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Guess who arrived in the western tropical Atlantic? *Microcosmus squamiger* (Ascidiacea: Pyuridae) records on the Brazilian coast

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Abstract

Microcosmus squamiger, an ascidian with high invasion potential, is recorded for the first time in the Brazilian western Atlantic, between Rio de Janeiro and Espírito Santo. The species was found near ports and marinas, and its introduction may have been favoured by intense nautical activity and climatic events such as La Niña. Coexistence with *Microcosmus exasperatus*, a morphologically similar species, was observed in all localities where *M. squamiger* was recorded. This discovery implies that a more rigorous process of species identification is necessary during monitoring activities, given that both species can be easily confused (only the syphon spinules differentiate them) and *M. exasperatus* is widely distributed with collection records dating back more than half a century on the Brazilian coast. The preference of *M. squamiger* for colder waters suggests that researchers in the Southeast and South Brazil, Uruguay, and Argentina should closely monitor the arrival and possible environmental impacts of this species. The identification of *M. squamiger* in locations close to bivalve mariculture areas in Rio de Janeiro raises concern, as the species has the potential to compete with bivalves. This study highlights the importance of continuing to monitor the potential spread and the implications of the introduction of *M. squamiger* into Brazilian waters, as well as its relationship with *M. exasperatus*, a species already established in this same region.

Introduction

Globalized maritime trade has facilitated the transport of numerous species through fouling on ship hulls or floating platforms (Frey *et al.*, 2014), enabling the establishment of non-native species outside their natural distribution range (Lambert, 2001; Marins *et al.*, 2010). On such vectors, sessile benthic species gain an opportunity to disperse over long distances and different marine environments as adults (Dias *et al.*, 2016), which generates significant environmental and economic implications (Lins and Rocha, 2020; Pires-Teixeira *et al.*, 2021). Ascidians can demonstrate a remarkable capacity for tolerance to wide variations in temperature, salinity, and high levels of pollution (Rocha *et al.*, 2017). This highlights the susceptibility of regions located in areas of intense maritime activity to the introduction of non-native species (Lins *et al.*, 2018). Ascidians have gained increasing recognition as prominent invaders in marine ecosystems, being able to compete effectively with other sessile organisms and potentially disrupting ecosystem functioning in various ways (Zhan *et al.*, 2015).

Microcosumus squamiger Michaelsen 1927 was described from Australia and recent genetic studies considered it a native species of this region, where populations have the greatest local genetic variability and a significantly higher number of unique haplotypes (Rius *et al.*, 2008). Its occurrence is associated with calm and shallow habitats close to the coast. In the last 20 years, this species has been identified in several parts of the world, including the Pacific coast of North America (Lambert and Lambert, 2003), South Africa (Monniot *et al.*, 2001; Holman *et al.*, 2022), the Iberian Peninsula and the Mediterranean Sea (Turon *et al.*, 2007), the Tyrrhenian Sea (Mastrototaro and Dappiano, 2008), the Eastern Aegean Sea (Önen, 2021), and South Korea (Bae *et al.*, 2022) indicating the notable dispersal capability of this species. Due to this dispersal capability and its ability to colonize both natural and artificial substrates, *M. squamiger* has been identified as an exceptional model for studies on the dispersal and establishment of invasive species (Rius *et al.*, 2009; Ordóñez *et al.*, 2013).

Lins *et al.* (2018) predicted the arrival of *M. squamiger* to the South West Atlantic coast of the Americas and this study reports the first record of many individuals in a large stretch of the Brazilian coast. We present a morphological description of those individuals and discuss their spatial distribution and the environmental risk associated with their presence.

