

Retraction

Retraction: Evaluating the secondary bioactive metabolites in *Geodia corticostylifera*

The editorial board of Brazilian Journal of Biology, announces the formal Retraction of the following article:

- Abdelbasset, W.K., Bokov, D.O., Jasim, S.A., Yasin, G., Abbas, H., Alkadir, O.K.A., Taifi, A., Jalil, A. T. and Aravindhana, S., 2024. Evaluating the secondary bioactive metabolites in *Geodia corticostylifera*. *Brazilian Journal of Biology*, vol. 84, e260090. <https://doi.org/10.1590/1519-6984.260090>.

This decision was made based on the fact that:

- the article presents a corresponding author information that does not belong to the actual authorship of this article;
- the article presents data that was previously published by Clavico et al. (2006) and used without permission by the authors and without the proper citation.

Profa. Dra. Takako Matsumura Tundisi
Editor in chief

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Original Article

Evaluating the secondary bioactive metabolites in *Geodia corticostylifera*

Avaliação dos metabólitos bioativos secundários em *Geodia corticostylifera*

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Abstract

Ophiactis savignyi could be discovered all over the world in tropical marine environments. People could have aided in the spread of *O. savignyi*, particularly in the western and eastern populations of Panama's Isthmus. The brittle star *Ophiactis savignyi*, often known as *savignyi's* brittle star, coexists alongside the sponge *Geodia corticostylifera*. The focus of this research has been to assess the functional relevance of *G. corticostylifera* secondary metabolites as antifoulant against mussels, protection against generalist fish and chemical cues to affiliated brittle stars. Both in flow-through and static seawater laboratory studies, *O. savignyi* which has previously been connected with sponges, was given both treated and control mimics at the same time. The sponge extract was also tested for its ability to protect fish against predators and fouling. Deterrence test using chemicals indicated that the normal level of the sponge extract may also suppress generalist fish predation in the field as well as the mussel *Perna perna's* normal attachment in clinical contexts. According to the findings, *G. corticostylifera* crude extract has many roles in the aquatic environments, apparently being accountable for this sponge's tight relationship with *O. savignyi*, which protects the ophiuroid and inhibits epibionts on itself.

Keywords: marine sponges, symbiotic relationships, endobiotic bacteria, aquiferous system, *G. corticostylifera*.

Resumo

Ophiactis savignyi pode ser descoberta em todo o mundo em ambientes marinhos tropicais. A população pode ter contribuído para a propagação de *O. savignyi*, particularmente as populações ocidentais e orientais do istmo do Panamá. A estrela-quebradiça *O. savignyi*, muitas vezes conhecida como estrela-quebradiça de *savignyi*, coexiste com a esponja *Geodia corticostylifera*. O foco desta pesquisa foi avaliar a relevância funcional dos metabólitos secundários de *G. corticostylifera* como anti-incrustante contra mexilhões, proteção contra peixes generalistas e sinais químicos para estrelas-quebradiças afiliadas. Em estudos de laboratório com fluxo contínuo e estático de água do mar, *O. savignyi*, que anteriormente havia se ligado a esponjas, recebeu mimetizadores tratados e controle ao mesmo tempo. O extrato de esponja também foi testado por sua capacidade de proteger os peixes contra predadores e incrustações. Testes de dissuasão usando produtos químicos indicaram que o nível normal de extrato de esponja também pode suprimir a predação de peixes generalistas no campo, bem como a fixação normal de mexilhão *Perna perna* em ambientes clínicos. De acordo com os achados, o extrato bruto de *G. corticostylifera* tem diversas funções em ambientes aquáticos, aparentemente responsáveis pela relação mais próxima dessa esponja com *O. savignyi*, protegendo o ofiuroide e inibindo os epibiontes.

Palavras-chave: esponjas marinhas, relações simbióticas, bactérias endobióticas, sistema aquífero, *G. corticostylifera*.

