



Brief Report

New Evidence of the Freshwater Sponge *Radiospongilla inesi* (Nicacio & Pinheiro, 2011) in Net Cage Aquaculture Systems: A Case Study from Southeastern Brazil

Daercy Maria Monteiro de Rezende Ayroza ¹ , Mauro Parolin ² , Bruna Larissa Maganhe ^{3,*} and Eduardo Gomes Sanches ¹

¹ Fisheries Institute, Av Rodrigues Alves 1252, Vila Mariana, São Paulo 04014-002, Brazil

² Paleoenvironmental Studies Laboratory, State University of Maringá, Campo Mourão Campus, Av. Comendador Norberto Marcondes 733, Centro, Campo Mourão 87302-060, Brazil

³ Postgraduate Program in Diversity and Evolution, Federal University of ABC, Al. da Universidade, s/n Anchieta, São Bernardo do Campo, São Paulo 09606-045, Brazil

* Correspondence: bruna.maganhe@ufabc.edu.br

Abstract

We investigated the occurrence of *Radiospongilla inesi* in a tilapia aquaculture facility located at the Chavantes Reservoir, Paranapanema River, Brazil. Specimens were collected from both artificial (net cages) and natural substrates along the reservoir margins in October and November 2024. Morphological analyses of 8 sponge samples, including 20 structures per sample (gemmules, megascleres, microscleres and spicules), identified the species as *Radiospongilla inesi* (Spongillidae). This is the third documented record of *R. inesi* in Brazil, and the first within the Paraná River Basin and in aquaculture net cage systems. Morphological features were consistent between individuals from natural and artificial substrates, although gemmules were absent in specimens colonizing the cages. The proliferation of *R. inesi* poses biofouling challenges by obstructing cage mesh openings, reducing water flow and dissolved oxygen levels, and potentially compromising fish welfare and production efficiency. These impacts increase operational costs and highlight the need for sustainable management strategies in freshwater aquaculture. Additionally, this study raises questions regarding the species' native status in the Paraná Basin versus potential invasive dispersal, emphasizing the need for further ecological and distributional investigations. Potential dispersal mechanisms and possible biofouling impacts are discussed, with recommendations for future quantitative and molecular studies.

Keywords: biofouling; fish farming; net cage mesh; Paraná River Basin; freshwater sponge



Academic Editor: Aires Oliva-Teles

Received: 18 August 2025

Revised: 26 September 2025

Accepted: 15 October 2025

Published: 21 October 2025

Citation: Ayroza, D.M.M.d.R.; Parolin, M.; Maganhe, B.L.; Sanches, E.G. New Evidence of the Freshwater Sponge *Radiospongilla inesi* (Nicacio & Pinheiro, 2011) in Net Cage Aquaculture Systems: A Case Study from Southeastern Brazil. *Aquac. J.* **2025**, *5*, 20. <https://doi.org/10.3390/aquacj5040020>

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1. Introduction

Fish farming represents an important economic activity in Brazil, primarily focused on non-native species, particularly Nile tilapia (*Oreochromis niloticus*). In 2024, tilapia production reached 662,230 tons, positioning Brazil as the second-largest exporter of fresh fillets to the United States [1]. In this context, the state of São Paulo ranks as the second-largest producer of tilapia in the country, with production dominated primarily by small and medium-scale fish farmers [2,3]. A significant portion of this production relies on cage-based aquaculture systems, with approximately 75,346 net cages installed in continental waters throughout the country [1]. The prevalence of medium- and large-sized reservoirs,

initially constructed for hydroelectric power generation, has facilitated the expansion of cage-based aquaculture, which is further supported by government incentives [4].

Cage-based aquaculture benefits from the continuous flow of water, which helps remove aquaculture waste and provides fish with dissolved oxygen [5]. However, this practice also results in the continuous input of organic matter into the aquatic environment via uneaten feed and metabolic waste, contributing to sedimentation and increased biochemical oxygen demand [6,7]. These inputs can promote eutrophication, characterized by increased algal biomass and shifts in phytoplankton abundance [8,9]. The conditions created by cage-based aquaculture can favor the establishment of invasive species such as the golden mussel (*Limnoperna fortunei*) in reservoirs [10] and may also provide favorable conditions for the proliferation of other aquatic organisms, including freshwater sponges.