Methodology

The research was carried out from the southern coast of Bahia (SBA) – 16°24'S, down to the centre-west of the State of Rio de Janeiro (RJ) – 23°03'S (Figure 1). The region is characterized by the presence of coral reefs in the SBA, and the abundance of rocky substrates in Espírito Santo and Rio de Janeiro. There is a marked change in the direction of the coast in Cabo Frio, RJ, which favours a seasonal upwelling of the South Atlantic Central Water (SACW) during the austral summer (Andrade and Dominguez, 2002; Coe and Carvalho, 2013). Furthermore, these regions experience intense maritime traffic, especially within Ilha Grande Bay, RJ, and Tartaruga Bay, ES (ANTAQ, 2023; MARINETRAFFIC, 2023). During 2022 and 2023, collections were carried out at 25 locations (Table 1), both by free diving in places with depths less than 3 m and by SCUBA diving in places with depths greater than 3 m. Most collections took place on natural substrates, to inventory native species or those well adapted to local conditions.

Microcosmus specimens were collected from substrates that included rocks, corals, and artificial structures such as shipwrecks and breakwaters. To ensure preservation and adequate analysis, the specimens were anaesthetized with menthol diluted in seawater at the time of collection and were subsequently transported to the laboratory, where they were fixed in 96% ethanol. The specimens were dissected and described using routine methods which also included mounting slides of the spinules present in the syphons for observation under an optical microscope (Zeiss Stemi 305), equipped with a digital camera (Zeiss Axiocam ERc 5s), and under scanning electron microscopy (JEOL-JSM-6390-LV) after the samples underwent dehydration in an alcohol concentration gradient (70%, 80%, 90% and 100%). Voucher specimens were deposited in the Zoology Collection of the Faculdade de Formação de Professores (CZFFP), part of the Universidade do Estado do Rio de Janeiro (UERJ).

Results

A total of 36 *M. squamiger* individuals were collected, 18 in Rio de Janeiro (11 in Ilha Grande Bay and 7 between Cabo Frio and Rio das Ostras) and 18 in Espírito Santo, near Vitória/Vila Velha (Table 1). The specimens were collected from natural and artificial substrates.

Microcosmus squamiger Michaelsen, 1927

Material examined: CZFFP-607: 2 individuals, Araraquara Island, RJ, 06 July 2022; CZFFP-608: 4 individuals, Brandão Island, RJ, 06 July 2022; CZFFP-610: 3 individuals, Jorge Grego Island, RJ, 18 October 2022; CZFFP-609: 2 individuals, Lopes Mendes, RJ, 18 October 2022; CZFFP-612: 4 individuals, Comprida Island, RJ, 13 April 2022; CZFFP-611: 4 individuals, Coqueiros Island, RJ, 24 March 2023; CZFFP-613: 1 individual, Itatiaia Island, ES, 21 March 2023; CZFFP-615: 13 individuals, Sereia Beach, ES, 21 March 2023; CZFFP-614: 4 individuals, Tartaruga bay, ES, 20 March 2023.

Description: The animals were found in isolation or aggregations with encrusting algae and bryozoans growing on the tunic (Figure 2A). The animals range from 3 to 5 cm in diameter and are globular with a rigid and thick tunic. Externally, they are reddish brown while internally the tunic is purple. Some of this colour fades after preservation in ethanol. The body wall is beige, with well-separated apical syphons with similar diameters (Figure 2B, C). The syphons have small spinules (between 15–20 μm), shaped as 'roof tiles' or 'fingernails' with serrated edges (Figure 2G).

There are around 14–16 large branched oral tentacles and some smaller ones, all branched to the third order (Figure 2D). The dorsal tubercle is very prominent and large, with the opening forming two spiral loops in opposite directions, as described by Bae *et al.* (2022) (Figure 2E). The dorsal lamina is simple, with a smooth margin, and extends towards the oesophageal opening. The digestive tract is positioned on the left side, the stomach is covered by a brown digestive gland. The intestine is isodiametric and strongly adhered to the body wall, without endocarps, and the anus rim is smooth. Gonads are present on both sides, well adhered to the body wall, and divided into three lobes each, with short gonoducts. On the right side, the gonads are close to the endostyle. The pharynx has 8 to 10 folds on each side and 8 to 12 straight stigmata per mesh. Parastigmatic vessels are present (Figure 2B, F). The distribution of longitudinal vessels for a large individual is as follows: right side _ E 4 *6* 4 *15* 4 *16* 5 *16* 5 *20* 6 *19* 4 *19* 3 *20* 4 *13* 6 3 DL; left side _ DL 4 *14* 5 *13* 4 *18* 4 *20* 4 *19* 3 *17* 4 *15* 4 *15* 4 *16* 3 *12* 3 *6* 3 E.