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1. Introduction

One of the prominent constituents of the marine benthos is marine sponges, which are found all over the world in temperate and polar waters, as well as deep-sea and intertidal environments (Pereira et al., 2002; Bouzon and Leire, 2007; Lo Giudice and Rizzo, 2018; Ereskovsky et al., 2019; Lhullier et al., 2020; Strano et al., 2020). In coral reef habitats, where predation and herbivory are frequent, these benthic organisms, accompanied by alcyonarian and cerata-like coelenterate, play a key role (Paul, 2019; Johnson and Mallock, 2020). Scleractinia, often known as hard or stony corals, are Cnidaria phylum aquatic creatures that produce a hard bone structure (Cairns, 2007; Cairns et al., 2009). Alcyonaria, which includes roughly three thousand water-based species made up of eight-fold symmetrical colonial polyps, is an Anthozoa subgroup (Ivanova, 2019). Scleractinia corals make up the majority of the Alcyonaria subclass (Ghallab et al., 2020). Sponges are a crucial group for ecological research, and they are among the most diverse and common groups of sessile marine invertebrates in Caribbean coral reefs (Diaz and Rützler, 2001).

Sponge species abound throughout the Brazilian coast, much as they do in the Caribbean (Randall and Kertman, 1968; Ferreira et al., 2004), yet the greater part of the species found along this southwestern Atlantic region's large coastline are undiscovered. The major contributors of marine organisms' unique secondary metabolites are sponges (Amsler et al., 2001; Rinehart, 2008; Li et al., 2020). Such primitive multicellular aquatic organisms are considered to have a wide range of biological effects, including antibacterial, antiviral, antifungal and antitumoral properties. Regarding this, ecological investigations conducted lately have shed some insight on the real ecological significance of marine sponges' secondary metabolites (He et al., 2020; Lee et al., 2021).

Earlier research employed feeding trends of organisms and proper methods to assess the efficacy of pure chemicals or crude extracts from sponges as predator-defense mechanisms, mostly in the temperate and tropical Caribbean, Mediterranean, Pacific, and Southwestern Atlantic (McGrath et al., 2018; Gökalp, 2021; Maslin et al., 2021).

It was found that reef fish *Thalassoma bifasciatum* (blue-headed wrasse) were put off by the crude extracts that were generated by a lot of the 71 Caribbean sponge species (69%), but that deterrence varied greatly across and within species (Pawlik et al., 1995). While chemical resistance was thought to be a useful tactic among many Caribbean sponges, certain dominant organisms in the Caribbean and the Red Sea seem to employ alternative strategies such as spicules (Burns and Ilan, 2003). Even though defenses against predation appears to be the most prevalent ecological activity linked to sponge secondary metabolites, additional functions have been identified. On Guam, for instance the sponge *Cacospongia* sp. is outgrown by the sponge *Dysidea* sp. as a result of necrosis.

A groundbreaking field research has revealed that sponge secondary metabolites could operate as kairomones, promoting overgrowth by others, or as allomones,

suppressing overgrowth (Engel and Pawlik, 2000). Furthermore, several investigations also demonstrated that marine sponges' secondary metabolites could reduce fouling in the research lab or even in field experiments, indicating that sponges produce allelochemicals to keep themselves free of epibionts (Adnan et al., 2018; Paul, 2019; Puglisi et al., 2019; Angulo-Preckler et al., 2020; Bryan et al., 2020; Levert et al., 2021). Many sponges, on the other hand, have symbiotic partnerships with a variety of invertebrates, microalgae, protozoa, autotrophic and heterotrophic bacteria, and this interconnectedness grow to the stage where the symbionts provide more biomass than the sponge for certain individuals (Haygood et al., 1999; Pochon et al., 2001; Totti et al., 2010). Sponges have such a large and varied affiliated fauna on their inner tissues, canals, and surfaces that they are regarded as one of the wealthiest benthic biotopes and living lodges in the ocean (Ribeiro et al., 2003). Different sponge properties, including geographic distribution, anatomy, morphology, and size, as well as seasonality, and depth, may influence the makeup of the sponge-associated fauna (Poore et al., 2000; Amsler et al., 2009). Few extensive studies on sponges' generation of chemical cues which could lure or deter certain species of animals have been conducted (Poore et al., 2000; Ribeiro et al., 2003; Amsler et al., 2009; Totti et al., 2010). Amphipods can detect antibiotic compounds from the *Halichondria panicea* sponge. *Halichondria panicea* is a shallow-subtidal or intertidal sponge exhibiting distinctive volcano-shaped oscular chimneys (Poore et al., 2000). It is occasionally branched, huge, or heavily encrusting. It can take many different forms, making identification challenging. The common name comes from the fact that certain forms have a grainy surface, but others have smooth, uniform glassy surfaces.