Although sponges are widely distributed across diverse environments, freshwater species remain among the least studied animal groups worldwide. Of the more than 8000 described sponge species, fewer than 200 occur in freshwater ecosystems [11]. In Brazil, approximately 25% of these freshwater species have been recorded, with most occurrences reported in the state of Rio Grande do Sul and the Amazon region [12]. These organisms are sessile and benthic, remaining attached to a variety of substrates, including rocks, submerged vegetation, macrophyte roots, and even artificial structures such as cages commonly used in intensive fish farming practices [13]. Recently, the freshwater sponge *Radiospongilla inesi*, originally known from northeastern Brazil, was reported for the first time in the Paranapanema River basin, southeastern Brazil, in late 2019 [14]. Although there are only a few records of the species in the country, its occurrence on artificial substrates, including ponds and channels, was documented by Nicácio, Severi & Pinheiro [15] and Calheira, Lanna & Pinheiro [16]. Nevertheless, significant knowledge gaps remain regarding its distribution and potential impacts on artificial environments.

Although *R. inesi* has previously been reported in natural and artificial environments, its colonization of aquaculture structures has not been documented. Here, we report the occurrence of *R. inesi* on net cages in a local fish farming facility and provide a detailed morphological identification of the species. We also discuss the potential ecological and economic implications of this finding.

2. Methods

2.1. Area of Study

The occurrence of freshwater sponges was investigated in net cages of an aquaculture facility located in the Chavantes Reservoir (23°07'31.3" S, 49°37'40.6" W). This river forms the natural boundary between the Brazilian states of São Paulo and Paraná, and hosts several reservoirs constructed for hydroelectric power generation. Among these, the Chavantes Reservoir covers an area of 400 km² and is classified as an accumulation-type reservoir, designed to store water and regulate downstream flow. Due to its regulatory function, the reservoir experiences substantial seasonal fluctuations in water level and discharge, both through turbines and overflow, largely driven by operational demands [17,18]. The aquaculture facility occupies a water surface area of approximately 100,000 m² and is dedicated to tilapia production. Net cages are rectangular, measuring 2.0 × 2.0 × 2.0 m and 6.0 × 3.0 × 2.0 m, respectively (Figure 1).

According to managers of the local aquaculture facility, the appearance of freshwater sponges coincided with the severe drought that affected southeastern Brazil in 2020. By March 2021, during one of the region's most critical drought periods, sponge presence was documented on net cages and experimental structures through photographic records. Since then, sponge proliferation has intensified and now represents a significant problem in the area (Figure 2).