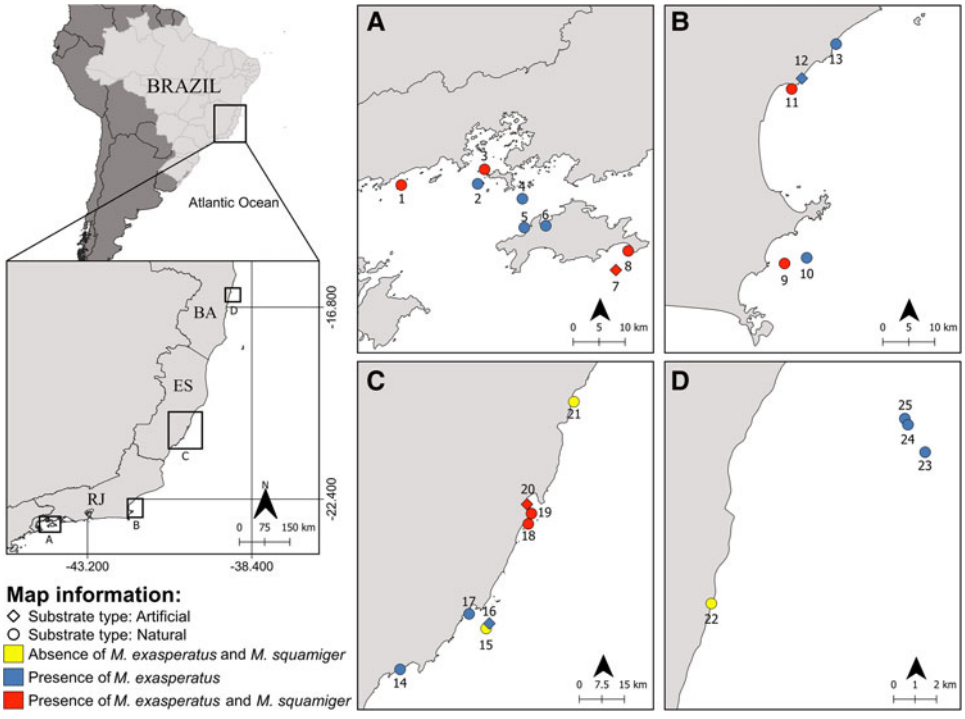

Figure 1. Sampling locations of *Microcosmus squamiger* and *Microcosmus exasperatus* in the studied section of the Brazilian coast. (A and B) Rio de Janeiro, (C) Espírito Santo and (D) South Bahia. Check Table 1 for more information on locations.

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Table 1. Information about the 25 locations investigated in Rio de Janeiro (RJ), Espírito Santo (ES), and Bahia (BA)