In laboratory experiments, *H. panicea* attracted the related amphipods *Jassa falcata* and *Caprella linearis* (Puglisi et al., 2019). Females' ability to swim of *Tritiaeta gibbosa* was inhibited when the same sponge species was present (Lavan et al., 2006). Hymeniacion perleve and *H. panicea* attracted the amphipods *Corophium* sp., *Microdeutopus anomalus*, *Microdeutopus damnosus*, and the crab *Carcinus maenas* (Gerovasileiou and Vouktsiadou, 2012; Strano et al., 2020). Such instances suggest that the host sponges might release chemical pheromone which serve as signals to the fauna around them. It was recently proposed that brittle stars that live in sponge environments have developed a way for identifying sponges (Henke and Pawlik, 2005; Dahihande and Thakur, 2017). Given the overwhelming body of work on the ecological significance of sponge compounds in the Caribbean, Bermuda, Mediterranean, and tropical Pacific sea (Pawlik et al., 1995; Claudio et al., 2006; Ribeiro et al., 2010; Gerovasileiou and Vouktsiadou, 2012; de Goeij et al., 2017; Schönberg et al., 2017; Avila et al., 2018; Pomponi et al., 2019; Bo et al., 2020; Mauro et al., 2020), such studies are scarce in the South Atlantic. With a variety of pharmacological effects, including antimicrobial, neurotoxic, cytotoxic, and hemolytic activities, *Geodia corticostylifera* has uncharacterized secondary metabolites (Lee et al., 2021). Including *Ophiactis savignyi*, this sponge is home to a variety of brittle

star species, which in densities of more than two-hundred individuals per sponge, or four individuals per cm³ of sponge tissue, are located inside the sponge's aquiferous canals (Figure 1) (Ereskovsky et al., 2019; Wulff, 2021).

According to our hypothesis, chemical cues may attract *O. savignyi* to *G. corticostylifera*, which may also prevent symbionts and predators (Pochon et al., 2001; Gökalp, 2021). The focus of this research has been to assess the functional relevance of *G. corticostylifera* secondary metabolites as antifouling agents against mussels, protection against generalist fish, and chemical cues to affiliated brittle stars.

2. Materials and Methods

In our search for bioactive compounds from marine organisms, we chose the sponge *G. corticostylifera*, whose extracts showed antifouling and antibacterial activities. Chemical defense systems are indicated by its orange color and the apparent lack of insect predators and fouling species. On the eastern coast of Arraial do Cabo, *G. corticostylifera* is a sponge that could be discovered in depths of 3–39 meters. However, it could also be observed in Venezuela, as well as along the whole southeastern Brazilian coast (Almeida et al., 2011). It is beige on the inside and orange on the outside, with a cerebral, firm, massive-globose shape and a hard consistency with deep grooves at the surface (Calcinai et al., 2017). In warm sides of the Atlantic, eastern Pacific, Indo-Pacific, and subtropical and tropical areas, the ophiuroid *O. savignyi* may be found. In Brazil, the Gulf of Mexico, the Caribbean, Bermuda, and South Carolina are all located in the Western Atlantic. It may be observed down to a depth of 518 meters, but it prefers shallow waters (Stöhr et al., 2012; Hendlar and Brugneaux, 2013; Granja-Fernández et al., 2017).