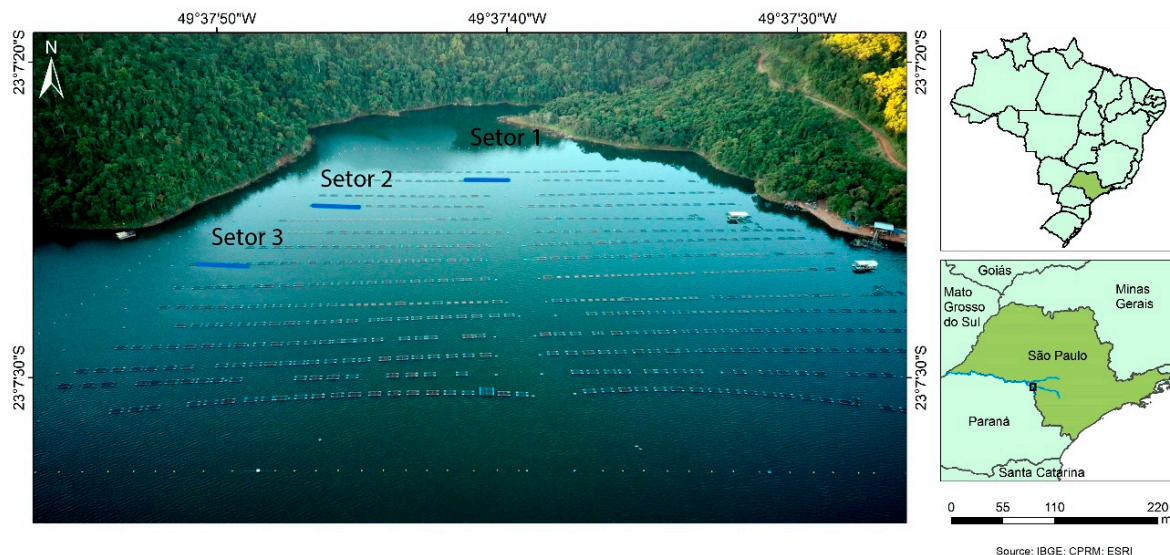


Figure 1. Location of the aquaculture area in the Chavantes reservoir, Paranapanema river, São Paulo, Brazil. The three sampling sectors represent the outer, central, and inner portions of the aquaculture area.



Figure 2. Freshwater sponges densely colonizing the mesh of tilapia net cages at Chavantes Reservoir. Multiple sponge individuals are visible, illustrating the extent of biofouling on artificial substrates.

2.2. Sample Collection

Freshwater sponges were collected from net cages ($n = 3$) (Figure 2) and submerged branches near the reservoir margins ($n = 5$) (Figure 3) in October and November 2024. After each sampling event, specimens were naturally dried under sunlight and subsequently transported to the Laboratory of Paleoenvironmental Studies (LEPAFE) at the State University of Paraná (UNESPAR), Campus Campo Mourão, Paraná.

At LEPAFE, specimens were examined under a stereomicroscope at $80\times$ magnification, and gemmules (Figure 4A) were carefully isolated. Sponge disaggregation followed the protocol described by Volkmer-Ribeiro [19]: gemmules and small sponge fragments were placed in test tubes containing 4 mL of 65% nitric acid (HNO_3). The material was gently heated over low heat by an alcohol flame, ensuring the formation of only small bubbles without vigorous boiling. Low heating was maintained for 1–2 min, after which the sample

was allowed to cool to room temperature. The solution was then centrifuged at $100\times g$ for 3 min with distilled water, and this washing step was repeated until the pH stabilized at 7, as measured with a benchtop pH meter. Subsequently, 70% ethanol was added to the solution. Drops of the resulting material were mounted on microscope slides and then covered with Entellan® (Merck KGaA, Darmstadt, Germany) and a coverslip.



Figure 3. Freshwater sponges and golden mussels colonizing natural substrates along the margins of Chavantes Reservoir. The image illustrates typical sampling sites and the attachment of sponges to natural structures.

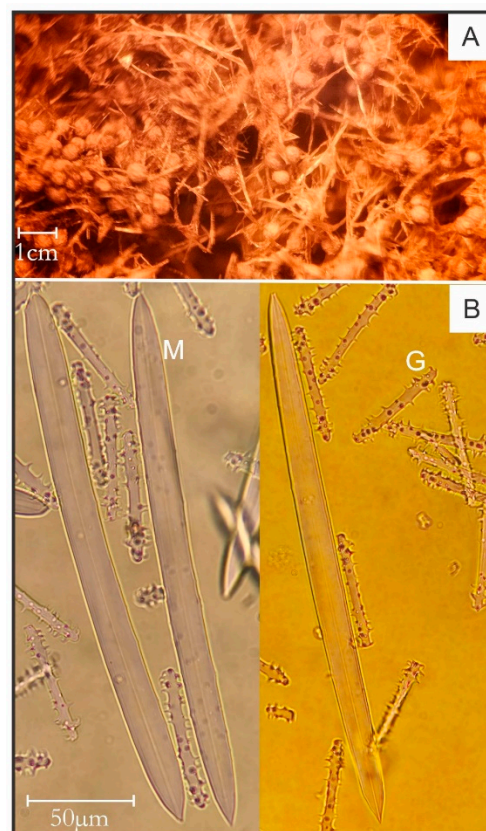


Figure 4. Light microscopy of *Radiospongilla inesi* structures used for morphological identification: (A) gemmules; (B) spicules, with M = megascleres and G = gemmuloscleres. Images highlight the key structural features essential for species confirmation.