State	Sampling sites	ID	GPS coordinate	Date (MM/DD/YY)	Depth (m)	Substrate Type	Abundance
RJ	Araraquara Island	1	23°03'35.0"S 44°33'23.0"W	07/06/2022	5	RR	2
RJ	Búzios Island	2	23°03'27.0"S 44°24'47.3"W	07/05/2022	9	RR	-
RJ	Brandão Island	3	23°01'48.1"S 44°23'59.6"W	07/06/2022	7	RR	4
RJ	Imboassica Island	4	23°05'07.8"S 44°19'45.5"W	07/05/2022	4	RR	-
RJ	Longa Island	5	23°08'20.2"S 44°19'22.0"W	10/19/2022	5	RR	-
RJ	Sítio Forte	6	23°07'47.6"S 44°17'08.9"W	10/19/2022	4	RR	-
RJ	Jorge Grego Island	7	23°13'12.2"S 44°09'47.5"W	10/18/2022	7	S	3
RJ	Lopes Mendes Beach	8	23°10'47.5"S 44°07'28.9"W	10/18/2022	5	RR	2
RJ	Comprida Island	9	22°52'12.5"S 41°57'20.0"W	04/13/2022	18	RR	4
RJ	Pargos Island	10	22°51'24.7"S 41°54'28.6"W	04/13/2022	9	RR	-
RJ	Coqueiros Island	11	22°32'19.4"S 41°56'10.5"W	03/24/2023	2	RR	3
RJ	Submarine Outfall	12	22°31'08.0"S 41°55'01.6"W	03/23/2023	2	P	-
RJ	Mar do Norte Beach	13	22°27'15.3"S 41°51'10.2"W	03/23/2023	2	RR	-
ES	Iriri Beach	14	20°50'04.9"S 40°41'46.1"W	03/22/2023	2	RR	-
ES	Escalvada Island	15	20°42'00.7"S 40°25'00.1"W	03/18/2023	25	RR	-
ES	Victory 8-B	16	20°41'27.8"S 40°23'06.5"W	03/18/2023	28	S	-
ES	Cerca Beach	17	20°39'11.9"S 40°28'17.0"W	03/21/2023	2	RR	-
ES	Itatiaia Island	18	20°21'52.3"S 40°16'51.2"W	03/21/2023	2	RR	1
ES	Sereia Beach	19	20°20'03.2"S 40°16'15.2"W	03/21/2023	2	RR	13
ES	Tartaruga Bay	20	20°18'12.9"S 40°17'11.1"W	03/20/2023	2	B	4
ES	Biologia Beach	21	19°58'23.1"S 40°08'15.7"W	03/19/2023	1	RR	-
BA	Mucugê Beach	22	16°29'45.0"S 39°04'07.4"W	07/28/2022	2	RR	-
BA	Ponta Norte	23	16°25'55.1"S 38°58'07.5"W	07/27/2022	15	CR	-
BA	Recife de Fora I	24	16°25'02.7"S 38°58'58.7"W	07/26/2022	4	CR	-
BA	Recife de Fora II	25	16°24'53.1"S 38°59'03.6"W	07/26/2022	3	CR	-

The numbers present in (ID) correspond to the localities in Figure 1. Substrate type refers to natural substrates (rocky reef (RR) and coral reef (CR)), and artificial ones (piers (P), shipwrecks (S), and breakwaters (B)). The abundance corresponds to the number of *Microcosmus squamiger* individuals collected at each location, and (-) indicates the absence of the species at the location

The description of the specimens here is in agreement with the descriptions of Kott (1985) and Bae *et al.* (2022) and they were well differentiated from *M. exasperatus* that have larger, pointed syphon spinules (Figure 2H). The difference in these structures between both species is even more evident when viewed in scanning electron microscopy (Figure 3).

Discussion

In this study, *M. squamiger* was recorded in approximately 46% of the locations investigated in the State of Rio de Janeiro. None of these locations were within or in direct proximity to ports or marinas. However, it is important to highlight that the areas chosen for investigation in Rio de Janeiro are strategically located on the route of intense international maritime and cabotage traffic, making them potential gateways for several exotic and invasive species (Castro *et al.*, 2017). In Espírito Santo (ES), specimens of *M. squamiger* were collected in locations adjacent to port and marina areas, which also increases the chance of finding exotic species (Clarke Murray *et al.*, 2011). In Southern Bahia (SBA), we did not find *M. squamiger* although *Microcosmus exasperatus* was recorded inhabiting the coral reefs (sites 23, 24, and 25 in Figure 1). In all locations where *M. squamiger* was documented, coexistence with *M. exasperatus* was observed. These two species share remarkable morphological similarities, with the main distinction being based on the shape and size of the spinules found in the syphons of both species (Mastrototaro and Dappiano, 2008). While *M. exasperatus* exhibits larger spicules with a pointed shape, *M. squamiger* has smaller spicules, fewer in number, and with a conformation that resembles nails or shingles (Kott, 1985). The subtlety of the morphological differences is the main reason these two species are confused with each other in different regions of the globe. It is important to highlight that the record of *M. squamiger* represents a recent and unprecedented introduction, not only in Brazilian waters but for the entire western Atlantic. This species had not been previously recorded by any of the research groups dedicated to Ascidiacea in Brazil, taking into account that research groups in Paraná and Rio de Janeiro have more than a decade of biological data in the region where this species was recently found. By 2010, surveys to the north (Rocha *et al.*, 2012) and neither to the south (Dias *et al.*, 2013) ever retrieved *M. squamiger*. On the other hand, *M. exasperatus* is considered a cryptogenic species in Brazil, with collection records dating back to more than half a century of presence on the Brazilian coast (Millar, 1958). Two other species of *Microcosmus* have already been recorded for Brazil: *M. helleri* Herdman, 1881, which differs from *M. squamiger* mainly due to a lower number of pharyngeal folds (6 on each side) and the absence of spinules on the syphons, and *M. anchylodeirus* Traustedt, 1883, which differs in quantity, shape, and position of the gonads (Rocha *et al.*, 2012).