The tropical peninsula of Arraial do Cabo protrudes from Brazil's southeast coast. The predominant eastern and northeastern winds produce a coastal upwelling in this region. *O. savignyi* and *G. corticostylifera* specimens were obtained manually by SCUBA diving from

5–8 meters depth at Saco dos Cardeiros and Forno beach. The upwelling phenomenon has no bearing on any collecting location. Throughout collecting, samples were encased in polyethylene bags to avoid the destruction of accompanying fauna, which was later isolated from the sponges in the lab. The density of related *O. savignyi* was assessed in the lab after a number of sympatric sponge species were collected (based on each species' natural abundance, n=1–12) and entangled in polyethylene bags. Briefly after collection, *G. corticostylifera* samples were washed thoroughly in saltwater to eliminate any attached animals that could tamper with the sponge's endobiotic bacteria or secondary metabolites. Sponge tissues were sliced into little bits, and organic solvents were used to remove them one after the other. To improve the efficacy of the initial extraction, sponge pieces were homogenized in methanol before being exposed to ultrasonic waves for about fifteen minutes. Utilizing pieces of dry spongin skeleton (commercial marine sponges), treatment and control sponge mimics were created, and then a solvent-only gel (controls) or an extract-containing gel (treatments) were added. The extract's natural volumetric content was determined by employing a recently made crude organic extract to create 20 ml of gel (consistent with 20 ml of *G. corticostylifera*). In advance of heating to the boiling point, an amount of 1.01 g phytigel was dissolved in deionized water of 20 ml and vigorously stirred. The extract aliquot was added after cooling to around 60°C and was then poured onto treatment (one spongin piece). With dichloromethane (DCM) added simply as solvent control, control mimics were generated in the very same way.

In a way analogous to that which is thought to occur naturally, phytigel was employed to facilitate the regulated diffusive extract diffusion to saltwater from the sponge mimic. Flow-through and static experiments were utilized.

With one *O. savignyi* specimen, a plastic container with a rectangle spongin in the initial experimental trials was used to store the treatment and control mimics. Without water flow, with a 10-meter depth, all recipients were put in



Figure 1. A variety of brittle star species live in *Ophiactis savignyi*.

a 2-m circular pool made of fiberglass. In each container, one brittle star was put evenly between treatment and control mimics (Figure 2).

A new group of tests was conducted using a seawater flow-through configuration to imitate more realistic dilution and flow of chemical cues since static seawater tests are implausible. With a seawater input and output, mimics of treatment and control were set in a rectangular plastic tray at opposite angles during an analogous test. Allowing the water to pass to the ophiuroid, the water input was placed on both sides of the treated and control mimics (Figure 2).

Experiments in the field and the preparation of artificial food were carried out (Burns et al., 2003; Helber et al., 2017; Wulff, 2021) to replicate the normal amount of chemical present in *G. corticostylifera*. 0.4 g of water, tuna fish, and alginic acid was used with an extract corresponding to 60 ml of fresh *G. corticostylifera* tissue. Before being placed into a rectangular mold, the mixture was vigorously stirred to form pellets. The same procedure was used to make control pellets but without the inclusion of extract.

Individuals demonstrating substrate exploring activity were chosen for trials after the mussels were collected. A variation of the process initially employed by Ina et al. (1989) and Goto et al. (1992), which was the method described by Da Gama et al. (2003), was used to measure antifouling activity. The results obtained in the field have been highly correlated with those acquired from this laboratory experiment. Filter paper with a water-resistant coating has been immersed insolvent after being sliced into 3-cm radius circles. With a 3-cm radius, one more set of filter papers has been immersed in *G. corticostylifera* crude extract at a natural concentration after being sliced into a chess board design.

Each assay's experimental design dictated the statistical analysis. The number of attached byssal threads in antifouling tests was studied, and the pellet quantity devoured (not as % as illustrated in graphs) in field predation was evaluated. Yates' correction for continuity (or Yates' chi-square test) (χ^2) was employed to assess chemotaxis

findings. The Yates' Correction is a modification to the Pearson's Chi-Squared test that is used to determine if two categorical variables are related. Because the variables did not follow the assumption of normality, the Wilcoxon signed ranks test was used to assess the predation and antifouling outcomes.