Microscopic analysis was performed using a Nikon E200[®] (Nikon Corporation, Tokyo, Japan) light microscope, with a 40× objective, combined with wide-field (WF) eyepieces of 10×, 20×, 22×, or 25×, resulting in total magnifications ranging from 400× to 1000×. Sponge identification was conducted using the LEPAFE reference collection (C136L12 and C142L12—*Radiospongilla* sp. Itaipu—PR) and following the taxonomic criteria outlined by Nicácio, Severi & Pinheiro [15]. A total of 20 structures were measured, comprising gemmules, megascleres (length and width), microscleres (length and width), and spicules (number of visible spines). Measurements were conducted using Capture 2.4[®] software.

3. Results and Discussion

The genus *Radiospongilla* [20] is characterized by the radial arrangement of gemmoscleres on gemmules and the absence of microscleres. Gemmules were observed in sponges attached to branches and macrophytes along the reservoir margins, whereas no gemmules were detected in specimens collected from the net cages. However, a comparison of megascleres from specimens collected at the reservoir margins and from the net cage revealed morphological similarities, indicating that they belong to the same species: oxeote megascleres bearing small central spines (42–61 µm; Figure 4B) and acanthostrongyle gemmuloscleres (Figure 4A,B). To date, no genetic data are available for *Radiospongilla inesi*, highlighting the need for future molecular studies to confirm species identification and investigate its ecology. However, based on morphological analysis, the observed spicules were identified as *Radiospongilla inesi* [12,15], family Spongillidae. The results of the measurements and their comparison with the study conducted by Nicacio, Severi & Pinheiro [15] are presented in Table 1.

Table 1. Measurements of *Radiospongilla inesi* recorded by Nicacio, Severi & Pinheiro [15] and in the present study.

Structure	Nicacio, Severi & Pinheiro [15] (µm)	Present Study (µm)
Gemmules	300–338–384	Minimum 320, maximum 400
Megasclere oxea	228–260–288/ 9–12	220–283/ 10–14
Gemmulosclere acanthostrongyle	51–69–78/ 3–3.2–4	48–65/ 2.8–4.2
Spines *	47–55–68	42–61

* The spines were counted only in the visible part of the gemmulosclere.

The measurements obtained in the present study (gemmules 320–400 µm, megasclere oxea 220–283 µm, gemmulosclere acanthostrongyle 48–65 µm, spines 42–61; Table 1, Figure 4) are consistent with those reported by Nicacio, Severi & Pinheiro [15] for *Radiospongilla inesi* (gemmules 300–384 µm, megasclere oxea 228–288 µm, gemmulosclere 51–78 µm, spines 47–68), with minor variation within expected intraspecific ranges. Nicacio [12] also reported measurements for other *Radiospongilla* species obtained in different regions, such as *R. crateriformis* and *R. amazonensis*, which exhibit notably larger gemmule and spicule dimensions. For example, *R. crateriformis* typically exhibits megascleres up to 450 µm and gemmules up to 123 µm, while *R. amazonensis* shows megascleres 276–401 µm and gemmules 75–94 µm. These comparisons clearly support the morphological identification of the specimens as *R. inesi* and distinguish them from congeners, reinforcing the third Brazilian record and the first report in the Paraná Basin.

The absence of gemmules in cage-associated sponges may be linked to seasonal or environmental factors. Gemmules, which are dormant propagules produced by most

freshwater sponges, are typically induced under adverse conditions and play a key role in enhancing colonization, survival, long-term persistence, and dispersal [21,22]. In this sense, the presence of gemmules exclusively along the reservoir margins may be attributed to the greater environmental variability in shallow natural areas, such as fluctuations in temperature, oxygen, and water level, which act as classical triggers for gemmulation. This pattern likely reflects a combination of factors, including the type of environmental stress (episodic versus chronic), the allocation of energy by the sponge, substrate suitability, and physical or biological disturbance. Natural substrates, such as roots, branches, and rocks, provide protected microhabitats that favor the deposition and retention of gemmules. In contrast, conditions in the production cages favor sponge survival and growth but do not trigger gemmulation, nor do they allow for stable retention of gemmules, due to routine cleaning, bioturbation by fish, and the artificial nature of the substrate.