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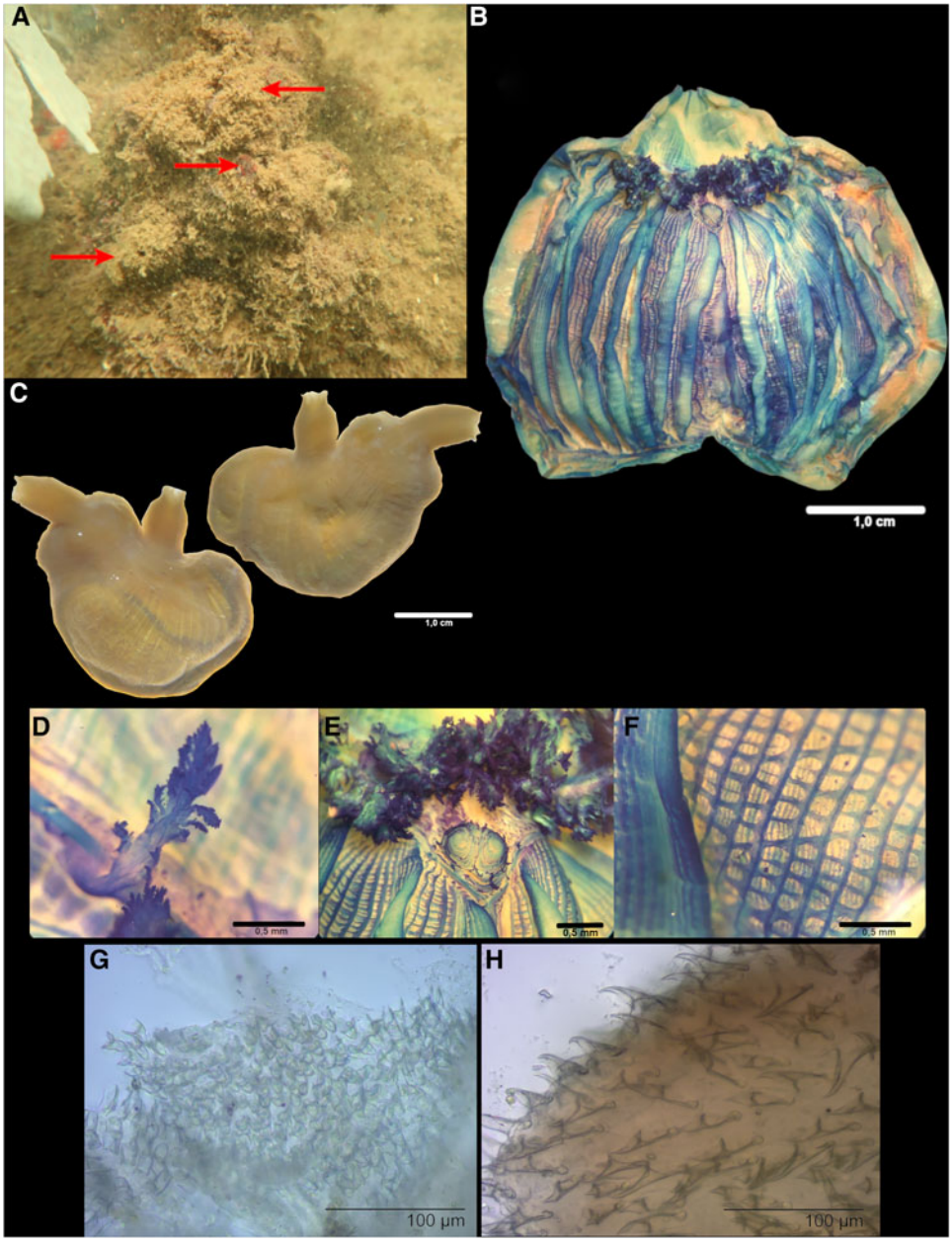