3. Results and Discussion

Sponge species were sampled out of a total of 27 specimens: *Paraleucilla magna*, *G. corticostylifera*, *Dysidea robusta*, *Aplysina fulva*, *Amphimedon viridis*. Per 100 ml⁻¹ sponge *O. savignyi* density varied from 0 to 385 ind. (Figure 3).

G. corticostylifera had the largest densities, whereas *A. fulva* had the lowest. In static seawater experiments, *O. savignyi* had a strong inclination to migrate near *G. corticostylifera* than the control, but the difference was not statistically noticeable (Figure 4a; χ^2 , $p < 0.37$).

However, in flowing seawater experiments, considerably more *O. savignyi* were drawn to sponge mimics with *G. corticostylifera* crude extract (χ^2 , $p < 0.03$). This finding suggests that compounds capable of causing positive taxis conduct in *G. corticostylifera*'s linked ophiuroid *O. savignyi*.

The artificial food comprising the *G. corticostylifera* crude extract was substantially less eaten than the control in the experiments for a chemical protective effect against predation by generalist fish (Figure 4b; Wilcoxon, $p < 0.001$). This finding showed that this sponge species crude extract may protect against a wide range of generalist fish, including *Kyphosus sectatrix*, *H. steindacneri*, *Haemulon aurolineatum*, and *Abudefduf saxatilis*, the area's most frequent species.

Indicating a considerable antifouling effect in comparison to the control (Wilcoxon, $p < 0.01$), the *G. corticostylifera* crude extract strongly prevented byssal thread attachment in juvenile mussels of the genus *Perna* (Figure 4c). The mussels earlier introduced

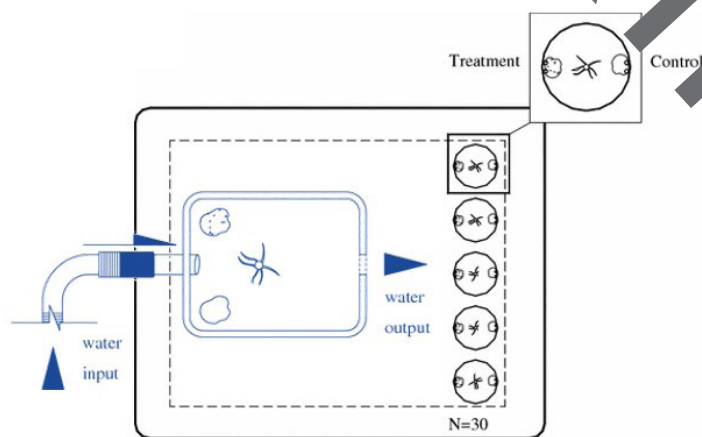


Figure 2. The static seawater test's experimental unit schematic. The brittle star receives seawater from the mimics as is demonstrated in this schematic of chemotaxis experiment.

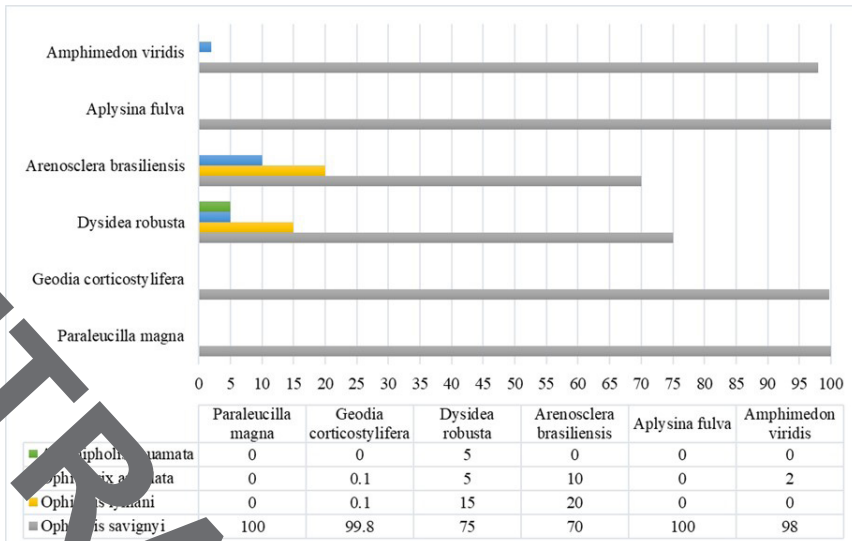


Figure 3. Ophiroid species on each sponge species has a different dominance (%).

