective sediment feeders, which is probably the case of most *Branchiosyllis* species associated with sponges (Jumars et al., 2015), followed by carnivores and feeders on suspended matter (Kaag et al. 1998; Venturini and Tommasi, 2004; Dean, 2008). In the present study, the peak in the abundance of *Branchiosyllis* in August, when individuals of small size predominated, indicates that these polychaetes were reproducing at this time, that is, the end of the rainy season in this region, when the salinity of the water increases, as observed at a nearby beach on the Pernambuco coast by Santos et al. (2003). Any environmental impact that occurs during the reproductive season, when the population is dominated by juveniles, which are more sensitive to pollutants than adults (Gosselin and Qian, 1997; Kutihara, 2008), will likely cause mass mortality and a consequent reduction in population size (Qian and Chia 1994; Jurgens et al., 2015), as observed at Paiva Beach.

In January 2020, the number of *Branchiosyllis* increased significantly in comparison with December 2019, which indicates that the populations may be recovering. Despite the predominance of invertebrates in marine environments, where they represent approximately 80% of all organisms, the impacts of oil pollution on these organisms in coastal ecosystems tend to be overlooked in comparison with vertebrates (Yuewen and Adzigbli, 2018). Oil spills may sometimes have a limited impact on marine ecosystems, but they often have long-lasting effects on certain species or whole communities, depending on a series of factors, including the characteristics of the organisms and the local ecosystem, the timing

and duration of the oil spill, and the type and amount of oil contaminated material (Weston, 1990; Weston et al., 2000). In polychaetes, the uptake of hydrophobic compounds from ingesta has been reported as a major contributor to the animal's total toxin load (Kaag et al., 1998; Selck et al. 2003). In polychaetes, the accumulation of organic contaminants is highest in sediment feeders, in particular selective sediment feeders, which is probably the case of most *Branchiosyllis* species associated with sponges (Jumars et al., 2015), followed by carnivores and feeders on suspended matter (Kaag et al. 1998; Venturini and Tommasi, 2004; Dean, 2008). In the present study, the peak in the abundance of *Branchiosyllis* in August, when individuals of small size predominated, indicates that these polychaetes were reproducing at this time, that is, the end of the rainy season in this region, when the salinity of the water increases, as observed at a nearby beach on the Pernambuco coast by Santos et al. (2003). Any environmental impact that occurs during the reproductive season, when the population is dominated by juveniles, which are more sensitive to pollutants than adults (Gosselin and Qian, 1997; Kutihara, 2008), will likely cause mass mortality and a consequent reduction in population size (Qian and Chia 1994; Jurgens et al., 2015), as observed at Paiva Beach.

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released into the environment (Yu et al., 2013; Yim et al., 2020).

Some organisms found off Paiva beach, such as the macrobenthos associated with macroalgae (Craveiro et al., 2020), would be expected to recover rapidly following the 2019 incident, given that this is an open, sandy oceanic beach with intense wave action (significant wave heights of up to 1.8 m in October - Pereira and Nogueira Neto, 2010). This would help to detach the oil from the surface of rocks and other substrates, and transport it to higher levels on the shore (Grande et al., 2012). The semidiurnal tidal regime at Paiva (Holanda et al., 2020) also favors the gradual, but constant transfer of the oil from the lower shore to the upper shore. In addition, the water temperatures of over 26°C in October (Domingues et al., 2017) may have enhanced the microbial breakdown of the oil (Zekri et al., 2005; Ribicic et al., 2018). Clean-up processes, that can have strong impacts on the recovery of the biota after oil spills (Dave and Ghaly, 2011; Castège et al., 2014; Shigenaka, 2014), were not used in Paiva Beach, where the spill was controlled by the rapid manual removal of the oil, both at sea and from the beach itself (Soares et al., 2020b). This may have been crucial to minimize the contact between local organisms and the oil, and thus limit the impacts of the disaster on the local biota. In addition, communities that inhabit rocky bottoms in the intertidal zone, like the reefs at Paiva beach, tend to recover from oil spills more quickly than those in coastal ecosystems with soft substrates, such as sandy beaches, seagrass meadows, and mangroves, where oily residues accumulate more easily, and are more difficult to remove (Bejarano and Michel, 2016; Rustandi et al., 2020; Magalhães et al., 2020).

## Conclusions

Following the 2019 oil spill on the tropical Brazilian coast, sponges (*Cinachyrella* sp.) on Paiva beach in Pernambuco state were found to be contaminated with oil in the form of superficial stains and droplets in their internal channels and in the sediments they contain. Oil contamination was also observed in the symbiotic polychaete *Branchiosyllis* spp. associated with these sponges, including stains on the surface of their bodies and droplets in their pharynxes. The results of the solubility tests and the Raman spectra indicated that the oil contaminating the two organisms was similar to the crude oil that washed up on the Brazilian coast following the oil spill. It seems likely that the polychaetes ingested oil droplets captured by the sponges when filtering sea water. The contamination of the polychaetes with this oil resulted in increased mortality, which caused a significant reduction in the *Branchiosyllis* populations of the coral reefs of Paiva beach following the oil spill.

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### Author contributions

**Anny Lira:** Conceptualization, Methodology, Writing- Original and revised manuscript.  
**NykonCraveiro:** Conceptualization, Data analysis, Map and photos preparation, Writing- Original and revised manuscript.  
**Fausthonda Silva:** Chemical analyses (solubility and Raman analyses), data analysis, writing - revised manuscript.  
**José Souto Rosa Filho:** Supervision, Writing- Reviewing and Editing.

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## 6 CONSIDERAÇÕES FINAIS

Como esperado, o presente estudo revelou que a diversidade de *Branchiosyllis* ainda é desconhecida para os recifes costeiros do estado de Pernambuco. Apenas com identificações baseadas em características morfológicas identificou-se uma espécie para o conhecimento científico. Uma abordagem interessante, a partir do conhecimento da distribuição das espécies proporcionada por este estudo, seria testar se a distribuição dessa espécie ao longo do litoral pernambucano.

Embora pouco se saiba sobre a associação dos *Branchiosyllis* com o seus hospedeiros, estudos mais complexos com a utilização de experimentos e análises temporais em períodos mais longos assim como análises químicas podem ser o próximo passo para aprimorar o conhecimento desta associação.

Em decorrência da realização deste estudo entre o ano de 2019 e 2020, houve-se a possibilidade de estudar as consequências agudas do derramamento de petróleo ocorrido no ano de 2019, no Brasil, podendo enfatizar a importância de conhecimento da fauna bentônica costeira para estudos ambientais marinhos, visto que na praia do Paiva no estado de Pernambuco foram encontradas esponjas contaminadas com petróleo também observou-se contaminação por óleo na faringe dos *Branchiosyllis* associados e na porção superficial do corpo, sendo sugerido que os poliquetas digeriram as partículas óleo após a filtração realizada nas esponjas (Lira et al 2021).

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