Figure 2. *Microcosmus squamiger* Michaelsen, 1927. (A) Three specimens in the natural environment forming clusters on Sereia beach (ES). (B) Dissected animal with oral tentacles and pharynx stained with methylene blue. (C) Animal removed from the tunic, making it possible to see the muscles. (D) Branched oral tentacle. (E) Dorsal tubercle with aperture in U with enrolled ends. (F) detail of the pharynx with stigmata and some parastigmatic vessels. (G) Syphon spinules of *M. squamiger*. (H) Syphon spinules of *M. exasperatus*, under a light microscope.

Detailed analyses of the life cycle of *M. squamiger* in the Mediterranean Sea indicate that this species is not particularly adapted to high water temperatures, with the optimum temperature for larvae varying between 20 and 25 °C, becoming unviable above 30 °C (Rius *et al.*, 2009, 2014). This might explain its absence in South Bahia, where the water temperature can exceed 27.5 °C in summer, reaching 31.4 °C in shallower reefs (PO.DAAC, 2023). We assume that the records of this species represent a recent introduction on the Brazilian coast, potentially facilitated by climatic events, such as the recent period of three consecutive years of La Niña (2020–23) (Fang *et al.*, 2023). This assumption is based on observations of expanded records of other ascidian species in the same region and during the same period, which might be influenced by a decrease in seawater temperature due to La Niña. For instance, the expanded occurrences of *Rhodosoma turcicum* (Savigny, 1816) and *Cnemidocarpa irene* (Hartmeyer, 1906), species originating or predominantly occurring in the Western Indo-Pacific Ocean (Nishikawa, 1992; Monniot and Monniot, 1994) were observed (Barboza and Skinner, 2021). Additionally, that study has also registered *Ciona robusta* Hoshino & Tokioka, 1967, which had not been found in the Ilha Grande Bay region since 2016 despite continuous monitoring. It is important to highlight that such climatic events, with global repercussions, have the potential to affect population dynamics and directly influence the distribution and survival capacity of benthic and pelagic species (Paes and Moraes, 2007; Wernberg *et al.*, 2012).

The presence of *M. squamiger* in many sites on natural substrates in a large stretch of coast suggests a fast spread of this species and raises concern about the potential impact of the population expansion of this invasive ascidian. Except for Sereia Beach, in Espírito Santo, local abundance now is low, probably as a result of the initial phase of establishment or non-ideal environment conditions, or both. Additionally, the presence of *M. squamiger* in the Baía da Ilha Grande (Figure 1A) raises concerns about population expansion because this region presents several areas designated for mariculture. *M. squamiger* has been reported in other locations as a potential competitor of bivalves, as well as colonizing aquaculture structures such as ropes and cages, thus increasing production costs due to maintenance (Rodriguez and Ibarra-Obando, 2008; Rius *et al.*, 2009; Chebbi *et al.*, 2010; Ali *et al.*, 2014). Its presence in mariculture facilities