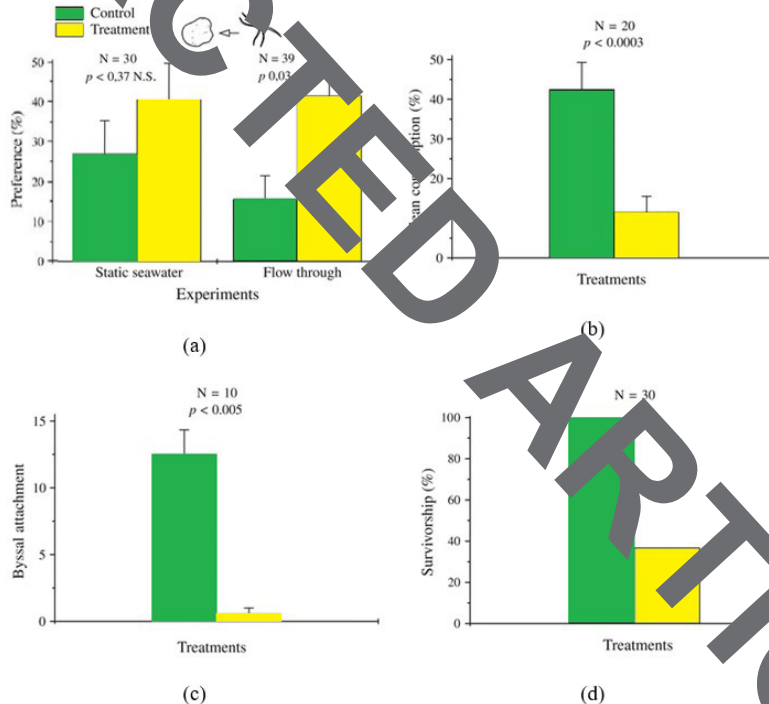


Figure 4. In static seawater, *O. savignyi* the brittle star approaches a treatment or control sponge mimic and pass through chemotaxis experiments, (a). After the field experiment, generalist fish ate a certain percentage of the treatment and control pellets, (b). *P. perna* mussel attachment after 12 hours in treatment and control, (c). Percentage of *P. perna* mussels that survived 24 hours after the experiments ended, (d).

to the sponge extract had a significant rate of death following the 24-hour recovery period, but none of the controls perished (Figure 4d). As a result, the toxicity of the *G. corticostylifera* crude extract to mussels is most likely connected to the nature of attachment inhibition in antifouling experiments.

4. Conclusion

Living organisms such as sponges, polychaete mats, and algae are common habitats for the brittle star *O. savignyi*. *Ophiomixis fragilis*, which were already common in several sponge species, showed a similar pattern, and

clearance trials demonstrated that these brittle stars might pick their environment after settling. According to our results, the ophiuroid *O. savignyi* is attracted to secondary metabolites produced by *G. corticostylifera*. Chemical cues are likely to initiate the relationship between *O. savignyi* and *G. corticostylifera* in nature, confirming prior data that ophiuroids' behavior is predominantly governed by chemoreception. *O. savignyi* is likely looking for a relationship with *G. corticostylifera* for defense, feeding, or both purposes. Because the *G. corticostylifera* crude extract readily deters generalist fish in the environment, the sponge may help protect *O. savignyi* from predation. A quick and cost-effective screening procedure for antifouling activity is the mussel test, with findings that are usually replicated in field experiments. Our findings revealed that *G. corticostylifera* crude extract serves a variety of ecological functions.

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