The genus *Radiospongilla* exhibits a broad global distribution, occurring in all zoogeographical regions except Antarctica [22,23]. Among the 18 species described to date, only three have been recorded in the Neotropical region. One of these, *R. amazonensis* [24], is endemic to Brazil, with occurrences documented in seven of the country's twelve hydrographic basins [11]. Another species, *R. crateriformis* [25], is considered cosmopolitan and has been reported from Mexico, the United States, Suriname, Canada, Barbados, Cuba, Costa Rica, China, and Japan [15]. It was first recorded in Brazil in the Ribeirão do Prata, Pernambuco State, in the northeastern region, and is regarded as an invasive species in the country. *R. inesi* [12,15], originally described from the Eastern Atlantic Basin in Pernambuco, was initially thought to be geographically restricted to northeastern Brazil. However, a more recent record by da Silva Leite & Cobo [14] confirmed its presence in southeastern Brazil, expanding its known distribution.

This study represents the third record of *Radiospongilla inesi* in Brazil, and the first report for the Paran  River Basin, as well as its first occurrence in aquaculture net cages in the Southeastern region. Nic cio, Severi & Pinheiro [15] initially recorded *R. inesi* in shallow, perennial freshwater habitats such as lagoons and streams, with depths ranging from 5 cm to 2 m, within the Dois Irm os State Park in Pernambuco, Northeastern Brazil. Later, da Silva Leite and Cobo [14] reported the species in a lotic environment, attached to pebbles in the Para ba River valley (Ribeir o dos Mottas), located more than 700 km from the present record at the Chavantes reservoir. Both previous records documented *R. inesi* occupying natural or modified habitats, on in situ substrates. In contrast, this is the first report of the species establishing on artificial structures used in fish farming.

The first record of freshwater sponges colonizing aquaculture net cage systems was reported by Volkmer-Ribeiro et al. [19] in the Itaipu Reservoir, Paran  State. In their study, *Convospongilla seckti* was observed on the net cages, while *Tubella repens* (formerly *Thochospongilla repens*) occurred on aquaculture floaters. Notably, the authors reported that these sponges often used *Limnoperna fortunei* as a substrate. A similar interaction was described by de Medeiros Fortunatto and Figueira [26], although their study was not conducted in an aquaculture setting. They documented *Drulia brownii* competing with the mussel in macrofouling interactions. In our study area, we observed a comparable pattern, with sponges generally predominating over *L. fortunei* (Figure 2).

Economic impacts were not assessed in this study; however, several freshwater sponges are known to contribute to biofouling of aquatic infrastructure, resulting in negative consequences for economically important activities [27]. Although often considered harmless, extensive sponge growth in reservoirs has been recognized as a problem since the late nineteenth century [28,29]. For example, in water supply reservoirs, *Trochospongilla leidii* substantially increased flow resistance in pipes, causing a 25% reduction in delivery capacity [30]. Attempts to remove these biofoulers using high doses of chlorine have

proven unsuccessful [31]. This persistent problem also affects net cage aquaculture, where macrofouling can severely obstruct mesh openings, leading to the accumulation of feces and uneaten feed, as well as reduced dissolved oxygen levels due to limited water exchange within the cages [32]. In addition, the added weight from encrusting organisms can deform the net cages and compromise mesh integrity, resulting in inappropriate stocking densities and negatively impacting both fish well-being and zootechnical performance [33,34].

In this context, various biofouling control methods have been tested and are currently applied across different industries, each presenting specific advantages and limitations [35]. Biofouling control can be broadly classified into accumulation preventives and restorative measures. Accumulation preventives, such as antifouling coatings, copper alloys, ultraviolet light, and low-dose chemicals, are used to inhibit biofouling establishment on surfaces. Restorative measures, including mechanical cleaning, heat, freezing, or desiccation, are employed to remove established biofoulers and restore equipment function, although practical challenges such as operational downtime and disposal of removed material must be considered [27]. For instance, Costa et al. [36] recommended high-pressure devices to control the golden mussel in tilapia cultured in net cages. However, in the case of freshwater sponges, the application of high-pressure treatments may inadvertently facilitate the dispersal of *R. inesi* through mechanical fragmentation, as the resulting fragments can serve as new propagules, enabling colonization of additional areas and further spread. To minimize this risk, detached fragments should be promptly collected and properly disposed of after cleaning to prevent their reintroduction and further spread. However, Sievers et al. [37] reported that high-pressure washing only moderately reduced biofouling, with efficacy varying depending on timing and frequency, highlighting that no single biofouling control strategy is perfect. In general, aggressive treatments can effectively remove biofouling but may compromise stock health, whereas gentler or biological methods are often less effective or difficult to implement at scale, underscoring the need to combine approaches, monitor stock performance, optimize timing and intensity, and consider economic costs.