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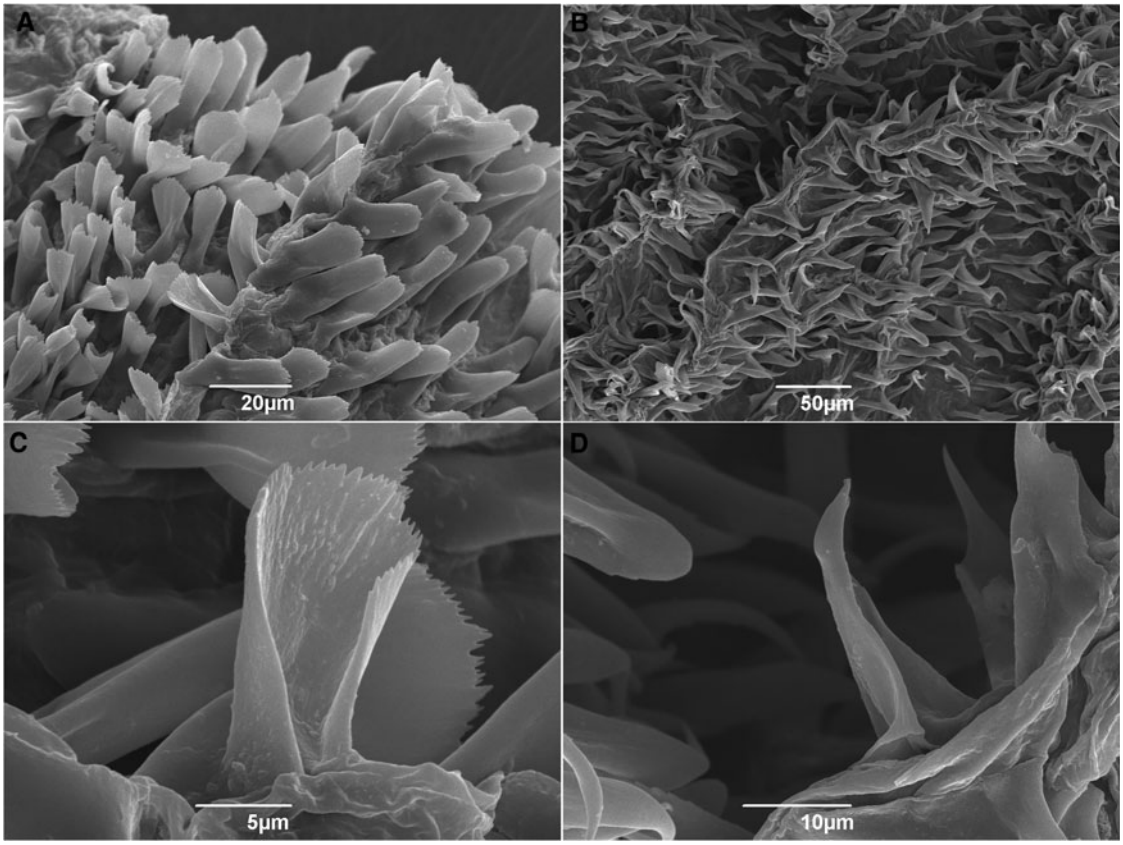

Figure 3. Images of the syphon spines of *Microcosmus squamiger* (A and C) and *Microcosmus exasperatus* (B and D) under scanning electron microscope.

could allow its spread through secondary introductions outside the regions where the species was initially found, as observed for other species (Lins and Rocha, 2023). Lins *et al.* (2018) predicted the arrival of *M. squamiger* on the southwest coast of the Atlantic at latitudes higher than those studied here; thus, we anticipate observing a distribution expansion towards the south. Another outcome could be the disappearance of the species or its limitation to greater depths as a consequence of the predicted El Niño 2023–2024 (NOAA, 2023), because strong El Niños in the past have raised the sea surface temperature in the Ilha Grande Bay to 33 °C (Barboza and Skinner, 2021). Therefore, we recommend that researchers and managers in the states south of Rio de Janeiro, as well as Uruguay and Argentina, remain vigilant regarding the arrival, expansion, and potential environmental impacts associated with the presence of this species in the natural environment.

Data. The data supporting the findings of this study are available upon request from the corresponding author.

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Author contributions. PCAS: Conceptualization, Formal analysis, Data curation, Writing – original draft.

LFS: Formal analysis, Project administration, Resources, Writing – Review and editing.

RMR: Formal analysis, Writing – Review and editing.

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Competing interests. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical standards. All samples were collected following Brazilian environmental laws and animal welfare guidelines for anaesthesia and sacrifice, adhering to best practices. This study was conducted under the ICMBio Licenses #36194-9 and #87660-1, and INEA License #016/2022.

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