While the most apparent issue caused by biofouling is the clogging of cage nets, these organisms also form complex three-dimensional structures that can provide habitats for pathogenic bacteria and other opportunistic organisms [28,38]. Although these aspects were not evaluated in the present study, they underscore potential future sanitary challenges in fish farming, including an increased risk of disease outbreaks. Moreover, biofouling may present occupational hazards for workers involved in aquaculture maintenance and water treatment operations [28], highlighting important aspects for future investigation.

Given the limited information available on *Radiospongilla inesi*, it remains unclear whether this record represents part of the species' natural range or a case of dispersal into the Paraná Basin as a potential invasive species. The presence of *R. inesi* in the region may be explained by several potential dispersal mechanisms, as numerous studies have demonstrated the dispersal capabilities of freshwater sponges, particularly via gemmules. However, these mechanisms have yet to be directly confirmed for *R. inesi* through field observations or experimental studies. Freshwater sponge dispersal is likely mediated primarily by gemmules, resistant propagules capable of floating (hydrochory) due to their buoyant pneumatic layer and durable coating, potentially allowing long-distance drift, especially during flood events [39,40]. Gemmules might also adhere to feathers or scales (zoochory), possibly enabling passive transport by migratory birds, mammals or fish [41,42]. Endozoochory has been demonstrated through observations of intact sponge gemmules regurgitated by birds that consumed fish carrying sponge material [43,44]. Green and Figuerola [45], for example, have recorded 186 gemmules of the freshwater sponge *Ephydatia fluviatilis* in a single pellet of a great cormorant, highlighting the dispersal potential of freshwater sponges. Although unconfirmed, wind dispersal (anemochory) could also occur

in extreme droughts, as observed in “arboreal sponges” from desiccated regions of the Amazon and Pantanal [17,45,46]. Additionally, gemmules may potentially hitchhike on other invasive species, such as mussels (e.g., *Mytilopsis leucophaeata* and *Limnoperna fortunei*), facilitating spread via human-mediated transport or waterfowl movements [47].

Invasive species can significantly alter habitats and disrupt ecosystem functions, posing significant risks to both environmental integrity and socio-economic interests [48]. Biological invasions are increasingly recognized as a global concern, driven by expanding human activity and an increasingly interconnected world [49]. Considering the potential impacts of *R. inesi* on aquaculture described in this study, further research is needed to determine whether *R. inesi* is native to the Paraná Basin or represents an emerging invasive species, for which molecular methods would be crucial, highlighting an avenue for future investigation. Meanwhile, a key recommendation for fish farmers is that macrofouling removal should be conducted in a manner that prevents cleaning residues from re-entering the reservoir; thereby avoiding additional organic loading and the spread of propagules. If this is not feasible, nets should be left to dry for at least one day prior to cleaning.

Author Contributions: Conceptualisation: D.M.M.d.R.A., M.P. and E.G.S. Developing methods: D.M.M.d.R.A., M.P. and E.G.S. Data analysis: D.M.M.d.R.A., M.P. and E.G.S. Preparation of figures and tables: D.M.M.d.R.A. and M.P. Conducting the research, data interpretation, writing: D.M.M.d.R.A., M.P., E.G.S. and B.L.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Fundação de Amparo à Pesquisa of the State of São Paulo (FAPESP) grant number 2013/08026-0 and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) grant number 3080983/2023-2. The APC was funded by FAPESP.

Institutional Review Board Statement: In accordance with Normative Resolution 50 of the National Commission for the Control of Animal Experimentation (CONCEA), dated 13 May 2021, which provides guidelines for the use of animals in scientific research and education in Brazil, this project is exempt from formal review by an Institutional Animal Ethics Committee. Article 2 of the Resolution specifies that Institutional Accreditation for Animal Activities in Teaching or Research (CIAEP) applies solely to animals of the phylum Chordata, subphylum Vertebrata (excluding humans).

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: The authors thank the Fundação de Amparo à Pesquisa do Estado de São Paulo of the State of São Paulo (FAPESP) for funding the project and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the research productivity grant.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

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